Supplement Review

Immunotherapy of type 1 diabetes: lessons for other autoimmune diseases

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Chapter summary

The nonobese diabetic (NOD) mouse is a well-recognised animal model of spontaneous autoimmune insulin-dependent diabetes mellitus. The disease is T-cell mediated, involving both CD4 and CD8 cells. Its progress is controlled by a variety of regulatory T cells. An unprecedented number of immunological treatments have been assessed in this mouse strain. This chapter systematically reviews most of these therapeutic manoeuvres, discussing them in the context of their significance with regard to the underlying mechanisms and the potential clinical applications. The contrast between the surprisingly high rate of success found for a multitude of treatments (more than 160) administered early in the natural history of the disease and the few treatments active at a late stage is discussed in depth. Most of the concepts and strategies derived from this model apply to other autoimmune diseases, for which no such diversified data are available.

Keywords: autoimmune diseases, immunotherapy, insulin-dependent diabetes mellitus

Introduction

Insulin-dependent diabetes mellitus (IDDM), or type 1 diabetes, is a T-cell-mediated autoimmune disease. Much effort has been devoted over the past two decades to establishing an immunological treatment that could substitute for insulin therapy. In this chapter, I provide an update of the noteworthy preclinical data obtained in the spontaneous animal models of the disease and of clinical trials in progress. These data are presented with particular attention to lessons that could benefit the immunotherapy of other autoimmune diseases, notably rheumatoid arthritis.

IDDM as an autoimmune disease

It is now firmly established that in the vast majority of cases, IDDM has an autoimmune origin [1]. This does not preclude the possible aetiological role of a triggering environmental factor, notably a pancreatotropic virus, but the fact remains that the β-cell lesion is mediated by β-cell-specific autoreactive T cells.

No consensus has been reached on the nature of the effector T cell(s). Research on the nonobese diabetic (NOD) mouse has shown that both CD4 and CD8 clones could induce the disease separately, but it is likely that the two cell types cooperate in the β-cell lesion. CD8 T cells could act through a direct cytotoxic mechanism, although this has not been proven. CD4 cells could act either as helper T cells or as effector cells through cytokine production.

Increasing importance is given to various subsets of regulatory T cells that have been shown to control the onsets of diabetes in both the NOD mouse and the BioBreeding

A glossary of specialist terms used in this chapter appears at the end of the text section.

(BB) rat. Three main types of regulatory T cell have been described [2]: Th2 cells, which appear after administration of soluble β-cell autoantigens, $CD4^+CD25^+$ T cells, and natural killer T cells, which probably appear spontaneously during ontogeny. It is not yet clear whether the onset of diabetes results from the decline of T-cell-mediated regulation or, what is more likely, from the overriding of the regulation by activation of β-cell-specific effector T cells. Another major uncertainty relates to the nature of the events that trigger such activation. Antigen mimicry or pancreatic inflammation are the most likely, but not necessarily the only, mechanisms.

Strengths and limitations of the NOD mouse model

More than 100 reports have been published using the NOD mouse to set up new immunotherapeutic strategies. Table 1 presents a nonexhaustive list of the main products or strategies tested so far.

The large number of successful results in this mouse has raised the question of the validation of the model as a preclinical tool for identifying strategies to be applied ultimately to humans. For several substances, the success in the NOD mouse has been confirmed in humans, e.g. cyclosporin A [3,4], heat shock protein (hsp)60 peptide [5], and anti-CD3 antibody (K Herold, unpublished observations). For others, however, such confirmation was not obtained, e.g. nicotinamide [6], oral insulin [7,8], and BCG (bacille Calmette–Guérin) [9]. It is important to realise that, contrary to human diabetes, which is essentially seen in the clinic when the disease is overt, diabetes in the NOD mouse can be studied at all stages of its natural history, including the preclinical stages. It is interesting in this context that the three drugs shown to be effective in human diabetes were still efficient in the NOD mouse at an advanced stage, whereas nicotinamide, BCG, and oral insulin worked only at the preclinical stage.

An intriguing question is whether the preventive effects observed after administration of a drug at a very early stage (e.g. 4–6 weeks of age) are specific. An attractive hypothesis is that early intervention resets the homeostasis of the immune system before the disease starts to progress irreversibly. It could be postulated that there is a checkpoint before which the disease outcome is not yet fixed. An agent that would inhibit the triggering event or boost immunoregulation could then show a long-term effect. Applied after this checkpoint, the agent would not show any significant therapeutic effect. To illustrate this concept, it may be suggested that if a virus causes the initial insult that triggers the onset of the diabetogenic process and that virus can be eliminated, an antiviral treatment could be effective if applied very early but would be ineffective once the initial inflammation had occurred and induced a sustained immune response to β-cell autoantigens.

The NOD mouse is one of the few spontaneous models of T-cell-mediated autoimmune diseases, and as such it is of special interest to all students of autoimmunity. This mouse strain is also of major interest because it has been used to generate many genetically modified models in which various genes have been deleted or overexpressed as transgenes in various tissues including the β cells (using the rat insulin promoter). Such mice provide invaluable help in discerning the mode of action of the various therapeutic strategies shown to operate in wild NOD mice.

A weakness of the NOD mouse model is that the putative target β-cell autoantigen(s) is (are) unknown. Several candidates have been proposed, such as glutamic acid decarboxylase, insulin, hsp60, and IA-2 [1], but no firm evidence has shown any of them to be primary autoantigens. This is not necessarily a major pitfall, since data have been accumulated to indicate that such a primary autoantigen may not exist. Even if it exists, diversification of autoimmune specificities (antigen spreading) occurs so fast that the primary antigen may not be crucial. Additionally, at the level of cytokine-dependent immunoregulation (cytokines are discussed further in section 6 below), the occurrence of bystander suppression [10] allows the suppression initially directed against a given β-cell antigen, whether it is a primary autoantigen or not, to be extended to most β-cell-specific T-cell responses.

Preclinical studies: a unique array of approaches

As mentioned above, a wide spectrum of agents or manipulations has been shown to prevent, and more rarely to cure, IDDM in NOD mice. They are listed here according to the factors postulated to contribute to the development of the disease. The various strategies that have been reported are presented below, and Table 1 lists the reference or references relevant to each product or strategy.

1. T-cell depletion or sequestration/diversion

The most straightforward approach to immunotherapy of a T-cell-mediated autoimmune disease such as IDDM is the removal of T cells, either targeted as a whole or as subsets. This has been accomplished in the NOD mouse using several approaches.

Anti-T-cell depleting antibodies offer the easiest strategy. One may thus delay the onset of diabetes by administration of depleting CD4 antibodies such as GK 1.5 and, to a lesser extent, CD8, CD44, CD45RA, or CD45RB antibodies. However, although the onset of diabetes can be prevented in the best cases, there is no clear effect on overt disease, even when it is only recently established. In recently established disease, besides anti-CD3 antibodies, which essentially act independently of major T-cell depletion (see below), only a mixture of depleting CD4 and CD8 antibodies or polyclonal antilymphocyte antibodies have been

Table 1

Immunotherapeutic agents or other treatments used in NOD mice

1 T-cell depletion or sequestration/diversion
- 1.1 Depletion
  - Anti-CD3 [28]
  - Anti-CD4 [40]
  - Anti-CD8 [41]
  - Anti-CD44 [42]
  - Anti-CD45RA [43]
  - Anti-CD45RB [44]
  - Anti-Thy l.2 [45]
  - Antilymphocyte globulin [11,45]
  - Neonatal thymectomy [46]
- 1.2 Sequestration/diversion
  - Anti-CD43 [47]
  - Anti-VLA-l [48]
  - Anti-VLA-4 [48,49]
  - VLA-4/Ig fusion protein [50]
  - Anti-CD62L [49]

2 Blockade of T-cell activation
- 2.1 Chemical immunosuppressants
  - Cyclosporin A [51]
  - FK-506 [52]
  - Azathioprine [53]
  - Rapamycin [54]
  - Deoxyspergualin [55]
- 2.2 γ Irradiation [56]

3 Targeting of T-cell receptors
- 3.1 TCRαβ antibody [13]
- 3.2 CD3 antibody [28]
- 3.3 Vβ8 antibody [57]
- 3.4 T-cell vaccination
  - Polyclonal activated T cells [58]
  - Glutaraldehyde-treated T cells [59]
  - Activated T cells
  - Vβ8 T cells [60]
  - Anti-hsp60 T-cell clone [61]
- 3.5 Blocking peptides [62]

4 Targeting of MHC molecules
- 4.1 Anti-class-I [63]
- 4.2 Anti-class-II [64]
- 4.3 MHC transgenic mice
  - Class I [65]
    - I-A [16,66]
    - I-E [67]

5 Targeting of costimulation and adhesion molecules
- 5.1 Costimulation molecules
  - Anti-CD28 [68]
  - CTLA-4–Ig fusion protein [69]
  - Anti-B7.2 [69]
  - Anti-CD40L [70]
- 5.2 Adhesion molecules
  - Anti-ICAM-1 [71]
  - Soluble ICAM-1
    - Recombinant protein [72]
    - Gene therapy (P Lemarchand, unpublished observations)
  - Anti-Mac [73]
  - Anti-LFA-l [71]

6 Cytokine blockade
- 6.1 IFN-γ
  - Anti-IFN-γ [74,75]
  - IFN-γR/IgG1 fusion protein [76]
- 6.2 IL-2
  - Anti-IL-2R [77]
  - IL-2R/Ig fusion protein [78]
  - IL-2 diphtheria-toxin protein [79]
- 6.3 IL-12
  - Anti-IL-12 [80]
  - IL-12 antagonist (p40)2 [81]
- 6.4 IFN-α (oral) [82]
- 6.5 IL-1
  - IL-1 antibody [83]
  - IL-1 antagonist [84]
- 6.6 IL-6 [75]
- 6.7 Lymphotoxin receptor [85]

7 Pharmacologically active cytokines
- 7.1 IL-4 [86]
- 7.2 IL-10 [87,88]
- 7.3 IL-13 [89]
- 7.4 IL-3 [37]
- 7.5 G-CSF (F Zavala, unpublished observations)
- 7.6 Lymphotoxin [90]
- 7.7 IL-11 [91]
- 7.8 IL-1α [92]
- 7.9 TNF-α [26]

8 Tolerance to soluble β-cell autoantigens
- 8.1 Insulin
  - Oral [93]
  - Oral + IL-10 [94]
  - Intranasal [34,95]
  - Subcutaneous
    - Native protein [96]
    - B chain [96]
    - Inactive analogue [95,97]
  - DNA vaccination [98]
  - Gene-transfer delivery [99] (proinsulin gene)
  - Cholera-toxin conjugate [100]
- 8.2 Glutamic acid decarboxylase (GAD)
  - Oral [101]
  - Intranasal [102]
  - Subcutaneous [103]
  - Intrathymic [104]
  - DNA vaccination [105]
  - Anti-GAD antibody [106]
- 8.3 Heat shock protein 60 (hsp60)
  - Subcutaneous or intraperitoneal
    - Protein [107]
    - P277 peptide [108,109]
  - Gene-transfer delivery [110]
- 8.4 Pancreatic extracts (oral) [111]

9 Stimulation of regulatory T cells
- 9.1 Pathogens
  - Bacteria
    - Mycobacteria
      - *Mycobacterium bovis* [112]
      - *M. avium* [113]
      - Complete Freund's adjuvant [114]
    - *Lactobacillus casei* [115]
    - Streptococcal extract [116]
    - Klebsiella extract [117]
    - *Escherichia coli* (+ oral insulin) [118]
  - Viruses
    - Mouse hepatitis virus [119]
    - Lactate dehydrogenase virus [120]
    - Lymphocytic choriomeningitis virus [121]
  - Parasites
    - Filariae [122]
    - Schistosomes [123]
- 9.2 Stimulation of innate immunity
  - α-Galactosylceramide [33,124]

Continued overleaf

Table 1 continued

Immunotherapeutic agents or other treatments used in NOD mice

- 9.3 Nondepleting anti-T-cell antibodies
  - Anti-CD3 [28]
  - Anti-CD4 [30]
  - Superantigens [125]
- 10 Gene therapy
  - 10.1 β-cell antigens
    - DNA vaccination [98,105]
    - GAD immunoglobulin [126]
  - 10.2 IL-4
    - Retrovirus (T-cell transfection) [127]
    - Biolistic [128]
    - Adenovirus [129]
    - IL-4/IgG1 fusion protein [130]
  - 10.3 IL-10
    - T-cell transfection [131]
    - Local [132]
    - Systemic [133]
  - 10.4 ICAM-1 (P Lemarchand, in preparation)
  - 10.5 IFN-γR/IgGl fusion protein [76,130]
  - 10.6 TGF-β [134]
  - 10.7 Calcitonin [135]
- 11 Cell therapy
  - 11.1 Islet or segmental pancreas transplantation (+ immunosuppression)
    - Syngeneic [12]
    - Allogeneic [136] (+ immunosuppression)
  - 11.2 Intrathymic islet transplantation [38]
  - 11.3 Bone marrow transplantation
    - Allogeneic [137,138]
    - Syngeneic [37]
  - 11.4 Dendritic cells [139,140]
  - 11.5 Natural killer T cells [141]
  - 11.6 CD4 cell lines
    - Polyclonal [142]
    - Anti-Ia^{g7} [143]
  - 11.7 Allogeneic cells
    - Macrophages [144]
    - Spleen cells [36]
- 12 Inhibition of β-cell lesion
  - 12.1 Nicotinamide [145]
  - 12.2 Antioxidants
    - Vitamin E [146]
    - Probucol analog [147]
    - Probucol + deflazacort [148]
    - Aminoguanidine [149]
  - 12.3 Anti-inflammatory agents
    - Pentoxifylline [150]
    - Rolipram [150]
- 13 Miscellaneous
  - 13.1 Immunomodulators
    - Linomide [151]
    - Ling-zhi-8 [152]
    - D-Glucan [153]
    - Multi-functional protein 14 [154]
    - Ciamexon [155]
    - Cholera toxin B [156,157]
    - Vanadate [158]
    - Vitamin D_3 analogue [159]
  - 13.2 Hormones and related proteins
    - Androgens [160]
    - IGF-I [153]
  - 13.3 Immunomanipulation
    - Natural antibodies [161,162]
    - Lupus idiotype [163]
    - Lipopolysaccharide [164,165]
  - 13.4 Diet
    - Casein hydrolysate [166,167]
  - 13.5 Other
    - Sulfatide [168]
    - Bee venom [90]
    - Kampo formulation [169]
    - Silica [170]
    - Ganglioside [171]
    - Antiasialo GM-1 antibody [172]
    - Hyaluronidase [42]
    - Concanavalin A [173]

CD45RA(B), CD45 receptor A(B); CDXXL, CDXX ligand; CFA, complete Freund's adjuvant; GAD, glutamic acid decarboxylase; G-CSF, granulocyte-colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; MHC, major histocompatibility complex; TCR, T-cell receptor; V, variable region (of immunoglobulin); VLA, very late antigen.

found to reverse the disease [11]. Immunosuppression is not specific to β-cell antigens and may be prolonged, thus exposing the patient to the hazards of generalised immunosuppression. A more subtle approach, which is probably less hazardous but also less efficient, targets T-cell homing molecules, aiming at diverting pathogenic T cells or their precursors from migrating to the islets. This is the putative mode of action of anti-VLA-1, anti-VLA-4, anti-CD43, and anti-L-selectin (CD62L) antibodies.

2. Blockade of T-cell activation

A less radical but similar approach to the previous one is to reversibly block T-cell activation. At present, this is achieved using chemical immunosuppressants.

Most drugs used in organ transplantation where T cells are also incriminated have been used, and these include, notably, cyclosporin A, azathioprine, rapamycin, FK506, and deoxyspergualin. Again, these drugs essentially worked when given early in the course of the disease as a preventive, but not a curative, treatment. This point is illustrated by results reported by Wang and Lafferty and their coworkers, showing that in diabetic NOD mice transplanted with syngeneic islets, recurrence of diabetes could be prevented by a depleting CD4 antibody (GK 1.5) but not by cyclosporin A [12].

3. Targeting of T-cell receptors

T-cell-receptor(TCR)-mediated recognition of β-cell autoantigens is a central step in the diabetes pathogenesis, at both the triggering and the effector phases. It was thus logical to attempt to block TCRs. This has been successfully achieved using a number of approaches.

Global TCR blockade can be obtained by administering antibodies directed against the constant portion of αβ TCRs or to the CD3 complex with which TCR is tightly associated both physically and functionally. In the case of CD3, though, the blockade effect is only part of the antibody mode of action, which also involves depletion (at least when the entire antibody molecule is used) and especially T-cell activation notably of regulatory T cells (see below). Here again, at least for TCRαβ antibody, immunosuppression is of the global type and works only preventively. Regression of diabetes was observed in mice with recently manifested diabetes [13], which is interesting inasmuch as it provides strong support to the argument that reversible T-cell-mediated inflammation takes place in the islets. However, such regression was inconsistent and transient (at variance with that induced by anti-CD3 as described below).

A more selective approach is to target T-cell subsets using selective TCR Vβ antibodies, on the assumption that pathogenic T cells preferentially use selective Vβ genes. Some encouraging but as yet unconfirmed results have been reported for Vβ8.1 and Vβ6. In fact, the experimental model in which such Vβ gene restrictive usage was initially reported, namely experimental allergic encephalomyelitis [14], has not been confirmed for other experimental autoimmune diseases. When whole myelin antigens are used, no clear Vβ gene restrictive usage has been found in human autoimmune diseases. A special case might be made for human diabetes for Vβ7 (and perhaps Vβ13), which are seemingly preferentially used by T cells present in islet infiltrates [15].

A last and even more specific TCR blockade could be obtained by immunising against idiotypes of pathogenic T cells, ideally T-cell clones. This has been attempted in the NOD mouse either using polyclonal T cells or T-cell clones, notably clones of anti-hsp60 T cells. Some effect was reported, but the results, which were often only partial, require confirmation.

4. Targeting of MHC molecules

Peptides of β cells are presented to T cells in the context of MHC molecules. It was thus logical to attempt to modulate the course of β-cell-specific autoimmunity in NOD mice targeting MHC molecules. Administration of either class-I-specific or class-II-specific monoclonal antibodies in young NOD mice (less than 2 months old) but not older ones prevents the onset of diabetes. The protection afforded by class II antibodies is long lasting and resistant to cyclophosphamide and can be transferred to nonantibody-treated mice by T cells. Its precise mode of action, however, remains elusive. It is noteworthy that NOD transgenic mice overexpressing mutated MHC non-NOD class II genes are protected from diabetes and, again, the protection can be transferred to wild NOD mice by T cells from transgenic mice [16,17]. Collectively, these data suggest that targeting MHC molecules might lead to stimulation of regulatory class II restricted CD4 T cells, which are as yet uncharacterised.

MHC molecules could also be targeted by blocking peptide binding to those molecules; this possibility is suggested by the prevention of diabetes that is afforded by the administration of Ia^{g7} immunogenic but not tolerated peptide binder. Again, one would have to demonstrate that the Ia^{g7} binder in question does not act as an altered peptide ligand (APL) known to stimulate regulatory T cells in these models.

5. Targeting of costimulation and adhesion molecules

The activation of autoreactive T cells specific to β-cell antigens involves a number of costimulation and adhesion molecules. Thus, antibodies to B7.1 or to CD40L prevent the onset of diabetes. CTLA-4–Ig, a fusion protein of CTLA-4 and IgG Fc, which inhibits the binding of CD28 to B7, also delays the onset of diabetes. A similar preventive effect has been reported for an anti-CD28 antibody, but here the mechanism of action of the antibody probably relates to an agonistic effect leading to signalling of regulatory T cells. In fact, this therapeutic approach is more generally complicated by the dual effect of some of the agents used, depending when they are administered. Thus, CTLA-4–Ig fusion protein prevents the onset of diabetes when administered late but accelerates the progression of the disease when administered early [18].

Note also that $CD28^{-/-}$ and $B7^{-/-}$ NOD mice show fulminant diabetes, probably because of the absence of regulatory T cells [18,19].

Diabetes has also been prevented by blocking adhesion molecules, particularly using antibodies against intercellular adhesion molecule (ICAM)-1 and LFA-1. Workers in this laboratory have recently found that administration of adenovirus-infected cells producing soluble recombinant ICAM-1 also protected NOD mice against diabetes. We have even shown that such gene therapy can reverse recently established diabetes (P Lemarchand, unpublished observations).

6. Cytokine blockade

A wide array of cytokines are involved in the differentiation and activation of the various T-cell subsets contributing to diabetes pathogenesis in NOD mice. All antibodies directed at cytokines or cytokine receptors inhibiting the onset of diabetes relate to Th1 cells. Thus, the onset of diabetes is prevented by antibodies directed against IFN-γ, IL-2 receptor (an association with low-dose cyclosporin A is required), or IL-12. Interestingly, a similar effect was obtained by blocking the cytokine receptor with a receptor/immunoglobulin fusion protein or by destroying the

receptor-bearing cell with a cytokine-toxin conjugate. The preventive effect of orally administered IFN-α is interesting but is difficult to interpret. Also intriguing is the absence of diabetes prevention in NOD mice genetically deficient in IFN-γ, IFN-γ receptor, or IL-12 [20–22], a paradox probably explained by a redundancy of the genes coding for these cytokines and their receptors. Prevention of diabetes has been reported after blockade of proinflammatory cytokines, namely IL-1, IL-6, and tumour necrosis factor (TNF)-α. In the latter case, the effect was observed only when the neutralising antibody was administered at a very young age.

7. Pharmacologically active cytokines

Many of the strategies resulting in stimulation of regulatory cells may be assumed to involve the suppressive effect of cytokines acting either systematically or locally at the islet level. The onset of diabetes may also be prevented by the direct administration of regulatory cytokines.

IL-4

Systemic administration of IL-4 can delay the onset of diabetes. The effect is not as dramatic as that of other procedures described here, but is nevertheless quite significant. In fact, the effect is more clear cut when the cytokine is directly delivered in the islet using either gene therapy or β-cell-targeted transgenesis.

IL-10

Findings similar to those reported for IL-4 have been reported for IL-10 after systemic administration of the recombinant cytokine. Paradoxically, however, the onset of diabetes is accelerated by intra-islet delivery of IL-10 in transgenic mice [23] or by systemic administration of an IL-10–Ig fusion protein [24], possibly due in the latter case to an unexpected Th2 polarization.

IL-13

A modest but significant delay in the onset of diabetes has been reportedly achieved by IL-13, another Th2 cytokine.

G-CSF

Granulocyte-colony-stimulating factor (G-CSF) has been used successfully to protect NOD mice from diabetes, following previous results in this laboratory showing that G-CSF could prevent systemic lupus erythematosus in $(NZB \times NZW)F_1$ mice [25]. Data collected in these various models suggest that the effect of G-CSF could involve Th2 polarisation.

TNF

Contrasting results have been reported for TNF. Given in the adult NOD mouse, TNF prevents the onset of diabetes [26] (an observation in keeping with the insulitis acceleration brought about by anti-TNF antibodies). Conversely, given to newborn NOD mice, TNF accelerates disease progression [27].

IL-1

IL-1 has been reported to protect NOD mice from the onset of diabetes. This is a surprising observation, because IL-1 has been shown to be exquisitely toxic to β cells and because an Il-1 antagonist has been reported to protect against diabetes.

IL-12

Again depending on the protocol of administration, IL-12 may accelerate or slow down the progression of diabetes.

Lymphotoxin

Diabetes protection has also been reported for lymphotoxin and lymphotoxin–receptor fusion protein.

8. Tolerance to soluble β-cell autoantigens

Many efforts have been made to induce tolerance to candidate β-cell autoantigens. Prevention of disease (but not cure of established disease) has been obtained with insulin, glutamic acid decarboxylase, and hsp60. In the case of insulin, evidence indicated that the effect was not exclusively linked to the hormone's metabolic activity, since the disease could be prevented with insulin, metabolically inactive B chain, or inactive analogues. In the case of hsp60, the antigen is not, strictly speaking, β-cell-specific, but its overexpression in inflamed β cells leads to some β-cell-selective expression.

With each of these three antigens, diabetes was prevented by using various routes of administration: subcutaneous (+ adjuvant), oral, nasal, intravenous, intrathymic. Tolerance was also induced by vaccination with antigen-specific DNA, as well as by transgenic overexpression of the autoantigen.

At the level of underlying mechanisms, there is no true antigen-specific tolerance, since the downregulation of autoimmunity extends to antigens other than the tolerogen. Accumulated data show that soluble β-cell autoantigens induce a deviation in immunity towards Th2, with bystander suppression probably involving local release of immunosuppressive cytokines [2].

9. Stimulation of regulatory T cells

The diabetogenic autoimmune response is tightly controlled by a variety of regulatory T cells. I have pointed out how the administration of soluble β-cell autoantigens could stimulate Th2 cells and prevent the onset of diabetes if given when the mice are young enough. Many other strategies have been used to prevent the onset of diabetes targeting non-Th2 regulatory T cells. One may assume, a priori, that most of these strategies are not β-cell-specific, since they use non-β-cell-related agents. The possibility cannot be excluded that, at least in some cases, the induced regulation is β-cell-specific at the effector level. One may postulate that a nonspecific stimulation

leads to the activation or boosting of β-cell-specific regulatory T cells, whether or not they are of the Th2 type. The strategies for stimulating regulatory T cells may be classified according to whether they make use of nondepleting anti-T-cell monoclonal antibodies, stimulation of innate immunity, or pathogens, as discussed below.

Nondepleting anti-T-cell monoclonal antibodies

Administration of anti-CD3 antibodies to NOD mice with recently manifested IDDM induces long-term remission of the disease. The effect is obtained after brief treatment (5 days) and does not require the use of the mitogenic whole autoantibody molecule (nonactivating F(ab′)2 fragments are tolerogenic) [28,29]. My colleagues and I have recently obtained data indicating that the effect is mediated by active tolerance involving TGF-β-dependent $CTLA^{+}CD25^{+}$ T cells (L Chatenoud, unpublished observations).

Similar, though less well documented, data have been reported for nondepleting anti-CD4 antibodies [30], in keeping with the analogous effect of the same antibodies in transplantation. [31].

Stimulation of innate immunity

NOD mice show an early deficit in NK (natural killer) T cells, both quantitatively and qualitatively (deficient IL-4 production) [32]. It was thus logical to attempt to prevent IDDM in such mice by stimulating the function of NK T cells. This was recently done by administering a selective NK-T-cell ligand, the glycolipid α-galactosylceramide. Interestingly, the protection still applies in some protocols when the glycolipid is given late, and can inhibit the recurrence of disease in diabetic mice with grafts of syngeneic islets [33].

Stimulation of γδ regulatory T cells has been reported after intranasal administration of insulin [34]. It will be interesting to learn whether such T cells that protect against diabetes after nasal administration of insulin are insulin specific.

Pathogens

Bacteria. A whole array of bacteria have been shown to prevent the onset of diabetes in NOD mice. Mycobacteria have been extensively studied, particularly *Mycobacterium bovis* (the source of BCG vaccine) and *M. avium*. The effect is also obtained with mycobacteria extracts (in complete Freund's adjuvant). The role of regulatory T cells in protection induced by complete Freund's adjuvant or vaccination with BCG is demonstrated by the transfer of protection that is achieved when CD4 T cells from protected mice are transferred to naive mice [35]. The nature of the regulatory cells in question is open to speculation (are they Th2 cells? CD25 cells?). Other bacterial-cell extracts have also been shown to prevent the onset of diabetes in NOD mice, notably extracts of streptococcus or klebsiella.

Viruses. The onset of diabetes in NOD mice can be prevented by infection with various viruses, in partucular lymphochoriomeningitis virus (LCMV), murine hepatitis virus (MHV), and lactate dehydrogenase virus (LDHV).

Parasites. Diabetes can also be prevented by deliberate administration of parasites, such as schistosomes or filariae.

10. Gene therapy

Gene therapy may be used in many ways to prevent or cure diabetes in NOD mice. Insulin gene therapy and related strategies are not discussed in this chapter.

Immune-based gene therapy has been developed along several lines. One possibility is to overexpress cytokines or cytokine receptors with the aim of reproducing the pharmacological effect of the particular molecules. Various experimental settings have been considered, including local intra-islet delivery of the cytokine (using transgenic mice or islet-specific T-cell transfection) and systemic delivery. Various vectors (viral and nonviral) have been used. IL-4, IL-4–Ig fusion protein, IL-10, IFN-γ receptor, Ig, and TGF-β all protected the mice from diabetes.

We recently reported that systemic delivery of soluble ICAM-1 using a recombinant adenovirus vector could also be protective and even curative in mice that had recently developed diabetes (P Lemarchand, unpublished observations).

Less expected is the protective effect of calcitonin gene therapy.

11. Cell therapy

Islet transplantation

Syngeneic islet transplantation is really a palliative procedure, not an immunotherapeutic one. However, unlike insulin therapy, it poses the problems of the prevention of disease relapse on the graft and consequently requires associated immunotherapy. Many of the procedures described above have been used to prevent such disease relapse, e.g. anti-CD3 and anti-CD4 antibodies, soluble glutamic acid decarboxylase, α-galactosylceramide, and BCG vaccination. Similar immunological problems will be met with attempts to regenerate islet cells from ductal stem cells, as has been recently described. The problem is even more serious in the case of allogeneic islet transplantation, in which two problems – relapse and allograft rejection – are combined.

Bone marrow transplantation

Allogeneic bone marrow transplantation. Another approach consists in replacing the bone marrow T (and B) cell precursors. This is not an easy approach, because of the associated allogeneic reaction (graft versus host and host versus graft). Such alloimmune response could have a protective effect, probably through the production of

immunoregulatory cytokines: this possibility is suggested by the protection afforded by induction of (usually partial) allogeneic tolerance in newborn NOD mice, which also totally protects from diabetes [36].

Syngeneic bone marrow transplantation. More unexpectedly, syngeneic bone marrow transplantation may also afford protection (in conjunction with IL-3), possibly by resetting immunoregulatory mechanisms that override effector ones [37].

Infusion of mononuclear cells
Prevention of diabetes has been reported after infusion of dendritic cells and $CD4^{-/-}CD8^{-/-}$ thymocytes presumably enriched in NK T cells. It has also been extensively demonstrated that the onset of diabetes in NOD mice is prevented by administering mature CD4 T cells (either polyclonal, notably of the CD25 type, or monoclonal).

Intrathymic islet transplantation
Diabetes has been prevented in NOD mice upon intrathymic grafting of syngeneic or allogeneic islets, either at birth or within 4 weeks of age. The preventive effect was associated with a complete absence of insulitis in most animals. The observations that spleen cells from tolerant islet-grafted NOD mice did not transfer diabetes into immunoincompetent hosts [38] and that cyclophosphamide did not break the tolerance in one study [39] are compatible with a preferential deletional mechanism.

12. Inhibition of β-cell lesion
Inhibition of the effector mechanisms leading to destruction of β cells has been attempted with limited success.

Nicotinamide has some protective effect but only at relatively high doses and early in the disease history. Nitric oxide (NO) inhibitors have also shown some effects as do antioxidants, pentoxifylline, and rolipram.

Anti-TCR antibodies and CD3 antibodies also deserve mention here. They probably act, at least in part, by inactivating effector T cells, as is suggested by virtually immediate reversal of hyperglycaemia after the first injection of such antibodies [13,28].

13. Miscellaneous
Immunomodulation
Some products known to modulate immune responses (without showing a clear overall suppressor or stimulator pattern of activities) prevent the onset of diabetes in NOD mice. These include linomide, ciamexon, vanadate, vitamin D3, and D-glucan.

Hormones
Some hormones or related compounds can also prevent insulitis and the progression of diabetes in NOD mice. This has notably been reported for androgens, a finding in keeping with the acceleration of disease seen after castration in males and the high female/male ratio of affected mice. The onset of diabetes is also prevented by IGF-I.

Immunomanipulation
Unexpectedly, immunisation against the lupus-associated idiotype 16/6 protects NOD mice from diabetes. The protective effect of natural antibodies presumably has a similar mode of action. The effect of such antibodies is interesting, but their mode of action is poorly defined.

Diet
Various diets have been shown to slow the progression of diabetes in NOD mice, notably the low-protein diets. It has been reported that casein hydrolysate formula does likewise.

Other products
A number of products listed in Table 1 that have an ill-defined action on the immune system have also been reported to prevent the onset of diabetes in NOD mice.

Concluding remarks
The number and variety of therapeutic interventions capable of preventing diabetes represents an unprecedented observation in immune pathology. The number of interventions that work in mice with advanced disease, and particularly with established diabetes, is much more limited, indicating that the majority of efficacious treatments are active only at the very early stages of a chronic process progressing from insulitis to clinical diabetes. As has been mentioned above, the only products that have been shown to arrest the destruction of β cells in man are those shown to act late in the natural history of the disease in NOD mice. Nevertheless, the early-acting procedures may prove useful in combination with late-acting drugs. One might envision treating patients who have recently diagnosed diabetes with the late-acting drugs, followed by administration of early-acting drugs, which would regain their activity once the immune homeostasis has been reset. Alternatively, these numerous early-acting compounds could be applied in man very early if valid prediction could identify subjects at risk of developing the disease. However, the logistic problems associated with such prediabetes trials should not be overlooked (for example, the number of subjects to be screened and enrolled and the duration of the trial). Lastly, many of the concepts and therapeutic strategies described above for IDDM could probably be extrapolated correctly to other autoimmune diseases, notably rheumatoid arthritis.

Glossary of terms
BB = BioBreeding (rat); BCG = bacille Calmette–Guérin; NOD = nonobese diabetic (mouse).

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