Rat Blood Neutrophils Express Very Late Antigen 4 and it Mediates Migration to Arthritic Joint and Dermal Inflammation

By Thomas B. Issekutz,* Masayuki Miyasaka,† and Andrew C. Issekutz‡

From the *Departments of Medicine, Pediatrics and Immunology, University of Toronto, Toronto, Ontario, M5G 2C4 Canada; the †Department of Bioregulation, Osaka University Medical School, Yamadaoka, Suita, 565 Japan; and the ‡Departments of Pediatrics and Microbiology-Immunology, Dalhousie University, Halifax, Nova Scotia, B3J 3G9 Canada

Summary

Blood neutrophils contribute to joint injury in human and experimental models of arthritis. Neutrophil migration out of the blood in joint inflammation involves both the CD18 (β2) integrins and a CD18 integrin-independent pathway. To investigate this migration, radiolabeled rat blood neutrophils were used to measure neutrophil accumulation in the inflamed joints of rats with adjuvant arthritis and the role of leukocyte integrins in migration to these joints and to dermal inflammation was determined. Neutrophils migrated rapidly (<2 h) to the inflamed joints 14–18 d after immunization with adjuvant. Blocking monoclonal antibodies (mAbs) to both LFA-1 and Mac-1 together, as well as a mAb to CD18, inhibited neutrophil accumulation in the inflamed joints by 50–75%. However, migration to dermal inflammation induced by C5aΔArg, tumor necrosis factor α, lipopolysaccharide, and polyinosine:cytosine was inhibited by ~90%. Flow cytometry revealed the expression of low levels of very late antigen 4 (VLA-4) on nearly all rat blood neutrophils. Treatment with anti-VLA-4 plus anti-LFA-1 but neither mAb alone, strongly (60–75%) inhibited neutrophil accumulation in arthritic joints. This mAb combination also inhibited neutrophil migration to dermal inflammatory reactions by 30–70%. Blocking VLA-4 together with the CD18 integrins inhibited neutrophil accumulation by 95–99%, virtually abolishing neutrophil accumulation in cutaneous inflammation. A similar blockade of VLA-4 and CD18 decreased neutrophil accumulation in the inflamed joints by 70–83%, but a significant portion of the neutrophil accumulation to these joints still remained. In conclusion, rat blood neutrophils express functional VLA-4 that can mediate neutrophil migration to both inflamed joints and dermal inflammatory sites. VLA-4 appears to be able to substitute for LFA-1 in this migration and is particularly important for accumulation in inflamed joints. However, there exists an additional CD18- and VLA-4-independent pathway of neutrophil migration to arthritic joints that is not involved in acute dermal inflammation.
washed and incubated for 10 min with 1 b~Ci 111In oxine (Amersham, Inc., Oakville, ON, Canada) per 10^7 cells. These cells were washed twice and resuspended for i.v. injection. Each rat received 10^7 labeled PMNs carrying 2–5 × 10^5 cpm.

The functional integrity of these PMNs was demonstrated by very active accumulation of labeled cells in dermal inflammatory reactions and the retention of >95% of the label on the cells in the circulation after i.v. injection, as reported previously (21). The purified neutrophils also showed no significant (<6%) increase in Mac-1 expression as measured by immunofluorescence flow cytometry compared with PMNs in blood.

mAbs and Ab Treatment. The mAb MRC OX-42 was a kind gift from Dr. D.W. Mason (University of Oxford, Oxford, UK). It is a mouse IgG2a mAb that reacts with CD11b of rat Mac-1 and blocks its adhesive functions, as previously shown (13). It was used as the F(ab)_2 fragment generated by pepsin digestion. The TA-3 mAb is an IgG1 mAb that reacts with the α chain of rat LFA-1 and blocks its function (6). The TA-2 mAb is an IgG1 mAb that reacts with α and blocks in vitro adhesion and in vivo lymphocyte and monocyte migration mediated by VLA-4 (8, 9, 22, 23). The WT.3 mAb is an IgG1 mAb that reacts with the β chain (CD18) of the rat β_2 integrins and blocks its function (24).

Control mAbs included 2CB4E1 (IgG2a) which was generated in our laboratory and reacts with rat neutrophils to approximately the same intensity as a saturating concentration of OX-42 and TA-3, as determined by flow cytometry. It immunoprecipitates a 65-kD polypeptide from rat leukocytes. mAb B9 (IgG1), which reacts with pertussis toxin, was also used as a nonbinding control mAb (25). Since the results with these control mAbs were comparable, these measurements were pooled. All of the mAbs were grown in ascites in mice, and ammonium sulphate–precipitated IgG was used.

The effect of the mAbs on neutrophil migration in vivo was determined by giving animals an i.v. injection of 1–2 mg TA-2, TA-3, or WT.3 IgG, or the F(ab)_2 fragment of OX-42, immediately before i.v. injection of labeled neutrophils. None of the mAb treatments caused neutropenia or clearance of the 111In-labeled neutrophils from the circulation when compared with animals not receiving any Abs.

Dermal Inflammatory Reactions. Inflammatory reactions, which we have previously shown to induce neutrophil accumulation, were induced in rats by intradermal (i.d.) injection (13, 26). Recombinant mouse TNF-α was kindly provided by Genentech, Inc. (South San Francisco, CA). Escherichia coli LPS was from List Biologicals (Campbell, CA). Zymosan-activated serum (ZAS), a source of C5a and chemotactic factor, was generated by activating C5 in normal rat serum with 5 mg/ml of Zymosan A (Sigma Chemical Co., St. Louis, MO) for 60 min at 37°C and removing the zymosan as described previously (27). Polyinosine:cytosine (poly I:C) was obtained from Sigma Chemical Co.

Measurement of Neutrophil Migration. Rats previously immunized with M. butyricum to induce adjuvant arthritis were anesthetized and injected intravenously with 111In-labeled neutrophils. Immediately afterward, the skin on the back of the animals was shaved and inflammatory stimuli in a volume of 0.05 ml were injected intradermally into several sites along with diluent controls. 2 h later, the animals were euthanized and blood was collected in acid citrate dextrose anticoagulant. The dorsal skin, including the area of dermal inflammatory reaction, was removed and frozen; the injected skin sites were punched out with a 12-mm punch and counted in a gamma spectrometer. The forelimbs were sectioned, leaving the forepaws (containing the metacarpal and phalangeal joints) and the carpals joints as separate samples for gamma counting. Similarly, the hind limbs were sectioned just above and below the tibiotaral joint, providing hindpaws (containing metatar-
sophalangeal and phalangeal joints) and talar joint samples. The red blood cells in 1 ml of blood were lysed with 0.84% NH₄Cl, the leukocytes pelleted by centrifugation, and the number of neutrophils and ¹¹¹In content of the cells determined. The accumulation of ¹¹¹In-neutrophils is expressed as the number of neutrophils $\times 10^6$ per tissue calculated from the blood neutrophil-specific activity.

Statistical Methods. Differences between groups were tested using a one-way analysis of variance and posthoc testing with the Tukey-Kramer Multiple Comparisons Test.

Results

Effect of mAbs to LFA-1, Mac-1, and CD18 on Neutrophil Accumulation in Arthritic Joints. To evaluate the role of the CD18 integrins in neutrophil accumulation in arthritic joints, animals were studied 14-18 d after immunization, when the joints were markedly inflamed. Animals received an i.v. injection of mAb OX-42 to Mac-1, mAb TA-3 to LFA-1, the combination of both of these mAbs, or mAb WT.3 to CD18, or a control mAb, and immediately afterward, an injection of ¹¹¹In-labeled neutrophils. Animals were killed 2 h later. As shown in Fig. 1, there was considerable neutrophil accumulation in the joints of the animals receiving the control mAb treatment with ~16 million neutrophils accumulating in the talar joint, which was the most severely affected, and 2-4 million in the other joints. Treatment with mAb to Mac-1 or to LFA-1 had no significant effect on neutrophil accumulation in any of the joints, although there was some decrease in neutrophil accumulation in the talar joints of animals treated with anti-LFA-1. The combination of mAbs to Mac-1 and LFA-1 strongly inhibited neutrophil accumulation to the large talar joints and also significantly reduced accumulation in the hind-paw, carpal, and forepaw joints. Treatment of rats with mAb to CD18 had a similar inhibitory effect. Both combined LFA-1 and Mac-1 blockade, or CD18 blockade, inhibited neutrophil accumulation in the talar joints by ~75%, and in the hind-paw and carpal joints by ~50%.

Effect of mAb to LFA-1, Mac-1, and CD18 on Neutrophil Migration to Cutaneous Inflammation. To compare the role of the CD18 integrins in neutrophil migration to inflamed joints with inflammation in the skin, the same animals injected with mAb and labeled neutrophils for the experiments in Fig. 1 were injected intradermally with stimuli to induce a dermal inflammatory reaction. As shown in Fig. 2, rats were injected with ZAS, a source of C5a des, Apr, TNF-α, LPS, and poly I:C, a potent cytokine inducer. Anti-Mac-1 had no effect on neutrophil accumulation to any of the inflammatory reactions. Anti-LFA-1 alone caused a small inhibition of neutrophil accumulation to poly I:C, but much more dramatic inhibition was induced by blocking the combination of both Mac-1 and LFA-1. The combination of anti-Mac-1 and anti-LFA-1 inhibited 80-90% of the neutrophil accumulation, and treatment with anti-CD18 inhibited >90% of the migration to the four inflammatory stimuli. Doubling the dose of the mAb treatment did not further suppress neutrophil accumulation. Thus, neutrophil accumulation to dermal inflammation was strongly suppressed (>90%) by blocking CD18 integrins, whereas accumulation in inflamed joints was inhibited only by 50-75%. Therefore, the contribution of VLA-4 to this latter migration was investigated.

Immunofluorescence Analysis of Neutrophil Staining with Anti-VLA-4. Fig. 3 shows that the TA-2 mAb to VLA-4 stained nearly all blood neutrophils. Although the intensity of the staining was relatively low, it was clearly greater than that of the isotype-matched negative control mAb.

Effect of mAb to VLA-4, LFA-1, and Mac-1 on Neutrophil Accumulation in Arthritic Joints. Rats were treated with anti-VLA-4, anti-LFA-1, both of these mAbs, or control mAb, and neutrophil migration to the talar, hindpaw, and carpal joints was determined (Fig. 4). Anti-α₄ by itself had no effect on neutrophil accumulation, but when combined with anti-LFA-1, it strongly inhibited neutrophil accumulation.
in all three joints. The talar and carpal joints were inhibited by 75%, and the hindpaw by 60%. In all cases, blocking \( \alpha_4 \) was more inhibitory than anti-LFA-1 alone.

Fig. 5 shows that blocking Mac-1 in combination with anti-VLA-4, unlike blocking LFA-1, did not significantly inhibit neutrophil accumulation in the inflamed joints.

There was a tendency for less neutrophil accumulation in the presence of dual Mac-1 VLA-4 blockade, but this effect was not significant.

**Effect of mAbs to VLA-4 and LFA-1 on Neutrophil Accumulation in Dermal Inflammatory Reactions.** The effect of blocking VLA-4 and LFA-1 on PMN migration to inflamed skin was also determined. As shown in Fig. 6, treatment with anti-\( \alpha_4 \) had no effect on neutrophil accumulation to ZAS, LPS, and poly I:C, and produced a small decrease in migration to TNF-\( \alpha \). Anti-LFA-1 alone also caused only a small decrease in neutrophil accumulation to some of the lesions, whereas the combination of blocking both VLA-4 and LFA-1 inhibited neutrophil accumulation by 35–70%, with the greatest effect observed in response to TNF-\( \alpha \). The combination of blocking both VLA-4 and Mac-1 was no more effective than VLA-4 alone (data not shown). Although blocking both VLA-4 and LFA-1 was not as effective as inhibiting LFA-1 and Mac-1 in migration to cutaneous inflammation (Fig. 2), these results showed that VLA-4 on neutrophils could mediate part of the accumulation in cutaneous inflammation, especially when LFA-1 was blocked.

**Effect of VLA-4 and Combined CD18 Blockade on Neutrophil Accumulation in Arthritic Joints.** Blockade of the CD18 integrins only partially inhibited neutrophil migration to inflamed joints, therefore the effect of blocking VLA-4, as well, was also examined (Fig. 7). Blocking VLA-4, LFA-1, and Mac-1 was not different from blocking the combination of LFA-1 and Mac-1. However, the addition of anti-VLA-4 to anti-CD18 treatment further decreased neutrophil accumulation to the talar, hindpaw, and carpal joints, and this decrease was significantly greater in the case of the larger joints of the hind limbs. This combined VLA-4 and CD18 blockade inhibited neutrophil accumulation into these joints by 70–83%, and suggested that VLA-4 contributed to a component of the CD18-independent neutrophil
migration into arthritic joints. Furthermore, there remained a component of ~25% that might be independent of these four integrins.

**Effect of VLA-4 and CD18 Blockade on Neutrophil Accumulation in Dermal Inflammation.** The effect of VLA-4 and CD18 integrin blockade on neutrophil migration to dermal inflammation is shown in Fig. 8. Treatment with anti-VLA-4 plus anti-Mac-1 and anti-LFA-1 was significantly more inhibitory than blockade of the combination of LFA-1 and Mac-1. Similarly, treatment with anti-VLA-4 and anti-CD18 was significantly more effective than blocking CD18 alone. Anti-VLA-4 plus anti-CD18 inhibited 95–98.5% of the neutrophil accumulation in the cutaneous inflammatory sites, suggesting that virtually all of the neutrophil migration was mediated by these integrins.

It should also be pointed out that in spite of this profound suppression of neutrophil accumulation in the dermal inflammatory sites, migration in these same animals to arthritic joints was only partially suppressed, and the level of circulating labeled blood neutrophils was actually slightly (~13%) increased.

**Discussion**

Neutrophil migration out of blood vessels is thought to involve an initial tethering and rolling adhesion to the vascular endothelium, a G-protein mediated activation of the neutrophil, and firm adhesion mediated by neutrophil integrins (1, 2). Several studies, including our own, have shown that the CD18 integrins, notably LFA-1 and Mac-1,
can mediate neutrophil adhesion and in vivo neutrophil migration to inflammatory sites (11–14). As reported here, 80–90% of the neutrophil infiltration in dermal inflammatory lesions could be inhibited by blocking LFA-1 and Mac-1 or all three members of the CD18 family. In contrast, a few investigations have also shown that neutrophils can utilize CD18-independent mechanisms for migration to the lung (14) and peritoneum (10), and to the inflamed joint (16). These studies show that 25–50% of the neutrophil accumulation in adjuvant arthritis was not inhibitable by anti-CD18 mAb treatment, even though this treatment inhibited >90% of the accumulation in dermal inflammation in the same animals (Figs. 1 and 2). Our previous studies also showed that neutrophil migration to an intra-articular delayed-type hypersensitivity reaction was also CD18 independent whereas migration to intra-articular IL-1 injection was CD18 dependent (26). These findings suggested that neutrophils utilized additional adhesion pathways for infiltration in adjuvant arthritis. Since monocyte migration to inflamed joints was highly dependent upon VLA-4, we wished to determine whether VLA-4 on neutrophils might contribute to the CD18-independent neutrophil accumulation in arthritis.

The findings reported here are novel in that they are the first to demonstrate the expression of α4 integrins on rat blood neutrophils, the first to demonstrate that the VLA-4 on neutrophils is functional and mediates, together with the CD18 (β2) integrins, part of the migration of neutrophils to inflamed joints and dermal inflammation, and that in addition to the CD18 and α4 integrin pathways, neutrophil accumulation in arthritis involves at least one additional CD18- and VLA-4-independent pathway.
Our studies showed that virtually all of the neutrophils in the blood react with an anti-α₄ mAb, TA-2 (Fig. 3). Although α₄ can associate with β₁ and β₇ to form VLA-4 and α₄β₇, LPAM-1, the latter has only been found on lymphocytes and is absent from both neutrophils and monocytes (28). Therefore, the α₄ integrin on the neutrophil in our studies is most likely VLA-4.

Eosinophils, monocytes, and lymphocytes all express VLA-4, which can mediate adhesion to cytokine-stimulated endothelium by binding to the vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells (8, 22, 29–34). The TA-2 mAb reacts with VLA-4, blocks its adhesive function, and inhibits the migration of T lymphocytes and monocytes to inflammatory reactions (8, 22, 23). Blocking α₄ with TA-2 alone produced only a small decrease in neutrophil migration to i.d. TNF-α and had no effect on accumulation in inflamed joints. This suggests that, by itself, VLA-4 plays a minor role in neutrophil accumulation in most of the inflammatory sites examined. However, the functional importance of VLA-4 could be seen when LFA-1 was also blocked with an α₆ (LFA-1)-specific mAb. Blocking VLA-4 significantly decreased neutrophil migration to inflamed joints by 60–75% if LFA-1 was also blocked (Fig. 4). Similarly, neutrophil migration to the dermal inflammatory sites was also inhibited by blocking VLA-4 together with LFA-1, although the magnitude of this effect depended on the inflammatory stimulus in the skin (Fig. 6). This suggests that VLA-4 on neutrophils is functional. In addition, VLA-4 can act as an alternate adhesion pathway to LFA-1, since blocking both VLA-4 and LFA-1 was required for significant inhibition of neutrophil accumulation in most of the inflammatory tissues. The effect of dual VLA-4 and LFA-1 blockade appeared to be greater on neutrophil accumulation in inflamed joints and TNF-α--injected skin than in the other dermal inflammatory sites.

Since previous studies have shown that TNF-α is a key mediator of neutrophil migration to joints in adjuvant arthritis (35), the marked effect of dual blockade of VLA-4 and LFA-1 in adjuvant arthritis and cutaneous TNF-α reactions and anti-VLA-4 alone on i.d. TNF-α might be the result of more efficient utilization by neutrophils of VLA-4 to bind to VCAM-1, which is strongly induced on endothelium by TNF-α (36, 37).

These findings regarding neutrophil migration in adjuvant arthritis are similar to those observed with monocyte recruitment to arthritic joints. Both VLA-4 and the CD18 integrins, particularly LFA-1, mediate monocyte accumulation in inflamed joints (9). In addition, monocyte migration to dermal inflammation is much more strongly inhibited by blocking CD18 integrins than migration to arthritic joints, where VLA-4 appears to be more important.

Blocking Mac-1 alone, or in combination with VLA-4, did not significantly inhibit neutrophil accumulation to either inflamed joints or skin (Figs. 1 and 2), suggesting that LFA-1 is sufficient to allow normal neutrophil migration. However, blocking both LFA-1 and Mac-1, or all three CD18 integrins (with anti-β₇ mAb) inhibited >90% of the neutrophil migration to dermal inflammation, and 50–75% of the migration to arthritic joints. Anti-VLA-4 treatment further inhibited neutrophil migration to dermal inflammation, such that neutrophil accumulation in cutaneous sites was nearly abolished (Fig. 8). Blocking VLA-4 also enhanced the effect of anti-CD18 treatment on neutrophil accumulation in arthritic joints (Fig. 7). This effect, however, was only partial. The increased inhibition of neutrophil accumulation with combined CD18/VLA-4 blockade further supports the contribution of VLA-4 to neutrophil accumulation in arthritic joints and dermal inflammation. The contribution of the CD18 integrins and VLA-4 in neutrophil migration to skin again parallels their roles in monocyte migration, although a larger proportion of monocyte migration to dermal inflammation is VLA-4 dependent (9).

Interestingly, anti-VLA-4 in combination with anti-LFA-1 and anti-Mac-1 was no more effective at inhibiting neutrophil accumulation in joints than blockade of the latter two integrins, whereas the addition of anti-VLA-4 to anti-CD18 treatment was more effective than anti-CD18 alone (Fig. 7). Although differences in Ab affinity and
vascular endothelium, are not thought to be able to mediate cell adhesion before transendothelial migration. The adhesion pathway mediating this migration is not clear. The selectins, L-, E-, and P-selectin, which can mediate initial tethering of leukocytes to the vascular endothelium, are not thought to be able to mediate the firm leukocyte adhesion required for transendothelial migration. VLA-5 and VLA-6 have recently been reported to be also present on neutrophils, and to mediate binding to fibronectin and laminin and migration across synovial fibroblast barriers. Neutrophil adhesion via these integrins to extracellular matrix proteins might contribute to part of the neutrophil infiltration in a chronically inflamed tissue such as the joint in adjuvant arthritis, in contrast to an acute dermal inflammatory reaction. However, there is no evidence that these integrins mediate cell adhesion before transendothelial migration.

A recent in vitro study has suggested that human blood neutrophils, stimulated with chemotactic factors in the presence of dihydrocytochalasin B, can express VLA-4 (39).

Similarly, transendothelial migration by neutrophils could also result in increased VLA-4 expression. Our studies suggest that in vivo, even in the absence of a microfilament disrupting agent such as cytochalasin, rat neutrophils have functional VLA-4, and that this VLA-4 may be particularly important in migration to selected inflammatory sites.

Other investigations have also demonstrated CD18-independent neutrophil migration to some types of inflammation in the lung and the peritoneal cavity (10, 14). In the lung, the role of VLA-4 in neutrophil accumulation has not been examined, whereas in peritoneal inflammation in rabbits, blocking both VLA-4 and CD18 inhibited neutrophil accumulation, although the basis of this effect is unknown (10). Recent studies by A. Issekutz (40) have demonstrated that human blood neutrophils in vitro can migrate across unstimulated endothelium in response to C5a in a CD18-independent manner, but that migration across cytokine-stimulated endothelium in the presence of C5a was partially CD11/CD18 independent and was not inhibitable by mAb to VLA-4. This suggests that, similar to the migration of rat neutrophils to inflamed joints, human neutrophils also demonstrate a CD18- and VLA-4-independent migration under the appropriate conditions. The mechanism operative in this migration, with human or rat neutrophils, awaits further characterization.

In conclusion, our studies have demonstrated that rat blood neutrophils express α4 integrins and that these are functional and mediate, together with β2 integrins, virtually all of the migration to cutaneous inflammation, but migration to inflamed joints involves not only β2 and α4 integrins, but also another pathway. The identification of this additional pathway and the full range of receptors mediating the migration of neutrophils in different inflammatory situations is yet to be fully clarified.

The authors gratefully acknowledge the excellent technical assistance of Ms. N. MacPhee, C. Jordan, and J. Stoltz. We also thank Ms. S. Gruman for her expert secretarial help in manuscript preparation.

This work was supported by grant 89036 from The Arthritis Society and grant MA8575 from the Medical Research Council of Canada. T.B. Issekutz is a recipient of a Research Chair supported by the MRC/PMAC Health Program and Sandoz Canada, Inc.

Address correspondence to Dr. Thomas B. Issekutz, Conacher Research Wing, 2-855, The Toronto Hospital, 101 College Street, Toronto, Ontario M5G 2C4, Canada.

Received for publication 31 October 1995 and in revised form 19 February 1996.

References
1. Butcher, E.C. 1992. Leukocyte-endothelial cell adhesion as an active, multi-step process: a combinatorial mechanism for specificity and diversity in leukocyte targeting. Adv. Exp. Med. Biol. 323:181–194.
2. Springer, T.A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. 76:301–314.
3. Hynes, R.O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. Cell. 69:11–25.
4. Haskard, D., D. Cavender, P. Beatty, T. Springer, and M. Ziff. 1986. T lymphocyte adhesion to endothelial cells: mechanisms demonstrated by anti-LFA-1 monoclonal antibodies. J. Immunol. 137:2901–2906.
5. Dustin, M.L., and T.A. Springer. 1988. Lymphocyte associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mecha-
nisms for lymphocyte adhesion to cultured endothelial cells. 
*J. Cell Biol.* 107:321–331.

6. **Issekutz, T.B.** 1992. Inhibition of lymphocyte endothelial adhesion and *in vivo* lymphocyte migration to cutaneous inflammation by TA-3, a new monoclonal antibody to rat LFA-1. 
*J. Immunol.* 149:3394–3402.

7. **Scheinman, A., R.L. Camp, and E. Puré.** 1993. Reduced contact sensitivity reactions in mice treated with monoclonal antibodies to leukocyte function-associated molecule-1 and intercellular adhesion molecule-1. 
*J. Immunol.* 150:655–663.

8. **Issekutz, T.B.** 1995. *In vivo* blood monocyte migration to acute inflammatory reactions, IL-1α, TNFα, IFN-γ, and C5α utilizes LFA-1, Mac-1 and VLA-4: the relative importance of each integrin. 
*J. Immunol.* 154:6533–6540.

9. **Issekutz, A.C., and T.B. Issekutz.** 1995. Monocyte migration to arthritis in the rat utilizes both CD11/CD18 and VLA-4 integrin mechanisms. 
*J. Exp. Med.* 181:1197–1203.

10. **Winn, R.K., and J.M. Harlan.** 1993. CD18-independent neutrophil and mononuclear leukocyte emigration into the peritoneum of rabbits. 
*J. Clin. Invest.* 92:1168–1173.

11. **Price, T.H., P.G. Beatty, and S.R. Corpuz.** 1987. *In vivo* inhibition of neutrophil function in the rabbit using monoclonal antibody to CD18. 
*J. Immunol.* 139:4174–4177.

12. **Nourshargh, S., M. Rampart, P.G. Hellewell, P.J. Jose, J.M. Harlan, A.J. Edwards, and T.J. Williams.** 1989. Accumulation of 111In-neutrophils in rabbit skin in allergic and non-allergic allergic reactions in vivo. Inhibition by neutrophil pre-treatment in *vitro* with a monoclonal antibody recognizing the CD18 antigen. 
*J. Immunol.* 142:3193–3198.

13. **Issekutz, A.C., and T.B. Issekutz.** 1992. The contribution of LFA-1 (CD11a/CD18) and MAC-1 (CD11b/CD18) to the *in vivo* migration of polymorphonuclear leukocytes to inflammatory reactions in the rat. 
*Immunology.* 76:655–661.

14. **Doerschuk, C.M., R.K. Winn, H.O. Coxson, and J.M. Harlan.** 1990. CD18-dependent and -independent mechanisms of neutrophil emigration in the pulmonary and systemic microcirculation of rabbits. 
*J. Immunol.* 144:2227–2233.

15. **Vedder, N.B., R.K. Winn, C.L. Rice, E.Y. Chi, K.-E. Arfors, and J.M. Harlan.** 1990. Inhibition of leukocyte adherence by anti-CD18 monoclonal antibody attenuates reperfusion injury in the rabbit ear. 
*Proc. Natl. Acad. Sci. USA.* 87:2643–2646.

16. **Issekutz, A.C., and T.B. Issekutz.** 1993. A major portion of polymorphonuclear leukocyte and T lymphocyte migration to arthritic joints in the rat is via LFA-1/MAC-1-independent mechanisms. 
*Clin. Immunol. Immunopathol.* 67:257–263.

17. **Bohnscak, J.F.** 1992. CD11/CD18-independent neutrophil adherence to laminin is mediated by the integrin VLA-6. 
*Blood.* 79:1545–1552.

18. **Bohnscak, J.F., and X.-N. Zhou.** 1992. Divalent cation substitution reveals CD18- and very late antigen-dependent pathways that mediate human neutrophil adherence to fibronectin. 
*J. Immunol.* 140:1340–1347.

19. **Kert, J.M., J.B. Sanders, I.C.M. Slaper-Cortenbach, M.C. Doorakkers, B. Hoolbrink, R.H.J. van Oers, A.E.G.K. Von dem Borne, and C.E. Van der Schoot.** 1993. αβ2 and αβ1 are differentially expressed during myelopoiesis and mediate the adherence of human CD34+ cells to fibronectin in an activation-dependent way. 
*Blood.* 81:344–351.

20. **Williams, J.H., K.M. Moser, T. Ulich, and M.S. Cairo.** 1987. Harvesting the noncirculating pool of polymorphonuclear leukocytes in rats by heterastarch exchange transfusion (HET): yield and functional assessment. 
*J. Leukocyte Biol.* 42:455–462.

21. **Issekutz, A.C., and T.B. Issekutz.** 1991. Quantitation and kinetics of polymorphonuclear leukocyte and lymphocyte accumulation in joints during adjuvant arthritis in the rat. 
*Lab. Invest.* 64:656–663.

22. **Issekutz, T.B., and A. Wykretowicz.** 1991. Effect of a new monoclonal antibody, TA-2, that inhibits lymphocyte adherence to cytokine stimulated endothelium in the rat. 
*J. Immunol.* 147:109–116.

23. **Issekutz, T.B.** 1991. Inhibition of *in vivo* lymphocyte migration to inflammation and homing to lymphoid tissues by the TA-2 monoclonal antibody: a likely role for VLA-4 *in vivo*. 
*J. Immunol.* 147:4178–4184.

24. **Tamatani, T., M. Kotani, T. Tanaka, and M. Miyasaka.** 1991. Molecular mechanisms underlying lymphocyte recirculation II. Differential regulation of LFA-1 in the interaction between lymphocytes and high endothelial cells. 
*Eur. J. Immunol.* 21:627–633.

25. **Halperin, S.A., T.B. Issekutz, and A. Kasina.** 1991. Modulation of *Bordetella pertussis* infection with monoclonal antibodies to pertussis toxin. 
*J. Infect. Dis.* 163:355–361.

26. **Gao, J.-X., A.C. Issekutz, and T.B. Issekutz.** 1994. Neutrophils migrate to delayed-type hypersensitivity reactions in joints, but not in skin. 
*J. Immunol.* 153:5689–5697.

27. **Issekutz, A.C., H.Z. Movat, and K.W. Movat.** 1980. Enhanced vascular permeability and haemorrhage-inducing activity of rabbit C5αneo-α probable role of polymorphonuclear leukocyte lysosomes. 
*Clin. Exp. Immunol.* 41:512–520.

28. **Erle, D.J., C. Rüegg, D. Sheppard, and R. Pyrela.** 1991. Complete amino acid sequence of an integrin β subunit (β2) identified in leukocytes. 
*J. Biol. Chem.* 266:11009–11016.

29. **Weller, P.F., T.H. Rand, S.E. Goetzl, G. Chi-Rosso, and R.R. Lobb.** 1991. Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1. 
*Proc. Natl. Acad. Sci. USA.* 88:7430–7433.

30. **Walsh, G.M., J.-J. Mermod, A. Hartnell, A.B. Kay, and A.J. Wardlaw.** 1991. Human eosinophil, but not neutrophil, adherence to IL-1–stimulated human umbilical vascular endothelial cells is αβ1 (very late antigen-4) dependent. 
*J. Immunol.* 146:3419–3423.

31. **Dobrni, A., R. Menegazzi, T.M. Carlos, E. Nardon, R. Kramer, T. Zacchi, J.M. Harlan, and P. Patriarca.** 1991. Mechanisms of eosinophil adherence to cultured vascular endothelial cells. Eosinophils bind to the cytokine-induced endothelial ligand vascular cell adhesion molecule-1 via the very late activation antigen-4 integrin receptor. 
*J. Clin. Invest.* 88:20–26.

32. **Bochner, B.S., F.W. Luscinskas, M.A. Gimbrone, Jr., W. Newman, S.A. Sterbinsky, C.P. Derse-Anthony, D. Klunk, and R.P. Schleimer.** 1991. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1–activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. 
*J. Exp. Med.* 173:1553–1556.

33. **Elices, M.J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M.E. Hemler, and R.R. Lobb.** 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. 
*Cell.* 60:577–584.

34. **Carlos, T., N. Kovach, B. Schwartz, M. Rosa, B. Newman, E. Wayner, C. Benjamin, L. Osborn, R. Lobb, and J. Harlan.** 1991. Human monocytes bind to two cytokine-induced adhesion ligands on cultured human endothelial cells: endothelial-leukocyte adhesion molecule-1 and vascular cell adhesion
molecule-1. *Blood.* 77:2266–2271.

35. Issekutz, A.C., A. Meager, I.G. Otterness, and T.B. Issekutz. 1994. The role of tumour necrosis factor-alpha and IL-1 in polymorphonuclear leukocyte and T lymphocyte recruitment to joint inflammation in adjuvant arthritis. *Clin. Exp. Immunol.* 97:26–32.

36. Pober, J.S., M.A. Gimbrone Jr., L.A. Lapierre, D.L. Mendrick, W. Fiers, R. Rothlien, and T.A. Springer. 1986. Overlapping patterns of activation of human endothelial cell by interleukin-1, tumor necrosis factor and immune interferon. *J. Immunol.* 137:1893–1896.

37. Pober, J.S., and R.S. Cotran. 1990. Cytokines and endothelial cell biology. *Physiol. Rev.* 70:427–452.

38. Gao, J.X., J. Wilkins, and A.C. Issekutz. 1995. Migration of human polymorphonuclear leukocytes through a synovial fibroblast barrier is mediated by both β2 (CD11/CD18) integrins and the β1 (CD29) integrins VLA-5 and VLA-6. *Cell. Immunol.* 163:178–186.

39. Kubes, P., X.-F. Niu, C.W. Smith, M.E. Kehrli, Jr., P.H. Reinhardt, and R.C. Woodman. 1995. A novel β1-dependent adhesion pathway on neutrophils: a mechanism invoked by dihydrocytochalasin B or endothelial transmigration. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 9:1103–1111.

40. Issekutz, A.C., H.E. Chuluyan, and N. Lopes. 1995. CD11/CD18-independent transendothelial migration of human polymorphonuclear leukocytes and monocytes: involvement of distinct and unique mechanisms. *J. Leukocyte Biol.* 57:553–561.