Virus-induced autoimmune disease
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The breaking of tolerance or unresponsiveness to self-antigens, involving the activation of autoreactive lymphocytes, is a critical event leading to autoimmune diseases. The precise mechanisms by which this can occur are mostly unknown. Viruses have been implicated in this process, among other etiological factors, such as genetic predisposition and cytokine activity. Several ways have been proposed by which a viral infection might break tolerance to self and trigger an autoreactive cascade that ultimately leads to the destruction of a specific cell type or an entire organ. The process termed 'molecular mimicry' and the use of transgenic models in which viral and host genes can be manipulated to analyze their effects in causing autoimmunity have been particular focuses for research. For example, there is a transgenic murine model of virus-induced autoimmune disease, in which a known viral gene is selectively expressed as a self-antigen in β cells of the pancreas. In these mice, insulin-dependent diabetes develops after either a viral infection, the release of a cytokine such as IFN-γ, or the expression of the costimulatory molecule B7.1 in the islets of Langerhans. Recent studies using this model have contributed to the understanding of the pathogenesis of virus-induced autoimmune disease and have furthered the design and testing of novel immunotherapeutic approaches.

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Abbreviations

APC	antigen-presenting cell
CMV	cytomegalovirus
CTL	cytotoxic T lymphocyte
GP	glycoprotein
IDDM	insulin-dependent diabetes type II
IFN	interferon
IL	interleukin
LCMV	lymphocytic choriomeningitis virus
MBP	myelin basic protein
MS	multiple sclerosis
NP	nucleoprotein
RIP	rat insulin promoter
TCR	T cell receptor

Introduction

Autoimmune disorders in humans are characterized by the breaking of immunological tolerance to self-antigens [1,2]. Many autoimmune diseases involve specific target cells or organs, such as pancreatic β-cells being destroyed in insulin-dependent diabetes type 1 (IDDM) [3] or the destruction of axonal myelin sheaths in multiple sclerosis (MS) [4,5]. Although the pathological picture has been well documented, knowledge of the precise etiology for triggering the autoimmune process, the mechanisms of pathogenesis and the initial self-antigen(s) involved is still vague. In many instances, autoantibodies or autoreactive T cells are found to precede the onset of clinical disease by several years. For example, antibodies to glutamate decarboxylase or other islet cell proteins are detectable in prediabetic individuals [6]. This opens the possibility of detecting early 'self'-antigens that may be involved in the autoimmune process and of designing specific therapeutic approaches to prevent development of disease even after the destructive process has started [1]. Two new strategies, oral tolerance induction and the use of MHC class I restricted peptides to modulate the autoreactive immune response, have shown promise in this regard [7–9].

Viruses and other infectious agents have been implicated in autoimmunity on the basis of several findings. First, although most autoimmune diseases are associated with one or multiple host genes [2,10], environmental factors also play a role [11,12]. Support for this concept comes from studies performed in monozygotic twins. For example, although studies link several haplotypes of the MHC with an increased risk for either IDDM or MS [3,4], the incidence of MS and IDDM has been shown to vary in monozygotic twins [4,13,14] and is also dependent on geographic location and lifestyle [1,15]. Hence, autoimmune diseases have a multifactorial etiology. Second, autoimmune responses and autoimmune diseases are newly generated or enhanced by infection with a variety of human DNA and RNA viruses in humans and experimental animals [16–19]. Antibody to DNA, to RNA, and to red blood cells can be elicited (New Zealand white [NZW] mice) or levels enormously enhanced (New Zealand black [NZB] × [NZB × NZW] F1 mice) by persistent infection with a DNA virus such as polyomavirus or with an RNA virus such as lymphocytic choriomeningitis virus (LCMV) [19]. Superantigens have also been implicated in triggering autoimmune responses [20].

One potential mechanism whereby viruses could cause autoimmunity is 'molecular mimicry' [19,21•,22]. Viruses, as well as microbial agents, may possess protein structures or shapes that mimic normal host self-proteins. An immune response elicited against the pathogen will eliminate it, but can also cross-react with one or more self-antigens that share determinants with the agent. The immunopathological process could continue chronically or be reinitiated by multiple viral infections. Hence, disease could continue after the triggering agent has been eliminated (i.e. so its presence is no longer detectable); a 'hit and run' phenomenon. Evidence for such cross-reactive immune

responses between viruses/microbes and self-antigens has been noted by many investigators [19,21•,22–24,25••,26]. The influence of host genetic factors in the susceptibility to autoimmune diseases is taken into account as well in molecular mimicry, since host genes control immune responses to various infectious agents, play a role in the expression of viral receptors, and can influence viral replication.

A major difficulty in establishing a correlation between triggering events such as a viral infection and the actual autoimmune disease is the usually long lag period that often precedes the clinical onset of disease. This makes recovery of virus or microbes difficult later on when autoimmune disease has developed. To circumvent part of this difficulty and allow a detailed analysis of the kinetics of a developing disease, transgenic technology can be employed to place a viral gene into specific cells of interest (β cells, oligodendrocytes, etc.) using a tissue-specific promoter. The foreign (viral or microbial) gene is integrated into the host gene, passed on to progeny, and in essence becomes a self-antigen. This allows critical assessment of the immune response and cellular factors, that is, cytokines, that play a role in breaking tolerance to the 'self-marker protein' and thereby cause autoimmune disease. In addition, such models serve as useful tools for developing and testing novel therapeutic approaches and for critically evaluating the role of other self-antigens in the autoimmune process. This review provides recent data gathered *in vitro* and *in vivo* illustrating how viruses can trigger autoimmune diseases. Concepts for understanding the pathogenesis of virus-induced autoimmune diseases as well as novel immunotherapeutic approaches will be discussed.

How viruses can influence autoimmune responses and diseases

The induction and development of autoimmune processes involves the breaking of tolerance (unresponsiveness) to self-antigens that are present in the periphery. Although the vast majority of T cells bearing TCRs with high affinities for self-antigens are eliminated during their development in the thymus (through a process termed negative selection [27]), naive/resting precursors of potentially autoreactive cells with lower-affinity TCRs can escape this selection process and travel to the periphery [28–33]. These cells are not activated under normal conditions. Viruses, during the course of infection, induce the release of multiple cytokines such as IFN-γ, which induces elevated levels of MHC class I and class II expression. Tough, Borrow and Sprent [34••] have shown that memory T cell proliferation can be initiated through the release of type I interferons (IFN-α and -β). Viruses can also polyclonally activate lymphocytes and, if these include potentially autoreactive lymphocytes, such effector cells can then (cross)-react with potential self-antigens [35]. Along this line, infection with one virus activates memory T lymphocytes that have been generated in

response to other, earlier (different) viral infections [36]. Certain populations of these cross-activated cells may be potentially autoreactive and able to induce autoimmune diseases.

There are multiple examples of either antibodies or T cells, or both, cross-reactive with self-antigens in individuals with autoimmune diseases (reviewed in [19,21•,22]). For example, Dan Kaufman and his colleagues [37] provided evidence for an association of Coxsackie B4 virus (P2-C protein) and glutamate decarboxylase-65 reactivity in the onset of autoimmune diabetes mellitus in humans. Recently, examples of cross-reactivities or abnormal immune responses between viruses and self-proteins have been described for rheumatoid arthritis [38], celiac disease [39], primary biliary cirrhosis [21•], myocarditis [40], systemic lupus erythematosus [41], and MS [42]. Among viruses implicated in autoimmunity [19,22] are Epstein–Barr virus [18], human T lymphotropic virus type I [43,44], hepatitis C virus [45], cytomegalovirus (CMV) [46], HIV [47], corona viruses [48,49], mumps, Coxsackie [16,37] and herpesvirus-6 [17]. Furthermore, epidemiological evidence has linked rubella, CMV, and Coxsackie viruses with clusters of IDDM cases.

When predicting potentially cross-reactive sequence matches for proteins and/or peptides, in addition to linear sequence homologies, conformational considerations need to be taken into account. For instance, a one amino acid substitution in a nine amino acid peptide can completely abrogate binding to the MHC or recognition by the TCR [50,51••]. Computer models and information obtained from crystal-structure analysis have facilitated these studies. In elegant studies, Wucherpfennig and Strominger [25••] evaluated the cross-reactivity of known myelin basic protein (MBP)-reactive T-cell clones derived from MS patients by using computer-predicted peptides from a variety of viruses (herpes simplex virus, Epstein–Barr virus, adenovirus, influenza A virus) and one bacterium that infects humans, *Pseudomonas aeruginosa*. Peptides were selected on the basis that their primary and secondary structures would fit into the HLA-DR MHC groove [25••,26]. The data showed that these peptides were all able to bind to and cause proliferation of the clonal MBP-reactive T cell with affinities either greater than or equivalent to that of the known MBP peptide. Thus a single TCR has the capacity to be activated by peptides from five different exogenous sources and one self-peptide.

In other studies, T cell lines established from MS patients were reactive to both MBP and a sequence from the human respiratory coronavirus 229E [49]. Additionally, molecular mimicry between human transaldolase, which in the brain is expressed selectively in oligodendrocytes, and HTLV-I/HIV-1 Gag proteins has also been described [44]. Human transaldolase stimulated proliferation of peripheral blood lymphocytes from patients with MS; in addition,

autoantibodies to human transaldolase were detected in the serum and cerebrospinal fluid of these patients. Thus, molecular mimicry between viruses and oligodendrocyte proteins may be important in the etiology of a human central nervous system autoimmune disease such as MS.

Activation of autoreactive lymphocytes by one or multiple viral infections

Ray Welsh and his colleagues [36] found that memory T cells reactive to one virus can be activated by other viruses. These investigators showed that repeated viral infections, even with different and unrelated viruses, stimulated the activation of memory lymphocytes produced in response to previous viral infections. This work suggests that memory lymphocytes may be activated more easily, express a different TCR repertoire than naive lymphocytes or have a lower-affinity TCR, thereby facilitating cross-activation. An example is a virus-induced autoimmune oligodendrocyte disease recently established by Evans *et al.* (CF Evans, MS Horwitz, MV Hobbs, MBA Oldstone, unpublished data). In this model, the nucleoprotein (NP) or glycoprotein (GP) of LCMV was expressed in oligodendrocytes using the MBP promoter. When replicating LCMV was given parenterally, LCMV-specific T lymphocytes (predominantly CD8+ but also CD4+) were activated in the periphery, crossed the blood–brain barrier, entered the central nervous system, and caused disease. As in to the findings by Welsh and co-workers [36], central nervous system disease was enhanced when LCMV and Pichinde or vaccinia viruses were administered sequentially in intervals of six weeks to several months,

Figure 1
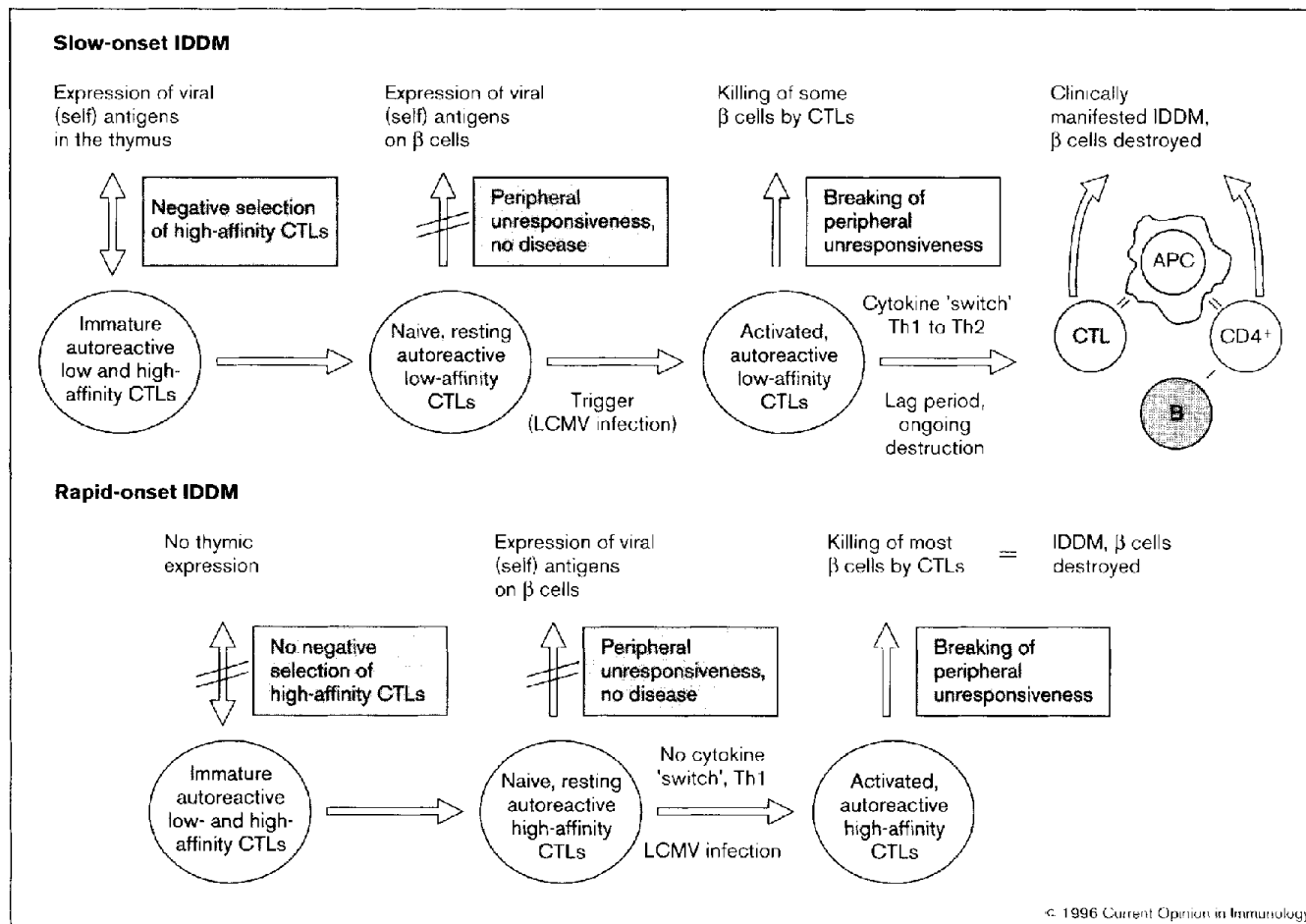
Induction of slow-onset and rapid-onset IDDM by LCMV in RIP–LCMV transgenic mice. Slow-onset IDDM occurs in mouse lines with thymic expression of the viral (self) antigen and is dependent on CD4+ and CD8+ lymphocytes. Transgenic mice with slow-onset IDDM develop islet infiltration as early as seven days after LCMV infection. The islet-infiltrating lymphocytes are predominantly (>95%) found around the islets, not inside the islets. This peri-insulitis persists for up to six months in RIP–NP H-2b mice, and lymphocytes found around the islets produce more Th2 than Th1 cytokines. Approximately six months post LCMV infection, the cytokine profile 'switches' rather suddenly from Th2 to Th1, associated with infiltration into the islets and development of IDDM one to two weeks after this switch has occurred. Rapid-onset IDDM occurs in lines without such thymic expression and depends on CD8 CTLs.

whereas the inoculation of Pichinde or vaccinia viruses in the absence of LCMV challenge did not cause disease.

Transgenic models for the study of virus-induced autoimmunity

A number of transgenic animal models have been created to study the pathogenesis of autoimmune diseases. In some models, the influence of environmental factors or pathogens was discovered serendipitously; the incidence of spontaneous autoimmune disease increased when transgenic mice were housed in quarters not free of specific pathogens, in the presence of naturally occurring bacteria and/or viral flora. For example, Hammer, Taurog and colleagues [52] generated HLA-B27 transgenic rats that spontaneously developed an autoimmune syndrome resembling ankylosing spondylitis. When the rats were transferred into germ-free conditions, most signs of the disease disappeared. Similarly, with the TCR-transgenic model for experimental allergic encephalomyelitis that resembles MS (developed by Goverman and colleagues [53]), the incidence of experimental autoimmune encephalomyelitis increased in pathogen-containing rooms. At present, for neither model is the precipitating agent(s) or event(s) known. Other laboratories have specifically generated transgenic models to study the role of infection in autoimmune diseases [32,33,54•]. Of these, models that use the rat insulin promoter (RIP) to express LCMV proteins in β-cells of the islets of Langerhans have proven to be very informative. Such transgenic mice were engineered independently by our laboratory [32] and by that of Hengartner and Zinkernagel [33]. In neither case did the transgenic mice develop IDDM or any other immunopathology of the β-cell islets unless infected with LCMV, after which more than 90% of the mice developed IDDM.

The RIP–LCMV transgenic model comes in two 'flavors'. In the first, the viral transgene is expressed only in the β-cells of the islets of Langerhans. IDDM following LCMV challenge develops rapidly and nearly all β-cells are destroyed within two weeks [33,55]. Anti-LCMV (self) CD8+ lymphocytes are mandatory for generating IDDM. Depleting CD8+ lymphocytes with antibodies or crossing these rapid-onset IDDM mice on backgrounds lacking the CD8 or β2-microglobulin genes results in the abrogation of IDDM [55]. This indicates that β-cells in the islets can be destroyed by cytotoxic T lymphocytes (CTLs), either directly or indirectly. To determine whether direct killing of β-cells by CTLs is dependent on the perforin pathway, IDDM in RIP–LCMV transgenic mice was studied in mice in which the perforin gene had been knocked out [56]. In the absence of a functional perforin gene, IDDM did not occur. Other factors, however, such as cytokines, are also involved in the process in which perforin and CD8+ cells are necessary for β-cell destruction. For example, when perforin-competent CD8+ CTLs are present in RIP–LCMV mice, no β-cell destruction occurs in the absence of IFN-γ, indicating that the destruction of

β-cells is a multifactorial process (our unpublished data). In addition to perforin-mediated killing, lysis has been observed in the perforin-independent Fas pathway. No other direct killing pathways are known, but indirect 'bystander' death of β-cells through the secretion of cytokines and other factors is worthy of future investigations.

In the second type of RIP–LCMV model, a slowly progressive injury of β-cells produces IDDM within six months of viral infection. The delay until IDDM occurs depends on the MHC background [55]. H-2^d mice develop IDDM within two months, but H-2^b mice require three to six months. In these slow-onset IDDM mice, the viral transgene is expressed in both the pancreas and the thymus but not in other tissues [55]. Because of transgene expression in the thymus, high-affinity antiself (viral) CTLs are eliminated by negative selection. The low-affinity antiself CTLs 'escape' thymic negative selection, traffic to the periphery, and are identifiable in islet infiltrates. To confirm and expand these findings, an LCMV antigen was purposely expressed as a transgene in the thymus by using a thymus-specific promoter [57]. Such transgenic mice also removed high-affinity anti-LCMV (self) CTLs and low-affinity cells passed to the periphery, supporting the proposed model of affinity dependence of thymic positive and negative selection [27,28]. The RIP–LCMV model is illustrated in Figure 1.

Depletion of CD4+ and/or CD8+ lymphocytes using monoclonal antibodies showed that, although CD4+ T cells are not required for inducing IDDM in transgenic lines with fast-onset IDDM [55], they are required in the slow-onset lines where high-affinity T cells are deleted. Hence, the low-affinity anti-LCMV (self) CD8+ CTLs need CD4+ T cells to generate IDDM, although the CD4+ cells when tested *in vitro* did not lyse LCMV-infected target cells directly. These studies show that the expression of viral or self antigens in the thymus is not sufficient to induce complete tolerance and that a slowly progressive autoimmune process can be triggered when such low-affinity CTLs that escaped negative selection become activated. CD4+ memory cells that can react with LCMV antigens might be required locally to drive the autoimmune process if autoreactive CTLs are of lower affinity. It is not known whether infiltrating lymphocytes found in insulitis lesions are mainly of the naive or of the memory phenotype and how many of them are fully activated. Other studies have shown that generation or maintenance of CD8+ memory CTLs can be impaired in the absence of CD4+ lymphocytes [58–60,61••]. Low-affinity, self-reactive CTLs demonstrated in the RIP–LCMV transgenic model [55] have also been found in other transgenic models of autoimmune disease and viral infections [31,57]. Viral infections can induce both high-affinity CTLs that are important for viral clearance and lower-affinity CTL responses. Potentially, these low-affinity CTLs can recognize different viral peptides, other viruses and several

MHC alleles; hence, such lower-affinity CTLs display less specificity than high-affinity CTLs and might be prone to more cross-reactivity with other antigens [30,35–37].

Numbers of antiviral (self) CTLs correlate with the incidence and onset of autoimmune disease [23,61••]. Following LCMV infection in RIP–LCMV transgenic mice that express the transgene only in β-cells, specific anti-LCMV CTLs are generated with a precursor frequency of approximately 1/100 during the primary burst (day 6–8 post intraperitoneal infection). This number drops to a frequency of 1/1000 by 30–40 days post intraperitoneal infection and represents LCMV-specific memory CTLs present in the host [61••,62]. When, instead of being inoculated with replicating LCMV virus, the transgenic mice are given vaccinia virus recombinants expressing the LCMV transgene, the numbers of LCMV-specific CTLs induced are 1–2 logs less [60], and IDDM does not occur. When the numbers of self-reactive CTLs increase, however, or when these cells proliferate locally in the islets of Langerhans, through the presence of the costimulatory molecule B7.1 or of cytokines such as TNF-α in β-cell islets [61••,63,64] for example, then IDDM occurs after infection with vaccinia virus–LCMV recombinants. Thus, a critical number of activated autoreactive CTLs is needed to induce IDDM [54•]. When there are insufficient numbers of CTLs, IDDM does not develop unless other factors are present in the islets, which expand the CTL numbers and/or enhance the inflammatory process [60,63,64]. This quantitative aspect in the induction of autoimmune diseases is also observed in TCR transgenic models in which either spontaneous disease is induced or the incidence of disease is enhanced when all or most T cells are engineered to bear the same self-antigen-reactive TCR [33,53,54•]. Other evidence for this concept comes from a report by Genain *et al.* [29], who showed that myelin-reactive cells isolated from the blood of normal, healthy marmosets caused encephalomyelitis when activated and amplified *in vitro* and infused back into the same animal, caused encephalomyelitis.

Breaking of peripheral tolerance and regulation of autoimmune diabetes by cytokines

Expression of LCMV transgenes or of B7.1 alone in β-cells using the RIP does not induce autoimmune diabetes [32,33,61••]. When both B7.1 and LCMV transgenes are coexpressed in β-cells, however, antiself (viral) CTLs are activated spontaneously in the absence of viral infection, and spontaneous IDDM occurs [61••]. In contrast, when the LCMV transgene is expressed both in the thymus and in the islets, and B7.1 is expressed in the islets, no activation of anti-LCMV (self) CTLs or IDDM occurs. When these mice are given a challenge of LCMV, however, rapid-onset IDDM develops within 14 days after viral challenge. In contrast, in their singly transgenic RIP–NP littermates, IDDM develops only four to five months after viral challenge. The rapid IDDM is associated with increased numbers of antiviral (self) CTLs and a predominance of IFN-γ-producing lymphocytes in the islets. In the singly transgenic RIP–NP littermates with slow-onset IDDM, fewer antiself CTLs are made, and more IL-4 and IL-10-producing T lymphocytes are found in the islets.

Immune responses are believed to be regulated by a balance of Th1 and Th2 cytokines [65]. IL-2 and IFN-γ are grouped as Th1-type cytokines and have proinflammatory effects. IL-2 is involved in the differentiation and activation of CD4+ and CD8+ T cells, natural killer cells and APCs, while IFN-γ is involved in antiviral defense mechanisms and upregulates the expression of MHC class I and II molecules. IL-4 and IL-10 are Th2 cytokines and along with TGF-β have been shown to inhibit Th1 cells *in vitro* and are thought to have an inhibitory effect on certain immune responses *in vivo*. When IFN-γ is coexpressed in the islets with the LCMV transgene, unresponsiveness to the viral transgene is broken, as is apparent when LCMV (self)-specific CTLs arise spontaneously and IDDM occurs without the usually required LCMV infection [66•]. In contrast, when the IFN-γ gene is knocked out, such RIP–LCMV mice do not develop autoimmune diabetes and MHC molecules are not upregulated in their islets following LCMV infection (MG von Herrath, MBA Oldstone, unpublished data). When IL-2 is coexpressed with the LCMV transgene in β cells, neither the spontaneous generation of anti-LCMV (self) CTLs nor IDDM follows, despite spontaneous infiltration by CD4+ cells and upregulation of MHC class I on islet cells. The kinetics of IDDM quickens and the severity of disease increases, however, when RIP–LCMV×RIP–IL-2 mice are challenged with LCMV [67]. Yet, when transforming growth factor-β or IL-10 is coexpressed with LCMV antigens in β-cells, unexpectedly no variation in the incidence of IDDM is observed [68]. A common theme emerges from these studies. Mice that do not develop IDDM have more IL-4-producing lymphocytes in their islets, whereas those developing IDDM have more IFN-γ- and fewer IL-4-producing cells. Preliminary studies in collaboration with Nora Sarvetnick indicate that RIP–LCMV transgenic mice that also express IL-4 in their β-cells do not develop IDDM after viral challenge. Recently, expression of IL-4 in islets of nonobese diabetic mice has been shown to prevent IDDM [69•]. These findings not only suggest a major immune regulatory role for these cytokines in IDDM, but also show an interesting correlation between destructive or nondestructive autoimmune infiltration in relationship to the local cytokine milieu. A role for other cytokines, such as IL-12, remains undetermined at this point.

Therapeutic approaches for virus-induced autoimmune diseases

Antigen-specific therapy that tolerizes antiself T cells and thereby eliminates the driving force of T-cell-mediated autoimmune diseases can be achieved by two different

mechanisms. First, peripheral tolerance can be induced by clonal deletion, anergy induction or exhaustion of autoreactive T cells through the presence of large amounts of self-antigens in the periphery and/or through the lack of costimulation when antigen is presented. Experimental treatment has been designed based on these observations [9]. Aichele *et al.* [7] successfully prevented IDDM in RIP–LCMV-GP transgenic mice by immunizing them with a specific LCMV-GP peptide. This treatment resulted in the tolerizing of LCMV GP-specific CD8+ CTLs. A second mechanism employs the approach of 'bystander suppression' or immune deviation [8]. Here, tolerance is initiated by immunization with an antigen that induces cells that can mediate suppression by secreting suppressive cytokines such as transforming growth factor-β and IL-4 [8]. The idea is to turn on a Th2-type profile response in the target area. Th2 immunosuppressive cytokines are made primarily by CD4+ lymphocytes, but may also be produced by CD8+ cells and γ/δ T lymphocytes. Low doses of orally administered antigen are believed to favor active suppression through the activation of Th2 lymphocytes, whereas higher doses favor clonal anergy of autoreactive inflammatory Th1 lymphocytes, for example, lymphocytes reacting with glutamate decarboxylase in IDDM [70]. By this means, it is possible to treat an organ-specific disease (e.g. IDDM [β-cells in the pancreatic islets]) even when the initiating autoantigen is not known. Perhaps antigen-specific regulatory T cells are activated in Peyer's patches of the gut, home to the specific tissues where the oral antigen 'resides' naturally (e.g. insulin in β-cells) and is presented by APCs, if inflammation is ongoing. As a consequence, their local secretion of immunosuppressive cytokines suppresses the autoimmune response and disease. Despite the controversy over whether this therapy works by active suppression [8] (the generation of transforming growth factor-β and IL-4), or by clonal anergy or 'exhaustion' [71] of self-reactive T lymphocytes, treatment has proven effective for a variety of T-cell-mediated experimental autoimmune diseases [8]. Interestingly, oral insulin therapy also prevents virus-induced IDDM in RIP–LCMV transgenic mice [72]. This therapy is successful when begun before the initiation of IDDM, but is also effective when administered during the course of diabetes. The therapy only succeeds in the model, in which the viral transgene is expressed on both β-cells and in the thymus, however, and, hence, works only against lower-affinity antiself (viral) effector T cells [55]. The therapy is not effective when the transgene is expressed only on β-cells and when IDDM is caused by high-affinity effector T lymphocytes [72].

Finally, since viruses can interfere at different levels in the antigen-presentation pathway, expression of such genes in the area targeted for autoimmune activity can be expected to block autoimmune disease. The adenoviral E3 complex genes interfere with transport of MHC class I molecules to the cell surface. Coexpression of E3

complex genes, specifically gp19, in β-cells of the islets of Langerhans (RIP–E3 transgenic mice) together witfh LCMV proteins using the RIP prevents virus-induced IDDM (MG von Herrath, S Efrat, MBA Oldstone, MS Horwitz, unpublished data). Herpes simplex virus ICP47 (infected cell protein) proteins and CMV US (unique short) 2 and US11 proteins [73] both prevent antigen presentation *in vitro*, although at present there is no proof that these viral proteins interfere with antigen presentation *in vivo*.

Conclusions

Based on the transgenic models, a likely scenario for the sequence of events occurring in virus-induced autoimmune disease is that, first, peripheral unresponsiveness is broken through the activation of cross-reactive (probably low-affinity) T lymphocytes. These cells then home to the respective target organ (for example, the β-cells in IDDM or oligodendrocytes in MS), where the self-antigen is recognized. In response, the activated lymphocytes release cytokines and possibly other mediators and then, for example, CD8+ CTLs begin to kill target cells bearing the respective MHC class I peptide complex. Depending on the precursor frequencies of the cells initially triggering the autoimmune process, the local cytokine milieu, and the MHC background genes involved, autoimmune disease can occur either fast, slow, or not at all. Damage is probably mediated either indirectly through the release of cytokines or directly through CTL-mediated lysis of the target cells. Once damage has occurred, a chain reaction is set into place, during which more potential self-antigens may be released, presented by professional APCs, and the autoimmune response is extended to other self-antigens in the target site. This sequence of events would explain the occurrence of autoantibodies that precede the clinical onset of disease, such as islet-cell antibodies found in IDDM. If cross-reactivity between a virus 'A' and a self-antigen 'A' is the initial triggering event, subsequent infections with different viruses 'B' through 'X' (which do not necessarily have to cross-react with self-antigen 'A'), could activate and expand virus-'A'-specific memory T (i.e. CD8+) lymphocytes, thus enhancing disease severity ([36]; CF Evans, MS Horwitz, MV Hobbs, MBA Oldstone, unpublished data). This scenario would explain the epidemiological findings that associate autoimmune diseases with multiple viral infections and may also explain why, in a disease like MS, the cerebrospinal fluid contains elevated gamma-globulin levels (antibodies) specific for multiple viruses. Specific antiviral therapies that inhibit viral replication and spread or tolerize the antiviral immune response in model systems may eventually provide a guide for curing autoimmune diseases. If viruses or microbial agents are a major contributing factor to human autoimmune diseases, as several studies reviewed here suggest, and if these agents can be identified, then one would expect a reduction in the incidence of autoimmune diseases when more effective and broadly applicable antiviral therapies become available.

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- of outstanding interest

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