

## Invited Review

# Role of Human Parvovirus B19 in the Pathogenesis of Rheumatoid Arthritis

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**SUMMARY:** Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown origin. It has been speculated that infectious agents are responsible for triggering RA. Persistent infection with human parvovirus B19 and its induction of immunopathology are hypothesized to explain the initiation and perpetuation of the disease process.

### 1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease which affects approximately 1% of the world population. RA is characterized by hyperplastic synovial membranes in joints with pronounced infiltration of macrophages and leukocytes and progressive destruction of cartilage and bone after invasion by inflammatory pannus (1).

In the initiation of the pathological process of RA, unknown antigen(s), perhaps derived from infectious agents, is supposed to be responsible for inducing the activation of immune cells in rheumatoid synovium. Synovial macrophages produce significant amounts of cytokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin-1 (IL-1), IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF), platelet-derived growth factor (PDGF), IL-8, and monocyte chemoattractant protein-1 (MCP-1) (2-5). Tissue-damaging proteases including collagenase (matrix metalloproteinase-1, MMP-1), gelatinase (MMP-2), and stromelysin (MMP-3) are also produced by macrophages at the cartilage-pannus junction (6-8). Macrophage-derived TNF $\alpha$  and IL-1 further activate the fibroblasts and lead to the release of cytokines, MMPs, and growth factors including fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) (9-11). Cytokines produced by macrophages and fibroblasts induce angiogenesis (e.g., TNF $\alpha$ , IL-8, FGF, and VEGF), the expression of adhesion molecules (e.g., TNF $\alpha$  and IL-1), and chemotactic stimulation to attract circulating cells (e.g., IL-8 and MCP-1) (12). A complex paracrine and autocrine network of cytokines working through the interaction between synovial macrophages and fibroblasts perpetuates and expands the inflammation, leading to joint destruction (13).

The specific antigen(s) that activates the immune response in immunogenetically susceptible hosts has not been isolated. Bacteria and viruses have been suggested as pathogens that initiate RA. The involvement of bacteria in the etiology of RA was based on findings including the efficacy of some antimicrobial agents in RA, alterations in the interstitial flora

of the patients with RA, and the detection of humoral or cellular immune responses against certain bacteria in patients with RA (14). Currently, polymerase chain reaction (PCR), a suitable technique for detecting organisms present at low concentrations or in a non-cultivable state, has been used to search for candidate pathogens. This technique, however, has yielded negative results for *Mycobacteria* sp. and variable results for *Mycoplasma* sp. and *Chlamydia* sp. (15). Human T cell lymphotropic virus type 1 (HTLV-1) was implicated in chronic joint inflammation. Viral mRNA and proteins were detected in the synovial cells from patients infected with HTLV-1 (16) and the development of a form of arthritis was observed in mice transgenic for the Tax protein of HTLV-1 (17). Epstein-Barr virus (EBV) was also demonstrated to express EBV-encoded small RNA (EBER) and latent membrane protein-1 (LMP-1) in synovial lining cells from patients with RA (18). The expression of viral products in synovial cells can be a trigger of inflammation.

Human parvovirus B19 causes arthropathy, which resembles RA (19, 20). In our study, a typical RA developed in 3 out of 12 patients with parvovirus arthritis (21). DNA and a capsid protein of parvovirus B19 were detected in the synovial tissue and bone marrow while arthritis was present. Treatment of patients with a high dose of immunoglobulin was effective in improving their symptoms and reducing signals of the virus. We further found that the target cells of parvovirus B19 were immune cells in the synovium and bone marrow, B19 in the synovial membrane was infectious, and infection with B19 stimulated the immune cells to produce TNF $\alpha$  and IL-6 (22). Based on these findings, we will discuss a mechanism that explains how human parvovirus B19 function in RA pathology.

### 2. Parvovirus B19 and rheumatoid arthritis

Parvovirus B19 is a small virus, classified in the genus, *Erythrovirus* in the *Parvoviridae* family. The genome of parvovirus B19 is about 5.6 kb of single-stranded DNA. Parvovirus B19 encodes non-structural protein NS1, capsid proteins VP1 and VP2, and other small proteins. All the transcripts are transcribed from the unique promoter P6.

Parvovirus B19 is the only parvovirus known to be pathogenic in humans. The virus was first found in serum from asymptomatic blood donors. B19 is now known to cause a wide variety of clinical symptoms, including erythema infectiosum in children (23), arthralgia/arthritis in adults

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(19,24), transient aplastic crisis in persons with underlying hemolysis (reviewed in 25), nonimmune hydrops fetalis by infection in utero (26), and pure red cell aplasia in immunocompromised patients (27).

Parvovirus B19 arthropathy is transient in most cases but occasionally becomes chronic. Some cases have arthropathy that is clinically indistinguishable from RA and fulfills the diagnostic criteria for definite RA (19,20). RA is defined by the presence of 4 or more of the following criteria: 1) morning stiffness in and around joints lasting at least 1 h before maximal improvement; 2) soft tissue swelling of 3 or more joint areas as observed by a physician; 3) swelling of the proximal interphalangeal, metacarpophalangeal, or wrist joints; 4) symmetric swelling; 5) rheumatoid nodules; 6) the presence of rheumatoid factor; and 7) radiographic erosions and/or periarticular osteopenia in hand and/or wrist joints (28). Following parvovirus B19 infection, rheumatoid factor at low-to-moderate titer was detected in some cases (19,29-33) and the development of rheumatoid nodules and/or bone erosions was also observed (34-36). Therefore, the etiologic role of parvovirus B19 in RA has been a subject of great interest. In early studies, anti-B19 antibodies were used to demonstrate parvovirus B19 infection in patients with RA. PCR has recently been applied to demonstrate the presence of parvovirus B19 DNA in blood and synovial materials. However, the results obtained so far are controversial (31,37-40). Since the incidence of exposure to parvovirus increases rapidly with age, carefully selected control samples are required (41). Recent studies have shown that parvovirus B19 DNA can persist in the synovial membranes not only in patients with chronic arthropathy but also in healthy immunocompetent individuals (42,43). The presence of microbial DNA in the synovium, however, cannot be taken as proof of the causation of arthritis. Metabolic products of parvovirus B19 such as viral mRNA and proteins, and a B19-specific immune response or inflammation should be detected to demonstrate the role of

parvovirus B19 in the pathogenesis of RA.

### 3. Prospective studies of patients with parvovirus B19 arthritis

In order to elucidate whether RA is triggered by infection with parvovirus B19, prospective studies have been conducted. Tyndall et al. demonstrated a case of erosive polyarthritis which developed after musculoskeletal disease associated with definite parvovirus infection (34). Gran et al. reported that among 7 patients with RA, a patient positive for anti-B19 IgM antibody showed radiological signs of bone destruction (35). Nikkari et al. presented the case of a patient with rheumatoid nodules and bone erosions as well as rheumatoid factor. Among 61 patients with early RA, this patient was the only one positive for parvovirus B19 DNA in the synovial tissue (36).

We examined 67 patients with acute inflammatory polyarthritis for parvovirus B19 infection (21). Twelve patients were positive for anti-B19 IgM antibody at the onset of arthritis. Joint pain remained in one third (4/12) of anti-B19 IgM-positive patients at 6 months after onset, compared with a rapid decrease in anti-B19 IgM-negative patients (2/52). Three out of four rheumatoid factor-positive patients fulfilled the criteria for definite RA. Active polyarthritis had developed in their fingers, wrists and elbows along with the development of rheumatoid nodules and destructive changes in the joints. The clinical course of one of the patients is shown in Figure 1.

The presence of parvovirus B19 genome DNA in these patients was examined by a method of nested PCR amplifying a region of NS1 (21). Parvovirus B19 DNA was detected in the peripheral blood cells during the first 4 months, but only in the synovial tissue and bone marrow after 2 years. No DNA of EBV or cytomegalovirus was detected in the same samples. Persistent parvovirus B19 infection in the synovium and bone marrow was evidenced through detection

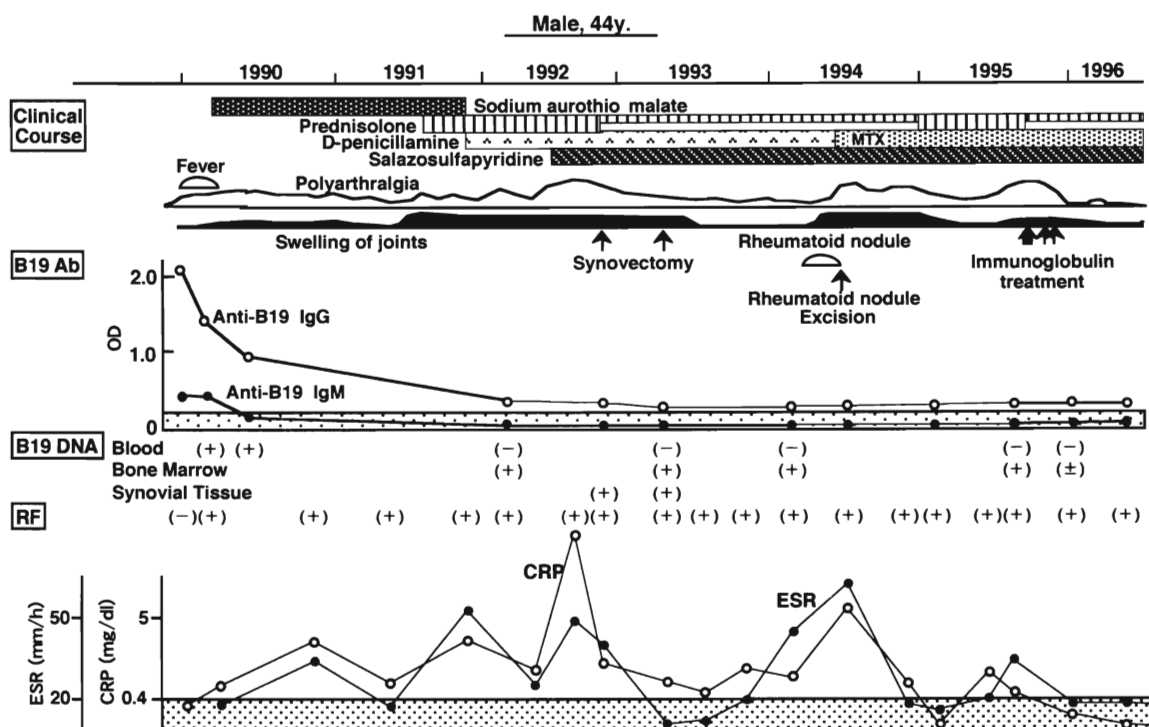


Fig. 1. Clinical course of a male patient with rheumatoid arthritis (RA). MTX, methotrexate; RF, rheumatoid factor; ERS, erythrocyte sedimentation rate.

of signals for VP1 mRNA and VP1 antigen using techniques of in situ hybridization (ISH) with labeled probes and immunofluorescence using anti-VP1 monoclonal antibody (PAR3) (22), respectively. The persistent presence for months or even years of parvovirus B19 DNA in the synovial membrane and bone marrow has also been reported for chronic arthritis (38, 44-48).

The effects of anti-rheumatic drugs were transient, and complete remission was not observed (21). Since the persistence of parvovirus B19 infection in the synovial tissue and bone marrow was considered to be a cause of chronic arthropathy, a high dose of immunoglobulin therapy was performed at 5 or 6 years after the onset of polyarthritis. Joint pain, swelling, and the values of C-reactive protein were improved. Parvovirus B19 DNA in the bone marrow turned out to be almost undetectable by PCR and the number of VP1-positive cells decreased. The association between clinical improvement and decrease in viral expression supports a link between chronic inflammation and parvovirus B19 infection, as indicated in a patient who recovered from parvovirus B19-associated pure red cell aplasia after immunoglobulin therapy (49).

#### 4. Parvovirus B19 in synovial tissue of rheumatoid arthritis

The investigation of patients with RA that developed after parvovirus B19-associated acute polyarthritis (21) suggests

that persistent infection with B19 is responsible for the process of RA. We further examined the synovium of patients with RA at an active stage for the presence of genome DNA, NS1 mRNA, and the VP1 antigen of parvovirus B19 (22) (Table). Parvovirus B19 DNA was found by nested PCR in the synovial tissue from 77% of patients with RA, from 15% of patients with osteoarthritis (OA), and from 16% of patients with trauma. The detection of parvovirus B19 DNA in the synovial membrane of immunocompetent healthy individuals is consistent with recent reports (42, 43). NS1 mRNA by ISH were positive in 73% of the synovial tissue sections from patients with RA but negative in all from patients with OA and trauma. Similarly, immunohistochemical staining with the antibody PAR3 detected VP1 antigen in the samples from 80% of patients with RA but not in those of patients with OA and trauma. The parvovirus B19 genome occasionally exists even in the non-RA synovium, but the persistent expression of parvovirus B19 genes seems to be required for the develop-

Table. Detection of genome DNA, NS1 mRNA, and VP1 antigen of parvovirus B19 in the synovium

| Disease | genome DNA | NS1 mRNA | VP1 antigen |
|---------|------------|----------|-------------|
| RA      | 30/39      | 11/15    | 32/40       |
| OA      | 4/26       | 0/25     | 0/36        |
| Trauma  | 5/31       | 0/16     | 0/22        |

RA: rheumatoid arthritis, OA: osteoarthritis

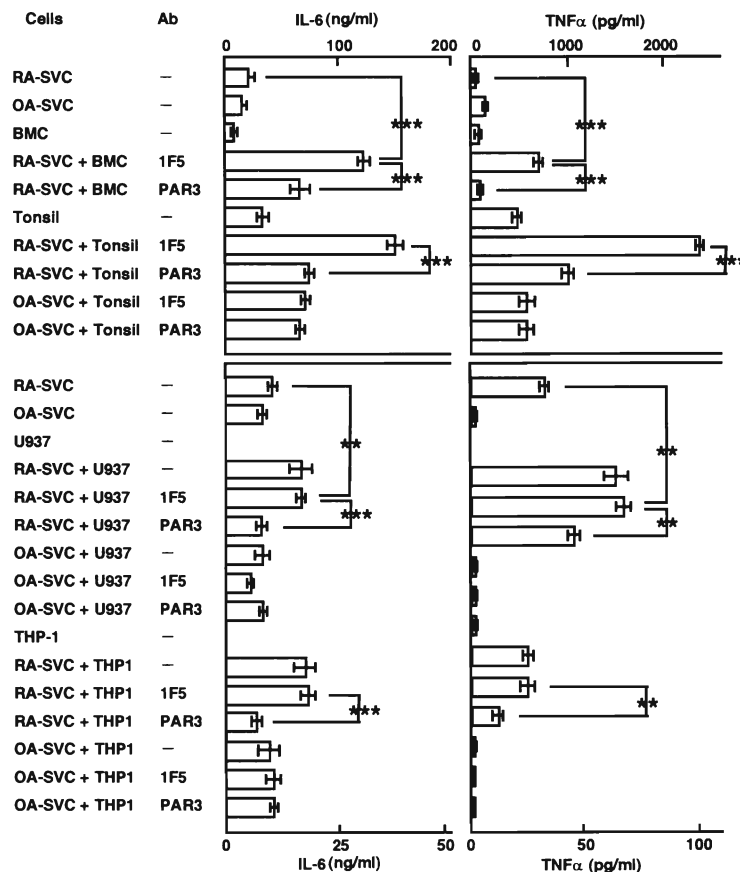


Fig. 2. The effect of parvovirus B19 infection on the production of IL-6 and TNF $\alpha$  in a coculture system. B19-negative bone marrow cells (BMC), tonsil cells (Tonsil), U937 cells, and THP-1 cells were cultured with synovial cells (SVC) from patients with rheumatoid arthritis (RA) or osteoarthritis (OA) for 3 days in a double-chamber system. Inhibition of the production of IL-6 and TNF $\alpha$  was examined by adding anti-VP1 antibody PAR3 in the coculture medium. An antibody 1F5 was used as a control antibody. The concentrations of IL-6 and TNF $\alpha$  in the culture medium were measured by ELISA (Medgenix Fleurus, Fleurus, Belgium). \*\*\*,  $P < 0.01$ ; \*\*,  $P < 0.02$ .

ment of RA.

VP1-positive signals in RA synovial tissue were found in the lymphoid follicle, germinal center, perivascular lymphocytes, and sublining macrophages, but not in the synovial lining cells, neutrophils, or vessels. Immunohistochemical staining using a combination of antibodies against VP1 and a cell surface marker identified the parvovirus B19-infected synovial cells as immune cells, including macrophages, follicular dendritic cells, T cells, and B cells, but not synovial fibroblasts or endothelial cells (22). In early studies, it was shown that parvovirus B19 has a very narrow target cell range and is highly tropic for human erythroid cells (50). A receptor of parvovirus B19 was identified to be globoside (blood group P antigen) (51), and its tissue distribution was shown to be consistent with the known tropism of parvovirus B19 (52). However, the synovium has recently been shown to contain globoside (53), and parvovirus B19 has been detected in peripheral blood leukocytes, macrophages, and myocardial cells *in vivo* (54, 55). It is necessary to elucidate whether globoside and/or other parvovirus B19-specific receptors are expressed on the surface of those immune cells in the RA synovial membrane.

Shimomura et al. reported serial appearance of RNA for nonstructural proteins, replicative forms of parvovirus B19 DNA, and RNA for capsid proteins, in synchronized and B19-infected cells from a megakaryoblastoid cell line, UT-7 (56). The expression of VP1 in the RA-synovial cells suggests the production of complete progeny in these cells. The ability of parvovirus B19 in the synovial immune cells to cause infection in B19-negative immune cells was evaluated by incubating the cells from patients (donor cells) and one of a variety of immune cells (recipient cells) in the same well partitioned by a filter membrane (22). The recipient cells, such as tonsil cells and cells from the human mononuclear cell lines, U937 and THP-1, became VP1-positive when cultured with the collagenase-treated cells from RA synovium as donor cells. Similar results were obtained when bone marrow mononuclear cells from patients with RA were used as donor cells. The virus transmission was blocked by anti-B19 VP1-specific antibody PAR3. No VP1-positive cells were detected in the combination of synovial tissue cells from patients with OA used as donor cells and tonsil cells as recipient cells and the combination of RA synovial cells as donor cells and synovial fibroblasts as recipient cells. These results indicate that the synovial cells and bone marrow cells from patients with RA produce complete parvovirus B19 which causes infection in immune cells but not in fibroblasts.

The continuous and enhanced production of a variety of inflammatory cytokines, as observed in the RA joint, is essential for the development of RA (57). We examined the effect of parvovirus B19 infection on the production of TNF $\alpha$  and IL-6 in the same system as described above (Fig. 2) (22). A significant increase in cytokine levels was observed when the synovial cells from patients with RA were cultured with B19-negative immune cells, compared with the cytokine secretion in each culture alone. PAR3 inhibited the increase in cytokine secretion. OA synovial cells failed to stimulate recipient cells to secrete the cytokines. These results suggest that parvovirus B19 released from cells of the RA synovium can cause infection in immune cells and stimulate the newly infected cells to produce proteins, including inflammatory cytokines.

## 5. A possible mechanism for the development of rheumatoid arthritis

Persistent infection with an infectious agent and its induction of immune-mediated inflammatory responses may be essential for the disease process of RA. We found that cells in the synovium and bone marrow from patients with RA which developed after parvovirus B19 arthritis were persistently infected with B19 and expressed the viral capsid protein VP1 for years (21). Evidence of persistent infection with parvovirus B19 was also obtained from an analysis of the synovium from other patients with RA (22).

The persistent expression of VP1 in infected synovial immune cells suggests a persistent production of B19 NS1 in these cells, since the production of NS1 precedes that of VP1 (56). Moffatt et al. demonstrated that NS1 can induce the production of IL-6 in THP-1 cells, as a *trans*-acting transcription activator on the IL-6 promoter (58). NS1 is expected to control the expression of other cytokine genes in B19-infected synovial immune cells. We observed the enhanced secretion of TNF $\alpha$  and IL-6 in a coculture system of RA synovial cells and B19-negative immune cells including U937 and THP-1 cells representing macrophages (22). In addition, the production of TNF $\alpha$  mRNA was stimulated by the induction of NS1 in an inducible expression system with U937 cells (Ishii et al., unpublished data). NS1 probably functions as a transcription-activating factor also on the TNF $\alpha$  promoter in B19-infected macrophages.

Synovial macrophages play important roles in the process of RA through the release of TNF $\alpha$  and IL-1, both of which stimulate the secretion of cytokines and tissue-damaging enzymes from synovial fibroblasts and chondrocytes (59). Among the macrophage-derived cytokines, the apparent importance of TNF $\alpha$  as an inflammatory mediator in RA is suggested (2), by the findings that the unregulated production of TNF $\alpha$  in transgenic mice leads to spontaneous arthritis (60) and that the treatment of RA patients with anti-TNF $\alpha$  monoclonal antibodies leads to an attenuation of the severity of inflammation and joint destruction (61). Up-regulation of TNF $\alpha$  expression by NS1 in macrophages may be a key process in the pathogenesis of RA and an available target for new therapeutic methods.

The mode of parvovirus B19 infection in immune cells of the synovial tissue and bone marrow from patients with RA is unknown. NS1 of parvovirus B19 has been shown to have cytotoxic activity which may be responsible for the lytic infection in erythroid lineage cells (50) and abortive infection in nonpermissive cells (62). The cytotoxicity of NS1 has recently been understood to be a function of apoptosis induction (63). Quantitative control of NS1 and/or modification of its cytotoxic activity by some host factors might be responsible for the persistent infection with parvovirus B19. The reason why cells expressing the viral capsid protein can escape immune surveillance for years is also unknown. Elucidation of these issues will provide clues to virus-host interactions which cause autoimmune disease.

## 6. Conclusions

We observed patients with RA which developed after the polyarthritis associated with parvovirus B19. Parvovirus B19 was persistently present in their synovium and bone marrow and continued to express its gene products. Infection with parvovirus B19 probably stimulates the synovial immune cells

to produce inflammatory cytokines by the activity of NS1. Once a paracrine and autocrine network of cytokines is induced, inflammation persists and tissue destruction, including cartilage and bone erosion, progresses. Treatment with disease-modifying anti-rheumatic drugs or immune suppressors did not provide complete remission to patients with RA. Removal of the cause of the disease was required. Treatment with immunoglobulin reduced parvovirus B19 in the synovium and improved the arthritis at the same time. These findings suggest that parvovirus B19 is at least one of the causative agents of RA. Identification of the etiology provides an opportunity to target RA using specific therapies.

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