The Journal of Immunology
RESEARCH ARTICLE | DECEMBER 15 1991

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Y Iigo; ... et. al

J Immunol (1991) 147 (12): 4167–4171.
https://doi.org/10.4049/jimmunol.147.12.4167

0022-1767/91/14712-4167$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 147, 4167–4171, No. 12, December 15, 1991
Printed in U.S.A.

ICAM-1-DEPENDENT PATHWAY IS CRITICALLY INVOLVED IN THE PATHOGENESIS OF ADJUVANT ARTHRITIS IN RATS

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Intercellular adhesion molecule 1 (ICAM-1) plays important roles in immune responses. In order to examine whether ICAM-1 is involved in pathogenesis of adjuvant arthritis (AA), we investigated the effect of anti-ICAM-1 mAb, 1A29, on AA in rats. In vivo administration of 1A29 exerted a very strong suppressive effect on the development of arthritis and induced a marked reduction of inflammatory parameters. 1A29 suppressed the Ag-specific proliferative response of lymph node cells from AA rats, suggesting that the mAb blocked the Ag recognition phase. The study using adoptive transfer of AA revealed that 1A29 completely inhibited production of arthritogenic lymphocytes in donors and partially suppressed progression of arthritis in recipients caused by these lymphocytes. These findings indicated that the inhibitory effect of 1A29 on development of arthritis was at least twofold, i.e., 1) interference with cell-cell interaction between APC and T cells, which resulted in abrogation of effector cell generation; and 2) blocking of effector cell migration to inflammatory lesions. These results indicated that ICAM-1-dependent pathway is critically involved in the pathogenesis of AA. The data support the concept that ICAM-1-dependent pathways are important in chronic inflammatory disease.

Adhesion of leukocytes to vascular endothelium is a crucial process in inflammatory reactions. It has been reported that adhesion of leukocytes to endothelium occurs through the interaction of cell surface molecules expressed on respective cells (1, 2). One of these adhesion molecules is ICAM-1,[2] which is a highly glycosylated protein of the Ig superfamily (3), and one of the ligands for LFA-1 (4). Other recent reports indicated that ICAM-1 is a counter-receptor for Mac-1 (5). ICAM-1 is expressed on the surface of a variety of hematopoietic cells and also non-hematopoietic cells such as vascular endothelial cells, thymic epithelial cells, tracheal epithelial cells, and fibroblasts (6). ICAM-1 expression is quite low in most cell types under normal conditions, but its expression is upregulated by inflammatory cytokines, IL-1, TNF-α, and IFN-γ at a transcriptional level (6). ICAM-1 participates in T cell infiltration and accumulation within the thyroid gland in autoimmune thyroid disease (7). In RA, ICAM-1 is markedly expressed on synovial cells, tissue macrophages, and endothelial cells in inflamed synovium (8). It is speculated that ICAM-1/LFA-1-dependent cellular interaction is critically involved in inflammatory responses.

AA is an experimental model of chronic inflammation, which is induced in rats by injection of CFA (9). Histologically, some pathologic features of AA have many similarities to and some differences from those of RA or Reiter's syndrome in humans (10). Although pathogenesis of AA has not been well understood, there is some lines of evidence to indicate that T cells reactive with cartilage components, which have the same antigenic determinant as heat shock protein, have a critical role in the development of arthritis (11–13). Histologic examination of AA revealed that severe leukocyte infiltration was observed in subsynovial tissue and synovial cavity (14). However, there is no evidence that ICAM-1 is involved in T cell priming and effector cell migration in AA.

In this paper, in order to directly investigate the role of ICAM-1 in the pathogenesis of AA, we attempted to block ICAM-1 by systemic administration of anti-ICAM-1 mAb at various stages of inflammation and examined the effect on the development of AA.

MATERIALS AND METHODS

Animals. Female 6-wk-old Lewis rats were purchased from Charles River Japan, Inc. (Atsugi, Japan). They were maintained under standard pathogen-free conditions and used at 7- to 9-wk of age.

Induction and evaluation of AA. On day 0, rats were injected intradermally at left hindpaw with 0.1 ml of CFA containing 6 mg of heat-killed *Mycobacterium butyricum* (Difco, Detroit, MI) in 1 ml of liquid paraffin (Merck, Rahway, NJ). The volume of paw was measured with an electronic water plethysmometer (Ugo Basile, Valese, Italy). On day 0, the volume of paw was determined immediately after CFA injection. Subsequent measuring of the same paw was carried out at various time points and compared with that of day 0.

Antibody. Characterization of anti-rat ICAM-1 mAb (1A29, mouse IgG1) was described elsewhere (15). Antinitrophenol mAb (C6–8–2, mouse IgG1) was used as an isotype control (16) (kindly provided by Dr. T. Azuma, Medical School, Nagoya City University). All antibodies

Received for publication July 12, 1991.
Accepted for publication October 1, 1991.
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[2] *Abbreviations* used in this paper: ICAM-1, intercellular adhesion molecule 1; AA, adjuvant arthritis; LFA-1, lymphocyte function-associated antigen 1; RA, rheumatoid arthritis; ESR, erythrocyte sedimentation rate.

were purified from ascites by capryic acid and ammonium sulfate precipitation (17).

Antibody administration. Each rat was injected i.p. with 5 mg/kg of antibody, 3 times per week from day 0 to day 26. Rats were bled on day 27 and ESR and serum concentration of sialic acid were determined. ESR was measured in the capillary tube (VC-C110P, Terumo, Tokyo, Japan). Sialic acid was measured using Sialic Acid Test Reagents (Kyokuto, Tokyo, Japan). Radiographic analysis was performed with HITEX HA-80 (HITEX, Osaka, Japan). The hind limbs removed from rats were x-rayed with a 90° projection from the medial aspect on Fuji x-ray film (Fuji Photo Film, Tokyo, Japan).

Proliferative response of lymph node cells in AA rats. On day 14, inguinal lymph nodes were removed from AA rats. Single cell suspensions were prepared in RPMI 1640 (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% FCS (Flow Laboratories, North Ryde, Australia), 2 mM glutamine, 2×10^{-5} M 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells (5×10^5/ml) were cultured in 96-well flat-bottomed plates for 96 h with various concentrations of heat-killed *M. butyricum* and pulsed with 1 μCi of tritiated TdR for the last 6 h. Cultures were harvested and TdR incorporation was measured with a liquid scintillation counter (Beta Plate, Pharmacia, Turku, Finland).

Adoptive transfer of arthritis from AA rats and antibody administration. Adoptive transfer of arthritis was done by the method of Taurog et al. (18) with a minor modification. Ten days after sensitization, spleens were removed and 2×10^8 spleen cells were transferred i.v. into 500-rad irradiated syngeneic rats. To assess the effect of 1A29 on the production of arthritogenic lymphocytes in donors or the development of arthritis caused by these lymphocytes in recipients, we administered 1A29 or control antibody into donors or recipients. Donors were given 1 mg/kg of antibody on days 0, 2, 4, 7, and 9 after CFA injection. Recipients received the same dose of antibody three times per week from day 0 to day 23 after transfer. The method to evaluate the severity of arthritis was used as described above.

Statistical analysis. Results of all experiments were subjected to Student's *t* test. The p values of less than 0.05 were considered significant.

RESULTS

Effect of 1A29 on development of AA. Figure 1 shows the time course of progression of AA. In rats sensitized with CFA, nonspecific inflammatory reaction produced a peak in swelling of injected paw on day 3, and a secondary rise in the paw volume was seen about day 10. The first clinical signs of arthritis in noninjected paws were observed at this time. 1A29 caused significant suppression of development of chronic arthritis, which became conspicuous from day 14. Figure 2*A* shows clinical manifestation of AA, characterized by erythema and swelling, which was almost completely suppressed by administration of 1A29 (Fig. 2*B*). The inhibitory effect of 1A29 on AA was also demonstrated by radiographic examination of hindlimbs of AA rats. Hindlimbs of control rats showed bone enlargement with active osteophytosis (Fig. 3*A*). This osteophytosis was characteristic of severe subperiosteal bony proliferative changes in the intertarsal joint. 1A29 suppressed these bony changes (Fig. 3*B*). In patients with inflammation such as RA, ESR and serum concentration of sialic acid are useful for monitoring the severity of the disease (19), in that both parameters correlate well with the level of acute phase protein and the severity of inflammation. In AA, tissue sialic acid content was increased both in acute phase and chronic phase of the disease (20). For determination of the effect of 1A29 on inflammatory parameters, ESR and serum concentration of sialic acid were examined on day 27. The results were summarized in Table I. In control, ESR and serum concentration of sialic acid were 15.9 mm and 160.2 mg/dl, respectively. Treatment of 1A29 restored these parameters to normal levels, 3.6 mm of ESR and 116.6 mg/dl of serum concentration of sialic acid.

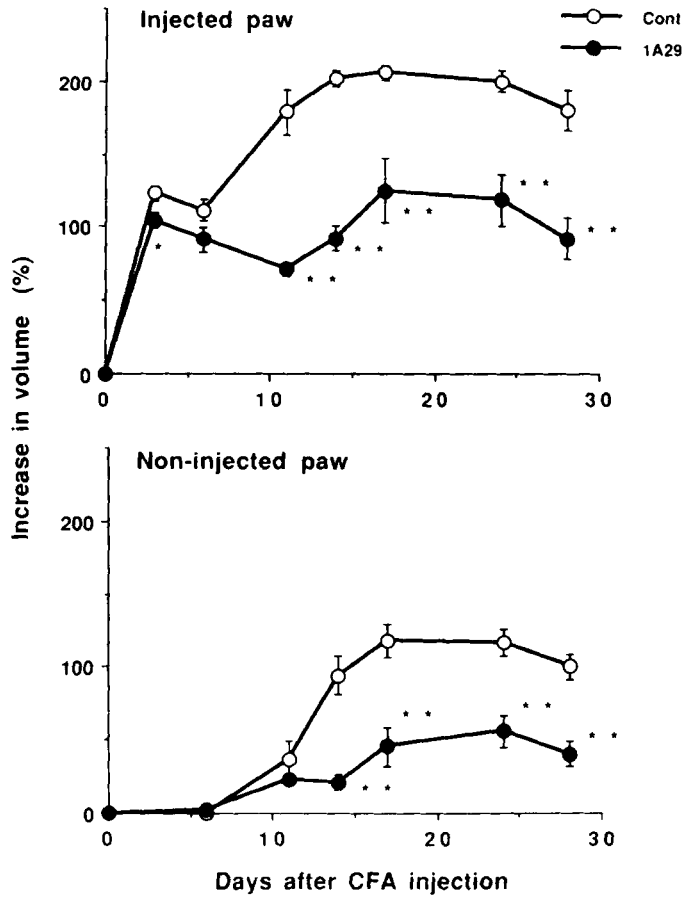

Figure 1. Time course of development of arthritis in AA rats treated with 5 mg/kg of 1A29 i.p. three times per week from day 0 to day 26. 1A29 inhibited the resultant swelling in both adjuvant injected paw (*top*) and non-injected paw (*bottom*). The number of rats consists of 7 in each group. The data is expressed as the mean of percentage of swelling and error bars represent the standard error of the mean. The difference in the severity of arthritis between 1A29 treated versus control is significant (*, $p < 0.05$ and **, $p < 0.01$, respectively).

In vivo effect of 1A29 on Ag-specific proliferation of lymph node cells. To examine whether 1A29 affected priming of T cells, in vitro Ag-specific proliferation assay was performed using lymph node cells taken from AA rats. As shown in Figure 4, lymph node cells from control rats responded to mycobacterial Ag in a dose dependent manner. Treatment with 1A29 suppressed the proliferative response dependently on the dosage of 1A29, and more than 80% inhibition was observed at 5 mg/kg.

Effect of 1A29 on adoptive transfer of AA. It was reported that AA could be transferred to irradiated inbred rats by injection of spleen cells from AA donor rats (18). We examined the effect of 1A29 on the production of arthritogenic lymphocytes in donor rats and the development of arthritis caused by these lymphocytes in recipients. The protocol of adoptive transfer of AA is illustrated in Figure 5. Flow cytometric analysis demonstrated that spleen cells from 1A29-treated donor rats showed the same proportions of $CD3^+$, $CD4^+$, $CD8^+$ cells as those from control rats (data not shown), suggesting that treatment of 1A29 did not affect the composition of T cell populations. However, as shown in Figure 6, development of arthritis in the rats injected with lymphocytes from 1A29-treated rats was completely inhibited, indicating that 1A29 suppressed the production of arthritogenic lymphocytes in donors. Moreover, the progression

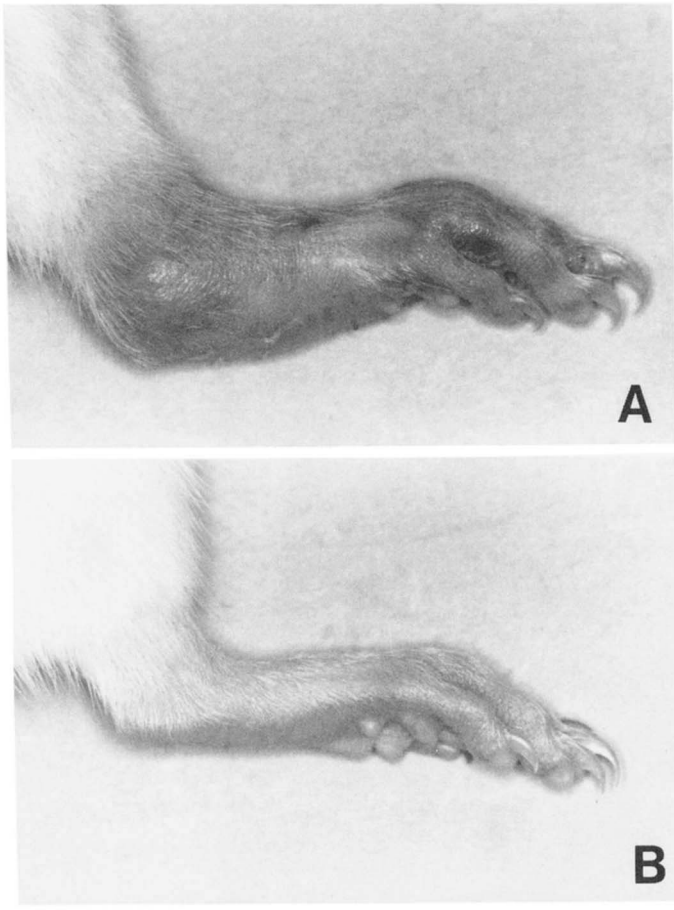

Figure 2. Macrophotographs of AA rats on day 27. Groups of rats were treated i.p. with either 5 mg/kg of 1A29 or isotype control three times per week starting on day 0. Diffuse swelling and erythema of the hindpaw were seen in the control rat (*A*) and this clinical manifestation of the disease was suppressed by 1A29 (*B*).

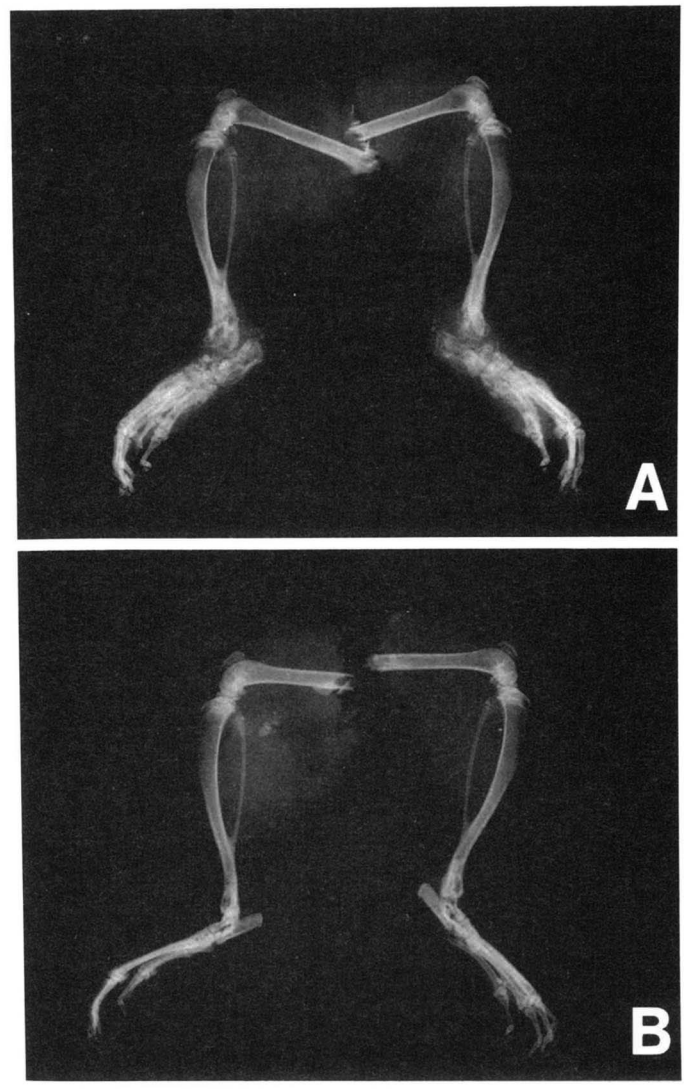

Figure 3. Radiographic examination of AA rats on day 27. Hindlimb of control rat showed soft tissue swelling and bone enlargement with active osteophytosis (*A*). 1A29 suppressed this bony change (*B*).

of arthritis in recipients injected with arthritogenic lymphocytes was partially suppressed by administration of 1A29 (Fig. 6), suggesting that 1A29 partially inhibited effector cell function. Morphologic examination revealed that infiltration of leukocytes was significantly reduced in affected joints, indicating that the suppression of effector cell function is mainly due to inhibition of leukocyte extravasation (data not shown). Collectively, these data indicate that 1A29 has inhibitory effects both on induction phase of inflammation, i.e. cell-cell interaction between APC and T cells, and on effector phase, i.e. leukocyte migration from the circulation to inflammatory lesions.

TABLE I

Inhibitory effect of 1A29 on ESR and serum concentration of sialic acid[a]

Treatment	ESR (mm)	Concentration of sialic acid (mg/dl)
Control	15.9 ± 4.3[b]	160.2 ± 1.8
1A29	3.6 ± 1.8[c]	116.6 ± 11.3[c]
Normal	3.9 ± 2.0	100.7 ± 2.4

[a] Rats were given 5 mg/kg of 1A29 or isotype control antibody three times per week from day 0 to day 26. On day 27, rats were bled and ESR and serum concentration of sialic acid were measured as described in *Materials and Methods*.

[b] Data are expressed means ± SD.

[c] ESR and concentration of sialic acid in 1A29-treated rats were statistically significant compared to those of control rats ($p < 0.01$).

DISCUSSION

AA can be divided into two phases, the induction phase and the effector phase, based on its pathogenesis. In the induction phase of AA, T cells are primed by bacterial Ag and arthritogenic T cells are generated, propagated by specific interleukins. In the effector phase of AA, tissue damage should be caused by arthritogenic T cells itself or polymorphonuclear leukocytes, which emigrated into the inflammatory site. As shown in Figure 2, 1A29 caused strong inhibitory effect on the development of AA. To elucidate which phase of inflammation was suppressed effectively by the treatment of 1A29, the effect of 1A29 on adoptive transfer model of AA was studied. It was demonstrated that 1A29 inhibited not only production of arthritogenic lymphocytes in donors, but also development of arthritis caused by these lymphocytes in recipients (Fig. 6). Ag-specific proliferation of lymph node cells in AA rats was also suppressed by in vivo treatment of 1A29 (Fig. 4). It can be speculated that inhibition of Ag-specific proliferation by 1A29 resulted in the decrease in the number of arthritogenic lymphocytes generated in donors. Dang et al. (21) reported that ICAM-1 was a major molecule in cell-cell interaction in Ag presentation from the study using ICAM-1-defective mutants. Altman et al.

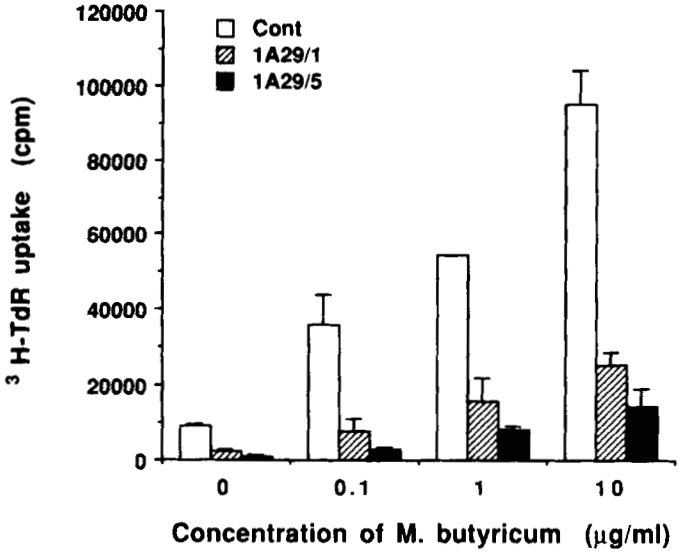

Figure 4. In vivo effect of 1A29 on the Ag-specific proliferation of lymph node cells from AA rats. 1A29 was administered i.p. to AA rats at 1 mg/kg (▨) or 5 mg/kg (■) three times per week from day 0 to day 14. On day 14, in vitro thymidine incorporation by lymph node cells was assayed. The proliferative response was expressed as the mean count per minute of triplicate cultures. Error bars represent the standard error of the mean. The difference in TdR incorporation between 1A29 treated vs control is significant ($p < 0.01$).

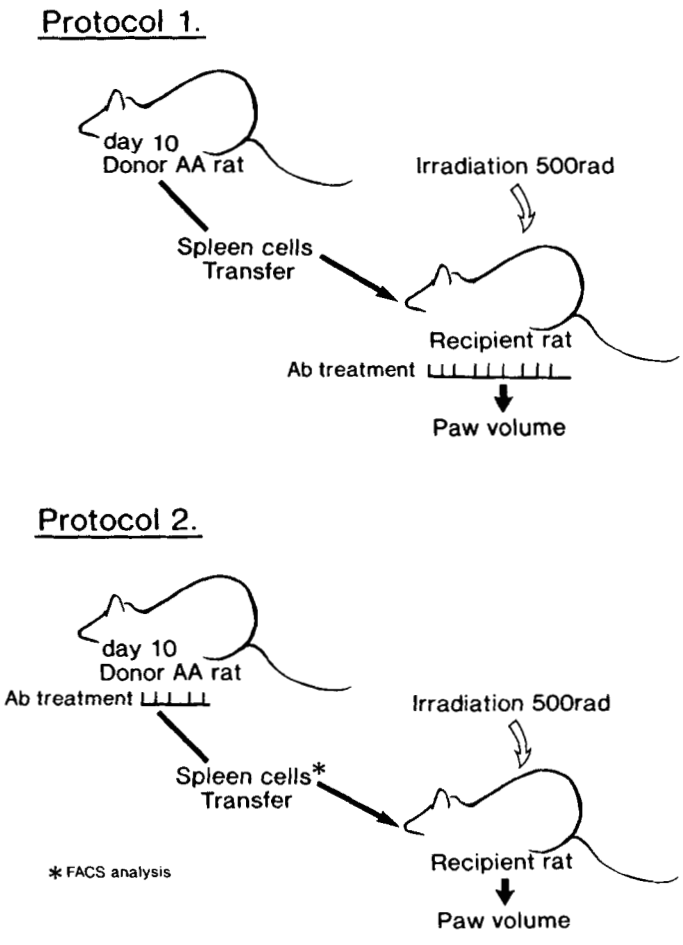

Figure 5. Protocol of adoptive transfer of AA. See *Materials and Methods.* for details.

(22) reported the importance of ICAM-1 in APC function of L cells cotransfected with ICAM-1 and HLA-DR. Other reports also demonstrated the involvement of ICAM-1/

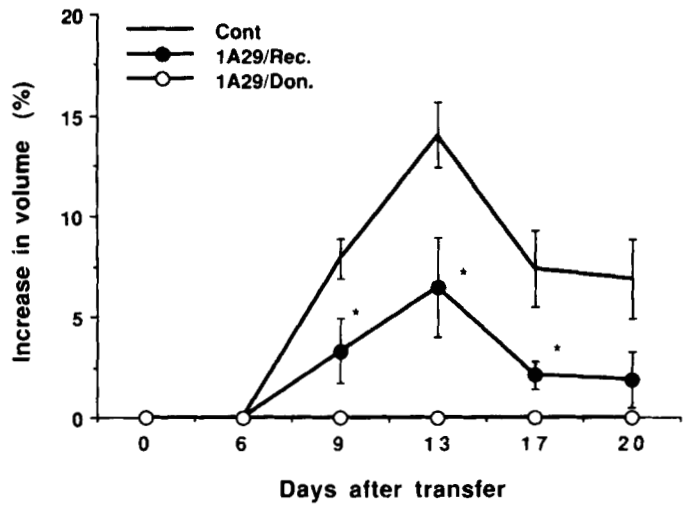

Figure 6. Effect of 1A29 on the development of arthritis was determined by the adoptive transfer model of AA as shown in Figure 5. 1A29 was administered to either recipients (●) or donors (○) at a dose of 1 mg/kg. The schedule of antibody administration is described in *Materials and Methods.* The data is expressed as the mean of percentage of swelling and error bars represent the standard error of the mean. The differences in paw volume between each of two groups and control are statistically significant (*, $p < 0.05$).

LFA-1 pathway in the interaction of Ag-specific T cells and APC (23). Considering these studies together with ours, which showed inhibitory effect of 1A29 on mixed lymphocyte reaction (24), it is conceivable that inhibition of AA by 1A29 was primarily due to the blocking of interaction between APC and T cells.

Leukocytes must attach to endothelium by virtue of some adhesion molecules, as a prerequisite for extravasation into inflammatory foci. As shown in Figure 6, 1A29 suppressed the progression of arthritis in recipients received arthritogenic lymphocytes. A significant inhibitory effect of 1A29 was also observed in effector phase of AA, although the extent of inhibition was noticeably less than that in induction phase of AA. In our recent study, 1A29 inhibited neutrophil extravasation in carrageenan-induced pleurisy (unpublished observations), which represents an acute inflammatory model caused mainly by extravasation of neutrophils. Although we have been unable to show reproducibly an inhibitory effect of 1A29 on in vitro binding of neutrophils from AA rats to cultured endothelial cell line (25), recent studies by others showed that the adhesion of human neutrophils to human umbilical vein endothelial cells is partially dependent on ICAM-1-CD11/CD18 pathway (26, 27). Further study will certainly be required to elucidate the exact role of ICAM-1 in leukocyte extravasation.

Recent preclinical trials showed that blocking of leukocyte adhesion provided an effective approach to immunosuppression or anti-inflammatory reactions. Regulation of ICAM-1-dependent pathway in nonhuman primates using anti-ICAM-1 antibody was effective on allograft rejection (28) and airway eosinophilia in asthma (29). In our present study, it was clearly demonstrated that ICAM-1 was involved both in induction of AA and in progression of arthritis. The data support the concept that ICAM-1-dependent pathways are important in chronic inflammatory disease.

Acknowledgment. We thank Masao Tsubokawa for expert technical assistance.

REFERENCES

1. **Osborn, L.** 1990. Leukocyte adhesion to endothelium in inflammation. *Cell 62:3.*
2. **Springer, A. T.** 1990. Adhesion receptors of the immune system. *Nature 346:425.*
3. **Staunton, E. D., S. D. Marlin, C. Stratowa, M. L. Dustin, and T. A. Springer.** 1988. Primary structure of ICAM-1 demonstrates interaction between members of immunoglobulin and integrin supergene families. *Cell 52:925.*
4. **Rothlein, R., M. L. Dustin, S. D. Marlin, and T. A. Springer.** 1986. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J. Immunol. 137:1270.*
5. **Diamond, S. M., D. E. Staunton, A. R. de Fougerolles, S. A. Stacker, J. Garcia-Aguilar, M. L. Hibbs, and T. A. Springer.** 1990. ICAM-1 (CD54): a counter-receptor for Mac-1 (CD11b/CD18). *J. Cell Biol. 111:3129.*
6. **Dustin, L. M., R. Rothlein, A. K. Bhan, C. A. Dinarello, and T. A. Springer.** 1986. Induction by IL 1 and interferon-γ: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J. Immunol. 137:245.*
7. **Martin, A., G. K. Huber, and T. F. Davies.** 1990. Induction of human thyroid cell ICAM-1 (CD54) antigen expression and ICAM-1-mediated lymphocyte binding. *Endocrinology 127:651.*
8. **Hale, P. L., M. E. Martin, D. E. Mccollum, J. A. Nunley, T. A. Springer, K. H. Singer, and B. F. Haynes.** 1989. Immunohistologic analysis of the distribution of cell adhesion molecules within the inflammatory synovial microenvironment. *Arthritis Rheum. 32:22.*
9. **Pearson, M. C.** 1956. Development of arthritis, periarthritis and periostitis in rats given adjuvants. *Pro. Soc. Exp. Biol. Med. 91:95.*
10. **Pearson, M. C., B. H. Waksman, and J. T. Sharp.** 1960. Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant: V. Changes affecting the skin and mucous membranes. Comparison of the experimental process with human disease. *J. Exp. Med. 113:485.*
11. **Eden, V. W., J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman, and I. R. Cohen.** 1985. Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. *Proc. Natl. Acad. Sci. USA 82:5117.*
12. **Helfgott, M. S., R. I. Kieval, F. C. Breedveld, E. Brahn, C. T. Young, R. Dynesius-Trentham, and D. E. Trentham.** 1988. Detection of arthritogenic factor in adjuvant arthritis. *J. Immunol. 140:1838.*
13. **Gaston, J. S. H., P. F. Life, P. J. Jenner, J. M. Colston, and P. A. Bacon.** 1990. Recognition of Mycobacteria-specific epitope in the 65-kD heat-shock protein by synovial fluid-derived T cell clones. *J. Exp. Med. 171:831.*
14. **Pearson, M. C., and F. D. Wood.** 1959. Studies of polyarthritis and other lesions induced in rats by injection of mycobacterial adjuvant. I. General clinical and pathological characteristics and some modifying factors. *Arthritis Rheum. 2:440.*
15. **Tamatani, T., and M. Miyasaka.** 1990. Identification of monoclonal antibodies reactive with the rat homolog of ICAM-1, and evidence for a differential involvement of ICAM-1 in the adherence of resting versus activated lymphocytes to endothelial cells. *Int. Immunol. 2:165.*
16. **Azuma, T., N. Sakato, and H. Fujio.** 1987. Maturation of the immune response to (4-hydroxy-3-nitrophenyl)-acetyl (NP) haptens in C57BL/6 mice. *Mol. Immunol. 24:287.*
17. **Russo, C., L. Callegaro, E. Lanza, and S. Ferrone.** 1983. Purification of IgG monoclonal antibody by caprylic acid precipitation. *J. Immunol. Methods 65:269.*
18. **Taurog, D. J., G. P. Sandberg, and M. L. Mahowald.** 1983. Cellular basis of adjuvant arthritis: I. Enhancement of cell-mediated passive transfer by concanavalin A and by immunosuppressive pretreatment of the recipient. *Cell. Immunol. 75:271.*
19. **Stefenelli, N., H. Klotz, A. Angle, and P. Bauer.** 1985. Serum sialic acid in malignant tumors, bacterial infections, and chronic liver diseases. *J. Cancer Res. Clin. Oncol. 109:55.*
20. **Reddy, K. G., and S. C. Dhar.** 1988. Studies on carbohydrate moieties of glycoproteins in established adjuvant induced arthritis. *Agents Actions. 25:63.*
21. **Dang, H. L., M. T. Michalek, F. Takei, B. Benaceraff, and K. L. Rock.** 1990. Role of ICAM-1 in antigen presentation demonstrated by ICAM-1 defective mutants. *J. Immunol. 144:4082.*
22. **Altmann, M. D., N. Hogg, J. Trowsdale, and D. Wilkinson.** 1989. Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cell. *Nature 338:512.*
23. **Harding, V. C., and E. R. Unanue.** 1991. Modulation of antigen presentation and peptide-MHC-specific, LFA-1-dependent T cell-macrophage adhesion. *J. Immunol. 147:767.*
24. **Tamatani, T., M. Kotani, and M. Miyasaka.** 1991. Characterization of the rat leukocyte integrin, CD11/CD18, by the use of LFA-1 subunit-specific monoclonal antibodies. *Eur. J. Immunol. 21:627.*
25. **Ise, Y., K. Yamaguchi, K. Sato, Y. Yamamura, F. Kitamura, T. Tamatani, and M. Miyasaka.** 1988. Molecular mechanisms underlying lymphocyte recirculation. 1. Functional, phenotypical and morphological characterization of high endothelial cells in vitro. *Eur. J. Immunol. 18:1235.*
26. **Smith, W. C., S. D. Marlin, R. Rothlein, C. Toman, and D. Anderson.** 1989. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J. Clin. Invest. 83:2008.*
27. **Luscinskas, W. F., M. I. Cybisky, J-M. Kiely, C. S. Peckins, V. M. Davis, and M. A. Gimbrone, Jr.** 1991. Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. *J. Immunol. 146:1617.*
28. **Cosimi, A. B., D. Conti, F. L. Delmonico, I. F. Preffer, S. Wee, R. Rothlein, R. Faanes, and R. B. Colvin.** 1990. In vivo effects of monoclonal antibody to ICAM-1 (CD54) in nonhuman primates with renal allografts. *J. Immunol. 144:4604.*
29. **Wegner, D. C., R. H. Gundel, P. Reilly, N. Haynes, G. Letts, and R. Rothlein.** 1990. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science 247:456.*