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 auto-immune 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 hemato-poietic 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 immuno-compromised 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 immuno-surveillance 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 herpes-viruses, 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.

References

1. 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.
2. Benoist C, Mathis D: **Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry?** *Nat Immunol* 2001, **2:**797-801.
3. Hafler DA: **The distinction blurs between an autoimmune versus microbial hypothesis in multiple sclerosis.** *J Clin Invest* 1999, **104:**527-529.
4. Yarilin DA, Valiando J, Posnett DN, **A mouse Herpesvirus induces relapse of experimental autoimmune arthritis.** *J Immunol* 2004, **173:**5238-5246.
5. Peacock JW, Elsawa SF, Petty CC, Hickey WF, Bost KL: **Exacerbation of experimental autoimmune encephalomyelitis in rodents infected with murine gammaherpesvirus-68.** *J Immunol* 2003, **33:**1849-1858.
6. Du C, Yao SY, Ljunggren-Rose A, Sriram S: *Chlamydia pneumoniae* **infection of the central nervous system worsens experimental allergic encephalitis.** *J Exp Med* 2002, **196:**1639-1644.
7. Tugizov SM, Berline JW, Palefsky JM: **Epstein–Barr virus infection of polarized tongue and nasopharyngeal epithelial cells.** *Nat Med* 2003, **9:**307-314.
8. Thorley-Lawson DA: **Epstein–Barr virus: exploiting the immune system.** *Nat Rev Immunol* 2001, **1:**75-82.
9. Lucht E, Brytting M, Bjerregaard L, Julander I, Linde A: **Shedding of cytomegalovirus and herpesviruses 6, 7, and 8 in saliva of human immunodeficiency virus type 1-infected patients and healthy controls.** *Clin Infect Dis* 1998, **27:**137-141.
10. Gautheret-Dejean A, Aubin JT, Poirel L, Huraux JM, Nicolas JC, Rozenbaum W, Agut H: **Detection of human Betaherpesvirinae in saliva and urine from immunocompromised and immuno-competent subjects.** *J Clin Microbiol* 1997, **35:**1600-1603.
11. Ianelli CJ, De Lellis R, Thorley-Lawson DA: **CD48 binds to heparan sulfate on the surface of epithelial cells.** *J Biol Chem* 1998, **273:**23367-23375.
12. Johansson EL, Rudin A, Wassen L, Holmgren J: **Distribution of lymphocytes and adhesion molecules in human cervix and vagina.** *Immunology* 1999, **96:**272-277.

13 Krieger JN, Ross SO, Riley DE: **Chronic prostatitis: epidemiology and role of infection.** *Urology* 2002, **60:**8-12.

14 Billis A, Magna LA: **Inflammatory atrophy of the prostate. Prevalence and significance.** *Arch Pathol Lab Med* 2003, **127:** 840-844.

15 Nickel JC, Downey J, Young I, Boag S: **Asymptomatic inflammation and/or infection in benign prostatic hyperplasia.** *BJU Int* 1999, **84:**976-981.

16 Harrison JD, Epivatianos A, Bhatia SN: **Role of microliths in the aetiology of chronic submandibular sialadenitis: a clinico-pathological investigation of 154 cases.** *Histopathology* 1997, **31:**237-251.

17 Roth MD, Arora A, Barsky SH, Kleerup EC, Simmons M, Tashkin DP: **Airway inflammation in young marijuana and tobacco smokers.** *Am J Respir Crit Care Med* 1998, **157:**928-937.

18 Sherwin RP, Richters V, Everson RB, Richters A: **Chronic glandular bronchitis in young individuals residing in a metropolitan area.** *Virchows Arch* 1998, **433:**341-348.

19 Joseph AM, Babcock GJ, Thorley-Lawson DA: **EBV persistence involves strict selection of latently infected B cells.** *J Immunol* 2000, **165:**2975-2981.

20 Decker LL, Klaman LD, Thorley-Lawson DA: **Detection of the latent form of Epstein–Barr virus DNA in the peripheral blood of healthy individuals.** *J Virol* 1996, **70:**3286-3289.

21 Deacon EM, Matthews JB, Potts AJ, Hamburger J, Bevan IS, Young LS: **Detection of Epstein–Barr virus antigens and DNA in major and minor salivary glands using immunocytochemistry and polymerase chain reaction: possible relationship with Sjogren's syndrome.** *J Pathol* 1991, **163:**351-360.

22 Jones K, Rivera C, Sgadari C, Franklin J, Max EE, Bhatia K, Tosato G: **Infection of human endothelial cells with Epstein–Barr virus.** *J Exp Med* 1995, **182:**1213-1221.

23 Lindhout E, Lakeman A, Mevissen ML, de Groot C: **Functionally active Epstein–Barr virus-transformed follicular dendritic cell-like cell lines.** *J Exp Med* 1994, **179:**1173-1184.

24 Lay JD, Tsao CJ, Chen JY, Kadin ME, Su IJ: **Upregulation of tumor necrosis factor-alpha gene by Epstein–Barr virus and activation of macrophages in Epstein–Barr virus-infected T cells in the pathogenesis of hemophagocytic syndrome.** *J Clin Invest* 1997, **100:**1969-1979.

25 McClain KL, Leach CT, Jenson HB, Joshi VV, Pollock BH, Parmley RT, Di Carlo FJ, Chadwick EG, Murphy SB: **Association of Epstein–Barr virus with leiomyosarcomas in children with AIDS.** *N Engl J Med* 1995, **332:**12-18.

26 Koide J, Takada K, Sugiura M, Sekine H, Ito T, Saito K, Mori S, Takeuchi T, Uchida S, Abe T: **Spontaneous establishement of an Epstein–Barr virus infected fibroblast line from the synovial tissue of a rheumatoid arthritis patient.** *J Virol* 1997, **71:** 2478-2481.

27 Takeda T, Mizugaki Y, Matsubara L, Imai S, Koike T, Takada K: **Lytic Epstein–Barr virus infection in the synovial tissue of patients with rheumatoid arthritis.** *Arthritis Rheum* 2000, **43:** 1218-1225.

28 Saal JG, Krimmel M, Steidle M, Gerneth F, Wagner S, Fritz P, Koch S, Zacher J, Sell S, Einsele H, *et al.*: **Synovial Epstein–Barr virus infection increases the risk of rheumatoid arthritis in individuals with the shared HLA-DR4 epitope.** *Arthritis Rheum* 1999, **42:**1485-1496.

29 Soderberg-Naucler C, Fish KN, Nelson JA: **Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors.** *Cell* 1997, **91:**119-126.

30 Jarvis MA, Nelson JA: **Human cytomegalovirus persistence and latency in endothelial cells and macrophages.** *Curr Opin Microbiol* 2002, **5:**403-407.

31 Kondo K, Xu J, Mocarski ES: **Human cytomegalovirus latent gene expression in granulocyte-macrophage progenitors in culture and in seropositive individuals.** *Proc Natl Acad Sci USA* 1996, **93:**11137-11142.

32 Slobedman B, Mocarski ES: **Quantitative analysis of latent human cytomegalovirus.** *J Virol* 1999, **73:**4806-4812.

33 Halary F, Amara A, Lortat-Jacob H, Messerle M, Delaunay T, Houles C, Fieschi F, Arenzana-Seisdedos F, Moreau JF, Dechanet-Merville J: **Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection.** *Immunity* 2002, **17:**653-664.

34 Raftery MJ, Schwab M, Eibert SM, Samstag Y, Walczak H, Schonrich G: **Targeting the function of mature dendritic cells by human cytomegalovirus: a multilayered viral defense strategy.** *Immunity* 2001, **15:**997-1009.

35 Hummel M, Abecassis MM: **A model for reactivation of CMV from latency.** *J Clin Virol* 2002, **Suppl 2:**123-136.

36 Forbes BA: **Acquisition of cytomegalovirus infection: an update.** *Clin Microbiol Rev* 1989, **2:**204-216.

37 Ho M: **Epidemiology of cytomegalovirus infections.** *Rev Infect Dis* 1990, **12** (Suppl 7):701-710.

38 Streblow DN, Nelson JA: **Models of HCMV latency and reactivation.** *Trends Microbiol* 2003, **11:**293-295.

39 Reddehase MJ, Podlech J, Grzimek NK: **Mouse models of cytomegalovirus latency: overview.** *J Clin Virol* 2002, **Suppl 2:** 23-36.

40 Saederup N, Mocarski ES Jr: **Fatal attraction: cytomegalovirus-encoded chemokine homologs.** *Curr Top Microbiol Immunol* 2002, **269:**235-256.

41 Saederup N, Aguirre SA, Sparer TE, Bouley DM, Mocarski ES: **Murine cytomegalovirus CC chemokine homolog MCK-2 (m131-129) is a determinant of dissemination that increases inflammation at initial sites of infection.** *J Virol* 2001, **75:**9966-9976.

42 Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K: **Latent human herpesvirus 6 infection of human monocytes/macrophages.** *J Gen Virol* 1991, **72:**1401-1408.

43 Kondo K, Kondo T, Shimada K, Amo K, Miyagawa H, Yamanishi K: **Strong interaction between human herpesvirus 6 and peripheral blood monocytes/macrophages during acute infection.** *J Med Virol* 2002, **67:**364-369.

44 Luppi M, Barozzi P, Morris C, Maiorana A, Garber R, Bonacorsi G, Donelli A, Marasca R, Tabilio A, Torelli G: **Human herpesvirus 6 latently infects early bone marrow progenitors in vivo.** *J Virol* 1999, **73:**754-759.

45 Lusso P: **Human herpesvirus 6 (HHV-6).** *Antiviral Res* 1996, **31:** 1-21.

46 Levy JA, Ferro F, Greenspan D, Lennette ET: **Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in the population.** *Lancet* 1990, **335:**1047-1050.

47 Fox JD, Briggs M, Ward PA, Tedder RS: **Human herpesvirus 6 in salivary glands.** *Lancet* 1990, **336:**590-593.

48 Di Luca D, Mirandola P, Ravaioli T, Dolcetti R, Frigatti A, Bovenzi P, Sighinolfi L, Monini P, Cassai E: **Human herpesviruses 6 and 7 in salivary glands and shedding in saliva of healthy and human immunodeficiency virus positive individuals.** *J Med Virol* 1995, **45:**462-468.

49 Black JB, Pellett PE: **Human herpesvirus 7.** *Rev Med Virol* 1999, **9:**245-262.

50 Mirandola P, Secchiero P, Pierpaoli S, Visani G, Zamai L, Vitale M, Capitani S, Zauli G: **Infection of CD34+ hematopoietic progenitor cells by human herpesvirus 7 (HHV-7).** *Blood* 2000, **96:**126-131.

51 Kempf W, Adams V, Wey N, Moos R, Schmid M, Avitabile E, Campadelli-Fiume G: **CD68+ cells of monocyte/macrophage lineage in the environment of AIDS-associated and classic-sporadic Kaposi sarcoma are singly or doubly infected with human herpesviruses 7 and 6B.** *Proc Natl Acad Sci USA* 1997, **94:**7600-7605.

52 Sada E, Yasukawa M, Ito C, Takeda A, Shiosaka T, Tanioka H, Fujita S: **Detection of human herpesvirus 6 and human herpesvirus 7 in the submandibular gland, parotid gland, and lip salivary gland by PCR.** *J Clin Microbiol* 1996, **34:**2320-2321.

53 Lautenschlager I, Lappalainen M, Linnavuori K, Suni J, Hockerstedt K: **CMV infection is usually associated with concurrent HHV-6 and HHV-7 antigenemia in liver transplant patients.** *J Clin Virol* 2002, **Suppl 2:**S57-S61.

54 Monini P, Colombini S, Sturzl M, Goletti D, Cafaro A, Sgadari C, Butto S, Franco M, Leone P, Fais S, *et al.*: **Reactivation and persistence of human herpesvirus-8 infection in B cells and monocytes by Th-1 cytokines increased in Kaposi's sarcoma.** *Blood* 1999, **93:**4044-4058.

55 Blasig C, Zietz C, Haar B, Neipel F, Esser S, Brockmeyer NH, Tschachler E, Colombini S, Ensoli B, Sturzl M: **Monocytes in Kaposi's sarcoma lesions are productively infected by human herpesvirus 8.** *J Virol* 1997, **71:**7963-7968.

56 Pauk J, Huang ML, Brodie SJ, Wald A, Koelle DM, Schacker T, Celum C, Selke S, Corey L: **Mucosal shedding of human herpesvirus 8 in men.** *N Engl J Med* 2000, **343:**1369-1377.

57 Morrison RP: **New insights into a persistent problem – chlamydial infections.** *J Clin Invest* 2003, **111:**1647-1649.

58. Cotter TW, Miranpuri GS, Ramsey KH, Poulsen CE, Byrne GI: **Reactivation of chlamydial genital tract infection in mice.** *Infect Immun* 1997, **65:**2067-2073.

59. Koehler L, Nettelnbreker E, Hudson AP, Ott N, Gerard HC, Branigan PJ, Schumacher HR, Drommer W, Zeidler H: **Ultrastructural and molecular analyses of the persistence of *Chlamydia trachomatis* (serovar K) in human monocytes.** *Microb Pathog* 1997, **22:**133-142.

60. Villareal C, Whittum-Hudson JA, Hudson AP: **Persistent Chlamydiae and chronic arthritis.** *Arthritis Res* 2002, **4:**5-9.

61. Rottenberg ME, Gigliotti-Rothfuchs A, Wigzell H: **The role of IFN-gamma in the outcome of chlamydial infection.** *Curr Opin Immunol* 2002, **14:**444-451.

62. Caldwell HD, Wood H, Crane D, Bailey R, Jones RB, Mabey D, Maclean I, Mohammed Z, Peeling R, Roshick C, *et al.*: **Polymorphisms in *Chlamydia trachomatis* tryptophan synthase genes differentiate between genital and ocular isolates.** *J Clin Invest* 2003, **111:**1757-1769.

63. Cotter TW, Ramsey KH, Miranpuri GS, Poulsen CE, Byrne GI: **Dissemination of *Chlamydia trachomatis* chronic genital tract infection in gamma interferon gene knockout mice.** *Infect Immun* 1997, **65:**2145-2152.

64. Schumacher HR: **Reactive arthritis.** *Rheum Dis Clin North Am* 1998, **24:**261-273.

65. Gerard HC, Wang Z, Whittum-Hudson JA, El-Gabalawy H, Goldbach-Mansky R, Bardin T, Schumacher HR, Hudson AP: **Cytokine and chemokine mRNA produced in synovial tissue chronically infected with *Chlamydia trachomatis* and *C. pneumoniae*.** *J Rheumatol* 2002, **29:**1827-1835.

66. Young NS: **Parvoviruses.** In *Fields Virology*. 3rd edition. Edited by Fields BN, Knipe DM, Howley PM. Philadelphia: Lippincott–Raven; 1995:2199-2220.

67. Young NS, Brown KE: **Mechanisms of disease: parvovirus B19.** *N Engl J Med* 2004, **350:**586-597.

68. Cossart YE, Field AM, Cant B, Widdows D: **Parvovirus-like particles in human sera.** *Lancet* 1975, **i:**72-73.

69. Cassinotti P, Burtonboy G, Fopp M, Siegl G: **Evidence for persistence of human parvovirus B19 DNA in bone marrow.** *J Med Virol* 1997, **53:**229-232.

70. Kerr JR, Curran MD, Moore JE, Coyle PV, Ferguson WP: **Persistent parvovirus B19 infection.** *Lancet* 1995, **345:**1118.

71. Musiani M, Zerbini M, Gentilomi G, Rodrigo G, De Rosa V, Gibellini D, Venturoli S, Gallinella G: **Persistent B19 parvovirus infections in haemophilic HIV-1 infected patients.** *J Med Virol* 1995, **46:**103-108.

72. Cotmore SF, Tattersall P: **The autonomously replicating parvoviruses of vertebrates.** *Adv Virus Res* 1987, **33:**91-174.

73. Foto F, Saag KG, Scharosch LL, Howard EJ, Naides SJ: **Parvovirus B19-specific DNA in bone marrow from B19 arthropathy patients: evidence for B19 virus persistence.** *J Infect Dis* 1993, **167:**744-748.

74. Saal JG, Steidle M, Einsele H, Muller CA, Fritz P, Zacher J: **Persistence of B19 parvovirus in synovial membranes of patients with rheumatoid arthritis.** *Rheumatol Int* 1992, **12:**147-151.

75. Soderlund M, von Essen R, Haapasaari J, Kiistala U, Kiviluoto O, Hedman K: **Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy.** *Lancet* 1997, **349:**1063-1065.

76. Kerr JR, Cartron JP, Curran MD, Moore JE, Elliott JR, Mollan RA: **A study of the role of parvovirus B19 in rheumatoid arthritis.** *Br J Rheumatol* 1995, **34:**809-813.

77. Nikkari S, Lappalainen H, Saario R, Lammintausta K, Kotilainen P: **Detection of parvovirus B19 in skin biopsy, serum, and bone marrow of a patient with fever, rash, and polyarthritis followed by pneumonia, pericardial effusion, and hepatitis.** *Eur J Clin Microbiol Infect Dis* 1996, **15:**954-957.

78. Takasawa N, Munakata Y, Ishii KK, Takahashi Y, Takahashi M, Fu Y, Ishii H, Fujii H, Saito T, Takano H, *et al.*: **Human parvovirus B19 transgenic mice become susceptible to polyarthritis.** *J Immunol* 2004, **173:**4675-4683.

79. Lunardi C, Tiso M, Borgato L, Nanni L, Millo R, De Sandre G, Severi AB, Puccetti A: **Chronic parvovirus B19 infection induces the production of anti-virus antibodies with autoantigen binding properties.** *Eur J Immunol* 1998, **28:**936-948.

80. Wagner AD, Goronzy JJ, Matteson EL, Weyand CM: **Systemic monocyte and T-cell activation in a patient with human parvovirus B19 infection.** *Mayo Clin Proc* 1995, **70:**261-265.

81. Kerr JR, Barah F, Chiswick ML, McDonnell GV, Smith J, Chapman MD, Bingham JB, Kelleher P, Sheppard MN: **Evidence for the role of demyelination, HLA-DR alleles, and cytokines in the pathogenesis of parvovirus B19 meningoencephalitis and its sequelae.** *J Neurol Neurosurg Psychiatry* 2002, **73:**739-746.

82. Barash J, Dushnitzki D, Barak Y, Miron S, Hahn T: **Tumor necrosis factor (TNF)alpha and its soluble receptor (sTNFR) p75 during acute human parvovirus B19 infection in children.** *Immunol Lett* 2003, **88:**109-112.

83. Li Y, Sun GR, Tumang JR, Crow MK, Friedman SM: **CDR3 sequence motifs shared by oligoclonal rheumatoid arthritis synovial T cells. Evidence for an antigen-driven response.** *J Clin Invest* 1994, **94:**2525-2531.

84. Curran SA, Fitzgerald OM, Costello PJ, Selby JM, Kane DJ, Bresnihan B, Winchester RJ: **Nucleotide sequencing of psoriatic arthritis tissue before and during methotrexate administration reveals a complex inflammatory T cell infiltrate with very few clones exhibiting features suggesting they are likely to drive the inflammatory process.** *J Immunol* 2004, **172:**1935-1944.

85. David-Ameline J, Lim A, Davodeau F, Peyrat MA, Berthelot JM, Semama G, Pannetier C, Gaschet J, Yie H, Even J, *et al.*: **Selection of T cells reactive against autologous B lymphoblastoid cells during chronic rheumatoid arthritis.** *J Immunol* 1996, **157:**4697-4706.

86. Scotet E, David-Ameline J, Peyrat M-A, Moreau-Aubry A, Pinczon D, Lim A, Even J, Semana G, Berthelot J-M, Reathnach R, *et al.*: **T cell response to Epstein–Barr virus transactivators in chronic rheumatoid arthritis.** *J Exp Med* 1996, **184:**1791-1800.

87. Tan LC, Mowat AG, Fazou C, Rostron T, Roskell H, Dunbar PR, Tournay C, Romagne F, Peyrat MA, Houssaint E, *et al.*: **Specificity of T cells in synovial fluid: high frequencies of CD8+ T cells that are specific for certain viral epitopes.** *Arthritis Res* 2000, **2:**154-164.

88. Fazou C, Yang H, McMichael AJ, Callan MF: **Epitope specificity of clonally expanded populations of CD8+ T cells found within the joints of patients with inflammatory arthritis.** *Arthritis Rheum* 2001, **44:**2038-2045.

89. Scotet E, Peyrat MA, Saulquin X, Retiere C, Couedel C, Davodeau F, Dulphy N, Toubert A, Bignon JD, Lim A, *et al.*: **Frequent enrichment for CD8 T cells reactive against common herpes viruses in chronic inflammatory lesions: towards a reassessment of the physiopathological significance of T cell clonal expansions found in autoimmune inflammatory processes.** *Eur J Immunol* 1999, **29:**973-985.

90. Berti R, Soldan SS, Akhyani N, McFarland HF, Jacobson S: **Extended observations on the association of HHV-6 and multiple sclerosis.** *J Neurovirol* 2000, **Suppl 2:**S85-S87.

91. Swanborg RH, Whittum-Hudson JA, Hudson AP: **Infectious agents and multiple sclerosis – are *Chlamydia pneumoniae* and human herpes virus 6 involved?** *J Neuroimmunol* 2003, **136:**1-8.

92. Newkirk MM, Watanabe Duffy KN, Leclerc J, Lambert N, Shiroky JB: **Detection of cytomegalovirus, Epstein–Barr virus and herpes virus-6 in patients with rheumatoid arthritis with or without Sjogren's syndrome.** *Br J Rheumatol* 1994, **33:**317-322.

93. Newkirk MM, Shiroky JB, Johnson N, Danoff D, Isenberg DA, Shustik C, Pearson GR: **Rheumatic disease patients, prone to Sjogren's syndrome and/or lymphoma, mount an antibody response to BHRF1, the Epstein–Barr viral homologue of BCL-2.** *Br J Rheumatol* 1996, **35:**1075-1081.

94. Edinger JW, Bonneville M, Scotet E, Houssaint E, Schumacher HR, Posnett DN: **EBV gene expression not altered in rheumatoid synovia despite the presence of EBV antigen-specific T cell clones.** *J Immunology* 1998, **162:**3694-3701.

95. Mehraein Y, Lennerz C, Ehlhardt S, Remberger K, Ojak A, Zang KD: **Latent Epstein–Barr virus (EBV) infection and cytomegalovirus (CMV) infection in synovial tissue of autoimmune chronic arthritis determined by RNA- and DNA- in situ hybridization.** *Mod Pathol* 2004, **17:**781-789.

96. Gordon PA, George J, Khamashta MA, Harats D, Hughes G, Shoenfeld Y: **Atherosclerosis and autoimmunity.** *Lupus* 2001, **10:**249-252.

97. Shi Y, Tokunaga O: **Herpesvirus (HSV-1, EBV and CMV) infections in atherosclerotic compared with non-atherosclerotic aortic tissue.** *Pathol Int* 2002, **52:**31-39.

98. Belland RJ, Ouellette SP, Gieffers J, Byrne GI: *Chlamydia pneumoniae* **and atherosclerosis.** *Cell Microbiol* 2004, **6:**117-127.

99. Fabricant CG, Fabricant J: **Atherosclerosis induced by infection with Marek's disease herpesvirus in chickens.** *Am Heart J* 1999, **138:**S465-S468.

100. Alber DG, Powell KL, Vallance P, Goodwin DA, Grahame-Clarke C: **Herpesvirus infection accelerates atherosclerosis in the apolipoprotein E-deficient mouse.** *Circulation* 2000, **102:**779-785.

101. Kleer CG, Tseng MD, Gutsch DE, Rochford RA, Wu Z, Joynt LK, Helvie MA, Chang T, Van Golen KL, Merajver SD: **Detection of Epstein–Barr virus in rapidly growing fibroadenomas of the breast in immunosuppressed hosts.** *Mod Pathol* 2002, **15:**759-764.

102. Papageorgiou PS, Sorokin C, Kouzoutzakoglou K, Glade PR: **Herpes-like Epstein–Barr virus in leprosy.** *Nature* 1971, **231:** 47-49.

103. Papageorgiou PS, Sorokin CF, Kouzoutzakoglou K, Bonforte RJ, Workman PL, Glade PR: **Host responses to Epstein–Barr virus and cytomegalovirus infection in leprosy.** *Infect Immun* 1973, **7:**620-624.

104. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D: **Organ-specific disease provoked by systemic autoimmunity.** *Cell* 1996, **87:**811-822.

105. Horwitz MS, Bradley LM, Harbertson J, Krahl T, Lee J, Sarvetnick N: **Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry.** *Nat Med* 1998, **4:**781-785.

106. Horwitz MS, Ilic A, Fine C, Rodriguez E, Sarvetnick N: **Presented antigen from damaged pancreatic beta cells activates autoreactive T cells in virus-mediated autoimmune diabetes.** *J Clin Invest* 2002, **109:**79-87.

107. Rodriguez-Garcia MI, Fernandez JA, Rodriguez A, Fernandez MP, Gutierrez C, Torre-Alonso JC: **Annexin V autoantibodies in rheumatoid arthritis.** *Ann Rheum Dis* 1996 **55:**895-900.

108. Lettesjo H, Nordstrom E, Strom H, Moller E: **Autoantibody patterns in synovial fluids from patients with rheumatoid arthritis or other arthritic lesions.** *Scand J Immunol* 1998, **48:**293-299.

109. Goldbach-Mansky R, Lee J, McCoy A, Hoxworth J, Yarboro C, Smolen JS, Steiner G, Rosen A, Zhang C, Menard HA, Zhou, *et al.*: **Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset.** *Arthritis Res* 2000, **2:**236-243.

110. Van Venrooij WJ, Pruijn GJ: **Citrullination: a small change for a protein with great consequences for rheumatoid arthritis.** *Arthritis Res* 2003, **2:**249-251.

111. Shrivastav M, Mittal B, Aggarwal A, Misra R: **Autoantibodies against cytoskeletal proteins in rheumatoid arthritis.** *Clin Rheumatol* 2002, **21:**505-510.

112. Smolen JS, Hassfeld W, Graninger W, Steiner G: **Antibodies to antinuclear subsets in systemic lupus erythematosus and rheumatoid arthritis.** *Clin Exp Rheumatol* 1990, **Suppl 5:**41-44.

113. Menard HA, Lapointe E, Rochdi MD, Zhou ZJ: **Insights into rheumatoid arthritis derived from the Sa immune system.** *Arthritis Res* 2000, **2:**429-432.

114. Brito J, Biamonti G, Caporali R, Montecucco C: **Autoantibodies to human nuclear lamin B2 protein. Epitope specificity in different autoimmune diseases.** *J Immunol* 1994, **153:**2268-2277.

115. Konstantinov K, Halberg P, Wiik A, Hoier-Madsen M, Wantzin P, Ullman S, Galcheva-Gargova Z: **Clinical manifestations in patients with autoantibodies specific for nuclear lamin proteins.** *Clin Immunol Immunopathol* 1992, **62:**112-118.

116. Paulus HE, Wiesner J, Bulpitt KJ, Patnaik M, Law J, Park GS, Wong WK: **Autoantibodies in early seropositive rheumatoid arthritis, before and during disease modifying antirheumatic drug treatment.** *J Rheumatol* 2002, **29:**2513-2520.

117. Kamel OW: **Iatrogenic lymphoproliferative disorders in non-transplantation settings.** *Semin Diagn Pathol* 1997, **14:**27-34.

118. Mariette X, Cazals-Hatem D, Warszawki J, Liote F, Balandraud N, Sibilia J: **Lymphomas in rheumatoid arthritis patients treated with methotrexate: a 3-year prospective study in France.** *Blood* 2002, **99:**3909-3915.

119. Callan MFC: **Epstein–Barr virus, arthritis, and the development of lymphoma in arthritis patients.** *Curr Opin Rheumatol* 2004, **16:**399-405.

120. Balandraud N, Meynard JB, Auger I, Sovran H, Mugnier B, Reviron D, Roudier J, Roudier C: **Epstein–Barr virus load in the peripheral blood of patients with rheumatoid arthritis: accurate quantification using real-time polymerase chain reaction.** *Arthritis Rheum* 2003, **48:**1223-1228.

121. Mottram PL: **Past, present and future drug treatment for rheumatoid arthritis and systemic lupus erythematosus.** *Immunol Cell Biol* 2003, **81:**350-353.

122. Slifkin MS, Doron S, Snydman DR: **Viral prophylaxis in organ transplant patients.** *Drugs* 2004, **64:**2763-2792.

123. Pereyra F, Rubin RH: **Prevention and treatment of cytomegalovirus infection in solid organ transplant recipients.** *Curr Opin Infect Dis* 2004, **17:**357-361.

124. Boeckh M, Nichols WG, Papanicolaou G, Rubin R, Wingard JR, Zaia J: **Cytomegalovirus in hematopoietic stem cell transplant recipients: Current status, known challenges, and future strategies.** *Biol Blood Marrow Transplant* 2003, **9:**543-558.

125. Griffiths PD: **Tomorrow's challenges for herpesvirus management: potential applications of valacyclovir.** *J Infect Dis* 2002, **Suppl 1:**131-137.

126. Hammerschlag MR: **Advances in the management of *Chlamydia pneumoniae* infections.** *Expert Rev Anti Infect Ther* 2003, **1:** 493-503.

127. Kang I, Quan T, Nolasco H, Park SH, Hong MS, Crouch J, Pamer EG, Howe JG, Craft J: **Defective control of latent Epstein–Barr virus infection in systemic lupus erythematosus.** *J Immunol* 2004, **172:**1287-1294.

128. Kang I, Park SH: **Infectious complications in SLE after immunosuppressive therapies.** *Curr Opin Rheumatol* 2003, **15:** 528-534.

129. James JA, Neas BR, Moser KL, Hall T, Bruner GR, Sestak AL, Harley JB: **Systemic lupus erythematosus in adults is associated with previous Epstein–Barr virus exposure.** *Arthritis Rheum* 2003, **44:**1122-1126.

130. Louthrenoo W, Kasitanon N, Mahanuphab P, Bhoopat L, Thongprasert S: **Kaposi's sarcoma in rheumatic diseases.** *Semin Arthritis Rheum* 2003, **32:**326-333.

131. Iwasaki T, Satodate R, Masuda T, Kurata T, Hondo R: **An immunofluorescent study of generalized infection of human cytomegalovirus in a patient with systemic lupus erythematosus.** *Acta Pathol Jpn* 1984, **34:**869-874.

132. Bulpitt KJ, Brahn E: **Systemic lupus erythematosus and concurrent cytomegalovirus vasculitis: diagnosis by antemortem skin biopsy.** *J Rheumatology* 1989, **16:**677-680.

133. Tsai YT, Chiang BL, Kao YF, Hsieh KH: **Detection of Epstein–Barr virus and cytomegalovirus genome in white blood cells from patients with juvenile rheumatoid arthritis and childhood systemic lupus erythematosus.** *Int Arch Allergy Immunol* 1995, **106:**235-240.

134. Gerard HC, Schumacher HR, El-Gabalawy H, Goldbach-Mansky R, Hudson AP: ***Chlamydia pneumoniae* present in the human synovium are viable and metabolically active.** *Microb Pathog* 2000, **29:**17-24.

135. Perrot S, Calvez V, Escande JP, Dupin N, Marcelin AG: **Prevalences of herpesviruses DNA sequences in salivary gland biopsies from primary and secondary Sjogren's syndrome using degenerated consensus PCR primers.** *J Clin Virol* 2003, **28:**165-168.

136. Fillet AM, Raguin G, Agut H, Boisnic S, Agbo-Godeau S, Robert C: **Evidence of human herpesvirus 6 in Sjogren syndrome and sarcoidosis.** *Eur J Clin Microbiol Infect Dis* 1992, **11:**564-566.

137. Bowles NE, Ni J, Kearney DL, Pauschinger M, Schultheiss HP, McCarthy R, Hare J, Bricker JT, Bowles KR, Towbin JA: **Detection of viruses in myocardial tissues by polymerase chain reaction. evidence of adenovirus as a common cause of myocarditis in children and adults.** *J Am Coll Cardiol* 2003, **42:**466-472.

138. Cioc AM, Nuovo GJ: **Histologic and in situ viral findings in the myocardium in cases of sudden, unexpected death.** *Mod Pathol* 2002, **15:**914-922.

139. Schonian U, Crombach M, Maser S, Maisch B: **Cytomegalovirus-associated heart muscle disease.** *Eur Heart J* 1995, **Suppl O:**46-49.

140. Sriram S, Stratton CW, Yao S, Tharp A, Ding L, Bannan JD, Mitchell WM: ***Chlamydia pneumoniae* infection of the central nervous system in multiple sclerosis.** *Ann Neur* 1999, **46:**6-14.

141. Gieffers J, Pohl D, Treib J, Dittmann R, Stephan C, Klotz K, Hanefeld F, Solbach W, Haass A, Maass M: **Presence of *Chlamydia pneumoniae* DNA in the cerebral spinal fluid is a common phenomenon in a variety of neurological diseases and not restricted to multiple sclerosis.** *Ann Neurol* 2001, **49:**585-589.

142. Simmons A: **Herpesvirus and multiple sclerosis.** *Herpes* 2001, **8:**60-63.

143. Jun HS, Yoon JW: **A new look at viruses in type 1 diabetes.** *Diabetes Metab Res Rev* 2003, **19:**8-31.

144. Gregory MC, Hammond ME, Brewer ED, **Renal deposition of cytomegalovirus antigen in immunoglobulin-A nephropathy.** *Lancet* 1988, **i:**11-14.

145. Iwama H, Horikoshi S, Shirato I, Tomino Y: **Epstein–Barr virus detection in kidney biopsy specimens correlates with glomerular mesangial injury.** *Am J Kidney Dis* 1998, **32:**785-793.

146. Hughes RA, Hadden RD, Gregson NA, Smith KJ: **Pathogenesis of Guillain–Barré syndrome.** *J Neuroimmunol* 1999, **100:**74-97.

147. Hadden RD, Karch H, Hartung HP, Zielasek J, Weissbrich B, Schubert J, Weishaupt A, Cornblath DR, Swan AV, Hughes RA, *et al.*: **Preceding infections, immune factors, and outcome in Guillain–Barré syndrome.** *Neurology* 2001, **56:**758-765.

148. Graninger W, Arocker-Mettinger E, Kiener H, Benke A, Szots-Sotz J, Knobler R, Smolen J: **High incidence of asymptomatic urogenital infection in patients with uveitis anterior.** *Doc Ophthalmol* 1992, **82:**217-221.

149. Holland GN: **Immune recovery uveitis.** *Ocul Immunol Inflamm* 1999, **7:**215-221.

150. Hida M, Shimamura Y, Ueno E, Watanabe J: **Childhood idiopathic thrombocytopenic purpura associated with human parvovirus B19 infection.** *Pediatr Int* 2000, **42:**708-710.

151. Yenicesu I, Yetgin S, Ozyurek E, Aslan D: **Virus-associated immune thrombocytopenic purpura in childhood.** *Pediatr Hematol Oncol* 2002, **19:**433-437.

152. Wagner AD, Gerard HC, Fresemann T, Schmidt WA, Gromnica-Ihle E, Hudson AP, Zeidler H: **Detection of *Chlamydia pneumoniae* in giant cell vasculitis and correlation with the topographic arrangement of tissue-infiltrating dendritic cells.** *Arthritis Rheum* 2000, **43:**1543-1551.

153. Helweg-Larsen J, Tarp B, Obel N, Baslund B: **No evidence of parvovirus B19, *Chlamydia pneumoniae* or human herpes virus infection in temporal artery biopsies in patients with giant cell arteritis.** *Rheumatology* 2002, **41:**445-449.

154. Hamamdzic D, Kasman LM, Le Roy EC: **The role of infectious agents in the pathogenesis of systemic sclerosis.** *Curr Opin Rheumatol* 2002, **14:**694-698.

155. Magro CM, Nuovo G, Ferri C, Crowson AN, Giuggioli D, Sebastiani M: **Parvoviral infection of endothelial cells and stromal fibroblasts: a possible pathogenetic role in scleroderma.** *J Cutan Pathol* 2004, **31:**43-50.

156. Tanawattanacharoen S, Falk RJ, Jennette JC, Kopp JB: **Parvovirus B19 DNA in kidney tissue of patients with focal segmental glomerulosclerosis.** *Am J Kidney Dis* 2000, **35:**1166-1174.

157. Wierenga KJ, Pattison JR, Brink N, Griffiths M, Miller M, Shah DJ, Williams W, Serjeant BE, Serjeant GR: **Glomerulonephritis after human parvovirus infection in homozygous sickle-cell disease.** *Lancet* 1995, **346:**475-476.

158. Iwafuchi Y, Morita T, Kamimura A, Kunisada K, Ito K, Miyazaki S: **Acute endocapillary proliferative glomerulonephritis associated with human parvovirus B19 infection.** *Clin Nephrol* 2002, **57:**246-250.

159. Munoz MG, Witkin SS: **Autoimmunity to spermatozoa, asymptomatic *Chlamydia trachomatis* genital tract infection and gamma delta T lymphocytes in seminal fluid from the male partners of couples with unexplained infertility.** *Hum Reprod* 1995, **10:**1070-1074.

160. Witkin SS, Jeremias J, Grifo JA, Ledger WJ: **Detection of *Chlamydia trachomatis* in semen by the polymerase chain reaction in male members of infertile couples.** *Am J Obstet Gynecol* 1993, **168:**1457-1462.

161. Asadullah K, Prosch S, Audring H, Buttnerova I, Volk HD, Sterry W, Docke WD: **A high prevalence of cytomegalovirus antigenaemia in patients with moderate to severe chronic plaque psoriasis: an association with systemic tumour necrosis factor alpha overexpression.** *Br J Dermatol* 1999, **141:**94-102.

162. Watanabe T, Kawamura T, Jacob SE, Aquilino EA, Orenstein JM, Black JB, Blauvelt A: **Pityriasis rosea is associated with systemic active infection with both human herpesvirus-7 and human herpesvirus-6.** *J Invest Dermatol* 2002, **119:**793-797.

163. Saha K, Sehgal VN, Sharma V: **High incidence of IgG class of Epstein–Barr virus capsid antibody in Indian patients of lepromatous leprosy.** *Trans R Soc Trop Med Hyg* 1982, **76:**311-313.

164. Nalesnik MA: **The diverse pathology of post-transplant lym-** phoproliferative disorders: the importance of a standardized approach. *Transpl Infect Dis* 2001, **3:**88-96.

165. Montone KT, Friedman H, Hodinka RL, Hicks DG, Kant JA, Tomaszewski JE: **In situ hybridization for Epstein–Barr virus NotI repeats in posttransplant lymphoproliferative disorder.** *Mod Pathol* 1992, **5:**292-302.

166. Andersen CB, Ladefoged SD, Lauritsen HK, Hansen PR, Larsen S: **Detection of CMV DNA and CMV antigen in renal allograft biopsies by in situ hybridisation and immunohistochemistry.** *Nephrol Dial Transplant* 1990, **5:**1045-1050.

167. Appleton AL, Sviland L, Peiris JS, Taylor CE, Wilkes J, Green MA, Pearson AD, Proctor SJ, Hamilton PJ, Cant AJ, *et al.*: **Role of target organ infection with cytomegalovirus in the pathogenesis of graft-versus-host disease.** *Bone Marrow Transplant* 1995, **15:**557-561.

168. Barkholt L, Reinholt FP, Teramoto N, Enbom M, Dahl H, Linde A: **Polymerase chain reaction and in situ hybridization of Epstein–Barr virus in liver biopsy specimens facilitate the diagnosis of EBV hepatitis after liver transplantation.** *Transpl Int* 1998, **11:**336-344.

169. Dolstra H, Van de Wiel-van Kemenade E, De Witte T, Preijers F: **Clonal predominance of cytomegalovirus-specific CD8+ cytotoxic T lymphocytes in bone marrow recipients.** *Bone Marrow Transplant* 1996, **18:**339-345.

170. Ljungman P: **Beta-herpesvirus challenges in the transplant recipient.** *J Infect Dis* 2002, **Suppl 1:**S99-S109.

171. Loren AW, Porter DL, Stadtmauer EA, Tsai DE: **Post-transplant lymphoproliferative disorder: a review.** *Bone Marrow Transplant* 2003, **31:**145-155.

172. Dockrell DH, Paya CV: **Human herpesvirus-6 and -7 in transplantation.** *Rev Med Virol* 2001, **11:**23-36.

173. Luppi M, Barozzi P, Rasini V, Torelli G: **HHV-8 infection in the transplantation setting: a concern only for solid organ transplant patients?** *Leuk Lymphoma* 2002, **43:**517-522.

174. Wang X, Huong SM, Chiu ML, Raab-Traub N, Huang ES: **Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus.** *Nature* 2003, **424:**456-461.

175. Santoro F, Kennedy PE, Locatelli G, Malnati MS, Berger EA, Lusso P: **CD46 is a cellular receptor for human herpesvirus 6.** *Cell* 1999, **99:**817-827.

176. Lusso P, Secchiero P, Crowley RW, Garzino-Demo A, Berneman ZN, Gallo RC: **CD4 is a critical component of the receptor for human herpesvirus 7: interference with human immunodeficiency virus.** *Proc Natl Acad Sci USA* 1994, **91:**3872-3876.

177. Secchiero P, Sun D, De Vico AL, Crowley RW, Reitz MS Jr, Zauli G, Lusso P, Gallo RC: **Role of the extracellular domain of human herpesvirus 7 glycoprotein B in virus binding to cell surface heparan sulfate proteoglycans.** *J Virol* 1997, **71:**4571-4580.

178. Wang FZ, Akula SM, Pramod NP, Zeng L, Chandran B: **Human herpesvirus 8 envelope glycoprotein K8.1A interaction with the target cells involves heparan sulfate.** *J Virol* 2003, **75:**7517-7527.

179. Su H, Raymond L, Rockey DD, Fischer E, Hackstadt T, Caldwell HD: **A recombinant *Chlamydia trachomatis* major outer membrane protein binds to heparan sulfate receptors on epithelial cells.** *Proc Natl Acad Sci USA* 1996, **93:**11143-11148.