Human parvovirus B19 as a causative agent for rheumatoid arthritis

(Interleukin 6/tumor necrosis factor α)

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Abstract

Human parvovirus B19 (B19) DNA was detected in the synovial tissues in 30 of 39 patients with rheumatoid arthritis (RA), and infrequently in those with osteoarthritis and traumatic joints. On the other hand, the expression of B19 antigen VP-1 was specific (27/27) in RA synovium with active synovial lesions, but not in osteoarthritis and controls. The target cells of B19 were macrophages, follicular dendritic cells, T cells, and B cells, but not synovial lining cells in the synovium. B19-negative bone marrow cells, tonsil cells, and macrophage cell line U-937 cells became positive for the expression of VP-1, and more productive for interleukin 6 and tumor necrosis factor α when cocultured with RA synovial cells. The expression of VP-1 and the production of interleukin 6 and tumor necrosis factor α was significantly inhibited by the addition of neutralizing antibody for B19, suggesting that B19 detected in RA synovial cells is infective. B19 is involved in the initiation and perpetuation of RA synovitis, leading to joint lesions.

Rheumatoid arthritis (RA) is a chronic inflammatory synovitis characterized by marked hyperplasia of the synovial lining cells and extensive infiltration of macrophages, lymphocytes, fibroblasts, and leukocytes in joints, where a variety of cytokines, including interleukin (IL) 1β, IL-6, tumor necrosis factor α (TNFα), granulocyte/macrophage colony-stimulating factor, IL-8, and proteolytic enzymes, are responsible for the process of the inflammation. The arthritis is often progressive and may result in destructive change of the affected joints. The etiology for RA is unknown. Many studies, including paleopathological analysis, have indicated the role of an infective agent in the etiology of RA. One of the suspects for such an agent is a virus because viral infections such as rubella, human parvovirus B19 (B19), cytomegalovirus (CMV), human T cell leukemia virus 1, and HIV often cause an acute onset of polyarthritis (1–5). Some cases with acute B19 infection are well known to present clinical symptoms resembling RA (1–3), and the presence of B19 DNA has been demonstrated in autoimmune diseases such as RA, systemic lupus erythematosus, adult-onset Still’s disease, and polyarteritis nodosa (6–8). Although the expression of B19 protein or its isolation from the affected organ is critical to know the role of the virus in vivo, these have remained unelucidated. In addition, the virus-associated arthropathy is usually transient and self-limiting. Therefore, the prevalence of IgM anti-B19 antibody at an early stage of RA was assessed to be incidental (9).

We have shown the persistence of B19 for more than 6 years in a patient with recurrent episodes of B19 viral infection (10). In addition, three cases of acute infection developed into active RA with destructive alterations in joints, rheumatoid nodules, and rheumatoid factor. In these cases of RA, B19 DNA and the VP-1 antigen, the structural protein of B19, were persistently positive in their bone marrow and synovial tissues for more than 7 years after the initial episodes of joint involvement (11). Treatment with intravenous Ig brought a marked reduction of joint signs and a decrease of B19-positive cells in the bone marrow, indicating a link of B19 and RA symptoms in these cases. This prompted us to study the role of B19 infection in the etiopathology of RA.

Materials and Methods

Patients and Methods. Blood and bone marrow were obtained from patients with RA or hematomal diseases. Synovial samples came from 16 RA patients with synovectomy, 23 with RA, and 26 osteoarthritis (OA) patients with total knee joint replacement, along with 31 normal individuals who were undergoing knee arthroscopy for traumatic ligament lesion. The obtained samples were fixed in 4% paraformaldehyde for 2 hr at room temperature, dehydrated in alcohol, embedded in paraffin, and then used for microscopy, in situ hybridization (ISH), and immunohistochemistry. All patients with RA met the American College of Rheumatology criteria for the diagnosis of RA (12). Among 35 RA samples, 31 showed positive tests for IgG anti-B19 antibodies in sera (13) and 12 of 20 OA samples included IgG anti-B19 antibodies. Tonsil samples were obtained from patients without RA at the tonsillectomy. All patients gave their informed consent.

Detection of B19 DNA by PCR and ISH. B19 DNA was amplified from serum, bone marrow cells (BMC), or synovial tissues by a nested PCR, and confirmed by a Southern blot analysis. Synthetic oligonucleotides used in PCR were as follows: for the primary amplification of NS-1 region, 5′-ATTCATACAGACTTGTAGC-3′ (1263–1282) and 5′-TCAGAGCTTTCACCACAC-3′ (1988–1970); for the secondary amplification of NS-1 region, 5′-CAGACATTTGAGCAGGT-TATG-3′ (1271–1290) and 5′-AGTGATGTATTGCTTGCCAG-G-3′ (1884–1866); for the primary amplification of VP region, 5′-AAGTGTGGCCAGGTCCCG-3′ (1970–2054) and 5′-GATCAGAGCTGATCTCC-3′ (3478–3458); for the secondary amplification of VP region, 5′-TGTCACAAGCATTGTGAGG-3′ (3184–3207) and 5′-AACACCT-ATAATGTGTGCCTGGG-3′ (3294–3271). The probe for a Southern blot analysis of the NS-1 region is a 483-bp fragment (nucleotides 586–1069), and that of VP-1 is a B19 DNA genome. B19 DNA/RNA in the bone marrow and synovial tissue also was investigated by ISH. Namely, a 480-bp fragment

Abbreviations: B19, human parvovirus B19; RA, rheumatoid arthritis; IL, interleukin; TNFα, tumor necrosis factor α; CMV, cytomegalovirus; OA, osteoarthritis; ISH, in situ hybridization; BMC, bone marrow cells; SVC, synovial cells.

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8227
between nucleotides 590 and 1090 of parvovirus B19 was used as a probe, which was labeled with \(^{35}\)S-dCTP or with digoxigenin 11-dUPT by the randomly primed labeling technique (Boehringer Mannheim). Pretreatment of the specimen was carried out as described previously (14). A 25-\(\mu l\) hybridization solution containing 50% formamide, 0.3 M NaCl, 1 mM EDTA, 10 mM Tris-HCl, 1% Denhardt’s solution, 80 \(\mu g/ml\) of salmon sperm DNA, 550 \(\mu g/ml\) of yeast tRNA, and 10% dextran sulfate was added on each slide with \(^{35}\)S-dCTP-labeled probe (1 \(\times 10^6\) cpm/ml), or 20 \(\mu g/ml\) of digoxigenin-labeled probe. After 18-hr hybridization at 42°C, slides were washed twice for 10 min in 3\(\times\) standard saline citrate (SSC), once for 60 min in 2\(\times\) SSC at room temperature, and finally twice for 30 min in 0.1\(\times\) SSC at 60°C. For autoradiography, the slides then were dehydrated in ethanol, dried in air, and immersed in Kodak NTB-2 nuclear track emulsion in a Kodak D-19 developer for 3 min at 18°C. The sections were stained with Wright-Giemsa. The other slides were developed according to the instructions of the supplier of the digoxigenin kit (Boehringer Mannheim).

**Immunofluorescence and Immunohistochemistry.** The cells were fixed in 50% acetone/methanol (vol/vol) for 20 min at \(-20°C\). Then, F(ab\(^\prime\))\(^2\) fragment of monoclonal anti-VP-1 antibody PAR3 (13), anti-CMV antibody DDG9, CCH2 (Dako), and monoclonal mouse IgG antibody 1F5 were applied on the slides, and specimens were incubated for 30 min at 37°C. After being rinsed with PBS and reacted with fluorescein isothiocyanate-labeled anti-mouse IgG, they were washed and then analyzed for the expression of VP-1 in the cells.

Immunohistochemistry was performed by a biotin-avidine method (Vectastain ABC kit, Vector Laboratories) (15). Two-color immunohistochemical staining also was performed as described previously (16). Briefly, 2-\(\mu m\) paraffin sections were blocked with serum, and the endogenous peroxidase was inactivated in 0.3% \(\text{H}_2\text{O}_2\) in methanol. The sections were labeled with F(ab\(^\prime\))\(^2\) fragment of anti-VP-1 antibody PAR3 overnight at 4°C, washed in PBS, incubated with biotinized anti-mouse IgG followed by biotin-avidine-anti-peroxidase, and developed with 3-amino-9-ethylcarbazole to produce a red color. Next, sections were blocked with serum as above, incubated sequentially with L26, KP-1, anti-human CD20 (Dako), CD3 (NovaCastra, Newcastle, U.K.) or Ki-M4P, anti-human follicular dendritic cell (17), and alkaline phosphatase-conjugated goat anti-mouse IgG and mouse alkaline phosphatase anti-alkaline phosphatase complexes, and then developed with Fast Blue BB Salt (Vector Blue, Vector Laboratories).

**Cell Separation and Tissue Culture.** BMC were obtained from heparinized aspiration of the sternum bone marrow and separated by a Ficoll-Hypaque (Pharmacia) density-gradient centrifugation. Tonsil cells finely minced and then separated by Percoll density gradient centrifugation (Pharmacia) were prepared as a single-cell suspension, which includes 50–60% CD20\(^+\), 30–50% CD3\(^+\), 1–5% CD68\(^+\), or Ki-M4P\(^+\) cells. Synovial specimens obtained at the time of synovectomy or joint replacement were treated with 1 ml/ml of collagenase (type 1; Sigma) for 4 hr in DMEM at 37°C in 5% \(\text{CO}_2\). The dissociated cells then were centrifuged at 500 \(\times\) g, resuspended with 10% fetal calf serum (GIBCO/BRL), 2 \(\mu M\) L-glutamine, 100 \(\mu M\) of penicillin, and 100 \(\mu g/ml\) of streptomycin, and used as freshly prepared cells. Synovial cells (SVC) from normal individuals with traumatic joints were obtained as described above, plated in 75-\(cm^2\) flasks, passaged twice, and then used as synovial fibroblasts.

**Infectivity Study in Double-Chamber Culture.** Infectivity of B19 was studied in identical parallel cultures in which 2 \(\times 10^5/ml\) of the BMC or SVC from RA or other diseases were separated from 2.5 \(\times 10^5/ml\) of BMC, tonsil cells, synovial fibroblasts, UT-7 cells, U937 cells, or THP-1 cells by a culture insert (Falcon, Becton Dickinson), and cultured at 37°C for 1–5 days. The culture medium included 1 unit/ml of erythropoietin and 20 units/ml of IL-3 in the case of BMC, 3 \(\mu l/ml\) of phytohemagglutinin in the case of tonsil cells. In inhibition studies with antibody, F(ab\(^\prime\))\(^2\) fragments of anti-VP-1 antibody, PAR3, or 1F5 with irrelevant specificity were added at 2 \(\mu g/ml\) to the culture. Each culture supernatant was obtained for cytokine assay, and after being washed three times, the separated cells were tested for the expression of VP-1.

**Measurement of Cytokines.** The levels of IL-6 and TNF\(\alpha\) in the culture supernatant were measured by ELISA (Medgenix, Fleurus, Belgium) according to the indicated protocol.

**Statistical Analysis.** Differences were compared by using the Student’s \(t\) test. A two-sided value of \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Detection of B19 DNA and B19 Protein VP-1 in RA.** We first analyzed the presence of B19 DNA in blood, bone marrow, and synovial tissues in a variety of diseases. B19 DNA was negative in blood from 54 normal subjects and 40 bone marrow samples from patients with hematological diseases such as acute leukemia or malignant lymphoma, when tested with nested PCR. Among 14 patients with RA tested for the presence of B19 DNA, five cases were positive in the bone marrow, but rarely in blood, with the exception of four cases obtained in the initial episode of RA. On the other hand, B19 DNA was detected in 30 of 39 RA synovial tissues, which were obtained at synovec-tomy or at the operation of knee joint replacement, and in four of 26 samples taken from OA and five of 31 from traumatic joints. The specificity of B19 DNA was confirmed by a Southern blot analysis after the nested PCR, with the reproducibility on the samples obtained at different times. We also tried to amplify the DNA of CMV and Epstein–Barr virus by PCR (18, 19), but failed to detect them in 10 synovial samples taken from RA patients.

We then evaluated the localization of B19 DNA and/or RNA by using an ISH. Morphologically, B19 DNA-positive cells in RA bone marrow appeared as lymphoid cells and erythroid cells. ISH studies using B19 probe also revealed the presence of B19 DNA and/or RNA in lymphoid follicles, sublining macrophages, and perivascular lymphocytes, but not in the synovial lining cells of the RA synovium (Figs. 1 and 2). Treatment with RNase to synovial specimen caused marked reduction of the binding signals with B19 probe in 18 samples taken from RA and OA synovium. The B19 sequences were neither detected in any cells of the bone marrow, nor in SVC of the other disease categories at ISH studies (Table 1 and Fig. 1).

We next investigated the expression of B19 protein VP-1 to determine whether or not B19 is biologically functional in RA cases. Approximately 2.5–8% of BMC in two patients with RA reacted with F(ab\(^\prime\))\(^2\) fragments of the monoclonal anti-VP-1 antibody PAR3 (14), but not with that of irrelevant antibodies such as the mAb to CMV, or monoclonal mouse IgG antibody 1F5 by an indirect double immunofluorescence staining. As shown in Fig. 1, VP-1-positive cells were also evident in RA SVC when demonstrated by an immunohistochemical method, but no evidence for B19 VP-1 was obtained in 18 samples taken from bone marrow, nor was there any evidence found in 58 samples of synovial tissues in the control group (OA and traumatic joints). The studies for the expression of early antigen of CMV also produced negative results in 10 synovium tissues of RA. Fig. 1 suggests that the distribution of VP-1-positive cells may be similar to that of B19 DNA-positive cells in RA synovium. We therefore tried to identify VP-1-positive cells in the synovium by using double-staining methods. In general, RA SVC are thought to include synovial lining cells, macrophages, T and B lymphocytes, follicular dendritic cells, neutrophils, and vascular endothelial cells. VP-1 was positive
in CD20+ (a B cell marker) cells, in Ki-M4P+ (a follicular dendritic cell marker) cells, CD68+ (a macrophage marker) cells, or CD3+ cells, respectively (Table 2 and Fig. 3), whereas no synovial lining cells, neutrophils, and endothelial cells were stained with PAR3. It was noted that all synovial specimen with active lesion (above 2 in inflammatory score) showed positive results (27/27) for VP-1 expression, but B19 antigen was detected infrequently (3/12) in those with a low inflammatory score (1 or 0). The results were reproducible in all samples from five RA cases, which had synovectomy or joint replacement twice at different times, indicating the persistence of B19 in RA joints.

**Infectivity of RA-Associated B19.** To provide direct evidence of the persistence and activation of B19 in RA, B19 infectivity was tested in a double-chamber culture system (Culture Insert, Falcon) of human cells and RA SVC. Table 3 and Fig. 4 show that B19-negative BMC, tonsil cells, UT-7, a macrophage cell line U937 cells, and THP-1 cells became positive for the expression of VP-1, when cocultured with the SVC or BMC derived from RA. The infection was markedly blocked by the coexistence of anti-VP-1 antibody, PAR3, which has a neutralizing activity for B19 infection (14), whereas 1F5 with irrelevant specificity did not inhibit the infectivity of RA-associated B19. Synovial fibroblasts, however, showed negative results for the expression of VP-1 at the coculture system. No evidence for B19 DNA and VP-1 antigens was obtained in the immunocytes, when B19-negative BMC or SVC derived from OA were cocultured.

**Cytokines Produced by SVC and B19.** The continuous and markedly enhanced production of a variety of inflammatory cytokines is a characteristic feature of RA joints and assessed to play an essential role in the pathogenesis of joint lesion in RA. Moffatt et al. (20) have demonstrated that the in vitro transfer of the gene encoding nonstructural protein 1, NS-1, of B19 induces the production of IL-6 in a variety of cell types, indicating the role of B19 on cytokine production. We therefore tested the influence of B19 on the production of IL-6 and TNFα in the coculture system used for the infectivity. The results showed spontaneous secretion of IL-6 and TNFα at significant levels in the culture of RA or OA SVC alone. RA-derived SVC caused an enhanced production of both TNFα and IL-6 when cocultured with B19-negative BMC, tonsil cells, U937 cells, or THP-1 cells, whereas OA-derived SVC did not. It also was revealed that the addition of neutralizing anti-VP-1 antibody PAR3 to the culture system induced the inhibition of IL-6 and TNFα production to the same levels as those in synovial cell culture alone. The representative case is shown in Fig. 5. The inhibition of the cytokine production was not affected with the coexistence of an irrelevant antibody 1F5. On the other hand, PAR3 had no effect on the production of IL-6 or TNFα in the coculture

<table>
<thead>
<tr>
<th>Disease</th>
<th>Specimen</th>
<th>PCR</th>
<th>ISH</th>
<th>VP-1 Ag</th>
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<td>Blood</td>
<td>5/25*</td>
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<td>0/16*</td>
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<td>0/5</td>
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<td>11/15</td>
<td>32/40</td>
</tr>
<tr>
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<td>Synovium</td>
<td>4/26</td>
<td>0/25</td>
<td>0/36</td>
</tr>
<tr>
<td>Trauma</td>
<td>Synovium</td>
<td>5/31</td>
<td>0/16</td>
<td>0/22</td>
</tr>
</tbody>
</table>

*Positive cases/total cases.
system of OA SVC and tonsil cells. This strongly supports the role of B19 in the process of an enhanced production of IL-6 and TNFα in RA synovium.

DISCUSSION

The results presented herein show the persistence of B19 in the synovium of RA joints as well as in the bone marrow in a high frequency. Furthermore, the evidence of the infectivity of B19 from RA SVC was provided through the coculture system; expression of B19 antigen VP-1 and enhanced production of IL-6 and TNFα were induced in cocultured tonsil cells or macrophage cell lines; they were significantly suppressed by the addition of a neutralizing anti-VP-1 antibody PAR3 to the coculture system.

Recently Soderlund et al. (21) demonstrated the detection of B19 DNA not only in the synovial tissues of chronic arthritis, but also in those of nonarthropathy controls, as confirmed in this paper. It is, however, noteworthy that the detection of B19 RNA and B19 antigen VP-1 in synovium is specific in RA, but not in other disease categories. Subsequently, data including previous case reports (11) provide important information toward the etiopathology of RA.

We examined three patients, who developed RA with destructive joint alterations after an acute B19 infection (ref. 11; C.M., unpublished results). The occurrence of RA studied was associated with the presence of IgM anti-B19 antibodies, rheumatoid factors, and the persistence of the virus during the

![Fig. 3. Immunohistochemical detection of VP-1 and cell markers in RA SVC by double staining. (A) Synovial lining cells are stained for KP-1 (blue), but not for anti-VP-1 (red). Original magnification ×400. (B) Mononuclear cells in the sublining layer are stained for both KP-1 (blue) and anti-VP-1 (red) antibody. Original magnification ×1,000. (C) Lymphocytes in lymphoid follicle are stained for both CD20 (blue) and anti-VP-1 (red) antibody. Original magnification ×400. (D) Mononuclear cells in the germinal center are stained for both Ki-M4P (a follicular dendritic cell marker, blue) and anti-VP-1 (red) antibody. Antigen-presenting cells (macrophages and follicular dendritic cells) and lymphocytes are positive for VP-1. Original magnification ×400.](image-url)
course of disease not only in the bone marrow but also in
synovial tissues. The association of the decrease of VP-1-
positive cells in the bone marrow and their clinical improve-
ment after iv. Ig therapy also correlates with our view of a
relationship between B19 infection and RA, as suggested in the
patients who recovered from B19-associated hemolytic anemia
after Ig therapy (22).

A second observation is the presence of B19 in immunocytes
such as macrophages, T cells, and B cells in RA. So far it has
been shown that the target for B19 is erythroblasts and possibly
megakaryocytes in bone marrow (23). Although granulocytic
precursors are resistant to B19 in vitro, precursors of granu-
locytes and monocytes in bone marrow may be permissive to
B19 in vivo. Recently, the receptor for B19 was reported to be
globoside, the P antigen of red cell membranes (24). Cooling
et al. (25) exposed the distribution of globoside in red cells,
granulocytes, kidney, liver, synovium, and others, indicating a
possible tissue tropism of B19 observed clinically in B19-
associated diseases.

In this paper, we demonstrated the expression of VP-1 as
well as the presence of B19 in follicular dendritic cells,
macrophages, T cells, and B cells in RA synovium. Our observa-
tion in vivo is compatible with that in the in vitro
infectivity experiments of RA-associated B19 to a macrophage
cell line U-937, THP-1, and tonsil cells, which include mac-
rophages, follicular dendritic cells, T cells, and B cells. The
results were reproducible in synovial samples from five pa-
tients obtained at different times, and both B19 DNA and VP-1
were positive in the synovial samples from 30 patients of 39
patients with RA at different stages, indicating the persistence
of B19 in RA synovium.

The question remains as to why B19 is persistently present
in RA cases, because B19, especially NS-1 protein, has a potent
cytotoxic effect to the host cells, and causes apoptosis in the
infected cells (ref. 26; S. Moffatt, personal communication). It
should be appropriate to note here that the Aleutian mink
disease parvovirus (ADV) can cause a persistent infection in
mink, characterized by hypergammaglobulinemia, autoanti-
odies, glomerulonephritis, and polyarthritis (27). Diseases
caused by other types of parvovirus may be attributable to a
fast replication of the virus and an increased gene expression
in the infected cells to cell death (28). On the other hand, ADV
replicates slowly in lymphoid tissues involving follicular den-
dritic cells and macrophages (29). The slow replication of ADV
may be associated with weak viral promoters, leading to
persistent infection in vivo. In acute B19 infection in humans,
the main target of B19 is erythroblasts, in which B19 induces
apoptosis soon after infection, whereas RA-associated B19 is
present in lymphocytes, macrophages, and follicular dendritic
cells. Although the virulence of RA-associated B19 in the
immunocytes remains to be clarified, B19 may be nontoxic
for these cells.

Lastly, it should be noted that RA-associated B19 in RA
SVC had an infectivity to macrophages and lymphocytes,
indicating that B19 is functionally active and may have an
ability to play a biological role in the immunocytes, possibly
affecting the development of RA. The fact that the expression
of VP-1 antigen was evident in active lesions of RA, but rare
in the joint lesion with a mild activity, supports this hypothesis.
Furthermore, the anti-VP-1 antibody-mediated suppression of
IL-6 and TNFα production by RA-associated B19-infected
cells is suggestive of an association with the inflammatory
cytokine production and cell proliferation in RA joints. Al-
though there may be marked variations in the synovial cell
infiltrate among the biopsy specimens from different RA
patients, the characteristic histological feature in early RA was
the increase of macrophages and the expression of IL-6 and
TNFα (30).

It also has been assessed that histological changes in the
initial stages of RA are indicative of an infiltration of mono-
nuclear cells, especially T cells at the perivascular area, fol-
lowed by the increase of macrophages, lymphocytes, and
synovial fibroblasts in the synovium. These cells secrete a variety
of inflammatory cytokines to cause marked proliferation of
the inflammatory cells. Recently, McInnes et al. (31) demon-

<table>
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<tr>
<th>Source</th>
<th>Ab</th>
<th>BMC</th>
<th>Tonsil</th>
<th>Fib</th>
<th>U937</th>
<th>THP-1</th>
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B19-negative BMC, tonsil cells (tonsil), synovial fibroblasts (Fib), U937 cells, THP-1 cells, or
B19-permissive cell line UT-7 cells were cocultured with SVC or BMC in double-chamber culture system.

Fig. 4.  An immunofluorescence analysis of the infectivity of B19.
B19-negative tonsil cells or U937 cells were cocultured with SVC at
double-chamber culture system using Cell Culture Insert (Falcon),
washed three times at 3 days, and then tested for the reactivity with
F(ab)2 fragments of VP-1 (14). Tonsil cells (A) or U937 cell (B)
cocultured with RA-derived SVC are positive for VP-1. But U937 cells
(C) do not show immunoreactivity for VP-1 when cocultured with
OA-derived SVC.
stratified that IL-15 secreted from activated T cells is essential for the production of TNFα, subsequently leading to the activation of macrophages, synovial lining cells, and others. We showed that B19 in the immunocytes, which may infiltrate the RA synovium, is at the initial stage, as well as active stages, had the ability to infect lymphocytes and macrophages in vitro, and also associated with the enhanced IL-6 and TNFα production by RA SVC. We have demonstrated that B19 enhanced IL-6 production in human hematopoietic cell lines and endothelial cells (20). In this experiment, NS-1 of B19, was shown to activate NF-κB, which binds and activates the promoter of the IL-6 gene.

In essence, we hypothesize that B19-positive T cells and macrophages infiltrate into the synovium and recruit circulating immunocytes. Synovial T cells and macrophages continuously activated by B19 secrete TNFα or others to stimulate a variety of SVC via the autocrine and paracrine way. This leads to an excessive synthesis of inflammatory cytokines and proteolytic enzymes and finally causes the destructive alteration of the joints.

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