2. Springer, T. A., Dustin, M. L., Kishimoto, T. K., and Marlin, S. D. *Ann. Rev. Immunol.* **5**, 223 (1987).
3. Dustin, M. L., Selvaraj, P., Mattaliano, R. J., and Springer, T. A. *Nature* **329**, 846 (1987).
4. Hollander, N., Selvaraj, P., and Springer, T. A. *J. Immunol.* **141**, 4283 (1988).
5. Wallner, B. P., Frey, A. Z., Tizard, R., Mattaliano, R. J., Hession, C., Sanders, M. E., Dustin, M. L., and Springer, T. A. *J. exp. Med.* **166**, 923 (1987).
6. Seed, B. *Nature* **329**, 840 (1987).
7. Selvaraj, P., Dustin, M. L., Mitnacht, R., Hünig, T., Springer, T. A., and Plunkett, M. L. *J. Immunol.* **139**, 2690 (1987).
8. Le, P. T., Vollger, L. W., Haynes, B. F., and Singer, K. H. *J. Immunol.* **144**, 4541 (1990).
9. Webb, D. S. A., Shimizu, Y., van Seventer, G. A., Shaw, S., and Gerrard, T. L. *Science* **249**, 1295 (1990).

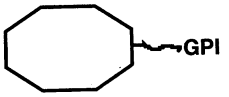
# AS1.5 CD59 cluster report

LLOYD B. KLICKSTEIN and TIMOTHY A. SPRINGER

**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

AS

[Klickstein and Springer, AS1.1, Table 1], defining two to three epitopes (see introductory diagram).

## Function

An extensive literature supports the identification of CD59 as an inhibitor of complement-mediated lysis. CD59 acts to inhibit the C5b-8 catalysed insertion of C9 into the lipid bilayer [8,9]. *In vitro* studies have demonstrated binding of purified CD59 protein to purified C8 and C9 [10]. CD59 is highly expressed on all haemopoietic cells and mAb to CD59 as well as to CD48 on lymphocytes were shown to be the most sensitive and specific for diagnosis of paroxysmal nocturnal haemoglobinuria (PNH) (Alfinito) by flow cytometry. Genetic deficiencies in CD59 [11] or CD55 [12,13] have shown that the CD59-deficient patient had clinical PNH, while the CD55 patients were apparently normal. Although the number of patients studied was small, CD59 is apparently the most important inhibitor of complement-mediated lysis on the erythrocyte surface.

Cells transfected with CD2 and purified CD59 protein have been used to demonstrate that CD59 is also a counterreceptor for CD2 [14].

## References


1. Holguin, M. H., Fredrick, L. R., Bernshaw, N. J., Wilcox, L. A., and Parker, C. J. *J. clin. Invest.* **84**, 7 (1989).
2. Sugita, Y., Nakano, Y., and Tomita, M. *J. Biochem.* **104**, 633 (1963).
3. Okada, N., Harada, R., Fujita, T., and Okada, H. *J. Immunol.* **143**, 1772 (1989).
4. Groux, H., Huet, S., Aubrit, F., *et al. J. Immunol.* **142**, 3013 (1989).
5. Davies, A., Simmons, D. L., Hale, G., Harrison, R. A., Tighe, H., Lachmann, P. J., and Waldmann, H. *J. exp. Med.* **170**, 637 (1989).
6. Štefanovà, I., Hilgert, I., Kristofova, H., Brown, R., Low, M. G., and Hořejší, V. *Mol. Immunol.* **26**, 153 (1989).
7. Okada, H., Nagami, Y., Takahashi, K., *et al. Biochem. Biophys. Res. Commun.* **162**, 1553 (1989).
8. Meri, S., Morgan, B. P., Davies, A., Daniels, R. H., Olavesen, M. G., Waldmann, H., and Lachmann, P. J. *Immunology* **71**, 1 (1990).
9. Whitlow, M. B., Iida, K., Štefanová, I., Bernard, A., and Nussenzweig, V. *Cell. Immunol.* **126**, 176 (1990).
10. Ninomiya, H. and Sims, P. J. *J. biol. Chem.* **267**, 13675 (1992).
11. Yamashina, M., Ueda, E., Kinoshita, T., Takami, T., Ojima, A., Ono, H., Tanaka, H., Kondo, H., Orii, T., Okada, M., *et al. New Engl. J. Med.* **323**, 1184 (1990).
12. Telen, M. J., Hall, S. E., Green, A. M., Moulds, J. J., and Rosse, W. F. *J. exp. Med.* **167**, 1993 (1988).
13. Daniels, G. L., Tohyama, H., and Uchikawa, M. *Transfusion* **22**, 362 (1982).
14. Hahn, W. C., Menu, E., Bothwell, A. L. M., Sims, P., and Bierer, B. E. *Science* **256**, 1805 (1992).


# AS1.6 CDw108 cluster report

LLOYD B. KLICKSTEIN and TIMOTHY A. SPRINGER

Two monoclonal antibodies (mAb), S015 (MEM-121) and S017 (MEM-150), both IgM from Hořejší's laboratory but derived from different fusions, defined the CDw108 cluster in this Workshop.

## Cellular distribution

CDw108 is expressed highly only on HPB-ALL cells, and weakly on some lymphoid, myeloid, and stromal cells. One study found CDw108 to be regulated during B-cell development, with B-cell chronic lymphocytic leukaemia (B-CLL) cells negative, Epstein–Barr virus (EBV)-transformed B cells and hairy leukaemia cells positive, and myelomas and plasmacytomas negative (Halperin).

## Immunochemisty

CDw108 mAb immunoprecipitate and detect a 75 kDa band in Western blots of non-reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels [Angelisová *et al.*, AS1.8]. The