Biomarkers
Friday, June 12
2:45 pm-4:45 pm

OR.9. Detection of Islet-specific Autoreactive T Cells by a Novel Robust and High Throughput Screening Strategy in Stored Blood Samples of Type 1 Diabetic Patients

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Type 1 diabetes (T1D) results from selective destruction of pancreatic β cells by cytotoxic T cells. In this process, β cells presenting islet-specific epitopes are recognized and destroyed by autoreactive CD8+T cells. Therefore, tools to reliably detect and monitor β cell specific CD8+T cells are essential. Current approaches to screen blood of T1D patients suffer from the large number of candidate epitopes, their lower HLA binding affinity, low precursor frequencies of circulation autoreactive T-cells, the presence of regulatory T-cells and the need for *ex vivo* culture. We developed a fast and high-throughput assay to determine HLA-binding properties and monitor antigen-specific T cell responses in stored blood of T1D patients. In this assay, conditional HLA ligands are cleaved upon UV-irradiation resulting in HLA molecules that are loaded with arrays of peptide ligands. Peptide-HLA monomers are then labeled with distinct fluorochromes and the blood of T1D patients is screened. We investigated the use of quantum dots (Qdots) as fluorochromes to generate peptide-HLA multimers instead of conventional tetramers. The use of Qdots allowed the simultaneous screening of eight different epitopes derived from Insulin, Pre-pro-insulin, IA2, GAD65, IGRP and ppIAPP, with high specificity and sensitivity. We demonstrate that this assay is of particular interest when monitoring specific T cell responses during the course of treatment trials and after clinical islet transplantation. In addition, the development of this technique allows the discovery and detection of multiple islet epitopes requiring minimal amounts of patient material.

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OR.10. Using Immune Response Data from a Lymphoma Vaccine Clinical Trial to Define Novel Endpoints for an Immunotransplant Trial

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Background: Cancer vaccines need to be more powerful and practical. We have completed a clinical trial using the practical approach of intratumoral injection of an "off-the-shelf" TLR9-agonist for patients with lymphoma. To increase the power of vaccination, we have developed an 'immunotransplant' maneuver- the transfer of vaccine-primed lymphocytes to lymphodepleted recipients. The obstacle to demonstrating the clinical benefit of immunotransplant is that the anti-lymphoma effect of 'lymphodepletion' confounds conventional clinical response measurements. However, correlating multiplex immune-response measurements with clinical response in the lymphoma vaccine trial could allow us to validate an immune endpoint to assess the benefit of immunotransplant. Results: Our lymphoma vaccine trial demonstrated 1 CR, 2 PR, and 8 SD amongst 15 patients as well induction of tumor-specific, memory CD8 T cells correlating temporally with tumor regression. In the supernatant of tumor-lymphocyte co-cultures, we have performed a multiplex high throughput assay measuring 'cytokine-response profiles'. We have tested these profiles for intra-patient reproducibility and inter-patient variability in the completed vaccine trial cohort. These, together with profiles of patients in our ongoing iteration of that trial will be used to correlate a specific signature with clinical outcome. Conclusions: An intratumoral TLR9-agonist-based vaccine for lymphoma is feasible and induces tumor-specific CD8 T cell responses and clinical responses. Immunotransplant significantly increases the anti-tumor effect of this vaccine maneuver pre-clinically. The validation of 'cytokine-response profiles' in the lymphoma vaccine trial may allow us to demonstrate the benefit of immunotransplant in early stage trials.

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OR.11. Discovery of Novel Antigenic Targets and Autoantibody Markers in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is the most common autoimmune disease characterized by chronic inflammation of synovial joints. The aim of this study was to identify novel target (auto)antigens and antibody biomarkers for early RA patients and RA patients that are seronegative for the 2 diagnostically applied serological RA markers, rheumatoid factor (RF) and anti-CCP (cyclic citrullinated peptides) antibodies (ACPA), by applying an autoantibody profiling procedure based on cDNA phage display. We report the identification of 14 novel target antigens and autoantibody markers for RA. Inclusion of the detection of antibodies against these antigens to the diagnostic testing for RF and ACPA presence increased the sensitivity of serological tests for RA by 25%. Moreover, antibodies against our panel were detected in an early disease phase and were associated with higher inflammatory disease activity, demonstrating the diagnostic and prognostic potential of the panel. Furthermore, we identified several novel antigens with exclusive immunoreactivity in ACPA negative RA patients. An increased expression of several of the identified antigens in RA synovial tissue was demonstrated, providing evidence for a biologically relevant role of the antigen-antibody systems in the RA disease process. In conclusion, the identified markers are proven to be valuable for improved RA diagnosis and prognosis prediction, especially for RA patients that are seronegative based on current diagnostic laboratory tests. Moreover, the identified antigen-antibody systems provide

important information regarding underlying disease processes in RA which can ultimately lead to the discovery of novel therapeutic targets.

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OR.12. Antibody and Antigen Complexes Based on Cohesin and Dockerin Interaction are Versatile Tools for Eliciting and Monitoring Specific Immune Responses

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Targeting antigens to dendritic cells (DCs) is a new approach in vaccine development- in mouse models small antigen doses linked to anti-DC receptor monoclonal antibodies evoke potent and broad immunity. But, application of this technology to human vaccines faces two barriers- identifying appropriate DC receptors to target, and delivering protective antigens. Many desirable viral antigens, e.g., Influenza A matrix proteins M1 and avian Influenza hemagglutinin HA-1 are not efficiently secreted as antibody fusion proteins, thus impeding their development as DC antigen-targeting vaccines. Dockerins and cohesins are interacting domains that assemble bacterial cellulosomes- structures for degrading cellulose. Recombinant antibodies with a dockerin domain fused to the heavy chain C-terminus are efficiently secreted by mammalian cells. We have adapted these modules to permit non-covalent assembly of recombinant anti-DC receptor antibody-antigen complexes. Many antigens are readily produced as cohesin-antigen fusion proteins either via secretion from mammalian cells, or as soluble cytoplasmic E. coli products, and they form very stable and homogeneous complexes with antibody-dockerin fusion proteins. These complexes bind efficiently to DC surface endocytic receptors and their antigen peptides are processed and effectively presented to antigen-specific CD4+and CD8+T cells. Furthermore, cohesin-antigen fusion proteins can be bound to dockerin-coated fluorescent beads, permitting multiplexed detection of antigen-specific serum antibodies. Thus, a set of anti-DC receptor antibody-dockerin fusion proteins can be readily configured with a set of cohesin-antigens, providing a very useful toolkit for comparative analysis of antigen-specific T and B cell responses in the context of DC antigen-targeting vaccine development.

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OR.13. Identification of a Peripheral Blood Gene Expression Classifier of Chronic Antibody-mediated Rejection in Kidney Transplant Recipients

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Chronic antibody-mediated rejection (CAMR) is an elusive phenomenon with no consensus in terms of treatment strategy. The current diagnosis of CAMR is based on the presence of certain histological features within the graft biopsy together with diffuse C4d deposition in peritubular capillaries and circulating donor-specific antibodies. A less invasive means of diagnosing this phenomenon would facilitate the setting up of clinical trials aimed at testing novel immunosuppressive regimens. Here we employed an exhaustive gene profiling strategy using pangenomic microarrays (Affymetrix HGU133Plus2) to identify a peripheral blood gene expression signature of CAMR in kidney transplant recipients. A total of 153 independent individuals were included in the discovery set. Patients classified as CAMR were all sampled at greater than one year post transplant (n = 19) and were compared to 127 controls including patients with various other clinical and histological diagnoses at>1yr post transplant. A two-group comparison based on the microarray data identified 55 individual genes whose expression significantly varied between the two groups as well as an optimal subset of 23 genes that together best discriminated between the two groups (a genetic signature). A list of candidate genes was also generated based on publicly available data as well as the literature. Technical validation of this exhaustive list of genes was confirmed by qPCR and a genomic classifier was developed. Validation of our genomic classifier on 150 independent and prospectively recruited patients having a blood sample simultaneous to a biopsy for cause or for protocol is now underway.

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OR.14. A Novel Immunologic Test for Active TB

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Rationale: Reducing transmission and limiting the burden of tuberculosis is a priority of global health politics. A rapid blood test which permits the prompt identification of clinically active pulmonary tuberculosis including sputum smear negative cases would be a major step forward. Objectives: The goal of this work was to examine whether a simple flow-cytometry based test examining the phenotype (CD27 expression) of tuberculin-activated CD4 T-cells can meet this requirement. Methods: Flow-cytometry was applied to examine tuberculin-stimulated (16 h) PBMC from patients with clinically active pulmonary tuberculosis, including several sputum-smear negative cases, and hospital staff with prolonged exposure to active pulmonary tuberculosis, including a number of cases of latent infection. Surface expression of CD27 was measured on tuberculin specific CD4 T-cells identified by one of the following