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Unscrambling the role of human parvovirus B19 signaling in systemic autoimmunity

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ANCA, antibody to neutrophil cytoplasmic antigens
β2GPI, β2 glycoprotein I
CL, cardiolipin
B19, parvovirus B19

ABSTRACT

Despite enormous progress in understanding how the immune system works, the pathogenesis of autoimmune diseases still remains unclear. Growing evidence indicates that infectious agents can be potent initial triggers, subverting and exploiting host cell signaling pathways. This role is exemplified by the association of parvovirus B19 (B19) with human autoimmune disease. Infection with this common virus exhibits striking similarities with systemic autoimmune diseases, and can be associated with elevated serum autoantibody titers. The B19 virus produces proline-rich, 11-kDa proteins that have been implicated in modulation of host signaling cascades involved in virulence and pathogenesis. Additionally, B19 produces a non-structural protein (NS1) that functions as a transcription regulator by directly binding the p6 promoter and the Sp1/Sp3 transcription factors. The protein is also involved in DNA replication, cell cycle arrest and initiation of apoptotic damage, particularly in erythroid cells. When transfected to non-permissive cells, NS1 recruits the mitochondria cell death pathway. It is even more remarkable that NS1 functions as a *trans*-acting transcription activator for the IL6 promoter, up-regulating IL6 expression in host cells. Hence, B19 infection may play a pivotal role in triggering inflammatory disorders. By promoting apoptotic damage and *trans*-activating pro-inflammatory cytokine promoters, B19 may break the delicate balance between cell survival and apoptosis, and may contribute to immune deregulation. Understanding the mechanisms used by B19 to alter the cell signaling machinery may provide further insight into the mechanism by which autoimmune diseases develop.

1. Introduction

The breakdown of tolerance to self-antigens is a common feature to autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjögren's syndrome. The resulting autoimmune responses attack various organs, resulting in tissue damage. Accumulating data suggests that such a tolerance break may be attributed to a number of inherited and/or acquired factors [1,2]. For a number of years, the role of infectious agents has been extensively investigated, and several potential pathogenic mechanisms have been proposed. Here, we discuss the potential role of a common virus that makes use of some unique strategies to alter signaling pathways in host cells, and impairs immune functions, with important implications towards our understanding of the

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induction and perpetuation of autoimmune phenomena in humans.

2. Human B19 parvovirus, a common infectious agent

Parvovirus B19 (B19) is the only member of the Parvoviridea family known to be pathogenic in humans [3]. It is a small non-enveloped icosahedral virus with a single-stranded linear 5.6-kb DNA genome. The only functionally active promoter within the viral genome, the p6 promoter, regulates the synthesis of a non-structural protein, (called NS1), two capsid proteins (VP1 and VP2, that are encoded by overlapping reading frames) and several smaller polypeptides with still unknown functions. VP1 contains a domain that interacts with the cellular receptor, and VP2 is involved in recognition by neutralizing antibodies.

Like that of other non-enveloped DNA viruses, the life cycle of B19 includes binding to host cell receptors, internalization, translocation of the genome to the host nucleus, DNA replication, RNA transcription, assembly of capsids and packaging of the genome, and, finally, cell lysis with release of mature virions [3]. The virus targets erythroid cells and uses as host cell receptor a glycolipid globoside, also known as the blood group P antigen [4]. Consistent with the observed tropism of B19, the P antigen is expressed on erythroid progenitors. However, the presence of P antigen is almost certainly not sufficient to explain the tropism of B19 to erythroid cells. In fact, P antigen is also present on cells that are not permissive for B19 replication, including megakaryocytes, endothelial cells and fetal myocytes. In addition, the level of P antigen expression does not correlate with the efficiency of viral binding, providing further evidence for the existence of a putative cellular co-receptor for efficient entry of B19 into human cells [5]. In addition to erythroid cells, B19 virus has been shown to infect and persist in both B and T cells [6].

During the course of infection, the patient develops specific immune reactions against B19 viral proteins [3]. Acute infections are initially characterized by a viremic phase which has its onset about 6 days after infection, reaches concentrations of 10^{10}–10^{13} particles per milliliter of blood, and persists for about 1 week. Viremia declines with the appearance of VP1- and VP2-specific IgM antibodies that appear approximately 9–12 days after infection, and are the first detectable immune reactions that indicate an acute B19 infection. Peak levels of IgM are reached during the third week after infection, decline rapidly with time, and become undetectable after 6–10 weeks. With the decrease of B19-specific IgM, antibodies of the IgG isotype become detectable. At this stage, the rash typical for parvovirus B19 infection may be observed. Approximately 4–6 months after infection, antibodies specific for linear VP2 epitopes begin to decline, whereas IgG against conformational epitopes persist together with VP1-specific antibodies. Not surprisingly, the majority of parvovirus B19 neutralizing antibodies that impart life-long protection against re-infection are part of this IgG subfraction. With time, VP2-specific antibodies may further decline after B19 infection, and, eventually, become undetectable. A high percentage of persistently infected patients have been reported to harbor NS1-specific IgGs antibodies that, in most cases, first become detectable about 4 weeks after infection and about 2 weeks after the VP1/VP2-specific immune response.

As in other viral diseases, a compromised immune system favors persistence of B19 infection. Thus, in immunocompetent hosts, specific IgG antibodies appear several days following IgM and in most cases persist for years. Their presence is a serological marker of past infection. In general, presence of IgG conveys protective immunity, but re-infections have been described in the presence of a low level of B19-specific IgG. Reversibly, persistent parvovirus B19 infections have been shown to be frequent in immunodeficient subjects.

3. Human B19 parvovirus-associated autoimmunity

B19 is a global and common infectious pathogen in humans. Its transmission occurs via the respiratory route, through blood-derived products administered parenterally, and vertically from mother to fetus. Generally, B19 infection is without any clinical symptoms or may result in a flu-like disease. It can also result in a variety of manifestations, including erythema infectiosum (particularly in children), hydrops fetalis, fetal or congenital anemia, abortion, thrombocytopenia, transient erythroblastopenia of childhood, neurologic disease and hepatitis.

In 1985, an association between B19 infection and arthropathies was made [7,8]. In children with erythema infectiosum, the incidence of arthralgia is approximately 10%. In adults, arthralgia and arthritis are the most common manifestations of primary B19 infection, affecting 60% of females and 30% of males. Remarkably, the onset of joint manifestations coincides with the appearance of circulating antibodies, suggesting that the arthropathy is immune-mediated. The symptoms appear as an acute, moderately severe polyarthritis, and about 50% of patients with chronic B19 arthropathy meet the criteria of the American College of Rheumatology for RA [9].

The ability of B19 to cause sub-acute or chronic polyarthritis mimicking early RA raises the possibility that this virus may be among the causes of RA, and it has been postulated that B19 is involved in the initiation and perpetuation of RA [6]. Consistently, B19 DNA can be detected in synovial fluid [10], cells [11] and synovial biopsy specimens [12] of affected joints. In addition to RA, B19 infection has been associated with a variety of autoimmune diseases [13], including juvenile idiopathic arthritis [14], SLE [15,16], reactive arthritis [11], Lyme disease [17], Sjögren's syndrome, primary biliary cirrhosis, polymyositis, dermatomyositis, autoimmune neutropenia, immune thrombocytopenia, vasculitis [18] and autoimmune hemolytic anemia [19–21]. Significantly, B19 infection has been associated with elevated levels of autoantibodies to nuclear antigens (e.g. ANA), double-stranded DNA, ANCA, mitochondrial antigen, smooth muscle, gastric parietal antigen, and phospholipids and CL [20,22].

As discussed above, sera from persistently infected patients show a strong specific antibody response to NS-1. Specific antibodies can be detected in sera from patients suffering from severe parvovirus B19-associated arthritis

using Western blot analysis and an ELISA. In one study, patients were followed for 3–18 months, during which IgM titers declined, but IgG directed to the nonstructural protein remained detectable [23]. This has led to the proposal that NS1-specific IgGs are associated with an altered course of disease, and it has been argued that this antibody subset is primarily found in patients with arthritis or persistent B19 infection [23]. Accordingly, prolonged viremia may lead to infection of cells that are outside the erythroid lineage, and that are not permissive. Such infection would shift gene expression towards the preferential transcription of the *NS1* gene, rather than the *VP1* and *VP2* genes. The cytotoxic and apoptotic effects of NS1 may result in cell lysis and release of NS1 protein, thereby rendering this non-structural viral component accessible to the host's immune system. However, in another study, NS1-specific antibodies were present frequently in parvovirus B19 infected individuals, contradicting claims that B19 NS1 IgGs are detected primarily in patients with arthralgia or chronic infection [24].

Epidemiological evidence also indicates that B19 is associated with arthritis. Based on examination of writings, paintings [25] and skeletons [26,27], it has been suggested that arthritis is a relatively new disease in Europe, where it appeared after the return of the explorers from the new world toward the end of the 15th century, shortly after the first contacts with the American continent. By contrast, arthritis has existed in North America for several thousands of years in the pre-Columbian era [28]. Since the first description of a disease compatible with erythema infectiosum was traced back to 1797 [29], B19 was, at this time, probably a new virus to Europe. These observations are consistent with a role for the virus in the pathogenesis of arthritis.

4. Potential mechanisms of autoantibody induction by the B19 parvovirus

Although the association between infection and autoimmune disease is well known, there is little evidence for a specific mechanistic link [30–33]. Naturally, the produced autoantibodies can play a role in tissue injury by a variety of mechanisms [34]. For instance, peripheral nerve abnormalities coincide with the detection of anti-B19 IgG [35]. Rash and joint symptoms are known to occur in chronically infected subjects treated with Ig [36]. In volunteer studies, appearance of rash and joint symptoms was concomitant with disappearance of vireamia and appearance of specific IgG [37]. These observations suggest an immune mediated mechanism.

As for other autoimmune diseases, molecular mimicry has been suggested to be an underlying mechanism. It proposes that the widespread cellular damage inflicted by infection might expose hidden self-antigens to auto-reactive lymphocytes. In this view, cross-reactive epitopes bind disease-associated HLA class II molecules, leading to a T cell-mediated immune response and resultant autoimmune damage. Consistently, it has been shown that in patients with skin rashes, RA and chronic B19 arthritis, affinity-purified anti-VP1 IgG react with human keratin, collagen type II, denatured DNA and CL [38]. Another more recent hypothesis stems from observations showing that damaged DNA can trigger an innate response. Specifically, it has been demonstrated that DNA damage initiates a cellular signaling pathway that alerts the immune system to the presence of potentially dangerous cells [39]. It is possible that this mechanism also operates to trigger an immune response to B19 parvovirus-infected cells.

There are also indications that the pathogenic role of B19 is, at least in part, due to its propensity to directly trigger apoptotic damage. In one study, an experimental in vitro system was established in which healthy primary human synovial fibroblasts were treated with or without B19-containing human sera and, then, tested for their ability to degrade reconstituted cartilage matrix [40]. Incubation with B19 induced an invasive phenotype in fibroblasts, and pre-incubation of viremic serum with a neutralizing antibody to B19 eliminated the observed effect.

5. B19 parvovirus-induced apoptotic degradation by transcriptional regulation

Since apoptosis is an active process of programmed cell death that regulates embryonic development and maintenance of homeostasis of multicellular organisms, it is important to discuss its potential role in B19-associated autoimmune phenomena. As with other viruses, infection triggers apoptosis of host cells, which can limit virus production and can serve as a defense mechanism of the host against intracellular microbes. Alternatively, apoptosis can contribute to viral pathogenesis and, possibly, to autoimmune diseases. In SLE, for example, there is an accelerated apoptosis [41] and the repertoire of autoantigens includes apoptotic bodies that may serve as a stimulator of an autoimmune response in predisposed subjects [42–44].

The mechanisms leading to induction and progression of apoptosis are complex and include extra-cellular stimuli, intra-cellular signals, and cleavage of proteins. Ultimately, there is migration of intra-cellular components to the cell membrane and phagocytosis of apoptotic cells by macrophages. For B19, induction of apoptotic damage seems to be directly related to the cytotoxicity of the NS1 protein, particularly to erythroid cells [45]. It could account for thrombocytopenia, leucopenia and arthritis. NS1 has been reported to function as a transcription regulator that acts by directly binding the p6 promoter and the Sp1/Sp3 transcription factors [46]. It has also been shown to be involved in DNA replication, cell cycle arrest and the initiation of apoptosis in erythroid lineage cells [45,46]. Additionally, NS1 has been identified to play a critical role in G1 arrest by significantly increasing expression of $p21^{WAF1/CIP1}$, a possible prerequisite to apoptotic damage in cells of the erythroid lineage [47].

6. NS1-induction of apoptosis through the mitochondria cell death pathway

Studies of cell death revealed that apoptosis involves activation of cysteine proteases, called caspases, present in most cell types in an inactive form. Upon activation, caspases function as effectors, or executioners, cleaving substrates at aspartic acid residues and leading to nuclear fragmentation and other

apoptotic changes [48]. If cells are deprived of necessary survival stimuli, there is a rapid increase in the permeability of mitochondrial membranes and release of several proteins, including cytochrome *c*, into the cytoplasm. This latter molecule functions as a cofactor with Apaf-1 to activate caspase-9 that initiates the apoptotic pathway. Other proteins released from the mitochondria may directly block the normal anti-apoptotic activities of Bcl family members, including Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and A-1.

It is intriguing that, in addition to erythroid lineage cells, human B19 has been found in various tissues. To understand the mechanism of its potential cytotoxicity in non-permissive cells, a construct of the *NS1* gene was made in a cytomegalovirus episomal vector (pEGFP-C1) and transfected into monkey epithelial cells (COS-7). Then, EGFP-NS1 expression was assessed in transfected cells by fluorescence microscopy, RT-PCR and Western blotting. Flow cytometry showed that NS1-transfected cells were arrested at the G1 phase by paclitaxel (a natural anti-tumor molecule) treatment, and apoptosis was increased. In addition, expression of p53 (an important molecule in apoptosis and cell cycle regulation), and its downstream cell cycle kinase inhibitors (p16INK4 and p21WAF1uCIP1) were up-regulated in NS1-transfected cells. There was also increased expression of the pro-apoptotic Bcl-2 family members Bax and Bad, and activation of caspase-3 and caspase-9, but not caspase-8 or Fas. Since activation of caspase-9 was suppressed by a p53 inhibitor (pifithrin-α hydrobromide), and because apoptosis was significantly inhibited by a caspase-9 inhibitor (Z-LEHD-FMK), the apoptotic pathway triggered by *NS1* transfection probably involves p53-induced Bax expression and subsequent activation of caspase-9. These results indicate that cell death of the NS1-transfected cells is associated with mitochondria-related apoptosis, suggesting that B19 infection of non-permissive cells may recruit the mitochondria cell death pathway. While the role of this viral cytopathic effect in the pathogenesis of B19-associated organ involvement remains unclear, these observations provide useful information to further characterize the role of B19 NS1 protein in B19 non-permissive cells [49].

7. Modulation of host normal signaling cascades by parvovirus B19

The small 11-kDa proteins of B19 parvovirus contain three proline-rich regions that conform to the consensus Src homology 3 (SH3) ligand sequences present in signaling molecules within the cell. It has been shown that these proteins specifically interact with the growth factor receptor-binding protein 2 (Grb2) in vitro [50], suggesting a possible mechanism of B19 to manipulate the host cell environment by modulating signaling pathways. This could, for example, affect down-stream signaling events, including those stemming from mitogenic stimuli. Other examples of virally encoded non-structural proteins that interact with SH3 domain proteins and/or disrupt mitogenic signaling include the hepatitis C virus NS5A protein [51,52] and the HIV-1 Nef protein [53,54]. These two latter proteins have been implicated in modulation of host signaling cascades involved in virulence and pathogenesis. The B19 parvovirus 11-kDa proteins may function in a similar way to enhance viral replication and propagation.

8. *Trans*-activation of host cell genes by NS1 of human parvovirus B19

In addition to being an activator of the viral p6 promoter, the NS1 protein can transactivate cellular promoters. Transfection of human hematopoietic cell lines with DNA encoding the NS1 protein induces secretion of IL-6, and the NS1 effect is mediated by the NF-κB site in the IL-6 promoter region [55,56]. This up-regulation of IL-6 transcription was specific, and TNF-α, IL-1β or IL-8 were not affected [55]. This observation implies that NS1 functions as a *trans*-acting transcription activator on the IL-6 promoter.

In further studies, the expression profiles of cytokines and chemokines were examined in B19 *NS1*-transfected epithelial cells [57]. A construct of the *NS1* gene in the pEGFP-C1 vector, called EGFP-NS1, was prepared, and COS-7 cells were transfected with either EGFP or EGFP-NS1 plasmids. Using ELISA or RT-PCR, the expression of IL-1, IL-5, IL-6, IL-10, TNF-α, TGF-β, GM-CSF, IL-8 (CXCL8), GRO-α (CXCL1), IP-10 (CXCL10), SDF-1 (CXCL12), MIP-1α (CCL3), MCP-1 (CCL2) and its receptor (CCR2), RANTES and its receptor (CCR5), the chemokine fractalkine (FKN) and its receptor (CX3CR1), and CCR11 (that recognizes members of the MCP family) were examined. Expression levels of IL-6, but not those of the other cytokines and chemokines tested, were increased in EGFP-NS1 transfected cells. Naturally, the cytokines and chemokines induced in epithelial cells are not representative of all cells, and other cells might very well induce some of these factors in response to the viral protein. Nonetheless, these results suggest that increased expression and secretion of IL-6 in B19 NS1-transfected epithelial cells may play a role in the pathogenesis of autoimmune diseases, in particular arthropathies. In as much as IL-6 can stimulate polyclonal B cell activation [58], these findings provide a mechanism that may account for the B cell activation in RA. The elevation of IL-6 production via the viral transactivator protein NS1 may contribute to the production of autoantibodies. Consistently, elevated IL-6 levels have been found in infants with B19 infection and lymphocytic myocarditis [59]. It is also remarkable that IL-6 secretion is known to be upregulated at the transcription level in rheumatoid synoviocytes [60].

Since, hematopoietic cells, but also epithelial cells, transfected with B19 *NS1* show increased expression and secretion of IL-6, it is likely that a wide variety of permissive and non-permissive cells can secrete IL-6 when B19 *NS1* is present. In fact, P antigen, the B19 cell receptor, is present on several human cells, including lymphocytes [61].

Further studies disclosed that the viral NS1 protein can transactivate the promoter controlling expression of TNF-α [62], a cytokine that plays a role in RA joint erosion. Consistently, elevated levels of TNF-α have been shown to be present in patients during the acute and convalescent phases of B19 infection [63]. Thus, the propensity of B19 to upregulate IL6 and TNF-α expression in host cells [55] suggests that it may alter immunity of the host. Through NS1 expression, B19 may contribute to immune deregulation

and induce B19-associated disorders. Hence, NS1 transactivation of proinflammatory cytokine promoters may be pivotal in triggering the various inflammatory disorders. The results suggest that, by inducing host cell genes, B19 NS1 may play a role in cytokine modulation, in clinical manifestations of B19 NS1 infection, and in the pathogenesis of autoimmune disease [55].

It must be emphasized that *trans*-activation of host cells is not unique to B19. For example, HTLV-1 tax protein and HIV-1 tat protein also *trans*-activate IL-6 production [64,65]. HIV-1 is known to cause a chronic inflammatory arthropathy in humans [66] and HTLV-1 tax protein leads to overgrowth of human synovial cells [67].

9. Conclusions

The observations discussed above and elsewhere [68,69] indicate that studying the interactions between infectious agents and the immune system often provides clues to our understanding of the immune defense mechanisms. As regards human B19 parvovirus, this pathogen is common and widespread, and causes several distinct clinical manifestations. A significant number of reports clearly implicate B19 in the pathogenesis of certain cases of rheumatic diseases, such as RA, juvenile idiopathic arthritis, SLE and vasculitis. The fact that viral proteins can *trans*-activate host cell genes may have considerable significance in the proposed link between B19 infection and autoimmunity. This could represent a strategy used by the virus to subvert immune functions and to persist in the host. In addition to providing insight into pathogen–host interactions [70], continued investigation of the pathogenesis of B19 parvovirus infections and arthritis may further our understanding of autoimmune diseases.

REFERENCES

[1] Rose NR. Mechanisms of autoimmunity. Semin Liver Dis 2002;22:387–94.
[2] Zouali M, editor. Molecular autoimmunity. New York: Springer Science/Business Media, Inc.; 2005. p. 437.
[3] Heegaard ED, Brown KE. Human parvovirus B19. Clin Microbiol Rev 2002;15:485–505.
[4] Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. Science 1993;262:114–7.
[5] Weigel-Kelley KA, Yoder MC, Srivastava A. Recombinant human parvovirus B19 vectors: erythrocyte P antigen is necessary but not sufficient for successful transduction of human hematopoietic cells. J Virol 2001;75:4110–6.
[6] Takahashi Y, Murai C, Shibata S, Munakata Y, Ishii T, Ishii K, et al. Human parvovirus B19 as a causative agent for rheumatoid arthritis. Proc Natl Acad Sci USA 1998;95:8227–32.
[7] White DG, Woolf AD, Mortimer PP, Cohen BJ, Blake DR, Bacon PA. Human parvovirus arthropathy. Lancet 1985;1:419–21.
[8] Reid DM, Reid TM, Brown T, Rennie JA, Eastmond CJ. Human parvovirus-associated arthritis: a clinical and laboratory description. Lancet 1985;1:422–5.
[9] Naides SJ. Rheumatic manifestations of parvovirus B19 infection. Rheum Dis Clin North Am 1998;24:375–401.
[10] Dijkmans BA, van Elsacker-Niele AM, Salimans MM, van Albada-Kuipers GA, de Vries E, Weiland HT. Human parvovirus B19 DNA in synovial fluid. Arthritis Rheum 1988;31:279–81.
[11] Kandolf R, Kirschner P, Hofschneider PH, Vischer TL. Detection of parvovirus in a patient with ''reactive arthritis'' by in situ hybridization. Clin Rheumatol 1989;8:398–401.
[12] 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;12:147–51.
[13] Vigeant P, Menard HA, Boire G. Chronic modulation of the autoimmune response following parvovirus B19 infection. J Rheumatol 1994;21:1165–7.
[14] Rodrigo de Liria C, Mendez M, Olive A, Junca J, Prats J, Tena X. Parvovirus infection mimicking a systematic onset of juvenile chronic arthritis (Still's disease). Clin Exp Rheumatol 1996;14:105–6.
[15] Nesher G, Rubinow A, Sonnenblick M. Efficacy and adverse effects of different corticosteroid dose regimens in temporal arthritis: a retrospective study. Clin Exp Rheumatol 1997;15:303–6.
[16] Trapani S, Ermini M, Falcini F. Human parvovirus B19 infection: its relationship with systemic lupus erythematosus. Semin Arthritis Rheum 1999;28:319–25.
[17] Mayo DR, Vance Jr DW. Parvovirus B19 as the cause of a syndrome resembling Lyme arthritis in adults. N Engl J Med 1991;324:419–20.
[18] Finkel TH, Torok TJ, Ferguson PJ, Durigon EL, Zaki SR, Leung DY, et al. Chronic parvovirus B19 infection and systemic necrotising vasculitis: opportunistic infection or aetiological agent? Lancet 1994;343:1255–8.
[19] Kerr JR. Pathogenesis of human parvovirus B19 in rheumatic disease. Ann Rheum Dis 2000;59:672–83.
[20] Meyer O. Parvovirus B19 and autoimmune diseases. Joint Bone Spine 2003;70:6–11.
[21] Lehmann HW, von Landenberg P, Modrow S. Parvovirus B19 infection and autoimmune disease. Autoimmun Rev 2003;2:218–23.
[22] Chou CC, Chen YN, Li CS. Congener-specific polychlorinated biphenyls in cetaceans from Taiwan waters. Arch Environ Contam Toxicol 2004;47:551–60.
[23] von Poblotzki A, Hemauer A, Gigler A, Puchhammer-Stockl E, Heinz FX, Pont J, et al. Antibodies to the nonstructural protein of parvovirus B19 in persistently infected patients: implications for pathogenesis. J Infect Dis 1995;172:1356–9.
[24] Heegaard ED, Rasksen CJ, Christensen J. Detection of parvovirus B19 NS1-specific antibodies by ELISA and western blotting employing recombinant NS1 protein as antigen. J Med Virol 2002;67:375–83.
[25] Short CL. The antiquity of rheumatoid arthritis. Arthritis Rheum 1974;17:193–205.
[26] Rothschild BM, Turner KR, DeLuca MA. Symmetrical erosive peripheral polyarthritis in the Late Archaic Period of Alabama. Science 1988;241:1498–501.
[27] Rothschild BM, Woods RJ, Rothschild C, Sebes JI. Geographic distribution of rheumatoid arthritis in ancient North America: implications for pathogenesis. Semin Arthritis Rheum 1992;22:181–7.
[28] Altschuler EL. Parvovirus B19 and the pathogenesis of rheumatoid arthritis: a case for historical reasoning. Lancet 1999;354:1026–7.
[29] Chorba T, Anderson LJ. Erythema infectiosum (fifth disease). Clin Dermatol 1989;7:65–74.
[30] Hasler P, Zouali M. B cell receptor signaling and autoimmunity. FASEB J 2001;15:2085–98.

[31] Zouali M, Sarmay G. B lymphocyte signaling pathways in systemic autoimmunity: implications for pathogenesis and treatment. Arthritis Rheum 2004;50:2730–41.
[32] Hasler P, Zouali M. Immune receptor signaling, aging, and autoimmunity. Cell Immunol 2005;233:102–8.
[33] Peeva E, Zouali M. Spotlight on the role of hormonal factors in the emergence of autoreactive B-lymphocytes. Immunol Lett 2005;101:123–43.
[34] Lim P, Zouali M. Pathogenic autoantibodies: emerging insights into tissue injury. Immunol Lett 2006;103:17–26.
[35] Walsh KJ, Armstrong RD, Turner AM. Brachial plexus neuropathy associated with human parvovirus infection. Br Med J (Clin Res Ed) 1988;296:896.
[36] Frickhofen N, Abkowitz JL, Safford M, Berry JM, Antunez-de-Mayolo J, Astrow A, et al. Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. Ann Intern Med 1990;113:926–33.
[37] Anderson MJ, Higgins PG, Davis LR, Willman JS, Jones SE, Kidd IM, et al. Experimental parvoviral infection in humans. J Infect Dis 1985;152:257–65.
[38] Lunardi C, Tiso M, Borgato L, Nanni L, Millo R, De Sandre G, et al. Chronic parvovirus B19 infection induces the production of anti-virus antibodies with autoantigen binding properties. Eur J Immunol 1998;28:936–48.
[39] Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature 2005;436:1186–90.
[40] Ray NB, Nieva DR, Seftor EA, Khalkhali-Ellis Z, Naides SJ. Induction of an invasive phenotype by human parvovirus B19 in normal human synovial fibroblasts. Arthritis Rheum 2001;44:1582–6.
[41] Emlen W, Niebur J, Kadera R. Accelerated in vitro apoptosis of lymphocytes from patients with systemic lupus erythematosus. J Immunol 1994;152: 3685–92.
[42] Huck S, Deveaud E, Namane A, Zouali M. Abnormal DNA methylation and deoxycytosine–deoxyguanine content in nucleosomes from lymphocytes undergoing apoptosis. FASEB J 1999;13:1415–22.
[43] Casciola-Rosen L, Andrade F, Ulanet D, Wong WB, Rosen A. Cleavage by granzyme B is strongly predictive of autoantigen status: implications for initiation of autoimmunity. J Exp Med 1999;190:815–26.
[44] Zouali M. Taming lupus. Sci Am 2005;292:58–65.
[45] Ozawa K, Ayub J, Kajigaya S, Shimada T, Young N. The gene encoding the nonstructural protein of B19 (human) parvovirus may be lethal in transfected cells. J Virol 1988;62:2884–9.
[46] Raab U, Beckenlehner K, Lowin T, Niller HH, Doyle S, Modrow S. NS1 protein of parvovirus B19 interacts directly with DNA sequences of the p6 promoter and with the cellular transcription factors Sp1/Sp3. Virology 2002;293: 86–93.
[47] Gartel AL, Tyner AL. The role of the cyclin-dependent kinase inhibitor p21 in apoptosis. Mol Cancer Ther 2002;1:639–49.
[48] Pinkoski MJ, Waterhouse NJ, Green DR. Mitochondria apoptosis and autoimmunity. Curr Dir Autoimmun 2006;9:55–73.
[49] Hsu TC, Wu WJ, Chen MC, Tsay GJ. Human parvovirus B19 non-structural protein (NS1) induces apoptosis through mitochondria cell death pathway in COS-7 cells. Scand J Infect Dis 2004;36:570–7.
[50] Fan MM, Tamburic L, Shippam-Brett C, Zagrodney DB, Astell CR. The small 11-kDa protein from B19 parvovirus binds growth factor receptor-binding protein 2 in vitro in a Src homology 3 domain/ligand-dependent manner. Virology 2001;291:285–91.
[51] Tan SL, Nakao H, He Y, Vijaysri S, Neddermann P, Jacobs BL, et al. NS5A a nonstructural protein of hepatitis C virus, binds growth factor receptor-bound protein 2 adaptor protein in a Src homology 3 domain/ligand-dependent manner and perturbs mitogenic signaling. Proc Natl Acad Sci USA 1999;96:5533–8.
[52] Macdonald A, Crowder K, Street A, McCormick C, Harris M. The hepatitis C virus NS5A protein binds to members of the Src family of tyrosine kinases and regulates kinase activity. J Gen Virol 2004;85:721–9.
[53] Saksela K, Cheng G, Baltimore D. Proline-rich (PxxP) motifs in HIV-1 Nef bind to SH3 domains of a subset of Src kinases and are required for the enhanced growth of Nef + viruses but not for down-regulation of CD4. EMBO J 1995;14:484–91.
[54] Manninen A, Hiipakka M, Vihinen M, Lu W, Mayer BJ, Saksela K. SH3-Domain binding function of HIV-1 Nef is required for association with a PAK-related kinase. Virology 1998;250:273–82.
[55] Moffatt S, Tanaka N, Tada K, Nose M, Nakamura M, Muraoka O, et al. A cytotoxic nonstructural protein, NS1, of human parvovirus B19 induces activation of interleukin-6 gene expression. J Virol 1996;70:8485–91.
[56] Mitchell LA. Parvovirus B19 nonstructural (NS1) protein as a transactivator of interleukin-6 synthesis: common pathway in inflammatory sequelae of human parvovirus infections? J Med Virol 2002;14:267–74.
[57] Hsu TC, Tzang BS, Huang CN, Lee YJ, Liu GY, Chen MC, et al. Increased expression and secretion of IL-6 in human parvovirus B19 nonstructural protein (NS1) transfected COS-7 epithelial cells. Clin Exp Immunol 2006;144:152–7.
[58] Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 2003;374:1–20.
[59] Nigro G, Bastianon V, Colloridi V, Ventriglia F, Gallo P, D'Amati G, et al. Human parvovirus B19 infection in infancy associated with acute and chronic lymphocytic myocarditis and high cytokine levels: report of 3 cases and review. Clin Infect Dis 2000;31:65–9.
[60] Miyazawa K, Mori A, Okudaira H. IL-6 synthesis by rheumatoid synoviocytes is autonomously upregulated at the transcriptional level. J Allergy Clin Immunol 1999;103:S437–44.
[61] Cooling LL, Koerner TA, Naides SJ. Multiple glycosphingolipids determine the tissue tropism of parvovirus B19. J Infect Dis 1995;172:1198–205.
[62] Fu Y, Ishii KK, Munakata Y, Saitoh T, Kaku M, Sasaki T. Regulation of tumor necrosis factor alpha promoter by human parvovirus B19 NS1 through activation of AP-1 and AP-2. J Virol 2002;76:5395–403.
[63] Kerr JR, Barah F, Mattey DL, Laing I, Hopkins SJ, Hutchinson IV, et al. Circulating tumour necrosis factor-alpha and interferon-gamma are detectable during acute and convalescent parvovirus B19 infection and are associated with prolonged and chronic fatigue. J Gen Virol 2001;82:3011–9.
[64] Hall WW. Human T cell lymphotropic virus type I and cutaneous T cell leukemia/lymphoma. J Exp Med 1994;180:1581–5.
[65] Scala G, Ruocco MR, Ambrosino C, Mallardo M, Giordano V, Baldassarre F, et al. The expression of the interleukin 6 gene is induced by the human immunodeficiency virus 1 TAT protein. J Exp Med 1994;179:961–71.
[66] Nishioka K, Maruyama I, Sato K, Kitajima I, Nakajima Y, Osame M. Chronic inflammatory arthropathy associated with HTLV-I. Lancet 1989;1:441.

[67] Nakajima T, Aono H, Hasunuma T, Yamamoto K, Maruyama I, Nosaka T, et al. Overgrowth of human synovial cells driven by the human T cell leukemia virus type I tax gene. J Clin Invest 1993;92:186–93.
[68] Viau M, Longo NS, Lipsky PE, Bjorck L, Zouali M. Specific in vivo deletion of B-cell subpopulations expressing human immunoglobulins by the B-cell superantigen protein L. Infect Immun 2004;72:3515–23.
[69] Viau M, Longo NS, Lipsky PE, Zouali M. Staphylococcal protein a deletes B-1a and marginal zone B lymphocytes expressing human immunoglobulins: an immune evasion mechanism. J Immunol 2005;175:7719–27.
[70] Hasler P, Zouali M. Subversion of B lymphocyte signaling by infectious agents. Genes Immun 2003;4: 95–103.