# Intercellular Adhesion Molecule 3, a Third Adhesion Counter-Receptor for Lymphocyte Function-associated Molecule 1 on Resting Lymphocytes

By Antonin R. de Fougerolles and Timothy A. Springer

*From the Committee on Immunology, Department of Pathology, Harvard Medical School, and Center for Blood Research, Boston, Massachusetts 02115*

## Summary

Recent studies suggest that some T and B lymphocyte cell lines bind to the integrin lymphocyte function-associated molecule 1 (LFA-1) chiefly through a pathway independent of its two known counter-receptors, intercellular adhesion molecules (ICAMs)-1 and -2. A monoclonal antibody (mAb) was raised that, in combination with blocking mAb to ICAM-1 and ICAM-2, can completely inhibit binding of these cell lines to purified LFA-1. This third ligand, designated ICAM-3 based on its functional relatedness to ICAM-1 and -2, is a highly glycosylated protein of 124,000 $M_r$. It is well expressed on all leukocytes and absent from endothelial cells. In assays of adhesion of resting lymphocytes to purified LFA-1, ICAM-3 is by far the most functionally important ICAM, implying an important role for ICAM-3 in the generation of immune responses.

Lymphocyte function-associated molecule 1 (LFA-1)[1] is an integrin that mediates a wide range of leukocyte interactions with other cells in immune and inflammatory responses (1, 2). Two homologous immunoglobulin family counter-receptors for LFA-1 have been discovered: the inducible intercellular adhesion molecule 1 (ICAM-1) (3–5) and constitutively expressed ICAM-2 (6). Development of a mAb to ICAM-2 allowed several previously LFA-1-dependent, ICAM-1-independent phenomena to be analyzed and suggested that a third ligand for LFA-1 existed (7). Binding of several cell types such as epithelial and endothelial cells to purified LFA-1 could be completely blocked with a combination of ICAM-1 and ICAM-2 mAb, whereas an ICAM-1–, ICAM-2–independent pathway of adhesion to LFA-1 existed on many lymphoid cell lines, including the T cell lymphoma cell line, SKW3 (7).

We now report on the production and characterization of a mAb, CBR-IC3/1, that, in conjunction with anti-ICAM-1 and anti-ICAM-2 mAbs, can completely inhibit adhesion of a variety of cell lines to purified LFA-1. Due to its functional role as a LFA-1 ligand, we have termed this novel molecule ICAM-3. The biochemical characteristics and cell distribution of ICAM-3 are distinct from those of either ICAM-1 or ICAM-2.

[1] *Abbreviations used in this paper:* LFA-1, lymphocyte function-associated molecule 1; ICAM, intercellular adhesion molecule.

## Materials and Methods

*Monoclonal Antibodies.* The following previously described murine mAbs to human antigens were used: TS2/16 (anti-CD29, IgG1) (8), RR1/1 (anti-ICAM-1, IgG1) (4), CBR-IC2/1 (anti-ICAM-2, IgG2a) (7), CBR-IC2/2 (anti-ICAM-2, IgG2a) (7), and X63 (nonbinding antibody, IgG1). All mAbs were titered and used at saturating concentrations (1:200 dilution of mAb ascites).

*Cell Culture.* The maintenance and growth conditions of the murine myeloma P3X63Ag8.653 and hybridomas have been described (7). The human lymphoid cell lines were grown in RPMI 1640 supplemented with 10% FCS, 5 mM glutamine, and 50 $\mu$g/ml gentamicin at 37°C and in the presence of 5% $CO_2$.

PBMC and neutrophils were obtained as described (7). Lymphocytes and monocytes were separated by cytometric analysis using forward and perpendicular light scatter, and their identity was confirmed by monocyte- and T cell–specific mAbs. Resting T cells were isolated from whole blood by plastic adherence and nylon wool filtration and were 91% $CD2^+$, while PHA blasts were generated by culturing the cells for 3 d in the presence of 10 $\mu$g/ml PHA (Sigma Chemical Co., St. Louis, MO).

*Development of ICAM-3 Hybridoma.* SKW3 cells were used to immunize 3–12-wk-old BALB/c female mice (Charles River Laboratories, Wilmington, MA). Immunizations ($10^5$–$10^6$ cells per intraperitoneal immunization) were given three times at 3-wk intervals. 3 d before fusion with the murine myeloma P3X63Ag8.653, the mice were injected both intraperitoneally and intravenously with $5 \times 10^5$ SKW3 cells. The protocol for fusion and subsequent maintenance of hybridomas was described previously (9). 600 hybridomas were screened for the ability to inhibit SKW3 binding to purified LFA-1 in the presence of mAb to ICAM-1 and ICAM-2. On this basis one mAb, CBR-IC3/1, was selected for further


J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/01/0185/06 $2.00
Volume 175 January 1992 185–190

analysis. It was cloned three times by limiting dilution and isotyped by ELISA using affinity-purified antibodies to mouse immunoglobulins (Zymed Immunochemicals, San Francisco, CA).

*Flow Cytometric Analysis.* Immunofluorescence flow cytometry was performed on an EPICS V analyzer (Coulter Diagnostics, Hialeah, FL) after staining cells with mAb-containing supernatants followed by FITC-conjugated anti-mouse antibody (Zymed Immunochemicals) as described (7). Since both primary and secondary mAbs were used at saturating concentrations, membrane antigen expression could be quantitated as a measure of mean fluorescence intensity (10).

*Surface Iodination.* Surface labeling of cells with $^{125}I$ was performed as described using Iodogen (Pierce Chemical Co., Rockford, IL) (11). Triton X-100 (1%) lysates were cleared with bovine IgG-coupled-Sepharose and then incubated with appropriate mAb-bound Sepharose for 2 h. Beads were washed and heated at 100°C in sample buffer containing 50 mM Tris, 1% SDS, and 1% 2-ME or 20 mM iodoacetamide. Samples were subjected to SDS 7% PAGE (12) and autoradiography with enhancing screens. Treatment of samples with *N*-Glycanase (Genzyme, Boston, MA) was as previously described (13); samples were incubated with 10 U/ml *N*-Glycanase for 18 h at 37°C.

*Purification of LFA-1.* LFA-1 was purified from JY lysates on TS2/4-Sepharose as described previously (14). The LFA-1 bound to TS2/4-Sepharose was eluted with 50 mM triethylamine (pH 11.5), 150 mM NaCl, 2 mM $MgCl_2$, and 1% octyl β-D-glucopyranoside. Samples were neutralized and stored frozen at −70°C.

*Adhesion Assay.* Adhesion of cells fluorescently labeled with 2',7'-*bis*-(2-carboxyethyl)-5 (and -6)-carboxyfluorescein (BCECF; Molecular Probes, Inc., Eugene, OR) to plates coated with purified LFA-1 was performed as previously described (7, 14, 15). Cells were pretreated with a 1:200 dilution of mAb ascites for 45 min at 4°C, and $10^5$ cells were transferred to each well. Cell lines adhered to solid phase LFA-1 for 1 h at 37°C, and nonadherent cells were removed by six aspirations with a 23-gauge needle. Lymphocytes and blasts were sedimented by centrifugation (30 *g* for 5 min) and incubated at 37°C for 30 min. Unbound lymphocytes and blasts were removed by flicking media from the plate eight times, with 100 μl added between each wash. Flicking was more effective for thorough removal of unbound T lymphocytes, which were more difficult to remove by aspiration because of their small size. Fluorescence was quantitated from the 96-well plates using a Pandex fluorescence concentration analyzer (Baxter Healthcare Corp., Mundelein, IL).

## Results and Discussion

To characterize the LFA-1–dependent, ICAM-1–, ICAM-2–independent pathway of adhesion, mAbs were raised to SKW3

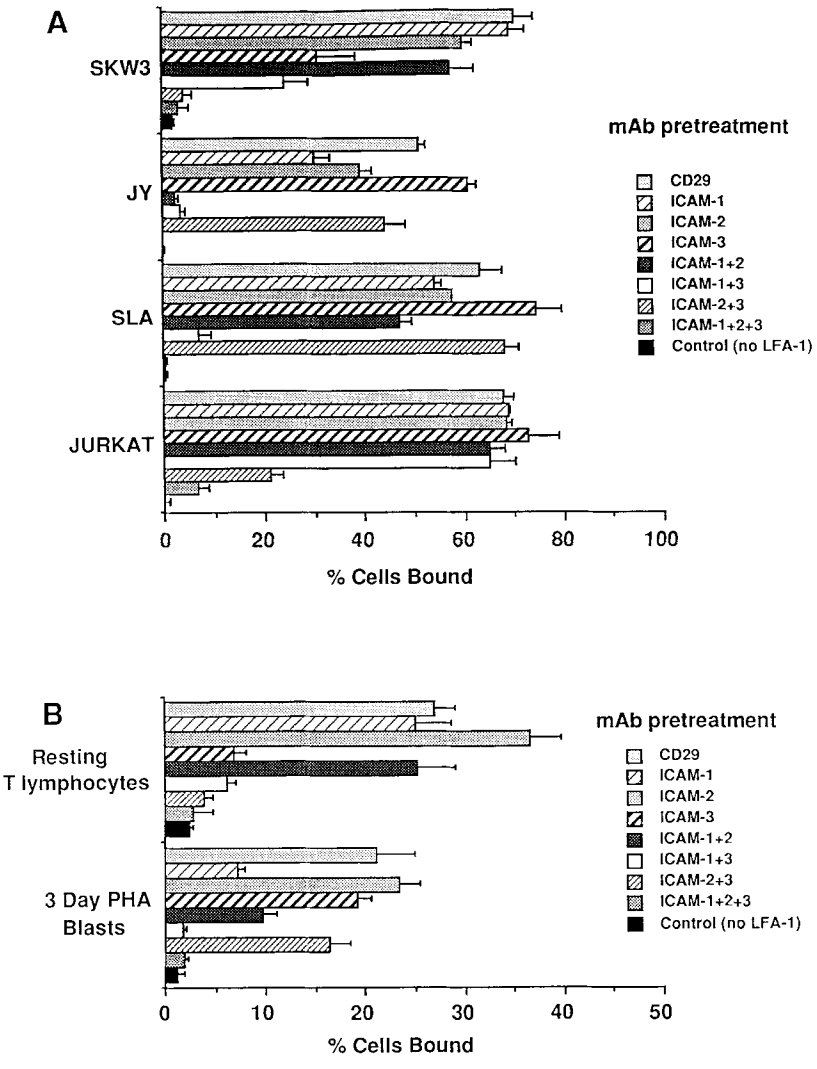


**Figure 1.** Adhesion of cell lines (*A*) and lymphocytes (*B*) to purified LFA-1 is accounted for by ICAM-1, ICAM-2, and ICAM-3. Cells were allowed to bind to LFA-1–coated microtiter wells in the presence of blocking mAbs specific for ICAM-1, ICAM-2, and ICAM-3. Site density of LFA-1 as determined by radioimmunoassay was 1100 sites/$\mu m^2$. Control wells were coated without LFA-1. mAbs used were TS2/16 (anti-CD29), RR1/1 (anti-ICAM-1), CBR-IC2/2 (anti-ICAM-2), and CBR-IC3/1 (anti-ICAM-3). One of four representative experiments is shown, and error bars indicate 1 SD.

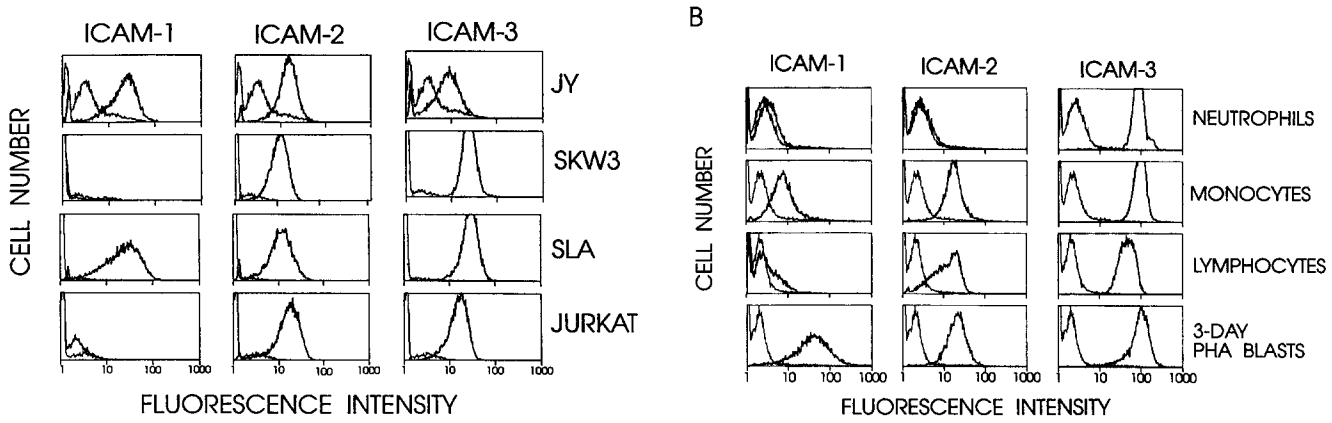


**Figure 2.** Flow cytometric analysis of ICAM-1, ICAM-2, and ICAM-3 expression. (*A*) Lymphoblastoid cell lines (*A*) or blood leukocytes and 3-d PHA activated lymphocytes (*B*) were labeled with saturating amounts of either mAb RR1/1 (anti-ICAM-1), mAb CBR-IC2/1 (anti-ICAM-2), mAb CBR-IC3/1 (anti-ICAM-3), or nonbinding control mAb X63 (*thin lines*), and were followed by FITC-anti-mouse immunoglobulin.

and were screened in combination with anti-ICAM-1 and anti-ICAM-2 mAbs for the ability to inhibit binding of this cell line to purified LFA-1. One mAb, CBR-IC3/1 (IgG1), was selected that completely inhibited this novel pathway of adhesion (Fig. 1 *A*). CBR-IC3/1 does not react with purified LFA-1 or COS cells transfected with either ICAM-1, ICAM-2, or LFA-1 cDNAs (data not shown), and it does react with the SLA cell line, which was derived from a LFA-1–deficient patient (16). The antigen recognized by CBR-IC3/1 is thus distinct from ICAM-1, ICAM-2, and LFA-1. By analogy to ICAM-1 and ICAM-2, whose names were based on their identification as ligands for LFA-1, we have designated this third counter-receptor ICAM-3; whether it also belongs to the Ig superfamily remains to be determined.

Adhesion of SKW3 to purified LFA-1 was only slightly inhibited by a combination of blocking anti-ICAM-1 and anti-ICAM-2 mAbs. When the anti-ICAM-3 mAb, CBR-IC3/1, was added alone, it significantly inhibited the adhesion, and this inhibition was complete when combined with blocking anti-ICAM-2 mAb (Fig. 1 *A*). Thus, adhesion of SKW3 to purified LFA-1 was mediated largely by ICAM-3 and also, in part by ICAM-2. In adhering to LFA-1, each of four cell lines utilized the three ICAMs to different degrees, as demonstrated by the different patterns of mAb inhibition (Fig. 1 *A*). The adhesion of the B lymphoblastoid cell line, JY, occurred through an ICAM-1 pathway with smaller contributions through the ICAM-2 and ICAM-3 pathways; inhibition was partial with ICAM-1 mAb and almost complete when combined with either ICAM-2 or ICAM-3 mAb. Another B lymphoblastoid cell line, SLA, utilized both ICAM-1 and ICAM-3, since adhesion was not inhibited by either ICAM-1 or ICAM-3 mAb alone and almost completely by the two mAbs together. The thymoma cell line, Jurkat, used both the ICAM-2 and ICAM-3 pathways of adhesion, with a small contribution by ICAM-1. There is considerable redundancy in use of ICAMs since for each of these cell lines, mAbs to at least two ICAMs were required to achieve substantial inhibition.

The pattern of distribution of ICAM-3 differed from that of ICAM-1 and ICAM-2 in several ways. Unlike ICAM-1 and ICAM-2, ICAM-3 was not expressed on either resting or stimulated endothelium (data not shown). This finding agrees with the observation that LFA-1–dependent binding of cells to both resting and stimulated endothelium was completely inhibited by a combination of mAbs to ICAM-1 and ICAM-2 (7). ICAM-3 was restricted to the hematopoietic lineage, being highly expressed on lymphoid and monocytic cell lines, with a few exceptions (Fig. 2 *A* and data not shown). In all cases examined thus far, expression of ICAM-3 was coordinate with the LFA-1-dependent, ICAM-1–, ICAM-2–independent pathway of adhesion. Cells binding LFA-1 solely through ICAM-1 and ICAM-2 did not express ICAM-3 (endothelium, Raji), while cell lines that bound weakly (JY, U937, Sup T) or strongly (SKW3, Jurkat, SLA) through this third pathway of adhesion had correspondingly low or high ICAM-3 surface expression. In all cases, the combination of all three anti-ICAM mAbs completely eliminated binding to LFA-1.

ICAM-3 differed markedly from ICAM-1 and ICAM-2 in its expression on leukocytes (Fig. 2 *B*). ICAM-3 was expressed at high levels on resting lymphocytes, monocytes, and neutrophils, whereas ICAM-1 and ICAM-2 were expressed much more weakly or were absent. Upon activation of lymphocytes with PHA, ICAM-3 expression increased two- to threefold, whereas expression of ICAM-1 was greatly increased (7, 17) (Fig. 2 *B*).

We tested the functional importance of the three ICAMs in adhesion of T lymphocytes to LFA-1 (Fig. 1 *B*). Resting lymphocytes were previously shown to bind strongly to purified LFA-1 (14), and we found that this binding is almost completely ICAM-3 dependent. After mitogenic activation with PHA, however, adhesion to LFA-1 occurred chiefly through ICAM-1, correlating with its increased surface expression, and in lesser degree through ICAM-3.

The relative affinity of the three ICAMs for LFA-1 can be examined by comparing their contributions to binding

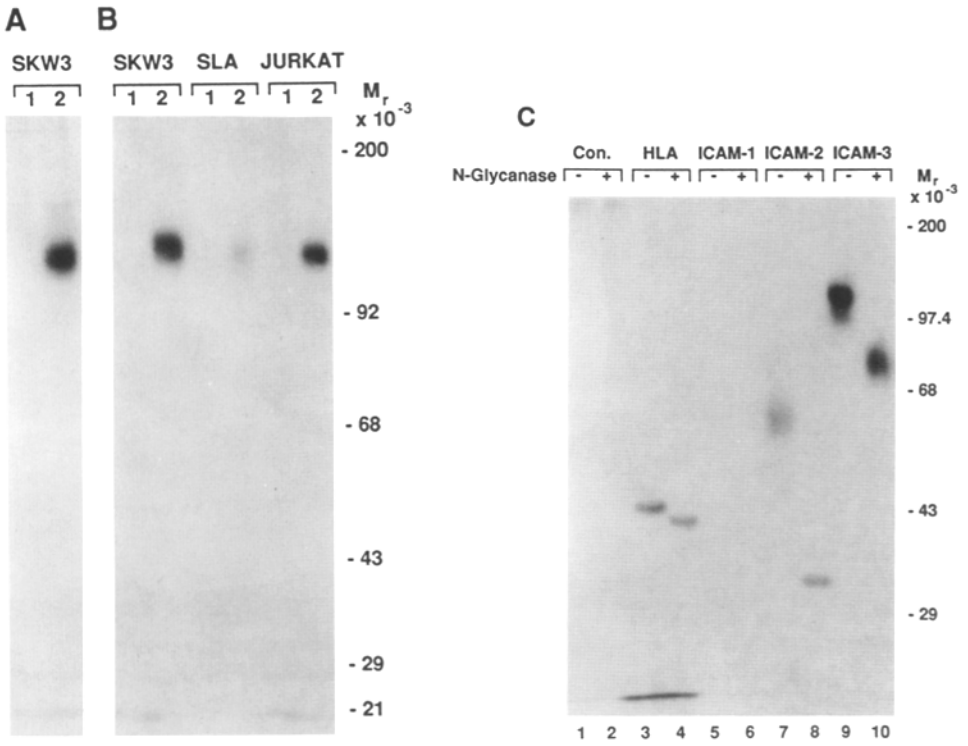


**Figure 3.** Immunoprecipitation of ICAM-3. (*A* and *B*) $^{125}$I-labeled cell lysates were immunoprecipitated with either (lanes *1*) nonbinding control X63 or (lanes *2*) mAb CBR-IC3/1, and subjected to SDS-PAGE under nonreducing (*A*) or reducing (*B*) conditions. (*C*) $^{125}$I-labeled SKW3 cell lysates were immunoprecipitated with either nonbinding control mAb X63, mAb W6/32 (anti-HLA-A,B,C), mAb RR1/1 (anti-ICAM-1), mAb CBR/IC2/1 (anti-ICAM-2), or mAb CBR-IC3/1 (anti-ICAM-3) and treated with (+) or without (−) *N*-glycanase before SDS-PAGE. All immunoprecipitates were analyzed by 7% SDS-PAGE and subjected to autoradiography; reduced molecular mass standards are shown to the right.

LFA-1 (Fig. 1) with their cell surface expression as measured by immunofluorescence flow cytometry (Fig. 2). This comparison revealed that ICAM-1 has the greatest affinity for LFA-1, and that ICAM-2 and ICAM-3 have similar, but lower, affinities. For instance, Jurkat cells expressed ICAM-2 and ICAM-3 at similar levels, and each contributed to binding to LFA-1. Where ICAM-2 expression was greater than that of ICAM-3, such as on JY cells, the ICAM-2 pathway of LFA-1 adhesion prevailed over ICAM-3. In contrast, SLA and SKW 3 expressed three- to fourfold more ICAM-3 than ICAM-2, and the ICAM-3 pathway of adhesion predominated over the ICAM-2 pathway. When ICAM-1 was expressed in comparable or somewhat lesser amount than ICAM-2 or ICAM-3, as was the case with SLA, JY, and the PHA-activated T cells, the ICAM-1 pathway of adhesion was dominant.

Immunoprecipitates of ICAM-3 from various $^{125}$I-labeled cell lines revealed a band of 124,000 $M_r$ under reducing conditions, with slightly increased mobility under nonreducing conditions (Fig. 3, *A* and *B*). Treatment with *N*-glycanase resulted in reduction of the ICAM-3 band to $M_r$ 87,000, indicating that ICAM-3, like ICAM-1 (17, 18) and ICAM-2 (7), is a highly glycosylated protein (Fig. 3 *C*). The biochemical characteristics, patterns of expression, and functional properties of ICAM-3 distinguish it from previously described adhesion molecules, including the human homing receptor LAM-1 (19), the inducible endothelial adhesion molecule VCAM-1 (20–22), and the VLA family of matrix receptors (23); no mAbs with similar cell distributions were found in the data bases from the third or fourth leukocyte workshop (24, 25).

The existence of three LFA-1 ligands suggests specialization for different aspects of LFA-1–dependent leukocyte interactions. ICAM-1 is basally expressed on endothelium and many epithelial cell types and is strongly induced in inflammation and immunity, where it is hypothesized to regulate cell localization (1) and facilitates recognition of specific antigens (26, 27). Since ICAM-2 is the predominant LFA-1 ligand on resting endothelium, this pathway of adhesion may have important consequences for normal recirculation of LFA-1–bearing lymphocytes through tissue endothelium (28–31). The finding that adhesion of resting T lymphocytes to LFA-1 occurs primarily via ICAM-3, combined with the fact that ICAM-3 is much better expressed than the other LFA-1 ligands on monocytes and resting lymphocytes, implies an important role in the initiation of immune responses. Recent studies have shown that B lymphocyte activation stimulates avidity of cell surface LFA-1 (32, 33) in a manner analagous to that reported for T lymphocytes (14, 34). Our studies predict that ICAM-3 on T lymphocytes would facilitate their interaction with antigen-presenting B lymphocytes. Furthermore, a role is suggested for LFA-1 ligand(s) other than ICAM-1 in both allogeneic and autologous mixed lymphocyte reactions (35) and in lysis by T cells of certain target cells (36).

The existence of multiple ICAMs may also have implication for therapy. ICAM-1 mAb is efficacious in vivo in prolonging renal (37) and cardiac (38) allografts. ICAM-3 mAb may be capable of inhibiting a distinctive and perhaps overlapping subset of immune responses in vivo, since it inhibits LFA-1-dependent adhesive interactions with a distinct subset of cell types.

Address correspondence to Dr. Timothy A. Springer, The Center for Blood Research and Department of Pathology, Harvard Medical School, 800 Huntington Avenue, Boston, MA 02115.

*Received for publication 13 September 1991.*

## References


1. Springer, T.A. 1990. Adhesion receptors of the immune system. *Nature (Lond.).* 346:425.
2. Kishimoto, T.K., R.S. Larson, A.L. Corbi, M.L. Dustin, D.E. Staunton, and T.A. Springer. 1989. The leukocyte integrins: LFA-1, Mac-1, and p150,95. *Adv. Immunol.* 46:149.
3. Marlin, S.D., and T.A. Springer. 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell.* 51:813.
4. Rothlein, R., M.L. Dustin, S.D. Marlin, and T.A. Springer. 1986. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J. Immunol.* 137:1270.
5. Dustin, M.L., D.E. Staunton, and T.A. Springer. 1988. Supergene families meet in the immune system. *Immunol. Today.* 9:213.
6. Staunton, D.E., M.L. Dustin, and T.A. Springer. 1989. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature (Lond.).* 339:61.
7. de Fougerolles, A.R., S.A. Stacker, R. Schwarting, and T.A. Springer. 1991. Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. *J. Exp. Med.* 174:253.
8. Sanchez-Madrid, F., A.M. Krensky, C.F. Ware, E. Robbins, J.L. Strominger, S.J. Burakoff, and T.A. Springer. 1982. Three distinct antigens associated with human T lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. *Proc. Natl. Acad. Sci. USA.* 79:7489.
9. Galfre, G., and C. Milstein. 1981. Preparation of monoclonal antibodies: strategies and procedures. *Methods Enzymol.* 73:3.
10. Kurzinger, K., T. Reynolds, R.N. Germain, D. Davignon, E. Martz, and T.A. Springer. 1981. A novel lymphocyte function-associated antigen (LFA-1): cellular distribution, quantitative expression, and structure. *J. Immunol.* 127:596.
11. Kishimoto, T.K., K. O'Connor, and T.A. Springer. 1989. Leukocyte adhesion deficiency: aberrant splicing of a conserved integrin sequence causes a moderate deficiency phenotype. *J. Biol. Chem.* 264:3588.
12. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.
13. Tarentino, A., C. Gomez, and T. Plummer. 1985. Deglycosylation of asparagine-linked glycans by peptide: *N*-glycosidase F. *Biochemistry.* 24:4665.
14. Dustin, M.L., and T.A. Springer. 1989. T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature (Lond.).* 341:619.
15. Dustin, M.L., J.G. Aguilar, M.L. Hibbs, R.S. Larson, S.A. Stacker, D.E. Staunton, A.J. Wardlaw, and T.A. Springer. 1989. Structure and regulation of the leukocyte adhesion receptor LFA-1 and its counter-receptors, ICAM-1 and ICAM-2. *Cold Spring Harbor Symp. Quant. Biol.* 54:753.
16. Hibbs, M.L., A.J. Wardlaw, S.A. Stacker, D.C. Anderson, A. Lee, T.M. Roberts, and T.A. Springer. 1990. Transfection of cells from patients with leukocyte adhesion deficiency with an integrin beta subunit (CD18) restores LFA-1 expression and function. *J. Clin. Invest.* 85:674.
17. Dustin, M.L., R. Rothlein, A.K. Bhan, C.A. Dinarello, and T.A. Springer. 1986. Induction by IL-1 and interferon, tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J. Immunol.* 137:245.
18. Staunton, D.E., S.D. Marlin, C. Stratowa, M.L. Dustin, and T.A. Springer. 1988. Primary structure of intercellular adhesion molecule 1 (ICAM-1) demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell.* 52:925.
19. Tedder, T.F., A.C. Penta, H.B. Levine, and A.S. Freedman. 1990. Expression of the human leukocyte adhesion/homing molecule, LAM-1: Identity with the TQ1 and leu-8 differentiation antigens. *J. Immunol.* 144:532.
20. Rice, G., J. Munro, and M. Bevilacqua. 1990. Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes: a CD11/CD18-independent adhesion mechanism. *J. Exp. Med.* 171:1369.
21. Carlos, T.M., B.R. Schwartz, N.L. Kovach, E. Yee, M. Rosso, L. Osborn, G. Chi-Rosso, B. Newman, R. Lobb, and J.M. Harlan. 1990. Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood.* 76:965.
22. Osborn, L., C. Hession, R. Tizard, C. Vassallo, S. Luhowskyj, G. Chi-Rosso, and R. Lobb. 1989. Direct cloning of vascular cell adhesion molecule 1 (VCAM-1), a cytokine-induced endothelial protein that binds to lymphocytes. *Cell.* 59:1203.
23. Hemler, M.E. 1990. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu. Rev. Immunol.* 8:365.
24. Gilks, W.R., D.J. Spiegelhalter, and S.P. Cobbold. 1988. Leukocyte Typing Database III. Oxford University Press, Oxford, England. 1–86.
25. Gilks, W.R., D.J. Spiegelhalter, and S.P. Cobbold. 1990. Leukocyte Typing Database IV. Oxford University Press, Oxford, England. 1–96.
26. Altmann, D.M., N. Hogg, J. Trowsdale, and D. Wilkinson. 1989. Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells. *Nature (Lond.).* 338:512.
27. Siu, G., S.M. Hedrick, and A.A. Brian. 1989. Isolation of the murine intercellular adhesion molecule 1 (ICAM-1) gene: ICAM-1 enhances antigen-specific T cell activation. *J. Immunol.* 143:3813.
28. Hamann, A., D.J. Westrich, A. Duijevstijn, E.C. Butcher, H. Baisch, R. Harder, and H.G. Thiele. 1988. Evidence for an accessory role of LFA-1 in lymphocyte-high endothelium interaction during homing. *J. Immunol.* 140:693.
29. Mackay, C.R., W.L. Marston, and L. Dudler. 1990. Naive and memory T cells show distinct pathways of lymphocytes recirculation. *J. Exp. Med.* 171:810.
30. Pals, S.T., A. Den Otter, F. Miedema, P. Kabel, G.D. Keizer, R.J. Scheper, and C.J.L.M. Meijer. 1988. Evidence that leu-

kocyte function-associated antigen-1 is involved in recirculation and homing of human lymphocytes via high endothelial venules. *J. Immunol.* 140:1851.
31. Nunoi, H., Y. Yanabe, S. Higuchi, H. Tsuchiya, J. Yamamoto, I. Matsuda, M. Naito, K. Takahashi, K. Fujita, M. Ushida, et al. 1988. Severe hypoplasia of lymphoid tissues in Mo1 deficiency. *Hum Pathol.* 19:753.
32. Dang, L.H., and K.L. Rock. 1991. Stimulation of B lymphocytes through surface Ig receptors induces LFA-1 and ICAM-1-dependent adhesion. *J. Immunol.* 146:3273.
33. Mourad, W., R.S. Geha, and T. Chatila. 1990. Engagement of major histocompatibility complex class II molecules induces sustained, lymphocyte function-associated molecule 1-dependent cell adhesion. *J. Exp. Med.* 172:1513.
34. van Kooyk, Y., P. van de Wiel-van Kemenade, P. Weder, T.W. Kuijpers, and C.G. Figdor. 1989. Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. *Nature (Lond.).* 342:811.
35. Bagnasco, M., G. Pesce, C. Pronzato, and G. Canonica. 1990. Functional involvement of the LFA-1/ICAM-1 adhesion system in the autologous mixed lymphocyte reaction. *Cell. Immunol. 128:362.*
36. Makgoba, M.W., M.E. Sanders, G.E. Ginther Luce, E.A. Gugel, M.L. Dustin, T.A. Springer, and S. Shaw. 1988. Functional evidence that intercellular adhesion molecule-1 (ICAM-1) is a ligand for LFA-1 in cytotoxic T cell recognition. *Eur. J. Immunol.* 18:637.
37. Cosimi, A.B., D. Conti, F.L. Delmonico, F.I. Preffer, S.-L. Wee, R. Rothlein, R. Faanes, and R.B. Colvin. 1990. In-vivo effects of monoclonal antibody to ICAM-1 (CD54) in non-human primates with renal allografts. *J. Immunol.* 144:4604.
38. Flavin, T., R. Rothlein, R. Faanes, K. Ivens, and V.A. Starnes. 1991. Monoclonal antibody against intercellular adhesion molecule (ICAM)-1 prolongs cardiac allograft survival in cynomologus monkeys. *Transplant. Proc.* 23:533.