

MHC Molecules and β -Cell Destruction

Immune and Nonimmune Mechanisms

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Hyperexpression of major histocompatibility complex (MHC) molecules by islet cells is a prominent, early feature of islet pathology in insulin-dependent diabetes mellitus and concomitant with β -cell failure after exposure of islets to specific cytokines or viruses. The transgenic expression of a class I MHC gene (*H-2K^b*) in the β -cells of either syngeneic or allogeneic mice leads to β -cell failure by a nonimmune mechanism. Several class II MHC transgenes, with one exception, have the same effect, but the expression of other transgenes that have products that are membrane proteins is not necessarily detrimental. Class I MHC molecules have been shown to interact directly with other membrane proteins. The inappropriate expression of MHC molecules could therefore interfere with key cellular functions. We postulate that the hyperexpression of MHC molecules in the β -cell, e.g., in response to viruses, is a primary, nonimmune mechanism of β -cell failure that precedes a secondary autoimmune response. *Diabetes* 38:815–18, 1989

It is generally accepted that insulin-dependent diabetes mellitus (IDDM) is due to the selective autoimmune-mediated destruction of β -cells within the islets of Langerhans. The disease process is probably triggered by environmental agents, e.g., viruses or chemical toxins, on a background of genetic susceptibility associated with particular alleles within the major histocompatibility complex (MHC) (1).

MOLECULAR PATHOLOGY OF ISLETS

In the animal models of IDDM, i.e., the BioBreeding (BB) rat and the nonobese diabetic (NOD) mouse, islet pathology is

characterized by a mononuclear cell infiltrate termed *insulinitis* (2,3). Although opportunities to study the pancreas in humans with IDDM are limited, the analysis of postmortem (4) and pancreas-isograft biopsy (5) specimens has revealed similar, although less florid, insulinitis in which CD8⁺ (cytotoxic/suppressor) T-lymphocytes predominate (4,5). In addition, Foulis et al. (6,7) have drawn attention to the fact that the human lesions exhibit hyperexpression of class I MHC molecules on islet cells in association with immunoreactive interferon- α (IFN- α). Whether this finding represents a non-specific response to injury or signifies a specific disease process such as a persisting virus infection remains to be determined.

Activated mononuclear cells are a source of cytokines, two of which, IFN- γ and tumor necrosis factor (TNF), significantly impair human β -cell function (8; Table 1) and potentially enhance islet cell immunoreactivity by upregulating the expression of MHC molecules (9–13). Human interleukin 1 (IL-1) has also been shown to impair β -cell function but apparently does not alter the expression of MHC molecules (14). One of the striking effects of these cytokines is their synergism: IFN- γ and TNF combined at lower concentrations almost completely abolish glucose-stimulated insulin secretion from cultured islets and, after several days, destroy the morphological integrity of the islets (8; Table 1). Either agent alone upregulates class I MHC molecules, whereas combined they induce the expression de novo of class II MHC molecules on islet cells (11,15).

The effects of IFN- γ , TNF, and IL-1 provide a molecular basis for β -cell destruction in autoimmune insulinitis but raise two important and related questions. Do the effects of these cytokines in vitro reflect the molecular pathology in vivo, and how is the specificity of β -cell destruction explained? Palmer et al. (16) report that IL-1 β selectively impairs β -cell function in rat islets in vitro, although we have been unable to demonstrate a differential effect of IFN- γ and TNF- α on β -cells. Whether β -cells compared with α -cells (glucagon) or δ -cells (somatostatin) are more susceptible to the effects of extracellularly applied cytokines may not be the question. In the presence of a specific autoantigen, cytokines could be de-

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TABLE 1
Effects of interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) on glucose-stimulated insulin release from mouse islets

| Culture treatment | Insulin released (ng \cdot 6 islets ⁻¹ \cdot 60 min ⁻¹) | |
|--------------------------------------|---|------------------|
| | 2 mM glucose | 20 mM glucose |
| None | 0.62 \pm 0.03 | 9.27 \pm 0.62 |
| IFN- γ (U/ml) | | |
| 200 | 0.61 \pm 0.02 | 12.03 \pm 1.04 |
| 2000 | 0.50 \pm 0.02 | 6.08 \pm 0.39* |
| TNF- α (U/ml) | | |
| 200 | 0.54 \pm 0.04 | 11.41 \pm 0.43 |
| 2000 | 0.70 \pm 0.04 | 4.81 \pm 0.51* |
| IFN- γ + TNF- α (U/ml) | | |
| 200 | 0.64 \pm 0.02 | 2.10 \pm 0.11* |
| 2000 | 0.54 \pm 0.01 | 0.64 \pm 0.02 |

* $P \leq .01$ compared with no treatment.

livered in a targeted fashion into the microenvironment of the β -cell or even directly into the β -cell by contiguous cytotoxic T-lymphocytes. Concomitant upregulation of class I MHC molecules would enhance the targeting of cytotoxic T-lymphocytes; the expression of class II MHC molecules might amplify the autoimmune response by enabling the β -cell to present autoantigen.

Several lines of evidence implicate viruses in initiating the islet lesion that results in β -cell destruction (17). We have observed that one of the consequences of direct infection of cultured mouse or human islet cells by reoviruses or Coxsackie viruses is the upregulation of class I MHC molecules (18). In the rat insulinoma line RINm5F, reovirus-induced upregulation of class I MHC mRNA and protein is accompanied by induction of the enzyme 2',5'-oligoadenylate synthetase, a marker of IFN action. However, we have not identified any IFN-like activity in the cell medium, suggesting that double-stranded RNA viruses might directly activate transcription of class I MHC genes (18).

HYPEREXPRESSION OF MHC MOLECULES—COMMON DENOMINATOR OF β -CELL DYSFUNCTION?

Hyperexpression of class I MHC molecules is a concomitant of impaired β -cell function in response to specific cytokines or viruses. It is also a prominent early feature of the islet pathology in human IDDM. Therefore, it seems logical to ask whether hyperexpression of class I MHC could be a mechanism not only for enhancing lymphocytotoxic immune responses but also for causing direct nonimmune impairment of β -cell function. The opportunity to examine this question was afforded by transgenic mice bearing the class I MHC gene *H-2K^b* linked to the rat insulin II promoter (19). The construct was expressed in mice of different class I MHC haplotypes: *kk*, *bb*, *bs*, or *ss*. The remarkable outcome was the development of diabetes in the absence of mononuclear cell infiltration of the islets, even in mice syngeneic for *H-2K^b*. Diabetes occurred only in mice expressing the *H-2K^b* transgene product. Islets expressing the transgene exhibited a progressive decrease in both insulin mRNA and protein and by 3–4 wk after birth were significantly shrunken but retained a normal complement of glucagon- and somatostatin-containing cells. The *H-2K^b* transgene product, al-

though expressed on the surface of β -cells as demonstrated by flow cytometry, was predominantly cytoplasmic, possibly because of the limiting concentrations of β_2 -microglobulin in the cells. The finding that neonatal thymectomy, which prevented the transgenic mice from rejecting allogeneic skin grafts, did not prevent them from developing diabetes confirmed the nonimmune nature of β -cell destruction. Furthermore, immunodeficient C57BL/6 nude mice expressing the transgene also developed diabetes (J. Allison and J.F.A.P. Miller, unpublished observations).

INFERENCES FROM TRANSGENIC EXPRESSION OF MHC MOLECULES

The results of the *H-2K^b* transgenic experiment raise two important questions: How does tolerance develop to a foreign class I MHC protein expressed in a peripheral site outside the thymus, and how is the hyperexpression of class I MHC associated with β -cell destruction? Neither question can be answered definitively at this time.

Tolerance to allogeneic *H-2K^b* might be explained by 1) cryptic expression of the transgene in the thymus, although this is unlikely because *Aspergillus* nuclease *S₁* mapping has failed to detect *H-2K^b* mRNA in the thymus; 2) shedding of peripherally derived *H-2K^b* and its uptake by thymus dendritic cells; 3) inability of β -cell-presented *H-2K^b* to elicit an active immune response; and 4) induction of suppressor activity by peripherally presented *H-2K^b*. Which, if any, of these possible mechanisms is operative is under investigation.

In addressing the nonimmune nature of β -cell destruction in these transgenic mice, the first consideration is whether the effect is specific. Would the expression of any transgene in the β -cell lead to impairment of its unique differentiated function, e.g., by nonspecifically impeding pathways for insulin processing and exocytosis? The answer to this question appears to be no. The studies cited in Table 2 demonstrate that the expression of a transgene in the β -cell is not always associated with β -cell destruction. They also demonstrate that an adverse effect of transgene expression cannot be attributed just to extra copies of the insulin promoter, which could bind essential transcription factors. Clearly, transgenic expression of a gene whose product, i.e., calmodulin, is tightly regulated in all cells is detrimental. On the other hand, the transgenic expression of a membrane protein, i.e., influenza hemagglutinin or herpes simplex glycoprotein D, is not necessarily detrimental. Transgenic expression of several MHC genes, excluding *I-A^k*, results in β -cell destruction. The exception suggests a degree of specificity based on the type of MHC gene product. Nevertheless, further controls are necessary to establish the specificity and mechanism of MHC transgene effects. These should include the transgenic expression of genes for other membrane proteins and MHC mutants linked to the insulin promoter and MHC genes linked to other tissue-specific promoters.

If the adverse effect of MHC transgene expression in the β -cell is a specific phenomenon, what mechanism could be involved? Conventionally, MHC molecules bind antigenic peptides and restrict immune responses. However, antigen binding might be a special case of a broader function for MHC molecules that antedated the evolution of the vertebrate immune system, i.e., the interaction with endogenous

TABLE 2
Transgenes expressed in mouse pancreatic β -cells

| Regulatory element | Transgene product | Diabetes | Ref. |
|--------------------------|--|--|-------------------------------|
| Rat insulin II | Class I MHC <i>H-2K^b</i> | Yes | 19 |
| | Class II MHC <i>I-E^a/E^b; I-E^b</i> | Yes | 21 |
| | Class II MHC <i>I-A^k</i> | No | D. Mathis* |
| | Influenza hemagglutinin | Disruption of islet architecture; most mice hyperglycemic but few overtly diabetic | |
| | Chicken calmodulin | Yes | J. Sambrook* P.C. Epstein* |
| | Oncogenes | | |
| | SV40 T antigen | No | 22 |
| | H-ras | Yes (males only) | D. Hanahan* |
| | Human p53 | No | |
| | Mouse p53 | No | |
| | Mouse <i>c-myc</i> | No | |
| | Mouse <i>v-fos</i> | No | |
| | Human placental lactogen | No | |
| | Polyoma large T antigen | No | |
| Polyoma middle T antigen | No | | |
| Human insulin | Class II MHC <i>I-A^g/A^g</i> | Yes | 20 |
| | Mouse interferon- γ | Yes | 20 |
| | Herpes simplex glycoprotein D | No | T. Stewart* |

*Personal communication.

molecules to modify intra- and intercellular signal recognition and transduction events. Class I MHC molecules have been shown to interact noncovalently with membrane receptors for insulin, glucagon, and EGF (23) and to associate, possibly via disulfide bonds, with the CD8 molecule of cytotoxic/suppressor T-lymphocytes (24,25). Although the physiological significance of these interactions is unknown, we hypothesize that the inappropriate expression of MHC molecules could interfere with key functions of other mole-

cules and ultimately impair cell viability. It would not matter whether the interactions were totally indiscriminate or restricted to particular endogenous molecules if the consequences were the same. Of possible relevance to a broader regulatory role for MHC molecules and our hypothesis is our observation that the expression of class I and class II MHC molecules is increased when the differentiated function of β -cells is incompletely expressed in the human fetal pancreas and in human insulinomas (26).

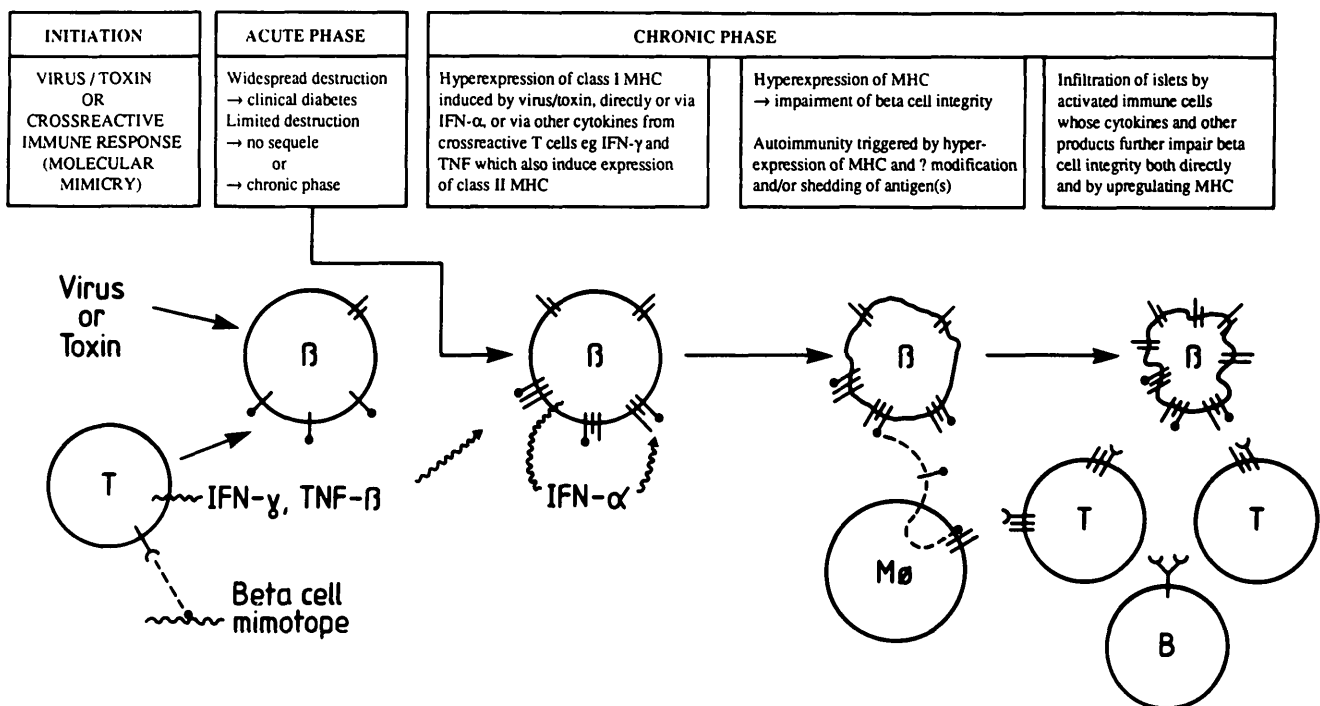


FIG. 1. MHC expression and molecular pathogenesis of β -cell destruction. Scheme is hypothetical and not inclusive of all possible elements or mechanisms. β -Cell destruction is presumed to be influenced by many genes in addition to those for MHC. Type and concentration of MHC molecule may be critical for nonimmune and immune effects. $\{$, T-lymphocyte receptor for antigen; $\}$, antigen; \parallel , class I MHC; $\|$, class II MHC; $M\phi$, macrophage; Υ , antibody.

CONCLUSIONS

The transgenic experiments demonstrate that the hyperexpression of MHC molecules leads to impairment of β -cell function by nonimmune mechanisms. Even if these mechanisms are not strictly specific, the results have important implications for the molecular pathogenesis of IDDM. Hyperexpression of MHC molecules, in response to certain virus infections or cytokines, and as demonstrated in the diabetic pancreas *in situ*, could be a primary event leading directly to β -cell destruction. We propose a scheme for the molecular pathogenesis of β -cell destruction in IDDM that emphasizes this primary, preautoimmune role for MHC hyperexpression in response to agents that might initiate β -cell destruction (Fig. 1). The specificity of β -cell destruction is attributed to the tropism of initiating agents, antigen-directed targeting of cytotoxic T-lymphocytes, or a differential susceptibility of β -cells to injury, e.g., mediated by cytokines and free radicals.

Finally, if hyperexpression of MHC molecules leads directly to β -cell death, why does autoimmunity not develop secondarily against residual β -cells in transgenic mice? This question may be another way to ask about the basis of tolerance to the transgene product in these mice. Perhaps the answer has to do with the autoantigen or the nature of its relationship with MHC and associated molecules in the natural models of IDDM versus experimental transgenic mice.

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