Etiology of organ-specific autoimmunity: Basic research and clinical implications in IBD

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GS EISENBARTH. Etiology of organ-specific autoimmunity: Basic research and clinical implications in IBD. Can J Gastroenterol 1996;10(2):121-125. Autoimmunity develops in the setting of genetic susceptibility and can be monogenic (eg, autoimmune polyendocrine syndrome type I with Addison's disease, mucocutaneous candidiasis and hypoparathyroidism, which is autosomal recessive with the causative gene on the tip of chromosome 21) or polygenic (usually with important alleles within the major histocompatibility complex [eg, type I diabetes]). In addition to genetic susceptibility, many autoimmune disorders can be classified into etiological categories (oncogenic, drug-induced, diet-induced, infectious or idiopathic). For most autoimmune disorders there are multiple target autoantigens and, for type I diabetes, a combinatorial approach (eg, expression of at least two autoantibodies of insulin, glutamic acid decarboxylase and/or ICA512/IA-2) is the best predictor of diabetes risk. Finally, antigen-specific therapies hold promise for the prevention and therapy of autoimmunity, eg, parenteral or oral therapy with insulin delays or prevents type I diabetes in animal models, and a small pilot trial of parenteral insulin in humans suggests that such therapy may similarly prevent diabetes in humans.

Key Words: *Autoantibodies, Combinatorial, Polyglandular failure, Type I diabetes*

Étiologie de l'auto-immunité spécifique à un organe : recherche fondamentale et implications cliniques dans les MII

RÉSUMÉ : L'auto-immunité s'installe dans le contexte d'une sensibilité génétique et peut être monogène (par exemple, syndrome auto-immun polyendocrinien de type I avec maladie d'Addison, candidose mucocutanée et hypoparathyroïdisme transmis selon un mode récessif autosomique et dont le gène causal se trouve sur l'extrémité du chromosome 21) ou polygénique (habituellement avec d'importants allèles à l'extérieur du complexe majeur d'histocompatibilité [par exemple, diabète de type I]). En plus d'une susceptibilité génétique, plusieurs troubles auto-immuns peuvent être classés selon des catégories étiologiques (oncogènes, induits par les médicaments ou l'alimentation, infectieux ou idiopathiques). Pour la plupart des maladies auto-immunes, il y a plusieurs auto-antigènes cibles et pour le diabète de type I, l'approche associative (par exemple expression d'au moins deux auto-anticorps de l'insuline, décarboxylase de l'acide glutamique et/ou ICA512/IA-2) est le meilleur facteur de prévisibilité du risque de diabète. Finalement, des traitements spécifiques aux antigènes semblent prometteurs pour la prévention et le traitement de l'auto-immunité, notamment le traitement parentéral ou oral à l'aide d'insuline retarde ou prévient le diabète de type I dans des modèles animaux et un petit essai pilote sur l'insuline administrée par voie parentérale chez l'humain suggère qu'un tel traitement puisse également prévenir le diabète chez l'humain.

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Fundamental questions concerning autoimmune disorders include:

- What genetic elements determine disease susceptibility?

- What activates/suppresses autoimmunity in genetically susceptible individuals?

- What are the target autoantigens (initiating, perpetuating, secondary)?

- What are the effector mechanisms?

- What are the clinical sequelae of answers to the above questions in terms of disease prevention with, in particular, antigen-specific therapies?

Answers to these questions are available in humans and spontaneous autoimmune animal models for a subset of the above questions in a subset of autoimmune disorders. For no disease are answers to all questions available. Nevertheless, the clear answers available for a few disorders will almost certainly be relevant to many autoimmune diseases. The linking of autoimmune disorders into characteristic disease syndromes affecting many target tissues provides evidence for the interrelatedness of disease pathogenesis. In particular, the polyendocrine autoimmune syndromes (1-3) indicate that disease susceptibility can lead to multiple organ-specific autoimmune diseases (Table 1) (5,6). In this report, selected organ-specific autoimmune diseases and syndromes with specific answers to the above questions will be reviewed with an emphasis on type I diabetes.

GENETIC ELEMENTS

For most (but not all) organ-specific autoimmune syndromes, genes within the major histocompatibility complex (MHC) contribute to susceptibility (4). An interesting exception is the polyendocrine autoimmune syndrome type I, with its associated mucocutaneous candidiasis, Addison's disease and hypoparathyroidism. This syndrome is inherited in an autosomal recessive manner. In contrast to Addison's disease of the type II autoimmune polyendocrine syndrome (APS), which is strongly associated with DR3 and DR4 haplotypes, the type I syndrome with Addison's disease has no class II human leukocyte antigen (HLA) association (7). In addition, approximately 5% of individuals with this syndrome develop type I diabetes. The DQ molecule DQA1*0102,DQB1*0602 is usually dramatically protective for type I diabetes (8,9). This molecule occurs in approximately 20% of Caucasians, but among approximately 250 patients with type I diabetes, we have observed only one individual with this allele, and that individual has APS type I. DQA1*0102,DQB1*0602 is associated with protection from type I diabetes even in the presence of cytoplasmic islet cell autoantibodies. Approximately 12% of islet cell autoantibody-positive first-degree relatives of patients with type I diabetes express this allele, but we have yet to observe one such relative progress to diabetes. One hypothesis is that protection by this class II allele requires an active immune response and this response cannot be generated in the pres-

TABLE 1
Autoimmune polyendocrine syndromes

Type I syndrome	Type II syndrome
Mucocutaneous candidiasis	
Hypoparathyroidism	
Addison's disease	Addison's disease
Type I diabetes (5%)	Type I diabetes (50%)
Chronic active hepatitis	
Graves' disease/thyroiditis	Graves' disease/thyroiditis
Vitiligo	Vitiligo
Asplenism	
	Celiac disease
	Serositis
Pernicious anemia	Pernicious anemia
	Stiff-man syndrome/ Parkinson's disease
	Myasthenia gravis
IgA deficiency*	IgA deficiency†

*See reference 5; †See reference 6. Ig Immunoglobulin

ence of the immunodeficiency associated with APS type I. DQA1*0102,DQB1*0602 does not protect from all autoimmunity; for example, this molecule is associated with multiple sclerosis. Protection from diabetes by class II alleles is most dramatically demonstrated by transgenic nonobese diabetic (NOD) mice, in which replacement of the missing functional DRalpha allele (I-E alpha of mouse) protects from diabetes. The mechanism underlying this protection is unknown.

The insulin autoimmune syndrome is characterized by high levels of insulin autoantibodies and is almost always associated with methimazole therapy for Graves' disease. Essentially 100% of individuals with this syndrome are DR4-positive with the specific DRB1*0406 allele (10). In patients developing type I diabetes, lower levels of anti-insulin autoantibodies are usually present, and in this case the autoantibodies are associated with lineage 1 DQalpha alleles (01* to 04*) (11). In particular, DR3 homozygous prediabetic and islet cell autoantibody-positive individuals homozygous for DQA1*0501 rarely express anti-insulin autoantibodies.

Celiac disease is of particular interest in that the disorder appears to be associated with a specific DQalpha and DQbeta heterodimer (DQA1*0501,DQB1*0201), produced either in trans with DR5 (DQA1*0501,DQB1*0301) and DR7 (DQA1*0201,DQB1*0201) or cis with DR3 (DQA1*0501,DQB1*0201) containing haplotypes (4).

Finally, myasthenia gravis is associated with different HLA haplotypes depending upon disease initiating factors. Idiopathic myasthenia gravis is associated with DR3, while myasthenia gravis developing after ingestion of penicillamine is associated with DR7 (12).

Class II alleles within the MHC have received the most attention relative to disease pathogenesis. Because these molecules are essential for antigen presentation, this emphasis is likely to be appropriate. Nevertheless, with more than 100 genes within this region and extensive linkage disequi-

librium between alleles, other genes important for immunological function may contribute to disease susceptibility (13-15).

Besides identifying genes within the MHC, intense efforts are underway to identify other loci contributing to disease susceptibility. Most of these efforts have not been successful or confirmed, including intense study of T cell receptor genes. For type I diabetes, polymorphisms of the insulin gene are clearly associated with risk of type I diabetes (16,17). In addition, several repositories have DNA and Epstein-Barr virus lines available from hundreds of families with type I diabetes. With the availability of appropriate family resources and polymorphic microsatellite markers spanning the human genome, it is very likely that additional important loci will be identified. We hypothesize that such loci, similar to mutations of the *fas* (apoptosis) gene in lpr mice (18), will globally influence ability to maintain tolerance and prevent autoimmunity. In association with such 'global' autoimmune genes, it is likely that alleles within the MHC contribute to targeting specific organs.

DISEASE ACTIVATION

Factors that trigger autoimmunity have been elegantly defined for a small number of autoimmune disorders such that an etiological classification of autoimmunity can be proposed (Table 2). It is noteworthy that for several forms of oncogenic autoimmunity, specific molecules are expressed only by the tumours associated with the remote autoimmunity (19-23). Several of these triggering molecules have been identified and sequenced (24). The existence of oncogenic autoimmunity suggests that presentation of self-antigens within inflammatory lesions can abrogate self-tolerance. It is clear from experimentally induced autoimmune disorders that immunization with self-antigens (25-27) and self-peptides can lead to autoimmunity and tissue destruction. Thus, lymphocytes reacting with self-antigens are present in normal animals.

The ability to 'break tolerance' to a series of molecules within inflammatory lesions may also contribute to the existence of linked autoimmune disorders such as Graves' thyroid disease associated with Graves' ophthalmopathy, and ovarian autoimmunity associated with myasthenia gravis. A recent report demonstrates that T cells recognize relatively few amino acids within peptides (27). Thus, T cell lines can react with peptides of both the acetylcholine receptor and ZP3, the ovarian sperm receptor sharing fewer than five of nine amino acids. Thus immunization with the appropriate peptide of the acetylcholine receptor can induce ovarian destruction (27).

Studies of type I diabetes have failed to identify a crucial environmental factor triggering type I diabetes. The three factors receiving the majority of study include congenital rubella infection (28), Coxsackie viral infection (29) and ingestion of milk within the first three months of life (30).

Congenital rubella infection is associated with a marked increase in diabetes risk. One hypothesis relates a 52 kDa islet protein recognized by diabetic sera (with homology to

TABLE 2
Etiological classification of autoimmunity

Etiology	Disease – Trigger
Oncogenic	Cerebellar degeneration – ovarian cancer
Drug-induced	Myasthenia gravis – penicillamine
Diet-induced	Celiac disease – gluten
Infectious	Rheumatic fever – streptococci
Idiopathic	Type I diabetes – unknown

heat shock protein 60) to a specific rubella capsid protein (31). The rubella and islet protein are both recognized by an antirubella monoclonal antibody. Rubella infection increases the risk of diabetes only with fetal infection. Thus an alternative hypothesis is that rubella infection increases the risk of diabetes secondary to lifelong T cell abnormalities (32). These T cell abnormalities may also contribute to the frequent occurrence of thyroiditis in such patients.

Coxsackie viral infections were originally associated with type I diabetes when it was assumed that the disorder was of acute onset. With accumulating data supporting that type I diabetes develops slowly in the majority of individuals, it is likely that acute infections at disease onset are not of direct pathogenic significance. Recently one of the most studied autoantigens associated with type I diabetes, glutamic acid decarboxylase (GAD) (33-36), was found to have homology with a peptide sequence of a Coxsackie protein. This region of homology is being studied for relevance to disease induction.

A large number of epidemiological studies suggests that either decreased breast-feeding or early introduction of cow's milk products increases diabetes risk (37-40). These epidemiological data are surprisingly consistent given the less than twofold increased risk. The risk ascribed to cow's milk ingestion may be higher in individuals with genetic susceptibility to type I diabetes. Trials to test the elimination of cow's milk from diets of infants are being designed. One hypothesis relating cow's milk to type I diabetes is that bovine albumin shares several small regions of homology with an islet molecule termed ICA69 or p69. Pietropaolo and co-workers (43) recently cloned, sequenced and expressed ICA69. Although there is considerable controversy over whether antibodies to albumin are associated with type I diabetes, several laboratories have now demonstrated that ICA69 autoantibodies are present in more than 50% of individuals developing type I diabetes (41,42) and in fewer than 5% of normal controls (43). A number of studies are now addressing T cell responses to albumin and ICA69.

AUTOANTIGENS

During the past several years investigators have characterized a large series of islet autoantigens. Similar to other organ-specific autoimmune disorders (44), islet enzymes are prominent islet autoantigens. Autoantigens include GAD (45), carboxypeptidase H (46) and a novel tyrosine phosphatase termed ICA512. In addition, the hormone insulin (47-51) and ICA69 (52) (unknown function) are recognized

by autoantibodies and T cells. Gangliosides, and in particular a GM2-1 islet ganglioside (53), are also recognized by anti-islet autoantibodies.

With an increasing number of islet autoantigens, whether any given autoantigen is primary to the disease process and whether any given autoantigen is primary for any individual developing type I diabetes are unanswered questions. I favour the hypothesis that if there is a primary autoantigen it will be insulin. Insulin is the only beta cell-specific target molecule. All other characterized autoantigens are expressed in nonislet cells (particularly neuroendocrine cells) or within the alpha and delta cells of islets, which are not destroyed in patients with type I diabetes. For example, GAD in the rat is beta cell-specific, but in humans GAD is expressed by non-beta cells (36).

Criteria to distinguish primary from 'secondary' autoantigens are being developed. The most important evidence that insulin plays an important role in the pathogenesis of type I diabetes comes from studies of insulin reactive T cell clones and therapy of NOD mice and humans with insulin. T cell clones that react with an insulin peptide have recently been isolated from NOD islets (personal communication). T cells reacting with a peptide of the B chain of insulin are a major component of intra-islet T cells; most important, such T cells are capable of transferring diabetes to NOD severe combined immunodeficiency disease mice, which are unable to generate B and T lymphocytes. Insulin reactive T cells are both present in the islet lesion of NOD mice and capable of producing beta cell destruction and type I diabetes (personal communication). In my studies and those of Ziegler et al (50) of the chronology of autoimmunity in humans, insulin autoantibodies frequently precede autoantibodies to other autoantigens, including GAD. Oral ingestion of insulin can delay or prevent diabetes in NOD mice (54), and pilot studies suggest that parenteral insulin administration may prevent type I diabetes in a subset of islet cell autoantibody-positive first-degree relatives of patients with type I diabetes (55). This pilot trial has been followed by a large multicentre National Institutes of Health trial for the prevention of type I diabetes with parenteral insulin therapy.

With a series of characterized autoantigens, and identification of susceptibility, alleles and two spontaneous animal models, studies of the pathogenesis of type I diabetes are accelerating. It is hoped that with increased knowledge, type I diabetes mellitus will be preventable and the lessons learned for this illness will have a positive impact on studies of other autoimmune disorders.

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