corticosteroid-resistant asthma.[40] The establishment of a correct T_H1/T_H2 balance and the induction of the IL-10 gene have been suggested as potential mechanisms of action.[40] It is also possible that induction of T_H1-like regulatory T cells might be involved in the IFN-α–mediated improvement of corticosteroid-resistant asthma. Further studies designed to explore the capacity of IFN-α to induce T_H1-like regulatory T cells would be important for a complete understanding of its role in the treatment of bronchial asthma and atopic diseases.

We to thank Drs A. Ishii, T. Sawada, Y. Morita, and K. Yamamoto for helpful advice and Ms C. Kawashima for preparing the manuscript.

REFERENCES

1. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature 1996;383:787-93.
2. Constant SL, Bottomly K. Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. Annu Rev Immunol 1997;15: 297-322.
3. Lichtman AH, Abbas AK. T-cell subsets: recruiting the right kind of help. Curr Biol 1997;7:R242-4.
4. Romagnani S. The Th1/Th2 paradigm. Immunol Today 1997;18:263-6.
5. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989;7:145-73.
6. Kamogawa Y, Minasi LA, Carding SR, Bottomly K, Flavell RA. The relationship of IL-4– and IFN gamma–producing T cells studied by lineage ablation of IL-4–producing cells. Cell 1993;75:985-95.
7. Coffman RL, Lebman DA, Rothman P. Mechanism and regulation of immunoglobulin isotype switching. Adv Immunol 1993;54:229-70.
8. Sanderson CJ. Interleukin-5, eosinophils, and disease. Blood 1992;79: 3101-9.
9. Ho IC, Hodge MR, Rooney JW, Glimcher LH. The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. Cell 1996;85:973-83.
10. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 1997;89: 587-96.
11. Kim JI, Ho IC, Grusby MJ, Glimcher LH. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. Immunity 1999;10:745-51.
12. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J Biol Chem 1997;272:21597-603.
13. Lavenu-Bombled C, Trainor CD, Makeh I, Romeo PH, Max-Audit I. Interleukin-13 gene expression is regulated by GATA-3 in T cells: role of a critical association of a GATA and two GATG motifs. J Biol Chem 2002;277:18313-21.
14. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 1999;17:189-220.
15. Rogge L, D'Ambrosio D, Biffi M, Penna G, Minetti LJ, Presky DH, et al. The role of Stat4 in species-specific regulation of Th cell development by type I IFNs. J Immunol 1998;161:6567-74.
16. Rogge L, Barberis-Maino L, Biffi M, Passini N, Presky DH, Gubler U, et al. Selective expression of an interleukin-12 receptor component by human T helper 1 cells. J Exp Med 1997;185:825-31.
17. Parronchi P, Mohapatra S, Sampognaro S, Giannarini L, Wahn U, Chong P, et al. Effects of interferon-alpha on cytokine profile, T cell receptor repertoire and peptide reactivity of human allergen-specific T cells. Eur J Immunol 1996;26:697-703.
18. Shibuya H, Nagai T, Ishii A, Yamamoto K, Hirohata S. Differential regulation of Th1 responses and CD154 expression in human CD4+ T cells by IFN-alpha. Clin Exp Immunol 2003;132:216-24.
19. Schandene L, Del Prete GF, Cogan E, Stordeur P, Crusiaux A, Kennes B, et al. Recombinant interferon-alpha selectively inhibits the production of interleukin-5 by human CD4+ T cells. J Clin Invest 1996;97:309-15.
20. Terabe M, Park JM, Berzofsky JA. Role of IL-13 in regulation of anti-tumor immunity and tumor growth. Cancer Immunol Immunother 2004; 53:79-85.
21. Geppert TD, Lipsky PE. Accessory cell independent proliferation of human T4 cells stimulated by immobilized monoclonal antibodies to CD3. J Immunol 1987;138:1660-6.
22. Hirohata S. Human Th1 responses driven by IL-12 are associated with enhanced expression of CD40 ligand. Clin Exp Immunol 1999;115:78-85.
23. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 2000;100:655-69.
24. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003;4:330-6.
25. Stock P, Akbari O, Berry G, Freeman GJ, DeKruyff RH, Umetsu DT. Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. Nat Immunol 2004;5:1149-56.
26. Wu CY, Gadina M, Wang K, O'Shea J, Seder RA. Cytokine regulation of IL-12 receptor beta2 expression: differential effects on human T and NK cells. Eur J Immunol 2000;30:1364-74.
27. Hibbert L, Pflanz S, De Waal Malefyt R, Kastelein RA. IL-27 and IFN-α signal via Stat1 and Stat3 and induce T-Bet and IL-12Rβ2 in naive T cells. J Interferon Cytokine Res 2003;23:513-22.
28. Kelly BL, Locksley RM. Coordinate regulation of the IL-4, IL-13, and IL-5 cytokine cluster in Th2 clones revealed by allelic expression patterns. J Immunol 2000;165:2982-6.
29. Cousins DJ, Lee TH, Staynov DZ. Cytokine coexpression during human Th1/Th2 cell differentiation: direct evidence for coordinated expression of Th2 cytokines. J Immunol 2002;169:2498-506.
30. Bohjanen PR, Okajima M, Hodes RJ. Differential regulation of interleukin 4 and interleukin 5 gene expression: a comparison of T-cell gene induction by anti-CD3 antibody or by exogenous lymphokines. Proc Natl Acad Sci U S A 1990;87:5283-7.
31. Jung T, Schauer U, Rieger C, Wagner K, Einsle K, Neumann C, et al. Interleukin-4 and interleukin-5 are rarely co-expressed by human T cells. Eur J Immunol 1995;25:2413-6.
32. Tendler CL, Burton JD, Jaffe J, Danielpour D, Charley M, McCoy JP, et al. Abnormal cytokine expression in Sezary and adult T-cell leukemia cells correlates with the functional diversity between these T-cell malignancies. Cancer Res 1994;54:4430-5.
33. Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinni MP, et al. IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. J Immunol 1992;149:2977-83.
34. Demeure CE, Wu CY, Shu U, Schneider PV, Heusser C, Yssel H, et al. In vitro maturation of human neonatal CD4 T lymphocytes. II. Cytokines present at priming modulate the development of lymphokine production. J Immunol 1994;152:4775-82.
35. Andreone P, Gramenzi A, Loggi E, Favarelli L, Cursaro C, Margotti M, et al. In vitro effect of indomethacin and interferon-α on Th1 and Th2 cytokine synthesis in patients with chronic hepatitis C. Cytokine 2004; 26:95-101.
36. Farrar JD, Asnagli H, Murphy KM. T helper subset development: roles of instruction, selection, and transcription. J Clin Invest 2002;109:431-5.
37. Seder RA, Paul WE, Davis MM, Fazekas DS. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice. J Exp Med 1992;176:1091-8.
38. Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. J Immunol 1990;145:3796-806.
39. Cogan E, Schandene L, Crusiaux A, Cochaux P, Velu T, Goldman M. Brief report: clonal proliferation of type 2 helper T cells in a man with the hypereosinophilic syndrome. N Engl J Med 1994;330:535-8.
40. Simon HU, Seelbach H, Ehmann R, Schmitz M. Clinical and immunological effects of low-dose IFN-α treatment in patients with corticosteroid-resistant asthma. Allergy 2003;58:1250-5.

Inhibition of CX3CL1 (Fractalkine) Improves Experimental Autoimmune Myositis in SJL/J Mice[1]

Fumihito Suzuki,* Toshihiro Nanki,[2]* Toshio Imai,† Hirotoshi Kikuchi,‡ Shunsei Hirohata,‡ Hitoshi Kohsaka,* and Nobuyuki Miyasaka*

Idiopathic inflammatory myopathy is a chronic inflammatory muscle disease characterized by mononuclear cell infiltration in the skeletal muscle. The infiltrated inflammatory cells express various cytokines and cytotoxic molecules. Chemokines are thought to contribute to the inflammatory cell migration into the muscle. We induced experimental autoimmune myositis (EAM) in SJL/J mice by immunization with rabbit myosin and CFA. In the affected muscles of EAM mice, CX3CL1 (fractalkine) was expressed on the infiltrated mononuclear cells and endothelial cells, and its corresponding receptor, CX3CR1, was expressed on the infiltrated CD4 and CD8 T cells and macrophages. Treatment of EAM mice with anti-CX3CL1 mAb significantly reduced the histopathological myositis score, the number of necrotic muscle fibers, and infiltration of CD4 and CD8 T cells and macrophages. Furthermore, treatment with anti-CX3CL1 mAb down-regulated the mRNA expression of TNF-α, IFN-γ, and perforin in the muscles. Our results suggest that CX3CL1-CX3CR1 interaction plays an important role in inflammatory cell migration into the muscle tissue of EAM mice. The results also point to the potential therapeutic usefulness of CX3CL1 inhibition and/or blockade of CX3CL1-CX3CR1 interaction in idiopathic inflammatory myopathy. ***The Journal of Immunology,*** **2005, 175: 6987–6996.**

Idiopathic inflammatory myopathy (IIM),[3] including polymyositis and dermatomyositis, is characterized by chronic inflammation of the voluntary muscles associated with infiltration of inflammatory cells, including CD4 and CD8 T cells and macrophages, in the skeletal muscle (1–3). Infiltrated CD4 and CD8 T cells express cytotoxic molecules, such as perforin and granzyme granules, and the T cells and macrophages express inflammatory cytokines, such as TNF-α and IFN-γ (4–8). Therefore, the infiltrated inflammatory cells might play an important role in the pathogenesis of IIM. The inflammatory cell migration into the muscle is thought to involve the interaction of chemokines and chemokine receptors (9–14).

Chemokines are involved in leukocyte recruitment and activation at the site of inflammatory lesion (15). Approximately 50 chemokines have been identified to date, and they are classified into four subfamilies, C, CC, CXC, and CX3C chemokines, based on the conserved cystein motifs (16). Although the majority of chemokines are small secreted molecules, CX3CL1 (fractalkine) is expressed on the cell surface as a membrane-bound molecule (17, 18). The membrane-bound CX3CL1 is expressed on endothelial cells stimulated with TNF-α, IL-1, and IFN-γ (19–21), induces adhesion of the leukocytes, and supports leukocyte transmigration into tissue (22, 23). The soluble form of CX3CL1 is generated by proteolytic cleavage at a membrane-proximal region of the membrane-bound CX3CL1 by TNF-α-converting enzyme (a disintegrin and metalloproteinase domain 17) and a disintegrin and metalloproteinase domain 10 (24, 25), and is known to induce leukocyte migration (23). In contrast, CX3CR1, a unique receptor for CX3CL1, is expressed on peripheral blood CD4 and CD8 T cells that express cytotoxic molecules and type 1 cytokines (26, 27). CX3CR1 is also expressed on monocytes/macrophages, NK cells, and dendritic cells (28, 29).

Based on the infiltration of CTLs and macrophages into the affected muscles in patients with IIM, we speculated that the CX3CL1-CX3CR1 interaction might contribute to the inflammatory cell migration. In the present study we induced experimental autoimmune myositis (EAM) in SJL/J mice and examined CX3CL1 and CX3CR1 expression in the affected muscle of EAM mice. Furthermore, we studied the effect of CX3CL1 inhibition on EAM mice.

Materials and Methods

Induction of EAM

Male 5-wk-old SJL/J mice were purchased from Charles River Japan. Purified myosin from rabbit skeletal muscle (6.6 mg/ml; Sigma-Aldrich) was emulsified with an equal amount of CFA (Difco Laboratories) with 3.3 mg/ml *Mycobacterium butyricum* (Difco Laboratories). Mice were immunized intracutaneously with 100 μl of emulsion into four locations (total, 400 μl) on the back on days 0, 7, and 14. On day 21, the mice were killed, and the quadriceps femoris muscles were harvested. The muscle tissues were frozen immediately in chilled isopentane precooled in liquid nitrogen, and then 6-μm-thick cryostat sections were prepared at intervals of 200 μm. The sections were stained with H&E or used for immunohistochemistry. The experimental protocol was approved by the institutional animal care and use committee of Tokyo Medical and Dental University.

Immunohistochemistry

Immunohistological staining was performed as described previously (26, 30) with some modifications. Briefly, 6-μm-thick sections were air-dried and fixed in cold acetone at −20°C for 3 min. After air-drying at room

*Department of Medicine and Rheumatology, Tokyo Medical and Dental University Graduate School, Tokyo, Japan; †KAN Research Institute, Kyoto, Japan; and ‡Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan

Received for publication January 4, 2005. Accepted for publication September 1, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by a grant-in-aid from the Japan Intractable Diseases Research Foundation; the Fund for Intractable Diseases Research by Atsuko Ouchi from Tokyo Medical and Dental University; the Ministry of Health, Labor, and Welfare; and the Ministry of Education, Science, Sports, and Culture, Japan.

[2] Address correspondence and reprint requests to Dr. Toshihiro Nanki, Department of Medicine and Rheumatology, Tokyo Medical and Dental University Graduate School, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. E-mail address: nanki.rheu@tmd.ac.jp

[3] Abbreviations used in this paper: IIM, idiopathic inflammatory myopathy; EAM, experimental autoimmune myositis; PTX, pertussis toxin.

0022-1767/05/$02.00

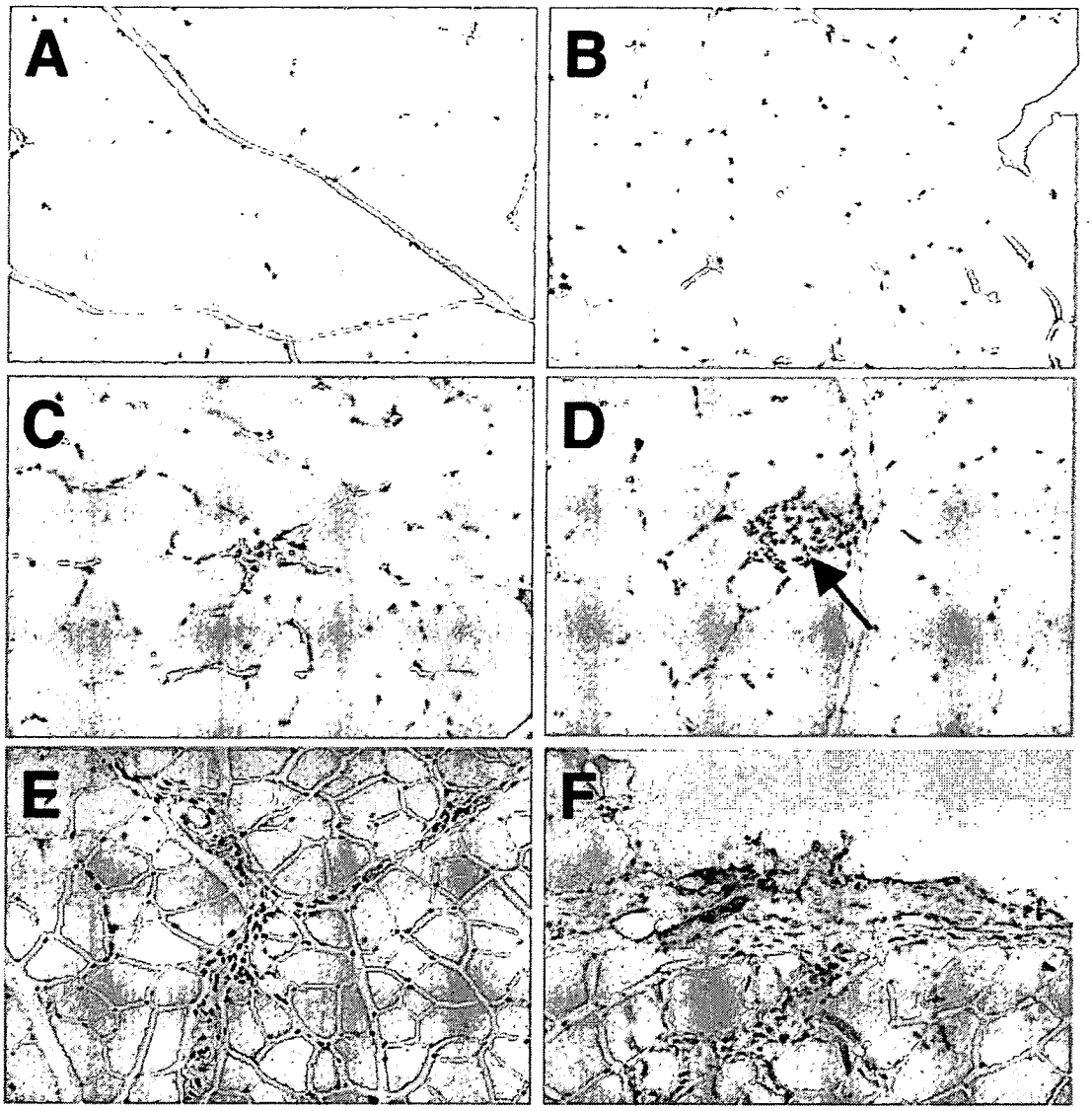

FIGURE 1. Histological changes found in the muscle of murine EAM. Quadriceps femoris muscle of normal mice and immunized mice on day 7 showed no inflammatory changes (*A* and *B*, respectively). On day 14, mild cellular infiltration in the muscle tissue was shown (*C*). Muscle tissues of EAM mice on day 21 showed cellular infiltration in the endomysium (*D*), perimysium (*E*), epimysium (*F*), and necrotic muscle fibers (arrow in *D*). H&E staining was used. Original magnification, ×200.

temperature, the slides were rehydrated in PBS for 2 min three times, and then the endogenous peroxidase activity was blocked by incubation in 1.0% H_2O_2 in PBS for 10 min, followed by rinsing for 2 min three times in PBS. Nonspecific binding was blocked with 10% normal rabbit serum in PBS for 30 min. For CD4, CD8, and F4/80 staining, the sections were incubated with 5 μg/ml rat anti-mouse CD4 mAb (GK1.5; Cymbus Biotechnology), 2 μg/ml rat anti-mouse CD8a mAb (53-6.7; BD Pharmingen), 5 μg/ml rat anti-mouse F4/80 mAb (C1:A3-1; Serotec), or normal rat IgG in Ab diluent (BD Pharmingen) overnight at 4°C. The samples were then washed three times in PBS for 5 min each time and incubated with biotin-conjugated rabbit anti-rat IgG (DakoCytomation) for 30 min at room temperature with 5% normal mouse serum. To analyze a time course of cell infiltration, numbers of $CD4^+$, $CD8^+$, and $F4/80^+$ cells in six randomly selected fields at ×200 were counted from three EAM mice on days 0, 7, 14, and 21.

For mouse vascular endothelial cell staining, we used a tyramide signal amplification kit (NEL700A; PerkinElmer). After blocking with 10% normal rabbit serum, the sections were incubated with 5 μg/ml rat anti-mouse vascular endothelial cadherin Ab (11D4.1; BD Pharmingen) or normal rat IgG overnight at 4°C. The samples were then washed three times in PBS for 5 min each time and incubated with biotin-conjugated rabbit anti-rat IgG for 30 min at room temperature with 5% normal mouse serum. After washing three times in PBS for 5 min each time, the sections were incubated with streptavidin-HRP for 30 min at room temperature and washed in PBS three times for 5 min each time. The samples were incubated with biotinyl tyramide amplification reagent at room temperature for 5 min, then washed three times in PBS for 5 min each time, and incubated again with streptavidin-HRP for 30 min. After washing three times in PBS for 5 min each time, diaminobenzidine tablets (Sigma-Aldrich) were used for visualization. The sections were counterstained in hematoxylin for 30 s and washed in tap water for 5 min.

For mouse CX3CL1 staining, the endogenous peroxidase activity was blocked by incubation in 1.0% H_2O_2 in methanol, and then the sections were incubated overnight at 4°C with goat anti-mouse CX3CL1 Ab (sc-7227; Santa Cruz Biotechnology) or normal goat IgG in Ab diluent at 5 μg/ml. The samples were then washed three times in PBS for 5 min each time and incubated with biotin-conjugated rabbit anti-goat IgG (DakoCytomation) for 30 min at room temperature with 5% normal mouse serum. After washing three times in PBS for 5 min each time, the sections were incubated with peroxidase-conjugated streptavidin (DakoCytomation) for 30 min at room temperature and washed three times for 5 min each time. For enhancing the expression of CX3CL1 on endothelial cells, a tyramide signal amplification kit was

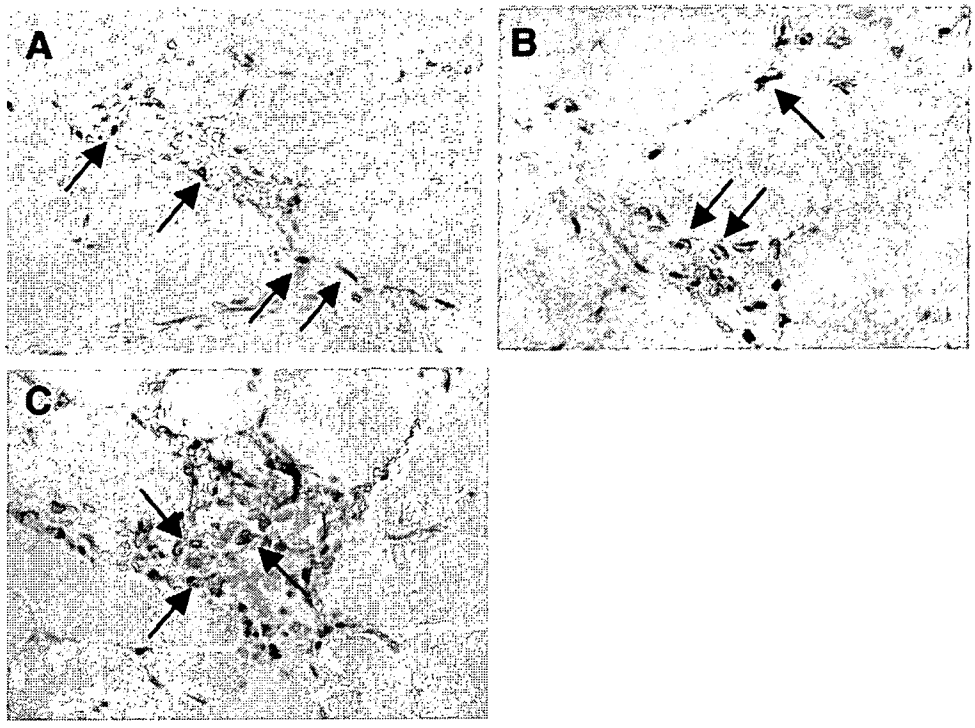

FIGURE 2. Infiltration of CD4 and CD8 T cells and macrophages in the muscles of EAM mice. Frozen sections of the quadriceps femoris muscle of EAM mice on day 21 were examined by immunohistochemistry using mAb against CD4 (*A*), CD8 (*B*), and F4/80 (*C*). The arrows indicate $CD4^+$, $CD8^+$, and $F4/80^+$ cells. Original magnification, ×200.

used as described above. Diaminobenzidine tablets were used for visualization. The sections were counterstained in hematoxylin for 30 s and washed in tap water for 5 min.

For CD4, CD8 or F4/80, and CX3CR1 double staining, the sections were incubated overnight at 4°C with 5 μg/ml rat anti-mouse CD4 mAb (GK1.5), 5 μg/ml rat anti-mouse CD8 mAb (53-6.7), 5 μg/ml rat anti-mouse F4/80 mAb (C1:A3-1), or normal rat IgG in Ab diluent. Subsequently, the samples were washed three times for 5 min each time in PBS and incubated with Alexa Fluor 488-conjugated goat anti-rat IgG (Molecular Probes) at 5 μg/ml for 1 h at room temperature. For CX3CR1 staining, the sections were washed three times in PBS for 5 min each time and then incubated with rabbit anti-mouse CX3CR1 Ab (30) or normal rabbit IgG at 5 μg/ml in Ab diluent for 2 h at room temperature. Next, the samples were washed three times for 5 min each time in PBS and incubated with Alexa Fluor 568-conjugated goat anti-rabbit IgG (Molecular Probes) at 5 μg/ml for 1 h at room temperature. The slides were examined using fluorescent microscopy (BZ-Analyzer; Keyence).

Treatment with anti-mouse CX3CL1 mAb

A mAb against murine CX3CL1 was generated from Armenian hamsters immunized with recombinant murine CX3CL1 by a standard method. One mAb, 5H8-4, was selected for additional studies. The specificity was examined by ELISA using a panel of murine CXC (MIP-2, keratinocyte-derived chemokine, and CXCL9, 10, 12, and 13), CC (CCL1–7, 9–12, 17, 19–22, 25, 27, and 28), C (XCL1), and CX3C (CX3CL1) chemokines. The mAb reacted specifically with murine CX3CL1. Five hundred micrograms of hamster anti-mouse CX3CL1 mAb (5H8-4) or control Ab (hamster IgG; ICN Pharmaceuticals) was injected into the mouse peritoneal cavity three times per week from day 0 for 3 wk. The injection of anti-CX3CL1 mAb did not affect the number of PBMC (data not shown).

The severity of inflammatory changes was classified using five grades according to the classification of Kojima et al. (31) with some modification: score 0, no inflammation; score 1, mild endomysial inflammatory changes; score 2, severe endomysial inflammatory changes; score 3, perimysial inflammatory changes in addition to score 2; and score 4, diffuse extensive lesion. If multiple lesions were found in one muscle specimen, 0.5 point was added to the indicated score. To evaluate the severity of inflammation using a different aspect, we counted the number of necrotic muscle fibers, and $CD4^+$, $CD8^+$, and $F4/80^+$ cells in continuous three sections. Each section examined six random fields at ×400. The evaluation of histopathological inflammatory changes was performed in a blind fashion for the experimental group identity.

Real-time RT-PCR

Total RNA was prepared from a 100 mg muscle block using RNA extraction solution, Isogen (Nippon Gene), and treated with DNase I (Invitrogen Life Technologies). The first-strand cDNA was synthesized using oligo$(dT)_{12-18}$ primers (Pharmacia Biotech) and SuperScript II reverse transcriptase (Invitrogen Life Technologies).

The relative quantitative real-time PCR was performed using SYBR Green I on ABI PRISM 7000 (Applied Biosystems) according to the instructions provided by the manufacturer. The cDNA was amplified with primers for TNF-α (5′, GTA CCT TGT CTA CTC CCA GGT TCT CT; 3′, GTG TGG GTG AGG AGC ACG TA), IFN-γ (5′, CCT GCG GCC TAG CTC TGA; 3′, CCA TGA GGA AGA GCT GCA AAG), perforin (5′, CCA CGG CAG GGT GAA ATT C; 3′, GGC AGG TCC CTC CAG TGA), and GAPDH (5′, ATG CAT CCT GCA CCA CCA A; 3′, GTC ATG AGC CCT TCC ACA ATG). These primers were designed using the ABI Primer Express Software program (Applied Biosystems). The reaction buffer contained the following components: 25 μl of SYBR Green PCR Master Mix (Applied Biosystems), 300 nM forward and reverse primers, 50 ng cDNA template, and RNA-free distilled water up to 50 μl of total volume. The PCR was conducted using the following parameters: 50°C for 2 min, 95°C for 10 min, and 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. GAPDH mRNA was used as an internal control to standardize the amount of sample mRNA. A validation experiment demonstrated approximately equal efficiencies of the target and reference. Thus, the relative expression of real-time PCR products was determined using the ΔΔCt method that compares the mRNA expression levels of the target gene and the housekeeping gene (32, 33). One of the control samples was chosen as a calibrator sample.

Statistical analysis

Differences in the score of tissue inflammation, number of necrotic muscle fibers, number of migrated cells, and relative expression levels of TNF-α, IFN-γ, and perforin between control Ab- and anti-mouse CX3CL1 mAb-treated EAM mice, and the relative expression levels of TNF-α, IFN-γ, and perforin between normal and EAM mice were examined for statistical significance using Mann-Whitney's *U* test. All data were expressed as the

mean ± SEM. The difference between two groups of mice was considered significant at $p < 0.05$.

Results

Development of EAM

SJL/J mice were immunized with purified rabbit myosin fraction and CFA on days 0, 7, and 14. On days 0, 7, 14, and 21, the quadriceps femoris muscles of these mice were histologically examined with H&E staining. All muscle specimens of normal SJL/J mice and immunized mice on day 7 showed normal appearance with no inflammatory changes (Fig. 1, *A* and *B*, respectively), whereas those of mice immunized with rabbit myosin fraction showed mild mononuclear cell infiltration at day 14 (Fig. 1*C*). On day 21, a significant number of mononuclear cells were infiltrated among the muscle fibers (endomysium; Fig. 1*D*), at perivascular areas (perimysium; Fig. 1*E*), and epimysium (Fig. 1*F*). Scattered lesions with aggregates of infiltrated mononuclear cells were formed, in which atrophic or necrotic muscle fibers were noted (arrow in Fig. 1*D*). Injection of PBS and CFA into SJL/J mice did not show infiltration of inflammatory cells in the quadriceps femoris muscles (data not shown).

To determine the subsets of infiltrating mononuclear cells in the quadriceps femoris muscles of EAM mice, we performed immunohistochemical analysis using mAbs against CD4, CD8, and F4/80. $CD4^+$ T cells were mainly located in the perimysium and some were found in the endomysium (Fig. 2*A*). $CD8^+$ T cells were predominantly detected in the endomysium and surrounded nonnecrotic muscle fibers (Fig. 2*B*). $F4/80^+$ macrophages were located in the endomysium as well and were especially present around the necrotic muscle fibers (Fig. 2*C*). Because these histological findings of inflammatory cell infiltration patterns resembled those of affected muscle lesions in IIM patients (34–36), we decided to use the EAM mice as an experimental model of IIM.

To evaluate a time course of cellular infiltration into the muscles, we counted the numbers of infiltrated $CD4^+$, $CD8^+$, and $F4/80^+$ cells on days 0, 7, 14, and 21 by immunohistochemical method. The majority of the infiltrating cells on day 14 were $F4/80^+$ macrophage (Fig. 3). In contrast, the number of $CD4^+$ and $CD8^+$ T cells was not increased until day 14, and they had significantly migrated into the muscles on day 21. These results were similar to previously reported data (37).

CX3CL1 and CX3CR1 expression in the muscle of EAM mice

We examined the expression of CX3CL1 in the muscle of normal SJL/J mice and EAM mice by immunohistochemistry. In the quadriceps femoris muscles of normal mice, no CX3CL1 expression was detected (Fig. 4, *A* and *G*). In contrast, CX3CL1 was expressed on infiltrated mononuclear cells predominantly in the endomysium and vascular endothelial cells of EAM mice on day 14 (Fig. 4, *B* and *H*, respectively) and day 21 (Fig. 4, *C* and *I*, respectively).

We next examined the expression of CX3CR1 on the infiltrated mononuclear cells in the quadriceps femoris muscle of EAM mice by double immunohistochemical staining. Some $CD4^+$ T cells expressed CX3CR1 (Fig. 5, *A–C*). The majority of $CD8^+$ T cells and most of the $F4/80^+$ macrophages expressed CX3CR1 (Fig. 5, *D–F* and *G–I*, respectively).

Effect of anti-mouse CX3CL1 mAb on EAM mice

To analyze the effect of anti-CX3CL1 mAb administration on EAM mice, we evaluated the histological changes in quadriceps femoris muscle using H&E staining. The incidence of inflammatory cell infiltration in control Ab-treated mice was 100% ($n = 10$). Treatment with anti-CX3CL1 mAb did not change the incidence of cellular infiltration (100%; $n = 10$). EAM mice treated with control Ab showed mononuclear cell infiltration with atrophy and necrosis of muscle fibers (Fig. 6*A*). In comparison, anti-CX3CL1 mAb-treated EAM mice showed milder histological changes (Fig. 6*B*). Analysis of histological scores of inflammatory changes in the quadriceps femoris muscles indicated that treatment with anti-CX3CL1 mAb significantly reduced inflammatory cell infiltration in the muscles of EAM mice compared with treatment with control Ab (Fig. 6*C*). Moreover, anti-CX3CL1 mAb treatment reduced the number of necrotic muscle fibers in muscles (Fig. 6*D*). A similar result was obtained in another independent set of experiments.

We next examined the effect of anti-CX3CL1 mAb treatment on the numbers of each subset of infiltrating cells. The numbers of $CD4^+$, $CD8^+$, and $F4/80^+$ cells in quadriceps femoris muscles were counted and compared between mice treated with control Ab and those with anti-CX3CL1 mAb. Anti-CX3CL1 mAb treatment significantly reduced the number of infiltrated $CD4^+$ T cells by ~30% (Fig. 7*A*), $CD8^+$ T cells by ~50%, and $F4/80^+$ macrophages by up to 50% (Fig. 7, *B* and *C*).

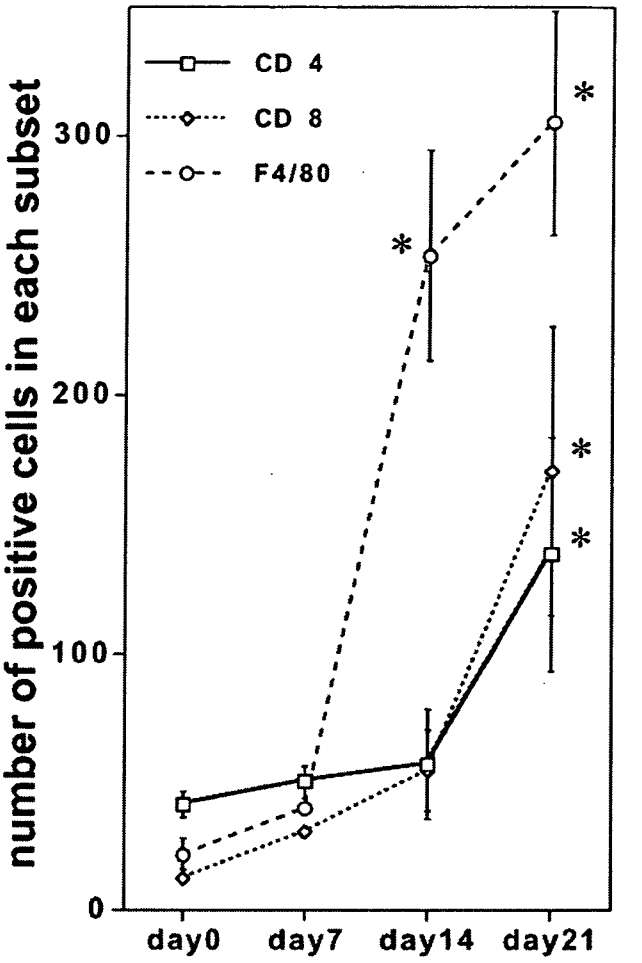

FIGURE 3. Time course of inflammatory cell infiltration into the muscle tissue of EAM mice. The numbers of infiltrating $CD4^+$, $CD8^+$, and $F4/80^+$ cells into the quadriceps femoris muscles were counted by immunohistochemistry. Data represent the mean ± SEM. *, $p < 0.05$.

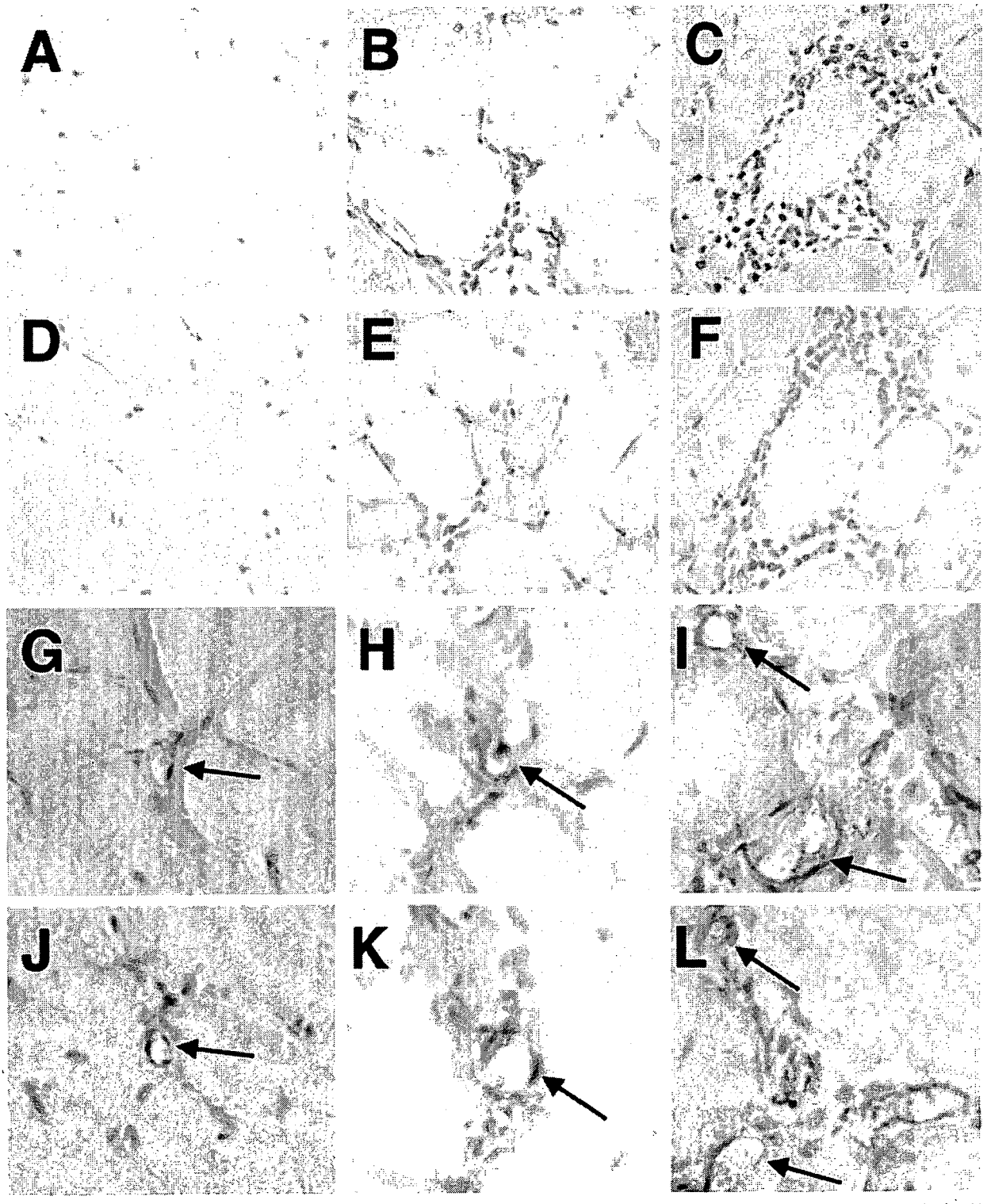

FIGURE 4. CX3CL1 expression in the muscles of EAM mice. Expression of CX3CL1 was examined by immunohistochemistry in normal mice (*A* and *G*) and EAM mice on day 14 (*B* and *H*) and day 21 (*C* and *I*). Vascular endothelial cadherin expression in the normal mice (*J*) and EAM mice on day 14 (*K*) and 21 (*L*) was also examined using serial sections with *G*, *H*, and *I*, respectively. Stainings with isotype control Ab for CX3CL1 are shown (*D*, normal mice; *E*, EAM on day 14; *F*, EAM on day 21). Arrows indicate vascular endothelial cadherin-positive endothelial cells (*J–L*), and corresponding endothelial cells (*G–I*). Original magnification, ×400.

We finally examined the effects of anti-CX3CL1 mAb treatment on the expression of cytokines and cytotoxic molecule in the quadriceps femoris muscle of EAM mice by quantitative RT-PCR. Although the relative quantities of TNF-α, IFN-γ, and perforin mRNA were very low in normal SJL/J mice, they were significantly up-regulated in EAM mice that received control Ab treatment ($p < 0.05$). Furthermore, treatment with anti-CX3CL1 mAb strikingly reduced mRNA expression (Fig. 8).

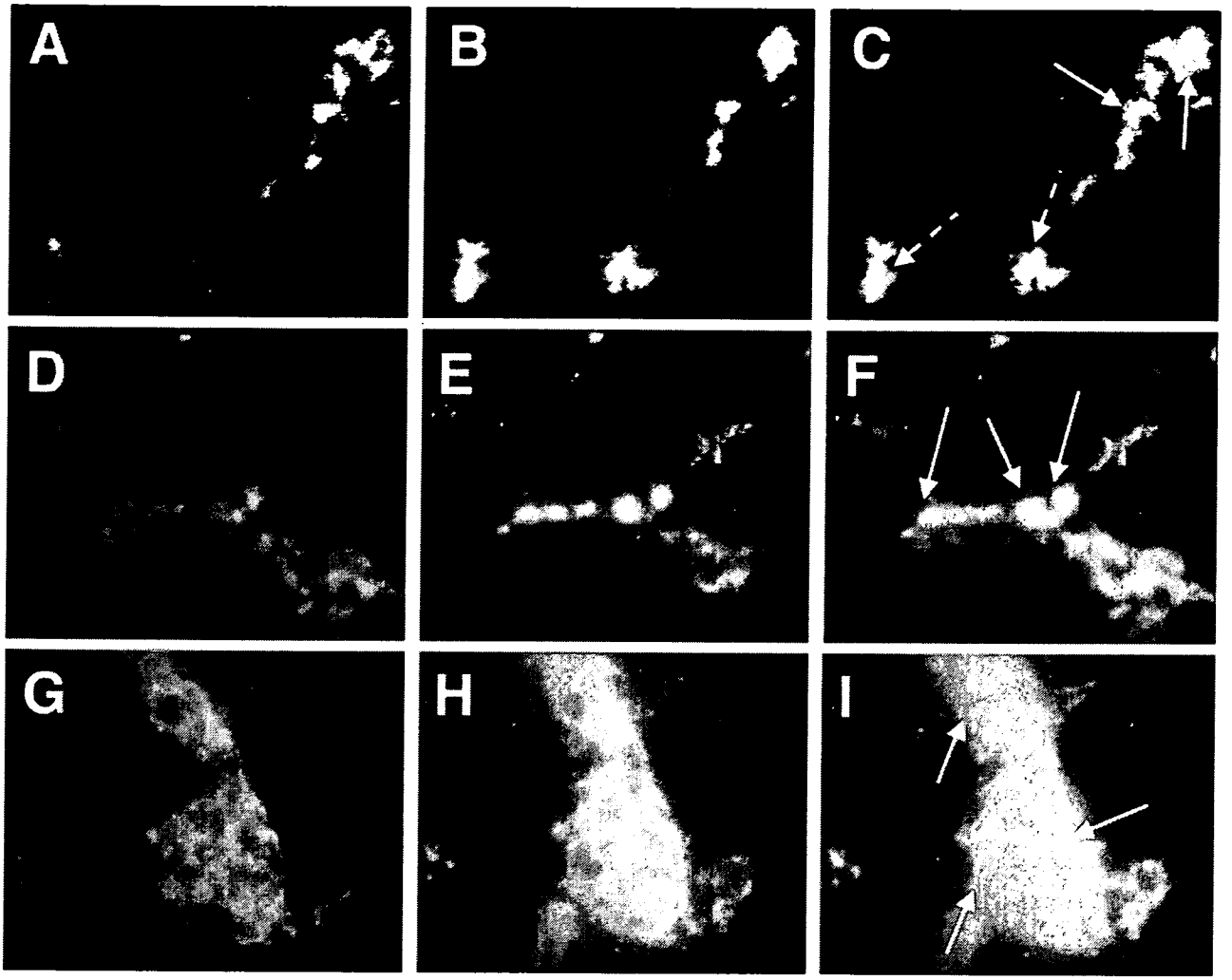

FIGURE 5. CX3CR1 expression on $CD4^+$, $CD8^+$, or $F4/80^+$ cells in the EAM muscle. Muscle tissues from EAM mice were double stained with CD4, CD8, or F4/80, and CX3CR1, and analyzed with fluorescent microscopy (*A*, CX3CR1; *B*, CD4; *C*, merged *A* and *B*; *D*, CX3CR1; *E*, CD8; *F*, merged *D* and *E*; *G*, CX3CR1; *H*, F4/80; *I*, merged *G* and *H*). Solid arrows indicate double-positive cells. Dotted arrows indicate CX3CR1-negative CD4 T cells. Original magnification, ×200.

Considered together, the above results indicate that treatment with anti-CX3CL1 mAb reduced infiltration of CD4 and CD8 T cells and macrophages and reduced the expression of various inflammatory cytokines and cytotoxic molecule in muscles.

Discussion

The major findings of the present study were the following. 1) CX3CL1 was expressed on infiltrated mononuclear cells and vascular endothelial cells, and its corresponding receptor, CX3CR1, was expressed on infiltrated inflammatory cells in the muscles of EAM. 2) Treatment with anti-CX3CL1 mAb ameliorated histological inflammatory changes in EAM mice, reduced the numbers of infiltrated CD4 and CD8 T cells and macrophages, and reduced the expression of TNF-α, IFN-γ, and perforin in the muscles. These results suggest that CX3CL1-CX3CR1 interaction seems to play an important role in inflammatory cell migration into the muscles of EAM mice.

Development of EAM in SJL/J mice by immunization with rabbit purified skeletal myosin fraction and CFA was previously reported (37–40). We modified the method by increasing the amount of immunized myosin and CFA and the addition of *Mycobacterium butyricum*. This modification shortened the period required for the development of myositis from 5 wk, which was thought to be appropriate for the induction (38), to 3 wk. Moreover, although pertussis toxin (PTX) injection into the peritoneal cavity increased the severity of inflammatory changes in the muscle (31), and thus, PTX was administered in the previous models (31, 36, 38), our modified method induces significant myositis without PTX injection. The EAM mice showed inflammatory cell infiltration in the endomysium, perimysium, and epimysium with muscle fiber necrosis. Immunohistochemical analysis showed that the invading cells surrounding nonnecrotic muscle fibers in the endomysium were mainly CD8 T cells, whereas macrophages were predominantly detected in necrotic fibers, and CD4 T cells were located in perimysium. Moreover, quantitative RT-PCR showed up-regulation of expression of TNF-α, IFN-γ, and perforin mRNA in the muscle of EAM mice. These findings in EAM mice are similar to those reported in IIM patients (4–8, 34–36).

Inflammatory cell migration into the affected muscle of IIM is thought to involve chemokine-chemokine receptor interaction

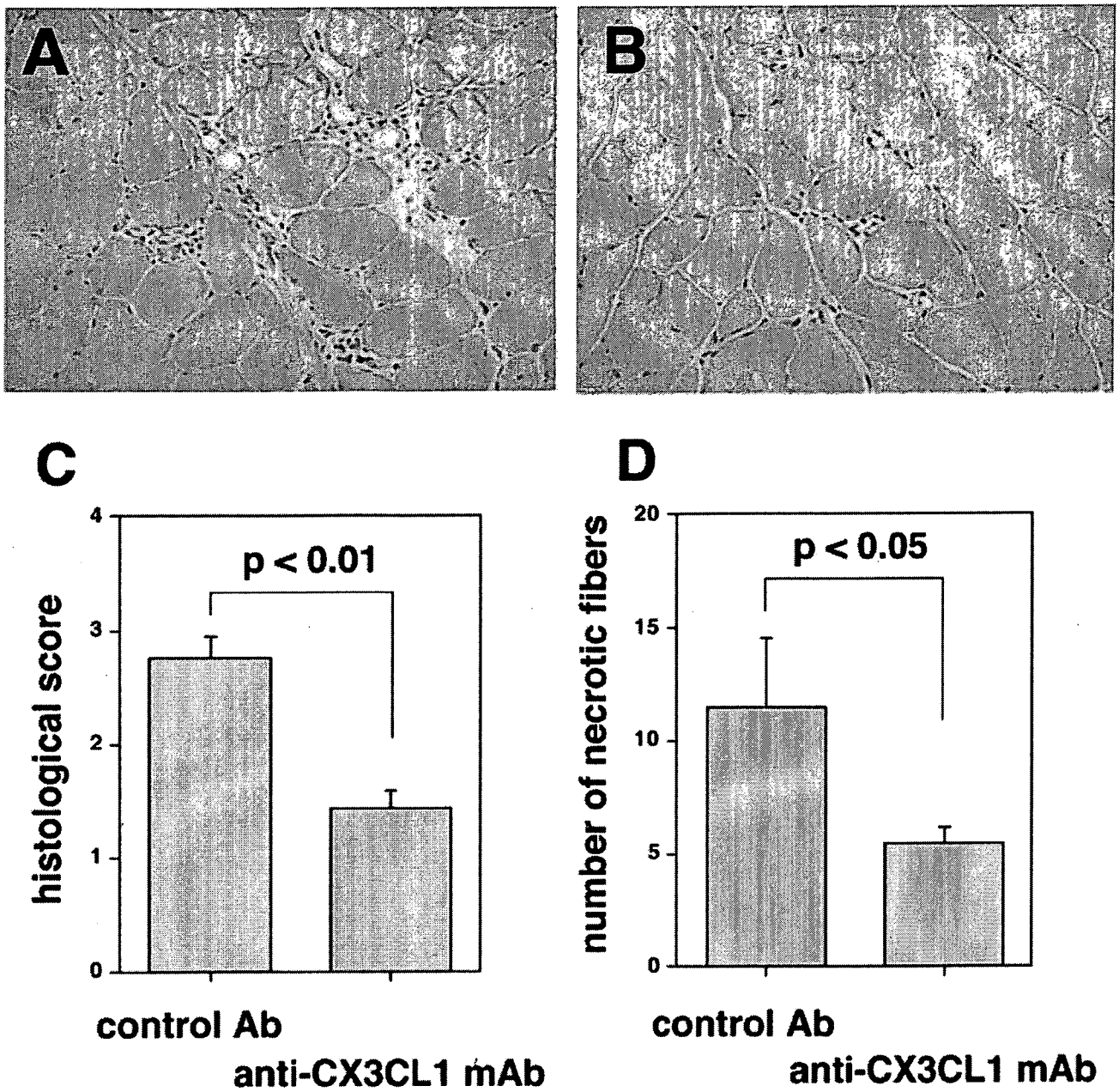

FIGURE 6. Inhibition of inflammatory changes in the muscle by treatment with anti-CX3CL1 mAb. Five hundred micrograms of hamster anti-mouse CX3CL1 mAb or control Ab was injected into the peritoneal cavity three times per week from day 0 for 3 wk. On day 21, the quadriceps femoris muscles of EAM mice were examined with H&E staining, histological scores were evaluated, and the numbers of necrotic fibers were counted. Mice treated with control Ab showed inflammatory cell accumulation (*A*). Mice treated with anti-CX3CL1 mAb showed milder inflammatory changes (*B*). Representative photomicrographs of histology from 10 animals in each group are shown. Histological scores of inflammatory changes in quadriceps femoris muscles were evaluated (*C*). The numbers of necrotic fibers were counted in the muscle tissues (*D*). Data represent the mean ± SEM.

(9–14). In the present study we focused on the role of CX3CL1-CX3CR1 interaction in the inflammatory cell migration. We showed the expression of CX3CR1 on some CD4 T cells and most CD8 T cells in EAM mice. It has been reported that CTLs including both $CD4^+$ and $CD8^+$ T cells invade the muscle fibers in IIM patients (3). These cells possess cytotoxic molecules, such as perforin and granzyme B, which are released into muscle cells (4, 5). Furthermore, type 1 cytokines, such as TNF-α and IFN-γ, were expressed in the inflammatory lesions of IIM patients (6–8). These findings suggest that the cytotoxic molecules and type 1 cytokines play important roles in the inflammatory lesions in IIM patients. In contrast, we reported previously that peripheral blood $CX3CR1^+$ T cells express cytotoxic molecules and type 1 cytokines (26, 27). Therefore, the interaction of CX3CL1 and CX3CR1 could induce the migration of T cells, which express cytotoxic molecules and type 1 cytokines, into the affected muscles.

The infiltrated macrophages into the affected muscle also express inflammatory cytokines (9, 41). They express TNF-α and IL-1β, which could stimulate T cells, macrophages, and

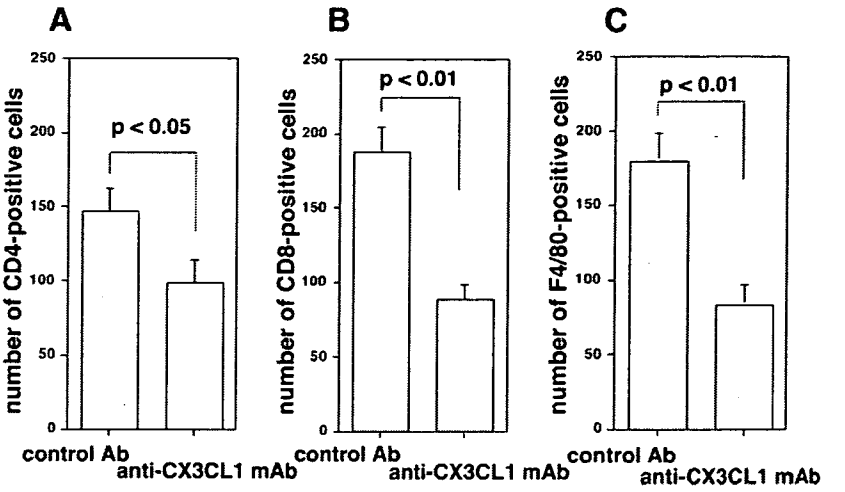

FIGURE 7. Decreased numbers of infiltrating cells of each subset by anti-CX3CL1 mAb treatment. Numbers of infiltrating $CD4^+$, $CD8^+$, and $F4/80^+$ cells were counted in the quadriceps femoris muscles from the experiment shown in Fig. 6. Data represent the mean ± SEM.

endothelial cells to produce various inflammatory cytokines, chemokines, and adhesion molecules. Moreover, these cytokines might have myocytotoxic effects (42–44). Our results showed that the majority of the $F4/80^+$ macrophages expressed CX3CR1 in the muscle of EAM mice. Thus, the CX3CL1-CX3CR1 interaction might also play an important role in macrophage migration into the affected muscle in addition to T cell migration.

CX3CL1 was expressed on infiltrated mononuclear cells in the affected muscles of EAM mice. Because CX3CL1 expression was located in the endomysium, infiltrated macrophages and/or CD8 T cells may express CX3CL1 in the muscles. Furthermore, we showed that CX3CL1 was also expressed on vascular endothelial cells in the EAM muscle tissue on days 14 and 21, but not in normal mice. It was reported that CX3CL1 was expressed on endothelial cells activated with TNF-α and IFN-γ in vitro (19–21). Expressed CX3CL1 on endothelial cells might recruit $CX3CR1^+$ cells, including macrophages and T cells, into muscle. These cells, in turn, express TNF-α and IFN-γ, which induce additional CX3CL1 expression on endothelial cells and also on recruited inflammatory cells. The enhanced expression of CX3CL1 may induce additional inflammatory cell migration. Consequently, these amplification cascades could contribute to the expansion of pathological changes in EAM mice. In fact, inhibition of CX3CL1 reduced the numbers of migrated CD4 and CD8 T cells and macrophages in the affected muscles of EAM mice and also reduced the expression of TNF-α, IFN-γ, and perforin. These results suggest that CX3CL1 blockade reduces the migration of inflammatory cells, which express cytotoxic molecules and cytokines, into the muscles. Thus, inhibition of CX3CL1-CX3CR1 interaction might be a potentially suitable therapeutic strategy for treatment of IIM.

Our data showed that mRNA expression of TNF-α, IFN-γ, and perforin was almost totally inhibited by anti-CX3CL1 mAb treatment, although the numbers of infiltrated monocytes were decreased by up to 50%. Recently it was reported that stimulation with CX3CL1 enhanced production of proinflammatory cytokines such as IFN-γ as well as the release of cytolytic granules by T cells (45). Thus, blockade of CX3CL1 might inhibit not only cellular migration, but also cytokine and cytotoxic molecule expression, by stimulation with CX3CL1 in the EAM muscle. Alternatively, because $CX3CR1^+$ T cells express type 1 cytokine and cytotoxic molecules (23, 26, 27), and $CX3CR1^{high}$ positive monocytes greatly produce inflammatory cytokines compared with $CX3CR1^{low}$ positive monocytes (46–48), treatment with anti-CX3CL1 mAb may selectively inhibit the migration of such specific T cells and macrophages. Therefore, anti-CX3CL1 mAb might be able to inhibit the expression of cytokine and cytotoxic molecules effectively in muscles, but additional study is required.

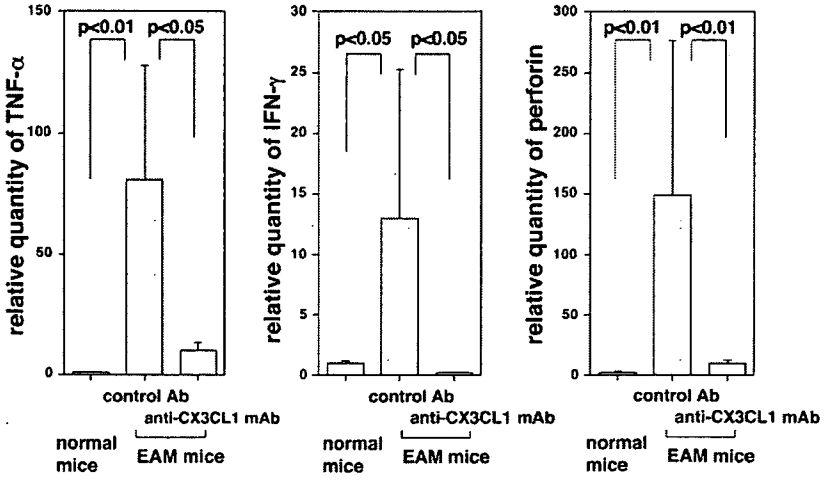

FIGURE 8. Reduction of TNF-α, IFN-γ, and perforin expression by anti-CX3CL1 mAb treatment. Expression of TNF-α, IFN-γ, and perforin mRNA in quadriceps femoris muscles from normal mice (n = 10) and from the experiment shown in Fig. 6 were measured using real-time RT-PCR. Data represent the mean ± SEM.

We recently reported that inhibition of CX3CL1 ameliorated collagen-induced arthritis in mice, probably by suppression of inflammatory cell migration into the synovium (30). Others reported that anti-CX3CR1 Ab treatment blocked inflammatory cell infiltration in the glomeruli, prevented crescent formation, and improved renal function in the Wistar-Kyoto crescentic glomerulonephritis model (49). Furthermore, the gene deletion of CX3CR1 resulted in an ~50% decrease in the formation of atherosclerotic lesions and the number of infiltrated macrophages in the lesion in experimental atherosclerosis mice (50, 51). These results together with our findings suggest that blockade of CX3CL1-CX3CR1 interaction might be therapeutically useful for several diseases associated with inflammatory cell infiltration. In this study we propose that such treatment is also suitable for IIM. To our knowledge, this is the first report demonstrating that a chemokine inhibitor could reduce the severity of myositis.

In conclusion, we demonstrated in the present study that inhibition of CX3CL1 significantly improved histopathological changes in the muscles of EAM mice, suggesting that blockade of CX3CL1 might be therapeutically beneficial for IIM.

Acknowledgments

We thank Dr. Hiroshi Nemoto (Toho University School of Medicine) for providing critical suggestions for the development of EAM mice. We also thank Miyuki Nishimura, Keiko Mizuno, and Yoko Inoue for their excellent technical support.

Disclosures

The authors have no financial conflict of interest.

References

1. Arahata, K., and A. G. Engel. 1984. Monoclonal antibody analysis of mononuclear cells in myopathies. I. Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. *Ann. Neurol.* 16: 193–208.
2. Engel, A. G., and K. Arahata. 1984. Monoclonal antibody analysis of mononuclear cells in myopathies. II. Phenotypes of autoinvasive cells in polymyositis and inclusion body myositis. *Ann. Neurol.* 16: 209–215.
3. Dalakas, M. C. 1991. Polymyositis, dermatomyositis and inclusion-body myositis. *N. Engl. J. Med.* 325: 1487–1498.
4. Cherin, P., S. Herson, M. C. Crevon, J. J. Hauw, P. Cervera, P. Galanaud, and D. Emilie. 1996. Mechanisms of lysis by activated cytotoxic cells expressing perforin and granzyme-B genes and the protein TIA-1 in muscle biopsies of myositis. *J. Rheumatol.* 23: 1135–1142.
5. Goebels, N., D. Michaelis, M. Engelhardt, S. Huber, A. Bender, D. Pongratz, M. A. Johnson, H. Wekerle, J. Tschopp, D. Jenne, et al. 1996. Differential expression of perforin in muscle-infiltrating T cells in polymyositis and dermatomyositis. *J. Clin. Invest.* 97: 2905–2910.
6. Tews, D. S., and H. H. Goebel. 1996. Cytokine expression profile in idiopathic inflammatory myopathies. *J. Neuropathol. Exp. Neurol.* 55: 342–347.
7. Lepidi, H., V. Frances, D. Figarella-Branger, C. Bartoli, A. Machado-Baeta, and J. F. Pellissier. 1998. Local expression of cytokines in idiopathic inflammatory myopathies. *Neuropathol. Appl. Neurobiol.* 24: 73–79.
8. Sugiura, T., Y. Kawaguchi, M. Harigai, K. Takagi, S. Ohta, C. Fukasawa, M. Hara, and N. Kamatani. 2000. Increased CD40 expression on muscle cells of polymyositis and dermatomyositis: role of CD40-CD40 ligand interaction in IL-6, IL-8, IL-15, and monocyte chemoattractant protein-1 production. *J. Immunol.* 164: 6593–6600.
9. Lundberg, I. E. 2000. The role of cytokines, chemokines, and adhesion molecules in the pathogenesis of idiopathic inflammatory myopathies. *Curr. Rheumatol. Rep.* 2: 216–224.
10. De Bleecker, J. L., B. De Paepe, I. E. Vanwalleghem, and J. M. Schroder. 2002. Differential expression of chemokines in inflammatory myopathies. *Neurology* 58: 1779–1785.
11. Liprandi, A., C. Bartoli, D. Figarella-Branger, J. F. Pellissier, and H. Lepidi. 1999. Local expression of monocyte chemoattractant protein-1 (MCP-1) in idiopathic inflammatory myopathies. *Acta Neuropathol.* 97: 642–648.
12. Confalonieri, P., P. Bernasconi, P. Megna, S. Galbiati, F. Cornelio, R. Mantegazza. 2000. Increased expression of β-chemokines in muscle of patients with inflammatory myopathies. *J. Neuropathol. Exp. Neurol.* 59: 164–169.
13. Bartoli, C., M. Civatte, J. F. Pellissier, and D. Figarella-Branger. 2001. CCR2A and CCR2B, the two isoforms of the monocyte chemoattractant protein-1 receptor are up-regulated and expressed by different cell subsets in idiopathic inflammatory myopathies. *Acta Neuropathol. (Berl.)* 102: 385–392.
14. De Rossi, M., P. Bernasconi, F. Baggi, R. de Waal Malefyt, and R. Mantegazza. 2000. Cytokines and chemokines are both expressed by human myoblasts: possible relevance for the immune pathogenesis of muscle inflammation. *Int. Immunol.* 12: 1329–1335.
15. Zlotnik, A., and O. Yoshie. 2000. Chemokines: a new classification system and their role in immunity. *Immunity* 12: 121–127.
16. Yoshie, O., T. Imai, and H. Nomiyama. 2001. Chemokines in immunity. *Adv. Immunol.* 78: 57–110.
17. Bazan, J. F., K. B. Bacon, G. Hardiman, W. Wang, K. Soo, D. Rossi, D. R. Greaves, A. Zlotnik, and T. J. Schall. 1997. A new class of membrane-bound chemokine with a CX3C motif. *Nature* 385: 640–644.
18. Pan, Y., C. Lloyd, H. Zhou, S. Dolich, J. Deeds, J. A. Gonzalo, J. Vath, M. Gosselin, J. Ma, B. Dussault, et al. 1997. Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 387: 611–617.
19. Fraticelli, P., M. Sironi, G. Bianchi, D. D'Ambrosio, C. Albanesi, A. Stoppacciaro, M. Chieppa, P. Allavena, L. Ruco, G. Girolomoni, et al. 2001. Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses. *J. Clin. Invest.* 107: 1173–1181.
20. Imaizumi, T., H. Yoshida, and K. Satoh. 2004. Regulation of CX3CL1/fractalkine expression in endothelial cells. *J. Atheroscler. Thromb.* 11: 15–21.
21. Fujimoto, K., T. Imaizumi, H. Yoshida, S. Takanashi, K. Okumura, and K. Satoh. 2001. Interferon-γ stimulates fractalkine expression in human bronchial epithelial cells and regulates mononuclear cell adherence. *Am. J. Respir. Cell Mol. Biol.* 25: 233–238.
22. Fong, A. M., L. A. Robinson, D. A. Steeber, T. F. Tedder, O. Yoshie, T. Imai, and D. D. Patel. 1998. Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. *J. Exp. Med.* 188: 1413–1419.
23. Umehara, H., E. T. Bloom, T. Okazaki, Y. Nagano, O. Yoshie, and T. Imai. 2004. Fractalkine in vascular biology: from basic research to clinical disease. *Arterioscler. Thromb. Vasc. Biol.* 24: 34–40.
24. Garton, K. J., P. J. Gough, C. P. Blobel, G. Murphy, D. R. Greaves, P. J. Dempsey, and E. W. Raines. 2001. Tumor necrosis factor-α-converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX3CL1). *J. Biol. Chem.* 276: 37993–38001.
25. Hundhausen, C., D. Misztela, T. A. Berkhout, N. Broadway, P. Saftig, K. Reiss, D. Hartmann, F. Fahrenholz, R. Postina, V. Matthews, et al. 2003. The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX3CL1 (fractalkine) and regulates CX3CL1-mediated cell-cell adhesion. *Blood* 102: 1186–1195.
26. Nanki, T., T. Imai, K. Nagasaka, Y. Urasaki, Y. Nonomura, K. Taniguchi, K. Hayashida, J. Hasegawa, O. Yoshie, and N. Miyasaka. 2002. Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. *Arthritis Rheum.* 46: 2878–2883.
27. Nishimura, M., H. Umehara, T. Nakayama, O. Yoneda, K. Hieshima, M. Kakizaki, N. Dohmae, O. Yoshie, and T. Imai. 2002. Dual functions of fractalkine/CX3C ligand 1 in trafficking of perforin$^+$/granzyme B$^+$ cytotoxic effector lymphocytes that are defined by CX3CR1 expression. *J. Immunol.* 168: 6173–6180.
28. Imai, T., K. Hieshima, C. Haskell, M. Baba, M. Nagira, M. Nishimura, M. Kakizaki, S. Takagi, H. Nomiyama, T. J. Schall, et al. 1997. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 91: 521–530.
29. Combadiere, C., K. Salzwedel, E. D. Smith, H. L. Tiffany, E. A. Berger, and P. M. Murphy. 1998. Identification of CX3CR1: a chemotactic receptor for the human CX3C chemokine fractalkine and a fusion coreceptor for HIV-1. *J. Biol. Chem.* 273: 23799–23804.
30. Nanki, T., Y. Urasaki, T. Imai, M. Nishimura, K. Muramoto, T. Kubota, and N. Miyasaka. 2004. Inhibition of fractalkine ameliorates murine collagen-induced arthritis. *J. Immunol.* 173: 7010–7016.
31. Kojima, T., N. Tanuma, Y. Aikawa, T. Shin, A. Sasaki, and Y. Matsumoto. 1997. Myosin-induced autoimmune polymyositis in the rat. *J. Neurol. Sci.* 151: 141–148.
32. Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-DDC(T)) method. *Methods* 25: 402–408.
33. Gutala, R. V., and P. H. Reddy. 2004. The use of real-time PCR analysis in a gene expression study of Alzheimer's disease post-mortem brains. *J. Neurosci. Methods* 132: 101–107.
34. Dalakas, M. C. 2002. Muscle biopsy findings in inflammatory myopathies. *Rheum. Dis. Clin. North Am.* 28: 779–798.
35. Dalakas, M. C., and R. Hohlfeld. 2003. Polymyositis and dermatomyositis. *Lancet* 362: 971–982.
36. Ito, T., T. Kumamoto, H. Horinouchi, K. Yukishige, R. Sugihara, S. Fujimoto, and T. Tsuda. 2002. Adhesion molecule expression in experimental myositis. *Muscle Nerve* 25: 409–418.
37. Matsubara, S., T. Kitaguchi, A. Kawata, K. Miyamoto, H. Yagi, and S. Hirai. 2001. Experimental allergic myositis in SJL/J mouse: reappraisal of immune reaction based on changes after single immunization. *J. Neuroimmunol.* 119: 223–230.
38. Nemoto, H., K. Nemoto, H. Sugimoto, and M. Kinoshita. 2001. FK506 suppressed the inflammatory change of EAM in SJL/J mice. *J. Neurol. Sci.* 193: 7–11.

39. Rosenberg, N. L., S. P. Ringel, and B. L. Kotzin. 1987. Experimental autoimmune myositis in SJL/J mice. *Clin. Exp. Immunol.* 68: 117–129.
40. Matsubara, S., T. Shima, and M. Takamori. 1993. Experimental allergic myositis in SJL/J mice immunized with rabbit myosin B fraction: immunohistochemical analysis and transfer. *Acta Neuropathol. (Berl.)* 85: 138–144.
41. De Bleecker, J. L., V. I. Meire, W. Declercq, and E. H. Van Aken. 1999. Immunolocalization of tumor necrosis factor-α and its receptors in inflammatory myopathies. *Neuromuscul. Disord.* 9: 239–246.
42. Kalovidouris, A. E., and Z. Plotkin. 1995. Synergistic cytotoxic effect of interferon-γ and tumor necrosis factor-α on cultured human muscle cells. *J. Rheumatol.* 22: 1698–1703.
43. Shelton, G. D., N. A. Calcutt, R. S. Garrett, D. Gu, N. Sarvetnick, W. M. Campana, and H. C. Powell. 1999. Necrotizing myopathy induced by overexpression of interferon-γ in transgenic mice. *Muscle Nerve* 22: 156–165.
44. Kuru, S., A. Inukai, Y. Liang, M. Doyu, A. Takano, and G. Sobue. 2000. Tumor necrosis factor-α expression in muscles of polymyositis and dermatomyositis. *Acta Neuropathol. (Berl.)* 99: 585–588.
45. Sawai, H., Y. W. Park, J. Roberson, T. Imai, J. J. Goronzy, and C. M. Weyand. 2005. T cell costimulation by fractalkine-expressing synoviocytes in rheumatoid arthritis. *Arthritis Rheum.* 52: 1392–1401.
46. Ancuta, P., R. Rao, A. Moses, A. Mehle, S. K. Shaw, F. W. Luscinskas, and D. Gabuzda. 2003. Fractalkine preferentially mediates arrest and migration of $CD16^+$ monocytes. *J. Exp. Med.* 197: 1701–1707.
47. Thieblemont, N., L. Weiss, H. M. Sadeghi, C. Estcourt, and N. Haeffner-Cavaillon. 1995. $CD14^{low}CD16^{high}$: a cytokine-producing monocyte subset which expands during human immunodeficiency virus infection. *Eur. J. Immunol.* 25: 3418–3424.
48. Belge, K. U., F. Dayyani, A. Horelt, M. Siedlar, M. Frankenberger, B. Frankenberger, T. Espevik, and L. Ziegler-Heitbrock. 2002. The proinflammatory $CD14^+CD16^+DR^{++}$ monocytes are a major source of TNF. *J. Immunol.* 168: 3536–3542.
49. Feng, L., S. Chen, G. E. Garcia, Y. Xia, M. A. Siani, P. Botti, C. B. Wilson, J. K. Harrison, and K. B. Bacon. 1999. Prevention of crescentic glomerulonephritis by immunoneutralization of the fractalkine receptor CX3CR1 rapid communication. *Kidney Int.* 56: 612–620.
50. Lesnik, P., C. A. Haskell, and I. F. Charo. 2003. Decreased atherosclerosis in $CX3CR1^{-/-}$ mice reveals a role for fractalkine in atherogenesis. *J. Clin. Invest.* 111: 333–340.
51. Combadiere, C., S. Potteaux, J. L. Gao, B. Esposito, S. Casanova, E. J. Lee, P. Debre, A. Tedgui, P. M. Murphy, and Z. Mallat. 2003. Decreased atherosclerotic lesion formation in CX3CR1/apolipoprotein E double knockout mice. *Circulation* 107: 1009–1016.

関節リウマチの病態形成における骨髄異常について

Bone marrow abnormalities in rheumatoid arthritis

廣畑　俊成*
Hirohata Shunsei

はじめに

関節リウマチ(RA)は関節滑膜の増殖による進行性の骨・軟骨破壊を主徴とする原因不明の炎症性疾患である．増殖した滑膜は軟骨・骨に侵入しパンヌスとなってこれらを破壊してゆく．関節滑膜を構成する主要な細胞としては，マクロファージ様のA型滑膜細胞(M型滑膜細胞)と線維芽細胞様のB型滑膜細胞(F型滑膜細胞)が存在することが知られている[1]．これまで，このような滑膜増殖は関節滑膜局所に存在する滑膜細胞の異常増殖に起因すると考えられてきた．しかし，近年になって，A型滑膜細胞は骨髄に由来する可能性が示唆されている[2]．

一方，RA関節滑膜においては，すでにその早期より血管新生が存在していることが明らかになっている[3]．さらに，RA骨髄$CD34^+$細胞からの血管内皮細胞への分化能は変形性関節症に比し有意に亢進しているとから，これらの異常が関節滑膜の血管新生に関与している可能性も考えられる[4]．したがって，RAの病態形成においては骨髄の異常の果たす役割が非常に重要であることが示唆されるわけである．本稿においては，これまでわれわれが明らかにしてきたRAの骨髄の異常について述べるとともに，今後解明されるべき問題等についても触れてみたい．

Key words ▶
CD34
滑膜 (synovium)
血管新生 (angiogenesis)
CD14
TNF-α

*帝京大学医学部内科学教室
(〒173-8605 東京都板橋区加賀2-11-1)

Ⅰ．RAの関節滑膜の病理像の特徴

RAの関節滑膜の病理学的特徴は以下の点に集約される[1]．

①表層細胞(lining cell)の重層化，②表層下(sublining layer)へのリンパ球，形質細胞，マクロファージ，多形核白血球，樹状細胞の浸潤，③偽性リンパ濾胞の形成，④血管新生．

表層細胞ではA型およびB型の滑膜細胞の増生がみられるが，A型滑膜細胞の増生がより顕著であるといわれる[1]．表層下には種々の細胞の浸潤がみられるが，特徴的なのはしばしば偽性リンパ濾胞の形成がみられる点である(図1)．また，完成されたRA関節滑膜においては血管新生が顕著である(図2)．しかし，滑膜に炎症性細胞浸潤や表層細胞の重層化がないRAの極めて早期において，すでに血管新生が認められることが明らかになっている[3]．したがって，RAの発症において

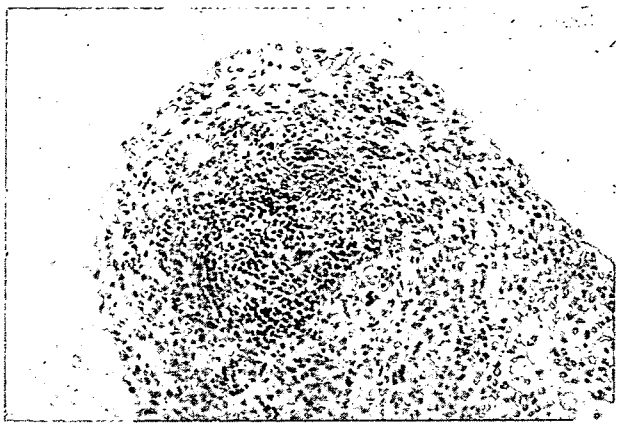

図1　関節リウマチ滑膜の病理組織像（1）
偽性リンパ濾胞を認める．（H&E，原倍率×25）

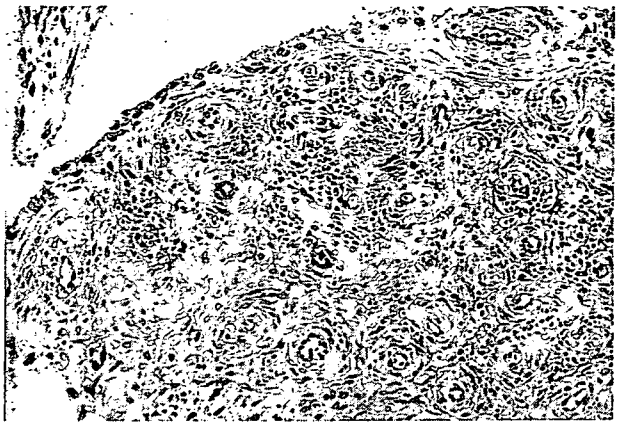

図2　関節リウマチ滑膜の病理組織像（2）
著明な血管新生を認める．（H&E，原倍率×25）

は関節滑膜での血管新生がprimaryの病変である可能性が十分に考えられる．

Ⅱ．A型滑膜細胞と骨髄

1．RA末梢血単球の異常

RAにおいては関節滑膜および皮下結節内に単球系細胞が存在することが確認されており，前者がA型滑膜細胞であると考えられる[1,5,6]．これらの単球系細胞は，骨髄から産生された単球系細胞が体循環を介して局所にrecruitされたものであると考えられる．この点においては，動物の実験関節炎モデルにおいては骨髄と関節腔を結ぶcanalを通じて細胞が侵入する経路も確認されているが[7]，ヒトではいまだ証明されていない．われわれは，これまでにRAの末梢血の単球表面のCD14がRAの疾患活動期に上昇していること，さらに末梢血単球表面のFcγRI，FcγRIIの発現はRAの活動期・非活動期のいずれにおいても上昇していることを示した[8]．すなわち，RAにおいては単球系細胞の活性化のみならず何らかの内因性の異常があり，これにより末梢血単球の表面抗原の異常を生じている可能性が考えられる．

2．RA骨髄$CD14^-$細胞からの$CD14^+$細胞の分化の亢進

前項で述べたRA末梢血$CD14^+$細胞の異常の機序を明らかにするためには，骨髄レベルでの異常の有無を明らかにする必要がある．通常，骨髄血由来の単核球成分より$CD14^-$細胞を精製して単純に培養しておくだけで$CD14^+$細胞が分化してくる[9]．この際，RA骨髄$CD14^-$細胞からは培養開始後24～72時間でOA骨髄$CD14^-$細胞に比し，有意に多くの$CD14^+$細胞に分化することが明らかになった[9]．さらに，RAにおいては，骨髄$CD14^-$細胞より分化した$CD14^+$細胞上のHLA-DR発現が増強していることも示されている[9]．したがって，RAにおいては骨髄からの単球系細胞の分化の亢進が存在し，これが病態に深く関与している可能性が考えられる．一方，活動期・非活動期のRAおよび健常人の間では末梢血中の$CD14^+$細胞数には一般には差が認められない．これは，RAでは組織への$CD14^+$細胞のrecruitmentが亢進しているため骨髄からの$CD14^+$細胞の産生が亢進していても，末梢血中ではさほどの増加を示さないためではないかと考えられる．事実，電顕上も，RAにおいては滑膜に単球

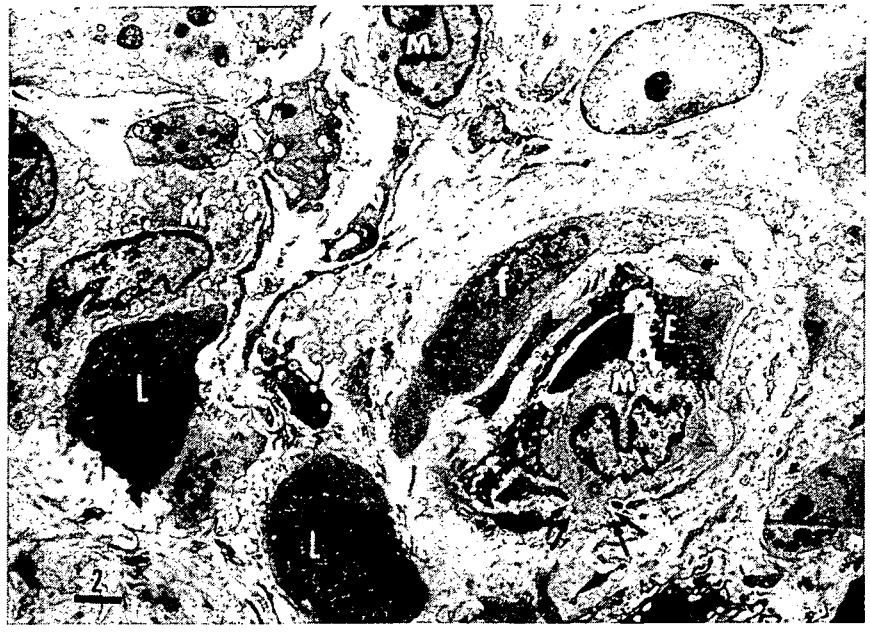

図3 関節リウマチ滑膜組織の電子顕微鏡像
単球系細胞が滑膜内に侵入しようとしている（矢印）．
M：単球，f：fibroblast-like cell，L：リンパ球，E：血管内皮細胞
（スケールは2μMを示す）

系細胞が入り込む像が認められる（図3）．さらに，RA患者の偽関節においても関節滑膜に酷似した組織が形成される．元々偽関節を発生する骨折部分には滑膜組織は存在しないことから，この組織の構成細胞は，骨髄由来細胞が体循環を経由して局所に流入した可能性が高いと考えられる．

このように，RAにおいては骨髄からのCD14$^+$細胞の分化が亢進し，全身の組織へとこれらが循環していることがわかる．しかしながら，単球表面上のFcγRI・FcγRIIの発現の異常がいかなる機序で生じているのかについてはいまだに明らかにはなっていない．

3. RA単球系細胞の機能の異常—特にBリンパ球活性化能について

RAの関節滑膜においては，リンパ球の浸潤のみならず，形質細胞の浸潤が顕著に認められる[1]．こうした形質細胞はリウマトイド因子をはじめとする自己抗体を産生していると考えられる[10]．RAにおいては，多量のリウマトイド因子の産生がみられるが，その機序については十分明らかにされていない．一般に健常人の末梢血Bリンパ球をautologousのCD4$^+$ Tリンパ球とともに抗CD3抗体の存在下に培養するとリウマトイド因子(IgM-RF)の産生が誘導される[11]．ここにRA患者骨髄CD14$^-$細胞から誘導したCD14$^+$細胞を加えると，IgMの産生量は変化させずにIgM-RFの産生量だけが特異的に増加することをわれわれは明らかにしてきた(図4)[12]．したがって，RA患者骨髄由来CD14$^+$細胞は，おそらくは直接の細胞間相互作用を介して，特にリウマトイド因子産生Bリンパ球を活性化し，Tリンパ球からのヘルパーシグナルにより容易にIgM-RFを産生するように至らしめるものと考えられる．しかしながら，現在のところはいかなる細胞間相互作用がリウマトイド因子

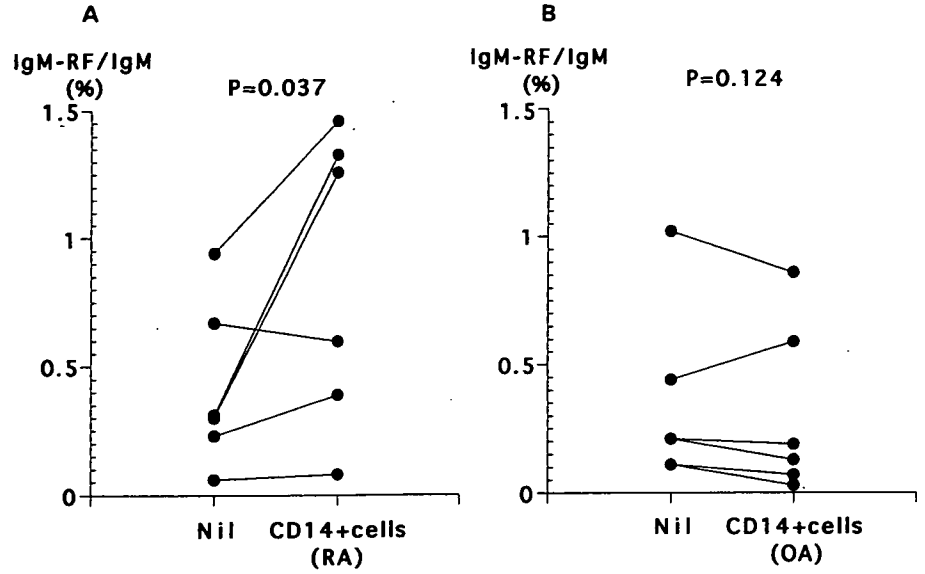

図4　関節リウマチ骨髄由来CD14⁺細胞によるIgMリウマトイド因子(IgM-RF)産生の増強効果

関節リウマチ(RA)骨髄由来CD14⁺細胞は健常人末梢血Bリンパ球によるIgM-RFを選択的に増強している（文献12より引用).

産生に関与するかについては明らかになっておらず，この点については今後解明してゆく必要がある.

Ⅲ．B型滑膜細胞と骨髄

前項で述べたように，RAの関節滑膜のA型滑膜細胞が骨髄由来であるということについてはすでにコンセンサスが得られている．一方，線維芽細胞様のB型滑膜細胞の由来についてはなお，さまざまな説があり[13]，意見の一致をみていない．われわれはRA骨髄のCD34⁺細胞からB型滑膜細胞様の細胞が誘導できるかについて検討を行った．対照群(OAあるいは健常人)の骨髄CD34⁺細胞をstem cell factor(SCF)＋granulocyte-macrophage colony stimulating factor(GM-CSF)の存在下に培養を行い，そこにIL-4あるいはTNF-αを添加したところ，TNF-αの存在下にやや線維芽細胞様細胞が多くなる傾向がみられた[14]．一方，IL-4を添加した場合は，すべての細胞は円形の細胞となり，culsterを形成し，さらにIL-4とTNF-αの両者を添加した場合にはapoptosisにより多くの細胞が死滅する傾向がみられた．RA骨髄CD34⁺細胞を同様に培養した際には，SCF+GM-CSFに加えてIL-4あるいはIL-4+TNF-αを添加した場合の形態的変化は，OA骨髄CD34⁺細胞とほぼ同様であった．SCF+GM-CSFのみの存在下ではRA骨髄CD34⁺細胞からは線維芽細胞様細胞への分化がやや多くみられたが，ここにTNF-αを添加するとこの傾向は一層著明となった(図5)[14]．図6に示すように，flow cytometryでこれらの培養細胞の表面抗原を検索すると，TNF-αの添加時に，RA骨髄CD34⁺細胞からの線維芽細胞への分化の亢進に一致して，CD14抗原，HLA-DR抗原の発現の著明な低下を認めた[14]．IL-4あるいはIL-4+TNF-αの添加によりCD14抗原の発現は低下するものの，HLA-DR抗原の発現はむしろ増加し，これはdendritic cellへの

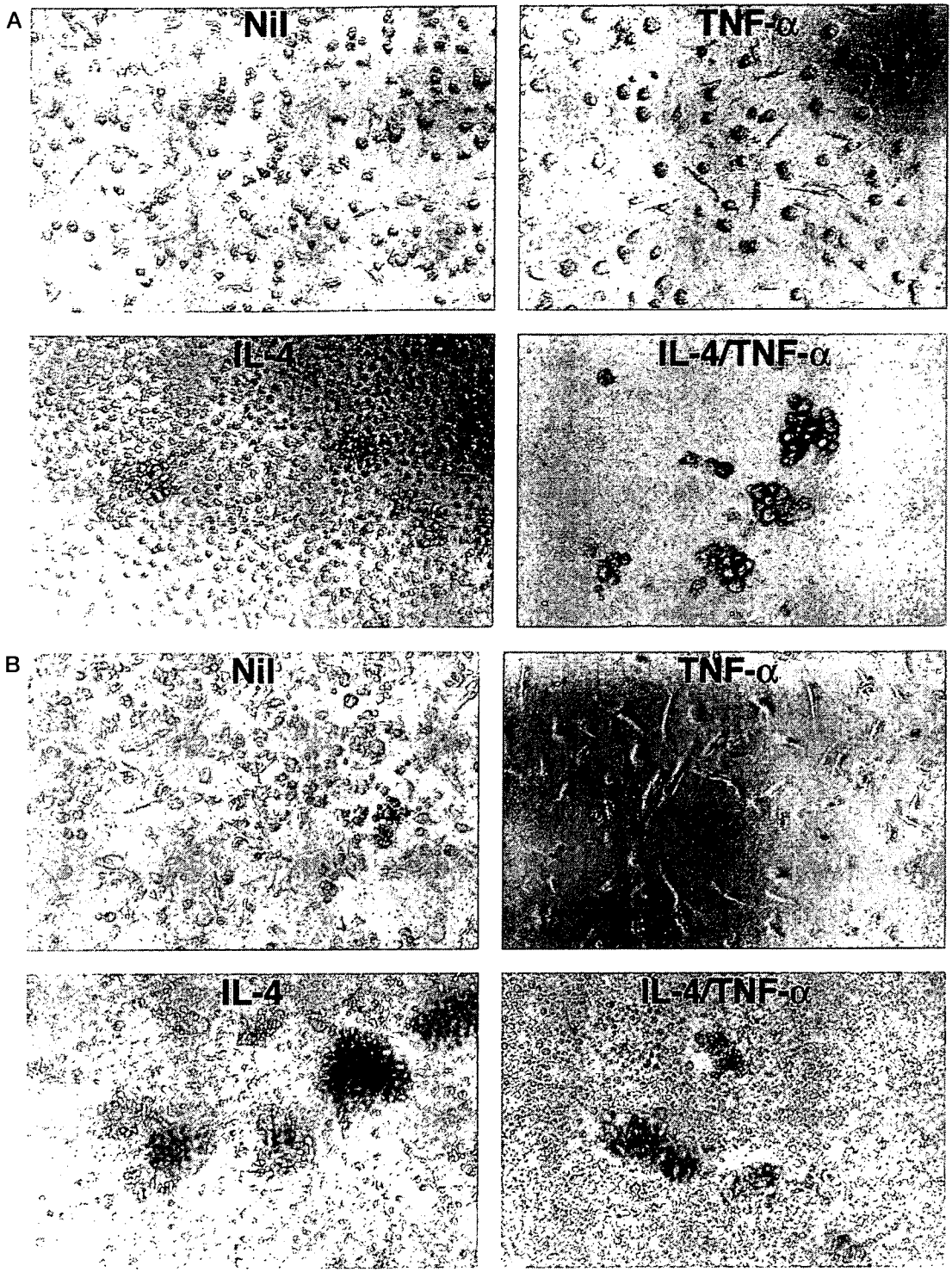

図5　変形性関節症(A)および関節リウマチ(B)骨髄CD34⁺細胞からの各種細胞の分化
骨髄CD34⁺細胞をSCF+GM-CSFの存在下に，各種サイトカインとともに4週間培養した時の形態変化を示す（文献14を改変）.

分化の誘導を示唆している．これらの結果をまとめてみると，表1に示すように，SCF+GM-CSF+TNF-αの存在下でのRA骨髄CD34⁺細胞からの線維芽細胞様細胞への分化は，OA骨髄CD34⁺細胞に比し有意に亢進していた．同時に，SCF+GM-CSF+TNF-

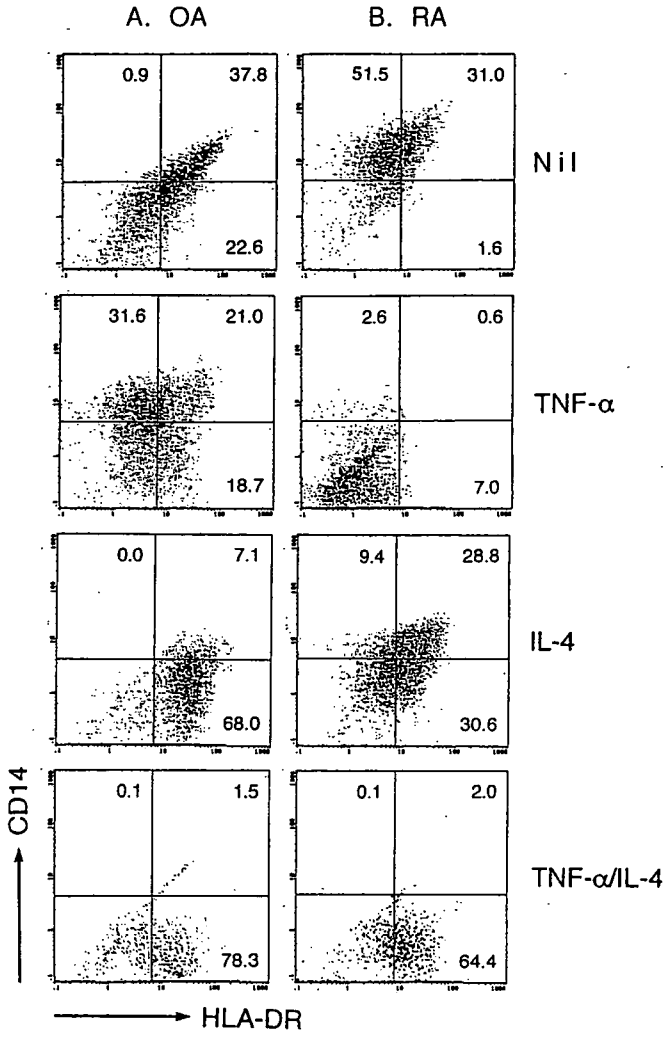

図6　変形性関節症(A)および関節リウマチ(B)骨髄CD34⁺細胞からの各種細胞の分化

骨髄CD34⁺細胞をSCF+GM-CSFの存在下に，各種サイトカインとともに4週間培養した時の表面抗原(CD14，HLA-DR)の変化を示す(文献14を一部変更)．

α で刺激した培養上清中のmatrix metalloproteinase 1 (MMP-1) の産生も，RA骨髄CD34⁺細胞においてはOA骨髄CD34⁺細胞に比して有意に亢進していた．したがって，RA骨髄CD34⁺細胞からSCF+GM-CSF+TNF-α の存在下に分化してくる線維芽細胞様細胞はMMP-1を産生するというB型滑膜細胞の性質を有していることが明らかになった[14]．以上のデータより，RA関節滑膜に存在するB型滑膜細胞も骨髄に由来する可能性が高いことが示唆された．

昨今いろいろの生物学的製剤がRAの治療に有用であることが示されており，中でも特に抗TNF-α 抗体や可溶性TNFレセプター-Ig融合蛋白の有用性が優れていることから，TNF-α がRAの病態形成上極めて重要な役割を果たすことが確認されている[15]．上記のわれわれの検討結果は，TNF-α に対する反応性がRA骨髄CD34⁺細胞においては明らかにOA骨髄CD34⁺細胞とは異なっていることを端的に示している[14]．すなわち，骨髄CD34⁺細胞のTNF-α に対する異常反応がRAの病態形成上重要な役割を果たしていることが示唆される．今後，このTNF-α に対する骨髄CD34⁺細胞の反応性の異常がいかなる

表1　関節リウマチ患者骨髄CD34⁺細胞からの各種細胞の分化

Cytokines	Morphology	RA patients (n=21)	Control (n=18)	Significance*
SCF+	M+D	9	13	
GM-CSF	M+D+F	12	5	P>0.05
	F dominant	0	0	
SCF+	M+D	1	12	
GM-CSF	M+D+F	9	5	P<0.001
TNF-α	F dominant	11	1	
SCF+	M+D	21	18	
GM-CSF	M+D+F	0	0	P>0.05
IL-4	F dominant	0	0	

*Chi-square test for independence　　(文献14より引用)

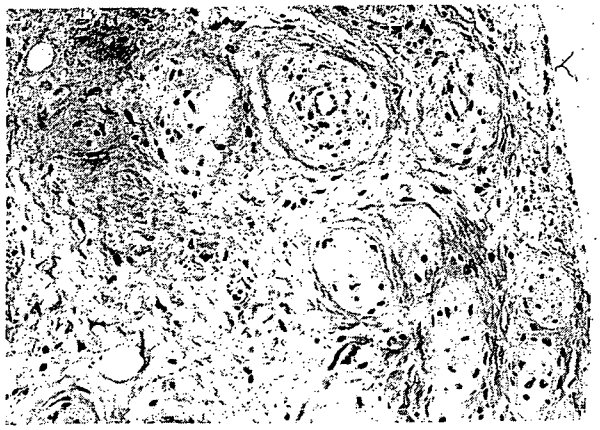

図7　早期関節リウマチ患者の関節滑膜にみられた血管新生（H&E，原倍率×40）
（文献3より引用）

機序によって生じているのかについて検討してゆく必要があろう．

Ⅳ．RAにおける血管新生と骨髄

RAの典型的な臨床像を示す以前に採取された患者の滑膜組織を検索すると，図7に示すように，全く滑膜細胞の増殖や炎症細胞の浸潤がみられないにもかかわらず，血管新生が生じている像が観察される[3]．これまで，成人にみられる血管新生（angiogenesis）においては，胎生期の血管形成（vasculogenesis）とは異なり，既存の血管から新生血管が枝分かれして増生してくるものと考えられていた．しかし，末梢血中にも骨髄由来の血管内皮前駆細胞が存在することが証明されて以来，こうした骨髄由来の血管内皮前駆細胞の関与する機転（vasculogenesis）が炎症・腫瘍などにおける血管の増生に関与することが明らかにされた[16,17]．

骨髄$CD34^+$細胞をSCF+GM-CSFの存在下に培養するとvWF^+細胞への分化がみられるが，RAにおいてはOAに比して，この骨髄$CD34^+$細胞からのvWF^+細胞への分化が有意に亢進していることが最近明らかになった[4]．さらに，この骨髄$CD34^+$細胞からのvWF^+細胞への分化は関節滑膜の毛細血管密度と正の相関を示していたことから，関節滑膜での血管新生において骨髄由来の血管内皮前駆細胞が重要な役割を果たすことが示唆された[4]．こうしたvWF+細胞の分化の亢進は培養中におけるTNF-αやVEGFなどのサイトカイン産生の亢進に基づくものではなく，RA骨髄$CD34^+$細胞自身のintrinsicな異常に基づくものと考えられる[4]．すなわち，RA骨髄$CD34^+$細胞はOA骨髄$CD34^+$細胞に比し，VEGFR2（vascular endothelial growth factor receptor 2）/KDR（kinase domain receptor）のmRNAの発現が有意に亢進していることが明らかになった[4]．したがって，RA骨髄$CD34^+$細胞はVEGFに対する反応性が亢進しており，これにより同じレベルのサイトカインの存在下においてOA骨髄$CD34^+$細胞に比しより効率的にvWF^+細胞へと分化し，関節滑膜での血管新生を支持することにより，関節滑膜の増殖に寄与しているもと考えられる．

おわりに

以上，本稿においてはRAの骨髄からはA型滑膜細胞だけでなく，B型滑膜細胞も産生される可能性の高いことを明らかにした．さらに，RA骨髄由来A型滑膜細胞様細胞は機能的にもRF産生Bリンパ球を活性化するという独特の異常を有していることを述べた．さらに，こうした関節滑膜の主要な構成細胞のみならず，これらの細胞が滑膜に侵入するために必要な血管の新生においても骨髄の異常が関与することが明らかになった．このような骨髄の異常は少なくとも$CD34^+$細胞というhematopoietic stem cellレベルでみられ

ており，こうした細胞においてVEGFR2/KDR mRNAの発現亢進という遺伝子異常が存在することも明らかになった．今後は，骨髄$CD34^+$細胞のもう1つの異常であるTNF-αに対する反応性の異常がいかなるメカニズムで生じているのかについて解析するとともに，さらにこうした骨髄$CD34^+$細胞レベルの異常がいかなる機序で生じているのかといった問題について検討を加えてゆくことが，RAの病態のみならず病因を解明する上で重要であると考えられる．

文　献

1) Kyogoku M, Sawai T, Murakami K et al : Histopathological characteristics of rheumatoid arthritis –as a clue to elucidate its pathogenesis–. Nippon Rinsho 50 : 483–489, 1992
2) Burmester GR, Stuhlmüller B, Keyszer G et al : Mononuclear phagocytes and rheumatoid synovitis. Mastermind or workhorse in arthritis? Arthritis Rheum 40 : 5–18, 1997
3) Hirohata S, Sakakibara J : Angioneogenesis as a possible elusive triggering factor in rheumatoid arthritis. Lancet 353 : 1331, 1999
4) Hirohata S, Yanagida T, Nampei A et al : Enhanced generation of endothelial cells from $CD34^+$ cells of the bone marrow in rheumatoid arthritis: possible role in synovial neovascularization. Arthritis Rheum 50 : 3888–3896, 2004
5) Hogg N, Palmer DG, Revell PA : Mononuclear phagocytes of normal and rheumatoid synovial membrane identified by monoclonal antibodies. Immunology 56 : 673–681, 1985
6) Hedfors E, Klareskog L, Lindblad S et al : Phenotypic characterization of cells within subcutaneous rheumatoid nodules. Arthritis Rheum 26 : 1333–1339, 1983
7) Nakagawa S, Toritsuka Y, Wakitani S et al : Bone marrow stromal cells contribute to synovial cell proliferation in rats with collagen induced arthritis. J Rheumatol 23 : 2098–2103, 1996
8) Shinohara S, Hirohata S, Inoue T et al : Phenotypic analysis of peripheral blood monocytes isolated from patients with rheumatoid arthritis. J Rheumatol 19 : 211–215, 1992
9) Hirohata S, Yanagida T, Itoh K et al : Accelerated generation of $CD14^+$ monocyte-lineage cells from the bone marrow of rheumatoid arthritis patients. Arthritis Rheum 39 : 836–843, 1996
10) Wernick RM, Lipsky PE, Marban-Arcos E et al : IgG and IgM rheumatoid factor synthesis in rheumatoid synovial membrane cell cultures. Arthritis Rheum 28 : 742–752, 1985
11) Hirohata S, Inoue T, Miyamoto T : Frequency analysis of human peripheral blood B cells producing IgM-rheumatoid factor. Differential effects of stimulation with monoclonal antibodies to CD3 and Staphylococcus aureus. J Immunol 145 : 1681–1686, 1990
12) Hirohata S, Yanagida T, Koda M et al : Selective induction of IgM rheumatoid factors by $CD14^+$ monocyte-lineage cells generated from bone marrow of patients with rheumatoid arthritis. Arthritis Rheum 38 : 384–388, 1995
13) Firestein GS : Invasive fibroblast-like synoviocytes in rheumatoid arthritis. Passive responders or transformed aggressors? Arthritis Rheum 39 : 1781–1790, 1996
14) Hirohata S, Yanagida T, Nagai T et al : Induction of fibroblast-like cells from CD34(+) progenitor cells of the bone marrow in rheumatoid arthritis. J Leukoc Biol 70 : 413–421, 2001
15) Feldmann M, Brennan FM, Maini RN : Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 14 : 397–440, 1996
16) Asahara T, Murohara T, Sullivan A et al : Isolation of putative progenitor endothelial cells for angiogenesis. Science 275 : 964–967, 1997
17) Gehling UM, Ergun S, Schumacher U et al : In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood 95 : 3106–3112, 2000

*　　*　　*

特集・抗リウマチ薬の使い方

ブシラミン

廣畑俊成*

Summary

ブシラミンは1つの分子内に2つのSH基を有する化合物で，分子内S-S結合を有するSA981を生体内で生ずることで，強力な抗リウマチ作用を発揮すると考えられる．その作用は，メトトレキサートと相補的であり，この両者を併用することで，より強力な治療効果が期待できる．ブシラミンの副作用としては，特に顆粒球減少，腎障害，肺障害などに対して注意が必要である．

はじめに

ブシラミン(bucillamine[BUC])は本邦で開発されたSH化合物であり，メトトレキサート(methotrexate [MTX])とともに関節リウマチ(Rheumatoid arthritis [RA])に対する有効な疾患修飾性抗リウマチ薬(Disease modifying anti-rheumatic drug [DMARD])としての地位を確立したと言っても過言ではない．本稿においては，BUCの薬理作用，有効性，副作用，使い方などについて概説するとともに，MTXとBUCの併用効果についても触れてみたい．

A. 薬理作用

1. BUCの構造上の特徴

D-ペニシラミン(D-penicillamine [DPC])とBUCはいずれも分子内にSH基を有するいわゆるSH化合物である(図1)[1]．事実，この両薬剤の効果発現

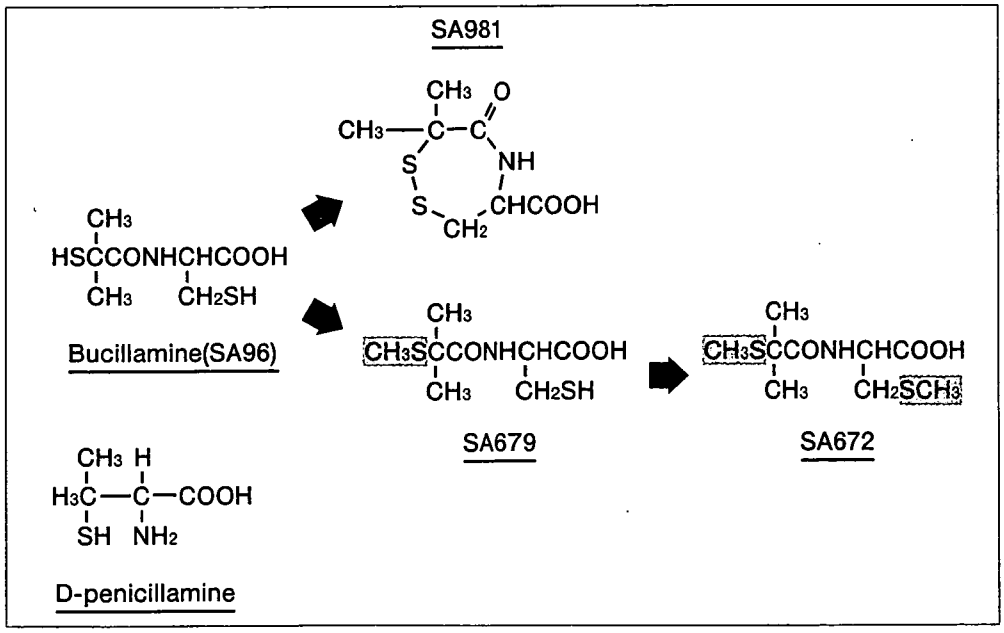

図1 DPC，BUCおよびその代謝体の化学構造式

*Shunsei HIROHATA（助教授）：帝京大学医学部内科

(0388-7332/05/￥180/論文/JCLS)