UNIVERSITÄTS KLINIKUM FREIBURG

CENTRUM FÜR CHRONISCHE IMMUNDEFIZIENZ

verstehen - erkennen - behandeln

Centrum für Chronische Immundefizienz

GEFÖRDERT VOM
Bundesministerium für Bildung und Forschung

Aus Gründen der Lesbarkeit wird im Folgenden zumeist nur die männliche Form verwendet. Frauen und Männer sind damit gleichermaßen gemeint.

For reasons of the legibility only the male form is used in the following mostly. Women and men are equally meant with it.

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CCI VERSTEHEN - ERKENNEN - BEHANDELN

CCI FORSCHUNGSGRUPPEN

GEMEINSAM FORSCHEN UND BEHANDELN - VONEINANDER LERNEN - FÜR UNSERE PATIENTEN

RESEARCH AND TREAT TOGETHER - LEARN FROM EACH OTHER - FOR OUR PATIENTS

CCI UNDERSTAND - DIAGNOSE - TREAT

CCI RESEARCH GROUPS

Prof. Dr. Stephan Ehl
Medizinischer Direktor CCI

Medical Director CCI

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EDITORIAL

Das Immunsystem ist eines der faszinierendsten Systeme des menschlichen Körpers. Es ist uns angeboren und gleichzeitig fähig, sich ein Leben lang weiterzuentwickeln und immer neuen Herausforderungen zu begegnen. Es ist wie mit den Abwehrspielern einer erfolgreichen Fußballmannschaft: Das Talent ist angeboren, aber nur durch ständiges Training, die Fähigkeit zur blitzschnellen Reaktion auf unterschiedliche Gegner und der Zusammenarbeit im Team, kann die Abwehr erfolgreich verteidigen.
Wenn das Immunsystem fehlerhaft funktioniert, ist der Mensch nicht nur Krankheitserregern ausgeliefert, sondern das Immunsystem kann sich auch gegen den eigenen Körper richten. Welche Auswirkungen es hat, wenn nur ein einzelner Bauteil des Immunsystems nicht richtig funktioniert, zeigt sich bei angeborenen Immundefekten. Die Behandlung und Erforschung dieser seltenen Erkrankungen erlaubt es, Prinzipien zu verstehen, die auch für häufigere Immunerkrankungen von Bedeutung sind.

Das Schicksal von David Vetter, einem amerikanischen Jungen, der in den 70er Jahren mit einem schweren kombinierten Immundefekt (SCID) geboren wurde, hat erstmals auf diese Art von Erkrankung in der breiten Öffentlichkeit aufmerksam gemacht. Da es damals keine Behandlungsmöglichkeiten gab, wuchs David zum Schutz vor Keimen in einem Plastikzelt auf – in der Hoffnung auf die Entwicklung besserer Therapien. David wurde nur 12 Jahre alt.

Heute können wir Patienten wie David besser helfen. Durch intensive Forschung ist es heute möglich, betroffenen Patienten früher eine Diagnose zu geben. Neue Medikamente sowie die Stammzelltransplantation oder die Gentherapie bieten neue Behandlungsmöglichkeiten. Trotz aller Fortschritte werden aber immer noch viele Immundefekte oft zu spät erkannt.

Die Diagnose und Behandlung von Patienten mit immunologischen Erkrankungen stellt eine außerordentliche interdisziplinäre Herausforderung dar. Mit der Einrichtung des Centrums für Chronische Immundefizienz (CCI) am Universitätsklinikum Freiburg wurden die ärztlichen und wissenschaftlichen Kräfte am Standort gebündelt, um das Immunsystem besser zu verstehen und dadurch neue Behandlungen zu entwickeln. Ärzte, Pflegepersonal und Wissenschaftler des CCIs arbeiten täglich daran, diese Erkrankungen zu verstehen, sie früher zu erkennen und die beste Therapie zu ermöglichen.

Das CCI wurde im November 2008 als ein vom Bundesministerium für Bildung und Forschung (BMBF) gefördertes integriertes Forschungs- und Behandlungszentrum gegründet. Die gemeinsamen Anstrengungen des Universitätsklinikums Freiburg und der Fakultät für Medizin, der Fakultät für Biologie und des Max-Planck-Instituts für Immunbiologie und Epigenetik haben den Erfolg des CCI möglich gemacht. Das CCI konnte sich in den letzten Jahren zu einem Referenzzentrum für die Patientenversorgung und Forschung auf dem Gebiet der Immunschwäche und Immunologie entwickeln.

Im Namen des CCI danken wir allen für ihr Vertrauen und ihre Unterstützung auf unserem Weg zu einem einzigartigen, national wie international anerkannten Forschungs- und Behandlungszentrum, das sich der Verbesserung des Verständnisses, der Diagnose und der Therapie von Immundefekten zum Wohle unserer Patienten widmet.

Beginn der Förderung des CCI im November 2008. Offizielles Eröffnungssymposium des CCI im Juni 2009.

Start of CCI funding in November 2008. Official opening symposium of the CCI in June 2009.

EDITORIAL

The immune system is one of the most fascinating systems of the human body. It is innate and at the same time able to evolve over a lifetime to meet always new challenges. It is like the defenders of a successful football team: The talent is innate, but only through constant training, rapid responses to different opponents and team collaboration, can the defense vitally contribute to team achievement. When the immune system fails to function properly, humans are not only susceptible to pathogens, but the immune system may also be directed against the body's own cells. The impact of even a single component of the immune system not functioning properly becomes apparent in congenital immunodeficiencies, which are often caused by a single genetic alteration. The treatment and research of these rare diseases allows for the understanding of principles that are also important for more common immune diseases.

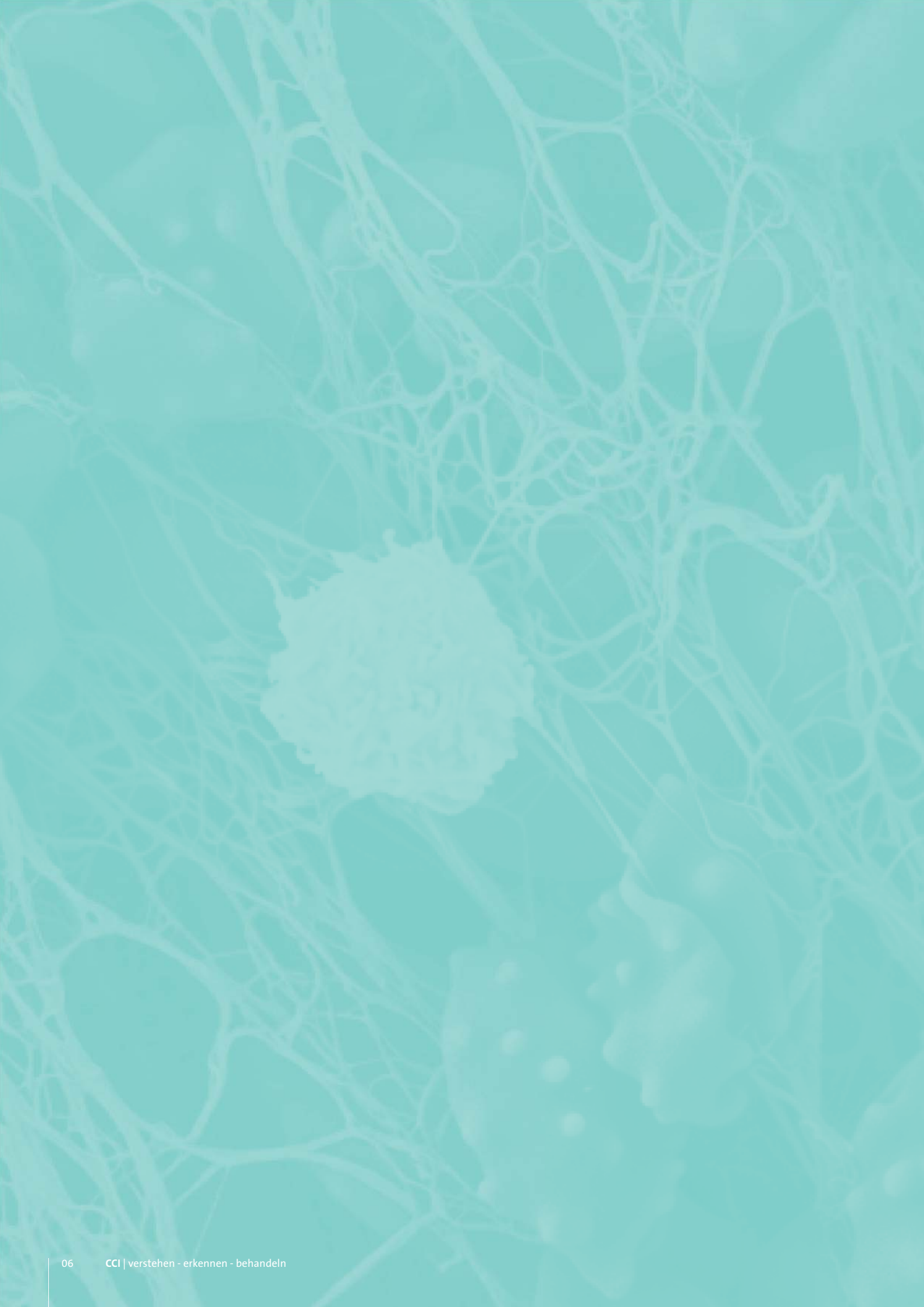
The fate of David Vetter, an American boy who was born in the 1970s with a severe combined immunodeficiency (SCID), brought this kind of disease to the general public's attention for the first time. Since at that time there were no treatment options, David grew up in a plastic tent to be protected against germs - in the hope of the development of better therapies. Yet, David lived only to the age of 12 years.

Today, we can better help patients like David. Through intensive research, it is now possible to detect the diseases earlier. New drugs as well as stem cell transplantation or gene therapy offer new treatment options. Despite all the advances, many immunodeficiencies are still detected too late.

The diagnosis and treatment of patients with immunological diseases represents an extraordinary interdisciplinary challenge. With the establishment of the Center for Chronic Immunodeficiency (CCI) at the Medical Center of the University of Freiburg, the medical and scientific forces were bundled into one institution to better understand the immune system and thereby develop new treatments for immune disorders. The doctors, nurses and scientists of the CCI work every day to understand these diseases, to detect them earlier and to enable the best treatment.

In November 2008, the CCI was funded by the Federal Ministry of Education and Research as an integrated research and treatment center. The joint efforts of the Medical Center and the Medical Faculty, the Faculty of Biology, and the Max Planck Institute of Immunobiology and Epigenetics have made the success of the CCI possible. The CCI has developed into a reference center for patient care and research in the field of immunodeficiencies in recent years.

On behalf of the CCI, we thank everyone for their trust and support on our way to become a unique, nationally and internationally recognized research and treatment center dedicated to improving the understanding, diagnosis and treatment of immunodeficiencies for the benefit of our patients.



CCI | VERSTEHEN - ERKENNEN - BEHANDELN

CCI | understand - diagnose - treat

Prof. Dr. Dr. h.c. mult. Jörg Rüdiger Siewert
Leitender Ärztlicher Direktor des Universitätsklinikums Freiburg

Chief Medical Director of the Medical Center - University of Freiburg

VORWORT

Das Centrum für Chronische Immundefizienz – heute und in der Zukunft

Die Diagnose von Immundefekten und ihre Behandlung sind große Herausforderungen. Nur durch Spitzenleistungen auf dem Gebiet der Patientenversorgung und der Grundlagenforschung, und durch Menschen, die beide Bereichen miteinander verknüpfen, können innovative Ansätze entwickelt werden.

Mit der Gründung des CCI im Jahr 2008 wurden bewusst die traditionellen Grenzen zwischen Erwachsenen- und Kindermedizin, zwischen Immunologie, Hämatologie und Infektiologie, und zwischen Grundlagen- und klinischer Forschung aufgehoben. Die Zusammenarbeit verschiedener Disziplinen ermöglicht Behandlung und Forschung auf höchstem Niveau - zum Nutzen der Patienten. Seit seiner Gründung hat sich das CCI zum größten Zentrum Deutschlands für Patienten mit Immundefizienz aller Altersstufen entwickelt. Das CCI bietet Ärzten und Forschern das Wissen und die technischen Möglichkeiten, ihre Ideen in klinische und experimentelle Projekte umzusetzen, um Patienten mit Immundefekten bessere Behandlungsmöglichkeiten zu bieten.

Zur Unterstützung dieses Exzellenzzentrums wurden sechs Professuren, darunter auch eine Professur für Zell- und Gentherapie, unter dem Schirm des CCI vereinigt. Anfang 2016 wird ein neues Forschungsgebäude den Forschungsgruppen des CCI und des Comprehensive Cancer Center (CCCF) eine neue Heimat bieten. Das Universitätsklinikum Freiburg ist stolz auf das CCI und seine Entwicklung in den letzten Jahren. Wir werden weiterhin das CCI auf seinem Weg zu einem der weltbesten Referenzzentren für Immundefizienz und Immunpathologie begleiten und unterstützen.

Prof. Dr. Dr. h.c. mult. Jörg Rüdiger Siewert

FOREWORD

The Center for Chronic Immunodeficiency – Today and in the future

The diagnosis of immunodeficiencies and their treatment are major challenges. Only through excellence in the fields of patient care and basic research, and by people familiar with both areas linking them together, can innovative approaches be developed.

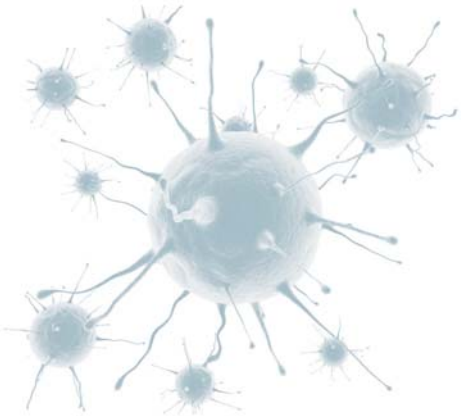
With the establishment of the CCI in 2008, the traditional boundaries between adult and pediatric medicine, between immunology, hematology and infectious disease, and between basic and clinical research were intentionally transcended to make intensive interdisciplinary cooperation possible. This collaboration of different disciplines enables treatment and research at the highest level - for the benefit of patients. Since 2008, the CCI has developed into the largest reference center for patients with immunodeficiencies of all ages. The CCI provides clinicians and researchers with the knowledge and technical capabilities to implement their ideas in clinical and experimental projects in order to provide patients with immunodeficiencies better treatment.

To support this center of excellence, six professorships, including a professorship for cell and gene therapy, were united under the umbrella of the CCI. A research building is under construction that will provide, at the beginning of 2016, a new home to the research groups of the CCI and the Comprehensive Cancer Center (CCCF). The Medical Center - University of Freiburg is proud of the CCI and its development in recent years. We will continue to accompany and support the CCI on its way to become one of the world's best reference centers for immunodeficiency and immunopathology.

Prof. Dr. Dr. h.c. mult. Jörg Rüdiger Siewert

März 2016: Zusammenführung aller Forschungsgruppen des CCI gemeinsam mit Gruppen des Tumorzentrums (CCCF) im Zentrum für Translationale Zellforschung (ZTZ).

March 2016: Housing of all research groups of the CCI together with cancer research groups of the CCCF at the Center for Translational Cell Research.



Am Universitätsklinikum Freiburg legte Prof. Dr. Hans-Hartmut Peter den Grundstein für die Erforschung von Immundefizienz.

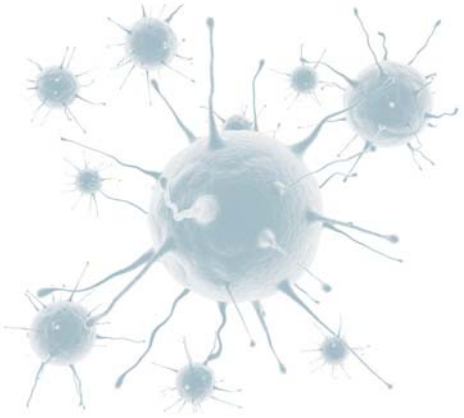
At the Medical Center - University of Freiburg, Prof. Dr. Hans-Hartmut Peter laid the foundation for the study of immunodeficiency.

IMMUNOLOGIE HAT IN FREIBURG TRADITION

In Freiburgs akademischer Geschichte hat die Immunologie schon lange einen besonderen Stellenwert. Sie reicht zurück zu dem Chirurgen Johan Matthias Alexander Ecker, der 1801 in Freiburg die erste erfolgreiche Studie zur Kuhpockenimpfung durchführte. Die erste Beschreibung einer Autoimmunerkrankung gelang 1866 durch die Zusammenarbeit des Freiburger Internisten Adolf Kußmaul mit dem Pathologen Rudolf Robert Maier. Es folgte die Entdeckung der Mastzellen durch Paul Ehrlich in 1877, und die Vorstellung seiner wichtigen Seitenkettentheorie, dem ersten Modell des adaptiven Immunsystems, in 1897. Ludwig Aschhoff, Direktor des Freiburger Pathologischen Instituts, veröffentlichte 1942 das Modell des Retikuloendothelialen Systems (RES). Helmut Schubothe beschrieb 1949 die „Chronische kalte Agglutinin Erkrankung“ als eigenständige Krankheit.

1962 gründete Otto Westphal das Max-Planck-Institut (MPI) für Immunbiologie in Freiburg. In den folgenden Jahren wurde das MPI zur Wiege der modernen immunologischen Ausbildung in Deutschland. Einer der Direktoren des MPI war Georges Köhler, der 1984 zusammen mit Caesar Milstein, für die bahnbrechende Arbeit zur Herstellung monoklonaler Antikörper, den Nobelpreis für Medizin erhielt. Eine weitere Errungenschaft von Georges Köhler war die Einrichtung der Professur für molekulare Immunologie an der Freiburger Fakultät für Biologie, die 1997 mit Michael Reth besetzt wurde. Unter seiner Führung entstand das Centre of Biological Signalling Studies (BIOSS) in Freiburg, das heute eng mit dem CCI kooperiert.

Auf der klinischen Seite übernahm 1984 Hans-Hartmut Peter von Helmut Schubothe die Leitung einer neuen Abteilung für Rheumatologie und klinischer Immunologie am Universitätsklinikum Freiburg. Unter seiner Leitung wurden spezialisierte Ambulanzen für HIV-Patienten und für Patienten mit primärer Immundefizienz eingerichtet. In den späten 90er Jahren gründete Hans-Hartmut Peter das erste europäische Konsortium zur Erforschung von primären Antikörpermangelsyndromen, 2002 etablierte er mit Hanspeter Pircher (Lehrstuhl Immunologie) und Thomas Boehm vom MPI für Immunbiologie und Epigenetik den Sonderforschungsbereich (SFB) 620 zum Thema Immundefizienz. Aus der Verbindung von klinischer Immunologie und der Grundlagenforschung im SFB 620 ging 2008 das Centrum für Chronische Immundefizienz (CCI) als integriertes Forschungs- und Behandlungszentrum hervor. Die Entstehung des CCI ist somit die Konsequenz aus der langjährigen Tradition der wissenschaftlichen und klinischen Immunologie in Freiburg.

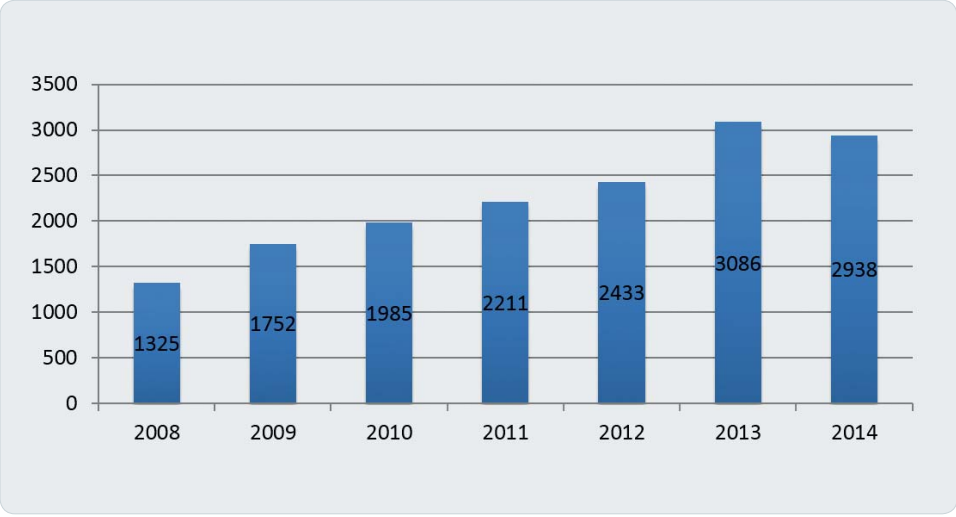


IMMUNOLOGY HAS TRADITION IN FREIBURG

Immunology has long held a special place in Freiburg's academic history. It stretches back to the surgeon Johan Matthias Alexander Ecker, who in 1801 conducted the first successful study on cowpox vaccination in Freiburg. The first description of an autoimmune disease occurred in 1866 through the collaboration of the Freiburg internist Adolf Kußmaul with the pathologist Rudolf Robert Maier. It was followed by the discovery of mast cells by Paul Ehrlich in 1877 and by the presentation of his important side-chain theory, the first model of the adaptive immune system in 1897. Ludwig Aschoff, director of the Institute of Pathology in Freiburg, published in 1942, the model of the reticular endothelial system (RES). Helmut Schubothe described in 1949 „Chronic cold agglutinin disease" as a disease.

In 1962, Otto Westphal founded the Max Planck Institute for Immunobiology (MPI) in Freiburg. In the following years, the MPI became the cradle of modern immunological education in Germany. One of the MPI directors was Georges Köhler who, along with Caesar Milstein, received the Nobel Prize for Medicine in 1984 for the groundbreaking work on producing monoclonal antibodies. Another achievement of Georges Köhler was the establishment of the professorship for Molecular Immunology at the Faculty of Biology, to which Michael Reth was appointed in 1997. Under his leadership, the Centre of Biological Signalling Studies (BIOSS) was born, which today closely cooperates with the CCI.

On the clinical side, in 1984 Hans-Hartmut Peter took over the leadership of a new Department for Rheumatology and Clinical Immunology at the Medical Center from Helmut Schubothe. Under his guidance specialized outpatient clinics for HIV patients and for patients with primary immunodeficiencies were established. In the late 90's, Hans-Hartmut Peter initiated targeted research on the causes of immunodeficiency in Freiburg. A growing cohort of patients with common variable immunodeficiency (CVID) justified the creation of the first European consortium for the study of primary antibody deficiency syndromes. In 2002, Hans-Hartmut Peter, Hanspeter Pircher (Chair of Immunology) and Thomas Boehm (MPI of Immunobiology and Epigenetics) established the Collaborative Research Center (CRC) 620 on immunodeficiency. From the combination of clinical immunology and basic research of the CRC 620 the CCI emerged as an integrated research and treatment center in 2008. The formation of the Center for Chronic Immunodeficiency (CCI) as an integrated center with a strong focus on translational research is thus the consequence of the long tradition of scientific and clinical immunology in Freiburg.



Entwicklung der Patientenkontakte seit Gründung des CCI im Jahr 2008.

Development of patient contacts since the founding of the CCI in 2008.

IMMUNDEFIZIENZ: OFT VERKANNT

„... auf die Abwehr kommt es an"

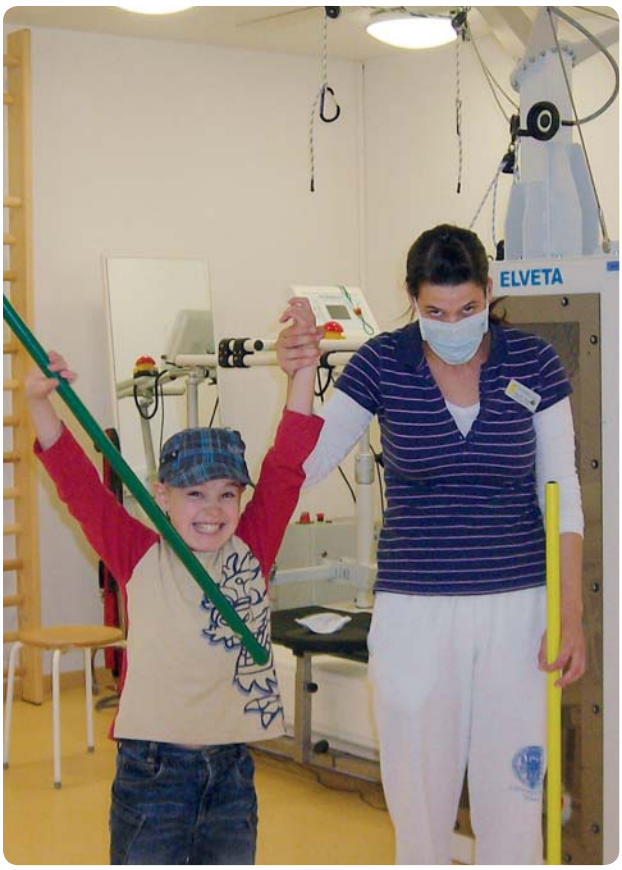
Das Immunsystem ermöglicht dem Körper, gefährliche Erreger zu bekämpfen und Tumorzellen schadlos zu machen, ohne gesunde Zellen zu schädigen. Um diese Aufgaben zu erfüllen, müssen alle Bestandteile des Immunsystems reibungslos zusammenarbeiten. Fällt nur ein Baustein aus, kann das schwerwiegende Folgen haben und ein an sich harmloser Infekt kann zur Gefahr werden. Immunschwäche, Immundefizienz oder Immundefekt sind Bezeichnungen für die mangelnde Fähigkeit des Immunsystems, seine Abwehraufgaben optimal zu erfüllen.

Bei der sekundären oder erworbenen Immundefizienz wird das Immunsystem durch eine äußere Einwirkung geschädigt. Das kann Folge einer Viruserkrankung (z.B. HIV), einer Chemotherapie während einer Krebsbehandlung oder Immunsuppression bei Autoimmunerkrankungen sein. Die erworbene Immunschwäche kann viele Menschen betreffen.

Primäre Immundefekte (PID), von denen es mehr als 250 verschiedene Varianten gibt, sind hingegen angeborene Erkrankungen des Immunsystems. Bei Menschen mit einem angeborenen Immundefekt ist oft eine Veränderung in einem einzigen Gen die Ursache für schwerwiegende Fehlfunktionen des Immunsystems. Durch die Genmutation ist die Bildung oder die Funktion wesentlicher Bestandteile des Immunsystems gestört. Normalerweise harmlose Infekte werden dann zur Bedrohung.

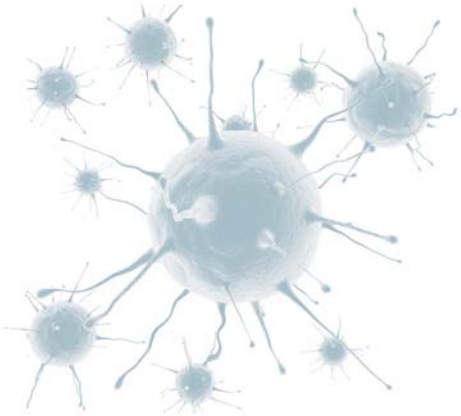
Immunschwache Menschen sind z.B. häufiger, länger und schwerer erkältet. Es kommt zu schweren, oft lebensbedrohlichen Lungenentzündungen und Infektionskrankheiten anderer Organe. Auch eine Fehlsteuerung des Immunsystems mit Fieberschüben, Milz- und Lymphdrüsenschwellungen, entzündlichen Darmerkrankungen und Autoimmunerkrankungen kann Ausdruck eines Immundefekts sein.

Angeborene Immundefekte können schon unmittelbar nach der Geburt zu lebensbedrohlichen Komplikationen führen, zeigen sich aber nicht immer schon im Kindesalter. Auch bei Erwachsenen kann, wie beim variablen Immundefektsyndrom (CVID), ein angeborener Immundefekt Ursache für schwere, häufig wiederkehrende Infektionen sein.



Die Abwehr stärken.

Training the defense.



IMMUNODEFICIENCY: OFTEN UNRECOGNIZED

„... it depends on the defence"

Every day the immune system enables the body to distinguish friend from foe to effectively combat dangerous pathogens and eliminate tumor cells without damaging healthy cells. To fulfill these tasks, the components of the immune system must all work smoothly together. The malfunction of just one component can have severe consequences and a normally harmless infection can become a danger. Immunodeficiency or immune defect are terms for the lack of ability of a person's immune system to perform its defense tasks.

In secondary or acquired immunodeficiency, the immune system is damaged by an external factor. This can be consequence of an infection (such as HIV), chemotherapy in cancer treatment or immunosuppression in autoimmune diseases. Acquired immunodeficiencies can affect many people.

Primary immunodeficiencies (PID), of which there are more than 250 different variants, however, are inborn errors of the immune system. In people with congenital immunodeficiency, it is often a change in a single gene that causes serious malfunctions of the immune system. As a result of the gene mutation, the formation and function of essential components of the immune system is disturbed. Normally harmless infections will then be a threat.

Congenital immunodeficiencies can lead to life-threatening complications almost immediately after birth, but do not always show up in childhood. Sometimes, the symptoms first arise later in life. Even in adults, a congenital immunodeficiency, as in the case of common variable immunodeficiency (CVID), can be the cause of severe, recurring infections often triggered by pathogens that are harmless to healthy immune people.

Immunocompromised individuals are harder hit by colds, catching them more frequently and with longer-lasting symptoms than healthy individuals. Serious, life-threatening infections can occur. An immunodeficiency may also manifest as a dysregulation of the immune system with fever, swollen lymph glands and spleen, inflammatory bowel disease or various autoimmune diseases.

Auf die Abwehr kommt es an.

It depends on the defence.

Primäre Immundefekte sind eine Gruppe von genetischen Erkrankungen, deren einzelne Formen bei 1 in 5.000 bis 1 in 100.000 Menschen auftreten. Es sind inzwischen mehr als 250 Gendefekte beschrieben, die zu Immundefekten führen können. Die Erkrankungen manifestieren sich vor allem mit Infektanfälligkeit, aber auch mit Störungen der Immunregulation (entzündliche Erkrankungen, Autoimmmunerkrankungen). Da diese Krankheitszeichen auch bei Patienten ohne Immundefekt auftreten (beispielsweise die erhöhte Infektanfälligkeit bei Kindern), wird oft viel zu spät an eine zugrunde liegende genetische Erkrankung gedacht.

Die Warnzeichen für einen Immundefekt erkennen:

BEI KINDERN UND ERWACHSENEN

- **Krankhafte Infektanfälligkeit: „ELVIS“**
  Erreger: opportunistische Erreger
  Lokalisation: mehrere Infektionsstellen oder für den Erreger ungewöhnliche Stellen
  Verlauf: unerwartet chronischer oder wiederkehrender Verlauf der Infektion oder unzureichendes Ansprechen auf Behandlung mit Antibiotika
  Intensität: ungewöhnlich schwerer Verlauf
  Summe: ungewöhnlich hohe Anzahl an Infektionen

- **Immundysregulation: „Garfield“**
  Granulome: Gewebeknoten beispielsweise in der Haut, im Darm oder in den Lymphknoten
  Autoimmunität: Überschießende Immunreaktionen gegenüber körpereigenem Gewebe
  Rezidivierendes **Fi**eber: häufig wiederkehrendes Fieber
  Ungewöhnliche **E**kzeme: ungewöhnliche Hautentzündungen
  Lymphoproliferation: krankhafte Vergrößerung von Milz, Leber und Lymphknoten
  Chronische **D**armentzündung: beispielsweise chronische Durchfälle

- Bei Kindern Gedeihstörung: verzögerte körperliche Entwicklung.
  Beispielsweise geringe Gewichtszunahme, geringes Wachstum, Gewichtsverlust
  Bei Erwachsenen Gewichtsverlust, meist mit Durchfall

- Hinweise in der Familiengeschichte: Blutsverwandtschaft der Eltern, Immundefekt, ungewöhnliche Infektanfälligkeit

- Laborbefund: geringe Anzahl von Immunzellen, niedriger Antikörperspiegel

Quelle: www.awmf.org/leitlinien/detail/ll/027-050.html

Primary immunodeficiencies are a group of genetic diseases, whose individual forms occur in 1:5.000-1:100.000 people. There are now more than 250 genetic defects described that can lead to immunodeficiencies. The diseases manifest themselves primarily with susceptibility to infection, but also with disorders of immune regulation (inflammatory diseases, autoimmmune diseases).
As these symptoms also occur in persons that do not suffer from an immunodeficiency (for example, the increased susceptibility to infection in children), an underlying genetic disorder is frequently considered too late.

The warning signs of an immunodeficiency:

IN CHILDREN AND ADULTS

- **Pathological susceptibility to infection, „T-CLIP“**
  Total: unusually high number of infections
  Course: unexpected chronic or recurring infections or incomplete response to antibiotic treatment
  Localization: several sites of infection or unusual location
  Intensity: unusually severe infections
  Pathogens: opportunistic pathogens

- **Immune dysregulation, „Garfield“**
  Granulomas: nodules of tissue, for example in the skin, the gut or the lymph nodes
  Autoimmunity: overactive immune responses against the body‘s own tissues
  Recurrent **F**ever: frequently recurring fevers
  Irregular **E**czema: abnormal skin infections
  Lymphoproliferation: abnormal enlargement of the spleen, liver and lymph nodes
  Chronic intestinal inflammation: for example, chronic **D**iarrhea

- In children, failure to thrive: delayed physical development. For example, poor weight gain, poor growth, weight loss
  In adults, weight loss, usually with diarrhea

- Indications in the family history: parental consanguinity, immunodeficiency, unusual susceptibility to infection

- Laboratory findings: small number of immune cells, low levels of antibodies

Source: www.awmf.org/leitlinien/detail/ll/027-050.html

Selten heißt auch selten zeitig erkannt

„Wir kennen die Schwächen Ihrer Abwehr ...“

Die unspezifischen Anzeichen und die Seltenheit der Erkrankungen erklären, warum die primäre Immundefizienz zu selten erkannt wird. Man geht derzeit davon aus, dass bei ungefähr 80% der Betroffenen der Immundefekt nicht diagnostiziert ist. Patienten, Familien und Hausärzte sind oft ratlos, was die häufigen Infekte und Entzündungen verursacht. Durch Antibiotika, Kortison und andere Medikamente wird versucht, die wiederkehrenden Infektionen und Entzündungen zu behandeln. So vergehen häufig mehrere Jahre, bis der Immundefekt erkannt und zielgerichtet behandelt wird.

Dabei kann eine schnelle Diagnose den Leidensweg verkürzen und Leben retten. Durch die regelmäßige Gabe von Immunglobulinen (Antikörperlösungen) als Infusion oder subkutan in das Unterhautfettgewebe, lässt sich die Immunabwehr vieler Patienten verbessern. Nicht selten kommen Patienten erst dann in die richtige Behandlung, wenn ihre Organe durch die häufigen Infektionen und Entzündungen schon geschädigt sind. Der schwere kombinierte Immundefekt (SCID) muss besonders schnell erkannt werden, denn ohne eine Stammzelltransplantation haben die Kinder kaum Überlebenschancen.

AM CCI STEHT IMMUNSCHWÄCHE IM MITTELPUNKT

„... und machen uns für Ihr Immunsystem stark “

Das CCI widmet sich sowohl der Diagnose und Behandlung von Immundefekten, als auch der Erforschung des Immunsystems. Die Zusammenführung dieser Bereiche in einem spezialisierten Behandlungszentrum ist ein entscheidendes Qualitätsmerkmal des CCI‘s. Nur durch die Zusammenarbeit von Experten verschiedener Fachrichtungen an einem Zentrum lassen sich seltene und gleichzeitig komplexe Erkrankungen behandeln. Das Kernstück des altersübergreifenden Behandlungskonzepts bilden eine Kinder- und eine Erwachsenenambulanz für Patienten mit Immundefizienz. Weiterhin ist auch ein spezialisiertes HIV-Zentrum am CCI angesiedelt. Eine moderne Diagnostik-Einheit, die unmittelbar von den neuesten Forschungsergebnissen profitiert, ermöglicht eine schnelle und genaue Diagnose, neue Therapieansätze kommen dem Patienten unmittelbar zu gute. Betroffenen Patienten können am CCI alle etablierten Behandlungsmöglichkeiten für Immundefizienz, einschließlich der Stammzelltransplantation, angeboten werden. Patienten können wiederum mit Teilnahme an klinischen Studien durch die Bereitstellung von biologischen Proben zur weiteren Erforschung von Immunerkrankungen beitragen. Die intensive klinische Zusammenarbeit unterschiedlicher Fachrichtungen, sowie der wissenschaftliche Austausch werden durch die regelmäßigen interdisziplinären CCI-Konferenzen gewährleistet. Als Deutschlands einziges integriertes Forschungs- und Behandlungszentrum (IFB) für Immundefekte wird das CCI seit 2008 vom Bundesministerium für Bildung und Forschung gefördert.

Rare also means rarely recognized in time

„We know the weaknesses of your defense ..."

The non-specific signs and the rarity of the disease explain why primary immunodeficiency is still seldom recognized. We assume that about 80% of people with an immunodeficiency remain undiagnosed. Patients, families and general practicioners are often at a loss as to what could cause frequent infections and inflammation. Antibiotics, cortisone and other drugs are used to treat the constantly recurrent infections and inflammation. Thus, often several years pass before the immune defect is detected and treated effectively.

Importantly, a rapid diagnosis can shorten the suffering of those affected and save lives. Regular doses of antibody solution (immunoglobulins), given as an infusion or injection under the skin, can improve the immune system of many patients. Unfortunately, the late diagnosis means that many patients only begin the correct treatment when their organs have already been damaged by the frequent infections and inflammation. Severe combined immunodeficiency (SCID) has to be diagnosed particularly rapidly, since without a stem cell transplant in the first year, the children have virtually no chance of survival.

IMMUNODEFICIENCIES TAKE CENTER STAGE AT THE CCI

„... and engage for your immune system"

The CCI is dedicated to both diagnosis and treatment of immunodeficiencies as well as the study of the immune system. The merging of these areas in a specialized treatment center for immunodeficiencies is a crucial attribute of the CCI: only through the integration of experts from various fields at one institution rare and at the same time complex diseases such as congenital immunodeficiencies can be optimally treated. The centerpiece of a patient care concept that spans all ages is formed by a pediatric and an adult outpatient clinic for patients with immunodeficiency. Furthermore, a specialized HIV center is also established at the CCI. A modern diagnostic unit that benefits directly from the latest research results enables rapid and accurate assessment of whether a disease can be explained by an immunodeficiency. At the CCI, patients are offered all established treatment options for immunodeficiencies, including stem cell transplantation for both children and adults. Patients may in turn contribute to the further study of immunodeficiencies by participating in clinical trials or with biological samples. The intensive clinical collaboration between different disciplines and scientific exchanges are ensured by regular interdisciplinary CCI conferences. As Germany's only integrated research and treatment center for immunodeficiencies, the CCI has been funded by the Federal Ministry of Education and Research since 2008.

GEMEINSAM DIE ABWEHR STÄRKEN

PATIENTENVERSORGUNG AM CCI

Patienten, die an einem primären Immundefekt leiden, sind chronisch krank. Sie benötigen eine lebenslange Behandlung und Betreuung, um mit ihrer Krankheit leben zu können. Diagnose und Behandlung erfordern die Kompetenz eines Teams von Experten. Mit den CCI-Ambulanzen wurden verschiedene auf Immundefekte spezialisierte Ambulanzen eingerichtet. Hierzu arbeitet ein konstantes Team aus Fachärzten, Assistenten, Krankenschwestern und Studienassistentinnen zusammen, die auf die Behandlung von Immundefekten spezialisiert sind.

Auch bei einem stationären Aufenthalt werden die Patienten auf immunologisch/infektiologischen Schwerpunkt-Stationen durch das CCI-Team betreut. Eine enge und gut strukturierte Zusammenarbeit mit den Station-Teams ermöglicht schnelle und reibungslose Abläufe. Der Erfolg des Konzepts des CCI zeigt sich in der Zahl der Ambulanzbesuche, die sich von 2009 bis 2013, sowohl bei erwachsenen Patienten als auch bei Kindern, mehr als verdoppelt haben.

Neben den spezialisierten Sprechstunden für Immundefekte beteiligt sich das CCI auch an der ambulanten und stationären Versorgung von Patienten mit sekundären Immunschwächen, die Infektionen, Transplantation oder immunsuppressive Behandlung zur Ursache haben.

Kinderambulanz

Die pädiatrische Immundefekt-Ambulanz des CCI ist räumlich und strukturell fest in das Zentrum für Kinder- und Jugendmedizin eingebunden. Kinder und Jugendliche, die zur Abklärung wiederkehrender Infekte, unklarer Entzündungskrankheiten oder Fieber oder mit bereits erkannten Immundefekten kommen, werden bis zum 18. Lebensjahr durch ein konstantes Team der CCI-Kinderambulanz betreut. Neben dem Leiter, Prof. Dr. Stephan Ehl, kümmern sich zwei Fachärzte und eine spezialisierte Studienschwester um die Patienten - von der ersten Anfrage bis zur komplexen Therapie, wie z.B. eine Knochenmarkstransplantation. Anfragen zur Diagnostik und Therapie von Kindern mit Immundefizienz kommen zunehmend aus der ganzen Welt.

Kinder und ihre Familien sind beim Team der CCI-Kinderambulanz in guten Händen.

Children and their families are in good hands at the CCI pediatric outpatient clinic.

STRENGTHENING THE DEFENSE TOGETHER

PATIENT CARE AT THE CCI

Patients suffering from a primary immunodeficiency are chronically ill. They require lifelong treatment and care in order to live and work with their illness. Diagnosis and treatment require the expertise of a team of experts. With the CCI outpatient clinics, different outpatient clinics specialized in immunodeficiencies were established. This includes a constant team of specialist doctors, assistants, nurses and study nurses who are specialized in the treatment of immunodeficiencies.
Also during a hospital stay, patients are cared for by the CCI team. A close and well-structured cooperation with the inpatient teams in the Pediatric and Internal Medicine departments allows for fast and smooth procedures. The success of the CCI concept can be seen in the number of outpatient clinic visits, which have almost doubled from 2009 to 2013.

In addition to specialized consultations for immunodeficiencies, the CCI is also involved in the inpatient and outpatient care of patients with secondary immunodeficiencies caused by infection, transplantation or immunosuppressive treatment.

Pediatric Outpatient Clinic

The CCI pediatric immunodeficiency outpatient clinic is spatially and structurally embedded in the Center for Pediatric and Adolescent Medicine. Children and adolescents, who present with recurrent infections, unclear inflammatory diseases or fever or with already diagnosed immunodeficiencies are cared for by a constant CCI pediatric outpatient team. Two specialist doctors and a specialized study nurse work alongside the head, Prof. Dr. Stephan Ehl, to care for patients - from the initial inquiry through to the complex therapy, such as bone marrow transplantation. Requests for diagnosis and treatment of children with immunodeficiencies increasingly come from all over the world.

Feste Ansprechpartner am CCI und der enge Austausch mit anderen Spezialisten ermöglichen eine optimale Betreuung von Patrick.

Reliable contacts the CCI and the close collaboration with other specialists provide optimal care for Patrick.

Patrick kann wieder davon träumen, Arzt zu werden

„Wir sind froh, letztendlich in Freiburg gelandet zu sein“

Als Patrick 7 Jahre alt war, wurde bei ihm ein Hyper IgE Syndrom (HIES) entdeckt, ein angeborener Immundefekt. Patienten mit HIES leiden unter einer Multisystemerkrankung, bei der neben dem Immunsystem auch das Bindegewebe, Knochen und Zähne betroffen sind. Es treten oft wiederkehrende Hautinfektionen auf, Hautabszesse und Pilzinfektionen. Schwerwiegender noch sind Lungeninfektionen, die oft einen komplizierten Verlauf zeigen. Damit hatte auch Patrick immer wieder zu kämpfen.

Patrick ist heute 14 Jahre alt und wird seit 2010 am CCI behandelt. Patrick ist medikamentös gut eingestellt und im Großen und Ganzen beschwerdefrei. Momentan gibt es für HIES keine Heilung. Als Behandlung stehen vorbeugende Antibiotika, sowie eine vorbeugende Behandlung gegen Hautpilze zur Verfügung. So nimmt auch Patrick regelmäßig ein Antibiotikum ein. Zusätzlich behandelt er sich homöopathisch mit Schüssler Salzen und achtet sehr auf seine Körperhygiene. „Ich dusche täglich und creme mich immer gut ein, damit meine Haut nicht zu trocken wird“, erzählt Patrick. „Mittlerweile lebe ich sehr gut mit meiner Erkrankung, früher musste ich mich beim Sport und Schwimmen einschränken“, resümiert Patrick.

Bei der halbjährlichen Untersuchung im CCI werden der allgemeine Gesundheitszustand und das Blut von Patrick untersucht, aber auch die Haut und die Lungenfunktion genau geprüft. „Obwohl wir einen relativ weiten Anfahrtsweg haben, kommen wir immer sehr gerne nach Freiburg“, erzählt Patricks Mutter. „Die Ärzte hier sind sehr kompetent, nehmen sich Zeit für uns und wir schätzen die gute Zusammenarbeit der Ärzte in den verschiedenen Kliniken wie HNO, CCI und Kinderklinik sehr. Auch ist immer jemand für uns per E-Mail oder Telefon im Notfall erreichbar.“

Patrick lässt sich von seiner Erkrankung nicht unterkriegen und hat bereits einen festen Berufswunsch: „Ich möchte auch mal Arzt werden.“

Patrick can dream of becoming a doctor again

„We are glad to have landed in Freiburg after all“

When Patrick was 7 years old, he was discovered to have hyper-IgE syndrome (HIES), a rare primary immunodeficiency. Patients with HIES often suffer from a multisystem disorder, in which not only the immune system, but also connective tissue, bones and teeth are affected. Frequently recurring skin infections appear; there are abscess formations on the skin and fungal infections. Worse yet are infections of the lung, which often have a very complicated progression. This is what Patrick had to battle again and again.

Patrick is now 14 years old and has been treated at the CCI since 2010. Patrick's condition is well managed with medication and he is generally symptom-free. Currently, there is no cure for HIES.

The treatment of choice available is prophylactic antibiotics, as well as a preventive treatment against fungal skin infections. So Patrick regularly takes an antibiotic. Additionally, he is treated homeopathically with Schuessler salts and pays close attention to his personal hygiene. „I shower daily and moisturize well, so my skin does not become too dry,“ says Patrick. „I now live quite well with my illness, whereas earlier I had to limit sports and swimming,“ says Patrick.

During his semi-annual visit at the CCI Freiburg, Patrick's general health and blood are checked, and any developments or changes in the skin, ears and lung function are carefully examined. „Although we have a relatively long drive, we are always very happy to come to Freiburg,“ says Patrick's mother. „The doctors here are highly competent, taking time for us, and we appreciate the cooperation of doctors in various clinics such as ENT, the CCI and the Children's Hospital very much. Also, someone is always reachable by e-mail or telephone in an emergency.“

Patrick doesn't let his illness get him down and he already has a solid career aspiration: “I would like to become a doctor.”

Familiensprechstunde

„Wir sind für die ganze Familie da …“

Damit Familien mit mehreren von Immundefizienz Betroffenen eine einheitliche Versorgung erhalten, wurde eine Familiensprechstunde eingerichtet. In dieser Sprechstunde sehen Kinderärzte und Erwachsenenmediziner gemeinsam die ganze Familie. Das erspart den Familien, von Arzt zu Arzt gehen zu müssen. Mit dieser Sprechstunde werden die Bedürfnisse aller Familienmitglieder erfasst.

In der Familiensprechstunde werden Betroffene einer Familie in einer gemeinsamen Sprechstunde von Kinderärzten und Erwachsenenmedizinern betreut.

In the family clinic, pediatricians and physicians take care of all affected persons in one family.

Transitionssprechstunde

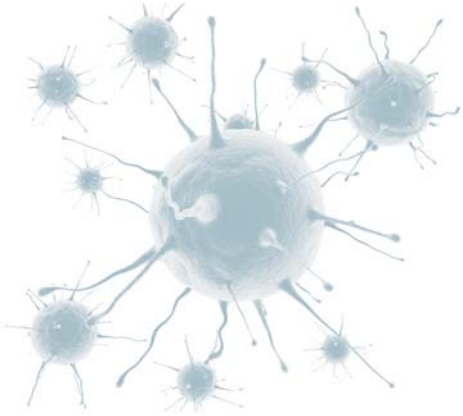
„… und begleiten Sie auf dem Weg zum Erwachsenen“

Da Immundefekte alle Altersstufen betreffen können, wurde am CCI eine Transitionssprechstunde eingerichtet, die Jugendlichen den Übergang von der Kinder- zur Erwachsenenambulanz erleichtert. Am CCI wird Jugendlichen auf dem schwierigen Weg ins Erwachsenenalter geholfen, Verantwortung für ihre chronische Erkrankung zu übernehmen. Das CCI unterstützt die jugendlichen Patienten mit speziellem Informationsmaterial und Patientenschulungen.

Transition bedeutet einen geplanten und strukturierten Übergang von jungen Erwachsenen mit chronischen Erkrankungen von einer Kinder-zentrierten zu einer Erwachsenen-orientierten Gesundheitsversorgung.

Family Consultations

„We are here for the whole family …“



In order to allow families with multiple affected members' consistent care, a family clinic was established. In this consultation, pediatricians and physicians together see the entire family. This saves families from needing to go from doctor to doctor. With this consultation, the needs of all family members are covered.

Transition Consultations

„… and take care of you while becoming an adult“

Since immunodeficiencies can affect all ages, a special transition clinic facilitates the transition of young adults from the pediatric to the adult outpatient clinic. At the CCI, adolescents are accompanied on this often difficult path to adulthood so they can start taking responsibility for their chronic disease. The CCI supports the adolescent patients using special information and patient education material.

Transition means a planned and structured transition of young adults with chronic diseases from child-centered to adult-oriented healthcare.

In der Transitionssprechstunde werden Jugendliche auf den Wechsel von der Kinder- in die Erwachsenensprechstunde vorbereitet.

In the transition clinic, adolescents are prepared for the change from pediatric to adult outpatient clinic.

Erwachsenenambulanz

In Freiburg wurde lange vor Gründung des CCI die erste spezialisierte Immundefektambulanz in Deutschland eingerichtet. Heute ist die CCI-Ambulanz für Erwachsene eine Ambulanz mit großer Erfahrung und Anlaufstelle für Patienten aus dem gesamten Bundesgebiet und darüber hinaus. Erwachsene Patienten mit Immunschwäche werden am CCI durch ein interdisziplinäres Team aus Immunologen und Infektiologen betreut.

Patienten mit angeborenen und erworbenen Immundefekten, oder mit ungewöhnlich verlaufenden Infektionserkrankungen finden hier die richtigen Ansprechpartner. Die unmittelbare Zusammenarbeit mit den Kinderärzten ist ein besonderes Merkmal der klinischen Arbeit und erleichtert nicht nur die Transition, sondern auch die Bewertung der komplexen Krankengeschichten, die oft weit ins Kindesalter zurückreichen.

„Das CCI ist die Anreise wert“

Dirk W., 52 Jahre, lebt in Nordrhein-Westfalen. Mit 37 Jahren kam er mit Verdacht auf Tuberkulose ins Krankenhaus. Vorausgegangen waren häufige Erkältungen und eine Lungenentzündung. Erst in der Klinik wurde in seinem Blut ein niedriger Immunglobulinspiegel entdeckt und die Diagnose eines Variablen Immundefekts gestellt. Eine Antikörper-Ersatztherapie wurde begonnen.

2013 kam Dirk W. auf Empfehlung eines Essener Rheumatologen an das CCI in Freiburg, da er unter Erbrechen ohne klare Ursache litt. Das CCI ist auf die Versorgung sekundärer Komplikationen bei Patienten mit primären Immundefekten spezialisiert. In enger Zusammenarbeit mit Gastroenterologen, Hämatologen und Pathologen konnte ein seltenes Lymphom des Dünndarms als Ursache für das Erbrechen gesichert und operativ erfolgreich entfernt werden.

Er kommt gerne für die halbjährlichen Kontrolluntersuchungen ans CCI. „Ich schätze das gute Verhältnis zu den Schwestern und Ärzten und ich fühle mich gut betreut. Das ist mir die weite Reise wert“, erklärt Dirk W.

Ein kompetentes Team betreut die erwachsenen Patienten am CCI.

A highly competent team looks after the adult patients at the CCI.

Adult Outpatient Clinic

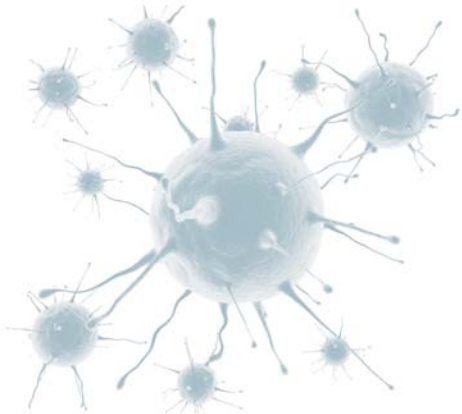
In Freiburg, the first specialized immunodeficiency clinic in Germany was established long before the foundation of the CCI. Today, the CCI outpatient clinic for adults is a clinic with enormous experience and the point of contact for patients from all over Germany and beyond. Adult patients with immunodeficiencies are cared for at the CCI by an interdisciplinary team of immunologists and infectious disease specialists. Patients with congenital and acquired immunodeficiencies or infectious diseases with an unusual course find the right contacts here. Direct cooperation with pediatricians is a particular feature of the clinical work and not only facilitates the transition, but also the assessment of highly complex medical histories, often going back well into childhood.

"The CCI is worth the journey"

Dirk W., 52 years old, lives in North Rhine-Westphalia. At the age of 37, he came to the hospital with suspected tuberculosis. This was preceded by frequent severe colds and pneumonia. Only in the hospital it was discovered that he had low immunoglobulin levels and the diagnosis of common variable immunodeficiency was made. Antibody substitution was initiated.

In 2013, on recommendation of an rheumatologist in Essen, Dirk W. came to the CCI in Freiburg, as he was suffering from vomiting without a clear cause. The CCI is specialized in the clinical care of secondary complications in patients with primary immunodeficiencies. In close cooperation with gastroenterologists, hematologists and pathologists a rare lymphoma of the small intestine was found as the cause of the vomiting, which could subsequently and successfully be removed surgically.

He gladly continues coming for semi-annual checkups to the CCI outpatient clinic. „I appreciate the good relationship with the nurses and doctors of the CCI and I feel well cared for. To me the long journey is worth it", explains Dirk W.



Es sind Patienten wie Silke Braun, die uns immer wieder ermutigen, weiter zu forschen.

Patients like Silke Braun encourage us to continue our research.

Ein starkes Team

Passivität ist nichts für Silke Braun (43). Die Mutter einer 16-jährigen Tochter geht auf Menschen zu. Das gilt auch für Ärzte. Sie wartet nicht darauf bis ihr die Ärzte sagen, was sie tun kann, sondern sie fragt nach. Über E-Mail ist sie mit ihren Ärzten in regelmäßigem Kontakt: Ihrem Internisten Dr. Ralf Weinert in Bad Salzuflen und ihren Ärzten am Centrum für Chronische Immundefizienz (CCI) in Freiburg. Patientin, niedergelassene Ärzte und Klinikärzte bilden ein Team. Dieser engen Zusammenarbeit ist es zu verdanken, dass es Silke Braun heute wieder gut geht, trotz einer schweren Immunerkrankung.

Mehr als 40 Jahre lang konnte sich kein Arzt erklären, was genau mit Silke Braun nicht stimmt. Sie litt an immer wiederkehrenden, schweren Durchfällen und häufigen, lange andauernden Infekten. Die 1,73 m große Frau war schließlich auf 45 kg abgemagert. Sie musste über eine Infusion ernährt werden, da sich ihr Körper weigerte, Nahrung bei sich zu behalten. Als Nierenprobleme und ein Magenkarzinom hinzukamen, war sie mit ihren Kräften beinahe am Ende. Dr. Oliver Pech, Internist in der HSK Wiesbaden, äußerte schließlich den Verdacht auf einen Immundefekt und überwies sie 2012 nach Freiburg ans CCI. Dort war sich Prof. Dr. Bodo Grimbacher aufgrund des Krankheitsbilds und ihrer Familiengeschichte schnell sicher, dass es sich tatsächlich um einen Immundefekt handeln muss und begann eine Therapie mit Kortison und immunsuppressiven Medikamenten. Parallel dazu wurden genetische Untersuchungen durchgeführt, um die Genmutation zu finden, die für ihre Erkrankung verantwortlich ist.

Im Sommer 2014 rief Prof. Dr. Bodo Grimbacher seine Patientin an. Erst kurz zuvor hatte seine Forschungsgruppe am CCI entdeckt, dass eine Mutation im CTLA-4-Gen für mehrere Fälle von schwerer Immundysregulation in einer anderen großen Familie verantwortlich war. Das Krankheitsbild glich dem von Silke Braun. Ein genetischer Test brachte die Gewissheit, dass auch bei Silke Braun eine CTLA-4-Mutation vorliegt. CTLA-4 ist ein wichtiges Molekül einer Signalkette, die für die Abschaltung der Immunantwort nach erfolgreicher Abwehr von Krankheitserregern sorgt. Ohne dieses Molekül ist die Immunsteuerung schwer gestört und richtet sich gegen das körpereigene Gewebe.

Damit hatte der Immundefekt von Silke Braun nicht nur einen Namen, sondern es gab auch eine neue mögliche Therapie durch ein Medikament, das ursprünglich für die Behandlung von rheumatoider Arthritis entwickelt wurde. Dieses Medikament kann genau das CTLA-4 Molekül ersetzen, welches im Immunsystem von Silke Braun defekt ist. In enger Absprache mit ihren behandelnden Ärzten zuhause, setzte Silke Braun andere immunsuppressive Medikamente ab und fing unter Anleitung von Prof. Dr. Bodo Grimbacher mit der neuen Therapie an. In der Hoffnung, dass sich durch diese neue, zielgerichtete Behandlung ihr Zustand bessern würde. Silke Braun und ihr Ärzteteam mussten warten. Die Wirkung solcher Medikamente setzt nicht unmittelbar ein und für die Behandlung von seltenen Immundefekten gab es kaum Erfahrung. Zeit, die sich qualvoll dehnte. Der Patientin ging es nicht gut. Wird das neue Medikament wirken? Wie lange kann es noch dauern? Wie lange darf es noch dauern? Dann, nach beinahe 12 Wochen, kam der Umschwung: Der Gesundheitszustand von Silke Braun besserte sich. Die Darmbeschwerden ließen nach und sie nahm an Gewicht zu. Mit dem neuen Medikament, das sie sich nur einmal in der Woche unter die Haut spritzen muss und einer begleitenden Therapie mit Immunglobulinen hat sie zum ersten Mal seit vielen Jahren ihre Krankheit im Griff. Im Januar konnte sie mit ihrer Familie in den Skiurlaub fahren. Zweimal im Jahr reist sie auf eigene Kosten die 600 km von Herford nach Freiburg zu Kontrolluntersuchungen im Uniklinikum. Beim nächsten Aufenthalt will Silke Braun ihrer Familie Freiburg und das Dreiländereck zeigen.

A Strong Team

Passivity is nothing for Silke Braun (43). The mother of a 16-year-old daughter approaches people. This also applies to doctors. She does not wait until her doctors tell her what they can do, but instead asks them. She is in regular contact with her doctors via e-mail: her internist Dr. Ralf Weinert in Bad Salzuflen and her doctors at the Center for Chronic Immunodeficiencies (CCI) in Freiburg. Patient, private practitioners and clinicians form a team. It is thanks to this close cooperation that Silke Braun is now well again, despite a severe immune disorder.

For more than 40 years, no doctor could explain what exactly was wrong with Silke Braun. She suffered from recurrent, severe diarrhea and frequent, long-lasting infections. The 1.73 m tall woman was eventually emaciated to 45 kg. She had to be fed through an IV line because her body refused to keep food down. As kidney problems and gastric carcinoma developed, she was almost out of strength. Dr. Oliver Pech, internist at the HSK Wiesbaden, finally expressed suspicion of an immunodeficiency and in 2012 referred her to the CCI in Freiburg. There, Prof. Dr. Bodo Grimbacher was quickly certain because of the clinical picture and her family history, that it must in fact be an immunodeficiency, and began treatment with corticosteroids and immunosuppressive drugs. In parallel, genetic studies were carried out to find the gene mutation responsible for her disease.

In the summer of 2014 Prof. Dr. Bodo Grimbacher called his patient. Just shortly before, his research group at the CCI had discovered that a mutation in the CTLA-4 gene was responsible for several cases of severe immune dysregulation in another large family. The clinical picture resembled that of Silke Braun. A genetic test provided the certainty that a CTLA-4-mutation was also present in Silke Braun. CTLA-4 is an important molecule of a signaling cascade that ensures the immune response switches off after successful defense against pathogens. Without this molecule, the immune control is severely disrupted and directs itself against the body‘s own tissues.

Thus, the immunodeficiency of Silke Braun had not only a name, but there was also a new potential therapy using a drug that was originally developed for the treatment of rheumatoid arthritis. This medication can precisely replace the CTLA-4 molecule that is defective in the immune system of Silke Braun. In close consultation with her physicians at home, Silke Braun discontinued other immunosuppressive drugs and started with the new therapy under the guidance of Prof. Dr. Bodo Grimbacher. In the hope that her condition would improve with this new, targeted treatment, Silke Braun and her medical team had to wait. The effect of these drugs does not start immediately and for the treatment of rare immunodeficiencies, there was little experience. Time stretched painfully. The patient was not doing well. Would the new drug work? How much longer can it take? How long should it take? Then, after nearly 12 weeks, the turnaround came: The health status of Silke Braun improved. The intestinal complaints subsided and she gained weight. With the new drug, which she has to inject under the skin once a week, and a concomitant therapy with immunoglobulin, she has for the first time in many years her disease under control. In January, she was able to go on a skiing holiday with her family. Twice per year she travels at her own expense the 600 km from Herford to Freiburg for check-ups at the Medical Center. The next time, Silke Braun wants to show her family Freiburg and the tri-border region.

Therapieschulung

Spezielle Schulungsangebote des CCI helfen Patienten, den Umgang mit der Erkrankung im Alltag zu erleichtern, die Lebensqualität zu steigern und den bestmöglichen Therapierfolg zu erzielen. Die Mitarbeiter des CCI unterstützen Patienten dabei, Heimtherapien zu erlernen, um den Alltag wieder selbstbestimmter leben und die Zahl der Klinikaufenthalte verringern zu können. Das CCI beteiligt sich auch an deutschlandweit stattfindenden Schulungswochenenden für Patienten mit Immundefekten.
www.pid-schulung.de.

Die subkutane Gabe der Antikörpertherapie erleichtert den Alltag für große und kleine Patienten mit Antikörpermangelerkrankungen.

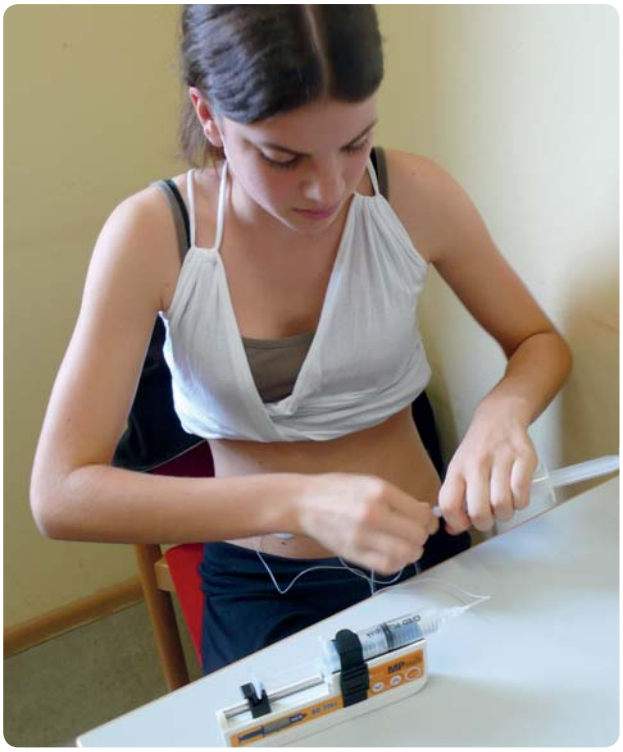
The subcutaneous administration of antibody therapy makes life easier for young and old patients with antibody deficiency diseases.

Antikörperersatztherapie als Heimtherapie

Liegt ein Antikörpermangel vor, wird eine Antikörperersatztherapie notwendig, was bedeutet, dass die Antikörper (Immunglobuline) regelmäßig ersetzt werden müssen, um den Körper vor Infektionen zu schützen. Die Antikörperersatztherapie kann über die Vene (intravenös) in der Klinik oder Arztpraxis durchgeführt werden, oder als Heimtherapie über das Unterhautfettgewebe (subkutan) gegeben werden. Für die Heimtherapie bietet das CCI Schulungen an, um den Patienten die nötige Sicherheit im Umgang mit den Hilfsmitteln und Medikamenten zu geben.

Therapy education

Special training courses of the CCI help patients to deal more easily with their disease in everyday life, to enhance the quality of life and provide the best possible therapeutic success. The staff of the CCI supports patients to learn home therapies in order to live more self-sufficiently again and reduce the number of hospital stays. In addition to patient education in the clinic, the CCI is also involved in weekend courses held throughout Germany for patients with primary immunodeficiencies.
www.pid-schulung.de.



Die Heimtherapie trägt für viele Patienten wesentlich zu einer Steigerung der Lebensqualität bei.

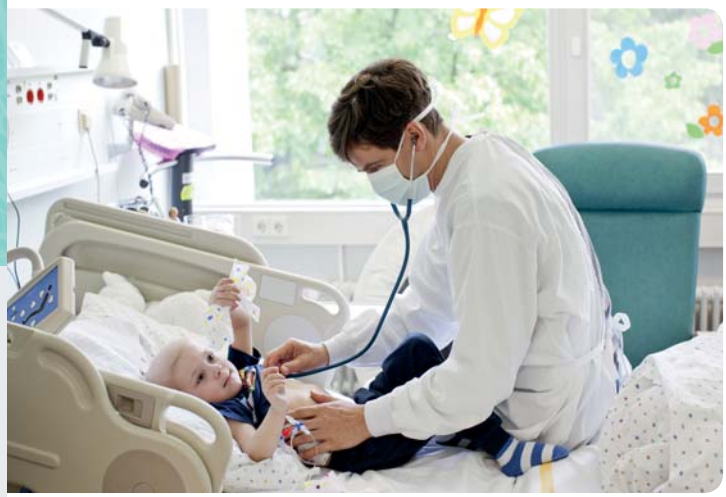
The home therapy helps many patients to enhance their quality of life significantly.

Antibody replacement therapy as home therapy

If there is a lack of antibodies, antibody replacement therapy is needed. In this case, antibodies (immunoglobulins) have to be supplied periodically to protect the body against infection. The antibody replacement therapy can be given via a vein (intravenously) in the hospital or doctor's office, or be given as a home therapy via the adipose tissue (subcutaneously). The CCI provides training to show the patients how to handle the equipment and medicines required for home therapy safely, thus creating a safe environment for home therapy.

Stammzelltransplantation

„Wir bieten moderne Therapien an“



täglichen Visiten. Die unmittelbare Verfügbarkeit spezialisierter immunologischer Diagnostik und die Zusammenarbeit von Immunologie, Infektiologie und Hämatologie in einer Einrichtung erlaubt es, eine optimale Versorgung anzubieten. Aktuell wird in Freiburg jährlich bei rund zwölf Kindern und zwei Erwachsenen mit Immundefekt eine Stammzelltransplantation durchgeführt. Die Anfragen nehmen deutlich zu. Die Weiterentwicklung der Zell- und Gentherapie ist ein wichtiger Schritt, um Patienten mit Immundefekten auch in Zukunft modernste Therapien anzubieten.

Für einige Formen der angeborenen Immundefekte, bei denen Immunglobuline nicht ausreichen, kann eine Stammzelltransplantation lebensrettend sein. Dies betrifft vor allem schwere Immundefekte im Säuglingsalter, manchmal aber auch bei Erwachsenen. Hierbei wird das defekte Immunsystem durch das intakte Immunsystem eines passenden Spenders ersetzt. Das Besondere am CCI ist die Tatsache, dass höchste Kompetenz in den Bereichen der klinischen Immunologie und der Stammzelltransplantation zusammenkommen. Die Immunologen begleiten die Transplantationen, die in den hämatologisch-onkologischen Abteilungen der Kinderklinik und der Inneren Medizin durchgeführt werden, in

In den vergangenen Jahren wurde für folgende Immundefekte eine Stammzelltransplantation am CCI durchgeführt:

- SCID (schwere kombinierte Immundefekte)
- CID (kombinierte Immundefekte)
- HLH (Hämophagozytose Syndrom)
- Griscelli- und Chediak-Higashi Syndrom
- IPEX (Immundysregulation, Polyendokrinopathie X-linked)
- CGD (septische Granulomatose)
- WAS (Wiskott Aldrich Syndrom)
- SAP und XIAP Defekte
- CD40 und CD40 Liganddefekte
- NEMO Defekt
- Knorpel-Haar Hypoplasie

Stem cell transplantation

„We offer modern therapies“

For some forms of congenital immunodeficiencies in which the use of immunoglobulins are not sufficient, a stem cell transplantation can be life-saving. This mainly applies to severe immunodeficiencies in infancy, but sometimes also in adults. Here, the defective immune system of the patient is replaced by a healthy immune system of a matched donor. The special feature of the CCI is that here the highest expertise in the fields of clinical immunology and stem cell transplantation converges. The immunologists accompany the transplantations which are performed in the Hematology/ Oncology Departments of the Children's Hospital and Internal Medicine in daily patient rounds. The immediate availability of specialized immunological diagnostics and the collaboration of immunology, infectious diseases and hematology in one institution allows for optimal patient care. Currently, about twelve children and two adults with immunodeficiencies receive a stem cell transplant in Freiburg each year. The inquiries are markedly increasing. The further development of cell and gene therapy is an important step to offer cutting-edge therapies to patients with immunodeficiencies also in the future.

Moderne Therapien helfen Patienten wieder ein normales Leben zu führen.

Modern therapies help patients to lead a normal life again.

In recent years, stem cell transplantation was performed for the following immunodeficiencies at the CCI:

- SCID (severe combined immunodeficiencies)
- CID (combined immunodeficiencies)
- HLH (hemophagocytic syndrome)
- Griscelli and Chediak-Higashi Syndrome
- IPEX (immune dysregulation polyendocrinopathy enteropathy x-linked)
- CGD (Chronic granulomatous disease)
- WAS (Wiskott Aldrich Syndrome)
- SAP und XIAP Defects
- CD40 und CD40 ligand deficiencies
- NEMO Deficiency
- Cartilage–hair hypoplasia

Die enge Zusammenarbeit der Kinderambulanz des CCI mit der Klinik für Pädiatrische Hämatologie und Onkologie ist entscheidend, wenn es, wie bei Moritz, um eine Stammzelltransplantation geht.

The close cooperation of the CCI's pediatric outpatient clinic with the Department of Pediatric Hematology and Oncology is crucial when, as with Moritz, a stem cell transplant is in question.

Eine Stammzelltransplantation hilft Moritz

„Freiburg ist zu einer zweiten Heimat für uns geworden"

Moritz kam 2009 zum ersten Mal mit seiner Mutter zu Prof. Dr. Stephan Ehl in die CCI-Ambulanz. Der heute 16-jährige Junge litt seit seiner Geburt an septischer Granulomatose. „Wir sind glücklich, nach langen Irrfahrten in Freiburg gelandet zu sein. Moritz wurde hier in Freiburg so gut behandelt, dass man sagen kann, er ist heute geheilt", erklärt die Mutter von Moritz. „Wir fühlten uns hier im CCI und der Kinderklinik sofort gut aufgehoben. Besonders aufgefallen ist mir die interdisziplinäre und sehr kompetente Betreuung – und in Freiburg scheut man sich nicht, auch den Rat von Spezialisten aus anderen Kliniken einzuholen. Wir hatten von Anfang an ein gutes Gefühl", resümiert sie ihren ersten Eindruck. Aufgrund der Schwere der Infektionen, die Moritz immer wieder durchlitt und dem schweren Verlauf einer begleitenden entzündlichen Darmerkrankung, entschloss sich das Expertenteam 2010 zu einer Stammzelltransplantation. Doch auch hier musste die Familie von Moritz noch einmal große Unsicherheiten durchstehen: Zu der Angst vor Komplikationen, die eine Stammzelltransplantation mit sich bringen kann, kam hinzu, dass die ersten beiden passenden Fremdspender ihre lebensrettende Teilnahme zurückzogen. „Doch der Dritte machte mit", strahlt Moritz noch heute. Nach zehn Tagen vorbereitender Chemotherapie, während der Moritz seine Haare verlor, wurde die Gabe der Stammzellen vorgenommen und nach fünf Monaten wurde Moritz entlassen. „Die Zeit in der Kinderklinik war einzigartig, die Ärzte und Schwestern und das Personal nahmen sich viel Zeit für uns. Wir lernten in dieser schweren Zeit im Elternhaus viele andere Familien kennen, mit denen wir noch heute befreundet sind", erzählt die Mutter von Moritz und fügt hinzu: „Besonders wertvoll war die psychologische und soziale Betreuung oder die Kunsttherapie für Moritz. Das hat uns sehr viel Rückhalt gegeben." Seit dem Eingriff ist der Defekt der Granulozyten behoben und Moritz lebt beschwerdefrei. Er half nun schon zum zweiten Mal bei der Heuernte auf dem Bauernhof seiner Eltern mit, während es ihm vorher wegen der Infektionsgefahr nicht einmal möglich war, unbeschwert im Garten zu spielen. „Für uns ist es wie ein geschenktes neues Leben", sagt seine Mutter. Zwei Mal im Jahr reist Moritz mit seiner Mutter über 600 km zur Untersuchung im CCI nach Freiburg. „Wir freuen uns immer wieder auf Freiburg. Freiburg ist zu einer zweiten Heimat für uns geworden".

Die septische Granulomatose (CGD) ist ein seltener Gendefekt, bei dem die Funktion der neutrophilen Granulozyten gestört ist. Granulozyten erkennen Krankheitserreger, die in den Körper eingedrungen sind, nehmen sie auf und machen sie unschädlich. Bei Patienten mit CGD breiten sich Krankheitserreger wie Bakterien und Pilze weitgehend ungehindert im Körper aus und verursachen schwere Infektionen, die nur durch die dauerhafte Einnahme von Antibiotika und anderen Medikamenten bekämpft werden können. Ungefähr eines von 100.000 Neugeborenen wird mit dieser schweren Störung des Immunsystems geboren. In Deutschland sind rund 150 Patienten bekannt.

Stem cell transplantation helps Moritz

„Freiburg has become a second home to us“

Moritz came for the first time with his mother to Prof. Dr. Stephan Ehl at the CCI outpatient clinic in 2009. The now 16-year-old boy has suffered from chronic granulomatous disease since birth. “We are so happy to have landed in Freiburg after a long journey with many wrong turns. Moritz was treated so well here in Freiburg that one can say he is now cured,” explains his mother with relief. “We felt immediately well cared for here at the CCI and Children’s Hospital. What particularly strucked me was the interdisciplinary and highly competent support – and in Freiburg one does not shy away from seeking advice from specialists from other hospitals. From the beginning, we had a good feeling,” she says, summing up her first impression. Due to the severity of repeated infections and the severe progression of an accompanying inflammatory bowel disease, the expert team decided in 2010 to perform a stem cell transplantation. However, even here, Moritz’s family once again had to endure major uncertainties: In addition to the fear of complications that come with a stem cell transplant, the first two matching unrelated donors withdrew their lifesaving participation. “But the third participated,” beams Moritz today. After ten days of preparatory chemotherapy, during which Moritz lost his hair, the procedure was performed and after five months Moritz was released from the hospital. “The time in the Children’s Hospital was exceptional: The doctors, nurses and other staff took a lot of time for us and during this difficult time we met many other families in the housing for parents, with whom we are still friends today,” says Moritz’s mother and then adds, “Especially valuable were the psychological and social care or art therapy for Moritz, which really gave us a lot of support.” Since the procedure, the defect of granulocytes has been corrected and Moritz lives symptom-free. He has already helped for the second time with the hay harvest on the farm of his parents, whereas previously it was not even possible for him to freely play in the garden for risk of infection. “For us it is like we have been given a new life,” says Moritz’s mother emotionally. Twice a year Moritz travels with his mother over 600 km for his examination in Freiburg. “We always look forward to Freiburg. Freiburg has become a second home to us.”

Chronic granulomatous disease (CGD) is a rare genetic defect, in which the function of the neutrophilic granulocytes is disturbed. Granulocytes recognize pathogens that have invaded the body, engulf them and render them harmless. In patients with CGD, pathogens such as bacteria and fungi can spread widely throughout the body unhindered and severe infections can only be combatted with constant medication, such as antibiotics. Approximately one in 100,000 newborns is born with this serious disorder of the immune system. In Germany, there are roughly 150 known patients.

Neueste Forschungserkenntnisse und Medikamente werden den Patienten zeitnah zugängig gemacht.

Latest research findings and drugs are promptly made available to the patients.

HIV-Zentrum

Die Infektion mit dem HI-Virus ist dank einer lebenslangen medikamentösen Therapie zu einer behandelbaren, aber immer noch ernsten Erkrankung geworden. Das Auftreten des erworbenen Immundefektsyndroms „AIDS“ (englisch: Acquired Immune Deficiency Syndrome) kann durch Medikamente überwunden oder verhindert werden. Die Patienten sind HIV-infiziert, aber eben nicht AIDS-erkrankt.

Im HIV-Zentrum am CCI werden Kinder und Erwachsene betreut, die mit HIV infiziert und erkrankt sind. Dabei verwirklicht das HIV-Zentrum die vielseitigen Ansprüche an die Behandlung der HIV-Infektion mit einem neuen Konzept. Die Basisversorgung übernimmt das Team um Dr. Susanne Usadel in enger Kooperation mit dem CCI und den Ärzten der Abteilung Infektiologie unter Leitung von Prof. Dr. Winfried Kern. Die persönliche und effiziente Betreuung in einer niedergelassenen Praxis am Universitätsklinikum gibt den Patienten Sicherheit und erlaubt eine enge Verzahnung von Versorgung und Wissenschaft zum Wohle der Patienten.

Die Zusammenarbeit des HIV-Zentrums mit der Universitätsfrauenklinik macht es HIV-positiven Frauen möglich, eine von Diskriminierung und Stigmatisierung befreite Schwangerschaft zu erleben.
HIV-exponierte Neugeborene, die ein potentielles Ansteckungsrisiko während der Schwangerschaft hatten, sowie HIV infizierte Kinder werden von Kinderärzten im CCI gesehen.

Dank des interdisziplinären Teams im HIV-Zentrum können Patienten und ihre Familien individuell versorgt, behandelt und beraten werden - auch in psychischen und sozialen Aspekten. Neueste Forschungserkenntnisse und Medikamente werden den Patienten zeitnah zugängig gemacht.
Der Stellenwert des HIV-Zentrums spiegelt sich in mehr als 300 Patientenkontakten pro Jahr wider.

Die enge Zusammenarbeit der Ärzte des HIV-Zentrums, der Universitätsfrauenklinik und der Kinderärzte am CCI hilft HIV-positiven Frauen während der Schwangerschaft und nach der Geburt.
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The close collaboration of doctors of the HIV Center, the Department of Obstetrics and Gynecology, and the pediatricians at CCI helps HIV-positive women during pregnancy and after birth.
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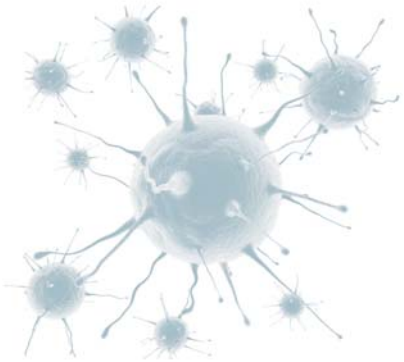


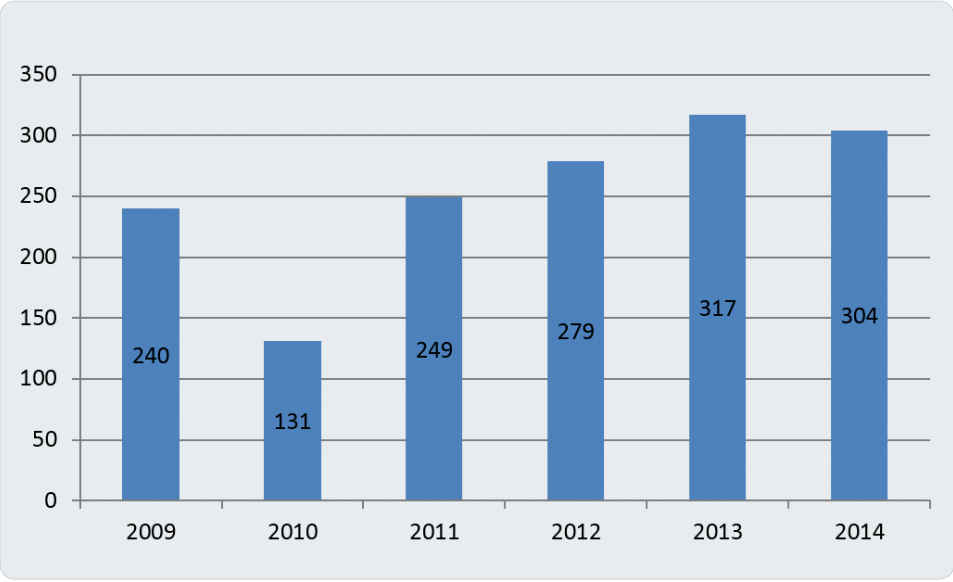
HIV Center

Thanks to new drugs, infection with HIV has become a disease that is treatable, though still potentially serious. The occurrence of the so-called secondary immunodeficiency or acquired immune deficiency syndrome „AIDS“ can thus be prevented. The patients are HIV-infected, but do not have AIDS. In the HIV Center at the CCI, children and adults that are infected with HIV and fallen ill are treated and cared for. The HIV Center thereby meets the various demands of a HIV infection treatment with a new concept. The team led by Dr. Susanne Usadel oversees the basic care of adult HIV patients in close collaboration with the CCI and the doctors of the Infectious Diseases Department, headed by Prof. Dr. Winfried Kern. The personal and efficient service in an established practice at the Medical Center gives patients security. The combined effort of doctors at the HIV Center enables a strong interplay of care and science for the benefit of patients.

The cooperation of the HIV Center with the Department of Obstetrics and Gynecology makes it possible for HIV-positive women to experience a discrimination- and stigma-free pregnancy.

HIV-exposed newborns who had a potential risk of infection during pregnancy and HIV-infected children and adolescents are seen by pediatricians at the CCI.

Thanks to the interdisciplinary team at the HIV Center, patients and their families receive individualized treatment, care and advice- also for psychological and social aspects. The latest research findings and medicines are promptly made accessible to the patients. The importance of the HIV Center is reflected in more than 300 patient contacts per year.



Entwicklung der Besuche im HIV-Zentrum.

Progression of visits to the HIV Center.

Der Austausch der Ärzte am CCI mit niedergelassenen Ärzten und Spezialisten anderer Kliniken ermöglicht eine rasche Diagnose und die bestmögliche Therapie für Patienten.

The exchange between the doctors at CCI with general practitioners and specialists from other clinics enables rapid diagnosis and the best treatment for patients.

Das CCI als Partner

„Wir unterstützen Sie und beantworten Ihre Fragen“

Das CCI ist die zentrale Anlaufstelle für zuweisende Ärzte, die bei der Diagnose und Behandlung von Patienten mit Immundefizienz Unterstützung suchen. Die niedergelassenen Ärzte und zuweisenden Kliniken sind wichtige Partner für das CCI. Ihre erste Verdachtsdiagnose auf einen möglichen Immundefekt ist entscheidend für das Schicksal der Patienten. Das CCI kann durch die Erfahrung mit diesen seltenen Erkrankungen bei der Diagnose helfen und ein individuelles Therapiekonzept erstellen. Die weitere Betreuung erfolgt dann in engem Kontakt mit den einweisenden Ärzten.

Gespräch mit Dr. Holger Hauch, Oberarzt in der Abteilung Hämato-Onkologie an der Universitätskinderklinik Gießen und Dr. Marcus Dahlheim, niedergelassener Kinderpneumologe und Allergologe in Mannheim.

Seit wann arbeiten Sie mit dem CCI zusammen? Haben Sie einen direkten Ansprechpartner?

Dr. Dahlheim: Wir in der Praxis Kinderlunge arbeiten seit drei Jahren mit dem CCI zusammen, wobei ich immer entweder Herrn Prof. Dr. Stephan Ehl oder Dr. Carsten Speckmann als Ansprechpartner habe.

Dr. Hauch: Ich empfinde es auch als sehr angenehm, immer denselben Ansprechpartner zu haben, das erleichtert die Kommunikation für uns zuweisende Ärzte enorm. Wir arbeiten seit Jahren mit dem CCI zusammen.

Wie kam es zu der Zusammenarbeit mit dem CCI?

Dr. Hauch: Ich kannte bereits Frau Prof. Gritta Janka, Klinik für Pädiatrische und Hämatologie und Onkologie des Universitätsklinikum Hamburg-Eppendorf, die sich bereits seit 30 Jahren mit der seltenen, aber lebensbedrohlichen Immunerkrankung Hämophagozytäre Lymphohistizytose (HLH) beschäftigt. Durch sie kam der Kontakt zu Prof. Dr. Stephan Ehl und Dr. Carsten Speckmann zustande.

Dr. Dahlheim: Bei mir war die Situation etwas anders. Ich habe gezielt nach regionalen klinischen Ansprechpartnern im Bereich Immundefekte gesucht. Leider gibt es aber im Mannheimer Raum nichts in dieser Art. Daher kamen entweder die Charité Berlin oder das CCI in Freiburg in Frage. Wir haben uns damals aufgrund der kürzeren Entfernung für Freiburg entschieden und den Schritt nie bereut.

Welche Erfahrungen haben Sie mit dem CCI gemacht?

Dr. Dahlheim: Nur die besten! Die Zusammenarbeit mit dem CCI ist mehr als unkompliziert, wir bekommen immer eine schnelle Reaktion auf E-Mails, die klinischen Ansprechpartner stehen uns jederzeit mit Rat und Tat zur Seite und die Empfehlungen haben Hand und Fuß.

Dr. Hauch: Auch wir haben durchweg positive Erfahrungen gemacht. Wir arbeiten auf verschiedenen Ebenen zusammen, das reicht von der Einsendung von Laborproben ans CCI mit anschließender telefonischer Beratung bis zur Verlegung von Patienten mit schwerem Immundefekt, die am CCI behandelt werden.

Warum senden Sie Patienten ans CCI?

Dr. Hauch: Die Universitätsklinik Gießen beinhaltet verschiedene Abteilungen, was zu einem breiten Patientenspektrum führt. Bei immunologischen Problemen werden meistens wir aus der hämato-onkologischen Abteilung angesprochen, allerdings kommen wir bei speziellen immunologischen Fragestellungen auch an unsere Grenzen und wenden uns dann gerne an das CCI.

Bei welchen Verdachtsfällen wenden Sie sich ans CCI?

Beide: Bei schweren, kombinierten Immundefekten und im Prinzip bei allen Immundefekten oder Autoimmunitätsphänomenen, die wir nicht einordnen können.

Was zeichnet Ihrer Meinung nach das CCI aus?

Dr. Hauch: Das CCI liefert eine Expertise, die es hier an der Universitätsklinik Gießen und in der Region nicht gibt und es bietet spezialisierte Diagnostik an.

Dr. Dahlheim: Das CCI bietet eine erweiterte immunologische Kompetenz an, die ein Allgemeinpädiater, aber auch ein Kinderpneumologe und Allergologe mit gewissem immunologischen Schwerpunkt, so nicht hat.

The CCI as a partner

„We support you and answer your questions"

The CCI is the focal point for referring physicians who seek support in the diagnosis and treatment of patients with immunodeficiencies. Medical practitioners and referring hospitals are important partners for the CCI. Their first suspected diagnosis of a possible immunodeficiency requiring further evaluation is crucial for the fate of the patient. Using the experience with these rare diseases, the CCI can help with diagnosis and create an individualized treatment plan. Further care is then provided in close contact with the referring physicians.

Interview with Dr. Holger Hauch, consultant in the Department of Hemato-Oncology at the University Children's Hospital Giessen and Dr. Marcus Dahlheim, registered pediatric pneumologist and allergist in Mannheim.

Since when have you worked with the CCI? Do you have a direct contact?

Dr. Dahlheim: We at the Praxis Kinderlunge have been working for 3 years with the CCI, where I have always had either Prof. Dr. Stephan Ehl or Dr. Carsten Speckmann as contact person.

Dr. Hauch: I also feel it is really nice to always have the same contact persons; this facilitates communication for us referring physicians tremendously. We have been working together with the CCI for years.

How did the collaboration with the CCI come about?

Dr. Hauch: I already knew Prof. Gritta Janka, Department of Pediatric Hematology and Oncology at the University Medical Center Hamburg-Eppendorf, who has been working for 30 years with the rare but life-threatening autoimmune disease hemophagocytic lymphohistiocytosis (HLH). The contact to Prof. Dr. Stephan Ehl and Dr. Carsten Speckmann came about through her.

Dr. Dahlheim: For me, the situation was somewhat different. I was looking specifically for regional clinical contacts in the area of immunodeficiencies. Unfortunately, there is nothing of this sort in the Mannheim area. Therefore, either the Charité in Berlin or the CCI in Freiburg came into question. At that time, we decided on Freiburg based on the shorter distance and we never regretted this move.

What experiences have you made with the CCI?

Dr. Dahlheim: Only the best! The collaboration with the CCI is more than straightforward, we always get a quick response to emails, the clinical point of contact is always ready with advice and assistance, and the recommendations have rhyme and reason.

Dr. Hauch: We also have consistently made positive experiences. We work together on different levels, ranging from sending laboratory samples to the CCI with subsequent telephone consultation to the transfer of patients with severe immunodeficiencies to be treated at the CCI.

Why do you send patients to the CCI?

Dr. Hauch: The University Hospital of Giessen is comprised of various Departments, which leads to a broad spectrum of patients. For immunological problems, we from the hemato-oncology Department will mostly be approached, though we also reach our limits with specific immunological questions and then we happily contact the CCI.

For which suspected cases do you contact the CCI?

Both: For severe combined immunodeficiencies and in principle all immunodeficiencies or autoimmunity phenomena that we cannot classify.

In your opinion, what distinguishes the CCI?

Dr. Hauch: The CCI provides an expertise that is not available here at the University Hospital Giessen or in the region, and it offers specialized diagnostics.

Dr. Dahlheim: The CCI offers a wider immunological knowledge that a general pediatrician or even a pediatric pneumologist and allergist with an immunological emphasis does not have.

Der beste Therapieansatz für den einzelnen Patienten wird in der Diskussion unter verschiedenen Spezialisten in der CCI Konferenz festgelegt.

The best treatment approach for each patient is determined through the discussion between different specialists in the CCI Conference.

EXPERTEN AUS KLINIK UND FORSCHUNG AN EINEM TISCH

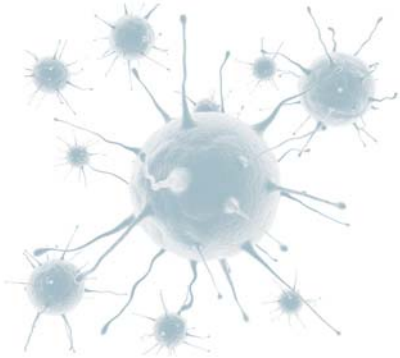
Die enge Kooperation unterschiedlicher Fachrichtungen zeigt sich in den wöchentlichen CCI-Konferenzen, Entzündungskonferenzen und translationalen Konferenzen. Die Konferenzen ermöglichen Therapieentscheidungen, die vom Wissen und der Erfahrung aller Experten getragen werden und die Diskussion innovativer Therapieansätze auf der Basis neuer wissenschaftlicher Erkenntnisse.

Klinische Konferenz

Die klinische Konferenz, unter der Leitung von Prof. Dr. Stephan Ehl, ist das wichtigste Forum zur fachübergreifenden Fallbesprechung. In dieser Konferenz sind alle im CCI zusammengeschlossenen medizinischen Disziplinen, Spezialisten in der Diagnostik sowie kliniknahe Wissenschaftler vertreten. Mittels Videokonferenz nimmt Dr. Klaus Schwarz vom Universitätsklinikum Ulm als Spezialist für Genetik regelmäßig teil. Weitere Experten, aber auch Ärzte anderer Kliniken können zur Vorstellung ihrer Patienten zugeschaltet werden. Neben den Fallbesprechungen finden auch Kurzvorträge über behandlungsrelevante wissenschaftliche Erkenntnisse statt.

Diagnostik Konferenz

Bei der wöchentlichen Diagnostik Konferenz werden immunologische Untersuchungsergebnisse von bis zu 20 Patienten besprochen. Die Einsendungen kommen aus den CCI-Ambulanzen, zum größeren Teil aber aus ganz Deutschland und darüber hinaus (2012 aus 25 einsendenden Ländern). An den Diagnostikbesprechungen nehmen Ärzte der Kinder- und Erwachsenenambulanz teil, um die Befunde im Zusammenhang mit den klinischen Informationen zu bewerten. Die Teilnahme von Wissenschaftlern erlaubt es, jederzeit die diagnostischen um experimentelle Tests zu erweitern und damit eine Tiefe der immunologischen Diagnostik anzubieten, die in Europa vorbildhaft ist. Wissenschaftler bekommen in den Diagnostik Konferenzen Anregungen für translationale Forschungsansätze.
Freiburg ist Referenzzentrum für Patienten mit hämophagozytischer Lymphohistiozytose (HLH), einer lebensbedrohlichen Immunerkrankung. Ca. 100 Patienten pro Jahr aus ganz Deutschland werden aus klinischer, immunologischer und genetischer Sicht in einer 3-wöchentlichen HLH-Videokonferenz gemeinsam mit Ärzten des Universitätsklinikums Hamburg-Eppendorf besprochen, mit einer schriftlichen Rückmeldung an die einsendenden Kliniken. Diese Struktur ist vorbildhaft im Bereich der seltenen Erkrankungen und wird von fast allen deutschen Kliniken in Anspruch genommen.



EXPERTS FROM THE CLINIC AND RESEARCH AT ONE TABLE

The close collaboration between different disciplines becomes obvious in the weekly CCI conferences, inflammation and translational conferences. These conferences allow treatment decisions that are supported by the knowledge and experience of all experts, and the discussion of innovative therapies based on new scientific evidence.

Clinical Conference

The weekly Clinical Conference, under the direction of Prof. Dr. Stephan Ehl, is the central communication platform for multidisciplinary case review. All medical disciplines brought together in the CCI, as well as diagnostic specialists and scientists from the clinical sector are represented in this conference. Dr. Klaus Schwarz from the Institute for Clinical Transfusion Medicine and Immunogenetics (ICT) at the University Hospital Ulm regularly participates via videoconference. More experts, including doctors from other clinics can be connected to present their patient cases. Apart from the case discussions, there are short talks on new scientific knowledge relevant for patient treatment.

Diagnostic Conference

During the weekly Diagnostic Conference of the CCI, immunological test results from up to 20 patients are discussed. The submissions come from the CCI clinics, and to a greater extent from all over Germany and beyond (in 2012 from 25 other countries). Doctors from the pediatric and adult outpatient clinics participate in order to evaluate the test results in the context of clinical information. Moreover, staff from the scientific working groups allows to extend diagnostic tests by experimental tests any time und thus offer a depth of immunological diagnostics that is exemplary in Europe. Conversely, the scientists get ideas for translational research approaches.

Freiburg is reference center for patients with hemophagocytic lymphohistiocytosis (HLH), a life-threatening immune-mediated disease. About 100 patients per year from all over Germany are discussed from a clinical, immunological and genetic point of view in the 3-weekly HLH Conference, with a written response to the referring clinics. This structure is exemplary in the field of rare diseases and is made use of by almost all German children's hospitals.

Bei der translationalen Konferenz werden Ergebnisse aus den Forschungslaboren im Hinblick auf ihre klinische Anwendung diskutiert.

In the translational conference, scientific results from the research laboratories are discussed regarding their potential clinical application.

Translationale Konferenz

Bei der translationalen Konferenz sind Beobachtungen an einzelnen Patienten der Ausgangspunkt für die wissenschaftliche Diskussion eines übergreifenden immunologischen Themas. Zu diesem Thema werden in der Regel Arbeiten von einem klinischen Wissenschaftler und einem Grundlagenforscher dargestellt. Nationale und internationale Experten werden als Sprecher für die Veranstaltung gewonnen, auch Industriepartner können Gäste sein. Die translationale Konferenz dient als Forum zur Diskussion neuer Erkenntnisse in der Pathogenese von Immundefekten und immunologischen Erkrankungen sowie von neuen Therapiekonzepten, die auf diesen molekularen Erkenntnissen aufbauen. Translationale Konferenzen finden alle drei Monate statt und haben mehr als 50 Teilnehmer aus allen Bereichen des CCI und darüber hinaus.

Translationale Forschung wendet die Erkenntnisse der Grundlagenforschung an, zielt jedoch auf konkrete Anwendungsgebiete, wie beispielsweise die Entwicklung eines neuen diagnostischen Tests oder eines neuen Medikaments.

Themen der Translationalen CCI Konferenzen im akademischen Jahr 2014/2015:

- Gentherapie der HIV-Infektion
- CTLA-4 als Therapeutikum
- Interleukin 17-Blockade beim Menschen
- Die chronisch-entzündliche Darmerkrankung

CTLA-4 – ein Gen mit Potential

Die Forschungsgruppe von Prof. Dr. Bodo Grimbacher entdeckte 2014 bei einer Patientengruppe Mutationen im CTLA-4 Gen, die die regulatorische Funktion von T-Zellen beeinträchtigen. Regulatorische T-Zellen haben die wichtige Aufgabe, die Immunantwort anderer T-Zellen zu hemmen. Das ist deshalb von großer Bedeutung, weil nur so gewährleistet werden kann, dass die Immunzellen ihren Angriff gegen Infektionserreger nach erfolgreicher Arbeit wieder einstellen und nicht gegen gesunde körpereigene Zellen richten. Bei Patienten mit diesem Gendefekt zeigen sich nicht nur Antikörpermangel und häufige Infekte der Lunge und der oberen Atemwege, sondern auch chronisch-entzündliche Darmerkrankungen. Vieles deutet darauf hin, dass CTLA-4 nicht nur bei seltenen Immundefekten, sondern vielleicht auch bei vielen anderen Immunerkrankungen eine Rolle spielen könnte. Dieses Wissen hilft dabei, gezielt Medikamente zu entwickeln und einzusetzen, die die Wirkung von CTLA-4 verstärken, um überschießende Immunreaktionen zu hemmen.

Die enge Verknüpfung von Forschung und Diagnostik ermöglicht die ständige Weiterentwicklung der diagnostischen Methoden.

The close interaction of research and diagnostics allows progressive development of diagnostiv methods.

Entzündungskonferenz

Seit 2014 führt das CCI eine Entzündungskonferenz durch, geleitet durch Prof. Dr. Bodo Grimbacher, Prof. Dr. Reinhard Voll und Prof. Dr. Robert Thimme. Immundefekterkrankungen gehen neben Infektionen oft mit chronischen Entzündungen einher, deren Behandlung schwierig sein kann und paradoxerweise den Einsatz von immunsuppressiven Medikamenten erfordert. Ausgehend von diesen Erfahrungen bietet das CCI mit der Entzündungskonferenz ein Forum für Patienten mit entzündlichen Erkrankungen aus allen Fachbereichen (beispielsweise Rheumatologie, Hämatologie, Dermatologie, Gastroenterologie und Neurologie). Neben der gemeinsamen Diskussion von klinischen Fällen wird auch die Pharmakologie immunsuppressiver Substanzen besprochen.

In der Entzündungskonferenz kommen klinische und wissenschaftliche Expertise Patienten mit entzündlichen Erkrankungen zugute.

In the inflammation conference clinical and scientific expertise benefits patients with inflammational disorders.

Translational Conference

In the Translational Conference, observations on an individual patient are the starting point for the scientific discussion of a comprehensive immunological topic. As a general rule, work of a clinical scientist and a basic researcher are presented on the subject. National and international experts are recruited as speakers for the event; industry partners can also be guests. The Translational Conference serves as a forum to discuss new insights into the pathogenesis of immunodeficiency and immunological disorders, and new therapeutic concepts that build on these molecular findings. Translational Conferences are held every three months and have more than 50 participants from all areas of the CCI and beyond.

Translational research applies the findings of basic research, however, focuses on specific application areas, such as the development of new diagnostic tests or a new drug.

Topics of the Translational CCI Conference for the 2014/2015 academic year:

- Gene therapy of HIV infection
- CTLA-4 as a therapeutic agent
- Interleukin 17-blockade in humans
- Chronic inflammatory bowel disease

CTLA-4 – a gene with potential

The research group of Prof. Dr. Bodo Grimbacher discovered in 2014 in a group of patients CTLA-4 gene mutations that impair the function of regulatory T cells. Regulatory T cells have a key role in inhibiting the immune response of other T cells. This is of great importance because it is the only way to guarantee that the immune cells cease their attack against infectious agents after a successful job and do not direct themselves against the body‘s own healthy cells. In patients with this genetic defect not only antibody deficiencies and frequent infections of the lungs and upper respiratory tract manifest, but also chronic inflammatory bowel diseases. There is good evidence that CTLA-4 may play a role not only in rare immunodeficiency, but perhaps also in many other immune diseases. This knowledge helps to specifically develop and implement medications that reinforce the effect of CTLA-4 to inhibit excessive immune reactions.exemplary in the field of rare diseases and is made use of by almost all German children‘s hospitals.

Inflammation Conference

Since 2014, the CCI offers an Inflammation Conference, led by Prof. Dr. Bodo Grimbacher, Prof. Dr. Reinhard Voll and Prof. Dr. Robert Thimme. Like many other immune disorders, immunodeficiency diseases are often accompanied by chronic inflammation and infection, for which treatment can be difficult and, paradoxically, requires the use of immunosuppressive drugs. Based on these experiences, the CCI offers with the Inflammation Conference a forum in which experiences with inflammatory diseases and immune-modulating drugs benefit patients from all disciplines (e.g., rheumatology, hematology, dermatology, gastroenterology and neurology). In addition to a common discussion of clinical cases, also the pharmacology of immunosuppressive agents is discussed.

Die Forschung bildet gemeinsam mit der Patientenversorgung das Fundament des CCI.

Research together with patient care is the foundation of the CCI.

VON DER FORSCHUNG IN DIE KLINISCHE ANWENDUNG

Translationale Forschung im Hinblick auf eine Anwendung der wissenschaftlichen Erkenntnisse zum Wohle des Patienten ist neben der Patientenversorgung das Fundament des CCI. Die immunologische und genetische Diagnostik, die sich durch die Erkenntnisse der Forschungsgruppen des CCI ständig weiterentwickelt, dient dem Ziel, für jeden Patienten mit Immundefekt eine Diagnose stellen zu können. Die Kenntnis des auslösenden Gendefekts, bildet die Basis spezifischer Therapieempfehlungen. Die Clinical Research Unit (CRU) und die Biobank des CCI sind weitere wichtige Werkzeuge der patientenzugewandten Forschung am CCI.

Routine Diagnostic Unit

Im Labor der Klinik für Rheumatologie und klinische Immunologie unter der Leitung von Dr. Ulrich Salzer wird serologische Autoimmundiagnostik und durchflusszytometrische Zelldiagnostik bei immunologischen Erkrankungen sowie bei autoimmunen und entzündlichen Erkrankungen durchgeführt.

Advanced Diagnostics Unit

Mit der Advanced Diagnostic Unit (ADU), unter der Leitung der Biotechnologin Ilka Fuchs, möchte das CCI die Brücke von der experimentellen Wissenschaft zur validierten Diagnostik schlagen. Das Grundkonzept der ADU ist der ständige Transfer von neuen Methoden aus den Forschungsgruppen in die immunologische Diagnostik. Durch diese enge Zusammenarbeit sind die Anzahl der Testverfahren für die Diagnose von Immunstörungen von 22 Tests im Jahr 2009 auf 35 Tests in 2013 angewachsen. Einige dieser Test werden in Deutschland und darüber hinaus nur am CCI angeboten. 2014 wurden 1274 Proben eingesandt, davon 10% aus dem Ausland.

Neben der Durchflußzytometrie und Zellkulturverfahren kommen eine Reihe von Aktivierungsassays zum Einsatz, bei denen kann unter anderem auch die Proteinphosphorylierung gemessen wird. Auch andere Methoden wie ELISA, Western Blot oder Laserscan Mikroskopie stehen der ADU zur Verfügung.

Seit 2011 wird ein Workshop zur Diagnostik bei Immundefizienz, vorrangig für junge Ärzte, Wissenschaftler und Laborleiter angeboten. Gemeinsam mit internationalen Experten werden Fälle durch die Teilnehmer präsentiert und mit den 40 Teilnehmern interaktiv diskutiert.

FROM RESEARCH TO CLINICAL APPLICATIONS

Translational research, which aims at the application of scientific knowledge for the benefit of patients, forms the foundation of the CCI next to patient care. The immunological and genetic diagnostics, which are constantly evolving with the findings of the research groups of the CCI, serve the goal of being able to provide a diagnosis to each patient with an immunodeficiency. Knowledge of the responsible genetic defect forms the basis counseling and specific treatment recommendations. The Clinical Research Unit (CRU) and the Biobank of the CCI are the basis of the patient-facing research at the CCI and enable the development of new therapeutic approaches.

Routine Diagnostic Unit

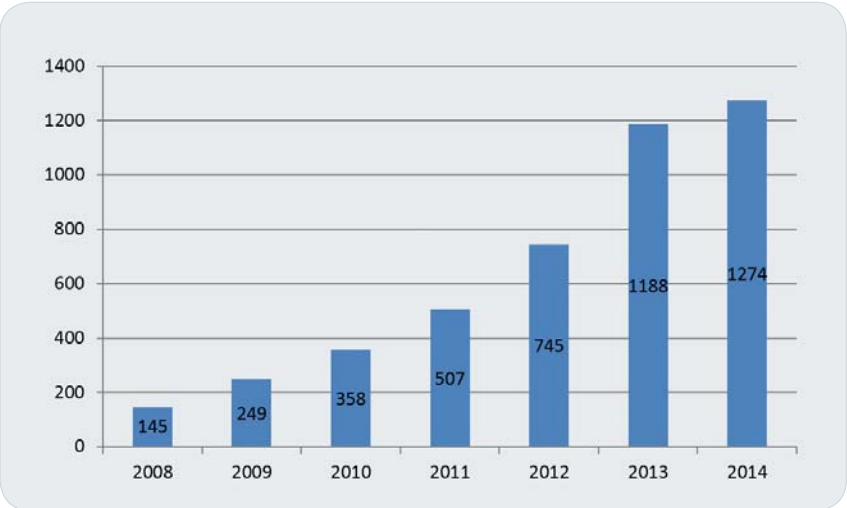
In the laboratory of the Department of Rheumatology and Clinical Immunology, under the direction of Dr. Ulrich Salzer, serological autoimmunity diagnostics and flow cytometric cell diagnostics are performed in patients with autoimmune and inflammatory diseases.

Advanced Diagnostics Unit

With the Advanced Diagnostic Unit (ADU), under the direction of the biotechnical engineer Ilka Fuchs, the CCI seeks to bridge the gap between experimental science and validated diagnostics. The basic concept of the ADU is the constant transfer of new methods from the research groups to the immunological diagnosis. Through this close cooperation, the number of test methods for the diagnosis of immune disorders has grown from 22 tests in 2009 to 35 tests in 2013. In Germany and beyond, some of these tests are only available at the CCI. 2014, 1274 samples were sent, of which around 10% originated from abroad.

The ADU works primarily with the method of flow cytometry combined with cell culture methods, but also functional assays such as protein phosphorylation assays are performed.

Since 2011, a workshop on the diagnosis of immunodeficiency, primarily aimed at young doctors, scientists and laboratory manager is offered every 2 years. Together with international experts, the participant present cases which are discusses interactively with the 40 participants.

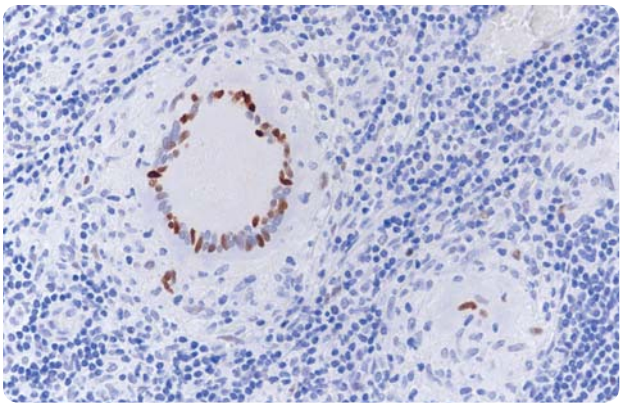


Entwicklung der Anzahl am CCI analysierten Proben.

Increase in the Number of Samples Analyzed at the CCI.

Immunopathology Unit

Das Institut für Pathologie (Leitung: Prof. Dr. Martin Werner) unterstützt mit einer speziellen Immunopathologie Unit (Koordination Dr. Max Seidl) das CCI bei der Untersuchung von Gewebeproben. In der Unit werden die mit Immundefizienz einhergehenden Gewebemerkmale erforscht und Diagnoseprotokolle erstellt. Experten auf dem Gebiet der Pathologie sind unerlässlich, wenn Patienten bei der CCI-Konferenz besprochen werden.

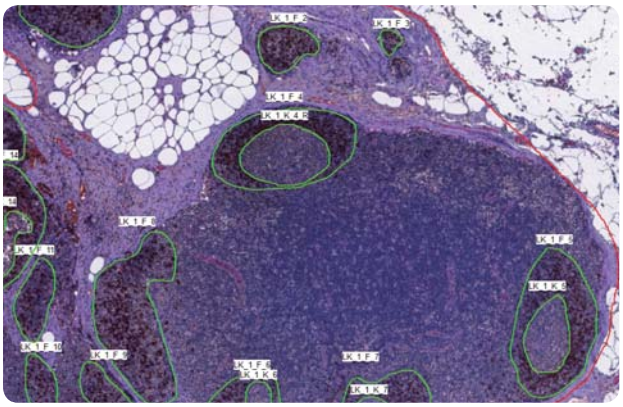


Lymphadenitis durch atypische Mykobakterieninfektion. Mittig ist eine Riesenzelle im Bereich eines Granuloms zu sehen.

Lymphadenitis by atypical mycobacteria infection. In the midlle, a giant cell in the area of a granuloma can be seen.

Genetics and Genomics Unit

Für die Auswahl der optimalen Therapie für Immundefekt-Patienten ist es wichtig zu wissen, welches Gen betroffen ist und welche Abwehr- oder Regulationsmechanismen dadurch beeinträchtigt werden. Die Genetics and Genomics Unit am CCI bietet für alle Immundefekterkrankungen eine genetische Diagnostik an. Diese wird sowohl in Freiburg (Prof. Dr. Bodo Grimbacher), als auch am Institut für Klinische Transfusionsmedizin und Immungenetik (IKT) in Ulm (Dr. Klaus Schwarz) durchgeführt. Durch diese Zusammenarbeit können mehr als 250 einzelne Gene untersucht werden, die primäre Immundefekte verursachen. Beide Einrichtungen nutzen moderne Next Generation Sequencing Verfahren, unterstützt durch eine leistungsfähige Bioinformatik, für eine raschere Diagnostik.

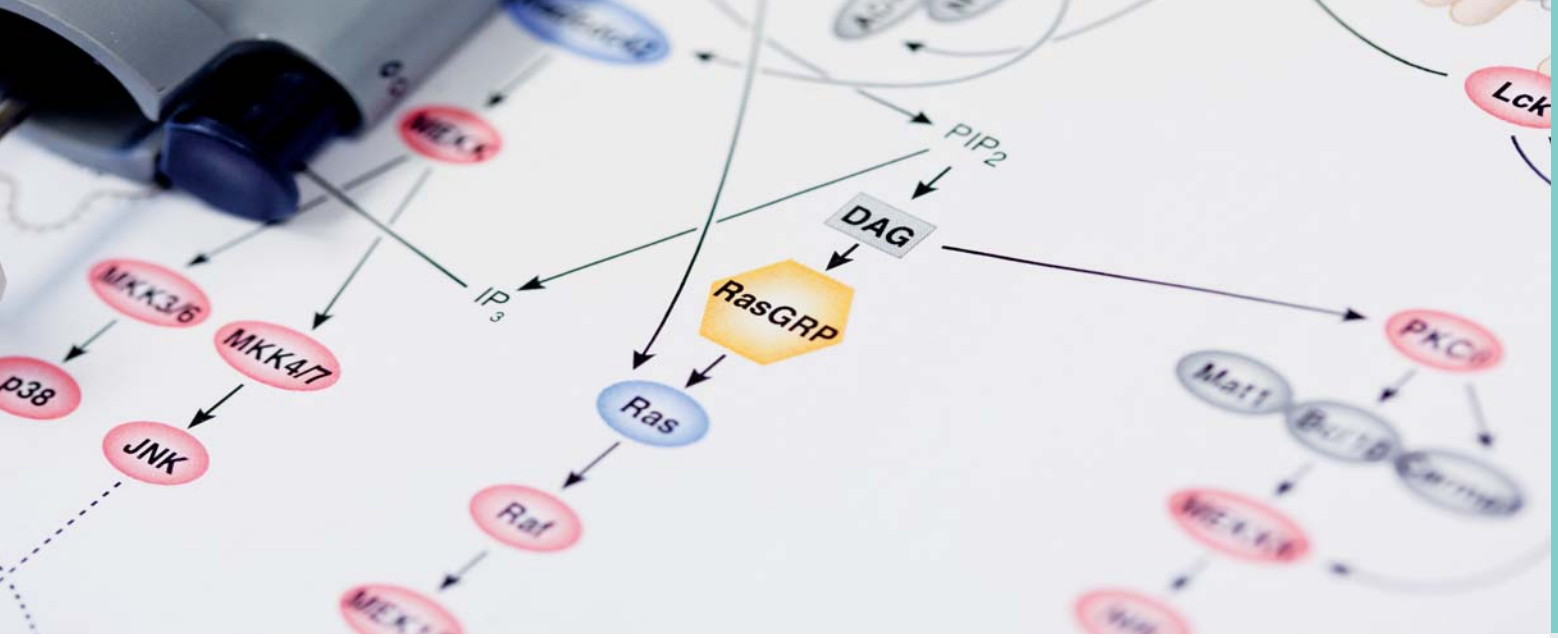


Lymphknoten aus einem Tumorabflussgebiet mit Vermessung der Follikel und Keimzentren.

Lymph node from a tumor draining area with surveying of follicles and germinal centers.

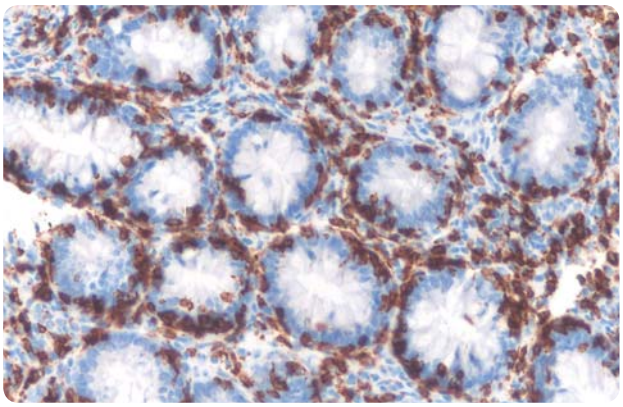
Eine frühe und genaue Diagnose ermöglicht eine zielgerichtete Therapie.

An early and accurate diagnosis allows for targeted therapy.



Immunopathology Unit

The Department of Pathology (Director: Prof. Dr. Martin Werner), supports the CCI in the investigation of tissue changes with a special Immunopathology Unit (coordination Dr. Max Seidl). In this unit, particular tissue abnormalities are the focus of research and diagnostic protocols are established. Experts in the field of pathology are essential when it comes to the discussion of patient cases at the interdisciplinary CCI conference.

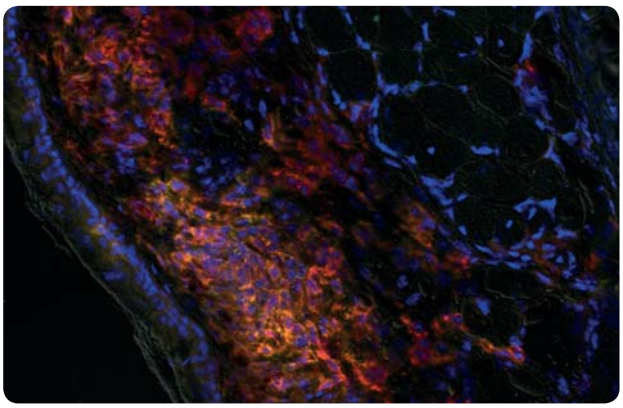


Gewebeprobe aus dem Dickdarm bei autoimmuner Entzündung des Darmes. Die braun gefärbten Entzündungszellen sind auch zwischen den Epithelzellen zu sehen.

Tissue sample from the colon during autoimmune inflammation of the colon. The brown coloured inflammatory cells can be seen also between the epithelial cells.

Genetics and Genomics Unit

For choosing the best treatment for patients with immunodeficiency, it is relevant to know which gene is affected and which defense or regulatory mechanisms are impaired. The Genetics and Genomics Unit offer a genetic diagnosis for all immunodeficiency disorders. This is done both in Freiburg (Prof. Dr. Bodo Grimbacher), and at the Institute for Clinical Transfusion Medicine and Immunogenetics (ICT) in Ulm (Dr. Klaus Schwarz). Working together both sites can analyze samples for more than 250 individual genes associated with primary immunodeficiencies. Both facilities use the modern Next Generation Sequencing method allowing a more rapid diagnosis.



Fluoreszenzmikroskopische Untersuchung einer bakteriellen Entzündungsreaktion im Ohr (Maus). Die verschieden leuchtenden Farbstoffe markieren Entzündungszellen des Immunsystems.

Fluorescence microscopic examination of a bacterial inflammation in the ear (mouse). The different luminous dyes mark inflammatory cells of the immune system.

iPs/Genome Editing Unit

Das CCI will mit seiner Forschung auch zur Entwicklung neuer gentherapeutischer Behandlungsansätze für angeborene und erworbene Immundefekte beitragen.
Die Gentherapie kann bei lebensbedrohlichen Formen der Immundefizienz eine Behandlungsmöglichkeit darstellen, wenn kein passender Spender für eine Stammzelltransplantation gefunden wird. Voraussetzung für eine Gentherapie ist, dass die Genmutation, welche die Immundefizienz verursacht, bekannt ist. Dann kann versucht werden, diese Mutation in den Stammzellen des Patienten zielgerichtet zu reparieren. Anders als die konventionelle Stammzelltransplantation kann Gentherapie den genetischen Fehler in den eigenen Zellen des Patienten beheben und es müssen keine körperfremden Zellen transplantiert werden. In der Vergangenheit wurden meist retrovirale Vektoren verwendet, um das gesunde Gen einzubauen. Allerdings birgt der Einsatz retroviraler Vektoren Gefahren: In den ersten Gentherapie-Studien wurde das Auftreten von Leukämien beobachtet. Man vermutet, dass eine ungewollte Aktivierung benachbarter Genabschnitte durch die Retrovirus-DNA dafür verantwortlich sein könnte.

Inzwischen werden neue, sicherere Wege in der Gentherapie entwickelt. Besonders vielversprechend ist dabei die Entwicklung induzierter pluripotenter Stammzellen (iPS-Zellen). Für die Entdeckung, dass mittels Gentechnik ausdifferenzierte Körperzellen in Stammzellen zurückverwandelt können, die fähig sind, sich zu ganz unterschiedlichen Gewebezellen zu entwickeln, erhielten John Gurdon und Shinya Yamanaka 2012 den Nobelpreis für Medizin.

Eine große Rolle bei der therapeutischen Nutzung von iPS-Zellen spielt die Entwicklung von maßgeschneiderten Designer-Nukleasen. Sogenannte TALEN-Designer-Nukleasen schneiden die Zell-DNA an der gewünschten Stelle auf. Dann kann eine neue DNA-Sequenz eingebaut werden und so ein defektes Gen zielgerichtet repariert werden. Prof. Dr. Toni Cathomen leitet am CCI die Forschungsgruppe zur Gentherapie und die iPSC/TALEN Plattform.

Team der Clinical Research Unit.

Team of the Clinical Research Unit.

Clinical Research Unit

Klinische Studien sind unverzichtbar, um Erkenntnisse aus der Forschung in neue Therapieansätze für Patienten umzusetzen. Epidemiologische Studien können entscheidende Hinweise für neue Forschungs- und Therapieansätze geben. Am CCI werden klinische und epidemiologische Studien auf dem Gebiet der Immundefizienz durch die Clinical Research Unit (CRU), unter der Leitung von PD Dr. Alexandra Nieters durchgeführt. Die CRU vereint Experten in der Organisation von Studien, der Versorgungsforschung, im IT-Bereich sowie der Statistik.

Die CRU leitet derzeit 13 Beobachtungs- und Interventionsstudien, darunter beispielsweise die Profound Combined Immunodeficiency (P-CID) Studie zur Transplantationsindikation bei kombinierten Immundefekten bei Kindern, die STILPAD Studie zur interstitiellen Lungenerkrankung bei primärem Antikörpermangel und die AWIS-Studie, eine epidemiologische Studie zum Thema Infektanfälligkeit in der Bevölkerung. Unterstützt wird die CRU dabei vom Studienzentrum am Universitätsklinikum Freiburg.

Patienten des CCI wird durch die CRU die Möglichkeit geboten, an für sie geeigneten Studien teilzunehmen. Durch die Studienteilnahme tragen die Patienten zur Forschung über Immundefizienz bei und erhalten eventuell Zugang zu neuen Therapieansätzen. Das CCI kann für Studien sowohl auf eigene Patientenkollektive zurückgreifen, als auch auf etablierte nationale (PID-Net) und internationale (ESID) Register für chronische Immundefizienz.

iPs/Genome Editing Unit

The CCI will also contribute with its research to the development of new gene therapy approaches for congenital and acquired immunodeficiencies.

Gene therapy for life-threatening forms of immunodeficiency can be a treatment option if no suitable donor for a stem cell transplant is found. A prerequisite for gene therapy is that the gene mutation that causes the immunodeficiency is known. Then one can attempt a targeted repair of this mutation in the stem cells of the patient. Unlike conventional stem cell transplantation, gene therapy can correct genetic defects in the patient's own cells and no foreign cells need to be transplanted. In the past, mostly retroviral vectors have been used to incorporate the healthy gene. However, the use of retroviral vectors can be dangerous: in the first gene therapy trials, the appearance of leukemia was observed. It is believed that an unintended activation of adjacent gene segments by the retrovirus DNA could be responsible.

Meanwhile, new, safer ways in gene therapy have been developed. Particularly promising is the development of induced pluripotent stem cells (iPS cells). For the discovery that somatic cells differentiated by genetic engineering can reconvert into stem cells capable of developing into very different tissue cells, John Gurdon and Shinya Yamanaka received the Nobel Prize for Medicine in 2012.

The development of customized designer nucleases plays a major role in the therapeutic use of iPS cells. So-called TALEN designer nucleases cut open the cell's DNA at the desired location. A novel DNA sequence can then be incorporated and thus a defective gene can be repaired in a targeted manner. Prof. Dr. Toni Cathomen leads the CCI research groups for gene therapy and the iPSC /TALEN platform.

Clinical Research Unit

Clinical trials are indispensable when it comes to turn research findings into new therapies for patients. Epidemiological studies can provide important clues for new research and therapeutic approaches. At the CCI, clinical trials and epidemiological studies in the field of immunodeficiency are carried out by the Clinical Research Unit (CRU), under the direction of PD Dr. Alexandra Nieters. The CRU brings together experts in the organization of studies, healthcare research, as well as in the areas of IT and statistics.

The CRU is currently running 13 observational and interventional studies, including for example the profound combined immunodeficiency (P-CID) study on the indication for transplantation in children with combined immunodeficiency, the STILPAD study for interstitial lung disease in primary antibody deficiency, and the AWIS study, an epidemiological study on susceptibility to infection in the population. The CRU is supported by the Study Center at the Medical Center - University of Freiburg.

CCI patients are offered by the CRU the opportunity to participate in studies suitable for them. By participating in studies, patients contribute to the research of immunodeficiency and possibly gain access to new therapeutic approaches. For studies, the CCI can build on its own patient cohorts of multiple PIDs, as well as on established national (PID-Net) and international (ESID) registries for chronic immunodeficiency.

In Klinischen Studien wird die Wirksamkeit neuer Medikamente geprüft.

In clinical trials the efficacy of new drugs is tested.

Biobank

Biobanken sichern die langfristige Lagerung und Zugänglichkeit biologischen Probenmaterials und der dazugehörigen Datensätze. Sie sind ein unverzichtbares Instrument der patientenorientierten klinischen Forschung. Es ist die Aufgabe des CCI, mit von Patienten zur Verfügung gestelltem Probenmaterial mit größter Sorgfalt umzugehen, und es zum Ziel der Verbesserung der medizinischen Versorgung von Patienten zu erforschen.

Die Anlage einer zentralen Biobank für die Lagerung von Zellen- und Serumproben von Immundefekt-Patienten wurde 2009 am CCI begonnen und seither erfolgreich vergrößert und professionalisiert. Der Aufbau dieser Biobank hilft bei der systematischen Erforschung der Schwächen des Immunsystems. Der Schutz der Patientendaten wird dabei immer berücksichtigt.

Biobanken dienen der Lagerung von biologischem Probenmaterial wie Zellen und Blut zu Forschungszwecken.

Biobanks are used for storage of biological samples such as cells and blood for research purposes.

KLINISCHE STUDIEN AM CCI

Was macht Menschen anfällig für Infekte? Die AWIS Studie

Infektanfälligkeit gehört zu einem der Symptome einer chronischen Immundefizienz. Abgesehen von den schweren Genmutationen bei primären Immundefekten ist vergleichsweise wenig bekannt, welche anderen Faktoren die Infektneigung beeinflussen und wie sich infektanfällige Menschen von wenig infektanfälligen Menschen unterscheiden. Um sich diesem Thema zu nähern, wurde Ende 2011 am CCI eine epidemiologische Atemwegsinfektanfälligkeitsstudie (AWIS) mit Teilnehmern aus Freiburg und der Region Südbaden begonnen.

Zunächst wurden an etwa 70.000 Erwachsene in Südbaden Fragebögen versendet mit dem Ziel, das Spektrum der Atemwegsinfektanfälligkeit zu erfassen.

Von mehr als 12.000 Personen wurden die Fragebögen ausgefüllt und zurückgeschickt. Daraus konnte ein Atemwegsinfektanfälligkeits-Score (Respiratory Tract Infection (RTI) Score) ermittelt werden. Anschließend wurden ausgewählte Personen eingeladen, ausführlichere Fragebögen auszufüllen, um die Merkmale infektanfälliger Personen mit denen von Personen ohne Infektneigung vergleichen zu können. Erste statistische Analysen dieser Daten zeigen, dass Menschen mit vielen Atemwegsinfekten auch häufiger von Infekten anderer Organe, wie beispielsweise der Haut oder des Magen- und Darmtraktes, berichteten. Allergische Erkrankungen und vor allem Asthma sind eine häufige Begleiterscheinung einer Infektneigung, ebenso wie chronisch obstruktive Atemwegserkrankungen. Teilnehmer mit einem höheren Bodymass Index zeigten auch einen höheren Atemwegsinfektanfälligkeits-Score.

Die Erkenntnisse, die aus epidemiologischen Studien wie der AWIS-Studie gewonnen werden, sind von allgemeinem Interesse, wenn es um die mögliche Vermeidung von Atemwegsinfekten geht. Darüber hinaus wird erwartet, dass sich aus einigen Ergebnissen zu Lebensstilfaktoren im Hinblick auf Infekthäufigkeit, für Patienten wichtige Empfehlungen zum Leben mit der erhöhten Infektanfälligkeit ableiten lassen.

Durch die Teilnahme an klinischen Studien tragen Patienten zur Entwicklung besserer Therapien bei.

Through participation in clinical trials, patients contribute to the development of better therapies.

Biobank

Biobanks secure the long-term storage and accessibility of biological samples and the associated records. They are a vital instrument for patient-oriented clinical research. It is the task of the CCI to handle patient material with the greatest care, and to investigate it with the goal of improving medical care for patients.

The creation of a central biobank for storage of cell and serum samples from immunodeficiency patients was started in 2009 at the CCI and has since been successfully augmented and professionalized. The setup of this biobank helps to systematically study immunodeficiencies. The protection of patient data is always taken into account.

CLINICAL STUDIES AT THE CCI

What makes people vulnerable to infections? The AWIS Study

Susceptibility to infection is one of the symptoms of a chronic immunodeficiency. Apart from the major gene mutations in primary immunodeficiencies, comparatively little is known about which other factors influence the susceptibility to infections and how infection-prone people differ from those who are not. In order to approach this subject, an epidemiological respiratory infection susceptibility study (German: Atemwegsinfektanfälligkeitsstudie, AWIS) with participants from Freiburg and the region of South Baden began at the end of 2011 at the CCI.

Initially, questionnaires were sent to approximately 70,000 adults in South Baden with the aim to capture the spectrum of respiratory infection susceptibility.

More than 12,000 people filled out and sent back the questionnaires. From these a Respiratory Tract Infection (RTI) score could be determined. Then, selected individuals were invited to fill out detailed questionnaires so that the characteristics of individuals susceptible to infections could be compared with those of people without susceptibility to infections. The first statistical analysis of these data shows that people with many respiratory infections reported also frequent infections of other organs such as the skin or gastrointestinal tract. Allergic diseases, especially asthma, are a common side effect of a tendency to infection, as well as chronic obstructive pulmonary disease. Participants with a higher body mass index also showed a higher RTI score.

The insights gained from epidemiological studies such as the AWIS study, are of general interest when it comes to the possible prevention of respiratory infections. Furthermore, it is expected that important recommendations for patients on living with the increased susceptibility to infection can be derived from some of the results of lifestyle factors in relation to infection incidence.

Stammzelltransplantation beim kombiniertem Immundefekt - die P-CID Studie

Profunde kombinierte Immundefekte (P-CID) beruhen auf einer Störung der T-Zell Immunität, was zu einer erhöhten Infektanfälligkeit und Störung der Immunregulation mit entzündlichen Erkrankungen und Autoimmunität führt. Typisch sind schwere Virusinfektionen, chronische Lungenentzündungen, Autoimmunerkrankungen, Milz- und Lymphknotenvergrößerung, chronische Ekzeme und Darmentzündungen. Die Erkrankungen sind potenziell lebensbedrohlich, so dass eine Behandlung durch Stammzelltransplantation (SZT) erwogen werden muss. Eine SZT ist aber immer auch mit Risiken verbunden. Deshalb ist es wichtig, die Entscheidung zur Stammzelltransplantation nach sorgfältiger Abwägung auf der Basis fundierter, wissenschaftlicher Daten zu treffen.

Ziel der prospektiven P-CID Studie ist es, den Verlauf der Krankheit künftig besser vorhersagen zu können und prognostische Marker zu identifizieren. Auf Basis der im Studienverlauf erhobenen klinischen Daten und Labordaten soll ein Risiko-Modell für die Patienten entwickelt werden. Damit kann in Zukunft besser entschieden werden, ob eine Stammzelltransplantation versucht werden sollte. Darüber hinaus bietet die Studie eine internationale Plattform für die Identifikation neuer Gendefekte und deren immunologische Analyse.

Durch die Teilnahme an klinischen Studien tragen Patienten zur Forschung über Immundefizienz bei.

Through participation in clinical trials, patients contribute to research about immunodeficiency.

Welche Therapie hilft beim variablen Immundefektsyndrom mit interstitieller Lungenerkrankung? Die STILPAD-Studie

Beim variablen Immundefektsyndrom (Common Variable Immunodeficiency, CVID), dem häufigsten angeborenen Immundefekt, ist die Bildung von Antikörpern zur Abwehr von Erregern beeinträchtigt. Dadurch leiden die betroffenen Patienten häufig an bakteriellen Atemwegsinfektionen. Zwischen 10% und 20% der Patienten haben zusätzlich entzündliche Veränderungen des Lungengewebes, die als interstitielle Lungenerkrankung bezeichnet werden. Diese Veränderungen erschweren den Sauerstoffaustausch in der Lunge und verursachen so Luftnot bei den betroffenen Patienten. Bis heute sind die Ursache und die Tragweite dieser interstitiellen Lungenerkrankung nur wenig verstanden. Die herkömmliche Behandlung mit Ersatz der fehlenden Antikörper (Immunglobulintherapie) zeigt bei CVID Patienten mit interstitieller Lungenerkrankung nur wenig Erfolg. Deshalb werden die Patienten oft zusätzlich mit Kortikosteroiden und immunsuppressiven Medikamenten behandelt. Allerdings gibt es bis heute keinen Beweis dafür, dass diese Therapie Erfolg bringt. STILPAD ist eine Beobachtungsstudie, die dieser Frage nachgeht. Bei insgesamt 149 Patienten soll herausgefunden werden, ob und wenn ja, welche Patienten von der zusätzliche Therapie profitieren. Der Verlauf der Erkrankung wird über weitere fünf Jahre beobachtet. Die ersten Ergebnisse werden 2018 erwartet.

Stem cell transplantation for combined immunodeficiency - The P-CID study

Profound combined immunodeficiencies (P-CID) are based on a disturbance in T cell immunity, which leads to an increased susceptibility to infection, and disorders of immune regulation with inflammatory diseases and autoimmunity. Severe viral infections, chronic lung inflammation, autoimmune diseases, spleen and lymph node enlargement, chronic eczema and intestinal inflammation are all typical. The diseases are potentially life-threatening, so that treatment by stem cell transplantation should be considered. However, stem cell transplantation carries its own risks. Therefore, it is important to make the decision on stem cell transplantation after careful consideration based on sound scientific data.
The goal of the P-CID study is to better predict the course of the disease in future and to identify prognostic markers. Based on the clinical and laboratory data collected during the study, a risk model is to be developed for patients. Then it can be better decided in the future, whether a stem cell transplant should be attempted. In addition, the study provides an international platform for the identification of new genetic defects that can cause combined immunodeficiencies and for their immunological analysis.

Which therapy in common variable immunodeficiency helps for interstitial lung disease? The STILPAD study

In common variable immunodeficiency (CVID), the most common congenital immunodeficiency, the formation of antibodies to fight off pathogens is impaired. As a result, the affected patients often suffer from bacterial respiratory infections. Between 10% and 20% of the patients also have inflammatory changes in the lung tissue that are referred to as interstitial lung disease. These changes complicate the exchange of oxygen in the lungs causing shortness of breath in the affected patients. To date, the cause and the extent of this interstitial lung disease are poorly understood. The conventional treatment with replacement of lacking antibodies (immunoglobulin therapy) shows only little success in CVID patients with interstitial lung disease. Therefore, patients are often treated additionally with corticosteroids and immunosuppressive drugs. However, there is still no proof that this therapy is successful. STILPAD is an observational study, which explores this question. A total of 149 patients will be followed to find out whether patients benefit from additional therapy and if so which patients. The course of the disease will be observed over five years. The first results are expected in 2018.

FORSCHEN UND LERNEN

Das CCI sieht die Forschung zu primären Immundefekten als einen wichtigen Schwerpunkt seiner Arbeit. Die Erforschung von Ursachen angeborener Immundefekte, die oft auf ein einziges fehlerhaftes Gen zurückgehen, bietet hervorragende Möglichkeiten, das komplexe Immunsystem und seine Schwächen besser zu verstehen. Diese Erkenntnisse kommen nicht nur Patienten mit primärer Immundefizienz zugute, sondern helfen auch bei der Behandlung nicht erblicher Immunschwächen, wie beispielsweise der HIV-Infektion. Sie können darüber hinaus bei der Suche nach neuen Therapien für Immunerkrankungen wie Lymphome, Rheumatoide Arthritis, Systemischer Lupus Erythematodes, Diabetes, Entzündliche Darmerkrankung oder Multiple Sklerose wertvoll sein.

Inzwischen kann durch die Sequenzierung der gesamten genetischen Information eines Patienten innerhalb von kurzer Zeit eine einzelne Genveränderung entdeckt werden. Auch auf diesem innovativen Gebiet arbeiten die Forscher des CCI, um die genetischen Ursachen von verschiedenen Arten von Immundefizienz bestimmen zu können. Für einige primäre Immundefekte bietet vielleicht in Zukunft die Gentherapie eine Chance auf Heilung. Zu diesem Ziel bereitet das CCI Patientenstudien vor, damit die Gentherapie zu einer sicheren klinischen Anwendung entwickelt werden kann.

Die Entwicklung von Gentherapie und neuer Diagnoseverfahren sind nur zwei Beispiele für die Vorteile, die eine Integration von Forschung und Behandlung, wie sie am CCI gelebt wird, für die Medizin bietet. Bei vielen Erkrankungen spielt das Immunsystem eine Rolle. Viele Krankheiten könnten vielleicht in Zukunft besser behandelt werden, wenn Forscher und Ärzte die Regelmechanismen des Immunsystems kennen und daraus lernen, seine Stärken zu unterstützen und seine Schwächen auszugleichen.

Wissenschaftliche Exzellenz kann langfristig aber nur durch die Förderung begabter Nachwuchswissenschaftler erreicht werden. Dem CCI ist es daher ein besonderes Anliegen, begabte junge Ärzte und Wissenschaftler zu fördern.

Das CCI bietet einen strukturierten Ausbildungsplan für Studierende, Ärzte und Wissenschaftler an, die sich in den Bereichen Immundefizienz und Infektiologie spezialisieren möchten. Grundlagenforschung und klinische Forschung werden gleichermaßen gefördert. Ziel ist es, die Vereinbarkeit einer kombinierten klinischen und wissenschaftlichen Ausbildung zu erhöhen, die Attraktivität einer wissenschaftlichen Karriere zu steigern und durch spezielles Mentoring frühe wissenschaftliche Unabhängigkeit zu ermöglichen.

Für die Ausbildung von jungen Wissenschaftlern arbeitet das CCI mit dem Max-Planck Institut für Immunbiologie und Epigenetik, der Abteilung für Molekulare Immunologie an der Biologischen Fakultät und der Spemann Graduierten Schule für Biologie und Medizin zusammen.

Die Erforschung der Signalwege des Immunsystems ist ein Schlüssel für die Entwicklung neuer Therapien.

Exploring the signaling pathways of the immune system is the key to developing new therapies.

RESEARCH AND STUDY

The CCI views research on primary immunodeficiencies as an important focus of its work. The investigation of the causes of congenital immunodeficiencies, which often go back to a single faulty gene, provides excellent opportunities to better understand the complex immune system and its weaknesses. These findings benefit not only patients with primary immunodeficiency, but also help in the treatment of nonhereditary immunodeficiencies such as HIV. Furthermore, they can be valuable in finding new treatments for allergies and immunologic diseases such as lymphoma, rheumatoid arthritis, systemic lupus erythematosus, diabetes, inflammatory bowel disease or multiple sclerosis.

Through sequencing the entire genetic information of the patient, a single gene mutation can be detected within a short time. Also in this innovative field, researchers at the CCI are working to determine the genetic causes of various types of immunodeficiency. For some PIDs, gene therapy may offer in the future a chance for a cure. With this goal in mind, the CCI is preparing patient studies to develop gene therapy for safe clinical application.

The development of new diagnostics and gene therapy are just two examples of the benefits for medicine offered by an integration of research and treatment, as found at the CCI. In many diseases, the immune system plays a role. Numerous diseases could perhaps be better treated in the future if researchers and doctors know the regulatory mechanisms of the immune system and learn to support its strengths and compensate for its weaknesses.
Scientific excellence can only be achieved long term by promoting talented young scientists. The CCI is therefore particularly concerned with fostering talented young physicians and scientists.

The CCI offers a structured training plan for students, doctors and scientists who wish to specialize in the field of infectious diseases and immunodeficiency. Basic science and clinical research are equally encouraged. The aim is to increase the compatibility of a combined clinical and scientific education, to increase the attractiveness of a scientific career and to enable early scientific independence with specialized mentoring.

For the training of young scientists, the CCI works together with the Max Planck Institute of Immunobiology and Epigenetics, the Department of Molecular Immunology at the Faculty of Biology and the Spemann Graduate School of Biology and Medicine.

Prof. Dr. Walter Hitzig (1922-2012), Mitbegründer der Kinderimmunologie.

Prof. Dr. Walter Hitzig (1922-2012), pioneer of pediatric immunology.

Walter Hitzig Programm

Prof. Dr. Walter Hitzig (1922-2012) gehört zu den Begründern der Kinderimmunologie in Europa. Zu seinen wesentlichen Leistungen zählt die Teilnahme an der Erstbeschreibung des schwersten bekannten Immundefekts, der SCID-Erkrankung. Durch seine Initiative entwickelte sich 1990 die Arbeitsgemeinschaft Pädiatrische Immunologie (API), die in den folgenden Jahren das Spezialgebiet prägen sollte. Hitzig ist Namensgeber der „Walter-Hitzig Stipendien“ die vom CCI an junge klinische Nachwuchswissenschaftler der Universität Freiburg im Fachbereich Immunologie vergeben werden. Mit der internationalen Ausschreibung der Stipendien will das CCI jungen, talentierten Ärzten die Forschung im Bereich Immundefizienz ermöglichen und ihre Karriere als forschende Mediziner fördern.

Ausbildung von Studierenden

Im Rahmen des MOTIVATE Programms der medizinischen Fakultät können herausragende Studierende der Medizin Stipendien für wissenschaftliche Arbeiten am CCI erhalten. Im Rahmen dieser Stipendien haben die Studierenden die Gelegenheit, ihre medizinische Doktorarbeit zu gestalten.

Für naturwissenschaftliche Doktoranden bietet das CCI in Zusammenarbeit mit den Studiengängen Immunbiologie unter der Leitung von Prof. Dr. Wolfgang Schamel und Molekulare Medizin, geleitet von Prof. Dr. Christoph Peters, ein strukturiertes Betreuungs-Programm. Dieses beinhaltet methodisches Training, Seminare, Workshops und Klausuren. Das CCI hat sich auch dem MD-PhD Programm der Spemann Graduierten Schule für Biologie und Medizin angeschlossen, die im Rahmen der Exzellenzinitiative in Freiburg etabliert wurde. Studenten, die ihre Doktorarbeit in einer Forschungsgruppe des CCI oder einer mit dem CCI verbundenen Gruppe durchführen, können an allen CCI-Veranstaltungen teilnehmen. Angeboten werden spezielle CCI-Seminare, Grundlagenforschungsseminare während des Sommersemesters und ein Advanced Immunology Kurs während des Wintersemesters. Zusätzlich bietet das CCI den Nachwuchswissenschaftlern die Möglichkeit, an den jährlichen Klausurtagungen „Immunologie in Freiburg“ teilzunehmen, die dem wissenschaftlichen Austausch dienen. In 2014 wurden 29 biologische und medizinische Doktoranden durch die Forschungsgruppen des CCI betreut.

Walter Hitzig Program

Prof. Dr. Walter Hitzig (1922-2012) is one of the pioneers of pediatric immunology in Europe. Among his major achievements is the initial description of the most severe known immunodeficiency, SCID. Through his initiative, the Working Party of Pediatric Immunology (API) developed in 1990, which in the following years shaped the specialty. Hitzig is the namesake of the „Walter Hitzig scholarships“ awarded by the CCI to young clinical scientists of the University of Freiburg in the Department of Immunology. With the international bid for the scholarships, the CCI seeks to enable talented young doctors to research in the field of immunodeficiency and to further promote their careers as research physicians.

Student Education

As part of the MOTIVATE program of the Medical Faculty, outstanding medical students receive scholarships for scientific work at the CCI. Within the framework of these scholarships, students have the opportunity to work on their medical doctoral thesis.

For PhD students, the CCI offers a structured guidance program in collaboration with the Departments of Immunobiology headed by Prof. Dr. Wolfgang Schamel and Molecular Medicine, led by Prof. Dr. Christoph Peters. This includes training in methods, seminars, workshops and retreats. The CCI is also affiliated with the MD-PhD program of the Spemann Graduate School of Biology and Medicine, which was established as part of the Excellence Initiative in Freiburg. Students who complete their thesis in a research group of the CCI or one associated with the CCI can participate in all CCI events. Special CCI seminars, basic research seminars during the summer term and an Advanced Immunology course during the winter term are offered. In addition, the CCI offers young researchers the opportunity to participate in the annual meeting „Immunology in Freiburg“ which is in the interest of scientific exchange. In 2014, the research groups of the CCI supervised 29 biological and medical doctoral students.

Wissenschaftliche Exzellenz braucht Austausch und gezielte Nachwuchsförderung.

Scientific excellence requires exchange and focused promotion of young talents.

Zu Gast am CCI

Das Gastwissenschaftler-Programm des CCI zielt darauf, die Zusammenarbeit internationaler Experten auf dem Gebiet der primären Immundefizienz weiter auszubauen und Labortechniken mit anderen Forschungsgruppen auszutauschen. Naturwissenschaftler und wissenschaftlich tätige Ärzte aus unterschiedlichen Ländern werden für eine Zeitdauer zwischen einer Woche und einem Jahr durch dieses Programm gefördert. Das CCI möchte auch weiterhin internationale Wissenschaftler dazu ermutigen, ihr Wissen und ihre Erfahrung in der Forschung zur Immundefizienz mit einem Aufenthalt am CCI zu erweitern.

Ales Janda, Gastwissenschaftler am CCI (Mai 2010-Mai 2011)

Nach meinem Abschluss in Medizin und einem Masterstudium in Immunologie in Oxford arbeitete ich als Forscher und Arzt mit Immundefizienz-Patienten in Prag.

Ich entschloss mich 2009, mehr Zeit der Forschung zu widmen und ging im Mai 2010 als Gastwissenschaftler an das CCI. In der Forschungsgruppe von Prof. Dr. Stephan Ehl konnte ich mich auf die Erforschung des autoimmunen lymphproliferativen Syndroms konzentrieren.

Die Integration von Forschung und Patientenversorgung wie sie das CCI lebt, eröffnete mir die Möglichkeit, nicht nur neue Labortechniken zu erlernen, sondern auch die klinische Behandlung von pädiatrischen und erwachsenen Patienten mit Immundefizienz. Ich bin sehr dankbar und glücklich, dass ich die Möglichkeit hatte, die pulsierende und motivierende Atmosphäre am CCI erleben zu dürfen. Diese Atmosphäre hat entscheidenden Einfluss auf meine Karriere als Arzt und Wissenschaftler gehabt.

A Guest at the CCI

The visiting scientist program of the CCI aims to further strengthen cooperation of international experts in the field of primary immunodeficiency and to share laboratory techniques with other research groups. Scientists and scientifically active doctors from different countries will be funded for a period of one week up to one year through this program. Furthermore, the CCI also wishes to encourage international scientists to expand their knowledge and experience in researching immunodeficiencies with a stay at the CCI.

Gastwissenschaftler am CCI, wie Dr. Ales Janda, tragen zum internationalen Austausch zwischen Wissenschaftlern und Ärzten bei.

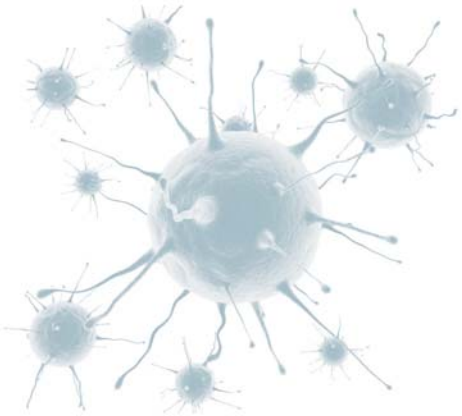
Visiting Scientist at the CCI, like Dr. Ales Janda, contribute to the international exchange between scientists and doctors.

Ales Janda, Guest Scientist at the CCI (May 2010-May 2011)

After graduating in medicine and earning a master's degree in immunology at Oxford, I worked as a researcher and doctor with immunodeficiency patients in Prague.

In 2009, I decided to devote more time to research and went in May 2010 as a visiting scientist to the CCI. In the research group of Prof. Dr. Stephan Ehl I could concentrate on the study of autoimmune lymphoproliferative syndrome.

The integration of research and patient care as it exists at the CCI opened the opportunity to learn not only new laboratory techniques, but also the clinical treatment of pediatric and adult patients with immunodeficiency. I am very grateful and happy that I had the opportunity of experiencing the vibrant and motivating atmosphere at the CCI. This atmosphere has had a decisive influence on my career as a physician and scientist.



Walter Hitzig übergibt CCI-Stipendiaten ihre Urkunden.

Walter Hitzig presents scholarship awards to young CCI scientists.

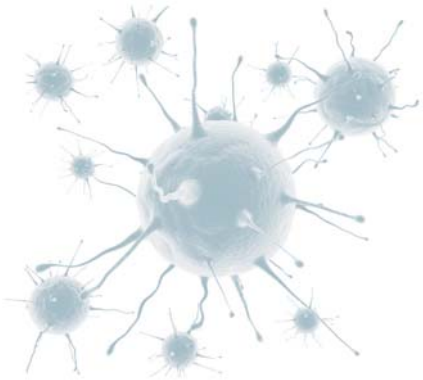
Nominierungen, Preise und Ehrungen

Wissenschaftler und Ärzte des CCIs wurden schon mehrfach für ihre Arbeit mit Preisen und Stipendien ausgezeichnet. So erhielt beispielsweise Dr. André Hennigs 2011 den Albrecht-Fleckenstein Nachwuchs-Preis. Der Helmut-Holzer-Forschungspreis der wissenschaftlichen Gesellschaft Freiburg, der für herausragenden Leistungen auf dem Gebiet der medizinischen Forschung vergeben wird, wurde 2012 an Dr. Christoph Neumann-Haefelin für seine Arbeiten auf dem Gebiet der Hepatitis-C Forschung verliehen. Der Hans-Hench-Preis, der von der Deutschen Gesellschaft für Immunologie (DGfI) für die beste Dissertation auf dem Gebiet der Klinischen Immunologie vergeben wird, wurde im Jahr 2012 an Dr. Lukas Kimmig für seine Arbeiten zur Bedeutung des menschlichen Komplement-Rezeptors 2 (CD21) für die humorale Immunantwort verliehen. Dr. Maximilian Seidl hat 2014 für das Projekt "Morphologische Zeichen der Immundysregulation als Prädiktoren der Lymphknotenmetastasierung" den Forschungsförderpreis der zweiten Nachwuchsakademie der Deutschen Gesellschaft für Pathologie erhalten. Ebenfalls in 2014 wurde Dr. Marta Rizzi mit einem Stipendium im Rahmen des Margarete von Wrangell Habilitations-Programms ausgezeichnet.

Als eines von vier Projekten wurde das CCI 2014 für sein Projekt: „Das Immunsystem verstehen – Immundefekte behandeln" für den Achse-Central Preis nominiert. Prof. Dr. Stephan Ehl und Henrike Ritterbusch hatten sich mit 20 anderen Kandidaten um den Preis beworben, den die Allianz Chronischer Seltener Erkrankungen (ACHSE e.V.) in Zusammenarbeit mit der Central Krankenversicherung vergibt. Ziel dieser Auszeichnung ist die Förderung und Verbreitung innovativer Projekte, die sich für die Versorgung von Menschen mit chronischen seltenen Erkrankungen einsetzen.

Dr. Marta Rizzi ist eine der Ärztinnen und Wissenschaftlerinnen am CCI, deren Forschungsarbeiten mit Preisen und Stipendien ausgezeichnet wurden.

Dr. Marta Rizzi is one of the physicians and scientists at the CCI, whose research has been awarded prizes and scholarships.



Nominations, Awards and Honors

Scientists and doctors of the CCIs have already been distinguished multiple times for their work with awards and scholarships. For example, Dr. André Hennig received in 2011 the Albrecht Fleckenstein Prize. The Helmut Holzer Research Award of the scientific community Freiburg, which is awarded for outstanding achievements in the field of medical research, was awarded in 2012 to Dr. Christoph Neumann-Haefelin for his work in the field of hepatitis C research. The Hans-Hench-Prize awarded by the German Society for Immunology (DGfI) for the best dissertation in the field of clinical immunology was given to Dr. med. Lukas Kimmig in 2012 for his work on the importance of human complement receptor 2 (CD21) for the humoral immune response. Dr. Maximilian Seidl received in 2014 the research grant from the second junior researcher academy of the German Society of Pathology for the project „Morphological signs of immune dysregulation as predictors of lymph node metastasis“. Also in 2014, Dr. Marta Rizzi was awarded a scholarship under the Margarete von Wrangell Habilitation Program.

As one of four projects, the CCI was nominated in 2014 for the Achse-Central Prize for its project: „Understand the immune system - treat immunodeficiencies“. Prof. Dr. Stephan Ehl and Henrike Ritterbusch had applied with 20 other candidates for the prize, which the Alliance for Chronic Rare Diseases (ACHSE) in collaboration with the Central Health Insurance awards. The aim of this award is to promote and disseminate innovative projects that promote the care of people with chronic rare diseases.

DIE PARTNER DES CCI

Klinische Partner

Der Erfolg des CCI in den letzten Jahren war nur möglich, weil es fest verzahnt ist mit vielen Abteilungen des Klinikums, die einerseits eine eigenständige Entwicklung des CCI gefördert haben, andererseits jederzeit mit ihrer Infrastruktur dem CCI zur Verfügung stehen.

Die exzellente Zusammenarbeit und das gewachsene Vertrauen wird auch nach der Förderung durch das Bundesministerium für Bildung und Forschung (BMBF) 2018 eine entscheidende Voraussetzung für die weitere Entwicklung des CCI sein. Die gemeinsame Ausbildung und Entwicklung von Personal mit spezifischer Expertise im Bereich Immundefizienz sowie gemeinsame Forschungsprojekte sind hierfür eine wichtige Grundlage.

Eine vollumfängliche Versorgung der CCI-Patienten ist nur durch die enge Zusammenarbeit im stationären Bereich der Kinderklinik (Prof. Dr. Charlotte Niemeyer, Prof. Dr. Ute Spiekerkötter) und der Inneren Medizin (Prof. Dr. Reinhard Voll, Prof. Dr. Winfried Kern, Prof. Dr. Robert Thimme) möglich.

Die Entwicklung im Bereich der Stammzelltransplantation von Immundefekten in der Pädiatrie (PD Dr. Brigitte Strahm) und zunehmend auch im Erwachsenenbereich (Prof. Dr. Jürgen Finke) ist ein sichtbarer Ausweis dieser Zusammenarbeit, ergänzt durch gemeinsame Sprechstunden und Forschungsprojekte vor allem im Überlappungsbereich zwischen Hämato/Onkologie und Immunologie.

In der Diagnostik ermöglicht die hervorragende Zusammenarbeit mit der Pathologie (Prof. Dr. Martin Werner) Spezialuntersuchungen im Bereich der Immundefekte, die zunehmend Referenzcharakter bekommen.

Die Unterstützung der Clinical Research Unit des CCI durch biometrische und biostatistische Expertise (Prof. Dr. Werner Vach) und das Studienzentrum (Rainer Bredenkamp) bettet die klinische Studienaktivität des CCI in einen grösseren Kontext ein und erlaubt die Umsetzung ambitionierter Projekte.

Prof. Dr. Niemeyer

Prof. Dr. Spiekerkötter

Prof. Dr. Voll

Prof. Dr. Kern

Prof. Dr. Thimme

PARTNERS OF THE CCI

Clinical Partners

The success of the CCI in recent years has only been possible because it firmly interacts with many Departments of the Medical Center of the University of Freiburg. These departments on the one hand promoted independent development of the CCI, on the other hand provide at any time their infrastructure to the CCI.

The excellent cooperation and the grown trust will be an essential prerequisite for the further development of the CCI, in particular after the end of BMBF funding in 2018. For this, the joint training and development of staff with specific expertise in immunodeficiency and joint research projects are an important basis.

Complete care of CCI patients would be impossible without the close cooperation in the inpatient management at the Center for Pediatrics (Prof. Dr. Charlotte Niemeyer, Prof. Dr. Ute Spiekerkötter) and the Department of Internal Medicine (Prof. Dr. Reinhard Voll, Prof. Dr. Winfried Kern, Prof. Dr. Robert Thimme)

Recent developments in the field of stem cell transplantation of immunodeficiencies in pediatrics (PD Dr. Brigitte Strahm) and, increasingly, also in the adult area (Prof. Dr. Jürgen Finke) is a visible identification of this cooperation, complemented by joint consultations and research projects especially in the overlap area between hemato/oncology and immunology.

In the diagnostic setting, the excellent cooperation with the Center for Pathology (Prof. Dr. Martin Werner) allows specialized studies in the field of immunodeficiencies that increasingly achieve reference character.

The support of the Clinical Research Unit of the CCI by biometric and biostatistical expertise (Prof. Dr. Werner Vach) and the Clinical Study Center (Rainer Bredenkamp) embeds the clinical trial activities of the CCI in a larger context and allows the realization of ambitious projects.

PD Dr. Strahm

Prof. Dr. Finke

Prof. Dr. Werner

Prof. Dr. Vach

R. Bredenkamp

Wissenschaftliche Partner

Die wissenschaftliche Grundlage für die Gestaltung des CCI wurde mit dem Sonderforschungsbereich (SFB) 620: „Immundefizienz - Klinik und Tiermodelle“ gelegt (Prof. Dr. Hans-Hartmut Peter, Prof. Dr. Hanspeter Pircher).

In diesem SFB haben sich langjährige Zusammenarbeiten mit dem Max-Planck-Institut (MPI) für Immunbiologie und Epigenetik (Dr. Thomas Boehm) und der Fakultät für Biologie (Prof. Dr. Michael Reth) entwickelt, die zur festen Einbindung dieser beiden Einrichtungen in die Aktivitäten des CCI geführt haben.

Die Integration einer CCI Juniorgruppe am MPI in die Abteilung von Dr. Thomas Boehm und die intensive Beteiligung der CCI-Arbeitsgruppen an der Gestaltung des Studiengangs Immunbiologie sind Beispiele für die vielfältigen Interaktionen.

Darüber hinaus ist die Interaktion mit dem Department für Medizinische Mikrobiologie und Hygiene, insbesondere mit dem Institut für Immunologie (Prof. Dr. Hanspeter Pircher), aber auch mit dem Institut für Virologie (Prof. Dr. Hartmut Hengel) und dem Institut für Mikrobiologie (Prof. Dr. Georg Häcker) Grundlage für die gemeinsame Weiterentwicklung des wissenschaftlichen Schwerpunkts „Immunologie und Infektiologie“ der medizinischen Fakultät, bei der das CCI eine führende Rolle übernimmt.

Die kürzliche Neueinrichtung des SFB1160: „Immunpathologie als Folge eingeschränkter Immunreaktionen“ zeigt, dass das integrative Potential dieser wissenschaftlichen Zusammenarbeiten auch weiterhin genutzt und ausgebaut wird.

Prof. Dr. Pircher

Dr. Boehm

Scientific Partners

The scientific basis for the design of the CCI was set by the Collaborative Research Center (CRC) 620: “Immunodeficiency – Clinical Manifestations and Animal Models” (Prof. Dr. Hans-Hartmut Peter, Prof. Dr. Hanspeter Pircher).

In this CRC, longstanding collaborations with the Max Planck Institute (MPI) of Immunobiology and Epigenetics (Dr. Thomas Boehm) and the Faculty of Biology (Prof. Dr. Michael Reth) have developed, which led to the solid integration of these two institutions in the activities of the CCI.

The integration of a CCI junior group at the MPI in the Department of Dr. Thomas Boehm and the intensive involvement of the CCI Research Groups in the design of the Master Studies in Immunobiology are examples of the various interactions.

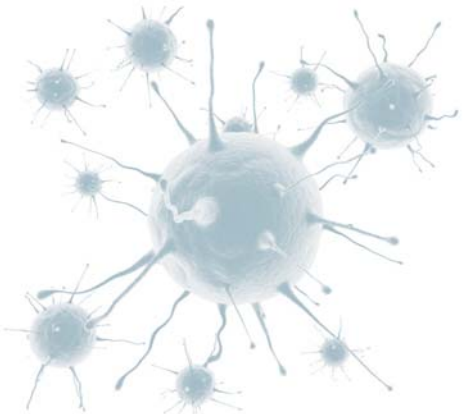
In addition, the interaction with the Center for Microbiology and Hygiene, in particular with the Institute for Immunology (Prof. Dr. Hanspeter Pircher), but also with the Institute for Virology (Prof. Dr. Hartmut Hengel) and the Institute for Microbiology and Hygiene (Prof. Dr. Georg Häcker) are the basis for the joint development of the scientific focus “Immunology and Infectious Diseases” of the Faculty of Medicine, in which the CCI is taking a leading role.

The recent establishment of the CRC1160: “Immune-mediated pathology as a consequence of impaired immune reactions” shows that the integrative potential of these scientific collaborations will continue to be used and developed.

Prof. Dr. Reth

Prof. Dr. Hengel

Prof. Dr. Häcker



DIE PROFESSUREN AM CCI

Der Auftrag des CCI, Medizin und Forschung zum Wohle von Patienten mit Immunerkrankungen zu integrieren, zeigt sich auch in der Leitung, die sich Prof. Prof. Dr. Stephan Ehl als Medizinischer Direktor und Prof. Dr. Bodo Grimbacher als Wissenschaftlicher Direktor teilen. Neben ihnen gibt es am CCI sechs weitere Professuren oder vergleichbare Führungsstellen, die mit herausragenden Ärzten und Wissenschaftlern aus unterschiedlichen Disziplinen besetzt sind.

Professur für Rheumatologie und Klinische Immunologie

Prof. Dr. Reinhard Voll ist für erwachsene Immundefizienz-Patienten am CCI und für Patienten mit rheumatologischen Krankheitsbildern verantwortlich. Er leitet die Forschungsgruppe Autoimmunität, die die Entstehung von entzündlichen Autoimmunerkrankungen, die oft bei primären Immundefekten auftreten, untersucht.

Professur für Pädiatrische Immunologie

Prof. Dr. Stephan Ehl ist medizinischer Direktor und Sprecher des CCI. Er ist außerdem am CCI für Kinder mit Immundefizienz-Erkrankungen verantwortlich. Weiterhin übersieht er den Bereich Klinische Studien und zusammen mit Prof. Dr. Klaus Warnatz die Advanced Diagnostic Unit. Er leitet die Forschungsgruppe zum Thema Immundefizienz bei Kindern.

Professur für Experimentelle Immundefizienz

Prof. Dr. Bodo Grimbacher ist Arzt und wissenschaftlicher Direktor des CCI und leitet die grundlagenwissenschaftliche Ausbildung im CCI. Seine Forschungsgruppe studiert die Auslöser von Immundefekten. Über die (mono)genetischen Ursachen der Defekte möchte er die Erkrankung der Patienten besser verstehen lernen, um daraufhin bessere, gezieltere und individualisierte Therapeutika zu entwickeln und anbieten zu können.

Professur für Zell- und Gentherapie

Prof. Dr. Toni Cathomen leitet das Institut für Zell- und Gentherapie am Universitätsklinikum und die gleichnamige Forschungsgruppe am CCI. Der Professur ist die Juniorgruppe „Genome Engineering“, die Research Unit Gene Therapy sowie die Forschungsplattform iPSC/TALEN unterstellt. Schwerpunkt der Forschungstätigkeit ist die Entwicklung, Evaluierung und Herstellung von therapeutisch wirksamen T-Zell- und Stammzellpräparaten mittels Gentherapie. Mittelfristig sollen Gentherapeutika zur Behandlung von angeborenen Immundefekten sowie zur Therapie von HIV-Infektionen und Leukämien entstehen.

THE CCI PROFESSORS

The mission of the CCI, to integrate medicine and research for the benefit of the patient, can also be seen in the leadership, which is shared by Prof. Dr. Stephan Ehl as the medical director and Prof. Dr. Bodo Grimbacher as the scientific director. Alongside them, there are six other professorships or similar leadership positions at the CCI, which are held by outstanding physicians and scientist from various disciplines.

Professor of Rheumatology and Clinical Immunology

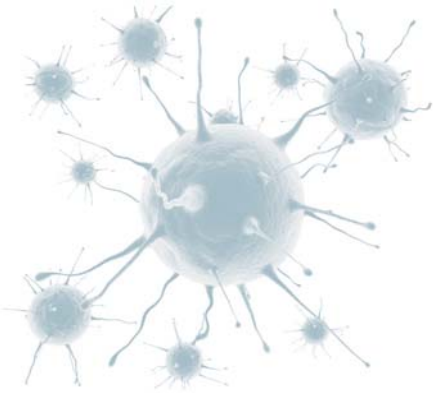
Prof. Dr. Reinhard Voll is responsible for adult immunodeficiency patients at the CCI as well as for patients with rheumatic diseases. The autoimmunity research group examines under his leadership the development of inflammatory autoimmune diseases, which often occur in primary immunodeficiency.

Professor of Pediatric Immunology

Prof. Dr. Stephan Ehl is medical director and spokesman of the CCI. In addition to these duties, he is responsible for children with immunodeficiency diseases at the CCI. Furthermore, he oversees the clinical studies and together with Prof. Dr. Klaus Warnatz the Advanced Diagnostic Unit. He heads the research group on immunodeficiency in children.

Professor of Experimental Immunodeficiency

Prof. Dr. Bodo Grimbacher is a physician and scientific director of the CCI. He also runs the basic scientific training program at the CCI. His research group studies the triggers of immunodeficiencies. In order to develop and provide better, more targeted and individualized therapies, he seeks to improve the understanding of the patients' diseases using the (mono)genetic causes of the defects.



Professor of Cell and Gene Therapy

Prof. Dr. Toni Cathomen heads the Institute for Cell and Gene Therapy at the Medical Center – University of Freiburg and the research group of the same name at the CCI. The Junior Group „Genome Engineering", the Research Unit of Gene Therapy, and the Research Platform iPSC/TALEN also fall under the professorship's direction. The focal point of the research is the development, evaluation and production of therapeutically effective T-cell and stem cell preparations using gene therapy. In the near future, gene therapy should develop as the treatment for congenital immunodeficiencies as well as for HIV infections and leukemia.

Professur für Klinische Infektionsimmunologie

Prof. Dr. Philipp Hennekes Spezialgebiet ist die infektionsimmunologische Betreuung von Kindern am CCI. Er leitet die Forschungsgruppe zum Thema Infektion und Immunität und betreut das Walter-Hitzig-Programm für Nachwuchs-Wissenschaftler aus dem Bereich der Medizin.

Professur (APL) für Klinische Immunologie

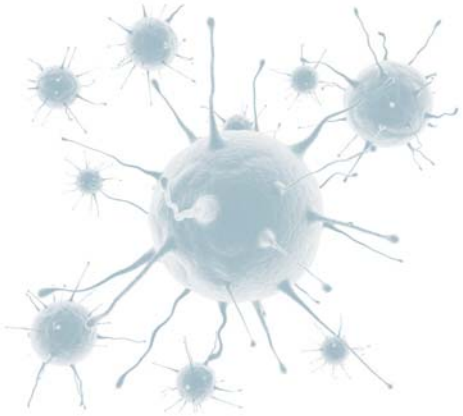
Prof. Dr. Klaus Warnatz leitet die Ambulanz für erwachsene Immundefizienz-Patienten und zusammen mit Prof. Dr. Stephan Ehl die Advanced Diagnostics Unit. Er ist Leiter des Freiburger Jeffrey Model Foundation-Zentrums für Immundefekte. Seine Forschungsgruppe untersucht Ursachen und Folgen von Störungen des erworbenen Immunsytems des Menschens.

Professur für Immunbiologie

Prof. Dr. Wolfgang Schamel ist Professor für Immunbiologie an der biologischen Fakultät der Universität Freiburg und leitet den Master-Studiengang Immunbiologie. Seine Arbeitsgruppe erforscht molekulare Mechanismen der T-Zell-Aktivierung. Durch Prof. Schamel ist die Fakultät für Biologie im Vorstand des CCI vertreten.

Juniorgruppe für Molekulare Immunologie

Dr. Ana Izcue leitet die Arbeitsgruppe Molekulare Immunologie am CCI, die am Max-Planck-Institut für Immunbiologie und Epigenetik (MPI) angesiedelt ist. Dr. Izcue stellt die Verbindung zwischen MPI und CCI her.



Professor of Clinical Infectious Immunology

Prof. Dr. Philipp Henneke's specialty is the infectious immunological pediatric care at the CCI. He heads the research group on infection and immunity and is in charge of the Walter-Hitzig Program for young scientists in the field of medicine.

Professor (adjunct) of Clinical Immunology

Prof. Dr. Klaus Warnatz runs the outpatient clinic for adult immunodeficient patients and together with Prof. Dr. Stephan Ehl, the Advanced Diagnostics Unit. He is also the director of the Freiburg Jeffrey Model Foundation-Center for Immunodeficiencies. His research group studies the causes and consequences of disorders of the acquired immune system of the human being.

Professor of Immunobiology

Prof. Dr. Wolfgang Schamel is a professor of immunobiology at the Faculty of Biology of the University of Freiburg and manages the immunobiology master's program. His research group investigates the molecular mechanisms of T cell activation. Prof. Schamel represents the Faculty of Biology in the board of the CCI.

Junior Group for Molecular Immunology

Dr. Ana Izcue leads the CCI Research Group of Molecular Immunology located at the Max Planck Institute of Immunobiology and Epigenetics (MPI). Dr. Izcue forges the link between the MPI and the CCI.

Das Management Team des CCI (von links nach rechts): Cornelius Struck, Gabriele Hofmann, Heike Ufheil, Günther Storz, Dorit Arlt

The CCI management team (left to right): Cornelius Struck, Gabriele Hofmann, Heike Ufheil, Günther Storz, Dorit Arlt

SELBSTVERWALTET, UNABHÄNGIG UND INTEGRIERT

Am CCI arbeiten Ärzte und Wissenschaftler unterschiedlicher Fachrichtungen zusammen. Seit seiner Gründung im Jahr 2008 ist das CCI eine selbstverwaltete und budgetär eigenständige Einrichtung des Universitätsklinikums Freiburg. Das CCI wird von einem Medizinischen und einem Wissenschaftlichen Direktor geleitet. Im CCI-Vorstand sind neben den CCI-Professoren die wichtigsten Partner des CCI am Universitätsklinikum Freiburg vertreten sowie mit Dr. Thomas Boehm das Max-Planck-Institut für Immunbiologie und Epigenetik und mit Dr. Klaus Schwarz das Institut für Transfusionsmedizin in Ulm. Der wissenschaftliche Beirat des CCI besteht aus acht Mitgliedern. Er garantiert einen kritischen, unabhängigen Blick auf die Arbeit des CCI und wird in Grundsatzentscheidungen des CCI mit einbezogen.

Der Wissenschaftliche Beirat begleitet in unterschiedlicher Zusammensetzung die klinische und wissenschafltiche Entwicklung des CCI. Er garantiert einen kritischen, unabhängigen Blick auf die Arbeit des CCI und wird in Grundsatzentscheidungen des CCI mit einbezogen.

Die derzeitigen Mitglieder des wissenschaftlichen Beirats des CCI sind:

- Prof. Dr. Stefan Meurer, Institut für Immunologie, Universitätsklinikum Heidelberg (Vorsitz)
- Prof. Dr. Maria Blettner, Institut für Medizinische Biometrie, Epidemiologie und Informatik (IMBEI), Johannes Gutenberg-Universität Mainz
- Prof. Dr. Andrew Cant, Great North Children‘s Hospital, Newcastle, UK
- Prof. Dr. David Nadal, Leiter Infektiologie, Kinderspital Zürich, Schweiz
- Prof. Dr. Martina Prelog, Kinderklinik, Universitätsklinikum Würzburg
- Prof. Dr. José Regueiro, Complutense Universidad Madrid, Spanien
- Prof. Dr. Antonius Rolink, Department Biomedizin, Universitätsspital Basel, Schweiz
- Prof. Dr. Adrian Thrasher, Center for Immunodeficiency, University College London, UK

SELF-GOVERNED, INDEPENDENT AND INTEGRATED

At the CCI, doctors and scientists from different disciplines work together on an equal footing. Since its foundation in 2008, the CCI is a self-governing, independent institution of the Medical Center at the University of Freiburg. The CCI is managed by a Medical and a Scientific Director. Apart from the CCI professors, representatives of the main partners of the CCI at the University Medical Center are represented as well as Max Planck Institute for Immunobiology (Dr. Thomas Boehm) and the Institute for Transfusion Medicine in Ulm (Dr. Klaus Schwarz).

The Scientific Advisory Board consists of eight members and accompanies the clinical and scientific development of the CCI. It guarantees a critical, impartial look at the work of the CCI and is involved in landmark decisions of the CCI.

Current members of the Advisory Board of the CCI are:

- Prof. Dr. Stefan Meurer, Institute of Immunology, Heidelberg University Hospital (Chair)
- Prof. Dr. Maria Blettner, Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), Johannes Gutenberg University Mainz
- Prof. Dr. Andrew Cant, Great North Children's Hospital, Newcastle, UK
- Prof. Dr. David Nadal, Head of Infectious Disease, University Children's Hospital Zurich, Switzerland
- Prof. Dr. Martina Prelog, Department of Pediatrics, University Hospital of Würzburg
- Prof. Dr. José Regueiro, Complutense University of Madrid, Spain
- Prof. Dr. Antonius Rolink, Department of Biomedicine, University Hospital of Basel, Switzerland
- Prof. Dr. Adrian Thrasher, Center for Immunodeficiency, University College London, UK

Das erste Advisory Board des CCI. Von links nach rechts:
David Nadal, Luigi Notarangelo, Martin Krönke, Christine Kinnon, Christian Bogdan, Hans Ochs (Vorsitz)

The first advisory board of the CCI. Left to right:
David Nadal, Luigi Notarangelo, Martin Krönke, Christine Kinnon, Christian Bogdan, Hans Ochs (Chair)

Mit vielen Aktionen in der Öffentlichkeit ist das CCI bemüht auf Erkrankungen des Immunsystems aufmerksam zu machen.

With many campaigns in the public, the CCI is working to draw attention to immune disorders.

Durch die Mitgliedschaft in internationalen Netzwerken, z.B. über die International Nursing Group for Immunodeficiencies (INGID) oder die European Society for Immunodeficiencies (ESID), arbeitet das CCI mit internationalen Organisationen zusammen, um Standards in der Patientenversorgung zu etablieren. Das CCI hat eine AWMF (Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften) Leitlinie zur Diagnostik primärer Immundefekte koordiniert, die Nicht-Spezialisten eine Unterstützung bieten soll, Immundefekte schneller zu erkennen und zu diagnostizieren.

ZUSAMMEN ÖFFENTLICHKEIT SCHAFFEN

„Wir machen aufmerksam“

Patienten mit seltenen Erkrankungen fühlen sich oft alleingelassen und durch die Unkenntnis der Öffentlichkeit missverstanden. Es gibt nur wenige Menschen mit der gleichen Erkrankung und häufig ist ein Kontakt für den Austausch untereinander schwierig. Zusätzlich gibt es nur wenige zuverlässige Informationen zu den Erkrankungen. Die Suche im Internet führt oft eher zu Frustration und Verunsicherung.
Die Ärzte und Wissenschaftler des CCI setzen sich durch Vorträge und Artikel für ein besseres Verständnis von Immundefekten ein. Durch Ihre Sachkenntnis und ihr Engagement möchten sie dazu beitragen, diesen Erkrankungen mehr Öffentlichkeit zu verschaffen. Patienten mit Immundefekten sollen in Zukunft die Chance auf eine schnellere und bessere Behandlung erhalten. Für dieses Ziel arbeitet das CCI mit deutschen und internationalen Patientenorganisationen, wie der Deutschen Selbsthilfe Angeborene Immundefekte (dsai) und der Jeffrey Modell Foundation (JMF) zusammen.

DSAI

Die Deutsche Selbsthilfe Angeborene Immundefekte (dsai) engagiert sich seit mehr als 20 Jahren für die Aufklärung im Bereich der Immundefekte. Sie hat das Ziel, Menschen mit Immundefekten und ihren Angehörigen den Erfahrungsaustausch außerhalb der medizinischen Betreuung zu ermöglichen. Unter dem Motto „Defektes Immunsystem? Starke Patientenorganisation“ macht sich die dsai für eine breite Aufklärung von Ärzten aller Fachgebiete und der Öffentlichkeit stark, engagiert sich für die Forschung und setzt sich für die Einrichtung von Immundefektambulanzen an deutschen Kliniken ein. Die Vereinigung ist ein kompetenter Partner in einem Netzwerk aus Betroffenen, Spezialisten, Behörden und Forscherteams. Seit seiner Gründung kooperiert das CCI mit der dsai in Form von gemeinsamen Informationsbroschüren, Schulveranstaltungen und der Durchführung von Patienten- und Ärztetagen.
www.dsai.de

Through membership in international networks, e.g. the International Nursing Group for Immunodeficiencies (INGID) and the European Society for Immunodeficiencies (ESID), the CCI is working with international organizations to establish standards in patient care. The CCI coordinated an AWMF (Association of the Scientific Medical Societies) guideline for the diagnosis of primary immunodeficiencies to provide support to non-specialist for faster detection and diagnosis of immunodeficiencies.

CREATING PUBLIC AWARENESS TOGETHER

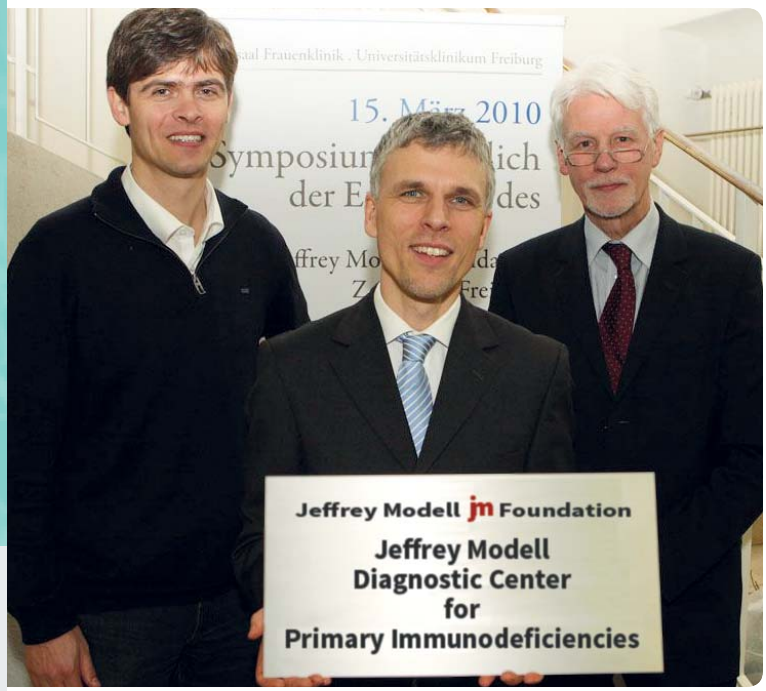
„We raise attention“

Patients with rare diseases often feel left alone and misunderstood due to the ignorance of the public. There are very few people with the same disease, and often it is difficult to establish contact to interact with each other. On top of that, dealing with the disease can be aggravating because there is little reliable information about these diseases. Searching the Internet often leads to frustration and uncertainty.
The doctors and scientists of the CCI campaign for a better understanding of diseases of the immune system through lectures and articles. With their expertise and commitment they want to contribute to an increased public awareness of these diseases. Patients with immunodeficiencies should be given the chance to receive faster and better treatment in the future. To reach this goal, the CCI works together with German and international patient organizations, such as the German Patient Support Group for Congenital Immunodeficiencies (German: Deutsche Selbsthilfe Angeborene Immundefekte, dsai) and the Jeffrey Modell Foundation (JMF).

DSAI

The German Patient Support Group for Congenital Immunodeficiencies (German: Deutsche Selbsthilfe Angeborene Immundefekte, dsai) has been involved for more than 20 years in awareness in the field of immunodeficiencies. Its aim is to enable people who suffer from a primary immunodeficiency and their family members to exchange experiences outside of medical care. Under the motto „Defective immune system? Strong patient organization” the dsai makes a strong case for a comprehensive education of physicians of all specialties and the public, is dedicated to the development of research and advocates the establishment of immunodeficiency outpatient clinics at German hospitals. Since its inception, the CCI cooperates with the dsai in the form of shared information brochures, school events and events for patients and physicians.
www.dsai.de

dsai
Defektes Immunsystem?
Starke Patientenorganisation!



Offizielle Eröffnung des JMF-Zentrums am CCI im März 2010.

Official opening of the JMF Center at the CCI in March 2010.

Jeffrey Modell Foundation Netzwerk

Vor 17 Jahren wurde die Jeffrey Modell Foundation (JMF) von Vicki und Fred Modell aus New York ins Leben gerufen. Beide haben sich, geprägt durch den Verlust ihres an einem schweren Immundefekt erkrankten Sohnes, zum Ziel gesetzt, die Früherkennung von Patienten mit angeborenen Störungen der Immunabwehr zu verbessern. Das CCI ist als JMF-Zentrum Mitglied des JMF-Netzwerks. Gemeinsam mit JMF veranstaltet das CCI Aktionen für mehr Öffentlichkeit für angeborene Immundefizienz, beispielsweise während der weltweiten primären Immundefizienz Woche (22.-29. April) und dem internationalen Tag der Immunologie (29. April).
www.info4pi.org

Informationsbroschüren

Das CCI hat zu einer Reihe von Immundefekten Informationsbroschüren für Patienten verfasst, in denen viele Fragen der Patienten aufgegriffen und beantwortet werden. Diese Broschüren wurden auch ins Türkische, Arabische und Englische übersetzt.
www.uniklinik-freiburg.de/cci/informationen-fuer-patienten/informationsblaetter.html

Schulveranstaltungen

Zusammen mit der dsai hat das CCI mehrfach Schülerfortbildungen veranstaltet, an denen jeweils mehr als 200 Gymnasiasten teilnahmen. In der halbtägigen Veranstaltung werden die Grundlagen des Immunsystems, mögliche Erkrankungen wie angeborene und erworbene Immundefizienz und Autoimmunität von Professoren des CCI anschaulich und praxisnah dargestellt.

Science Days

Der Förderverein Science und Technologie e.V. organisiert jedes Jahr in Zusammenarbeit mit dem Europa-Park die Science Days. Dabei werden Wissenschaft und Technik durch anschauliche Experimente und Workshops für jeden greifbar vermittelt. Das CCI hat mehrmals mit Informationsständen über das Immunsystem mitgewirkt.

Mitarbeiter und Wissenschaftler des CCI wollen die Faszination des Immunsystems weitergeben.

The scientists of the CCI want to share the fascination of the immune system.

Jeffrey Modell Foundation Network

Seventeen years ago, Vicki and Fred Modell from New York launched the Jeffrey Modell Foundation (JMF). Both, marked by the loss of their son to SCID, aim to improve the early identification of patients with congenital disorders of the immune system, so as to enable the early employment of appropriate therapeutic measures. As JMF Center, the CCI is member of the JMF Network. Together with JMF, the CCI organizes campaigns to increase publicity for congenital immunodeficiency, for example during the global Primary Immunodeficiency Week (April 22 to 29) and the International Day of Immunology (April 29).
www.info4pi.org

Für Kinder wie Jeffrey arbeitet das CCI mit Organisationen wie der dsai und der JMF zusammen. Die Zusammenarbeit schafft mehr Öffentlichkeit und dient der Verbesserung der Diagnose und Therapie von Immundefekten.

For children like Jeffrey, the CCI works together with organizations such as the dsai and the JMF. The collaboration creates more publicity and serves to improve the diagnosis and treatment of immunodeficiencies.

Information Brochures

The CCI has written a series of patient information brochures on immunodeficiencies, in which many patients' questions are addressed and answered. These brochures were also translated from German into Turkish, Arabic and English.
www.uniklinik-freiburg.de/cci/informationen-fuer-patienten/informationsblaetter.html

School Events

Together with the dsai, the CCI has repeatedly held student education courses, which more than 200 high school students attended each time. At the half-day event at the Medical Center in Freiburg, students in the middle and upper years of school can become informed about the basics of the immune system, as well as potential diseases such as congenital and acquired immunodeficiency and autoimmunity.

Science Days

The Association for Science and Technology (in German: Förderverein Science and Technology e.V.) organizes every year in collaboration with the Europa-Park, the Science Days. Here, science and technology are shared in a tangible and interesting way. The CCI has participated in the Science Days on several occasions with information booths on the immune system.

Viele Interessierte nutzen die Gelegenheit, sich bei den Freiburger Abendvorlesungen über Mikroorganismen und das Immunsystem zu informieren.

Many interested people took the opportunity to inform themselves about microorganisms and the immune system at the Freiburg evening lectures.

Abendvorlesungen

2014 veranstaltete das Universitätsklinikum in Kooperation mit der Badischen Zeitung drei Abendvorlesungen zum Thema Immunsystem. Ärzte und Wissenschaftler des CCI erklärten mehr als 200 interessierten Zuhörern in zwei Vorträgen, warum Mikroorganismen sowohl der beste Freund als auch der ärgste Feind des Menschen sein können. Im dritten Vortrag wurde über Immunschwächen, Autoimmunerkrankungen und ihre Therapien informiert.

Weltwoche angeborene Immunschwäche und Tag der Immunologie

Jedes Jahr wird weltweit vom 22. – 29. April die Weltwoche angeborene Immunschwäche (englisch: Worldwide Primary Immunodeficiency Week, WPIW) begangen. Während dieser Woche klären Patientenorganisationen, Ärzte und Kliniken über primäre Immundefekte auf. Mit Aktionen, Informationsständen und Veranstaltungen werden diese Krankheiten der Öffentlichkeit näher gebracht. Das CCI beteiligt sich mit Informationsständen, Aktionen und Pressearbeit.

Evening Lectures

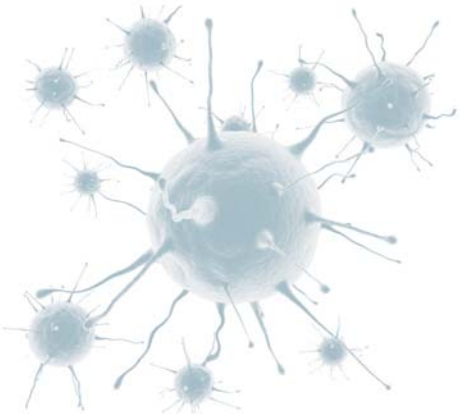
In 2014, the Medical Center - University of Freiburg, in cooperation with the Badische Zeitung, held a series of three evening lectures for the public on the subject of the immune system. Physicians and scientists from the CCI explained to more than 200 interested listeners each time why microbes can be both best friend and worst enemy of man. In the third lecture the audience was informed about immunodeficiencies, autoimmune diseases and their therapies.

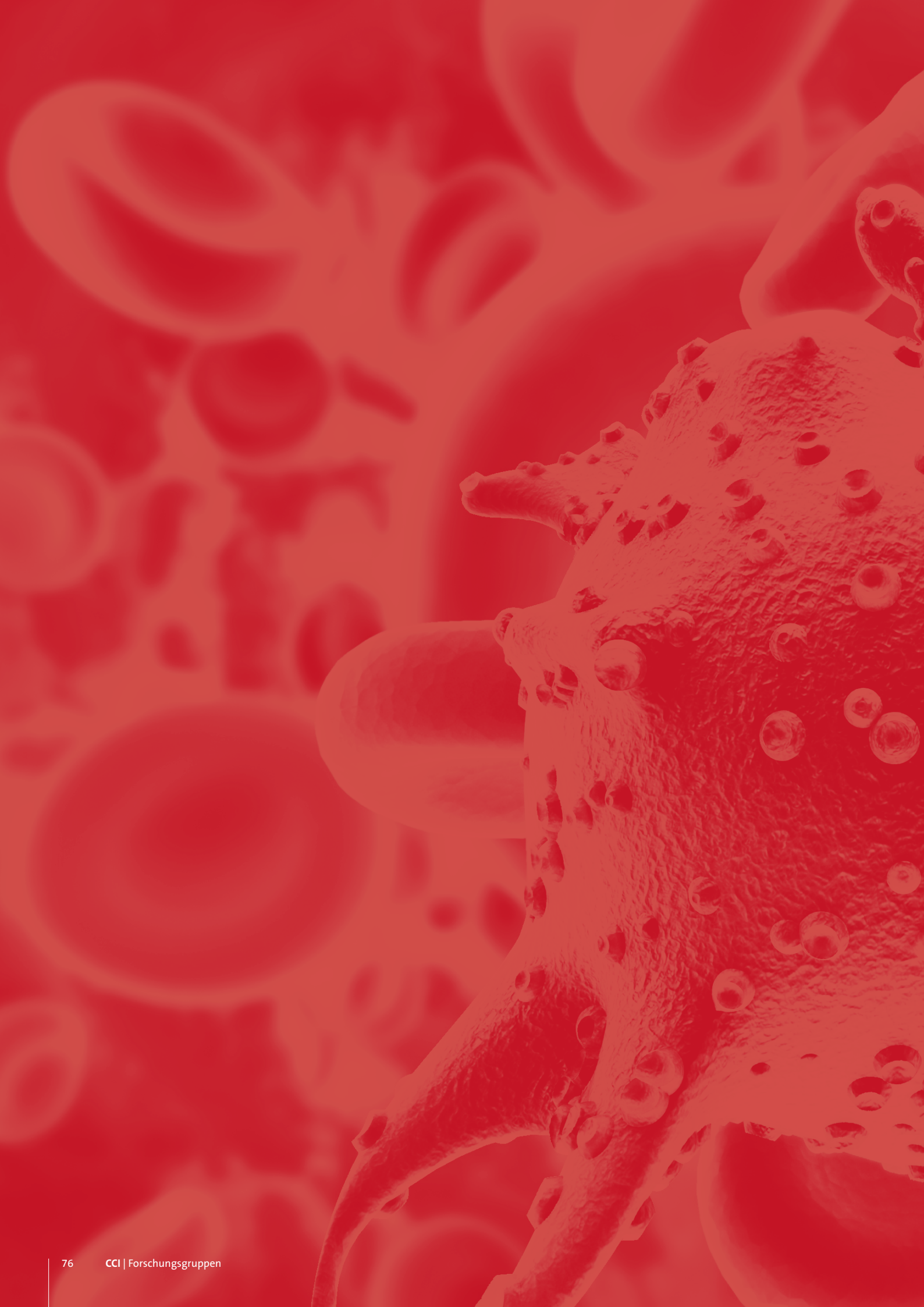
Worldwide Primary Immunodeficiency Week and Day of Immunology

Every year from April 22-29 the Worldwide Primary Immunodeficiency Week (WPIW) is celebrated around the world. During this week, patient organizations, doctors and clinics shed light upon primary immunodeficiencies. With activities, information booths and events, these diseases are brought closer to the public's attention. The CCI partakes in both events with information stands, activities and public relations.

Während der Weltwoche für angeborene Immunschwäche wird mit Aktionen und Veranstaltungen die Öffentlichkeit auf Erkrankungen des Immunsystems aufmerksam gemacht.

During the Worldwide Primary Immunodeficiency Week, the public's attemtion is called to disorders of the immune system by actions and events.





CCI | FORSCHUNGSGRUPPEN

CCI | research groups

Prof. Dr. Toni Cathomen
Director
Institute for Cell and Gene Therapy
Center for Chronic Immunodeficiency (CCI)
Medical Center – University of Freiburg

CELL & GENE THERAPY

Developing novel treatment options for chronic immunodeficiencies

KEYWORDS

Primary Immunodeficiencies
Acquired Immunodeficiencies
Targeted Genome Editing
Designer Nucleases
Induced Pluripotent Stem Cells

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GROUP LEADER
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Dr. rer. nat. Tatjana Cornu
Dr. med. Florian Emmerich
Dr. rer. nat. Markus Hildenbeutel
Dr. rer. nat. Barbara Timm
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Ilona Skatulla

OTHERS
Ruba Hammad (M.Sc. student)
Kristina Semukhina (M.Sc. student)
Hoai-Thanh Vu (PA)

Several patients suffering from severe forms of immunodeficiency have been effectively treated with procedures that combine cell and gene therapy. While gene therapy aims at modifying the genome of patient cells to achieve a therapeutic benefit, cell therapy is defined as the administration of "therapeutic" cells in a patient for the treatment of a disease. Overall, the combination of cell and gene therapy to treat primary immunodeficiencies has been highly successful but a few serious adverse events in some trials are prompting the development of safer protocols.

The research group "Cell and Gene Therapy" is affiliated with both the CCI and the Institute for Cell and Gene Therapy at the University Medical Center Freiburg. Its major goals include the generation of novel cellular models to understand primary immune deficiencies and the development of safe cell and gene therapy protocols to treat immunodeficiency patients. In particular, the laboratory focuses on:

1. The generation of cellular disease models and cell therapeutics based on induced pluripotent stem cells (iPSCs).
2. The development of safe gene therapeutics based on targeted genome editing in human stem cells using designer nucleases.
3. The translation of the gene therapy efforts into the clinic.

INDUCED PLURIPOTENT STEM CELL TECHNOLOGY

Induced pluripotent stem cells (iPSCs) offer an unprecedented access to human biology with opportunities to fundamentally advance the fields of drug development, disease research and regenerative medicine. To fully exploit the potential of pluripotent stem cells, we have established robust protocols that allow us to modify the genome of these cells in a targeted fashion and to differentiate iPSCs into specific immune cell types (Rahman et al., 2015; Bobis-Wozowicz et al., 2014; Osiak et al., 2011).
Using the non-integrating Sendai virus based system for reprogramming, we have generated and fully characterized several iPSC lines from patients suffering from functional defects in the lymphoid or myeloid lineages (Fig. 1). To comprehensively study the disease-causing genotypes that may prompt a differentiation block and/or lead to a functional deficiency of the effector cells, we have established protocols to commit iPSCs towards the blood lineage, like T-cells and monocytes. A second focus is the generation of genetically corrected autologous immune effector cells from iPSCs for transplantation in future therapeutic settings.

SAFE GENE THERAPEUTICS

Many primary immunodeficiencies (PID), such as severe combined immune deficiency (SCID), familial hemophagocytic lymphohistiocytosis (FHL) and hyper-IgE-syndrome (HIES), are caused by well-defined mutations in the human genome. Likewise, the etiology of many acquired forms of immunodeficiency is well known. They arise, for instance, from chronic infections with the human immunodeficiency virus (HIV). Often the only therapeutic option for PID patients is allogeneic stem cell transplantation (SCT). In order to help PID patients without a matching donor, autologous SCT after genetic intervention is the only promising alternative. An increasing number of clinical studies attest to the therapeutic potential of gene therapy and many immunodeficiency patients have been successfully treated. On the other hand, some serious adverse events in these trials, including the development of

leukemia after retroviral gene transfer in hematopoietic stem cells, have prompted the development of safer gene therapy protocols, such as targeted gene editing with designer nucleases.
Therapeutic genome editing using designer nucleases has permitted the modification of the human genome in various cell types. For instance, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and RNA-guided nucleases have been employed to mediate the targeted genome editing in iPSCs, hematopoietic stem cells and CD4+ T-cells. Although we have shown that ZFNs can be efficiently employed in multipotent and pluripotent stem cells (Rahman et al., 2015; Bobis-Wozowicz et al., 2014; Höher et al., 2011; Osiak et al., 2011), the lack of specificity can induce significant toxicity (Cornu et al., 2008; Szczepek et al., 2007). In particular, the ZFN pair, which is currently employed in clinical trials to disrupt the CCR5 locus in patient-derived CD4+ T lymphocytes to generate HIV-resistant cells, revealed a high degree of off-target activity (Mussolino and Cathomen, 2011). Hence, it is important to further develop designer nuclease platforms to improve their safety profile. To this end, we have employed in collaboration with the Mussolino lab the superior TALEN platform to generate CCR5-specific designer nucleases for clinical translation (Mussolino et al., 2014; Mussolino et al., 2011). Furthermore, the Sleeping Beauty transposon system, which has evolved into a safe and acceptable alternative to viral vectors for stable gene transfer (Voigt et al., 2012), has been used to transfer a therapeutic *UNC13D* expression cassette into T-cells and CD34+ cells of FLH patients.

CLINICAL TRANSLATION

Adaptation of preclinical treatment protocols to standards of "good manufacturing practice" (GMP) and the subsequent clinical translation constitute two major hurdles in human gene therapy. To overcome these obstacles and to promote clinical translation of the CCI gene therapy efforts, the This Unit works in close collaboration with the clinical partners in the CCI, the Clinical Trials Unit, the regulatory experts of the Institute for Cell and Gene Therapy, and the clean-room facility to produce cell and gene therapeutics under GMP conditions. Within the next five years we will lay the foundation for three phase I/II clinical trials by establishing the conditions for GMP-compatible manufacturing of genetically modified CD4+ and CD34+ cells for the treatment of HIV, FLH and autosomal dominant HIES.

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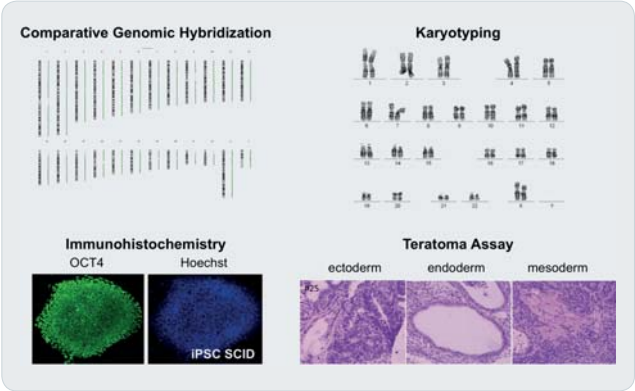

Fig. 1:
Characterization of induced pluripotent stem cells (iPSCs).
After reprogramming, the generated iPSCs are fully characterized to verify pluripotency of the cells and to ensure genomic integrity of the selected clones. Tests for genetic stability include microarray based comparative genomic hybridization (arrayCGH) and karyotyping, while pluripotency is confirmed by expression analysis of pluripotency markers and the ability to form teratomas.

CLINICAL TRANSLATION OF GENE THERAPY

The development of safe advanced therapy medicinal products (ATMPs) to treat chronic immunodeficiency patients is a major goal of the CCI. However, adaptation of preclinical manufacturing protocols to standards of "good manufacturing practice" and ensuing clinical translation represent two of the most difficult hurdles in human gene therapy. To overcome these barriers, we have established a Research Unit "Gene Therapy" that combines expertise in molecular biology and stem cell biology with an understanding of the regulatory issues and clinical translation. The mid-term goal of the Unit is to lay the foundation for three phase I/II clinical trials by establishing the conditions for GMP-compatible manufacturing of genetically modified CD4+ and CD34+ cells for the treatment of familial hemophagocytic lymphohistiocytosis (FHL), hyper-IgE-syndrome (HIES) and HIV infection.

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OBJECTIVES

One of the major difficulties in human gene therapy is to translate genetically modified cells into a GMP grade product that is approved by the regulatory authorities. In order to receive manufacturing approval, the rooms, the equipment, the reagents, and the individual production procedures have to be GMP compliant. Moreover, the manufacturing processes need to be carried out by highly skilled and trained personnel. Hence, the generation of clinical grade products differs in many aspects from research grade products. Regulatory guidelines issued by the European Medicine Agency (EMA; Regulation (EC) No 1394/2007 on ATMPs) have to be respected as to secure appropriate product quality, safety and efficacy. This is achieved by employing specifically designed quality control tests and protocols generated for this purpose during the preclinical phase. Moreover, in-process controls have to be applied throughout the whole production process by implementing proper equipment qualification, process validations of every single step in the production chain, as well as a complete product characterization.

The overall objective is to promote clinical translation of the CCI gene therapy efforts, which will be realized in three defined steps:

1. To develop in a preclinical setting gene transfer and designer nuclease-mediated genome editing for three disease entities (FHL, HIES, HIV) in pat ent-derived T-cells and CD34+ cells, respectively.
2. To adapt the established protocols to GMP standard for the production of ATMPs for the clinical trials.
3. To support clinicians in establishing the clinical protocols for the planned phase I/II clinical trials.

To achieve these goals, the Unit works in close collaboration with the clinical partners in the CCI, the Clinical Research Unit of the CCI, the Clinical Trials Centre, the clean-room facility to produce the gene therapeutics under GMP conditions, and the relevant regulatory experts in the Institute for Cell and Gene Therapy.

RELEVANCE AND STATE OF THE SCIENCE

Many congenital disorders of the immune system, such as Severe Combined Immune Deficiency (SCID), Wiskott-Aldrich Syndrome (WAS) and Chronic Granulomatous Disorder (CGD), are caused by well-characterized mutations in the human genome. Often, the only therapeutic option is allogeneic stem cell transplantation (SCT), but it is not always possible to find a suitable HLA-matched donor. In order to help immune deficient patients without a matching donor, autologous SCT after genetic intervention is frequently the only promising alternative. The concept of gene therapy is simple. It has the goal to overcome the physiological consequences of the underlying mutation in the genome by genetic manipulation or by modulation of specific gene expression in order to re-establish a normal equilibrium. In spite of the straightforward concept, the underlying gene therapy technologies constitute a complex medical treatment, which is subject to vast research efforts in developing efficient and safe curative clinical protocols. Fortunately, an increasing number of studies attest to the therapeutic potential of gene therapy. For example, immune deficient patients (X-SCID, ADA-SCID, WAS, X-CGD) have been successfully treated by autologous SCT after genetic intervention in stem cells with retroviral/lentiviral vectors. Patients suffering from Leber congenital amaurosis have benefited from injecting a recombinant adeno-associated virus (rAAV) in the patients' eye. Moreover, encouraging results have been seen in early phase I/II clinical trials using the systemic administration of rAAV coding for factor IX as a therapy for hemophilia type B or autologous SCT after lentiviral vector mediated gene addition has been developed as therapies for β-thalassemia and X-linked adrenoleukodystrophy. Finally, cases of refractory leukemia have been successfully treated by transfusion of T-cells expressing chimeric antigen receptors (CARs) introduced by lentiviral gene transfer. On the other hand, setbacks and side effects have been reported, in particular the development of leukemia after retroviral gene transfer in hematopoietic stem cells.

Hence, safer methods to modify the human genome in relevant target cells are required. Therapeutic genome editing using designer nucleases has permitted the modification of complex genomes in a targeted fashion. For instance, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and RNA-guided nucleases have been employed to mediate the targeted genome editing in various human cells, including induced pluripotent stem cells (iPSCs), hematopoietic stem cells (HSCs) and CD4+ T-cells. Although we have recently shown that ZFNs can be efficiently employed in multipotent and pluripotent stem cells (Rahman et al., 2015; Höher et al., 2011), their lack of specificity can induce significant toxicity (Cornu et al., 2008; Szczepek et al., 2007). In particular, the ZFN pair, which is currently employed in clinical trials to disrupt the CCR5 locus in patient-derived CD4+ T-lymphocytes, revealed a high degree of off-target activity (Mussolino et al., 2014; Mussolino et al., 2011). Alternative ZFN designs have been explored by us and others because the development of more specific designer nucleases to improve their safety profile is imperative, especially when applied to HSCs, as their high proliferative potential renders them susceptible to transformation (Mussolino et al., 2013; Mussolino et al., 2012).

EXAMPLE HIV GENE THERAPY

The identification of CCR5 as the major co-receptor of human immunodeficiency virus type 1 (HIV1) provided an explanation for the previously noted HIV1-resistance of frequently exposed, but uninfected individuals, who were found to carry two copies of the *CCR5Δ32* allele. These observations have been subsequently exploited in the development of drugs that aim at blocking the interaction of HIV1 with CCR5. In 2007, an allogeneic HSCT was performed in a HIV1-infected patient with acute myeloid leukemia using progenitor cells from a donor especially selected for homozygosity of the *Δ32* deletion. More than 3 years after treatment, the patient was still off antiretroviral medication and without any evidence of viral replication. However, *Δ32* donors are scarce and heterologous HSCT is not exempt of risks. Recent successes achieved in studies conducted in humanized mouse models have provided pre-clinical evidence that designer nucleases can be used to knockout *CCR5 ex vivo* and to suppress HIV1 *in vivo,* when used to modify the genome of human T-cells or HSCs. This designer ZFN has now been evaluated in a phase I/II clinical trial of *ex vivo* expanded autologous T-cells (*N Engl J Med* 370, 901-10; 2014). The results of this study demonstrate that the modified CD4$^+$ T-cells were durably engrafted in the peripheral blood of HIV1 patients for over a year. Moreover, the observed relative survival benefit of genetically modified cells during interruption of HAART implies that ZFN-induced disruption of the *CCR5* locus confers a selective advantage to CD4$^+$ cells in patients infected with HIV. We intend to capitalize on these observations and propose to develop an approach that prevents virus entry into T-cells through targeted disruption of the *CCR5* gene using a superior TALEN designer nuclease platform that we have developed (Fig. 1). Using this platform, a panel of *CCR5*-specific TALENs has been produced and their activities and specificities compared side-by-side with the ZFN currently used in the clinical trial (Mussolino et al., 2014). Our results demonstrate that we have produced two TALEN pairs, which were as active as the benchmark ZFN but revealed a considerably higher specificity than the ZFN and were significantly less cytotoxic and less genotoxic. Moreover, other than in the current clinical trials, in which the ZFNs have been expressed from adenoviral vectors, we express the TALEN by transfecting mRNA into the respective target cells. As of today, several clinical trials have used *ex vivo* electroporation of nucleic acids to genetically modify target cells, including electroporation of RNA into CD14$^+$ cells to generate tumor reactive dendritic cells (NCT00834002).

Notably, TALEN-mediated genome editing offer several advantages over the currently applied technologies in the phase I/II clinical gene therapy trial, especially in terms of safety (TALEN-encoding mRNAs vs. adenoviral mediated expression of ZFNs), cost (GMP-grade mRNA vs. GMP-grade viral vector production), and regulatory affairs (RNA/DNA vs. viral vectors).

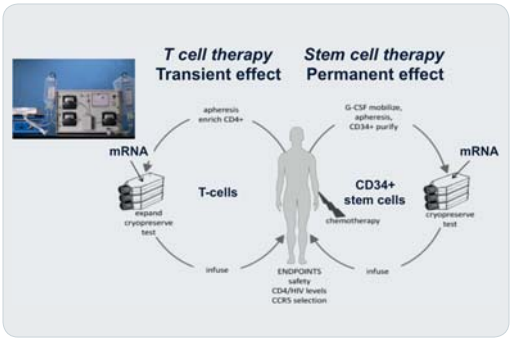

Fig. 1:
GMP-grade production of HIV-resistant cells.
Autologous CD4$^+$ T-cells or CD34$^+$ HSCs are transfected with mRNA encoding *CCR5*-specific TALEN using a GMP-grade electroporator (left). The modified T-cells are expanded *ex vivo* for 10 days before adoptive transfer. For permanent treatment, autologous CD34$^+$ cells are enriched from peripheral blood following G-CSF mobilization and then electroporated. Chemotherapy assists engraftment of the modified CD34$^+$ cells (*figure modified from Curr Opin HIV AIDS* 6, 74-9; 2011).

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PEDIATRIC IMMUNOLOGY

Human genetic disorders of T cell immunity: understanding the role of T cells in control of viral infections and immune homeostasis

KEYWORDS

Primary Immunodeficiencies
T Cell Immunity
Antiviral Immunity
Hemophagocytosis
Immunopathology

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T cell immunity is important for the control of most infections. A successful T cell response involves antigen recognition, cellular activation and differentiation, rapid expansion and exertion of effector functions. These potent and highly dynamic processes must be tightly regulated in order to avoid inappropriate or uncontrolled inflammatory responses. Human genetic diseases provide a fascinating window to understand T cell immunity and its relevance for the control of infectious diseases as well as for diseases of immune dysregulation.

The research group „Pediatric Immunology“ works at the interface between clinical and basic immunology with a particular focus on T cell immunity. The research activities cover a large spectrum from basic research in animal models or the identification of new genetic defects to international diagnostic and clinical studies. The group integrates both physicians and basic scientists.

We study three immunodeficiency states representing models for different aspects of T cell immunity:

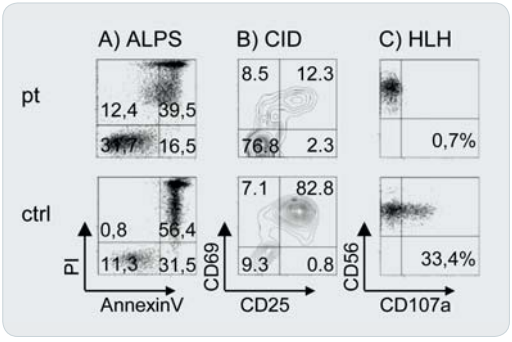

Fig. 1:
A) Impaired T cell apoptosis in a patient with CD95 mutation.
B) Impaired T cell activation in a patient with IKKbeta deficiency.
C) Impaired NK cell degranulation in a patient with a novel cytotoxicity defect.

LYMPHOPROLIFERATION AND AUTOIMMUNITY

The phenotype of benign lymphoproliferation and autoimmunity, in particular autoimmune cytopenia, is observed in a number of primary immunodeficiencies. The most prevalent disorder is autoimmune lymphoproliferative syndrome (ALPS), which is mostly associated with germline or somatic mutations in CD95. CD95 is involved in lymphocyte apoptosis, but has also additional non-apoptotic signaling functions.

We have established a registry for patients with lymphoproliferation and autoimmunity. Based on a careful clinical, immunological and genetic analysis of these patients, we (i) evaluate biomarkers for diagnosis and prognosis in ALPS patients, (ii) study the role of Fas in T cell differentiation and B cell differentiation (collaboration with Marta Rizzi) and (iii) identify novel genetic causes of lymphoproliferation and autoimmunity using whole exome sequencing.

(SEVERE) COMBINED IMMUNODEFICIENCY

SCID is usually the consequence of loss-of-function mutations in genes involved in lymphocyte development. In contrast, patients with hypomorphic (leaky) mutations in these genes have a more variable phenotype. In addition to infection susceptibility, patients show signs of impaired immune regulation such as cytopenias, eczema, granulomas or inflammatory bowel disease. A similar picture can be observed in genetic diseases affecting T cell activation and differentiation (combined immunodeficiencies). Characteristic immune phenotypes include T cell lymphopenia and repertoire alterations, γ/δ T cell expansions, altered T cell differentiation and proliferation defects.

We have established a prospective observational outcome study on patients with profound combined immunodeficiency. The goal is to provide data on the natural history that can help in the decision, if and when to transplant these patients. In the laboratory, we perform a careful phenotypic and functional immunological analysis. In some cases, we identify mutations in known genes and can then analyze the consequence of specific mutations for protein and cellular immune function. In other cases, we use functional assays in combination with exome sequencing (collaboration with Klaus Schwarz, Ulm) to elucidate novel genetic causes for CID. These findings are related to the particular clinical phenotypes with the goal to better understand infection control and immune regulation in humans.

We also use mouse models of "leaky" SCID that show features of infection susceptibility and immune dysregulation similar to human patients. In particular, we study T cell development and differentiation and T cell mediated control of viral infections. The goal is to understand how human T cell immunity works under limiting conditions.

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Familial hemophagocytic lymphohistiocytosis (FHL) represents a severe disturbance of immune homeostasis characterized by prolonged fever, cytopenia, splenomegaly, liver dysfunction, characteristic biomarker alterations and hemophagocytosis. Most cases of FHL are due to genetic defects in perforin or other genes involved in lymphocyte cytotoxicity. In about 10% of patients with FHL, the genetic basis of the disease remains unresolved.

In collaboration with G. Janka and K. Lehmberg (Hamburg), we are currently setting up an international registry and clinical study platform for treatment studies on HLH (TREAT-HLH). We provide functional immunological evaluation of HLH patients from German speaking countries. On the basis of these diagnostic studies, we characterize the T cell response in HLH and identify novel biomarkers for HLH activity. Patients without a genetic diagnosis are evaluated by whole exome sequencing (collaboration with U. zur Stadt, Hamburg and HC. Hennies, Köln) for novel genetic causes.

We have also established an HLH mouse model based on infection of perforin-deficient mice with lymphocytic choriomeningitis virus (LCMV) to address the following questions: (i) Which initial triggers are required for the induction of HLH? (ii) What is the relevance of persisting antigen for HLH? (iii) Which cell types and cytokines drive the disease? (iv) Is gene therapy a safe and effective approach to treat FHL? A second specific cohort of interest are patients with XIAP deficiency who may present with HLH, but also other, highly variable signs of immune dysregulation (e.g. colitis). We combine observations in human patients with murine experiments to better understand the role of XIAP in immune regulation and the characteristic susceptibility to EBV infection.

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(SEVERE) COMBINED IMMUNODEFICIENCY - (S)CID

"determining the limiting factors for T cell mediated control of infections and of immune homeostasis"

Combined immunodeficiency (CID) is the phenotypic presentation of a large number of genetic disorders of the immune system. Clinical manifestations include a wide range of infectious diseases including persistent viral and opportunistic infections and manifestations of impaired immune regulation such as autoimmunity, eczema, granulomas or inflammatory bowel disease. The common immunological abnormality is impaired T cell immunity, but other immune and epithelial cells can also be affected. The study of CID offers a unique opportunity to understand the limiting factors of protective T cell immunity. We approach this problem by the study of individual patients, patient cohorts and mouse models. To transfer this knowledge to patient care, a prospective clinical study has been initiated to better define the threshold when stem cell transplantation should be performed in affected patients.

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CURRENT FUNDING

BMBF
DFG
EU (E-Rare)

LESSONS FROM INDIVIDUAL PATIENTS

Stromal interaction molecule 1 (STIM1) deficiency is a rare genetic disorder of store-operated calcium entry, associated with a complex syndrome including immunodeficiency and immune dysregulation. The link from the molecular defect to these clinical manifestations is incompletely understood. We identified two patients with a homozygous R429C point mutation in STIM1 completely abolishing store-operated calcium entry in T cells.

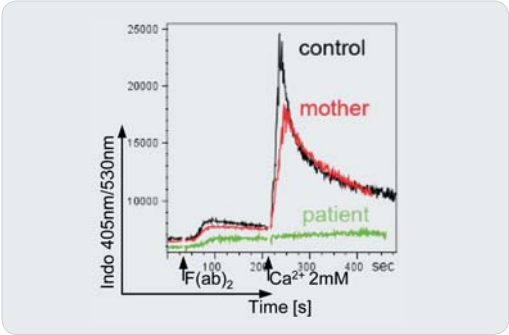

Fig. 1:
Impaired Ca2+ flux in T cells of a patient with STIM1 deficiency

Immunological analysis revealed that despite the expected defect of T cell proliferation and cytokine production in vitro, significant antiviral T cell populations were generated in vivo. These T cells proliferated in response to viral Ags and showed normal antiviral cytotoxicity. However, antiviral immunity was insufficient to prevent chronic CMV and EBV infections with a possible contribution of impaired NK cell function and a lack of NKT cells. Furthermore, autoimmune cytopenia, eczema, and intermittent diarrhea suggested impaired immune regulation. FOXP3-positive regulatory T (Treg) cells were present but showed an abnormal phenotype. The suppressive function of STIM1-deficient Treg cells in vitro, however, was normal.

Given these partial defects in cytotoxic and Treg cell function, impairment of other immune cell populations probably contributes more to the pathogenesis of immunodeficiency and autoimmunity in STIM1 deficiency than previously appreciated.
Collaboration with Stefan Feske, New York

Severe combined immunodeficiency (SCID) comprises a heterogeneous group of heritable deficiencies of humoral and cell-mediated immunity. Mutations of various genes, which perturb normal lymphocyte development and function, result in susceptibility to a wide array of infections. However, a major cohort of SCID patients with lymphocyte activation defects remains uncharacterized. We investigated four patients from four families of Northern Cree First Nations ancestry who display clinical characteristics of SCID including early onset of severe viral, bacterial and fungal infections despite normal B and T cell numbers. The patients were hypo-/agammaglobulinemic and peripheral B and T cells were almost exclusively of naïve phenotype. Treg and γ/δ T cells were absent.

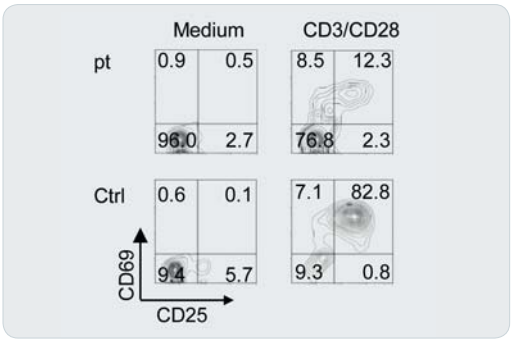

Fig. 2:
Impaired T cell activation in a patient with IKKbeta deficiency

All patients carried a homozygous insertion c.1292dup (exon13) in IKBKB encoding IKK2 (IKKκβ), leading to loss of IKK2 protein expression, a component of the IKK2/NF-κB signal transduction and gene regulation system. Immune cells showed impaired responses upon stimulation by TCR, BCR, TLRs, inflammatory cytokine receptors and mitogens. This study defines a novel class of human SCID characterized by normal lymphocyte development despite a loss of IKK2 function. The IKK2 loss results in an impaired stimulation response in a variety of immune cells, leading to significant impairment of adaptive and innate immunity. While IKK2 deficiency is embryonic lethal in mice, our observations suggest a more restricted function of IKK2/NF-κB signaling in humans.
Collaboration with Klaus Schwarz, Ulm

LESSONS FROM MOUSE MODELS

Signaling via the TCR is critical for T cell development, activation and effector functions. Here, we analyzed which of these TCR-mediated processes is limiting during antiviral immunity. We used a mouse strain with impaired canonical TCR signaling due to a point mutation leading to reduced expression of Src homology 2 domain-containing protein of 76 kDa (SLP-76) and a partial reduction in peripheral T cells (twp mice). Following infection with Lymphocytic Choriomeningitis Virus (LCMV), the impaired TCR signal allowed the generation of a normal proportion of antiviral effector T cells. Moreover, TCR dependent antiviral T cell effector functions including cytokine production and cytotoxicity were largely normal. The main limiting factor in the antiviral response of twp mice was impaired T cell proliferation leading to a 10-fold reduction of antiviral T cells at the peak of the immune response.

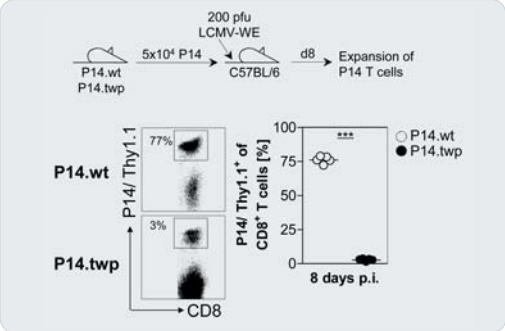

Fig. 3:
Impaired expansion of virus-specific CTL in mice with reduced TCR signaling

This was still sufficient to control infection with LCMV-ARM, but the more rapidly replicating LCMV-WE induced T cell exhaustion and viral persistence. Thus, under conditions of impaired canonical TCR signaling, reduced T cell proliferation is the limiting factor in antiviral immunity. These findings have implications for understanding and evaluating antiviral defense in human "leaky" SCID patients.
Collaboration with Chris Goodnow, Canberra and Hanspeter Pircher, Freiburg

THE P-CID STUDY

Currently, there are no treatment guidelines for patients with profound combined immunodeficiency (P-CID), in particular with respect to the question, if and when they should undergo hematopoetic stem cell transplantation (HSCT). We initiated an international long-term prospective observational cohort study with the goal to provide natural history data on patients with P-CID. We determine survival, the frequency of severe infections, manifestations of immune dysregulation and malignancies and quality of life for at least 5 years after study inclusion. The decision whether the patients undergo HSCT or not is left with the treating center, but is well documented on a yearly basis.
As of November 2013, 50 patients fulfilling the entry criteria of impaired T cell immunity and at least one severe event have been included. About 50% of the patients have a molecular diagnosis, half of them have a diagnosis of "leaky" SCID.

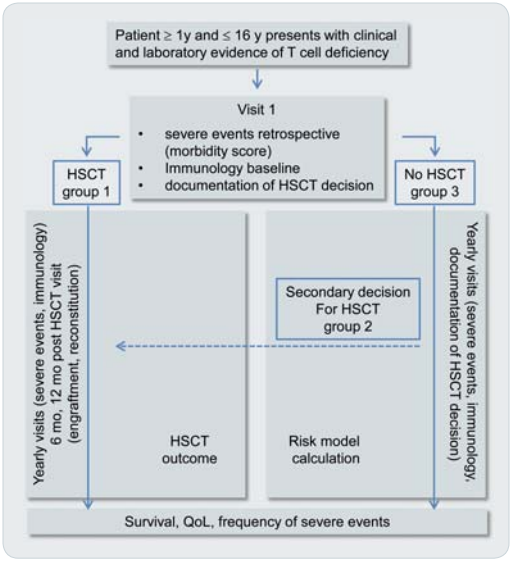

Fig. 4:
Study design of the P-CID study

The primary endpoint of the study is overall survival determined after year 5. The event analyzed is death from any cause. The time to this event is the time from the first major infection or major manifestation of immune dysregulation (documented retrospectively at the time of study entry) to death. In a matched pair analysis based on clinical and immunological risk factors, the impact of HSCT on survival will be estimated. The secondary objective is to develop a risk model for P-CID patients.

Parameters that will be evaluated for their predictive value (with respect to death and to "need to transplant") include the genetic diagnosis, the severity of the T cell deficiency at the time of diagnosis and its evolution over time and the presence of additional immunological or non-immunological factors.
The main hypothesis is that P-CID patients undergoing early HSCT have a better 5-year survival than patients who undergo late HSCT or are not transplanted.
Collaboration with the EBMT Inborn Errors Working Party

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B LYMPHOCYTE DEVELOPMENT

In vivo and in vitro models of human B cell development

KEYWORDS

B cell development
In vitro models
Humanized mice
BAFF-receptor
B cell survival

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B lymphocytes are central elements of the adaptive immune system. After terminal differentiation into plasma cells they produce antibodies that protect efficiently against infections by viruses, bacteria by neutralizing pathogens, by labeling them for opsonization or for complement-mediated destruction. Mouse models generated by genetically modifying the genome of mice by specific gene inactivation have provided deep insights into the molecular regulation of B lymphocyte development and function. However, comparison between genetically modified mice and the clinical manifestations and the immunophenotypes of human primary immunodeficiencies delivered increasing evidence that B cell development and B lymphocyte subpopulations differ significantly between men and mice (reviewed in 5,7). As we are trying to understand the genetic alterations and molecular changes that cause primary immunodeficiencies resulting in impaired B cell function we developed and optimized *in vivo* and *in vitro* models for human B cell development and function. Combined with the analysis of genetically defined primary immunodeficiencies, we use these models to understand the biology of human B lymphocytes.

IN VIVO AND IN VITRO MODELS FOR HUMAN B CELL DEVELOPMENT

B cells develop in the bone marrow from hematopoietic stem cells. During development, they proceed from the pro-B cell stage over the stage of pre-B cells to the stage of IgM+ immature B cells. These cells leave the bone marrow and migrate through the circulation to the spleen where they complete maturation as naïve follicular or as marginal zone (MZ) B cells.

We studied the development of human B cells experimentally by developing and optimizing two complementary in vivo and in vitro models. Xenotransplantation of human cord blood or bone marrow-derived CD34+ HSCs by intravenous injection into newborn Rag2-/-IL-2rg-/- mice allowed us to analyze the development of human B lymphocytes *in vivo*. Besides of differentiation into B cells (Figure 1), engrafted CD34+ stem cells were also found to develop into myeloid lineage cells and T cells. Comparison between B cell precursors isolated from human bone marrow and human precursor B cells isolated from the bone marrow of humanized mice revealed that the mouse model exactly recapitulated all developmental stages found in adult bone marrow.

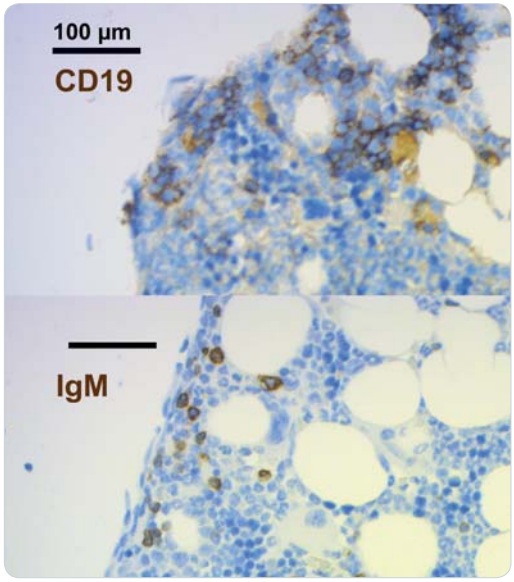

Fig. 1:
Bone marrow of humanized mice.
Human CD19+ pre-B and immature B cells (upper panel) and IgM+ immature B cells (lower panel) in the bone marrow of a 20 weeks old humanized mouse.

In addition to the in vivo model of humanized mice, we developed a novel, feeder cell-free in vitro differentiation system allowing the differentiation of human CD34+ HSC into IgM+ immature B cells (1). Allowing for the precise control of growth and differentiation, the analysis of B cells developing *in vitro* provided strong evidence that human immature B cells can develop from lymphocyte progenitors independent from IL-7, FLT3L and other exogenously

added cytokines. In contrast to murine B cell precursors, human cells proliferate by autonomous replication As these models for human B cell development provide the direct access to all stages of B cell precursors and the controlled manipulation of the growth and differentiation parameters, in vitro differentiation systems combined with in vivo analyses of humanized mice provide ideal tools to study the function of genes regulating early phases of human B cell development in the bone marrow.
T cell-dependent activation mature B cells and differentiation into plasma cells has been analyzed by an optimized in vitro system mimicking T-dependent B cell responses. Using this approach, defects in plasma cell development, the role of histone deacetylase-induced protein modifications and IL-21 / IL-21-dependent signaling has been analyzed by us (6, 11) and in collaboration with others (3, 9).

B LYMPHOCYTE SURVIVAL AND SELECTION

B cell activating factor receptor (BAFFR)-induced signals are essential for the survival of peripheral B cells as well as for the transition from immature to transitional and to mature B cells (10). BAFFR is a member of the tumor necrosis factor receptor superfamily binds a TNF-like ligand named BAFF. BAFF serum concentrations are regulated by the number of circulating B cells carrying BAFF-binding receptors (8). In humans, BAFFR-deficiency arrests B cell development at the stage of immature B cells (10). Thus, BAFFR-deficient humans suffer from hypogammaglobulinemia and do not mount B cell responses against T-independent antigens like pneumococcal cell wall polysaccharides. A frequent SNP changing the BAFFR proline residue 21 to arginine (P21R) was shown to locate in the pre-ligand assembly domain of the extracellular part of BAFFR (2). The mutation disturbs ligand-independent BAFFR multimerization. As BAFFR multimers strongly enhance BAFF binding, B cells expressing P21R BAFFR respond only poorly to synergistic activation by BAFFR, TLR9 and BCR signals. As the P21R-encoding SNP is found significantly more frequently among CVID patients than in healthy control populations, disturbed BAFFR function predisposes to CVID (2). Thus, the crosstalk between BCR, TLR9 and BAFFR plays an important role in T-independent B cell responses.

CIRCULATION OF HUMAN B CELLS

At least 3 different types of shingosine-1-phosphate (S1P) receptors play an important role in regulating the distribution of B cells in follicles, retention in GCs, and egress from secondary lymphoid organs. Attracted by high S1P concentrations in vessels, resting lymphocytes expressing S1P-receptor 1 leave primary and secondary lymphoid organs into the circulation. We were able to show that that the different human B cell subsets have a characteristic pattern of S1P-receptor expression resulting in differential responses to S1P. Studying mutations in three genes linked to primary immunodeficiencies, *LRBA*, *DOCK8* and *WASP*, we demonstrated that the proteins encoded by these genes form part of the signaling cascade induced by S1P binding to S1P-receptor 1. These data suggest that clinical manifestations found in these deficiencies may result from impaired responses to S1P-receptor signaling (4).

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PRIMARY IMMUNODEFICIENCIES CHANGING B CELL SURVIVAL, DIFFERENTIATION AND CIRCULATION

Molecular studies of primary immunodeficiencies have yielded new and exciting insights into the biology of human B cells. Compared to the development of other cell types, B lymphocyte development has its own characteristics like the somatic recombination and hypermutation of immunoglobulin gene segments during assembly and affinity maturation of B cell antigen receptors. Moreover, the early and late phases of B cell development are locally separated as early steps take place in the bone marrow whereas late phases are confined to the spleen and lymph nodes where memory B cells and plasma cells develop. Mature and memory B cells scan the body for pathogens and terminally differentiated plasma cells home back to the bone marrow. Therefore, B cell migration is a well-regulated process. As antibody concentrations are low in primary antibody deficiencies, mutations interfering with the development of B and plasma cells may result in antibody deficiency. In addition to the regulation of B cell migration, survival signals play an important role in maintaining a functional compartment of circulating B cells. We analyzed circulation and survival of human B cells by studying different forms of primary antibody deficiencies.

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CURRENT FUNDING

DFG

SIGNALS REGULATING THE CIRCULATION OF HUMAN B CELLS

Mouse models revealed that sphingosine-1-phosphate receptors (S1P-receptors) play an important role in regulating the distribution of B cells in follicles, retention in GCs, and egress from secondary lymphoid organs.

Stored in erythrocytes and produced by vascular endothelial cells high S1P concentrations are higher in blood and lymph than in lymphoid tissues. Attracted by high S1P concentrations, resting B cells leave lymphoid tissues and enter into the circulation. As little was known about the expression and function of the five different S1P-receptors in human B cells, we studied their expression and signaling in different human B cell subsets using cells and cell lines from primary immunodeficiency patients suffering from defined genetic defects (2).

Follicular and marginal zone, as well as memory B cells were found to express S1P1 and S1P4 but very low levels of S1P2, whereas cycling non-recirculating GC B cells and extrafollicular plasma cells express high levels of S1P2 and S1P4. In lentiviral gene transfer experiments with vectors expressing different S1P-receptor-GFP, -RFP and -BFP fusion proteins we were able to demonstrate by a combination of transwell assays, time-lapse fluorescence and TIRF microscopy that S1P-receptor-1 (S1P1) induced B cell migration against a S1P gradient, whereas S1P2 inhibited S1P1-dependent signaling and chemotaxis.

Beta-arrestin2, LRBA, DOCK8 and WASP were identified as downstream components of the S1P1-dependent signaling cascade suggesting that changes in the immunophenotype as well as clinical manifestations found in patients suffering from LRBA, DOCK8 and WASP deficiency may be caused by impaired S1P1 signaling.

BAFF-RECEPTOR SIGNALING AND B CELL SURVIVAL

BAFFR is a member of the tumor necrosis factor receptor superfamily and binds the TNF-like ligand BAFF (B cell activating factor of the tumor-necrosis-factor family). BAFFR is expressed by follicular, marginal zone and memory B cells but not by activated B cells in the germinal center (Figure 1).

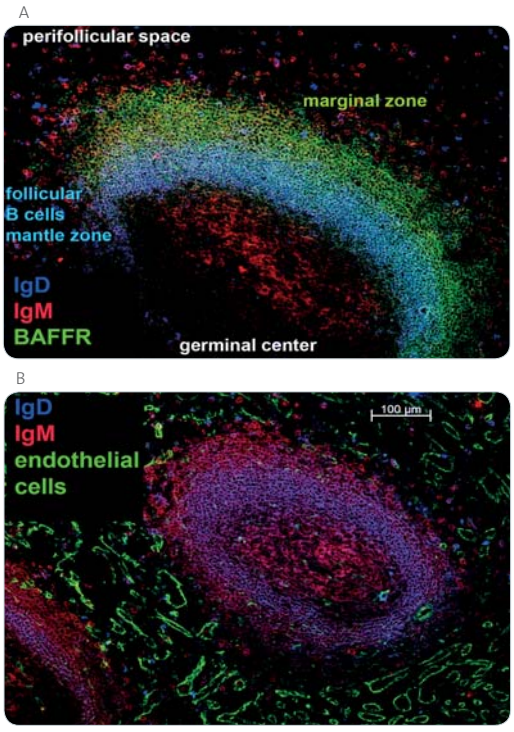


Fig. 1:
BAFF-receptor expression in human spleen.
A) BAFFR is expressed by IgMhi IgDlo marginal zone B cells and by IgMlo IgDhi follicular / mantle zone B cells but not by germinal center cells.
B) Germinal center, follicular and mantle zone B cells. B cells and antigens circulate through sinus endothelial cells (green)

BAFF binding to BAFFR activates the non-canonical NF-κB2 pathway and, more downstream, pro-survival genes like BCL2 regulating mitochondrial function and energy supply. In addition, BAFF binding activates in a PI3-kinase dependent manner AKT (protein kinase B), a key regulator of cell cycle, survival and protein synthesis.

Studying patients with primary antibody deficiencies and low numbers of circulating B cells we identified the first case of BAFFR deficiency in humans. Caused by the homozygous deletion of the codons encoding most of the BAFFR transmembrane region, BAFFR deficiency arrests B cell development at the stage of transitional B cells. As marginal zone B cells are lacking, humoral immune responses against pneumococci cannot be mounted.

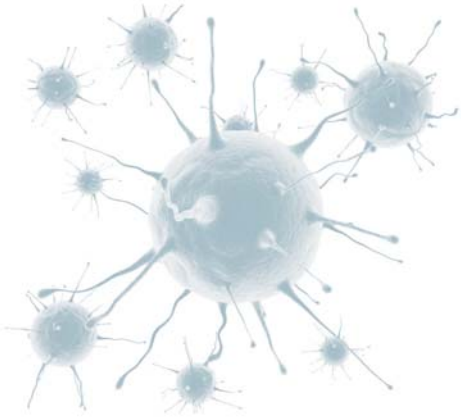
Caused by the very severe reduction of plasma cells and switched memory B cells T-dependent B cell responses are impaired. Interestingly, IgA serum levels and IgA-dependent immune responses are normal in BAFFR deficiency demonstrating that BAFFR function is dispensable to maintain the function of mucosal B cells (4).

Studying the functional consequences of BAFFR missense mutations found in the human population we found that a SNP with a minor allele frequency of about 5% impairs ligand-independent BAFFR multimerization (1). The SNP changes the proline residue 21 into arginine (P21R). We showed that P21 defines the pre-ligand assembly domain of BAFFR. The change to R21 prevents ligand-independent BAFFR clustering and because BAFFR clustering strongly enhances BAFF binding, the P21R BAFFR variant disturbs BAFF-binding to BAFFR in a dominant-negative manner.

Thus, B cells from P21R carriers respond only poorly to BAFFR activation. As the P21R-encoding SNP is found significantly more frequently among CVID patients than in populations of healthy controls, disturbed BAFFR function seems to predispose to CVID (1).

The essential role of BAFFR and of NF-κB as a key transcription factor in B cell activation is further underlined by the severely compromised function of the immune system and B cell responses in immunodeficient patients carrying mutations in CARD11(3).

Interacting with MALT1 and BCL-10, CARD11 is a positive regulator of NF-κB activity and of BAFFR expression thus linking BCR signaling and BAFFR-dependent B cell survival. Therefore, cooperation between BCR- and BAFFR-dependent signaling pathways is essential for the maturation and survival of B cells.



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EXPERIMENTAL IMMUNODEFICIENCY

Identifying the Genetic and Molecular Basis of Primary Immunodeficiencies

KEYWORDS

Hyper-IgE Syndrome
Chronic mucocutaneous Candidiasis
Common Variable Immunodeficiency
Chronic inflammatory bowel disease
Genetic cause

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Primary immunodeficiencies due to monogenetic defects in humans are an experiment of nature. Their investigation enables physicians and researchers to dissect the immune system and evaluate the respective components in human immunology. The identification of new disease genes leads to improved diagnosis and disease management, and ultimately, by illuminating the pathophysiology, to the development of new therapeutic strategies. Currently my group studies the following four immune deficiency phenotypes:

HYPER-IMMUNOGLOBULIN E (IGE) SYNDROMES

Hyper-IgE syndrome (HIES) is a complex chronic primary immunodeficiency found to be quite rare (incidence 1:1,000,000). It presents as a defect of the immune response and is characterized by both immunological and non-immunological manifestations. Immunological manifestations include highly elevated serum IgE levels, eosinophilia, recurrent bacterial and fungal infections, eczema, skin infections, and pneumonia. Non-immunological manifestations include a characteristic facial appearance, scoliosis, retained primary teeth, joint hyperextensibility, recurrent bone fractures following minimal trauma, and craniosynostosis. Most cases of HIES are sporadic, but both autosomal dominant (AD-HIES) and autosomal recessive (AR-HIES) inheritance has been observed.

Autosomal Dominant HIES. We and others discovered in 2007 that heterozygous mutations of the *Signal Transducer and Activator of Transcription 3 (STAT3)* are a causal factor for most AD-HIES cases. These mutations lead to the production of STAT3 mutant forms, which function in a dominant-negative way over the wild-type STAT3. STAT3 is involved in multiple JAK-STAT signaling pathways and plays a key role in the production of a broad range of cytokines, one of which is IL-6, a regulator of Th17 cells. Defective signaling due to mutations in STAT3 therefore leads to the failure of Th17 cell development in AD-HIES patients. Interestingly, 30% of AD-HIES patients do not show STAT3 mutations; hence, further genetic causes of AD-HIES remain to be elucidated.

Autosomal Recessive HIES. Homozygous mutations of the *Dedicator Of Cytokinesis 8 (DOCK8)* gene have been shown to be responsible for many, although not all, cases of autosomal recessive HIES (AR-HIES). DOCK8 belongs to a family of guanine nucleotide exchange factors (GEFs) that are responsible for the activation of small G proteins, therefore a crucial part of intracellular signaling networks that when defective lead to phenotypes seen in HIES patients. Recently, the lab has identified a novel gene termed phosphoglucomutase 3 (PGM3), which is involved in protein glycosylation, as a causal factor for AR-HIES. Further research regarding its pathogenesis is currently being performed.

CHRONIC MUCOCUTANEOUS CANDIDIASIS

Chronic mucocutaneous candidiasis (CMC) is a heterogeneous immunological disorder and may be either congenital (primary CMC) or due to HIV infection or inhaled corticosteroid use (secondary CMC). CMC is characterized by persistent or recurrent Candida infections of the skin, nails and mucous membranes and an increased susceptibility to dermatophyte fungi of the genera Trichophyton and Microsporum. Most cases of CMC are sporadic, but both autosomal dominant and autosomal recessive traits of inheritance have been described. Genetic causes for an increased susceptibility to candidiasis may lie in defects affecting the interleukin (IL) 17 axis of host defense such as the IL-17 cytokine, the IL-17 receptor, or in defects affecting the signaling pathways leading naïve T cells to differentiate into IL17-producing Th17 cells, such as the JAK-STAT signaling pathway. Our group has collected a variety of CMC families with different traits of inheritance

and unknown genetic cause. genetic cause. We are currently carrying out methods such as linkage analysis, whole exome sequencing, and candidate gene analysis, in order to discover the genetic cause in patients with CMC.

COMMON VARIABLE IMMUNE DEFICIENCY

Common Variable Immunodeficiency (CVID) is the most frequent primary immunodeficiency in humans with a prevalence of about 1:25.000. It is characterized by hypogammaglobulinemia of at least two immunoglobulin isotypes, poor or absent vaccination responses, and an age of diagnosis of at least two years of age. It is a diagnosis of exclusion when all other causes of hypogammaglobulinemia such as known genetic disorders, transient hypogammaglobulinemia, infectious diseases, or malignancy have been ruled out. The clinical picture of CVID patients is highly variable. In addition to recurrent infections, CVID patients may suffer from noninfectious complications like enteropathy, autoimmunity, granulomatous disease, lymphoproliferation or malignancy. While only 10 % of CVID patients have a familial background, the majority of the cases are sporadic. Among the familial cases, around 75% display an autosomal dominant and 25% an autosomal recessive trait. We are working on the identification of novel mutations in families affected by CVID, as the genetic mutations causing the disease are mostly unknown.

Autosomal-recessive mutations in ICOS were the first monogenetic defects identified in CVID patients. Since then, autosomal recessive or compound heterozygous mutations in ten genes have been found to cause CVID or CVID-like phenotypes. Five of those have been either described by our group (ICOS, TACI, and LRBA) or in collaboration with us (CD19 and BAFF-R).

Mutations in TNFRSF13B encoding TACI have been found to be associated with an **autosomal dominant** form of CVID. The most common mutation amongst patients diagnosed with a TACI mutation is the C104R mutation. However, this polymorphism exists in both affected and healthy individuals. Therefore, we assume that there are additional genes which act as modifiers in patients with TACI mutations. We are working towards the identification of such modifier genes.

INFLAMMATORY BOWEL DISEASE

Inflammatory Bowel Diseases (IBD) constitute immune-mediated disorders characterized by a chronic and relapsing inflammation of the gastrointestinal tract. The two most common entities of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Although the symptoms of UC and CD may be very similar, the location of disease varies in both types. In CD patients the inflammation can range from mouth to anus and may present as skip lesions, i.e. affected areas alternating with healthy sections. However, the most commonly affected region is the terminal ileum. On the other hand, UC presents with continuous inflammation and only affects the colon.

There is a common consent that the development of IBD results from a complex interplay of genetic susceptibility, immune dysregulation and environmental factors, which renders the etiology multifactorial. More than 150 genetic loci have been described to be associated with IBD, nevertheless the contribution to genetic heritability is low. However, besides the "classic" IBD forms that have a peak age of onset in the mid-twenties, several monogenic defects causing early-onset IBD (eoIBD) have been described during recent years. Early-onset IBD is characterized by an onset during the first months to years of life as well as a severe and often treatment-refractory course of disease. These monogenic defects associated with IBD-like pathology can be divided into defects in the intestinal epithelial barrier, impaired granulocyte and phagocyte activity, hyper- and autoinflammatory disorders, as well as disturbed T and B cell selection and activation, which also comprise defects in immune regulation of regulatory T cells and IL-10 signaling. Most of these monogenic defects affect key functional components within inflammatory networks and may also deliver general insights into inflammatory disorders. Of special interest for our group are mutations in the Interleukin-10 signaling pathway.

Our objective is to perform genetic testing and to screen our patient cohort with IBD-like disease manifestations of early onset for the existence of variants in certain susceptibility genes using targeted next generation sequencing. Subsequently, we will perform functional analyses with the patients' peripheral blood mononuclear cells (PBMCs) to further elucidate the pathophysiology and gain a better understanding of disease processes. In the long term we will apply the findings in a translational setting to identify new therapeutic approaches.

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LRBA DEFICIENCY

Learning about a novel Primary Immunodeficiency

Common variable immunodeficiency (CVID) is the most frequent form of primary immune deficiency with a prevalence of approximately 1:25,000 and 1:50,000. CVID is characterized by low levels of IgG, IgA and/or IgM (at least two standard deviations below the mean for age) and defective specific antibody production, associated with an increased number of infections, most often bacterial respiratory tract infections. In addition, CVID is associated with chronic lung disease, lymphoproliferation and autoimmune complications. Although the diagnosis is not typically made around 30 years of age, about 28% of patients are less than 21 years at the age of diagnosis. Even though several genetic CVID-causing defects have been identified in Freiburg (ICOS-deficiency, TACI-deficiency, CD19-deficiency, BAFF-receptor-deficiency and CD21-deficiency) most (>90%) of the genetic causes of CVID are still unknown.

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HUNTING A CVID-CAUSING GENE

Genetic linkage analysis, which is a powerful tool to detect the chromosomal location of disease-causing genes, is based on the observation that genes/loci that reside physically close on a chromosome remain linked during meiosis. By using linkage analysis, we were able to identify two intervals on chromosome 4q and 5q that segregated perfectly with the disease status in a consanguineous Israeli family who had two affected children. Genotyping analysis with microsatellites confirmed a perfect segregation only on chromosome 4q. Within the linkage interval, twenty genes were sequenced and a homozygous mutation c.7970T>G in both affected children in a gene known as *LRBA* was identified. This mutation was found in heterozygosity in the unaffected family members. In addition, the *LRBA* gene was sequenced in 14 additional CVID families and three new homozygous deleterious mutations were found in three CVID patients (Figure 1).

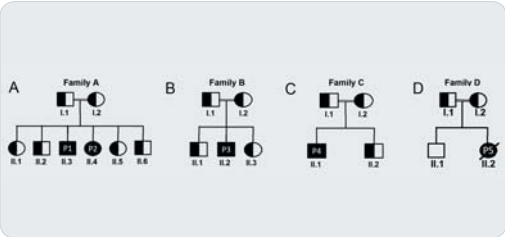
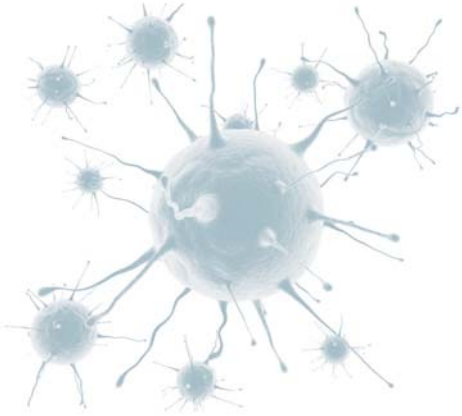


Fig. 1:
Pedigrees and *LRBA* mutations in 4 CVID families. Shaded figures are affected individuals homozygous for a mutation in *LRBA*. Half-shaded figures are unaffected individuals heterozygous for a mutation in *LRBA*.
(A) Family A with two affected individuals showing a homozygous missense p.Ile2657Ser substitution.
(B) Family B with one affected individual who had a homozygous nonsense substitution p.Arg1683*.
(C) In family C, the nucleotide substitution leads to a nonsense mutation: p.Glu59.
(D) The affected individual from family D has a large homozygous deletion including exons 1 and 2 and ~60 kb upstream and ~50 kb downstream. The parents in family D are heterozygous for the deletion, but the unaffected sibling does not carry the deletion.

LRBA DEFICIENCY

The lipopolysaccharide responsive beige-like anchor protein (LRBA) is part of the PH-BEACH-WD40 protein family, which is highly conserved among species and widely expressed in human tissues. Although little is known about the exact function of LRBA in human biology, members of this family including LYST, have been involved in the regulation of lysosome-related organelle size and movement.

The identification of these new mutations (Figure 1) led us to describe a novel PID, known since as LRBA deficiency. LRBA deficiency is caused by the loss-of-protein-expression of LRBA and is characterized by early-onset hypogammaglobulinemia, autoimmune manifestations, susceptibility to inflammatory bowel disease, and recurrent infections. However, recent LRBA case reports have shown LRBA patients without hypogammaglobulinemia and presenting with inflammatory bowel disease and a combined immunodeficiency. Thus, LRBA deficiency became a truly variable syndrome.



Moreover, LRBA-deficient patients present several *in vitro* abnormalities:

- Decreased proliferation, plasmablasts differentiation and IgG antibody production after stimulation with CD40L+anti-IgM+BAFF+CpG of B cells.
- Deficient T-cell activation (ICOS and CD69 expression) and reduced proliferation after PHA stimulation.
- Increased apoptosis after serum-deprivation (Figure 2). This observation supports previous reports in tumor cells suggesting that LRBA promotes proliferation and cell survival.
- Decreased autophagy in B-lymphocytes under starvation conditions (Figure 3). In addition, we observed an abnormal accumulation of autophagosomes in B cells by Electron Microscopy.

These data together suggest that LRBA plays a role in autophagy and that the increased susceptibility to apoptosis might be a consequence of defective autophagy. Supporting the effect of autophagy on B cell function, specifically in plasma cells, Atg5 mice (Atg 5 is an essential autophagic molecule) were unable to mount efficient T-cell dependent and T-cell Independent antibody responses. This was explained by the fact that the intense metabolic demand of synthesizing antibodies requires functional autophagy; since lack of this essential mechanism lead to high stress in the endoplasmic reticulum and therefore increase of cell death.

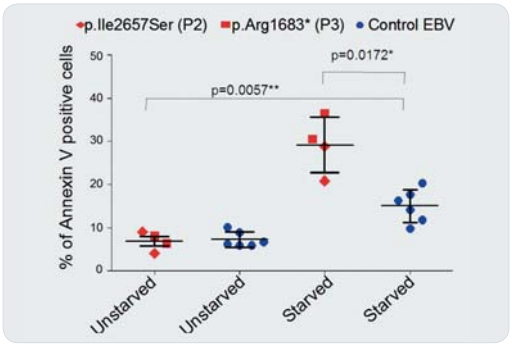


Fig. 2:
Apoptosis Test. EBV cell lines from two affected individuals (red bars) and three healthy donors (blue bars) were subjected to starvation. The proportion of early apoptotic cells was determined with Annexin V and propidium iodide staining. Both the percentage of early apoptotic EBV cells after starvation and the increase in apoptosis after starvation were significant (p values are 0.0172 and 0.0057, respectively, as determined by Welch t tests).

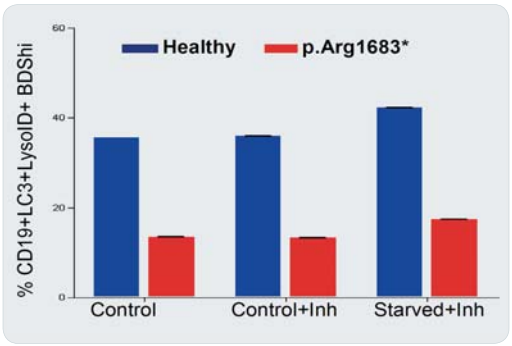


Fig. 3:
Autophagy measurement by co-localization of LC3 (autophagosome protein) and LysoID (lysosomal staining).
PBMCs from Healthy Donor (blue) and LRBA patients (red) were under starvation and starvation and in the presence or absence of the lysosomal inhibitors E64/pepstatin.

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INFECTION AND IMMUNITY

Successful and failing development of innate cellular immunity against bacteria: How the individual and its microbiome learn to coexist

KEYWORDS

Macrophages
Innate Immunity
Streptococcus
Mycobacteria
Neonatal Immunity

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Birth goes along with an immediate colonization by potentially harmful bacteria. Accordingly, the infant immune system needs to rapidly adapt to the microbial landscape that establishes at the mucosal and dermal surfaces. Cellular innate immunity, which utilizes germ line encoded recognition systems to sense microbial components, delivers the first line of defense against bacteria and guarantees protection against invasion of pathogens. At the same time, the cellular response has to be tightly regulated in order to prevent inflammation being triggered by bacteria that are in a "harmless" colonizing state.

The research group „Infection and Immunity" makes use of animal models, in vitro models and "human models", i.e. patients with defined defects in components of innate cellular immunity. Thereby, we improve understanding of beneficial and harmful interactions between microbial flora and mucocutaneous surfaces, both in higher dimensional models like genetically modified mice and on the single cell level between myeloid cells and bacteria. Monocytes and macrophages are at the heart of the group's interest, as are Gram-positive bacteria (streptococci and staphylococci) and mycobacteria. By definition this work is interdisciplinary including basic and clinical scientists, microbiologists, immunologists and cell biologists.
The following five projects form the core portfolio of our group.

CELL AUTONOMOUS RECOGNITION OF STREPTOCOCCI

In this project, the contribution of distinct pattern recognition systems, e.g. Toll-like receptors and the inflammasome, to the cell autonomous and global inflammatory program in response to streptococci is analyzed. The major focus is the response of the developing innate immunity and its contribution to neonatal streptococcal sepsis. This work has been productive both on the level of dissecting basic mechanisms of a) how streptococci are sensed, b) how this influences the response program of the cell and eventually the individual, and c) in preparing translational approaches to adjunctive sepsis therapy (e.g. Insulin, c-Jun kinase inhibition). The project on group B streptococci, which represent the most important pathogens in neonatal sepsis, is part of a longstanding international collaboration with Douglas Golenbock (UMass Medical School, Worcester, USA) and Patrick Trieu-Cuot (Institute Pasteur, Paris). Furthermore, we have analyzed the interaction of *Streptococcus pneumoniae* with respiratory epithelial cells and found that pneumococci themselves promote their capability to colonize. They actively interfere with laminar mucous transport through a mechanism that is independent of ciliary beat.

ROLE OF MONONUCLEAR MYELOID CELLS IN SKIN IMMUNITY TO STAPHYLOCOCCI

The observation underlying this project is that patients with inborn signaling defects in Toll-like receptor signaling, i.e. MyD88 and IRAK4 deficiency, suffer in particular from soft tissue infections with *S. aureus*. Here, the principal role of mononuclear phagocytes in orchestrating the skin response to *S. aureus* is analyzed in an intradermal mouse infection model (ear infection). The specific contribution of monocyte subsets is assessed in genetically modified mice (e.g. CCR2-ko, NR4A1-ko) and in bone-

marrow chimeras. Furthermore, the molecular contribution to the functional and structural host cell-bacteria interface is explored, in particular with respect to the recognition of bacterial nucleic acids, which involves MyD88 and IRAK4. This work is performed in close collaboration with Marco Prinz (Neuropathology, Freiburg).

ROLE OF MACROPHAGES IN GRANULOMA FORMATION IN MYCOBACTERIAL INFECTIONS

Multinucleated giant cells (MGC) are specialized macrophages in supercellular tissue structures called granulomas. Granuloma formation is a recognized mechanism by which the human immune system contains mycobacteria like *M. tuberculosis* at the site of invasion. In this project we aim at delineating the origin and function of MGC in disease, both in a mouse model of mycobacterial disease and in an in vitro model. We expect this basic project to identify molecules that may serve as targets for anti-mycobacterial therapy.
In a related project, we have established a case control study on children with lymphadenitis caused by non-tuberculous mycobacteria (NTM-Kids). Currently, over 100 NTM patients have been collected. Next to understanding the quality of their tissue reaction with a particular focus on the occurrence and function of MGC, we aim at identifying risk factors, such as life style and epigenetic modifications in candidate genes, for lymphadenitis caused by non-tuberculous mycobacteria.

ANALYSIS OF PATIENTS WITH SUSPECTED OR CONFIRMED DEFECTS IN INNATE IMMUNITY

The entry portal for this project is a diagnostic unit headed by P. Henneke, which annually analyzes about 50 patients, who are suspicious of defects in innate immunodeficiency. Moreover, cells from patients with defined genetic defects are analyzed for alterations in innate immunity. As examples, patients with defects in the IκB-kinase β, STIM-1, MyD88, CARD-11, complement component 8B (all in preparation), NEMO and G6Pc3 have been analyzed.
Moreover, we have initiated a cohort of infants with symptomatic CMV infections in the frame of a hospital-based study, that involves most of German children's hospitals (deriving its data via the "Erhebungseinheit für seltene pädiatrische Erkrankungen – ESPED) in order to identify risk factors for the failure to establish co-existence with this virus, which is ubiquitously found in children. In this study, several patients with underlying immunodeficiency have already been identified.

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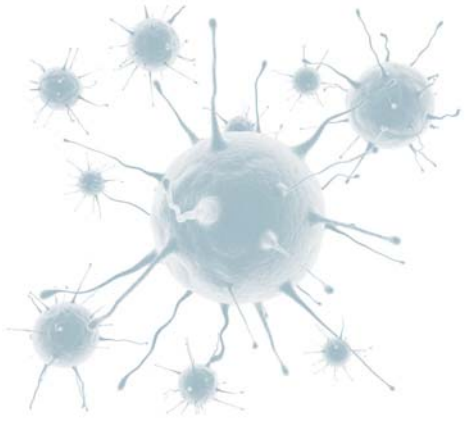

DEVELOPMENT OF CELLULAR INNATE IMMUNITY AGAINST STREPTOCOCCI

How the human body learns to contain bacteria at its surfaces

Invasive infections with bacteria, which are adapted to a harmless residence on human body surfaces are claiming millions of lives every year. These infections are a particular threat to infants and patients with primary or disease-induced immunodeficiency, such as malignant diseases, HIV and diabetes. As an example, Group B streptococci (GBS) are normal commensals on the skin of 10% of infants. At the same time, only 1% of colonized infants will develop sepsis, which still makes GBS the most common cause of neonatal sepsis. Currently, it is impossible to predict the risk in the individual baby. The research group "Infection and Immunity" has developed several in vitro and in vivo models to improve the understanding of successful and failing control of streptococci by the human immune system, which is paradigmatic for understanding antibacterial immunity altogether.

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INTERACTIONS BETWEEN GBS AND MONONUCLEAR PHAGOCYTES IN A CELL-BY-CELL FASHION

Mononuclear phagocytes, in particular tissue macrophages (MΦ) constitute the first line cellular defense system against bacteria that have breached the mucosal surfaces of the respiratory and intestinal tract. MΦ can be conceived as sentinel cells that communicate to the environment the invasion of bacteria, while taking up and digesting them. The uptake and digestion process involves wrapping of the bacteria into intracellular membrane vesicles, which provide an acidic milieu of highly proteolytic and oxidative (nitric oxide and H_2O_2) activity. This so called "phagolysosomal processing" of GBS, and in particular NO and protein nitration, are important for full host-cell activation by GBS. Furthermore, we have found that bacterial single stranded RNA (ssRNA) of GBS, other Gram-positive bacteria and mycobacteria in the phagolysosome is the essential bacterial component that activates MΦ. On the host-cell site, we identified the multispanning endoplasmatic reticulum protein UNC-93B and the adapter of most endosomal Toll like receptors (TLRs), MyD88, to be required for ssRNA recognition (Fig. 1).

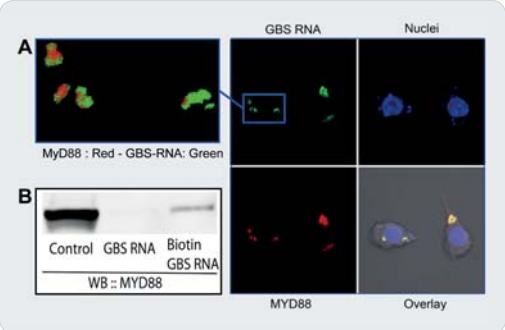


Fig. 1:
Streptococcal RNA interacts with the MyD88 complex in macophages.
A. RNA from ethyluridine-incorporated group B streptococci (GBS) was labelled with the Click-iT RNA labelling kit. MyD88-YFP-expressing RAW264.7 macrophages were stimulated with labelled GBS RNA and observed with confocal microscopy.
B. MyD88-YFP-expressing RAW264.7 macrophages were stimulated with biotinylated GBS RNA (1 µg/ml) for 2 h. Lysates of these cells were subjected to immunoprecipitation with avidin agarose and western blot analysis with MyD88-specific antibodies.

In addition to the MyD88-UNC-93B pathway, we have identified a second signaling cascade that is induced by GBS ssRNA and specifically activates the so-called NLRP3 inflammasome and therefore IL-1β. Activation of the NLRP3 inflammasome is important for survival of a systemic GBS infection, as demonstrated in NLRP3-deficient mice. Induction of IL1β requires cytolytic activity of the well established GBS virulence factor, β-hemolysin.
Collaboration with Douglas Golenbock, Worcester, USA

IDENTIFICATION OF NOVEL EXTRACELLULAR GBS TOXINS

Once GBS have invaded the tissue of a newborn infant, they can cause fulminant sepsis. The associated excessive inflammation throughout the body is, in a neonatal mouse model, induced in part by diacylated bacterial lipoproteins from GBS. Yet, whereas RNA carries most of the inflammatory activity of the GBS particle, lipoproteins are extracellular toxins of GBS. The receptors for diacylated GBS lipoproteins both in vitro and in vivo are TLR2 and 6. In contrast, lipoteichoic acid, which was previously suggested as an important effector in all Gram-positive bacteria, was found to be dispensable for cellular activation by GBS.
Furthermore, we unraveled a novel signaling pathway in the interaction of bacterial lipoproteins with TLR2, which does not adhere to the established model on TLR2-dependent signaling. This pathway leads to the activation of phospholipids (PIP3), which in turn are essential to put MΦ into an alarm state, the so-called MΦ polarization. Thus, at the beginning of invasion, recognition of extracellular GBS lipoproteins may be protective, since it helps the cellular first line defense to fight back. Indeed, this is what we could show in the neonatal mouse model. If only few bacteria are used for infection, lethality of mice is increased either by the absence of TLR2 expression in the mice or by the absence of lipoprotein expression in GBS. Yet, when a high dose of GBS is used, which corresponds to a model where lipoproteins are toxic, the absence of TLR2 protects the mice.

These studies have important therapeutic implications, since they helped to establish lipoproteins as disease-mediating toxins in Gram-positive bacteria, and particular in GBS.
Collaboration with Patrick Trieu-Cuot, Paris and Guiseppe Teti, Messina

INSULIN MODULATES THE RNA-INDUCED CYTOKINE RESPONSE IN GRANULOCYTES

The immune system and the endocrine system are interwoven on many levels. A prominent example for this is insulin, which has been assigned regulatory properties both for the antimicrobial and the inflammatory responses in infection. Insulin resistance is associated with increased susceptibility to invasive bacterial infections. Moreover, insulin concentrations are > 10 fold higher in tissue (e.g. kidney, liver) than in the plasma, thus insulin may be particularly important in modulating the first line defense in the tissue. GBS is particularly interesting in this context, since newborn infants and patients with type 2 diabetes, which are affected most by GBS infections, show peripheral insulin resistance. We found that the GBS-induced, MyD88-dependent chemokine formation in granulocytes is specifically down-modulated by insulin via insulin receptor mediated induction of phosphatidyl-inositol 3 kinase (PI3K). PI3K inhibits transcription of chemokine genes on the level of NFkB binding. Insulin specifically modulates the chemokine response without affecting antibacterial properties, such as migration, phagocytosis, bacterial killing and formation of reactive oxygen species. In summary, insulin may exert beneficial immunoregulatory functions, since it allows the individual cell to attack invading bacteria without escalating the inflammation cascade.
Collaboration with Taco Kuijpers, Amsterdam

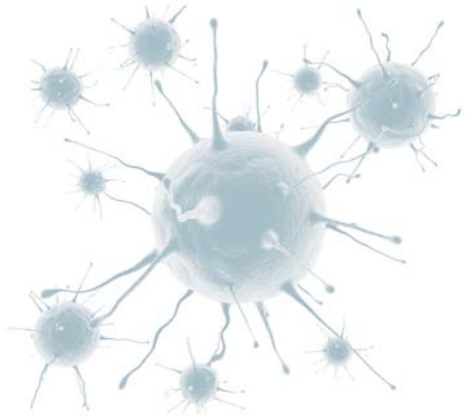
MECHANISMS OF STREPTOCOCCUS PNEUMONIAE TO INTERFERE WITH CLEARANCE FROM THE RESPIRATORY TRACT

Streptococcus pneumoniae is the most important cause of bacterial pneumonia and meningitis. At the same time, it is – like GBS – a normal mucocutaneous colonizer. Mucociliary airway clearance is an innate defense mechanism that protects the lung from harmful effects of potential airway pathogens including *Streptococcus pneumoniae*. In mouse tracheal epithelial cells grown in air-liquid interface cultures, we showed with high-speed video microscopy and live-cell imaging that the apical pneumococcal infection caused insufficient fluid flow along the epithelial surface. This resulted in a loss of efficient clearance, whereas ciliary beat frequency remained normal. Pneumococci caused specific morphologic aberrations of F-actin at the apical cortex of the lateral cell borders and F-actin, localized within the planes of the apical cell sides at the ciliary bases. This affected the columnar shape of the polarized respiratory epithelial cells and distorted the planar architecture of the ciliated respiratory epithelium. Hence, weakening of the mechanical support for effective cilial strokes by *S. pneumoniae* results in turbulent fluid flow at non-planar distorted epithelial surface areas, which enables bacteria to resist cilia-mediated clearance.

OBSERVATIONS IN HUMANS

As outlined above, it cannot be predicted, which infant will develop GBS sepsis, since many are exposed and colonized and only few will develop severe infections. It is an ongoing debate, whether sepsis usually originates from established mucocutaneous GBS colonization of the infant, or whether it results from an acute exogenous GBS infection. We analyzed twins, who both developed GBS sepsis twice in a strikingly parallel fashion originating from one hypervirulent GBS clone. The presentation as cervical soft tissue infection in all cases, the synchronicity of the episodes and the detection of GBS DNA in breast milk, strongly suggest that GBS sepsis can result from enteral transmission of GBS with short incubation period.

In further study we found that patients with an inborn defect in a single molecule (IκB kinase 2), which is central to many functions in phagocytes, results in lethal immunodeficiency to a host of microorganisms, including commensal bacteria.
Collaboration with Claire Poyart, Paris and with Klaus Schwarz, Ulm



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MOLECULAR IMMUNOLOGY

Control of the immune tolerance: how T cells achieve the balance between immunocompetence and tolerance to the extended self

KEYWORDS

Foxp3
CD4+ T cells
Regulatory T cells
Intestine
Epithelial cells

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The immune system has the dual task of defending the body against pathogens while remaining tolerant to harmless antigens. The latter include molecules from the body but also genetically foreign antigens that form part of the extended self, like the ones coming from the food and commensal microbiota. Specific tolerance is an active process that involves different immune cells and requires a functioning immune system. In immunodeficient disease not only the inflammatory arm, but also the tolerogenic arm of the immune response can be affected. As a paradoxical result, immunodeficient patients often suffer from autoimmunity or chronic inflammation.

In the research group "Molecular Immunology", we use mouse models to dissect the genetic and cellular components that regulate antigen-specific tolerance. We focus on a particular T cell subset, namely Foxp3$^+$ regulatory T cells or Treg that is key for the maintenance of tolerance in mice and humans.

GENERATION OF REGULATORY T CELLS

T cell tolerance can be imprinted at two sites. The thymus is the central organ for T lymphocyte maturation and the place where T cells are selected to prevent autoimmunity. When a T cell encounters self-antigen in the thymus, there are two possible outcomes: either cell death or differentiation into a regulatory T cell via induction of the transcription factor Foxp3. Cells that did not encounter their specific antigen in the thymus go to the periphery as naive T cells. When a CD4$^+$ lymphocyte encounters its antigen in the periphery, it has again the choice between two fates: becoming a T helper cell that will induce inflammation, or becoming a regulatory cell that will suppress the inflammatory activity of other T cells and of cells of the innate immune system. This decision is mediated by the induction of specific transcription factors such as Tbet, GATA3 and RORγt in proinflammatory helper T cells and of Foxp3 in regulatory T cells. Each of these transcription factors then promotes its own expression, stabilizing the lineage. Hence, the choice to express Foxp3 in a sustained way is crucial for the decision between immunity and tolerance. In collaboration with the group of S. Arnold in Freiburg, our group is studying transcription factors that modulate this fate choice and can determine if the encounter with antigen will result in inflammation or in tolerance.

MAINTENANCE OF REGULATORY T CELLS

The regulatory T cell population has a very high homeostatic capacity, which allows it to keep pace with the inflammatory proliferation during an immune response while returning to basal levels once the inflammation diminishes. This homeostasis can be disrupted in certain settings. In collaboration with H. Pircher, we are analyzing the role of inhibitory molecules in controlling the accumulation and function of Foxp3$^+$ regulatory T cells. We are also interested in the consequences of excessive Treg accumulation, as this could be a factor promoting immunodeficiency.

TISSUE-SPECIFIC CONTROL OF THE IMMUNE RESPONSE

It is becoming increasingly evident that the immune processes in the tissues are controlled in a different way to the ones in the lymphoid organs. One example for this is IL-23, a cytokine that prevents Treg induction and activity while favoring proinflammatory T cell responses in peripheral

tissues such as the gut and the nervous system. The mechanisms and implications of this control at the organ level are just starting to become apparent (Fig.1).

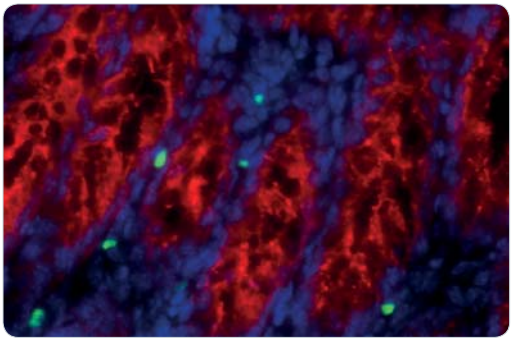

Fig. 1:
Foxp3+ cells in the intestine.
The picture shows a microscopy image of the gut from a wild-type mouse. Blue: cell nuclei stained with DAPI, Red: E-cadherin on intestinal epithelial cells, Green: Foxp3 in the nucleus of regulatory T cells.

In this context, the intestine is an ideal organ to study immune processes. It represents a major gateway for the entry of pathogens and therefore it has its own immune structures and a vast array of immune cells. At the same time, it is continuously confronted to exogenous antigens that need to be tolerated. The balance between inflammation and tolerance is locally controlled by several partially overlapping mechanisms. However, in conditions of immunodeficiency, this balance is often disrupted, and intestinal inflammation is frequent in immunodeficient patients. Similarly, colitis is the result of altered immune tolerance in a considerable number of mouse models.

In our group, we are addressing the role of local factors in the induction and maintenance of tolerance in the intestine. We are particularly interested in the role of intestinal epithelial cells in modulating immune responses. Intestinal epithelial cells form a single cell barrier separating the organism from the outside world. They carry nutrients and other molecules into the body and produce mucus and antimicrobial molecules to keep potential pathogens at bay. They also communicate with the immune system to modulate the immune response. Because they react to immune signals, they are also strongly affected by immune dysregulation, and patients with ongoing intestinal inflammation are at a higher risk of developing cancer. Our group has identified several candidates to mediate the communication of epithelial cells with the immune system and we are studying how these molecules impact on immune responses, especially on Treg homeostasis. In collaboration with K. Warnatz and M. Seidl, we are also analyzing the potential role of these molecules in human immunodeficiency. We hope that the understanding of T cell specific processes in their local environment will allow us to understand how immune responses are controlled at the organ level.

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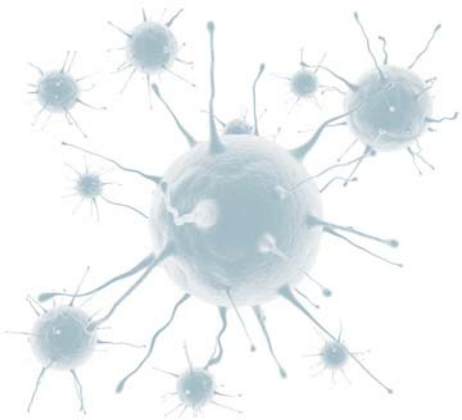

LOCAL REGULATION OF THE IMMUNE RESPONSE

"understanding the crosstalk between epithelial cells and the immune system"

To understand and treat immune disorders it is essential to know how the immune responses are controlled in the tissue. In this context we are analyzing the relationship of epithelial E-cadherin, expressed on epithelial cells, with its receptor KLRG1, expressed on Treg. To do that, we are taking a combined approach genetically deleting either molecule in mouse models. Our data suggest that interactions between epithelial cells and Treg shape the regulatory immune compartment in the gut.

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CURRENT FUNDING

BMBF
MPI-IE

E-cadherin is a key component of the intercellular epithelial junctions. Genome-wide association studies have linked a region containing the CDH1 gene, encoding E-cadherin, to increased susceptibility to chronic inflammatory bowel disease, suggesting that E-cadherin may also play a role in inflammation. In addition, a variant of E-cadherin associated to increased susceptibility to inflammatory bowel disease has been found to be expressed at lower levels at the cell surface, suggesting that defective E-cadherin expression on the cell membrane could favor intestinal inflammation. However, the immune roles of E-cadherin are not straightforward to study. Indeed, E-cadherin plays an essential role in establishing intercellular junctions in epithelia. Disrupting E-cadherin affects the structure of intercellular tight junctions that are core to the impermeable barrier in the intestine, and permeability to the intestinal contents can directly lead to intestinal inflammation, obscuring other potential roles of E-cadherin in the maintenance of immune homeostasis.

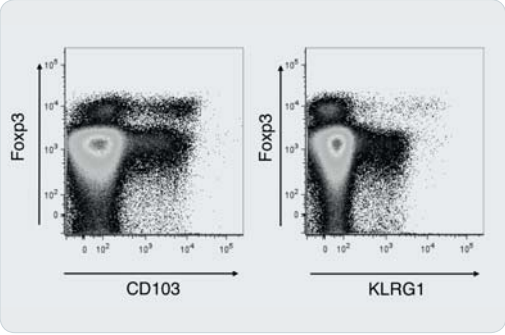

Fig. 1:
Foxp3⁺ Treg contain populations expressing high levels of CD103 or KLRG1.
The graph shows a FACS plot with CD103 and KLRG1 expression versus Foxp3 among total mouse spleen lymphocytes.

Besides forming homotypic interactions with other E-cadherin molecules, E-cadherin can be bound by two specific receptors on immune cells, the integrin $\alpha_E\beta_7$ (CD103) and the lectin KLRG1. CD103 can be found on different immune cell types including myeloid cells and T cells. Of note, gut dendritic cells expressing CD103 have been shown to induce Foxp3 from naive T cells, whereas E-cadherin-expressing gut dendritic cells are associated to inflammation in mice and patients suffering of inflammatory bowel disease. CD103 is also expressed on Treg; however, CD103-deficient mice do not present major immune problems, and CD103-/- Treg are functional in vivo.

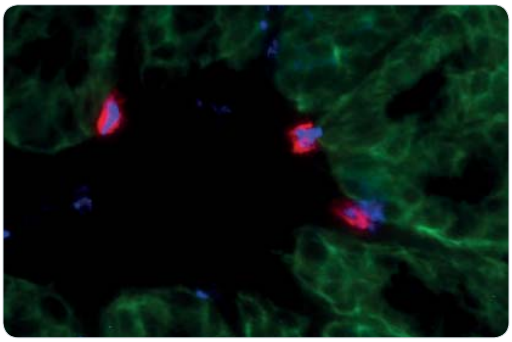

Fig. 2:
KLRG1⁺ Foxp3⁺ Treg are present in the mouse intestine.
Microscope picture showing KLRG1⁺ Treg in the colon of a wild-type mouse. Blue: Foxp3, Red: KLRG1, Green: E-cadherin

KLRG1 is an inhibitory receptor expressed by lymphocytes, especially by CD8+ cytotoxic T cells and some innate lymphoid cells, where it dampens activation. Among CD4+ T cells, KLRG1 is mostly expressed by subpopulations of Foxp3+ T cells, especially in the gut compared to lymphoid organs (Fig. 1). The function of KLRG1 on Treg is currently unknown.

Our group is currently studying the role of E-cadherin and KLRG1 in intestinal immune responses and, more specifically, in the control of Treg populations. We have characterized the phenotype of KLRG1+ Treg in the gut and other organs. KLRG1+ Treg are found at high frequency in the gut of young mice, decreasing in percentage afterwards. They are highly proliferative and have low levels of anti-apoptotic molecules, suggesting an effector phenotype (Fig. 2). In collaboration with H. Pircher, we are studying Treg cells from KLRG1-/- mice. KLRG1-/- mice are healthy and do not show signs of spontaneous autoimmune disease under steady state conditions. We are currently characterizing the frequency, distribution and phenotype of Treg populations in KLRG1-/- mice and challenging their mucosal immune system to determine the role of KLRG1 in Treg homeostasis. We are using immunodeficient mice as a model of T cell-mediated colitis. In this

model, colitis is induced upon naive CD4+ T cell transfer and prevented by the simultaneous transfer of CD4+ Foxp3+ regulatory T cells. The T cell transfer model allows us to dissect the effects caused by lack of KLRG1 on effector or on regulatory T cells. To better dissect the role of KLRG1 on Treg, we are also using bone marrow chimeras of KLRG1-deficient and wild-type cells. This setting will reveal the role of KLRG1 on Treg biology under competitive settings.

We are complementing this approach with in vitro studies of the response of Treg to T cell receptor signals in the presence or absence of KLRG1 coligation. T lymphocytes get activated through signals via their receptor for antigen. While Treg response to antigen is difficult to measure, it has become clear that antigen recognition is important for Treg accumulation and survival. KLRG1 has been shown to reduce activation of CD8+ T lymphocytes in response to antigenic signals, and we would like to see if it has similar effects on the Treg population. A problem in this approach is that traditional methods used to measure T cell responses to TCR signals, such as CD69, do not faithfully represent TCR stimulation in Treg. To circumvent this problem, we have characterized an alternative marker for TCR activation that is not induced in Treg by non-cognate stimuli. This method will allow us to verify if KLRG1 modifies the response to antigenic signals in Treg.

In parallel, we are tackling the interactions between Treg and intestinal epithelial cells from a complementary angle. In collaboration with M. Stemmler, we are analyzing the gut immune response in the absence of epithelial E-cadherin. To overcome the embryonic lethality caused by lack of E-cadherin, we have taken advantage of a model of epithelial to mesenchymal transition where intestinal E-cadherin is replaced by its close relative N-cadherin. This substitution also occurs spontaneously during disease settings in mice and humans, most notably as part of tumor progression to cancer. The model of E-cadherin replacement allows us to study the consequences of E-cadherin ablation in the presence of a functional epithelium. Despite having intact tight junctions, mice deficient for E-cadherin develop a severe intestinal inflammation after birth. This inflammation is mediated by the normal intestinal microbiota, and mice kept under germ-free conditions have a normal lifespan. Surprisingly, despite the ongoing intestinal inflammation observed under normal conditions, mice lacking intestinal E-cadherin have an exaggerated accumulation of Treg cells.

The combined approach of E-cadherin and KLRG1 deletion will allow us to understand the role of E-cadherin and its immune receptors in Treg function and gut tolerance, and to get an insight into the crosstalk between epithelial cells and T lymphocytes in the gut. Since E-cadherin expression is altered during tumor progression, and immunodeficient patients suffer from an increased tumor rate, we also want to check whether Treg are altered in the tumor environment (Fig. 3).

Our studies show that lack of either E-cadherin or KLRG1 promotes the accumulation of Foxp3+ T cells in the gut. These cells show high levels of CTLA4 and CD25, which can mediate suppression of immune responses, indicating that they are regulatory T cells. Our functional analyses have demonstrated that KLRG1 ligation reduces signaling through the T cell antigen receptor into regulatory T cells, providing a mechanistic explanation for the effect of KLRG1. Interestingly, lymphopenic mice in which regulatory T cells underwent expansion showed an increased frequency of KLRG1-expressing Foxp3+ cells, suggesting that KLRG1 interactions may play a stronger role during conditions of immunodeficiency than under normal settings. All in all, our data reveal a new level of local interaction in the gut that may be especially relevant in immunodeficient patients.

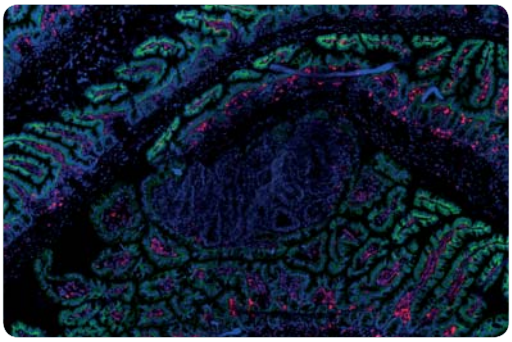

Fig. 3:
Tumor in the intestinal immune environment. Microscope picture showing hematopoietic cells around an intestinal adenoma in the mouse. Blue: cell nuclei; green: E-cadherin as marker for normal epithelial cells; Red: CD45 as marker for immune cells

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GENOME ENGINEERING

Genome editing using designer nucleases: from basic research to the specific correction of genetic mutation for human gene therapy

KEYWORDS

Gene editing
Gene knockout
HIV therapy
Transcription activator-like effector nuclease (TALENs)
RNA guided endonucleases (RGNs)

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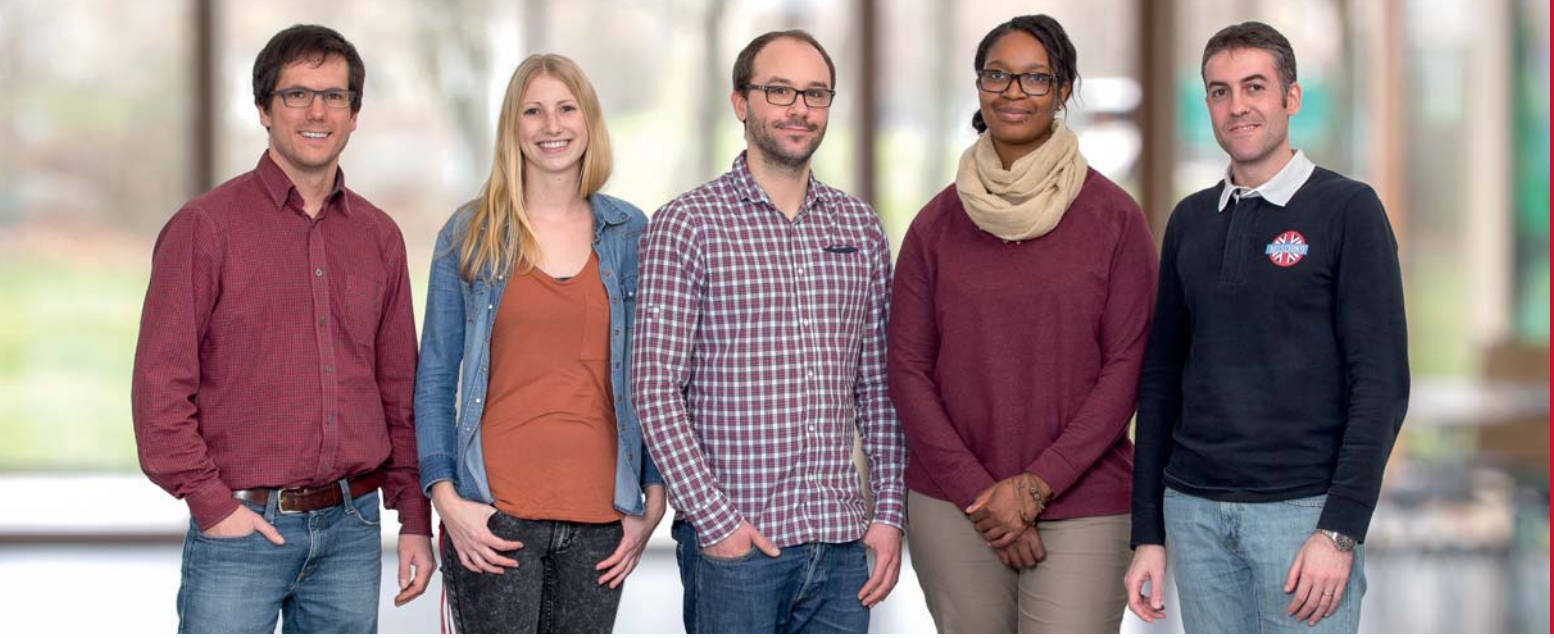
Genome engineering holds great promise for various applications, spanning from basic research to systems biology, biotechnology and human gene therapy. By using designer nucleases, the researcher can now create a targeted DNA double-strand break (DSB) in a locus of choice, triggering the cellular DNA repair pathways that can be harnessed to induce permanent modifications of the target genome.
The Junior Research Group "Genome Engineering" is affiliated with both the CCI and the Institute for Cell and Gene Therapy. It aims at further developing two promising designer nucleases platforms and to apply these technologies to expand the basic knowledge of immunological disorders and to set the stage for new approaches of gene therapy for primary and acquired immunodeficiencies.

DNA REPAIR AND GENOME EDITING

The creation of a targeted DNA double-strand break (DSB) in a locus of choice activates the cellular DNA repair pathways, including Non-Homologous End Joining (NHEJ) or Homologous Recombination (HR) (Kass et al., 2010) that can be harnessed to induce permanent modifications in genomes of different complexity. NHEJ is the major repair mechanism of DSBs in multicellular eukaryotes but it is imprecise and it leads to small deletion or insertion mutations at the break point. Thus, NHEJ can be harnessed to knockout a gene to achieve therapeutic benefit. On the other hand, the HR repair pathway relies on the intact sister chromatid or an exogenous homologous DNA fragment to repair a DSB, thus leading to the precise reconstitution of the broken chromosome and can be used for precise correction of a disease causing mutation (Segal et al., 2013).
We use genome editing techniques to create point mutations in healthy cells in order to study gene function or to generate cell line models of human disorders. This is particularly important to dissect the basic mechanisms underlying immunological disorders and for a proper evaluation of the cellular phenotype resulting from certain genetic mutations (collaboration with Bodo Grimbacher, CCI, Freiburg). On the other hand we aim to specifically correct genetic mutations in diseased cells to explore novel therapeutic options for patients suffering from primary and acquired immunodeficiencies *Collaborations with Toni Cathomen and Stephan Ehl, CCI, Freiburg*

DESIGNER NUCLEASES AS TOOL TO ALTER THE GENOME

Tailored DNA breaks can be induced at specific genomic locations by using designer nucleases. These artificial enzymes are composed of an engineered DNA-binding domain, which drives a DNA-cleavage domain to generate a DSB in a targeted manner. Zinc-finger nucleases (ZFNs) comprise the most successful class of engineered nucleases to date but a limited targeting range and time-consuming methods to generate ZFNs with novel specificities limit their wide-spread use (Cathomen at al., 2008). Recent development of customized TALENs (transcription activator-like effector nucleases) and RGNs (RNA guided nucleases) has allowed the rapid editing of genomic DNA in a variety of organisms and human cell types at very high efficiencies (1, 2).
We have developed an improved TALEN scaffold and generated tailored nucleases that combine high activity with low cytotoxicity (3). For clinical applications, designer nucleases should be specific



enough to induce only the desired genomic modification, thus we utilize cutting-edge high throughput sequencing technologies to dissect the specificity signature of these artificial enzymes (collaboration with TJ Cradick, Atlanta, USA). In addition, we have recently adopted the use of RGNs as an alternative versatile tool for genome editing with the aim to dissect and improve their specificity profile. To this end we are developing a novel *in vitro* system to identify designer nucleases-associated off-targets.

HIV INFECTION AND TREATMENT OPTIONS

The human immunodeficiency virus (HIV) is a major global health burden which has resulted in the death of 25 million people in the past 30 years. HIV infection is characterized by the depletion of CD4+ immune regulatory cells and may result in acquired immunodeficiency syndrome (AIDS). The immune system is not capable of efficiently clearing HIV infection and current treatments involve a combination of antiretroviral drugs referred to as highly active antiretroviral therapy (HAART). However, the elevated costs, the emergence of viral resistance and side effects of long-term therapy represent limitations for HIV treatment (Manjunath et al., 2013). In addition, HAART does not offer a permanent cure since interruption of therapy leads to rapid rebound of viremia from latent reservoirs. Gene therapy is an attractive method to derive HIV-resistant cells by disrupting the *CCR5* co-receptor used by HIV to infect its target cells. Indeed, a naturally occurring mutation in this gene (delta32 mutation) confers resistance to HIV infection (Hutter et al., 2009).

In the Genome Engineering lab, we aim to generate safe TALE-nucleases that can specifically inactivate the *CCR5* gene to create patient-derived HIV resistant immune cells that can be re-administered to the patient to establish a novel therapeutic opportunity for HIV infected people and to set the stage for clinical translation of genome engineering-based approaches for the treatment of rare and acquired immunodeficiencies.

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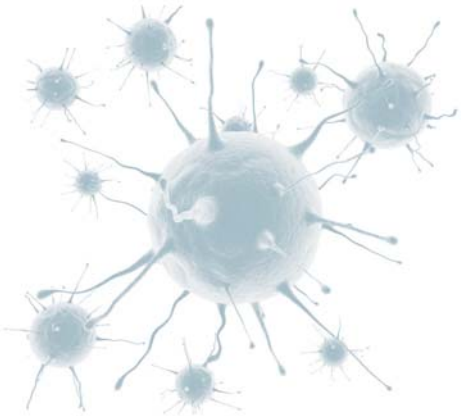

DESIGNER NUCLEASES

Identifying a safe and specific platform to tackle HIV infection

Designer nucleases, as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), have become increasingly popular for targeted genome modification in the last decade. These artificial enzymes can be used to introduce permanent genetic modification by inducing targeted double stranded breaks and have shown great potential for genome surgery in complex organisms including human stem cells (Segal et al., 2013). ZFNs are the most successful class of designer nucleases that have been applied in clinical trials for the modification of patient-derived CD4+ T cells to generate transplantable HIV-resistant cells by disrupting of the viral co-receptor *CCR5* (Perez et al., 2008). However, genome-wide assessment of their specificity has revealed off-target cleavage (2). TALENs seem to be better tolerated in human cell lines but whether this correlates with higher specificity and/or lower off-target cleavage has not been clearly addressed yet (3).

SCIENTISTS

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Nicola Bundschuh

CURRENT FUNDING

BMBF

Employing *in vitro* assays we dissected the cytotoxicity profile associated with the use of ZFNs and TALENs targeted to the *CCR5* gene. Moreover, giving the great potential of genome editing techniques for the treatment of HIV infection, we used high throughput sequencing to address the specificity of *CCR5*-specific designer nucleases and identified nucleases that combine high targeting activity with low cytotoxicity for future clinical translation (1).

CREATING HIV RESISTANCE

CCR5 is the major co-receptor for HIV entry into the target cells and a specific mutation, the 32 deletion, results in a truncated protein that has been associated with virus resistance. This observation has driven the development of anti-HIV medications to disrupt the HIV–CCR5 interaction. Small-molecules approaches that block this interaction have shown promise in clinical trials but such form of treatment does not represent a definitive cure to HIV infection. Gene therapy offers the potential to develop novel therapeutic strategies aimed at generating immune cells that lack the CCR5 receptor on their surface and that can be re-transplanted to the patient to reconstitute an immune system resistant to HIV infection. The potential of this approach is strengthened by the "Berlin Patient", an HIV-positive man who required a hematopoietic stem cell transplant to treat acute myeloid leukemia and who received cells from a donor homozygous for the CCR5delta32 mutation (Hutter et al., 2009). The transplantation resulted in the generation of donor derived *CCR5*-negative, HIV-resistant immune cells that were able to suppress viral infection/replication, thus representing a proof for a permanent cure.

This result points to the importance of a genetic knockout of *CCR5* for long-term resistance to HIV infection and this can be efficiently achieved in patient derived cells using designer nucleases, such as zinc finger nucleases (ZFNs), as currently under evaluation in clinical trials (e.g. NCT01044654). However, ZFNs are associated with a certain degree of cytotoxicity and off-target mutagenesis that may hamper their further development for clinical application. On the other hand, TALE-based nucleases (TALENs) seem to be better tolerated by human cells and are associated with high specificity, thus representing a powerful and potentially safer platform to edit the human genome(4). We therefore intend to perform an in-depth characterization of activity, toxicity and specificity of *CCR5*-specific TALENs and a side-by-side comparison with the ZFNs used in the clinic with the aim to establish a safer and more specific platform to be used for the permanent disruption of the endogenous *CCR5* in primary human CD4+ T cells for clinical translation.

CCR5-SPECIFIC DESIGNER NUCLEASES: ZFNS OR TALENS?

Designer nucleases targeted to a particular DNA sequence are arising as a powerful tool to precisely alter complex genomes for basic research, biotechnology, synthetic biology or human gene therapy. However, while the specificity of these artificial enzymes is only a minor concern in basic research, it represents a bottleneck for the translation of this reagent into the clinic since very rare mutagenic events in cells with high proliferative potential, such as hematopoietic stem cells, can lead to uncontrolled proliferation. Thus, the development of safer and more specific platforms to edit the human genome is imperative. In our lab, we have established a novel TALEN scaffold that allows the generation of functional designer nucleases in about two weeks. We have generated a panel of *CCR5*-specific TALENs in order to perform a side-by-side comparison with the ZFNs used in clinical trial to identify a safer and more specific platform to be used for the clinical development of a genome engineering approach for the treatment of HIV infection based on *CCR5* disruption.

First results in human cell lines have provided evidence that our *CCR5*-specific TALENs are as active

as the well-established ZFNs in inducing mutations at the *CCR5* gene. This is monitored using the T7 endonuclease 1 assay (T7E1) that allows to indirectly measuring the extent of nuclease-induced gene disruption at the target site (Fig. 1).

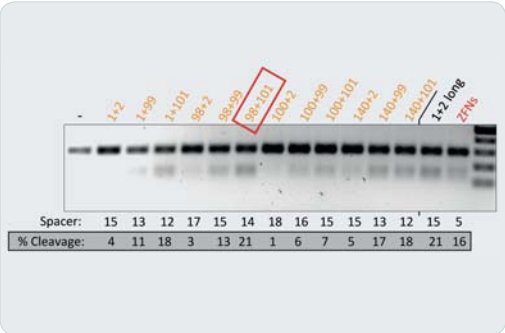


Fig. 1:
Disruption of endogenous human *CCR5* locus.
A panel of 13 TALEN pairs (orange) generated in our lab were tested by T7 endonuclease 1 assay for their cleavage activity at the *CCR5* locus and compared with ZFNs (red) used in a clinical trial. The spacer length between the two target half-sites and the percentage of modified alleles is indicated on the bottom.

However, an important parameter for a successful designer nuclease is its specificity. A major off-target locus of the *CCR5*-specific ZFN has been identified in *CCR2*, which shares a high degree of sequence identity with the *CCR5* locus. Indeed, both the ZFN and TALEN target sites in *CCR5* differs from the corresponding site in *CCR2* in only two positions. When the T7E1 assay was used to monitor off-target cleavage at *CCR2*, we could show that our *CCR5*-specific TALENs were able to efficiently discriminate between very similar sequences as opposed to ZFNs with mutation rates as high as 21% in *CCR5* and only 3% at the highly homologous *CCR2* locus (Fig. 2).

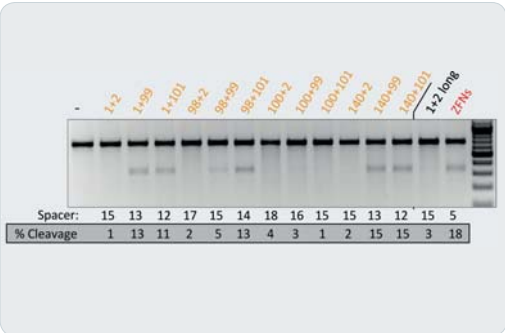


Fig. 2:
Off-target cleavage at the *CCR2* locus.
The *CCR5*-specific nucleases were tested for their cleavage activity at the *CCR2* locus using the T7 endonuclease 1 assay. The percentage of modified alleles is indicated on the bottom.

In contrast, *CCR5*-specific ZFN had almost comparable activity at the two loci, highlighting the great potential of TALENs to discriminate between highly identical target sites in the human genome. Concomitant assessment of cytotoxicity, as measured by cell survival, revealed an almost 2-fold increase when comparing the *CCR5*-specific TALEN versus the ZFN pair, thus further highlighting the TALEN platform as a platform similarly effective as the ZFNs but likely more specific and less cytotoxic (Fig. 3).

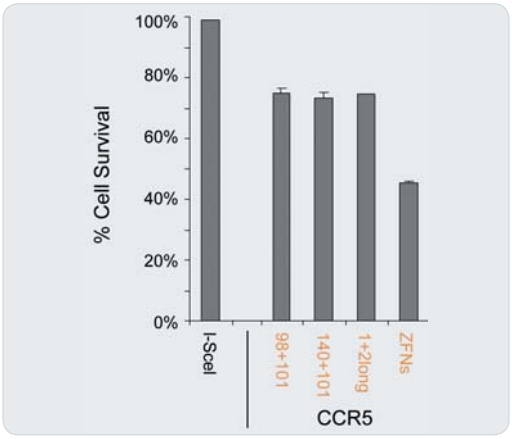
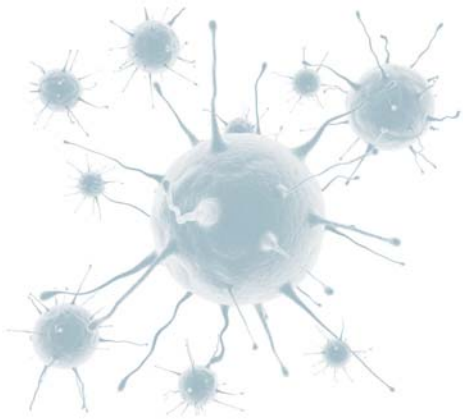


Fig. 3:
Cytotoxicity of *CCR5*-specific designer nucleases.
The graph shows the percentage of cell survival 5 days post transfection with the indicated designer nucleases compared to the non-toxic meganuclease I-*Sce*I

We are currently investigating the specificity signature of our *CCR5*-specific TALENs on a larger scale by using high throughput sequencing methods to analyze *in silico* predicted off-target sites and we have generated additional TALENs in order to identify the most efficient and specific for further clinical translation.



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EPIDEMIOLOGY

Population-level investigations into immune deficiency and proneness to infections

KEYWORDS

Infectious disease susceptibility
Airway infections
Vitamin D
Genetic Epidemiology
Epigenetic markers

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Population-based research of immunodeficiency phenotypes is an important addition to the clinical and experimental research offered by the CCI. The Research Group Epidemiology has the overall goal to identify host factors driving predilection to respiratory tract infections (RTI) and to better characterize individuals with moderate to mild forms of immunodeficiency in the general population. We have established a population-based study on the susceptibility to airway infections (AWIS) in the local population and are engaged in collaborative projects such as the Ulm Birth Cohort to study biomarkers, in particular DNA methylation markers, for infection-proneness in adulthood and childhood. Moreover, we participate actively in the development of a sophisticated program on host and pathogen interaction in the context of a prospective epidemiological study with the acronym NaKo coordinated by the Helmholtz Center for Infectious Diseases that will be initiated in 2014 and recruit 200.000 individuals across Germany.

AIRWAY INFECTION SUSCEPTIBILITY (AWIS) STUDY:
Little epidemiological research has been conducted so far to identify host factors that are associated with the recurrent RTI phenotype. Therefore, the AWIS team (Ilona Geist, Stefan Wolfram, Melanie Bäurle, Karin Rau, Hans-Hartmut Peter and Livia Maccioni) have initiated a cross-sectional epidemiological study in the local South Baden population to estimate the frequency of RTI and to identify infection-prone individuals and those rarely infected for subsequent comparative investigation. We have mailed pre-screening questionnaires to assess RTI frequency and severity, comorbidities, hospitalizations due to infections and selected lifestyle habits to about 70.000 randomly selected individuals in the age range of 18 - 69 years. About 12.800 of the selected individuals participated in this study and agreed to be approached for the embedded AWIS-PLUS study aimed at a comparison of epidemiological, genetic and immunological factors of individuals with frequent RTI and those rarely affected by RTI despite similar exposure likelihood to RTI pathogens. A score (RTI score) was calculated to identify infection-prone individuals and a comparison group of individuals with few or no RTI. The distribution of the RTI score in individuals is presented in Fig. 1.

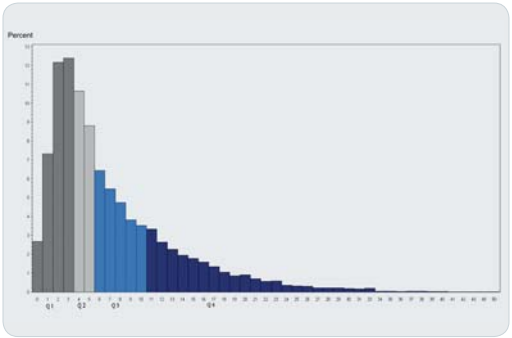

Fig. 1:
Quartiles (Q1-Q4) of the RTI score in the AWIS study population.

In our epidemiological analysis of the pre-screening questionnaires we found frequent contact to small children, current smoking, obesity, airway allergies and prior surgery of immune organs strongly associated with a high score, whereas educational level did not correlate with material differences in the RTI score.
Subsequently, we selected from all quartiles of the RTI score around 2000 individuals for more detailed inquiries on their lifestyle and environmental exposures as well as on co-morbidities. We also

obtained saliva samples using Oragene Kits and extracted DNA for genotyping to identify putative genetic differences between infection-prone people and those unaffected. First statistical analysis of questionnaire data revealed an overall higher infectious burden of those with frequent RTI and greater co-morbidity especially with allergic diseases. To better understand how lifestyle and environmental factors impact upon infection risk in the susceptible population, we are currently inviting more than 1200 AWIS participants to enroll in a prospective cohort. Various biospecimens including blood, nasal swabs and urine will be collected to establish a biobank to identify biomarker profiles of infection-prone individuals.

VITAMIN D AND RESPIRATORY TRACT INFECTIONS

Vitamin D is an essential nutrient with immunoregulatory functions, and a potential biomarker to assess susceptibility to common infections. Basal and epidemiologic evidence suggest that vitamin D may protect against respiratory viruses, in particular respiratory syncytial virus (RSV), the leading cause of lower respiratory tract infection in neonates. In collaboration with Prof. Dietrich Rothenbacher (University of Ulm) and Prof. Hermann Brenner (German Cancer Research Center in Heidelberg), Anna Luczynska explored in 777 samples of the Ulm Birth Cohort whether cord blood vitamin D concentrations are associated with subsequent risk of lower respiratory tract infections (LRTI) in the first year of life. Our study provides evidence of a high prevalence of vitamin D deficiency in neonates born in Germany. Our findings further indicate that vitamin D deficiency at birth is associated with increased risk of LRTI, particularly in infants born in autumn. In addition, the association appears to be stronger in mothers without a history of allergy. This association supports the idea that especially children born in autumn might benefit from an intervention to increase cord blood vitamin D values in order to reduce risk of subsequent LRTI.

DNA METHYLATION AND RISK OF EARLY CHILDHOOD INFECTIONS

Host, environment and pathogen factors contribute to risk of infectious disease in early childhood. Epigenetic mechanisms constitute an interface for interaction between the genome and the environment and are particularly susceptible to impact in the pre-implantation phase and in subsequent intra-uterine development. The main aim of this project conducted by Magdeldin Elgizouli is the identification of alternations in DNA methylation associated with risk of common infections in childhood. Using a combined epigenome-wide and candidate-gene approach we investigated cord blood DNA methylation patterns in the Ulm Birth Cohort, a longitudinal study of newborns, in relation to prospective frequency of lower respiratory tract infections. So far differential methylation patterns were identified and subsequently validated in two key immune response genes. Replication of the findings in an independent cohort and further characterization of differential loci in target tissues will follow. Methylation patterns in cord blood were independently influenced by maternal age, maternal education and number of siblings in support of the proposition that the physical and social environment dynamically mold the epigenome.

GENE AND ENVIRONMENT INTERACTION IN THE ETIOLOGY OF LYMPHOMAS

In addition to our activities in the field of immunodeficiency and infection susceptibility, the group continues its involvement in the InterLymph consortium, a large international effort to elucidate risk and protective factors for lymphomas. Research in molecular cancer epidemiology as performed by the scientists of the InterLymph consortium serves as a successful model for collaborative projects in the area of rare heterogeneous diseases. Currently, the main focus of the Consortium is to finalize and publish the InterLymph GWAS initiatives with genome-wide data on thousands of cases of the main NHL subtypes followed by various initiatives on gene-by-environment interactions. Funded by the José Carreras Foundation, the focus of Anne-Sophie Stöhlker in Freiburg will be on the secondary data analysis of genetic variability of epigenetic regulators and its role in lymphomagenesis. Beyond the new insights into the implication of epigenetic mechanisms in the etiology of lymphomas, we expect an added value of this project also for the field of infection susceptibility. Statistical methods and study principles of this lymphoma-related work may very well be adapted and transferred to the field of immunodeficiency.

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MOLECULAR GENETICS OF ANTIBODY DEFICIENCIES

Analysis of primary antibody deficiencies – genetic and functional analysis of defects in human B cells

KEYWORDS

Primary Antibody Deficiency
B-cell immunity
TACI
micro-RNA
GATA-2

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The research focus of our clinically and genetically oriented laboratory is the molecular genetics of primary immunodeficiencies. Among these, we are mostly interested in studying adult patients with antibody deficiencies, who are clinically summarized under the diagnostic term "Common variable immunodeficiency – CVID". The systematic functional and genetic screening of several candidate genes revealed gene defects which present with CVID-like phenotypes. Currently, we aim at understanding how these genetic defects cause the disease and influence its immunological and clinical presentation. In this respect, the further analysis of genetic defects in the candidate gene TACI and their functional evaluation are of great importance.

BAFFR, CD21 AND TACI DEFICIENCY

Primary human antibody deficiencies such as CVID may result from mutations and epigenetic changes that interfere with normal development and activation of B cells. Recently, we found that homozygous mutations in the BAFF-R gene correlate with CVID and prevent the formation of a normal B cell compartment. In collaboration with Hermann Eibel and Marta Rizzi, we were further able to delineate the interdependence of BAFF serum levels and peripheral B-cell numbers and the role of a common polymorphism in the BAFFR extracellular domain. We further contributed to the identification of the first human gene defect in the CD21B cell coreceptor. Our studies explore the role of genetic variants in the TACI receptor for CVID disease pathogenesis. In this respect, we could identify new families carrying biallelic mutations in TACI and have contributed to several cohort studies investigating TACI mutations in antibody deficient patients or related disorders. In a recent study, we investigate the role of the complement protein family of Ficolins in CVID and found that low levels of Ficolin-2 were strongly correlated with bronchiectasis.

ANALYSIS OF MICRO-RNAS IN NORMAL HUMAN PERIPHERAL B-CELL DEVELOPMENT AND IN PATIENTS WITH PRIMARY ANTIBODY DEFICIENCIES

Recently, microRNAs (miRNAs) were described as posttranscriptional regulators of gene expression in mammals. MiRNAs control a variety of biological processes, including development, differentiation and function of the immune system. Several miRNAs have been shown to be involved in B-cell development and differentiation. These findings motivated us to investigate the possible involvement of miRNAs in the pathogenesis of CVID. We found 21 miRNAs differentially regulated in CVID patients compared to controls (14 up, 7 down). Out of these, we chose the most significantly dysregulated miR-193b~365 pair for further analysis. This miRNA pair shows a restricted, activation-dependent expression pattern in terminal B-cell stages and targets important factors involved in terminal B-cell differentiation.

ANALYSIS OF PATIENTS WITH B-CELL LYMPHOPENIA AND GATA-2 DEFICIENCY

About 10% of CVID patients have low or absent peripheral B-cells. We could identify a BAFFR gene defect in one of our CVID B^{low} patients and several male patients with btk deficiency. In a subset of CVID patients, an abnormal distribution of B-cell precursors in the bone marrow was shown. A newly developed in vitro culture system for cultivation of human B cell precursors (developed by M. Rizzi) and whole exome sequencing (collaboration with H. Eibel) will help to understand the CVID B^{low} patient cohort better in the future. Another recently described primary immunodeficiency

caused by mutations in GATA-2, the MonoMAC syndrome (aka DCML deficiency), is also characterized by low to absent peripheral B-cells. Although GATA-2 deficient patients have generally normal total Immunoglobulin levels, we found that IgG subclasses and pneumococcal polysaccharide antibody responses may be diminished in some patients. In a new research project (in collaboration with M. Wlodarski and C. Mussolino) we aim to better characterize the defect of the B cell system in human GATA-2 deficiency.

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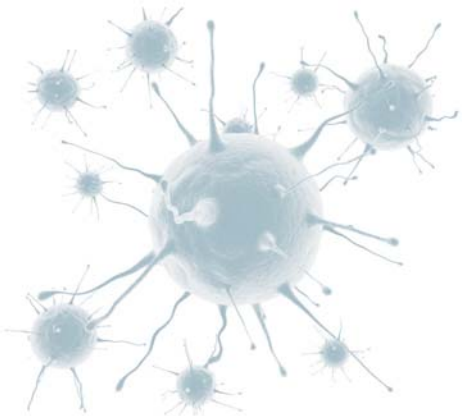

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Understanding the transcriptional regulation of lymphocyte differentiation

KEYWORDS

E proteins
Id proteins
B cell development
T cell differentiation
Transcriptional regulation
Chromatin modifications

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B and T cells have to differentiate into highly specialized cells in order to fulfill their specific functions in the immune system. Immunodeficiency often results from defective lymphocyte differentiation, resulting in the complete absence of certain cells, their non-functionality or aberrant activation. Cellular differentiation is tightly controlled by the action of various transcription factors. In order to understand the underlying cause of immunodeficiency with defective lymphocyte differentiation, the research group "Immunobiology" is interested in the molecular mechanisms governing B and T cell differentiation. Specifically, we aim to deciphering the molecular events of transcription factor activity, such as protein-protein interactions, histone modifications and DNA methylation. Ultimately, our goal is to identify novel and feasible targets, such as chromatin modifying enzymes, for manipulating cell fate decisions and thus contribute to the development of novel prophylactic and therapeutic approaches, e.g. for patients with immunodeficiency.

We are particularly interested in the transcriptional regulation of lymphocyte differentiation by the E protein transcription factors and their regulators, the Id proteins. In mammals the family of the E protein transcription factors includes E2A, HEB and E2-2 (Fig. 1). They belong to the basic region helix-loop-helix transcription (bHLH) factors and thus form homo- or heterodimers with cell-type specific bHLH factors, such as MyoD or NeuroD in order to regulate target gene transcription. In the immune system mainly homo- or heterodimers among E proteins are active. The transcriptional activity of all E proteins can be inhibited by the Id (Inhibitor of differentiation) proteins. These proteins lack the basic DNA binding domain, however can form heterodimers with any of the E proteins via their HLH domain (Fig. 1) and in turn prevent their DNA binding. Of the four Id proteins (Id1-4) mainly Id2 and Id3 are expressed and functionally important in lymphocytes.

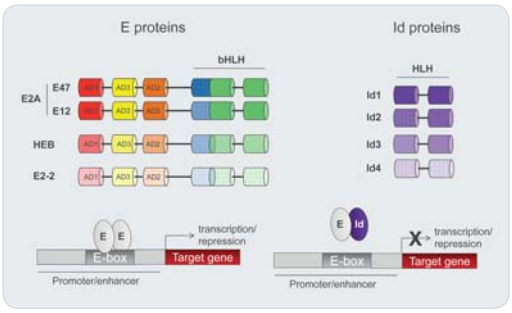

Fig. 1:
Schematic diagram of E and Id proteins, depicting different protein domains (AD: activation domain); blue: basic DNA binding region; green/violet: helix-loop-helix (HLH) domain) and their mode of action.

VARIANTS E12 AND E47 IN B CELL DEVELOPMENT

The differentiation of highly specialized B cells from hematopoietic stem cells (HSC) is tightly regulated by the concerted action of several transcription factors. In the adult B cells develop in the bone marrow during a multistep process from HSCs into committed B cells that express a B cell receptor (BCR) (Fig. 1). The expression of a BCR requires the rearrangement of the immunoglobulin heavy chain (*IgH*) and light chain (*IgL*) gene loci, which is facilitated by the RAG proteins RAG1 and RAG2 at different stages of B cell development. After commitment to the B cell lineage at the pre-pro B cell stage these pro B cells undergo *IgH DJ* rearrangements followed by *IgH VDJ* joining. Productive *IgH VDJ* gene rearrangements will result in the surface expression of a pre-BCR. Pre-BCR signaling leads to the suppression of RAG activity and promotes the survival and proliferation of these large pre B cells. After a period of clonal expansion RAG activity is again induced to permit *IgL* gene rearrangements. Here the *Igκ* gene locus is rearranged first generating a complete BCR at the cell surface. In the presence of BCR self-reactivity continued *Igκ* rearrangement will replace the self-reactive *Igκ VJ* joints in a process called receptor

editing. If the pre B cell does not succeed to generate an innocuous BCR upon rearrangement of both *Igκ* loci, the second *IgL* loci, called *Igλ* will be rearranged. Finally, surface expression of a functional BCR, which lacks self-reactivity, will suppress *Rag* gene expression and these positively selected mature B cells will migrate into peripheral lymphoid organs.

The E2A proteins E12 and E47, which arise through mutually exclusive splicing of two exons in the *Tcfe2a* gene, have been shown to be important for several developmental checkpoints during B cell development (Fig. 2). In pre-pro B cells E2A proteins act upstream of the B lineage determining transcription factors Ebf1 and Pax5 and together with these proteins activate the expression of B cell specific genes leading to B cell commitment. Consequently, E2A deficient mice fail to generate mature B cells. Furthermore, in pro B cells E2A is important for maintaining the B cell specific gene expression program. Later on during B cell specification E2A proteins directly activate the rearrangement of the *IgH* and *IgL* genes. Although the role of E2A proteins in B cell development is well established, it is less clear whether E12 and E47 have overlapping and redundant roles or whether they perform distinct functions.

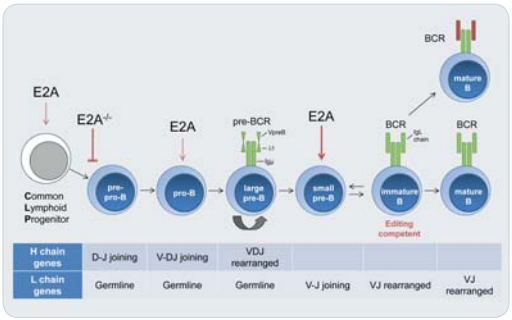

Fig. 2:
Schematic diagram depicting the functions of E2A during B cell development.

In order to investigate the specific functions of E12 and E47 during B cell development, we generated mice specifically lacking either E12 or E47, while retaining normal expression of the other splice variant. Analysis of the B cell compartment in the bone marrow and spleen of wt, E12-/- and E47-/- mice revealed an almost complete absence of B cells in E47-/- mice, while total numbers of B cells in the E12-/- mice were almost normal. Thus, we conclude that while E47 is essential for developmental progression at the pre-pro–B cell stage, E12 is dispensable for early B cell development, commitment, and maintenance. However, further phenotyping of different B cell subsets revealed that E12-/- mice have fewer *Igλ* expressing B cells in the bone marrow and spleen as compared to wt mice. Interestingly, also E47+/- and E12+/- mice have reduced numbers of *Igλ*-expressing B cells. Thus, both E12 and E47 play critical roles at the pre-B and immature B cell stage and importantly, the abundance of E47 and E12 is limiting during the development of *Igλ*-expressing B cells. Additionally, quantification of germline transcripts of the *Igλ* locus, which are an indicator of locus accessibility, revealed that E12 and E47 promote *Igλ* locus accessibility. Chromatin Immunoprecipitation analysis further showed that E12 and E47 directly bind to the *Igλ* enhancers and modulate histone modifications (H3 ac and H3K4me3) at the *Igλ* enhancer. Based on these and previously published data, we proposed a model for the sequential rearrangement of the *IgL* genes. Attenuation of IL-7 signaling upon migration of pre–B cells away from IL-7–expressing stroma, leads to an increase in E12 and E47 expression. Early on E47 abundance is sufficient to initiate *Igκ* light chain gene rearrangement. It has been shown before, that signaling through a functional BCR suppresses E12/E47 expression to prevent further rearrangements and promote positive selection and maturation. However, in the absence of such a signal, the ratio of E12 to E47 reaches a certain threshold, which enables the cell to rearrange the *Igλ* loci. Failure to generate a functional BCR during a certain time window will lead to cell death, presumably caused by high E12 and E47 levels as it has shown before.

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IMMUNOLOGY

B and T cell antigen receptor signaling in primary immunodeficiency

KEYWORDS

Primary immunodeficiency
Antigen receptor
CD3γ-deficiency
Signaling
Boolean models

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The adaptive immune system is indispensable for the control of pathogenic microorganisms such as bacteria and viruses. B and T cells are key players of the immune response that encompasses antigen recognition, cellular activation, differentiation into effector cells, pathogen clearance and the formation of an immunological memory. Development, activation as well as function of B and T cells depend on their antigen receptors with the underlying signaling networks. Mutations of the receptors or the signaling molecules involved in antigen receptor signaling can therefore lead to severe immune defects. In fact, an increasing number of primary immunodeficiencies has been identified exhibiting aberrances in B or T cell receptor signal transduction.

The activities of the research group „Immunology“ include basic research in mice and humans as well as systems biology approaches to describe, analyze and characterize primary immunodeficiencies with a strong focus on the B and T cell antigen receptors and their signaling machineries. In addition, the CCI junior group “Immunobiology” hold by K. Schachtrup is affiliated to the research group “Immunology”. Research focus of this group is the role of E and Id proteins in the transcriptional regulation during lymphocyte differentiation (see page 110). At the Faculty of Biology the junior group “Lymphocyte Signalling” headed by S. Minguet, focusses on the proximal signalling events of T cell activation and on T cell migration.

THE ROLE OF TCR SIGNALING IN CD3γ IMMUNODEFICIENCIES

One of our projects deals with the role of signaling pathways downsteam of the TCR in CD3γ immunodeficiency. The T cell receptor (TCR) plays a crucial role in the activation of T cells and is composed of the TCRαβ antigen-binding subunits as well as the signal-transducing CD3εγ, CD3εδ and CD3ζζ dimers, that contain cytoplasmic tyrosines. Whether the different tyrosines have redundant roles, or specific roles coupling to different signaling pathways is unknown.

Patients defective in CD3γ expression are immunodeficient, although reasonable numbers of T cells that express a CD3γ-deficient TCR are present. Previously, we showed that CD3 replaces CD3γ in this TCR (9). We hypothesized that the phenotype is caused by a difference in the signal transduction capabilities of the wt compared to the CD3γ-/- TCR. Weaker signaling by CD3δ in the CD3γ-/- TCR would cause reduced T cell activation contributing to the immunodeficient phenotype in CD3γ-deficient patients (Fig. 1).

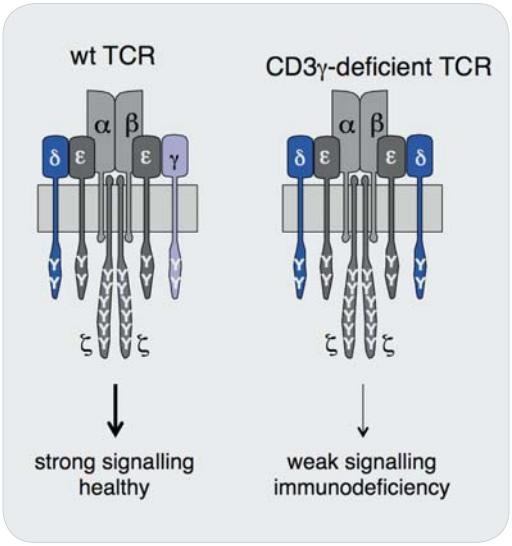

Fig. 1:
In contrast to the wt TCR, the CD3γ-/- TCR contains two CD3δ which leads to reduced signaling by the mutant TCR.

To study TCR signaling, we utilized our humanized CD3γ-deficient mouse strain (5,10). This strain neither expresses the murine CD3γ nor the CD3δ isoforms but it contains a human CD3δ transgene, so that a hybrid TCR as shown in Fig.1 is expressed. The T cells of this mouse strain have a very similar phenotype as the ones from the CD3γ-deficient patients (5,10).
Collaboration with Jose Ramon Regueiro, Madrid; Matthias Gstaiger and Ruedi Aebersold, Zürich

ANALYSES OF IMMUNE-DEFICIENT PATIENTS

Our long-standing research focus involves the investigation of the molecular mechanisms leading to immune cell activation. In this context, we collaborated with Stephan Ehl to analyze NK cells of an ORAI-deficient patient and found the ORAI-mediated calcium influx was required for NK cell function (5). Similarly, we helped to analyze T cells of two STIM-deficient patients and showed that cytotoxic and regulatory T cell display partial defects in their functions (4). We also collaborated with the group of Jose Ramon Regueiro in Madrid to carefully analyze two new patients with a partial CD3δ immunodeficiency (6). In these patients, small amounts of wt CD3δ are produced that had a differential impact on the αβ TCR compared to the γδ TCR.
In addition, we established protocols for the flow cytometric measurement of different signaling molecules to improve analysis of B and T cell signal transduction in immune-deficient patients. We are now able to address the activity of important signaling proteins by this approach including Ig-alpha, Syk, Zap-70, Lyn, Lck, PLC1/2, Akt, Erk, IB, and p38.
Collaborations with Stephan Ehl, CCI; Hermann Eibel, CCI; Klaus Warnatz, CCI; Jose Ramon Regueiro, Madrid

ACTIVATION OF B AND T LYMPHOCYTES

In our "basic science" projects we investigate the molecular mechanisms with which B and T cells are activated by foreign antigens, such as proteins from bacteria or viruses. In particular, we are interested in the very early steps of activation that occur directly after antigen has bound to the BCR and TCR. A deep understanding of the underlying molecular details might help to open new avenues for the treatment of patients with immunological disorders.
(i) We have shown that the TCR occurs in different forms on the cell surface (7). For instance, the αβ TCR exists in two distinct conformations (8). In the inactive conformation the αβ TCR cannot be phosphorylated. Thus, the T cell stays quiescent. The active conformation is stabilized by antigen-binding and allows the αβ TCR to be phosphorylated, to recruit the adaptor protein Nck and to transmit the signal of antigen-binding further downstream. As a consequence the αβ T cell is activated. We also showed that the γδ TCR is different in that it does not require adopting the active conformation, in order to transmit signals for cellular activation (2).
(ii) Using quantitative proteomics approaches we could identify the novel protein Kidins220 as an interaction partner for the BCR and the TCR (1, 3). Kidins220 is a transmembrane scaffold protein that regulates activation of the MAP kinase pathways as induced by the BCR and TCR. Using mice that are deficient for Kidins220, we found that this scaffold protein is not only involved in B and T cell activation, but also in the development of those cells. Currently, we address the molecular mechanism with which Kidins220 regulates B and T cell activation.
Collaborations with Stephan Ehl, CCI; Maximilian Seidl, CCI; Bodo Grimbacher, CCI; Balbino Alarcon, Madrid; Oreste Acuto, Oxford.

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AUTOIMMUNITY

Autoimmune inflammatory diseases: pathogenesis and development of therapeutic strategies

KEYWORDS

Inflammation
Cell Targeting
Signaling Pathways
Plasma Cells
Apoptosis

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Systemic autoimmune diseases including systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) cause disability, significantly compromises quality of life and also life expectancy. RA is a chronic inflammatory disorder which affects multiple joints, causes pain, swollen joints and erosions of the cartilage and bone. SLE is characterized by inflammation of multiple organs including joints, skin, lung, blood vessels, brain and kidneys. A hallmark of SLE is the production of pathogenic autoantibodies against nuclear antigens, especially double stranded (ds) DNA and nucleosomes. During the disease course the development of co-morbidities like infections, malignancies and atherosclerosis are increased in SLE patients and represent are responsible for the majority of the mortality related to SLE. Moreover, patients suffering from primary immune deficiencies (PID) paradoxically often develop signs and symptoms of autoimmune diseases.

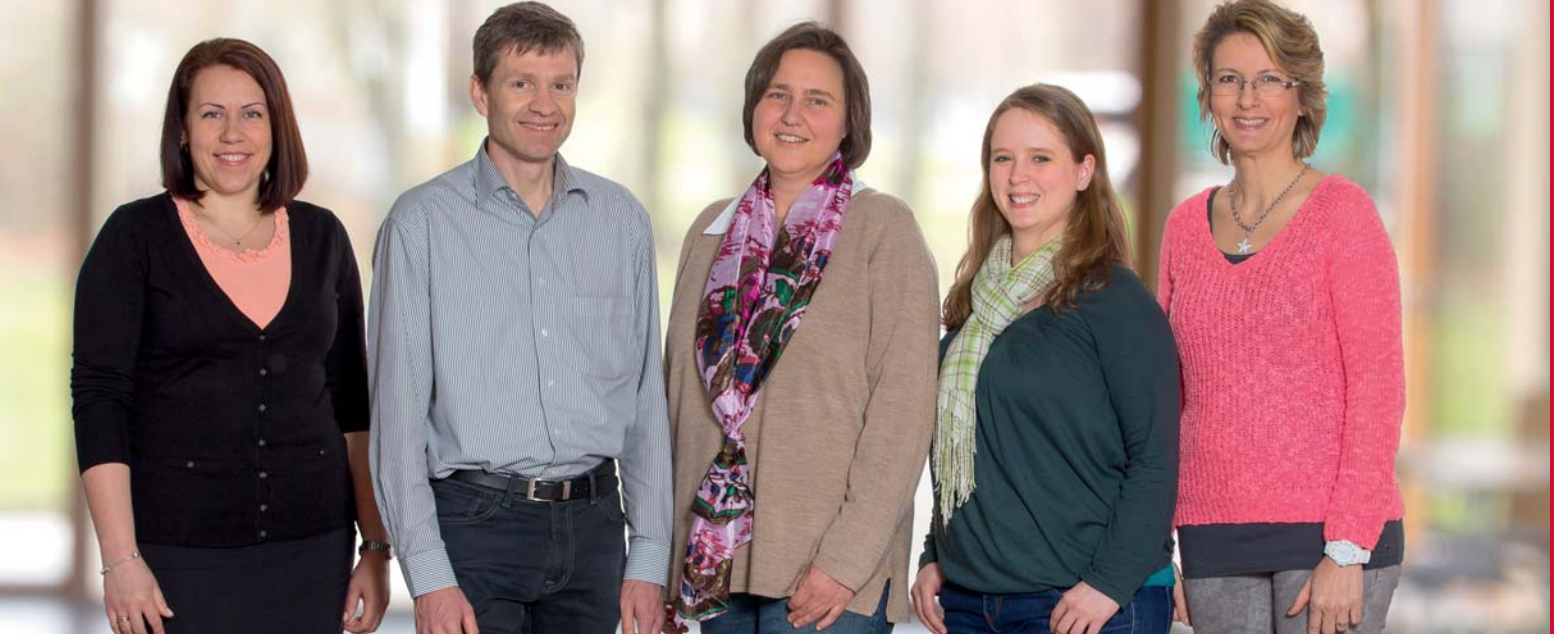
The research group „Autoimmunity" is interested in the etiology and pathogenesis of inflammatory autoimmune diseases, also in connection with primary immune deficiencies. The research aims to get insights in selective cell-types, signaling molecules and transcription factors (e.g. nuclear factor kappaB, NF-κB) that are crucially involved in pathogenesis and, hence, represent potential targets for therapy. The close interaction of physicians and scientists enables to establish biomedical laboratory research from bench to bedside and vice versa. Our research comprises several projects: the role of NF-κB in SLE and RA and the development of cell-type specific therapeutic interventions, the characterization of renal plasma cells and the IgG glycovariants in lupus, impaired clearance of dying cells and its immunological consequences.

DEVELOPMENT OF STRATEGIES TO INHIBIT SIGNALING PATHWAYS IN A CELL-TYPE SPECIFIC MANNER

In order to determine specific intracellular signaling pathways involved in the pathogenesis of RA, SLE and other autoimmune diseases, we are primarily investigating the intracellular signaling in those cell types that may contribute to disease pathogenesis. For that reason we developed the so-called "sneaking-ligand" approach in collaboration with Prof. Dr. Stefan Dübel (Braunschweig). The "sneaking ligand constructs" are fusion proteins consisting of three modules. The fusion protein is internalized in a receptor-specific manner, and then the effector domain is released from the endosomal compartment into the cytoplasm to interact with its respective partner molecule. Recently we developed an E-selectin-specific NF-κB inhibitor which ameliorates clinical and histological manifestations in mouse models of arthritis. Importantly, the "ligand sneaking" approach may reduce adverse treatment effects and maintain the homeostatic function of the cell. Besides identifying disease-relevant signaling pathways, this powerful approach could represent an attractive tool for the development of new treatment strategies.

PLASMA CELL SURVIVAL AND THEIR INFLAMMATORY SURVIVAL NICHES IN MURINE LUPUS NEPHRITIS

Plasma cells (PC) are major players in the pathogenesis of SLE due to the secretion of pathogenic autoantibodies. Whereas short-lived plasma cells undergo apoptosis within a few days, long-lived plasma cells can most likely survive life-long if not dislodged from their niches. Long-lived (memory) PC are extremely resistant to conventional treatments including high dose



corticosteroids and even high dose chemotherapy with autologous stem cell transplantation. Therefore, memory plasma cells secreting pathogenic antibodies may be responsible for many refractory disease courses of antibody-driven diseases such as SLE. Recently, we found that memory PC secreting pathogenic antibodies to dsDNA can be found also in the inflamed kidneys of NZB/W lupus mice. To further investigate the function and differentiation of memory PC in lupus, our group utilizes different lupus mouse models such as the NZB/W F1 model, pristane-induced lupus and the MLR/lpr lupus model. In these models, we demonstrated that proteasome inhibition efficiently eliminates plasma cells, including long-lived ones. Furthermore, ELISPOT and secretion assays revealed that the IgG secretion rate of autoreactive antibody-producing cells from inflamed kidneys is markedly higher than the secretion rate of bone marrow- or spleen-derived plasma cells. The underlying mechanisms of increased IgG secretion by renal plasma cells are currently under investigation.

Moreover, the pathogenicity of IgG antibodies could be strongly influenced by their subclass and glycosylation pattern which affects the affinity to activating versus inhibitory Fcγ-receptors. Therefore we are investigating the glycosylation patterns of anti-dsDNA antibodies (Fig. 1).

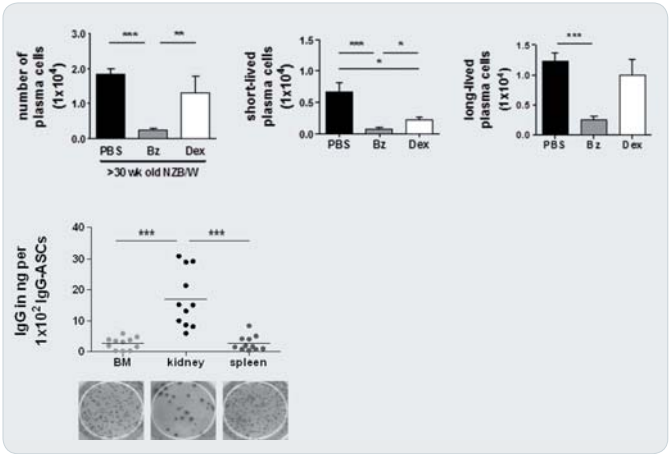


Fig. 1:
Treatment of lupus mice with the proteasome inhibitor bortezomib (Bz) showed markedly decreased numbers of short- and long-lived plasma cells (PC) within the nephritic kidneys. IgG-secretion rates from bone marrow, renal and splenic IgG-ASCs of NZB/W F1 mice.

IMMUNOLOGICAL CONSEQUENCES OF CELL DEATH IN AUTOIMMUNE DISORDERS

Autoantibodies against dsDNA and nucleosomes represent a serological hallmark of SLE. It is well known that impaired phagocytosis of apoptotic cells with consecutive release of nuclear antigens occur in SLE and may contribute to the pathogenesis of the disease. We discovered that nucleosomes from late apoptotic cells contain the high-mobility group box 1 protein (HMGB1). HMGB1 is a non-histone, DNA-binding nuclear protein that fulfills two functions. Located in the nucleus, HMGB1 binds to DNA and regulates transcription and chromosome architecture. In the extracellular milieu HMGB1 acts as endogenous ligand of RAGE, TLR2, and TLR4 and induces the secretion of pro-inflammatory cytokines.

Primary Sjögren syndrome (pSS) is an inflammatory autoimmune disease that mainly affects the tear and saliva glands. The immunological mechanisms of this disease have not yet been fully clarified. pSS shares some pathogenic features with SLE. Therefore, we asked the question if HMGB1 is implicated in the pathogenesis of pSS.

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THE "SNEAKING-LIGAND" APPROACH - CELL-TYPE SPECIFIC MODULATION OF INTRACELLULAR SIGNALING PATHWAYS

Attenuation of experimental arthritis using an endothelium selective NF-κB inhibitor

The transcription factor NF-κB plays a major role in the pathogenesis of inflammatory autoimmune diseases such as rheumatoid arthritis (RA) [1]. Proinflammatory cytokines, chemokines and adhesion molecules are transcriptionally regulated through activation of the classical NF-κB pathway. Hence, interference with NF-κB activation represents an attractive target for therapeutic intervention. However, a systemic blockade of NF-κB by small molecule inhibitors is associated with severe adverse effects, because NF-κB is involved in multiple cellular functions in various organs including liver, heart and brains [2, 3]. Moreover, small molecule NF-κB inhibitors or cell penetrating peptides conjugated to factor NF-κB inhibiting molecules are not cell-specific and enter the cell in a receptor-independent manner [4]. To target selectively cell types involved in disease pathogenesis we developed the so-called *"sneaking-ligand" approach* [5].

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CURRENT FUNDING

DFG

THE "SNEAKING LIGAND" APPROACH

Powerful tools to investigate the involvement of certain cell types and molecules in disease pathogenesis are based on the generation of transgenic and knockout mice [6]. However, therapeutic interventions during periods of active disease are limited in these models. An alternative approach is the use of cell penetrating peptides (CPPs) like TAT or the transduction domain of Antennapedia (Antp) which could be conjugated to effector proteins. One of those CPPs that inhibit the classical NF-κB pathway is Antp-linked to the Nemo-binding peptide (Antp-NBP) developed and characterized by Michael May and coworkers [4]. However, Antp-NBP cannot selectively be targeted to certain cell types. Therefore, we established our *"sneaking-ligand constructs"* which are based on the conjugation of three domains displaying similar functions like the domains of *Pseudomonas Exotoxin A*. Since activated endothelial cells play a pivotal role in transmigration of leukocytes into the sites of inflammation, we were interested to study the function of NF-κB in the activated endothelium in mouse models of arthritis. The prototypic *sneaking-ligand* construct 1 (SLC1) comprises three domains: 1) three repeats of an E-selectin-binding ligand (EBL) [7]; 2) the translocation domain of *Pseudomonas exotoxin A* (*ETAII*) [8], that enables the endosomal release of 3) the Nemo-binding peptide (NBP) encompassing amino acids 644–756 of IKK2 into the cytosol to block IKK assembly [4] (Fig.1) [5].

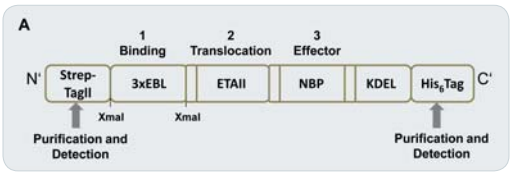


Fig.1:
A) Schematic illustration of the functional SLC1.
Three repeats of E-selectin–specific peptide (DITWDQLWDLMK) connected with a S_4G linker; ETA II: translocation domain of Pseudomonas exotoxin A domain II; NBP as effector domain. StrepTagII and His6Tag is included for purification and detection. B) Transport of KDEL containing SLC1 to the endoplasmatic reticulum (ER) in a retrograde manner. Cleavage of SLC1 in the ER and released of the NBP domain into the cytosol.

The *"sneaking-ligand constructs"* are cloned in an expression vector for recombinant expression in E. coli. The purified and refolded functional SLC1 and the respective non-functional control proteins MutEBL (including a scrambled binding domain), DelEBL (lacking the binding domain) and MutNPP2 (including a mutated NBP domain) were tested for their functionality in *in vitro* assays and *in vivo* models.

Collaboration with Stefan Dübel, Braunschweig and Harald Burkhardt, Frankfurt am Main

SNEAKING LIGANDS INHIBIT NF-KB IN THE ACTIVATED ENDOTHELIUM

The function of cytoplasmic SLC1 was investigated in a CHO (Chinese hamster ovary cells) expressing mouse E-selectin (CHO-E cells) using a NF-κB dependent Luciferase reporter gene assay. First the CHO-E and wildtype CHO cells are transiently transfected with pB2xLuc vector DNA an then pretreated for 90 min at 37 °C with SLC1 (500 nM). NF-κB activation was initiated by cytokine application. The transcriptional NF-κB activity was significantly reduced in SLC1-treated CHO-E cells (Fig. 2 left panel). No significant effect was observed in SLC1 treated wildtype CHO cells confirming the E-selectin specific uptake and processing of the fusion protein (Fig. 2 right panel).

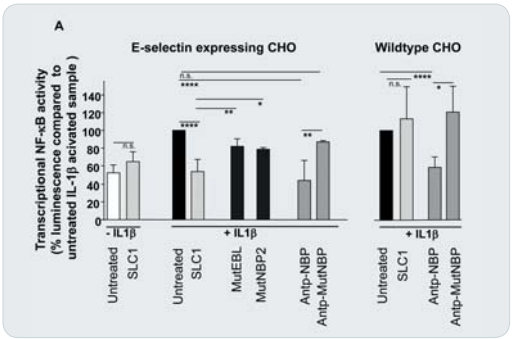


Fig.2:
Internalized SLC1 selectively inhibit NF-κB activation *in vitro* analyzed by luciferase gene reporter assay.
Confocal microscopy of internalized SLC1.

Antp-NBP (10 µM), which is internalized via a receptor-independent process, reduced the transcriptional NF-κB activity in both CHO-E and wildtype cells. Further, SLC1 treatment blocks NF-κB activation in comparison to the non-functional SLCs and MutNPP2.

Collaboration with Klaus Warnatz, Freiburg

IN VIVO IMAGING OF SLC1 BINDING TO CYTOKINE-ACTIVATED VASCULAR ENDOTHELIUM

To examine the specific binding of SLC1 to E-selectin *in vivo* whole mouse *in vivo* imaging confirmed the specific binding of SLC1 to the cytokine activated endothelium (Fig.3) [9]. Inflammation was induced in the skin of transgenic green fluorescent protein (GFP)-expressing mice by cytokine induction followed by an intraperitoneal injection of an infrared labelled SLC1 (SLC1-FP653). A skin-flap was prepared and the anesthetized mouse was imaged. SLC1-FP653 interacted selectively with cytokine-activated endothelium, whereas the control with a mutated binding domain did not (Fig. 3).

Collaboration with Agnes Schröder, Erlangen and Johannes Wessels Göttingen

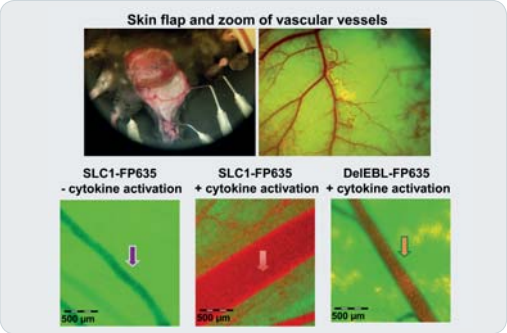


Fig.3:
In vivo imaging of vascular vessels in the skin.
Infrared labelled SLC1 (SLC1-FP635) binds to the cytokine-activated endothelium resulting in an orange staining of the endothelium border (middle panel).

ENDOTHELIUM SPECIFIC NF-KB INHIBITION ATTENUATED EXPERIMENTAL ARTHRITIS

Next we investigated the role of NF-κB activation in endothelial cells in mouse models of arthritis. We used the models of antigen-induced arthritis (AIA), collagen-induced arthritis CIA and serum transfer arthritis to test the effect of SLC1. For the latter, mice received an injection of serum from K/BxN mice containing antibodies against glucose-6-phosphoisomerase (anti-G6PI) which cause an acute polyarthritis [10]. Simultaneously with induction, mice were injected intraperitoneally with SLC1 and control SLC proteins (100 µg/injection). Treatment was repeated 7 and 24 h later. The paw swelling was visually assessed and expressed as a clinical arthritis score. SLC1-treated mice showed a significantly lower arthritis score compared to the control groups (PBS-, MutNBP2-, and DelEBL) (Fig.4).

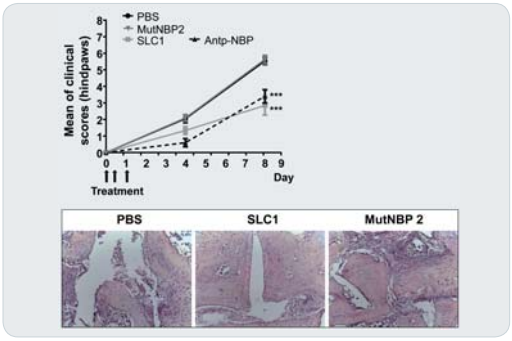
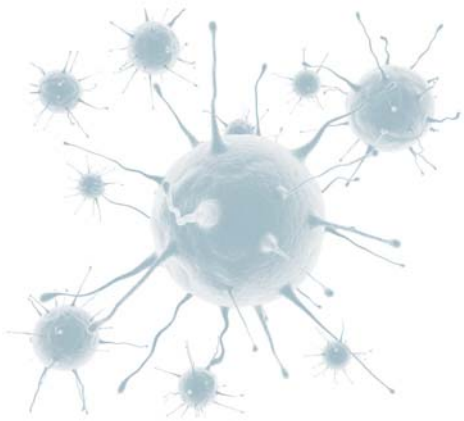


Fig.4:
E-selectin specific NF-kB inhibition attenuated clinical and histological manifestations in the K/BxN serum transfer model.
Clinical score are significantly lowered in SLC1 treated mice (upper panel). Representative images of HE stained section presenting the blocked migration of leukocytes into the affected joints after SLC treatment (lower panel, middle).

The observed therapeutic effect of SLC1 was comparable to Antp-NBP. The histological manifestations were assessed in HE-(hematoxylin/eosin), toludinineblue-, and Trap-(tartrate-resistant acid phosphatase)-stained joint sections. SLC1 application suppressed the migration of leukocytes into the joint and protected against cartilage and bone degradation.

Taken together, using the "sneaking ligand" approach we identified the essential role of NF-κB activation in endothelial cells in arthritis. Moreover, this approach represents an attractive tool to study disease-relevant cell types and signaling pathways. Further, SLCs may improve effectiveness as well as minimize the risks of adverse effects of therapeutic interventions by selective cell targeting.

Collaboration with Georg Schett, Erlangen; Falk Nimmerjahn, Erlangen and Michael J. May, Philadelphia, USA



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INFECTION AND IMMUNITY

Mycobacterial diseases: Infiltration of the immune system

KEYWORDS

Nontuberculous mycobacteria
NTM-NET
Tuberculosis
Diagnosis
Host-Pathogen-Interaction
Epidemiology

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Mycobacteria, e.g. mycobacteria of the *M. tuberculosis* complex as well as nontuberculous mycobacteria (NTM), have the ability to survive and replicate within macrophages after phagocytosis. Severe infections with nontuberculous mycobacteria are marker diseases for several defined immunodeficiency disorders that involve the INF-y and IL12-axis. These disseminated infections usually occur in early childhood. On the other hand host and environmental factors that predispose humans without overt immunodeficiency to reactivation of latent tuberculosis or localized NTM diseases have not been sufficiently elucidated.

At the same time, mycobacterial diseases are of growing importance worldwide: tuberculosis due to the enormous amount of infected patients with impaired local or systemic immunity (especially HIV-infected patients) being at risk for reactivating or developing this disease. Pulmonary disease due to NTM-PD is an emerging infection mainly in regions with a decreasing prevalence of tuberculosis. Patients with existing pulmonary diseases or patients with local or systemic immunosuppression are at risk to develop NTM-PD. Mycobacterial diseases thus occur from early childhood to seniority and require competence across pediatric and adult infectious diseases and immunology subspecialties.

Therefore, the CCI has decided early on to build up cohorts of patients with mycobacterial diseases and foster their study. So far our group has focused on building up structures, epidemiological studies and evaluation of diagnostic tools.

NTM-NET

NTM infections are considered rare diseases that seldom are treated within a single center. Cooperation across regions within a country, but also across countries thus is important to more efficiently allow studies. In 2009, the CCI organized an international meeting in Freiburg, where the NTM-NET was founded as an international network to promote clinically oriented research in the field of nontuberculous mycobacterial diseases around the globe by sharing and developing ideas and research protocols. Embedded as a branch in the European oriented TBnet, the NTM-NET has grown since then and comprised in 2013 more than 170 members from 23 European and 28 Non-European countries. For further informations see www.ntm-net.org.

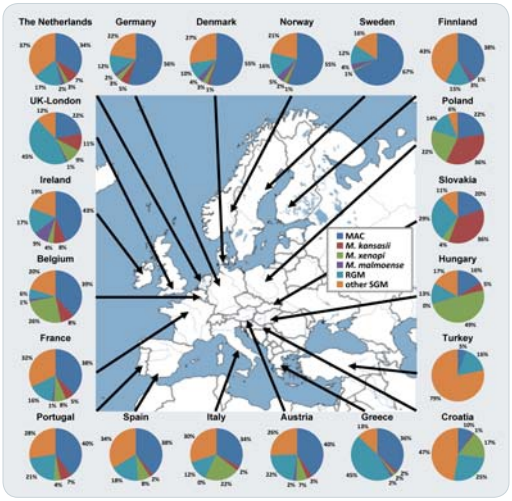

Fig. 1.:
Distribution of different nontuberculous mycobacteria from pulmonary samples in 2008 in Europe.
MAC: Mycobacterium avium complex; RGM: rapid-growing mycobacteria; SGM: slow-growing mycobacteria (from Hoefsloot et al. 2013, ERJ December 1, 2013 vol. 42 no. 6 1604-1613; copyright ERS).

NTM EPIDEMIOLOGY

NTM are a heterogeneous group of mycobacteria that are unevenly distributed around the world.

The clinical significance of the different strains varies from unimportant commensal to strains almost always causing disease in the respective host. The epidemiology of these strains worldwide, their distribution (3), the burden and the trends of NTM pulmonary disease-associated hospitalizations in Germany (2) as well as their prevalence, incidence, treatment modalities and factors influencing the treatment decisions in different European countries including Germany (cooperation with INSMED, INSMED Incorporated, New Jersey, USA) are key to better steer research and launch prospective studies (Fig. 1).

DIAGNOSTIC TOOLS FOR MYCOBACTERIAL DISEASES

The group currently focusses on the evaluation of new tools for the diagnosis of mycobacterial diseases. Tuberculosis specific IFN-γ release assays of patients with different types of immunosuppression were evaluated both in a single center study as well as in a large multicenter-TBnet-trial. We also participate in a trail evaluating IP10 instead of IFN-γ in the same release assay in patient cohorts with active tuberculosis (collaboration with M. Ruhwald, Staten Serum Institute, Copenhagen, Denmark). In Tanzania, we conducted a retrospective analysis of the use of a computer-aided detection system for pulmonary tuberculosis in chest radiographs (collaboration with K. Reither, Basel, Switzerland and Bagamoyo, Tanzania).

Diagnosis of NTM-PD in adults is hampered by the difficulties to distinguish colonization and infection and requires the isolation of the same mycobacterium from respiratory secretions at two separate occasions. The need for a diagnostic method for measurements of specific T-cell immunity may be helpful in this regard. We therefore start to use an ELISPOT assay detecting T-cell response to specific *M. avium* proteins in both pediatric and adult cohorts of patients (collaboration with J. Dominguez, Barcelona, Spain).

Given the need to diagnose mycobacterial infections in patients with granulomatous diseases, a semiquantitative dot-blot array specific for several mycobacterial species was retrospectively evaluated in formalin-fixated paraffin-embedded tissue of patients with known tuberculous and NTM diseases (collaboration with Chipron, Berlin).

Given the need to distinguish tuberculous from non-tuberculous lymphadenitis in children we also pursue a diagnostic trial for the detection of specific antibodies for the diagnosis of NTM-lymphadenitis in children (collaboration with Kozo Morimoto, Japan, and J.v.Ingen, The Netherlands).

The CCI (PIs A. Nieters, P. Henneke) has also launched a nationwide multi-center study (NTM-Kids) including children with NTM-lymphadenitis and controls to study molecular-epidemiological interactions of host and pathogen, and (epi)genetic as well as environmental factors on the etiology and disease manifestations.

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CLINICAL IMMUNOLOGY

Common variable immunodeficiency (CVID): A human model of disturbed B cell memory response: From monogenic disease to complex immune dysregulation

KEYWORDS

Common variable immunodeficiency (CVID)
B cell differentiation
Immunological memory
Germinal center
Autoimmunity
Immune dysregulation

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Common variable immunodeficiency (CVID) is the most common form of primary immunodeficiency in humans. It was defined as an antibody deficiency syndrome with heterogeneous immune pathogenesis and variable clinical presentation. While some patients present only with an increased susceptibility towards bacterial infections, nearly half of the patients show additional signs of immune dysregulation as lymphoproliferation, autoimmunity and chronic organ inflammation. Currently, in <10% of CVID patients a monogenic cause has been identified. The majority of patients present as sporadic cases and a polygenic and multifactorial pathogenesis is assumed.
The research group „Clinical Immunology" therefore set out to

1) classify CVID patients based on immunological phenotypes into more homogeneous groups.
2) describe new monogenic defects associated with CVID.
3) dissect the pathogenesis of the CVID subgroup presenting with immune dysregulation (s. next chapter).
4) use CVID as perturbation model of the human humoral immune system in order to investigate late B cell differentiation in the germinal center response.
5) transfer our knowledge of primary immunodeficiency onto treatment associated secondary forms of immunodeficiency.

This research is integrated into cohort studies and clinical trials exploring the different clinical manifestations and their treatment options.

CLASSIFYING A HETEROGENEOUS DISEASE

Classification has been based on in-depth clinical and immunological characterization of patients. Phenotyping circulating B and T cells of CVID patients has demonstrated a good correlation with the clinical distinction of "infection only" patients and patients with signs of immune dysregulation. The "Freiburg" (and later EUROClass) classification has become a standard in the diagnostic evaluation of CVID patients. Over 80% of patients have a poor germinal center output of switched memory B cells and plasmablasts and about 20% show an expansion of an activated CD21^{low} B cell population strongly associated with a reduction of circulating naïve CD4 T cells and clinical signs of immune dysregulation.
In collaboration with the group of Stephan Ehl and international collaborators, criteria for adult patients with a late onset form of combined immunodeficiency (LOCID, Malphettes et al, CID 2009) are currently developed, since these will impact the treatment of patients.
Collaboration with several European centers

IDENTIFYING NEW MONOGENIC DEFECTS IN PATIENTS WITH HYPOGAMMAGLOBULINEMIA

Monogenic defects serve as pathogenic models for the heterogeneous pool of antibody deficiency. In collaboration with the group of Bodo Grimbacher we described a mutation in the Inducible CoStimulator (ICOS) as a prototype of antibody deficiency due to germinal center defect. Since then several monogenic defects have been identified interfering with the activation of B cells at different maturation stages. These monogenic defects not only help to define pathogenetically relevant phenotypes, but are of interest because the affected molecules present potential targets for immunosuppressive therapies in autoimmune disease. In recent years, in collaboration with P. Stepensky and O. Elpeleg (Hadassah University Jerusalem, Israel) we have investigated children of consanguineous decent with hypogammaglobulinemia. After the initial discovery of CARD11 deficiency, new genetic defects

have been identified and are under current investigation.
Collaboration with Polina Stepensky and Orly Elpeleg, Jerusalem, Israel; Bodo Grimbacher, Freiburg; Kaan Boztug, Vienna, Austria

USING CVID PATIENTS AS A MODEL FOR DISTURBED GERMINAL CENTER (GC) RESPONSE

The severely reduced output of post GC cells is a hallmark of CVID. While ICOS deficiency served as model for disturbed GC formation, most CVID patients with lymphadenopathy show a regular initiation of GC formation. A study on ten lymph nodes of CVID patients characterized the presence of ill-defined GCs and the absence of plasma cells as hallmarks of the disturbed histoarchitecture in CVID lymphadenopathy (Fig. 1). Further studies currently investigate the pathomechanism of the disturbed output.
Collaboration with Institute of Pathology, Freiburg

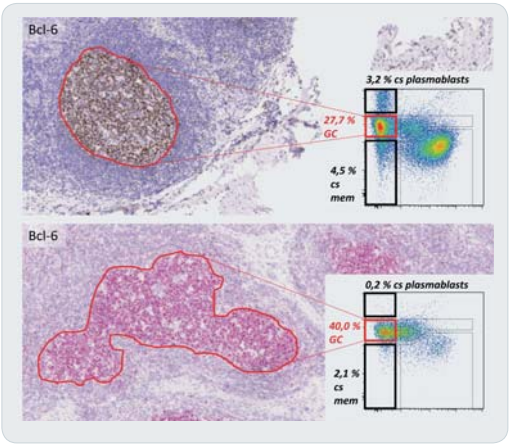

Fig. 1:
Histological sections and flowcytometric plots of lymph nodes of a healthy control (A) and a patient with CVID Freiburg Ia (B).
Germinal centers are marked by Bcl-6 stain in the immunohistochemistry. B cell populations of the lymph nodes are dissected according to their IgD (X-axis) and CD38 (Y-axis) expression.
Cs mem = class switched memory B cells, GC = GC B cells, cs plasmablasts = class switched plasmablasts

TRANSFERRING OUR KNOWLEDGE IN PRIMARY IMMUNODEFICIENCY ONTO TREATMENT ASSOCIATED ANTIBODY DEFICIENCY

With the development of an increasing number of aggressive and targeted therapies in the management of autoimmune disease or tumors the description and surveillance of secondary immunodeficiency becomes an important part in the long term care of treated patients. In collaboration with nephrologists, hematologists and rheumatologists we have investigated the impact of several forms of immunosuppression on the adaptive immune system. Currently, we have described the alteration of the immune system under high dose steroids, in organ transplantation and post stem cell transplantation.
Collaboration with Reinhard Marks/Jürgen Finke, Freiburg; Reinhard Voll, Freiburg; a.o.m.

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B CELL DYSREGULATION IN COMMON VARIABLE IMMUNODEFICIENCY – MORE THAN JUST A VICTIM

Common variable immunodeficiency (CVID) is defined by the reduction of at least two serum immunoglobulin isotypes, a poor specific antibody response and the exclusion of other differential diagnoses. Based on this definition CVID is a heterogeneous antibody deficiency syndrome including nearly 50% of patients presenting with immune dysregulation. This dysregulation manifests in autoimmunity, lymphoproliferation and chronic inflammation mainly of the lung, gut and liver. While patients presenting with "infection only" profit tremendously from immunoglobulin replacement therapy, patients with additional manifestations often require immunosuppressive therapy. In order to identify patients, to understand the underlying immune dysregulation and to develop better treatment we investigate the immune function on a molecular and cellular level in correlation to the clinical course in a well-defined CVID cohort.

SCIENTISTS

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DISSECTING THE CLINICAL PRESENTATION AND PATHOGENESIS OF CVID PATIENTS WITH IMMUNE DYSREGULATION

Nearly half of the CVID patients suffer from autoimmune phenomena, lymphoproliferation, lung, liver or gut inflammation. These patients present a therapeutic challenge since immunoglobulin replacement alone does not correct the secondary manifestations in most patients and a better comprehension of the pathogenesis and an early recognition of these patients is crucial for better management.

A multicenter survey has demonstrated the lack of sufficient clinical evidence in the application of immunosuppressive treatment for most secondary complications. Claudia Wehr and Marta Rizzi demonstrated a high mortality rate in a worldwide survey on stem cell transplantation in selected patients with CVID, indicating the need for better selection, timing and protocols of the procedure.

Worldwide collaboration with other immunodeficiency centers

ASSOCIATION OF CD21^{low} B CELLS WITH IMMUNE DYSREGULATION

The regulation of the homeostasis of the healthy peripheral immune system is age-dependent. The B cell homeostasis of an adult person is usually reached during the teenage years, especially characterized by the expansion of a switched memory B cell pool. In CVID the severe reduction of switched memory B cells is a very common finding. A subgroup of CVID patients is characterized by the additional expansion of an activated B cell population characterized by the absent expression of CD21 (Freiburg Ia, EUROClass 21low). This B cell phenotype is part of a more complex alteration of the immune system comprising a severe reduction of naïve CD4 T cells and regulatory T cells suggestive for a combined immunodeficiency in (at least) some of these patients. Clinically, CD21^{low} patients have an increased prevalence of splenomegaly, autoimmunity and granulomatous disease. Remarkably, this expanded CD21^{low} B cell population is also seen in patients with viremic HIV, hepatitis C and systemic autoimmune diseases.

The analysis of different compartments revealed a strong expansion of CD21^{low} B cells at peripheral inflammatory sites like the bronchoalveolar space in CVID patients with interstitial lung disease and synovial space in patients with rheumatoid arthritis while they were rare in secondary lymphoid tissues (Fig. 1).

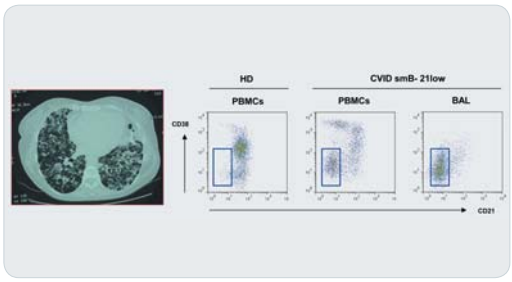

Fig. 1:
CD21^{low} B cells (blue gate) are enriched in the bronchoalveolar space of CVID patients with interstitial lung disease.

CD21^{low} B cells are functionally impaired. While BCR stimulation *in vitro* did not lead to cell cycle entry of CD21^{low} B cells, KREC analysis of these cells demonstrated a previous history of increased proliferation *in vivo*. Despite similarities to anergic B cells, CD21^{low} B cells express higher levels of CD86 and can still produce antibodies after *in vitro* stimulation. Therefore, given the preserved antibody production *in vitro* and the potential co-stimulatory function of these cells, the high percentage of auto-/poly reactive clones among CD21^{low} B cells represents a relevant autoimmune threat. The effect of this B cell population on the local inflammation *in vivo,* however, remains to be determined.

Collaboration with Mirjam van de Burg, Rotterdam; Eric Oksenhendler, Paris and the EUROClass group

CD21^{low} B CELLS ALLOW IN-SIGHT INTO CELL INTRINSIC MECHANISMS OF TOLERANCE

While certain effector functions of B cells are still preserved especially the proliferative expansion of CD21^{low} B cells is clearly abrogated. This defect is associated with a decreased store operated Ca^{2+}

entry (SOCE) after BCR activation, but increased PLCγ2 phosphorylation and the Ca^{2+} store release upstream of the SOCE. Several inhibitory receptors are overexpressed on the surface of $CD21^{low}$ B cells and have been suggested to be involved in the reduced Ca^{2+} response, but the exact mechanisms of the regulation of chronically activated B cells at this level remains to be elucidated. Currently, we are investigating the signaling network and transcriptome in $CD21^{low}$ B cells in comparison to naïve B cells of the same patient and healthy controls (Fig. 2).

Collaboration with Stephan Feske, New York; Michael Reth, Freiburg, Wolfgang Schamel, Freiburg, Kaan Boztug, Wien

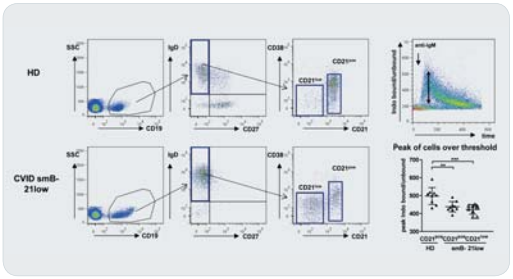

Fig. 2:
Impaired Ca^{2+} mobilisation in B cell subpopulations of CVID patient of the EUROClass smB-21^{low}
After gating on CD19+ B cells (left) and then on naive $CD21^{pos}$ and $CD21^{low}$ B cells (second from right) intracellular calcium flux was determined by flowcytometric detection of the ratio of bound to unbound INDO1 (right top) after stimulation with anti IgM. The peak response is indicated by the arrow. B cells of CVID smB-21^{low} patients had significantly lower Ca^{2+} flux after IgM stimulation than control (HD) B cells (right bottom).

THE STUDY OF INTERSTITIAL LUNG DISEASE IN PRIMARY ANTIBODY DEFICIENCY (STILPAD)

Interstitial lung disease (ILD) is associated with a poor prognosis in CVID. Usually, the regular immunoglobulin replacement does not affect the progressive loss of lung function. Therefore, immunosuppressive therapies are often initiated without current evidence of benefit of the various forms of treatment. In STILPAD a total of 149 patients have been recruited in an international multicenter observational trial. Currently, the retrospective arm of the study is analyzed for the different forms of interstitial lung disease, the natural course of changes in lung structure and function, the indication and different forms of treatment and their effect on lung disease. These data will be extended into a five-year prospective phase, when additional peripheral blood and bronchoalveolar parameters are collected in order to gain insight into potential immune mechanisms and define biomarkers for progressive lung disease. Interestingly, unlike sarcoidosis B cells are often found in the bronchoalveolar space of CVID with ILD. More than 80% of these cells are $CD21^{low}$ B cells. The recent evidence for B cell depletion (Rituximab) as a successful treatment strategy underlines a potentially pathogenic role of these cells.

The data gained by this observational trial will provide the basis for an interventional trial necessary to finally address the management of one of the major morbidities in CVID (Fig. 3).

Collaboration with 14 European immunodeficiency centers; H. Tiddens, Rotterdam, I. Lipkins, New York

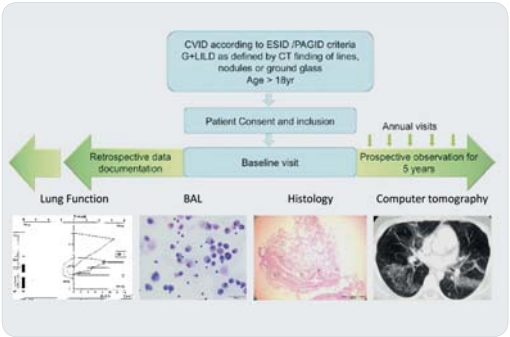

Fig.3:
Study design of the observational study on interstitial lung disease in CVID (STILPAD). Lung pathology will be monitored by the indicated methods over a period of five years.

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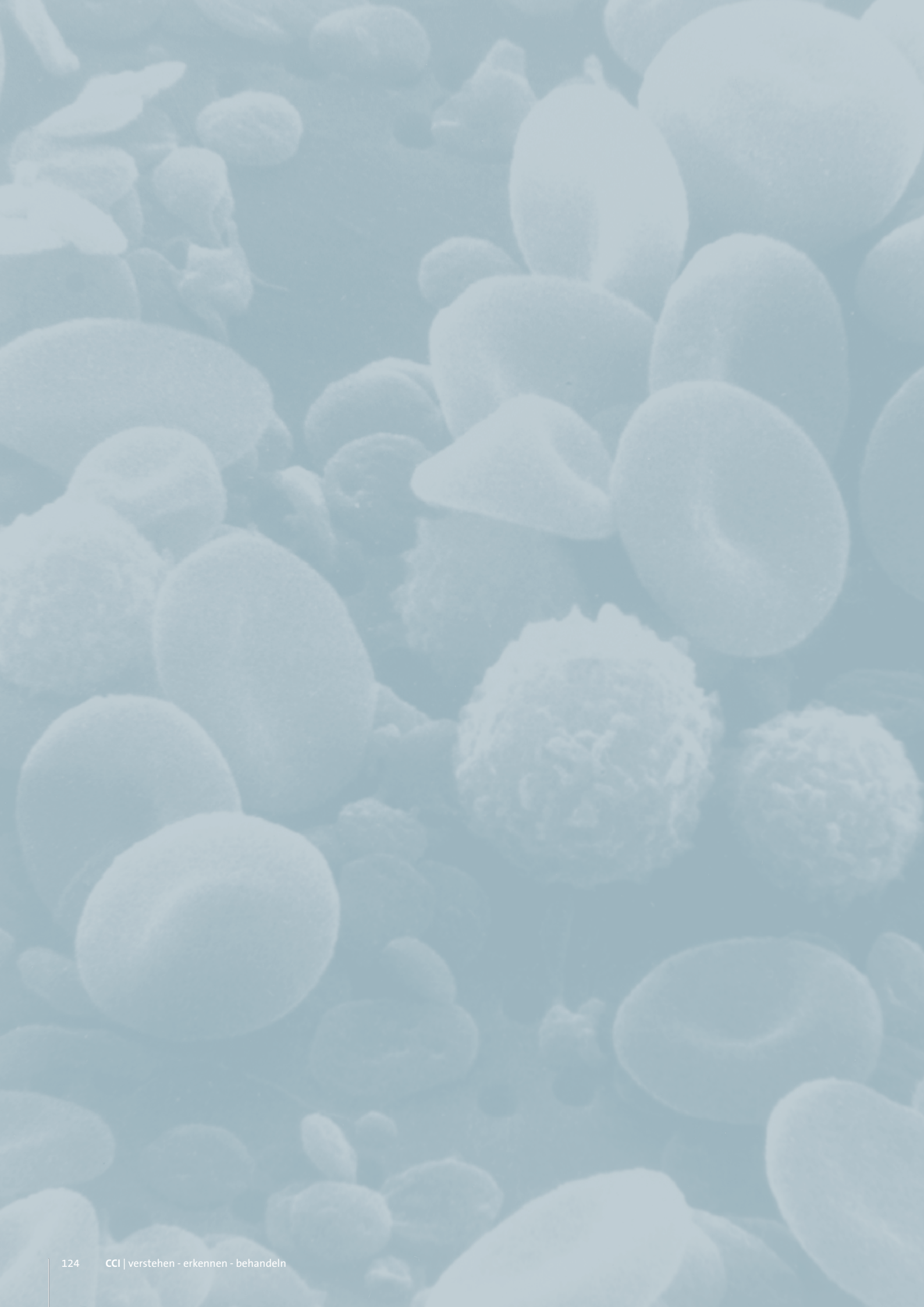

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