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Molecular Mimicry, Bystander Activation, or Viral Persistence: Infections and Autoimmune Disease

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INTRODUCTION

Virus infections have been long associated with autoimmune diseases, whether it is multiple sclerosis, diabetes, or myocarditis. We summarize our perspectives on three potential mechanisms for virus-induced autoimmune disease or virus-induced immunopathology. These include molecular mimicry, bystander activation, and persistent virus infection. Infection of the host and the interactions between the immune response and virus set the stage for a "fertile field" where the host and/or target organ is "primed" for subsequent immunopathology. Each of these mechanisms are covered in the context of a disease setting.

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MECHANISMS OF IMMUNOPATHOLOGY THAT COULD LEAD TO AUTOIMMUNE DISEASE THROUGH A FERTILE FIELD

Molecular Mimicry

Molecular mimicry, bystander activation, and viral persistence with or without epitope spreading are three mechanisms that can initiate immunoreactivity leading to autoimmune disease. It is relatively easy to envisage how molecular mimicry could induce autoimmunity. Molecular mimicry represents a shared immunologic epitope with a microbe and the host (33). For example, individuals with rheumatic fever can develop an autoimmune disease due to infections with group A beta-hemolytic streptococci. Sera from infected individuals can have antibodies reactive with heart, joints, brain, and skin (158). Heart reactive autoantibodies can be removed by absorption with whole group A streptococci or cell wall preparations. Monoclonal antibodies derived from rheumatic fever patients

cross-react with streptococcal antigens such as the group A carbohydrate antigen and the M protein (a virulence factor associated with streptococci) and myosin. Cross-reactive peptides from M protein and cardiac myosin can induce autoimmune disease in mouse models of rheumatic heart disease (reviewed in reference 21). This is one of the best examples of molecular mimicry in autoimmune disease (21).

In a viral system, viruses have been shown to have cross-reactive epitopes with host self proteins (33). One of us (R.S.F.), with colleagues, produced various monoclonal antibodies to measles virus and herpesviruses (33). As expected, most of the monoclonal antibodies reacted with cellular proteins from uninfected cells; some of the antibodies were viral specific (reacting with only viral antigens) and a few monoclonal antibodies reacted with both viral and cellular proteins. An extension of this observation was published in a study by Srinivasappa et al. (125) showing that almost 4% of antiviral monoclonal antibodies also reacted with self proteins.

Mimicry can also take place at the level of the T-cell. We had previously shown that the hepatitis B virus polymerase shared an immunologic epitope with myelin basic protein (MBP) (32). When the viral peptide was injected into rabbits, some of the animals developed an experimental autoimmune (allergic) encephalomyelitis (EAE)-like disease, had T-cell reactivity, and developed antibodies to MBP. In subsequent years, Wucherpfennig and Strominger (155) showed that viral peptides could activate autoreactive T cells against MBP. Similarly, Hemmer et al. (51, 52), using combinatorial libraries, found that MBP-specific T cells reacted to a variety of viral and bacterial proteins. Therefore, cross-reactive immune responses between viruses and host are relatively common; but, in order for autoimmune disease to occur, we predict that the cross-reaction takes place between the virus and host at a "disease-related" epitope. If this does not occur, autoimmunity may arise but no disease transpires.

Disease-inducing epitopes are those peptides of autoantigens that can be presented by major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APCs) to autoreactive $CD4^+$ T cells. Some of the MBP peptides that can induce EAE in different animal species are reviewed by Alvord (5). These epitopes, when injected with complete Freund's adjuvant (CFA) into the appropriate species and strains, can induce EAE. Use of peptides with slightly different amino acid compositions can result in protection or a downmodulation of disease. This is often known as the altered peptide ligand strategy to modulate disease. This approach was validated in EAE models of multiple sclerosis (MS) (reviewed by Martin et al. [89]); but when used in MS patients, it met with mixed success (12, 65).

In most if not all the models where molecular mimicry has been used to induce an autoimmune disease, an adjuvant such as CFA or an actual infection is required. This suggests that, in addition to having a cross-reacting disease, inducing epitope-sufficient activation of APCs is required.

Bystander Activation

Bystander activation/killing as a mechanism leading to autoimmune disease has gained support through the use of experimental animal models mirroring some of the features of autoimmune disease such as the nonobese diabetic (NOD) mouse for type 1 diabetes (T1D) and EAE for MS. Virus infections lead to significant activation of APCs such as dendritic cells. These activated APCs could potentially activate preprimed autoreactive T cells, which can then initiate autoimmune disease (bystander activation of autoreactive immune T cells). In addition to this mode of bystander activation of autoreactive T cells, virus-specific T cells also might initiate bystander activation. For example, virus-specific T cells migrate to areas of virus infection/antigen such as the heart, pancreas, or central nervous system (CNS), where they encounter virus-infected cells that present viral peptides in the context of MHC human leukocyte antigen (HLA) class I molecules to virus-specific T cells. The $CD8^+$ T cells recognize these infected cells and release cytotoxic granules resulting in the killing or death of the infected cells. Under these circumstances the dying cells, the $CD8^+$ T cells and inflammatory cells (macrophages) within the inflammatory focus release cytokines such as tumor necrosis factor (TNF), TNF-β, lymphotoxin (LT), and nitric oxide (NO), which can lead to bystander killing of the uninfected neighboring cells. This results in additional immunopathology at sites of infection (25, 123). This also appears to be true for $CD4^+$ T cells that can recognize peptide in the context of class II molecules (156). Here cytokines released by the $CD4^+$ T cells can directly kill uninfected cells but also macrophages can kill uninfected cells in a bystander manner (94).

Persistent Virus Infections

Persistent viral infections can lead to immune-mediated injury due to the constant presence of viral antigen driving the immune response. Many of these aspects will be discussed in the myocarditis section. One example of a persistent CNS infection is Theiler's murine encephalomyelitis virus infection of susceptible mice. Following infection, an acute disease develops where neurons are infected and an encephalitis ensues. Most mice recover from this acute disease phase and develop a persistent infection. In the CNS Theiler's murine encephalomyocarditis virus is able to persist in glial cells, particularly astrocytes, microglial cells, and oligodendrocytes, and macrophages (137). Infectious virus, viral proteins, and the viral genome can be detected for the life of the animal. Much of the demyelinating disease is driven by the presence of virus and viral antigens in oligodendrocytes or associated glial cells. T-cell responses against virus-infected cells lead to inflammation and demyelination. Antiviral antibodies can also play a role in immune-mediated disease. Antiviral immune responses initiate disease (34). However, later during the chronic phase, immune reactivity to CNS myelin antigen can be detected and is thought to enhance the extent of demyelination (139).

Virus-Host Interactions

With most microbes and their hosts, there is a balance between the virus and the host. From the perspective of the virus, if it is too virulent it will either kill the host prior to being able to spread to other susceptible hosts or it will kill all susceptible hosts; in either case, the virus will disappear from nature. However, if the virus is not virulent enough, the host's

immune system will eliminate it before it can spread to other hosts, and the virus will become extinct. From the host's perspective, too weak an immune response may allow rapid viral dissemination, leading to death; but too strong an immune response may cause dramatic immunopathology which, in some cases, may also be lethal. For example, CNS infection by lymphocytic choriomeningitis virus (LCMV) leads to an intense antiviral T-cell response and consequent fatal choriomeningitis. Thus, the virus is trying to evade the host's immune response and spread to other hosts, and the host is attempting to eliminate the virus without causing too much tissue damage. The longer the virus and the host interact, the more the two seem to adapt towards peaceful coexistence. For example, herpesviruses are carried by almost all adult humans but cause only a sporadic (and usually very mild) disease; and the papovavirus JC virus can persist for the life of the host, usually without ever causing disease.

Antigen-Processing Pathways

There are two basic pathways used to "present" viral antigens to host T cells. Endogenous proteins are processed and presented through the MHC class I pathway. Entry into the class I pathway begins when intracellular self or viral proteins are ubiquitinated via lysine amino acids within the protein. Additional ubiquitins are then attached to the original ubiquitin, and the resulting polyubiquitinated protein is targeted to the proteasome, where it is cleaved into short peptides. The peptides proceed into the endoplasmic reticulum via a transporter system where the peptides encounter MHC class I molecules (class I protein and β_2 microglobulin). Depending on the conformation, charge, and sequence of the peptide, it will associate with the MHC class I molecule with different affinities. These peptide-MHC class I complexes proceed through the Golgi apparatus and are subsequently transported to the surface of the cell. These peptide-MHC class I complexes are then recognized by T-cell receptors (TCRs) on $CD8^+$ T cells.

$CD8^+$ T cells could induce immunopathology that has the potential to initiate autoimmune disease by two nonmutually exclusive mechanisms. The first would be that the virus and the host contain a cross-reactive CD8 epitope. In the rat insulin promoter (RIP)-transgenic models for diabetes, the LCMV glycoprotein (GP) and nucleoprotein (NP) epitopes have been inserted into the genome of certain strains of mice. The expression of the viral epitopes is driven by the RIP such that the epitopes are found in the pancreas and regarded as self. Depending on the levels of expression in the pancreas and in the thymus, diabetes is induced in an acute or slow time frame. If no expression of the epitope is observed in the thymus, epitope-specific $CD8^+$ T cells are not negatively selected against and are present in the periphery. Upon infection with LCMV (encoding the cross-reactive epitope), an acute inflammatory response is mounted with diabetes appearing in a matter of days or weeks postinfection. If the epitope is found in the thymus, high-affinity $CD8^+$ T cells are mostly deleted. Upon infection with LCMV, these mice develop diabetes in weeks to months following infection. The generation of sufficient numbers of $CD8^+$ T cells requires $CD4^+$ T-cell help for expansion. This is reviewed in references 102, 104, and 142.

The second mechanism would be due to $CD8^+$ T cells killing virus-infected cells such as in the CNS (Fig. 1). Self antigens contained in dead or dying cells could then be presented by APCs to $CD4^+$ or $CD8^+$ T cells, leading to the autoimmune disease. Cytokines, particularly gamma interferon (IFN-γ), would be required to activate the APCs with upregulation of MHC class II molecules for efficient presentation to $CD4^+$ T cells. IFN-γ is essential for the development of diabetes in the RIP models (144).

In both instances above, self proteins would be released into the environment and engulfed by additional dendritic cells and/or macrophages. These self antigens could then, with the appropriate stimulatory signals, such as costimulatory molecules and Toll-like receptor (TLR) signaling. on the APCs, stimulate autoreactive T cells, leading to further damage at the original site of infection or within the organ or tissue containing the self antigen (Fig. 1).

Generally, antigenic epitopes in exogenous proteins are presented via the MHC class II pathway. Exogenous autoantigens are taken up by specialized APCs by endocytosis and ultimately enter endosomes where they are degraded into peptides. Here, the peptides associate with MHC class II molecules. These peptide-MHC class II complexes are then transported to the surface of the cell. These complexes can then be recognized by TCRs on $CD4^+$ T cells. Class II molecules are only found on selected cell-types within the body, whereas class I molecules are found on most cells with the possible exception of neurons, but this is still somewhat controversial. Viral antigens could be phagocytosed by macrophages and dendritic cells and processed through the MHC class II pathway leading to activation of viral or self $CD4^+$ T cells (Fig. 1). There is some "cross talk" between the two pathways: occasionally, endogenous proteins can be presented via class II molecules and exogenous antigens can be presented by class I molecules on APCs (1, 16, 48, 81, 150).

Generation of autoreactive T cells. Depending on the experimental model or disease, $CD8^+$ T cells can be the effector cells, cytotoxic T lymphocytes (CTLs), that can cause cell destruction (138), whereas $CD4^+$ T cells can also be the effector cell (CTL); but, conventionally the $CD4^+$ T cells activate macrophages, and macrophages are the effector cells (delayed type hypersensitivity response). In the diabetes model discussed below, effector cells are mainly $CD8^+$ T cells. Here, $CD8^+$ T cells can directly kill islet cells and secrete proinflammatory cytokines. In the model of CNS autoimmune disease, EAE, the cells that can transfer disease are conventionally thought of as $CD4^+$ T cells (95, 108, 113, 114, 130). These cells can secrete myelinotoxic cytokines that damage the oligodendrocyte and generate an inflammatory focus to which macrophages are recruited that in turn cause demyelination. Thus, in these instances, the actual mechanism of killing or tissue damage can be the $CD8^+$ T cells, where these cells can kill target cells directly, while $CD4^+$ T cells can initiate damage more by a bystander mechanism.

There is some debate whether oligodendrocytes or myelin can express MHC class II molecules in vivo. If they do not, then autoreactive $CD4^+$ T cells would recognize self peptide on microglial or other class II-positive cells in the CNS and produce cytokines and chemokines resulting in the recruitment and activation of macrophages (cells of the innate immune system). Macrophages would release interleukin (IL)-1 and

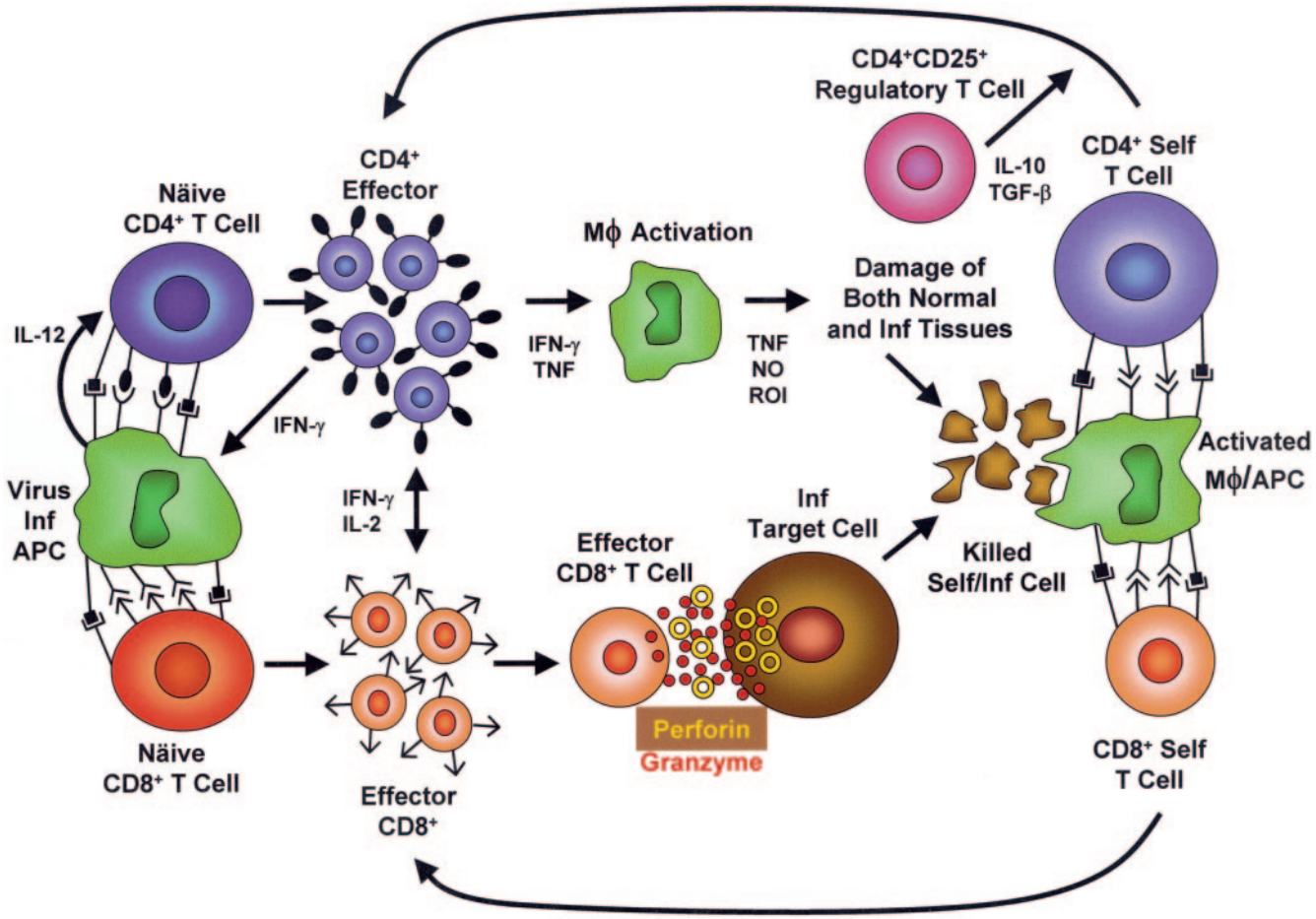

FIG. 1. Virus-infected APCs present viral peptides in the context of MHC class I or II to naive $CD8^+$ T cells or $CD4^+$ T cells, respectively. Activation of T cells leads to IFN-γ production, which will further activate APCs, leading to IL-12 production, a potent T-cell-differentiating cytokine. Effector $CD4^+$ T cells release proinflammatory cytokines such as IFN-γ and IL-2, stimulating T cells to differentiate into effector T cells. Activated T cells can also secrete IFN-γ and TNF, which can lead to macrophage activation. The activated macrophages in turn release TNF, nitric oxide, and reactive oxygen intermediates (ROI), which can kill infected cells and uninfected cells. The dead and dying cells are then phagocytosed by macrophages and dendritic cells that can present self antigens to autoreactive $CD4^+$ T cells. Similarly, effector $CD8^+$ T cells can kill infected cells via perforin and granzyme granules. Cell debris is taken up by APCs, which can present self antigens to autoreactive $CD8^+$ T cells. The generation of such cells could lead to autoimmune responses with enhanced inflammation if not modulated by regulatory T cells releasing IL-10 and/or TGF-β. Bracketed squares, costimulatory molecules and ligand. Bracketed ovals, MHC class II peptide complex and T-cell receptor. Arrow-Y, MHC class I peptide complex and T-cell receptor. Double open circle, perforin. Shaded circle, granzyme.

TNF and start engulfing myelin. Some of the targeting of macrophages to the myelin could also be due to myelin-specific antibodies. Macrophages would recognize myelin-antibody immune complexes via Fc receptors and begin engulfment of myelin.

The Fertile Field

The fertile field concept has been recently reviewed (143) and may involve all three mechanisms: molecular mimicry, bystander activation and viral persistence. In brief, we proposed that any given individual may be repeatedly exposed to a potential immunogen without any untoward consequences; but that under some circumstances, for example, if the person had a viral infection at the time of exposure, infection would alter the immunological environment in which the antigen was encountered, leading to a profound immune response. In other words, the virus, even if it contained no cross-reactive antigens, would give rise to a fertile field in which immune responses to any exogenous antigen might flourish. A fertile field could be generated in other ways. For example, an infection with a virus having molecular mimicry to self CNS proteins can potentially prime autoreactive T cells but not to the point where they can initiate autoimmune inflammatory CNS disease; later events may trigger these cells to cause disease. In the diabetes and Theiler's murine encephalomyocarditis virus models, inflammation due to aberrant cytokine expression or inflammation induced by infection of the target organ appears to be a requisite for the creation of a fertile field.

In the following sections, the potential mechanisms for immune mediated diseases will be discussed for three organs: the CNS, heart, and pancreas.

Multiple sclerosis: autoimmunity. Some of the first descriptions of MS are credited to the French physician Charcot

(reviewed in reference 6). In 1868, he described the classical form of the disease. There are several proposed mechanisms for the etiology of MS. These are: a persistent viral infection; a strictly autoimmune mechanism where the mechanism is similar to the experimental animal model, EAE; and a mechanism where a virus having molecular mimicry with a self CNS protein can prime animals for disease induced by a totally different virus infection later in life (prime challenge model).

The hallmark of MS is white matter lesions that evolve into plaques. Active plaques contain perivascular infiltrates of mononuclear cells including lymphocytes, macrophages and occasional plasma cells. $CD4^+$ T cells are found around the periphery of the plaque, and $CD8^+$ T lymphocytes are observed in perivascular regions. Perivascular and interstitial edema can be seen, often by magnetic resonance imaging. Axonal loss and/or axonal damage with microglial and astrocytic changes are often observed.

In MS the target for immune mediated damage is the myelin producing cell, the oligodendrocyte, and the axon. Loss of oligodendrocytes either by direct viral infection or immune attack can lead to large areas of demyelination, since an oligodendrocyte can myelinate multiple axons with myelin. From EAE studies it is presumed that MS is mediated by $CD4^+$ Th1 T cells, and that the effectors are activated macrophages that can strip and engulf myelin from the axons (79). In addition, the $CD4^+$ T cells and macrophages can produce vast arrays of proinflammatory cytokines that result in oligodendrocyte death and myelin vesiculation. The release of various toxic cytokines can also lead to axonal loss with axonal bulb or torpedo formation (147). In the end this is a disease of nerve conduction in the CNS.

Several factors are involved in MS, myocarditis, and diabetes. At the top of the list are genetic contributions to disease. HLA DR 1501 is the most prevalent component in Northern Europeans with MS. Presumably, this is due to the antigens the class II molecule can present to autoreactive T cells. These antigens could include viral and self antigens or peptides. Also twin studies demonstrate that in MS, similar to other autoimmune diseases, the concordance rate is about 30% for monozygotic twins, whereas the concordance rate for dizygotic twins is around 5%, which is similar to that for siblings (26, 97, 116). Therefore, genetics do contribute to susceptibility to MS. Another major component is gender. There is a sexual dimorphism in immune responsiveness in humans. Females are overrepresented for relapsing-remitting MS by about 2.7 females to 1 male. This is most likely due to a better or more active immune system possessed by women (reviewed in reference 13). Age is another factor. There is a window between ages 20 to 40 within which most individuals are diagnosed with MS. Lastly, environmental factors such as infections play a big role.

Epidemiologic studies indicate that MS is not found uniformly over the Earth. As one moves from the equator to the north and south, the incidence of MS increases (75). Part of this could be the HLA (genetics) of the populations inhabiting various parts of the Earth; but it could also be interpreted as the kinds, types, or timing of various infections being dissimilar in the different parts of the world. In addition, migration studies suggest that if one moves from a high-risk area to a low-risk area after age 15, an individual keeps the high MS risk (2–4, 23, 76, 77). However, if a genetically susceptibly individual moves prior to age 15, he or she would acquire the lower MS risk rate of the area to which he or she moved. The reverse also appears to hold true, with moving from a low-risk area to a high-risk area. One interpretation of these data is that a virus or microbe could either prime or protect individuals for autoimmune disease (MS) later in life.

TABLE 1. Viruses recovered from patients with multiple sclerosis[a]

Agent	Yr	Agent	Yr
Rabies virus	1946	Herpes simplex virus type 2	1964
Scrapie agent	1965	MS-associated agent	1972
Parainfluenza virus 1	1972	Measles virus	1972
Simian virus 5	1978	Chimpanzee cytomegalovirus	1979
Coronavirus	1980	SMON-like virus	1982
Tick-borne encephalitis flavivirus	1982	HTLV-1	1986
		Herpes simplex virus type 1	1989
LM7 (retrovirus)	1989	Borna disease virus	1998
Human herpesvirus 6	1994		

[a] Adapted from reference 82a with permission of the publisher. MS, multiple sclerosis; SMON, subacute myelo-opticoneuropathy; HTLV, human T-cell lymphotrophic virus.

We know that viruses have been associated with MS for about the last 60 years. Almost two dozen viruses have been isolated from the brains of MS patients (reviewed in reference 63). These include herpesviruses, paramyxoviruses, and retroviruses (Table 1). Further, virus infections often precede MS exacerbations.

We have an evolving model that we feel recapitulates what is observed in MS. This is a "fertile field" model, where the first infection sets up or tills the field. Young (3 to 4 weeks old prior to puberty) female (gender) SJL/J mice (genetically susceptibility) were injected with a cDNA encoding myelin proteolipid protein (PLP), where ubiquitin is encoded at the 5′ end of the PLP coding region to form ubiquinated PLP (uPLP). This was to simulate infections early in life by a virus having molecular mimicry with a self CNS protein (setting up the fertile field), a protocol that by itself does not lead to CNS pathology or clinical signs of CNS disease. Different virus infections have been reported to induce exacerbations of disease, and therefore, we decided to give the mice a nonspecific immunologic stimulus, simulating a second virus infection at a later time. Mice were then challenged with CFA (Fig. 2A). About 10 to 14 days postchallenge, some of the mice developed clinical signs similar to those of mice with EAE. Lymphoproliferation assays indicated that there was T-cell reactivity to $PLP_{139–151}$. Examination of the CNS tissue from the CFA-challenged mice found T-cell infiltration and lesions in about 20% of mice. Control mice injected with CFA alone or mice primed with a cDNA encoding PLP without ubiquitin and challenged with CFA did not have any lesions (136).

We then asked whether an actual virus infection having mimicry with self CNS proteins could prime for autoimmune disease later in life. Recombinant vaccinia viruses were constructed that encoded either PLP, myelin-associated glycoprotein (MAG), or an astrocyte protein, glial fibrillary acidic protein. SJL/J mice were infected with these viruses. The viruses by themselves did not cause clinical signs or inflammatory lesions, but did set up a fertile field. After the virus was cleared, mice were given CFA (Fig. 2B). From 80 to 90% of the mice developed disease (136). Control mice infected with a recom-

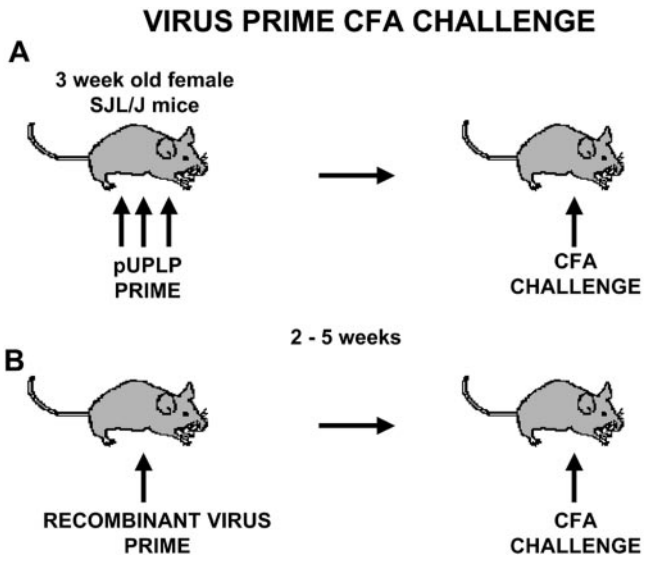

FIG. 2. (A) Three-week-old female SJL/J mice were primed with a cDNA encoding ubiquitin in frame with PLP (three times). Two weeks after the last injection, mice were challenged with CFA. Some of these animals developed CNS inflammatory lesions typical of EAE. (B) Three- to 4-week-old female SJL/J mice were primed with recombinant vaccinia virus encoding self CNS proteins. The vaccinia virus is cleared by about 2 weeks postinfection. After 5 weeks mice were challenged with CFA. Most of the animals primed with the recombinant vaccinia viruses encoding self CNS proteins developed CNS inflammatory lesions, while those infected with a recombinant virus encoding β-galactosidase and challenged with CFA did not.

binant vaccinia virus encoding β-galactosidase (VV_{SC11}) and challenged with CFA did not develop clinical or pathological disease. Interesting lesions were more localized to the brain rather then the spinal cords, whereas mice with EAE have more spinal cord lesions than brain lesions.

Most individuals do not receive a bolus of CFA during their lifetime. Therefore, we asked whether CFA could be replaced by a viral infection. In the next set of studies we primed mice with the uPLP and then challenged the mice with our recombinant virus encoding β-galactosidase (VV_{SC11}). Only 20% of animals developed CNS disease. None of the control animals primed with a cDNA encoding β-galactosidase or nonubiquitinated PLP and challenged with VV_{SC11} developed disease.

At this time we were somewhat puzzled as to why we were not seeing more disease. Therefore, we asked whether the type of virus infection mattered. Young female SJL/J mice were primed with vaccinia virus encoding PLP (VV_{PLP}). After the virus was cleared, the mice were challenged with wild-type vaccinia virus (WR strain), LCMV (Armstrong strain), or murine cytomegalovirus (MCMV), Smith strain). Interestingly, mice challenged with wild-type vaccinia virus or LCMV did not develop lesions. In contrast, mice infected with MCMV developed lesions in white matter regions in the brains such as the internal capsule and pontine base and near the hippocampus. MCMV-challenged mice were also impaired in their righting reflex responses and did not gain as much weight as the controls.

We can explain the experiments in the following manner. The first is that the priming infection (setting up the fertile field) increases the number of autoreactive T cells but not sufficiently to cause disease. We have previously demonstrated that a critical number or mass of autoreactive T cells must be generated in order for diabetes to develop (121). Below this number, diabetes does not develop or a secondary event is required. There are at least two possibilities to explain the exacerbations or what secondary events are required for disease. The first is the autoreactive T cells were sufficiently activated and proliferation was initiated by bystander activation. In the context of MCMV infection, interleukin-12 is produced by infected dendritic cells (22) with the production of IFN-γ by natural killer (NK) cells leading to the activation of the autoreactive T cells which were previously expanded during the first infection having molecular mimicry with self CNS proteins. These T cells proliferate to sufficient numbers, above the disease threshold, and now disease or pathology ensues.

Another potential mechanism is a variation of the theme of heterologous immunity (119, 146). Virus infection A leads to the generation of memory T cells specific for A. Mice immune to virus A are now infected with virus B. Interestingly, not only are B memory T cells generated, but a subset of A memory T cells are stimulated, maintained, and expanded. This expansion is due to, in some instances, unrecognized cross-reactive epitopes common to both viruses A and B (15). An extension of this would be that infection with virus A has molecular mimicry with a self protein, such as infection with the VV_{PLP}. Infection with virus B, MCMV, would have an unrecognized cross-reactive epitope with the self protein and would then lead to engagement of the autoreactive T-cell receptor in the context of infection. This interaction would lead to the proliferation of these autoreactive T cells (above a critical mass) and disease would ensue. The two mechanisms are not mutually exclusive. One prediction of this model is that infections can occur in the periphery (outside the CNS). In this model there is no need for infection of the target organ, be it CNS or pancreas. In this model virus infection can silently prime for autoimmune disease early in life that is triggered by other infections later in life.

Viruses can vaccinate against autoimmune disease. Viruses having molecular mimicry with self proteins can be used to vaccinate against autoimmune disease. An encephalitogenic region from MBP for the PL/J strain of mouse is the first 9 to 11 amino acids [acetylated (Ac)1–11)] (159). We have made two recombinant viruses which encode the first 23 amino acids of MBP. The first vaccinia virus encoding glycoprotein (GP) amino acids 1 to 23 of MBP ($VV_{GP/M1-23}$) fuses the MBP sequence to the 3′ end of the first 218 amino acids of the LCMV GP. The second was made as a minigene construct that encodes only the first 23 amino acids from MBP, a vaccinia virus encoding amino acids 1 to 23 of MBP (VV_{M1-23}). In both recombinant viruses, the first amino acid of MBP is not acetylated as in the native molecule. It is important for that the first amino acid be acetylated in order for the peptide to be encephalitogenic. When mice were vaccinated with either $VV_{GP/M1-23}$ or VV_{M1-23} and studied, no disease resulted. However, when we attempted to induced EAE in these mice using (Ac)1–20, the mice were protected. Interestingly, when the vaccinated mice were sensitized with whole MBP, the majority of mice were also protected from disease. Mice were not protected against EAE when whole spinal cord homogenate was used,

demonstrating that the protection is antigen specific. Delayed-type hypersensitivity to MBP was also statistically reduced in mice vaccinated with $VV_{GP/M1-23}$ compared with control mice infected with VV_{SC11}. Lymphocytes from vaccinated and MBP-sensitized mice could not adoptively transfer EAE to naïve mice whereas lymphocytes from control mice could (10). A potential mechanism is that these viruses protect animals by presenting an "altered peptide ligand" which activates regulatory cells that modulate the disease. Such experiments are ongoing. These data suggest that viruses that have molecular mimicry with self proteins may be used as vaccines to prevent autoimmune disease later in life.

Myocarditis: Autoimmune or Immune-Mediated Pathology?

Several forms of cardiac insult can result in myocarditis; but, we shall focus on virus induced myocarditis, and on whether the myocarditis is caused by (i) the infection itself; (ii) the immune response to the infection; or (iii) autoimmunity. Myocarditis is surprisingly common, as revealed by a necropsy study of more than 12,000 victims of violent or accidental deaths (that is, deaths which were, presumably, unrelated to heart disease); myocarditis was present in approximately 1% of these individuals (41) indicating that, at any given time, ~2 million Americans have inflammatory infiltrates in the heart. However, myocarditis is often asymptomatic; only a subset of cases, probably around 10%, exhibit clinical disease, developing symptoms such as chest pains, palpitations, or signs of heart failure. Individuals in the larger, symptom-free, group usually recover without obvious sequelae, but are by no means free of risk; acute myocarditis, even when asymptomatic, predisposes to catastrophic dysfunction of the electrical pathways in the heart and can lead to the collapse and death of young and vigorous individuals, especially during exertion (11, 145).

Although the majority of symptomatic patients recover well from acute myocarditis, the disease can have serious long-term sequelae; some 10 to 20% of people with symptoms (i.e., ~20,000 to 40,000 patients per year in the United States) will develop chronic disease, and a substantial proportion of these individuals progress over time to dilated cardiomyopathy (DCM) (101, 124), which is thought to have an incidence (new cases per year) of 3.5 to 8.5 cases per 100,000 population (~9,000 to 20,000 new cases annually in the United States) (39). DCM is a serious condition in which one or both ventricles dilate and decompensate, with resulting cardiac failure. There is a 50% mortality in the 2 years following diagnosis (40), and the most effective treatment is heart transplantation; indeed, DCM is the condition underlying almost half of all heart transplants (49). In many cases, histological examination reveals extensive cardiac fibrosis suggestive of prior myocardiocyte destruction (87).

Here, we shall focus on myocarditis induced by an enterovirus, type B coxsackievirus (CVB), which, as discussed below, is known to replicate in the heart tissue and to induce strong inflammatory responses therein. Therefore, the damage to heart muscle may be most simply explained by direct microbial cytolysis and/or by the immunopathological consequences of the antimicrobial immune responses. However, in addition to these straightforward explanations, autoimmunity has been invoked to explain the acute and chronic diseases mentioned above.

Coxsackievirus myocarditis. Several viruses cause myocarditis, but the role of enteroviruses is very well established. Cardiovascular signs and symptoms are present in 1.5% of all enteroviral infections, and CVB is the commonest cause of infectious myocarditis; the incidence of cardiovascular symptoms is 3.5% for CVB and 0.7% for type A coxsackievirus and for another enterovirus, echovirus (44). CVB has been isolated from the hearts of patients with myocarditis, CVB-related nucleic acid signals have been found (by PCR and in situ hybridization) in the myocardium, and serologic studies implicate CVB in the acute disease. Furthermore, CVBs isolated from stool or pharyngeal specimens of patients with acute myocarditis have been administered to mice and have infected the heart (38, 153).

Demonstration of infectious CVB in the human myocardium has been more difficult, since myocardial biopsy remains unusual, but necropsy specimens have yielded infectious CVB (38, 128, 129), which is cardiotropic in mice (128). Slot blot hybridization studies have shown positive signal for CVB RNA in myocardial biopsy specimens of approximately 45% of patients with myocarditis or DCM compared with none of the controls (90), and ~43% of patients with healed myocarditis or DCM remained positive for CVB signal (7). High levels of neutralizing antibodies are found in about 50% of patients, and serial antibody studies show a fourfold or greater change in paired sera in approximately half of patients (90). As further evidence that enteroviruses may cause DCM, this chronic disease occurs in 10 to 20% of patients with proven prior enteroviral myocarditis, while its incidence in the total population is approximately 0.005%, and a large study confirmed this strong correlation ($P < 0.001$) between prior coxsackievirus infection and DCM (115). Acute myocarditis and DCM are, therefore, significant contributors to human morbidity and mortality, and the role of CVB has been clearly demonstrated. Several CVB3 isolates, when inoculated into normal mice, causes myocarditis (37, 53, 70), pancreatitis (92, 122), and neonatal CNS infections (31) and thus faithfully recapitulate many aspects of CVB infection and disease in humans.

What Mechanisms Might Underlie CVB Myocarditis?

While there is no doubt that CVB3 can cause myocarditis in mice, the precise mechanism underlying this pathogenic outcome remains controversial. Five possible pathogenic mechanisms are outlined in Table 2. From this table, it is clear that, although both the acute and chronic diseases induced by CVB almost certainly have a large immunopathological component, this does not necessarily imply autoimmunity; mechanisms 1 and 2 are sufficient to explain the observed clinical phenomena, as long as the virus (or, at least, some viral materials) can persist in the host animal. So, what is the evidence for viral persistence?

In tissue culture, CVB can establish long-term persistent infection in a variety of cell types, including human myocardial cells (50, 64) and human and murine lymphoid cells (91, 157); infectious virus can be recovered over a period of weeks to months. The in vivo situation is less well understood. CVB RNA can persist for many months in skeletal muscle, apparently as double-stranded RNA, and RNA persistence corre-

TABLE 2. Mechanisms[a]

Mechanism	In theory, could mechanism explain:	
	Acute disease	Chronic disease
Direct virus-driven cell death (1) (cytolysis, apoptosis)	Yes	If CVB persists
Immune responses (immunopathology)		
(2) Against CVB3 antigens on infected cells	Yes	If CVB persists
(3) Against self antigens expressed only on CVB3-infected cells	Yes	If CVB persists
(4) Against self antigens that share cross-reactive immune determinants ("molecular mimicry") and, therefore, could be present on uninfected cells	Yes	Yes (does not require viral persistence)
(5) Against self antigens rendered more immunogenic by infection ("epitope spreading")	Yes	Yes (does not require viral persistence)

[a] Note: mechanisms 2 to 5 are all immunopathological, but only 3 to 5 are autoimmune.

lates with the degree of myositis observed (133–135). However, in these and other in vivo studies, infectious virus could not be isolated at the later stages, despite the presence of CVB-related RNA sequences. It is important to draw a clear distinction between viral RNA and infectious virus; the two are not necessarily equivalent, and terminology such as "CVB persistence," often used to describe the presence of CVB-related nucleic acid signal, should be employed only if infectious virus can be identified within, or reactivated from, the tissues.

Do CVB materials also persist in heart muscle? In vivo, CVB has been detected by in situ hybridization in biopsy specimens of human DCM patients; one could argue that this represented an acute infection, present by coincidence at the time of biopsy, but the failure to detect infectious virus suggests that an acute infection was not present. Recent studies in several mouse strains have shown long-term persistence of CVB-related nucleic acid signal in the heart, associated with chronic myocarditis and fibrosis (68); the signal is found in several organs, including heart and is often highly localized, being found near regions of inflammation (68, 69). The identification of CVB RNA long after the primary infection provides several potential explanations for chronic myocarditis: first, it remains possible that infectious virus may be sporadically reactivated; second, viral protein expression alone can be toxic to cells (148, 149); and third, the upregulation of viral protein expression could lead to a recrudescent immunopathology. Thus, in principle, chronic myocarditis and DCM may be explained by persistent CVB materials and, as in the acute phase, there may be immunopathology, but there is no need to invoke autoimmunity.

But how might CVB materials persist, especially if infectious virus is not detectable beyond ~14 days postinfection? Recent findings from several laboratories indicate that there are interactions between CVB and the infected cell; in particular, CVB may respond to, and may regulate, the cell cycle. A cell cycle effect on picornaviral replication was suggested by studies carried out some two to three decades ago (27, 78, 86, 126), but it has not been clearly delineated, and, judging from its omission from recent reviews on virus-cell cycle interactions (106, 131), appears not to be widely appreciated. These studies are described in several recent publications (8, 30, 35, 82, 83, 93, 105, 134) and will be summarized only briefly here.

We have found that the outcome of infection of tissue culture cells depends on their cell cycle status; infection of quiescent cells (G_0) or cells blocked at the G_2/M phase leads to low levels of viral protein synthesis and inefficient production of infectious virus; but "release" of the cell, allowing it to pass through G_1, results in increased viral gene expression and infectious virus production. Thus, the virus appears to respond to the cell cycle status. Others have shown the reciprocal; the virus can affect the cell cycle, arresting cells at the G_1/S boundary, by increasing the degradation of cyclin D1 (85). Therefore, the virus seems to have evolved (i) to arrest the cell at the stage most beneficial to the virus's replication and (ii) to remain quiescent in cells that fail to enter the G_1 stage.

What viral component might allow the virus to "sense" the cell status and to respond appropriately? Picornaviruses contain, in their 5′ untranslated region, an internal ribosome entry site (IRES), to which cell cycle-regulated proteins may bind, regulating picornaviral protein expression (109), and some viral IRESs appear to respond to the cell cycle status in tissue culture (140). IRES elements have been identified in cellular mRNAs, and many of the encoded cellular gene products are associated with the cell cycle (111), although these cellular IRESs are most active in G_2/M, when CVB gene expression is low. Perhaps CVB has incorporated a cellular IRES, but subsequent modifications have allowed it to operate best when host translation is almost entirely cap dependent. In that way, the virus can kill two birds with one stone: it can shut down cap-dependent translation at a time when the host most relies on it and at the same time can very efficiently translate its own proteins in the absence of competing host IRESs.

Thus, many of the requirements are in place to explain CVB-induced myocarditis, without invoking autoimmunity. However, this merely shows that autoimmunity may not be required; it does not directly address whether or not it actually is responsible for the disease. We believe that three key questions must be asked. First, are autoreactive responses induced by CVB infection? If not, then autoimmunity can be dismissed as a cause of myocarditis. However, even if autoreactive responses are found, their mere presence does not prove that they are pathogenic; a second question must be asked, do the autoreactive responses contribute to disease? Only if the answer is affirmative should we approach the third question, what is the underlying mechanism of autoimmune disease? One might expect that myocarditis would result from a single autoimmune mechanism; but, over the past three decades, at least four distinct mechanisms have been proposed: autoantibodies, autoreactive MHC class I-restricted $CD8^+$ T lymphocytes, autoreactive MHC class II-restricted $CD4^+$ T lymphocytes,

and most recently, T cells carrying γδ T-cell receptors. Further complicating the issue, it has been suggested that the mechanism of autoimmune postviral myocarditis may be dependent on the mouse strain; for example, that autoimmune T cells may be responsible in BALB/c mice and autoantibodies in the DBA/2 strain (72).

Cardiac autoantibodies induced by CVB were first described in 1 of 55 sera that were screened for antimyosin antibodies; the serum that scored positive was from an individual who had coxsackievirus-caused pericarditis (29). Since then, a large number of autoantibodies have been described in the sera of patients with myocarditis (summarized in reference 36), but the clinical relevance for many is unclear, because many of the target proteins are intracellular (107). Autoreactive antibodies against cardiac myosin were identified in mouse models of CVB infection (152), and an association was found between susceptibility to chronic myocarditis and the presence of autoreactive antibodies (151). One possible explanation of these data was molecular mimicry; perhaps CVB infection induced antiviral antibodies that cross-reacted with myosin, but this was shown not to be the case (98). In studies of chronic myositis, both CVB-specific antibodies and autoantibodies were found, but there was no statistically significant association with the extent of myopathy; rather, the autoantibodies appeared to be an independent reflection of the damage done by the virus infection (132). Taken together, these data indicate that CVB myocarditis favors the induction of autoantibodies, but these may be the consequence of disease rather than its cause.

T lymphocytes have long been implicated in CVB-induced myocarditis (154), and adoptive transfer studies have identified cytolytic $CD8^+$ T cells (at that time, known as Lyt-2^+ cells) as major players (46). Subsequent analyses have confirmed and extended these findings; there is no doubt that $CD8^+$ T cells contribute substantially to the myocarditis that is induced by CVB3. But are the cells autoreactive (potentially causing autoimmune disease), or are they specific for viral materials? Adoptive transfer data identified $CD8^+$ T cells that appeared to recognize uninfected myocardiocytes, but the antigen target of these autoreactive cells was not identified (57, 58), and subsequent analyses revealed two types of cytolytic T cells induced by CVB infection; autoreactive $CD8^+$ T cells and virus-specific $CD4^+$ T cells (28).

Ongoing studies of the nonviral (and, almost certainly, autoimmune) myocarditis induced by inoculation of cardiac myosin had now progressed to a point at which autoantibodies were no longer considered a likely mediator of disease, and suspicion focused on T cells (99, 100), in this case, and in contrast to the earlier report regarding CVB, the major autoreactive population of T cells were $CD4^+$. MHC class II-restricted peptides from cardiac α-myosin have been identified that, when inoculated with adjuvant, induce myocarditis in susceptible mice (24, 110). However, a link to CVB-induced disease remains tenuous, because the T cells induced by those peptides do not cross-react with CVB, and these peptide-specific $CD4^+$ T cells have not been identified as a factor in CVB-induced myocarditis.

The most recent T-cell family to be implicated contains γδ T cells. Despite their having been discovered some time ago, the biological function of these cells remains unclear. They play a protective role in various noninfectious models of chronic stimulation (73), wound healing (61), and tumor immunity (62), and they can be activated by nonspecific stimuli (96), suggesting that foreign (e.g., viral) antigens may not be required for activation of many of these cells; their TCRs are, presumably, activated by unidentified endogenous materials and, as such, they may be categorized as autoreactive. Several functions have been ascribed to γδ T cells during CVB3 infection. The cells appear to directly interact with myocardiocytes and have been proposed as the main effector population responsible for myocardial injury associated with DCM-like signs during CVB3-induced myocarditis (55). One population of γδ T cells appears to suppress CVB-induced myocarditis (56), while another (expressing a different Vg receptor) exacerbates disease by secreting IFN-γ (56), thereby activating $CD4^+$ T cells, which in turn are required to activate autoreactive $CD8^+$ T cells (59). These data represent one of the few cases in which the biological role(s) of γδ T cells has been investigated during microbial infection, and it will be interesting to identify the antigen(s) recognized by these cell populations.

In summary, there is no doubt whatever that autoreactive antibodies and T cells can be induced during CVB infection. However, the evidence that these virus-induced autoreactive responses are themselves pathogenic is relatively scant, and the invocation of different mechanisms of autoimmunity in different hosts may not be necessary. Furthermore, it may be significant that immunosuppression is not a recommended treatment for myocarditis. If the chronic disease were autoimmune in nature, one would predict that immunosuppression might have been an effective treatment; that this treatment is not recommended indicates that an autoimmune mechanism is unlikely.

Using Occam's razor gives a simpler explanation: that the long-term disease results from reactivation of viral materials that have persisted in host cells, with consequent viral cytolysis and/or immunopathology. This concept is exemplified by the lifelong infection established by herpes simplex virus in dorsal root ganglia. Contrary to the prevailing wisdom, which holds that herpes simplex virus is truly latent for much of the time, it appears that herpes simplex virus does not remain silent within the ganglia; rather, it is constantly "trying" to reactivate, and this recrudescence is actively suppressed by $CD8^+$ T cells that recognize viral antigens (66). This explains why immunosuppression leads to more frequent herpes simplex virus eruptions, because the immune system is unable to hold the virus in check. Perhaps CVB myocarditis should be viewed in the same light, and further studies should be focused on the mechanism by which CVB establishes persistence or latency and the circumstances that may lead to viral reactivation.

Type 1 Diabetes: an Autoimmune Disease?

T1D is a disease, similar to MS, which is presumably autoimmune mediated, resulting in selective destruction of insulin-producing β-cells in the pancreatic islets of Langerhans. Individuals exhibit autoantibodies to several islet antigens prior to clinical disease onset that function as an excellent predictor of disease risk (17). Their pathogenetic role is still under debate (42, 71, 141) since plasmapheresis has not conclusively alleviated T1D in humans (88, 112, 127) and autoantibodies alone cannot transfer disease in animal models. In addition, differ-

ences in cytokine production by islet antigen-reactive T lymphocytes have recently been described (9) whereby individuals with T1D produce more IFN-γ in response to naturally processed proinsulin peptides than healthy controls who generate higher amounts of the regulatory cytokine IL-10. These data coupled with observations from animal models allow the mechanistic hypothesis that autoaggressive T cells such as IFN-γ-producing $CD4^+$ and $CD8^+$ T lymphocytes are dysregulated in T1D and are the main cause of β-cell destruction.

Etiologically, investigations have shown that the genetic risk of developing autoimmunity reflected by the development of islet cell antibodies is almost 100% in monozygotic twins, whereas the risk of developing clinical disease exhibits merely about 50% concordance. Therefore, not autoimmunity but disease penetrance appears to strongly depend on additional environmental factors or modulators that would act upon an existing, yet preclinical, autoimmune process. Microbial infections are excellent candidates, since they affect the immune system on multiple levels and their effects were extensively examined using animal models. Interestingly, the answers from these studies have painted an increasingly complex picture that we will discuss in more detail in the following. It has become clear that viruses in particular can accelerate or stop ongoing autoimmune processes. This dichotomy makes defining agents that impact the pathogenesis of human T1D much more difficult. In the following section we will discuss these problems based on the insight gained from studies in animal models. Better understanding will lead to rational identification of crucial infectious events and could in the long run help to form strategies to avoid detrimental infectious events.

Triggering of Autoimmunity by Infections: a Likely Scenario?

Is precipitation of autoimmune diabetes in nonpredisposed, naïve individuals a likely scenario? Based on evidence gathered in various models, we would argue that the answer is no. In the following we will discuss a few key findings that are helpful for understanding what could occur in vivo.

TLRs and the triggering of autoimmunity. The family of TLRs are instrumental in activating APCs and initiating inflammation, and they are triggered by a variety of microbial components. For example, double-stranded RNA binds to TLR3 and lipopolysaccharide activates TLR4. Some recent investigations shed more light on the potential role of TLR cross-linking in offsetting autoimmune processes. In these studies, TLR agonists were administered with model autoantigens to trigger autoimmunity in non-diabetes-prone animals that expressed the same antigen in their β-cells as a transgene. Intriguingly, divergent outcomes were observed. In one study, autoimmune disease occurred readily (80), whereas in the other one, autoimmune responses were invoked but were transient (47). The crucial difference was the need for autoantigen-specific CD4 helper responses. If added to the latter model, autoimmune disease developed. Thus, for autoantigenic immunizations in conjunction with TLR ligation to precipitate disease in naïve animals, a variety of factors likely need to coincide. Therefore, we believe that an effect of TLR ligation on autoimmunity is more likely to occur if an autoimmune process is already established, as would be the case in prediabetic individuals. Indeed, investigations from the BB rat model support this notion. If TLR agonists were administered during the prediabetic phase in this genetically determined model of autoimmune diabetes, T1D development was strongly accelerated (160). Thus, breaking of tolerance to autoantigens requires very strong inflammatory stimuli, unless autoreactive T cells are already activated. Ultimately, the development is strictly dependent on numbers of autoaggressive T cells available and thymic tolerance can prevent development of autoimmunity even in the presence of TLR agonists.

Inflammation and conditioning of the target organ. Inflammation can condition the β-cell downstream of TLR signaling events. One very important pathway is the upregulation of MHC class I by means of IFNs α/β and γ signaling. In a noninflamed state, β-cells express very few MHC class I molecules, which renders them essentially nonrecognizable for killing by CTLs. In contrast, any viral infection leading to the release of α/β IFNs will "unmask" them to the immune system. This event alone can be transient and will not lead to their destruction unless a significant number of activated autoaggressive CTLs are able to reach the islets (118). These will have to be activated and driven by APCs that are primed. In addition to genetic factors, viral infections can accomplish this. Thus, unmasking of target cells and activation of APCs can be major pathogenetic events elicited by viruses. These can occur downstream of TLR signaling events or through other causes (80).

Is the glass half full or half empty? If one takes a close look at the experimental systems in which viral infections can precipitate T1D in an otherwise naïve, nonpredisposed animal, one comes to the conclusion that, in addition to TLR stimuli and inflammatory mediators such as IFNs described in the previous section, a relatively large number of autoaggressive T cells are required. This raises the question whether a scenario similar to those reflected in the RIP-LCMV (102, 104), RIP-hemagglutinin (HA) (84), or RIPm ovalbumin (Ova) (74) models would likely occur in human patients.

Our assessment is that the glass is half empty. The reason is that in all of these models a significant number of activated $CD8^+$ and/or $CD4^+$ T cells are required to destroy a sufficient amount of β-cells to result in diabetes. Precursors reach levels up to 1/10 during the peak of the inductive response, a frequency that has never been detected for autoaggressive T cells in human peripheral blood of diabetes-prone individuals. We, therefore, would like to suggest that lesser numbers are more likely present in prediabetic humans. This, in turn, raises the question how such lower frequencies of autoreactive cells can play a role in disease pathogenesis. The solution is to postulate that they would act in concert with an existing inflammatory state that is chronic and, at least in part, genetically predetermined. Indeed, this proposition is in agreement with a significant amount of experimental evidence, which is described in the next section. Thus, one should consider the pathogenetic potential of autoaggressive T cells in context with the fertile field (143) they encounter in the target organ.

Enhancement of T1D by Infections

Molecular mimicry in T1D. Cross-reactivity between foreign and host components is one mechanism that could explain a viral influence on autoimmunity. Indeed, there is ample evi-

dence that such cross-reactivities can occur on the T- and B-cell levels (125, 155) and some scenarios have already been described above. A question emerging from considerations presented in the previous section is whether molecular mimicry alone is sufficient to lead to T1D. One would in general acknowledge that mimicry can break tolerance to autoantigens, but is this alone sufficient to result in disease? Recent studies provided the answer: it is very unlikely.

Mice expressing a defined viral protein as a self antigen in β-cells were infected with viruses expressing the antigen itself or molecular mimics. Autoimmunity occurred readily in all cases, however, diabetes did not develop (19) unless TCR signal transduction was enhanced (45). Thus, especially if one takes into account that most individuals will exhibit some degree of tolerance to autoantigens by restricting their autoreactive repertoire in the thymus, it becomes unlikely that molecular mimics contained within foreign proteins would precipitate autoimmune disease unless other factors are provided.

We recently investigated whether one could attribute a more significant role for mimicry if it occurred in an individual with an existing autoimmune process established in the islets of Langerhans. We used the RIP-LCMV model for T1D, in which mice express a protein of LCMV specifically in the pancreatic β-cells (104). Such mice only develop disease when infected with LCMV. Secondary infection of LCMV-immune RIP-LCMV mice with Pichinde virus (PV), which shares a structural similarity in a normally subdominant epitope (14), massively accelerated the autoimmune process (19). Lymphocytes with specificity to the mimicking epitopes on LCMV and PV are normally of low frequency after single infection with LCMV or PV (14, 19). Apparently, after heterologous, sequential infection with both viruses, such autoaggressive T cells are expanded to a frequency high enough to significantly impact the autodestructive process resulting in acceleration of disease (19). We conclude from these investigations that mimic events can indeed play a significant role in individuals with subclinical autoimmunity, for example, those with a strong genetic predisposition.

TLRs and inflammation. Antigen-nonspecific events can enhance autoimmune processes as well, if these have been established previously in an antigen-specific manner. One recent example shows that TLR agonists can accelerate diabetes development in the BB rat (160). Another example was provided through investigations in the NOD mouse, where CBV can accelerate diabetes when given during a crucial prediabetic phase (54, 120). However, in the latter studies, abrogation of T1D was also intriguingly observed.

Prevention of Diabetes by Infections

Trafficking. In contrast to initiation and/or acceleration of autoimmunity, virus infections have also been found to abrogate ongoing autoimmune processes. An interesting example is the prevention of T1D in the RIP-LCMV and NOD mouse models (18). Infection of prediabetic RIP-LCMV-NP or NOD mice with ongoing insulitis but not clinically manifested T1D with LCMV, a well-characterized mouse pathogen, results in substantial viral growth in the pancreatic draining lymph node and other lymphoid organs, but not as much in the pancreas or islets. Such a strong inflammation at sites other than the target

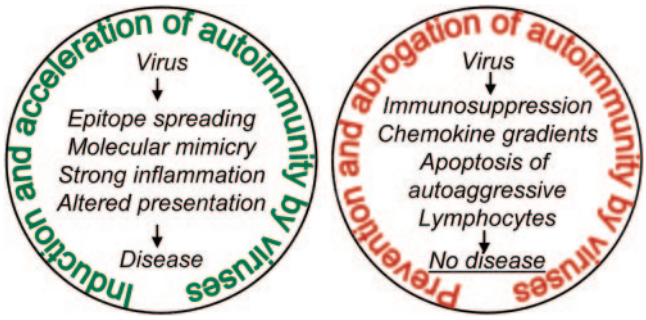

FIG. 3. Virus infection can initiate or accelerate autoimmune disease via epitope spreading and molecular mimicry, leading to the development of an inflammatory region with activated APCs and possible presentation of self antigens. On the other side of the coin, virus infection could lead to immunosuppression and chemokine gradients of anti-inflammatory cytokines such as IL-10 or TGF-β with activation-induced cell death of autoreactive cells.

site of autoimmune destruction had a significant impact on trafficking of autoaggressive lymphocytes: as early as day 1 after the abrogative infection, the chemokine CXCL10 (IP-10, interferon-γ-inducible protein of 10 kDa) was induced to much higher levels in the pancreatic draining lymph node than the pancreas itself (18). As a result, cellular infiltrates in the islets of Langerhans were drastically reduced at day 3 after secondary infection (18), which indicated that autoaggressive T cells had recirculated from the islets to peripheral lymphoid sites where stronger inflammatory signals, among them IP-10, were present. Interestingly, at the same time, a significant increase of apoptosis of antigen-specific autoaggressive lymphocytes was noted in the pancreatic draining lymph node, suggesting hyperactivation-induced cell death of autoaggressive lymphocytes (18). These data could explain earlier findings that demonstrated a lower frequency of disease in NOD mice that were infected with LCMV (103). In addition, these observations could explain the geographic distribution of autoimmune diseases worldwide by fitting well into the concept of the "hygiene hypothesis," which suggests that cleaner living conditions will lead to an enhanced incidence of autoimmune disorders, asthma, and allergies (117).

Apoptosis of autoaggressive lymphocytes. Similar to virus infections, overexpression of cytokines during an ongoing autoimmune destruction would be a possible means to induce apoptosis of autoaggressive lymphocytes. Indeed, β-cell-specific expression of TNF under the control of a tetracycline-sensitive promoter (tTA-system) (67) late during LCMV-induced T1D in the RIP-LCMV mouse abrogated disease irreversibly (20). In these experiments mice that were already diabetic reverted to a permanent nondiabetic state if TNF was expressed at a critical time at the beginning of clinically overt disease. Interestingly, TNF expression caused only apoptosis in experienced T cells that were in a stage of high activation, whereas inexperienced T cells remained in the lymphocyte pool (20). A similar effect had been noted in the NOD mouse model (60). Thus, in analogy to viruses that can induce and abrogate autoimmune diseases, TNF, which is traditionally referred to as a "proinflammatory" cytokine with the potential to boost an immune response, can indeed abrogate an ongoing autoimmune process when expressed at a critical time. Similar

data have been reported by Richard Flavell's group, which found that there is a crucial time window of TNF expression that determines whether an ongoing subclinical autoimmune process will cause disease or not (43).

CONCLUSION

The occurrence of autoimmunity and some forms of myocarditis is clearly a consequence of genetic factors coupled with exposure to environmental factors. Viruses have been shown to be one of the environmental factors that are capable of precipitating autoimmune disease by a variety of possible mechanisms discussed here. On the other side of the coin, viruses have the potential to abrogate an ongoing autoimmune reaction by inducing apoptosis of autoreactive cells, by influencing cellular trafficking, or by immune suppression (see Fig. 3 for an overview). However, it has been difficult to provide direct evidence for the involvement of viruses in human autoimmune diseases, perhaps because the causative virus has been cleared by the time of diagnosis. Further, it will be more difficult to obtain direct evidence for virus-induced protection from disease, since we are all infected by multiple viruses. The total infectious history of each individual and exposure to other environmental agents have to be considered and tracked. Some of the factors might be disease promoting, whereas others might be protective. In the future it will be important to monitor such environmental factors individually to assess their relative contributions to diabetes and other autoimmune diseases.

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