Use of Anti-Platelet-Endothelial Cell Adhesion Molecule-1 Antibody in the Control of Disease Progression in Established Collagen-Induced Arthritis in DBA/1J Mice

Jun Ishikawa¹*, Yohei Okada¹, Ian N. Bird², Bharat Jasani³, Julia H. Spragg⁴ and Toshimitsu Yamada¹

1Inflammation Research, Pharmacology Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21, Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan
2Yamanouchi Research Institute, Yamanouchi Pharmaceutical Co., Ltd., Littlemore Park, Oxford OX4 4SX, United Kingdom
3Department of Pathology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, United Kingdom
4PPP Healthcare Medical Trust, 13 Cavendish Square, London W1M 9DA, United Kingdom

Received September 20, 2001 Accepted December 19, 2001

ABSTRACT—Platelet-endothelial cell adhesion molecule-1 (PECAM-1) is expressed on the membrane of leukocytes and vascular endothelial cells. PECAM-1 has been shown to play an important role in the process of leukocyte transmigration in various animal models of acute inflammation. We investigated the role of PECAM-1 in the progression of arthritis by systemically administrering anti-murine PECAM-1 monoclonal antibody, 2H8, to DBA/1J mice with collagen-induced arthritis (CIA). Subcutaneous administration of dexamethasone (0.5 mg/kg per 2 days) significantly reduced hindpaw swelling and the clinical score of established CIA. Intraperitoneal administration of 2H8 (0.25 mg/mouse per 2 days) significantly inhibited hindpaw swelling in a time-dependent manner. 2H8 also significantly prevented further deterioration in the clinical score, but failed to reverse joint destruction discernible at the histological level. Both dexamethasone and 2H8 inhibited body weight decrease by preventing the further development of arthritis. Histopathological assessment revealed that 2H8, as well as dexamethasone, inhibited inflammatory cell transmigration into the synovium of the hind paw joint and ameliorated synovitis and cartilage erosion. These results suggest that PECAM-1 plays an important role in the progression of CIA and that an inhibitor of PECAM-1 might have therapeutic value for clinical treatment of rheumatoid arthritis.

Keywords: CD31, Anti-platelet-endothelial cell adhesion molecule-1, Collagen-induced arthritis, Neutrophil infiltration, DBA/1J mouse

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation in the joint including synovial tissues. In the synovitis of rheumatoid arthritis, leukocytes that have migrated into synovial tissue generate cytokines (e.g., tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6) which enhance inflammation (1). In particular, TNF-α produced by synovial monocytes including macrophages is of key importance to the progression of rheumatoid arthritis (2, 3). This cytokine activates endothelial cells and circulating leukocytes and causes expression or upregulation of several adhesion molecules including E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (4), which are known to play important roles in the adhesion of leukocytes to vascular endothelium and their trans-endothelial migration into inflammatory sites.

Platelet-endothelial cell adhesion molecule-1 (PECAM-1), which is constitutively expressed on the surface of endothelial cells and some inflammatory leukocytes, appears to play an important role in the transmigration of leukocytes into tissues (5 – 7). Normally, PECAM-1 is found at intercellular borders of endothelial cells and forms endothelial cell-to-cell contacts (8). TNF-α induces a redistribution of PECAM-1 on human endothelial cells without changing the total number of cell surface molecules (9). This redistribution may serve to regulate the transmigration of leukocytes across vascular endothelial cells. Results from various in vivo studies using models of acute inflammation suggest a major role for PECAM-1 in the accumulation of leukocytes at inflammatory sites. It has been reported that although anti-PECAM-1 antibody (Ab) had no signifi-
cant effect on the rolling or adhesion of leukocytes (10), it significantly inhibited their extravasation into surrounding tissue in IL-1β-activated mesenteric preparations. Similarly, it has been reported that anti-PECAM-1 Ab inhibits the migration of neutrophils in acute inflammation in rats and in immunodeficient mice transplanted with human skin grafts (11, 12). Moreover, it has been reported that anti-PECAM-1 monoclonal Ab (mAb) inhibited the migration of monocytes in a model of acute inflammation in mice (13). In addition anti-PECAM-1 Ab was shown to have a protective effect against myocardial ischemia and reperfusion injury in cats (14). The synovial expression of PECAM-1 is increased in rheumatoid arthritis and osteoarthritis, and this could facilitate the recruitment of inflammatory cells to the site of inflammation (15). Whilst there is no evidence to suggest the involvement of PECAM-1 in acute inflammatory exacerbation of chronic inflammatory diseases, PECAM-1 derived peptide has also been shown to prevent death from acute graft-versus-host disease in mice (16, 17), and soluble domain 1 of PECAM-1 has been reported as sufficient to block transendothelial migration in vivo (18). Blocking of PECAM-1 may therefore have therapeutic potential in the prevention of active inflammation in chronic inflammatory disorders such as rheumatoid arthritis. To investigate the anti-inflammatory role of a PECAM-1 blockade in rheumatoid arthritis, we have examined the effect of the anti-murine PECAM-1 mAb 2H8 on established CIA in mice, using dexamethasone as a positive control for an anti-inflammatory agent.

MATERIALS AND METHODS

Materials

Hamster anti-murine PECAM-1 mAb (clone 2H8) was purchased from Serotec (Oxford, UK). Normal hamster IgG was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Complete Freund’s adjuvant H37 Ra (CFA) was provided by Difco Laboratories (Detroit, MI, USA). Bovine-derived type II collagen was purchased from Cosmo Bio Co., Ltd. (Tokyo). Type II collagen was dissolved in 0.05 M acetic acid (4 mg/ml), and emulsified in an equal volume of CFA by repeated passage through a nearly closed stopcock mounted between two 10-ml syringes following sonication by ultrasonic transducer (H20; Cho-Onpa Kogyo, Tokyo). Sonication (19 kHz for 15 s) was performed five times on ice to avoid denaturation.

Induction of arthritis

Male DBA/1J mice (6-week-old) were purchased from Seiwa Experimental Animals, Ltd. (Fukuoka). Animals were injected intradermally at the base of the tail with type II collagen (0.2 mg) in 100 µl of CFA emulsion and given a booster injection of the same amount of type II collagen 21 days later (19).

Assessment of arthritis

Clinical severity was characterized by palpation and observations of joint properties and inflammation of surrounding tissues; and it was assessed on a scale of 0 – 3, using a previously published scoring system (2): 0 = normal, 1 = slight swelling and/or erythema of the fingers, 2 = pronounced edematous swelling, and 3 = joint rigidity with edematous swelling or joint ankylosis. Scores of 1 and 2 mainly reflect reversible edematous inflammation, but a score of 3 reflects irreversible components like established joint ankylosis. Each limb was graded with a maximum score of 12 per mouse (2). Paw swelling was evaluated by measuring the thickness of each hind paw using a dial thickness gauge (Peacock™; Ozaki Mfg, Tokyo). Body weight was monitored as a parameter for inflammation onwset.

Experimental protocols

In the primary study, to determine the pattern of arthritic induction by type II collagen immunization and to select the right time point for administration of anti-PECAM-1 and dexamethasone during the exacerbation phase, we observed arthritic parameters for 4 weeks after booster challenge using collagen-immunized (n = 45) and non-immunized animals (n = 8). In the next study, to investigate the effect of anti-PECAM-1 mAb on arthritis, the immunized mice with positive signs of arthritis were selected and divided into three groups seven days after booster immunization (day 7). The animals were treated with antibodies or dexamethasone every two days from day 8 to day 20. Group I: non-immunized animals (n = 8); Group II: type II collagen-immunized animals treated with hamster IgG (0.25 mg/mouse per 2 day, n = 8); Group III: type II collagen-immunized animals treated with 2H8 (0.25 mg/mouse per 2 day, n = 8); Group IV: type II collagen-immunized animals treated with subcutaneous dexamethasone (0.5 mg/kg per 2 day, n = 8). Body weight, clinical score and hindpaw swelling were monitored on day 7, 11, 14, 17 and 21. There was no significant difference in the arthritic parameters between Group II, III and IV on day 7. The dose of 2H8 was determined based on the blocking dosage for inflammatory cell migration in acute inflammation in vivo (13). In that experiment, 0.25 mg/mouse of 2H8 completely inhibited thioglycollate-induced peritoneal leukocyte migration in mice.

Histological assessment

Mice were sacrificed by hyper-anesthetization with dimethyl ether (Kanto Chemical Co., Inc., Tokyo) in a closed glass chamber the day after completion of treatment.
From the right legs of every animal, arthritic joints of the ankles were removed and fixed in 10% (wt/vol) buffered formalin (10% formalin neutral buffer solution, pH 7.4; Wako Pure Chemical Industries Ltd., Osaka), and then decalcified with 20% (wt/vol) ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA) (Sigma Chemical Co., St Louis, MO, USA) for 1 month. Paw tissues were embedded in paraffin, sectioned at about 5 μm with a microtome (HM400RM; Meiwa, Toyota) and stained with hematoxylin and eosin (H&E) for microscopic evaluation. Inflammatory cells that had migrated into the synovial tissues were counted by microscopy (at ×40 magnification) and classified into four groups (0, 1–50, 51–100 and >100 cells). Neutrophil infiltration into the synovial space was scored in the following manner: 0 = no infiltration, 1 = mild, 2 = moderate, and 3 = severe infiltration. The severity of arthritis in each joint was classified at four levels based on the following criteria: normal, no synovitis; mild, minimal synovitis without cartilage or bone erosion; moderate, synovitis and erosions with maintained joint architecture; severe, synovitis, extensive erosion and loss of joint integrity (2). Neutrophils were identified microscopically from cell-figure and nuclear shape stained with hematoxylin and eosin.

**Statistical analyses**

Values were expressed as the mean ± S.E.M. Statistical analysis between Group I and Group II was performed by Student’s t-test for body weight and hindpaw swelling and by the Wilcoxon rank sum test, for clinical score. The data from control, anti-PECAM-1 mAb, and dexamethasone were analyzed using one-way ANOVA with Dunnett’s multiple range test for body weight and hindpaw swelling and the Steel test, for clinical score. The correlation between inflammatory cell migration and the severity of arthritis was analyzed by simple linear regression with Pearson correlation coefficients. All statistical analyses were performed by SAS. A P value of <0.05 vs control (Group II) was considered significant.

**RESULTS**

**Typical pattern of pathogenesis of collagen-induced arthritis (CIA) in DBA/1J mice**

By the day of booster immunization, slight symptoms of arthritis were already expressed in 33% of collagen-immunized animals (Fig. 1). The incidence of arthritis increased time-dependently until seven days after booster immunization, and finally arthritis was expressed in all immunized animals except one (in which oscheitis-like inflammation was induced probably due to mis-administration of collagen emulsion). After booster immunization, body weight decreased, and clinical score and hindpaw swelling increased in a time-dependent manner (Fig. 2). The changes in body weight and hindpaw swelling were most pronounced in the immunized group at day 10 compared to the non-immunized control group. Clinical score also peaked at day 10 and remained high.

---

**Fig. 1.** Time course of the incidence of arthritis induced by type II collagen-immunization in DBA/1J mice. Closed squares, non-immunized animals (n = 8); closed circles, type II collagen-immunized animals (n = 45).

**Fig. 2.** Typical pattern of arthritis induced by type II collagen-immunization in DBA/1J mice. Each graph indicates the time course of the change in body weight (a), clinical score (b) and hindpaw thickness (c), respectively. Closed squares, non-immunized animals (n = 8); closed circles, type II collagen-immunized animals (n = 45).
Selection of animals for evaluation

The apparent onset of arthritis was observed in type II collagen-immunized animals seven days after the booster injection (day 7, Table 1). The parameters for assessment of arthritis in control animals (i.e., body weight, clinical score and paw swelling) were significantly changed compared with non-immunized animals throughout the experiment.

Effect of anti-PECAM-1 mAb, 2H8, on CIA mice

In view of the above results mAb and dexamethasone treatment was initiated on day 8 after booster immunization during the exacerbation phase. Body weight in the IgG control group (Group II) decreased at day 11 and 14 and then reversed at day 17 or 21, but no decrease in body weight was observed in the 2H8- or dexamethasone-treated mice (Fig. 3). In the control group, the clinical score

| Group No. | Immunization | N   | Clinical score (index) | Hindpaw swelling (mm) | Body weight (g) |
|-----------|--------------|-----|------------------------|-----------------------|-----------------|
| I         | None         | 8   | 0.0 ± 0.0              | 2.41 ± 0.03           | 22.1 ± 0.5      |
| II        | Collagen II  | 8   | 6.4 ± 0.3**            | 2.98 ± 0.09**         | 17.9 ± 0.3**    |
| III       | Collagen II  | 8   | 6.4 ± 0.3**            | 2.76 ± 0.12**         | 17.9 ± 0.3**    |
| IV        | Collagen II  | 8   | 6.4 ± 0.3**            | 2.90 ± 0.21**         | 18.5 ± 0.4**    |

Group I, non-immunized animals; Group II, type II collagen-immunized animals treated with hamster IgG; Group III, type II collagen-immunized animals treated with 2H8; Group IV, type II collagen-immunized animals treated with subcutaneous dexamethasone. Values are the mean ± S.E.M. **P<0.01 compared with Group I. No significant difference in any parameters between Group II, III and IV (one-way ANOVA with the Duncan test for body weight and hind paw swelling and the Steel test for clinical score, n = 8).

Fig. 3. Effects of anti-PECAM-1 mAb (2H8) and dexamethasone on the body weight gain of arthritic mice. Closed squares, non-immunized animals (n = 8, Group I); open circles, intraperitoneal administration of control hamster IgG (0.25 mg/mouse per 2 day, n = 8, Group II); closed circles, intraperitoneal administration of anti-PECAM-1 mAb, 2H8 (0.25 mg/mouse per 2 day, n = 8, Group III); closed diamonds, subcutaneous administration of dexamethasone (0.5 mg/kg per 2 day, n = 8, Group IV). *P<0.05, **P<0.01 vs control (one-way ANOVA with Dunnett’s test for body weight and hind paw swelling and the Steel test for clinical score).

Fig. 4. Effects of anti-PECAM-1 mAb (2H8) and dexamethasone on the clinical progression of established collagen-induced arthritis in mice. Upper and lower graphs indicate time-dependent change of clinical score and hindpaw thickness, respectively. Closed squares, non-immunized animals (n = 8, Group I); open circles, intraperitoneal administration of control hamster IgG (0.25 mg/mouse per 2 day, n = 8, Group II); closed circles, intraperitoneal administration of anti-PECAM-1 mAb, 2H8 (0.25 mg/mouse per 2 day, n = 8, Group III); closed diamonds, subcutaneous administration of dexamethasone (0.5 mg/kg per 2 day, n = 8, Group IV). *P<0.05, **P<0.01 vs control (one-way ANOVA with Dunnett’s test for body weight and hind paw swelling and the Steel test for clinical score).
reached a peak on day 11, and then dropped slightly until day 21 (Fig. 4). In contrast, the clinical score in the 2H8-treated mice did not change throughout the treatment period (day 7 to day 21). The animals that received 2H8 showed a time-dependent improvement in hindpaw thickness recovering to normal on day 17 and day 21. Dexamethasone quickly improved both clinical score and hindpaw swelling at day 11, and the effect lasted until day 21.

**Histological evaluation**

Typical joint histological sections are presented in Fig. 5. In the non-immunized animals, no inflammatory cell migration or synovitis symptoms were observed in the hindpaw joint tissue (Fig. 5A). However, in the type II collagen-immunized animals treated with control IgG, a large leukocyte infiltrate in the synovium and some cartilage erosion (arrow) was observed in the hindpaw joint (Fig. 5B). The major inflammatory cells in synovial tissues were neutrophils. The leukocyte infiltration significantly correlated with the severity of arthritis ($R^2 = 0.873$, $P = 0.0001$, Fig. 6). Chronic administration of 2H8 or dexamethasone apparently inhibited this infiltration (Table 2) and histopathological damage (Fig. 5, C and 5D; Table 3). Assessment of neutrophil infiltration into the joint space revealed little infiltration in hindpaw sections from mice treated with 2H8 mAb or dexamethasone compared to IgG control (Table 4).

![Fig. 5. Histopathological assessment of arthritic joints. A: Joint from non-immunized mouse hind paw. B, C and D: Joint from collagen-immunized mouse hind paw. Hamster IgG was administered intraperitoneally (B). Anti-hamster PECAM-1 mAb was administered intraperitoneally (C). Dexamethasone, as a positive control, was administered subcutaneously (D). The arrow indicates the site of cartilage damage. Each figure is a microscopic photograph taken at $\times 100$ magnification (stained by hematoxylin/eosin).](image-url)
Rheumatoid arthritis progresses generally by repeated exacerbation and remission over ten or more years (20). In collagen-immunized mice, the symptoms of arthritis, hindpaw swelling and clinical score, are exacerbated by day 10, after which periods of stabilization and remission occur, mimicking one phase of the chronic pattern. In this study, to investigate the role of PECAM-1 in the exacerbation of CIA, we started Ab treatment from day 8, in the middle of the exacerbation phase. Furthermore, to determine the magnitude of the anti-inflammatory effect of anti-PECAM-1 mAb on synovitis and cartilage damage, dexamethasone was used as a positive control.

Treatment with anti-PECAM-1 mAb prevented a worsening of the clinical score of the joints and ameliorated hindpaw swelling. Hindpaw thickness was reduced in a time-dependent manner by anti-PECAM-1 mAb treatment, but clinical score did not change until the end of the evaluation. Clinical score reflects mainly the condition of the joint such as ankylosis. Thus, when anti-PECAM-1 mAb ameliorates hindpaw edema without affecting joint ankylosis, the clinical score of the joints does not change. There-

![Graph](Image)

**Fig. 6.** Correlation between severity of arthritis and synovial leukocytes. The severity significantly correlated with inflammatory cell migration into the synovium ($R^2 = 0.873, P = 0.0001, n = 24$). In this analysis, the range of cell numbers (cells/view field at 40x, Table 2) was indicated by a score as follows: 0, 0; 1 – 50, 1; 51 – 100, 2; >101, 3; and the severity of arthritis was indicated by the sum of scores for inflammatory cell migration, cartilage erosion, and synovium fibrosis. The number in parentheses indicates the number of animals.

| Treatment | No. of joints assessed | 0 | 1 – 50 | 51 – 100 | >101 |
|-----------|------------------------|---|-------|----------|------|
| CONT      | 8                      | 0 (0) | 4 (50) | 3 (37.5) | 1 (12.5) |
| 2H8       | 8                      | 4 (50) | 3 (37.5) | 1 (12.5) | 0 (0) |
| Dexamethasone | 8                  | 4 (50) | 3 (37.5) | 1 (12.5) | 0 (0) |

The number in parentheses indicates the percentage of joints positive. CONT = control hamster IgG.

| Treatment | No. of joints assessed | Normal | Mild | Moderate | Severe |
|-----------|------------------------|--------|------|----------|--------|
| CONT      | 8                      | 0 (0) | 1 (12.5) | 6 (75) | 1 (12.5) |
| 2H8       | 8                      | 2 (25) | 4 (50) | 2 (25) | 0 (0) |
| Dexamethasone | 8                  | 4 (50) | 2 (25) | 2 (25) | 0 (0) |

The number in parentheses indicates the percentage of joints positive. CONT = control hamster IgG.

| Treatment | No. of joints assessed | 0 | 1 (Mild) | 2 (Moderate) | 3 (Severe) |
|-----------|------------------------|---|---------|--------------|------------|
| CONT      | 8                      | 1 (12.5) | 1 (12.5) | 2 (25) | 4 (50) |
| 2H8       | 8                      | 4 (50) | 3 (37.5) | 1 (12.5) | 0 (0) |
| Dexamethasone | 8                  | 6 (75) | 1 (12.5) | 1 (12.5) | 0 (0) |

The number in parentheses indicates the percentage of joints positive. CONT = control hamster IgG.
fore, this result may indicate that anti-PECAM-1 mAb ameliorates edematous swelling, but not already established joint ankylosis.

Both anti-PECAM-1 mAb and dexamethasone inhibited symptoms of arthritis in CIA mice. However, there was an apparent difference between their inhibition patterns. Anti-PECAM-1 mAb prevented further progression of CIA compared with control antibody, whereas dexamethasone ameliorated arthritis symptoms to virtually normal levels as measured by hindpaw thickness or clinical score. In the course of the exacerbation phase of rheumatoid arthritis, inflammatory cells invade the synovial cavity and produce inflammatory cytokines such as TNF-α, IL-1β and IL-6 (1), and growth factors like platelet derived growth factor (21), basic fibroblast growth factor (22) and vascular endothelial growth factor (23, 24). These cytokines and growth factors accelerate pannus formation and finally cause cartilage damage and bone destruction. Glucocorticoids are generally known to induce anti-inflammatory effects by inhibiting cytokine production (25, 26), adhesion molecule expression (27, 28) and chemical mediator release (29) at various stages of the inflammation process. The inhibitory effect in the CIA mice could be explained by dexamethasone halting disease progression by inhibiting cellular infiltration into joints and improving clinical scores by inhibiting the production of cytokines and other inflammatory mediators by leukocytes already resident in the joint. Since anti-PECAM-1 is unlikely to have direct anti-inflammatory activity, it is likely that the antibody prevented further progression of the disease by inhibiting the infiltration of inflammatory cells into the synovial cavity. The histological finding that both anti-PECAM-1 and dexamethasone suppress cell infiltration into synovial tissues to the same degree supports this idea. Moreover, the inhibitory effects of mAb and dexamethasone on arthritis were correlated with leukocyte infiltration into the synovial cavity. Although both 2H8 and dexamethasone apparently inhibited cell infiltration, the mode of their actions is quite different. Dexamethasone may suppress inflammatory cell infiltration by inhibiting the production of chemoattractants such as chemokines (30) like monocye chemoattractant protein-1 (MCP-1) and IL-8 and blocking the expression of adhesion molecules (31) like ICAM-1 and E-selectin that play an important role in binding of leukocytes to endothelial cells (32). CD31/PECAM-1 is constitutively expressed on the surface of various leukocytes and concentrated in the lateral borders between endothelial cells (5). Thus PECAM-1 probably plays a key role in trans-endothelial migration. In fact, the inhibitory effect of anti-PECAM-1 on cell migration was as potent as that of dexamethasone in CIA mice. Again, it is likely that a blockade of PECAM-1 interaction by the Ab caused the inhibition of cell infiltration. The homophilic interaction of PECAM-1 is thought to be a common feature of trans-endothelial migration irrespective of type of cell or stimulus (6).

It has been reported that several anti-adhesion antibodies have therapeutic effects in the murine CIA model. Anti-mouse ICAM-1 mAb (33), anti-mouse lymphocyte function-associated antigen-1 (LFA-1) mAb (33), and anti-rat Mac-1 mAb (34) have all been reported to suppress the development of CIA without affecting the production of anti-type II collagen antibody. In these reports, each mAb reduced the severity, but did not prevent the exacerbation of collagen-induced arthritis. However, in the present study, both anti-mouse PECAM-1 mAb, 2H8, and dexamethasone ameliorated symptoms of arthritis in mice in which CIA was already established. Treatment of these mice with anti-PECAM-1 mAb prevented a worsening of the clinical score of the joints and reduced hindpaw swelling. Dexamethasone ameliorated both the clinical score and hindpaw swelling. Therefore, these results suggest that PECAM-1 functionally differs from other adhesion molecules. PECAM-1 is a member of the immunoglobulin (Ig)-immunoreceptor tyrosine-based inhibitory motif (ITIM) family, whereas ICAM-1 and VCAM-1 are of the Ig-superfamily (35). PECAM-1 has cytoplasmic Src homology 2 binding domains that can mediate cell function through the binding of protein tyrosine phosphatase similar to other Ig-ITIM family members such as the cytotoxic T lymphocyte antigen-4 and CD22 (35). The difference in function between PECAM-1 and the other adhesion molecules cited above may be reflected in the results obtained with the Abs in the CIA model.

Recently, it was reported that Abs against the first Ig-like domain of human PECAM-1 that inhibited PECAM-1-dependent homophilic adhesion also blocked neutrophil recruitment in vivo (36). It is known that the anti-PECAM-1 mAb 2H8 binds to domain 1 of murine PECAM-1 (36) and blocks neutrophil recruitment in a murine model of acute inflammation (13). Thus, it is tempting to speculate that the mechanism of leukocyte inhibition seen in the CIA model is due to inhibition of PECAM-1 homophilic interaction and this clearly warrants further investigation. However, it must be noted that neutrophils of PECAM-1 knock-out mice were arrested between the vascular endothelium and the basement membrane of inflammatory site mesenteric microvessels, but that normal numbers of leukocytes were recovered from inflammatory sites, suggesting that the defect in migration is compensated for by other PECAM-1-independent mechanisms or by increased migration (37). At present, it is unclear exactly what role PECAM-1 has in acute inflammation. Recently, another role for PECAM-1 was described. It was reported that anti-PECAM-1 mAb inhibited human T-cell-receptor-mediated signal transduction by stimulating inhibitory mechanisms,
which suggests that PECAM-1 acts not only as an adhesion molecule, but also as an immune modulator (38). Whether the PECAM-1 mAb used in this study is capable of operating in a similar manner is unclear, but requires investigation. The stimulation of inhibitory mechanisms in this way could be a new therapeutic approach for the prevention of joint destruction in rheumatoid arthritis.

PECAM-1 is constitutively expressed and concentrated in the lateral borders between endothelial cells. Thus, the blocking of PECAM-1 may well induce bleeding. Indeed, it was reported that the knock out of PECAM-1 caused a prolongation of bleeding time in mice (39). However, in the present study, no apparent sign of bleeding was observed. Treatment with the anti-PECAM-1 antibody is different from the case of PECAM-1 knock-out in mice, and it may not be sufficient to change or affect the structure of the junction of endothelial cells. Several inflammatory cytokines, including TNF-α and IL-1β, are important to the progression of rheumatoid arthritis, and these cytokines are mainly produced by infiltrated inflammatory cells. Thus, it is likely that the inhibitory effect on CIA of anti-PECAM-1 mAb is the result of a blocking of the migration of inflammatory cells, which produce TNF-α or IL-1β.

The present study is the first to show a role for PECAM-1 in the disease progression of collagen-induced arthritis in mice. The most important point in the treatment of rheumatoid arthritis is the prevention of synovitis and pannus formation followed by cartilage damage and bone destruction (40). It has been reported that in a rodent model of arthritis, the expression of PECAM-1 increased as the inflammation worsened in the synovial lining, endothelial cells and leukocytes (41). It is likely that anti-PECAM-1 mAb inhibited neutrophils from infiltrating the synovial space by preventing them from interacting with endothelial cells at the synovial site. Dexamethasone, meanwhile, is thought to inhibit arthritis through multiple anti-inflammatory effects such as the inhibition of production of cytokines, chemokines and chemical mediators, as well as adhesion molecules including PECAM-1.

The biological function of PECAM-1 in inflammatory diseases has not been fully elucidated, but it is hoped that the present study will contribute to the development of clinical treatments for chronic inflammatory diseases like rheumatoid arthritis.

Acknowledgment
We thank Dr. M. Hiruma of Experimental Biomedical Research Inc. for carrying out the pathophysiological analysis.

REFERENCES
1. Feldmann M, Brennan FM and Maini RN: Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 14, 397 – 440 (1996)
2. Williams RO, Feldmann M and Maini RN: Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. Proc Natl Acad Sci USA 89, 9784 – 9788 (1992)
3. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Bijl H and Woody JN: Repeated therapy with monoclonal antibody to tumor necrosis factor α (cA2) in patients with rheumatoid arthritis. Lancet 344, 1125 – 1127 (1994)
4. Collins T, Read MA, Neish AS, Whitley MZ, Thanso D and Maniatis T: Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. FASEB J 9, 899 – 909 (1995)
5. Liao F, Huynh HK, Eiroa A, Greene T, Polizzi E and Muller WA: Migration of monocytes across endothelium and passage through extracellular matrix involve separate molecular domains of PECAM-1. J Exp Med 182, 1337 – 1343 (1995)
6. Bianchi E, Bender JR, Blasi F and Pardi R: Through and beyond the wall: late steps in leukocyte transendothelial migration. Immunol Today 18, 586 – 591 (1997)
7. Newman PJ: The biology of PECAM-1. J Clin Invest 100, S25 – S29 (1997)
8. Ferrero E, Ferrero ME, Pardi R and Zocchi MR: The platelet endothelial cell adhesion molecule-1 (PECAM-1) contributes to endothelial barrier function. FEBS Lett 374, 323 – 326 (1995)
9. Lewis HR, McLean NV, Yan H-C, Daise M, Sun J and Delisser HM: IFN-γ and TNF-α induce redistribution of PECAM-1 (CD31) on human endothelial cells. J Immunol 154, 6582 – 6592 (1995)
10. Wakelin MW, Sanz M-J, Dewar A, Albelda SM, Larkin SW, Boughton-Smith N, Williams TJ and Nourshargh S: An anti-platelet-endothelial cell adhesion molecule-1 antibody inhibits leukocyte extravasation from mesenteric microvessels in vivo by blocking the passage through the basement membrane. J Exp Med 184, 229 – 239 (1996)
11. Vaporesyan AA, DeLisser HM, Yan H-C, Mendiguren II, Thom SR, Jones ML, Ward PA and Albelda SM: Involvement of platelet-endothelial cell adhesion molecule-1 in neutrophil recruitment in vivo. Science 262, 1580 – 1582 (1993)
12. Chosay JG, Fisher MA, Frahood A, Ready KA, Dunn CJ and Jaeschke H: Role of PECAM-1 (CD31) in neutrophil transmigration in murine models of liver and peritoneal inflammation. Am J Physiol 274, G776 – G782 (1998)
13. Bogen S, Pak J, Garifallou M, Deng X and Muller WA: Monoclonal antibody to murine PECAM-1 (CD31) blocks acute inflammation in vivo. J Exp Med 179, 1059 – 1064 (1994)
14. Muñoz-Torrepa, Delyani JA, Albelda SM and Lefer AM: Blockade of platelet endothelial cell adhesion molecule-1 protects against myocardial-ischemia and reperfusion injury in cats. J Immunol 156, 3550 – 3557 (1996)
15. Szekaneanes Z, Haines GK, Harlow LA, Shah MR, Fong TW, Fu R, Lin SJ-W and Koch AE: Increased synovial expression of the adhesion molecules CD66a, CD66b, and CD31 in rheumatoid and osteoarthritis. Clin Immun Immunopathol 76, 180 – 186 (1995)
16. Chen Y, Schlegel PG, Tran N, Thompson D, Zehnder JL and Chao NJ: Administration of a CD11-derived peptide delays the onset and significantly increases survival from lethal graft-versus-host disease. Blood 84, 1452 – 1459 (1997)
17. Behar E, Chao NJ, Hiraki DD, Krishnaswamy S, Brown BW, Zehnder JL and Grumet FC: Polymorphism of adhesion mole-
cule CD31 and its role in acute graft-versus-host disease. N Engl J Med 334, 286 – 291 (1996)
18 Liao F, Ali J, Greene T and Muller WA: Soluble domain I of platelet-endothelial cell adhesion molecule (PECAM) is sufficient to block transendothelial migration in vitro and in vivo. J Exp Med 185, 1349 – 1357 (1997)
19 Courtenay JS, Dallman MJ, Dayan AD, Martin A and Mosedale B: Immunization against heterologous type II collagen induces arthritis in mice. Nature 283, 666 – 668 (1980)
20 Gordon DA and Hastings DA: Rheumatoid arthritis: clinical feature: early, progressive and late disease. In Rheumatology, Edited by Klippel JH and Dieppe PA, pp 3.4.1.(1994)
21 Waguri Y, Otsuka T, Sugimura I, Matsui N, Asai K, Moriyama J, Ishikawa et al. 340, 269 – 270 (1996)
22 Matey DL, Dawes PT, Nixon NB and Slater H: Transforming growth factor beta 1 and interleukin 4 induced α smooth muscle actin expression and myofibroblast differentiation in human synovial fibroblasts in vitro: modulation by basic fibroblast growth factor. Ann Rheum Dis 56, 426 – 431 (1997)
23 Jackson JR, Minton JA, Ho ML, Wei N and Winkler JD: Expression of vascular endothelial growth factor in synovial fibroblasts is induced by hypoxia and interleukin 1β. J Rheumatol 24, 1253 – 1259 (1997)
24 Paleolog EM, Young S, Stark AC, McCloskey RV, Feldmann M and Maini RN: Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor α and interleukin-1 in rheumatoid arthritis. Arthritis Rheum 41, 1258 – 1265 (1998)
25 Tobler A, Meier R, Seitz M, Dewald B, Bagggiolini M and Fey MF: Glucocorticoids down regulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6, but not of M-CSF in human fibroblasts. Blood 79, 45 – 51 (1992)
26 Bleecker MW, Netea MG, Kullberg BJ, Van der Ven Jongekrijg J and Van der Meer JW: The effects of dexamethasone and chloropromazine on tumor necrosis factor-α, interleukin-1β, interleukin-1 receptor antagonist and interleukin-10 in human volunteers. Immunology 91, 548 – 552 (1997)
27 Burke-Gaffney A and Hellewell PG: Regulation of ICAM-1 by dexamethasone in a human vascular endothelial cell line EAhy926. Am J Physiol 270, C552 – C561 (1996)
28 Wheller SK and Perretti M: Dexamethasone inhibits cytokine-induced intercellular adhesion molecule-1 up-regulation on endothelial cell lines. Eur J Pharmacol 331, 65 – 71 (1997)
29 Lindén M: The effects of β2-adrenoceptor agonists and a corticosteroid, budesonide, on the secretion of inflammatory mediators from monocytes. Br J Pharmacol 107, 156 – 160 (1992)
30 Mehndate K, al-Daccak R, Schall TJ and Mourad W: Induction of chemokine gene expression by major histocompatibility complex II ligands in human fibroblast-like synoviocytes. Differential regulation by interleukin-4 and dexamethasone. J Biol Chem 269, 32063 – 32069 (1994)
31 Cronstein BN, Kimmel SC, Levin RJ, Martiniek F and Weissmann G: A mechanism for the anti-inflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. Proc Natl Acad Sci USA 89, 9991 – 9995 (1992)
32 Bevilacqua MP: Endothelial-leukocyte adhesion molecules. Annu Rev Immunol 11, 767 – 804 (1993)
33 Kakimoto K, Nakamura T, Ishii K, Takashi T, Igou H, Yagita H, Okumura K and Onoue K: The effect of anti-adhesion molecule antibody on the development of collagen-induced arthritis. Cell Immunol 142, 326 – 337 (1992)
34 Taylor PC, Chu CQ, Plater-Zyberk C and Maini RN: Transfer of type II collagen-induced arthritis from DBA/1 to severe combined immunodeficiency mice can be prevented by blockade of Mac-1. Immunology 88, 315 – 321 (1996)
35 Newman PJ: Switched at birth: a new family for PECAM-1. J Clin Invest 103, 5 – 9 (1999)
36 Nakada MT, Amin K, Christofidou-Solomidou M, O’Brien CD, Sun J, Guruhagavatula I, Heaveran GA, Taylor AH, Paddock C, Sun QH, Zehnder JL, Newman PJ, Albelda SM and Delisser HM: Antibodies against the first Ig-like domain of human platelet endothelial cell adhesion molecule-1 (PECAM-1) that inhibit PECAM-1-dependent homophilic adhesion block in vivo neutrophil recruitment. J Immunol 164, 452 – 462 (2000)
37 Duncan GS, Andrew DP, Takimoto H, Kaufman SA, Yoshida H, Spellberg J, de la Pompa JL, Elia A, Wakeham A, Karan-Tamir B, Muller WA, Senaldi G, Zukowski MM and Mak TW: Genetic evidence for functional redundancy of platelet/endothelial cell adhesion molecule-1 (PECAM-1): CD31-deficient mice reveal PECAM-1-dependent and PECAM-1-independent functions. J Immunol 162, 3022 – 3030 (1999)
38 Newton-Nash DK and Newman PJ: A new role for platelet-endothelial cell adhesion molecule-1 (CD31): inhibition of TCR-mediated signal transduction. J Immunol 163, 682 – 688 (1999)
39 Mahooti S, Graesser D, Patil S, Newman P, Duncan G, Mak T and Madri JA: PECAM-1 (CD31) expression modulates bleeding time in vivo. Am J Pathol 157, 75 – 81 (2000)
40 Zvaifler NJ: Etiology and pathogenesis of rheumatoid arthritis. In Arthritis and Allied Conditions, 20th edition, Edited by McCarty DJ and Koopman WJ, Vol 1, pp 723 – 736, Lea and Febiger, Philadelphia (1993)
41 Volin MV, Szekanecz Z, Halloran, MM, Woods JM, Magua J, Damergis JA Jr, Haines GK III, Crocker PR and Koch AE: PECAM-1 and leukosialin (CD43) expression correlate with heightened inflammation in rat adjuvant-induced arthritis. Exp Mol Pathol 66, 211 – 219 (1999)