Cell Death Mediators in Autoimmune Diabetes—No Shortage of Suspects

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A hallmark of many organ-specific autoimmune diseases is an exquisitely specific destruction of one of the cell types that makes up the organ. Over the years, much interest has been focused on the initiator phase of such disorders, exploring the factors that permit or provoke the autoimmune attack, and devising means to interfere with them. More recently, no doubt a reflection comes from a variety of observations: that CD8+ T cells provoke the autoimmune attack, and devising means to molecules. Evidence consistent with such a mechanism is an exquisitely specific destruction of one of direct recognition of a target on the CD8+ T cell. A consensus seems to be emerging that if part of the secretory machinery, or picked up from the target, has not proven a simple task. Mice provoke diabetes upon transfer into T cell±T cell receptor (TCR) transgenic (tg) models. The nature of the maximum destruction of the maximum destruction of the target cells. So far, this has not proven a simple task.

Studies on insulin-dependent diabetes mellitus can be taken as an example (for reviews, see Bach, 1994; Tisch and McDevitt, 1996). This disorder is characterized by specific destruction of the insulin-producing β cells of the islets of Langerhans of the pancreas, resulting in insufficient insulin production, eventually leading to hyperglycemia. Much of what we know (or at least suspect) about the etiology and pathogenesis of autoimmune diabetes comes from studies on small animal models—in particular the nonobese diabetic (NOD) mouse, which spontaneously develops a disease with many of the features of the human disorder. There has emerged a complex picture of pathogenesis in the NOD mouse, involving distinct phases and multiple lymphocyte populations. It has been suggested that disease progression is regulated at two checkpoints (reviewed in Andrè et al., 1996): first, no signs of pathology are evident until 3–5 weeks of age, when leukocyte infiltration of the islets (or insulitis) begins; second, the islet infiltrates remain rather harmless until about 12–15 weeks of age, when active destruction of the β cells (culminating in diabetes) ensues. It is known that T lymphocytes are critical throughout the unfolding of disease, but the role of particular subsets has been rather controversial. A consensus seems to be emerging that CD8+ T cells somehow initiate the process, CD4+ T cells are the predominant islet invader during the early stages, and both CD4+ and CD8+ cells are required for the maximum destruction of β cells (discussed at length in Wang et al., 1996). To aid in elucidating the roles of the different subsets, several groups have developed transfer or T cell receptor (TCR) transgenic (tg) models that focus on a particular CD4+ or CD8+ T cell specificity directed at either a natural or artificial diabetogenic antigen.

Although the destruction of pancreatic islet β cells is the defining characteristic of autoimmune diabetes, we are still rather ignorant about the events immediately preceding and directly responsible for β cell death. It is known that the process ultimately depends on T cells, but it is not at all clear whether they promote destruction primarily by direct or indirect means. Two general mechanisms have been proposed, as reviewed by Bach (1994) and Tisch and McDevitt (1996) and illustrated in Figure 1. The first (recognition-linked) attributes the specificity of destruction to cytotoxic T cell recognition of autoantigens displayed by major histocompatibility complex (MHC) molecules on β cells. Thus killing is provoked by direct recognition of a target on the β cell, and necessitates T cell±β cell contact. In mice, this would imply recognition of class I-restricted antigens by CD8+ cells because β cells appear not to express MHC class II molecules. Evidence consistent with such a mechanism comes from a variety of observations: that CD8+ cells follow CD4+ cells into the islets when splenocytes from diabetic donors are transferred to healthy recipients; that both CD4+ and CD8+ T cells are required to transfer diabetes even though the former, alone, can invade host islets; that certain CD8+ clones isolated from diabetic mice provoke diabetes upon transfer into T cell-deficient hosts; that diabetes but not insulitis is reduced when adult (insulitic) NOD mice are injected with anti-MHC class I or anti-CD8 monoclonal antibodies (MAbs); and that CD8+ T cells from diabetic animals can specifically attach to and lyse β cells in vitro. It might also be worth mentioning that CD8+ T cells often, though not always, dominate islet infiltrates in diabetes patients. However, there exists evidence against this scenario, notably that some CD4+ T cell clones isolated from diabetic mice can transfer diabetes in the absence of CD8+ cells in transfer or transgenic systems. In addition, it was recently reported that splenocytes from diabetic donors can transfer disease into hosts lacking MHC class I molecules.

The second proposed mechanism for islet β cell destruction (activation-linked) is an indirect one, wherein the proximity of β cells to angry T cells leads to their death. It stemmed from a pair of perplexing observations: CD4+ T cells can promote diabetes in NOD mice in the absence of CD8+ cells, yet MHC class II molecules do not appear to be expressed on β cells. Thus, it has been proposed that potentially pathogenic CD4+ cells are stimulated by recognition of autoantigens encountered on more typical antigen-presenting cells (APCs) within the islets, such as macrophages and dendritic cells (pancreatic autoantigens being shed from β cells, if part of the secretory machinery, or picked up from damaged or phagocytized β cells, perhaps following primary CD8-mediated cytolysis); as a result of such T±APC interactions, the activated T cell may directly kill the bystander β cell (for example, through Fas/FasL interaction) (a) in Figure 1), may produce soluble mediators that induce β cell death (b), or may activate the cytotoxic functions of macrophages (c). The nature of critical soluble mediator(s) is controversial: interferon (IFN)γ, interleukin (IL)-1, tumor necrosis factor (TNF)α, IL-6, and nitric oxide have all been implicated. An additional complexity is that some of these molecules have
potent synergistic effects when in combination, and that
certain of them are active, sometimes differentially, as
either membrane or soluble forms. Also debated is at
what point the β cells, themselves, enter the process
ultimately leading to destruction: i.e., are they induced
to synthesize certain of these mediators, hastening their
own death? Interestingly, it has been reported that in
vitro incubation of islets with certain cytokines leads
to destruction of β cells preferentially, providing a potential
explanation for the β cell specificity of killing during
diabetes, although this has been disputed. At present,
we are far from being able to draw a coherent picture of the
events immediately preceding β cell death during auto-immune attack. It will probably turn out that they
can die by multiple means during the development of
diabetes and this could evolve through the course of
disease.

This issue of Cell brings new information on this impor-
tant matter. Chervonsky et al. (1997 [this issue of Cell])
report experiments suggesting a role for Fas/Fas ligand
(FasL) interactions in β cell death in at least one form
of autoimmune diabetes. Three points are most relevant.
First, NOD lpr/lpr mice, deficient in Fas expression be-cause of an incapacitating mutation in the fas gene did
not develop spontaneous diabetes. Second, transfer of a particular NOD-derived, islet-reactive CD8⁺ T cell
clon (Wong et al., 1996) into young, irradiated NOD
animals led to diabetes several days later, but a parallel
transfer into NOD lpr/lpr recipients, did not provoke dis-
ease. Transfer into animals engineered to express FasL constitutively on islet β cells promoted diabetes unusu-
ally rapidly. Third, after transfer of the CD8⁺ T cell clone
into NOD mice, expression of Fas was rapidly induced on β cells. These data provide strong evidence that Fas
is a major mediator of β cell death in this CD8⁺ T cell
transfer model of diabetes. It is not yet clear whether
Fas-mediated death plays an important role in the sponta-
nous NOD model. The significance of the reported
block in spontaneous diabetes in NOD lpr/lpr mice is
obscured by the known pleiotropic effects of fas
gene mutation—e.g., lymphadenopathy, dysregulation
of T cell populations, polyclonal B cell stimulation,
strong constitutive up-regulation of FasL on lympho-
cytes (Chu et al., 1995, and references therein). This
problem could have been circumvented at least partially
by transfer experiments infusing polyclonal populations
of effector T cells from diabetic NOD mice into irradiated
NOD versus NOD lpr/lpr recipients.

The possibility that distinct mechanisms of β cell
death might reign in the different diabetes models is
underlined by recent observations made by other
groups. Zinkernagel, Hengartner, and colleagues have
studied a model of autoimmune diabetes based on a
transgenic mouse line that expresses the glycoprotein
of lymphocytic choriomeningitis virus (LCMV) specifi-
cally in islet β cells (Ohashi et al., 1991). These mice are
free of pathogenesis until infected with LCMV, when
they develop rampant insulitis and diabetes. By crossing
in a null mutation of the perforin gene, this group
demonstrated that their diabetes model is dependent on per-
forin-mediated cytotoxicity (Kági et al., 1996), consistent
with the fact that CD8⁺ T cells are known to be the
primary effector cells. Evidence that perforin was actu-
ally playing its role at the stage of β cell destruction
was two-fold: (1) that only diabetes, not insulitis, was
affected; and (2) that activated T cells from a TCR tg
mouse line expressing an LCMV glycoprotein-specific
TCR and also carrying the perforin mutation could trans-
fer insulitis but not diabetes into wild-type recipients,
while cells from TCR tg littermates not bearing the muta-
tion transferred both. Katz and colleagues have argued
for the importance of still another mediator of β cell
death on the basis of results with a third diabetes model
(Pakala et al., 1997). These experiments consisted of
grafting islets from wild-type or various mutant mice
into animals previously treated with streptozotocin to
induce diabetes, and monitoring the integrity of the graft
over time. They found that islet grafts from wild-type
mice or mutants lacking Fas, the α chain of the IFNγ
receptor (R), or TNF-R2 were destroyed, while those
from mutants devoid of TNF-R1 survived. This would
seem to implicate a TNF/TNF-R1 interaction in the death
of β cells in this model, in line with the many reports
implicating TNFα in the progression to diabetes in NOD
mice (see discussion in Sarvetnick, 1996).

So the picture we have at the moment is a cloudy
one: data from three different diabetes models implicat-
ing three distinct death effector systems—Fas/FasL,
perforin, and TNF/TNF-R1—and not yet clearly indicat-
ing which of them (or others) are most important in the

Figure 1. Two Proposed Mechanisms for Is-
let β Cell Destruction in Autoimmune Dia-
betes

[Diagram of two proposed mechanisms for islet β cell destruction in autoimmune diabetes, labeled as Recognition-Linked and Activation-Linked, with arrows and labeled components such as APC, IL-1, IFNγ, NO, IL-6, TNFα, and Mφ.]
NOD model, not to mention diabetes in human patients. With regard to humans, it is important to keep in mind that patients can present with quite heterogeneous clinical parameters (Bach, 1994), and thus diabetes in man could be a set of related disorders with possible differences in inciting antigen, primary effector cell type, and, most relevant here, mechanism of β cell destruction. It is not known at present which of the mouse models will prove the best for studying which of the human variations. Neither is it known whether islet grafts are subject to the same or different mechanisms of destruction, an important question given the increasing interest in using islet xenografts to attenuate disease.

Although this is clearly a complicated issue, and the data reported so far suggest that its resolution will be complex, it is an issue well worth tackling because of the obvious therapeutic implications: the potential to engineer death-defying β cells, an achievement which could significantly advance islet graft technology. One might attempt to block the different candidate death effector molecules one by one, and in combination, in the various mouse models. This could be achieved by treatment with the appropriate blocking reagents (MAbs, soluble receptors), by introducing the relevant null gene mutations or dominant negative mutants in a time-controlled fashion and specifically on islet β cells, or only on the relevant attacking population of lymphocytes. (Wholesale knockout or transgenic approaches to manipulate such molecules have pleiotropic effects, and would be difficult to unravel.) The technology to accomplish this is available, if heavy (reviewed in Spencer, 1996). Even more rigor and ingenuity will need to be applied to the human system. Although correlations between enhanced expression of particular death effector molecules and autoimmune destruction (e.g., Dowling et al., 1996; Giordano et al., 1997) are tantalizing, they cannot be taken as proof of causality. Here too, other strategies have to be devised—for example, more effectively exploiting the potential of humanized severe combined immunodeficiency (SCID) mice (Möller, 1991).

Given the importance of the task to be accomplished, the difficulties raised above should not be viewed as encumbrances, but as stimuli.

**Selected Reading**


