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B22.6 B-cell and Activation Workshop mAb to phosphatidylinositol-anchored proteins

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Certain cell-surface proteins lack a membrane-spanning segment and are anchored to the cell surface via a glycosyl-phosphatidylinositol (PI) moiety. So far only a few human leucocyte differentiation antigens have been identified as PI-anchored proteins. We have recently selected human B lymphoblastoid mutant cells, which lack expression of PI-anchored cell-surface proteins due to a defect in assembly or attachment of the glycosyl-PI anchor [1]. The mutant clones, derived from the JY cell line, provide a convenient probe for identification of PI-linked proteins.

Antibodies of the B-cell and Activation Sections were screened by immunofluorescence flow cytometry for binding to PI-anchor-deficient mutant cells and their wild-type parent cells. Five antibodies which bind to wild type but not to mutant cells were identified (Table 1). To

Table 1. PI-anchored antigens identified by Workshop antibodies

Workshop no.	mAb name	Binding (SLFI)*		Decrease of fluorescence intensity following PI–PLC treatment (per cent)†
		Wild-type cells	Mutant cells	
Control				
—	W6/32 (anti-HLA)	153.3	152.9	0
—	TS1/18 (CD18 mAb)	9.6	9.8	0
—	1A10 (anti-DAF)	5.4	0.0	69
B-cell Section				
B43	AD2	0.7	0.0	69
B121	1E9.28.1	1.2	0.0	48
B124	7G2.2.11	1.2	0.0	47
B128	BLAST-1	1.7	0.0	70
Activation Section				
A87	JML-H105	2.6	0.0	77

*SLFI, specific linear fluorescence intensity.
†Wild-type cells were incubated with Bacillus thuringiensis PI-PLC (1 μmol/min/ml) for 1 h at 37°C and analysed by flow cytometry. The antibody staining on cells which were treated in the same way but without PI–PLC was taken as 100 per cent.

exclude the possibility of a coincident quantitative difference in cell-surface expression between different clones, binding assays were repeated with five different wild-type clones and five different mutant clones. In spite of the weak reactivity, correlation between antibody binding activity and the PI-anchor mutation was demonstrated. To verify that these antibodies recognize PI-anchored proteins, wild-type cells were treated with phosphatidylinositol-specific phospholipase C (PI–PLC) and subsequently tested for antibody binding. The reactivity of the five antibodies was reduced following PI–PLC treatment (Table 1), suggesting that they recognize PI-anchored proteins. Thus, antibodies B43 (AD2), B121 (1E9.28.1), B124 (7G2.2.11), and B128 (BLAST-1) of the B-cell Section and antibody A87 (JML-H105) of the Activation Section showed consistent binding to the five wild-type clones but no binding to the five mutant clones, and their antigen was released by PI–PLC from the surface of the five different wild type clones. The results indicate that these five antibodies recognize PI-anchored proteins. Decoding revealed that mAb B43 (AD2), B121 (1E9.28.1), and B124 (7G2.2.11) have similar tissue distribution, which differs from that of mAb B128 (BLAST-1). mAb B128 defines BLAST-1, a cell-surface antigen previously shown to be PI-anchored [2]. The three antibodies B43 (AD2), B121 (1E9.28.1), and B124 (7G2.2.11) are weakly reactive with subpopulations of resting and activated B-cells, and in immunoenzyme histology are positive with follicle mantle, dendritic reticulum cells, epithelial cells, and vascular endothelium. This suggests that these antibodies form a new cluster which defines a PI-anchored antigen.

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B22.7 New germinal-centre compartments defined by mAb

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Two lymphoid cell populations are generally recognized in germinal centres (GC)—centroblasts which produce intense basophilic staining in the lower or dark zone and centrocytes which occupy the upper or light zone [1]. They are surrounded by a follicular mantle of small lymphocytes which is located eccentrically and forms a prominent cap overlying the upper pole (Fig. 1).

In previous Workshops we reported that the entire follicular dendritic cell (FDC) network within the GC, as identified by the FDC-specific antibody BU-10, is revealed by CD21 monoclonal antibodies (mAb), whereas the FDC showing strongest reactivity with CD23 mAb are located in the area of the light zone that is furthest from the dark zone [2, 3]. In the current Workshop we confirmed these findings and have identified additional clusters of antibodies which show topographically-restricted reactivity within FC.

The CD19 mAb reacted with most B-cells in the mantle and GC; CD39 antibodies stained mantle B- but not GC-cells; CD45-like mAb identified a subset of the mantle cells; CD38 mAb reacted with GC-cells and not mantle B-cells (Table 1). FDC in the sub-apical parts of the light zone were preferentially stained by the CD23 mAb B78

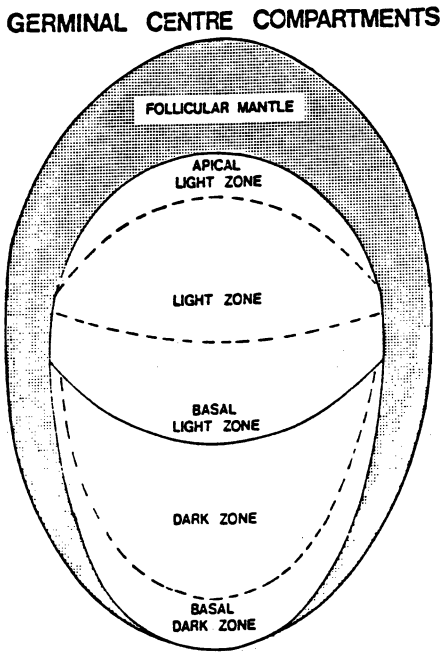

Fig. 1.