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Review

Viruses, cytokines, antigens, and autoimmunity

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ABSTRACT To explain the pathogenesis of autoimmunity, we hypothesize that following an infection the immune response spreads to tissue-specific autoantigens in genetically predisposed individuals eventually determining progression to disease. Molecular mimicry between viral and self antigens could, in some instances, initiate autoimmunity. Local elicitation of inflammatory cytokines following infection probably plays a pivotal role in determining loss of functional tolerance to self autoantigens and the destructive activation of autoreactive cells. We also describe the potential role of interleukin 10, a powerful B-cell activator, in increasing the efficiency of epitope recognition, that could well be crucial to the progression toward disease.

Organ-Specific Autoimmunity

Autoimmune diseases are characterized by tissue destruction and functional impairment caused by autoreactive cells or antibodies. Although the target antigens and genetic bases of several autoimmune diseases are now better understood, the initial events leading to loss of tolerance toward self-constituents remain unknown. Typical of organ-specific autoimmunity is the selective targeting of a single organ or individual cell type, whereas gross anomalies of the immune system are usually absent.

Type 1 Diabetes: Antigens and Autoimmunology of the Pancreas

Type I diabetes is among the most-studied of the organ-specific autoimmune diseases. This disease develops as insulin-producing beta cells of the pancreatic islets are infiltrated by macrophages and lymphocytes (1, 2). Ultimately, the process completely destroys the beta cells. But, years before type I diabetes is clinically evident, usually as an acute and sudden event, autoantibodies to islet antigens can be identified in individuals who subsequently progress to this disease. Therefore, a beta cell-specific virus is unlikely to be the direct cause of this islet destruction. Additionally, the fine specificity of beta cell destruction in type I diabetes is so precise that the adjacent non-insulin-secreting endocrine cells of the pancreas are spared.

Autoantigens in Type I Diabetes

Humoral and cellular autoimmunity to islet antigens has been detected both in new-onset diabetic patients and in their first degree relatives (3–6) and in animal models of this disease (7, 8). In particular, immune responsiveness to each of several individual islet constituents, such as glutamic acid decarboxylase (GAD), has been linked repeatedly to type I diabetes. Two research teams (9, 10) have shown that an immune response to GAD precedes the onset of reactivity to other islet molecules, such as insulin and carboxypeptidase, in nonobese diabetic (NOD) mice. These authors also found that intrathymic or intravenous injection of GAD into such mice precluded the spreading of reactivity to other islet molecules and prevented diabetes. Moreover, a T-cell clone specific for an islet-secreted protein accelerated diabetes in young NOD mice (11), as did insulin-specific T-cell clones (12). Oral feeding with insulin (13) or immunization with insulin (14) along with both isoforms of GAD (15, 16) also prevented diabetes in this mouse strain. All these data indicate that antigen-specific T-cell reactivity has a fundamental role in the effector phase of type I diabetes.

However, we believe that antigen-specific T cells and antibodies represent epiphenomenons of a primary destructive event (such as a viral infection) that results in local inflammation of the islet. These antigen-primed effectors are probably responsible for the majority of the islet destruction. Conceivably, as an aftermath of local cytokine secretion, a number of self molecules in the pancreas become antigenic. This hypothesis would explain why such an assortment of molecules assumes antigenicity in type I diabetes and why it is possible to induce disease with several T-cell clones, specific for different molecules. The prevention of this disease by both GAD and insulin could well result from a bystander suppression of the immune response. If so, the protective effect of GAD or insulin would not necessarily reflect a primary pathogenic role for the immune response to these molecules.

Of course, epidemiology studies tell us that the vast majority of virus infections do not lead to devastating autoimmune diseases such as diabetes. The potential for autoimmunity is limited to a small subset of antigens since most self-responsive lymphocytes are eliminated within the thymus. Additionally, the organism has the ability to counterregulate such destructive processes in the periphery through feedback within intricate cytokine networks. However, in some small subset of genetically predisposed individuals these "stop gap" measures of the body fail, allowing the effectors generated to destroy self.

Viruses as Triggers of Autoimmunity

The question of what determines the initial loss of tolerance to a self antigen still remains to be answered. We favor the theory that infection can act as a trigger of organ-specific autoimmunity in predisposed individuals. An interesting example of the association between a viral infection and autoimmunity is offered by subacute thyroiditis, a disease whose clinical presentation in humans strongly suggests a viral pathogenesis (17). Although subacute thyroiditis is not an autoimmune disease, these patients have autoantibodies and T-cell reactivity to thyroid autoantigens (18, 19). These autoimmune phenomena are transient, and most of the patients recover completely. But, a few of these individuals progress from subacute thyroiditis to autoimmune thyroid disease (20–22).

The clinical history of subacute thyroiditis may represent a general paradigm of organ-specific autoimmunity with autoimmune disease following viral infections in only a subset of patients. A potential role of viruses and other infectious agents as inducers of autoimmunity is also suggested by the association of chronic infections such as malaria, tuberculosis, syphilis, and schistosomiasis with autoimmune phenomena (23). However, the loss of tolerance to self antigens in these patients does not usually lead to autoimmune disease, suggesting that other factors must participate.

The main difficulty in assigning a pathogenic role to any virus in type I diabetes is that during the long prodromal phase pre-

Abbreviations: GAD, glutamic acid decarboxylase; NOD, nonobese diabetic; IFN-γ, interferon γ.

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ceding overt type I diabetes the infecting agent would have disappeared. That is, any related viral infection in the pancreatic islets that triggered a long-term, chronic autoimmune response would have occurred years earlier. Subsequently, the virus would have been eliminated by an anti-viral immune response, making its direct identification at the clinical onset of diabetes virtually impossible.

However, suggestive evidence for a viral pathogenesis of type I diabetes comes from its development as a sequel of congenital rubella infection (24). Interestingly, several other endocrine diseases (25–27), thought to be autoimmune, can also develop following congenital rubella infection. Autoantibodies to tissue-specific antigens have been identified in the sera of patients with rubella-associated endocrine disease, suggesting their autoimmune pathogenesis (28, 29).

Several years after intrauterine infection, type I diabetes was diagnosed in patients expressing the HLA class II *DR3* or *DR4* haplotypes (30); these haplotypes also confer an increased risk of developing type I diabetes when rubella is not involved (31). Because congenital rubella infection can directly induce immunologic abnormalities (32), rubella-associated autoimmunity may result. However, this pathogenic mechanism would not explain why a dysfunction so induced would cause organ-specific rather than systemic autoimmunity.

An alternative hypothesis is that an initial immune response directed to rubella virus-infected cells could activate reactivity not only to the viral antigens but also to self antigens on the cells. The presence of particular HLA class I or class II molecules could affect the ability of antigen presenting cells to present self peptides specific for the target tissue. This, in turn, would determine the efficiency of an immune response to these autoantigens, a response that presumably could destroy the target tissue. Compelling data for this "mimicry" mechanism as a potential explanation for multiple sclerosis (33) has recently been presented. T-cell clones to the immunodominant myelin basic protein were activated by a variety of viral and bacterial peptides. Interestingly, alignment of the primary amino acid sequences did not indicate the potential for activation in most of the cases. This work elegantly establishes the fact that molecular mimicry may be an underappreciated mechanism for activation of subsets of T cells that can mediate autoimmune tissue destruction (34).

Pathogenic Events Leading to Loss of Self Tolerance Following a Viral Infection

Several mechanisms have been proposed to explain how an initial anti-viral or bacterial response could allow the recognition of self antigens of the infected tissue. We have investigated, in particular, the role of cytokines secreted within target tissues in determining the loss of tolerance to self antigens. The expression of interferon γ (IFN-γ) in islet cells of transgenic mice led to destruction of the cells and development of type I diabetes (35). Local expression of IFN-γ in the islets abolished tolerance to islet cell antigens in a specific manner, since the IFN-γ transgenic mice rejected histocompatible islet grafts but not pituitary transplants. Loss of tolerance to self antigens can, therefore, occur not only as a result of a systemic immune defect but also as a result of local cytokine secretion. While the vast majority of self reactive, high-affinity T cells are destroyed in the thymus, some lower-affinity clones escape selection. Indeed, under normal conditions, these autoreactive cells reside in a quiescent state in the periphery (36). Possibly, the induction of costimulatory signals by local secretion of cytokines could activate these otherwise quiescent T cells. Our theory of this process is that, following viral infection and local cytokine secretion, immune reactivity undergoes dysregulation in specific target tissues of genetically predisposed individuals. In this manner, a relatively common viral infection with tropism for a given tissue could produce organ-specific autoimmune disease in some individuals.

Regional factors within tissues are also clearly an important factor. IFN-γ is a Th1-promoting cytokine that activates T cells but no detectable humoral response to islet antigens (37). However, the expression of IFN-γ in the motor end plate led to development of antibodies to motor end plates and a disease clinically similar to human myasthenia gravis (38). Therefore, this factor induces a distinct response pathway in different target tissues. The importance of tissue-specific factors in the induction of autoimmunity has been stressed by Barrett and coworkers (39). These authors expressed H/K ATPase β subunit, an autoantigen in thymectomy-induced autoimmune gastritis of BALB/c mouse. Three days after undergoing thymectomies, the transgenic mice they used developed autoimmune gastritis and periinsulitis but not diabetes. The induction of insulitis in this model was antigen specific and absolutely dependent on the T-cell response to the transgene product in the islets. Lack of diabetes in the presence of periinsulitis strongly suggests that tissue-specific factors determine the pathogenicity of a local immune response. The inducibility of an autoimmune response could be critically affected by a localized feature, making the target cells more visible for the activated immune system. Results from a recent study (40) showed the hyperinducibility of class II molecules in thyrocytes obtained from patients with Graves disease and cultured in the presence of IFN-γ.

Autoimmunity and Diversity

The search for target antigens in insulin-dependent diabetes mellitus has identified a large and increasing number of pancreatic islet molecules. The anti-islet response that causes diabetes is diverse from the point of T cells as well, with no T-cell receptor predominating the islet reactive repertoire. Thus, significant diversification occurs and is probably essential for enough damage for clinical disease to develop. This notion is supported by the fact that induction of immune sensitization by immunization to any of these islet antigens individually does not lead to diabetes. Thus, from the initial "insult" there is a gradual recognition of increasing numbers of islet antigens and recruitment of more islet reactive T-cell specificities into the pancreas. Evidence for the inter- and intramolecular spreading process in insulin-dependent diabetes mellitus has been presented recently (9, 10). The molecular processes governing determinant spreading in insulin-dependent diabetes mellitus are of significant interest, since they allow a unique axis for intervention. B lymphocytes may be critical for this diversification process since they have the unique capability to concentrate proteins and present nondominant determinants to T lymphocytes. A role for B lymphocytes in the development of autoimmune diabetes in the NOD mouse was demonstrated by experiments where B lymphocytes were abrogated by anti-IgM administration (41). Factors that regulate activation of B lymphocytes and enhancement of their antigen presenting ability may be very important in the disease process (42). Interestingly, the cytokine interleukin 10 is a potent B-cell activator and enhances major histocompatibility complex II expression on B cells. We have found that this cytokine accelerates disease when overexpressed in the pancreatic islets (43) and it has also been found that it is essential for disease to progress (M. S. Lee, R. Mueller, L. S. Wicker, L. B. Peterson, and N.S., unpublished data). We now hypothesize that this molecule is important in the diversification process that precedes disease. A further understanding of cytokine regulation of diversification, processing enzymes and antigen presenting cells involved will be an important area of upcoming investigation.

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