REVIEW

Viruses and autoimmunity: an affair but not a marriage contract

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SUMMARY

Viruses are considered as causative agents and contributors to lesion expression in autoimmune disease, notions best supported by studies in animal model systems. This review discusses relationships between virus infection and autoimmunity focusing on mechanisms by which they could induce autoreactivity. The popular idea of molecular mimicry is viewed skeptically with the reviewers taking the viewpoint that viruses contribute to autoimmunity mainly by inducing several nonspecific inflammatory events that together are sufficient to trigger autoreactivity in genetically receptive hosts. Copyright © 2002 John Wiley & Sons, Ltd.

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BACKGROUND

Autoimmune diseases (AID) affect 3%–5% of the population and represent a significant cause of ill health and shortened life expectancy. Many named diseases are recognised with most having a highly varied clinical presentation indicating the likely involvement of numerous factors in lesion pathogenesis. To be accepted as an autoimmune disease, tissue damaging lesions or metabolic dysfunctions must be the consequence of either an antibody or T cell orchestrated response to one or more host components. In some diseases the spectrum of autoreactivity is focused and a single cell type or organ may be involved. A good example is insulin-dependent diabetes mellitus. In others, such as systemic lupus erythematosus (SLE), multiple self components are targets of autoreactivity and these may increase with time. The pathogenesis and control of autoimmune disease are far better understood than is their aetiology.

Autoimmune diseases do not occur under normal circumstances since lymphocytes with

high affinity receptors for self antigens are removed from the immune repertoire during central and peripheral tolerance induction. However, in reality, potentially autoreactive lymphocytes are present in normal individuals, but usually these cells are either not activated or are tolerised. Furthermore, it has become apparent that lymphocyte receptors, particularly on T cells, may be highly degenerate in their recognition specificity [1,2]. Accordingly, a single receptor may react with multiple epitopes, many of which may not be closely related in primary amino acid sequence with each other. Indeed, by some estimates, a single T cell receptor may react with up to 10^6 different ligands [1,2]. Such degeneracy means that a T cell clone expanded following antigen triggering could react with numerous other epitopes including self peptides presented by self MHC. Given this circumstance, a central question arises: why doesn't autoimmunity occur all of the time? In fact it does, but tissue damaging autoreactivity (i.e. autoimmune disease), is a relative rarity requiring a complex series of events to occur.

This complex induction process involves multiple factors. In humans, as well as animal models, there is a strong genetic susceptibility component that always involves multiple genes. In humans at least ten genes have been implied to influence susceptibility with genes of the MHC class II complex usually playing the major role [3]. Nevertheless, with AID genetics alone never accounts

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Abbreviations used

AID, autoimmune disease; APC, antigen presenting cell; EAE, experimental allergic encephalomyelitis; HSK, herpetic stromal keratitis; IDDM, insulin dependent diabetes mellitus; LCMV, lymphocytic choriomeningitis virus; MM, molecular mimicry; MS, multiple sclerosis; SLE, systemic lupus erythematosus; TCR, T-cell receptor; TMEV, Theiler's murine encephalomyelitis virus

for disease induction or expression. For instance, an autoimmune disease of the same type never occurs in monozygous siblings more frequently than 50% and this frequency is lower if such siblings are raised in different environments [4,5]. Accordingly, AID occurs in genetically susceptible individuals but many other factors must also be involved. These nongenetic factors are assumed to include environmental components, dietary constituents and infectious agents, particularly viruses. Viruses, in fact, have been implicated as initiators, perpetuators and most recently as terminators of AID [6–10].

However, the affair between viruses and AID has never matured into a legal contract. Currently, save possibly post-measles encephalitis no human AID can be proven as the direct consequence of infection with a known virus. Furthermore, if viruses are involved in the aetiology of AID, the agents involved are likely to be removed or contained by the immune system long before the disease becomes clinically recognised. Hence, any affair between viruses and autoimmunity is unlikely to be 'till death do us part', a scenario that makes the task of incriminating any virus in the aetiology of an autoimmune disease daunting.

The best evidence available linking virus infection to human autoimmune disease is only circumstantial. The most studied situation is with the common autoimmune disease multiple sclerosis (MS). With MS, for example, lesion induction and recurrence has been associated

with many named virus infections [10]. Of the more than ten candidates, the current favourite is HHV6 [11]. However, like all previous candidates, this agent's causative role has not been substantiated or confirmed. Most likely, as further discussed subsequently, many viruses could contribute to MS pathogenesis via their induction of an inflammatory reaction especially if this occurs directly in the CNS.

Insulin dependent diabetes mellitus (IDDM) is the other AID for which viruses are strong suspects as aetiologic agents. Coming closest to proving Koch's postulates was an isolate of Coxsackie B virus from the pancreas of a patient with IDDM that was subsequently shown to induce diabetes upon experimental infection of mice [12]. Moreover, some epidemiological evidence shows that Coxsackie B infection is common in people who eventually develop IDDM [13], but the case for Coxsackie B virus as a cause of human IDDM is weak.

Rubella virus may be a more likely candidate. Thus in former prevaccination times, when rubella was more prevalent, up to 12% persons who had clinical rubella as an infant went on to develop some form of diabetes [14]. Other viruses linked to human AID include EBV with SLE [15] and HCV with various rheumatoid diseases [16]. Table 1 summarises some associations of viruses and human AID. The most convincing evidence that links viruses with autoimmunity comes from observations on animal model systems. Several

Table 1. Natural virus infections of humans associated with autoimmunity (compiled from [6] and [10])

Disease	Viruses
Multiple sclerosis	Measles, simian virus 5, HSV, parainfluenza 1, canine distemper virus, coronavirus, HTLV1, human herpesvirus 6, MS associated retrovirus, EBV
Insulin dependent diabetes mellitus	Rubella, Coxsackie B4, CMV, mumps
Myocarditis	Coxsackie B3, adenovirus, HCV
Systemic lupus erythematosus	EBV
Herpes stromal keratitis	HSV
Myasthenia gravis	HCV
Various rheumatoid diseases	HCV
Guillain-Barre syndrome	CMV, EBV

models exist most of which deal with experimental allergic encephalomyelitis (EAE) or IDDM. An excellent comprehensive review of this topic was recently published [6].

MECHANISMS BY WHICH VIRUSES CAUSE AUTOIMMUNITY

Numerous mechanisms have been advocated to explain how virus infections could induce and cause expression of AID (Table 2). These fall basically into two categories. (A) They are the consequence of a specific recognition event set off by cross-reactivity between the infecting virus and host autoreactive T cells. This concept is usually referred to as molecular mimicry (MM), and is the most popular idea to explain how viruses induce autoimmunity. (B) They are the consequence of nonspecific inflammatory events set off by viral infections that usually, but not invariably, infect the target organ. This general mechanism has several subcategories. These include unveiling of host autoantigens, bystander activation of autoreactive T cells by inflammatory cytokines, adjuvant effects of virus on antigen presenting cells, superantigen activation of autoreactive T cells and pattern receptor stimulation of autoreactive T cells. These models can all be supported by experiments in various animal models, many of which are highly contrived and express viral antigens, cytokines or costimulator molecules as transgenes or use infecting viruses that express autoantigens as recombinant proteins.

For natural AID, especially with human disease, we still do not understand how (or even if) viruses initiate autoimmunity.

The concept of molecular mimicry was conceived 20 years or so ago when it was recognised that certain autoantibodies could additionally react with some viral antigens [17]. Subsequently,

Table 2. Postulated mechanisms of virus induced autoimmune disease

Molecular mimicry
Non-specific inflammation
Unveiling host autoantigens
Activating autoreactive T-cells
Superantigen production
Pattern receptor stimulation
Adjuvant effects

the emphasis shifted to T cell mediated immunity since autoreactive T cells appear more often involved in experimental autoimmunities. Moreover, T cell receptor recognition shows far more degeneracy than does Ig recognition [1,2]. Several experimental approaches make a powerful argument for MM and the topic has received frequent reviews by its advocates [7,8,18]. However, as sanguinely reviewed recently by Benoist and Mathis [19], the case for MM is compelling but as yet by no means conclusive. We note in passing that the MM hypothesis makes such good sense that it should be correct!

Some of the most convincing evidence supporting the MM hypothesis comes from studies in transgenic mouse models of diabetes or encephalitis in which a viral protein is engineered to be a surrogate self antigen [20,21,22]. Most studied are models using proteins from lymphocytic choriomeningitis virus (LCMV), expressed in the pancreatic islet cells using a rat insulin promoter. Such animals fail to spontaneously develop diabetes although they readily do so if subsequently infected with LCMV [21,22]. LCMV fails to replicate in the islets of normal mice, so direct viral damage to islets should not complicate issues. Most interpret the model to indicate that the virus infection breaks tolerance and triggers the induction of activated antiviral (but now anti-surrogate self) CD8⁺ T cells which migrate to the islets and destroy β cells that express viral (self) antigens.

Alternative ways of achieving the same effect are to additionally express a costimulator on islet cells such as B7.1 or to damage the cells with toxic drugs [23]. These observations are not consistent with MM. Furthermore, although the transgene model is taken to support MM, in reality the antigens involved are facsimiles rather than mimics. Moreover, IDDM seems to be rather easy to trigger since the transgene expression of certain cytokines in β cells can also result in IDDM [24,25]. It will be of interest to unravel the respective roles of specific and nonspecific inflammatory effects on the islets in the transgenic models.

Another experimental model that strongly supports MM was recently developed by the Miller laboratory [26]. This model uses a nonpathogenic variant of the Theiler's picornavirus (TMEV) which, when injected intracerebrally into mice,

fails to cause an EAE-like syndrome. However, if the virus is engineered to express PLP 139-151, a peptide autoantigen derived from myelin and involved in EAE, then acute disease does occur. Even more interesting was the observation that a molecular mimic of PLP 139-151 derived from the bacterium *Haemophilus influenzae* also caused EAE when expressed by the virus [26]. These lesions were judged to represent autoimmunity, although in reality were the result of infection by a virus that continued to replicate in the CNS. Thus it is still not possible to discount nonspecific inflammatory effects of the virus in lesion pathogenesis. In fact, peptide expression in TMEV appears to have a potent adjuvant effect. Thus immunisation with the PLP 139-151 requires strong adjuvants to induce EAE and immunisation with the *Haemophilus influenzae* peptide fails to cause such lesions [6]. Potent adjuvant effects of infectious agents even outside of the target organ for AID can often result in autoimmunity [27]. Thus in a model recently described by the Fujinami group, animals were primed with viruses that expressed autoantigens from the CNS (so called primed for MM). Subsequent challenge after several weeks with nonspecific stimuli such as Freund's adjuvant or viruses that failed to express the relevant peptide and replicated outside the target organ could induce inflammatory effects in the CNS. Although the model was presented as evidence for MM, the triggering effect was in fact entirely nonantigen specific and not in support of MM.

Natural infection models have also been described which lend support to the MM hypothesis. The one considered as the most compelling by several writers is herpetic stromal keratitis (HSK), a lesion of the mouse cornea that occurs as a sequel to infection HSV [18,19,28]. HSV is of course a major pathogen of mankind infecting around 80% of the population and causing HSK lesions in around 500 000 persons in the USA [29]. Mice show major strain differences in susceptibility to HSV infection, an effect attributable to multiple genes and still poorly understood. In the model described by the Cantor group, the basis of resistance and susceptibility to HSK was shown to depend on alleles expressed at the IgH locus [30]. Strains possessing the IgH^a/d/e alleles were susceptible whereas others, most notably IgH^b, were resistant. Resistance appeared to be explained by tolerance to the IgH^b gene product,

i.e. the IgG2a^b isotype of Ig. Thus tolerisation of IgH^b mice to IgG2a^b Ig or to a peptide derived from this protein (292-308), which was taken to represent the target autoantigen expressed in the cornea during HSK [30], resulted in resistance. More interestingly a search of the gene bank revealed a peptide in the UL6 protein of HSV that was a potential molecular mimic of the G2a 292-308 peptide [30]. Hence the idea arose that HSK in susceptible strains was the consequence of the UL6 peptide breaking tolerance and inducing autoreactive T cells responsible for orchestrating HSK.

Several other elegant experiments were presented in further support of this model, but most provocative were those showing that the mutant viruses lacking expression of the UL6 peptide, but not other proteins, were minimally effective at inducing HSK in a reconstitution model [28,30].

Moreover, a transgenic mouse was developed that expressed the T-cell receptor (TCR) for the UL6 peptide as a transgene [31]. In such mice, wild type HSV was a potent inducer of HSK, but in contrast a virus with a single amino acid change that resulted in the UL6 peptide being unable to bind to the restricting MHC molecule, was without virulence [31]. These experiments together make a powerful argument for MM.

Nevertheless, the marriage has not been consummated, and many doubts remain. Objections include the fact that replicating virus is always required to induce HSK and lesions cannot be elicited by expressing the MM peptide in the eye [32]. The UL6 peptide itself is not an immunodominant epitope and in the hands of another group is not recognised at least by BALB/c mice (also IgH^d) following infection with HSV [32]. Furthermore, immunisation with the UL6 peptide, induces a response to itself but this neither cross-reacts with HSV nor to the G2a 292-308 peptide [32]. Other aspects of the MM hypothesis for HSK also could not be confirmed [32]. Thus the Cantor model for MM and autoimmunity, just like others cannot be taken as gold standard proof of MM. Indeed, in the case of HSK, it is highly debatable if the lesion is solely or even principally an autoimmune lesion [33]. Other explanations abound including the logical idea that the ocular inflammation represents an immunopathological response to viral antigens [33].

IF NOT MOLECULAR MIMICRY, THEN HOW DO VIRUSES INDUCE AUTOIMMUNITY?

Assuming viruses can incite autoimmunity, then one or more mechanisms must be occurring if MM is not the cause. Unfortunately, most models that implicate nonspecific mechanisms as responsible for AID are often imprecise and usually confounded by the likelihood that multiple nonspecific activation events could be ongoing. Balanced reviews on the mechanisms by which viruses might induce AID other than by MM have been published [10,34]. One model which persuasively argues for AID induction other than by mimicry involves Theiler's virus induced encephalomyelitis of mice [35]. In this model, intracerebral injection of virus results initially in the induction of inflammatory lesions that appear to represent a CD4+ T cell mediated immunopathological reaction against expressing cells viral antigen. However, the resultant damage (direct or perhaps bystander) to tissues releases myelin components which somehow induce a progressive wave of autoreactive T cell responses against myelin antigens. Characteristically, the first target autoantigen is PLP 131-159 and a much later one MOG 92-106 [35]. This progressive event is referred to as epitope spreading. A variety of events could be involved in epitope spreading. These include release of sequestered antigens, enhanced antigen presenting cell (APC) activity and the bystander activation effects of cytokines such as gamma interferon and IL-12 [6]. As a consequence of epitope spreading, late lesions resulting from TMEV infection appear to be the consequence of autoreactivity rather than virus induced demyelinating disease.

Another well studied model of autoimmunity that involves non-antigen specific mechanisms is Coxsackie B virus induced IDDM in a transgenic mouse with a diabetogenic TCR transgene [34].

In this model, bystander activation which likely involved non TCR mediating activation of autoreactive cells by inflammatory cytokines, was considered to be the primary mechanism occurring in autoreactive lesions [34]. This non-specific effect occurs as a consequence of virus affecting the exocrine part of the pancreas. The inflammatory cytokines generated activate beta cell autoreactive cells already in the endocrine pancreas (or migrants from the draining lymph node) which

destroy beta cells. A similar bystander mechanism has been suggested to explain how HSV infection of the eye induces lesions of HSK [36]. Other more exotic means by which viruses could cause, or exacerbate, autoimmunity include superantigen activation of autoreactive T cells [37] and viral induced transformation of autoantigen producing B cells. The latter mechanism has been suggested as a possible means by which EBV could induce SLE [15] and HCV could induce mixed cryoglobulinaemia [38].

WHERE DO WE STAND?

It is probably a safe bet that virus infections can act as triggering factors in the aetiology of some autoimmune diseases. Equally likely is the fact that virus infection alone is not the principal cause of any AID in an outbred animal and that when viruses are involved multiple pathways of immune activation operate. We also imagine that pathways that involve nonantigen specific activation of already existent autoreactive T cells are more common events than is induction by MM. However, the relative importance of MM and nonspecific activation could vary according to the frequency of autoreactive T cells. Thus at low frequency MM could be important, but irrelevant if a high frequency of autoreactive T cells are present. Assuming that viruses that express molecular mimics are as rare as white elephants, persons exposed to others with autoimmunity, or incubating disease, need not be fearful of contracting a MM virus that will set off disease in themselves even if they are closely related to the patient. On the downside, however, we may never have a useful prophylactic viral based vaccine to protect against AID. Research needs to continue to further define mechanisms by which viruses participate in the cause, progression and even termination of AID. The best outcome might be to discover pathways of lesion expression that can be successfully neutralised by smart immunotherapies that include strategically designed recombinant viruses. Perhaps a binding contract between viruses and autoimmunity will one day be written. We hope to be around to witness the ceremony.

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REFERENCES

1. Wilson DB, Pinilla C, Wilson DH, Houghton RA. Immunogenicity. I. Use of peptide libraries to identify epitopes that activate clonotypic CD4+ T cells and induce T cell responses to native peptide ligands. *J Immunol* 1999; **163**: 6424–6434.
2. Mason D. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol Today* 1998; **19**: 395–404.
3. Vyse TJ, Todd JA. Genetic analysis of autoimmune disease. *Cell* 1996; **85**: 311–318.
4. Kumar D, *et al.* North American twins with IDDM. Genetic etiological and clinical significance of disease concordance according to age, zygosity and the interval after diagnosis in first twins. *Diabetes* 1993; **42**: 1351–1363.
5. McFarland HF. Twin studies and multiple sclerosis. *Ann Neurol* 1992; **32**: 722–723.
6. Olson JK, Croxford JL, Miller SD. Virus-induced autoimmunity: potential role of viruses in initiation, perpetuation and progression of T-cell mediated autoimmune disease. *Viral Immunol* 2001; **14**: 227–250.
7. Oldstone MBA. Molecular mimicry and immune mediated disease. *FASEB J* 1998; **12**: 1255–1265.
8. Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest* 2001; **108**: 1097–1104.
9. Fujinami RS. Viruses and autoimmune disease – two sides to a coin. *Trends Microbiol* 2001; **9**: 377–381.
10. Whittton JL, Fujinami RS. Viruses as triggers of autoimmunity: facts and fantasies. *Curr Opin Microbiol* 1999; **2**: 392–397.
11. Soldan SS, *et al.* Association of human herpes virus 6 (HHV 6) with multiple sclerosis: increased IgM response to HHV 6 early antigen and detection of serum HHV 6 DNA. *Nat Med* 1997; **3**: 1394–1397.
12. Yoon JW, Onodera T, Notkins AL. Virus induced beta cell damage and insulin dependent hyperglycaemia in mice infected with Coxsackie virus B4. *J Exp Med* 1978; **148**: 1068–1080.
13. Clements GB, Galbrath DN, Taylor KW. Coxsackie B virus and onset of childhood diabetes. *Lancet* 1995; **346**: 221–223.
14. Ginsberg-Fellner F, *et al.* Diabetes mellitus and autoimmunity in patients with congenital rubella syndrome. *Rev Infect Dis* 1985; **7** Suppl: S170–S176.
15. James JA, Harley JB. B-cell epitope spreading in autoimmunity. *Immunol Res* 1998; **164**: 185–200.
16. Romero PM, *et al.* Rheumatologic and autoimmune manifestations in patients with chronic hepatitis C virus infection. *Rev Esp Enferm Diag* 1997; **89**: 591–598.
17. Fujinami RS, *et al.* Molecular mimicry in virus infection: cross reaction of measles virus phosphoprotein and herpes simplex virus protein with human intermediate filaments. *Proc Natl Acad Sci U S A* 1983; **80**: 2346–2350.
18. Mavesakil E, Elzen P, Sercarz EE. Self reactive T cell and degeneracy of T cell recognition: Evolving concepts – from sequence homology to shape mimicry and TCR flexibility. *Autoimmunity* 2001; **16**: 201–209.
19. Benoist C, Mathis D. Autoimmunity provoked by infection: How good is the case for T cell epitope mimicry? *Nature Immunol* 2001; **2**: 797–801.
20. Oldstone MBA, Nerenberg M, Southern P, Price J, Lewicki H. Virus infection triggers insulin dependent diabetes mellitus in a transgenic model. Role of antiself immune response. *Cell* 1991; **65**: 319–331.
21. Ohashi P, *et al.* Ablation of tolerance and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 1991; **65**: 305–317.
22. Evans CF, Horwitz MS, Hobbs MF, Oldstone MBA. Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. *J Exp Med* 1996; **184**: 2371–2384.
23. Von Herrath MG, Guerde S, Lewicki H, Flavell RA, Oldstone MBA. Coexpression of B7.1 and viral (self) transgene in pancreatic β cells can break peripheral ignorance and lead to spontaneous autoimmune diabetes. *Immunity* 1995; **3**: 727–738.
24. Campbell I, *et al.* Islet inflammation and hyperplasia induced by the pancreatic islet specific overexpression of IL-6 in transgenic mice. *Am J Pathol* 1994; **145**: 157–166.
25. Sarvetnick N, *et al.* Insulin dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon gamma. *Cell* 1998; **52**: 773–782.
26. Olson JK, *et al.* A virus-induced molecular mimicry model of multiple sclerosis. *J Clin Invest* 2001; **108**: 311–318.
27. Theil DJ, *et al.* Virus can silently prime for and trigger central nervous system autoimmune disease. *J Neurovirol* 2001; **7**: 220–227.
28. Panoutsakopoulou V, Cantor H. On the relationship between viral infection and autoimmunity. *J Autoimmunity* 2001; **16**: 341–345.
29. Dawson C, Togni B. Herpes simplex eye infections. Clinical manifestation, pathogenesis and management. *Eur Ophthalmol* 1976; **21**: 11–29.
30. Cantor H. T-cell receptor crossreactivity and autoimmune disease. *Adv Immunol* 2000; **75**: 209–233.
31. Panoutsakopoulou V. Analysis of the relationship between viral induction and autoimmune disease. *Immunity* 2001; **15**: 137–147.
32. Deshpande S, *et al.* Herpes simplex virus induced

- keratitis: evaluation of the role of molecular mimicry in lesion pathogenesis. *J Virol* 2001; **75**: 3077–3088.
33. Streilen JW, Dana MR, Ksander BR. Immunity causing blindness: five different paths to herpes stromal keratitis. *Immunol Today* 1997; **18**: 443–449.
34. Horwitz MS, Fine C, Ilic A, Sarvetnick N. Requirements of viral-mediated autoimmune diabetes: β -cell damage and immune infiltration. *J Autoimmunity* 2001 2001; **16**: 211–217.
35. Miller SD, *et al.* Persistent infection with Theiler's viruses leads to autoimmunity via epitope spreading. *Nat Med* 1997; **3**: 1133–1136.
36. Gangappa S, Babu JS, Thomas J, Daheshia M, Rouse BT. Virus induced immunoinflammatory lesions in the absence of viral antigen recognition. *J Immunol* 1998; **61**: 4289–4300.
37. Kotzin BC, Leung DY, Kappler J, Marrack P. Superantigens and their potential role in human disease. *Adv Immunol* 1993; **55**: 99–166.
38. Ferri C, Zignego AL. Relation between infection and autoimmunity in mixed cryoglobulinemia. *Curr Opin Rheumatol* 2000; **12**: 53–60.