Monocyte Migration to Arthritis in the Rat Utilizes both CD11/CD18 and Very Late Activation Antigen 4 Integrin Mechanisms

By Andrew C. Issekutz and Thomas B. Issekutz

From the Departments of Pediatrics and Microbiology–Immunology, Dalhousie University, Halifax, Nova Scotia, Canada B3J 3G9

Summary

In human and experimental models of arthritis, blood monocytes migrate into the inflamed synovium and joint space. The mechanisms required for monocyte migration across the vascular endothelium in joints is poorly defined. Radiolabeled rat blood monocytes were used to measure monocyte migration to the inflamed joints of rats with adjuvant arthritis, and the role of monocyte adhesion molecules was analyzed. Monocyte accumulation in the inflamed joints was maximal 14–21 d after immunization with adjuvant, when arthritis had fully developed. Blocking mAbs to lymphocyte function-associated antigen 1 (LFA-1), Mac-1, and very late activation antigen 4 (VLA-4) were used to evaluate the role of these integrins in the migration. Migration to the joints was not inhibited by treatment of the animals with mAb to LFA-1, Mac-1, or VLA-4 alone, and was partially (50%) inhibited in only the most arthritic joint, the talar joint, by the combination of mAb to LFA-1 plus Mac-1. In contrast, this combination inhibited migration to dermal inflammation induced by C5a$_{des\ Arg}$, endotoxin, tumor necrosis factor α, and poly-inosine-cytosine by 60–70%. When mAbs to LFA-1 and VLA-4 were combined, migration to all the inflamed joints was strongly inhibited (80–98%, depending on the joint). Treatment with the combination of the three mAbs to LFA-1, Mac-1, and VLA-4 completely eliminated monocyte migration to all joints and dermal inflammation. The results show that ^{51}Cr blood monocytes can be used to quantify monocyte migration to arthritic joints in the rat. LFA-1 alone or VLA-4 alone is sufficient to mediate most of this migration, and either LFA-1 or VLA-4 is required for monocyte migration to joint inflammation. These results indicate that both the VLA-4 and LFA-1 integrins should be therapeutic targets for suppression of monocyte infiltration of joints in arthritis.

Inflammation is characterized by PMN and mononuclear cell margination in the microvascular bed and the migration of these leukocytes into the extravascular space. This process is in part initiated by chemotactic factors, which directly stimulate adhesion and migration of leukocytes via leukocyte receptors (1–3). A number of cytokines, in particular IL-1 and TNF-α, have been shown to stimulate vascular endothelium to express adhesion molecules, which interact with counterreceptors on leukocytes including monocytes, resulting in their adhesion and transendothelial migration (4–6). The accumulation of blood monocytes in sites of inflammation is a common feature in many inflammatory conditions, including human rheumatoid arthritis, and in experimental models of arthritis (7–9). These cells differentiate into macrophages in the tissues and are believed to contribute to the inflammatory reaction through their capacity to present antigen and secrete cytokines, growth factors, oxygen radicals, and proteolytic and degradative enzymes (10–12). Therefore, the factors involved in recruiting monocytes from the blood into the tissues and the actual mechanisms used by these cells for migrating across the vascular wall are important to understand if one is to develop strategies for modulating chronic inflammation.

The β_2 integrin family of leukocyte membrane glycoproteins also known as CD11/CD18, are recognized as important surface molecules for the adhesion of all types of leukocytes to vascular endothelium and extracellular matrix (4, 5, 13). Monocytes express all three forms of the CD11/CD18 complex, namely LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), and p150/95 (CD11c/CD18) (13). Each of these share the common β_2 integrin chain (CD18) but have different α chains (CD11a, CD11b, or CD11c), which form $\alpha\beta$ heterodimers with some unique and some overlapping functions (13, 14). CD11/CD18 glycoproteins are recognized as essential for PMN migration, as illustrated by patients who have leukocyte adhesion deficiency (LAD) disease, a condition in which synthesis of the β chains is abnormal or completely deficient, resulting in virtual absence of the CD11/CD18 mol-

ecules on leukocytes (15). These patients have a dramatic impairment in the mobilization of leukocytes, especially PMN, from blood into sites of infection. Investigation of leukocyte adhesion deficiency disease patients, plus in vitro and in vivo studies using mAb to block the CD11/CD18 adhesion molecules, have demonstrated a nearly absolute requirement for this adhesion glycoprotein complex in mediating the migration of PMN in vivo and across vascular endothelium in vitro (4, 15–19). However, the mechanisms used by monocytes for migration appear to be somewhat more complex. Recent studies by ourselves and others indicate that monocytes may also utilize the $\alpha_4\beta_1$ integrin, also known as very late activation antigen 4 (VLA-4),[1] for migration across vascular endothelium in vitro (20, 21) and to peritoneal inflammatory reactions in vivo (22).

We have recently developed techniques in the rat for quantitating blood monocyte migration to sites of inflammation in the skin, induced by chemotactic factors and inflammatory cytokines (23). Here, we have investigated the migration of rat blood monocytes to inflamed joints of rats with adjuvant arthritis, which is one model of human arthritis. Using this model, we have used mAb to subunits of the CD11/CD18 complex, i.e., to LFA-1 and Mac-1, and mAb to VLA-4 to define the role of these adhesion molecules on monocyte migration to chronic joint inflammation. Our results indicate that monocytes migrate actively into the inflamed joints of rats with arthritis at the height of clinical inflammation. This migration is mediated by both the CD11/CD18 adhesion molecules, in particular LFA-1, and by VLA-4. Since monocyte migration to arthritis is completely inhibited (>98%) when both of these adhesion molecule complexes are blocked, monocyte infiltration to inflamed joints appears to absolutely require these mechanisms.

Materials and Methods

Animals. Adjuvant arthritis was induced in 6–8-wk-old inbred male Lewis strain rats by immunization on the lower back with 0.5 mg *Mycobacterium butyricum* (Difco Laboratories, Inc., Detroit, MI) in 0.05 ml mineral oil in two sites subcutaneously at the base of the tail. All the animals developed polyarticular arthritis between days 11 and 13 after immunization. For clinical scoring, 0–4 points were assigned per limb and tail, based on the severity of erythema, swelling, and limitation of movement. The maximum possible score was 20 (9).

Monocyte Isolation and Labeling. Rat blood monocytes for migration studies were obtained using the hydroxyethyl starch exchange transfusion technique of Williams et al. (24) as modified by us previously for blood monocyte migration studies (23). Briefly, the total blood volume of a donor rat was exchanged using 50 ml 6% hydroxyethyl-starch–saline (DuPont Chemical Co., Dorval, Canada). After recovery of 45–50 ml blood hydroxyethyl starch perfusate, the red cells were allowed to sediment and the leukocyte rich plasma was harvested. The leukocytes were recovered by centrifugation,

resuspended in calcium-magnesium–free Tyrode's solution plus 10% platelet-poor plasma (Tyr-10% PPP), and layered onto 63% isotonic Percoll (Pharmacia Fine Chemicals, Dorval, Canada) layered above 74% Percoll. After centrifugation, the mononuclear layer on the top of the 63% Percoll was removed, washed, and resuspended in Tyr-10% PPP. The osmolality was slightly increased by the addition of 9% NaCl to improve the separation of monocytes from lymphocytes as reported previously (23). The cell suspension was layered onto a 40%/55%/58% Percoll gradient. After centrifugation, monocytes were harvested at the 40/55% and the 55/58% interphases. The purified monocytes were washed and labeled with 75 μCi $Na_2{}^{51}CrO_4$ (Amersham, Oakville, Canada) in 1 ml Tyr-10% PPP at 37°C for 30 mins. Monocytes were then washed and 4–6 × 10^6 monocytes, carrying 1–1.5 × 10^5 cpm ^{51}Cr were injected intravenously into each rat. The injected cells contained minimal platelets and were >85%, and usually >90% monocytes as reported previously (23). The viability of labeled cells was confirmed by: (A) >95% Trypan blue dye exclusion and staining with the vital dye, neutral red; (B) very active accumulation of labeled cells in dermal inflammatory reactions; and (C) retention of >95% ^{51}Cr on the labeled monocytes in the circulation 2 h after intravenous injection of the cells as reported previously (23). The purified, labeled monocytes also showed no significant (<6%) increase in Mac-1 expression measured by immunofluorescence flow cytometry compared to monocytes in blood.

Monoclonal Antibodies and Antibody Treatment. The mAb MRC OX-42 was a kind gift from Dr. D.W. Mason (University of Oxford, Oxford, United Kingdom). It is a mouse IgG_{2a} mAb which reacts with the α chain of rat Mac-1 (CD11b/CD18; CR3) and blocks its adhesive functions as shown previously (18). It was used as the $F(ab)_2$ fragment generated by pepsin digestion using standard techniques. The TA-3 mAb is an IgG_1 mAb produced in our laboratory. It reacts with the α chain of rat LFA-1 and blocks its function (18, 25). The TA-2 mAb is an IgG_1 mAb described previously (26). It reacts with α_4 and blocks in vitro adhesion and in vivo lymphocyte migration mediated by VLA-4 (26–28).

Control mAbs included 1G8 and 2CB4E1, which were generated in our laboratory and react with rat monocytes to approximately the same cell surface density as a saturating concentration of OX-42 and TA-3, as determined by flow cytometry. Antibody 1G8 (IgG_3) immunoprecipitates 160, 45, and 30 kD polypeptides, and 2CB4E1 (IgG_{2a}) immunoprecipitates a 65 kD polypeptide from rat leukocytes. Additionally, mAb B9 (IgG_1), which reacts with pertussis toxin and is, thus, an irrelevant, nonbinding antibody, was also employed as reported previously (18, 29). All of the mAbs were grown in ascites in mice, and ammonium sulfate–precipitated IgG was used.

The effect of the mAbs on monocyte migration in vivo was assessed by giving animals an intravenous injection of 1–2 mg TA-2, or TA-3 IgG, or the $F(ab)_2$ fragment of OX-42 immediately before intravenous injection of labeled monocytes. None of the mAb treatments caused neutropenia, lymphopenia, or clearance of the ^{51}Cr monocytes from the circulation, when compared to animals not receiving any antibodies.

Dermal Inflammatory Reactions. Inflammatory reactions, which we have previously shown to induce monocyte accumulation (23), were induced in the rats by intradermal (id) injection. Recombinant mouse TNFα was a gift from Genentech Inc. (South San Francisco, CA). Poly-I:C was from Sigma Chemical Co. (St. Louis, MO), and *Escherichia coli* 0111 endotoxin (LPS) was from List Biological Laboratories, Inc. (Campbell, CA). Zymosan-activated serum (ZAS) generated by activating complement in normal rat serum with zymosan A (Sigma Chemical Co.) and followed by removal of the

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[1] *Abbreviations used in this paper:* poly I:C, polyinosine-cytosine; Tyr-10% PPP, Tyrode's solution plus 10% platelet poor plasma; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late activation antigen 4; ZAS, Zymosan-activated serum.

zymosan by centrifugation, was used as a source of the chemotactic factor C5a$_{des Arg}$ (30).

Measurement of Monocyte Migration. Rats were anesthetized and injected intravenously with ^{51}Cr-labeled monocytes. Immediately afterward, the skin on the back of the animals was shaved and the inflammatory stimuli injected intradermally into two sites. 2 h later, blood was collected to measure blood ^{51}Cr, and the animals were killed. The dorsal skin, including the area of dermal inflammatory reactions, was removed and frozen, and the injected skin sites were punched out with a 12-mm punch and counted in a gamma spectrometer (1280; LKB Instruments, Inc., Gaithersburg, MD). The forelimbs were sectioned, leaving the forepaws (containing the metacarpal and phalangeal joints) and the carpal joints as separate samples for gamma counting. Similarly, the hindlimbs were sectioned just above and below the tibiotalar joint, providing hindpaws (containing metatarsal phalangeal and phalangeal joints) and talar joint samples for gamma counting. The ^{51}Cr content of all the tissues is expressed as ^{51}Cr cpm per 10^6 cpm injected.

Statistical Methods. Statistical significance was determined using the Student's t test.

Results

Monocyte Migration to Joints of Animals with Arthritis. In initial experiments, the migration of ^{51}Cr-labeled blood monocytes to joints was measured at various times after immunization with *M. butyricum* to induce adjuvant arthritis (Fig. 1). The monocyte accumulation was determined during a 2-h period at each time point. Thus, the results indicate the rate of migration at different stages after immunization. There was no increase in the migration of ^{51}Cr-labeled monocytes in the first week after immunization, but, between 7 and 14 d, there was a marked increase, most notably in the talar joint, which in this model is the most severely affected joint. Polyarticular arthritis was present in all of the animals immunized and developed on day 10 or 11 after immunization. The clinical score of animals at 14 d was 11.8 ± 0.4 and, at 21 d, 13.6 ± 1.0. Fig. 1 shows that monocyte migration to the talar joints decreased by 21 d but remained high in the hind paws, carpal joints, and tail, which is affected by spondylitis in this model.

Effect of mAb to LFA-1 and Mac-1 on Monocyte Migration to Arthritic Joints. To evaluate the role of LFA-1 and Mac-1 on monocytes in mediating migration into inflamed joints, animals were treated 14–16 d after immunization when monocyte migration to all the joints was at or near its maximum. Animals received an intravenous injection of mAb OX42 to Mac-1, mAb TA-3 to LFA-1, or a combination of both mAbs or control mAbs, and, immediately afterward, an injection of ^{51}Cr-labeled monocytes. The animals were then killed 2 h later. As shown in Fig. 2, monocyte accumulation in the lesions was not affected by the control mAb treatment, namely the migration was comparable to that in which no mAbs were used (Fig. 1). Treatment with mAb to Mac-1 or to LFA-1 had no significant inhibitory effect on monocyte accumulation in any of the joints or in the tail. The combination of antibody to Mac-1 and to LFA-1 inhibited only the most severe joint involved, namely the talar joint, and, here, monocyte accumulation was suppressed by only 50%.
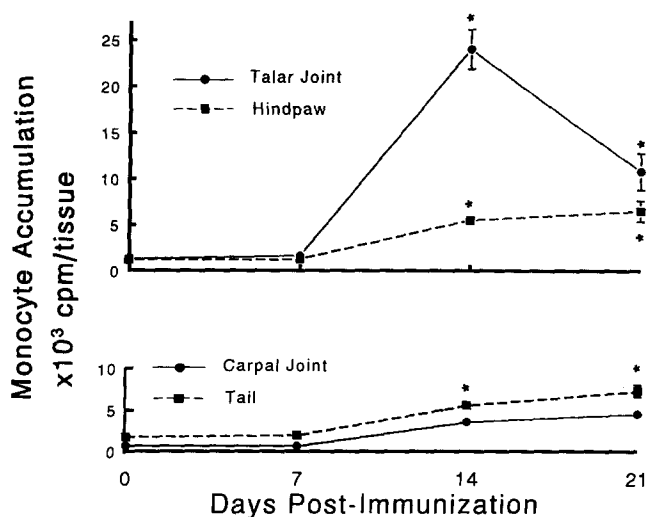

Figure 1. The kinetics of monocyte migration to joints after induction of adjuvant arthritis. Rats were immunized with *M. butyricum* and, at various times thereafter, as indicated, monocyte migration to the inflamed joints was quantified. For this, ^{51}Cr-labeled blood monocytes were injected intravenously, and the animals were killed 2 h later. Monocyte accumulation is expressed as the cpm accumulated in the tissue during the 2 h-period. Values are mean ± SEM of 8–15 animals in each group. *P <0.001.

Effect of mAb to VLA-4 and to LFA-1 and Mac-1 on Monocyte Migration to Joints. In addition to LFA-1 and Mac-1, monocytes also express the $\alpha_4\beta_1$ integrin, VLA-4, which, in vitro, can mediate monocyte adhesion (4, 6, 13, 31, 32).
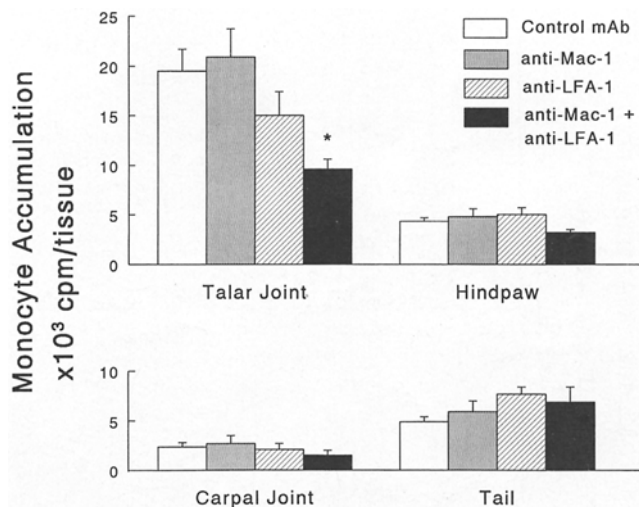

Figure 2. The effect of treatment with mAb to Mac-1 and LFA-1 on monocyte migration to arthritic joints. Rats with severe arthritis at day 14 to 16 after immunization were injected intravenously with control mAb (2CB4E1, B9, or 1G8), anti-Mac-1 (mAb MRC OX-42), anti-LFA-1 (mAb TA-3), or a combination of both these antibodies as indicated. Immediately thereafter, the animals were injected with ^{51}Cr-labeled blood monocytes. 2 h later, the rats were killed and ^{51}Cr monocyte accumulation was determined as in Fig. 1. Values are mean ± SEM of 5–15 animals in each group. Values for background monocyte accumulation in corresponding joints of normal nonarthritic animals were subtracted from each value. These corresponded to 1,311 cpm in talar joint, 1,201 cpm in the hindpaw, 648 cpm in the carpal joint, and 1,715 cpm in the tail. *P <0.05.

Therefore, the effect of blocking the α_4 chain of VLA-4 on in vivo monocyte migration to joints at 14–16 d after immunization was investigated. As Fig. 3 shows, treatment with anti–VLA-4 had no significant inhibitory effect on monocyte accumulation in the talar joint, carpal joint, or tail, but it inhibited, by ~60%, monocyte accumulation in the hindpaw (i.e., in the small joints of the foot). When anti–VLA-4 was combined with anti–Mac-1, there was some inhibition of monocyte accumulation in the joints, which reached statistically significant levels only in the talar joint. In contrast, when anti–VLA-4 was combined with anti–LFA-1, monocyte migration was much more profoundly inhibited. This combination resulted in at least 80% inhibition of monocyte accumulation in the talar joint, hindpaw, and tail, and nearly complete inhibition of monocyte accumulation (i.e., 98%) in the carpal joint. Experiments were also performed with a combination of all three mAbs to block VLA-4, Mac-1, and LFA-1. Essentially, this combination of mAbs completely blocked monocyte migration to all of the inflamed joints. Furthermore, the combination of the three mAbs completely inhibited monocyte migration to joints of animals 21 d after immunization (not shown).

Effect of mAb to Mac-1, LFA-1, and VLA-4 on Monocyte Migration to Dermal Inflammation. The migration of monocytes to dermal inflammatory reactions was also evaluated in the same animals which were used for the arthritis studies in Figs. 2 and 3. Inflammation in the skin was induced by intradermal injection of ZAS (as a source of C5a$_{des\ Arg}$ [30]),
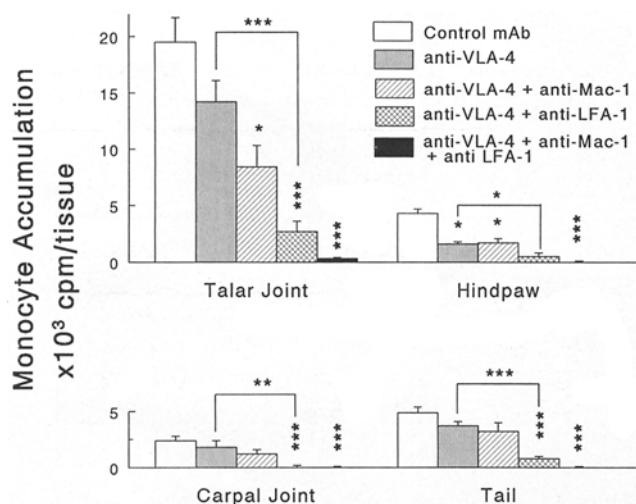

Figure 3. The effect of treatment with antibody to VLA-4 and to Mac-1, or LFA-1 on monocyte migration to arthritic joints. Animals with severe arthritis as in Fig. 2 were injected intravenously with anti–VLA-4 (mAb TA-2), or anti–VLA-4 + anti–Mac-1 (TA-2 + OX-42), or anti–VLA-4 + anti–LFA-1 (TA-2 + TA-3) mAbs, or a combination of all three mAbs, as indicated before intravenous injection of ^{51}Cr-labeled blood monocytes. Monocyte accumulation in the joints was measured after 2 h and is expressed as in Figs. 1 and 2. Values are mean ± SEM of 5–15 animals in each group, and values for normal, noninflamed joints were subtracted as in Fig. 2. *P <0.05; **P <0.01; ***P <0.001.

LPS, TNFα, and the interferon inducer, polyinosine-cytosine (poly I:C). Each of these stimuli induced marked monocyte accumulation in the cutaneous lesions, which was not affected by treatment with control mAbs (Fig. 4). Treatment of rats with the mAb to Mac-1 in combination with mAb to LFA-1 inhibited 60–75% monocyte accumulation to each of the stimuli. In contrast, treatment with mAb to VLA-4 had no affect on monocyte accumulation to any of the four inflammatory stimuli. However, when the antibodies to Mac-1 and LFA-1 were combined with the mAb to VLA-4, monocyte accumulation in the dermal inflammatory reactions was virtually completely inhibited (>98%), as was the case in the arthritis joints.

Discussion

In the experiments reported here, we have shown that a recently developed technique, using radiolabeled blood monocytes harvested from rats, can be used to quantitate monocyte migration and accumulation in the joints of rats with adjuvant arthritis. This accumulation increased during, and roughly correlated with the clinical signs of, joint inflammation (Fig. 1). Interestingly, the results also suggest that LFA-1 and Mac-1 (CD11/CD18) are not required for monocyte migration to the inflamed joints (Fig. 2). Migration to the smaller joints (carpal, hindpaw, and tail) was not significantly affected by treatment with mAbs to LFA-1 and Mac-1, and was only partially inhibited to the talar joint. This contrasts with the 60–75% inhibition by the mAb combination of monocyte migration to dermal inflammation induced by four different stimuli (ZAS, LPS, TNFα, and poly I:C) in the same animals (Fig. 4). The cutaneous results demonstrate that the mAbs to Mac-1 and LFA-1 were able to block migration in the arthritic animals. This is also in accordance with previous investigations in this model in which the mAbs, used here to Mac-1 and LFA-1, blocked 90–95% of PMN accumulation in acute dermal inflammatory reactions and inhibited PMN accumulation in inflamed joints by 50% (18, 29). The mAbs used here have also been shown, in vitro, to block PMN and lymphocyte adhesion to rat vascular endothelium, confirming that they recognize epitopes involved in adhesion functions (18, 25, 26). Furthermore, in the presence of anti–VLA-4, anti–Mac-1, and anti–LFA-1, monocyte accumulation in the skin was completely inhibited, suggesting that Mac-1 and LFA-1 were completely blocked by these mAb treatments. The conclusions which can be drawn from these findings are that blood monocytes, in migration to chronically inflamed joints, use mechanisms distinct from LFA-1 and Mac-1 and, in fact, distinct from all CD11/CD18 family members, because, in recent experiments, identical results were obtained with an anti-CD18 mAb (WT-3, kindly provided by Dr. M. Miyasaka) that blocks all members of the CD11/CD18 complex (33).

Lymphocytes, eosinophils, and monocytes also express the β_1 integrin, VLA-4 ($\alpha_4\beta_1$), which is involved in adhesion, especially to cytokine-activated endothelium, by binding to vascular cell adhesion molecule-1 (VCAM-1) on the endothelial cell (4–6, 13, 32, 34). The TA-2 mAb reacts with α_4 of rat
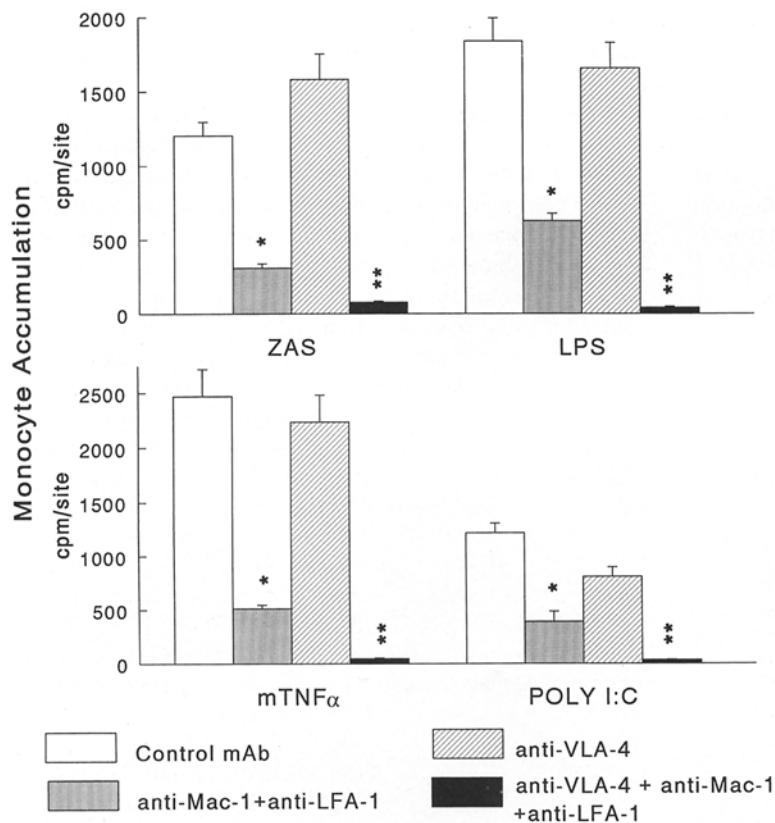
Figure 4. The effect of antibody to VLA-4, and to Mac-1 and LFA-1, on monocyte migration to dermal inflammation. In the animals in Figs. 2 and 3, immediately after intravenous injection of the mAbs, ^{51}Cr-labeled blood monocytes were injected intravenously and dermal inflammatory reactions were induced by intradermal injection of 50% ZAS, 1 ng of endotoxin (LPS), 10 ng mouse TNF-α, or 100 ng poly I:C per site. The labeled cells were allowed to accumulate in the skin sites for 2 h. At the same time they were also migrating to the inflamed joints. Values in control diluent–injected sites averaged 75 cpm and were subtracted from all the values. Values are mean ± SEM of 5–15 animals in each group. *$P < 0.001$; **$P < 0.001$, relative to anti–Mac-1 and anti–LFA-1.

$\alpha_4\beta_1$ (VLA-4), blocks its adhesion function, and inhibits the migration of T lymphocytes to inflammatory reactions (26–28). TA-2 also binds to VLA-4 on rat blood monocytes. Blocking VLA-4 caused only a mild, and in most joints, a statistically insignificant effect on monocyte accumulation. This treatment also had no effect on monocyte migration to the dermal inflammatory reactions. These findings suggest that, by itself, VLA-4 plays a minor role in monocyte migration to inflammation in these arthritic animals. However, the importance of VLA-4 in this migration became apparent when it was found that blocking VLA-4 in animals treated with anti–LFA-1 and anti–Mac-1 mAbs completely eliminated monocyte migration to the joints and to dermal inflammation. This suggests that VLA-4 can function as an alternate adhesion/migration molecule to the CD11/CD18 complex in mediating monocyte migration. In acute inflammation in skin, the CD11/CD18 complex appears to play a significant role by itself in monocyte accumulation, but in the chronic inflammation associated with arthritis, the balance between VLA-4 and CD11/CD18 (LFA-1 and Mac-1) usage is such that either VLA-4 or CD18 integrins can mediate near-maximal monocyte accumulation. These conclusions are in agreement with a recent report by Winn and Harlan (22), showing that mononuclear cell accumulation in inflamed peritoneum in rabbits was not inhibited by mAb to CD18 or to VLA-4 treatment alone, but was almost completely blocked by a combination of these two antibodies.

The findings reported here are unique in that they are, to our knowledge, the first demonstration of the kinetics of radiolabeled blood monocyte migration to joints of rats with adjuvant arthritis, the first to demonstrate that both CD11/CD18 and VLA-4 are involved in this migration process, and the first to use CD11 subunit specific antibodies (i.e., to the CD11a subunit of LFA-1 and the CD11b subunit of Mac-1) to dissect the relative contribution of each of these integrins to monocyte migration. The contributions of LFA-1 and Mac-1 were only apparent when the VLA-4 adhesion/migration mechanism was blocked. As shown in Fig. 3, addition of anti–LFA-1 to anti–VLA-4 inhibited monocyte migration almost completely to the carpal joint and by 80% to the other joints. These observations suggest that Mac-1 plays a lesser role than LFA-1 in monocyte migration to arthritis. Similar conclusions were reached in a previous study regarding PMN migration to joint inflammation, which showed that anti–LFA-1 mAb inhibited as well as a combination of anti–LFA-1 plus anti–Mac-1 mAbs did, despite the fact that, for complete inhibition of migration to dermal inflammation, both LFA-1 and Mac-1 on the PMN had to be blocked (18, 29).

The findings here also suggest that, in chronic joint inflammation, the relative contribution of VLA-4, LFA-1, and Mac-1 to monocyte migration are different from that observed with acute dermal inflammation. The increased VLA-4 usage in monocyte migration to chronic arthritic joints may be explained, in part, by our recent in vitro studies, in which mono-

cyte migration across unstimulated vascular endothelium (in response to C5a chemotactic factor) was largely dependent on CD11/CD18. Anti-CD18 inhibited 70–75% of the migration and monocytes from a patient with CD18 deficiency who had a similar impairment of monocyte transendothelial migration. However, activation of the endothelium with cytokines such as IL-1, TNF-α, or LPS resulted in monocyte migration to C5a, which was CD11/CD18-independent and was mediated by VLA-4 on the monocyte interacting with VCAM-1 on activated endothelium (20). These conditions are likely to be operative during chronic joint inflammation in arthritis, since vascular endothelium in rheumatoid synovium expresses the adhesion molecules E-selectin, intercellular adhesion molecule 1 (ICAM-1), and VCAM-1, and synovial fluid from such joints contain chemotactic factors for monocytes, such as C5a, monocyte chemotactic protein (MCP)-1, and LTB-4 (2, 12, 35–38). In addition, the alternatively spliced CS-1

fragment of fibronectin may also act as a ligand for VLA-4 and contribute to this migration (31, 39). The inflammatory reactions induced in the skin lasted only 2 h and may have been too short for adequate expression of VCAM-1 on endothelium for optimal VLA-4–mediated migration. Later time points were not studied. Nevertheless, even at 2 h a small VLA-4 component of the monocyte migration appeared to be present in acute dermal reactions when both LFA-1 and Mac-1 were blocked.

In conclusion, these results demonstrate that monocyte migration to both acute dermal inflammatory reactions and to arthritis involves both the CD11/CD18 (LFA-1 and Mac-1) and the VLA-4 adhesion molecules, and that, in chronic joint inflammation, either mechanism can be alternatively used to maintain normal or near normal monocyte migration. These conclusions may apply to monocyte infiltration in other chronic inflammatory conditions as well.

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