Gene-environment interaction in type 1 diabetes mellitus

MASSIMO TRUCCO

Division of Immunogenetics. Children's Hospital of Pittsburgh. Rangos Research Center. Pittsburgh, Pa. USA.

Type 1 diabetes (T1D) mellitus can best be characterized as a disorder of gluco-regulation due to the insufficient production of a single critical hormone: insulin. Since the middle of the last century the most efficient pharmacologic solution has been to administer the hormone to the patient daily. Increasingly sophisticated dosing schedules together with the availability of recombinant variants of the hormone have succeeded in granting normal lifespan to type 1 diabetics. Nevertheless, no matter the degree of sophistication, current even aggressive regimens have not proven capable of faithfully recapitulating the normal performance of the endogenous insulin producing beta cells in response to glucose. This limit leads to the inevitable principal causes of morbidity and mortality associated with T1D, namely the complications of kidney and heart together with ocular and neural diseases.

While insulin replacement continues to be the primary treatment, the need to establish physiologic gluco-regulation in order to avoid complications has led to multiple avenues of alternative interventions, most of which are at the experimental stage. What all of these interventions have in common, however, is the hurdle imposed by the immune system at the level of ongoing autoimmunity and, in some cases, at the level of transplant rejection by the host[1,2]. Autoimmunity in T1D is characterized by an inflammatory response against the insulin-producing beta cells of the pancreas, a chronic inflammation around and in the pancreatic islets of Langerhans termed "peri-insulitis" and "insulitis", respectively. As studied in the two classic rodent models of the disease (diabetes-prone BioBreeding, DP-BB, rat and non obese diabetic, NOD, prone mouse), early on in the acute phase of the immune attack the islets exhibit an abundant cell infiltration by mononuclear cells, macrophages and dendritic cells (DC). With time, T-cells become the major constituent of the insulitis and are responsible for the greatest beta cell damage and destruction. The clinical onset manifests once the number of surviving beta cells cannot secrete sufficient insulin to satisfy the body's needs.

A strong genetic predisposition is a *conditio sine qua non* of T1D and a large body of studies support that key genetic susceptibility loci affect the genesis, function and survival of immune cell subsets. To understand the critical role played by the genetic predisposition in T1D, it is necessary to consider the processes that shape

Correspondence: Dr. M. Trucco.
Hillman Professor of Pediatric Immunology.
Head, Division of Immunogenetics.
Children's Hospital of Pittsburgh.
Rangos Research Center.
Pittsburgh, Pa, USA.
E-mail: mnt+@pitt.edu

the immune system: A randomized pool of immature cells continuously generated in the bone marrow (BM) eventually reach the thymus. Once in the thymus, these immature cells, individually expressing unique receptors, undergo positive and negative selection through receptor interaction with fragments of proteins (peptides) present in our bodies (the "self") by antigen-presenting cells (APC) once properly inserted in the peptide-binding groove of Major Histocompatibility Complex (MHC) molecules. Indeed, the epithelial thymus is now known to express a wide array of self-antigens, including insulin, all of which are normally produced by cells targeted in a number of autoimmune disorders. Human leukocyte antigens (HLA), the human MHC molecules, anchored in the membrane of thymic epithelial cells and other APC, display HLA/self-peptide complexes for T-cell receptor (TCR) interaction. A cell that interacts strongly with the HLA/self-peptide complex dies in the thymus and is thus eliminated, i.e., negatively selected. On the contrary, cells that interact poorly with the complex do not proliferate sufficiently or become unable to function (i.e., anergic) and are eventually lost. The cells showing affinities between these two extremes proliferate modestly, receive a positive signal (positive selection), survive and emerge from the thymus to circulate in the periphery. Once in the periphery, the cells that matured in the thymus (T cells) can be engaged by circulating APC. DC are extremely powerful APC that collect foreign or "ignored" (i.e., not previously exposed to the immune system) material, to present it as "new" antigens to T cells through their HLA molecule. These T cells interact with these new antigens promoting the phenomenon of epitope spreading (i.e., the expansion of newly recognized antigens) observed in the islet inflammation. The pathologic vicious circle of continuous presentation of old and new antigens, collected by the DC from the newly destroyed beta cells, to naive T cells in the pancreatic lymph nodes that eventually go back to the pancreas to kill other beta cells, is what eventually brings to clinically-overt diabetes[3,4].

The reduced expression of certain self-antigens, like insulin, at the thymus epithelium cell surface may interfere with a successful negative selection. Also, even in the case in which self-antigens are normally expressed, allelic forms of the HLA class II molecules (like the HLA-DQ that lack a charged amino acid at position 57 of its beta chain) were shown to be strongly correlated with the development of T1D. Conversely, resistance to the disease was found to be associated with the inheritance of an HLA-DQ allelic form with an aspartic residue at the same position (Asp57).The importance of this amino acid change has to do with the physical structure of the non-Asp57 alleles constituting class II molecules with a suboptimal functional groove. In fact, the molecular interactions that normally drive positive and negative selection are altered by the disease-associated HLA molecules so that even strongly self-reactive T cell clones are allowed to escape to the periphery[5-7].

Evidence of beta cell regeneration promoted by bone marrow or stem cell allo-transplantation in new-onset disease NOD mice has been observed by several groups. On this basis we were not surprised to see that in the NOD mouse as well, abrogation of autoimmunity is sufficient to promote regeneration or rescue of the insulin-producing beta cells in the host endocrine pancreas even after the onset of the disease[8]. These studies suggest that, although the physiological state of islet cells tends towards a fully differentiated phenotype, the lack of autoimmune aggression, together with the "danger" signals generated by massive beta cell destruction may trigger processes inside progenitors (whether islet-resident or ductal epithelium-resident) that result in some degree of islet cell regeneration[9-12]. T1D pathogenesis is then a dynamic process. Once self tolerance is lost and beta cells begin to be destroyed, the system reaches a new equilibrium in which the newly-differentiated beta cells are in turn eliminated by the ongoing autoimmune process.

Interestingly, we have recently shown that the *in vitro* treatment of DC with CD40, CD80, and CD86 antisense oligodeoxyribonucleotides (AS-ODN), reduce co-stimulatory molecule levels at their surface, producing functionally-immature DC capable of preventing or reversing new onset diabetes in the NOD mouse[13]. This was accomplished while maintaining T-cell responsiveness to alloantigens in animals that received repeated injections of modified DC. Co-stimulatory depleted DC also augmented the number of T regulatory cells (Treg) that were CD4+ CD25+ Foxp3+ through short-range IL-7 signaling[14]. We also are currently conducting an NIH-funded, FDA-approved phase I clinical trial that is designed to test the safety of AS-ODN-treated autologous DC into T1D patients with established disease (fig. 1). Leukocytes of the patient are obtained by apheresis and DC are generated *in vitro* from them and engineered in GMP facilities with the addition of AS-ODN. In turn, these DC, which express low levels of CD40, 80, and CD86, are injected into the patient by intradermal administration at an anatomical site proximal to the pancreas[13-15]. DC will migrate to the nearest, i.e., pancreatic, lymph nodes, where they are able to interrupt the vicious circle that maintains islet-specific inflammation, i.e., insulitis. In the pancreas, DC acquire beta cell specific antigens from apoptotic cells, leading to the eventual display of these antigens to naïve T-cells in the pancreas-draining lymph nodes. The lack of co-stimulatory molecules will result in an anergizing signal to the T-cells, induce regulatory immune cells (like Foxp3+ Treg), and interrupt the T-cell mediated anti-beta cell epitope-spreading phenomenon. Within the endocrine pancreas, once the insult of autoimmunity is abrogated, the physiologic process of regeneration might continue efficiently, eventually replenishing the population of insulin-producing cells to a number sufficient to maintain euglycemia, thus curing the diabetic recipient.

Thus far, we have not observed adverse events of any

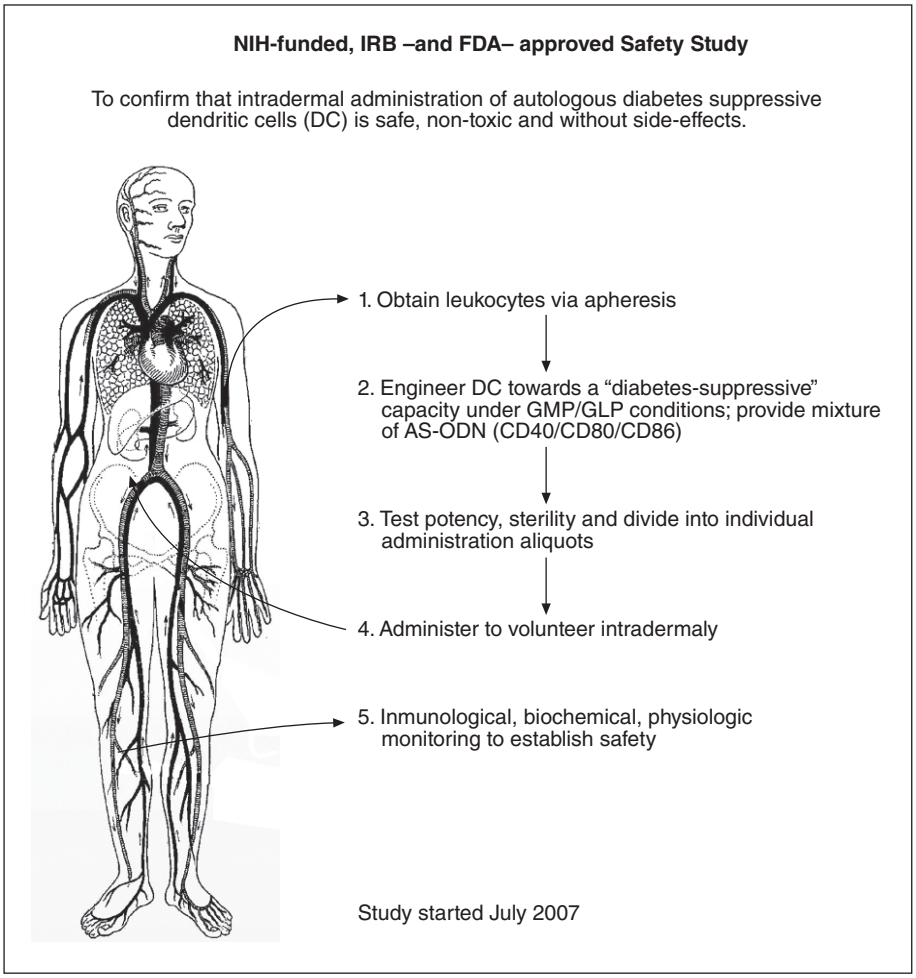

Fig. 1. Clinical trial for type 1 diabetes. Schematic of the procedures involved in the phase I of the trial, currently underway at the University of Pittsburgh, to prove the safety of the living DC-based vaccine. (Used by permission of Pediatric Diabetes, Giannoukakis et al[4].) AS-ODN: antisense oligodeoxyribonucleotides; DC: dendritic cells; GLP: good laboratory practices; GMP: good manufacturing practice.

sort, nor did the patients experience even subjective discomforts; the hematological and immunologic profiles after DC administration in all of the first six treated diabetic patients are similar if not identical to those measured at baseline pre-screening; there is no evidence of latent viral activation; there is no evidence of induction of any additional autoimmune reaction; there is no worsening of glycemia or increased insulin requirements; physical examinations and all biochemistry is within the normal range.

Conflict of interest

The author declares he has no conflict of interest.

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