

Inmunological effectors of beta cell damage in type 1 diabetes

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EFFECTORES INMUNITARIOS DE LA DESTRUCCIÓN DE CÉLULAS BETA EN LA DIABETES MELLITUS TIPO 1

RESUMEN

Gran parte de lo que sabemos sobre el desarrollo de la diabetes mellitus tipo 1 (T1D), se lo debemos a los estudios realizados en el ratón NOD, actualmente uno de los mejores modelos experimentales de esta enfermedad. Estudios realizados en esta cepa murina han demostrado que los linfocitos T son los principales efectores de la destrucción de las células beta pancreáticas. Sin embargo, también se ha observado que otras células del sistema inmune están implicadas en el desarrollo de la enfermedad. Entre ellas, las células dendríticas, los macrófagos y los linfocitos B, son imprescindibles tanto en el inicio como en fases más avanzadas de la enfermedad. El objetivo de la presente revisión es sintetizar los recientes conocimientos sobre el papel de estas poblaciones celulares como células efectoras en el desarrollo de la T1D.

PALABRAS CLAVE: T1D / Célula beta / Islo de Langerhans / Autoinmunidad / Linfocito / Autoanticuerpo / Célula Dendrítica / Macrófago.

ABSTRACT

To a large extent, our knowledge of the development of type 1 Diabetes Mellitus (T1D) comes from studies carried out in NOD (non-obese diabetic) mice, a diabetes-prone mouse model. Studies with this animal model have shown that islet-infiltrating T-lymphocytes are major effectors of β cell damage in T1D and that diabetogenesis is not only involved in the recruitment of autoreactive T-lymphocytes, but also of other cellular elements of the immune system. Of these, dendritic cells, macrophages and B-lymphocytes are believed to play a crucial role in the onset and/or progression of T1D, at least in NOD mice. The aim of the present review is to outline the current knowledge on the effector role of these cellular subsets in the development of T1D.

KEY WORDS: T1D / Beta cell / Islet of Langerhans / Autoimmunity / Lymphocyte / Autoantibody / Dendritic cell / Macrophage.

INTRODUCTION

Type 1 Diabetes Mellitus (T1D) becomes manifest after extensive loss of pancreatic β cells (Fig. 1) through an autodestructive (autoimmune) mechanism mediated by the patient's own immune system^(1,2). To date this is an irreversible process, and in consequence the patients depend on exogenous insulin administration to survive. Currently one of the best experimental models to study this autoimmune disease is the NOD mouse that spontaneously develops a

form of diabetes that in many aspects resembles human T1D⁽³⁾. NOD mice develop insulinitis (islet infiltration by mononuclear cells) at 3-5 wk of age and become diabetic at 3-4 months of age (Fig. 2 and 3). The incidence of the disease is about 60-90% in females and 10-40% in males^(4,5). Susceptibility of humans and NOD mice to T1D is under complex genetic control, including both Major Histocompatibility Complex (MHC) and non-MHC linked genes⁽⁶⁻¹⁰⁾. In humans, approximately 20 T1D loci are

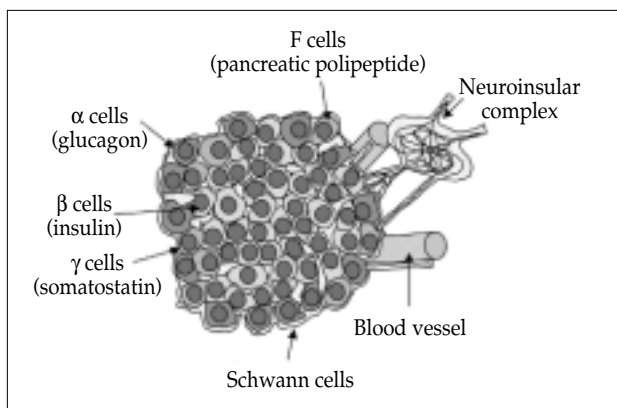


Figure 1. Schematic drawing of an islet of Langerhans. The islets consist of four major endocrine cell types: insulin-producing β cells (60%, the highest percentage of cells in the islets), glucagon-producing α cells (25%), somatostatin-secreting δ cells (10%), and F cells (or PP cells, 1%). Between the islet cells, a dense network of neurons and Schwann cells provide innervation from sympathetic, parasympathetic or sensorial autonomic nervous systems. The islet also contains a polar neuroinsular complex constituted by neurons and glial cells where most of the autonomic nerves regulating islet endocrine cell function converge.

associated with susceptibility to the disease^(7, 9-10). Only three of these loci are well characterised: the IDDM1 locus inside the class II MHC genes⁽¹¹⁾, the IDDM2 locus linked to the polymorphism in the promoter region of the preproinsulin gene^(12, 13), and the IDDM12 locus associated to the T-cell regulatory gene CTLA4⁽¹⁴⁾. In NOD mice, also about 20 loci linked to the disease have been described so far^(8, 15-19). Like in humans, the molecular nature and mechanism of action of most of the putative genes present in those loci are still unknown. In this regard, a clear association has been found only between the *Idd1* locus

and the H-2^{s7} haplotype, and also between *Idd5.1* locus and the CTLA4 gene^(14, 20). Moreover, a possible linkage of NRAMP1, and IL-2 or IL-21 genes with the *Idd5.2*, and *Idd3* loci, respectively, has also been established^(9, 21). In addition to the genetic control, other environmental factors play an important role in preventing or provoking the disease. A sterile environment, low room temperature and fiber rich diets raise the incidence and accelerate the onset of the disease^(4, 9, 22-28). Moreover, other stochastic factors also seem to play a major role in the incidence of the disease; some of them are linked to age (e.g., hormones) and/or to the state of maturation of the immune system (e.g., T and B-lymphocyte repertoire)^(9, 23, 29).

CD4⁺ AND CD8⁺ T LYMPHOCYTES IN THE COURSE OF T1D

Transfer experiments using splenocytes from prediabetic NOD mice showed that T1D transfer into immunodeficient NOD mice requires both CD4⁺ and CD8⁺ T-lymphocytes⁽³⁰⁻³³⁾. However, other studies have demonstrated that either CD4⁺ or CD8⁺ β cell-reactive T-lymphocyte clones isolated from diabetic NOD mice could transfer T1D⁽³³⁻³⁵⁾.

Since β 2-microglobulin-deficient (β 2m⁻) NOD mice do not develop insulinitis, it has been suggested that the first insult against β cells could be somehow mediated by cytotoxic CD8⁺ T-lymphocytes (CTLs)⁽³⁶⁻³⁸⁾. Additional experiments seem to support this idea: on the one hand reestablishment of MHC class I expression on β cells in β 2m-NOD mice restores susceptibility to insulinitis⁽³⁹⁾, and on the other splenocytes from healthy NOD mice cannot transfer insulinitis into β 2m-NOD.SCID mice⁽⁴⁰⁾. Although the mechanism by

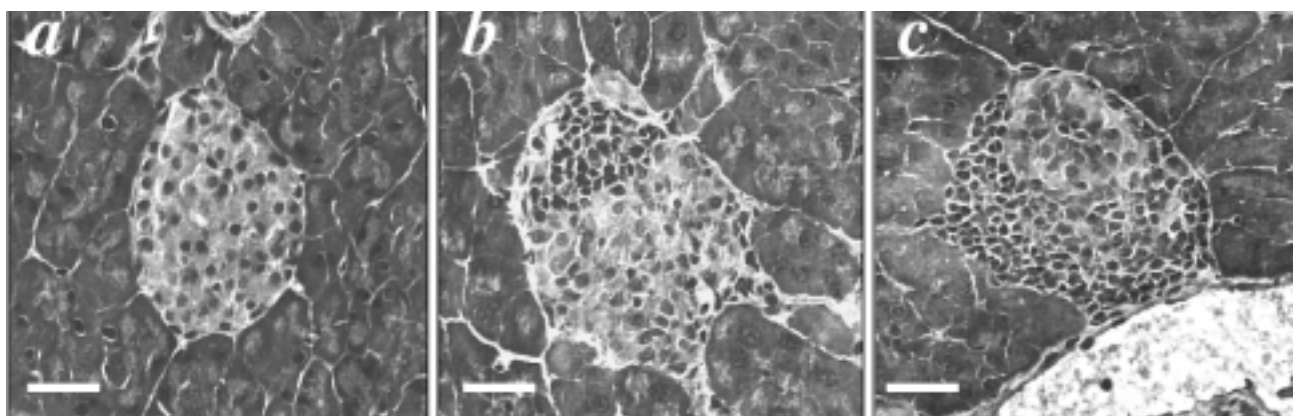


Figure 2. Several degrees of insulitis in islets from NOD mice. a) Islet of Langerhans from a 3 wk old female without infiltration. b) Islet from a 7 wk old female with mild to moderate insulitis. Mononuclear cell islet infiltration usually starts at the pole nearest to the capillary. c) Severe insulitis of an islet from a 12 wk old female (severe infiltration of mononuclear cells inside the islet). Images were obtained with a confocal microscopy (Axioskop2; ref.# 800779, Zeiss) from cryosections (5 μ m) of pancreata from NOD mice (3, 7 and 12 wk old females, respectively) stained with hematoxylin and eosin. Bar = 50 μ m (Puertas MC et al., unpublished observations).

which those CD8⁺ CTLs will initiate the disease is not known, it is possible that their recruitment to the islets could be mediated by the recognition of one or very few autoantigens in the context of the MHC class I H-2K^d. It has been shown that CD8⁺ CTLs present in the pancreatic islets of prediabetic and acutely diabetic NOD mice are usually H-2K^d-restricted and tend to use highly homologous TCR α -CDR3 sequences, suggesting recognition of immunodominant autoantigen/K^d complexes on β cells⁽⁴¹⁻⁴³⁾. This MHC restriction is not found on CD4⁺ T-lymphocytes, thus supporting the idea that their recruitment could occur by the shedding of β cell antigens after the initial β damage.

ROLE OF ANTIGEN PRESENTING CELLS (APCs) IN THE INITIATION OF THE AUTOIMMUNE DIABETES

Although T-lymphocytes are the major effectors of β cell damage, the cooperation of other cells of the immune system is required for the initiation and posterior development of the disease. These cells include dendritic cells, macrophages and B-lymphocytes⁽⁴⁴⁻⁴⁷⁾, which share the common function of presenting antigens to T-lymphocytes in the context of the MHC. In order to activate T-lymphocytes, the antigen needs to be presented with at least one additional co-stimulatory signal via membrane receptors (CD28/B7) and usually in the presence of stimulatory cytokines (ex: IL-2, IL-4). This first contact between APCs and T-lymphocytes takes place in the regional lymph nodes, where antigens and surveillant APCs from body tissues (e.g., pancreas) are driven by the lymph^(48, 49). Usually, APCs present antigens produced by themselves (cell catabolism) or coming from the tissular environment. This presentation is carried out in the absence of the additional co-stimulatory signal so that the putative T-lymphocytes that can recognise those autoantigens are anergised. It is well known that antigens coming from the intracellular metabolism are presented by class I MHC molecules to CD8⁺ T-lymphocytes. Contrarily, presentation of exogenous antigens takes place through class II MHC molecules to CD4⁺ T lymphocytes. However, exogenous antigens can also be processed and presented by the class I MHC pathway, a process known as «cross presentation»⁽⁵⁰⁾. In some cases cross presentation results in tolerance, whereas in others it activates T-lymphocytes^(51, 52). Factors involved in that decision include the antigen dose, timing, the state of activation of the APC and the presence of T helper cells in the environment^(35, 53-55). It has been suggested that this could represent a general mechanism involved in the initiation of autoimmune diseases⁽⁵⁰⁾.

At present time it is unclear why or when β cell specific pre-cytotoxic T-lymphocytes are primed in T1D, and which

APCs are involved in this activation. Several studies have shown that all three types of APCs could be involved in the presentation of putative autoantigens, rendering T-lymphocytes either tolerant or autoreactive to them. The type of APC, its state of activation and the environment where the presentation takes place may determine whether antigen presentation will result in activation or suppression⁽⁵⁶⁾.

Dendritic cells (DCs) preferentially capture soluble antigens (including proteins and peptides) and constitutively express co-stimulatory molecules (CD40L)⁽⁵⁷⁾. This gives DCs the capacity to induce T-lymphocyte responses, either Th1 or Th2. DCs are not only the most potent activators of naive T-lymphocytes; they also contribute significantly to the establishment of central and peripheral tolerance. Two main types of DC populations are found in mouse lymphoid organs: conventional DCs and plasmacytoid DCs (pDCs)^(58, 59). Conventional DCs comprise two subsets that are phenotypically differentiated by the presence in their surface of the CD8 molecule. Both subsets can activate T-lymphocytes, but only lymphoid-derived CD8⁺ DCs can induce apoptosis and produce cytokines characteristic of T-lymphocytes^(60, 61). On the other hand, pDCs are defined by the expression of B220 and Ly6-C, and produce large amounts of type I interferons during viral infections. Initially, it was believed that CD8⁺ DCs (CD8 α ⁺ Dec-205⁺ CD11b⁻) have their origin in a lymphoid lineage, whereas CD8⁻ DCs (CD8 α ⁻ Dec-205⁻ CD11b⁺) derive from myeloid precursors. At the present time, it is accepted that conventional and plasmacytoid DCs can differentiate from both common lymphoid and myeloid progenitors^(58, 59). Immunohistochemical studies in NOD mice have shown that DCs are already present in pancreatic islets before the development of insulinitis and persist throughout the disease⁽⁶²⁾. Recently it has been shown that these early DCs are of the CD8⁻ phenotype⁽⁴⁹⁾. Moreover, those early infiltrating DCs are able to activate T-lymphocytes *in vitro*⁽⁶³⁾. Thus, DCs could initiate autoimmunity and recruit autoreactive T-lymphocytes to the islets. Adoptive transfer of DCs constitutively expressing the immunodominant cytotoxic T-lymphocyte epitope of the Lymphocytic Choriomeningitis Virus Glycoprotein (LCMV-GP) induces autoimmune diabetes in RIP-GP transgenic mice expressing this glycoprotein in islet β cells. In RIP-GP mice, development of T1D was dependent on the dose and timing of antigenic stimulation⁽⁴⁷⁾. Some studies suggest that DCs from NOD mice have a defect in the maturation process⁽⁶⁴⁻⁶⁸⁾ and/or in the transcription factor NF- κ B⁽⁶⁹⁾ that results in an abnormal activation, which contributes to the development of a pathogenic Th1 immune response.

As DCs, macrophages also appear in pancreatic islets before the recruitment of T-lymphocytes^(62, 70). Macrophages can pick up protein complexes, including bacteria, cell

fragments, and apoptotic and necrotic cells. However, unlike DCs, macrophages do not express co-stimulatory molecules constitutively⁽⁵⁷⁾. Therefore, before priming T-lymphocytes, macrophages have to be activated. Although the role of macrophages in T1D seems to be more related to their scavenger function rather than to their APC function, a defective antigen presentation cannot be ruled out. In fact, defects in macrophage maturation and function have been described in NOD mice⁽⁷¹⁻⁷³⁾. This defect could result in an abnormal antigen presentation⁽⁷⁴⁾. Moreover, macrophages have been shown to be absolutely necessary for the development and activation of β -CTLs^(75, 76). In addition, T1D and insulinitis can be prevented in NOD mice by transferring allogeneic thymic macrophages, through a mechanism that is still unclear⁽⁷⁷⁾.

B LYMPHOCYTES AS APCs AND AS AUTOANTIBODY-SECRETING CELLS IN THE T1D

B-lymphocytes are thought to play a critical role in the initiation and/or progression of T1D, at least in NOD mice^(46, 78, 79). Although the mechanisms by which B-cells contribute to diabetes development remain poorly understood, it has been proposed that they do so by capturing β cell autoantigens via cell surface autoreactive immunoglobulins, and by presenting these antigens to autoreactive T-lymphocytes (80-82, Puertas M.C., et al., unpublished observations). Several lines of evidence support this notion. First, T1D development in both human and NOD mice is associated with presence of circulating autoantibodies against several β cell autoantigens^(83, 84). Second, transmission of β cell-reactive autoantibodies from pregnant NOD females to foetuses during pregnancy contributes to the progression of diabetes in the offspring⁽⁸⁵⁾.

Our current appreciation of the antigenic repertoire of autoreactive B-lymphocytes in T1D almost exclusively stems from studies of the antigenic specificity of circulating islet-reactive autoantibodies⁽⁸³⁾, and from analyses of the antigenic specificity of peripheral B-cell hybridomas (from human blood⁽⁸⁶⁻⁸⁸⁾ or rodent spleen⁽⁸⁹⁻⁹⁴⁾). In general, these studies have shown the existence, in both pre- and acutely diabetic individuals, of an extensive peripheral B-lymphocyte response against a wide range of β cell autoantigens, including GAD, IA-2, insulin, carboxypeptidase H, ICA69, and ganglioside GM2-1.

TOLERANCE TO PUTATIVE AUTOANTIGENS IN T1D

Several islet β cell autoantigens have been described as putative triggers of T1D, including insulin (or proinsulin), tyrosine phosphatase, IA-2, glutamic acid decarboxylase

(GAD), and heat shock protein 60 (hsp 60). How and why these autoantigens are recognised by the immune system as dangerous or foreign is still poorly understood. Defects in both central and peripheral tolerance to these autoantigens have been widely described. In thymic APCs, impaired antigen presentation by an unstable H-2 IA^{g7} molecule⁽⁹⁵⁾ or by a proteasome defect⁽⁹⁶⁾ is a possible cause of the lack of central tolerance (negative selection). In this context, it is interesting that diabetes and insulinitis can be prevented by intrathymic grafting of syngenic or allogenic islets^(97, 98). These results support the hypothesis of an altered mechanism of antigen presentation by thymic APCs that could only be overcome by the presence of β cell autoantigens in the thymus. It has also been suggested that a defect involving both Fas-dependent and Fas-independent apoptosis pathways may also be involved in the lack of central (as well as peripheral) tolerance⁽⁹⁹⁾.

Despite those major defects in thymic negative selection, a «normal» thymus also allows thymocytes with low grade of autoreactivity to survive negative selection and progress to mature T-lymphocytes. In fact, very often islet-reactive T-lymphocytes are found in the peripheral blood of healthy individuals. Those autoreactive T-lymphocytes are silenced by peripheral tolerance mechanisms that include peripheral deletion, immunologic ignorance, anergy, and suppression⁽¹⁰⁰⁾. Interestingly, suppression is mediated by a heterogeneous group of regulatory T-lymphocytes (*Treg*), some of which are naturally occurring suppressor T cells, whereas others are induced by specific ways of antigenic stimulation⁽¹⁰⁰⁻¹⁰⁸⁾. The naturally occurring suppressor T cells are present constitutively, and their regulatory effect is mediated by a cell-contact-dependent, and less by a cytokine-dependent mechanism. This group includes CD4⁺CD25⁺ T-lymphocytes, natural killer (NK) T-lymphocytes, and probably $\gamma\delta$ T-lymphocytes^(102, 103). By contrast, the population of acquired regulatory T cells can be obtained from peripheral T cells *in vitro* after antigen-engaging and in presence of IL-10 and TGF- β , and also after induction of oral tolerance⁽¹⁰⁹⁻¹¹¹⁾. This group of *Treg* cells is composed mainly by two subpopulations of CD4⁺ T cells, the Tr1 and Th3 cells, that depend on IL-10 and/or TGF- β to exert their suppressive function^(101, 102, 112). Finally, three different subsets of CD8⁺ T lymphocytes have been defined that elicit suppressor functions on antigen-specific T cells⁽¹⁰⁴⁾. These CD8⁺ *Treg* cells act by preventing the expression of co-stimulatory molecules on APCs (type 1), by inhibiting the secretion of IFN- γ and IL-6 (type 2), or through the secretion of cytokines, most importantly of IL-10 (type 3). In the last years, many studies have proved how important *Treg* cells are in the maintenance of natural self-tolerance, and thus to prevent autoimmune diseases including T1D^(101, 102, 106-116).

FUNCTIONAL STATE OF PANCREATIC β CELLS AND AUTOIMMUNE DIABETES.

Besides the autoimmune response, the functional condition of islet β cells may play a decisive role in the pathogenesis of T1D. Beta-cell dysfunction and death, early during the prediabetic stage of T1D has been underlined to contribute to the autoimmune process^(49, 117, 118). Also, increased functional activity of islet β cells has been related with a higher incidence of the disease^(27, 63, 117). In NOD mice, preventive treatments with insulin that reduce the functional activity of islet β cells result in a lower incidence of diabetes⁽¹¹⁹⁾. These results suggest that an increase in islet β cell activity may drive islet β cells to stress, which will ultimately induce: i) an increased antigen presentation on islet β cells; ii) a possible production of new autoantigens (neoantigens); and/or iii) an increased β cell death and the consequent production and release of danger signals (heat-shock proteins, uric acid, interferons, nucleotides, extracellular matrix breakdown products...). In fact, β cells from NOD mice may have constitutive components that determine a higher susceptibility to be destroyed by cytotoxic cytokines (IL-1 β , TNF, and IFN- γ)^(120, 121). Thus, it is likely that in NOD mice the chances of autoantigen presentation by APCs to islet β cell autoreactive T-lymphocytes is higher than in other related strains.

CRITICAL CHECKPOINTS DURING THE PROGRESSION OF THE DISEASE

Despite our limited knowledge of the onset of this disease, we can draw a close picture of the progression of the disease regarding insulinitis and clinical outcome. In this disease context, two crucial checkpoints have been established⁽¹²²⁾ (Fig. 3). The first coincides with the onset of insulinitis, and the second takes place later, with progression from severe insulinitis to overt diabetes. In NOD mice, the onset of the disease occurs at three to five weeks of age with a few islets being affected by an incipient insulinitis. At this period, the islet infiltrate is mainly constituted by dendritic cells and macrophages, but also by a few B- and T-lymphocytes^(49, 62, 70, 123). What triggers the initiation of insulinitis remains uncertain. On the one hand, β cell malfunction or death, which would induce the expression and release of neoantigens and some endogenous danger signals, could trigger the activation of the innate immunity that would finally result in an immune response specifically targeted against autoantigens^(49, 117, 118, 124). On the other hand, functional defects of the APCs may be responsible for an inappropriate APC/T-lymphocyte interaction that would lead to the initiation of the adaptive immune response^(64-68, 71-74, 124). Moreover, the presence of maternal transferred autoantibodies against β cell autoantigens,

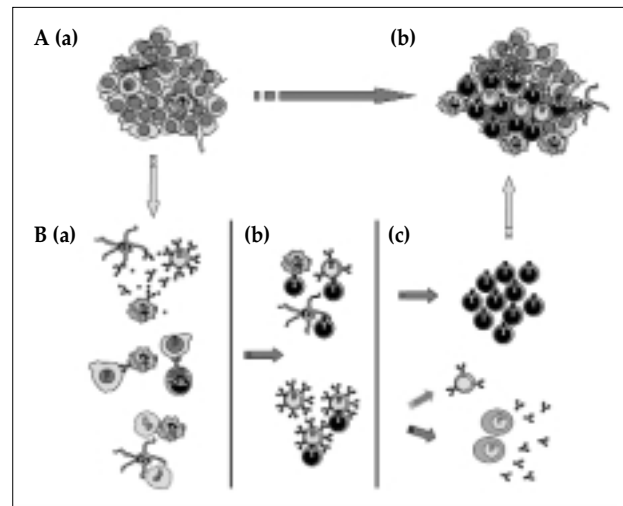


Figure 3. Scenario of several steps (critical checkpoints) during islet inflammation (122). A (a) Schematic drawing of an islet before the beginning of islet infiltration (3 wk old NOD mice). At this period only a few dendritic cells and macrophages accumulate around ducts and inside the islets^(49, 62, 123). The development of the infiltration marks the first critical checkpoint of the disease. (b) Severely infiltrated islet composed mainly by B- and T-lymphocytes during disease progression (characteristically found in islets from 12 wk NOD mice). At this period, the islet recruitment of a pathogenic T-lymphocyte population with high avidity for β cell autoantigens represents progression from benign inflammation to highly destructive infiltration (29, 127, 128). In this period the action of NK cells may also be decisive (129). This step marks the second critical checkpoint of the disease. B (a). During the perinatal period, islet-surveilling APCs capture β cell autoantigens (by phagocytosing dead β cells, soluble autoantigens and probably immunocomplexes, constituted by autoantibodies, autoantigens, and complement)⁽⁴⁹⁾. Subsequently, the autoantigens are processed and presented in the context of the MHC. For unknown reasons, this capture leads to APC activation and maturation, and later to their migration to the regional lymph nodes. During this period of time, maternal-transferred autoantibodies may also be involved in antibody-dependent cellular cytotoxicity of β cells, and thus cause the release of cytoplasmic autoantigens⁽⁸⁵⁾. (b). In the pancreatic regional lymph node, activated APCs present the autoantigens to autoreactive T-lymphocytes, therefore inducing their activation and proliferation. Later, activated T-lymphocytes (CD4⁺ Th2) induce the activation of autoreactive B-lymphocytes and subsequently form germinal centers at the follicular lymphoids. (c) Activated autoreactive CD4⁺ and CD8⁺ T-lymphocytes leave the pancreatic regional lymph nodes through the efferent lymphatic vessels. Once in the bloodstream, the pathogenic T-lymphocytes go to the pancreatic islets driven by chemokine signals where they carry out their cytotoxic activity selectively on β cells^(29, 126, 127). Islet antigen specific B-lymphocytes become either APCs, autoantibody-secreting plasma cells or memory B-cells probably with the specific function to perpetuate autoantibody production⁽⁷⁹⁻⁸³⁾. B-cells with a memory cell phenotype are found in the infiltrated islet, thus suggesting a possible role as APC in situ (Puertas MC et al., unpublished observations).

may facilitate the formation of immunocomplexes and their subsequent capture, processing and presentation by APCs. The existence of this mechanism has been confirmed in other autoimmune pathologies^(125, 126). This may explain at least in part, the decisive role of maternal transferred autoantibodies in the initiation or acceleration of the autoimmune response⁽⁸⁵⁾.

As the disease progresses, most pancreatic islets are being affected by an insidious and progressive insulinitis (62, 122,

123, Puertas MC, et al., unpublished observations). The characteristics of the infiltrated islets change over this period, and at twelve to fifteen weeks of age most islets are affected by severe inflammation composed primarily by T- and B-lymphocytes. The presence of severe insulinitis does not always imply β cell destruction. The progression of benign inflammation to overt diabetes is preceded by islet recruitment of a pathogenic T-lymphocyte population with high avidity for β cell autoantigens^(29, 127, 128). This highly reactive T-lymphocyte population is a more efficient destroyer of β cells than the preceding infiltrated mononuclear cells, and thus β cell loss is accelerated after its recruitment. Recent findings in some NOD transgenic models suggest that NK cells can also play a decisive role in the loss of β cells during this last period of the disease (129; Alba A et al., personal communication).

Clinical disease approximately appears when ninety percent of the insulin secreting cell population has been destroyed. The absence of recruitment of the highly pathogenic T-lymphocytes and possibly NK cells will delay or prevent the final development of diabetes. This feature may explain at least in part why diabetes never appears in some individuals despite the existence of massive insulinitis.

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