Cell, Vol. 89, 1–3, April 4, 1997, Copyright ©1997 by Cell Press

Cell Death Mediators in Autoimmune Diabetes—No Shortage of Suspects

Minireview

Christophe Benoist and Diane Mathis
Institut de Génétique et de Biologie Moléculaire et Cellulaire
(CNRS/INSERM/ULP)
1 rue Laurent Fries
67404 Illkirch
C. U. de Strasbourg
France

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 of the impressive advances in the cell death field (reviewed in Vaux and Strasser, 1996), greater attention has been paid to the effector phase, the goal being to delineate the mechanism(s) by which the autoimmune attackers promote death 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 André 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 invaders 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 can provoke 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 just 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 cytotoxicity); as a result of such T–APC interactions, the actived 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 cytocidal functions of macrophages (c). The nature of critical soluble mediator(s) is controversial: interferon (IFNγ), interleukin (IL)-1, tumor necrosis factor α (TNFα), IL-6, and nitric oxide have all been implicated. An additional complexity is that some of these molecules have

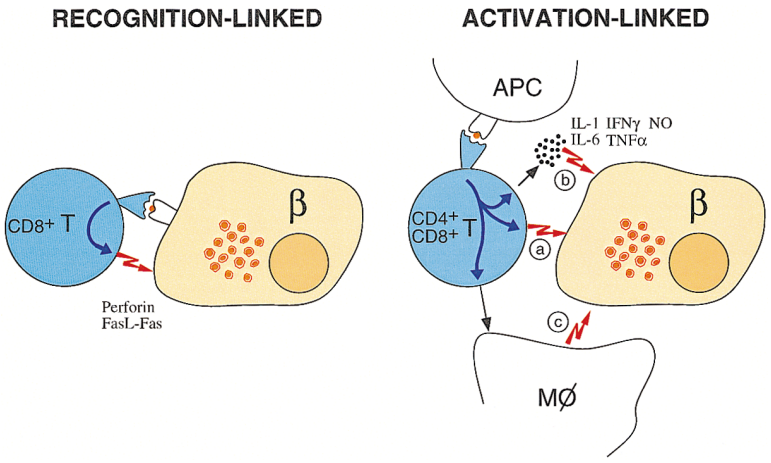

Figure 1. Two Proposed Mechanisms for Islet β Cell Destruction in Autoimmune Diabetes

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 autoimmune 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 important 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 because 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 clone (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 disease. Transfer into animals engineered to express FasL constitutively on islet β cells promoted diabetes unusually 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 spontaneous NOD model. The significance of the reported block in spontaneous diabetes in NOD *lpr/lpr* mice is obscured by the known pleiotropic effects of the *fas* gene mutation—e.g., lymphoadenopathy, dysregulation of T cell populations, polyclonal B cell stimulation, strong constitutive up-regulation of FasL on lymphocytes (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) specifically 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 perforin-mediated cytotoxicity (Kägi et al., 1996), consistent with the fact that $CD8^+$ T cells are known to be the primary effector cells. Evidence that perforin was actually 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 transfer insulitis but not diabetes into wild-type recipients, while cells from TCR tg littermates not bearing the mutation 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 implicating three distinct death effector systems—Fas/FasL, perforin, and TNF/TNF-R1—and not yet clearly indicating which of them (or others) are most important in the

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 can not 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

André, I., Gonzalez, A., Katz, J., Wang, B., Benoist, C., and Mathis, D. (1996). Proc. Natl. Acad. Sci. USA *6*, 2239–2264.

Bach, J.F. (1994). Endocrine Rev. *15*, 516–542.

Chervonsky, A.V., Wang, Y., Wong, F.S., Visintin, I., Flavell, R.A., Janeway, Jr., C.A., and Matis, L.A. (1997). Cell, this issue.

Chu, J.L., Ramos, P., Rosendorff, A., Nikolic-Zugic, J., Lacy, E., Matsuzawa, A., and Elkon, K.B. (1995). J. Exp. Med. *181*, 393–398.

Dowling, P., Shang, G., Raval, S., Menonna, J., Cook, S., and Husar, W. (1996). J. Exp. Med. *184*, 1513–1518.

Giordano, C., Stassi, G., De Maria, R., Todaro, M., Richiusa, P., Papoff, G., Ruberti, G., Bagnasco, M., Testi, R., and Galluzzo, A. (1997). Science *275*, 960–963.

Kägi, D., Odermatt, B., Ohashi, P.S., Zinkernagel, R.M., and Hengartner, H. (1996). J. Exp. Med. *183*, 2143–2152.

Möller, G., ed. (1991). The Scid mouse. Immunol. Rev. *124*, 5–220.

Ohashi, P.S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C.T., Odermatt, B., Malissen, B., Zinkernagel, R.M., and Hengartner, H. (1991). Cell *65*, 305–317.

Pakala, S.V., Chivetta, M.L., and Katz, J.D. (1997). J. Allergy Clin. Immunol. *99*, 6380.

Sarvetnick, N. (1996). J. Exp. Med. *184*, 1597–1600.

Spencer, D.M. (1996). Trends Genet. *12*, 181–187.

Tisch, R., and McDevitt, H. (1996). Cell *85*, 291–297.

Vaux, D.L., and Strasser, A. (1996). Proc. Natl. Acad. Sci. USA *93*, 2239–2244.

Wang, B., Gonzalez, A., Benoist, C., and Mathis, D. (1996). Eur. J. Immunol. *26*, 1762–1769.

Wong, F.S., Visintin, I., Wen, L., Flavell, R.A., and Janeway, C.A. (1996). J. Exp. Med. *183*, 67–76.