Review

Amplification of autoimmune disease by infection

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Abstract

Reports of infection with certain chronic persistent microbes (herpesviruses or Chlamydiae) in human autoimmune diseases are consistent with the hypothesis that these microbes are reactivated in the setting of immunodeficiency and often target the site of autoimmune inflammation. New experimental animal models demonstrate the principle. A herpesvirus or *Chlamydia* species can be used to infect mice with induced transient autoimmune diseases. This results in increased disease severity and even relapse. The evidence suggests that the organisms are specifically imported to the inflammatory sites and cause further tissue destruction, especially when the host is immunosuppressed. We review the evidence for the amplification of autoimmune inflammatory disease by microbial infection, which may be a general mechanism applicable to many human diseases. We suggest that patients with autoimmune disorders receiving immunosuppressing drugs should benefit from preventive antiviral therapy.

What do herpesviruses, Chlamydiae and parvovirus B19 have in common?

The question of how infectious organisms contribute to autoimmunity has continued to be of interest to clinical rheumatologists and basic immunologists. Recent reviews have considered the possible contributions of different non-mutually exclusive mechanisms, including molecular mimicry, bystander activation, cryptic antigens, and epitope spreading [1–3]. However, current understanding, as reflected by these reviews, does not account for the skewed list of infectious organisms often quoted as being associated with various autoimmune disorders. As outlined in Table 1, certain organisms are repeatedly mentioned as being linked to different autoimmune disorders. These are human herpesviruses (HHVs), in particular the non-neurotropic herpesviruses such as Epstein–Barr virus (EBV), cytomegalovirus (CMV) and HHV6 (the group also includes HHV7 and HHV8), Chlamydiae and parvovirus B19. As these organisms are mentioned in the context of so many different diseases it is unlikely that they would have specific etiologic roles. Moreover, there is a large, controversial and often contradictory literature on these associations, which suggests that pathogenic mechanisms might be redundant and non-specific. New data demonstrate a role for such microbes in augmenting disease expression in several experimental mouse models [4–6].

One approach is to examine relevant similarities between Chlamydiae, parvovirus B19 and non-neurotropic herpesviruses. Although, superficially, they have nothing in common, they may share cell tropisms (Table 2) in that they have a predilection for hematopoietic cells and endothelial cells. The ability of these organisms to 'hitch a ride' and get around in hematopoietic cells might actually serve a vital function. For instance, the infectious life-cycle of herpesviruses includes three functions for infected host cells: first, initial viral replication; second, a long-term latency reservoir; and third, the production of infectious virus at a convenient mucosal or skin site. The initial host cell for productive lytic infection, for example with EBV, may be an oral mucosa epithelial cell [7], but it is quickly replaced by the major target cell, the B lymphocyte, during acute infectious mononucleosis. For EBV the same cell serves as the latency reservoir. Conveniently, herpesvirus latency is frequently established in circulating hematopoietic cells. To complete the infectious life cycle, virus must be produced and transmitted to uninfected individuals. This occurs at mucosal sites: salivary glands, buccal mucosa and urogenital mucosa [8–10]. It is assumed that, at intervals, productive infection occurs in mucosal epithelial cells even in normal individuals and that these mucosal cells are infected in turn via circulating hematopoietic cells after local reactivation of latent virus. For this purpose EBV-infected B cells may use the CD48 molecule to bind heparan sulfate on epithelial cells [11,12]. This may occur at sites of chronic or intermittent inflammation. Indeed, the lymphoid organs of Waldeyer's ring, where EBV is thought to reactivate, are sites of

CMV = cytomegalovirus; EAE = experimental autoimmune encephalomyelitis; EBV = Epstein–Barr virus; HHV = human herpesvirus; IFN = interferon; MHC = major histocompatibility complex; MHV = murine herpesvirus; PCR = polymerase chain reaction; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; TCR = T-cell receptor; TNF = tumor necrosis factor.

Table 1

Disease associations

Disease	EBV	CMV	HHV6	HHV7	HHV8	*Chlamydia trachomatis*	*Chlamydia pneumoniae*	PV B19	References
SLE	+[a]	+[b]	+		+			+	[127–133]
RA	++	++			+	++	++	++	[64,95,130,134]
Sjoegren's disease	++		++						[135,136]
Myocarditis	+	+					+	++	[137–139]
MS	+	+	++				++		[91,140–142]
T1DM[c]	+	+							[143]
IgA nephritis	++	++							[144,145]
Guillain–Barré syndrome	+	+							[146,147]
Uveitis		++				+			[148,149]
Reiter's syndrome	+	+				++			[64,89]
Polymyositis dermatomyositis		+			+				[130]
Aplastic anemia								++	[66]
ITP	+	+						+	[150,151]
Vasculitis	+	+			+			++	[130]
Behcet's disease					+				[130]
Giant cell arteritis							++?	+	[152,153]
Scleroderma		+						+	[154,155]
Glomerulonephritis	++							++	[144,145,156–158]
Autoimmune infertility						+			[159,160]
Psoriais		+							[161]
Pityriasis rosea			++	++					[162]
Atherosclerosis	+	+					++		[98]
Leprosy	+	+							[102,103,163]
After transplant	++[d]	++[d]	+	+	+	+	+		[123,124,164–173]

[a]Associations that include some form of documented presence (by culture, electron microscopy, immunohistochemistry, PCR or *in situ* hybridization) of the microbe in autoimmune target tissues are indicated by ++. Other associations are indicated by +. Note that the references are not comprehensive and omit most of the contradictory literature; the purpose was to look for evidence of microbial presence specifically in the autoimmune target tissues.

[b]CMV in SLE is often a complication from immunosuppressive therapy causing colitis, ileitis, retinitis, pneumonitis or vasculitis, but infection can also occur before therapy. It is unclear whether infection occurs on top of a pre-existing autoimmune lesion in an autoimmune tissue (for example skin or kidney). In settings of viral reactivation due to immunosuppression, the virus may be expressed ubiquitously and we were therefore more interested in reports in which expression was limited to an autoimmune target tissue.

[c]A recent review lists up to six viruses associated with type I diabetes mellitus (T1DM), but we focus here only on those mentioned repeatedly in association with a wide variety of autoimmune disorders.

[d]PTLD (post-transfusion lymphoproliferative disease) represents a spectrum of disorders in which lymphocytes (predominantly B cells) infiltrate the allo-transplant organ. PTLD can evolve from a condition that is reversible upon cessation of immunosuppression, to an irreversible monoclonal lymphoma. Productive herpesvirus infections, especially EBV and CMV, occur *in situ* in allotransplants. By contrast, EBV is not usually present in rejected transplant tissues. Chlamydiae can cause infectious complications in severely immunodeficient transplant patients but do not directly infiltrate the transplanted tissues.

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV, human herpesvirus; ITP, immune thrombocytopenia; MS, multiple sclerosis; PV, parvovirus; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

physiologic chronic inflammation. Other such sites of physiologic inflammation include the gastrointestinal mucosa and certain types of urogenital mucosa such as the cervical transitional mucosa [12].

Low-grade histological inflammation of the prostate may be more common than is generally thought [13], and was noted in 66% of autopsies of men over the age 40 in one study [14] and in all men with benign prostate hypertrophy

Table 2

Characteristics of human herpesviruses, Chlamydiae and parvovirus

Organism	Receptors	Main cellular tropism	Proposed latency cell	Other tropism	References
EBV	CD21, MHC-II, α5β1 integrin	B	B	EPC, EC	[8]
CMV	EGFR	M/M	M/M; EC	N, EPC, EC	[174]
HHV6	CD46+	M/M, T, B	M/M	N, EPC	[175]
HHV7	$CD4^+$ heparan sulfate receptor	T	M/M	N, EPC, EC	[176,177]
HHV8	Heparan sulfate receptor, EGFR	EC, M/M, B, T	B	N, EPC	[174,178]
Chlamydia pneumoniae	Heparan sulfate receptor	M/M		EC,EPC	[179]
Chlamydia trachomatis		M/M		EC,EPC	
Parvovirus B19	Erythrocyte P antigen	Erythroid precursors		EC	[66,67]

B, B cells; CMV, cytomegalovirus; EBV, Epstein–Barr virus; EC, endothelial cells; EGFR, epidermal growth factor receptor; EPC, epithelial cells; HHV, human herpesvirus; MHC, major histocompatibility complex; M/M, myelomonocytic cells; N, neural cells; T, T cells.

[15]. Discrete focal inflammation of clinically normal salivary glands has also been noted [16]. Finally, asymptomatic airway inflammation is common and can be elicited by ubiquitous stimuli such as smoke or smog [17,18]. Herpesviruses must have evolved a way of migrating to such mucosal sites, perhaps by taking advantage of inflammatory cells that go there naturally. A possible unintended sequel is that inflammatory cells may also migrate to internal sites of inflammation, such as the synovium of an arthritic patient. Reactivation of virus at these sites does not serve the purpose of the virus but may aggravate the disease process. The prediction from this model is that any organism that uses hematopoietic inflammatory cells to migrate to a site of inflammation can be reactivated in autoimmune target tissues. Thus, there need not be a specific organism associated with a specific disease.

Herpesviruses

How well does this model fit for the organisms listed in Table 1? EBV (HHV4) is well known to infect resting B lymphocytes. $CD27^-$, $CD5^-$, IgD^- memory B cells later provide a latency reservoir [8,19]. There are estimates that 1 in 10^5 to 1 in 10^6 B cells carry latent EBV in normal adults [20]. Upon reactivation of EBV in the lymphoid tissue of Waldeyer's ring [8], shedding occurs from the oral mucosa. Although not yet proven, it is possible that mucosal epithelial cells adjacent to these lymphoid organs produce infectious virus [11,21]. Indeed, EBV can infect several cell types other than B cells, including endothelial cells [22], follicular dendritic cell lines [23], T lymphoma cells in hemophagocytic syndrome [24], smooth muscle tumor cells in immunosuppressed hosts [25] and synoviocytes from patients with rheumatoid arthritis (RA) [26–28].

Acute lytic infection with CMV (HHV5) occurs in monocytes in the blood, and a latency reservoir is established in circulating myelomonocytic cells and their $CD33^+$ $CD34^+$ bone marrow progenitors [29–31]. About 0.01 to 0.004% of mononuclear cells from peripheral blood donors, who had received granulocyte colony-stimulating factor mobilization for transplant purposes, contained the viral genome [32]. CMV can also infect dendritic cells [33,34] and endothelial cells, and may establish a latency reservoir in these cells too [30]. Lytic infection can involve epithelial cells, fibroblasts, stromal cells, neuronal cells, smooth muscle cells and hepatocytes in infected target tissues. CMV seems to be reactivated from latency by allostimulation [29,35]. Perhaps reactivation also occurs by immune stimulation at a mucosal site where CMV is excreted, such as the salivary glands, the lactating mammary glands or the urogenital tract [10,36,37], allowing both horizontal sexual transmission and vertical transmission to the newborn infant. Tumor necrosis factor-α (TNF-α) can substitute for allostimulation in inducing expression of the CMV IE-1 gene [29,38], but for complete CMV reactivation it is likely that other checkpoints must be overcome [39], perhaps regulated by other cytokines such as interleukin-13 and granulocyte/macrophage colony-stimulating factor, which are known to promote the replication of human CMV [38]. Moreover, CMV has evolved its own specialized CC-chemokine gene, MCK-2. The presence of MCK-2 results in greater inflammatory responses and enables CMV shedding in the salivary glands [40,41].

HHV6 infects cells of the myelomonocytic lineage both acutely and then latently. This includes bone marrow progenitors and myelomonocytic cells in peripheral blood [42–45]. HHV6 also has tropism for T cells, B cells, natural killer cells (viral subgroup A) and dendritic cells [45]. Finally, lytic infection can occur in many other cell types including neurons, muscle cells and epithelial cells. The last of these probably allow productive infection at a mucosal site, such as the salivary glands [46–48].

HHV7 may infect predominantly T cells but also myelomonocytic cells [49–51]. Like other herpesviruses it can infect epithelial and endothelial cells. Salivary glands are a major site of production of HHV7 [9,52]. HHV6 and HHV7 antigenemia occurs in the setting of CMV reactivation in transplant patients [53].

Finally, HHV8 targets myelomonocytic cells, lymphocytes and endothelial cells [54,55]. There may be a latency reservoir in B cells and circulating monocytes. Epithelial cells can also be infected and HHV8 is detected in the saliva of asymptomatic persons [9,52,56].

The cellular receptors used for herpesviral entry and fusion are often expressed ubiquitously (Table 2) and do not completely explain the targeted cell types. Just because a receptor is known does not mean it is the only one. CD21 and major histocompatibility complex (MHC) class II are known EBV receptors on B cells but $\alpha_5\beta_1$ integrin is a receptor for entry into polarized tongue and nasopharyngeal epithelial cells [7]. Nevertheless, there is a recurrent pattern in that these β- and γ-herpesviruses establish latency in hematopoietic cells and are reactivated for production of infectious virus at a suitable mucosal site. To some extent this may also apply to α-herpesviruses, although their distinguishing feature is tropism for, and latency in, neuronal cells and host-to-host transmission through skin lesions.

Chlamydiae

Chlamydiae are bacteria that live within vacuoles in eukaryotic cells. Acute infections target mucosal cell surfaces (lung, genital tract or eye). Persistence for many years is common, and studies in mouse models have shown that quiescent organisms can be reactivated [57,58]. Host cells include endothelial cells (*Chlamydia pneumoniae*) and epithelial cells (*Chlamydia trachomatis*). Circulating monocytes also carry Chlamydiae and may serve to disseminate the organism [59,60]. *In vitro*, small amounts of interferon-γ (IFN-γ) arrest chlamydial development and promote a morphologically distinguishable persistent form. This is reversible in the presence of excess tryptophan [57,61]. Thus, it is thought that IFN-γ limits available intracellular pools of tryptophan for the bacteria without affecting their viability and that this occurs via the tryptophan decyclizing enzyme indoleamine 2,3-dioxygenase. However, not all Chlamydiae are dependent on exogenous tryptophan: serovars D–K of *Chlamydia trachomatis*, with urogenital rather than ocular tropism, possess *trp*RBA, a tryptophan synthase gene cluster, and can synthesize tryptophan from indole substrates produced by vaginal microbial flora [62]. In IFN-γ knockout mice, and even more so in mice with severe combined immunodeficiency, *C. trachomatis* (strain MoPn) disseminates to various tissues from the genital tract and infection fails to resolve [63]. Thus, as with the Herpesviruses, the host inflammatory response can control the persistence of Chlamydiae, although the mechanistic details differ. The proinflammatory cytokine mix present in the arthritic synovium may promote the local persistence of Chlamydiae [57,61,64,65].

Parvovirus B19

With parvovirus B19, acute infection occurs in the upper respiratory tract [66,67]. At least 50% of the general population have been exposed and have detectable IgG antibodies. There are three clinical syndromes: fifth disease (erythema infectiosum), hydrops fetalis, and transient aplastic crisis/pure red cell aplasia. The latter led to the discovery that parvovirus B19 has exquisite cell tropism for early erythroid cells and progenitors, resulting in a cytopathic effect in giant pro-normoblasts [66]. However, anemia develops primarily when red cell turnover is increased, as in patients with chronic hemolysis. The virus uses globoside or erythrocyte P antigen to gain entry to these cells. Although the receptor is present on other cells, including megakaryocytes and endothelial cells, productive infection is restricted to pronormoblasts [66]. Parvoviruses of other animal species infect lymphocytes and monocytes, but this has not been shown for B19 in humans. A reticuloendothelial site for B19 infection remains a possibility (N. Young, personal communication). Parvovirus B19 is a single-stranded DNA virus that does not enter typical latency or become integrated in the host cell genome. However, persistence of the organism does occur.

In the original description [68], viremia was described in healthy asymptomatic blood donors. By nested PCR, parvovirus DNA was found in bone marrow from 4 of 45 random cadavers [69]. It is also known that the virus can be transmitted by blood products [70]. Virus can 'persist' in normal and immunodeficient patients without clinical evidence of disease [70,71]. Patients with congenital immunodeficiency, children with leukemia during or after chemotherapy, patients with AIDS, and transplant recipients may suffer persistent parvovirus B19 infection and the viral DNA load can be as high as in acute infection [66]. Cryptic infection with low-grade viral replication in normal hosts [72] may explain why B19 DNA is found in the bone marrow of patients with arthritis [73], some of whom may have B19 DNA in the synovium and the synovial fluid [74–76] and occasionally viral DNA is widespread including in the serum and skin [77].

The pathogenic role of viral DNA in the synovium is debated because control samples from osteoarthritis patients, or patients with recent joint trauma, also contained B19 DNA. While transgenic expression of nonstructural protein-1 (NS1) of parvovirus B19 in C57Bl/6 mice did not result in spontaneous arthritis, it did render mice of a resistant genetic background susceptible to collagen-induced arthritis [78]. In these mice NS1 was

expressed in the synovium after arthritis induction. There are further associations where B19 DNA has been found in the relevant tissues, for example hepatitis, myocarditis and various types of vasculitis [67].

Perhaps cryptic infection is normally contained in the presence of neutralizing antibodies, which are present in many sera and can be administered therapeutically in the form of intravenous immunoglobulin to immunodeficient patients [66]. It is not known whether this virus uses hematopoietic cells for dissemination within the body, nor is it known where or how the virus is excreted for dissemination to new hosts. Data are also lacking on whether the inflammatory milieu might influence viral replication. Autoimmunity associated with parvovirus B12 infection (Table 1) is thought to be due to immune complexes, cross-reactive antibodies, immune dysregulation or the production of inflammatory cytokines [79–82]. Overall, the data on this virus are not as strong as those for herpesviruses and Chlamydiae in support of the hypothesis proposed herein.

Circumstantial evidence for the hypothesis

In summary, it is possible that both herpesviruses and Chlamydiae gain access to sites of chronic tissue inflammation through a Trojan horse mechanism, because the influx of inflammatory hematopoietic cells will include a small number of cells that carry these organisms in dormant forms. There is some circumstantial evidence for this hypothesis. First, several studies aimed at discovering the autoantigen in human autoimmune diseases have used TCR repertoire analysis. In several instances, expanded CD4 and CD8 clones were found. Although investigators had invariably been hunting for autoantigen-reactive clones, the only specificities that have been uncovered are herpesvirus antigens! For example, CD4 clones from RA synovia examined by Li and colleagues were 'auto-reactive' with EBV-transformed B cell lines [83]. CD8 clones in psoriatic arthritis bore the signature TCR BV CDRIII region of T cells specific for BMLF1 of EBV [84]. CD8 clones from RA synovia characterized by Bonneville and colleagues in a series of elegant studies were reactive with latent and lytic viral antigens, including BZLF1 and BMLF1 [85,86]. Curiously, the EBV antigens identified were often lytic gene products. This implied that productive viral infection might have occurred in the synovium.

These results were corroborated by using MHC class I tetramers, specifically EBV and CMV peptides bound to HLA-A2. Synovial T cells specific for herpesvirus antigens were found enriched in the synovium in comparison with blood obtained at the same time from the same patient [87,88]. Finally, these studies revealed that the concentration of herpes-specific T cells in the inflammatory synovium was not disease specific. This phenomenon was observed in RA, in psoriatic arthritis, in ankylosing spondylitis, in uveitis, and in multiple sclerosis, where target tissues were also enriched in CMV-specific and EBV-specific T cells [89]. In this context the much touted association of a disease such as multiple sclerosis with HHV-6 or *Chlamydia* [90,91] is less puzzling. As with CMV and EBV, these organisms may reactivate within the autoimmune target tissue.

Whether herpesviruses are produced *in situ* in autoimmune target tissues has been examined in several studies [26–28]. Koide and colleagues were able to culture infectious EBV from RA synoviocytes obtained *ex vivo* [26]. Takeda and colleagues provided immunohistological and *in situ* hybridization studies in support of productive viral infection in RA synovia [27]. Many studies have provided serological evidence of productive EBV infection in RA, and also for HHV6 and CMV [92,93]. Productive infection by EBV in the oral mucosa is significantly increased in RA in comparison with normal subjects [92]. Finally, PCR studies for viral DNA and RNA in RA synovia have yielded contradictory results [28,94,95]. However, negative results can easily be explained by the rapid and efficient clearance of virus-infected cells by a competent immune system. Some samples that were negative by PCR were nevertheless enriched for EBV-specific CD8 cells [94].

As discussed, T cells specific for lytic viral antigens can accumulate in the inflammatory target tissues in several autoimmune diseases. However, this is not specific to autoimmunity. It might also occur in other inflammatory lesions, including atherosclerotic plaques for example [96–98]. The association between herpesvirus infection of the arterial wall and atherosclerosis is striking for Marek's disease in chickens [99]. Infection of apoE$^{-/-}$ mice with a murine γ-herpesvirus accelerates atherosclerosis, and viral mRNA is present in the aorta [100]. There may be other examples, as suggested by unusual reports such as the detection of EBV by PCR and immunohistochemistry in fibroadenomas of the breast in immunosuppressed hosts [101], and the association of EBV with leprosy [102,103].

The key question is whether this matters for disease progression. If these microbes aggravate disease by superimposed infection, antimicrobial therapy would be predicted to halt disease progression. This question has now been addressed in animal models.

Murine models to test the hypothesis

Murine herpesvirus (MHV)-68 is a mouse gamma herpesvirus. It most closely resembles EBV and HHV8 and is a natural pathogen of small rodents. This virus has now been used to infect mice with transiently induced arthritis [4] using serum transferred from K/BxN mice [104]. Normally, a clinically severe but transient inflammatory arthritis develops within 2 days and resolves after 3 to 4 weeks.

The course of this transient arthritis was aggravated and prolonged by infection with MHV-68 given 2 to 5 days after arthritis induction [4]. In immunocompetent mice the disease remained transient, but in severely immunocompromised mice a relapse of arthritis was observed. The relapse was due to lytic viral infection in synovial tissues of recovering arthritic, but not normal, joints in the same animal. Infection was demonstrated by PCR, immunohistochemistry and electron microscopy. Virus-specific T cells were enriched in the affected joints. Clinical relapse of arthritis could be inhibited with an antiviral drug, cidofovir, known to be active against MHV-68. Latent infection could be reactivated in the synovium when normal mice, latently infected with MHV-68, were treated with Cytoxan. This was associated with increased arthritis and viral antigens in the synovium by immunohistochemistry. These data strongly suggest that a herpesvirus infection can be imported to the inflammatory site of an autoimmune target tissue. Genuine viral infection is established, and this alters the course of the autoimmune disease.

MHV-68 infection is also known to exacerbate experimental autoimmune encephalomyelitis (EAE) in mice, an experimental mouse model for multiple sclerosis [5]. The mechanism by which the virus altered disease expression was not uncovered in this study. Although viral DNA was not detected in the diseased spinal cords, this might have been due to insufficient sensitivity of the assays. In an immunocompetent host, as in these mice, virus-infected cells are instantly removed and only the telltale viral antigen-specific T cells remain as proof of what has happened.

C. pneumoniae was used to infect mice (intraperitoneally) on day 7 of EAE induction. *C. pneumoniae*, but not *C. trachomatis*, resulted in more severe neurological disease [6]. *C. pneumoniae*, usually present only in spleen and lungs, was found in the central nervous system by reverse transcriptase PCR and by immunohistochemical staining associated with perivascular lymphocytic infiltrates. In conclusion, several animal models, using herpesviruses or Chlamydiae, support our hypothesis.

Mechanisms of amplification of autoimmunity

Imported infection as described above can theoretically have one of three effects: first, it can exacerbate ongoing disease leading to greater severity and duration; second, it can induce a relapse; or third, it can lead to chronic progressive disease. In the KxN arthritis model using the γ-herpesvirus MHV-68 [4], exacerbation of transient arthritis was observed in immunocompetent mice. Disease was also exacerbated in Cytoxan-treated immunodeficient mice, and in severely immunocompromised RAG1$^{-/-}$ mice a relapse due to lytic viral infection in the synovia was observed. In EAE the same virus (MHV-68) exacerbated disease [5]. Only immunocompetent mice were examined and the observation period was not long enough to assess relapse or chronicity. These authors did not find lytic viral infection in the central nervous system by viral plaque assays or by PCR. For *C. pneumoniae* and EAE [6], exacerbation was also noted in immunocompetent mice, but relapse or chronicity was not examined. In that paper, *in vitro* responses to myelin basic protein, such as T cell proliferation and γ-IFN production, were measured. Mice with EAE plus *C. pneumoniae* infection had larger responses to myelin basic protein than mice with EAE alone, suggesting that autoimmune responses were amplified by the infection.

Our data from immune-suppressed mice showed extensive viral infection, with MHV-68 in the synovium involving all cell types including fibroblasts and synovial lining cells [4]. By electron microscopy many of these cells were lytically destroyed, extracellular free viral particles were abundant and polymorphonuclear cells ingesting viral particles were seen. This picture suggests lytic viral infection. In an immunocompetent mouse, this infection would presumably be contained by a cellular and a humoral immune response. A local antiviral immune response would no doubt contribute to autoimmune inflammation. Cytotoxic tissue damage, whether induced by cytotoxic T cells or due to lytic viral infection, would result in a proinflammatory milieu. Cytokines and chemokines could contribute to inflammation in a non-specific way. However, infection might also release sequestered autoantigens and thus spread the repertoire of targeted autoantigens.

Indeed, Horwitz and colleagues have demonstrated bystander tissue destruction by Coxsackie virus in autoimmune diabetes [105]. As a result, sequestered autoantigen was released, which re-stimulated resting auto-reactive T cells in TCR transgenic mice, containing an overabundance of such T cells specific for an islet autoantigen. Both Coxsackie virus and the drug streptozotocin, an islet-damaging agent, had this effect [106]. Coxsackie virus is not a persistent or latent virus of hematopoietic cells. Mechanisms pertaining to the amplification of autoimmunity by MHV-68 or Chlamydiae might therefore differ and have not yet been rigorously examined.

In RA, studies need to be performed to examine whether viral infection with herpesviruses contributes to the emergence of new autoimmune responses. Of interest are responses to the following: collagen type II, proteoglycans and chondrocyte glycoprotein 39; nuclear lamins, topoisomerase II and RA33 antigen (heterogeneous nuclear ribonucleoprotein A2); cytoplasmic antigens such as anti-neutrophil cytoplasmic antibodies; extracellular

antigens such as keratin and IgG, the target of typical rheumatoid factors; and apoptosis-related proteins such as annexin V, calpastatin, vimentin and filaggrin [107–115]. For the last two antigens, arginine is replaced by citrulline, a process that occurs during apoptosis and is catalyzed by peptidyl arginine deiminase [110]. One indication that immunosuppressive therapy, with potential reactivation of endogenous herpesviruses, is associated with the emergence of new antibody specificities, has been published. In patients with RA (726 paired samples), initial drug therapy (often methotrexate) was associated with a change from a negative to a positive antinuclear antibody test in 12.5% [116].

Antimicrobials for autoimmunity?

The implication from these studies is that it may be time to design trials of antimicrobial drugs for selected patients with autoimmune diseases such as RA. It is already common practice to treat transplant patients and cancer patients receiving strong immunosuppressive drugs with acyclovir or valacyclovir, to prevent the reactivation of CMV and EBV. Whether patients with autoimmune diseases, such as RA and systemic lupus erythematosus (SLE), on immunosuppressive drugs such as methotrexate, azathioprine or cytoxan could also benefit from antiviral drugs need to evaluated. The occurrence of EBV-related lymphomas in methotrexate-treated patients with RA [117,118] suggests that EBV-specific immunosurveillance is deficient [119]. EBV genomic DNA, measured by real-time PCR, was increased in the peripheral blood mononuclear cells of patients with RA by about 1 log over controls [120]. However, fluctuations of EBV DNA in the blood mononuclear cells were not correlated with immunosuppressive therapy (either methotrexate alone or methotrexate plus anti-TNF-α) in small groups of patients. EBV DNA in the affected joints was not measured. Whether those patients with higher viral load did worse than others was also not reported.

The use of antimicrobials for autoimmunity is not without precedent, and successes have been reported. In most cases antibiotics have been used for their non-antimicrobial effects. Dapsone (which inhibits neutrophil function), tetracyclines (which block collagenase) and chloroquine (which blocks antigen presentation and cytokine secretion) have all been used in treating RA and SLE [121]. However, organisms like Chlamydiae are susceptible to antibiotics including tetracyclines, raising the possibility that some of these drugs might have been beneficial as a result of antimicrobial activity.

To optimize chances of therapeutic success, we suggest that patients first be screened for reactivated herpesviruses, parvovirus B12 and persistent Chlamydiae. Screening for CMV or EBV reactivation by quantitative PCR is standard practice in bone marrow transplant patients. This helps to guide the clinical use of antiviral drugs, which are now often used for prophylaxis [122-125]. These include acyclovir, gancyclovir and the oral prodrugs valacyclovir and valgancyclovir. We propose the same approach for autoimmunity. Depending on the organism(s) present in the analyzed autoimmune tissues, antiviral drugs for EBV or CMV, tetracycline or other antibiotics for Chlamydiae, or intravenous immunoglobulin for parvovirus could be tried. Note that there are few data on the efficacy of antibiotics for chronic *Chlamydia* infections [126]. Careful monitoring for the presence of the microbial organism in the relevant tissue (synovial fluid in RA) will be desirable to monitor the effectiveness of the drug. For example, quantitative PCR assays for herpesviruses, parvovirus and Chlamydiae could be used. Cultures might also be helpful. Finally, prophylactic antiviral therapy in patients receiving immunosuppressive drugs such as low-dose methotrexate in RA should be considered.

Competing interests

The author(s) declare that they have no competing interests.

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