Restoration of immune tolerance in Type I diabetes

Project Summary:

Type I diabetes (T1D) is a chronic autoimmune disease in which the immune system attacks a normal tissue. In the case of T1D, the target tissue is the pancreas 'beta cells', which are the cells that produce the hormone insulin that controls how most cells in the body adsorb glucose from the blood. [Glucose is the primary molecule of chemical energy and is derived from dietary food although not usually in the exact chemical form of glucose. Instead, other carbohydrates more commonly found in the diet (either sugars or starches) are converted into glucose for immediate usage (and also into a storage form called glycogen). The amount of glucose in the blood thus influences how quickly and thoroughly the cells of the body are able to receive the energy that they need.]

In non-diabetic persons the function of insulin is to mediate a finely-tuned control mechanism which carefully regulates the amount of glucose made available for cells which need energy. In a non-diabetic the amount of insulin released from the pancreas beta cells is exactly the right amount that is needed to control usage of blood glucose. Over the course of a typical day, glucose levels in the blood rise and fall depending upon the amount and types of food eaten as well as the amount of exercise undertaken. In turn the amount of insulin that is released from the pancreas rises precisely in response to how much is needed to control the removal of glucose from the blood.

Because of the loss of insulin (due to the death of the pancreas beta cells), it is impossible for persons with T1D to properly regulate glucose levels in the blood. In T1D, even if taking injections of insulin, the level of glucose in the blood is imperfectly controlled such that over the course of time the average glucose level is higher than in a non-diabetic. Biochemical reactions occur in which the elevated levels of glucose in the blood becomes attached to other blood proteins. The body tries to eliminate those 'glucose-modified' proteins but cannot do so properly and cells become damaged. Thus, over time, the blood vessel cells become damaged and cannot function properly to deliver adequate blood to tissues and organs. Without adequate blood supply cells and tissues die. That is why major long-term consequences of diabetes involve those tissues where damage to the smallest blood vessels is the most clinically significant: the eye, the kidney, and the extremities (where the pressure that makes the blood flow is the lowest exasperating the consequences of insufficient blood supply).

This complex side effect of excess blood glucose is the biochemical basis of the long-term consequences of Type I diabetes.

Experimental curative therapy of T1D has several different strategies including the attempt to reverse the immune assault on the pancreas beta cells. In essence this research strives to restore the body's own normal ability to control its immune system, termed 'immunological tolerance'. Unfortunately, the mechanistic details concerning tolerance are incompletely understood but is of paramount importance to master before realistically being able to inform a therapy.

The current project investigates different aspects of immunological tolerance in a mouse model of T1D with the ultimate goal of development of a strategy to increase tolerance in T1D such that the immune system is...
unable to kill pancreas beta cells. If anti-beta cell immune response can be curtailed by restoring tolerance, islet cell function may be able to be restored either by transplantation or self renewal from the body's own islet stem cells.

Our strategy is to target elimination of those T cells that kill beta cells. We expect to discover if tolerance can be manipulated in T1D to cause reversal of immune-mediated beta cell destruction.

We have evaluated, by predictive algorithms, several known protein antigens expressed in T1D for candidate peptide epitopes that may be recognized by CD8+ T cells in Non Obese Diabetic mice (NOD). Candidate peptide epitopes were synthesized and tested for the ability to stimulate proliferation of spleen cells and IFN-g secretion by (and separately) islet-infiltrating lymphocytes in vitro. Peptides from several known antigens predicted to bind to MHC Class I have been shown to stimulate proliferation of CD8+ T cells from pre- and overtly diabetic NOD mice. We have tested several peptides for the ability to induce tolerance in NOD mice by testing if mice injected with those peptides resist development of disease (which implies deviation from immune-mediated killing of islet cells). We found several that can significantly delay development of disease. This means that T cells that recognize those peptides are causally involved in disease development.

In addition, candidate peptides that stimulate T cells have informed the creation of a type of recombinant protein called a 'tetramer' (which in this case mimic antigen expression in beta cells and are thus identify T cells that are able to react with beta cells). The tetramers have been used to quantify islet-reactive T cells in pre- and overtly diabetic mice. Using tetramers the development of antigen-specific T cells with the onset of disease will be determined. This analysis compliments the in vivo peptide-based induction of tolerance data.

Ultimately selected candidate tetramers will form the basis of reagent preparation that will be designed to target and eliminate diabetogenic T cells in pre-diabetic mice and recently-diagnosed diabetic mice. The intention is to create a monoclonal antibody or a synthetic single-stranded DNA molecule (an 'aptamer') that is reactive with a death receptor fused to an antigen-specific tetramer and to test the combinatorial reagent for the ability to eliminate specific T cells in vitro and in vivo.

Regulation of activation of tumor-infiltrating CD8+ T cells

Project summary:

CD8+ Cytolytic T Lymphocytes (CTL) play an essential role in killing of virus-infected and transformed cells but in unmanipulated hosts fail to control tumor growth. Although the frequency of antigen-specific T cells in cancer patients is low, demonstrable priming occurs in response to tumor growth. Investigation of animal models and tumor-bearing patients show production of antigen-specific CTL in the periphery but whose effector phase T cell function is suppressed upon entrance to the tumor, a phenotype postulated to contribute to tumor escape from immune-mediated eradication. This implies
the tumor microenvironment induces Tumor Infiltrating Lymphocyte (TIL) lytic dysfunction, a conclusion that was substantiated by several experimental approaches.

In murine colorectal carcinoma (MCA38) nonlytic TIL were shown to be recently-activated effector memory cells (CD44+CD62LloCD69+CD95L+CD122+CD127+). The dysfunctional lytic phenotype was subsequently shown to be due to a tumor-induced block in proximal TCR-mediated signaling that obviates a key kinase involved in T Cell Receptor signal transduction, ZAP70, in turn due to rapid inactivation of p56\(^{ck}\) upon contact with cognate tumor cells. During analysis of p56\(^{ck}\) isolated from TIL we observed that when nonlytic TIL form conjugates \textit{ex vivo} with cognate tumor cells, p56\(^{ck}\) co-immunoprecipitates with a 120kD protein, but whose identity and potential role in regulation of TIL function was unknown.

We have identified this novel p56\(^{ck}\) interacting partner: the adhesion molecule Protocadherin-18 (‘pcdh18’). We show that in cells of the hematopoietic lineage pcdh18 is expressed in activated central memory CD8\(^+\) T cells (CD44hiCD62LhiCD127hi) coincident with differentiation to the effector memory phenotype: CD8\(^+\)CD44hiCD62LloCD127hi. pcdh18 is expressed both in endogenous CD8\(^+\) memory T cells that accumulate as mice age, and those elicited by prior immunization with various antigens. (Furthermore, by gene array analysis we found that more than two dozen different inhibitory signaling receptor genes <ISR> are expressed in activated memory cells and TIL.) In addition, transfection of pcdh18 into primary CD8\(^+\) T cells (which do not express pcdh18) imparts the nonlytic TIL phenotype: defective proximal signal transduction, loss of effector phase functions, and Activation Induced Cell Death. Thus, these data reconcile prior observations concerning p56\(^{ck}\) activation status in TIL and identifies a novel activation marker of CD8\(^+\) effector memory T cells which can also function as a negative regulator of proximal TCR signaling and therein effector phase function.

Expression of several dozen ISR genes during activation of Central memory cells raises the question how can Central memory cells be efficiently activated? The fact that Central memory cells are indeed rapidly activated and expand \textit{in vivo} upon re-exposure to antigen supports the notion that either: not all ISR transcripts are translated, that the encoded proteins are unable to function, or the inhibitory signal is superseded by the activation signal (antigen recognition). It is also possible that ISR ligands are not expressed on antigen presenting cells during Central memory cell re-activation thus Ag-dependent activation is unimpeded. The notion that multiple ISR are expressed upon T cell activation but the availability of any given ligand controls ISR activity is supported by the observations that dendritic cells and endothelial cells can express ligands for multiple ISR and tumors commonly express ISR ligands (e.g. MCA38 tumors express pcdh18, B7-1 and possibly additional ISR ligands). Such redundancy in this system that restricts effector T cell function argues its physiological importance in governing the response to re-activation of the memory response.

Our current efforts strive to understand the dynamics of interaction between Inhibitory Signaling Receptors expressed on memory T cells and their ligands that are expressed on dendritic cells, endothelial cells, or tumor cells.
Relevant publications:


