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Viruses, host responses, and autoimmunity

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Summary: Conceptually, the initiation of autoimmune disease can be described as a three-stage process involving both genetic and environmental influences. This process begins with the development of an autoimmune cellular repertoire, followed by activation of these autoreactive cells in response to a localized target and, finally, the immune system's failure to regulate these self-reactive constituents. Viruses have long been associated with inciting autoimmune disorders. Two mechanisms have been proposed to explain how a viral infection can overcome immunological tolerance to self-components and initiate an organ-specific autoreactive process; these mechanisms are molecular mimicry and bystander activation. Both pathways, as discussed here, could play pivotal roles in the development of autoimmunity without necessarily excluding each other. Transgene technology has allowed us and others to examine more closely the roles of these mechanisms in mice and to dissect the requirements for initiating disease. These results demonstrate that bystander activation is the most likely explanation for disease development. Additional evidence suggests a further role for viruses in the reactivation and chronicity of autoimmune diseases. In this scenario, a second invasion by a previously infecting virus may restimulate already existing autoreactive lymphocytes, and thereby contribute to the diversity of the immune response.

Organ-specific autoimmunity

The organ-specific autoimmune diseases insulin-dependent diabetes mellitus (IDDM) and multiple sclerosis (MS) are characterized by chronic inflammation, tissue destruction, and loss of function to the pancreas or central nervous system (CNS), respectively, without the obvious presence of a pathogen. The challenge of working backwards to identify and predict common denominators involved in initiating such complex chronic diseases of humans is immense. A great deal of research has gone into generating animal models that resemble some of the clinical manifestations of these two complex diseases.

Organ-specific autoimmunity is thought to result from individuals' loss of tolerance to self-antigens. Appropriate tests can identify autoreactive T cells and antibodies preceding the onset of clinical disease in, for example, IDDM (1–4). Then, as organ-specific autoimmune disease progresses, an immune response directed at a single antigen of that organ targets particular cell types for destruction. Rarely do additional aberran-

cies in the immune response occur outside of the affected tissue. In IDDM, pancreatic β cells and their antigens are the targets of destruction; in MS, the myelin sheath produced by oligodendrocytes of the CNS is affected, and in rheumatoid arthritis, collagen is attacked as a self-antigen present in the synovial membranes of joints. Many autoimmune diseases are multifactorial with associations to both genetic and environmental factors. Although strong evidence links some autoimmune diseases with inherited genes, including those of the major histocompatibility complex (MHC) (5, 6), the participation of environmental influences (7, 8) has been well documented. In fact, viral infections often precede both the onset of diabetes (9–14) and relapses of MS (15–17). In experimental animals, viruses can actually cause diabetes (14, 18, 19) as well as demyelination like that in MS (20, 21).

Understanding the etiology of autoimmune diseases thoroughly enough to account for the complexity of such multifactorial processes is difficult. To simplify and group the many components associated with the progression of these diseases, we can separate their onset into three sequential steps. First, a repertoire of immune cells with the capacity for autoreactivity is established. Appropriate MHC and T-cell receptor (TCR) alleles must be available both to present and to recognize self-antigens, thereby generating an autoreactive response. For example, susceptibility to diabetes is inherited by humans via MHC alleles like HLA DQ8 or by non-obese diabetic (NOD) mice through I-A g7, but the presence of these alleles alone does not lead to disease. Therefore, an environmental event like infection is apt to be a contributory factor. Second, potentially autoreactive T cells must be activated. Target tissue inflammation, possibly through viral infection, is the most likely mediator for T-cell activation. An initial, localized infection would not only serve to release sequestered antigens that stimulate autoreactive cells, but would also create a high local concentration of cytokines and chemokines that would further attract immune cells to the organ. Once an infection is neutralized (i.e. viral clearance), a chronic response to the target tissue by these newly activated autoreactive lymphocytes could remain. Third, a failure of the immune system to counter-regulate the autoreactive response would result in further chronicity. Without proper regulation, an excess of activated lymphocytes would remain after the infection had dissipated, resulting in a breakdown of tolerance to specific self-antigens. The lack of effective regulation could be genetically determined so that the individual's immune response is out of control. A similarly plausible scenario is that a sufficiently strong inflammatory response could activate an equally strong autoreactive T-cell population for which normal lymphocyte counter-regulation would be insufficient.

Inflammation as an autoimmune process

Infections and inflammatory processes can elicit cytokine and/or chemokine release, immune cell infiltration, and tissue destruction, as well as release and presentation of both foreign and self-antigens. Consequently, self-reactive lymphocytes are activated. Normal immune regulation minimizes the impact of these autoreactive T cells by limiting their migratory capability and lifespan after infection. Logically, then, defects in this regulatory process lead to autoimmune disease as is the case in the lpr/lpr mouse, which develops a lupus-like syndrome (22). These mice lack functional Fas/FasL interactions that regulate targeted cell death via apoptosis as a mechanism of controlling and reducing lymphocyte numbers after a pathogen infection. Without this mechanism, autoreactive lymphocytes generated as a consequence of inflammation persist to react with self-tissue. Chronic infection such as that observed in HIV can also promote autoreactivity (23). Similarly, prolonged inflammation in a specific locale can cause the autoreactive response to spread from one antigen to another (epitope spreading) as has been observed in experimental autoimmune encephalitis (EAE) (24) and Theiler's virus infection of the CNS (25–27).

Local increases in specific cytokines during viral infection are most likely a major element in controlling the activation of self-reactive lymphocytes and/or loss of tolerance to self-antigen. The expression of both Th1 and Th2 cytokines like interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-4, IL-10, and transforming growth factor (TGF)-β in the pancreata of transgenic mice incite autoimmune diabetes (28–33) but, conversely, IL-4, IL-6 and TGF-β suppress spontaneous diabetes in NOD mice (34–36). Similarly, transgenic expression of IFN-γ and TNF-α in the CNS (37, 38) leads to the development of demyelinating disease and, in the case of IFN-γ, enhances disease after EAE induction (39). Therefore, increased local cytokine levels that mimic and induce organ-specific inflammation can result in tissue damage and release of tissue-specific self-antigens that ultimately activate autoreactive lymphocytes. Likewise, a viral infection can disturb the balance of immune regulation and lead to organ-specific autoimmune disease. For instance, in recent work with herpes simplex virus type 1 (HSV-1), which induces herpes stromal keratitis (HSK), an autoimmune disease of the eye, this virus was found to induce disease without the presence of an autoreactive T-cell population (40). The virus's presence in the eye is enough to stimulate cytokine production and, in turn, inflammation. However, this inflammatory response is largely non-specific, comprised of transgenic T cells specific to ovalbumin, an antigen not present in either the virus or the infected mouse. The

influx of non-specific T cells is sufficient to induce tissue damage and clinical disease, most likely through both the action of cytokines action and the inflammation itself, which increases pressure within the eye. Additionally, in Theiler's virus infection of mice, CNS demyelination is chronic and associated with viral persistence within the CNS tissue. This persistent infection continuously stimulates the immune response and recruits cytokine-producing viral-specific and autoreactive T cells (27, 41). Although not really an autoimmune disease, Dengue hemorrhagic fever is believed to result from a viral infection in which the immune system responds to virus by producing cytokines so vigorously that overwhelming tissue destruction follows (42). Lastly, after rubella virus infection, a number of autoimmune diseases have developed in the endocrine system, including IDDM (43). Although no known cross-reactivity exists between rubella and endocrine autoantigens, these diseases are most likely a consequence of rubella's tropism for endocrine tissue in susceptible individuals.

Viral initiation of organ-specific autoimmunity

The strength of the epigenetic hypothesis is that viruses and other pathogens can act at all three stages of disease induction. By breaking down host tissues or mimicking host antigens, viruses can establish an autoreactive immune response. Infections of specific tissue can lead to the activation, amplification, and recruitment of autoreactive lymphocytes. A strong, virally induced immune response can then overwhelm local counter-regulatory mechanisms. In this manner, virus infection can induce the loss of self-tolerance, the well documented slow progression and chronic state of diseases like diabetes and MS, and most importantly, the seeming inability to discover infectious agents responsible for the disease.

Molecular mimicry

The concept that a pathogen can initiate autoimmune disease by activating lymphocytes or antibodies with the capacity to recognize cross-reacting viral and host determinants is termed molecular mimicry (44–49). To date, molecular mimicry has been assigned a presumptive role in the pathogenesis of several human diseases, including IDDM (3), ankylosing spondylitis (50), Guillain-Barre syndrome (51), primary biliary cirrhosis (52), and MS (53–55). Numerous publications have reported cross-reactive immune responses present during autoimmunity. One of the strongest associations between this form of immunity and disease is the example of bacterial streptococcal M protein cross-reacting with myocardial tissue antigens in both man and mouse (56). In MS patients, Wucherpfennig & Strominger (53) found many cross-reactive T-cell determinants between myelin basic protein (MBP) and epitopes from such common viruses as HSV, Epstein–Barr virus (EBV), adenovirus, and influenza virus. In other studies, T-cell lines originating from MS patients were reactive to both MBP and a sequence from the human respiratory coronavirus, 229E (54). Additionally, the oligodendrocyte protein, transaldolase, has molecular similarity to human T-cell leukemia virus-1 Gag (55). The CB3 and CB4 strains of coxsackie virus have small regions of amino acids that resemble those in the myocardium or the pancreatic islet antigen, glutamic acid decarboxylase (GAD), respectively, and infection with these viruses is associated with the clinical onset of myocarditis (57–59) or IDDM (13), respectively, in mouse and man.

Particularly interesting is the association between coxsackie viruses and IDDM. Epidemiological studies have shown that coxsackie viral infection is a frequent event in patients who ultimately suffer from IDDM (2, 14, 60–64). In patients and NOD mice with IDDM, autoreactive responses to islet antigens like GAD and heat shock protein 60 are observed preceding the clinical onset of disease (2, 3, 65, 66). Suppression of the autoimmune response in NOD mice following the injection of GAD intrathymically or intravenously in 3-week-old mice was successful in delaying and/or preventing the onset of disease (67–69). These results further underscore the important link between tolerance to GAD and susceptibility to IDDM. Moreover, the identification of a six-amino-acid stretch of similarity between coxsackie virus CB4 P2C protein and GAD generated support for the mimicry hypothesis as the mechanism by which coxsackie virus infection might initiate IDDM in susceptible individuals (3, 4). Immunization with peptides from both GAD and CB4 P2C protein in mice also demonstrated that both peptides could generate cross-reactive T-cell responses (70–72); however, these responses were restricted to the NOD MHC allele. Additionally, in patients with IDDM, the predominantly recognized determinant contains the cross-reactive GAD sequence (3). The ability of coxsackie virus to infect the pancreas and inflict a strong, local inflammatory response further complicates the distinction between possible causes for the related diabetes: either the virally induced cross-reactivity or the induced inflammatory processes. In fact, despite all the circumstantial evidence supporting mimicry between GAD and CB4 P2C, the coxsackie-induced inflammatory disease appears to drive the autoimmunity.

Unlike bystander activation described below, molecular mimicry between epitopes common to the virus and host may result in an activated immunity whose purpose is to clear virus,

but results in an attack on cells presenting similar self-epitopes. In this manner, a virus would induce responsiveness to a self-epitope systemically and be cleared before the onset of clinical disease. The role of molecular mimicry may be limited to the activation of the autoreactive response and this, as we will describe later, may not be sufficient to initiate clinical disease. Additional environmental influences may be required to recruit the autoreactive response to the target tissue and induce disease.

Bystander damage and activation

The concept of bystander damage and activation following a viral infection requires destruction of specific tissue, release of sequestered antigen and increased local immune inflammation. Lymphocytes would be recruited to the tissue and those reactive to the released, sequestered self-antigen would in turn be restimulated in the inflammatory response. Thus, autoreactive lymphocytes would gain access to the target tissue without being directly involved in the initial viral insult or reactive to viral antigens. Successive targeted viral infections over a lifetime would fulfill the requirement for both the generation and activation of autoimmune lymphocytes and their targeted recruitment. The role of virus in this mechanism is not only to select the tissue, but also to induce a strong inflammatory response.

As we stated previously, recent data support the existence of molecular mimicry and bystander activation as two probable mechanisms of virus-induced autoimmune disease. Although bystander activation requires tissue-specific damage, molecular mimicry allows for systemic infection to break tolerance to tissue-specific antigens. By either mechanism, viruses can initiate an autoimmune process but are not required at the clinical onset of disease. So, even though the mechanisms of molecular mimicry and bystander activation are somewhat different and dependent on the tropism of the virus, they are certainly not mutually exclusive.

Transgenic mouse models

Experimental models to test both these mechanisms have been established in transgenic mice. Among the first of these models was one in which mice manipulated to express the viral gene products of lymphocytic choriomeningitis virus (LCMV) in their pancreatic β cells did not develop spontaneous diabetic disease. However, after LCMV infection of the mice, viruses were cleared by a vigorous cellular immune response that subsequently led to an attack on β cells and resulted in diabetes (73, 74). This sequence of events was not limited to the pancreas, because other transgenic mice were eventually generated whose CNS specifically expressed LCMV and, after infection by that virus, developed a disease similar to MS (75). In neither case did the investigators find any evidence of infectious LCMV at the onset of clinical disease. However, virus-specific memory T lymphocytes and antibodies were found. Thus, in these experimental systems, any virus with molecular identity to an infected host's "self" antigen could initiate disease. An important emphasis is that these experimental models reflect molecular identity rather than true molecular mimicry. Efforts to discriminate between mimicry and identity have been hindered by the inability to generate mutations in LCMV *in vitro*. Nevertheless, in subsequent experiments with these transgenic mice, challenges with recombinant vaccinia viruses encoding the well-described cytotoxic T-lymphocyte epitopes of LCMV did not induce diabetes. Although these vaccinia virus infections did indeed generate significant numbers of anti-self (viral) specific lymphocytes (1/6,000), a threshold of 1/1,500 cytotoxic T cells appears to be required to induce clinical disease (49, 76–78). Thus, even in these idealized circumstances of molecular identity, not enough autoreactive lymphocytes are generated to induce disease; therefore, molecular mimicry on its own is probably inadequate to induce autoimmune disease.

A second model that reflects chronic inflammatory disease similar to that observed following an environmental insult, such as a virus, is tissue-specific IFN-γ expression. β-cell specific IFN-γ expression in transgenic mice led to the spontaneous development of autoreactive lymphocytes and diabetes (28), while similarly, a CNS-driven IFN-γ transgene specific for oligodendrocyte expression induced CNS lymphocytic infiltration and demyelination (37). IFN-γ has the ability, directly as well as indirectly, to upregulate a number of the immune system's regulatory molecules like cytokines, including TNF-α and IL-12, (79–81) and also cell surface molecules including B7 (79–81), intercellular adhesion molecule (82, 83), and MHC class I and II (84–88). In this manner, a virus can infect a specific organ, thereby eliciting an inflammatory response regulated by local cytokine expression; in turn, this event allows a large number of activated immune cells to enter the tissues and further increase the local presentation of antigens including self antigens. Self-reactive immune cells are, thus, stimulated as part of the bystander response. Ordinarily this aberrant response can be controlled, but in the susceptible individual, immune dysregulation may allow these autoreactive cells to persist. By virtue of infecting a specific organ, a virus can elicit an autoimmune response resulting in clinical manifestations well after viral clearance.

In the case of coxsackie virus-induced diabetes, which is complicated by this virus's unique tropism for the pancreas as well as by the molecular similarity between virus and pancreas, we can experimentally identify the mechanistic role of the virus. To discriminate between the molecular mimicry and bystander activation hypotheses in this disease, three strains of mice were infected with coxsackie virus CB4 and assessed for the development of disease (89). The first of these strains were the NOD mice, which carry the NOD MHC allele to which presentation of the cross-reactive epitope is restricted; these mice develop spontaneous IDDM by 16 to 20 weeks of age. The CB4-infected NOD mice showed no enhancement or change in the onset or progression of diabetes. Additionally, no proliferation to either of the cross-reactive epitopes was observed. Therefore, the predominant immune response to the virus following such infection is most likely to other viral epitopes. The second strain tested, B10.H2g7 mice, carry the restricted MHC allele but lack many other susceptibility factors and do not develop spontaneous IDDM; again, these mice did not develop diabetes or insulitis after infection. The lack of development of diabetes or even proliferative responses to the cross-reactive epitopes in these mice strongly suggests that similar determinants are not involved in the response to coxsackie viral infection. Indeed, they may not be processed and presented at all. However, infection of the third experimental strain, BDC2.5 transgenic mice, which harbor a transgene encoding a diabetogenic TCR specific to an islet granule antigen that is distinct from GAD65 and does not cross-react with CB4, resulted in rapid onset of diabetes within 2 to 4 weeks of infection in two-thirds of the mice. Yet, these BDC2.5 mice do not otherwise develop spontaneous diabetes. Coxsackie virus infection of the BDC2.5 mice activated the resting islet-specific memory lymphocyte population, and this was measurable by increased cell surface expression of CD25 and CD44 molecules on the transgenic Vβ4$^+$ lymphocytes. Thus, infection and inflammation of the pancreas led to the activation of a significant population of islet-specific memory T lymphocytes through a bystander mechanism and resulted in clinical disease.

The rapid nature of the induced diabetes is consistent with the idea that the responding T cells in the pancreas were resting memory lymphocytes prior to activation by CB4 infection. Indeed, a primary response may not stimulate sufficient numbers of autoreactive lymphocytes, whereas reactivation of memory autoreactive T cells can generate a sufficiently intense response to produce clinical disease. Whether these memory cells were reactivated by release of sequestered antigen from the damaged tissue or simply by cytokine induction from the systemic immune response to the virus remained in question. Viruses like LCMV have been shown to cross-activate non-specific resting memory lymphocytes during infection, and those lymphocytes made up a significant portion of the newly expanded T-cell population (90–93). Although in recent, elegant studies (94, 95), others have questioned the magnitude of this non-viral response, it is agreed that a small yet substantial population of non-viral specific lymphocytes are activated. Viral expansion of non-specific T-cell responses can be mimicked by the injection of IFN-α or poly I:C (96). Therefore, we performed additional experiments to dissect the requirements for IDDM induction by infecting BDC2.5 mice with LCMV. Yet, LCMV infection did not induce diabetes, nor was there an observable increase in insulitis. Immunostaining of peripheral blood lymphocytes before and after infection of these BDC2.5 mice demonstrated preferential activation of the non-$V_\beta4$ (non-transgenic) population of CD4$^+$ lymphocytes, visible as increases in CD44 and CD25 staining, without the activation of the $V_\beta4^+$ (transgenic) population. During acute infection, LCMV rarely infects the islets, and no pancreatic tissue destruction was observed. Additionally, as expected, treatment of BDC2.5 mice with poly I:C did not induce diabetes (M. S. Horwitz, N. Sarvetnick, in preparation). Similarly, in work done with transgenic mice expressing an encephalitogenic TCR, disease was induced only after breakdown of the target tissue to allow entry of lymphocytes (97), which suggests a role for the pathogen in tissue damage and disease induction. This outcome further argues for the conclusion that release of sequestered antigen, not induction by cytokines from the systemic immune response, is responsible for reactivation of the resting anti-islet lymphocyte population and development of IDDM.

The ability of coxsackie virus to elicit IDDM in BDC2.5 TCR transgenic mice but not in NOD mice demonstrates that the mechanism was bystander damage and activation of T cells, and not an autoreactive response to GAD induced through molecular mimicry. Furthermore, the diabetic state in these mice stemmed from the pancreatic tropism of the CB4 viral strain and its capacity to direct tissue damage.

Although the ability of viruses to activate T-cell responses nonspecifically has been well documented (90, 92, 93), the role of this bystander amplified set of lymphocytes in autoimmune disease has been the subject of some debate. If, as suggested, these newly activated responses cannot act in vivo to cause disease (98, 99), how has non-specific cross-activation of lymphocytes yielded pathologic events in two distinct models of virus-induced demyelinating disease (25, 75). Following coxsackie viral infection, non-specific reactivation of autoreactive lymphocytes resulted in diabetes. This sequence suggests a

mechanism requiring direct tissue damage and release of sequestered antigen, which were not factors in the reports questioning whether non-specific T-cell activation plays a significant role in disease (98, 99). Nor did those reports focus on the consequences of activating autoreactive resting memory T cells. The difference in inducibility of diabetes by coxsackie virus versus LCMV in the BDC2.5 transgenic mouse again underscores not only the importance of breakdown and release of sequestered antigen in tissue that ultimately becomes the target for autoimmune disease but also the requirement for a pre-existing memory population of autoreactive lymphocytes.

Recent experiments on HSK, an autoimmune disease of the eye triggered by HSV-1, re-emphasize the complex events that enable a virus to inflict tissue-specific damage and also share antigenic similarity. Zhao et al. (100) used mutational analysis of the virus to demonstrate a requirement for the cross-reactive epitope in HSV-1 to induce HSK. However, in sharp contrast, Gangappa et al. (40) found that HSK could occur in mice incapable of generating a cross-reactive response to HSV-1. How can such conflicting results be reconciled? The tropism and replication of the virus itself may point to the answer. Replication of the virus in the eye can result in tissue damage and recruitment of lymphocytes that are ultimately required to induce disease. Mutational alterations in that virus would directly deter replication of that virus in the eye and, thereby, reduce its ability to induce disease. The apparent cross-reactive response may simply serve to amplify the disease process.

Is there a role for molecular mimicry in the development of autoimmune disease? Studies of large panels of monoclonal antibodies (>600) generated against viruses (45, 49, 101, 102) have measured the rate of cross-reactivity with host proteins to be roughly 4%. Although this is undoubtedly a higher rate than statistical analysis would predict, the biological relevance of this number is questionable, since the studies were made under *in vitro* conditions. More importantly, the generation and activity of T cells that cross-react has not been adequately demonstrated. In related research, Wucherpfennig & Strominger (53) tested a panel of 129 peptides from pathogens that mimicked the T-cell epitope of MBP on T-cell clones from MS patients. Only eight peptides efficiently activated some of these clones, of which only one could be easily identified as a molecular mimic of the original epitope. Missing from their analysis was a comparison to similar host peptides. Furthermore, the biological consequence of such cross-reactivity was not demonstrated. In fact, many reports have documented the appearance of self-reactive T cells in patients with autoimmune disease as well as in healthy individuals. The generation of cross-reactive T cells not only requires a similar structural sequence between the epitopes (which is difficult to predict), but these epitopes must be properly processed and presented by similar MHC molecules. These parameters are rarely tested preceding pronouncements of molecular mimicry. Of course, the requirements for MHC binding and the affinity necessary for activation are areas of intense immunological investigation. Yet, in the case of the putative epitopes from CB4 P2C and GAD, despite their similar amino acid sequences, no one had demonstrated that these are the actual immunogenic epitopes processed and presented by the MHC. In the somewhat convincing work of Fujinami & Oldstone (45), rabbits were injected with a peptide from the hepatitis B virus polymerase. This peptide's sequence closely resembled that of MBP, and the injected rabbits developed a CNS disease similar to EAE. Yet, to date, the infection of humans with hepatitis B has not been associated with encephalomyelitis-like symptoms. Regardless of repeated attempts to demonstrate molecular mimicry between sequences of host and pathogen at the level of T-cell recognition, only a small number of epitopes have proven capable of cross-activation, and virtually all these experiments bypass the critical steps of antigen processing and presentation. And more importantly, in no case have T cells specific to pathogens been able to mediate autoimmunity *in vivo*. So, even though some evidence exists to verify the involvement of molecular mimicry in autoimmune pathology, for the most part, even that evidence seems mere coincidence, leaving other mechanisms of disease promotion as stronger candidates.

Viruses as reactivators of autoimmune disease

Not only can viruses trigger autoimmune diseases but they are also likely, be important in the reactivation and chronicity of autoimmunity. After an initial event that generates and expands a population of self-reactive T cells, subsequent infections can serve to restimulate and further amplify this autoimmune response. Recent evidence supports two possible mechanisms for viral restimulation of autoimmune disease leading to relapses or exacerbation of disease: cross-activation of self-reactive memory lymphocytes and stimulation of self-antigen diversification.

Cross-activation of memory lymphocytes

Memory T lymphocytes are activated more easily and produce higher levels of cytokines than naive T lymphocytes. Repeated viral infections from distinct viruses with no known cross-reactivity have been shown to cross-activate memory lymphocytes from earlier infections (92, 93). This scheme of multiple infec-

tions not only accounted for the restimulation of unrelated memory cells but also for the enhancement of autoimmune CNS disease in the transgenic LCMV model (75). In that work, exacerbation of CNS disease occurred only after a second infection of mice that had previously been exposed to LCMV. Since these mice did not develop CNS disease after their first exposure to LCMV, clearly self-reactive (LCMV transgene-specific) memory lymphocytes established by the prior LCMV infection were required. Most important, once LCMV memory lymphocytes had been established, infections with viruses other than LCMV also caused disease, most likely, through cross- (non-specific) activation of the LCMV-specific memory T cells. During experiments on transgenic mice with MBP-specific T cell receptors, EAE developed spontaneously in animals housed in a non-sterile environment (103). Furthermore, disease required breakdown of the blood–brain barrier (97) and a role for a pathogen was indicated. These observations demonstrate that the TCR repertoire and exposure to environmental agents both influence susceptibility to CNS autoimmune disease. Also implied is that the development of a self-reactive repertoire and a history of viral infections are crucial to the development of autoimmune disease. In fact, the latter results are similar to the outcomes of coxsackie viral infections in BDC mice described earlier (89). In that case, unmanipulated mice did not develop spontaneous disease, although they harbored a T-cell population composed primarily of memory cells specific for an islet granule antigen. Only after tissue breakdown imparted by coxsackie viral infection was disease induced (89). Somewhat similarly, children who were vaccinated as protection from polio, and later received poliovirus booster immunizations developed immune responses to unrelated antigens, including reovirus and tetanus toxoid, thereby indicating that secondary activation of immune responses also occurs in humans (104). Additionally, in patients with MS, disease exacerbates following common viral infections (15–17). Therefore, viruses play a major part in restimulating and enhancing the autoimmune response during chronic or long-term autoimmune disease. Undoubtedly, repeated viral infections over a susceptible patient's lifetime could enhance the opportunity to develop autoimmune disease.

The cross-activation of memory T cells specific for an oligodendrocyte protein by viral exposures subsequent to the initiating infection may account for periodic exacerbations of disease in patients with MS. Such repeated infections may also explain both the long lag period before the symptoms of this disease manifest and the risk factor association to the first 15 years of life in MS patients. A cross-reactive immune response to oligodendrocyte-specific antigens through a process of molecular mimicry early in life could lead to the generation of memory T cells specific for a myelin antigen. These self-reactive T cells could then be reactivated from subsequent exposures to pathogens, eventually leading to clinically observable disease. Similar scenarios may be responsible for the long prodromal period in other autoimmune diseases like diabetes, rheumatoid arthritis and lupus.

Is activation of resting memory lymphocytes simply caused by non-antigenic stimulation following upregulation of cytokine expression in response to any viral infection? Recent evidence has implicated type 1 IFNs in the reactivation of specific memory lymphocytes (90), and, of course, viral infections are key mediators of type 1 IFNs (105). As stated earlier, the upregulation of systemic type 1 IFN was not enough to activate diabetogenic T cells in transgenic BDC2.5 mice infected with LCMV (89) or treated with poly I:C (M. S. Horwitz, N. Sarvetnick, in preparation). Results from other investigators (98, 99), imply that an additional factor is required to reactivate lymphocytes and drive autoimmunity. Merely activating potential autoimmune responses is not enough to cause disease. Yet, in some instances, viral cross-activation has increased disease (25, 75). Tough & Sprent (96) have described the potential for cross-activation during infection and indicated that $CD8^+$ T cells are the population predominantly reactivated by type 1 IFNs. $CD4^+$ T cells are not similarly reactivated with IFN type 1 stimulation. More specifically, this activation of $CD8^+$ T cells by IFN was induced by IL-15 and appeared to utilize the IL-2 receptor β that is found on $CD8^+$ T cells but not $CD4^+$ T cells (106). Therefore, this $CD8^+$-selected reactivation makes sense and is appropriate considering that, after viral intrusion, the cytolytic arm of the immune response is generally the most critical in clearing the infection. Therefore non-specific cross-activation may participate only when $CD8^+$ T cells and their epitopes are involved in disease development. However, in the coxsackie-induced IDDM of BDC2.5 transgenic mice (89), in HSV-1-induced HSK (40), and in pathogen-induced EAE (103), the activation of pathogenic $CD4^+$ lymphocytes was essential for disease induction, and target tissue breakdown was required. In systems dependent on $CD8^+$ lymphocytes, such as the LCMV transgenic models previously described, systemic cross-activation may be the only requirement to activate memory $CD8^+$ T cells and induce their pathogenic acts that produce disease. The actions of $CD8^+$ T cells may, in turn, be more important during relapses of human autoimmune disease, and this population whose function is considered critical to the development of both IDDM and MS. Certainly $CD4^+$ and $CD8^+$ T cells are both important participants in human autoimmune disease. Their reactivation, apparently via separate mechanisms, is clearly mutually dependent.

Alternatively, such reactivation may result from either release of sequestered antigen or cross-reactivities between the infectious agent and the previously stimulated antigen. The simplest explanation, since inflammation and access to the target tissue are both required, is release of sequestered antigens during the inflammatory process. These antigens would certainly reactivate both $CD4^+$ and $CD8^+$ lymphocytes and leave a population of autoreactive lymphocytes that recognizes a number of autoantigens, as is observed in human autoimmune disease. One could hypothesize that memory lymphocytes could reactivate through molecular mimicry; however, the induction of clinical disease would require a significant increase in the total number of these specific self-reactive lymphocytes, as Oldstone has described (49, 77, 78, 107). Considering the number of specific lymphocytes required, the viral epitope would need to cross-react with a self-epitope of a suitably high affinity and would most likely have to be the predominant epitope for viral clearance. Additionally, this cross-reactivation alone would undoubtedly be insufficient to cause disease without target tissue inflammation.

Stimulators and inhibitors of autodestruction

The search for target antigens in IDDM and MS has identified a multitude of islet- or myelin-related molecules, thus demonstrating the broad diversity of the autoimmune response. Certainly, a single epitope would be incapable of generating an autoreactive path to disease. Therefore, some mechanism must diversify an autoimmune response to include additional antigens so as to maintain chronic disease. In accord is evidence for epitope spreading in animal models of both diabetes and demyelinating diseases (24, 41, 68), most likely reflecting the situation in patients with autoimmune diseases. Antigen-presenting cells, specifically B cells, may be critical for this diversification process, since they have the capacity to concentrate proteins and present many determinants to T cells. Cytokines like IL-10 have been shown to have immunostimulatory properties with respect to antigen-presenting cells (108). In particular, IL-10, when overexpressed in pancreatic β cells of transgenic mice, has accelerated disease (109). Additionally, IL-10 expression is required for the development of insulitis (33). However, despite the necessity for IL-10 in the early progression of diabetes (33, 110), this cytokine also acts during diabetes by modulating $CD8^+$ lymphocytes and does not require B-cell participation (111). Although originally thought to be a suppressor of the inflammatory response similar in action to other Th2 type cytokines like IL-4, recent data have ascribed to IL-10 a function in the inflammatory pathway. Indeed, expression of IL-10 in the pancreatic islets leads to a $CD8^+$ T-cell-dependent, $CD4^+$ T-cell independent disease (111). The mechanism by which IL-10 modulates the potency of islet-specific $CD8^+$ cells is not yet fully understood however; recent studies demonstrate that IL-10 promotes CD8 function through a fas-independent mechanism (B. Balasa, N. Sarvetnick, in preparation). The requirement for IL-10 in the natural disease progression of the NOD mouse renders this area an important one for further investigation. Another intriguing observation is that several viruses, including HIV-1, EBV, Thieler's murine encephalomyelitis virus (TMEV) and respiratory syncytial virus, have been shown to induce IL-10 *in vivo* along with other inflammatory cytokines (112–116). Moreover, in comparisons of EAE and TMEV infection, both of which induce a demyelinating disease similar to MS, IL-10 expression was observed before or coinciding with the onset of clinical disease and continuing throughout its course (115). Elsewhere, IL-10 did not specifically accompany remissions of disease but, instead, was expressed continuously throughout all stages of chronic disease, including relapses as well as remissions (115). In these experiments and others by the Miller laboratory using EAE and TMEV to induce CNS disease, antigenic diversification and epitope spreading were found over the course of disease (25, 26, 115), and this change of epitopes clearly correlated with relapses in clinical disease. Additionally, the Rodriguez laboratory (117) showed that $CD8^+$ T cells are essential in the development of clinically evident demyelinating disease induced by TMEV. By infecting an individual in a way that produces a limited and controlled autoreactive response, a virus could induce local upregulation of a cytokine like IL-10 and consequently diversify the self response locally. We speculate that IL-10 upregulation could lead to an increased magnitude and efficiency of killing within the $CD8^-$ T-cell compartment, damaging tissue and, thus, releasing antigen to be presented by B cells so as to further diversify the response. The importance of IL-10 in association with antigenic diversification and disease relapse as well as its important mechanism of facilitating $CD8^+$ T-cell target destruction is a critical area of investigation.

Another cytokine involved in viral pathogenesis and possibly in diversifying the response from antiviral to antiself is TGF-β. TGF-β is pluripotent in its activity and has been implicated in the immune response as a suppressor of the inflammatory pathway and also in the regeneration of injured tissue. Expression of TGF-β in the pancreata of NOD mice protected them from IDDM (36). Of particular interest, this protection was mediated by alterations in preferences of the antigen-presenting cells resulting in polarization of the response towards a Th2 phenotype. Furthermore, TGF-β appeared to act in concert

with IL-4, suppressing autoimmune responses without killing or removing the inflammatory T cells (118). Yet, even though TGF-β can act to suppress the development of IDDM in the NOD mouse (36), it did not suppress or prevent virus-mediated autoimmune disease in the LCMV transgenic model (32). Such alterations could be important after a virus infects its host; at that time a local elevation in TGF-β with an imbalance of IL-4 could influence the responding T-cell subsets. This typically results in protection from autodestructive disease through this decrease in the population of autoreactive lymphocytes. However, by making a change in the T-cell subsets that respond following infection, it is hypothetically possible that autoreactive T cells could be inadvertently activated and lead to disease. As one might expect after infection by a number of different viruses, TGF-β is indeed upregulated (115, 119–122). In fact, TGF-β expression has been specifically observed after infection with TMEV, HSV, EBV and chronic hepatitis C viral infection (115, 119–122). Curiously, the viruses associated with increased TGF-β levels are also chronic or persistent pathogens. Specifically, the severity of HSV-1-induced pneumonia was supposedly governed by viral dysregulation of TGF-β rather than viral replication (119). The role, if any, of TGF-β in these diseases is conjectural so far but is worthy of clarification.

As a matter of course, immune responses follow viral infections. Counter-regulatory responses are critical to control damage to the host. Previous results, mainly from *in vitro* studies, indicate that cytokines can be classed as either immunostimulatory or immunosuppressive. However, during the past several years many cytokines, such as IL-4, IL-6, TGF-β, IFN-γ, IL-10, TNF-α, and TNF-β, have been associated with both induction and cessation of autoimmune diseases (28–39). The disease outcome from each individual signal varies depending upon whether the signal is experienced locally or systemically, and the specific stage of the disease during which the signal is present. Clearly, a balance exists, and ultimately many factors play into whether a particular cytokine acts to stimulate or suppress. Indeed, the progression toward disease is quite complex, and factors affecting diverse processes such as CTL killing might be disease promoting early or disease inhibiting later depending upon the state of differentiation of their tissue target. The cytokine IL-4, expressed in pancreatic islets, is able to completely block insulitis and clinical disease in the NOD mouse (34). However, co-expressed in conjunction with a TCR transgene that results in a monoclonal CD4 T-cell repertoire reactive toward islet antigen, clinical disease is actually promoted by IL-4 instead of inhibited (31). This discrepancy indicates that the ability to counter-modulate disease is dependent upon the content of the T-cell repertoire. Specific effects on the individual antigen-presenting cell populations may mediate the disease-promoting activity of IL-4 and other cytokines in the presence of a pathogenic T-cell repertoire (31). The cellular content of the specific target tissue may also govern the pathway of the immune response. Studies with IFN-γ *in vitro* and *in vivo* indicate its propensity to stimulate cellular autoimmunity (123, 124). However, interestingly, when this molecule is present in the neuromuscular junction, humoral autoimmunity not cellular autoimmunity results (125). Therefore the tissue and the specific signal co-participate in determining the ultimate pathway of the response. A further understanding of the diverse mechanisms of these effector molecules within individual tissues is clearly warranted. Increased understanding in this area will allow therapeutic intervention modulating these complex molecules.

Conclusions

Multiple factors from genetic and environmental sources underlie the pathogenicity of autoimmune diseases like IDDM and MS. These diseases have long prodromal phases before clinical onset and are, thereafter, characterized by numerous relapses and exacerbations. All these qualities are typical aftermaths of virus infection. Because viruses effectively activate autoreactive lymphocytes through both cross-reactive epitope stimulation (molecular mimicry), and bystander damage and activation, viral activities can break immunological tolerance and initiate autoimmunity. We have described mechanisms by which molecular mimicry and bystander activation could create an autoimmune state. Evidence from multiple systems demonstrates that bystander activation is a key factor in the viral induction of autoimmune disease, whereas the importance of molecular mimicry is less well established and comes under question. In addition, we present evidence that viruses have the power to reactivate an autoimmune response as disease exacerbates to the level of clinical onset. A better understanding of these mechanisms, viral cross-activation as well as local cytokine induction, may allow us to focus immunotherapeutic strategies not necessarily on preventing disease but on events that mitigate disease. Reducing or suppressing the antiviral response in susceptible individuals should enable us to decrease the severity or delay the onset of clinical autoimmune disease.

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