AS1.4 CD58 cluster report

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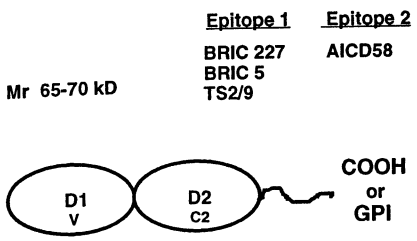
CD58 (LFA-3)



LFA-3 (CD58) was initially defined with mAb S024 (TS2/9) that blocked antigen-specific T-cell-mediated killing [1]. LFA-3 was subsequently found to be a counterreceptor for CD2 and the ligand on erythrocytes responsible for rosetting with T lymphocytes [2]. CD58 was clustered in the Fourth Workshop on the basis of three monoclonal antibodies (mAb) submitted by three laboratories. Four CD58 mAb were submitted to the Fifth Workshop, S029 (AICD58), NK15 (L306), S002 (BRIC5), and S024 (TS2/9).

Cellular distribution and immunochemistry

mAb to CD58 react with nearly all haemopoietic cells and many other cell types, including endothelium [2]. CD58 is a 60–70 kDa, heavily glycosylated protein [1,3]. The M_r is reduced to 26 kDa upon N-glycanase treatment [3]. The COOH terminal region is alternatively spliced to yield either a transmembrane form or a glycosylphosphatidylinositol (GPI)-linked form, and both forms are expressed equally on all cells studied to date, except erythrocytes. The GPI-anchored isoform is selectively expressed on erythrocytes [3]. The isoforms have identical functional activity as CD2 ligands [4].

Molecular cloning

cDNA clones for both transmembrane [5] and GPI-anchored [6] forms of CD58 have been isolated. The extracellular domain of LFA-3 contains two immunoglobulin superfamily (IgSF) domains [5,6].

Transfectant and epitope analysis

mAb submitted to the Adhesion Section were analysed on CHO cell transfectants to confirm specificity [Klickstein and Springer, AS1.1, Table 1]. A study by Anstee found that S002 (BRIC5) and S024 (TS2/9) blocked binding of ^{125}I-labelled BRIC227 mAb, while S029 (AICD58) did not, thus defining two epitopes on CD58 (see introductory diagram).

Function

CD58 (LFA-3) functions as an adhesion molecule in T-cell-mediated cytolysis and helper functions [1,2]. CD58 is the counterreceptor for CD2, and mediates rosetting of human erythrocytes with T lymphocytes. Activated T lymphocytes are more active than resting T lymphocytes in rosetting with human erythrocytes. The greater activity of sheep as compared to human erythrocytes in rosetting with T lymphocytes reflects the higher density of the homologue of LFA-3 on sheep erythrocytes [7]. Studies in this Workshop confirmed the findings of the Fourth Workshop that S002 (BRIC5) and S024 (TS2/9) blocked human erythrocyte (E^{hu}) rosetting and T-cell proliferation stimulated by activated T cells [Klickstein and Springer, AS1.1, Table 2] while S029 (AICD58) did not. These results were concordant with the epitope mapping described above. A signalling role has been defined for LFA-3 [8,9] and this was extended in this Workshop with the observation that only memory cells respond to costimulation with LFA-3 [Semani *et al.*, AS1.11].

References

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AS1.5 CD59 cluster report

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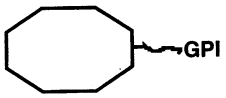
CD59

Mr 20 kD

Epitope 1	Epitope 2	Epitope 3
BRIC229	MEM-43/5	p282
BRA10G	MEM-125	
YTH53.1	C2G4	
MEM-43		



CD59 is a glycosylphosphatidylinositol (GPI)-anchored surface molecule that protects cells from lysis by homologous complement. It is known as membrane inhibitor of reactive lysis (MIRL), homologous restriction factor of 20 kDa (HRF20), and protectin, and was identified by several independent laboratories [1–7]. CD59 was clustered in the Fourth Workshop on the basis of two monoclonal antibodies (mAb) that were also submitted to this workshop as S011/T159 (YTH53.1) and S013/T103 (MEM-43). These mAb together with six new mAb were used to study CD59 in the Fifth Workshop [Klickstein and Springer, AS1.1, Table 1].

Cellular distribution

CD59 is very widely distributed and highly expressed. No normal cells have been reported to be CD59-deficient. The U937 monocyte-like line and monocytes were among the lowest expressing cells studied in this Workshop, apart from cells with a general defect in GPI anchoring.

Immunochemistry

CD59 is an 18–20 kDa protein, and migrates faster on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after treatment with endoglycosidases, suggesting the presence of N-linked carbohydrate. CD59 is GPI-anchored on all cells examined [1–7].

Molecular cloning

cDNA for CD59 [5,6] encodes a 128-residue open reading frame, including a 25-amino-acid NH_2-terminal signal peptide. The predicted molecular weight of the mature polypeptide is 11.5 kDa. The observed M_r of 18–20 in SDS-PAGE for the cell surface protein is probably accounted for by N-linked glycosylation and the GPI anchor.

Transfectant and epitope analysis

Chinese hamster ovary (CHO) cells transfected with the CD59 cDNA and a purified protein enzyme-linked immunosorbent assay (ELISA) were employed to identify CD59 mAb in Subpanel 1 [Klickstein and Springer, AS1.1, Table 1]. The Fletcher and Anstee laboratories performed epitope mapping