Mac-1 ($\alpha_M\beta_2$, CR3, CD11b/CD18), together with LFA-1 ($\alpha_L\beta_2$, CD11a/CD18), $\alpha_X\beta_2$ (CD11c/CD18, CR4) and $\alpha_D\beta_2$, constitute the β_2 subfamily of integrin adhesion receptors (1). Neutrophils, monocytes, natural killer cells, and certain subsets of lymphocytes express Mac-1. Integrins are notorious for their capacity to recognize multiple ligands, and Mac-1 is its most promiscuous member. Protein ligands for Mac-1 include numerous extracellular matrix proteins (fibronectin, laminin, collagen, and elastase); counterreceptors of the immunoglobulin superfamily (ICAM-1 and ICAM-2); blood coagulation proteins (fibrinogen, Factor X, and kininogen); and the complement pathway product, iC3b. Indeed, Mac-1 may recognize proteins merely as a consequence of their denaturation. Nonprotein ligands (LPS, zymosan, β-glycans, heparins, and proteoglycans) further broaden the repertoire of Mac-1 ligands. In addition, a variety of microorganisms produce Mac-1 ligands as a means of subverting or bypassing host defense mechanisms (2).

Ligand engagement by Mac-1 initiates a variety of intracellular signaling events, which, in turn, regulate many leukocyte responses, including phagocytosis, degranulation, adhesion, migration, aggregation, expression of procoagulant activity, and adherence to microorganisms. Mac-1 also cooperates with other leukocyte receptors, FcRIIIB, FcRIIA, and the u-PAR, to execute specific responses (1). Excessive activation of Mac-1 can have deleterious effects, including tissue destruction, ischemia-reperfusion injury, and autoimmune diseases (3). Of all the proposed physiological and pathophysiological functions ascribed to Mac-1, its contributions to the inflammatory response are viewed as being particularly important in vivo. Mac-1, together with its sister β_2 integrin, LFA-1, are thought to play central roles in mediating the firm adhesion of leukocytes to endothelial cells, a critical step to the subsequent leukocyte transmigration.

Given the ubiquitous and extremely diverse nature of the Mac-1 ligands and its capacity to initiate a multitude of cellular responses, Mac-1 is surmised to play innumerable roles in leukocyte biology. Nevertheless, in vivo studies to support this premise are limited, and no selective genetic deficiency of Mac-1 has been reported. In this issue of *The Journal*, Lu and colleagues (4) address this information gap. They report the generation of the Mac-1–deficient mice, thus providing a direct testing ground for the functions of Mac-1 in vivo. After systematically verifying that the homologous recombination approach had yielded mice deficient of Mac-1, the authors focus on an evaluation of neutrophil function. Neutrophils isolated from the Mac-1–deficient mice show defective adherence to keyhole limpet hemocyanin– and fibrinogen-coated surfaces; are unable to phagocytose C3bi-coated particles; are incapable of homotypic aggregation; and exhibit a reduced adhesion-dependent respiratory burst and degranulation. Thus, many of the purported functions of Mac-1 now can be unequivocally assigned to the receptor. Surprisingly, however, neutrophil emigration into the peritoneum in response to two different inflammatory stimuli, thioglycollate injection or implantation of fibrinogen-coated discs, was not compromised in the Mac-1–deficient animals. Thus, the role of Mac-1 in neutrophil transmigration has been directly examined and could not be corroborated.

Lu et al. (4) support these findings on neutrophil transmigration by administering a blocking LFA-1 mAb to Mac-1–deficient and wild-type animals. The mAb had a major effect; neutrophil emigration was reduced by 78% in the deficient animals and 58% in the wild-type mice. Thus, the authors conclude that "LFA-1 is sufficient in mediating neutrophil emigration in Mac-1–deficient mice." Furthermore, the authors found that administration of a blocking mAb to Mac-1 did not reduce neutrophil emigration significantly in wild-type mice and did not accentuate the effect of the LFA-1 mAb. Overall, these results are consistent with those of Schmits et al. (5), who found that neutrophil migration into the peritoneum of LFA-1–deficient mice was reduced by 60%. Thus, LFA-1 plays a major role but is not fully responsible for neutrophil extravasation in the inflammatory models studied. This conclusion has been supported in rabbits as well; an LFA-1 mAb prevented cellular recruitment but an Mac-1 mAb did not (6).

A potential problem associated with deficient mouse models is that redundancy or adaptation may mask roles of specific proteins. This complexity was recently illustrated in the study of Doerschuk et al. (7), who found that neutrophil sequestration in a C5a-mediated lung injury was reduced substantially by P-selectin and ICAM-1 mAbs but was not altered in P-selectin–, ICAM-1–, or P-selectin/ICAM-1–deficient mice. Lu et al. (4) were not confronted with such a dilemma; the data with blocking mAbs and mutant mice were entirely consistent.

Does the study of Lu et al. (4) establish that Mac-1 is not involved in leukocyte transmigration? The answer to this question is "no." A take-home message from this study by Lu et al. is that data obtained with blocking mAbs to the β_2 integrins support studies in deficient mice and vice versa. Indeed, Mac-1 has been shown to contribute significantly to neutrophil adhesion and transmigration in in vivo models using mAbs (3); both LFA-1 and Mac-1 blocking mAbs have been required to eliminate neutrophil migration in several different model systems. Thus, the roles of Mac-1 and LFA-1 in neutrophil adhesion and transmigration appear to be complementary under many circumstances.

The functions of adhesion receptors depend on the cell type analyzed, the degree of cellular activation, and the experimental model studied. For example, Arnaout et al. (8) showed that an LFA-1 mAb significantly inhibited unstimulated monocyte adhesion to endothelial cells, while only minor inhibition was observed with an Mac-1 mAb. In contrast, upon stimulation of neutrophils with PMA, their adhesion was significantly inhibited by the Mac-1 mAb, but not by the LFA-1 mAb. Thus, Mac-1 may play different roles in different tissues and different injury models (3). The Mac-1–deficient mouse provides an opportunity to test these possibilities, and the crossbreeding of these animals with other deficient animals is an obvious approach to dissect the contributions of the various adhesion receptors to the inflammatory response.

J. Clin. Invest.

0021-9738/97/03/1145/02 $2.00
Volume 99, Number 6, March 1997, 1145–1146

From the work of Lu et al. (4), the contribution of Mac-1 to phagocytosis, homotypic aggregation, adhesion, and the respiratory burst has been clearly established. Is this the extent of Mac-1 functions? Of the numerous Mac-1 ligands, which of these interactions is important in vivo? With the successful development of the Mac-1–deficient mouse model, such questions can now be systematically attacked. There may be many additional surprises awaiting us regarding Mac-1 and its functions.

Edward F. Plow and Li Zhang
Joseph J. Jacobs Center for Thrombosis and Vascular Biology
The Cleveland Clinic Foundation

References

1. Springer, T.A. 1995. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Annu. Rev. Physiol.* 57:827–872.

2. Isberg, R.R., and G. Tran Van Nhieu. 1994. Binding and internalization of microorganisms by integrin receptors. *Trends Microbiol.* 2:10–19.

3. Albelda, S.M., C.W. Smith, and P.A. Ward. 1994. Adhesion molecules and inflammatory injury. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 8:504–512.

4. Lu, H., C.W. Smith, J. Perrard, D. Bullard, L. Tang, S.B. Shappell, M.L. Entman, A.L. Beaudet, and C.M. Ballantyne. 1997. LFA-1 is sufficient in mediating neutrophil emigration in Mac-1–deficient mice. *J. Clin. Invest.* 99:1340–1350.

5. Schmits, R., T.M. Kundig, D.M. Baker, G. Shumaker, J.J.L. Simard, G. Duncan, A. Wakeham, A. Shaninian, A. van der Heiden, M.F. Bachmann, et al. 1996. LFA-1–deficient mice show normal CTL responses to virus but fail to reject immunogenic tumor. *J. Exp. Med.* 183:1415–1426.

6. Rutter, J., T.J. James, D. Howat, A. Shock, D. Andrew, P. De Baetselier, J. Blackford, J.M. Wilkinson, G. Higgs, B. Hughes, and M.K. Robinson. 1994. The in vivo and in vitro effects of antibodies against rabbit β2-integrins. *J. Immunol.* 153:3724–3733.

7. Doerschuk, C.M., W.M. Quinlan, N.A. Doyle, D.C. Bullard, D. Vestweber, M.L. Jones, F. Takei, P.A. Ward, and A.L. Beaudet. 1996. The role of P-selectin and ICAM-1 in acute lung injury as determined using blocking antibodies and mutant mice. *J. Immunol.* 157:4609–4614.

8. Arnaout, M.A., L.L. Lanier, and D.V. Faller. 1988. Relative contribution of the leukocyte molecules Mo1, LFA-1, and p150,95 (LeuM5) in adhesion of granulocytes and monocytes to vascular endothelium is tissue- and stimulus-specific. *J. Cell. Physiol.* 137:305–309.