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Rapid communication

A CD8+ T-lymphocyte-mediated and CD4+ T-lymphocyte-independent autoimmune diabetes of early onset in transgenic mice

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Summary While transgenic mice expressing tumour necrosis factor-alpha under the control of the beta-cell-specific insulin promoter display a marked lymphocytic infiltration of the islets, they never develop insulin-dependent diabetes mellitus (IDDM). In striking contrast, "double" transgenic mice whose beta cells express both tumour necrosis factor-alpha as well as the co-stimulatory B7-1 molecule all develop IDDM at an early age. Further, administration of anti-CD8 but not anti-CD4 immunoglobulins prevents diabetes onset. These results indicate that while tumour necrosis factor-alpha induced lymphocytic infiltration is not sufficient to effect beta-cell destruction, locally co-stimulated islet-infiltrating CD8+ T lymphocytes could play a critical role in the development of IDDM. [Diabetologia (1994) 37: 1277–1279]

Key words TNFα, B7-1, transgenic, mouse, diabetes, autoimmunity, lymphocytes.

Insulin-dependent diabetes mellitus (IDDM) is believed to result from the T-cell-mediated autoimmune destruction of the pancreatic islet insulin-producing beta cells. To better understand IDDM pathogenesis, transgenic mouse models have been generated to study the effect of either locally-produced cytokines or beta-cell surface-targeted antigen expression on diabetes susceptibility. For example, since NOD mouse islets contain tumour necrosis factor alpha (TNFα) mRNA [1], suggesting a role for this cytokine in disease pathogenesis, transgenic mice producing TNFα under the control of the beta-cell-specific insulin promoter have been studied [2]. Despite an intense lymphocytic infiltration of the islets, presumably resulting from the TNFα-induced local endothelial changes observed in these mice, they never spontaneously develop the disease. In fact, a number of immune manipulations, including the breeding of the TNFα transgenic mice with other mice whose beta cells express a transgene-encoded foreign antigen, failed to initiate a beta-cell-specific immune reaction [2]. Thus, while the locally produced powerful inflammatory effects of TNFα resulted in a lymphocytic infiltrate sufficiently intense to bring about a marked disruption of islet architecture, there was no immune response resulting in significant beta-cell damage. It is therefore likely that additional signals are required to activate self-reactive T cells leading to autoimmunity.

In addition to processed antigen, professional antigen-presenting cells express on their surface a number of accessory molecules that appear to be indispensable for T-cell activation. Among these important accessory molecules are the ligands for the T-cell CD28 co-stimulatory receptor [3]. The first co-stimulatory ligand family member identified and cloned was B7-1 (BB1, CD80) [4]. Transgenic mice expressing mouse B7-1 on their pancreatic beta cells under the control of the rat insulin I promoter have been reported [5]. These mice display no alteration in islet morphology or function, such that normoglycaemia is maintained throughout the life of the animals [5].

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Abbreviations: FACS, Fluorescence-activated cell sorter, IDDM, insulin-dependent diabetes mellitus; i.p., intraperitoneal injection; mAb, monoclonal antibody; NOD, non obese diabetic; TNFα, tumour necrosis factor alpha.

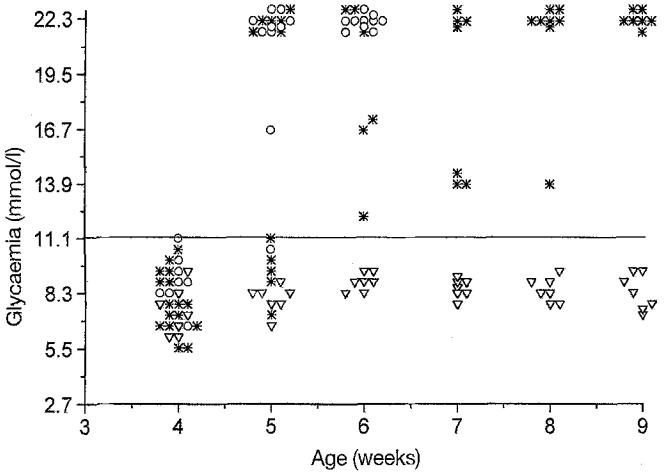

Fig. 1. Glycaemias of double transgenic TNFα/B7-1 mice. Untreated animals (*); anti-CD4 (GK1.5)-injected mice (o); anti-CD8 (H-35)-injected mice (▽)

In the present work we reasoned that pancreatic beta-cell expression of B7-1 might provide a co-stimulatory signal that would activate the islet-infiltrating lymphocytes in the TNFα transgenic mice.

Materials and methods

Mice: TNFα and B7-1 transgenic mice have been described previously [2, 5]. Doubly transgenic mice were produced by mating individuals (either males or females) of the two transgenic strains. Evolution of glycaemia was followed weekly, after at least a 4-h fast, using Haemo-Glukotest 20-800R strips (Boehringer Mannheim, Mannheim, Germany).

Treatment: When indicated, double transgenic mice were given either GK1.5 (anti-CD4) or H-35 (anti-CD8) monoclonal antibodies (for references see [2]). Twice-weekly treatment was initiated at birth at a dose of 0.25 mg mAb i.p. up to 4 weeks of age when the dose was increased to 0.5 mg i.p. The efficacy of the antibody treatments was verified by fluorescence activated cell sorter (FACS) analyses, which confirmed the complete absence of the immunodepleted T-cell subpopulation.

Immunostaining and electron microscopy: Histological examination of TNFα/B7-1 pancreata was performed by conventional light microscopy, immunofluorescence and electron microscopy, as described [2].

Statistical analysis

Body weights of the animals were compared between groups of mice using an unpaired t-test. Values are give as mean ± standard deviation.

Results

Breeding of the insulin promoter-B7-1 transgenic mice with the insulin promoter-TNFα transgenic mice yielded double-transgenic offspring (81 out of 305 pups, i.e. 27 %), all of which developed clinical signs of IDDM starting between 4 and 5 weeks of age. These signs included hyperglycaemia (≥ 11 mmol/l, Fig. 1), polyuria, polyphagia and weight loss (weight at 7 weeks of age: 18.7 ± 2.7 g for 31 double transgenic mice compared with 25.2 ± 3.6 g for 36 mice bearing either one of the two transgenes, $p < 0.001$). In addition, the double transgenic mice displayed a decreased longevity of only 20 ± 3.5 weeks ($n = 20$) compared with mice carrying only one transgene surviving more than 52 weeks. Histological examination of pancreata from double transgenic mice revealed, as early as 3 weeks of age, a similar degree of insulitis as in TNFα transgenic mice. Ultrastructural analysis provided evidence for cytoplasmic alterations of beta cells at contact points with activated lymphocytes, suggesting immune-cell-mediated killing, without the nuclear changes usually associated with apoptosis. Islets from older and severely diabetic double transgenic mice were dramatically reduced in size, with a near complete beta-cell absence (Fig. 2), while other islet endocrine cells remained detectable. No exocrine pancreas abnormality was observed. Thus, both the clinical symptoms and the histological evidence of beta-cell destruction were con-

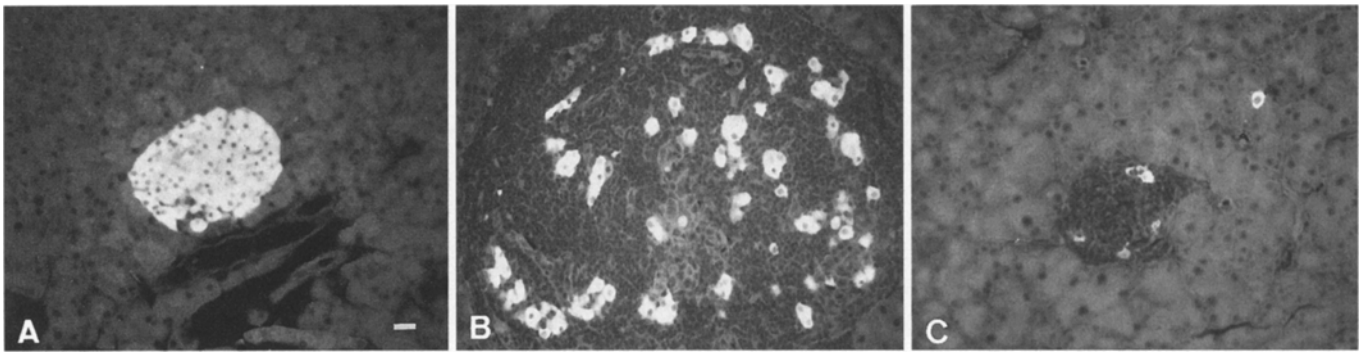

Fig. 2. Anti-insulin immunofluorescent staining of sections from paraffin-embedded pancreas of A) a non-transgenic mouse, B) a 10-week-old TNFα transgenic mouse, with a very large infiltrated islet (see reference [2]), C) a 10-week-old TNFα/B7-1 double transgenic mouse, with an atrophic islet containing only few beta cells. (Bar = 20 μm)

sistent with a rapidly progressive auto-immune IDDM-like process. These observed alterations, present only in the double transgenic mice, could be due to the locally-expressed B7-1-mediated activation of lymphocytes attracted to the islets in response to TNF-α-induced endothelial changes. Whether glutamic acid decarboxylase, which appears to be a major beta-cell antigen both in human IDDM and in NOD mice [6–8], is also involved in this form of autoimmune diabetes is presently being explored.

To evaluate the role of T-cell populations underlying the diabetes in this model, double transgenic mice were administered either GK1.5 (anti-CD4) or H-35 (anti-CD8) monoclonal antibodies. Blood glucose was measured weekly (Fig. 1). Mice administered GK1.5 ($n = 11$) (in which the $CD4^+$ T-lymphocyte subpopulation was decreased by more than 99 %, as determined by FACS analysis), developed diabetes as rapidly as untreated double transgenic mice. In striking contrast, the seven mice given H-35 remained normoglycaemic at 9 weeks; after the treatment was discontinued all mice became hyperglycaemic within 3–10 weeks (not shown). Whether this was associated with the reappearance of $CD8^+$ T lymphocytes, as seems likely, was not explored. Other clinical signs of diabetes were also prevented in H-35 treated mice; furthermore, they could carry out normal pregnancies, whereas no untreated double transgenic female ever delivered a litter. Pancreatic histologic evaluation of anti-CD8-treated double transgenic mice confirmed beta-cell survival amidst the marked lymphocytic infiltrate, and immunohistologic analysis verified the absence of $CD8^+$ T cells in the insulitis. We conclude that the diabetes in these double transgenic mice is not simply due to beta-cell toxic effect resulting from the expression of the two foreign transgene-encoded proteins; rather, the beta-cell destruction appears to result from a $CD8^+$ T-lymphocyte-mediated cytotoxic attack.

Discussion

The double transgenic IDDM model described herein provides further strong evidence for locally-expressed co-stimulatory molecules playing a critical role in autoimmunity [5]. Similar results with respect to the diabetogenic consequences of concomitant TNFα and B7-1 expression in islet beta cells have recently been reported [9]. The highly predictable, early onset, and dramatic expression of IDDM in this model makes it particularly attractive as a tool with which to study the immunological mechanisms underlying beta-cell destruction and to test therapies for their ability to modulate or prevent the disease process. Indeed, in these respects the available rodent IDDM models (i.e. the NOD mouse and the BioBreeding Wistar rat) are not ideal, because of the late and inconsistent disease onset and because of persistent uncertainty regarding pathogenic mechanisms. Our finding that immune ablation of the $CD8^+$, but not $CD4^+$, T lymphocytes prevents diabetes in this model points to a critical role for $CD8^+$ T cells in the disease, indicating that those cells may be the direct effectors of the autoimmune attack. These results are in accord with the demonstration that a CD28-B7 interaction in vitro is necessary and sufficient for the activation of $CD8^+$ cytotoxic T lymphocytes [10]. Further, our results suggest the possibility that the role of the $CD4^+$ T-cell sub-population in disease pathogenesis is the local release of TNFα and/or the attraction of professional antigen-presenting cells bearing co-stimulatory ligands such as B7-1. The precise mechanism of $CD8^+$ T-lymphocyte cytotoxicity in this double transgenic model, as well as the beta-cell antigens against which the immune response is directed, need to be determined. However, it is already apparent that particular attention should be given to therapeutic strategies aimed at decreasing co-stimulatory pathway activation and/or $CD8^+$ T-lymphocyte function.

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