Human Parvovirus B19 Transgenic Mice Become Susceptible to Polyarthritis

Naruhiko Takasawa, Yasuiko Munakata, Keiko Kumura Ishii, Yuichi Takahashi, Minako Takahashi, Yi Fu, Tomonori Ishii, Hiroshi Fujii, Takako Saito, Hiroshi Takano, Tetsuo Noda, Misao Suzuki, Masato Nose, Suzan Zolla-Patzner and Takeshi Sasaki

*J Immunol* 2004; 173:4675-4683; doi: 10.4049/jimmunol.173.7.4675
http://www.jimmunol.org/content/173/7/4675

References

This article cites 35 articles, 12 of which you can access for free at: http://www.jimmunol.org/content/173/7/4675.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Human Parvovirus B19 Transgenic Mice Become Susceptible to Polyarthritis

Naruhioko Takasawa,* Yasuhiko Munakata,* Keiko Kumura Ishii,† Yuichi Takahashi,* Minako Takahashi,* Yi Fu,* Tomonori Ishii,* Hiroshi Fujii,* Takako Saito,* Hiroshi Takano,† Tetsuo Noda,‡ Misao Suzuki,‡ Masato Nose,§ Suzan Zolla-Patzner,¶ and Takeshi Sasaki‡*  

Human parvovirus B19 (B19) often causes acute polyarthritis in adults. In this paper, we analyzed nucleotide sequences of the B19 genome of patients with rheumatoid arthritis (RA), and then introduced the nonsstructural protein 1 (NS1) gene of B19 into C57BL/6 mice that had a genetic origin not susceptible to arthritis. The transgenic mice developed no lesions spontaneously, but were susceptible to type II collagen (CII)-induced arthritis. B19 NS1 was expressed in synovial cells on the articular lesions that were histologically characteristic of granulomatous synovitis and pannus formation in cartilage and bone. Serum levels of anti-CII Abs and TNF-α increased in NS1 transgenic mice to the same levels as those of DBA/1 mice, which were susceptible to polyarthritis. Stimulation with CII increased secretion of Th1-type- and Th2-type cytokines in NS1 transgenic mice, indicating that a viral agent from the joints in humans can cause CII-induced arthritis resembling RA. The Journal of Immunology, 2004, 173: 4675–4683.

Rheumatoid arthritis (RA) is a chronic polyarthritis with destructive joints, where a variety of cytokines have an important role in the inflammatory process. The etiology of RA is unknown. In the past decade, extensive studies have focused on defining the genes associated with the occurrence of RA; the studies included genetic polymorphisms of immunologically related genes, such as HMC. Microenvironment is another factor that may be responsible for the etiopathogenesis of RA, and human parvovirus B19 (B19) is one candidate in the etiopathogenesis as indicated by epidemiological data showing that B19 infection and RA may be relatively new diseases in Europe (1–3). B19, a causative agent of erythema infectiosum in infants (4), often causes acute onset polyarthritis in adults that, in some cases, may resemble RA in terms of human leukocyte-associated Ag, rheumatoid factor production, and clinical signs including erosive change of joints (5–10). We previously described three patients who developed typical RA with destructive change in joints and rheumatoid nodules after acute B19 infection (11). The B19 RNA and B19 structure protein, viral protein 1 (VP1), are specifically and frequently detected in T and B cells, macrophages, and follicular dendritic cells of the rheumatoid synovium (12). Coculture study of rheumatoid synovial cells with infected cells using a double chamber system showed that TNF-α and IL-6 were secreted from the macrophage cell line, U937, or bone marrow cells of normal subjects, but the production of the cytokines was inhibited by anti-B19 Ab-recognizing VP1 (12). The studies have also shown a cross-reactivity of anti-B19 VP1 IgG with human collagen II (CII), and an increased invasiveness of B19-infected synovial fibroblasts (13, 14), indicating that B19 may participate in etiopathogenesis of RA. However, no data exist to show a direct role of microorganisms that originate from RA joints in the etiopathogenesis of polyarthritis or RA. We show here that the introduction of the nonstructural protein 1 (NS1) gene of B19 can cause mice to be susceptible to polyarthritis through up-regulation of inflammatory cytokines, which are characteristic of RA lesions.

Materials and Methods

Isolation of the B19 genome from the rheumatoid synovium

Synovial tissues were obtained from six patients with RA who fulfilled the criteria of the American College of Rheumatology 1987 to diagnose RA (15). The serum samples were obtained from 20 patients with acute B19 infection. All patients gave an informed consent. Synovial tissue was minced and treated with collagenase type I (1 mg/ml; Sigma-Aldrich, St. Louis, MO) for 4 h. DNA was extracted from synovial cells by treating with proteinase K (1 mg/ml; Takara, Kyoto, Japan) and phenol-chloroform, and then underwent PCR. The primer set used was 5′-TCGCCCTTATGCAAATGGGCAG-3′ and 5′-GTGTTAGGCTGTCTTATAGGTAC-3′. Almost the whole genome (4.7 kb) of B19, including the coding regions of NS1, VP1, and VP2, was amplified from DNA samples. The amplified fragment was cloned into pGEM-T (Promega, Madison, WI). The sequences of the cloned B19 genome were analyzed by using the 7-deaza-dGTP cycle sequencing kit (Amersham Life Sciences, Buckinghamshire, U.K.). Each sequence was based on the data of five plasmid clones. DNA from the synovium of the remaining four patients with RA and from the serum of patients with acute B19 infection was also used to amplify NS1 region by using a primer set of 5′-CGGCTGTGACACCTGAAACC-3′ and 5′-GAGCTGCACCTGAGGAGT-3′. The fragment of the NS1 region was analyzed by direct sequencing without cloning. The DNA sequence of the plasmid clone, N8, that included B19 genome from a patient with B19-associated aplastic crisis (16) was also determined. The published sequence of Au isolate from a patient with aplastic crisis (17) was

Copyright © 2004 by The American Association of Immunologists, Inc. 0022-1767/04/$02.00
FIGURE 1. Nucleotide sequence alignment of B19 NS1-coding region from the genome strains, Mi, Rm, N8, and Au. The nucleotide sequence of Mi is shown and homologous nucleotides in other strains are indicated as hyphen bars. Nucleotide triplets resulted in amino acid difference among strains are boxed. Amino acids at the position of 183 were of special interest. Arrows indicate the junction site of sequences from N8 (nt 436–576) and Mi (nt 577–2451) in the transgene for constructing NS1 transgenic mice.
used as a reference. GenBank accession numbers for the sequences reported here are AB030673 for Mi, AB030694 for Rm, and AB030673 for N8.

Construction of the NS1 transgene

The NS1 region of the B19 genome amplified from the synovial DNA of a patient with RA (Mi) was cloned into pGEM-T, and pGEM-T/B19-MiNS1 was generated. After a BamH1 restriction site was created by PCR at nt 708 of pGEM-T/B19-MiNS1, the AflII-Smal and Smal-Smal fragments of pGEM-T/B19-MiNS1 were replaced with the corresponding fragments of N8 (16). The BamH1 fragment including the NS1 gene was excised from the obtained construct, and was inserted into the plasmid pBstN (18) at the site upstream of the rabbit β-globin exon-intron sequence and SV40 poly(A) sequence. The resulting pBstN/B19-MiNS1 comprised the promoter regions from the N8 strain (nt 92–576, AB030673) and the NS1-coding regions from the Mi strain (nt 577-2478, GenBank accession no. AB030693). The transgene was prepared by digesting pBstN/B19-MiNS1 with XbaI and SalI.

Generation of NS1 transgenic mice

B19 NS1 transgenic mice of a C57BL/6 (H-2b) origin were generated in the Center for Animal Resources and Development of Kumamoto University (Kumamoto, Japan). The transgenic mice were kept and bred in the heterozygous form at the Animal Unit of Tohoku University School of Medicine (Tohoku, Japan), an environmentally controlled and specific pathogen-free facility. Male and female C57BL/6 mice were purchased from CLEA Japan (Tokyo, Japan) and male and female DBA/1 mice (H-2q) were obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan). We used 8- to 12-wk-old, age-matched mice in all experiments.

Induction of arthritis

Calf CII was obtained from Elastin Products (Owensville, MO) and was dissolved at a concentration of 4 mg/ml in 0.1 M acetic acid at 4°C. Mice were immunized at the tail base by injecting 200 μg of CII emulsified in CFA containing Mycobacterium tuberculosis strain H37 Rv (Wako Pure Chemical, Tokyo, Japan) and were boosted at the same location by injecting 200 μg of CII together with IFA (Wako Pure Chemical) 21 days later. The mice were observed for development of arthritis from day 10 after immunization and blood was drawn to measure anti-CII Abs or TNF-α. The clinical severity of arthritis was quantified and expressed as an arthritis index by using the following scoring system: 0, no change; 1, swelling in one joint (digitus, wrist, or ankle); 2, swelling in more than one joint or mild inflammation of paws; 3, severe swelling of the entire paw or ankylosis or both. Each paw was graded so that each mouse could achieve a maximum score of 12. At the end of the experiment, the joints were histopathologically examined for erosions, pannus formation, and synovium infiltrates.

Expression of B19 NS1 mRNA in various tissues of NS1 transgenic mice

Total RNA was isolated from the liver, kidney, spleen, thymus, and synovium of the knee joints of the mice immunized with CII by using an acid

FIGURE 2. Expression of the NS1 gene in transgenic mice. A, Map of the NS1 transgene. The 4.0 kb transgene consists of the B19 p6 promoter region, an NS1 coding region, part of the rabbit β-globin gene and an SV 40 poly(A) signal. The primers for PCR were set at positions 1.1 kb apart on the transgene. B, NS1 mRNA in the tissues of transgenic mice. NS1 mRNA was detected in total RNA preparation from the homogenates of the liver, kidney, spleen, and thymus. DNA-PCR amplified 1.1-kb fragment from tail DNA of transgenic mice (data not shown) and the transgene plasmid. RT-PCR was expected to amplify 539-bp product, as the region between the primers included two introns. Sequencing analysis of 539-bp product confirmed that the product lacked intron sequences (data not shown). Mouse γ-actin was amplified as a constantly expressing gene. C, NS1 mRNA in joints. NS1 mRNA was also detected in the ankles and knees. Fragments of expected size were amplified.
guanidium thiocyanate-phenol-chloroform extraction method (ISOGEN; Nippon Gene, Tokyo, Japan). NS1 mRNA was detected by using RT-PCR including 1/2H9262 g of total RNA using the RNA PCR kit Ver. 2.1 (Takara, Tokyo, Japan). The PCR primers used were 5’/H11032-GAGCCTGGAACACTGAAACCC-3’/H11032 and 5’/H11032-AGCCTGCACCTGAGGAGTGA-3’/H11032 for NS1, and 5’/H11032-ACCATTGGCAATGAGCG-3’/H11032 and 5’/H11032-CATCTGCTGGAAGGTGGACTGAGGCCAG-3’/H11032 for -actin. The primer set for NS1 is designed to amplify 539 bases of spliced mRNA and 1.1 kb of genomic DNA. The PCR condition was 35 cycles of a sequential reaction, 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min.

Histopathological study

The knee and foot joints were fixed in 10% neutral buffered formalin, were decalcified in a 5% EDTA-2Na solution, and then were embedded in paraffin. The specimens were cut into 2- to 3-μm thick sections and were stained with H&E. Specimens of the lungs, heart, liver, pancreas, kidneys, salivary gland, prostate, spleen, thymus, bone marrow, and brain were examined by using light microscopy.

Immunohistochemical staining of joints

The immunohistochemistry study was done by using a biotin-streptavidin method. Briefly, after deparaffinization, sections were treated with 0.3% H2O2 in methanol for 30 min to minimize endogeneous peroxidase activity. Then nonspecific protein binding was blocked with 10% normal goat serum for 30 min at room temperature, and 2.5/2H9262 g/ml human monoclonal anti-NS1 or anti-VP1 Ab (19) was applied to the sections which were incubated overnight at 4°C. A 1/1000-diluted biotinized anti-human IgG Fc fragment (Seikagaku, Tokyo, Japan) was added to the slides. The slides were incubated for 60 min at room temperature, and then were rinsed in PBS. The sections were reacted with streptavidin (Histofine; Nichirei, Tokyo, Japan) for 30 min at room temperature, and then were developed with a solution of 0.05% 3,3’-diaminobenzidine dehydrochloride (Grade III; Sigma-Aldrich) in 0.05 M Tris-HCl buffer, pH 7.6, and 0.01% H2O2.

Table I. Clinical signs of arthritis after immunization with type II collagen

<table>
<thead>
<tr>
<th>Mice</th>
<th>Incidence (%)</th>
<th>Onset (Days)</th>
<th>Arthritic Scores</th>
<th>No. of Arthritic Joints/No. of Total Joints (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS1</td>
<td>31/54 (57.4)</td>
<td>24.0 ± 7.8</td>
<td>2.94 ± 1.92</td>
<td>95/216 (43.9)</td>
</tr>
<tr>
<td>WT</td>
<td>3/46 (6.5)</td>
<td>31.0 ± 3.6</td>
<td>0.26 ± 0.12</td>
<td>3/184 (3.6)</td>
</tr>
<tr>
<td>DBA/1</td>
<td>43/55 (78.2)</td>
<td>30.0 ± 5.4</td>
<td>4.32 ± 2.56</td>
<td>129/220 (58.6)</td>
</tr>
</tbody>
</table>

* Fifty-four (25 males and 29 females) NS1 Tg mice, 46 (22 males and 24 females) WT C57BL/6 mice, or 55 (31 males and 24 females) DBA/1 mice were immunized with 200 μg of CII twice at 21-day intervals as described in Materials and Methods, and were checked for arthritis. The experiments were done as seven separated groups, of which each group included NS1 transgenic, WT C57BL/6, and DBA/1 mice.

FIGURE 3. The expression of NS1 protein in the transgenic mice. Immunostaining of NS1 using human monoclonal anti-NS1 Ab and biotinylated anti-human IgG Ab shows an expression of NS1 in synovial cells including immunocytes of the joint in the founder N66 of NS1 transgenic mouse (A), but NS1 expression is weak in the founder N36 of NS1 transgenic mouse (B) and negative in a wild type of C57/6 mice (C). Original magnification: A–D, ×200

FIGURE 4. CIA developed in transgenic mice. Incidence of arthritis (A) and severity of clinical signs (B) were evaluated in NS1 transgenic mice (n = 54), wild-type H-2 mice of C57BL/6 background (n = 46), and DBA/1 mice (n = 55) after immunization with CII in CFA. Results are expressed as a percentage of arthritic mice (A) or as mean arthritic scores in each group on a given day during the course of CIA (B): ○, NS1 transgenic mice (NS1 B6); ●, wild-type C57BL/6 mice (B6); △, DBA/1 mice (DBA/1).
Measurement of serum TNF and detection of serum anti-CII Abs

Sera were obtained from mice at days 10–11 and at days 32–38 after CII immunization. The TNF-α concentration in the sera was measured by using an ELISA plate (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. Serum Ab titers against CII or murine CII were measured by using a modified ELISA. In brief, a 96-well microplate (Falcon; BD Biosciences, Franklin Lakes, NJ) was coated with 50 μl of CII or murine CII (Elastin Products) at 20 μg/ml in sodium bicarbonate (pH 8.5) at 4°C overnight. After being washed three times with PBS containing 0.05% Tween 20 and 0.1% BSA, each well was blocked with 150 μl of PBS containing 1% BSA at room temperature for 1 h, and was washed three times with PBS containing 0.05% Tween 20. Fifty microliters of the diluted goat anti-mouse IgG (1/2000) together with HRP at room temperature for 1 h, and were further washed three times with PBS containing 0.05% Tween 20. The bound enzyme was reacted with 400 μg/ml o-phenylenediamine and 0.01% H2O2 in 50 μl of citrate buffer at room temperature for 10 min. The OD values at 492 nm were then read using a microplate reader.

In vitro production of cytokine by lymph node (LN) and spleen (Sp) cells

To measure the levels of cytokine production by LN or Sp cells, male mice were immunized with 200 μg of CII emulsified in CFA intradermally at both fore and hind footpads. Popliteal and axillary LN or SP cells were obtained from the immunized mice at ~10 days after immunization. The tissue was minced through a sterile wire mesh to give a single cell suspension. The cells (10^6 cell/ml) were cultured in a round tube (Falcon; BD Biosciences) in the presence or absence of 50 or 100 μg/ml CII or murine CII. After 72 h, the supernatants were collected and IFN-γ, IL-2, or IL-4 production was measured by using an ELISA plate (R&D Systems) according to the manufacturer’s instructions.

Proliferation response of LN cells

We also used popliteal and axillary LNs for cell proliferation assays. Cells (2.5 × 10^5 per well) were cultured in 96-well flat-bottom microplates (Falcon; BD Biosciences) in the presence or absence of 50 or 100 μg/ml denatured CII or murine CII at 37°C in 5% CO2 for 4 days. During the final 18 h of culture, cells were pulsed with 1 mCi of [3H]TdR. Cells were harvested on glass fiber filters by using an automated sample harvester. The incorporated radioactivity was measured by using a scintillation counter. The results of the [3H]TdR incorporation assay were expressed as the mean cpm ± SD of triplicated cell preparation derived from the LN and Sp of different mice.

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

Nucleotide sequence of B19 DNA from the rheumatoid synovium

We first amplified the almost whole genome of B19 isolated from two patients (Mi and Rm) with RA. The amplified sequences of B19 DNA from the rheumatoid synovium of Mi were completely coincided with those from bone marrow of the same individual (Mi, GenBank accession no. AB030673). Further analysis using DNA samples from four more patients with RA and from 16 patients with acute B19 infection revealed that no genetic change was specific among RA-derived isolates (data not shown).

Generation of B19 transgenic mice

To understand the role of B19 in polyarthritis, we generated transgenic mice expressing the B19 gene from rheumatoid joints, and...
investigated the ability of B19 to induce polyarthritis associated with inflammatory cytokines. The NS1 gene encodes a functional protein of B19, and, therefore, we used the NS1 gene of the Mi isolate derived from RA, to construct the transgene for B19 transgenic mice. As pGEM-T/B19-MiNS1 did not include the authentic promoter of B19, and, therefore, we used the NS1 gene of the Mi isolate derived from RA, to construct the transgene for B19 transgenic mice. As pGEM-T/B19-MiNS1 did not include the authentic promoter of B19, and, therefore, we used the NS1 gene of the Mi isolate derived from RA, to construct the transgene for B19 transgenic mice. As pGEM-T/B19-MiNS1 did not include the authentic promoter of B19, and, therefore, we used the NS1 gene of the Mi isolate derived from RA, to construct the transgene for B19 transgenic mice.

**Histopathological features of CIA in NS1 transgenic mice**

Histopathological examination of NS1 transgenic mice immunized with CII verified a destructive arthritis, which seems qualitatively similar to the arthritis induced in DBA/1 mice or human RA. We found no inflammatory lesions in other tissues by histopathological study. The NS1 protein was present in the inflamed synovium of the transgenic mice, which consists of fibroblasts, capillary endothelial cells, and infiltrating mononuclear cells (Fig. 3, A and B and Fig. 5E). However, immunostaining unexpectedly failed to show VPI, the capsid Ag of B19, in the joints of NS1 transgenic mice (data not shown). Further studies showed no NS1 was in the joints of DBA/1 mice and wild-type C57BL/6 mice (data not shown).

**Levels of TNF-α and Abs to CII in transgenic mice**

TNF-α and Abs specific to CII have a major role in the pathogenesis of CIA (20, 21). Neither TNF-α nor anti-CII Abs were detected in unstimulated NS1 transgenic mice or in DBA/1 mice.

**FIGURE 6.** Levels of TNF-α and titers of anti-CII Abs in sera. Sera were collected from each mouse on day 35 after immunization of CII and were evaluated for the levels of TNF-α by ELISA as described in Materials and Methods (A). The mean ± SD of the levels of TNF-α are shown for NS1 transgenic (○), wild-type C57BL/6 (●), and DBA/1 mice (△). Sera were also evaluated for the levels of IgG, IgG2a, and IgG2b anti-CII Abs by ELISA (B). The mean ± SD of the levels of IgG, IgG2a, or IgG2b anti-CII Abs is shown for NS1 transgenic (○), wild-type C57BL/6 (●), and DBA/1 mice (△).
However, blood samples showed that the levels of TNF-α increased in NS1 transgenic mice, but were low in wild-type mice when the mice were stimulated with CII (Fig. 6A). Anti-CII Ab activity of different forms of IgG was also significantly higher in NS1 transgenic mice than those in wild-type mice in response to CII (Fig. 6B). However, the Ab against murine CII was not detected in the mice of all groups when stimulated with murine CII (data not shown).

Immune response of LN cells to CII

We examined whether the existence of the NS1 protein alters the immune response to CII. LN cells were obtained from mice of each group at 10–11 days after CII immunization, and were tested for their ability to produce IFN-γ, IL-2, and IL-10, or to proliferate in the response to CII dose-dependently. Fig. 7A shows that in vitro antigenic stimulation with CII increased production of cytokines at higher levels in the LN cell culture from NS1 transgenic mice dose-dependently and the concentration of cytokine was almost comparable to the cytokine concentration of CIA-susceptible DBA/1 mice. CII stimulation also increased the proliferative response in NS1 transgenic mice (Fig. 7B).

Discussion

We first investigated the nucleotide sequences of the B19 genome in the rheumatoid synovium. Although it has been reported that unique segments were involved in B19 DNA isolated from patients with B19 encephalitis (22), the present data demonstrated no specific sequence in the NS1 region of B19 genome obtained from RA patients. However, we used the NS1 gene of Mi genome as a source of transgene, because the isolate originated from RA lesions.

The generated transgenic mice did not show a spontaneous clinical appearance of RA or autoimmune diseases. We could also detect neither autoantibodies such as Abs to murine CII nor TNF-α activity in sera of the unstimulated transgenic mice. At least two factors may be associated with the failure of spontaneous disease in transgenic mice. One is that we may have obtained only a progeny of the transgenic mice with low expression levels of the NS1 protein that are not enough to induce an immunological disorder. The reason why we have a difficulty to get transgenic mice with highly expressed NS1 may be the potent cytotoxicity of NS1 that may cause fetus loss (23). Another is a genetic factor associated with arthritis. The H-2 haplotype is a susceptibility factor in autoimmune diseases, and the DBA/1 mice with H-2d are susceptible.

FIGURE 7. Cytokine production and proliferative response of LN and Sp cells in response to CII. LN or Sp cells were obtained from mice on day 12 after primary immunization with CII in CFA, and 5 × 10⁵ cells in each group were stimulated in vitro with 100 μg/ml heat-denatured CII for 4 days. The culture supernatant was collected at day 4, and was assessed for the content of IFN-γ, IL-2, and IL4 by ELISA (A). ○, NS1 transgenic mice; ●, wild-type C57BL/6 mice; △, DBA/1 mice. LN cells (5 × 10⁵) from mice at day 12 after CII stimulation were also incubated with 50 or 100 μg/ml heat-denatured CII for 4 days in vitro. The cells were collected on day 4, and were assessed for uptake of ³H that was added to the culture on day 3 (B). The mean ± SD of the levels of ³H uptake are shown: NS1-transgenic (■), wild-type C57BL/6 (●), and DBA/1 mice (□).
to polyarthritis, but C57BL/6 mice with H-2b are not (20, 21). The NS1 transgenic mice are in C57BL/6 background, indicating the expression levels of NS1 in the transgenic mice might be not enough to cause spontaneous disease through breaking the immune regulation by a H-2 haplotype in C57BL/6 background.

However, CII stimulation caused polyarthritis in the NS1 transgenic mice despite the expression of H-2d. One may argue the possible role of the promoter in the transgene because, depending on the promoter used, transgenesis may happen by transcriptional activation of genes downstream of the promoter. However, this is unlikely because polyarthritis is uncommon in two lines of the transgenic mice with low levels of NS1 expression, whereas three lines that clearly expressed NS1 protein became susceptible to polyarthritis and to CII stimulation, supporting the role of B19 NS1 in the pathogenesis of polyarthritis. It should be mentioned here why NS1 transgenic mice became susceptible to polyarthritis when challenged with CII. One possibility is that the expression of the H-2 haplotype with the regulatory ability for the immune response might be impaired in NS1 transgenic mice, resulting in an enhanced response to CII stimulation. This mechanism has been demonstrated at the decreased or increased immune response in a variety of viral infections, such as HSV (24), adenovirus type 12 (25), or EBV (26), that induced altered DR expression on the infected cells. However, the phenotype in NS1 transgenic mice was still H-2d even after introducing the NS1 gene and no altered response was observed at the MLR by using spleen cells from NS1 transgenic and wild-type C57BL/6 mice (data not shown). Susceptibility to polyarthritis is not solely attributed to MHC, as shown in the case of human T cell leukemia virus type-1 env-pX region-introduced transgenic (pX-Tg) mice, which develop an inflammatory polyarthropathy in BALB/c background, but rarely in C57BL/6 background (27). In human T cell leukemia virus type-1 env-pX region-introduced transgenic mice, substitution of H-2d haplotype with H-2a did not decrease the frequency of arthritis, but abrogation of Fas-mediated apoptosis of T cells may be responsible for the development of arthritis (27). Therefore, further study should be made to test the ability of dendritic cells as an APC for the development of arthritis (27). Another possibility is that some genes associated with CIA may be activated in NS1 transgenic mice. CIA is a murine model of arthritis (27). Therefore, further study should be made to test the ability of dendritic cells as an APC or the expression of other phenotypes such as Fas in NS1 transgenic mice.

We believe this study is the first one to show that viral components originating from RA joints cause RA-like polyarthritis in vivo. Thus, discussing the results in association with the etiopathology of B19 polyarthritis and RA may be worthwhile. In most patients with acute B19 infection, B19 disappears soon after the clinical appearance of polyarthritis that is also transient (5, 6). One mechanism for acute B19 infection may be mediated by the immune complex (35). However, the clinical course of RA is long and different immune cells continuously proliferate and secrete marked amounts of cytokines in RA joints where TNF-α would have a central role in the inflammatory process of the disease. Thus, autoimmune disease is a result of dysregulation of immune responses associated with inappropriate activation of macrophages, and T and B cells. The results of this study agree with our previous data for B19 and RA (12). Namely, B19 protein VP1 and B19 DNA and RNA were detectable repeatedly at different times in macrophages, follicular dendritic cells, and T and B cells in the rheumatoid synovium, indicating persistent activation of B19 in the RA synovium. The secretion of TNF-α and IL-6 induced by the coculture of the macrophage cell line, U937, or THP1 with rheumatoid synovial cells was inhibited by coexistence of monoclonal anti-B19 Abs (12). We also showed that transduction of U937 cells with NS-1 gene-activated transcription factors, AP-1 and AP-2, resulted in the up-regulation of TNF-α gene expression and secretion of TNF-α from host cells (34). This indicates that persistent activation of B19 may constantly up-regulate the TNF-α gene in infected cells. Data of the NS1 transgenic model leads us to hypothesize that persistently activated B19 may induce an increased immune response, such as activating and proliferating synoviocytes by an autocrine and paracrine pathway in the joints, resulting in an inflammatory process resembling RA.

Acknowledgments

We thank K. Umene (Kyushu University, Fukuoka, Japan), T. Nounou (Nakamura Gakuen University, Fukuoka, Japan), and M. Kimura (Tokai University, Sagamihara, Japan) for providing plasmids.

References


