AS5.2 CD11b cluster report

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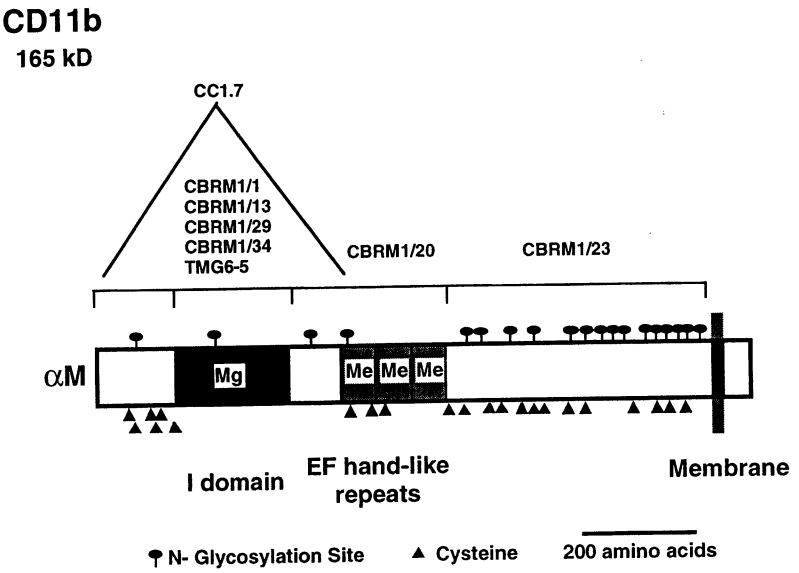

CD11b (Mac-1, Mo1, OKM1, complement receptor type 3 (CR3)) belonging to the leucocyte integrin family, was one of the earliest integrin molecules recognized. It was initially identified in the mouse [1] and later in the human [2–4] as a marker on myeloid cells. CD11b is the integrin α subunit (αM) of Mac-1 that is expressed non-covalently associated with the CD18 subunit (integrin β_2) as a heterodimer. CD11 was preliminarily clustered in the First Workshop as characterized by three different monoclonal antibodies (mAb), B2.12, Mo1, and M522, that reacted with mature cells of the myelomonocytic series but not with most immature cells. During the Third Workshop in 1986 the CD11b cluster was established with two mAb, Mo1 (Todd) and 44 (Hogg), that were found to be specific for CR3, the receptor for the complement component iC3b. These mAb reacted mainly with circulating monocytes and granulocytes plus dendritic reticulum cells.

Ten antibodies were submitted as CD11b mAb to the Adhesion Section in this Workshop. Eight of these mAb, S127 (CBRM1/13), S128 (CBRM1/1), S129 (CBRM1/20), S130 (CBRM1/23), S131 (CBRM1/29), S132 (CBRM1/34), S172 (CC1.7), and S174 (TMG6-5), were clustered as CD11b mAb based upon our specificity tests with transfectants expressing the LFA-1, Mac-1, and p150,95 antigens. In addition, two mAb submitted as possibly to integrins, S232 (PEN 2) (S232) and S231 (PEN 3), belong to the CD11b cluster.

Molecular cloning

cDNA clones encoding both the human and murine CD11b antigens have been isolated and sequenced [5–8], and the mRNA are 4.8 and 6.0 kb, respectively. The predicted human αM amino-acid sequence is 1153 residues long and contains a signal sequence of 16 residues, and a short cytoplasmic tail of 24 residues (see the diagram at the beginning of this chapter). The amino-terminal extracellular domain contains seven internal homologous repeats with an inserted or I domain of 187 residues found between repeats II and III, three divalent metal cation binding sites in repeats V–VII, and 19 potential N-linked glycosylation sites. Like the other β_2 integrin α subunits, the human αM gene is located on chromosome 16,

band p11–p13.1 [9]. The CD11b promoter region has been characterized and contains a single transcription initiation site. The 5′ flanking region, upstream of this site contains AP-1, AP-2, and AP-3-like binding sites, four *Alu* repeats, and an $(AC)_{16}$ sequence. The region 242 bp upstream and 71 bp downstream of the initiation site are sufficient to direct tissue and cell-specific expression of CD11b *in vitro* [10].

Immunohistochemistry

Tissue expression of CD11b is restricted to leucocytes, and mainly to myeloid and natural killer cells. Blind panel studies of two CD11b mAb, S131 (CBRM1/29) and S232 (PEN 2), showed by immunostaining of normal skin, psoriatic skin, tonsil, and melanoma that expression of the αM subunit was restricted to macrophages. No staining to other cell types, such as epidermis, Langerhans cells, melanocytes, endothelial cells, or T and B cells, was scored. Although there is little expression on lymphocytes *in vivo*, CD11b expression has been found on some Ly-1$^+$ peritoneal B lymphocytes [11], activated and virus-specific memory CD8$^+$ cytotoxic T lymphocytes [12], and on HIV-1 infected B-lymphoblastoid cells [Pocsik *et al.*, unpublished Workshop report].

Epitope analysis and transfection studies

All eight clustered CD11b mAb stained CHO cells transfected with CD11b/CD18 but not cells transfected with CD11a/CD18 or CD11c/CD18. Further epitope mapping was carried out using chimeric CD11b × CD11c α subunits coexpressed with CD18 in CHO cells. Results from flow cytometry suggested that mAb S127 (CBRM1/13), S128 (CBRM1/1), S131 (CBRM1/29), S132 (CBRM1/34), and S174 (TMG6-5) were directed against the I domain, and that S130 (CBRM1/23) bound to the extracellular C-terminal region [Luk *et al.*, AS5.9]. S172 (CC1.7) may recognize both the divalent cation-binding repeats and N-terminal region, and S129 (CBRM1/20) may recognize the divalent cation-binding repeats (see introductory diagram).

Cellular expression

CD11b antigen is expressed on monocytes, macrophages, granulocytes, NK cells, and phorbol myristate acetate (PMA)-induced myeloid cell lines [13,14]. Increased surface expression of αM was observed after differentiation or maturation of monocytic and myelomonocytic cell lines. CD11b expression on neutrophils and monocytes is upregulated by inflammatory stimuli such as interleukin-1 (IL-1), granulocyte–macrophage colony-stimulating factor (GM-CSF), and γ-interferon (IFN-γ). In response to chemoattractants such as formyl-Met-Leu-Phe (fMLP), C5a, and leukotriene B4, CD11b/CD18 stored in intracellular vesicles is rapidly mobilized to the cell surface [13–16]. Pocsik *et al.* [unpublished Workshop report] showed that the HIV-1 *tat* gene could induce upregulation of surface expression of CD11b (detected by mAb CBRM1/23) in a human B-lymphoblastoid cell line, Raji.

Functional studies

Mac-1 is a multifunctional receptor that binds an array of ligands including ICAM-1 (via its third Ig-domain), fibrinogen, iC3b, and coagulation factor X. Ligand binding to Mac-1 is divalent-cation-dependent. Mac-1 mediates adherence of polymorphonuclear leucocytes (PMN) and monocytes to endothelium and, subsequently, PMN extravasation to sites of inflammation. It also mediates neutrophil homotypic aggregation, chemotaxis, and phagocytosis of iC3b-coated particles [13,14,16]. Functional studies using different CD11b antibodies indicated that CBRM1/13, CBRM1/1, CBRM1/29, CBRM1/34, and TMG 6-5 (all of which are I-domain-specific mAb) were able to block phorbol ester-induced homotypic adhesion of PMN [Salcedo and Patarroyo, unpublished Workshop report].

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AS5.3 CD11c cluster report

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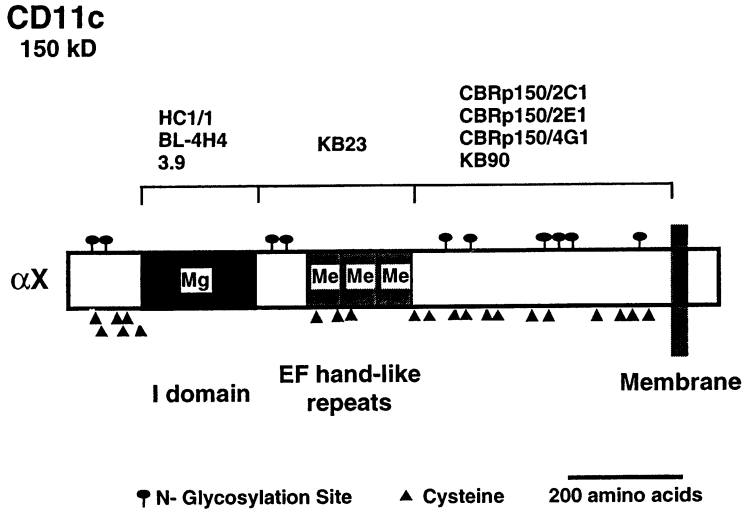

The leucocyte integrin CD11c/CD18 (p150,95, integrin $\alpha^x\beta_2$, complement receptor type 4 or CR4) was first biochemically identified as a protein containing a 150 kDa α^x subunit that was non-covalently associated with the 95 kDa β subunit common to Mac-1 and LFA-1 [1]. The antigen defined with 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