3. Fleit, H. G., Wright, S. D., and Unkeless, J. C. *Proc. nat. Acad. Sci., USA* 79, 3275 (1982).
4. Perussia, B., Kobayashi, M., Rossi, M. E., Anegón, I., and Trinchieri, G. *J. Immunol.* 138, 765 (1987).
5. Tetteroo, P. A. T., van der Schoot, C. E., Visser, F. J., Bos, M. J. E., and von dem Borne, A. E. G. K. In *Leucocyte typing III*, (ed. A. J. McMichael *et al.*), p.702. Oxford University Press, Oxford, (1987).
6. Lanier, L. L., Ruitenberg, J. J., and Phillips, J. H. *J. Immunol.* 141, 3478 (1988).
7. Ravetch, J. V., and Perussia, B. *J. exp. Med.* (in press).
8. Selvaraj, P., Rosse, F., Silber, R., and Springer, T. A. *Nature* 333, 565 (1988).
9. Huizinga, T. W. J., van der Schoot, C. E., Jost, C., Klaassen, R., Kleijer, M., von dem Borne, A. E. G. K., Ross, D., and Tetteroo, P. A. T. *Nature* 333, 667 (1988).
10. Simmons, D. and Seed, B. *Nature* 333, 568 (1988).
11. Titus, J. A., Perez, P. Kaubisch, A., Garrido, M. A., and Segal, D. M. *J. Immunol.* 139, 3153 (1987).
12. Anegón, I., Cuturi, M. C., Trinchieri, G., and Perussia, B. *J. exp. Med.* 167, 452 (1988).
13. Cuturi, M. C., Anegón, I., Sherman, F., Loudon, R., Clark, S. C., Perussia, B., and Trinchieri, G. *J. exp. Med.* 169, 569 (1989).
14. Cassatella, M. A., Anegón, I., Cuturi, M. C., Griskey, P., Trinchieri, G., and Perussia, B. *J. exp. Med.* 169, 549 (1989).
15. Trinchieri, G., Rosen, M., and Perussia, B. *Blood* 69, 1218 (1987).
16. Perussia, B., Acuto, O., Terhorst, C., Faust, J., Lazarus, R., Fanning, V., and Trinchieri, G. *J. Immunol.* 130, 2142 (1983).

N2.7 Reactivity of Workshop CD16 mAb with distinct membrane-anchored forms of CD16

PERIASAMY SELVARAJ, MARGARET L. HIBBS, OLLI CARPÉN, and TIMOTHY A. SPRINGER

CD16 is a low-affinity Fc gamma receptor for IgG expressed on granulocytes, macrophages, and large granular lymphocytes (LGL) [1]. CD16 on granulocytes is anchored to the membrane via a phosphatidylinositol glycan (PIG) moiety and is released from the cell surface upon phophatidylinositol-specific phospholipase C (PI–PLC) treatment [2, 3], whereas CD16 expressed on LGL and cultured monocytes is resistant to PI–PLC treatment [Selvaraj *et al.*, submitted]. After removal of glycans, the CD16 isolated from LGL and cultured monocytes is 5–7 kDa larger than granulocyte CD16 molecules strongly suggesting the presence of transmembrane and cytoplasmic protein domains [Selvaraj *et al.*, submitted].

Previous studies have demonstrated differences among CD16 mAb in reactivity with granulocytes and LGL [4, 5]. We have looked for differences between CD16 on neutrophils and LGL, and between the two alloantigens NA1 and NA2. NA1 and NA2 appear to be true alleles on neutrophils since they obey the Hardy–Weinberg law. Paradoxically, NA1 and NA2 are expressed on neutrophil CD16, but individuals who have $NA1^+$ neutrophil CD16 have $NA1^-$CD16 on their LGL. $NA2^+$ individuals express NA2 on both neutrophils and LGL.

Antibody binding to neutrophils, LGL, and COS cells transfected with a cDNA encoding PIG-anchored CD16 was analysed by immunofluorescence flow cytometry [2]. mAb N403 (CLB/FcGran1), N406 (BW209/2), N409 (3G8), and N411 (VEP13) showed strong and similar reactivity with NA1/NA1 and NA2/NA2 granulocytes whereas N407 (YFC120.5) and N410 (G7E11) showed weak reactivity. mAb N404 (CLB/Gran11) reacted strongly with NA1/NA1 granulocytes but less so with NA1/NA2 granulocytes, suggesting that it recognizes the NA1 form of CD16 [5]. mAb N408 (GRM1) reacted strongly with NA1NA2 granulocytes but not with NA1/NA1 granulocytes showing that it is specific for the NA2 form of CD16. mAb N401 (BL-LGL/1) did not react with NA1 granulocytes but reacted weakly with NA1/NA2 granulocytes. mAb N402 (B73.1) and N405 (Leu11C) reacted weakly with granulocytes. PI–PLC treatment of granulocytes decreased binding of all the CD16 mAb by 55–88 per cent.

Large granular lymphocytes showed reactivity with 10 of 11 CD16 Workshop mAb. The putative NA1-specific mAb N404 (CLB/Gran11) did not react with LGL. mAb N407 (YFC120.5) and N410 (G7E11) showed weak fluorescence as in the case of granulocytes. mAb N401 (BL-LGL/1), N402 (B73.1), N403 (CLB/FcGran1), N405 (Leu11C), N408 (GRM1), and N409 (3G8) showed strong

Table 1. Binding of CD16 panel antibodies to isoforms of CD16

Workshop no.	mAb name	SLFI (per cent decrease with PI–PLC)*			CD16⁻ COS cells Percentage positive cells (% decrease with PI–PLC)
		Neutrophils		LGL	
		NA1/NA1	NA1/NA2		
NA1+NA2					
N403	CLB/FcGran1	97.2 (77)	97.2 (78)	28.9 (0)	36 (91)
N406	BW 209/2	47.5 (55)	31.2 (54)	12.2 (0)	40 (90)
N407	YFC120.5	21.6 (70)	16.8 (73)	2.7 (0)	25 (100)
N409	3G8	77.8 (78)	68.6 (85)	24.1 (0)	32 (62)
N410	G7E11	19.4 (44)	9.0 (56)	4.0 (0)	21 (38)
N411	VEP13	77.8 (86)	79.3 (82)	7.3 (0)	12 (58)
NA1					
N404	CLB/Gran11	92.9 (79)	26.9 (73)	0 (–)	36 (67)
NA2					
N401	BL-LGL/1	0 (–)	4.6 (76)	28.9 (0)	3.5 (43)
N408	GRM1	0 (–)	62.9 (83)	38.4 (0)	4.0 (100)
N402	B73.1	13 (66)	3.1 (68)	24.7 (0)	30 (83)
N405	Leu11C	6.5 (66)	1.9 (84)	19.1 (0)	24 (100)

*SLFI, specific linear fluorescence intensity. Granulocytes and LGL were analysed at different times with different amplification settings and therefore the SLFI were not directly comparable. Granulocytes from two different donors were also analysed at different times with different amplification settings but the values were normalized (using N403 (CLB/FcGran1) for comparative purposes.

fluorescence with LGL. mAb N411 (VEP13) reacted strongly with both NA1 and NA2 granulocytes but weakly with LGL. PI–PLC treatment did not affect binding of any of the CD16 mAb to LGL.

We have further analysed the binding of mAb to COS cells transfected with a CD16 cDNA (clone pCD16.2) which is identical to the published pCD16 cDNA clone [6] in its hydrophobic C-terminal sequence [B. Seed, unpublished] but has an extracellular domain identical to that described by Petty *et al.* [7] [M. Hibbs, unpublished]. On SDS–PAGE, the CD16 expressed in COS cells showed an identical electrophoretic mobility to that of the NA1 form of granulocyte CD16 [Selvara *et al.*, submitted]. As shown in Table 1, the NA2-specific mAb N401 (BL-LGL/1) and N408 (GRM1) showed very little or no reactivity with the CD16 expressed on COS cells. mAb N403 (CLB/FcGran1), N406 (BW209/2), N407 (YFC120.5), N409 (3G8) and the NA1-specific mAb N404 (CLB/Gran11) showed strong reactivity. PI–PLC treatment reduced the binding of the CD16 mAb to COS cells. The reactivity of the NA1-specific mAb N404 (CLB/Gran11) [5] and the lack of reactivity of NA2-specific mAb N401 (BL-LGL/1) and N408 (GRM1) with the CD16 expressed on COS cells shows that the pCD16.2cDNA clone encodes the NA1 form of granulocyte CD16 which is in agreement with the electrophoretic mobility observed on SDS–PAGE [Selvaraj *et al.*, submitted].

mAb N408 (GRM1) reacts with NA2 granulocytes and LGL but not with NA1NA1 granulocytes, whereas mAb N404 (CLB/Gran11) reacts with NA1NA1 granulocytes but not with NA2/NA2 granulocytes or LGL [5] (also Table 1). These results along with the size difference observed on SDS–PAGE after deglycosylation [Selvaraj *et al.*, submitted] demonstrate that, apart from the differ-

ences in the membrane anchor, there exists structural heterogeneity between neutrophil NA1 CD16, neutrophil NA2 CD16, and LGL CD16.

Acknowledgements

We thank Drs M. Low for PI–PLC, B. Seed for CD16 cDNA clone, and Mr P. Lopez for flow cytometry anlysis.

References

1. Unkeless, J. C., Scigliano, E., and Freedman, V. H. *Ann. Rev. Immunol.* 6, 251 (1988).
2. Selvaraj, P., Rosse, W. F., Silber, R., and Springer, T. A. *Nature* 333, 565 (1988).
3. Huizinga, T. W. J., Van Der Schoot, C. E., Jost, C., *et al. Nature* 333, 667 (1988).
4. Perussia, B., Acuto, O., Terhorst, C., *et al. J. Immunol.* 130, 2142 (1983).
5. Tetteroo, P. A. T., Van Der Schoot, C. E., Visser, F. J., Bos, M. J. E., and Von dem Borne, A. E. G. In *Leucocyte typing III* (ed. A. J. McMichael, *et al.*), p.702. Oxford University Press, Oxford (1987).
6. Simmons, D. and Seed, B. *Nature* 333, 568 (1988).
7. Pettz, G. A., Grundy, H. O., Lebo, R. V., *et al. Proc. nat. Acad. Sci. USA* 86, 1013 (1989).

N3 Cluster report: CD18

PETER UCIECHOWSKI and R. E. SCHMIDT

The human leucocyte adhesion molecules consist of distinct α-chains but share a common β-chain CD18 with a molecular weight of 95 kDa. This β-subunit is an integral membrane polypeptide whose extracellular portion contains four cysteine-rich homologous repeats, and has been mapped to chromosome 21 [1]. Homologies of CD18 to other α/β heterodimeric receptor molecules defined a gene superfamily termed integrins involved in both cell–cell and cell–matrix receptors [2]. A disease called leucocyte adhesion deficiency (LAD) which is caused by a genetic defect of CD18 emphasizes its important role in the function of all three antigens. There is also evidence that the β-chain is involved in cytoskeletal interactions.

As described in the CD11 report, antibodies directed against CD18 were tested together with CD11a antibodies in the LFA panel since they were not distinguished during the prescreening procedure. Further CD18 antibodies were placed into the new antibody and myeloid panel as controls. The profiles are given in Fig. 1 of the CD11 report [N1].

Data on molecular weight based on information provided by submitters are shown in Table 1. Specificity for CD18 was assigned using transfectants, hybrids, and adhesion assays (Larson, Springer, Shaw, Prieto) as described in the CD11 report in detail.

In summary, eight antibodies were assigned as CD18 recognizing reagents (Table 1). One antibody of the CD11b panel, N306 (MJ5/1), was identified as a CD18 antibody using transfectants (Larson and Springer).

The effect of antibodies on functional properties of CD18 such as aggregation, and ligand binding was investigated by Diamond and Springer. They reported that all antibodies differ in inhibition of homotypic and neutrophil adhesion. In addition, the inhibitory effects of CD18 antibodies varied in binding to purified solid phase LFA-1 or p150,95, and also in binding of radiolabelled Mac-1 to iC3b-coated erythrocytes. Thus, the CD18 antibodies appear to recognize different or overlapping epitopes which are important in certain adhesive and binding functions of all adhesion antigens. Further functional studies are discussed in the CD11 report.

References

1. Corbi, A. L., Larson, R. S., Kishimoto, R. K., Springer T. A., and Morton, C. C. *J. exp. Med.* 167, 1597 (1988).
2. Kishimoto, T. K., O'Conner, K., Lee. A., Roberts, T. M., and Springer, T. A. *Cell* 48, (1987).