Improving treatment of infectious diseases

The Infection Biology research group explores host- and pathogen-specific aspects of infectious diseases in a translational setting together with the Division of Infectious Diseases and Hospital Epidemiology, the Laboratory of Clinical Microbiology, the Division of Hematology at the University Hospital Basel, the Biozentrum, ETH Zürich and Basel and the University of Fribourg. Our laboratory has two main topics: First, we aim at understanding and improving the host immune response towards viruses and fungi in immunocompromised patients. This also includes vaccine responses [1-5]. The second goal is to explore novel anti-infective approaches against foreign-body/implant-associated infections caused by staphylococci [6,7].

In this report, we focus on infections in immunocompromised patients, the host immune response to fungal infections, as well as pre-clinical and clinical studies of adoptive T-cell therapy for fungal and viral infections.

Infections in immunocompromised patients are common

Chemotherapies to treat leukemia and solid tumors and immunosuppressive treatments to reduce graft versus host diseases (GVHD) and rejection in transplant recipients have overall increased the short and mid-term survival of these patients. However, the loss of pathogen-specific immunity associated with these treatments increases the risk for infectious complications. Invasive fungal and viral infections belong to the most serious complications in these patients and are still associated with an exuberant mortality. Although antifungal and antiviral drugs are available for some infections, their therapeutic efficacy is often limited and depends on several factors including the immune status of the host and the extent of infection at the time of diagnosis. Due to the disease severity in these patients and the problem of accurate diagnosis, efforts have been made to implement prophylactic and preemptive drugs. Application of these drugs is however associated with toxicity, high

Foto: From left to right, front row: Claudia Bernardini, Anne-Kathrin Woischnig, Nina Khanna; middle row: Adrian Egli, Claudia Stühler, Justyna Nowakowska; back row: Richard Kühl, Fabrizia Ferracin, Pascal Forrer.
Missing people are David Burckhardt and Mohammedyaseen Syedbasha.

costs and the emergence of resistant or less susceptible pathogens. Therefore, efforts to improve diagnosis, development of novel immunological biomarkers to guide treatment duration and to decrease host immunosuppression while enhancing the reconstitution of immune defenses are crucial. Immunotherapeutic strategies such as adoptive transfer of virus- and fungus-specific T-cells could boost long-term immunity and thereby reduce toxicity and costs induced by preemptive and prophylactic drug regimens.

Opportunistic fungal infections are associated with high mortality

The most common opportunistic fungal pathogens causing disease in immunocompromised patients are the yeasts *Candida species* (spp.) and the molds *Aspergillus spp.* [2,8]. *Aspergillus fumigatus* is a saprophytic, filamentous mold that is mainly found in the soil. In humans it predominantly affects the lungs and is characterised by hyphal invasion and destruction of pulmonary tissue (Figure 1). In patients undergoing myeloablative chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT), the incidence of pulmonary invasive aspergillosis is between 10 and 20% and is associated with an attributable mortality of 20-50%. In recent years, due to the wide use of antifungal prophylaxis, rare molds such as *Fusarium spp.* and *Mucorales spp.* have emerged[8].

Host immune responses to fungi are not well understood

Neutrophils belong to the first line of defence and are key players to control fungal infections. In many patients at risk for fungal infection the total number of neutrophils is in a normal range, but little is known about the effector functions including migration, oxidative burst and degranulation. Moreover, the interaction with lymphocytes remains unclear. Distinct fungus-specific $CD4^+$ T-helper (T_H) subsets such as T_H1 and probably T_H17 cells, $CD8^+$ T cells and natural killer (NK) cells are important for pathogen control in mice, whereas activation of T_H2 cells often exacerbates disease (Figure 2)[2].

T_H1 cytokines interferon-gamma (IFN-γ) and/or GM-CSF are able to enhance the oxidative burst of neutrophils in response to fungi as well as increase the hyphal damage. We are currently establishing the mechanism by which T_H1 cytokines are able to boost the effector functions of neutrophils and are elucidating specific signaling pathways of these cytokines in neutrophils and their role in apoptosis.

As the recovery of the antifungal immunity in patients after HSCT is largely unknown, we have studied the quantitative and qualitative impairments in these patients.

Antifungal immune response is impaired after HSCT

Within a prospective study from 2011-2013 at the stem cell transplant unit at the University Hospital of Basel, we investigated the immune reconstitution of neutrophils, NK cells and T lymphocytes in 60 patients after HSCT with or without invasive aspergillosis over one year.

Overall post-transplant patients developing fungal in-

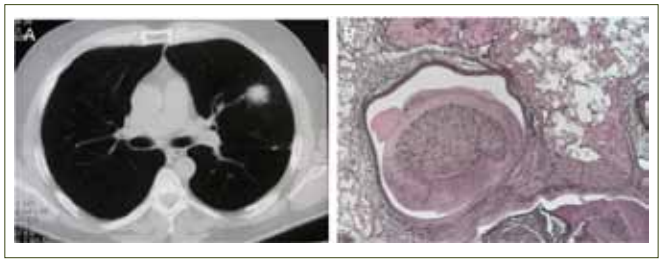

Figure 1. (A) Computed tomography of the chest with invasive pulmonary aspergillosis (Radiology, University Hospital of Basel). (B) Histological sections of invasive pulmonary aspergillosis stained with Grocott with a magnification of 2,5x (Pathology, University Hospital of Basel).

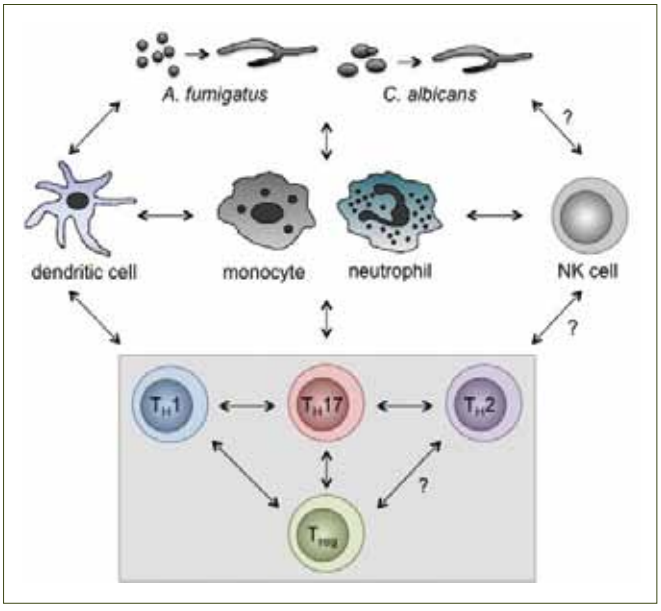

Figure 2. Crosstalk of the key players in anti-fungal immunity

fections showed low lymphocyte and NK cell counts whereas the numbers of neutrophils remained normal. Interestingly, the production of reactive oxygen species of the neutrophils was comparable to patients without fungal infections and to healthy individuals. In contrast, fungus-specific T-cell immunity showed significantly lower IFN-γ responses and almost no detectable IL-17 production up to 12 months post-transplantation when compared to healthy donors, indicating a dominant impairment of adaptive immunity after HSCT. These findings support the generation of T-cell therapy to boost long-term immunity and to control the devastating fungal infections.

T-cell Immunotherapy for viral and fungal infections

Previous studies have demonstrated that virus-specific T-cells are efficacious and safe with respect to prevention of GVHD [9] (Figure 3) but limited data for antifungal T-cell transfer are available in humans.

The generation of virus-specific T-cells is currently limited due to its elaborate production requiring *in-vitro* expansion over at least 14 days under good manufacturing practice (GMP) conditions. Therefore, more rapid approaches without the need of long-term *in vitro* expansion would be desirable. The selection of IFN-γ-producing T cells following stimulation with viral antigens by the GMP-approved Miltenyi® IFN-γ Capture System for direct infusion into patients is rapid (< 48 hours) and promising for cytomegalovirus (CMV), adenovirus and Epstein-Barr virus (EBV) [10]. The IFN-γ Capture System is however restricted to antigens with moderate to high memory T-cell frequencies in peripheral blood and can therefore not be used for the isolation of fungus-specific T cells. To increase the sensitivity for isolation of these rare pathogen-specific memory cells, other T-cell activation markers may be more suitable which enable capture of a greater number of antigen-specific T cells irrespective of cytokine production. Different T-cell surface molecules that are selectively expressed or strongly upregulated after T-cell activation such as CD25, CD69, CD71, CD134, CD137 and CD154 could be similarly useful for selection of antigen-specific T cells (Figure 4). CD154 and CD137 for example are transiently expressed on activated $CD4^+$ and $CD8^+$ T cells following antigen stimulation. We recently were able to show that CD154 is a promising candidate for selection of pathogen-specific T cells due to its high specificity and sensitivity[1].

We are currently performing pre-clinical adoptive T-cell transfer studies for EBV using the IFN-γ Capture System and are comparing different isolation methods for fungal pathogens. Furthermore, we are establishing a clinical protocol and have already used the CliniMACS® Cytokine Capture System in two patients suffering from treatment-refractory CMV infection at the University Hospital of Basel.

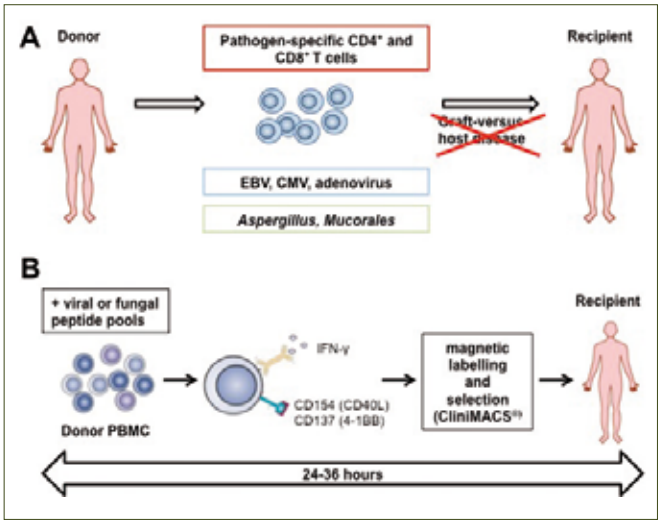

Figure 3. Adoptive T-cell transfer for viral and fungal infections. (A) Pathogen-specific T cells reduce the risk of graft-versus-host disease. (B) Pathogen-specific T cells can be selected based on IFN-γ secretion or activation-dependent expression of CD154 and CD137 within 36 hours for direct infusion into recipients.

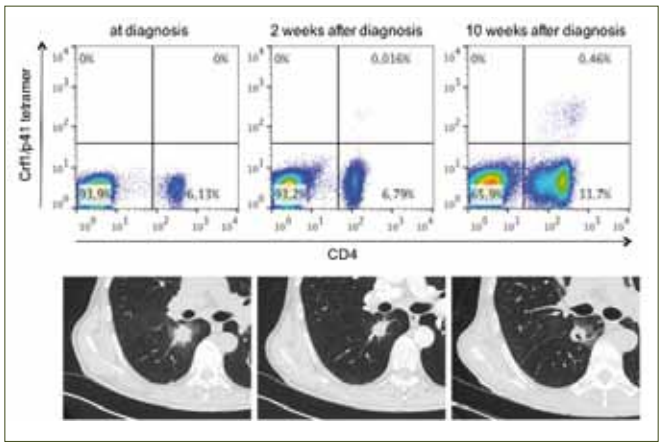

Figure 4. Peripheral blood mononuclear cells of a HLA-DRB1*04-positive HSCT recipient at different time points after diagnosis of IA were pre-stimulated with A. fumigatus Crf1/p41 peptide for 7 days and the frequency of Crf1/p41-specific T cells determined by MHC class II tetramer staining. Computed tomography of the chest at the respective time points is shown in the panels below.

Pre-clinical studies

T-cells specific for fifteen EBV proteins for clinical application

EBV-associated post-transplant lymphoproliferative disorders (PTLDs) belong to the most serious complications of immunosuppression occurring in approximately 5% of all HSCT recipients. In these patients the immunosuppression reduces the number and/or the function of the EBV-specific T cells leading to uncontrolled proliferation of EBV-infected B cells and tumor formation. Thus, reconstitution of antigen-specific T cells has the potency to provide immediate and also long-term protection against PTLDs. Since the T-cell response to EBV is directed against a set of viral proteins and strongly depends on the human leukocyte antigen (HLA) types, determination of protective EBV-derived antigens poses a great challenge. We therefore aimed at identifying immunogenic antigens most suitable for generation of a T-cell product using the CliniMACS® Cytokine Capture System. We are especially interested in designing an EBV-specific T-cell product consisting of both $CD4^+$ and $CD8^+$ cells covering a broad range of HLA types and in investigating its role in controlling PTLDs.

We demonstrated that a mix of $CD8^+$ and $CD4^+$ T-cell epitopes with known HLA restrictions derived from 15 EBV latent and lytic proteins induced significantly higher T-cell responses and allowed better IFN-γ-based selection than peptide pools of single EBV proteins including EBV nuclear antigen (EBNA)1, EBNA3c, latent membrane protein (LMP)2a and BZLF1 – all known to be expressed in PTLDs. Additionally, the EBV_{mix}-specific T cells recognized endogenously processed viral antigens, were devoid of alloreactive potential and controlled better the EBV-infected B cells in vitro. These findings indicate a clear advantage of combining defined T-cell epitopes derived from different EBV proteins over using single viral antigens for the generation of EBV-specific T cells for adoptive transfer.

Aspergillus-specific T cells for immunotherapy

As T cells, in particular $CD4^+$ T_H1 cells, appear to control invasive fungal infections in humans and mice, induction of fungus-specific $CD4^+$ T_H1 immunity is an appealing strategy to combat these infections. However, immunotherapeutic strategies are so far limited due to lack of antigens inducing protective T-cell responses in HSCT recipients and targeting a broad spectrum of pathogenic fungi and their elaborate production.

We identified three *A. fumigatus* proteins Crf1, Gel1 and Pmp20 that are strongly inducing T_H1 responses in healthy individuals and HSCT recipients. T cells specific for these antigens expanded in patients with well-controlled invasive aspergillosis after HSCT and this corresponded with a reduction of the fungal lesion in computed tomography indicating that these cells contribute to control the infection (Figure 5). We were also able to demonstrate that T_H1 cells specific for the three proteins can be selected within 24 hours based on activation-dependent expression of CD137 or CD154 with similar efficiency and specificity. These cells recognize endogenously processed *A. fumigatus* and the multi-specific T-cell lines, especially those selected by CD154, additionally cross-react to different *Aspergillus* and *Mucorales* spp.

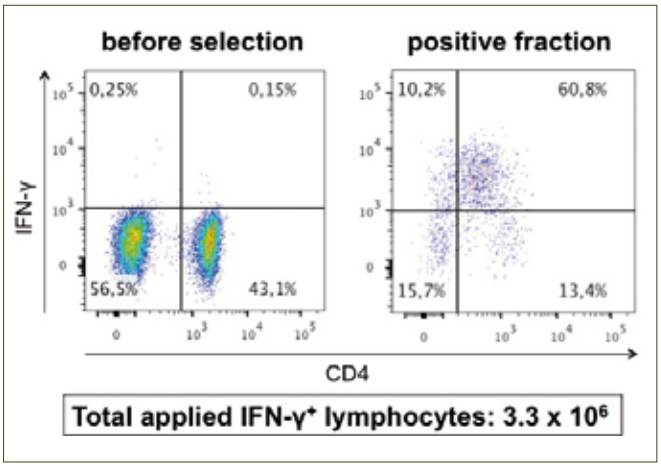

Figure 5. (A) IFN-γ positive enrichment for CMV pp65. IFN-γ positivity before selection (left). Positive lymphocyte fraction shows 71% purity ($CD4^+$ and $CD8^+$) after stimulation with CMV pp65 peptide pool (right). This experiment has been performed with $1x10^9$ PBMC of a CMV^+ leukapheresis of a healthy stem cell donor for the treatment of a patient with multi-resistant treatment refractory CMV retinitis at the Diagnostic Hematology at the University Hospital of Basel after approval by the Ethical Committee EKNZ (10/2014 "Zulassung für eine Einzelbehandlung"). Total cell yield was 3.3×10^6.

First patient experience using CMV-specific T cells to treat multi-resistant CMV infection and retinitis

In October 2013 we treated a 52-year-old CMV-seropositive patient that suffered after allogeneic HSCT from a multi-resistant CMV infection with retinitis with CMV-

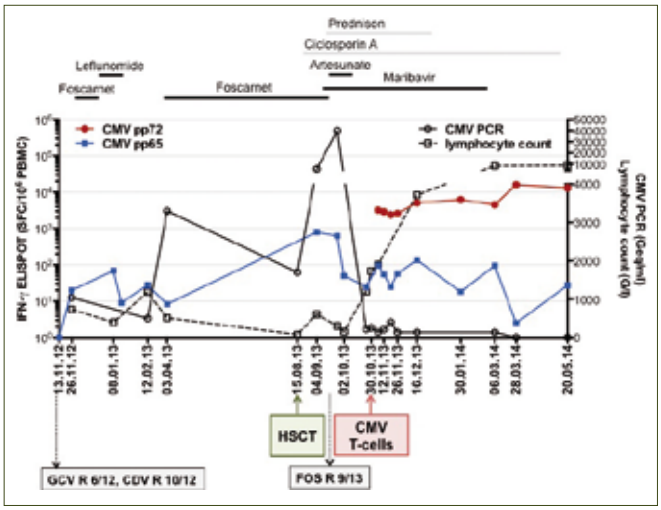

Figure 5. (B) Course of the patient

specific T cells. Using the CliniMACS® Cytokine Capture System after stimulation with CMV pp65 peptide pool 3.3 x 10^6 total $CD3^+$ cells with a purity of 70% of IFN-γ^+ $CD3^+$ were selected and applied to the patient.

The follow-up was favorable: the patient recovered from the CMV retinitis and cleared the virus in peripheral blood two months after adoptive T-cell transfer and concomitantly CMV pp65-specific but also pp72-specific T cells expanded in peripheral blood. Antiviral drug treatment with maribavir could be stopped after 3 months and no relapse occurred. This case illustrates for the first time in Switzerland that adoptive T-cell therapy for a viral infection is safe and feasible using the CliniMACS® Cytokine Capture System and might also lead to the control of the viral infection. A phase I/II study in recipients of HSCT with treatment refractory post-transplant viral infections to foster adoptive T-cell transfer is currently planned.

Nina Khanna and team

Literature

1 Khanna, N. *et al.* Generation of a multipathogen-specific T-cell product for adoptive immunotherapy based on activation-dependent expression of CD154. *Blood* **118**, 1121-1131, doi:10.1182/blood-2010-12-322610 (2011).

2 Stuehler, C. *et al.* Cross-protective TH1 immunity against Aspergillus fumigatus and Candida albicans. *Blood* **117**, 5881-5891, doi:10.1182/blood-2010-12-325084 (2011).

3 Egli, A. *et al.* Immunomodulatory Function of Interleukin 28B During Primary Infection With Cytomegalovirus. *J Infect Dis*, doi:10.1093/infdis/jiu144 (2014).

4 Egli, A. *et al.* Vaccine adjuvants--understanding molecular mechanisms to improve vaccines. *Swiss Med Wkly* **144**, w13940, doi:10.4414/smw.2014.13940 (2014).

5 O'Shea, D., Widmer, L. A., Stelling, J. & Egli, A. Changing face of vaccination in immunocompromised hosts. *Current infectious disease reports* **16**, 420, doi:10.1007/s11908-014-0420-2 (2014).

6 Nowakowska, J., Griesser, H. J., Textor, M., Landmann, R. & Khanna, N. Antimicrobial properties of 8-hydroxyserrulat-14-en-19-oic acid for treatment of implant-associated infections. *Antimicrob Agents Chemother* **57**, 333-342, doi:10.1128/AAC.01735-12 (2013).

7 John, A. K., Schmaler, M., Khanna, N. & Landmann, R. Reversible daptomycin tolerance of adherent staphylococci in an implant infection model. *Antimicrob Agents Chemother* **55**, 3510-3516, doi:10.1128/AAC.00172-11 (2011).

8 Marr, K. A. Fungal infections in hematopoietic stem cell transplant recipients. *Med Mycol* **46**, 293-302, doi:791304420 [pii] 10.1080/13693780701885552 (2008).

9 Leen, A. M., Tripic, T. & Rooney, C. M. Challenges of T cell therapies for virus-associated diseases after hematopoietic stem cell transplantation. *Expert Opin Biol Ther* **10**, 337-351, doi:10.1517/14712590903456003.

10 Feuchtinger, T. et al. Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. *Blood* **116**, 4360-4367, doi:blood-2010-01-262089 [pii] 10.1182/blood-2010-01-262089 (2010).