Expression of L-Selectin, but Not CD44, Is Required for Early Neutrophil Extravasation in Antigen-Induced Arthritis

Sándor Szántó, István Gál, Andrea Gonda, Tibor T. Glant and Katalin Mikecz

*J Immunol* 2004; 172:6723-6734; doi: 10.4049/jimmunol.172.11.6723
http://www.jimmunol.org/content/172/11/6723

References

This article cites 83 articles, 38 of which you can access for free at:
http://www.jimmunol.org/content/172/11/6723.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Expression of L-Selectin, but Not CD44, Is Required for Early Neutrophil Extravasation in Antigen-Induced Arthritis

Sándor Szántó, István Gál, Andrea Gonda, Tibor T. Glant, and Katalin Mikecz

L (leukocyte)-selectin (CD62L) and CD44 are major adhesion receptors that support the rolling of leukocytes on endothelium, the first step of leukocyte entry into inflamed tissue. The specific contribution of L-selectin or CD44 to the regulation of cell traffic to joints in arthritis has not been investigated. We used CD44-deficient, L-selectin-deficient, and CD44/L-selectin double knockout mice to determine the requirement for these receptors for inflammatory cell recruitment during Ag-induced arthritis. Intraperitoneal immunization resulted in similar activation status and Ag-specific responses in wild-type and gene-targeted mice. However, extravasation of neutrophil granulocytes, but not the emigration of T cells, into the knee joints after intra-articular Ag injection was significantly delayed in L-selectin-deficient and double knockout mice. Intravital videomicroscopy on the synovial microcirculation revealed enhanced leukocyte rolling and diminished adherence in mice lacking either CD44 or L-selectin, but CD44 deficiency had no significant effect on the recruitment of L-selectin-null cells. Compared with wild-type leukocytes, expression of L-selectin was down-regulated in CD44-deficient cells in the spleen, peripheral blood, and inflamed joints, suggesting that reduced expression of L-selectin, rather than the lack of CD44, could be responsible for the delayed influx of granulocytes into the joints of CD44-deficient mice. In conclusion, there is a greater requirement for L-selectin than for CD44 for neutrophil extravasation during the early phase of Ag-induced arthritis.

Extravasation of leukocytes during inflammation involves distinct steps of interaction with the endothelium of postcapillary venules (1). The interaction begins with the capture and tethering of the leukocyte to the endothelium, followed by rolling under blood flow. Rolling cells are exposed to locally produced chemokines that enhance the avidity of interaction, leading to leukocyte arrest and firm adhesion. Adherent cells detach and, ultimately, migrate across the vessel wall (1–3). Leukocytes that migrate to inflamed tissue and those that migrate (home) to lymphoid organs follow a similar sequence of adhesion steps.

Leukocyte capture and rolling are mediated via receptor-ligand interactions between the members of the selectin family and carbohydrate moieties on glycoproteins (4). L (leukocyte)-selectin (CD62L), which is constitutively expressed on the surface of leukocytes, supports lymphocyte rolling in peripheral lymph nodes (PLNs) (5) and neutrophil rolling in inflamed extralymphoid tissue (6). E- and P-selectins are expressed by endothelial cells under inflammatory stimuli (reviewed in Refs. 7 and 8). P-selectin glycoprotein ligand-1 (PSGL-1), the major leukocyte ligand for P-selectin, can mediate cell rolling on inflamed endothelium (4, 9). Exposure of rolling cells to proinflammatory or “homing” chemokines, via interaction with chemokine receptors and subsequent signaling, can result in the activation of β2 (e.g., very late activation Ag-4 (VLA-4)) or β3 (Mac-1 or LFA-1) integrins on leukocytes (10). Binding of leukocyte integrins to counterreceptors on endothelium (e.g., VCAM-1 or ICAM-1) leads to deceleration, arrest, and firm adhesion (3, 11, 12). Integrins are unable to establish an adhesion interaction between the leukocyte and endothelium under flow unless the cells have rolled first (2), with the exception of VLA-4, which is capable of initiating a rolling interaction (13).

CD44 is a transmembrane glycoprotein that has been shown to mediate lymphocyte rolling along the vessel wall by recognizing hyaluronan on the surface of activated endothelium (14, 15). CD44 is expressed by a wide variety of cell types, including peripheral blood leukocytes and endothelial cells. Both the cell surface density and the ligand (hyaluronan) binding capacity of CD44 are enhanced upon exposure of cells to proinflammatory stimuli (14, 16–19).

The requirement for L-selectin or CD44 for inflammatory responses has also been addressed by a number of studies using gene knockout (KO) mice. Targeted mutation of L-selectin had a negative impact on acute inflammatory reactions, including contact dermatitis (30), thioglycollate-induced peritonitis, and allograft rejection (31). Neutrophil recruitment in the postcapillary venules of inflamed cremaster muscle was severely impaired in L-selectin KO mice (6). Remarkably, T cell-mediated inflammation such as the delayed-type hypersensitivity reaction (32) or EAE (33) could not
be induced in L-selectin-deficient mice. Because expression of L-selectin in naive T cells is required for homing to PLNs and subsequent activation by Ag delivered to these lymph nodes, the failure of L-selectin mutant mice to develop inflammation could be due to insufficient T cell memory (32, 34).

Targeted disruption of the CD44 gene increased the resistance of DBA/1 mice to collagen-induced arthritis (35). In contrast, CD44 deficiency led to enhanced lung injury in bleomycin- and Escherichia coli-induced, but not in Streptococcus pneumoniae-induced, pneumonia in mice (36, 37). Although these observations suggest distinct roles for L-selectin and CD44 in various inflammatory conditions, they also reveal a considerable disparity between the observations made in Ab-treated and gene-targeted mice.

Ag-induced arthritis (AIA) can be induced in mice by s.c. or intradermal immunization with methylated BSA (mBSA) followed by intra-articular (i.a.) injection of the same Ag into the knee joint (38–41). AIA shows similarities with rheumatoid arthritis (RA), including the involvement of the adaptive immune system in the initiation of the disease, infiltration of the synovium by inflammatory leukocytes, synovial hyperplasia, and cartilage erosion (40, 41). Unlike the systemic autoimmune forms of murine arthritis, AIA has a well-defined onset, i.e., it starts after i.a. injection of the Ag. The mouse knee joint is accessible to intravital microscopy (42); therefore, this model offers an opportunity to study the kinetics of leukocyte-endothelial cell interactions in arthritic joints from the first hours of inflammation. Intravital videomicroscopy has not been performed on CD44-deficient mice and, to date, immune-mediated arthritis has not been induced in mice lacking L-selectin.

Using mice deficient in CD44, L-selectin, or both, we tested the hypothesis that CD44 and L-selectin are required for the recruitment and subsequent extravasation of inflammatory leukocytes into the joints during the course of AIA. We found that during the initial phase of arthritis, expression of L-selectin, but not of CD44, was necessary for the entry of neutrophil granulocytes into the joints.

Materials and Methods

Mice deficient in CD44, L-selectin, or CD44/L-selectin (double KO)

Mice deficient in CD44 were generated by targeted gene disruption as previously described (35), and L-selectin (CD62L)-deficient mice (43) were purchased from The Jackson Laboratory (Bar Harbor, ME). Both gene-deficient lines were backcrossed into BALB/c background for six generations using a genomic marker-assisted speed-congenic approach described elsewhere (44, 45). Wild-type (WT) BALB/c mice for backcross breeding were purchased from the National Cancer Institute (Frederick, MD). CD44 KO and L-selectin KO BALB/c mice were intercrossed to generate mice lacking both CD44 and L-selectin (double KO). All mice were genotyped by PCR using primer pairs specific for the cd44 and cd62l genes, as well as for the neo- (neo) and puromycin (puro) resistance genes present in the targeting constructs of CD44 (35) and cd62l (43) loci, respectively. Experiments were conducted on WT (cd44+/−/cd62l+/−), CD44 KO (cd44−/−/cd62l+/−), L-selectin KO (cd44+/−/cd62l−/−), and double KO (cd44−/−/cd62l−/−) littermates that shared the same genetic background except for the targeted loci. Because the gene-deficient mice were healthy, showing no evidence of increased susceptibility to infections, they were maintained under standard conditions.

Immunization of mice with mBSA, induction of AIA, and clinical and histopathological assessments of arthritis

All protocols involving animals were approved by the Institutional Animal Care and Use Committee. Methylated BSA (Sigma-Aldrich, St. Louis, MO) was dissolved in PBS, and the insoluble material was removed by centrifugation. After sterilization by gamma irradiation (20 Gy), the protein concentration in the mBSA solution was determined using the bicinchoninic protein assay (Pierce, Rockford, IL), and then the final concentration was adjusted to 1–5 mg/ml with sterile PBS. WT, CD44 KO, L-selectin KO, and CD44/L-selectin double KO mice received a total of 100 μg of mBSA emulsified in 100 μl of CFA (Difco, Detroit, MI) injected s.c. into the flanks and intradermally into the proximal tail. Alternatively, separate groups of mice were injected with the same amounts of mBSA in adjuvant i.p. Animals were boosted with the same doses of Ag and adjuvant injected into the corresponding sites 2 wk later. Two to three weeks after the boost, mice were anesthetized and injected with 30 μg of mBSA in 6 μl of PBS into the right knee and with 6 μl of PBS (without Ag) into the left knee (40, 41). The animals were monitored for joint swelling at defined time points between 4 h and 5 days postinjection by measuring the knee joint diameter (at 0.05-mm precision) with a constant-tension microcaliper (Bel-Art Products, Pequannock, NJ). Mice were used for various experiments at 4 h, 12 h, 1 day, or 5 days after the i.a. injections. For histopathology, the knees were dissected, fixed in formalin, decalcified, embedded in paraffin, sectioned, and stained with H&E.

Ag-specific immune responses

For lymphocyte stimulation assays, spleens were harvested 5 days after i.a. injection of mBSA. Spleen cells (3 × 10^5/well) were cultured in the presence or absence of 50 μg/ml BSA in 96-well plates in serum-free HL-1 medium (BioWhittaker, Walkersville, MD). Proliferation was determined by incorporation of [3 H]thymidine (PerkinElmer, Boston, MA). Expression of the early activation marker CD69 in lymphocytes was examined using two-color fluorescence labeling (anti-CD3-PE with anti-CD69-FITC for T cells and biotinylated anti-CD19 and streptavidin-PE with anti-CD69-FITC for B cells) as described previously (47). In brief, increasing dilutions of serum (1/50–1/12,500) were incubated with the immobilized Ag, and BSA-bound Abs were detected with peroxidase-conjugated rabbit anti-mouse IgG (Zymed, San Francisco, CA). Purified mouse IgG (Sigma-Aldrich), coated onto wells at increasing concentrations, served as a reference standard.

Flow cytometry

Abs for cell labeling were purchased from BD Pharmingen (San Diego, CA), except for the anti-CD4 mAb IRAWB14 (48, 49). Expression of CD44 and L-selectin was detected using mAbs against mouse CD62L (clone MEL-14) and CD44 (clone IRAWB14), respectively. Spleen cells, harvested 1 day after induction of AIA, were incubated with biotinylated MEL-14 and IRAWB14, followed by staining with PE-conjugated streptavidin (Molecular Probes, Eugene, OR). Expression of the early activation marker CD69 in lymphocytes was examined using two-color fluorescence labeling (anti-CD3-PE with anti-CD69-FITC for T cells and biotinylated anti-CD19 and streptavidin-PE with anti-CD69-FITC for B cells) as described previously (35). Mouse CD69 was expressed by cultured T cells, were harvested 1 day after i.a. injection of mBSA. After RBC lysis, leukocytes were suspended in Ca^2+–Mg^2+–free HBSS (Invitrogen, Carlsbad, CA), aliquoted, and subjected to fluorescence labeling. Granulocyte-specific expression of adhesion molecules, including L-selectin, Mac-1 (CD11b, e− integrin β2-chain; clone M1/70), LFA-1 (CD18, β2 integrin β2-chain; clone 7/116), VLA-4 (CD49d/Cd29, α4/β1 integrin chains; clones 9C10 and Ha25, respectively), and PSLG-1 (CD162, clone 2PH1), in peripheral blood cells was determined by two-color fluorescence labeling (blood pooled from two to three mice was used for each series), during which the cells were coincubated with biotinylated or PE-conjugated anti-Gr-1 mAb and either a biotinylated or PE-conjugated adhesion molecule-specific mAb. Binding of biotin-labeled Abs was visualized using AlexaFluor488-conjugated streptavidin (Molecular Probes). To compare expression levels of L-selectin and Mac-1 on circulating and inflammatory leukocytes, inflammatory cells were also harvested from the knees of mice bled at 24 h after i.a. Ag injection. Inflammatory cells were subjected to immunolabeling simultaneously with the blood leukocytes. One- and two-color flow cytometry was performed using a FACScan instrument and CellQuest analysis software (BD Biosciences, San Jose, CA).
Intravital videomicroscopy on the synovial microcirculation of the knee

The synovium of the mouse knee joint was accessed through an anterior opening, as described by Veihelmann et al. (42). The intravital microscopy procedure described here, however, used a substantially modified technical approach and a video recording system different from that used by Veihelmann et al. In our protocol, the mouse was anesthetized by i.m. administration of a ketamine/xylazine mixture and was placed on its back on a glass plate. To prevent limb movement during surgery or microscopy, the knee was held between two laterally positioned pieces of rubber, glued to the glass plate. After excision of the shaved skin above the joint, the patellar ligament (tendon) was transected below the patella using a sterile no.11 surgical blade. The ligament was lifted to expose the i.a. fatty synovial tissue (Fig. 1A). Because there is no direct vascular connection between the patellar tendon and the underlying soft tissue (Fig. 1C and Ref. 50), the tendon can be separated from the i.a. tissue without injury to the blood vessels. The mouse on the glass plate was then transferred to the stage of the intravital microscope (Eclipse E600FN; Nikon, Garden City, NJ). The synovial tissue of the open joint was continuously superfused with sterile warm (37°C) PBS through a fine silicon tube attached to the objective lens (Fig. 1B). This constant flow of warm PBS maintained physiological temperature, prevented tissue drying, and provided a liquid bridge between the water immersion lens (×40 magnification) and the tissue. A drop of blood was drawn from the retroorbital vein (to determine systemic leukocyte counts and differentials), followed by i.v. injection of rhodamine 6G (Molecular Probes) at a dose of 3 mg/kg body weight. Rhodamine 6G is a cell-permeable red fluorescent dye that rapidly stains nuclear and mitochondrial DNA in circulating leukocytes after i.v. administration (51).

Real-time recording and analysis of leukocyte-endothelial interactions

Leukocyte movement in postcapillary venules of the synovium (Fig. 1D) was recorded for 1 min by streamline acquisition of images with a digital camera (CoolSnap; RS Photometrics, Trenton, NJ) attached to the intravital microscope. Videorecording was performed on at least two postcapillary venules (ranging between 20 and 50 μm in diameter, as measured with an on-screen caliper) within each joint. Upon playback of the video, results were analyzed offline using the Metavue image analysis program (Universal Imaging, West Chester, PA). The frequency of rolling interactions (number of rolling cells/min) was determined by counting the fluorescent cells that rolled past a line perpendicular to the vessel axis at a speed much less than the velocity of freely flowing cells in the centerline (52, 53). Results were expressed as the frequency of rolling cells/min/mm vessel perimeter (2πr). Firm adherent cells (immobile for at least 30 s) were counted along a 100-μm (0.1-mm)-long segment of each venule and were expressed as adherent cells/mm² of vessel surface (2πr). The results were normalized to systemic leukocyte counts, determined in the blood samples taken before videomicroscopy. The velocity of rolling cells (20 cells per knee joint) was also measured and expressed in μm/s. After intravital videomicroscopy, the examined knee joints were processed for histological evaluation of leukocyte influx into the extravascular space. Leukocytes in peripheral blood samples were counted in a hemocytometer after nuclear staining with Turk’s solution (crystal violet in diluted acetic acid), and the percentages of mononuclear and polymorphonuclear cells (differentials) in the same samples were determined after staining of blood smears with Giemsa.

Statistical analysis

Data were analyzed using SigmaStat software (SPSS, Chicago, IL). The nonparametric Mann-Whitney rank sum test was used to determine significant differences (p < 0.05) between the WT and KO groups of mice.

Results

Cell surface expression of CD44 and L-selectin and immune responses in mBSA-immunized WT, CD44 KO, L-selectin KO, and CD44/L-selectin double KO mice

The phenotype of gene-targeted mice was confirmed by flow cytometry after immunostaining of spleen cells with mAbs against CD44 or L-selectin (Fig. 2). As expected, CD44-deficient, L-selectin-deficient, and Ref. 50, the tendon can be separated from the i.a. tissue without injury to the blood vessels. The mouse on the glass plate was then transferred to the stage of the intravital microscope (Eclipse E600FN; Nikon, Garden City, NJ). The synovial tissue of the open joint was continuously superfused with sterile warm (37°C) PBS through a fine silicon tube attached to the objective lens (Fig. 1B). This constant flow of warm PBS maintained physiological temperature, prevented tissue drying, and provided a liquid bridge between the water immersion lens (×40 magnification) and the tissue. A drop of blood was drawn from the retroorbital vein (to determine systemic leukocyte counts and differentials), followed by i.v. injection of rhodamine 6G (Molecular Probes) at a dose of 3 mg/kg body weight. Rhodamine 6G is a cell-permeable red fluorescent dye that rapidly stains nuclear and mitochondrial DNA in circulating leukocytes after i.v. administration (51).

Real-time recording and analysis of leukocyte-endothelial interactions

Leukocyte movement in postcapillary venules of the synovium (Fig. 1D) was recorded for 1 min by streamline acquisition of images with a digital camera (CoolSnap; RS Photometrics, Trenton, NJ) attached to the intravital microscope. Videorecording was performed on at least two postcapillary venules (ranging between 20 and 50 μm in diameter, as measured with an on-screen caliper) within each joint. Upon playback of the video, results were analyzed offline using the Metavue image analysis program (Universal Imaging, West Chester, PA). The frequency of rolling interactions (number of rolling cells/min) was determined by counting the fluorescent cells that rolled past a line perpendicular to the vessel axis at a speed much less than the velocity of freely flowing cells in the centerline (52, 53). Results were expressed as the frequency of rolling cells/min/mm vessel perimeter (2πr). Firm adherent cells (immobile for at least 30 s) were counted along a 100-μm (0.1-mm)-long segment of each venule and were expressed as adherent cells/mm² of vessel surface (2πr). The results were normalized to systemic leukocyte counts, determined in the blood samples taken before videomicroscopy. The velocity of rolling cells (20 cells per knee joint) was also measured and expressed in μm/s. After intravital videomicroscopy, the examined knee joints were processed for histological evaluation of leukocyte influx into the extravascular space. Leukocytes in peripheral blood samples were counted in a hemocytometer after nuclear staining with Turk’s solution (crystal violet in diluted acetic acid), and the percentages of mononuclear and polymorphonuclear cells (differentials) in the same samples were determined after staining of blood smears with Giemsa.

Statistical analysis

Data were analyzed using SigmaStat software (SPSS, Chicago, IL). The nonparametric Mann-Whitney rank sum test was used to determine significant differences (p < 0.05) between the WT and KO groups of mice.

Results

Cell surface expression of CD44 and L-selectin and immune responses in mBSA-immunized WT, CD44 KO, L-selectin KO, and CD44/L-selectin double KO mice

The phenotype of gene-targeted mice was confirmed by flow cytometry after immunostaining of spleen cells with mAbs against CD44 or L-selectin (Fig. 2). As expected, CD44-deficient, L-selectin-deficient, and C...
lectin-deficient, and double KO mice lacked cell surface expression of the corresponding adhesion receptor(s). As described earlier for CD44-deficient mice immunized with type II collagen (47), expression of L-selectin in the spleen cells of mBSA-immunized CD44 KO mice was significantly lower (Fig. 2F) than in the cells of WT littermates (Fig. 2E). In contrast, CD44 expression in L-selectin KO and WT lymphocytes was comparable, i.e., the absence of L-selectin did not alter the cell surface density of CD44 (compare Fig. 2, A and C).

Traditionally, AIA is induced by local challenge with Ag after s.c. and/or intradermal immunization (40, 54), where most of the T cell priming occurs within the PLNs that drain the injection sites. However, L-selectin-deficient mice that exhibit impaired homing of naive T cells to PLNs (5) may not be able to mount a full response to Ag delivered from the skin to the lymph nodes (32). Indeed, immunization with mBSA via the s.c./intradermal route resulted in significantly lower T cell stimulation in mice lacking L-selectin than in WT or CD44-deficient animals (Fig. 3A), although the BSA-specific IgG response did not seem to be affected by the absence of L-selectin (Fig. 3B). Intraperitoneal administration of mBSA, where the spleen is the major Ag-draining lymphoid organ, resulted in a normal T cell response, as well as normal production of BSA-specific IgG, in both L-selectin-deficient and double KO mice (Fig. 3, C and D), supporting the notion that lymphocyte homing to (and subsequent activation in) the spleen does not require L-selectin expression (55). As additional evidence of equivalent activation status of immune cells in this organ, we found that the percentages of splenic T or B cells that expressed the early lymphocyte activation marker CD69 (56) were very similar in L-selectin-deficient and WT mice 24 h after i.a. Ag challenge (CD69+/CD3+ cells: 35.7 ± 8.2% in L-selectin KO vs 38.1 ± 6.7 in WT; and CD69+/CD19+ cells: 62.4 ± 11.0% in L-selectin KO vs 58.5 ± 8.9% in WT). The activation status of spleen cells from CD44 KO mice was also close to WT (CD69+/CD3+ cells: 41.1 ± 12.4%; and CD69+/CD19+ cells: 56.9 ± 4.7%), which was essentially the same finding as reported in our previous study on CD44-deficient DBA/1 mice with collagen-induced arthritis and superantigen-induced peritonitis (47).

**Clinical and histological features of AIA in mice lacking CD44 and/or L-selectin**

After i.p. immunization, WT, CD44 KO, L-selectin KO, and double KO mice were injected with mBSA in the right knee and sterile PBS in the left knee. They were monitored for the development of arthritis at defined intervals between 4 h and 5 days (Fig. 4). Injections of PBS into the knee did not result in inflammation in mBSA-immunized mice, although a slight increase (<0.1 mm) in joint diameter could be detected at early time points. Joints injected with mBSA showed swelling in all four experimental groups, with a peak response at 24 h (Fig. 4). Knee swelling was significantly less in L-selectin KO and CD44/L-selectin double KO than in WT mice at all time points between 12 h and 5 days after mBSA challenge. Joint swelling was also reduced in CD44 KO mice, but significant reduction relative to WT was detected only 1 and 2 days after i.a. Ag injection (Fig. 4).

On histological sections, infiltration of the i.a. soft tissue by inflammatory cells was seen as early as 4 h after mBSA injection in WT (Fig. 5A) and CD44-deficient (Fig. 5B) mice, whereas the knee joints of L-selectin-deficient (Fig. 5C) and double KO (Fig. 5D) mice showed little evidence of inflammation at this time point. L-selectin KO (Fig. 5C) and CD44/L-selectin double-deficient (Fig. 5D) mice exhibited identical histopathological features. Knee

**FIGURE 3.** Immune responses in WT, CD44 KO, L-selectin KO, and CD44/L-selectin double KO mice immunized s.c. (A and C) or i.p. (B and D) with mBSA. Mice were sacrificed 8 days after the booster injection. Ag-specific T cell proliferation (A and B) and the serum concentrations of BSA-specific IgG (C and D) were determined. Data shown are the mean ± SEM (n = 12 in each group). Asterisks indicate significant differences (p < 0.05) between WT and KO groups of mice. SI, Stimulation index.

**FIGURE 4.** Kinetics of joint swelling during AIA in WT, CD44 KO, L-selectin KO, and double KO mice. Mice were immunized with mBSA i.p. and then received i.a. injections of either mBSA (right knee) or PBS (left knee), each given in a 6-μl volume. Knee joint diameter (in the frontal plane) was measured with a microcaliper. Results are expressed as the increase in joint diameter (mean ± SEM; n = 15 per group). Because the changes in the diameters of PBS-injected knees were identical in all four genotypes of mice, combined data of all PBS-injected knees are shown (control). Significant differences in knee swelling between WT and genetargeted mice (mBSA-injected knees only) are indicated with asterisks (*, p < 0.05; **, p < 0.01).
joints injected i.a. with PBS did not show any histological sign of inflammation irrespective of the presence or absence of either CD44 or L-selectin (an example of histology of a PBS-injected WT knee is shown in Fig. 1C).

Synovitis progressed rapidly in WT mice (Fig. 5E). One day after the i.a. Ag injection, massive cellular infiltrates were observed at the synovium-cartilage interface and underneath the patellar ligament (Fig. 5E, arrow), comprising predominantly polymorphonuclear (neutrophil) leukocytes (Fig. 5Ee, boxed area from Fig. 5E shown at higher magnification). The degree of inflammatory cell infiltration appeared to be somewhat lower in the synovium of CD44-deficient mice than in the WT controls (Fig. 5,

**FIGURE 5.** Histopathology of the knee joints in WT, CD44 KO, L-selectin KO, and double KO mice at different time points during the development of AIA. The knees were dissected 4 h (A–D), 24 h (E–H and Ee–Hh), or 5 days (I–L) after i.a. injection of mBSA into the right knees and of PBS into the left knees of mBSA-immunized mice. Arrows (E–H) indicate infiltrates of inflammatory leukocytes within the i.a. soft tissue; boxed areas are shown at approximately six times higher magnification (Ee–Hh). Because PBS-injected contra-lateral knee joints did not show histopathological evidence of inflammation in any group of mice, microphotographs of control knees are not shown, except for a PBS-injected joint of an immunized WT mouse (see Fig. 1C). H&E staining was used.
compare E and Ee with F and Ff). In L-selectin KO (Fig. 5, G and Gg) and double KO (Fig. 5, H and Hh) mice, only mild synovial inflammation could be seen at 24 h, with the participation of both mononuclear cells and neutrophils.

Whereas the histological appearance of the joints was clearly different on day 1, the inflammatory features of AIA became similar in all four genotypes of mice by day 5 after mBSA injection (Fig. 5, I–L). This stage of inflammation was characterized by synovial hyperplasia and heavy infiltration of the soft tissue by granulocytes (Fig. 5, I–L, and data not shown).

The effects of ablation of CD44, L-selectin, or both on leukocyte-endothelial cell interactions in the synovial microcirculation of the inflamed knee

Because both L-selectin and CD44 can support leukocyte rolling on activated endothelium, it was of interest to examine the effects of gene deficiencies on the rolling/adhesion behavior of leukocytes in the inflamed joints of mice using intravital videomicroscopy. As illustrated in Fig. 1, A and B, a minimally invasive surgical approach was used to access the synovial tissue without vascular injury, and physiological conditions were maintained by bathing the open joint in warm PBS. To gain insight into the kinetics of leukocyte recruitment throughout early and later phases of the inflammatory process, we performed intravital microscopy on the knee joints 4 h, 12 h, 1 day, and 5 days after i.a. injection of mBSA. In each case, the PBS-injected contralateral joint was used as a reference (noninflamed control joint; see Fig. 1C). A few rolling leukocytes (velocity > 30 μm/s), but essentially no firmly adherent cells, were observed in the postcapillary venules of control joints (data not shown), which argued against a significant trauma to the i.a. soft tissue caused by injection of PBS and/or surgery.

Numerous leukocytes interacted with the endothelium in the joints of WT mice (Fig. 6), and the vast majority of these cells adhered tightly to the vessel walls 4 h after Ag injection (Fig. 6B and see a snapshot from a video in Fig. 7A). At this early phase of AIA, CD44 deficiency had an opposite effect on leukocyte behavior: in the synovial venules of CD44 KO mice, significantly more cells rolled, but many fewer cells exhibited firm adhesion than in the vessels of WT joints (Fig. 6, A and B). The firm adhesion interactions were also reduced (to the same extent) in the joints of L-selectin-deficient and double KO mice (Fig. 6, A and B). At 12 h, the frequency of rolling cells increased slightly in the WT and decreased in the CD44 KO joints (Fig. 6A), whereas the number of adherent cells did not change (Fig. 6B). Differences between WT and KO animals in firm adhesion events could be still detected 1 day after the initiation of AIA. The overall rate of leukocyte recruitment (frequency of rolling and adherent cells), however, decreased by day 1 and was also low at day 5 (Fig. 6, A and B).

**FIGURE 6.** Kinetics of leukocyte-endothelial cell interactions in AIA as revealed by intravital videomicroscopy. The frequency of rolling cells (A) and the number of firmly adherent cells (B) in the synovial postcapillary venules of WT, CD44-deficient, L-selectin-deficient, and double KO mice are shown at 4 h, 12 h, 1 day, and 5 days after i.a. administration of mBSA. Each column represents the mean ± SEM of data from five mice (combined results from two to three venules in the mBSA-injected knee joint of each mouse). The frequency of rolling cells (A) and the number of adherent cells (B) were expressed as described in Materials and Methods. Asterisks indicate significant differences between WT and gene-targeted mice (*, p < 0.05; **, p < 0.01). C, Velocity distribution of rolling cells in the four genotypes of mice at 4 h after i.a. mBSA injection. The velocity of a total of 100 rolling cells (20 cells/joint, 5 joints/group) was measured for each group of mice. Cells were sorted into seven speed categories, ranging from 0.5 to >90 μm/s, as indicated in C.
Because peripheral blood leukocyte counts (as well as differentials) were remarkably similar in the different genotypes of i.p. immunized mice at each time point (data not shown), the results remained the same after normalization of recruitment data to the number of circulating cells available for interactions with endothelium.

Leukocytes in all gene-targeted mice showed a tendency to roll faster than WT cells, and this was most obvious at 4 h after i.a. mBSA injection (Fig. 6C). The average rolling velocity was higher in the KO groups than in WT mice (data not shown), but the differences did not reach statistical significance in any KO group at any of the time points. At 4 h of AIA, when the frequency of rolling interactions was significantly greater in the gene-targeted than in the WT mice (Fig. 6A), the rolling speed distribution profiles clearly demonstrated a shift toward higher velocity values in CD44 KO, and particularly in L-selectin and double KO, mice (velocity distribution of 100 rolling cells of each genotype is shown in Fig. 6C). The average rolling speed increased slightly from day 1 during the development of AIA; however, the magnitude of increase was similar in all mBSA-injected knees regardless of the presence or absence of CD44 or L-selectin expression (data not shown).

As illustrated by initial snapshots of representative video records, numerous fluorescent leukocytes interacted with the endothelium 4 h after mBSA injection in WT knee joints (Fig. 7A), whereas interacting cells were much less abundant in the vessels of CD44 KO (Fig. 7B), L-selectin KO (Fig. 7C), and double KO (Fig. 7D) mice. However, by looking at the single snapshots, the differences between WT and gene-targeted groups appear to be greater than expected from the results of analysis of full video records, in which the overall frequency of leukocyte-vessel interactions (rolling plus adhesion) did not differ dramatically in any KO group at any of the time points. At 4 h of AIA, when the frequency of rolling interactions was significantly greater in the gene-targeted than in the WT mice (Fig. 6A), the rolling speed distribution profiles clearly demonstrated a shift toward higher velocity values in CD44 KO, and particularly in L-selectin and double KO, mice (velocity distribution of 100 rolling cells of each genotype is shown in Fig. 6C). The average rolling speed increased slightly from day 1 during the development of AIA; however, the magnitude of increase was similar in all mBSA-injected knees regardless of the presence or absence of CD44 or L-selectin expression (data not shown).

As illustrated by initial snapshots of representative video records, numerous fluorescent leukocytes interacted with the endothelium 4 h after mBSA injection in WT knee joints (Fig. 7A), whereas interacting cells were much less abundant in the vessels of CD44 KO (Fig. 7B), L-selectin KO (Fig. 7C), and double KO (Fig. 7D) mice. However, by looking at the single snapshots, the differences between WT and gene-targeted groups appear to be greater than expected from the results of analysis of full video records, in which the overall frequency of leukocyte-vessel interactions (rolling plus firm adhesion) did not differ dramatically in the WT and KO groups at 4 h after i.a. mBSA injection (see Fig. 7A, and B). However, whereas most WT cells adhered tightly to the vessel walls (Fig. 7B), most KO cells rolled (Fig. 7A), and a high proportion of these cells rolled with high velocity (>60 μm/s; Fig. 6C). Cells rolling at a speed of 100 μm/s can be followed on the video record, but images of fast-rolling leukocytes (if captured at all) often appear blurry on a single video frame. Because slowly rolling and tightly adhering leukocytes, but not fast-rolling cells, appear as discrete fluorescent spots on single snapshots, the images shown in Fig. 7 seem to further discriminate between the WT and KO cells in favor of immobile and slowly moving cells that exhibit the most stable (shear-resistant) interactions with the endothelium.

A secondary adhesion mechanism, which can greatly enhance the efficiency of inflammatory cell recruitment, involves an interaction of cell surface L-selectin with PSGL-1 on other leukocytes (57). Indeed, tethering and rolling of leukocytes on adherent leukocytes was frequently observed in the venules of WT mice upon playback of video records. In most cases, this secondary interaction resulted in the arrest of the rolling cell near the adherent leukocyte. Although collisions between rolling cells still occurred in the vessels of the gene-targeted mice, the leukocyte-leukocyte contacts were short-lived and seldom led to the arrest of rolling cells (data not shown).

Histology of the i.a. soft tissue, dissected after intravital microscopy, showed numerous inflammatory cells outside the postcapillary venules in the mBSA-injected joints of WT mice (Fig. 8E), indicating that leukocyte migration into the extravascular space in these joints had started earlier than 4 h after i.a. Ag injection. Compared with WT, the frequency of extravasated cells was similar or lower in CD44 KO joints (Fig. 8F), but inflammatory cells were rarely seen outside the blood vessels of L-selectin KO (Fig. 8G) or double KO (Fig. 8H) mice. However, the number of extravasated cells increased in all mBSA-injected joints throughout the 5-day experimental period, and genotype-specific differences in the degree of leukocyte infiltration disappeared by day 5 of AIA (Fig. 5, I–L).

**Leukocytes in the arthritic joints and peripheral blood of WT, CD44-deficient, L-selectin-deficient, and double KO mice**

The cellular composition of infiltrates in the joints of mBSA-injected mice was quantitatively determined using flow cytometry. Four hours after i.a. Ag injection, 88–92% of the infiltrating cells expressed Gr-1 (a marker of granulocyte lineage) in the knee joints of WT (Fig. 8A) and CD44-deficient (Fig. 8B) mice, but only 29–36% of the cells were Gr-1 positive in the knees of L-selectin-deficient and double KO mice (Fig. 8, C and D). The total number of cells that could be harvested from the joints at this early time point was very low in each group (data not shown). At 24 h, >98% of joint-infiltrating cells were granulocytes in WT and CD44 KO mice (Fig. 8, A and B), whereas in L-selectin-deficient and double KO mice, the frequency of Gr-1-positive cells increased, but was still relatively low (48–76%) (Fig. 8, C and D). However, 5 days after mBSA injection, granulocytes represented >90% of the infiltrating cells in all arthritic joints (Fig. 8, A–D). The percentage of CD3-positive T cells was very low (<4% in each group) at 4 h postinjection (Fig. 8, E–H). The percentage of T cells increased in the joints...
of all KO mice and was the highest (>20%) in the double KO group at 24 h, but declined by day 5 postinjection (Fig. 8H).

We also compared the expression of L-selectin and CD44, respectively, in the cells harvested from the inflamed joints of WT and KO mice at 24 h (Fig. 8, I–P). As in splenocytes (Fig. 2), L-selectin expression was diminished in inflammatory cells (predominantly neutrophils) in CD44 KO (Fig. 8N) when compared with those in WT joints (Fig. 8M), whereas the absence of L-selectin did not influence CD44 expression (Fig. 8, I and K).

Because neutrophil activation is typically associated with down-regulation of L-selectin and simultaneous up-regulation of Mac-1 (CD11b) expression (58), reduced levels of L-selectin in CD44-null granulocytes raised the question of whether L-selectin loss was the consequence of a higher degree of neutrophil activation in CD44 KO than in WT mice. Flow cytometric analysis of peripheral blood leukocytes from mBSA-immunized and challenged WT and CD44 KO mice showed similar light scattering profiles (Fig. 9, A and E), but L-selectin-high granulocytes were virtually absent in the CD44 KO population (Fig. 9, compare boxed areas in C and G). Despite disparate L-selectin levels in WT and CD44-null neutrophils, there was no significant difference between these cells in the magnitude of CD11b expression (Fig. 9, D and H), arguing against enhanced granulocyte activation in CD44 KO mice. Moreover, 24 h after induction of AIA, granulocytes expressed L-selectin at significantly lower levels in the inflamed joints (Fig. 8, M and N, and data not shown) than in the circulation (Fig. 9, C and G) in both genotypes of mice, suggesting that L-selectin shedding occurred in both WT and CD44-null cells upon entry into the extravascular space at the inflammatory site. As in the case of CD11b (Fig. 9, D and H), CD18 (the β2 chain of LFA-1 and Mac-1), the α4 and β2 chains of VLA-4, and PSGL-1 were expressed in similar proportions of circulating neutrophils and at comparable cell surface densities in WT and all three groups of gene-targeted mice (data not shown).

**Discussion**

CD44 and L-selectin have been shown to be involved in leukocyte extravasation in different models of inflammation; however, little is known about the specific contribution of these adhesion molecules to the regulation of leukocyte traffic to joints in arthritis. Only a few studies used CD44-deficient mice to investigate the requirement for CD44 in inflammatory arthritis (35, 47) and, to date, there have been no reports describing arthritis in L-selectin KO mice. Here, using mice deficient in CD44, L-selectin, or both, we addressed the contribution of both adhesion receptors to leukocyte extravasation in joint inflammation.

In AIA, the inflammatory response is elicited in a single joint by i.a. injection of an Ag after s.c. or intradermal immunization. Expression of L-selectin is critical for the homing of naive T lymphocytes to PLNs (5), to which the Ag is transported via the lymph from the injection site. Due to the homing defect, the lymph nodes are underpopulated in L-selectin-deficient mice (5, 32). Thus, it has been uncertain whether full immunological memory can be generated by cutaneous immunization in these animals. We found that Ag (mBSA)-specific T cell responses were indeed significantly reduced in mice lacking L-selectin after s.c./intradermal Ag administration. However, T cells were adequately activated, and the magnitude of their responses to Ag was normal (WT-like) in L-selectin KO mice after i.p. immunization (Fig. 3). The most likely explanation for the emergence of adequate T cell memory in L-selectin KO mice in this case is that the Ag from the peritoneal cavity is delivered to splenic lymphocytes that could enter the white pulp of the spleen in an L-selectin-independent fashion (55). Despite comparable immune responses to BSA, the morphological features of AIA were different in WT, CD44-deficient, L-selectin-deficient, and CD44/L-selectin double KO mice. Joint swelling was diminished in CD44 KO animals for 2 days after i.a. Ag injection and was strongly reduced throughout the 5-day observation period in mice.
rapidly form between the locally injected mBSA and BSA-specific T cell-dependent model of arthritis is the presence of neutrophils in this disease (62). One of the possible explanations for the predominance of joint-homing neutrophils in this disease is the presence of immune complexes (ICs). As in the Arthus reaction (63), ICs can rapidly form between the locally injected mBSA and BSA-specific serum Abs (Fig. 3) that diffuse into the joint from the circulation. Indeed, an earlier study (39) demonstrated deposition of large amounts of radiolabeled mBSA in the form of ICs within the knee joints upon i.a. injection into immunized mice. ICs, besides activating the complement system, can stimulate the local production of chemokines that preferentially attract neutrophils (64, 65). In addition, ICs have been shown to promote neutrophil attachment via an adhesive interaction with the low-affinity Fc receptor (FcγIII) (66).

We found that extravasation of granulocytes, but not the recruitment of T cells, was diminished in the joints of mice lacking L-selectin at the early phase of AIA (Figs. 5 and 8). These data indicate that L-selectin is necessary for the early influx of neutrophils into the joints, whereas most T cells can enter these sites in an L-selectin-independent manner. Our results are in line with previous studies that have demonstrated a requirement for L-selectin for neutrophil rolling in various models of acute inflammation (5, 6, 30, 67, 68) and the lack of a strict requirement for L-selectin for T cell migration into inflammatory sites (22, 69, 70). However, we found that at a later phase of AIA (day 5 after i.a. Ag injection), neutrophils migrated into L-selectin-deficient and WT joints with similar efficiency (Figs. 5 and 8), suggesting that these cells were recruited to inflamed synovium via alternative rolling/adhesion mechanisms.

CD44 is another adhesion receptor that can promote inflammation by mediating leukocyte rolling on activated endothelium (14, 15, 23, 26, 71). We showed in the present study that in comparison with WT mice, CD44-deficient mice exhibited moderate reductions in joint swelling and neutrophil infiltration during the first day of AIA. As in the case of L-selectin KO mice, CD44-deficient inflammatory cells migrated to the joints with WT-like kinetics at a later phase of AIA (Figs. 5 and 8). In CD44-deficient mice, an interesting finding was the marked down-regulation of L-selectin on the surface of leukocytes (Figs. 2 and 8), which was first reported in collagen-induced arthritis (47). Compared with WT cells, peripheral blood neutrophils (Fig. 9) all exhibited reduced L-selectin expression in CD44-null mice. Loss of L-selectin (via shedding) is generally associated with leukocyte activation (58, 72), as is up-regulation of certain activation markers such as Mac-1 (CD11b) in neutrophils (58) or CD69 in lymphocytes (56). Expression of both Mac-1 and CD69 was similar in WT and CD44-null leukocytes, suggesting that L-selectin loss is not the result of excessive activation of the mutant cells, rather it is a consequence of an aberrant regulation of L-selectin expression in the absence of CD44 for a yet unknown reason. Because there is no change in cell surface levels of CD44 in L-selectin-deficient leukocytes (splenic lymphocytes, circulating, or inflammatory cells), this modulation of receptor expression is not reciprocal.

The absence of both L-selectin and CD44 was expected to have either additive or synergistic negative effects on joint inflammation in AIA. However, the combination of L-selectin and CD44 deficiency resulted in an inflammatory response indistinguishable from that exhibited by mice deficient in L-selectin only (Fig. 5). This observation confirmed a much greater requirement for L-selectin than CD44 during inflammatory cell extravasation in early AIA.

Intravital videomicroscopy was performed (Fig. 1) to detect alterations of leukocyte recruitment in the synovial microvessels of
mBSA-challenged knee joints, caused by the lack of expression of CD44, L-selectin, or both. Inflammatory cell recruitment into the joints was the most intense at the earliest time point (4 h) of video recording after i.a. Ag injection (Fig. 6). In WT mice, numerous leukocytes engaged in firm adhesion interactions with the endothelium, and only a few cells rolled (Fig. 6). The frequency of rolling leukocytes was significantly higher and the number of firmly adherent cells was significantly lower in the vessels of CD44-deficient mice than in those of WT mice. At first, the high frequency of rolling cells in CD44 KO mice suggested that rolling of CD44-deficient cells could be mediated by L-selectin. In L-selectin-deficient and double KO mice, however, rolling leukocytes were still more frequent than in WT mice (Fig. 6). The function of L-selectin was not compensated for by CD44, because leukocytes in L-selectin-deficient and double KO mice exhibited almost identical rolling and adhesion behavior (Figs. 6 and 7). The lack of any discernible effect of CD44 deficiency on the rolling of L-selectin-deficient cells and exaggerated down-regulation of L-selectin in CD44 KO, compared with WT cells, together suggested that the moderate reduction in leukocyte influx into the joints of CD44-deficient mice could be the result of diminished L-selectin expression rather than the consequence of a missing CD44 function.

In view of the almost universal success in reducing inflammation by CD44-specific Ab treatment in mice with different forms of arthritis (23–25), including AIA (K.M., unpublished observations), as well as other inflammatory reactions and diseases (22, 26–28), it is surprising that the complete lack of CD44 expression has only a weak influence on the development of AIA. Two studies, which directly compared the effects of gene (P-selectin and ICAM-1) deficiencies and Ab blockade in the same inflammation models, both reported a more robust suppression of inflammation by Ab treatment than gene ablation (73, 74). Although not by direct comparison, we have found similar (or perhaps greater) discrepancies between anti-CD44 Ab-treated (23) and CD44-deficient (35) mice with collagen-induced or other forms of arthritis. Anti-CD44 treatment suppresses disease severity to a much greater extent than does CD44 deficiency. However, unlike in CD44 KO mice, down-regulation of L-selectin does not seem to be responsible for arthritis suppression, in that L-selectin expression is unaltered in anti-CD44-treated mice (K.M., unpublished observations). In recent intravital studies (I. Gál, S. Szántó, E. Bajnok, and K. Mięczek, manuscript in preparation), we have found that certain CD44-specific mAbs (e.g., IM7) exhibit an instant effect on the adhesion behavior of circulating leukocytes in inflamed vessels of WT mice, but this behavior is different from that seen in CD44 KO animals. In addition, injection of IM7 results in a transient depletion of circulating granulocytes (an effect which could account for a disruption of leukocyte influx into inflammatory sites) (75), whereas granulopenia is uncommon in CD44-deficient mice (35). The differences between anti-CD44-treated and CD44 KO animals in the adhesion behavior of leukocytes might be, at least in part, related to the ability of certain Abs (particularly those preferably used for in vivo treatment) to elicit cytoskeletal signaling through CD44. In vitro studies have shown that engagement of CD44 (in leukocytes and other CD44-expressing cells) with these mAbs induces robust changes in morphology, actin cytoskeleton assembly, and adhesion (76–78), responses which cannot occur in cells lacking CD44. Collectively, these observations suggest that anti-CD44 mAbs and CD44 deficiency influence leukocyte adhesion behavior via different mechanisms in vivo. These mechanisms should be determined in further studies.

In comparison with WT mice, the frequency of rolling cells was elevated in all KO groups at the initial phase of AIA (Fig. 6A). Because L-selectin was either absent (in L-selectin KO and double KO) or reduced (in CD44 KO) and CD44 was not appreciably involved in the rolling interactions, the majority of these cells must have rolled in a PSGL-1- and/or VLA-4-dependent manner (9, 13). Apparently, rolling via PSGL-1 or VLA-4 was less “productive” than rolling via L-selectin, because many fewer leukocytes were arrested in the vessels of gene-targeted mice than in those of WT littermates (Fig. 6B). Thus, the increased frequency of rolling interactions in the mutant mice could be the consequence of repeated cycles of “futile” rolling of recirculating leukocytes. Our results, in line with previous studies (12, 79), suggest that L-selectin in neutrophils must be more effective than PSGL-1 (interacting with endothelial P/E-selectin) or VLA-4 (recognizing VCAM-1) in preparing these cells for integrin-mediated firm adhesion in vivo. In addition, the absence or low frequency of secondary leukocyte-leukocyte interactions via L-selectin-PSGL-1 binding (57) in the gene-targeted mice could contribute to the reduced/delayed recruitment of mutant cells in the inflamed joints.

At the early phase of AIA, the frequency of rolling interactions and the proportion of fast-rolling cells were elevated in L-selectin KO mice (Fig. 6C), a finding which deviates from the observations reported in previous in vivo studies using L-selectin-deficient mice with other types of inflammation (5, 30, 68). High-avidity binding of leukocyte PSGL-1 to P-selectin supports slow rolling interactions (80) that play a predominant role in inflammatory leukocyte recruitment, particularly in mice lacking L-selectin (43). The absence or reduced expression of L-selectin, therefore, was expected to result in decreased rolling velocity in AIA, but this was not the case (Fig. 6). The frequency of rolling interactions, as well as the velocity of rolling, are regulated by multiple factors, including the density of leukocyte and endothelial adhesion receptors and ligands/counterreceptors, the avidity and membrane distribution of adhesion molecules, and the specificity and concentration of locally released chemokines (6, 64, 81–83). Although we found that the proportion of leukocytes expressing PSGL-1, LFA-1/Mac-1, and VLA-4 was comparable in WT and gene-targeted mice and that these cells also expressed each molecule at similar levels, the density and distribution of endothelial ligands and the avidity of receptor-ligand interactions could not be determined in vivo. Additional factors, other than those listed above (e.g., the presence of ICs in the joint and expression of adhesion molecules, cytokines, and chemokines specific for the synovial microenvironment), could also influence the rolling behavior of leukocytes, whose adhesion interactions with endothelium have already been destabilized by the lack of CD44, L-selectin, or both adhesion receptors.

In summary, using mice deficient in CD44, L-selectin, or both, this study demonstrates the requirement for L-selectin for neutrophil recruitment in the early phase of AIA. Neither L-selectin nor CD44 appears to be necessary for the migration of T lymphocytes into the joint, cells which constitute a minor population of inflammatory leukocytes in this model of arthritis. As revealed by intravital microscopy on the synovial microcirculation and by histopathology, L-selectin expression is critical for establishment of firm adherence and subsequent extravasation of neutrophils in the joint. CD44 deficiency has only a moderate suppressive effect on granulocyte influx into the inflammatory site, and this effect might be the consequence of reduced L-selectin expression in CD44-deficient leukocytes. Paradoxically, the number of rolling leukocytes and the velocity of rolling are increased in the synovial circulation in the absence or decreased levels of L-selectin expression in AIA. This observation is somewhat in conflict with studies that have reported either a negative impact (5, 30, 68) or no effect (6) of L-selectin mutation on the frequency of rolling neutrophils and rolling velocity in other models of inflammation. Whether the unusual rolling behavior of leukocytes at the early phase of AIA in
L-selectin-deficient mice is attributable to compensatory interactions between nonconventional adhesion molecules, such as FevRIII on neutrophils (66) and ICs in the synovial blood vessels, or other factors unique to the synovial microenvironment (e.g., selective expression of endothelial adhesion receptors or chemokines) remains to be determined.

Neutrophil leukocytes are present in high numbers in the synovial fluid of RA patients during active phases of the disease (61). Neutrophils are a source of matrix-degrading enzymes, oxygen radicals, and proinflammatory mediators that, together, can inflict considerable tissue damage (84). Our results suggest that therapies targeting L-selectin expression have the potential to reduce damage by inhibiting or delaying neutrophil influx into the rheumatoid joint during acute episodes of inflammation.

Acknowledgments
We thank Bara Sarraj, Reinout Stoop, Tamás Bárdos, and Sonja Velins for expert assistance.

References


