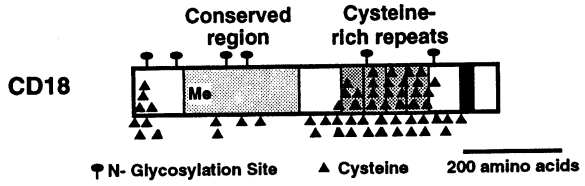
6. Corbi, A. L., Larson, R. S., Kishimoto, T. K., Springer, T. A., and Morton, C. C. *J. exp. Med.* **167**, 1597 (1988).
7. Corbi, A. L., Garcia-Aguilar, J., and Springer, T. A. *J. biol. Chem.* **265**, 2782 (1990).
8. Kishimoto, T. K., Larson, R. S., Corbi, A. L., Dustin, M. L., Staunton, D. E., and Springer, T. A. *Adv. Immunol.* **46**, 149 (1989).
9. Arnaout, M. A. *Immunol. Rev.* **114**, 145 (1990).
10. Stacker, S. A. and Springer, T. A. *J. Immunol.* **146**, 648 (1991).
11. Hogg, N., Takacs, L., Palmer, D. G., Selvendran, Y., and Allen, C. *Eur. J. Immunol.* **16**, 240 (1986).
12. Miller, L. J., Schwarting, R., and Springer, T. A. *J. Immunol.* **137**, 2891 (1986).
13. Freundenthal, P. S. and Steinman, R. M. *Proc. natl Acad. Sci., USA* **87**, 7698 (1990).
14. Keizer, G. D., Borst, J., Visser, W., Schwarting, R., de Vries, J. E., and Figdor, C. G. *J. Immunol.* **138**, 3130 (1987).
15. Postigo, A. A., Corbi, A. L., Sánchez-Madrid, F., and de Landazuri, M. O. *J. exp. Med.* **174**, 1313 (1991).
16. Caligaris-Cappio, F., Pizzolo, G., Chilosi, M., Bergui, L., Semenzato, G., Tesio, L., Morittu, L., Malavasi, F., Gobbi, M., Schwarting, R., Campana, D., and Janossy, G. *Blood* **66**, 1035 (1985).
17. Aklyma, H. and McGear, P. L. *Neuroimmunology* **30**, 81 (1990).
18. Miller, L. J., Bainton, D. F., Borregaard, N., and Springer, T. A. *J. clin. Invest.* **80**, 535 (1987).
19. Loike, J. D., Sodeik, B., Cao, L., Leucona, S., Weitz, J. I., Detmers, P. A., and Wright, S. D. *Proc. natl Acad. Sci., USA* **88**, 1044 (1991).
20. Keizer, G. D., te Velde, A. A., Schwarting, R., Figdor, C. G., and de Vries, J. E. *Eur. J. Immunol.* **17**, 1317 (1987).
21. Berton, G. *et al. J. cell Biol.* **118**, 1007 (1992).

AS5.4 CD18 cluster report

LILLI PETRUZZELLI and TIMOTHY A. SPRINGER



The CD18 cluster was designated during the Second Workshop and separated from CD11a in the Third Workshop. CD18 is also known as the β_2 subunit of the integrin family and is found on the cell surface of leucocytes complexed with either of three α subunits, CD11a, CD11b, or CD11c, to form the heterodimers, LFA-1, Mac-1, and p150,95, respectively [1]. LFA-1 is the receptor for three members of the Ig supergene family of proteins, ICAM-1 (CD54), ICAM-2 (CD102), and ICAM-3 (CD50) [2–4]. Mac-1 and p150,95 bind to ICAM-1, fibrinogen, and iC3b [5–9]. The importance of CD18 in the inflammatory function of leucocytes has been elucidated through the study of leucocyte adhesion deficiency patients who do not express any of the heterodimeric molecules on their cell surface and who were subsequently found to have a molecular defect in the CD18 molecule [10,11]. The monoclonal antibodies (mAb) clustered within this group are S123 (6.7), S137 (CBRM1/19), S147 (MAY.017), S153 (CBR LFA-1/7), S155 (CBR LFA-1/2), S162 (TS1/18), S164 (CLB-LFA1/1), and S166 (L130).

Molecular characterization

The molecular structure of CD18 has been defined [12]. It is a transmembrane protein of M_r 95 000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions [1]. Its features include an extracellular domain that is rich in cysteine residues, a transmembrane region, and a short cytoplasmic tail. The cytoplasmic region of CD18 is essential for adhesiveness of LFA-1 for ICAMs [13,14]. CD18 maps to chromosome 21 [15]. All antibodies submitted immunoprecipitated the 95-kDa species in complex with CD11, except for S137. The immunoblot results for mAb S153 and S155 were submitted by the donor.

Flow cytometry

LFA-1 is expressed on lymphocytes, monocytes, and more weakly on neutrophils, whereas Mac-1 and p150,95 are found on neutrophils, monocytes, and some activated lymphocytes [5]. The antibodies were clustered based on their reactivity with transfectants of CHO and K-562 cells expressing CD11a/CD18, CD11b/CD18, and CD11c/CD18. The antibodies reacted with transfected cell lines expressing the appropriate complex with the exception of mAb S137 (CBRM1/19), which reacted with CHO but not K-562 transfectants. Flow cytometry of neutrophils, lymphocytes, and T- and B-cell lines from human sources revealed cell surface staining by all of

the antibodies in this panel except for S137 (CBRM1/19). The S137 (CBRM1/19) epitope was enhanced when the monocytic cell THP-1 was stimulated with phorbol esters for 24 h and was present on THP-1 foam cells and on U937 cells transfected with c-*fgr*. CBRM1/19 has previously been shown to recognize activated but not resting neutrophils and recognized an activation epitope on CD18 [M. Diamond and T. Springer, personal communication]. In addition to the human cell lines examined in this panel, the antibodies were also tested for their reactivity on porcine, bovine and cynomologus monkey leucocytes. S123 (6.7) was the only antibody to recognize all three species. S147 (MAY.017) and S166 (L130) reacted with porcine and monkey cells, S155 (CBR LFA-1/2) recognized bovine and monkey cells, S153 (CBR LFA-1/7) stained monkey neutrophils, and S162 (TS1/18) reacted with porcine cells.

Functional studies

LFA-1 and Mac-1 have been shown to play a central role in homotypic and heterotypic cellular adhesion in immune and inflammatory responses [16]. Cellular stimulation activates adhesiveness of LFA-1 and Mac-1 independently of any change in surface density [17,18]. The β-subunit cytoplasmic domain is required for the inside–out signalling of adhesiveness [13,14]. In addition, the increased avidity of LFA-1 and Mac-1 for ligands can be mimicked by antibodies directed against the extracellular domain of CD18 [19; L. Petruzzelli and T. Springer, manuscript in preparation]. The antibodies in this group were examined for their effects on homotypic aggregation, ligand-binding, cytotoxic cell–cell interactions, and HIV-induced syncytia formation. Five members of this group, S123 (6.7), S147 (MAY.017), S162 (TS1/18), S164 (CLB-LFA1/1), and S166 (L130), were able to inhibit aggregation of both lymphoblastoid B-cell lines and granulocytes, and this correlated with the ability of the mAb to block binding to ICAM-1 or fibrinogen. This same group of antibodies was also inhibitory in the cytotoxic killing assay. mAb S155 (CBR LFA-1/2) was able to induce marked stimulation of binding to ICAM-1. HIV-induced syncytia formation was inhibited by S147 (MAY.017).

Immunohistochemistry

All antibodies in this CD cluster, with the exception of S137 (CBRM1/19), stained lymphocytes, granulocytes, and macrophages in tonsil; lymph nodes; lymph nodes with follicular hyperplasia; liver; and sections of small bowel with and without Crohn's disease. Skin was negative with the mAb except for S153 (CBR LFA-1/7) which stained Langerhan's cells. There was generally increased staining of inflammatory tissues. All antibodies reacted with synovial membranes from patients with and without rheumatoid arthritis.

References

1. Sánchez-Madrid, F., Nagy, J., Robbins, E., Simon, P., and Springer, T. A. *J. exp. Med.* **158**, 1785 (1983).
2. Marlin, S. D. and Springer, T. A. *Cell* **51**, 813 (1987).
3. Staunton, D. E., Dustin, M. L., and Springer, T. A. *Nature* **339**, 61 (1989).
4. de Fougerolles, A. R., Stacker, S. A., Schwarting, R., and Springer, T. A. *J. exp. Med.* **174**, 253 (1991).
5. Larson, R. S. and Springer, T. A. *Immunol. Rev.* **114**, 181 (1990).
6. Diamond, M. S., Staunton, D. E., de Fougerolles, A. R., Stacker, S. A., Garcia-Aguilar, J., *et al. J. Cell Biol.* **111**, 3129 (1990).
7. Wright, S. D., Weitz, J. I., Huang, A. J., Levin, S. M., Silverstein, S. C., *et al. Proc. natl Acad. Sci., USA* **85**, 7734 (1988).
8. Altieri, D. C. and Edgington, T. S. *J. biol. Chem.* **263**, 7007 (1988).
9. Stacker, S. A. and Springer, T. A. *J. Immunol.* **146**, 648 (1991).
10. Anderson, D. C. and Springer, T. A. *Ann. Rev. Med.* **38**, 175 (1987).
11. Kishimoto, T. K., Hollander, N., Roberts, T. M., Anderson, D. C., and Springer, T. A. *Cell* **50**, 193 (1987).
12. Kishimoto, T. K., O'Connor, K., Lee, A., Roberts, T. M., and Springer, T. A. *Cell* **48**, 681 (1987).
13. Hibbs, M. L., Xu, H., Stacker, S. A., and Springer, T. A. *Science* **251**, 1611 (1991).
14. Hibbs, M. L., Jakes, S., Stacker, S. A., Wallace, R. W., and Springer, T. A. *J. exp. Med.* **174**, 1227 (1991).
15. Corbi, A. L., Larson, R. S., Kishimoto, T. K., Springer, T. A., and Morton, C. C. *J. exp. Med.* **167**, 1597 (1988).
16. Springer, T. A. *Nature* **346**, 425 (1990).
17. Dustin, M. L. and Springer, T. A. *Nature* **341**, 619 (1989).
18. Diamond, M. S. and Springer, T. A. *J. Cell Biol.* **120**, 545 (1993).
19. Robinson, M. K., Andrew, D., Rosen, H., Brown, D., Ortlepp, S., *et al. J. Immunol.* **148**, 1080 (1992).