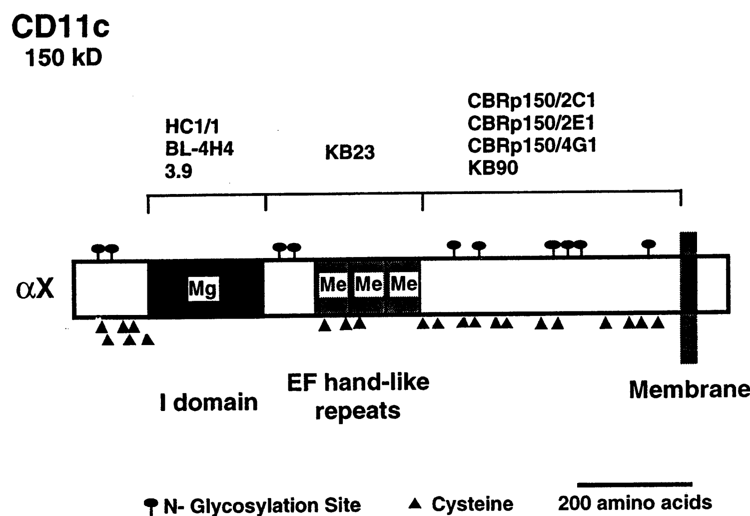
7. Arnaout, M. A., Remold-O'Donnell, E., Pierce, M. W., Harris, P., and Tenen, D. G. *Proc. natl Acad. Sci., USA* **85**, 2776 (1988).
8. Pytela, R. *EMBO J.* **7**, 1371 (1988).
9. Corbi, A. L., Larson, R. S., Kishimoto, T. K., Springer, T. A., and Morton, C. C. *J. exp. Med.* **167**, 1597 (1988).
10. Shelley, C. S. and Arnaout, M. A. *Proc. natl Acad. Sci., USA* **88**, 10525 (1991).
11. De la Hera, A., Alvarez-Mon, M., Sánchez-Madrid, F., Martínez-A, C., and Durantez, A. *Eur. J. Immunol.* **18**, 1131 (1988).
12. McFarland, H. I., Nahill, S. R., Maciaszek, J. W., and Welsh, R. M. *J. Immunol.* **149**, 1326 (1992).
13. Kishimoto, T. K., Larson, R. S., Corbi, A. L., Dustin, M. L., Staunton, D. E., and Springer, T. A. *Adv. Immunol.* **46**, 149 (1989).
14. Arnaout, M. A. *Immunol. Rev.* **114**, 145 (1990).
15. Miller, L. J., Bainton, D. F., Borregaard, N., and Springer, T. A. *J. clin. Invest.* **80**, 535 (1987).
16. Carlos, T. M. and Harlan, J. M. *Immunol. Rev.* **114**, 1 (1990).

AS5.3 CD11c cluster report

JOHN LUK and TIMOTHY A. SPRINGER



The leucocyte integrin CD11c/CD18 (p150,95, integrin $\alpha^x\beta_2$, complement receptor type 4 or CR4) was first biochemically identified as a protein containing a 150 kDa α^x subunit that was non-covalently associated with the 95 kDa β subunit common to Mac-1 and LFA-1 [1]. The antigen defined with mAb S-HCL3 as a marker of hairy leukaemic cells [2] was later shown to be identical to p150,95 [3,4]. Monoclonal antibodies (mAb) specific for the p150,95 $\alpha^x\beta_2$ integrin heterodimer are specific for its α^x or CD11c subunit. The CD11c cluster was established in the Third Workshop as defined by four different antibodies, KB23, 3.9, Ki-M1, and BU15. These anti-α^x mAb immunoprecipitated glycoprotein bands of 150/95 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained tissue macrophages in most types of tissues such as tonsil and skin.

Ten antibodies were submitted as CD11c mAb to the Adhesion Section of the Fifth Workshop and five of them were from previous Workshops, including S138 (BL-4H4), S143 (BU15), S144 (3.9), S157 (KB23), and S171 (S-HCL3). Antibody S171 (S-HCL3) was also studied in the NK panel (NK17) under its other name, Leu M5 [4]. Clustering of these

antibodies was based on reactivity with LFA-1, Mac-1, and p150,95 transfectants studied by flow cytometry. In addition to the above previously clustered mAb, all of the other submitted CD11c mAb, S124 (HC1/1), S133 (CBRp150/2C1), S135 (CBRp150/2E1), S136 (CBRp150/4G1), and S156 (KB90), were clearly clustered as CD11c mAb.

Molecular cloning

A human CD11c cDNA has been cloned and characterized [5] and the mRNA is 4.7 kb. The translated molecule is a typical transmembrane glycoprotein of 1163 residues (see the introductory diagram). It contains a short signal sequence, a large domain of 1088 residues, a transmembrane region, and a short cytoplasmic tail of 35 residues. The amino-terminal extracellular domain contains an inserted or I-domain (187 residues) not found in many other integrin α subunits, and three tandem putative divalent-cation-binding, EF hand-like repeats. The human α^x gene, located on chromosome 16, band p11–p13.1 [6], spans about 30 kb, and consists of 31 exons grouped in five clusters. Each of the three putative divalent-cation-binding sites is encoded by a separate exon, while the I-domain is distributed over four exons [7].

Immunohistochemistry

Tissue expression of CD11c is restricted to leucocytes, and is mainly on myeloid cells with the highest expression on macrophages. CD11c is also a marker for hairy cell leukaemia [8,9]. Blind panel studies of a reference CD11c mAb, S136 (CBRp150/4G1), showed restricted expression of α^x in macrophages by immunostaining of normal skin, psoriatic skin, tonsil, and melanoma. No staining of other cell types such as epidermis, Langerhans cells, melanocytes, endothelial cells, or T and B cells was observed. Immunochemical studies on sperm in the blind panel by Ziegler demonstrated that the anti-α^x mAb, CBRp150/4G1 stained about 11–75 per cent of spermatozoa, albeit weakly, with specificity against the midpiece. In contrast, mAb to CD11a, CD11b, and CD18 were negative on sperm.

Epitope analysis and transfection studies

All of the assigned CD11c antibodies strongly stained transfected cells expressing CD11c/CD18, but not transfected cells expressing CD11a/CD18 or CD11b/CD18. The epitopes recognized by these mAb were localized by flow cytometry of CHO cell lines stably transfected with CD11b × CD11c α subunit chimeras (see introductory diagram) [10; Luk *et al.*, AS5.9].

Cellular expression

The cellular expression of CD11c antibodies was mainly observed on macrophages, monocytes, granulocytes, NK cells [3,4,11,12], dendritic cells [13], activated T and B lymphocytes [14,15], hairy leukaemia cells [2], chronic lymphocytic leukaemia cells [16], and microglia [17]. In addition to cell surface expression, p150,95 is present in a large intracellular pool in granulocytes and monocytes, and rapid mobilization of this cytoplasmic store to the cell surface is induced by inflammatory mediators [3,12,18]. Activation of B lymphocytes with phorbol esters for several days upregulates p150,95 surface expression [15]. Following extravasation *in vivo*, p150,95 is upregulated during differentiation of peripheral blood monocytes into tissue macrophages [11,12]. Pocsik *et al.* [unpublished Workshop report] showed that HIV-1 *tat* gene could induce upregulation of surface expression of CD11c in a human B-lymphoblastoid cell line, Raji.

Functional studies

p150,95 mediates monocyte/granulocyte adhesion to endothelium during inflammatory responses [10], fibrinogen binding [19], neutrophil adhesion to serum-coated surfaces, and chemotaxis and adhesion of peripheral blood monocytes [20]. Recent studies have implicated p150,95 in B-cell activation and the respiratory burst in granulocytes and in cytotoxic T-lymphocyte (CTL)-mediated killing [14,15,21].

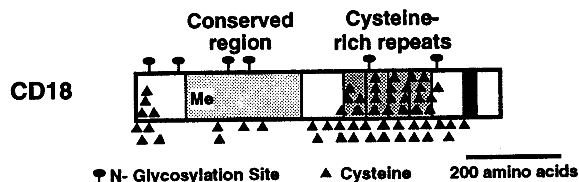
References

1. Sánchez-Madrid, F., Nagy, J., Robbins, F., Simon, P., and Springer, T. A. *J. exp. Med.* **158**, 1785 (1983).
2. Schwarting, R. H., Stein, H., and Wang, C. Y. *Blood* **65**, 974 (1985).
3. Springer, T. A., Miller, L. J., and Anderson, D. C. *J. Immunol.* **136**, 240 (1986).
4. Lanier, L. L., Arnaout, M. A., Schwarting, R., Warner, W. L., and Ross, G. D. *Eur. J. Immunol.* **15**, 713 (1986).
5. Corbi, A. L., Miller, L. J., O'Connor, K., Larson, R. S., and Springer, T. A. *EMBO J.* **6**, 4023 (1987).

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**, 313[illegible] (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., Berguis, 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