AS4.1 CD50 (ICAM-3) cluster report

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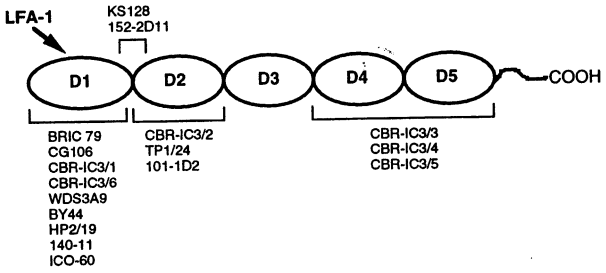
CD50 (ICAM-3)

Mr 120 kD



CD50 was provisionally clustered as CDw50 in the Fourth Workshop, on the basis of two monoclonal antibodies (mAb) from Vilella's laboratory, 101-1D2 and 140-11, submitted to the Fifth Workshop as S115 and S114, respectively. CDw50 was described as a 110-kDa leucocyte-specific antigen of unknown function. In the subsequent 4 years, ICAM-3 was described, found to be identical to CDw50 [1–3], and was independently cloned by three groups [4–6]. Improved characterization in the current Workshop has allowed the CDw50 designation to be changed to CD50. Sixteen mAb in Subpanel 4 of the Adhesion Structure Workshop are directed against CD50 and one mAb S121 (ICO-60), in Subpanel 5 was found to recognize CD50 as well. mAb S114 (140-11) was also studied in the T-cell panel. Many of the studies performed as part of the Fifth Workshop addressed the localization and characterization of LFA-1 binding sites and epitopes on ICAM-3 (CD50), function in HIV-induced syncytia formation, and a possible signalling role in the regulation of T-cell aggregation or adhesion.

Cellular expression

Histochemical analysis of lymphoid, gut, and other tissues [Autschbach *et al.*, AS2.5; Koretz *et al.*, AS4.16; Krajewski *et al.*, AS10.10; unpublished Workshop studies by Cerf-Bensussan, Cordell, Malizia, Staquet, and Timens] confirmed that CD50 expression is limited to leucocytes and some cells of probable haemopoietic origin, such as dermal Langerhans cells [Staquet, unpublished Workshop study]. Flow cytometric analysis by many participants confirmed the leucocyte-specific distribution of CD50.

Immunohistochemistry

Immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of surface-iodinated protein [Campanero *et al.*, AS4.7; Staquet *et al.*, unpublished Workshop study] as well as SDS-PAGE of S087 (CBR-IC3/1) immunoaffinity-purified CD50 protein [deFougerolles and Springer AS4.6; Klickstein *et al.*, AS4.8] confirm a single polypeptide chain with an M_r of 120 kDa, as previously published [1–3]. CD50 has been reported to be phosphorylated on serine residues upon treatment of T cells with various stimuli [2], which was confirmed in this Workshop, whereas CD50 protein, immunoprecipitated from granulocytes labelled with ^{32}P, migrated at 160–190 kDa [Skubitz *et al.*, unpublished Workshop study], which may reflect a different mobility of the phosphorylated form. CD50 is heavily glycosylated and treatment of purified ICAM-3 with N glycanase decreases the apparent M_r from 120 to 65 kDa, similar to the predicted M_r of the polypeptide chain [3].

Molecular cloning

CD50 was cloned by immunoaffinity protein purification and synthesis of degenerate oligonucleotides [4], by expression using mAb [5], and as an anonymous ICAM, ICAM-R, related to ICAM-1 and -2 with a degenerate polymerase chain reaction (PCR) primer technique [6]. All three groups predicted a 518-residue, amino-terminal extracellular region with five Ig superfamily domains, a 25-amino-acid transmembrane domain, and a 37-residue cytoplasmic domain (see introductory diagram). The predicted M_r of the polypeptide chain is 57 kDa and there are 15

consensus N-linked glycosylation sites. CD50 is most closely related to CD54 (ICAM-1) and CD102 (ICAM-2), with 52 and 34 per cent sequence identity in the extracellular regions, respectively [4–6]. The human CD50 gene is located on chromosome 19 and the coding sequence spans 12 kb of genomic DNA [Klickstein *et al.*, unpublished].

Transfectant and epitope analysis

The specificity of all CD50 mAb was confirmed with transfectants in three laboratories [Klickstein and Springer, AS4, Table 1]. Epitope mapping studies were performed employing immunoglobulin superfamily domain (IgSF) deletions of CD50, site-directed mutagenesis, and mAb cross-blocking [Klickstein and Springer, AS4, Table 1].

Function

A subset of IgSF domain 1 mAb stimulated aggregation of SKW3 cells [deFougerolles and Springer, AS4.6; Klickstein *et al.*, AS4.8], T cells [Campanero *et al.*, AS4.7; Bernard *et al.*, AS4.12], or U937 cells (Ikewaki). Interestingly, in the case of some T cells and T-cell-lines, this aggregation was not blocked with mAb to CD11a or CD18, suggesting a novel adhesion pathway. Only domain 1 mAb blocked binding of CD50-bearing cells to purified LFA-1 on plastic [Klickstein *et al.*, AS4.8; Holness *et al.*, unpublished Workshop report]; however, 101-1D2, a domain 2 mAb, was reported to block binding of NK1-L16 stimulated T cells to L cells expressing CD50 [Binnerts *et al.*, AS5.7]. A combination of mAb to domain 1 and 2 is required for complete inhibition of CD50-dependent cell–cell adhesion [deFougerolles and Springer, AS4.6]. A subset of CD50 IgSF domain 1 mAb were found to significantly inhibit HIV-induced syncytia formation of MOLT-4 cells [Ida *et al.*, AS5.8], while selected mAb recognizing other epitopes blocked to a lesser degree. A CD50 domain 1-CD21 chimera acquired domain 1 epitopes and mediated LFA-1 binding of transfected cells, proving that CD50 IgSF domain 1 is necessary and sufficient for binding to LFA-1 [Klickstein *et al.*, AS4.8]; however, the possibility of contributions from domain 2 to binding affinity has not been ruled out.

References

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