Journal of Clinical Immunology, Vol. 25, No. 1, January 2005 (© 2005)
DOI: 10.1007/s10875-005-0354-4

The Inhibitory Co-Receptor, PECAM-1 Provides a Protective Effect in Suppression of Collagen-Induced Arthritis

MAE-XHUM WONG,[1] JOHN D. HAYBALL,[2] P. MARK HOGARTH,[1] and DENISE E. JACKSON[1,3]

Submitted: April 13, 2004; revised June 22, 2004; accepted: August 23, 2004

Studies of PECAM-$1^{-/-}$ mice have identified that PECAM-1 functions as an inhibitory co-receptor to modulate immunological responsiveness. In this study, we describe the *in vivo* consequences of PECAM-1 deficiency in mouse models of collagen-induced arthritis (CIA) and K/BxN passive transfer model that resembles many of the features of human rheumatoid arthritis. Immunization of PECAM-$1^{-/-}$ C57BL/6 (H-2^b) mice with chicken collagen type II induced CIA with an incidence of 82% by day 49, while 33% of wild-type and 100% of DBA/1 mice developed arthritis in a similar time frame. The mean onset of disease for PECAM-$1^{-/-}$ C57BL/6 mice was day 32 compared to day 51 for wild-type C57BL/6 mice and day 18 for DBA/1 mice (H-2^q susceptible). In terms of disease severity, the mean maximal arthritic index for PECAM-$1^{-/-}$ C57BL/6 mice was comparable to DBA/1 mice (8.91 ± 0.91 *vs* 11.67 ± 0.82). This mean maximal index in PECAM-$1^{-/-}$ C57BL/6 mice was significantly higher than wild-type C57BL/6 mice (5.00 ± 0.73). IgG_1 and IgG_{2b} antibody responses against CII were elevated in arthritic PECAM-$1^{-/-}$ C57BL/6 mice compared to wild-type C57BL/6 mice. Histological examination of arthritic paws of PECAM-$1^{-/-}$ C57BL/6 mice revealed inflammatory infiltrates of lymphocytic/monocytic cells and cartilage/bone destruction similar to CIA-induced DBA/1 arthritic paws. In the K/BxN model, the arthritis was not augmented in PECAM-$1^{-/-}$ mice compared to wild-type mice. In contrast, in active CIA, PECAM-$1^{-/-}$ mice developed severe disease comparable to susceptible DBA/1 mice and profoundly more severe than C57BL/6 mice, where only one third developed a mild/moderate disease. Together these observations suggest that PECAM-1 plays a crucial role in the suppression of development of autoimmune arthritis.

KEY WORDS: Rheumatoid arthritis; collagen-induced arthritis; PECAM-1; knockout mouse.

[1]Austin Research Institute, Austin Hospital, Heidelberg, Victoria, Australia.
[2]Dame Roma Mitchell Cancer Research Laboratories, Hanson Institute, IMVS, Adelaide, South Australia.
[3]To whom correspondence should be addressed at Kronheimer Building, Austin Research Institute, Austin Hospital, Studley Road, Heidelberg, Victoria 3084, Australia; e-mail: d.jackson@ari.unimelb.edu.au.

INTRODUCTION

Murine models of active and adoptive passive transfer of collagen-induced arthritis (CIA) have been extensively used for defining new *in vivo* therapeutic targets in rheumatoid arthritis (RA) and understanding processes involved in the development of erosive autoimmune arthritis (1). The active CIA murine model resembles many of the features of human RA including pannus formation, synovitis, and susceptibility dependence on MHC class II alleles (2–4). Rheumatoid arthritis and CIA are characterized by development of pannus, together with a hyperplastic and chronic inflammatory tissue that invades the articular cartilage and bone of the joints (5). Macrophages located in the pannus tissue produce proinflammatory cytokines, IL-1β, and TNF-α that activate the pannocytes that produce adhesion molecules and matrix degrading enzymes (6). The culmination of these events is irreversible cartilage destruction, bone erosion, and a pathological remodeling of the bone structure.

Active CIA closely resembles the pathological expression of RA due to its immunoreactivity to multiple autoimmune targets, pathogenic mechanisms and the genetic predisposition based upon MHC alleles (3, 4). CIA requires a functional B-cell response and the development of autoantibodies to native helical collagen type II (2). In contrast, the adoptive passive transfer model of RA using arthritogenic K/BxN sera or anti-collagen type II antibodies is not MHC dependent, but is histologically similar to the postimmunization disease (7). This model requires the autoantibodies to target arthritogenic epitopes and bypasses the requirement for autoantibody development required in

Abbreviations used: CIA, collagen-induced arthritis; RA, Rheumatoid arthritis; GPI, glucose 6-phosphate isomerase; PECAM-1, Platelet Endothelial Cell Adhesion Molecule-1; SPF, specified pathogen-free; CII, chicken type II; CFA, complete Freund's adjuvant; MT, Mycobacterium tuberculosis.

the active CIA model (8). It is severe and rapid in onset usually within hours or days of transfer (7). Arthritis in the K/BxN mouse model has been attributed to pathogenic antibodies that recognize glucose 6-phosphate isomerase (GPI). Recent studies have indicated that there is a low prevalence (∼20%) of antibodies to GPI in patients with RA (9). In contrast, antibodies to collagen type II occur in ∼70% of patients with early RA and their frequency decreases with time after onset, indicating the importance of native helical collagen type II as an arthritogenic epitope in the initiation of RA (10).

Using either the active or adoptive passive transfer CIA model, mice deficient in complement factors, Fc receptor γ chain and the low-affinity IgG receptor, FcγRIII have been shown to be highly resistant to development of CIA (11–14). In contrast, mice deficient in the inhibitory Fc receptor, FcγRIIb1 have been shown to be more susceptible and have increased disease severity on an H-2^b genetic background in active models of CIA, while in passive transfer models of CIA, the FcγRIIb1-deficient mice are similar to wild-type mice in their kinetics and disease severity (8, 15, 16). Therefore, by using these two different models (active CIA and K/BxN) of RA induction, it can provide information about the importance of immunoreceptors in the two concomitant stages of CIA including the initial stage and the end stage of disease development.

Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1/CD31) is now classified into the Ig-ITIM superfamily and has the distinguishing features of six extracellular Ig-domains with the amino terminal Ig-domain mediating homophilic binding and two intracellular ITIM domains that modulate its cell-signaling properties primarily through SHP-2 protein—tyrosine phosphatase (17). Our studies with PECAM-1-deficient mice strongly suggest that it serves an important role in modulation of B-cell immunological responsiveness and regulation of peripheral tolerance (18). PECAM-1 is broadly expressed on the surface of both vascular and haematopoietic cells. PECAM-1 is also expressed in the synovial lining and its level of expression is upregulated in adjuvant-induced models of rat arthritis (19, 20). Previous studies have demonstrated that intravenous infusion of anti-PECAM-1 antibodies leads to a significant reduction in adherent, but not rolling leukocytes in arthritic animals (21). A more recent study has suggested that administration of an anti-PECAM-1 monoclonal antibody 2H8 modulated the disease course of established CIA in DBA/1 mice (22). As PECAM-1 is a multifunctional immunoreceptor that plays a pivotal role in integrin-dependent cell adhesion, cell migration, leukocyte transmigration, negative regulation of immune signaling, and autoimmunity, it is likely to be important in the pathogenesis of CIA. However, due to the heterogeneity of factors involved in onset and disease progression including complement components, Fc receptors and glucose phosphate isomerase in mouse models of RA, the precise role of PECAM-1 is not clearly defined. In order to assess the role of PECAM-1 in the pathogenesis of RA, we studied the onset and progression of inflammatory arthritis in PECAM-1-deficient mice compared to wild-type mice in both active and adoptive passive transfer models of RA.

MATERIALS AND METHODS

Mice

PECAM-1-deficient mice were backcrossed onto C57BL/6 background ($H-2^b$) for eight generations (23). The backcrossed mice were then intercrossed to generate mice homozygous PECAM-1 deficient mice (PECAM-$1^{-/-}$). PECAM-1 genotypes determined by PCR using tail derived genomic DNA and the following primer sets: 5′-GCCCAGTATTTTTCATGATGC-3′; 3′-CACCCTGACCCTCAGGATC-5′. PCR was performed in 25-μL volumes using 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.20 μM primers, and 2.5 U of AmpliTaq Gold polymerase (Applied Biosystems Division, Foster City, CA) for 40 cycles.

The Institutional Animal Care and Use Committee Approved All Procedures

All mice were backcrossed, bred, and maintained at the specified pathogen-free (SPF) facility in the Animal House of the Austin Research Institute, Heidelberg, Victoria. The animals were fed rodent chow and acidified water. Experiments were performed with age- and sex-matched mutant and wild-type mice.

K/BxN offspring were generated by mating KRN transgenic mice on a C57BL/6 (B) background with NOD/Lt (N) mice (24). Serum was collected from arthritic K/BxN mice at approx. 5–6 weeks of age (24).

Induction of CIA

Chicken collagen type II (Sigma Chemical Company, St. Louis, MO) or ultrapure, pepsin-free bovine collagen type II (Chrondrex, USA) were solubilized to a concentration of 4 mg/mL in 0.01 M acetic acid at 4°C with constant mixing overnight. 200 μg chicken collagen type II was emulsified with an equal volume of complete Freund's adjuvant (1:1) (Difco Laboratories, Detroit, MI), and 100 μL of the emulsion was injected intradermally

under halothane anesthesia, at the base of the tail of each 8-week-old mouse. Mice were boosted on day 21 and 42 with 200 μg of chicken collagen type II emulsified in an equal volume of incomplete Freund's adjuvant (1:1) (Difco Laboratories, Detroit, MI) (16, 25). Arthritis development was monitored every three times per week starting from day 7 post immunization. Arthritis development was monitored for 80 days or until mouse paws reached the maximum grades for swelling. Clinical severity of arthritis was quantified according to the following scoring criteria and grading from 0 to 4. Score 0, no swelling; score 1, the appearance of swelling of a single joint or mild edema; score 2, two or more joints and moderate swelling; score 3, severe swelling affecting most joints; score 4, severe swelling over the entire paw, summed over all four paws to give a maximum score of 16. Using this mouse model, the onset and severity in susceptible DBA/1 mice is usually between days 18 and 20. Each of these arthritis experiments was balanced in terms of the age, sex, genetic background, immunization protocol, and clinical scoring of the mice.

Histological Assessment of CIA

At the termination of the CIA experiment, the hind paws of wild-type C57BL/6, PECAM-$1^{-/-}$ C57BL/6 and DBA/1 mice were removed. The paws were fixed in phosphate buffer containing 4% (v/v) formaldehyde, decalcified in EDTA, and paraffin embedded. Sagittal sections (5 μm) were stained with hematoxylin and eosin and evaluated using a Leitz DMRBE microscope (Leica, Hawthorn East, Victoria).

Measurement of Anti-CII Antibodies

Mice were bled from the tails at four different time points (day 14, 28, 48, and 59) after immunization, and individual sera was analyzed for CII-specific IgG antibodies by ELISA. Microtiter plates (Nunc Maxisorb, Medos) were coated overnight at 4°C with 50 μL of native CII in PBS at 50 μg/mL. Plates were washed with PBS containing 0.05% (v/v) Tween 20 (PBS/Tween) and serially diluted serum samples in PBS/Tween were added and incubated for 2 h at room temperature. The plates were then washed and incubated for 2 h at room temperature with 50 μL of sheep anti-mouse conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted to 1:10,000 in PBS/Tween. Following washing, 50 μL of *p*-nitrophenyl phosphate disodium salt (PNPP) (Pierce Chemical Company, Rockford, IL) diluted in diethanolamine buffer at 1 mg/mL was applied. Absorbances were read at 405 nm once color development had occurred. A standard curve was generated from pooled sera obtained from CII hyperimmunized DBA/1 mice.

ELISA to detect anti-CII specific IgG isotypes was performed using a modification of the above protocol. Serially diluted serum samples were incubated at 4°C overnight followed by the addition of 50 μL of biotinylated rat anti-mouse IgG_1 (1:1,000), biotinylated rat anti-mouse IgG_{2a} (1:1,000); or biotinylated rat anti-mouse IgG_{2b} (1:1,000) (Caltag Laboratories, Burlingame, CA) were applied to each plate for 5 h at room temperature. After washing, 50 μL of streptavidin-alkaline phosphatase (diluted 1:1,000) (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was added and incubated for 1 h at room temperature. The plates were washed and PNPP substrate was added. The concentrations of each antibody isotype were calculated by comparison with a polyclonal anti-CII standard.

T-cell Proliferation Assay

Eight-week-old age and sex matched PECAM-$1^{-/-}$ and PECAM-$1^{+/+}$ mice were immunized in foot pads and base of tail with 400 μg bovine collagen type II emulsified 1:1 with complete Freund's adjuvant (CFA). After 21 days post immunization, mice were sacrificed and the popliteal lymph nodes were removed. Individual single cell suspensions were made DMEM supplemented with 50 μM 2-mercaptoethanol, 10 mM HEPES pH 7.2, 20 mM Glutamine, 100 U/mL Penicillin, 100 μg/mL Streptomycin, and 5% (v/v) foetal calf serum. 1×10^5 lymph node cells were plated in 96-well round bottom microtiter plates in triplicate and stimulated in the presence and absence of 5, 50, and 100 μg/mL heat-denatured ultrapure, pepsin-free bovine collagen type II in PBS. The cells were incubated at 37°C in 5% CO_2 for 4 days, and 1 μCi/well of [^{3}H]-TdR was added to the culture for the last 6 h. [^{3}H]-TdR incorporation was measured using a β-scintillation counter, and the results expressed as mean cpm $\pm$ SEM.

Arthritis Transfer with K/BxN Mouse Sera

Sera from K/BxN mice (40–60 days of age) were pooled and 100 μL injected intraperitoneally at time 0 and 48 h. Arthritis was scored twice daily by clinical examination (clinical scoring described above) and experiments were normally conducted over a two-week period or until mice reached maximal clinical scoring.

Statistical Analysis

The anti-CII IgG antibodies were analyzed at different time points after immunization using ANOVA.

RESULTS

PECAM-1$^{-/-}$ Mice on an H-2^b Background are Susceptible to CIA

Susceptibility to CIA and RA are clearly associated with the MHC class II polymorphism in the induction of the disease (26–28). In genetically resistant strains of mice, neither arthritis nor high levels of anticollagen antibodies are produced, including BALB/c (*H*-2^d), C3H/He (*H*-2^k), or C57BL/6 (*H*-2^b) mice (28–29). Although the PECAM-1$^{-/-}$ mice were generated and backcrossed eight generations onto *H*-2^b C57BL/6 background, a haplotype that is less susceptible to CIA induction, we wanted to determine if deletion of this inhibitory co-receptor would convert a nonsusceptible strain of mice into a susceptible one. In order to investigate if PECAM-1$^{-/-}$ mice on an *H*-2^b background with 200 μg chicken type II collagen (CII) in conjunction with CFA containing *Mycobacterium tuberculosis* (MT) strain H_{37}Rv and boosted at the same location with 200 μg CII plus incomplete Freund's adjuvant 21 and 42 days later. The mice were observed for the development of arthritis three times a week starting from day 7 after immunization and bled periodically for anti-CII antibody determination in comparison to age and sex-matched *H*-2^b wild-type C57BL/6 or DBA/1 mice. In addition, we have included wild-type littermates from C57BL/6/129 genetic background to control for any influence of residual 129 genes.

To our surprise, we found that PECAM-1$^{-/-}$ mice on a genetically resistant background were more susceptible to erosive progressive arthritis and had increased incidence of disease in an active CIA model. As shown in Fig. 1A,

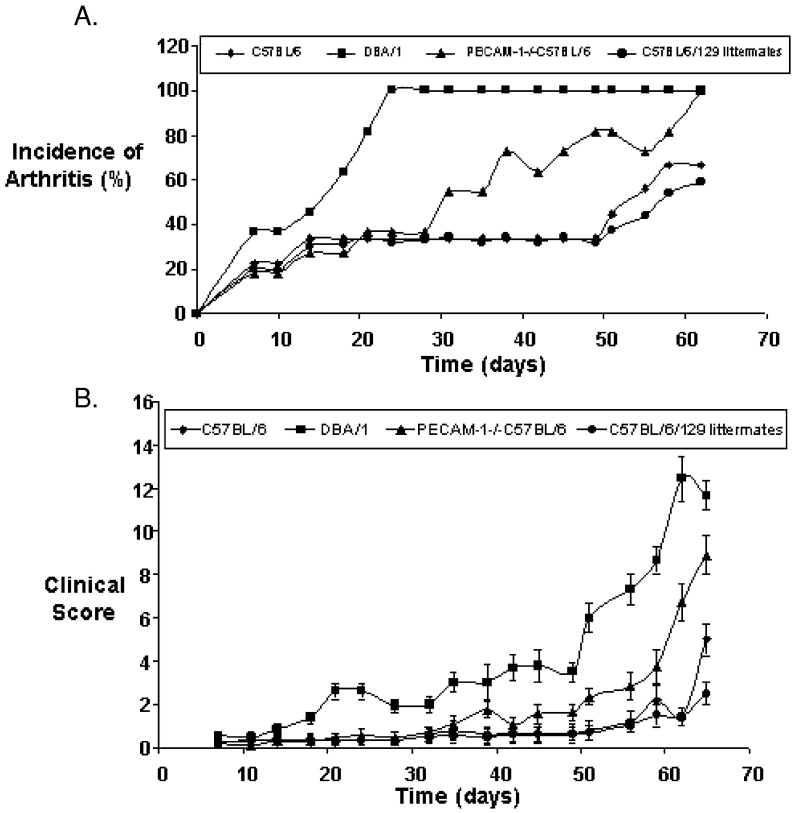

Fig. 1. Incidence and severity of collagen-induced arthritis in PECAM-1$^{-/-}$ mice on a H-2^b background. (A). Incidence of arthritis expressed as a percentage of mice that are assessed for clinical features of arthritis for each group determined on a given day. The figure shows results of three combined experiments performed ($n = 9$ per experiment). (B). Clinical scoring of PECAM-1$^{-/-}$ C57BL/6 H-2^b mice (▲, $n = 9$), BX8 C57BL/6/129 H-2^b littermate mice (●, $n = 9$), wild-type C57BL/6 H-2^b mice (◆, $n = 9$), and DBA/1 H-2^q mice (■, $n = 9$) after immunization with CII according to "Materials and Methods." Results are expressed as a clinical score based upon the mean arthritic score for each group determined on a given day during the course of collagen-induced arthritis.

PECAM-$1^{-/-}$ C57BL/6 mice developed a more accelerated arthritis over time than wild-type C57BL/6 mice and wild-type C57BL/6/129 littermate mice, but not as progressive as DBA/1 mice. By day 49, 82% of PECAM-$1^{-/-}$ C57BL/6 mice had developed arthritis, while 22% of wild-type C57BL/6, 22% of wild-type C57BL/6/129 littermates and 100% of DBA/1 mice had developed arthritis. The mean onset of the disease following CII immunization for PECAM-$1^{-/-}$ C57BL/6 mice was day 32 compared to wild-type C57BL/6 mice at day 51, PECAM-$1^{+/-}$ C57BL/6 littermates at day 53 and DBA/1 mice at day 18. In terms of disease severity, the mean maximal arthritic index for PECAM-$1^{-/-}$ C57BL/6 mice was significantly lower as compared to DBA/1 mice (11.67 ± 0.82 *vs* 8.91 ± 0.91 by Day 65; $P < 0.05$; $n = 9$). This mean maximal arthritic index in PECAM-$1^{-/-}$ C57BL/6 mice was significantly higher than wild-type C57BL/6 mice (5.00 ± 0.73; $P < 0.05$, $n = 9$) and wild-type C57BL/6/129 littermates (2.50 ± 0.34; $P > 0.05$, $n = 9$) (Fig. 1B).

Histopathological Features of CIA in PECAM-$1^{-/-}$ C57BL/6 Mice

The hind paws of CIA-induced wild-type C57BL/6, PECAM-$1^{-/-}$ C57BL/6, and DBA/1 mice were examined visually and histologically at the termination of the experiment by hematoxylin and eosin staining (Fig. 2A–I). PECAM-$1^{-/-}$ C57BL/6 and DBA/1 joints showed arthritic lesions consisting of inflammatory infiltrates of lymphocytic and monocyte/macrophage cells associated with cartilage-bone destruction (Fig. 2D, F, G, I). In contrast, the joints of nonarthritic wild-type C57BL/6 mice appeared histologically normal, with no significant inflammatory infiltrates or cartilage-bone destruction (Fig. 2E, H). Thus, histologically the arthritic lesions of CIA-induced PECAM-$1^{-/-}$ C57BL/6 mice showed similar features to the CIA-susceptible DBA/1 mouse strain.

Cellular and Humoral Immune Responses in CIA-Induced PECAM-$1^{-/-}$ C57BL/6 Mice

Previous studies have demonstrated that PECAM-$1^{-/-}$ mice have higher antibody levels in response to thymus-independent antigens (18). These studies suggested that PECAM-1 may serve a role in modulation of humoral immune responses. In order to investigate differences in the immune responses to collagen type II, we examined the cellular and humoral responses for PECAM-$1^{-/-}$ compared to PECAM-$1^{+/+}$ mice. As shown in Fig. 3, the T-cell proliferative response of popliteal lymph node cells derived from ultrapure, pepsin-free collagen type II primed PECAM-$1^{-/-}$ and PECAM-$1^{+/+}$ mice demonstrated that the PECAM-$1^{-/-}$ T cells had lower proliferative responses. These results suggest that the exaggerated CIA observed in the PECAM-$1^{-/-}$ mice is not explained by hyper-proliferative T-cell responses.

As immunization with CII leads to development of antibodies directed towards conformational CII-specific epitopes that play a major role in the pathogenesis of CIA, we wanted to determine the collagen-specific total IgG, IgG_1, IgG_{2a}, and IgG_{2b} antibody production in sera of PECAM-$1^{-/-}$ C57BL/6 mice compared to wild-type C57BL/6 and DBA/1 immunized mice. As shown in Fig. 4, PECAM-$1^{-/-}$ mice had higher anti-CII antibody titres than those of wild-type C57BL/6 and DBA/1 mice for anti-CII IgG_1 and IgG_{2b} isotypes ($P < 0.05$; $n = 9$). DBA/1 mice showed high anti-CII antibody titres for anti-CII IgG_{2a} compared to wild-type C57BL/6 and PECAM-$1^{-/-}$ C57BL/6 mice (Fig. 4) ($P < 0.01$; $n = 9$) (Fig. 4). Therefore, the enhanced antibody response to anti-CII IgG_1 isotype in the PECAM-$1^{-/-}$ C57BL/6 mice may suggest that PECAM-1 may be regulating Th2-biased help and IL-4 activities that are generally associated with IgG_1 isotype.

Arthritic Development is not Augmented by Transfer of Arthritogenic K/BxN Sera into PECAM-$1^{-/-}$ C57BL/6 Mice

In order to determine if PECAM-1 modulates the early stages of arthritis development or the later stages of progressive disease, we adoptively transferred arthritogenic monoclonal antibodies derived from pooled sera of K/BxN mice into both wild-type and PECAM-$1^{-/-}$ C57BL/6 mice. In these experiments, pooled (K/BxN) and normal C57BL/6 sera were transferred to wild-type and PECAM-$1^{-/-}$ C57BL/6 mice. All mice that received the pooled K/BxN sera initially developed arthritis by day 2 and reached maximum clinical scores by day 10 (Fig. 5). In contrast, both the wild-type and PECAM-$1^{-/-}$ C57BL/6 mice receiving normal C57BL/6 sera did not develop clinical signs of arthritis. Both PECAM-$1^{-/-}$ C57BL/6 and wild-type C57BL/6 mice developed inflammatory arthritis over time with similar kinetics and severity (Fig. 5). Therefore, based upon these results, it would appear that by circumventing the development of an antibody response by adoptive transfer of arthritogenic antibodies (K/BxN model), it is possible to bypass the requirement for PECAM-1 regulation of that response.

DISCUSSION

In this study we have utilized an active CIA mouse model and an adoptive passive K/BxN serum transfer

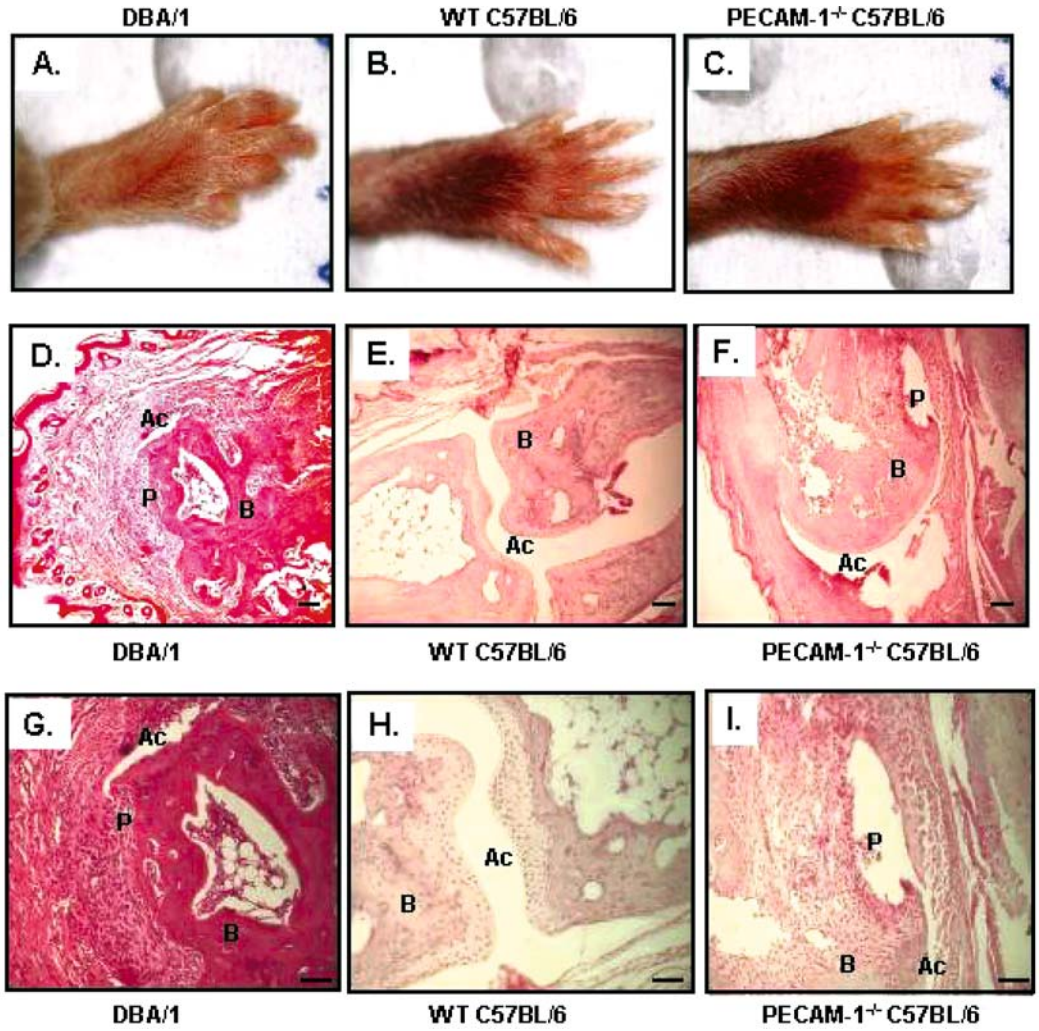

Fig. 2. Clinical and histological features of collagen-induced arthritis in PECAM-$1^{-/-}$ C57BL/6 H-2^b mice. (A–C) The appearance of paws from a typical DBA-1 (A), wild-type C57BL/6 (B), and PECAM-$1^{-/-}$ C57BL/6 (C) mice 60 days after CII immunization. Note that the PECAM-$1^{-/-}$ and DBA/1 paws are arthritic, while the wild-type mouse paw is normal in appearance. (D–F) Histological features of hind paws of DBA/1, wild-type C57BL/6, and PECAM-$1^{-/-}$ C57BL/6 mice 60 days after CII immunization. Severe arthritis is seen in DBA/1 (D) and PECAM-$1^{-/-}$ C57BL/6 (F) mice with inflammatory cellular infiltrate, invasive pannus, and cartilage-bone destruction. Joints of wild-type C57BL/6 (E) mice were normal in appearance with normal synovia and smooth intact cartilage. Representative sagittal paraffin sections with haematoxylin–eosin stain (original magnification, 10 × in D–F; bar = 0.1 mm). (G–I) Inflammatory cellular infiltrates and pannus formation is observed within hind paws of PECAM-$1^{-/-}$ C57BL/6 and DBA/1 mice, but not wild-type C57BL/6 mice. Magnification, 20 × in G–I; bar = 0.1 mm). Ac, articular cartilage, B, bone, P, pannus.

system to dissect the role(s) of PECAM-1 in the initial-stage and end-stage effector mechanisms in inflammatory arthritis. This study demonstrates a critical role of PECAM-1 as a key player in the pathogenesis of CIA. Several important findings have emerged from this study. Firstly, that the deletion of PECAM-1 renders the nonpermissive H-2^b mouse strain susceptible to CIA in an active CIA model. Secondly, by adoptive transfer of arthritogenic antibodies (K/BxN model), it is possible to bypass the requirement for PECAM-1 regulation of the antibody response. Thirdly, the antigen-specific T-cell proliferative response to collagen type II in PECAM-1-deficient H-2^b mice was reduced compared to wild-type H-2^b mice, suggesting that it does not account for the increased susceptibility of PECAM-1-deficient mice on a nonpermissive genetic background.

Using an active CIA model, we demonstrate that PECAM-1-deficient mice on an H-2^b genetic

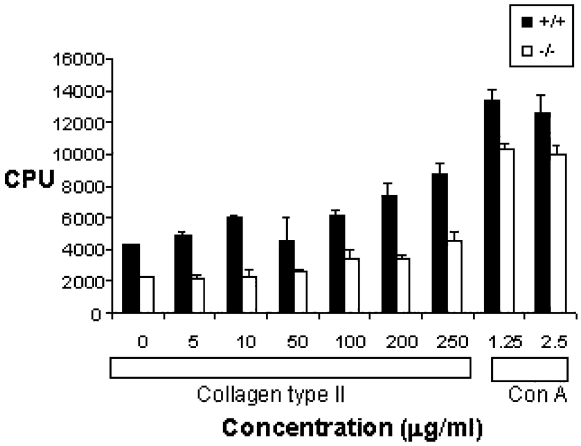

Fig. 3. T-cell proliferation of CII-primed lymph node cells in response to CII immunization. Popliteal draining lymph node cells from bovine collagen type II primed PECAM-$1^{+/+}$ (black bars) and PECAM-$1^{-/-}$ (white bars) ($n = 8$) were stimulated *in vitro* with different doses of heat denaturated collagen type II (0-250 μg/mL). ConA (1.25 and 2.5 μg/mL) was included as a positive control. Proliferation responses were determined after 6 h of culturing with [^{3}H]-TdR. These results are representative of three separate experiments.

background develop a progressive erosive arthritis and an increased incidence of disease compared to wild-type mice on a similar genetic background (Fig. 1). The histological characteristics of the arthritic paws of PECAM-1-deficient mice are similar to those observed in CIA-susceptible DBA/1 mice (H-2^q) (Fig. 2). CIA-induced PECAM-1-deficient mice also showed elevated levels of anti-CII IgG_1 and IgG_{2b} production (Fig. 4). In contrast, using the K/BxN model, the passively transferred antibodies induced erosive arthritis in wild-type and PECAM-1-deficient mice with similar kinetics and severity (Fig. 5). These results are consistent with the results obtained with FcγRIIb$^{-/-}$ mice, where deletion of FcγRIIb lead to an earlier onset and exaggerated CIA in susceptible mouse strains and permitted induction of CIA in resistant H-2^b mouse strain (11, 16). The fact that FcγRIIb-deficient mice are more susceptible to CIA induction upon immunization with type II collagen may reflect a failure to maintain peripheral tolerance to potentially cross-reactive autoantigens (30). This hypothesis is supported by recent studies that suggest that autoreactive B-cell clones

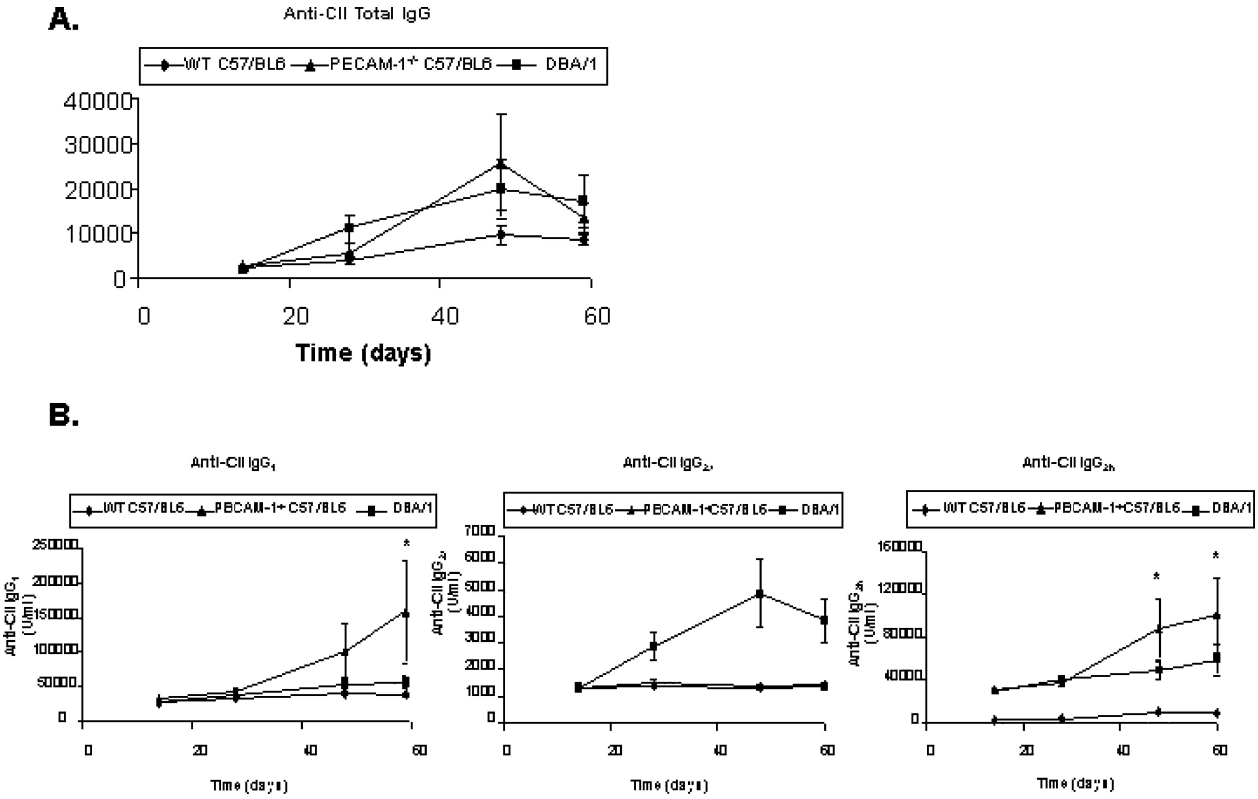

Fig. 4. Anti-CII IgG antibody levels in arthritic PECAM-$1^{-/-}$ C57BL/6 mice after CII immunization. Circulating anti-CII IgG total (A) and specific isotypes (B) were determined at day 14, 28, 48, and 59 after CII immunization in individual sera of nonarthritic wild-type C57BL/6, arthritic PECAM-$1^{-/-}$ C57BL/6, and arthritic DBA/1 mice. The mean ± standard deviation of antibody levels of total IgG and isotype-specific IgG anti-CII are shown. $^*P < 0.05$ compared with the wild-type group ($n = 9$).

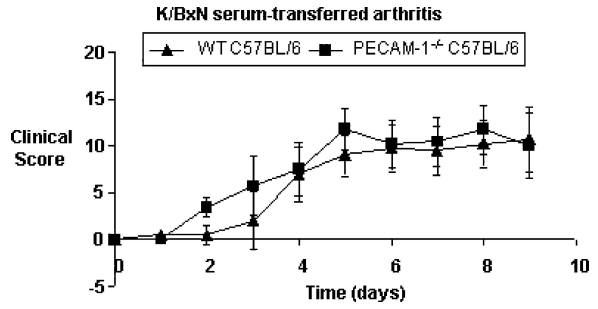

Fig. 5. Passive-transferred arthritis in wild-type versus PECAM-$1^{-/-}$ mice. (A) Arthritogenic pooled K/BxN sera was transferred into 6-week-old wild-type C57BL/6 and PECAM-$1^{-/-}$ C57BL/6 mice. Clinical scoring was recorded for both wild-type C57BL/6 (▲) and PECAM-$1^{-/-}$ C57BL/6 (■) mice over a two-week period ($n = 6$). Results are expressed as the mean clinical score (mean ± SD) for each group on a given day following induction of K/BxN serum-transferred arthritis. Results represent four combined experiments ($n = 6$ per group).

may be the important perpetrators in the pathogenesis of RA (31–33). Alternatively, the increased susceptibility of FcγRIIb-deficient mice to CIA induction could be due to lack of regulation of antibody effectors. CIA requires a functional B-cell response and the development of pathogenic anti-CII antibodies directed to target arthritogenic epitopes on native helical collagen (2). Therefore, based upon our study of active and passive transfer models of CIA using PECAM-1-deficient mice, it would appear that PECAM-1, like FcγRIIb, controls the development of arthritis by regulating antibody production.

The aetiology and pathogenesis of RA is still under considerable debate. Various studies have demonstrated that RA is a multifaceted disease involving autoimmune targets, different pathogenic mechanisms and a chronic inflammatory response. Multiple effector cells, cell surface receptors, and inflammatory mediators have been shown to be important players in modulation of the initial-stage and end-stage effector mechanisms of the disease process. These include B cells, T cells, neutrophils, macrophages and mast cells, complement factors (C5a, C5aR), inflammatory cytokines (IL-1β and TNF-α), Fc receptors (FcγRI, FcγRIII, FcγRIIa, FcγRIIb), Fcγ common chain and immune complex deposition (GPI–anti-GPI complexes, pathogenic immunoglobulins) in the joints (13, 14, 32–34).

Apart from PECAM-1's role in regulating immunological responsiveness, it also has a defined role in chronic inflammation. PECAM-1 has been previously shown to be an important mediator of transendothelial migration of leukocytes, neutrophil recruitment *in vivo*, and neutrophil and monocyte chemotaxis (35–38). These earlier studies utilized antibody blocking experiments and soluble recombinant PECAM–Ig chimeric molecules to implicate PECAM-1 in regulating leukocyte transmigration and neutrophil recruitment *in vivo* (39–41). The subsequent generation of the PECAM-1 knockout mouse revealed that its function was not essential during development and in acute transient inflammation (23). However, it was shown to be important in attenuating the chronic process of foreign body inflammation, transbasement membrane migration of leukocytes, and vascular permeability (23, 41–43).

Recent studies have demonstrated that PECAM-1 can negatively regulate ITAM-associated signaling pathways involving Fcγ common chain and FcγRIIa (44–46). Fc receptors are known to play an important role in the initiation phase of the pathogenesis of chronic inflammatory processes and are attractive targets for biological therapies for RA (11, 14). In this study, we demonstrate that CIA-induced PECAM-1-deficient $H\text{-}2^b$ mice have elevated anti-CII IgG isotype levels, primarily involving IgG_1 and to a lesser extent, IgG_{2b} antibodies in arthritic animals (Fig. 4). While elevated IgG_{2a} responses are normally associated with CIA, our findings of elevated IgG_1 and IgG_{2b} in CIA-induced PECAM-$1^{-/-}$ mice are consistent with deletion of an inhibitory co-receptor as observed in CIA-induced FcγRIIb-deficient mice (16). Previous studies have demonstrated that multimerized IgG_1 is sufficient at activating the FcγIII receptor in the effector pathway of arthritis (47). As the IgG_1 isotype is associated with Th2 promoting regimens, it would appear that PECAM-1 has a potential role in regulating Th2 biased help and IL-4 activities in RA. Future studies will be required to clarify this issue.

During submission of our manuscript, other workers reported similar findings that deletion of PECAM-1 on a CIA susceptible $H\text{-}2^q$ DBA/1 background accelerates the onset of CIA (48). The main difference in our studies is that we examined PECAM-$1^{-/-}$ mice on a nonsusceptible $H\text{-}2^b$ C57BL/6 genetic background. As PECAM-1 is widely expressed and upregulated in RA, it is likely to play an important role in regulating chronic inflammation throughout the body. Indeed, it would appear that in RA, PECAM-1 may prove to be an attractive target for therapy as it has a protective role in the suppression of the autoimmune process and modulation of the chronic inflammatory response. Therefore, future treatment strategies may exploit the ligand and inhibitory signaling properties of PECAM-1 in chronic inflammatory states to treat patients with established RA.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Patricia Mottram for her critical review of the manuscript and collection of K/BxN sera. We thank Drs. Tak Mak and Gordon

Duncan for providing the PECAM-1-deficient mice. This work was supported by Grant No. 250399 (to D.E.J.) from the National Health and Medical Research Council of Australia. She is the recipient of an NHMRC Senior Research Fellowship. M.-X. Wong received an NHMRC Dora Lush PhD Scholarship.

REFERENCES

1. Van den Berg WB: Lessons from animal models of arthritis. Curr Rheumatol Rep 4(3):232–239, 2002
2. Myers LK, Rosloniec EF, Cremer MA, Kang AH: Collagen-induced arthritis, an animal model of autoimmunity. Life Sci 61(19):1861–1878, 1997
3. Cremer MA, Rosloniec EF, Kang AH: The cartilage collagens: A review of their structure, organization, and role in the pathogenesis of experimental arthritis in animals and in human rheumatic disease. J Mol Med 76:275–288, 1998
4. Lindqvist AKB, Bockermann R, Johansson ACM, Nandakumar KS, Johannesson M, Holmdahl R: Mouse models of rheumatoid arthritis. Trends Genet. 18:S7–S13, 2002
5. Zvaifler NJ, Firestein GS: Pannus and pannocytes. Alternative models of joint destruction in rheumatoid arthritis. Arthritis Rheum 37(6):783–789, 1994
6. Ji H, Pettit A, Ohmura K, Ortiz-Lopez A, Duchatelle V, Degott C, Gravallese E, *et al*.: Critical roles for Interleukin 1 and tumour necrosis factor α in antibody-induced arthritis. J Exp Med 196(1):77–85, 2002
7. Maccioni M, Zeder-Lutz G, Huang H, Ebel C, Gerber P, Hergueux J, *et al*.: Arthritogenic monoclonal antibodies from K/BxN mice. J Exp Med 195(8):1071–1077, 2002
8. Kaplan CD, O'Neill SK, Koreny T, Czipri M, Finnegan A: Development of inflammation in proteoglycan-induced arthritis is dependent on FcγR regulation of the cytokine/chemokine environment. J Immunol 169:5851–5859, 2002
9. Matsumoto I, Lee DM, Goldbach-Mansky R, Sumida T, Hitchon CA, Schur PH, Anderson RJ, *et al*.: Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and a spectrum of other chronic autoimmune disorders. Arthritis Rheum 48(4):944–954, 2003
10. Cook AD, Rowley MJ, Mackay IR, Gough A, Emery P: Antibodies to type II collagen in early rheumatoid arthritis. Correlation with disease progression. Arthritis Rheum 39(10):1720–1727, 1996
11. Kleinau S, Martinsson P, Heyman B: Induction and suppression of collagen-induced arthritis is dependent on distinct Fcγ receptors. J Exp Med 191(9):1611–1616, 2000
12. Diaz de Stahl T, Andren M, Martinsson P, Verbeek JS, Kleinau S: Expression of FcγRIII is required for development of collagen-induced arthritis. Eur J Immunol 32:2915–2922, 2002
13. Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, *et al*.: Arthritis critically dependent on innate immune system players. Immunity 16:157–168, 2002
14. Corr M, Crain B: The role of FcγR signalling in the K/BxN serum transfer model of arthritis. J Immunol 169:6604–6609, 2002
15. Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV: Augmented humoral and anaphylactic responses in FcγRII-deficient mice. Nature 379:346–349, 1996
16. Yuasa T, Kubo S, Yoshino T, Ujike A, Matsumura K, Ono M, *et al*.: Deletion of Fcγ receptor IIb renders H-2^b mice susceptible to collagen-induced arthritis. J Exp Med 189(1):187–194, 1999
17. Jackson DE: The unfolding tale of PECAM-FEBS Lett 540:7–14, 2003
18. Wilkinson R, Lyons AB, Roberts D, Wong MX, Bartley PA, Jackson DE: Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) acts as a regulator of B-cell development, B-cell antigen receptor (BCR)-mediated activation, and autoimmune disease. Blood 100(1):184–193, 2002
19. Scekanecs Z, Haines GK, Harlow LA, Shah MR, Fong TW, Fu R, *et al*.: Increased synovial expression of the adhesion molecules CD66a, CD66b, and CD31 in rheumatoid and osteoarthritis. Clin Immunol Immunopathol 76:180–186, 1995
20. Volin MV, Szekanecz Z, Halloran MM, Woods JM, Magua J, Damergis JA, *et al*.: PECAM-1 and Leukosialin (CD43) expression correlate with heightened inflammation in rat adjuvant-induced arthritis. Exp Mol Pathol 66(3):211–219, 1999
21. Decking J, Mayer A, Petrow P, Seiffge D, Karbowski A: Antibodies to PECAM-1 and glucocorticoids reduce leukocyte adhesion in adjuvant arthritis of the rat knee synovium *in vivo*. Inflamm Res 50(12):609–615, 2001
22. Ishikaw J, Okada Y, Bird IN, Jasani B, Spragg JH, Yamada T: 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. Jpn J Pharmacol 88:332–340, 2002
23. Duncan GS, Andrew DP, Takimoto H, Kaufman SA, Yoshida H, Spellberg J, *et al*.: 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(5):3022–3030, 1999
24. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D: Organ-specific disease provoked by systemic autoimmunity. Cell 87:811–822, 1996
25. Campbell IK, Hamilton JA, Wicks IP: Collagen-induced arthritis in C57BL/6 (H-2b) mice: New insights into an important disease model of rheumatoid arthritis. Eur J Immunol 30:1568–1575, 2000
26. Wooley PH, Luthra HS, Stuart JM, David SC: Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I-region) linkage and antibody correlates. J Exp Med 154:688–700, 1981
27. Wooley PH, Luthra HS, Griffiths MM, Stuart JM, Huse A, David SC: Type II collagen-induced arthritis in mice. IV. Variations in immunogenetic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule. J Immunol 135:2443–2451, 1985
28. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B: Immunization against heterologous type II collagen induces arthritis in mice. Nature 283:666–668, 1980
29. Seki N, Sudo Y, Yoshioka T, Sugihara S, Fujitsu T, Sakuma S, *et al*.: Type II collagen-induced murine arthritis. Induction and perpetuation of arthritis require synergy between humoral and cell-mediated immunity. J Immunol 140:1477–1484, 1988
30. Ravetch JV, Lanier LL: Immune inhibitory receptors. Science 290(5489):84–89, 2000
31. Benoist C, Mathis D: A revival of the B cell paradigm for rheumatoid arthritis pathogenesis? Arthritis Res 2:90–94, 2000
32. Hirano T: Revival of the autoantibody model in rheumatoid arthritis. Nature Immunol 3(4):342–344, 2002
33. Galloway TS, Ray K, Malhotra R: Regulation of B lymphocytes in health and disease. Meeting review. Mol Immunol 39:649–653, 2003
34. Hogarth PM: Fc receptors are major mediators of antibody based inflammation in autoimmunity. Curr Opin Immunol 14(6):798–802, 2002

35. Muller WA, Weigl SA, Deng X, Phillips DM: PECAM-1 is required for transendothelial migration of leukocytes. J Exp Med 178:449–460, 1993
36. Vaporciyan AA, DeLisser HM, Yan HC, Mendiguren II, Thom SR, Jones ML, *et al.*: Involvement of Platelet Endothelial Cell Adhesion Molecule-1 in neutrophil recruitment *in vivo*. Science 262:1580–1582, 1993
37. Bogen S, Pak J, Garifallou M, Deng X, Muller WA: Monoclonal antibody to murine PECAM-1 blocks acute inflammation *in vivo*. J Exp Med 79:1059–1064, 1994
38. Ohto H, Maeda H, Shibata Y, Chen RF, Ozaki Y, Higashihara M, *et al.*: A novel leukocyte differentiation antigen: Two monoclonal antibodies TM2 and TM3 define a 120-kd molecule present on neutrophils, monocytes, platelets, and activated lymphoblasts. Blood 66(4):873–881, 1985
39. Laio F, Ali J, Greene T, Muller WA: Soluble domain 1 of platelet-endothelial cell–adhesion molecule (PECAM) is sufficient to block transendothelial migration *in vitro* and *in vivo*. J Exp Med 185(7):1349–1357, 1997
40. Laio F, Schenkel AR, Muller WA: Transgenic mice expressing different levels of soluble platelet/endothelial cell adhesion molecule-IgG display distinct inflammatory phenotypes. J Immunol 163(10):5640–5648, 1999
41. Nakada MT, Amin K, Christofidou-Solomidou M, O'Brien CD, Sun J, Gurubhagavatula I, *et al.*: 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(1):452–462, 2000
42. Graesser D, Solowiej A, Bruckner M, Osterweil E, Juedes A, Davis S, *et al.*: Altered vascular permeability and early onset of experimental autoimmune encephalomyelitis in PECAM-1-deficient mice. J Clin Invest 109(3):383–392, 2002
43. Solowiej A, Biswas P, Graesser D, Madri JA: Lack of platelet endothelial cell adhesion molecule-1 attenuates foreign body inflammation because of decreased angiogenesis. Am J Pathol 162(3):953–962, 2003
44. Jones KL, Hughan SC, Dopheide SM, Farndale RW, Jackson SP, Jackson DE: Platelet endothelial cell adhesion molecule-1 is a negative regulator of platelet–collagen interactions. Blood 98:1456–1463, 2001
45. Patil S, Newman DK, Newman PJ: Platelet endothelial cell adhesion molecule-1 serves as an inhibitory receptor that modulates platelet responses to collagen. Blood 97:1727–1732, 2001
46. Thai LM, Ashman LK, Harbour SN, Hogarth PM, Jackson DE: Physical proximity and functional interplay of PECAM-1 with the Fc receptor FcγRIIa on the platelet plasma membrane. Blood 102:3637–3745, 2003
47. Ji H, Gauguier D, Ohmura K, Gonzalez A, Duchatelle V, Danoy P, *et al.*: Genetic influences on the end-stage effector phase of arthritis. J Exp Med 194:321–330, 2001
48. Tada Y, Koarada S, Morito F, Ushiyama O, Haruta Y, Kanegae F, *et al.*: Acceleration of the onset of collagen-induced arthritis by a deficiency of platelet endothelial cell adhesion molecule-1. Arthritis Rheum 48(11):3280–3290, 2003