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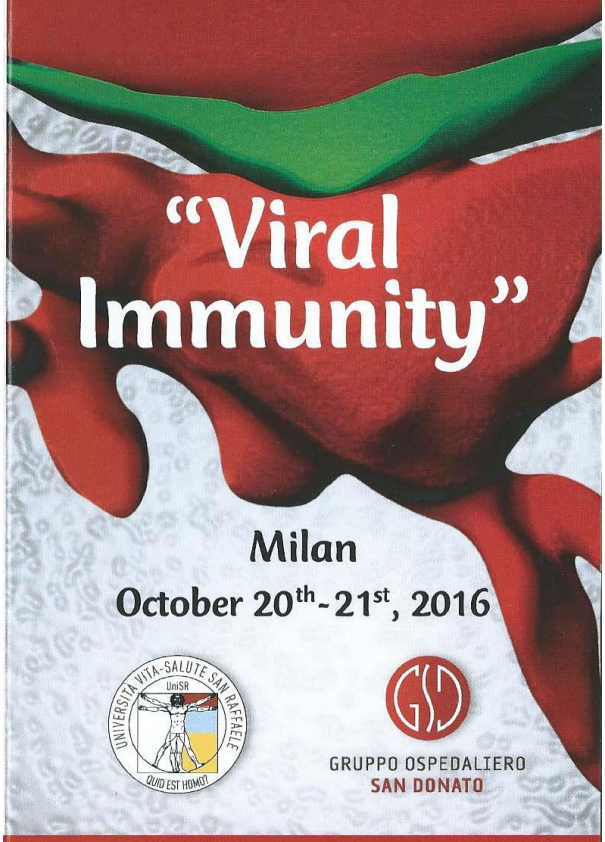

"Viral Immunity"

Milan
October 20th-21st, 2016

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Analysis of genetic determinants of healthy immune responses to common pathogens reveals dose-dependent associations with *HLA-DRB01*04* allele

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Healthy humans are regularly infected with pathogens that have the ability to persist through life after the acute phase. Although occurrence of infection is dependent on environmental factors influencing exposure to the microbe, our hypothesis is that host factors influence (i) the rate of seroconversion upon exposure, (ii) the intensity and characteristics of pathogen-specific as well as bystander immune responses. To address this question, we used a systems biology approach, integrating serological testing, genome-wide association studies (GWAS) and extensive immunophenotyping in a well-characterized population of 1000 healthy individuals recruited by the *Milieu Interieur* Consortium. Blood cell composition was assessed on whole blood by flow cytometry. Serums were used for qualitative (seropositive, seronegative) and quantitative assessment of IgG responses against (i) persistent or recurrent viruses - Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Herpes simplex virus 1 & 2 (HSV-1 & 2), Varicella zoster virus (VZV), and Influenza A virus; (ii) persistent bacteria - *Helicobacter pylori* (HP), (iii) persistent parasite - *Toxoplasma gondii*, and (iv) pathogens targeted by vaccine campaigns in France - Measles, Mumps, Rubella, and Hepatitis B virus. Illumina Omni Express and HumanExome arrays were used for genotyping. After imputation with IMPUTE2, logistic or linear regressions were performed to detect associations between 5 million human polymorphisms, immune phenotypes and antibody responses. No genome-wide significant associations were found for serostatus. In contrast, genome-wide significant associations ($P < 5 * 10^{-8}$) were observed within the MHC locus on chromosome 6 for the levels of IgG mounted against EBV and Rubella. By imputing classical HLA alleles and amino acids, we found that these associations correspond to variations in amino acid composition of HLA-DRβ1 and HLA-DPβ1 molecules respectively. In parallel, multiple regression approaches revealed a significant impact of CMV, EBV and HP on baseline immune system, in particular on T cell differentiation. By integrating phenotypic and genetic data, we uncovered previously unrecognized dose dependent associations between the *HLA-DRB1*04* allele and baseline immune responses. Together, our results provide new possible insights into mechanisms determining response to persistent pathogens, and encourage further genetic and functional work.

Regulation of T-cell response via modulation of NK cell function

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CD8+ T cells are central effectors of the adaptive immune response that serve the elimination of virus-infected cells. Fast-replicating virus strains or high viral burden are often associated with defective CD8+ T cell function, a condition that favors the establishment of chronic infection. Recently, natural killer (NK) cells have been shown to control CD8+ T cell responses at an early stage of infection, thereby contributing to virus persistence. While perforin plays a role in this process, it is not knows whether other apoptotic mediators may also contribute to this NK-cell dependent regulation of the CD8+ T cell response.
Using a model of hepatitis based on infection with lymphocytic choriomeningitis virus (LCMV), we found a role for *Tnfsf10/Trail* in modulating the immune response and the subsequent immunopathology. *Trail*-deficient mice showed higher LCMV-specific CD8+ T cell response than wild type animals, resulting in better virus clearance and reduced liver pathology. This was strictly dependent on NK cells, as indicated by depletion studies. Further investigation disclosed a *Trail*-mediated mechanism controlling cytokine and cytotoxic granule content production in NK cells following LCMV infection. Thus, reduced levels of granzyme B in NK cells likely explain the impaired NK cell-dependent restriction of the T cell response in LCMV-infected *Trail*$^{-/-}$ animals.
Taken together, our data reveal an unpredicted function of *Trail* in determining NK cell activation independently of apoptosis signaling. They also show the relevance of this novel regulatory mechanism for the immunomodulation of T cell-mediated liver disease.

Innocent Bystanders: How persistent viral infections modulate T cell homeostasis

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Immune responsiveness of a host towards microbial challenges or vaccines is given by the various constituents of the immune system which are continuously modulated by the previous infection/vaccination history of an individual, the constant encounter with commensal microorganisms at mucosal surfaces (the "microbiome") and the exposure of the immune system to persistent viral infections (the "virome"). Persistent viral infections are wide-spread in the human population with estimated 8-12 persistent viral infections per individual. Yet, in contrast to the "microbiome", there is currently limited knowledge on how these persistent infections impinge on immune homeostasis or immune responsiveness.

The aim of this project is to address the influence of two well-defined persistent viral infections in the mouse, Lymphocytic Choriomeningitis virus (LCMV) and murine cytomegalovirus (MCMV) on the long-term composition, phenotype and function of diverse innate and adaptive immune cells and how such alterations affect vaccine efficacy, susceptibility to infection by heterologous pathogens, predisposition to autoimmunity and immunological ageing.

We specifically studied the impact of these persistent infections on the phenotype and function of naïve or memory bystander T cells. Chronic LCMV infection, and to a lesser extent MCMV infection, had a profound impact on the maintenance, phenotype and function of bystander T cells which was not caused by Type I interferon signaling. There is a link between phenotypic changes, pSTAT1 signaling and prolonged viremia, which is mostly pronounced upon LCMV infection. We are currently delineating the mechanisms leading to these numerical, phenotypic and functional alterations and will assess their physiological consequences.

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Molecular determinants of hepatic effector CD8+ T cell intrasinusoidal crawling

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The liver is a vital organ in which CD8+ effector T cells (CD8 TE) determine pathogenesis and outcome of Hepatitis B virus (HBV) infection. Our group recently coupled advanced imaging and dedicated mouse models of HBV infection to show that after intrasinusoidal arrest circulating CD8 TE aimed at viral clearance crawl and probe underlying hepatocytes for presence of antigen (Ag) by extending filopodia-like protrusions through sinusoidal fenestrae. Both Ag-specific and non-specific CD8 TE actively crawl irrespective of bloodstream direction at a speed that is 500- to 1000-fold slower than that of sinusoidal blood flow. The molecular determinants used by hepatic CD8 TE to crawl in search of Ag are unknown. Herein, we used multiphoton intravital microscopy (IVM) coupled with blocking antibodies and pharmacological reagents to screen for molecules that control hepatic CD8 TE crawling. Our preliminary results indicate that blockade of integrin LFA-1 or VLA-4, as well as global inhibition of G protein-coupled chemokine receptors did not impact hepatic CD8 TE dynamic behavior. By contrast, CD8 TE crawling was halted in mice treated with hyaluronidase, an enzyme that cleaves hyaluronic acid off liver sinusoids. Whereas the hyaluronic acid receptor CD44 is not involved in this process we are currently testing another receptor called RHAMM also expressed by CD8 TE. We believe that findings emerging from this work will advance our knowledge on how adaptive immunity mediate viral clearance and liver pathology and may help in the design of rational immunotherapeutic strategies aimed at terminating chronic HBV infection.

Viral Immunity, Milano, 20-21 October 2016

Asymmetric cell division modulation impacts T cell fate determination.

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Activation of T lymphocytes leads to polarization of molecules such as TCRs, integrins and cytokine receptors towards the immunological synapse. This polarization leads to asymmetric distribution of proteins that is maintained post mitosis of activated T cells, resulting in two daughter cells with different phenotypes and fate determinations. While the proximal daughter adopts an effector cell fate, the distal daughter is destined to become a memory cell. We hypothesize that the capacity of T cells to divide asymmetrically depends on their differentiation stage, with effector cells potentially showing compromised asymmetric cell division potential compared to naïve or memory cells, and that interfering in asymmetry rates during mitosis would have an impact on the differentiation fate of these cells. To address this hypothesis we developed an efficient method to induce asymmetric cell division (ACD) through the co-culture of TCR transgenic CD8$^+$ T cells and dendritic cells previously loaded with the respective peptide antigen for which the T cells are specific. Alternatively, we also stimulated T cells in anti-CD3, anti-CD28, Fc-ICAM coated plates. Using these methods, CD8$^+$ T cells in various differentiation stages were assessed for their ability to asymmetrically segregate specific surface and cytoplasmic markers by confocal microscopy. Specifically, we compared the extent of asymmetric cell division upon activation of naïve, effector, memory or exhausted CD8$^+$ T cells isolated after acute (effector and memory) or chronic (exhausted) LCMV infection. Our data confirmed previous reports showing that naïve and memory T cells undergo asymmetric distribution of several markers upon stimulation or re-challenge. In contrast, short-lived effector cells isolated at the peak of an acute LCMV infection and exhausted CD8$^+$ T cells isolated from chronically infected mice largely lost their capacity to divide asymmetrically. These data suggest that "stem" cells endowed with the ability to undergo clonal (re)expansion (naïve and memory cells) show higher asymmetric cell division rates in comparison to more terminally differentiated effector or exhausted/senescent cells that are unable to form a memory reservoir. Interestingly, transient rapamycin treatment (inhibition of the mTOR pathway) increased asymmetry rates in all the tested experimental conditions and specifically recovered - to some extent - asymmetry in exhausted cells. Furthermore, exhausted cells treated with rapamycin showed better re-expansion capacity when transferred to new hosts and submitted to viral rechallenge. This suggests that interfering in ACD rates can possibly lead to an improvement in long-term survival and function of T cells during chronic infection and memory formation in vaccination setups. Ongoing studies investigate the molecular mechanisms responsible for the establishment and maintenance of the asymmetry of surface and cytoplasmic molecules during mitosis.

Key words: *asymmetric cell division, polarization, T cell fate*

Interferon-driven deletion of antiviral B cells at the onset of chronic infection

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Inadequate antibody responses and perturbed B cell compartments represent hallmarks of persistent microbial infections, but the mechanisms whereby persisting pathogens suppress humoral immunity remain poorly defined. Using adoptive transfer experiments in the context of a chronic lymphocytic choriomeningitis virus (LCMV) infection of mice, we have documented rapid depletion of virus-specific B cells that coincided with the early type I interferon response to infection. We found that the loss of activated B cells was driven by type I interferon (IFN-I) signaling to several cell types including dendritic cells, T cells and myeloid cells. Intriguingly, this process was independent of B cell-intrinsic IFN-I sensing and resulted from biased differentiation of naïve B cells into short-lived antibody-secreting cells. The ability to generate robust B cell responses was restored upon IFN-I receptor blockade or, partially, when experimentally depleting myeloid cells or the IFN-I-induced cytokines interleukin 10 and tumor necrosis factor alpha. We have termed this IFN-I-driven depletion of B cells "B cell decimation". Strategies to counter "B cell decimation" should thus help us better leverage humoral immunity in the combat against persistent microbial diseases.

Human cytomegalovirus (HCMV)-specific memory T-cell response and HCMV transmission to the fetus in pregnant women with primary HCMV infection

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Background: HCMV primary infection during pregnancy is the major cause of congenital infection, resulting in 20% of cases in sensory and developmental impairment. HCMV-specific T-cell response has a role in the control of infection and in the prevention of virus transmission to the fetus.

Patients and Methods: HCMV-specific T-cells were investigated in 40 pregnant women (12 of whom transmitted the infection to the fetus) at a median time of 45 (range 26-66) days after onset of primary HCMV infection. For comparison, 8 pregnant women with remote infection were enrolled. To detect HCMV-specific expandable T-cells, PBMC were stimulated for 10 days with overlapping 15-mer peptide pools of HCMV proteins immediate early (IE)-1, IE-2 and phosphoprotein (pp)-65, and subsequently re-stimulated with the corresponding peptide in a cultured-ELISPOT assay. Results were expressed multiplying the number of spots/million cells for the proliferation index (number of antigen-stimulated cells divided by the number of culture medium-stimulated cells). Cytokine flow cytometry was also performed to investigate IFN-γ production by $CD4^+$ and $CD8^+$ memory T-cells.

Results: While in remote infections no significant difference was observed among T-cell response to pp-65, IE-1 and IE-2, 1-2 months after onset of primary infection, pp65-specific T-cell response was significantly higher than IE-1- and IE-2-specific T-cell response ($p<0.05$). However, a significantly lower response was observed in primary infection with respect to remote infection for all the three proteins examined. Flow cytometry analysis showed that, in primary infection, HCMV-specific expandable T-cells directed to pp65 and, when detectable, to IE-1, were $CD4^+$. Strikingly, response to pp65 was significantly lower ($p<0.01$) in women transmitting the infection to the fetus. A cultured ELISPOT response ≤ 50 is associated with an odds ratio of 6.44 (95% CI 1.44 to 28.90), for virus transmission to the fetus.

Conclusions: Determination of pp65-specific expandable memory T-cells by cultured ELISPOT in pregnant women with primary HCMV infection is a promising tool to assess the risk of HCMV transmission to the fetus.

Protein tyrosine phosphatase PTPN22 controls T cell immune responses and drives viral persistence

Running title: PTPN22 in chronic viral infection

Authors

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Abstract

Protein tyrosine phosphatase PTPN22 is expressed exclusively by hematopoietic cells, and plays key roles in innate and adaptive immune responses. PTPN22 on one hand regulates positively the activation of the innate immune system whereas on the other, it inhibits T cell responses. PTPN22 is associated with a broad range of autoimmune diseases such as type 1 diabetes and rheumatoid arthritis. Studies performed in murine models have underscored the role of PTPN22 in immune regulation and its involvement in autoimmunity. Often genes associated with autoimmunity play significant role in host-pathogen interactions, suggesting that genetic polymorphisms conferring predisposition to autoimmunity may provide an advantage in immune responses against pathogens. For this reason, here we analyzed the role of PTPN22 in a chronic LCMV (*Lymphocytic Choriomeningitis Virus)* infection mouse model. We demonstrate that knockout mice (PTPN22-/-) are resistant to chronic LCMV infection, show less weight loss and faster viral clearance, which is associated with higher serum IFNα and IL-6 levels and less exhausted virus specific cytotoxic lymphocytes (CTLs) and Th1 lymphocytes. Furthermore, studies with TCR transgenic mice show that PTPN22-deficient virus-specific CD8 T cells display reduced expansion and higher levels of exhaustion phenotype compared to PTPN22-sufficient T cells after adoptive transfer in wild-type hosts infected with persistent virus. This suggests that whereas T-cell autonomous PTPN22 is required for CTL accumulation during chronic viral infection, resistance to chronic infection in the absence of PTPN22 is determined by CTL-extrinsic factors. In summary, our results show that PTPN22 promotes viral persistence by regulating both antiviral innate and adaptive immune responses.

T help in the setting of a chronic LCMV infection

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During chronic viral infections the CD4 T cell response is increasingly skewed towards T follicular helper (T_{FH}) cells. These cells possess important functions in B cell maintenance, differentiation and control of the Germinal Center (GC) response - as mainly shown in the context of acute infections or vaccination. In the context of chronic viral infection - such as chronic LCMV infection - complete absence of CD4 T cells or T_{FH} cells from the onset of infection abrogates the virus-specific antibody response as well as eventual viral control. Yet their relevance is less defined during the course of chronic viral infection, in particular their role in sustaining and shaping antiviral antibody responses in face of continued high antigen loads. Using a novel *in vivo* system that allows conditional ablation of T_{FH} cells or LCMV-specific CD4 T cells we show that T_{FH} cells - but not LCMV-specific CD4 T cells - are dispensable for the maintenance of LCMV-specific antibody titers after their initial establishment. However, sustained presence and activity of T_{FH} cells is mandatory for the late emergence of LCMV-specific antibodies that are capable of neutralizing the viral inoculum but also contemporary virus isolates. Importantly, the ability to generate these antibodies was responsible for eventual control of persistent infection. Thus, continued activity of T_{FH} cells during the course of chronic infection permits resolution of the infection in absence of overt immunopathology by supporting the generation of neutralizing antibodies.

CEACAM1 induces B-cell survival and is essential for protective antiviral antibody production

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Abstract

B cells are essential for antiviral immune defense because they produce neutralizing antibodies, present antigen, and maintain the lymphoid architecture. Here we show that intrinsic signaling of CEACAM1 is essential for generating efficient B-cell responses. Although CEACAM1 exerts limited influence on the proliferation of B cells, expression of CEACAM1 induces survival of proliferating B cells via the BTK/Syk/NF-κB-axis. The absence of this signaling cascade in naive *Ceacam1*$^{-/-}$ mice limits the survival of B cells. During systemic infection with cytopathic vesicular stomatitis virus, *Ceacam1*$^{-/-}$ mice can barely induce neutralizing antibody responses and die early after infection. We find, therefore, that CEACAM1 is a crucial regulator of B-cell survival, influencing B-cell numbers and protective antiviral antibody responses.

Molecular Mechanisms of Host-Virus Interactions in Chronic Infections

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Chronic human viral infections represent one of the most prominent global health problems. The identification of novel therapeutic targets is challenged by the limited understanding of the molecular mechanism underlying host-virus interactions. We aim to employ a well-established model of chronic viral infections, lymphocytic choriomeningitis virus (LCMV), for investigation of key molecular determinants of infection outcome.

For the current study we employ two LCMV strains that differ from each other in immunobiological characteristics: the Armstrong strain leads to an acute infection which is controlled by an intact immune response, whereas the Clone 13 strain results in persistent infection accompanied by generalized immunosuppression. One key genetic determinant for the differences in the infection courses lies in a single point mutation located in the L gene, which encodes the viral RNA-dependent RNA-polymerase (L protein). This model system helps to elucidate the early stages of molecular mechanisms, which determine the infection course. We use a multidisciplinary experimental approach to dissect host-virus interactions in viral persistence including reverse genetics tools, RNA sequencing, mass spectrometry and mouse infectious models.

Our project aims to reveal the differences in viral strategies during acute and persistent infections implicating a powerful infection model on some of the still poorly understood molecular mechanisms of viral persistence. A deeper understanding of this process may point out the direction for further investigations of antiviral therapeutic targets for the treatment of a wide range of human persistent infections, including hepatitis B, hepatitis C and HIV.

Influence of bacterial RNA on memory generation against virus-like particles

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Due to their highly repetitive nature and bacterial RNA content, virus-like particles (VLPs) derived from bacteriophage Qβ elicit strong and long lasting humoral immune responses. After immunization memory B cells (MBC) and plasma cells (PCs) are generated in germinal center (GC) responses. It has been reported that MBC differentiate to secondary plasma cells after challenge, which produce higher amounts of antibodies than PCs in primary responses[1]. To determine the influence of bacterial RNA, a ligand for TLR7/8 inside the VLPs, on MBC and secondary PC generation, adoptive transfers of MBCs generated in the presence or absence of TLR7/8 stimulation followed by challenge with Qβ VLPs were performed. The antibody response of secondary PCs, derived from Qβ RNA induced MBCs, is higher and starts earlier, than the primary response. In contrast, the response of secondary PCs, derived from MBCs induced by Qβ without RNA is more similar to the primary response. Thus, TLR7/8 signalling seems to drive differentiation of MBCs capable of generating secondary plasma cells, responsible for rapid IgG responses during secondary antibody responses.
Moreover, we are interested to determine differences in gene expression patterns between activated VLP specific GC center cells versus circulating memory and naïve B cells. Using fluorescent activated cell sorting VLP specific and naïve B cells were sorted to extract RNA. The gene expression pattern was determined using RNA sequencing. Vast differences in gene expression between Qβ specific and naïve, as well as between GC and MBC are observed. GC cells express higher amounts of genes related to cell cycle whereas in MBCs genes of various immune system categories are upregulated.

References:

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Inflammatory monocytes hinder antiviral B cell responses

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T cell regulation by NK cells via the activating receptor NCR1 (NKp46) during virus infections

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Natural killer (NK) cells are known for their function in recognizing and eliminating infected cells as well as "altered self" cells. By the expression of various inhibitory and activating receptors NK cells are able to sense sudden changes and exert their effector functions without further stimulation. There is raising evidence that NK cells have the ability to regulate T cell responses. In this study, we examine the role of the activating NK cell receptor Natural cytotoxicity receptor (NCR) 1 in regulating T cell responses during acute and chronic Lymphocytic Choriomeningitis virus (LCMV) infection.

In the absence of NCR1 virus-specific CD8 T cells are increased in number and exhibit a more activated phenotype. The increased presence of activated virus-specific CD8 T cells results in reduced viral titers early during chronic infection - at the expense of exacerbated immunopathology, implying that NCR1 and its ligands are involved in balancing antiviral immunity which is critical in the context of chronic infections. Interestingly, the increase of number and effector functions in absence of NCR1 is only apparent during the first two weeks of infection which is likely due to the short half-lives of fully differentiated effector cells. In absence of NCR1, CD4 T cells differentiate preferably into T_H1 cells and to a lower extent into CD4 follicular helper cells during chronic infection.

It is conceivable that abrupt cellular changes triggered by T cell activation might render highly stimulated T cells into NK cell targets via recognition by NCR1. We are currently addressing this hypothesis and we extend our analyses to T cells bearing self-reactivity.

The spectrum of antibodies induced by the current influenza vaccines

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The Influenza virus is still a major global health threat causing a contagious respiratory acute infection on an annual basis. The current trivalent vaccine (TIV) contains inactivated whole-viruses of three viral strains that are updated almost every year. Prevention efficacy of these TIVs in adults is approximately at 75% and drops sharply in the elderly population. We are aiming to get novel insights into low vaccine protection rates by analyzing the humoral immune response induced by them. Host protecting antibodies induced by the TIVs are mainly expected to be directed against the surface glycoproteins of the viral particle, primarily hemagglutinin (HA). However, our serological studies of 16 vaccinated donors showed that serum antibody titers specific for nucleoprotein (NP) are comparably high as the ones for HA in every donor tested. These findings are of particular interest since the protective abilities of NP specific antibodies are still a matter of debate. Here, we dissected the vaccine specific antibody response in vaccinated donors by isolating monoclonal antibodies reactive to the antigens of all viral strains contained in the TIVs. Analysis of the IgG memory cells in four donors vaccinated with different seasonal TIVs, showed that a large portion of the vaccine specific antibody response are directed against HA, as expected. Interestingly, significant numbers of IgG antibodies against NP were found only in the two elderly donors studied. These results suggest that the donor age and the different influenza TIVs may play an important role on the humoral immune response introduced. Therefore, further investigation of these aspects will give a better understanding of the current adaptive immune responses induced by TIVs and help establish more efficient influenza prevention methods.

Antibody-mediated virus control in "tolerant" congenitally infected virus carriers

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Neonatal exposure of mice to Lymphocytic choriomeningitis virus (LCMV) results in antiviral tolerance and life-long co-existence of virus and host. The role of antibodies in the control of such a congenital infection remains poorly defined. To this end, we infected newborn wt mice or B cell receptor repertoire-restricted (BCR^{res}) T11µMT mice with a recombinant, trisegmented reporter virus (r3LCMV). While viral titers dropped dramatically in wt mice after a phase of high-level viremia by week eight accompanied by production of GP-1-binding IgG antibodies, viremia remained at high levels in BCR^{res} mice. Moreover, histological analysis revealed that the virus had formed clearly demarcated foci of infected hepatocytes in the liver of BCR^{res} mice, whereas individual infected cells were scattered randomly throughout the liver of wt animals. This suggested antibody-dependent suppression of viremia in r3LCMV carrier mice, supposedly by inhibition of cell-to-cell spread.

Since trisegmented LCMV are somewhat attenuated, we extended our study to animals, which had been infected at birth with LCMVwt, comparing wt to BCR^{res} carriers. We found our carrier colony to segregate into "viremic controller" and "non-controller" animals. Viremic controller mice were significantly more frequent in wt than BCR^{res} carrier cohorts. Altogether, these observations supported the interpretation that neonatally infected carrier mice mounted antiviral antibody responses, which partially controlled viremia.

Antibody Protection in Chronic Viral Infections

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Passive antibody (Ab) transfer or Ab response inducing vaccinations are known as one the best treatments against chronic viral infections. However, the key mechanism behind Ab dependent protection is still debated. One of the most intriguing question in the field is how non-neutralizing antibodies protect against viral infections. In this study we used lymphocytic choriomeningitis virus (LCMV) to determine which immune components are required for *in vivo* protection by LCMV glycoprotein specific KL25 Ab. We observed that Fc gamma receptor and complement system activation are not mandatory for the clearance of infection by KL25. Additionally, infection caused by a neutralization resistant LCMV mutant can be cleared by F(ab')2 fragments in a Fc independent way. *In vitro*, KL25 and F(ab')2 fragments significantly inhibited viral budding whereas F(ab') fragments failed. Since the importance of F(ab') dimerization for viral budding inhibition was previously reported, we tried to asses role of such mechanism for *in vivo* viral clearance by engineering a monovalent KL25. Monovalent KL25 has comparable *in vivo* half-life and it can efficiently bind GP1 protein and neutralize the virus. Unexpectedly, monovalent KL25 treated mice successfully cleared the LCMV infection. Monovalent KL25 was also shown to inhibit viral release in vitro although it does not carry a dimeric F(ab'). Viral release inhibition might be induced by redundant mechanisms and those mechanisms might explain the protective effect of non-neutralizing Abs.

A novel role of lymph node fibroblasts in chronic viral infection

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Secondary lymphoid organs are sites where adaptive immune cells meet antigen-presenting cells to induce T cell tolerance or immunity. The backbone of these specialized organs consists of different types of non-hematopoietic cells that compartmentalize these organs into specific areas, like the T cell zone where the clonal selection and amplification of T cells occurs. Fibroblastic reticular cells (FRC) are the main non-hematopoietic cell type found in T zones, where they form a sponge like network. This cell type has been shown to be able to "communicate" with T cells and dendritic cells in order to maintain immune homeostasis but also to modulate immune responses. We and others have shown that FRCs attenuate antigen-specific T cell responses by the IFNg-dependent upregulation of inducible NO synthase (iNOS) followed by the release of nitric oxide (NO). Further we observed that FRC provide a second mechanism to dampen T cell responses by expressing cyclooxygenase 2 (COX2). The aim of the current study is to dissect the immune modulatory role of FRCs in more detail by focusing on the role of COX2.

In contrast to the inducible iNOS expression our data show that COX2 is constitutively expressed in FRC. We identified a Cox2-dependent lipid mediator produced by FRC and the corresponding receptors necessary on T cells that mediate a suppressive effect. By using a FRC specific COX2 deficient mouse model we will show data that this pathway is able to modulate cellular responses in chronic viral infection. Thus, we provide the first evidence that lipid mediators produced by FRC upon viral infection are responsible for dampening adaptive immunity in vivo. These findings suggest that the use of common non-steroidal anti-inflammatory drugs like aspirin and ibuprofen that block Cox1/2 enzymes may enhance adaptive immunity in chronic viral infection, similar to recent findings in the field of anti-tumor immunity.

Lack of terminally exhausted epitope-specific CD8+ T cells in chronically HBV-infected inactive carriers

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Persistent antigen recognition during chronic infection results in an impaired virus-specific CD8+ T-cell response characterized by a progressive loss of effector functions, a phenomenon called exhaustion. Exhausted CD8+ T cells in mice are defined by high levels of inhibitory receptors such as PD1, low levels of memory markers like CD127 and the transcription factor TCF1 and up-regulation of the ectonucleotidase CD39. Furthermore, differential expression of the transcription factors Eomes and T-bet has been shown to dissect distinct subsets of murine exhausted virus-specific CD8+ T cells. In humans, however, little is known about subsets within exhausted virus-specific CD8+ T-cell populations targeting one single epitope and the accompanying transcriptional programme.

In order to define subsets of exhausted virus-specific CD8+ T cells including their key transcriptional regulators in humans, we analyzed HLA-A*02-restricted epitope-specific CD8+ T-cell populations by tetramer-associated magnetic bead enrichment with subsequent flow cytometric analyses in chronically HBV-infected inactive carriers (n=12) or patients chronically infected with HCV (n=8).

Expression patterns of PD1 and CD127 revealed that exhausted epitope-specific CD8+ T cells in chronic HBV and HCV infection are heterogeneous populations and can be subdivided into PD1+ cells that either express or do not express CD127. Interestingly, in chronic HBV infection, the majority of exhausted epitope-specific CD8+ T cells co-expressed PD1 and CD127 whereas a significant proportion of PD1+ HCV-specific CD8+ T cells lacked CD127 expression. Of note, expression levels of PD1 on virus-specific CD8+ T-cell populations were significantly higher in chronic HCV compared to HBV infection. $PD1^{high}$ HCV-specific CD8+ T cells exhibited high Eomes and CD39 and low T-bet expression, defining terminally exhausted CD8+ T cells. In contrast, HBV-specific CD8+ T cells lacked CD39 expression and significantly up-regulated TCF1 suggesting a less differentiated "memory-like" population with significantly higher proliferative capacity compared to HCV-specific CD8+ T cells.

Taken together, epitope-specific CD8+ T cells in chronic viral hepatitis display a heterogeneous phenotype based on PD1 and CD127 co-expression that is linked to distinct transcriptional profiles suggesting the presence of distinct subsets of exhausted CD8+ T cells in humans. Interestingly, and in contrast to HCV infection, epitope-specific CD8+ T-cell populations in chronic HBV-infected inactive carriers lack terminally exhausted subsets and harbor a high proliferative capacity, rendering them a promising target to boost CD8+ T-cell responses in immunotherapeutic approaches.

The role of mitochondrial pro-fission protein Drp1 in T cell lineage

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Mitochondrial dynamics deeply affect the physiology of the cell and are strictly regulated by the mitochondria-shaping proteins that balance mitochondria fission and fusion processes in response to the cell needs. Apoptosis and cell proliferation are some of the cellular processes in which a crucial role for mitochondrial dynamics has been demonstrated in several cellular types, especially for the main fission-promoting factor, Drp1. In addition, Drp1-dependent fragmentation is required to fuel the cell migration process in mature T cells. Considering the importance that apoptosis, cell proliferation and migration have during T cell development and function, in our work we are investigating the role of mitochondrial dynamics in the T cell lineage. We took advantage of a conditional-KO mouse model in which Drp1 is removed specifically when precursor T cells enter the thymus, at the early steps of their development. We found that Drp1 modulates proliferation and migration of developing thymocytes. It also regulates clonal expansion, migration and extravasation across the endothelial barriers of mature T cell, so controlling their recirculation inside secondary lymphoid organs and accumulation inside target inflamed sites. These findings suggest that mitochondrial shaping is crucial in T cell development homeostasis and for mature lymphocytes' immuno-protective functions against different infections and tumours. Indeed, the absence of Drp1 in T cell lineage increases tumor growth in mice, and less CD8+ cells infiltrate them, so highlighting a defect in T cell immune-surveillance.

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The role of non-neutralizing antibodies in chronic viral infection

This project explores the protective mechanisms of binding but non-neutralizing antibodies (nnAb) in the prevention of chronic lymphocytic choriomeningitis virus (LCMV) infection. Similar to many human chronic viral infections, persistence of LCMV is associated with compromised T and B cell responses. The administration of nnAb prior to LCMV infection results in improved T cell response and subsequent viral clearance. However, recent studies excluded some of the main protective mechanisms triggered by nnAb leaving the mode of action of LCMV-specific nnAb in chronic infection unclear.

Here we show that provision of LCMV-binding antibodies prior to infection inhibits the establishment of viral chronicity. Transfer of LCMV-specific nnAb does not inhibit viral entry, but alters the early viral tropism to antigen presenting cells (APC). This preferred infection of APCs is associated with enhanced antiviral T cell response and subsequent viral clearance. Together, our data demonstrate the crucial interplay between antibodies, early virus tropism and the ensuing antiviral T cell responses in the control of a chronic viral infection. Notably, the fact that antiviral nnAb protect against chronic infections provides a rational to further dissect their mode of action, which supports the development of prophylactic vaccines against chronic viral infections.

IN VITRO STUDY OF TOLL-LIKE RECEPTORS SENSING BoHV-1

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Pathogen detection is crucial in the establishment of an immune response by the host immune system. Pathogen recognition is primarily carried out by cell surface or endosomal receptors, which trigger signaling cascades that result in the upregulation of inflammatory cytokines and type 1 interferon.

Toll-like receptors (TLRs) are the key innate immune receptors. Understanding the involvement of TLRs during pathogen infection helps to provide a clearer picture on the establishment of the innate immune response to the infectious agent. This issue is particularly important in studies of animal viruses, where knowledge is much lower compared to human viruses.

TLRs 3, 7, 8 and 9 are located in endosomal membranes and primarily serve as nucleic acid sensors for viruses. Herpesviruses are known for their ability to modulate host immune responses upon infection in order to establish latency. Bovine Herpesvirus type 1 (BoHV-1) is an alphaherpesvirus causing infectious bovine rhinotracheitis (IBR) in cattle.

We performed an *in vitro* study in order to profile the expression of TLRs 3, 7, 8 and 9 in bovine peripheral blood mononuclear cells (PBMCs) obtained by 4 heifers from an IBR-free herd and one heifer BoHV-1-free but persistently infected with Bovine Viral Diarrhea Virus (BVDV), a pestivirus with heavy economic impact on farms. Isolated PBMCs were *in vitro* infected with BoHV-1 and tested at 2, 18 and 48 hours post-infection for the expression of endosomal TLRs by Real-Time PCR assays. Negative controls were included for all the animals and time-points.

TLR 3 was very low expressed *in vitro* in infected as well as in control PBMCs at all time-points; TLRs 7, 8 and 9, differentially expressed at 2 and 18 hours post-infection, showed downregulation due to BoHV-1 at 48 hours post-infection. Interestingly, in our *in vitro* model, at 48 hours was detected the peak of BoHV-1 replication.

The *in vitro* infection with BoHV-1 of PBMCs isolated from a BVDV persistently infected heifer showed high expression levels of all the TLRs tested except TLR3 at all time-points and both in BoHV-1 infected and control PBMCs. This stimulation, not associated to BHV-1 infection, is presumably due to BVDV continuous replication.

These results, taken together, suggest that the differential expression of endosomal TLR genes is associated to the type of virus infection and the replication cycle. Deeper understanding of bovine innate immune responses following infection by these two viruses, which are responsible for the most costly bovine viral diseases, could help to identify the strategies by which the pathogens evade host defenses and to develop effective intervention strategies.

Tuning virus-specific T cell responses by infection induced inflammation

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Signals delivered by costimulatory molecules and pro-inflammatory cytokines are critical for driving T cell expansion and differentiation. However, the signals that are required for T cell activation are unique in distinct infections, resulting in considerable plasticity of the immune response. This is exemplified by the critical contribution of the CD28/B7 costimulatory pathway in driving MCMV-specific T cell expansion, whereas these signals are dispensable for the expansion of LCMV-specific T cells, due to redundancy with other costimulatory signals. Remarkably, even in the setting of a co-infection with LCMV, CD28/B7-mediated signals remain essential for MCMV-specific T cell expansion, indicating that MCMV and LCMV differentially modulate APCs and the provision of costimulatory signals. Here we follow-up on these observations and examine if the requirements for pro-inflammatory cytokines are fixed or plastic in an altered environment established during co-infections. We found that upon certain infections the pro-inflammatory cytokine IL-12 impacts T cell differentiation, but the necessity for this signal can be overruled in a type I IFN dependent manner. Future experiments will focus on identifying the APCs that directly engage with T cells and determining how pathogens differentially modulate APCs and their function.

ABSTRACT – VIRAL IMMUNOLOGY

TCF1 defines Hepatitis C virus-specific CD8+ T cells that are maintained after removal of chronic antigen stimulation

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Chronic viral infection results in impaired virus-specific CD8+ T-cell responses, a phenomenon called T-cell exhaustion. Exhausted CD8+ T cells exhibit reduced cytokine production and proliferative capacity, co-express inhibitory molecules (e.g. PD-1) and lack memory markers like IL-7R α-chain (CD127) or transcription factor TCF1. The mechanisms responsible for CD8+ T-cell exhaustion are not completely understood, however, one key feature seems to be the prolonged and continuous exposure to antigen. An open question of clinical importance is the fate of exhausted virus-specific CD8+ T cells after viral elimination. By taking advantage of the recently approved direct acting antivirals (DAA) in Hepatitis C virus (HCV) therapy we were able to address this issue for the first time in a highly relevant clinical setting. Here, we analyzed phenotype and function of HCV-specific CD8+ T cells prior, during and after successful DAA therapy in a cohort of 29 chronically HCV-infected patients by using a peptide/MHCI tetramer enrichment strategy. Our results can be summarized as follows:

First, we identified different subsets of HCV-specific CD8+ T cells co-existing during antigen persistence. We found a CD127-PD1hi subset that exhibited markers of terminal exhaustion and a less differentiated CD127+PD1+ subset that was characterized by TCF1 expression. Second, we could assign memory-like characteristics to the TCF1+CD127+PD1+ subset of HCV-specific CD8+ T cells including long-term antigen-independent survival after DAA-mediated antigen removal. Of note, we were able to monitor the HCV-specific CD8+ T-cell response in a case of viral relapse. In this patient, re-exposure to HCV led to robust expansion of HCV-specific CD8+ T cells and emergence of terminally exhausted CD127-PD1hi cells suggesting a progenitor-progeny relationship within this heterogeneous HCV-specific CD8+ T-cell population. Third, these memory-like TCF1+CD127+PD1+ HCV-specific CD8+ T cells share phenotypic, molecular and functional properties of both T-cell memory and T-cell exhaustion clearly demonstrating divergent T-cell differentiation in chronic compared to spontaneously resolved HCV infection. Finally, we could show that TCF1 expression by the CD127+PD1+ T-cell subset was linked to the proliferative potential of the overall HCV-specific CD8+ T-cell population. Thus, the TCF1+CD127+PD1+ subset contained the proliferative capacity of HCV-specific CD8+ T cells during and after antigen persistence and appears to be central for the maintenance of HCV-specific CD8+ T-cell populations.

In sum, our study reveals that memory potential of human virus-specific CD8+ T cells is not precluded by chronic infection even after years of chronic antigen exposure. These results have clear implications for protection from re-infection after antigen persistence and for future immunotherapeutic approaches.