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Rational vaccine and biosignature design for tuberculosis control

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Tuberculosis (TB) is a health threat of global dimension. Annually ca. 9 million individuals develop this disease, of whom 1/5 dies. At the same time tuberculosis is a challenging target for immunologic research since it reflects the outcome of a longstanding co-evolutionary process between the pathogen and the human host. Worldwide 2 billion individuals are infected with Mtb, of whom 90% will carry the pathogen lifelong. In 10% of infected individuals TB disease emerges due to dysbalanced immunity. The current vaccine BCG prevents disseminated childhood TB but fails to protect against pulmonary TB in all age groups. We have genetically modified BCG to improve its protective efficacy. Our vaccine has successfully completed phase IIa trial in newborn in Africa and will now be tested in newborn delivered by HIV+ mothers. Global gene expression profiling has provided deep insights into the host response in TB. Biosignatures have been defined which distinguish latent infection without disease from active TB disease. Currently, efforts are undertaken to define a prognostic biosignature which can predict risk of active TB disease in healthy infected individuals. Such biomarkers not only provide helpful tools for novel intervention measures in TB but also shed light on relevant host mechanisms of protection/pathology.
Cytomegalovirus vector expressing NKG2D ligand provides long-lived memory CD8 T cells with outstanding protective capacity

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Cytomegalovirus (CMV) establishes life-long infection of its host, ensuring continuous supply of effector memory CD8⁺ T cells. CMVs possess numerous immunoevasion genes able to modulate basically any part of immune response, including NK cell and CD8⁺ T cell response. It is well established that deletion of these viral inhibitors leads to virus attenuation in vivo. These features make CMV a very attractive CD8⁺ T cell vaccine-vector candidate. Control of CMV infection is in great part dependent on NKG2D, an activating receptor when expressed on NK cells and co-stimulatory one when expressed on CD8⁺ T cells. We have constructed highly attenuated mouse CMV (MCMV) expressing NKG2D ligand RAE-1γ inserted in place of its viral inhibitor (Slavuljica et al, 2010) and foreign CD8⁺ T cell epitope as well (Trsan et al, 2013). Such a recombinant vaccine-vector provided outstanding and long-lasting CD8⁺ T cell-mediated protection against challenge infections. Moreover, RAE-1γMCMV-vector circumvented MCMV interference of antigen presentation, improved antigen presentation to CD8⁺ T cells and potentiated memory CD8⁺ T cell response. Outstanding properties of RAE-1γ expressing MCMV vector were retained even in NKG2D deficient mice, pointing to additional NKG2D-independent immune function of RAE-1γ.
Adoptive cell therapy using primary or gene modified central memory T cells

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Adoptive transfer of primary (unmodified) or genetically engineered antigen-specific T cells has demonstrated astonishing clinical results in the treatment of infections and some malignancies. The definition of optimal targets and antigen receptors as well as the differentiation status of transferred T cells are emerging as crucial parameters for generating cell products with predictable efficacy and safety profiles. Our laboratory has demonstrated that defined subsets within the memory CD8+ T cell compartment fulfill all key characteristics of adult tissue stem cells and are essential for robust and long-term maintained responses upon adoptive transfer. We have developed clinical multi-parameter enrichment technologies to purify these memory stem cells for clinical applications. In my presentation I will also report on the status of ongoing clinical trials.

Infusing small numbers of T cells within a memory stem cell product can be highly effective therapeutically, but bears some risk of toxicity. Therefore, safeguards that allow selective depletion of transferred cells in the case of un-tolerable side effects may be needed. We are currently exploring the capacity of a truncated version of EGFR (EGFRt) co-expressed with adoptively transferred T cells to further improve adoptive immunotherapy.
Adoptive transfer of allogeneic natural killer (NK) cells is a relatively non-toxic approach which is gaining interest to combat cancer. We reported previously a GMP-compliant cytokine-based culture system for the generation NK cell products from umbilical cord blood CD34+ hematopoietic progenitor cells (HPC) with high cell numbers, purity and functionality, and importantly absence of T cell contaminants. Pre-clinical studies conducted in mice demonstrated that these HPC-NK cells have bone marrow homing capacity and potently prolong survival of K562-bearing mice. Currently, this HPC-NK cell product is being investigated in a phase I clinical trial in older acute myeloid leukemia (AML) patients who are not eligible for allo-SCT. In this study, escalating doses of allogeneic HPC-NK cells, ranging from 3x10^6 up to 30x10^6 NK cells/kg, are infused in cohorts of 3 patients following fludarabine/cyclophosphamide conditioning. In addition to the non-transplant cancer setting, it would be highly valuable to exploit HPC-NK cell products for adoptive immunotherapy after allo-SCT. However, isolation of high numbers of functional NK cells from donors is challenging. Hence, we adapted the cytokine-based ex vivo culture protocol to generate high numbers of functional NK cells from G-CSF mobilized CD34+ HPC that are devoid of T cell impurity. We demonstrated that addition of aryl hydrocarbon receptor (AhR) antagonist StemRegenin-1 (SR1) to the culture protocol potently enhances expansion of G-CSF mobilized CD34+ HPC, and induces expression of NK cell associated transcription factors promoting NK cell differentiation. Moreover, we observed that combining IL-15 with IL-12 drives the generation of more mature and highly functional HPC-NK cells. Collectively, these data show that adaptation of the culture protocol using the AhR antagonist SR1 and IL15/IL12 cytokine combination can lead to improved next-generation HPC-NK cell products from various CD34+ cell sources for adoptive cancer immunotherapy.
The Yin and the Yang of a bacterial toxin: Fooling host phagocytes and delivering immunotherapeutic T cell vaccines

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The whooping cough agent, Bordetella pertussis, secretes an adenylate cyclase toxin (ACT) that penetrates host phagocytes bearing the α5β2 integrin receptor Mac-1 (also known as CR3 or CD11b/CD18). It targets in particular the sentinel functions of neutrophils, macrophages and the professional antigen presenting dendritic cells (DC, CD11b\text{high}). ACT recognizes a positively charged loop of the CD11b subunit of CR3 near the hinge region outside of the I/domain of CD11b and inserts directly across phagocyte membrane. ACT-mediated Ca\text{2+} influx then induces calpain-mediated cleavage of talin, enabling ACT to hijack the receptor and mobilize it into membrane lipid rafts. There, translocation of the AC domain across cell membrane is completed across a tightly sealed protein-lipid interface. The AC binds cytosolic calmodulin and catalyzes conversion of ATP to cAMP, generating supraphysiologic cAMP levels that subvert phagocyte functions, causing phagocyte impotence due to inactivation of the Syk kinase and block of signaling of leukocyte receptors. Activation of PKA through cAMP next provokes transient inactivation of the small GTP-ase RhoA, causing rapid and unproductive cell ruffling. In parallel, transient activation of the tyrosine phosphatase SHP-1 occurs by an as yet unknown PKA-dependent mechanism and causes inhibition of oxidative burst and block of expression of iNOS, preventing bactericidal NO production in phagocytes. Simultaneously, activated SHP-1 causes stabilization of BimEL and activation of Bax, provoking induction of apoptosis.

A subpopulation of ACT molecules oligomerizes into small cation-selective pores that permeabilize cells for potassium efflux. This contributes to induction of maturation of dendritic cells that is, however, hijacked by cAMP signaling, which compromises the capacity of DCs to stimulate antigen-specific T cell immune responses. Migration of the incompletely mature DCs into lymph nodes then likely contributes to suppression of adaptive host immune responses to the pathogen and supports bacterial colonization of the host in early stages of infection. Later in infection, ACT action provokes NALP3 inflammasome activation in dendritic cells, which likely contributes to late inflammatory response and eventual development of Th1/Th17 polarized immune responses that support eventual clearance of the bacterial infection.

The amazing capacity of ACT to accommodate foreign antigenic polypeptides and the capacity of ACT to promote maturation of dendritic cells allowed development of genetically detoxified ACT (dACT) into a novel carrier for delivery of antigens for processing into both the MHC class I and II-restricted presentation pathways. This enables efficient induction of prophylactic as well as therapeutic antigen-specific CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell immune responses. I will conclude by reviewing recent applications of ACT technology for immunotherapy of certain tumors.
New direct acting antivirals for the treatment of hepatitis C - dream or reality?

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Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide. The number of chronically infected persons worldwide is estimated to be about 160 million. The long-term HCV infection can lead to extensive fibrosis and cirrhosis with or without hepatocellular carcinoma (HCC). The long-awaited all-oral therapy for hepatitis C virus infection has arrived with the registration of the hepatitis C nucleotide inhibitor sofosbuvir in a combination of the NS5A inhibitor ledipasvir (also in a single formulation with sofosbuvir,) and daclatasvir; the protease inhibitor simeprevir, and the 3D regimen based on the ritonavir boosted protease inhibitor paritaprevir; the NS5A inhibitor ombitasvir, and the non-nucleoside polymerase inhibitor dasabuvir. Novel regimens for hepatitis C virus (HCV) have shorter treatment durations and increased rates of sustained viral response, less side-effects compared to standard therapies with pegylated interferon-alfa and ribavirin. The main drawback is the extremely high-cost of those drugs, budget-breaking prices of the newer anti-HCV limits patient access to HCV therapy even in resource-rich countries. Therefore treatment should be prioritized for patients with significant fibrosis or cirrhosis (METAVIR score F3 to F4) and patients with decompensated cirrhosis (Child-Pugh B and C) should be urgently treated with an IFN-free regimen. However viral resistance patterns should be followed-up in a group of non-responders to protease inhibitors. Naive patients with mild and moderate fibrosis (F0-F2) and otherwise good predictors of response can still be offered a combination of pegylated interferon and ribavirin for 24 weeks.
Vaccines and immunotherapeutics for highly pathogenic microorganisms—how far we are from bench to bed?

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Last terrifying and still ongoing Ebola virus (EBOV) outbreak has shown to the scientific and medical community how fragile and unprepared for the challenges in the clash with dangerous pathogens are they. A number of cases and deaths are among other things, a result of years of ignorance and neglect of the wider scientific community in the development of vaccines and therapeutics for dangerous pathogens.

Only thanks to the enthusiasm of individual research groups, today we have a starting points for the development of vaccines and biologicals for highly pathogenic microorganisms that belong to the group of orphan microorganisms. The development of orphan drugs and vaccines to highly pathogenic microorganisms, however, requires close collaboration of top microbiologists, infectious diseases and immunology experts, which mostly still is not often the case. Of course, without the interests of the industry for the development of orphan drugs, we will not be able to finalize this demanding job.

The recent approach in development of antivirals to EBOV showed that EBOV glycoprotein (GP) plays critical roles in the early stage of virus infection, including receptor binding and membrane fusion. Two novel EBOV inhibitors targeting viral entry were reported recently. To identify small molecule inhibitors of EBOV entry, the group of experts carried out a cell-based high-throughput screening using human immunodeficiency virus-based pseudotyped viruses expressing EBOV-GP. Two compounds were identified, and mechanism-of-action studies were performed. United States Army Medical Research Institute of Infectious Diseases (USAMRIID) experts showed lately that EBOV-like particles can be reduced in size to a more amenable range for manipulation, and that these smaller particles retained their temperature stability, the structure of the GP antigen, and the ability to stimulate a protective immune response in mice. Also, it was shown by other group that aerosolized EBOV vaccine protect primates and elicits lung-resident T cell responses. Promising results were obtained with vaccination with a highly attenuated recombinant vesicular stomatitis virus vector which protects against challenge with a lethal dose of EBOV and a cytomegalovirus-based vaccine which provides long-lasting protection against lethal Ebola virus challenge after a single dose.

Besides the EBOV, there are other important emerging pathogens (e.g. hantaviruses, *Francisella tularensis*, Middle East Respiratory Syndrome –MERS, *Yersinia pestis* etc.) and ongoing research for the therapeutics/vaccines development which will be described from the preclinical models to clinical trial results in this area.
Accumulation of defective interfering particles as the mechanism of viral vaccine attenuation

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Immunization programs have implemented live attenuated vaccines as very effective means of protection against infectious diseases. Live attenuated vaccine against mumps reduced mumps incidence ≥97%. Some of the vaccine strains were abandoned due to unwanted side effects and the genetic marker of attenuation has not been identified so far. Our hypothesis was that non-infectious viral particles, in particular defective interfering particles (DIPs), contribute to neuroattenuation. We showed that non-infectious particles of mumps vaccine attenuated neurovirulence of wild type mumps virus. Furthermore, we attenuated a recent wild type mumps virus in Vero cells through 16 passages but already the fifth passage (p5) showed accumulation of DIPs and attenuated neurovirulence in a newborn rat model when compared to second passage (p2). Sequence analysis of p2 and p5 revealed a single mutation in the 5’ untranslated region of the HN gene. The analysis of the expression level of the HN protein showed that this mutation does not affect the expression of the protein. We conclude that passages of the mumps virus in Vero cells for only three passages accumulated DIPs which attenuate neurovirulence. The findings of this study reveal DIPs as a very promising and general neuroattenuating factor which should be considered in the rational design of the new mumps vaccine.