

Characteristics of Autoimmunity in Type 1 Diabetes and Type 1.5 Overlap With Type 2 Diabetes

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This presentation is an overview of mechanisms for developing and maintaining self-tolerance in mammalian organisms. Because this meeting is focused on type 1 diabetes and its mechanisms, the discussion deals primarily with mechanisms of T-cell tolerance, since type 1 diabetes in both effector and initiator phases is primarily a T-cell-mediated autoimmune disease. Emphasis is placed on more recently discovered mechanisms of maintaining self-tolerance (autoimmune regulator [AIRE]) and a new defect in T-cell negative selection. The emerging picture is that of a polygenic disease with various combinations of different alleles of many genes with important roles in the normal immune response or normal immune responses. *Diabetes* 54 (Suppl. 2):S4–S10, 2005

In recent years, it has become apparent that in some patients, there is a considerable overlap between type 1 and type 2 diabetes. A small subset of patients with type 2 diabetes (~10%) in the course of disease development produce autoantibodies characteristic of type 1 diabetes: antibodies to insulin, GAD, and IA-2. Although these patients have been studied in detail with respect to the nature of their diabetes (and the fact that they have lower C-peptide levels than most patients with type 2 diabetes), they have not been fully characterized.

The purpose of this presentation is to present a broad overview of the mechanisms responsible for susceptibility to, and pathogenesis of, autoimmune disease, with a particular emphasis on type 1 diabetes. The most salient generalizations concerning autoimmunity are presented in Table 1, which summarizes many of the findings that have been made in animal models of type 1 diabetes and in patients with type 1 diabetes.

Beginning in the early 1970s, after the demonstration of linkage of genetic control of the immune response to synthetic polypeptide antigens to the murine major histocompatibility complex (MHC), many investigators in laboratories around the world began a search for associations between particular alleles of MHC class I and class II genes and susceptibility to a variety of autoimmune diseases. Again in the early 1970s, investigators in Denmark

showed that patients with type 1 diabetes had an increased incidence of HLA B8 and B15. When homozygous typing cells for the different alleles of class II MHC molecules became available, it was apparent that in most patients with type 1 diabetes, there was a stronger association with the HLA DR3 and DR4 MHC haplotype. Over the years, it became apparent that these serologically and cellularly detected genetic polymorphisms detected structural alleles of MHC class I and class II molecules. These class I and class II MHC molecules were subsequently found to have as their primary function the presentation of peptide fragments of both self-proteins and foreign proteins to the developing T-cell receptor repertoire in the thymus and in the periphery. As a generalization, class I molecules present primarily intracellular proteins that have been digested by the proteasome to a length of 9–10 amino acids, which are then bound by class I MHC molecules in the endoplasmic reticulum, after which they are transported directly to the surface and presented for interaction with T-cell receptors “restricted” to MHC class I and specific for bound peptides in class I molecules. Class II MHC molecules present primarily exogenous peptides from self-proteins and foreign proteins that have been carried through the endosomal pathway, edited for removal of weakly binding peptides, and then transported to the surface for presentation to T-cells, which are “restricted” to the MHC class II alleles of the individual and specific for their bound peptides. Any one MHC class I or II molecule is capable of binding thousands of different peptides, but these form a definitely restricted subset of all the possible peptides from all proteins in both self-proteins and foreign proteins.

Since these early demonstrations of the association of type 1 diabetes susceptibility with particular MHC class II alleles (now known to be HLA DQ2 and DQ8) (1), more than 30 autoimmune diseases have been shown to have varying strengths of association (and in some cases genetic linkage) with particular MHC class II or class I alleles (HLA DR, DQ and HLA-A, HLA-B). This demonstration of genetic predisposition to autoimmune disease then sparked interest in applying newer techniques of molecular genetics to genome-wide screens for the detection of other genes that might influence susceptibility or resistance to the autoimmune disease under study. This resulted in the finding that most autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, multiple sclerosis, and a number of others, are polygenic in their inheritance pattern (2). Thus, a proper combination of normal alleles of many genes interact to determine individual susceptibility to a particular autoimmune disease. In some cases, these genetic tendencies are found for several autoimmune diseases, and diseases such as systemic lupus, rheumatoid arthritis, and other related diseases frequently appear in multiple individuals in one family tree, as do a number of endocrine deficiency

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AIRE, autoimmune regulator; APECED, autoimmune polyendocrinopathy associated with cutaneous abnormalities and ectodermal dysplasia; IL, interleukin; MHC, major histocompatibility complex; TCR, T-cell receptor.

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TABLE 1
Principles of autoimmunity

- Susceptibility to autoimmunity is predominantly genetic (due to combinations of normal variant alleles of many immune-related genes).
- The genotype of an individual's MHC class II molecules (HLA DR, DQ) is quantitatively the strongest genetic factor predisposing to the development of type 1 diabetes.
- MHC susceptibility is almost always "necessary but not sufficient" for autoimmune disease to develop.

diseases due to autoimmunity, which also have a definite familial incidence.

There is abundant evidence that the major genetic factor determining predisposition to the development of many autoimmune diseases is the genotype of the MHC class II molecules (1) (Table 2). In some diseases, such as ankylosing spondylitis, the dominant predisposing genetic factor is a class I MHC molecule, e.g., HLA B27. As noted in Table 1, the MHC genotype is a necessary but not sufficient precondition for the development of autoimmune disease. For example, in perhaps the most extensively analyzed animal model, the nonobese diabetic (NOD) mouse, there are 22 chromosome regions on 15 different chromosomes that contain a gene or genes that determine susceptibility to type 1 diabetes. Susceptibility at the MHC region alone, as shown in Fig. 1, is thus insufficient to develop autoimmune type 1 diabetes and requires definite contributions from other non-MHC genes. In findings in both mice and humans, a number of predisposing genes have been identified. These include interleukin (IL)-2, the insulin gene (INS-2), and CTLA-4 (2). A large number of these 22 regions contain candidate genes that are clearly involved in regulating or interacting in the immune response but that have not yet been proven to be a susceptibility gene or allele. Nonetheless, progress in this field is being made, and more and more non-MHC chromosome regions have been identified that predispose to a particular autoimmune disease, such as type 1 diabetes or systemic lupus erythematosus. Unfortunately, many of these non-MHC susceptibility regions require the analysis of a large number of

TABLE 2
HLA-associated risk factors for autoimmune disease

	HLA allotype	Frequency (%)		Relative risk
		Patients	Control	
Ankylosing spondylitis	B27	>95	9	>150
Narcolepsy	DQ6	>95	33	>40
Celiac disease	DQ2 and DQ8	95	28	30
Type 1 diabetes	DQ8 and DQ2	81	23	14
Subacute thyroiditis	B35	70	14	14
Multiple sclerosis	DQ6	86	33	12
Rheumatoid arthritis	DR4	81	33	9
Juvenile rheumatoid arthritis	DR8	38	7	8
Psoriasis vulgaris	Cw6	87	33	7
Addison's disease	DR3	69	27	5
Graves' disease	DR3	65	27	4
Myasthenia gravis	DR3	50	27	2
Type 1 diabetes	DQ6	<0.1	33	0.02

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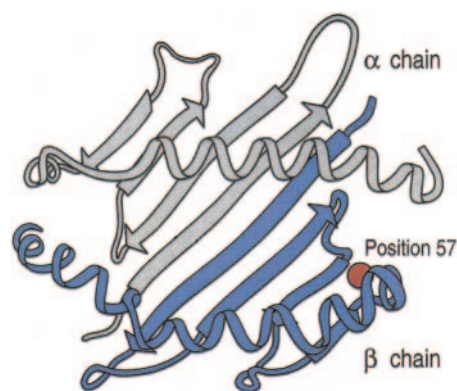


FIG. 1. Position 57 of the DQ β -chain affects susceptibility to type 1 diabetes. A schematic diagram of the structure of the HLA DQ molecule indicating position 57 of the DQ β chain (indicated by a filled circle) is shown here. Position 57 in the β -chain lies opposite a conserved arginine at position α 76 on the next to last turn of the helix of the α -chain. The positive charge of the arginine is neutralized by the negative charge of aspartic acid at position 57. In the absence of the aspartic acid position at 57, the arginine at α 76 causes a positive charge in this region, leading to a preference for this DQ allele to bind peptides with a negative residue in positions 9 or 10 in the peptide. ©2005 from *The Immune System*, 2nd edition, by Peter Parham. Reproduced with permission from Routledge/Taylor & Francis Books.

families and appear to have relatively small additive effects in determining susceptibility. If all of these additional susceptibility regions are only additive in effect, and particularly if they must be recessive to have their effect, then the realization of the hope that it would be possible to genotype and predict susceptible individuals may be only partially realized. However, some of the genetic effects that have been detected in the NOD mouse model and in the NZM 2410 murine model of systemic lupus have sufficiently strong effects to permit them to be major factors in predicting susceptibility in individuals of known genotype for these other genes as well as for the MHC genes.

These molecular genetic studies over the long term promise to lead to a detailed cellular and molecular understanding of the development of autoimmunity and the "breaking" of the normal state of self-tolerance.

NORMAL MECHANISMS MAINTAINING SELF-TOLERANCE

The immune system, in both its B-cell and T-cell arms, generates an enormous diversity of receptors in both compartments. This immediately raises the problem of how the organism manages to avoid developing autoreactivity to self-components among the very large number of new receptors generated in the course of B- and T-cell development. This problem—the prevention of autoreactivity—is solved in several ways. The first and possibly most important mechanism is the deletion of developing autoreactive B-cells within the bone marrow and the deletion of autoreactive T-cells in the thymus (3). A great deal has been discovered in the past two decades about the mechanisms of deletion of autoreactive B-cells in the bone marrow and the selective deletion of autoreactive T-cells in the thymus, because these cells develop within the thymus. Although the new findings in both B- and T-cell systems are too extensive and too detailed to discuss in this overview, there are several specific mechanisms of downregulating or deleting autoreactive B- and T-cells that are relevant to this discussion, because they

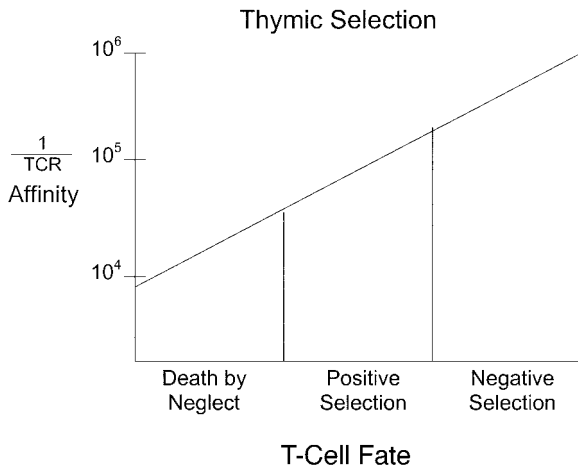


FIG. 2. A schematic diagram of the affinity differences between developing T-cells that are allowed to die by neglect, those that are positively selected to go on to the periphery, and those with very high affinity and potential autoreactivity that are programmed to die by apoptosis.

can be defective in individuals developing an autoimmune disease such as type 1 diabetes. For this reason, a brief discussion of T-cell development and the removal of autoreactive T-cells is necessary to understand mechanisms that can be defective in autoimmune disease.

First, during the course of T-cell development in the thymus, pro-thymocytes go through a complex and precise series of stages leading to the expression of T-cell receptor α and β genes and to the expression at the “double positive” stage of both CD4 and CD8 cell surface markers (3). CD4 is expressed by helper T-cells, which interact with and recognize MHC class II molecules on the surface of antigen-presenting cells. MHC class II molecules present peptides from many self-proteins and foreign proteins on the stromal cells of the thymic cortex and medulla. At this double-positive stage, developing T-cells are subjected to strict selection based on the affinity of their T-cell receptor for self-MHC molecules presenting a large variety of self-peptides in their peptide-binding grooves (3). This selective process is based on the affinity of the T-cell receptor for MHC/peptide complexes and is diagrammed in Fig. 2. T-cells with very low affinity for any self-peptide/MHC complex are programmed to undergo

apoptotic cell death by “neglect,” since they encounter no ligand that can activate the T-cells. T-cells with a somewhat higher affinity for self-MHC/peptide complexes are stimulated to proliferate and divide (positive selection) and proceed to the expression of only CD4 or CD8—the “single positive” stage of T-cell development—after which they move into the medulla. From there, after several days, the surviving T-cells migrate to the periphery (positive selection) (Fig. 3). A significant subset of developing T-cells has a very high affinity for some self-peptide/MHC complexes. T-cells with too high an affinity for a self-peptide/MHC (which are thus capable of inflicting damage when encountering the peptide/MHC complex in the periphery) are diverted back into the apoptotic cell death pathway and are deleted from the repertoire by negative selection (Fig. 3). Experimental observations have shown that there is a relatively narrow window of T-cell receptor (TCR) affinity, above which all T-cells undergo negative selection.

A great deal is known about this process of thymic positive and negative selection, and many of the proteins and gene products that are required for the successful triggering of positive selection on the one hand, and negative selection on the other hand, are listed in the diagram in Fig. 4 (3).

Extensive experimentation has shown that deletion of many of these genes, either in the positive or negative selecting pathways, can result in a failure to activate the positive and negative selection pathways, leading in the case of defective positive selection to a relative immune deficiency of the T-cell repertoire, or in the case of failure to activate the negative selection process to autoimmunity due to release from the thymus of autoreactive T-cells. In this regard, the proapoptotic protein Bim is of real importance, as will be shown below.

MECHANISMS FOR ENSURING SELF-TOLERANCE TO PROTEINS EXPRESSED PRIMARILY IN THE PERIPHERY

For decades, the possible mechanisms that determine lack of self-reactivity to proteins such as insulin, expressed primarily in the islet β -cells in the pancreas, lens crystallin expressed primarily in the eye, or myelin protein components expressed primarily in the central nervous system, were not fully understood. Recently (2000–2004), a series of new findings have demonstrated the existence of pre-

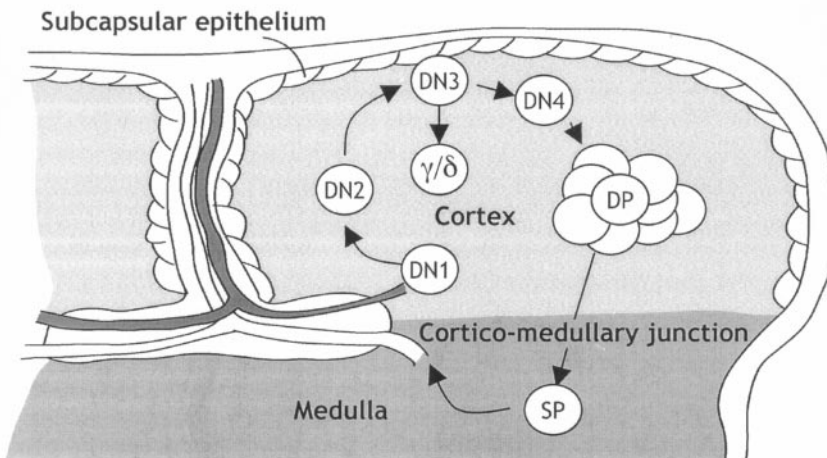


FIG. 3. A diagram showing the migration and development of T-cells as they enter the thymus as double-negative T-cells (lacking both CD4 and CD8) and migrate into the cortex area of the thymus. Once in the cortex area of the thymus, they go through a series of developmental stages, leading finally to the expression of both CD4 and CD8 in the mature double-positive T-cell, which then changes into the single-positive CD4 or CD8 T-cell and migrates to the medulla. Once in the medulla, the cells encounter medullary epithelial cells expressing a wide variety of peripheral proteins expressed in these cells, leading to deletion of T-cells, which have reactivity for these self-proteins in self-MHC class II molecules. Reprinted with permission from the *Annual Review of Immunology*, Volume 21, ©2003 by Annual Reviews (www.annualreviews.org).

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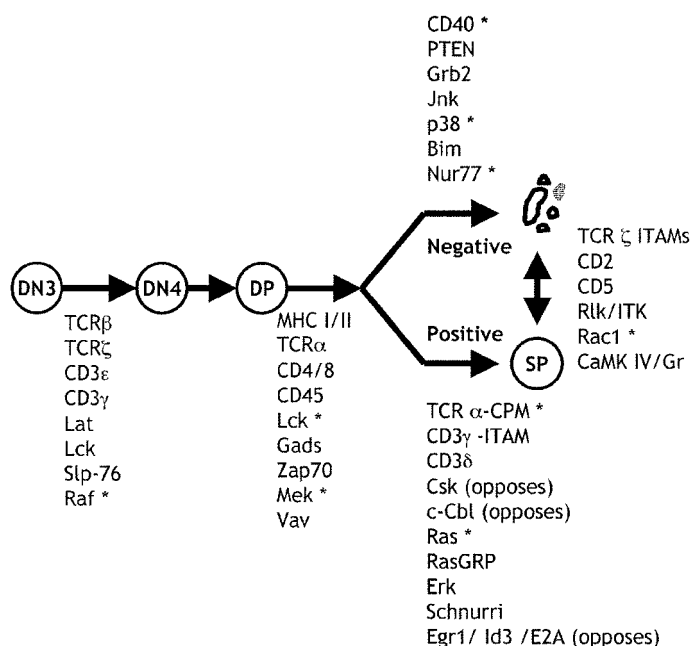


FIG. 4. A schematic diagram of the developmental stages and the genes expressed at these stages, or that influence the further development of these T-cells. At the double-positive stage, as indicated by the split arrows, T-cells with high affinity undergo negative selection due to the action of a large number of genes listed above the negative arrow. On the other hand, T-cells with intermediate levels of affinity for self-MHC molecules, binding a variety of self-peptides and foreign peptides, go on to be single positive cells, which are positively selected due to the action of a large number of genes, which are listed under the positive arrow in the figure. At the extreme right of the figure, there is a list of genes that are active in both positive and negative selection. Reprinted with permission from the *Annual Review of Immunology*, Volume 21, ©2003 by Annual Reviews (www.annualreviews.org).

viously unsuspected mechanisms for preventing reactivity to peripherally expressed self-proteins.

A mutant form of the recently identified autoimmune regulator (AIRE) gene has been shown to be the cause of the autoimmune polyendocrinopathy associated with cutaneous abnormalities and ectodermal dysplasia (APECED) syndrome. It is now known that the *AIRE* gene (which has sequence similarities to a number of transcription factors) determines (by an as yet unknown mechanism) the expression of many (but not all) peripheral proteins within the medullary epithelial cells in the thymic medulla (4,5). Thus, hundreds of self-proteins expressed primarily in the periphery are also expressed in small numbers of medullary epithelial cells in the center of the thymus. These proteins include insulin, GAD65 and 67, lens crystallin, myelin basic protein, α -fetoprotein, and many other peripheral proteins (4). The presently known genes under the control of the *AIRE* gene have a heavy bias toward proteins expressed in endocrine organs, including insulin, somatostatin, GAD65, and many others, which may explain the preponderance of autoimmune endocrine deficiency syndromes in the APECED syndrome. Inactivation of the *AIRE* gene in mice leads, several months after birth, to a polyendocrinopathy with lymphocytic infiltration and clear autoimmune lesions in the thyroid, islets of Langerhans, parathyroids, and other endocrine organs (5).

There are also additional peripheral mechanisms for removing or inactivating autoreactive T- and B-cells (Table 3). Thus, numerous studies have shown that when T-cells are exposed to a cognate peptide/MHC molecule on anti-

TABLE 3

Self-tolerance to proteins expressed primarily in the periphery

- AIRE
- T-cell anergy
- Activation-induced T-cell death/deletion
- Decreased TCR expression
- Regulatory T-cells

gen-presenting cells in the *absence* of expression of any co-stimulatory molecules such as CD28 or CD40, the T-cells develop an anergic state in which they do not respond to specific peptide/MHC complexes and cannot be activated to divide or proliferate. There are apparently precise genetic programs that lead to the development of this anergic state.

T-cells that have undergone excessive stimulation, or are exposed to large amounts of stimulating cytokines such as IL-2, also can undergo apoptosis, a process known as activation-induced T-cell death. Thus, T-cells in the periphery encountering large amounts of a peripheral antigen for which they are specific can in part be deleted by this mechanism.

Further, in a number of experimental systems, it has been observed that the expression of a self-antigen in the periphery in T-cell receptor transgenic mice expressing a T-cell receptor specific for that self-antigen (or neo-self-antigen) can lead to escape from programmed cell death by the mechanism of sharply decreasing the expression of the specific T-cell receptor. This mechanism has been demonstrated in a number of systems in which a transgene encoding a foreign protein is crossed on to a background in which there is a T-cell receptor transgene specific for the protein. One of the outcomes of this encounter is deletion of the T-cells specific for the protein, plus very extensive downregulation of the specific T-cell receptor.

Finally, since the mid 1990s, there have been a large number of reports that a subset of T-cells developing in the thymus and migrating to the periphery are capable of downregulating the immune response. Characteristics of this newly described subset of regulatory T-cells are presented in Table 4. Although there are some differences in the characteristics of these regulatory T-cells when studied in different strains of mice, in different antigenic systems, or in several different models of autoimmune diseases in mice, there is wide agreement on the major characteristics of this important regulatory T-cell subset (6). Most investigators have shown that for these cells to effectively suppress an autoreactive T-cell response, cell-to-cell contact is necessary. Separation by a permeable membrane preventing cell-to-cell contact has shown that there is no soluble factor that is responsible for this suppression. In almost all of these "suppressor" systems, the T-cells are CD4⁺ and CD25⁺, have an immature or resting phenotype, and are anergic to antigen stimulation even when the specific ligand has been presented. Further, these cells can suppress a T-cell response to an unrelated

TABLE 4

Characteristics of regulatory T-cells

- Require cell-to-cell contact to suppress a T-cell response
- Are anergic and antigen non-specific
- Express Foxp3, CD4, and CD25
- Have an immature/resting phenotype
- Different systems may use different effector molecules (CTLA-4, TGF- β , IL-10)

antigen simply by cell-to-cell contact. Again, most investigators have found that the CD4⁺CD25⁺ regulatory T-cells express the Foxp3 transcription factor and are anergic, requiring both IL-2 and anti-CD3 to become activated. These regulatory T-cells, in various autoimmune models (including responses to soluble protein antigens, and models of inflammatory bowel disease), have different effector molecules that appear to be mediating this effect. These include cytotoxic T lymphocyte-associated-4 antigen (CTLA-4), transforming growth factor (TGF)- β , and IL-10. Similarly, CD4⁺CD25⁺ anergic regulatory T-cells have recently been described in the synovial fluid of patients with rheumatoid arthritis (7). In rheumatoid arthritis, these CD4⁺CD25⁺ regulatory T-cells are incapable of suppressing an active T-cell proliferative response, but become capable when they have been exposed to anti-tumor necrosis factor (tumor necrosis factor is present in synovial fluid in relatively high concentrations).

Studies in both animal models of autoimmune disease and in patients with rheumatoid arthritis, and patients undergoing organ transplantation, have shown that the CD4⁺CD25⁺ regulatory T-cells are an important factor in suppressing the autoimmune damage that is apparently present in most individuals and can cause autoimmune lesions if CD4⁺CD25⁻ T-cells are present without the CD4⁺CD25⁺ regulatory T-cell subset. Thus, these regulatory T-cells are a major factor in maintaining the normal phenotype of a complete absence of autoimmunity in the normal animal or patient. This has led to a number of attempts to develop methods for using these regulatory T-cells in a therapeutic manner by first developing approaches for expanding the numbers of these regulatory T-cells (normally present in 5–10% of the T-cell population) to inject them into patients with autoimmunity to suppress the autoimmune process. It is clear that this is an area that will see increasing application in the coming years and offers a potentially new approach to the management of autoimmunity and of transplantation rejection.

OTHER GENETIC DEFECTS IN NEGATIVE SELECTION

Several laboratories, using mice transgenic for a T-cell receptor specific for a β islet cell protein, or for a neo-self-antigen (such as lysozyme or influenza hemagglutinin) expressed in the islets, have shown that the NOD mouse has a genetic defect in negative selection—the ability to delete autoreactive T-cells in the thymus (8). This defect has been shown in a comparison between B10 mice and NOD mice expressing the same neo-self-antigen and the same specific T-cell receptor plus the same MHC class II molecule. When mice express both a neo-self-antigen and a T-cell receptor specific for that antigen, which is expressed both in the periphery and in the medullary epithelial cells in the thymus, this leads to the normal process of negative selection of the “autoreactive” T-cells. This process is seen to proceed normally in B10 mice expressing these two transgenes, so that very few of the T-cells specific for the neo-self-antigen escape to the periphery. However, in the NOD mouse, a considerable number of these “autoreactive” T-cells escape to the periphery, revealing a clear defect in the ability to delete autoreactive T-cells in the thymus via negative selection.

Recently, using appropriate crosses between B10 and NOD strains expressing these two transgenes (above), combined with a genome-wide screen using a number of single nucleotide polymorphism (SNP) and variable num-

ber of tandem repeats (VNTR) markers, it was found that the defect in the NOD mouse is due in large part to a mutant form of the *Bim* gene (Fig. 4), which encodes a protein that normally is pro-apoptotic, and assists in the process of negative selection in the thymus. This is an excellent example of a variant allele of a normal gene resulting in a phenotypic defect (diminished thymic negative selection), which helps in predisposition to type 1 diabetes in this NOD animal model (8).

GENETIC, FUNCTIONAL, AND STRUCTURAL CHARACTERIZATION OF TYPE 1 DIABETES IN THE NOD MOUSE

Type 1 diabetes in the NOD mouse is very similar to the same disease in humans. Nonobese diabetic mice develop type 1 diabetes with an 80% incidence in females by 30 weeks of age and a variable lower incidence in males by this time. Extensive studies have shown that transfer of disease requires both CD4 and CD8 T-cells and is very strongly linked to the MHC class II molecule I-A^{g7}. In this regard, the one outstanding difference between NOD type 1 diabetes and that seen in humans is that the murine counterpart of HLA DR (I-E) is not expressed in the NOD mouse (and a number of other inbred strains, due to a 600 base pair deletion in the promoter region of the α -chain). NOD transgenic mice with a transgene expressing E α , and therefore expressing normal E α /E β heterodimers, do not develop diabetes, or develop it at an extremely low incidence. Using several site-specific mutants of the I-A^{g7} β -chain, susceptibility to diabetes in this model maps precisely to residues 56 and 57 in the I-A β -chain.

It is clear that both the initiating and effector mechanisms in type 1 diabetes are due to the actions of T-cells, because NOD mice with a knockout of membrane IgM, and consequently with complete absence of B-cells, still develop type 1 diabetes. Further, at least one patient has been seen with Bruton's α - γ -globulinemia and complete absence of B-cells; this patient developed type 1 diabetes at age 14.

The initial lesion in type 1 diabetes is insulinitis, which gradually increases in severity. The initial infiltrate is composed of a predominant T-cell population that produces both TH1 and TH2 cytokines, as well as B-cells, macrophages, and dendritic cells. As the process progresses over the next several weeks, cytokine production gradually shifts to a predominantly TH1 and cytotoxic T-cell response, which results in destruction of islet β -cells.

Principal autoantigens in type 1 diabetes in NOD mice. The principal autoantigens identified in NOD mice are insulin, GAD65, and heat shock protein 60. In addition, there are several other antigens that have not yet been identified, including those that are the target of the MHC class II-restricted T-cell receptor in the BDC 2.5 T-cell clone and resulting TCR transgenic mouse. Early reports have suggested that IA-2 is also an autoantigen in the mouse, as it is in human diabetes. In human diabetes, the predominant autoantigens, as identified by autoantibody screens, and with predictive ability, are insulin, GAD65, GAD67, and IA-2.

The 3D structure of DQ8 and I-A^{g7}. The crystal structure of I-A^{g7} with a peptide of GAD65 (residues 206–220) has been solved and reveals that the peptide binds in the predicted configuration, with the P9 residue of the peptide, glutamic acid, in a close interaction with the conserved

arginine at $\alpha 76$. This situation can develop because the peptide binding groove at the right-hand end of the groove has a predominant positive charge due to a conserved arginine at $\alpha 76$. The fact that there is no salt bridge formed between arginine $\alpha 76$ and aspartic acid $\beta 57$, which is replaced in I-A^{G7} by serine, is the major structural difference between I-A^{G7} and other I-A alleles. Correspondingly, the DQ8 crystal structure with a peptide from the principal epitope of the insulin B-chain in type 1 diabetes in humans reveals that the peptide has a glutamic acid at peptide position P9, which can make the same type of electrostatic bond with the conserved arginine at $\alpha 76$ (9).

However, even though the three-dimensional structure of known MHC/peptide epitopes of islet cell proteins (the targets of the T-cell autoimmune response in type 1 diabetes) has been solved, this does not yet explain how these particular peptide/MHC configurations permit or foster the development of the predominantly inflammatory response in islet β -cells. This is a subset of the general problem of how certain configurations of MHC molecules with bound peptides trigger a predominantly TH1 or TH2 response and has not yet been completely solved.

Treatment and prevention of type 1 diabetes in NOD mice and humans. It is beyond the scope of this brief review to discuss all of the many immunological manipulations that can lead to prevention of the disease in NOD mice. Many of these manipulations have unacceptable side effects and therefore have not been attempted in humans. However, a number of approaches that have been successful in the NOD mouse, particularly the administration of aqueous soluble GAD65 and/or aqueous soluble insulin B-chain peptide epitopes, have been successful in NOD mouse models in a number of laboratories. The monoclonal antibody to CD3 has also proven extremely effective in treating and maintaining an insulin-free state in NOD mice for a long period of time, when the therapy is begun at the first appearance of glycosuria. These three immunological manipulations are among the most promising for developing strategies that can treat type 1 diabetes at or just after onset and that offer the possibility of developing preventive therapies when individuals with susceptible HLA genotypes, autoantibodies, and sufficient susceptibility at other genetic loci are identified and become candidates for some type of preventive immunotherapy.

THE OVERLAP BETWEEN TYPE 1 AND TYPE 2 DIABETES IN HUMANS

As noted at the beginning of this article, it was recently found that $\sim 10\%$ of patients with well-established type 2 diabetes have several characteristics that are usually seen only in patients with type 1 diabetes. These include a very low C-peptide; autoantibodies to insulin, GAD, and IA-2 or some combination of these three; and the early development of a requirement for insulin replacement therapy. It is as yet unclear whether this is simply the coincidence of two relatively common diseases or whether there is some real interaction between type 2 diabetes and the development of a type 1 diabetes phenotype in susceptible individuals.

The fact that 10% of patients with type 2 diabetes are found to have autoantibodies characteristic of type 1 diabetes and to have lower C-peptide levels suggests that this is not a case of a simple coincidence of two relatively common diseases. This result is because of the known incidence of type 1 diabetes in the general population. In

the general population, $\sim 20\%$ lack aspartic acid at DQ β 57 on both chromosomes. Thus, at HLA, 20% of the population could be said to be potentially susceptible to type 1 diabetes. Despite this, only 0.5–0.8% of the population develop type 1 diabetes. Assuming that there is no association between HLA and type 2 diabetes (which has been shown in several early studies), the existence in 10% of type 2 diabetic patients of autoantibody characteristics of type 1 diabetes suggests that the additional stress produced on islet β -cells by the necessity to produce more than usual amounts of insulin can, in individuals who are susceptible at the HLA region, lead to the development of autoantibodies, insulinitis, and exhaustion of the insulin-producing β -cells, with a corresponding decrease in C-peptide and a distinctly different phenotype.

Clearly, it is important to determine HLA type in a sufficient population of type 2 diabetic patients to compare the distribution of HLA alleles to a control population. Presumably, there is a normal distribution of HLA genotypes in the normal population and in type 2 diabetic patients. The roughly 10% of type 2 diabetic patients developing autoantibodies characteristic of type 1 diabetes would also be HLA typed in the study. Presumably, these patients would show an excess access of HLA DR3 and DR4 haplotypes and a corresponding excess of HLA DQ2 and DQ8. If this is the case, this would mean that roughly half of the type 2 diabetic patients susceptible to type 1 diabetes at the MHC region (20%) develop the disease—a much larger number of patients than develop type 1 diabetes in the absence of type 2 diabetes. Further, the clinical characteristics of this patient population, their insulin requirement, their response to other therapies for type 2 diabetes, and the long-term prognosis of this subset of type 2 diabetic patients become of great interest. The interaction between the abnormal physiology of type 2 diabetes, leading to an unusual stress on islet β -cells to produce excess insulin, may be a major factor in leading to apoptosis in islet β -cells, presumably by the pathway of apoptosis arising from stress in the endoplasmic reticulum.

SUMMARY

The past 10 years have seen definite advances in our understanding of the mechanisms for maintaining self-tolerance and for the breakdown of self-tolerance due to genetic variations in MHC class II and I genes, in *AIRE* genes, and in alleles normally acting to promote apoptosis in a variety of cell types. Although many other genes are involved, it is possible that the analysis of MHC, *AIRE*, *Bim*, and a few other genes that are currently in the process of being identified could lead to a partial realization of the goal of accurate prediction of susceptibility to type 1 diabetes development. It is also likely that the same genes that are responsible for defects in negative selection of T-cells of particular specificity may also be operative in other autoimmune diseases, such as systemic lupus erythematosus. If this is the case, comprehensive genotyping offers the possibility of obtaining much greater insights into the pathogenesis of type 1 diabetes, other autoimmune diseases, and the promise of developing new therapeutic approaches based on this information.

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