FIG. 2. Plasma viral load in the infected monkeys. Plasma viral RNA of four monkeys (200, 944, 520, and 844) was analyzed by PCR for the presence of the SIVmac239 *gag* region.

shown that the reduction of the CD4/CD8 ratio correlates with the increase of SHIV viral copies (15, 25). These four monkeys showed that the peak of viral copies occurred on the 14th day after inoculation and declined to 500 copies or less on the 56th day (Fig. 2).

We arbitrarily defined a postinoculation period of day 1 through 3 as "early stage" (covering monkeys 4345, 1, and 2 in Fig. 3) and that of day 56 or later as "advanced stage" (covering monkeys from 90c to 039 in Fig. 3). Monkey 4345 showed a remarkable reduction in the number of colonies in 24 h (Fig. 3). Monkeys 1 and 2 also had such a dramatic decline on day 3 (Fig. 3). However, nine monkeys (90c through 0634 in Table 1 and Fig. 3) maintained colony formation during the advanced

stage at a level comparable to that of the control monkeys (Fig. 3). Compared with sham-inoculated controls, monkey 054 had a somewhat lower number of colonies on the 113th day. Monkey 039, which died of AIDS on day 238, showed more reduced colony formation, especially CFU-GM formation, than did monkey 054 or the sham-inoculated control monkeys (Fig. 3). At the advanced stage, no difference in the morphology or the number of colonies was noted between the noninfected and the infected monkeys (Fig. 4).

Taken together, a reduction of CD4/CD8 ratio and CFU-GM growth occurred in the early phase of the postinoculation period. However, the CFU-GM growth tended to increase following viremia while CD4$^+$ T lymphocytes continuously declined. The colony growth of the infected monkeys during the advanced stage recovered up to a level comparable to that of the control monkeys.

Infection of CFU-GM with SHIV C2/1 virus was tested by a PCR technique as described in Materials and Methods. Of the 14 cynomolgus monkeys infected with SHIV C2/1 virus, only three were positive, suggesting that the direct infection of bone marrow progenitor cells was minimal (Fig. 5). There was no positive case in the control monkey group.

DISCUSSION

Hematological abnormalities such as anemia, lymphopenia, and thrombocytopenia have been documented in a variety of retrovirus infections in both humans and experimental animals. While the precise mechanisms for such hematological abnormalities remain to be elucidated, several hypotheses have been postulated: (i) destruction of infected cells by a virus itself
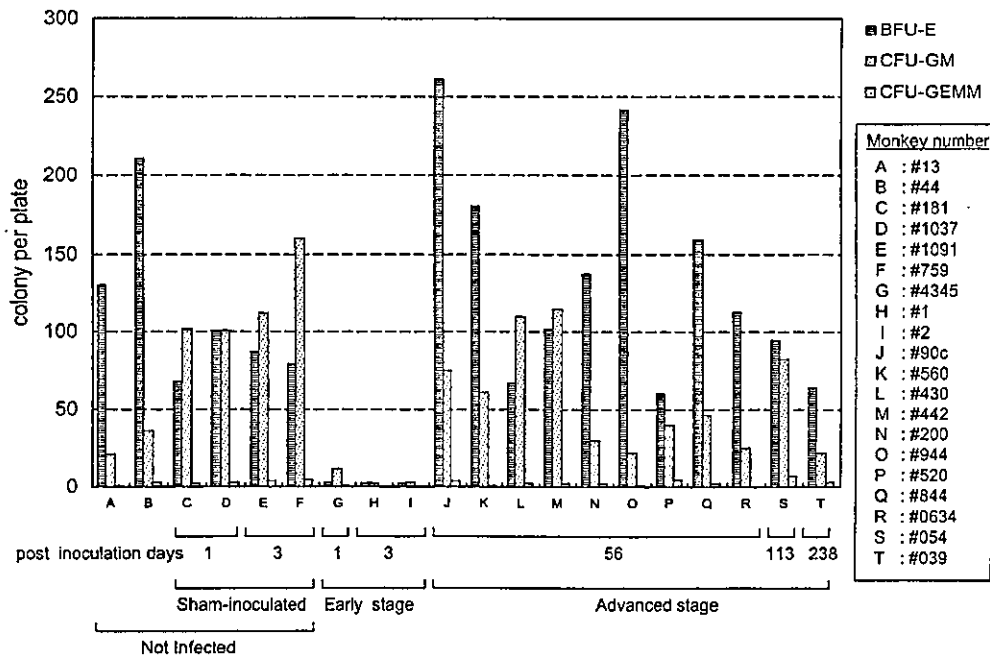

FIG. 3. Colony assay on monkeys inoculated with SHIV C2/1. A period of days 1 through 3 after inoculation was defined as early stage, whereas days 56 through 238 were defined as advanced stage. P was <0.005 for CFU-GM, and P was <0.02 for CFU-E in comparison of early stage and advanced stage and of virus-inoculated monkeys and sham-inoculated controls at days 1 and 3. P values were calculated according to Kruskal-Wallis analysis. There was no statistically significant difference between sham-inoculated controls and non-sham-treated controls by Mann-Whitney analysis.
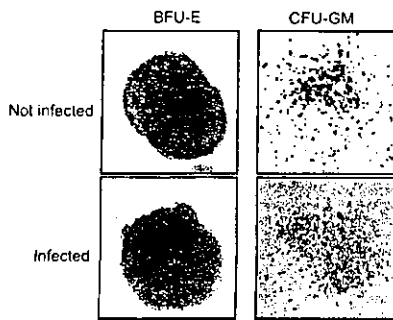
FIG. 4. Morphology of colonies produced by BFU-E and CFU-GM. Photographs of colonies cultured in nitrocellulose medium are shown at an ×75 magnification by a microscope. The left column shows BFU-E, and the right column shows CFU-GM. The upper section shows colonies from uninfected monkeys, while the lower shows colonies from monkeys infected with SHIV C2/1.

or by the antibody-dependent cell-mediated cytolytic mechanism, (ii) damage of the thymus or the lymphoid tissue, (iii) abnormal turnover of infected cells in the peripheral blood (i.e., apoptosis), and (iv) suppression of hematopoietic progenitor cells (23).

In this report, we showed that the remarkable decrease in the colony formation occurred during the early stage of infection with SHIV C2/1 (days 1 through 3 postinoculation). These results suggest that the hematopoietic progenitor cells are damaged or defective during such an early phase of infection. Furthermore, the CD4/CD8 ratio in monkey 4345 decreased within several hours, compared with controls (Fig. 1). We used ketamine for viral or saline inoculation, blood sampling, and autopsy. Ketamine has safely been used for bone marrow aspiration in humans and monkeys (21, 22, 26, 34, 35). It would be unlikely, therefore, that such bone marrow suppression occurred as a result of the anesthetic agent. However, our anesthetic procedure probably induces the release of corticosteroids in animals by the stress of capture and injection, which may have a negative impact on the colony formation. Our observation of the ability of bone marrow cultures from sham-inoculated controls to produce BFU-E and CFU-GM at days 1 and 3 proved otherwise.

After day 56, the ability of the bone marrow to form colonies recovered despite the preceding viremia and the continuing reduction of CD4/CD8 ratio. Furthermore, the colony formation was maintained at a level comparable to that of the control monkeys toward the terminal stage. Many reports have noted that CFU-GM growth continuously declines in SHIV infection, and such a decline appears to correlate with disease activity (20). However, CD4/CD8 ratio may not reflect the ongoing status of the bone marrow. A reason for the continuous reduction of CD4/CD8 ratio could be that infected T lymphocytes were destroyed in the peripheral blood more than they were produced in the bone marrow. This could be due to enhanced apoptosis or ongoing destruction of T cells by the antibody-dependent cell-mediated cytolytic mechanism in SHIV infection (24).

In contrast to previous reports, our results clearly showed that the decreasing CFU-GM growth recovered in the advanced stage, suggesting that the damage to colony formation during the early stage is reversible. We showed by PCR in this

report that the direct infection of bone marrow progenitor cells with SHIV C2/1 was minimal (3, 5, 13, 18). It is possible, however, that the number of colonies was too low for detection of SHIV C2/1 virus or that SHIV C2/1 virus-infected cells were already removed by the host immune system before the assays (6, 8, 13).

It has been reported that the cellularity of the bone marrow from patients with HIV does not always correlate with the peripheral blood abnormalities (4). The commonly seen pancytopenia is often associated with hypercellular bone marrow where the increased number of lymphocytes, plasma cells, or histiocytes is seen. The latter finding suggests either dysmyelopoiesis or increased peripheral destruction of blood cells. Yoshino et al. have recently reported that atypical lymphocytes and monocytes were observed in the peripheral blood following intense viremia on the 10th to 14th days of SHIV infection (33). They further found erythroid multinucleation and atypical mononuclear cells in the hypercellular bone marrow, suggesting direct viral infection of hematopoietic progenitor cells (33).

As mentioned above, the colony formation in the bone marrow of the infected monkeys recovered spontaneously following viremia, suggesting that the reduced colony formation capability was reversible. It has been reported that inhibition of SIV replication in bone marrow macrophages resulted in increased colony growth of progenitor cells (32), and administration of recombinant human GM colony-stimulating factor could reverse leukocytopenia (11). Our data thus support the concept that, in the early phase, production of inhibitory factors or a lack or an inhibition of stimulatory cytokine production from lymphoid cells may be responsible for some of the bone marrow kinetic defects previously described in HIV (14, 28, 29). It is necessary to determine and verify what factor is participating in the regulation and recovery of the bone marrow CFU-GM growth.

The highly pathogenic SHIV C2/1 virus is an interesting tool to study the effect of HIV and SHIV infection on hematopoietic progenitor cells (15, 17, 25). Such studies will help us understand the pathophysiology of AIDS and contribute to the development of vaccines in humans (12).
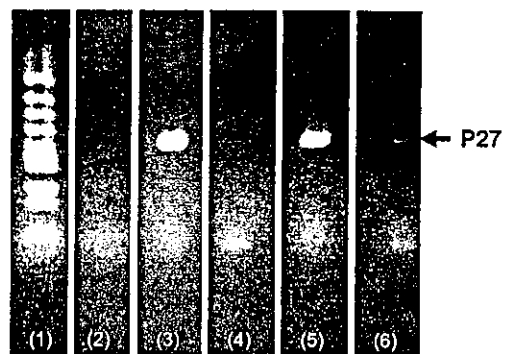

FIG. 5. Detection of SIVmac239 gag sequence in bone marrow colonies by PCR. Lanes: 1, a DNA ladder marker, HincII; 2, a negative control; 3, a positive control, a DNA sample from cell line M8166; 4, CFU-GM from monkey 4345 at the early stage; 5, CFU-GM from monkey 430 at the advanced stage; 6, CFU-GM from monkey 039 at the advanced stage (this monkey died of AIDS on day 238).

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AUTOIMMUNITY
REVIEWS

Epstein-Barr virus etiology in rheumatoid synovitis

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Abstract

The etiology of rheumatoid arthritis (RA) has remained unknown, although it has been investigated and speculated that both genetic and environmental components contribute to the cause of this disease.

Epstein-Barr virus (EBV) has been a strong candidate about for over 25 years as environmental infectious agent(s). There are many circumstantial evidence for association between EBV and RA, but definite evidence is wanting.

In present article, we review an increase circumstantial proof which has been investigated so far and demonstrate direct evidence for the presence of EBV in inflamed synovial cells in patients with RA and discuss on the recent finding of signaling lymphocytic-activation molecule (SLAM)-associated protein (SAP), which opened a new approach to understand on impaired function of cytotoxic T cell for EBV in patients with RA.
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Keywords: Epstein-Barr virus; Rheumatoid synovitis; EBV-encoded small RNA (EBER); Signaling lymphocytic activation molecule (SLAM); SLAM-associated protein (SAP)

Contents

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1. Why, Epstein-Barr virus and rheumatoid synovitis

The etiology of RA remains unclear, although the cause of RA has been attributed to several factors, such as genetic or environmental/infectious agents (bacteria, viruses) for over 25 years.

In twin studies, Silman et al. reported that the concordance rate for RA in a nationwide study of 91 monozygotic pairs was 15%, which is lower than the 30% figure usually quoted from a study 30 years ago and sets a limit on the potential genetic role in RA etiology [1]. Furthermore, they investigated the genetic contribution to the clinical features of RA by comparing RA-concordant monozygotic twin pairs, which highlighted the importance of non-genetic factors in RA susceptibility [2]. These data indicate that genetics is not the sole component and that both genetic and environmental/infectious factor(s) play important roles in the etiopathogenesis of RA. The current theory for the cause of RA is the RA shared epitope hypothesis, which is based on the fact that RA is associated with HLA-DRβ alleles containing the QKRAA amino acid sequence in their third hypervariable regions [3].

It was reported that RA patients have enhanced humoral and cellular immune responses to the EBV and to microorganisms that share the QKRAA sequence [4]. Immune reactivity to recombinant proteins encompassing the shared epitope was also seen in 22 monozygotic twin pairs discordant for RA, thus suggesting that either genetic or shared environmental/infectious agents were associated with the etiology of this disease.

Antibodies against EBV increased in patients with RA [5] and antibodies against an antigen in the nucleus of EBV-transformed B cells, designated RA-associated nuclear antigen (RANA), are also present in patients [6]. RANA is identical to EBNA-1 [7]. The antibody against EBNA-1 reacts with a 62-kDa protein in the synovium of patients with RA [8], and there is substantial homology in the amino acid sequences of gp110, which is a component of the EBV capsid protein, and HLA-DR4 [9]. These results suggest that EBV has a strong etiologic role in RA. Furthermore, the number of infected peripheral B lymphocytes in RA patients tends to be higher than in normal individuals [10,11] and RA patients show an impaired ability to generate EBV-specific cytotoxic T lymphocytes [11,12].

Taken together, the above results suggest that aberrant gene function and EBV may be present in patients with RA.

2. Evidence of Epstein-Barr virus in rheumatoid synovium

Fox et al. demonstrated that a monoclonal antibody, selected for reactivity with the EBV-encoded antigen EBNA-1, exhibited strong reactivity with synovial lining cells in joint biopsies from 10 of 12 patients with RA as well as with the adherent cells eluted from these tissues. No staining of RA synovial membrane or eluted synovial-lining cells was observed with monoclonal antibodies directed against antigens encoded by cytomegalovirus, herpes simplex viruses or human T cell leukemia virus type I. Among 12 osteoarthritis and normal synovial biopsies, few reactive cells were noted. These results suggest that the inflamed synovial cells in patients with RA contain EBV [8]. However, evidence of EBV DNA in rheumatoid synovial cells could not be demonstrated by Southern blotting [13]. The failure to detect EBV DNA may be due to insufficient sensitivity. Using polymerase chain reaction (PCR), EBV DNA was detected and confirmed in the synovial tissue of RA, including the RNA of several latent and lytic EBV genes [14–16] with also us, indicating that EBV is present in the RA synovial tissue. There were no differences in EBV gene expression between synovial tissues and peripheral blood when comparing RA with osteoarthritis and other disease controls because PCR is not able to identify the source of amplified signals and is typically a qualitative, rather than quantitative, form of analysis.

In order to identify of the cells in synovial tissue that EBV DNA was amplified from, we undertook the detection of EBV using in situ hybridization for the presence of EBV-encoded small RNA (EBER) in synovial cells and immunohistochemical staining for expression of CD21 molecules or latent membrane protein (LMP-1) and EBNA-2. EBER was identified in synovial cells and lymphocytes from RA patients (23.5%) but was not seen in any of the control synovial tissues (osteoarthritis and psoriasis). Interestingly, in some cases, we found that EBER was localized in the

synovial lining cells that were located at the apex of the villus proliferating lesions. Furthermore, LMP-1 molecules were also detected in synovial cells. However, CD19, CD21 and EBNA-2 were not observed. The incidence of EBV-positive cells in synovial cells with severe lymphocyte infiltration tended to be higher than in cells with moderate infiltration [17].

This study was very carefully performed in order to confirm EBV infection in patients with RA. Considerable evidence has been accumulated regarding the presence of EBV in synovial cells and lymphocytes in patients with RA [17–19], with the exception of a study by Niedobitek et al. [20].

EBV infection of synovial cells still eludes us, because CD21, a receptor for EBV, could not be detected on the synovial membrane. Cell fusion with EBV-infected lymphocytes has been suggested to play a role in viral infection of non-lymphoid cells [21]. It has recently been proposed that pre-synovial stem cells are recruited into the joints from bone marrow. Patients with rheumatoid arthritis may therefore have EBV-infected pre-synovial stem cells in their marrow and these cells move to the synovial membranes in the joints (Fig. 1).

3. Signaling lymphocytic activating molecule-associated protein (SAP) plays a crucial cytotoxic role in Epstein-Barr virus infection

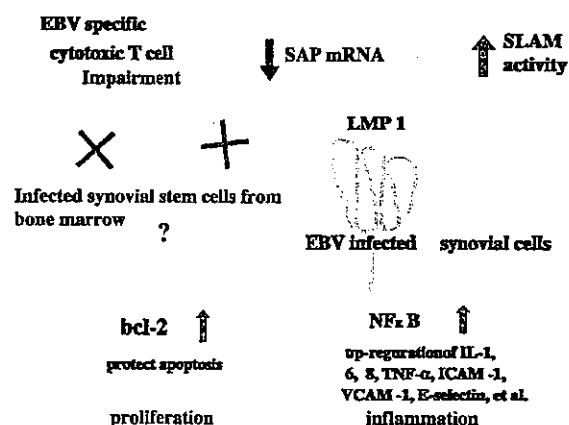In 1996, we cloned several cDNA clones from patients with IgA nephritis. The function of those

Fig. 1. Impaired SAP function may involve EBV infection to synovial cells and synovitis in patients with RA.

cloned gene has since been unclear. It was recently reported that Signaling lymphocytic activating molecule (SLAM) is a cell surface molecule (CD150) to which SAP binds, thus initiating a new T-cell signal-transduction pathway via the co-receptor molecule SLAM. One of these cDNA clones from patients with IgA was identified as SAP.

In T cells expressing SAP, SLAM–SLAM interactions trigger selective up-regulation of IL-4 secretion in response to antigen receptor stimulation [22]. Because defective SAP protein produced by a mutated SAP gene plays a crucial role in the pathogenesis of the inherited immunodeficiency X-linked lymphoproliferative syndrome (XLP) [23,24], we attempted to identify genes causing disturbances in the function of EBV cytotoxic T cells in RA patients, focusing particularly on the SAP encoded by the XLP gene.

Using quantitative real-time PCR, the expression levels of SAP transcripts in peripheral T lymphocytes or leukocytes were examined. SAP transcript levels in peripheral leukocytes of RA patients were significantly lower than those of normal individuals, those of inactive systemic lupus erythematosus patients, and those of chronic renal disease patients [25]. Decreased SAP transcript levels in patients with RA were also observed in peripheral CD2 T lymphocytes when compared with normal individuals. Furthermore, we found that the nucleotide sequence of SAP cDNA did not possess any mutations or deletions in the coding region when compared with wild-type SAP cDNA [25].

The role of this decrease in SAP transcripts is uncertain. Genomic polymorphism in the promoter or enhancer region might be present in RA patients or certain cytokines may inhibit SAP mRNA expression. Because SAP primarily exists in T cells, disturbances in SAP mRNA expression would affect SLAM-induced signal-transduction events in T cells. RA patients have impaired IFN-γ production by T cells, which indicative of Th2 type responses [26]. SLAM production during T cell activation induces IFN-γ production and redirects Th2 type cells to Th1 type cells, and an inadequate response in RA patients would result from impaired function of the SLAM/SAP pathway. An impaired SAP pathway may contribute to the failure to eliminate EBV-infected

cells by cytotoxic T lymphocytes or NK cells in patients with RA (Fig. 1).

This hypothesis is supported by the results of abnormally elevated EBV-infected B cells [10,11] and defective EBV-suppressive T cells in patients with RA [11,12]. The role of decreased SAP transcripts in patients with RA is unclear. Abnormal promoter or enhancer genes may contribute to this event in patients with RA. We investigated nucleotide mutations in the SAP promoter region in patients with RA, and identified a single nucleotide mutation (manuscript in preparation). This single nucleotide mutation lead to the failure of the immune system to eliminate Epstein-Barr virus infected synovial cells.

4. Summary

We are encouraged by Ollier, who stated that a causal link between EBV and RA cannot yet be supported, but it does seem increasingly likely that viruses, such as EBV, have a role in the progression or exacerbation of inflammatory responses within the RA joint, and if treatments can be developed that can limit or prevent reactivation of EBV, these may be beneficial for RA [27]. We believe that we are very close to identifying a link between EBV and RA.

Take-home messages

- Antibodies against Epstein-Barr virus (EBV) increased in patients with rheumatoid arthritis (RA).
- Number of infected peripheral B lymphocytes in RA patients tends to be higher than in normal individuals.
- Specific cytotoxic T lymphocytes against EBV show an impairment in patients with RA.
- EBV DNA was detected and confirmed in the synovial tissue in patients with RA by polymerase chain reaction.
- EBV gene and latent membrane protein were demonstrated and confirmed in synovial cells and lymphocytes in patients with RA by the methods of in situ hybridization and immunohistochemical staining.

- Decreased SAP transcript levels in patients with RA were observed in peripheral T lymphocytes when compared with normal individuals. An impaired SAP pathway may contribute to the failure to eliminate EBV-infected cells by cytotoxic T lymphocytes or NK cells in patients with RA.

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Anti-peroxiredoxin I and IV autoantibodies

Anti-oxidative enzymes are protective from various oxidative stresses. Karasawa et al. (Microbiol Immunol 2005;49:57) investigated whether autoimmunity to the anti-oxidative peroxiredoxin enzymes exists in patients having systemic autoimmune diseases. The authors found that 33% of 92 patients with autoimmune diseases had autoantibodies to peroxiredoxin I: 57% of systemic lupus erythematosus patients, 19% of rheumatoid arthritis, 5% of Behcet disease, and 46% of primary systemic vasculitides. Nonetheless, autoantibodies to peroxiredoxin IV were detected in only 17% of these patients. The presence of anti-peroxiredoxin I autoantibodies correlated with lower serum levels of CH50, C3, and C4. Based on their findings the authors suggest that anti- peroxiredoxin autoantibodies may be involved in the pathophysiology of systemic autoimmune diseases.

CD226 expression deficiency and apoptosis in NK T cells from lupus patients

Tao et al. (J Immunol 2005;174:1281) report that NK T cells from active lupus patients are highly sensitive to anti-CD95-induced apoptosis compared with those from normal subjects and inactive lupus patients. They present data supporting that deficient expression of CD226 and survivin in NK T cells from active lupus patients is a molecular base of high sensitivity of the cells to anti-CD95-induced apoptosis.

症例報告

全身性エリテマトーデスに合併した
ヒトパルボウイルス B19 感染症

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北村　　登　　松川　吉博　　澤田　滋正[1]　　堀江　孝至

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Human Parvovirus B19 Infection In Patients with Systemic Lupus Erythematosus

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Human parvovirus B19 (HPV-B19) infection has been documented in patients with systemic lupus erythematosus (SLE), and is particularly important in the onset of the disease. The medical records of four cases of HPV-B19 infection in patients with SLE were reviewed and studied in detail. All four patients showed improvement with steroid therapy, and HPV-B19 DNA was detected in each sample of peripheral blood or bone marrow. Our findings suggest that we should clinically distinguish between the symptoms of HPV-B19 infection from those of SLE. Follow-up studies of larger groups of patients with HPV-B19 infection associated with a clinical picture of SLE are needed to better determine the role of this virus.

Key words: Human parvovirus B19, systemic lupus erythematosus
ヒトパルボウイルス B19, 全身性エリテマトーデス

要旨　ヒトパルボウイルス B19 (HPV-B19) 感染と全身性エリテマトーデス (SLE) をはじめとする自己免疫疾患の発症との関連性が報告されてきた. 我々はパルボウイルス初感染あるいは持続感染を伴った SLE の診断基準を満たす興味深い症例を 4 例経験した. いずれもステロイドの治療に反応を示し, 末梢血や骨髄からパルボウイルスが検出された. 臨床的にパルボウイルス感染症と SLE との鑑別に注意が必要と考えられた. また病因との関連性に関しては今後, 多くの症例を集め統計学的に検討することが重要と思われた.

はじめに

膠原病をはじめとした自己免疫疾患の病因とウイルスとの関係は以前より様々な議論がなされているが, その機序の解明には未だ至っていない. サイトメガロウイルス, Epstein-Barr virus といったウイルス感染症と SLE との臨床像の類似性が報告されてきた[1~3]. 特に HPV-B19 は SLE との臨床像の類似性やその病因との関係が論じられてきた[4,5]. 今回我々は SLE の診断基準を満たした HPV-B19 感染症を 4 例経験した. それらの臨床像や免疫学的異常に関して, 文献的考察を加えて報告する.

I. 症　例

症例 1 : 21 歳, 女性.
主　訴：多関節痛
既往歴：特記すべきことなし
家族歴：高脂血症 (母)
現病歴：平成 14 年 1 月中旬より PIP 関節を中心に DIP, MP 関節, および手関節の腫脹と疼痛が出現. 持続, 増悪するため平成 14 年 2 月 13 日に日大板橋病院を受診した. 顔面の紅斑および指趾先端の皮疹が認められた. また, 血清学上, 白血球減少と低補体価, 抗核抗体の高値

Table 1　Laboratory findings and clinical examination on Admission

Hematology		Blood chemistry			
WBC	2800 /μl	TP	6.8 g/dl	Na	145 mmol/l
Neutro	53.0%	Alb	3.5 g/dl	K	3.9 mmol/l
Eosino	1.0 %	T-Bil	0.27 mg/dl	Cl	109 mmol/l
Lympho	22.0 %	AST	32 IU/l	ESR	92 mm/h
Hb	10.5 g/dl	ALT	33 IU/l	CRP	1.05 mg/dl
RBC	349×10⁴/μl	BUN	16.5 mg/dl	IgG	2023 mg/dl
Plt	11.9×10⁴/μl	Cr	0.76 mg/dl	IgM	234 mg/dl

Immunological examination

C3　　23 mg/dl
C4　　3 mg/dl
CH50　<13.0 U/ml
ANA　 1280 x
LE cell　(+)
Anti-ds DNA Ab　398.9 IU/ml
Anti-ss-DNA ab　673.6 AU/ml
Anti Sm Ab　97.9 Index
Anti SS-A Ab　135.3 Index
Anti SS-B Ab　19.4 Index
Anti cardiolipin IgG Ab 1.7 U/ml
Anti cardiolipin IgM Ab 20.0 U/ml

Other clinical findings

1)Urinalysis ; Normal

2)Chest XP ; pleural effusion

3)Abdominal CT ;
　ascites (＋), splenomegaly
　Lymph node swelling around spleen and pancreas

4)Biopsy of skin ; Discoid Lupus erythematosus

が認められ，SLE の疑いにて同年 2 月 25 日に入院となった．

入院時現症：身長 156 cm，体重 48.7 kg，血圧 116/78 mmHg，脈拍 86 bpm，整　体温 36.5°C 意識清明，眼瞼に浮腫，眼瞼結膜に軽度貧血を認めた．眼球結膜黄染なし．頸部に圧痛を伴う直径 5 mm 大のリンパ節を触知した．胸部聴診上，呼吸音，心音ともに異常なし．腹部，平坦軟，圧痛なし．肝脾触知せず．PIP 関節に関節炎を認めた．下腿に浮腫なし．

検査所見 (Table 1)：白血球減少と軽度の貧血，赤沈の亢進を認めた．また抗核抗体，抗 DNA 抗体，抗 RNP 抗体，抗 SS-A 抗体，抗 SS-B 抗体，LE 細胞陽性，抗カルジオリピン抗体陽性．補体価は低下していた．パルボウイルスの検索では，血清の IgG 型抗パルボウイルス抗体陽性で，IgM 型抗パルボウイルス抗体陰性と，既感染パターンを示したが，骨髄のパルボウイルス DNA PCR は陽性，血球貪食像も認められた．その他尿所見は異常なし．画像診断では胸膜炎．顔面の皮疹の生検は「Discoid Lupus」であった．以上の結果から①蝶形紅斑 ②多関節炎 ③抗核抗体陽性 ④漿膜炎 ⑤白血球減少より SLE と診断した．ステロイドの治療により改善傾向を認めた．

症例 2：19 歳，女性．
主　訴：発熱，咳嗽
既往歴：特記すべきことなし
家族歴：虚血性心疾患 (父母，祖母)
現病歴：平成 14 年 2 月 14 日より上記主訴が出現．近医にて抗生剤，感冒薬の投与を受けていたが改善せず．同年 2 月 27 日に当院を受診．胸部 X-P 上，左肺炎と胸

膜炎の所見を認め，精査加療目的に入院となった．

入院時現症：身長 153 cm，体重 46kg，血圧 120/80 mmHg，脈拍 100 bpm，整　体温 39.7°C 意識清明，眼瞼結膜貧血．眼球結膜黄染なし．表在リンパ節を触知せず．胸部聴診上ラ音を認め心音ともに異常なし．腹部平坦軟，圧痛なし．下腿に浮腫なし．皮疹なし．神経学的所見なし．

検査所見 (Table 2)：白血球の低下と赤沈の亢進を認めた．また異型リンパ球も認められた．また抗核抗体，抗 DNA 抗体，抗 RNP 抗体陽性．補体価は低下していた．パルボウイルスの検索では，血清 IgM 型抗パルボウイルス抗体陽性，IgG 型抗パルボウイルス抗体陰性で，初感染パターンを示した．骨髄のパルボウイルス DNA PCR は陰性．その他尿所見は異常なし．以上の結果から①抗核抗体陽性 ②漿膜炎 ③白血球減少 ④抗 DNA 抗体陽性より SLE と診断．経過中，意識障害が出現しステロイドのパルス療法を施行し症状の改善をみた．

症例 3：26 歳，女性．
主　訴：多関節痛，発熱
既往歴，家族歴：特記すべきことなし
現病歴：平成 13 年 10 月より手指の冷感と疼痛が出現．某病院の外科を受診．抗核抗体 2540 倍と高値を認め膠原病精査のため平成 14 年 2 月 13 日に当院を紹介受診した．外来にて精査中，同年 4 月頃より上記症状が増悪し，全身に紅斑が出現．プレドニン 10 mg 投与を開始．5 月初旬より 38°C 台の発熱が持続するため 5 月 21 日に入院となった．

入院時現症：身長 161 cm，体重 45 kg，血圧 110/56

Table 2　Laboratory findings and clinical examination on Admission

Hematology		Blood chemistry			
WBC	2300 /μl	T-Bil	0.39 mg/dl	Na	144 mmol/l
Neutro	83.0%	AST	37 IU/l	K	3.7 mmol/l
Mono	3.0 %	ALT	16 IU/l	Cl	107 mmol/l
Lympho	13.0 %	BUN	11.6 mg/dl	ESR	48 mm/h
Aty-Ly.	1.0%	Cr	0.62 mg/dl	CRP	0.84 mg/dl
Hb	10.2 g/dl			IgG	1822 mg/dl
Plt	10.0×10⁴/μl			IgM	94 mg/dl

Immunological examination		Other clinical findings
C3	16 mg/dl	1)Urinalysis ; Normal
C4	3 mg/dl	
CH50	<13.0 U/ml	
ANA	1280 x	
LE cell	(−)	
Anti-ds DNA Ab	400 IU/ml	
Anti Sm Ab	(−)	
Anti cardiolipin Ab	(−)	

Table 3　Laboratory findings and clinical examination on Admission

Hematology		Blood chemistry			
WBC	7200 /μl	TP	7.5 g/dl	Na	141 mmol/l
Neutro	90.0%	T-Bil	0.48 mg/dl	K	4.3 mmol/l
Mono	3.0 %	AST	20 IU/l	Cl	103 mmol/l
Lympho	7.0 %	ALT	16 IU/l	ESR	110 mm/h
Hb	12.0 g/dl	BUN	7.1 mg/dl	CRP	4.81 mg/dl
Plt	19.6×10⁴/μl	Cr	0.63 mg/dl		

Immunological examination		Other clinical findings
CH50	58.6 U/ml	1)Urinalysis ; Normal
ANA	1280 x	
LE cell	(−)	
Anti-ds DNA Ab	2.4 IU/ml	
Anti-ss-DNA Ab	11.0 AU/ml	
Scl-70 Ab	6.4 Index	
Anti cardiolipin IgG Ab 1.7 U/ml		
Anti cardiolipin IgM Ab 20.0 U/ml		
PR3 ANCA	<10 Index	
MPO ANCA	<10 Index	

mmHg, 脈拍 84 bpm, 整 体温 38.1°C 意識清明, 眼瞼
結膜貧血なし. 眼球結膜黄染なし. 頚部に直径 5 mm 大
のリンパ節を数個触知した. 腋窩, 鎖骨下, 鼡径リンパ
節は触知せず. 胸部聴診上, 呼吸音, 心音ともに異常な
し. 腹部, 平坦軟, 圧痛なし. 下腿に浮腫なし. 顔面,
前胸部, 手掌に紅斑を認める. 冷水によるレイノー症状
あり. 神経学的所見なし.

　検査所見 (Table 3)：リンパ球減少と CRP, 赤沈の亢進
を示した. また抗核抗体, 抗 ds DNA 抗体, 抗 RNP 抗
体陽性. 補体価は高値を示した. パルボウイルスの検索
では, 血清 IgM 型抗パルボウイルス抗体陽性, IgG 型抗
パルボウイルス抗体陰性で初感染パターンを示した. 末

梢血と骨髄のパルボウイルス DNA PCR は陽性. 以上の
結果から ①蝶形紅斑 ②関節炎 ③リンパ球減少 ④抗核抗
体陽性より SLE と診断. ステロイドの増量と抗生剤にて
改善した.

　症例 4：23 歳, 女性.
　主 訴：発熱
　既往歴, 家族歴：特記すべきことなし
　現病歴：平成 9 年 9 月より SLE の診断にて当科外来
を通院. プレドニン 5 mg 内服にて治療中であった. 平
成 14 年 4 月 9 日から 5 月 18 日まで発熱と全身性の皮疹
にて入院. 抗生剤の投与にて軽快した. その際の骨髄検

Table 4　Laboratory findings and clinical examination on Admission

Hematology		Blood chemistry			
WBC	3200 /μl	TP	8.3 g/dl	Na	140 mmol/l
Neutro	64.0%	AST	47 IU/l	K	3.3 mmol/l
Mono	9.0%	ALT	6 IU/l	Cl	102 mmol/l
Eosino	1.0 %	BUN	9.1 mg/dl	ESR	120 mm/h
Lympho	26.0 %	Cr	0.5 mg/dl	CRP	8.63 mg/dl
Hb	9.1 g/dl				
Plt	40×10⁴ /μl				

Immunological examination		Other clinical findings
C3	102 mg/dl	1)Urinalysis ; Normal
C4	25 mg/dl	
CH50	50.5 U/ml	2)Chest XP ; Consolidation at left lower lung field
ANA	1280 x	
LE cell	(−)	
Anti-ds DNA Ab	42.9 IU/ml	
Anti RNP Ab	61.0 Index	
Anti Sm Ab	97.9 Index	
Anti cardiolipin Ab	(−)	

査で血球貪食像が認められていた．退院後，再度発熱が認められ，胸部X-P上，左下肺野に肺炎像を認め入院となった．

入院時現症：身長 148 cm，体重 38.6 kg，血圧 110/56 mmHg，脈拍 66 bpm，整　体温 38.0℃ 意識清明，眼瞼結膜貧血なし．眼球結膜黄染なし．頚部その他にリンパ節を触知せず．胸部聴診上，左下肺野に湿性ラ音を聴取．心音に異常なし．腹部，平坦軟，圧痛なし．下腿に浮腫なし．皮疹を認めず．神経学的所見なし．

検査所見 (Fig. 4)：白血球減少と貧血，CRP の高値と赤沈の亢進を認めた．また抗核抗体陽性，抗 ds-DNA 抗体，抗 RNP 抗体陽性．補体価は高値を示した．パルボウイルスの検索では，血清 IgM 型抗パルボウイルス抗体陰性，IgG 型抗パルボウイルス抗体陽性で既感染パターンを示したが，骨髄のパルボウイルス DNA PCR は陽性であった．尿所見は正常．胸部レントゲンで左下肺野に肺炎像を認めた．以上より SLE の活動性に大きな変動はないものと判断しステロイドの増量はせず，抗生剤による肺炎の治療を行い改善した．

II. 考　　察

Human Parvovirus B 19 (HPV-B19) は，1975 年に発見された DNA ウイルスの一種でヒトに病原性を持つ唯一のパルボウイルスである．小児では伝染性紅斑を起こすウイルスとして有名であり，顔面の皮疹は SLE 様の紅斑である．また成人の急性感染では多発性関節炎を起こし，その症状は関節リウマチ様である[6,7]．また関節炎は数年にわたり遷延することもあり，骨髄での感染により赤芽球癆や再生不良性貧血を来すこともあると言われている．最近の報告例の中には高熱と関節炎で発症し，白血球減少，クームス陽性の溶血性貧血，血小板減少，自己抗体陽性，抗 ds-DNA 抗体陽性，低補体価が出現し，当初SLEの診断基準を満たしステロイド治療やγグロブリンの投与が行われた症例も報告されている[8]．

我々の症例におけるパルボウイルスの検索では，症例 1，4 は既感染型で症例 2，3 は IgM 抗体陽性の初感染型であった．一方，末梢血 PCR は症例 3 のみ陽性，骨髄の PCR は症例 1，3，4 で陽性であった (Table 5)．既感染型と初感染型に明らかな臨床症状の差は認められなかったが，症例 1，4 の骨髄中のウイルスの存在が確認され，かつ血球貪食像が認められた．またいずれの症例もステロイドの治療が効果を示した．Table 6 に示すように，4 症例の臨床症状では顔面の紅斑と散在性丘状紅斑が 3 症例に認められ，すべての症例が若年女性であり関節炎とともに抗核抗体，血液学的や免疫学的異常を認めたが，腎症状はいずれの症例も認めなかった (Table 6)．症例 2 では血清と骨髄のいずれもウイルスが検出されなかったが，4 症例中で最も重篤な臨床経過を辿り，生体内のウイルスの存在と臨床症状が必ずしも相関しなかった．つまりウイルスを排除しようとする宿主の免疫応答が自己免疫を引き起こした可能性が推測された．免疫学的異常に関して 4 症例はいずれも抗 U1-RNP 抗体が陽性であり，かつ腎症が認められなかった．以前より抗 U1-RNP 抗体陽性例の臨床像の特徴として腎症の発生頻度が少ないという経験から，今回の 4 症例も抗 U1-RNP 抗体が陽性であり，腎症の合併頻度の低下との関連が考えられた．またパルボウイルス感染が冬から春に集中する季節性や地域性が認められたとの報告もあるが，我々の症例においても 2 月から 4 月にかけて集中していた[9~12]．

Table 5　Summary of date for four cases with HPV B19 infection

		症例1	症例2	症例3	症例4
血清	IgG	(＋)	(−)	(−)	(＋)
	IgM	(−)	(＋)	(＋)	(−)
	PCR	(−)	(−)	(＋)	(−)
骨髄	PCR	(＋)	(−)	(＋)	(＋)
	血球貪食像	(＋)	(−)	(−)	(＋)
胸水	IgG	(＋)	/	/	/
	IgM	(−)			
	PCR	(−)			
治療		/	PSL 50mg	パルス PSL 60mg	PSL 30mg　PSL 4-5mg 隔日

IgG:IgG型抗パルボウイルス抗体　　IgM:IgM型抗パルボウイルス抗体
PCR:パルボウイルスDNA-PCR　　パルス：メチルプレドニゾロン1mg×3日間

Table 6　Clinical presentation in four cases of HPV B19
infection and SLE features

	症例1	症例2	症例3	症例4
1, 顔面紅斑	○	—	○	以前○
2, 円判状皮疹	非典型的	—	非典型的	非典型的
3, 光線過敏症	—	—	—	—
4, 口腔潰瘍	—	—	—	—
5, 関節炎	○	○	○	○
6, 漿膜炎	○	○	—	—
7, 腎病変	—	—	—	—
8, 神経学的異常	—	○	—	—
9, 血液学的異常	○	○	○	○
10, 免疫学的異常	○	○	○	○
11, 抗核抗体	○	○	○	○
リンパ節腫脹	(＋)	(−)	(＋)	(−)
血清パルボウイルス	(−)	IgM(＋)	PCR(＋)	(−)
骨髄パルボウイルス	PCR(＋)	(−)	PCR(＋)	PCR(＋)
ステロイド治療	改善	改善	改善	改善

　以上のように HPV-B19 感染は臨床的に SLE の初発症状と類似点が多く，関節炎や皮疹などの症状が認められた場合は，パルボウイルス感染症を念頭に置き，HPV-B19 IgG や IgM 抗体価の測定が必要と考えられた[12]．この 4 症例は，臨床症状が強くウイルス抗体価の結果を待たずに，緊急でステロイドの強力な治療に踏み切らざるえない症例が多かった．臨床的にパルボウイルス感染で一過性に症状が軽快するセルフ・リミティングな症例もある[13]．また免疫抑制剤の使用が臨床症状の悪化や持続感染を起こす可能性があるとの報告もあり，感染初期のステロイド投与の選択に慎重な検討が必要と考えられた[14]．

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CASE REPORT

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Wegener's granulomatosis complicated with intestinal ulceration

Abstract We report the case of a 32-year-old man who developed Wegener's granulomatosis complicated with refractory intestinal ulceration. In August 2001, he presented with a high fever, nasal bleeding, and bilateral leg numbness. These symptoms worsened, which prompted him to consult his home doctor on February 18, 2002. In spite of treatment with antibiotics, his symptoms did not improve. Furthermore, abdominal pain and melena occurred as additional symptoms in March 2002. He was admitted to our hospital on April 5, 2002. A deformed nose condition (the so-called saddle nose) was observed at this time. Laboratory data showed a high erythrocyte sedimentation rate (103mm/h) and a high level of serum C-reactive protein (14.98mg/dl), and hematuria and proteinuria were also observed. The patient was positive for an antineutrophil cytoplasmic antibody specific for proteinase-3 (PR3-ANCA). A chest computed tomography (CT) scan revealed multiple pulmonary nodules in the lung field. A biopsied-specimen from the nasal mucosa showed necrotizing granulomatosis with giant cells. Together with his symptoms and the laboratory and pathological findings, the patient was diagnosed as having Wegener's granulomatosis. A colon fiberscopy showed multiple ulcerations with bleeding from the terminal ileum to the ascending colon, and nodular lesions at the terminal ileum. We started a combination therapy of prednisolone (60mg/day) and cylophosphamide (100mg/day) orally. The patient's gastrointestinal symptoms disappeared and abnormal serological indicators improved. Although Wegener's granulomatosis complicated with refractory intestinal ulceration is rare, this case indicates that the gastrointestinal region is also a target organ of Wegener's granulomatosis.

Introduction

Wegener's granulomatosis is a systemic necrotizing vasculitis of unknown etiology with distinct clinical and histological features. Histologically, it consists of necrotizing vasculitis affecting mainly small to medium-sized arteries, and sometimes involving venous or capillary vessels.[1] The disease typically involves the upper and lower airway, lungs, and kidneys. Although inflammatory involvement of the disease has been reported in other organs, a gastrointestinal complication is relatively rare except for scattered case reports.[2]

We present a case of Wegener's granulomatosis complicated with peripheral neuropathy and refractory intestinal ulceration, and then discuss the possibility that gastrointestinal involvement may be an inherent clinical manifestation of Wegener's granulomatosis.

Case report

A 32-year-old man developed a high fever, nasal bleeding, and bilateral leg numbness in August 2001. He consulted his home doctor, and was treated with an oral antibiotic. The high fever improved temporarily, but his symptoms worsened again in December 2001, when he noticed a macrohematuria. On February 18, 2002, he was admitted to a hospital where he was given a drip infusion of antibiotics. He was transferred to our hospital on April 5, 2002, because of abdominal pain and melena, in addition to the above symptoms.

In the course of a physical examination, his blood pressure was found to be normal and his heart rate was 82/min. A high fever (38.8°C) was observed. Surface lymph nodes were not palpable. A deformed nose condition described as

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Fig. 1. The microscopic findings of nasal mucosa showing ulceration infiltrated with neutrophils and necrotizing granuloma with giant cells (*)

"saddle nose" was observed. His skin and conjunctiva were strikingly anemic. His heart and lung sounds were normal. Tenderness of the lower abdomen was found, but no organ enlargement or mass was palpable in the abdomen. Multiple sensory paralyses in his right and left feet, and the right-hand side of his face were observed.

Laboratory tests on admission revealed a leukocytosis (10300/μl) and normocytic anemia (6.9g/dl). His erythrocyte sedimentation rate (ESR) was 103mm/h and his serum C-reactive protein (CRP) level was 14.98mg/dl. Although renal function (serum blood nitrogen and creatinine level) was normal, a microscopic hematuria and proteinuria (0.98g/day) was observed. A mild elevation of serum transaminase levels (GOT 50U/l, GPT 64U/l) was found. In an examination for autoantibodies, antinuclear antibodies were found (40 dils) and an antineutrophilic cytoplasmic antibody (ANCA) specific for protease-3 (PR-3 ANCA) was positive (titer 102 EU.) However, an ANCA specific for myeloperoxidase (MPO-ANCA) and anti-ds DNA antibodies was not observed.

Several nodular shadows were revealed in the right upper and lower lobes on chest X-ray films. In a chest computed tomography (CT) scan, the nodular shadows in the lung were confirmed. In a head CT scan, the sinus and nasal mucosa were found to be infiltrated by the necrotizing granuloma. A biopsy of the nasal mucous membranes was performed and the microscopic findings of the specimen showed an ulceration, small to medium-sized vasculitis infiltrated with neutrophils, and necrotizing granuloma with giant cells in squamous epithelium and in stroma (Figs. 1 and 2).

A colon fiberscopy showed an elevated granulomatous lesion at the terminal ileum (Fig. 3A), and multiple, consecutive ulcerations with bleeding from the ascending colon to the terminal ileum (Fig. 3B–D). No ulcerations or granulomas were observed in the peripheral part of the small intestine, descending colon, and rectum. Microscopic findings from a biopsy specimen from the elevated lesion at the terminal ileum (Fig. 3A) showed nonspecific
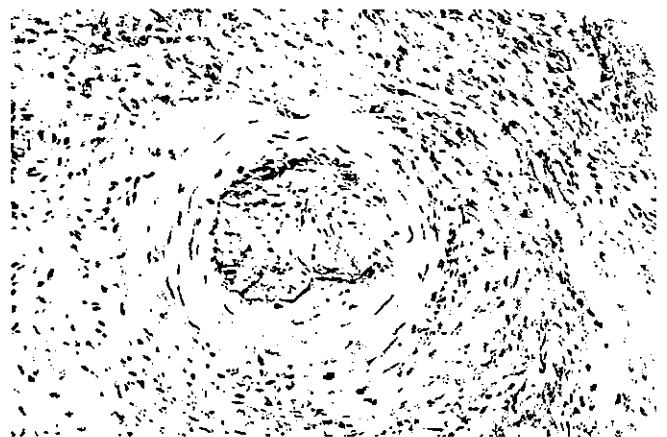

Fig. 2. The microscopic findings of nasal mucosa showing medium-size vasculitis

inflammation with granulomatous lesion, giant cells, and vasculitis without necrosis (Fig. 4). We diagnosed the patient as having Wegener's granulomatosis with gastrointestinal organ complications.

We started oral medication with 60mg/day prednisolone and 100mg/day cyclophosphamide (Fig. 5). The melena stopped immediately, and after 2 weeks of treatment the ESR, the serum level of CRP, and a titer of PR3-ANCA had normalized. Although bilateral leg numbness and nodular shadows on both lungs (as shown by a chest CT scan) partly remained, the granulomatous mass in the sinus (shown by a head CT scan), the multiple ulcerations of the ascending colon, and the elevated lesion of the terminal ileum disappeared within 3 months of the onset of treatment.

The patient was discharged on July 21, 2002. Two years after discharge, the patient had no nasal symptoms or bilateral leg numbness. The nodular shadows on the bilateral lungs and granuloma of the sinus were significantly reduced. Moreover, no recurrence of intestinal ulceration was observed by fiberscopy.

Fig. 3. Colon fiberscopy showing multiple ulcerations with bleeding (**) and elevated lesions (*) at the ascending colon.
A The terminal ileum.
B The descending colon
C Granulomatous lesion at the terminal ileum
D Nonconsecutive ulcer at the descending colon
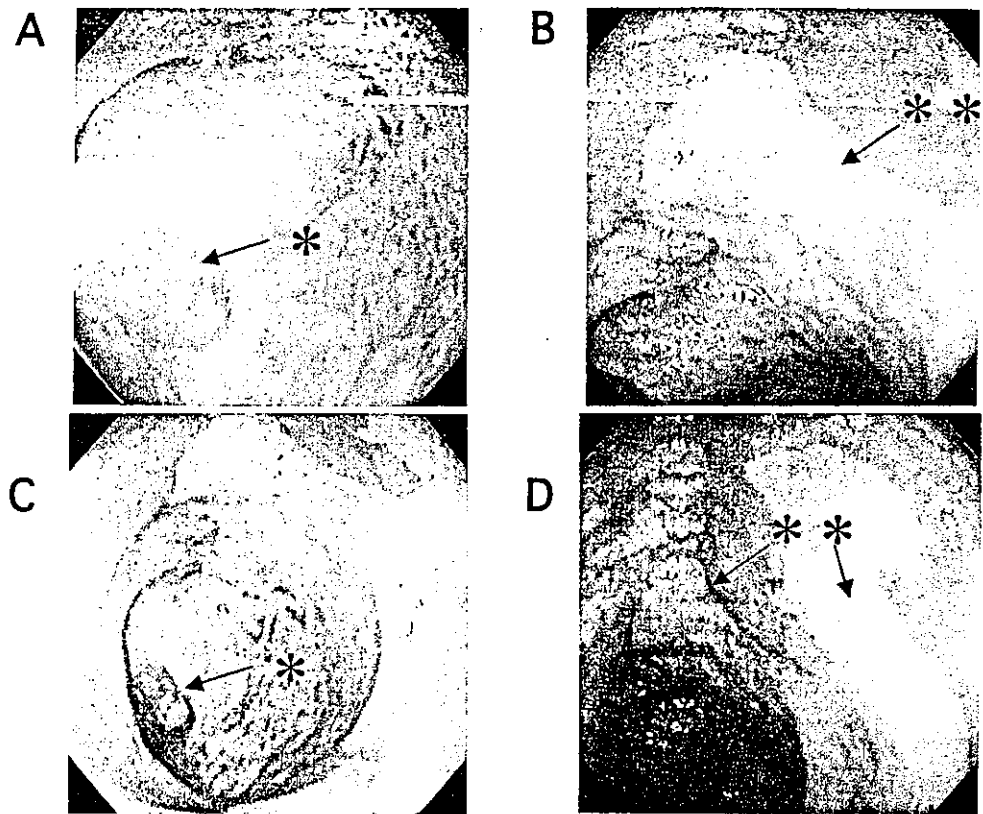
Fig. 4. The microscopic findings of the terminal ileum mucosa infiltrated with neutrophils and necrotizing granuloma with giant cells (*) and vasculitis without necrotizing lesion (**)
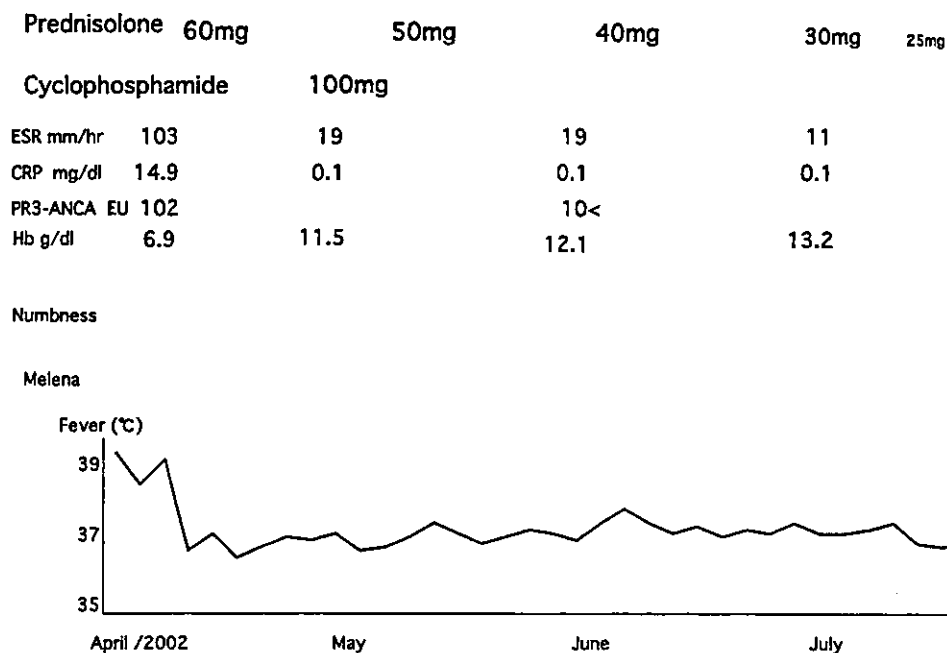
Discussion

Wegener's granulomatosis is a systemic granulomatous inflammatory disease which can affect a variety of organs. Histologically, it consists of necrotizing vasculitis affecting mainly small to medium-sized arteries, and sometimes involving venous or capillary vessels.[1] This granulomatous vasculitis involves primarily the upper and lower airway system, the lungs, and the kidneys. Since its description in the 1930s,[3,4] various sites of involvement have been identified, in a vast range of organ systems. The incidence of gastrointestinal involvement reported here is relatively rare.

Hashimoto et al.[5] reported that the initial symptoms of Wegener's granulomatosis in Japanese cases were nasal occlusion (51%), nasal bleeding (45%), and pyrexia (27%). Gastrointestinal symptoms were not referred to in this report. Moreover, in a study of 158 patients with Wegener's granulomatosis seen at the National Institute of Health, no gastrointestinal manifestations were reported.[2] Storesund et al.[6] reported that they found only six cases of Wegener's granulomatosis in the available medical literature that presented with severe intestinal involvement. In this report, any gastrointestinal involvement occurs in an earlier period of the Wegener's granulomatosis, and in most of the reported cases the disease had an active status. The intestinal involvement appears in any region of the large intestine, with skipped lesions which sometimes lead to perforation. Under microscopic examination, some of the cases showed ischemic changes and vasculitis in addition to ulcerations.[7-10] On the other hand, granulomatous lesions or typical necrotizing vasculitis are not usually recognized in this complication.

In our case, a biopsied specimen from the elevated lesion from the ascending colon showed nonspecific inflammation

Fig. 5. Clinical course on admission. *ESR*, erythrocyte sedimentation rate; *CRP*, C-reactive protein

Prednisolone	60mg	50mg	40mg	30mg	25mg
Cyclophosphamide	100mg				
ESR mm/hr	103	19	19	11	
CRP mg/dl	14.9	0.1	0.1	0.1	
PR3-ANCA EU	102		10<		
Hb g/dl	6.9	11.5	12.1	13.2	

Numbness

Melena

Fever (°C)

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39│
  │
37│
  │
35└_____
   April /2002    May      June      July
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with granulomatous lesions, giant cells, and vasculitis without necrosis. The most important differential diagnosis in our case is the overlapping of other inflammatory bowel diseases such as Crohn's disease. One case of the overlapping of Wegener's granulomatosis and Crohn's disease has been reported.[11] It is important to differentiate between our case and Crohn's disease. Typical visible colonoscopic features of Crohn's disease are liner ulcer, nonconsecutive ulcer, small intestinal ulcer, cobble stoning, and inflammatory pseudopolyposis.[12] In our case, these typical findings for Crohn's disease were lacking. Furthermore, in our case, a vasculitis and giant cells, not usually seen in Crohn's disease, occurred histologically, although there was no necrotic vasculitis. Therefore, in our case, the overlapping of Wegener's granulomatosis and Crohn's disease is not the same. Unfortunately, typical histological findings in the gastrointestinal tract for Wegener's granulomatosis are uncommon. In only one case, described by Richard and William,[13] has the presence of typical necrotizing vasculitis been found in a biopsy specimen.

PR-3 ANCA, as well as the colonoscopy findings, will be useful to differentiate Wegener's granulomatosis from other inflammatory bowel diseases.[6,11,14] Together with visible colonoscopic features, pathological findings in the colon biopsy specimen, and a positive titer of PR3-ANCA, we diagnosed Wegener's granulomatosis complicated with intestinal ulceration. Although some cases with perforation had poor prognoses, this gastrointestinal complication has usually been curable in most reported cases.[6,8,13,15] In our case, the patient has remained in remission for more than 2 years.

In conclusion, gastrointestinal involvement is a rare event in Wegener's granulomatosis. However, our case and some cases in the literature indicate that the gastrointestinal region is one of the target organs in Wegener's granulomatosis in the initial stages of the disease.

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17　サイトメガロウイルス持続感染経過中に肺好酸球症候群（PIE症候群）と側頭動脈炎を併発した一例

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座長（三﨑）　それでは，第17席，白岩先生お願いします。

白岩（日本大学医学部内科学講座血液・膠原病内科部門）　今回われわれは，サイトメガロウイルス持続感染経過中に肺好酸球症候群（PIE症候群）と側頭動脈炎を併発した一例を経験しましたので報告します。

〔スライド映写〕

(1)　症例です。

(2)　入院時の現症です。身長が175cm，体重が76kg，血圧が136/90mmHg，脈拍108，体温が40℃と高度の発熱を認めます。体表面に表在リンパ節は触知せず，特徴的な皮疹等は特に認めませんでしたが，皮膚および眼球結膜が黄染しておりました。口腔内の所見では，びらんを伴い腫大した扁桃を両側に認めました。心音・呼吸音とも異常なく，腹部所見で右季肋下に肝を3横指触知し，脾臓の腫大は明らかではありませんでした。

(3)　次に入院時6月19日の検査所見です。異常値のみ述べますと，まず血算で白血球が23600と高値を示し，そのうち異型リンパ球が50%近く占めております。また，血小板が約11万とやや低値を示しております。凝固系は明らか