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Review

Dissecting autoimmune diabetes through genetic manipulation of non-obese diabetic mice

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Abstract

Type 1 diabetes results from a genetically and immunologically complex autoimmune process that is specifically directed against the pancreatic beta cells. Non-obese diabetic mice spontaneously develop a form of autoimmune diabetes closely resembling the disease in humans. This happens because, like human diabetic patients, non-obese diabetic mice have an unfortunate combination of apparently normal alleles at numerous loci associated with Type 1 diabetes. In isolation, each of these allelic variants affords a small degree of susceptibility to diabetes. In combination, however, they set in motion a series of immunological events that lead to islet inflammation and overt diabetes. Type 1 diabetes is associated with defects in self-tolerance and immunoregulation. It involves presentation of beta cell antigens to autoreactive T lymphocytes by professional antigen-presenting cells, the recruitment of antigen-activated T cells into pancreatic islets, and the differentiation of these antigen-activated lymphocytes into beta cell killers. Understanding the precise sequence of events in the pathogenesis of Type 1 diabetes has been, and remains, a challenging task. Much of our understanding of the immunology of the disease stems from studies of genetically engineered, non-obese diabetic mice. These mice provide reductionist systems, with which the contribution of individual cellular elements, molecules or genes to the disease process can be dissected. This review focuses on the lessons that have been learned through studies of these mice. [Diabetologia (2003) 46:1447–1464]

Keywords Non-obese diabetic mice, autoimmune, immunology, lymphocytes, pathogenesis, Type 1 diabetes.

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Abbreviations: APCs, antigen-presenting cells; β2m, beta-2 microglobulin; CD62L, L-selectin; CDR3, complementarity-determining region 3; CTL, cytotoxic T lymphocyte; DC, dendritic cell; IA-2, insulinoma-associated protein 2; ICA69, islet cell antigen 69 KDa; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; LFA-1, leucocyte function-associated antigen-1; mAb, monoclonal antibody; NK, natural killer; MIP-1α, macrophage inflammatory protein 1α; NOD, non-obese diabetic; 8.3-NOD, 8.3-TCRαβ-transgenic NOD mice; PLN, pancreatic lymph node; rag, recombination-activating gene; RAG-2, recombination-activating gene 2; RIP, rat insulin promoter; TCR, T cell receptor.

Introduction

In humans and non-obese diabetic (NOD) mice Type 1 diabetes results from a chronic autoimmune process against the pancreatic beta cells. There is extensive evidence that for Type 1 diabetes to develop autoreactive $CD4^+$ and $CD8^+$ T lymphocytes have to be activated in the pancreatic lymph nodes (PLNs) and recruited into pancreatic islets. These antigen-activated T lymphocytes progressively destroy the pancreatic beta cells, bringing about clinical disease after a long period of islet inflammation. Macrophages, dendritic cells (DCs) and B lymphocytes are also key factors in the disease process, notably as antigen-presenting cells (APCs). These different types of immune cells contribute to the development of Type 1 diabetes by participating in complex multi-cellular interactions, which are directed by cytokines and chemokines, and modulated by co-stimulatory factors and adhesion

Table 1. TCR- and BCR-transgenic NOD mice

Mouse	Gene	Cell type	MHC restriction	Antigen/ligand	Effects on diabetes	References
BDC-2.5-NOD	TCR	CD4	I-A^{g7}	Decapeptides 1040 series	Reduced[a]	[13, 35]
4.1-NOD	TCR	CD4	I-A^{g7}	NI[4]	Accelerated[b]	[12, 15]
G286-NOD	TCR	CD4	I-A^{g7}	$GAD65_{286–300}$	Inhibited[c]	[17]
8.3-NOD	TCR	CD8	K^d	NRP-A7 NRP-V7 $IGRP_{206–214}$	Accelerated[b]	[15, 24, 26]
AI4	TCR	CD8	K^d	NI[d]	Accelerated[b]	[16]
9.33-NOD	TCR	CD8	K^d	NI[d]	Accelerated[b]	[18]
VH125-NOD	IgM	B	None	Insulin	Accelerated[b]	[55]
VH281-NOD	IgM	B	None	NI[d]	Inhibited[c]	[55]
NOD.*IgHEL*	IgM-HEL[e]	B	None	HEL[e]	Inhibited[c]	[56]

[a] Lower incidence of diabetes than wild-type NOD mice
[b] Earlier onset of diabetes than in wild-type NOD mice
[c] Lower insulitis scores (or absence of insulitis) and no diabetes
[d] NI, not identified
[e] HEL, hen egg lysozyme

Table 2. Transgenic NOD mice

Mouse	Gene(s)	Promoter	Effects on Type 1 Diabetes	References
NOD.*RIP-B7.1*	*Cd80*	Insulin	Accelerated[c]	[1]
NOD.*CTLA4-Ig*	*Ctla4-Ig*	Keratin	Increased[b]	[62]
NOD.*RIP-IL-2*	*Il2*	Insulin	Accelerated[c]	[85, 86]
NOD.*RIP-IL-4*	*Il4*	Insulin	Inhibited[e]	[88]
BDC2.5-NOD.*RIP-IL-4*	*Il4/BDC2.5-TCR*	Insulin	Accelerated[c]	[89]
NOD.*RIP-IL-6*	*Il6*	Insulin	Delayed[d]	[96]
NOD.*RIP-IL-10*	*Il10*	Insulin	Accelerated[c]	[1]
NOD.*RIP-TGFβ*	*Tgfb1*	Insulin	Inhibited[e]	[102]
NOD.*Glu-TGFβ*	*Tgfb1*	Glucagon	Inhibited[e]	[103]
NOD.*RIP-TNFα*	*Tnfa*	Insulin	Accelerated[c]	[44]
NOD.*RIP-IFN-γΔR*	*InfgΔR*	Insulin	None	[118]
NOD.*RIP-FasL*	*FasL*	Insulin	Accelerated[c]	[145]
NOD.*GAD65-Tg*	*GAD65*	MHC class I	Accelerated[c]	[32]
NOD.*AS-GAD*	Antisense-GAD65	Insulin	Inhibited[e]	[29]
NOD.*tet-ICA69*	*ICA69*	Tet07	Reduced[a]	[33]
NOD.*proinsulin-Tg*	*Proinsulin2*	MHC class II	Inhibited[e]	[34]

[a] Lower incidence of diabetes than in wild-type NOD mice
[b] Higher incidence of diabetes than in wild-type NOD mice
[c] Earlier onset of diabetes than in wild-type NOD mice
[d] Later onset of diabetes than in wild-type NOD mice
[e] Lower insulitis scores (or absence of insulitis) and no diabetes

molecules. Defects in self-tolerance and immunoregulation, brought forth by a combination of ill-defined environmental influences (in humans) and genetic elements (in humans and mice), set these complex cellular interactions in motion. The genetic and immunological complexity of Type 1 diabetes has necessitated the generation of genetically engineered models of Type 1 diabetes. This review summarises the contributions of studies in transgenic, gene-targeted and congenic NOD mice to our understanding of how diabetes develops. The effects of various transgenes, targeted mutations and gene polymorphisms on the natural history of diabetes in NOD mice are summarised in Tables 1, 2, 3, 4 [1, 2, 3, 4, 5].

To the best of our knowledge, the reviewed studies followed the "Principles of laboratory animal care" (NIH, publication no.85-23, 1985; http://www.grants1.nih.cgov/grants/onlaw/references/phspol.html), complied with specific national laws on animal care, were approved by the relevant ethics committees, and were carried out in accordance with the Declaration of Helsinki (http://www.wma.net/e/policy/17cnote.pdf).

Role of T cells in Type 1 diabetes

Early T cell transfer studies revealed that $CD4^+$ and $CD8^+$ T cells are necessary for Type 1 diabetes to develop in NOD mice [6]. However, the relative role of each T cell subset in the disease process remains unclear. Since beta cells do not express MHC class II molecules, it is generally believed that autoreactive

Table 3. Congenic gene-targeted NOD mice

Mouse	Gene	Effects on Type 1 diabetes	References
NOD.*Igμ*$^{-/-}$	*Igh-6*	Inhibited[d]	[47, 48]
NOD.*Igμ*$^{-/-}$	*Igh-6*	Reduced[a]	[49]
NOD.*CD28*$^{-/-}$	*Cd28*	Increased[b]	[62, 82]
NOD.*CD62L*$^{-/-}$	*Cd62L*	None	[62, 82]
NOD.*CD80*$^{-/-}$	*Cd80*	Accelerated[c]	[134]
NOD.*CD86*$^{-/-}$	*Cd86*	Accelerated[c]	[62, 82]
NOD.*CD154*$^{-/-}$	*Cd154*	Inhibited[d]	[44]
RIP-IL-10-NOD.*CD154*$^{-/-}$	*Cd154* RIP-IL-10 transgene	None	[2]
RIP-TNFα-NOD.*CD154*$^{-/-}$	*Cd154* RIP-TNFα transgene	None	[44]
4.1-NOD.*CD154*$^{-/-}$	*Cd154* 4.1-TCR transgene	Inhibited[d]	[28]
NOD.*IL-4*$^{-/-}$ *	*Il4*	None	[90, 91]
NOD.*IL-10*$^{-/-}$ *	*Il10*	None	[91]
NOD.*IL-12*$^{-/-}$	*Il12*	None	[111]
NOD.*IFN-γ*$^{-/-}$ *	*Infg*	None	[91, 114]
NOD.*IFN-γRB*$^{-/-}$ *	*Infgr2*	None	[115, 116]
NOD.*IRF-1*$^{-/-}$	*Irf1*	Inhibited[d]	[120]
NOD.*TNFR1*$^{-/-}$	*Tnfr-1*	Inhibited[d]	[127]
NOD.*Pfp*$^{-/-}$ *	*Prf1*	Reduced[a]	[143]
NOD.*Fas*^{lpr}	*Fas*	Inhibited[d]	[144]
NOD.*FasL*^{gld}	*FasL*	Inhibited[d]	[146]
NOD.*ICAM-1*$^{-/-}$	*Icam1*	Inhibited[d]	[133]
RIP-IL-10-NOD.*ICAM-1*$^{-/-}$	*Icam-1* RIP-IL-10 transgene	Inhibited[d]	[100]
NOD.*CD1d*$^{-/-}$	*Cd1d*	Accelerated[c]	[72, 73]
NOD.*β2m*$^{-/-}$	*B2m*	Inhibited[d]	[7, 8, 9]
β2m^{b}-NOD.*β2m*$^{-/-}$	*B2m* B2m^{b} transgene	Inhibited[d]	[168]
NOD.*CIITA*$^{-/-}$	*Ct2a*	Inhibited[d]	[150]
RIP-TNFα-NOD.*CIITA*$^{-/-}$	*Ct2a* RIP-TNFα transgene	Accelerated[c]	[163]
NOD.*CD4*$^{-/-}$ *	*Cd4*	Inhibited[d]	[16]
NOD.*MIP-1α*$^{-/-}$	*Mip-1a*	Reduced[a]	[129]
NOD.*ICA69*$^{-/-}$	*Ica-1*	None	[30]
NOD.*Proinsulin2*$^{-/-}$	*Proinsulin2*	Accelerated[c]	[31]

* Available from the Type 1 Diabetes Repository (T1DR) at The Jackson Laboratory <exref type = "URL" >http://www.jax.org/t1dr</exref>

[a] Lower incidence of diabetes than in wild-type NOD mice

[b] Higher incidence of diabetes than in wild-type NOD mice

[c] Earlier onset of diabetes than in wild-type NOD mice

[d] Lower insulitis scores (or absence of insulitis) and no diabetes

CD4+ T cells need to engage beta cell antigens on local APCs to undergo activation. Studies of NOD mice deficient in CD8+ T cells have suggested that the initial insult shedding these beta cell autoantigens into the milieu is mediated by CD8+ cytotoxic T lymphocytes (CTLs) [7, 8, 9], which invariably infiltrate the islets of NOD mice (reviewed in [10]). In contrast to this, another view holds that Type 1 diabetes is initiated by CD4+ T cells (or other cell types). Thus splenic CD4+ T cells from NOD mice can transfer islet inflammation (insulitis) into immunocompromised hosts, but splenic CD8+ T cells cannot do this [6]. Moreover, beta cells do not express key co-stimulatory molecules, suggesting that naive autoreactive CD8+ T cells also need to engage their target antigens on professional APCs to be able to differentiate into CTLs. Finally, genetic susceptibility and resistance to Type 1 diabetes are profoundly affected by polymorphisms of MHC class II genes (reviewed in [11]), which specifically control the development and function of CD4+ T cells.

The low peripheral precursor frequency and repertoire heterogeneity of pathogenic autoreactive T cells in NOD mice precludes detailed studies of their developmental biology. During the last decade a number of transgenic NOD mice expressing T cell receptors (TCRs) that target naturally occurring, non-transgenic beta cell autoantigens have become available [12, 13, 14, 15, 16, 17, 18]. The rearranged TCR-V(D)J genes expressed by these mice were isolated from islet-spe-

Table 4. Non-MHC*idd* loci and NOD mice congenic for non-MHC *idd* loci

Idd locus	Chromosome	Origin	Effects on Type 1 diabetes	Animal model	References
Idd2	9	NON	None	Not available	[3]
Idd2	9	B10	Reduced[a]	Not available	[170]
*Idd3**	3	B6	Reduced[a]	NOD.B6*Idd3*	[176]
Idd4	11	B6	Reduced[a]	NOD.B6*Idd4*	[4]
*Idd5**	1	B10	Reduced[a]	NOD.B10*Idd5*	[179, 180]
Idd6	6	PWK	Accelerated[c]	Not available	[170]
Idd6	6	C3H	Reduced[a]	NOD.C3H*Idd6*	[178]
Idd6	6	B10	Reduced[a]	Not available	[170]
Idd7	7	B10	Increased[b]	Not available	[170]
Idd8	14	B10	Increased[b]	Not available	[170]
*Idd9**	4	B10	Reduced[a]	NOD.B10*Idd9*	[181]
*Idd10**	3	B6	Reduced[a]	NOD.B6*Idd10*	[182]
Idd11#	4	B6	Reduced[a]	NOD.B6*Idd11*	[5]
Idd12	14	B6	Reduced[a]	Not available	[170]
Idd13	2	NOR	Reduced[a]	NOD.NOR*Idd13*	[167]
Idd14	13	NON	Reduced[a]	Not available	[171]
Idd14	13	B6	Accelerated[c]	NOD.B6*Idd14*	[177]
Idd15	5	NON	Reduced[a]	Not available	[171]
Idd16	17	CTS	Reduced[a]	NOD.CTS*Idd16*	[172]
Idd16	17	B10A	Inhibited[d]	NOD.B10AG3	[173]
*Idd17**	3	B6	Reduced[a]	NOD.B6*Idd17*	[182]
*Idd18**	3	B6	Reduced[a]	NOD.B6*Idd18*	[182]
Idd19	6	C3H	Reduced[a]	NOD.C3H*Idd6Idd19*	[178]
Idd20	6	C3H	Reduced[a]	NOD.C3H*Idd20*	[178]

Table does not include sub-congenic lines.
* Available from the Type 1 Diabetes Repository (T1DR) at The Jackson Laboratory http://www.jax.org/t1dr or from Taconic Research Farms http://www.taconic.com/emerging/nod-models/nodcongenics.htm
# Overlaps with *idd*9
[a] Lower incidence of diabetes than in wild-type NOD mice
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cific T cell clones, which had been propagated from NOD mice, cloned into TCR-expression shuttle vectors and introduced into the mouse germ line. As most of the lymphocytes that develop in these animals express a single TCR, it is possible to do detailed investigations of the mechanisms which control the development, regulation, activation, recruitment and effector function of diabetogenic T cells in genetic backgrounds prone to and resistant to Type 1 diabetes.

Autoreactive CD8+ T cells

The antigenic specificity or specificities of the CD8+ T cells assumed to be involved in the initiation of Type 1 diabetes are unknown. Several lines of evidence had previously suggested that the antigenic repertoire of these T cells is restricted. For example, many of the CD8+ T cells isolated from islets of acutely diabetic NOD mice are cytotoxic to beta cells in the context of the MHC class I molecule H-2K^d and use TCRα chains with homologous complementarity-determining region 3 (CDR3) sequences [19]. Moreover, most CD8+ T cells isolated from islets of transgenic NOD mice expressing the TCRβ chain of the CD8+ clone NY8.3 (which uses a representative CDR3α sequence) express endogenous TCRα chains which are identical to the chain used by the clonotype which donated the TCRβ transgene [15]. A considerable percentage of CD8+ T cells propagated from the earliest insulitic lesions of NOD mice use this particular TCRα chain, which employs Vα17 and Jα42 elements [20]. Studies of 8.3-TCRαβ-transgenic NOD mice (8.3-NOD) have shown that CD8+ T cells expressing this particular TCRα chain (along with an appropriate TCRβ chain) are highly diabetogenic [15].

The availability of 8.3-NOD mice afforded a unique opportunity to search for antigenic ligands of CD8+ CTLs relevant to Type 1 diabetes. It is important to note that differentiated CTLs derived from islets of pre-diabetic and acutely diabetic NOD mice are functionally and phenotypically unstable: they either do not survive repeated antigenic stimulation in vitro, or they lose cytotoxic activity within days or weeks in culture [19]. Repeated antigenic stimulation with antigen-pulsed DCs of 8.3-CTLs differentiated in vivo induces transient re-expression of the recombination-activating genes (*rag*) and TCR revision, leading to loss of beta cell reactivity [21]. The screening of H-2K^d-binding combinatorial peptide libraries with short-term-expanded 8.3-CTL lines led to the identification of two major peptide ligands for T cells expressing the

prevalent Vα17-Jα42 TCRα chain: NRP and NRP-A7, an alanine mutant analogue of NRP with superior agonistic properties [22]. Subsequent studies confirmed that these two peptides were recognised by a large proportion of islet-associated CD8+ T cells from wild-type NOD mice [22, 23]. Hyper-mutation of the NRP sequence subsequently led to identification of NRP-V7, a higher affinity ligand of the 8.3-TCR than NRP or NRP-A7 [24]. Using fluorescently labelled peptide/MHC tetramers carrying the NRP-V7 peptide, we later discovered that progression of insulitis to overt diabetes in NOD mice is associated with expansion of the circulating pool of NRP-V7-reactive T cells in peripheral blood [25]. More recently the identity of the naturally occurring ligand of the NRP-A7/V7-reactive CD8+ T cell subpopulation has been discovered using 8.3-CTLs as probes. This ligand is a beta-cell specific protein of unknown function, which resides in the endoplasmic reticulum [26]. The question of whether islet-associated CD8+ T cells in NOD mice target only one or several epitopes from this autoantigen and whether this autoantigen is also targeted by human MHC class I-restricted CD8+ T cells is one of the fundamental issues currently under investigation.

The fact that the NRP-A7/V7-reactive CD8+ T cell subpopulation is prevalent in T cell infiltrates of islets from NOD mice does not imply that the CD8+ T cell response in Type 1 diabetes is exclusively directed against a single autoantigen, let alone a single peptide. One study, for example, has reported that insulitic CD8+ T cells in young NOD mice recognise an insulin-derived peptide [27]. Although we find that these cells represent only a very small fraction of all islet-associated CD8+ T cells, even in young animals [23, 25], they could still play an important role in the disease process. However, there is also evidence, in islets of NOD mice, for the existence of autoreactive CD8+ T cells which recognise neither insulin nor the antigenic target of 8.3-CD8+ T cells [23, 25]. Explaining the repertoire of MHC class I-restricted CD8+ T cell responses in murine Type 1 diabetes has obvious diagnostic and therapeutic implications for human Type 1 diabetes.

CD4+ T-cell-assisted recruitment of CD8+ T cells

Comparative studies of 8.3-NOD mice which were competent or deficient in recombination-activating gene 2 (*rag-2*) have shown that efficient accumulation of 8.3-CD8+ T cells into islets requires the assistance of CD4+ T cells [15, 28]. This, however, is not a universal phenomenon. Thus AI4 CD8+ T cells are as diabetogenic in the absence of CD4+ T cells as they are in their presence. The reasons for these differences among strains are unknown, but one possibility is that the need for help from CD4+ T cells in MHC class I-restricted TCR-transgenic animals is a function of the affinity of the TCRs of CD8+ T cells for peptide/MHC. Whatever the mechanism, it is likely that the peripheral frequency of T-helper-independent, high-avidity, autoreactive CD8+ T cells in non-TCR-transgenic mice is too low for these cells to be able to initiate disease without CD4+ T cells (see below). This would explain why disease initiation in wild-type NOD mice requires the coordinated recruitment of CD4+ and CD8+ T cells.

Autoreactive CD4+ T cells

The antigenic specificity or specificities of autoreactive CD4+ T cells contributing to the initiation of and/or progression to Type 1 diabetes in the NOD mouse are unknown, but several candidates have been considered. Autoreactive CD4+ T cells from NOD mice recognise a number of islet autoantigens, including insulin, GAD65 and GAD67, the tyrosine phosphatase-like IA-2, phogrin, ICA69, and heat shock protein 60. NOD mice overexpressing or lacking some of these autoantigens, including GAD65, ICA69 and proinsulin, have been generated [29, 30, 31, 32, 33]. Systemic overexpression of a *GAD65* transgene has been found to promote diabetogenesis [32], suggesting that there is a breakdown of tolerance to this autoantigen in NOD mice. In contrast, overexpression of *ICA69* or *proinsulin 2* transgenes gives variable degrees of diabetes resistance [33, 34], possibly by inducing central tolerance of autoreactive lymphocytes. It should be noted that the results of these studies are difficult to reconcile with those obtained in NOD mice lacking these autoantigens (see below).

Islet-specific suppression of GAD65 or GAD67 expression by a rat insulin promoter (RIP)-driven, antisense *GAD* transgene inhibited the development of insulitis and Type 1 diabetes [29]. Although this result was taken to imply that this autoantigen plays a critical role in the pathogenesis of Type 1 diabetes, the authors didn't formally exclude the potentially confounding contribution of genetic contamination of the transgenic mice to the study's outcome [29]. Disruption of the *ICA69* gene in NOD mice did not noticeably change the natural history of Type 1 diabetes, indicating that this autoantigen is dispensable [30]. Interestingly, disruption of the *proinsulin 2* gene in NOD mice accelerated diabetes, suggesting that proinsulin-2-reactive T cells play a regulatory role in this strain [31]. Studies of transgenic NOD mice expressing a GAD65-reactive TCR [17] have also suggested a regulatory role for GAD65-reactive CD4+ T cells in NOD mice. Two other MHC class II (I-A^{g7})-restricted TCRs derived from NOD mice have been studied so far (BDC2.5 and 4.1). Although their natural antigenic ligands remain unknown [12, 13], there is evidence that they recognise novel autoantigens. Thus the

BDC2.5 TCR recognises a mimotope which is homologous to the 528–539 fragment of GAD65 and was defined using combinatorial peptide libraries, but it does not recognise the natural epitope [35]. According to our unpublished observations, 4.1-CD4+ T cells recognise a beta cell autoantigen other than proinsulin, phogrin, ICA69, IA-2, GAD65 or GAD67.

T cell priming

It has been recently shown that the PLNs are essential to the development of Type 1 diabetes [36]. Earlier experiments had suggested that diabetogenic T cells first encounter cognate peptide/MHC complexes in the PLNs [28, 37, 38]. These experiments, which used T cells labelled with carboxyfluorescein diacetate succinidimyl ester and expressing MHC class I (K^d)- or class II-restricted ($I\text{-}A^{g7}$), beta-cell-autoreactive TCRs (8.3-CD8+ and BDC2.5- or 4.1-CD4+), also showed that priming of these T cells does not occur in neonatal mice. This suggests that access of beta cell autoantigens to the cross-presentation pathway is developmentally regulated [37, 38]. In 8.3-CD8+ T cells, for example, priming in the PLNs is undetectable up to three weeks after birth. The amount of priming increases with the age of the animal and the extent of beta cell apoptosis in islets, implicating in situ beta cell death in the facilitation of this process. Interestingly, priming of 8.3-CD8+, 4.1-CD4+ and BDC2.5-CD4+ T cells in NOD mice is preceded by a physiological "wave" of beta cell apoptosis, which peaks at about 2 weeks of age and is independent of T cells [39]. These observations have led to suggestions that developmental remodelling of beta-cell mass in the post-neonatal period promotes access of beta-cell autoantigens to the cross-presentation pathway. In fact, the earliest T cell cross-priming event detectable in the PLNs of NOD mice is not affected by expression of a transgene protecting beta cells from cytotoxicity induced by CD8+ CTLs (Yamanouchi et al., submitted).

T-T collaboration

Productive collaboration between CD4+ T-helper cells and pre-cytotoxic CD8+ T cells requires the presentation of different epitopes by the same APC, usually a DC, in a CD40/CD154-dependent manner [40, 41, 42]. CD40 ligation on DCs induces the up-regulation of T cell co-stimulatory molecules, elicits the production of pro-inflammatory cytokines and endows DCs with the ability to differentiate CD8+ T cells. Blockade of the CD40-CD154 pathway inhibits the development of insulitis and diabetes in NOD mice [43, 44]. Whereas disruption of CD154 also protects monoclonal 4.1-TCR-transgenic NOD mice from diabetes, it has no effect on the T-helper-independent diabetogenic activity of 8.3-CD8+ T cells [28]. In fact, 8.3-CD8+ T cells do not need CD154 to undergo antigen-driven activation in vivo [28]. Despite this, the ability of T-helper cells to enhance the diabetogenic activity of 8.3-CD8+ T cells is CD154-dependent, because only CD154+ CD4+ T cells can have this enhancing effect [28]. Systemic activation of DCs with an agonistic anti-CD40 monoclonal antibody (mAb) or with CpG DNA (a ligand of toll-like receptor 9) was found to bypass the need for CD154+ CD4+ T cells in this system, suggesting that T-helper cells promote the recruitment and activation of autoreactive CD8+ T cells by activating DCs. When expressed locally, TNFα can also overcome the need for CD154 in a non-TCR-transgenic model of diabetes induced by CD8+ T cells [44]. However, CD154 does not simply act by ligating CD40 on DCs; ligation of CD154 co-stimulates CD4+ T cell responses in vitro [45] and in vivo [28].

B lymphocytes

The activation of CD8+ T cells in Type 1 diabetes is probably driven by DCs, which have the unique ability to process exogenous antigens via the endogenous pathway of antigen presentation (called "cross-presentation"). Activation, on the other hand, of autoreactive CD4+ T cells appears to involve the recruitment of B cells as APCs, at least in non-TCR-transgenic NOD mice. People with Type 1 diabetes bear autoreactive B cells that differentiate into plasma cells capable of secreting non-cytopathic autoantibodies against multiple islet cell antigens. These autoreactive B cells are thought to contribute to Type 1 diabetes development by capturing beta cell antigens via surface immunoglobulins (the B cell receptor) and by presenting epitopes derived from these antigens to autoreactive CD4+ T cells via MHC class II molecules on the cell surface. Several lines of evidence support the hypothesis that B cells play an important role in Type 1 diabetes: (i) maternal autoantibodies influence diabetes development in the offspring [46]; (ii) Igμ-gene-deficient NOD mice and NOD mice bearing B cells deficient in MHC class II molecules do not usually develop diabetes [47, 48, 49, 50, 51, 52, 53]; (iii) certain autoreactive CD4+ T cells do not proliferate in the PLNs of B-cell-deficient NOD mice [54]; and (iv) a reduced peripheral frequency of islet-specific B cells inhibits diabetogenesis, whereas an increased frequency accelerates it [55, 56]. The suggested ability of B cells to act as APCs in Type 1 diabetes could be age-dependent, because the adoptive transfer of B cells into adult B-cell-deficient NOD mice does not restore T-cell mediated autoimmunity [51]. In contrast, other findings suggest that the diabetogenic activity of B cells in NOD mice is not always necessary, as: (i) splenic T cells from 5-week-old NOD mice can trans-

fer diabetes into NOD.*scid* mice in the absence of B cells [57]; (ii) fetal thymic transplants can restore diabetes susceptibility in NOD.*scid* mice (that is, in the absence of B cells) [58]; and (iii) certain autoreactive CD4+ T cells can efficiently trigger diabetes in B-cell-deficient TCR-transgenic animals [15, 59]. Although these experimental systems do not fully repeat the natural history of Type 1 diabetes in wild-type NOD mice, they do make the contribution of B cells to Type 1 diabetes seem more complex than currently thought.

Immunoregulation

There is evidence suggesting that the autoimmune proclivity of NOD mice results from impaired development and/or function of regulatory T cells. However, other lines of evidence indicate that NOD mice do have functional regulatory cells. Splenocytes from diabetic NOD mice can efficiently transfer diabetes into young immunocompromised NOD mice. Yet when splenocytes from diabetic NOD mice are co-injected with splenocytes from pre-diabetic donors, they lose some of their diabetogenic potential. This suggests that the spleens of pre-diabetic animals contain anti-diabetogenic (regulatory) cells [60]. These putative regulatory cells probably develop in the post-natal period, since splenocytes from diabetic mice can transfer disease into neonatal, but not into older NOD hosts. The development of these cells must be thymus-dependent, because thymectomy at three weeks of age accelerates the onset of Type 1 diabetes in female NOD mice [61]. Three T cell subsets with regulatory properties have been identified in NOD mice: CD4+CD25+, CD4+CD62L+ and NK (natural killer) T cells [62, 63, 64]. In response to TCR ligation, CD4+CD25+ T cells do not proliferate (they are anergic), but they can efficiently inhibit the proliferation of naive T cells in vitro [65]. Both CD4+CD25+ and CD4+CD62L+ T cells can delay the onset of diabetes in recipients of diabetogenic T cells [62, 63, 66, 67]. Recent studies suggest that CD4+CD25+ T cells arise from high-avidity autoreactive T cells during thymocyte development, i.e. upon engagement of self-peptide/MHC complexes on thymic epithelium [68].

The development of NK T cells in NOD mice is impaired, but not completely abrogated [64, 69, 70]. Natural killer T cells are restricted by CD1d (an MHC class I-like molecule) and usually express an invariant TCRα chain (Vα14-Jα281) [71]. The fact that CD1d-deficient NOD mice develop diabetes earlier and more frequently than wild-type NOD mice [72, 73] shows that NOD mice bear functional NK T cells. Similarly, when α-galactosyl-ceramide, a glycolipid recognised by NK T cells, was given to NOD mice, this protected them from diabetes [72, 74, 75, 76]. Studies in BDC2.5 TCR-transgenic NOD mice provide additional evidence that NK T cells in NOD mice are functional. Massive insulitis develops at a very young age in BDC2.5-NOD mice [37, 77], but their intra-islet BDC2.5 CD4+ T cells do not differentiate into diabetogenic T cells in the presence of endogenous (non-transgenic) T cells. As a result, these mice have a very low incidence of diabetes [59]. Four lines of evidence suggest that diabetes resistance in BDC2.5-NOD mice is mediated by NK T cells. Firstly, BDC2.5 CD4+ T cells activated in vitro can readily transfer diabetes into NOD.*scid* recipients, which lack B and T cells [59]. Secondly, the BDC2.5-TCR efficiently triggers diabetes in mice deficient in TCR-Cα or *rag* and in mice with NOD.*scid* backgrounds (all of which lack endogenous T cells) [77]. Thirdly, the introduction of a CD1d deficiency into these mice abrogates their resistance to diabetes [73]. And finally, splenic DX5+ NK T cells actively suppress the development of diabetes in BDC2.5-NOD.*RAG-2*$^{-/-}$ hosts in the presence of CD4+ T cells from young, pre-diabetic NOD mice [77].

The mechanisms by which NK T cells inhibit diabetes have been examined in NOD mice expressing a *Vα14-Jα281 TCR* transgene. These mice have increased numbers of NK T cells [78]. It has been found that NK T cells do not inhibit the development of diabetes by blocking the activation of autoreactive T cells, but possibly by inhibiting the differentiation of antigen-activated T cells into effectors producing interferon-γ (IFN-γ) [79]. The reconstitution of NOD.*scid* mice with thymic pre-T cells from various strains of mice has shown that defective NK T cell development in NOD mice results from defects intrinsic to T cells, rather than from defects in thymic stromal cells [80]. Despite these observations, the role played by impaired NK T cell development in the pathogenesis of Type 1 diabetes remains unclear.

Co-stimulation versus autoreactivity and immunoregulation

Expression of transgenic B7.1 molecules in beta cells of NOD mice accelerates the onset of Type 1 diabetes, indicating that this co-stimulatory molecule, when expressed locally, can amplify the diabetogenic autoimmune response. Subsequent gene-targeting experiments, however, showed that the role of co-stimulatory molecules in Type 1 diabetes is more complex.

Although treatment of young NOD mice with CTLA-4-Ig inhibits the development of diabetes (presumably by blocking co-stimulatory molecules on APCs) [81], NOD mice deficient in CD28, as well as NOD mice deficient in B7.1/B7.2, have a higher incidence and faster onset of diabetes than wild-type NOD mice [62, 82]. This is because the development of regulatory CD4+CD25+ T cells is controlled, at least in part, by CD28/B7 interactions [62, 82]. So in the absence of CD28 or B7 molecules, these T cells do not develop properly.

Interactions between CD40 and CD154 also help maintain the balance between effector and regulatory T cells in immune responses, albeit through a different mechanism. Whereas CD154 blockade inhibits $CD4^+$ T cell help (see above), it has no effect on the development or function of regulatory $CD4^+CD25^+$ T cells, at least in NOD mice (Serra et al., submitted). As helper $CD4^+$ T cells oppose the immunosuppressive activity of regulatory $CD4^+CD25^+$ T cells, we proposed that CD154 blockade inhibits diabetogenesis by promoting "unopposed" suppression of diabetogenic T cell responses by these regulatory T cells. TNFα/TNFR [67] and TRANCE/RANK signals [83] have also been implicated in the development of $CD4^+CD25^+$ T cells. Hence, manipulation of these costimulatory pathways could be important for the therapy of Type 1 diabetes.

Cytokines

Cytokines play important roles in the development, activation, recruitment and function of T cells and APCs, but can also function as effectors of beta cell death. Although cytokines are typically classed as pro-inflammatory and anti-inflammatory, their effects on the natural history of Type 1 diabetes are more a function of the location, timing and duration of expression than a function of the subgroup they belong to.

Anti-inflammatory cytokines

IL-2 is a T cell growth factor. However, IL-2 deficiency causes a multi-organ inflammatory syndrome which results from impaired development of regulatory $CD4^+CD25^+$ T cells [84]. Thus IL-2 can be regarded as an anti-inflammatory cytokine. Expression of a RIP-driven *il-2* transgene in beta cells of NOD mice accelerated the development of diabetes, possibly because it promoted the growth of islet-infiltrating T cells in situ [85]. This transgene also induced diabetes in NOD.*scid* mice [86], suggesting that it promoted diabetes by impairing beta cell function.

Unlike IL-2, IL-4 is clearly an anti-diabetogenic cytokine [64, 87, 88]. Expression of a RIP-driven *il*-4 transgene protected NOD mice from insulitis and diabetes by inducing immunoregulatory Th2 cells [89]. Yet when this transgene was expressed in BDC2.5-NOD mice, it enhanced the pathogenicity of TCR-transgenic T cells, suggesting that, produced in situ, IL-4 can potentiate the growth and/or activation of autoreactive Th1 cells [89]. However, it does not seem likely that IL-4 plays a role in the natural history of Type 1 diabetes, as NOD mice deficient in IL-4 have a normal incidence of diabetes with normal kinetics [90, 91]. An alternative explanation is that the absence of IL-4 in these mice is compensated by other cytokines, e.g. IL-13, which can prevent diabetes in NOD mice [92] and shares a common receptor subunit with IL-4 [93]. Studies of NOD mice deficient in IL-4 receptor α chain may clarify this possibility.

Another anti-inflammatory cytokine initially thought to have diabetogenic activity is IL-6. The treatment of NOD mice with an anti-IL-6 antibody inhibited the development of Type 1 diabetes [94], whereas expression of a RIP-*il-6* transgene in non-diabetes-prone mice induced islet inflammation [95]. Surprisingly, expression of this transgene in NOD mice delayed the onset of diabetes [96], possibly by inducing Th2 cells [97]. Interleukin-10, another Th2-type cytokine, has been shown to have diabetogenic and anti-diabetogenic effects. Systemic treatment of NOD mice with recombinant IL-10 inhibited the development of Type 1 diabetes [99]. However, when this cytokine was expressed locally (in pancreatic islets), it promoted Type 1 diabetes [44, 99, 100]. Despite the above, NOD mice deficient in IL-10 develop diabetes normally, which suggests that IL-10 does not play a role in spontaneous development of the disease, or that its role is compensated by other mechanisms [91]. Expression of TGF-β, a powerful negative regulator of T-cell-mediated immune responses [101], in NOD islet cells altered APC preference and polarised islet antigen-specific responses towards a Th2 phenotype, inhibiting the development of insulitis and diabetes [102, 103].

Pro-inflammatory cytokines

Interleukin-12 is a pro-inflammatory cytokine necessary for the development of IFN-γ-producing Th1 cells. Several observations suggest that IL-12 plays an important role in diabetogenesis. Firstly, the progression of insulitis to overt diabetes in NOD mice is associated with increasing amounts of IL-12 in pancreatic islets [104]. Secondly, genetic susceptibility to human Type 1 diabetes is associated with *il-12 p40* gene polymorphism [105]. In addition, the administration of recombinant IL-12 promotes diabetes, and IL-12 blockade inhibits it [106, 107, 108, 109, 110], although this depends on when the treatment begins [108, 110]. However, like IL-4- and IL-10-deficient NOD mice, NOD mice deficient in IL-12 have a normal incidence of diabetes with normal kinetics [111], suggesting that the role of IL-12 in the disease process is compensated by other cytokines, e.g. IL-2 [112].

Interferon-γ is another Th1-type cytokine with pro- and anti-diabetogenic activities. Whereas IFN-γ blockade protects NOD mice from diabetes [113], NOD mice deficient in IFN-γ- and NOD mice deficient in IFN-γ receptor beta chain develop diabetes with normal incidence [92, 114, 115, 116, 117]. Although NOD mice deficient in IFN-γ receptor alpha chain do not develop diabetes [117], there is compel-

ling evidence that resistance to diabetes in these mice is not caused by IFN-γ receptor deficiency, but by the presence of a linked anti-diabetogenic 129 allele [115, 116]. Overexpression of a dominant negative IFN-γ receptor transgene in islet beta cells made them unresponsive to the effects of IFN-γ on beta cells, i.e. up-regulation of MHC class I expression, but did not inhibit diabetogenesis [118]. This indicates that IFN-γ receptor signalling in beta cells is not an essential component of the disease process. Interferon regulatory factor-1, a transcription factor induced by IFN-α, IFN-β and IFN-γ, plays a key role in Th1 cell development [119]. Unlike NOD mice deficient in IFN-γ, NOD mice which are deficient in interferon regulatory factor-1 develop neither diabetes nor insulitis [120], suggesting that lack of IFN-γ in IFN-γ-deficient NOD mice is compensated by IFN-α and/or IFN-β. The development of diabetes in *RIP-IFN-α*-transgenic mice supports this possibility [121].

Another pro-inflammatory cytokine which contributes to the pathogenesis of Type 1 diabetes in NOD mice is TNFα. Given to neonatal NOD mice, TNFα exacerbates Type 1 diabetes [122]. However, when given to older animals, it inhibits disease progression [123]. Studies in *RIP-TNFα*-transgenic mice [44, 124] have shown that local production of TNFα promotes diabetogenesis by enhancing APC function and the development of T-helper-independent $CD8^+$ effector cells. In contrast, prolonged treatment of adult NOD mice with recombinant TNFα attenuates TCR signals [125], induces T cell tolerance [126] and generates regulatory $CD4^+CD25^+$ T cells [67]. Studies in NOD mice deficient in TNF receptor-1 have also documented a role for TNFα as effector molecule in Type 1 diabetes [127, 128].

The pathogenesis of Type 1 diabetes also involves cytokines not discussed here. To the best of our knowledge, these cytokines have not been studied in the context of cytokine-transgenic or gene-targeted NOD mice and were therefore omitted. It is also likely that chemokines play essential, non-redundant roles in the pathogenesis of Type 1 diabetes, but very little is known about the specific nature of these roles. One exception is macrophage inflammatory protein 1α (MIP-1α), a member of the CC chemokine family that is expressed by activated islet-specific $CD4^+$ Th1 cells. NOD mice deficient in MIP-1α develop a significantly reduced incidence of diabetes [129]. (For detailed reviews on the role of cytokines, chemokines and their receptors in Type 1 diabetes, please see references [130, 131, 132]).

Adhesion molecules

To study the contribution of intercellular adhesion molecule-1 (ICAM-1) and L-selectin (CD62L) to the pathogenesis of Type 1 diabetes, NOD mice lacking these adhesion molecules have been produced [100, 133, 134]. Expressed on multiple cell types, including beta cells and vascular endothelial cells, ICAM-1 interacts with leucocyte-function-associated antigen-1 (LFA-1) on T cells and delivers a potent co-stimulatory signal which influences the fate of activated T cells in inflammatory lesions [135]. NOD mice deficient in ICAM-1 do not develop insulitis, even in the presence of a diabetogenic *RIP-il-10* transgene [100, 136]. Moreover, short-term treatment of young NOD mice with an anti-ICAM-1 mAb has been shown to inhibit the development of Type 1 diabetes [137]. Nevertheless, splenocytes from mAb-treated NOD mice did not transfer diabetes into NOD.*scid* recipients, suggesting that ICAM-1 blockade has tolerogenic properties [137]. Interestingly, transient blockade of the ICAM-1/LFA-1 interaction in diabetic NOD mice (with recombinant soluble ICAM-1) caused a sustained reversal of diabetes in a large number of animals, raising the possibility that interactions between ICAM-1 and LFA-1 contribute to diabetogenesis through more than one mechanism [137]. The other adhesion molecule for which gene-targeted NOD mice are available is CD62L. Non-obese diabetic mice given an anti-CD62L mAb developed neither insulitis nor Type 1 diabetes [138], whereas CD62L-deficient NOD mice developed diabetes normally [134].

Evolution of insulitis to overt diabetes

Non-obese diabetic mice begin to develop insulitis at about 3 weeks of age, but do not become diabetic until at least nine weeks later. Studies of the fate of NRP-A7/V7-reactive $CD8^+$ T cells in wild-type NOD mice using peptide/MHC tetramers showed that progression of insulitis to overt diabetes in NOD mice is associated with "avidity maturation" of this T cell subset [23]. As pre-diabetic NOD mice age, their islet-associated $CD8^+$ T cells contain increasing numbers of NRP-A7-reactive cells, and these cells bind NRP-A7/K^d tetramers with increasing avidity. Repeated treatment of pre-diabetic NOD mice with soluble NRP-A7 peptide blunted the avidity maturation of the NRP-A7-reactive $CD8^+$ T cell population by deleting clonotypes expressing high-affinity TCRs and expanding clonotypes expressing low-affinity TCRs. This inhibited production of CTLs and halted the progression of insulitis to diabetes. It is likely that avidity maturation is not unique to NRP-A7/V7-reactive T cells, but rather a general phenomenon in autoimmune T cell responses.

By comparing the fate of TCR-transgenic $CD8^+$ T cells expressing high- or low-affinity NRP-A7/NRP-V7-reactive TCRs in TCR-transgenic NOD mice, we recently showed that central and peripheral tolerance selectively decrease the size of the high-avidity T cell pool. This study also showed that autoimmune inflammation fuels the expansion of this pool (Han et al., un-

published observations). The results provide a developmental basis for T cell avidity maturation in the context of autoimmunity. They also support the idea that beta cell destruction in Type 1 diabetes is executed by a progressively expanding, but initially small population of high-avidity clonotypes that evade peripheral tolerance by localising into inflamed pancreatic islets.

Beta cell destruction

Several lines of evidence indicate that CD8+ CTLs, along with CD4+ T cells, are major effectors of beta cell destruction in Type 1 diabetes. The former are consistently present in islets of NOD mice [10], can transfer diabetes into NOD.*scid* mice [139, 140] and can kill beta cells in vivo of mice resistant to Type 1 diabetes [141]. Studies of NOD mice expressing the *8.3-TCRβ* transgene provided the first evidence that CD8+ CTLs contribute to beta cell loss in spontaneous Type 1 diabetes. These mice have a minor (but selective) increase in the frequency of beta-cell-reactive CD8+ T cells and owing to increased recruitment of CD8+ (but not CD4+) T cells into islets, develop accelerated Type 1 diabetes [14]. On the other hand, there is evidence that beta cell destruction in Type 1 diabetes is also effected by CD4+ T cells. Thus beta-cell-autoreactive CD4+ T cell clones can transfer disease into immunocompromised NOD mice [142], and monoclonal TCR-transgenic NOD mice expressing I-A^{g7}-restricted TCRs develop accelerated diabetes [15, 59]. However, the results of these studies in TCRαβ-transgenic NOD mice should be interpreted with caution, as the unusually high frequencies of autoreactive CD4+ or CD8+ T cells in these animals could overwhelm the mechanisms which, in non-transgenic mice, would prevent the relevant T cells from causing insulitis upon activation.

Various animal models have been developed to test the role of two major pathways of cell-mediated cytotoxicity in Type 1 diabetes, perforin and Fas. Perforin-deficient NOD mice develop insulitis but rarely become diabetic, implicating perforin-expressing CTLs as effectors of beta cell destruction in Type 1 diabetes [143]. In contrast, Fas-deficient NOD.Fas^{lpr} mice do not develop diabetes or insulitis [144], and transgenic NOD mice expressing FasL in islet beta cells develop an accelerated form of Type 1 diabetes (owing to beta cell fratricide) [145]. NOD mice which are heterozygous for the $FasL^{gld}$ mutation are also resistant to diabetes [146]. Taken together, these results suggest that the Fas/FasL pathway of cell-mediated cytotoxicity could, by mediating lysis of beta cells by disease-initiating CTLs, be key to the initiation of Type 1 diabetes. In support of this hypothesis, 8.3-CD8+ CTLs, which are representative of the earliest CD8+ T cells propagated from islets of wild-type NOD mice, kill beta cells exclusively via Fas [147]. However, Fas-deficient islet grafts are readily destroyed in spontaneously diabetic NOD mice [148], indicating that Fas-mediated beta cell cytotoxicity is not an essential mechanism of beta cell destruction at later stages of the disease process, where perforin and soluble effectors such as TNFα possibly play a more important role. Studies with TCR-transgenic mice have shown that different T cell clones can kill the same targets (beta cells) via different mechanisms. So whereas BDC2.5-CD4+ T cells kill beta cells through a pathway dependent on TNF receptors [128], 4.1-CD4+ CTLs do so via Fas [149].

Genetics

MHC-linked genes. MHC class II genes are a major component of genetic susceptibility and resistance to many autoimmune disorders, including Type 1 diabetes. NOD mice deficient in MHC class II expression owing to inactivation of the class II-transactivator do not develop diabetes, implying that MHC class II molecules are essential to the development of diabetes [150]. In humans MHC-linked susceptibility and resistance to Type 1 diabetes are primarily associated with the *HLA-DQB1* locus. Alleles encoding DQβ chains with Ser, Ala or Val at position 57 generate risk, whereas alleles encoding DQβ chains with Asp at this position provide different degrees of protection [11]. The NOD mouse is homozygous for a unique H-2 haplotype ($H\text{-}2^{g7}$). This haplotype carries a non-productive *I-Eα* gene and encodes an I-$A\alpha^{d}$/I-$A\beta^{g7}$ heterodimer in which the His and Asp found at positions 56 and 57 in most I-Aβ chains are replaced by Pro and Ser respectively. Studies of congenic NOD mice expressing non-NOD MHC haplotypes, and of NOD mice expressing $I\text{-}E\alpha^{d}$, $I\text{-}E\alpha^{k}$, modified $I\text{-}A\beta^{g7}$, $I\text{-}A\alpha^{k}/I\text{-}A\beta^{k}$ or $I\text{-}A\beta^{d}$ transgenes have proven that class II molecules play a direct role in providing susceptibility or resistance to Type 1 diabetes (reviewed in [11]). As MHC molecules play a pivotal role in T cell development, some authors have hypothesised that protective MHC molecules provide resistance to Type 1 diabetes by tolerising autoreactive T cells [151]. Studies in MHC-congenic NOD mice have not found evidence for T cell tolerance (the mice were resistant to diabetes despite exporting autoreactive T cells to the periphery). Consequently it was later proposed that MHC-associated resistance to Type 1 diabetes was caused by immunoregulation or immune deviation [13, 152, 153, 154]. This, however, assumes that "tolerance" would target all autoreactive T cells, regardless of pathogenicity, which means the presence of circulating autoreactive T cells in the animals would be taken to imply absence of deletional tolerance.

Evidence, at least in mice, for a relationship between deletion of certain highly diabetogenic CD4+ T cells and the MHC-linked resistance to Type 1 diabetes comes from our studies with 4.1-TCR-transgenic NOD mice [12, 155, 156]. Here thymocytes expressing the 4.1-TCR were found to undergo central deletion in *H-2*$^{g7/b}$, *H-2*$^{g7/k}$, *H-2*$^{g7/q}$ and *H-2*$^{g7/nb1}$ NOD mice resistant to Type 1 diabetes, a process which happened by engaging anti-diabetogenic MHC class II molecules on bone marrow-derived APCs [12]. Expression in 4.1-NOD mice of anti-diabetogenic MHC class II transgenes such as *I-E*α^k, *I-A*^{d} and *I-A*^{g7PD} (mutated I-A^{g7} encoding Pro and Asp at positions 56 and 57) also led to various degrees of 4.1-thymocyte tolerance and resistance to Type 1 diabetes [156]. This ability of anti-diabetogenic MHC class II molecules to tolerise autoreactive CD4+ thymocytes is not universal, as neither I-Eα^k nor I-A^{g7PD} molecules tolerised CD4+ thymocytes expressing the BDC2.5-TCR [157, 158]. Collectively, these data suggest that in non-TCR-transgenic NOD mice protective MHC class II alleles afford resistance to Type 1 diabetes by tolerising a group of highly pathogenic, MHC-promiscuous, 4.1-like CD4+ T cells which play a critical role in diabetogenesis. 4.1-CD4+ T cells would be representative of this T cell subpopulation. Although speculative, this interpretation is consistent with the observation that the anti-diabetogenic activity of class II molecules in both wild-type and 4.1-TCR-transgenic NOD mice maps to residues around I-Aβ and I-Eβ chain position 57 [156], which are also implicated in the DQB1-linked resistance to Type 1 diabetes in humans and non-TCR-transgenic NOD mice [11]. Whether protective MHC class II molecules also afford resistance to diabetes by selecting regulatory T cells remains to be determined.

A number of HLAs, including HLA-DR3, HLA-DR4, HLA-DQ6 and HLA-DQ8, have been expressed in NOD mice to evaluate the role of these molecules in the pathogenesis of Type 1 diabetes [150, 160, 161, 162, 163, 164]. The HLA-DQ8 molecule, which imparts susceptibility to human Type 1 diabetes, can present most of the major islet autoantigens to autoreactive CD4+ T cells in NOD mice both in vitro and in vivo [159, 160]. The fact that *HLA-DQ8*-transgenic NOD mice which are deficient in I-A^{g7} do not spontaneously develop Type 1 diabetes [161] does not imply that the HLA-DQ8 molecule is not diabetogenic. Probably resistance to diabetes in these mice results from the introduction into the NOD genetic background of non-NOD genetic elements linked to the I-Aβ mutation in the 129 background, which was introduced into transgenic mice to ensure that they do not express murine MHC class II molecules. In general, these studies of HLA-transgenic mice suggest that HLA-DQ8 and HLA-DQ3 do impart susceptibility and that HLA-DR4 and HLA-DQ6 molecules afford resistance to Type 1 diabetes [161, 164, 165].

Systemic expression of an *H-2L* ^{d} transgene (a non-NOD MHC class I gene) in NOD mice reduced the severity of insulitis and inhibited the development of diabetes, suggesting that MHC class I genes play a part in resistance to Type 1 diabetes [166]. It has since been shown that the *Idd13*-linked resistance to diabetes maps to the *β2m* locus, and that transgenic expression of the non-NOD allele of *β2m* in *β2m*-deficient NOD mice affords resistance to diabetes [167, 168]. Although the mechanisms by which the *β2m* dimorphism affords susceptibility or resistance to diabetes are unclear, these findings strongly support the idea that MHC class I-restricted T cells are necessary for diabetogenesis to occur. Importantly, a recent study in HLA-transgenic NOD mice has suggested that this is not peculiar to murine MHC class I molecules. When the *HLA-A2* allele, which is associated with Type 1 diabetes in humans, was transgenically expressed in NOD mice, it also accelerated the onset of diabetes [169].

Non-MHC-linked genes. Diabetogenesis in NOD mice results from complex interactions between the MHC and multiple (i.e. as many as 20) non-MHC-linked genetic elements (*Idd*) [170, 171, 172, 173, 174, 175, 176, 177, 178]. The diabetogenic and anti-diabetogenic activities of *Idd* loci have been studied in congenic NOD mice. In most cases, NOD alleles at *Idd* loci confer diabetes susceptibility, whereas non-NOD alleles at these loci impart resistance. In other cases, the non-NOD alleles (e.g. at *Idd7* and *Idd8*) confer susceptibility [170, 171] or susceptibility and resistance, depending on the strain of origin. Thus the non-obese normal allele at *Idd14* is protective, whereas the B6 allele is more diabetogenic than its NOD counterpart [171, 177]. For *Idd6* the B10 and C3H alleles are protective, whereas the PWK allele confers susceptibility [170, 178].

Studies of sub-congenic lines have shown that chromosomal intervals originally thought to contain single *Idd* loci, in fact contain several. For example, the original *Idd5* and *Idd9* intervals are now thought to contain at least three different *Idd* genes each [179, 180, 181]. Similarly, *Idd17* and *Idd18* loci were recently found near the *Idd3* and *Idd10* loci [177, 183]. Another example of a locus which was missed in initial mapping studies because of its close linkage to a previously identified *Idd* locus on the same chromosome is *Idd16*, which is located on mouse chromosome 17, near the MHC (*Idd1*). There is also evidence that at least two MHC-linked genes other than *I-A*, *I-E*, *K* and *D* contribute to MHC-linked susceptibility or resistance to diabetes [172, 173, 174, 175].

Most of the aetiological mutations responsible for *Idd*-linked susceptibility and/or resistance to diabetes remain a mystery, but there are some exceptions. As noted above, the *Idd13* chromosomal interval contains the dimorphic *β2m* gene, which has been linked conclusively to diabetes risk [167, 168]. A polymorphism

at *Ctla4* is an excellent candidate for one *Idd5* locus [179], and there is considerable evidence suggesting that *Idd3* corresponds to *il-2* or *il21* [183]. Some *Idd* loci afford susceptibility or resistance to more than one autoimmune disease. Thus B6 mice congenic for the NOD alleles at *Idd3* and *Idd5* loci develop Sjogren's syndrome [184, 185, 186], and NOD mice bearing the B6 alleles at the *Idd3* locus are resistant to experimental autoimmune encephalomyelitis [187]. A major challenge facing this field will be to determine the mechanisms through which individual *Idd* elements actually afford diabetes susceptibility or resistance.

Environmental factors

Although Type 1 diabetes is a genetic disease, disease concordance between monozygotic twins is close to 40 per cent [188], suggesting that environmental factors help trigger the disease process. About 13 viruses have been associated with Type 1 diabetes in humans and animal models [189]. One potential trigger of Type 1 diabetes in humans is enterovirus infections during childhood [190], which can induce inflammatory reactions capable of causing beta cell damage [191]. Another possible factor is molecular mimicry between viral and beta cell antigens. In this case infection of the host by the virus induces cross-reactive immune responses against beta cells [192]. The effects of enterovirus infections on the pathogenesis of Type 1 diabetes have also been explored in NOD mice [193, 194, 195]. Genetic predisposition to Type 1 diabetes was found to play a major role in the ability of viral infection to lead to overt disease. So whereas infection of the diabetes-resistant strain C57BL/10.*H2* ^{g7} failed to trigger insulitis, infection of diabetes-prone NOD mice accelerated disease progression [195]. In this model, infection of beta cells by the virus appears to accelerate disease by enhancing the shedding of "sequestered" beta cell autoantigens into the milieu [195]. However, viral infection is not invariably associated with disease exacerbation; other studies have shown that it can also dampen diabetogenic immune responses [196].

Concluding remarks

Type 1 diabetes results from a chronic, beta-cell-specific, T-cell-dependent autoimmune process that develops in genetically susceptible individuals. The nature of the immune cell types, genetic elements and autoantigens involved in this complex autoimmune response remains a matter of intense investigation. Fine dissection of the mechanisms underlying the development of spontaneous autoimmune diabetes has benefited tremendously from the availability of numerous lines of genetically manipulated NOD mice. Studies of the natural history of diabetes in NOD mice lacking or overexpressing molecules thought to play key roles in the disease process have provided an understanding of the pathogenesis of Type 1 diabetes in detail that was unthinkable a few years ago. One of the main developments has been the creation of transgenic strains of NOD mice expressing TCRs that target disease-relevant autoantigens. These models provide very useful tools with which to probe the effects of multiple genes, gene polymorphisms and certain environmental factors on the developmental biology and pathogenicity of autoreactive T cells. Nevertheless, it has become clear that a conclusive understanding of the contribution of specific genes to the pathogenesis of Type 1 diabetes will not be possible without the development of germ-line-competent embryonic stem cells from NOD mice. Studies with gene-targeted NOD mice derived from such lines will put an end to the potentially confounding contribution of linked diabetogenic or antidiabetogenic alleles to study outcome. Nevertheless the animal models already available today offer numerous possibilities for creating combinatorial models of varying complexity.

Sources

The studies reviewed in this manuscript were selected from periodical reviews of the literature as well as from PubMed seaches (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) using the following key words: NOD and transgene, IDDM and cytokine, NOD and co-stimulation, IDDM genetics, IDDM and immune regulation.

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