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Review article

Cytokines and beta-cell destruction

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Several genetic and environmental factors appear to cooperate to precipitate type1 diabetes, a spontaneous autoimmune disease in humans and in the nonobese diabetic (NOD) mouse. NOD mice, like type1 diabetic patients, develop insulitis, an early infiltration of leukocytes into the pancreas that leads to inflammatory lesions within the islets. However, overt type1 diabetes requires the subsequent destruction of the vast majority of insulin producing islet β-cells, a process mediated by activation of autoreactive T-helper type1$CD4^+$ and subsequently, $CD8^+$ T- cells[1].

ROLE OF CHEMOKINES

T-cells invading pancreatic islets are observed at the time of diagnosis of type 1 diabetes. However, timing for this invasion (long time or only shortly before diagnosis) is unknown. Knowledge about this timing is of importance for the planning of intervention trials aiming to prevent type 1 diabetes. Recently, a new class of small molecules guiding cellular migration has been described, the so-called chemokines. Chemokines have been divided into four subfamilies according to the position of cysteine residues: the CXC family with an amino acid between two cysteines, the CC family with none, the C family with only one cysteine, and the CX3C family with three amino acids between two cysteines. Chemokines act on their effectors via specific receptors that belong to a seven-transmembrane domain G protein-coupled family. These receptors are expressed on leukocytes, among them T-cells. Chemokine receptors are critically involved in the extravasation of cells into inflamed tissue (e.g., the insulitis in type 1 diabetes). Moreover, the repertoire of chemokine receptors is associated with the functional aspects of cells. Th1 cells are associated with CCR5 and CXCR3 and Th2 cells with CCR3 and CCR4 chemokine receptors. This finding is intriguing for the pathogenesis of type 1 diabetes since this disease is likely to be triggered by Th1 lymphocytes, whereas Th2 lymphocytes may be protective. Indeed, in another T-cell-mediated autoimmune disease, multiple sclerosis, Th1-associated chemokine receptors on peripheral blood lymphocytes were found to be a marker for immune activity of the disease. A striking reduction of the Th1-associated chemokine receptors CCR5 and CXCR3 on peripheral blood lymphocytes was found at the time of diagnosis of type 1 diabetes. This finding was reproduced for CD3 and CD4 cells and correlated with a reduced stimulation of the Th1-associated cytokines IFN-γ and TNF-α by phytohemagglutinin (PHA). It is assumed that Th1-associated peripheral T-cells are reduced in a narrow time window at the time of diagnosis of diabetes, possibly due to extravasation in the inflamed pancreas. Thus, chemokine receptor expression of peripheral blood lymphocytes may be a useful surrogate marker for the immune activity of type 1 diabetes (e.g., in intervention trials)[2].

ROLE OF CYTOKINES

An increased expression of cytokines in the insulitis has been reported, and different combinations of cytokines have been shown to decrease the function and viability of β-cells in vitro, partially through the production of nitric oxide and/or reactive oxygen species by the β-cells themselves or by cells in their vicinity, such as macrophages and endothelial cells. It has been hypothesized that initial damage to β-cells could result in the release of intracellular components, leading to inflammation and activation of a β-cell directed immune response, followed by destruction of all insulin-producing cells[3].

Interleukin 1 β

Interleukin (IL)-1β has been proposed as an effector in the immune-mediated destruction of pancreatic β-cells. Its putative role in the development of type 1 diabetes is based on in vitro observations indicating an IL-1–induced toxicity in isolated rodent islets. It is amplified by a simultaneous presence of IFN-γ and TNF-α. This combination of cytokines is also cytotoxic in human islet preparations. IL-1β can kill β-cells by necrosis as well as by apoptosis. However, induction of one or the other form of cell death depends on the presence of additional environmental factors. In vitro experiments do not provide information on the effectors and mode of β-cell death in type 1 diabetes. This objective is difficult to achieve with in vitro models. Data indicate that if IL-1β is released within the islets of the human pancreas, the cytokine will not necessarily cause the massive

necrosis seen in cultured rat islets. To achieve this effect in human islets, local IL-1 levels will have to be chronically elevated and lead to toxic intercellular nitric oxide (NO) concentrations; this will probably require sufficient inducible nitric oxide synthase (iNOS) induction in β-cells or neighboring non–β-cells as well as a reduced flow of the interstitial fluid. If these conditions are not met, the cytokine may cause apoptosis of β-cells if these are simultaneously and chronically exposed to other cytokines such as IFN-γ. Alternatively, IL-1β may influence the functional state of the β-cells by altering their phenotype and, hence, their responses to physiological and pathophysiological agents[4].

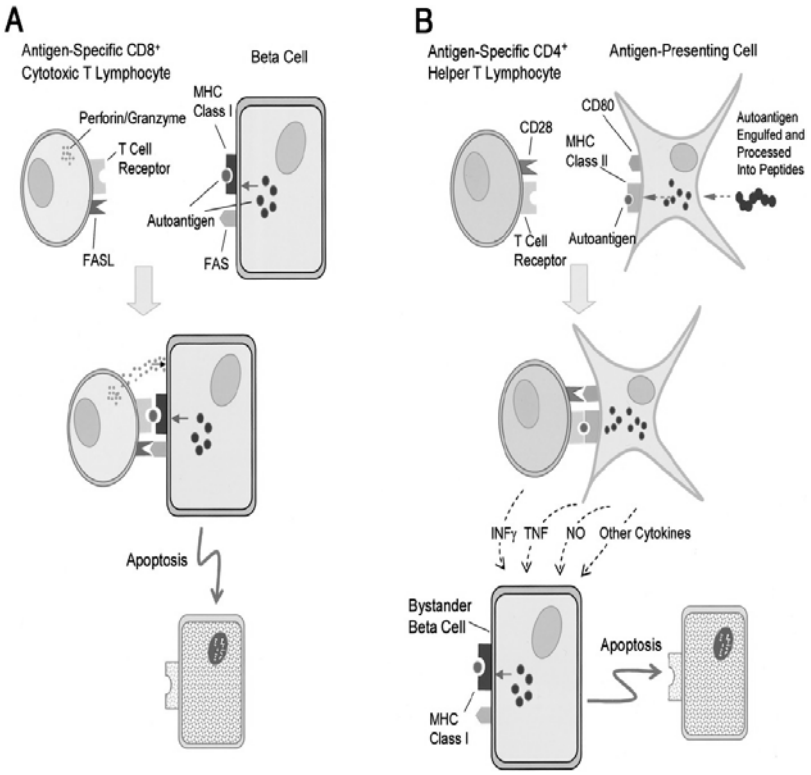

Figure 1: Mechanisms of β- cell destruction (Quoted from Notkins, 2002[17]).

Tumour necrosis factor-α

Tumor necrosis factor (TNF)- related apoptosis-inducing ligand (TRAIL) is expressed in different tissues and cells, including pancreas and lymphocytes, and can induce apoptosis in various tumor cells but not in most normal cells. TRAIL gene expression is upregulated in pancreatic islets during the development of autoimmune type 1 diabetes in nonobese diabetic (NOD) mice[5].

Studies have suggested that TNF-α expression is increased in the pancreatic β cells of NOD mice, and this localized TNF-α production plays a critical role in both the initiation of insulitis and the subsequent progression to β cell destruction. NOD mice deficient in TNFR1 receptor develop insulitis similar to that of wild-type NOD mice; however, progression to diabetes is not observed, indicating that β cell toxicity may occur via a TNFR1-dependent mechanism. Transcriptional inhibition in the liver with D-galactosamine increases by several thousand-fold the sensitivity of mice to the lethal effects of TNF-α. D-galactosamine selectively blocks transcription in hepatocytes by depleting uridine nucleotides necessary for the production of mRNA transcripts. Treatment with TNF-α in conjunction with D-galactosamine results in acute liver apoptosis and liver failure. This model of liver injury and lethality is mediated by TNF-α signaling through the TNFR1 receptor, resulting in activation of caspases and subsequent hepatocyte apoptosis[6].

Autoreactive $CD4^+$ T cells play a major role in the pathogenesis of autoimmune diabetes in nonobese diabetic (NOD) mice. Non-MHC genetic background controls enhanced entry into the IFN-γ pathway by NOD T cells. Increased IFN-γ, decreased IL-4, and decreased IL-10 production in NOD T cells is CD4 T cell intrinsic. Therefore, the non-MHC NOD genetic background controls the cytokine phenotype[7].

Understanding of the final effector phase of autoimmune diabetes is becoming an area of intense research, due to recent realization of the importance

of apoptosis in a variety of physiological or pathological conditions. Autoreactive T lymphocytes are the most important effector cells in autoimmune diabetes, and they ultimately induce apoptosis of β islet cells. CD4+ T lymphocytes are the final effector cells in β-cell destruction, whereas CD8+ T lymphocytes play a role in the initiation period of islet destruction. The role of macrophages as effector cells has also been suggested. Besides their role as APCs, they may also be a source of oxygen radicals or other soluble cytotoxic mediators. Although effector cells and their roles are rather clearly defined, it is far from clear which molecule(s) are the real effector(s) in autoimmune diabetes.

A combination of IFN-γ and TNF-α, but neither cytokine alone, induced classical caspase-dependent apoptosis in insulinoma and pancreatic islet cells. IFN-γ treatment conferred susceptibility to TNF-α-induced apoptosis on otherwise resistant insulinoma cells by STAT1 activation followed by IFN regulatory factor (IRF)-1 induction. IRF-1 played a central role in IFN-γ/TNF-α-induced cytotoxicity because inhibition of IRF-1 induction by antisense oligonucleotides blocked IFN-γ/TNF-α-induced cytotoxicity, and transfection of IRF-1 rendered insulinoma cells susceptible to TNF-α-induced cytotoxicity. STAT1 and IRF-1 were expressed in pancreatic islets of diabetic NOD mice and colocalized with apoptotic cells. Moreover, anti-TNF-α Ab inhibited the development of diabetes after adoptive transfer. Taken together, these results indicate that IFN-γ/TNF-α synergism is responsible for autoimmune diabetes in vivo as well as β-cell apoptosis in vitro and suggest a novel signal transduction in IFN-γ/TNF-α synergism that may have relevance in other autoimmune diseases and synergistic anti-tumor effects of the two cytokines[8].

Islet-specific expression of TNF-α can play a dual role in autoimmune diabetes, depending on its precise timing in relation to the ongoing autoimmune process. In a transgenic model (rat insulin promoter-lymphocytic choriomeningitis virus) of virally induced diabetes, TNF-α enhanced disease incidence when induced through an islet-specific tetracycline-dependent promoter system early during pathogenesis. Blockade of TNF-α during this phase prevented diabetes completely, suggesting its pathogenetic importance early in disease development. In contrast, TNF-α expression abrogated the autoimmune process when induced late, which was associated with a reduction of autoreactive CD8 lymphocytes in islets and their lytic activities. Thus, the fine-tuned kinetics of an autoreactive process undergo distinct stages that respond in a differential way to the presence of TNF-α. The precise duration of TNF-α expression is an important factor determining the progress of an ongoing autoimmune process to diabetes. However, later during pathogenesis, too much of such an inflammatory factor is not beneficial to the propagation of the autoimmune process. This could occur in vivo, for example, by a second infection by the same or another unrelated virus capable of inducing inflammatory factors. Indeed, such a scenario has been observed in rat insulin promoter-lymphocytic choriomeningitis virus (RIP-LCMV) model, in which secondary viral infection can abrogate ongoing autoimmunity by enhancing apoptosis of autoreactive lymphocytes. Furthermore, TNF-α was demonstrated in various other occasions to confer immunoregulatory activities by down-regulation of type 1 cytokines, suppression of T cell proliferation and cytokine production, or preventing the development of self-reactive T lymphocytes[9].

BREAKDOWN OF SELF-TOLERANCE

There is mounting evidence that $CD4^+CD25^+$ T cells represent an important mechanism for the maintenance of self-tolerance. $CD4^+CD25^+$ T cells prevent experimentally induced organ-specific autoimmune disease. Removal of these cells from mice that normally do not develop autoimmune disease can result in autoimmunity. The effects of TNF in disease, are often contradictory and puzzling. Elevation of TNF levels during the neonatal period in NOD mice intensifies the diabetic process, while injection of neutralizing anti-TNF into newborn NOD mice resulted in complete prevention of disease. Interestingly, if TNF treatment was started in the adult NOD mouse, disease onset was delayed, and the incidence of diabetes was decreased or abrogated. Anti-TNF administered during the young adult period did not yield complete protection from diabetes and may even accelerate the diabetic process. NOD mice have a relative deficiency of CD4+CD25+ T cells in thymus and spleen and administration of TNF or anti-TNF to NOD mice can modulate levels of this population consistent with their observed differential age-dependent effects on diabetes in the NOD mouse. These data suggest that alterations in the number and function of $CD4^+CD25^+$ T cells may be one mechanism by which TNF and anti-TNF modulate type I diabetes mellitus in NOD mice[10].

In the periphery, activation of self-reactive $CD8^+$ T cells usually involves cross-presentation of exogenous self-antigen by antigen presenting cells

(APCs) bearing MHC self-peptides. Such cross-presentation normally induces expansion followed by deletion of self-reactive $CD8^+$ T cells. Breakdown in tolerization of self-reactive $CD8^+$ T cells can lead to autoimmunity. It is unclear what governs whether APCs promote expansion and survival of activated self-reactive $CD8^+$ T cells or their expansion followed by deletion. Inflammatory cytokines have been implicated in the breakdown of tolerance to self-antigens[11].

ANTIGEN PRESENTING CELLS

The differentiation of naive $CD8^+$ T cells into effector cytotoxic T lymphocytes (CTLs) normally requires the interaction of three types of cells: APCs bearing peptide–MHC complexes, $CD4^+$ T cells, and $CD8^+$ T cells. $CD4^+$ T cell help the differentiation of naive $CD8^+$ T cells into effector CTLs is at the level of the APC, where initial engagement of $CD4^+$ T cells with APCs triggers the maturation of the APC into a cell fully competent to prime $CD8^+$ T cells. Interactions between CD40 on APCs and CD154 on activated $CD4^+$ T cells play a dominant role in the generation of T and B cell immunity. Several autoimmune conditions have been shown to be dependent on CD40–CD154 signals, and in diabetes, blockade of CD40–CD154 signals abrogates both insulitis and diabetes. TNF-α enhances autoimmunity in NOD mice by provoking islet-infiltrating APCs to cross-present exogenous islet antigen to $CD8^+$ T cells. Effector $CD4^+$ T cells are not required for the priming of islet-specific $CD8^+$ T cells. TNF-α can substitute for $CD4^+$ T cell help in the cross-presentation of exogenous islet antigen to $CD8^+$ T cells by overcoming the need for CD154 signals in the activation of islet-infiltrating APCs. These findings reveal that inflammatory stimuli may promote autoimmunity by thwarting normal $CD4^+$ T cell–dependent CD154 immune regulatory mechanisms[11].

NATURAL KILLER T LYMPHOCYTES

Extensive work in the early 90s established the importance of the Th1/Th2 paradigm and showed that Th2 $CD4^+$ lymphocytes can dampen Th1-mediated immune responses. Later experiments highlighted the importance of a new subset of lymphocytes, termed NKT cells, in the regulation of Th2 cell differentiation. These NKT cells are distinct from other thymus-derived lymphocytes in that they express some markers typical of NK cells, as well as a quasi-invariant T cell receptor (TCR) type. Following stimulation of this TCR, NKT cells produce large amounts of IL-4, which drives the differentiation and growth of Th2 cells. This finding suggested that NKT cells could directly control physiological Th2-driven immune responses and thus indirectly regulate Th1-mediated autoimmunity.

Because NKT cells are abundant in the liver and bone marrow but are present in very small numbers in peripheral lymphoid organs, this hypothesis was initially controversial, but it soon received strong support from experiments performed in genetically manipulated mice. In particular, work with mice lacking the β2 microglobulin chain, which are NKT cell-deficient because they lack the MHC class I-restricting element CD1d, indicated that NKT cells are indeed important players in Th2 differentiation and IgE production. Moreover, Vα14Jα281 transgenic mice, which harbour a large population of NKT cells, show heightened Th2 polarization and high serum levels of IgE. Data showed a major deficiency of NKT cell numbers in nonobese diabetic (NOD) mice, a well-established model of spontaneous, autoimmune T-cell mediated insulin-dependent diabetes. Both the number of NOD NKT cells and the functional capacity of these cells, as assessed by IL-4 release following TCR ligation, are dramatically reduced. Moreover, in vivo treatment with the specific NKT cell TCR ligand α-galactosyl ceramide (α-GalCer, a glycolipid derived from a marine sponge) prevents disease in NOD mice. Over-expression of NKT cell-associated Vα14Jα281 TCR chain in transgenic mice has a similar effect. Conversely, the onset of diabetes was reported to be accelerated in NOD mice lacking CD1d expression, consistent with a pathogenic role for NKT cell deficiency. Unfortunately, such studies in human patients are intrinsically limited by the poor representation of NKT cells among circulating mononuclear cells[12].

In their report, Lee et al (2002)[13] have characterized NKT cells using double staining with a CD1d–α-GalCer tetramer and a Vα24 specific antibody. They found that the numbers of NKT cells did not differ between diabetic patients, subjects at high risk for developing the disease, and normal controls. IL-4 production by NKT cells was also similar among these groups, as assessed by intracytoplasmic staining following short-term phorbol myristate acetate (PMA) and ionomycin stimulation. These observations are in clear distinction with previous reports showing both a numerical and a functional defect in the IL-4 producing ability of NKT cells in diabetic patients, as compared to both discordant twins and normal controls.

The discrepancies between these studies are difficult to explain, since the methods for detecting NKT cells and the functional assays employed differ significantly. While the use of CD1d tetramers in Lee et al[13] report adds considerably to the specificity of detection, this protocol may also identify low avidity tetramer-binding cells whose biological relevance is questionable. Moreover, the assumption that α-GalCer binds to all NKT cells may be incorrect in light of some reports showing that there are invariant and non-invariant CD1d-restricted T cells that do not respond to this glycolipid. Hence, the double-staining procedure applied may have missed some NKT cells. Conversely, since α-GalCer loaded CD1d tetramers have been reported to stain CD1d-restricted T cells not expressing the invariant Vα24 TCR chain, it is also possible that some of the cells identified by this procedure are not NKT cells. Finally, because Lee's group examined whole mononuclear cells that had been triggered with PMA and ionomycin, it is not possible to compare their data with those of earlier reports, where clones of NKT cells were expanded in culture and stimulated in a more physiological manner via the TCR[12].

PROTECTIVE CYTOKINES

The mechanism underlying suppression of immune responses by interleukin-4 (IL-4) has remained unexplained. Antigen-presenting dendritic cell (DC) is central to counter-regulation of autoimmune disease by IL-4. Stimulation of cytotoxic precursors by antigen pulsed dendritic cells induces their differentiation but the process is blocked by IL-4. IL-4-influenced DC to produce distinct effects on $CD8^+$ T cells depending on their state of activation. The molecular basis for this regulation is the alteration of the expression ratio of the costimulatory ligands B7.1/B7.2 on dendritic cells. B7.2 induces expansion of $CD8^+$ T cells and B7.1 governs their acquisition of cytolytic activity. IL-4 influences the dendritic cell to elicit qualitative differences in T-cell responses, providing the basis for the counter-regulation mediated by IL-4[14].

Using the biodegradable polymer, poly[alpha-(4-aminobutyl)-L-glycolic acid] (PAGA), interleukin-4 (IL-4) plasmid (pCAGGS-IL-4), was injected intravenously at the age of 4 weeks to prevent autoimmune insulitis in NOD mice. At 6 weeks after the injection, the grade of insulitis of the mice was evaluated by double blind methods. In vitro transfection assays showed that PAGA enhanced the expression of IL-4 in 293T cells. In the plasmid/PAGA complex injected group, the prevalence of severe insulitis in NOD mice was markedly improved, suggesting that PAGA enhanced the delivery of IL-4 plasmid. The pCAGGS-IL-4/PAGA complex is thus an effective system to prevent autoimmune insulitis in NOD mice and is applicable for the prevention of autoimmune diabetes[15].

Several findings have recently questioned the long held hypothesis that cytokines belonging to the Th2 pathway are protective in T-cell-mediated autoimmunity. It was found that pancreatic expression of IL-4 activated islet antigen-specific BDC2.5 T cells and rendered them able to trigger insulin-dependent diabetes mellitus in ins-IL-4/BDC2.5 mice. IL-4 is mainly known as the Th2-driving cytokine. However, IL-4 is also critical for DC maturation and upregulation of antigen uptake and presentation by macrophages. Pancreatic expression of IL-4 activated self-reactive BDC2.5 T cells by increasing islet antigen presentation by macrophages and dendritic cells. Therefore, IL-4 could have triggered self-antigen presentation within the pancreatic islets both by driving maturation of DC from a tolerizing to a priming state and by increasing self-antigen uptake by macrophages16.

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