The band is 80 kDa after reduction. The antigen contains about 20 per cent of N-linked carbohydrate as determined by endoglycosidase F treatment. The antigen identified by both CDw108 mAb is 100 per cent glycosylphosphatidylinositol (GPI)-anchored on HPB-ALL [Angelisová *et al.*, AS1.8] and JY cells [Klickstein *et al.*, AS1.7].

No specific functions were identified during the course of this Workshop; however, many GPI-anchored antigens have been shown to be associated with protein tyrosine kinases [Angelisová *et al.*, AS1.8] or to mediate cellular activation or proliferation of some cells. In HPB-ALL, CDw108 antigen is associated with several other GPI-linked molecules, glycolipids, and with protein tyrosine kinases [Angelisová *et al.*, AS1.8]. It is clear that mAb to CDw108 do not affect T-cell proliferation or human erythrocyte E^{hu} rosetting in the assays studied in this Workshop [Klickstein and Springer, AS1.1, Table 2].

AS1.7 Identification of novel GPI-anchored antigens by analysis of GPI-anchor-deficient cells with mAb in the Blind Panel

LLOYD B. KLICKSTEIN, MICHAEL R. YORK, ED LUTHER, and TIMOTHY A. SPRINGER

The glycosylphosphatidylinositol (GPI) anchor [1] is a posttranslational modification of a nascent polypeptide chain where a characteristic COOH-terminal signal peptide directs the concerted proteolytic cleavage and transfer of a GPI moiety [2–4]. The GPI-anchored protein is then transported via the endoplasmic reticulum to the cell membrane where the long-chain fatty acids of the GPI group attach the protein to the outer leaflet of the lipid bilayer. Some of the enzymes in the GPI biosynthetic pathway have been cloned [5].

To identify novel GPI-anchored antigens, clones of a B lymphoblastoid cell line, an erythroleukaemia cell line, and a fetal kidney epithelial cell line (Table 1) were analysed by indirect immunofluorescence and flow cytometry with the 480 monoclonal antibodies (mAb) of the Fifth Workshop Blind Panel and with the 31 mAb in Subpanel 1 of the Adhesion Section. The staining pattern of the normal phenotype clone was compared to that obtained with corresponding GPI-anchor-deficient cells and with that of the

Table 1 Established GPI-anchored antigens

CD	Antigen	Workshop mAb		Cell line*	% loss of staining on GPI-anchor-deficient clone
		Code	Clone name		
CD24	HSA, B7-2	CD24.3	HB-8	K-562	100
CD48	BLAST-1	S028	6.28	JY	100
CD52	CAMPATH-1	XB003	O97	K-562, JY	100
CD55	DAF	S031	IA10	K-562, JY, 293	100
CD58	LFA-3	S024	TS2/9	K-562, JY	50
CD59	MIRL	S013	MEM-43	K-562, JY, 293	100
CD14	P55-65	MR3	MoS39		
CD16	FcγRII	MR5	3G8		
CD56	NCAM	NK19	Leu 19		
CD67	CGM6	M40	MF25.1		
CD73	Ecto 5′NT	CD73.1	1E9.28.1		

*Cell line in which mAb stained normal cell clone but not the GPI-anchor-deficient clone.
†% of staining of normal cell clone absent on GPI-anchor-deficient clone.

normal cells treated with phosphoinositol-specific phospholipase C (PI-PLC). Human umbilical vein endothelial cells (HUVEC) were also studied with the 57 mAb from the Endothelial Section with and without PI-PLC treatment to identify endothelial-specific GPI-anchored antigens [Klickstein *et al.*, E6.29].

The JY25 (normal) and JY33 (GPI-anchor-deficient) clones were reported previously [6]. To prepare GPI-anchor-deficient K-562 and 293 cells, 5×10^7 cells in log phase growth were treated overnight with 200 μg/ml ethylmethanesulfonate. When the cells were again growing exponentially, they were stained with CD55 mAb IA10 and CD59 mAb YTH53.1 and allowed to bind to goat anti-mouse IgG immobilized on plastic Petri dishes. Non-adherent cells were harvested and cultured. This procedure was repeated 3–4 times until a population of cells that demonstrated background staining with IA10 and YTH53.1 was detected, at which point negative and positive cell clones were obtained by limiting dilution cloning. The K-562 and 293 normal and GPI-anchor-deficient clones chosen for this study were 4K1.3 and 5K2.5 and 293Tag1.6 and A293.2.2, respectively. These cell clones were biosynthetically labelled with [^{3}H]ethanolamine and the polar lipids were extracted and analysed by normal phase thin layer chromatography (TLC). The normal cell clones exhibit at least two spots corresponding to hydrophilic lipids that are absent in the mutant clones (Fig. 1), confirming that the 5K2.5 and A293.2.2 clones truly represent GPI-anchor-deficient cells, rather than CD55 and CD59 double mutations.

CD24, CD48, CDw52, CD55, CD58, and CD59 were confirmed as GPI-anchored antigens, which validated the method (Table 1). CD56 was not GPI-anchored on 293 cells, and was absent on the other two cell types. The GPI-anchored antigens CD14, CD16, CD67, and CD73 were not present at significant levels on the three cell types studied.

Three of the clusters assigned during the Fifth Workshop were found to be wholly or in part GPI-anchored (Table 2). CD93 is 50 per cent GPI-anchored on JY cells, as is CD58. CDw108 is 100 per cent GPI-anchored on JY cells and CDw109 is 100 per cent GPI-anchored on HUVEC and on 293 cells, although the staining of the latter cells was weak (Table 2). Three unclustered, or orphan, mAb were found to recognize GPI-anchored antigens; A049 (3F4) antigen was present and 100 per cent GPI-anchored on all three cell types, T010 (4dD8) antigen was strongly expressed and 90 per cent GPI-anchored on JY cells,

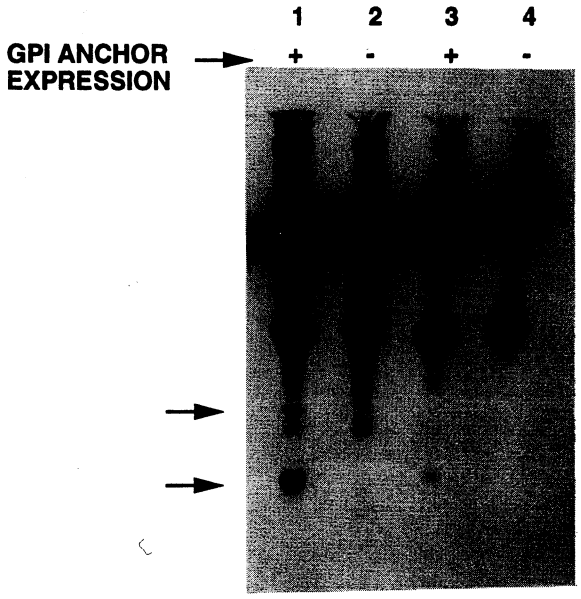

Fig. 1 Thin-layer chromatographic (TLC) analysis of [^{3}H]ethanolamine-labelled polar lipids. Cells biosynthetically labelled with [^{3}H]ethanolamine were extracted with $CHCl_3$:CH_3OH:H_2O, 10:10:3 (v:v:v). The dried lipids were taken up in butanol and extracted with H_20, dried again, and applied to an activated silica gel TLC plate. The plate was developed with $CHCl_3$:CH_3OH:H_2O, 10:10:3 (v:v:v), dried, and fluorographed. Lane 1, 4K1.3; lane 2, 5K2.5; lane 3, 293TAg1.6; lane 4, A293.2.2. The pair of arrows indicate polar lipids absent in the GPI-anchor-deficient cells.

and E057 (C4A9.2.3) antigen was 100 per cent GPI-anchored on HUVEC (Table 2).

The three mAb against S-ENDO 1 (Table 2) and the antigens recognized by NK28 (H5B2) and NK29 (H5D1) stained the 4K1.3 K-562 clone but not the GPI-anchor-deficient clone 5K2.5. This result could not be verified by PI-PLC treatment since K-562 cells, like erythrocytes, express a PI-PLC-resistant GPI anchor (data not shown). Therefore, because clonal variation in the cells unrelated to the ability to synthesize the GPI anchor is not ruled out, the assignment of a GPI-anchor to these antigens is tentative.

Interestingly, the reference mAb for Cdw75 and CDw76, two carbohydrate antigens that are dependent on β-galactoside-α-2, 6-sialyltransferase [7], did not stain the GPI-anchor-deficient K-562 clone (Table 3), although the staining of the normal cell clone gave a

Table 2 Novel GPI-anchored antigens

Workshop mAb Code	Clone name	Donor	M_r (reduced)	Cell line*	% of loss of staining on GPI-anchor deficient clone†	Confidence‡
CD93						
M16	VIMD2b	Knapp	140	JY; not found on K-562 or 293	50	High
M17	VIMD2	Knapp	140	JY; not found on K-562 or 293	50	High
M34	WDS 4.B4	De Smet	140	JY; not found on K-562 or 293	50	High
M48	X2	Peters	140	JY; not found on K-562 or 293	50	High
CDw108						
S015	MEM-121	Hořejší	80	JY; not found on K-562 or 293	100	High
S017	MEM-150	Hořejší	80	JY; not found on K-562 or 293	100	High
CDw109						
E045	8A3	Sutherland	150	HUVEC & 293; not found on JY or K-562	100	High
E046	7D1	Sutherland	150	HUVEC & 293; not found on JY or K-562	100	High
S-ENDO 1						
E053	F432G-3	George/Poncelet/Laurent/Sampol	100	K-562; not GPI on 293 or HUVEC; not found on JY	100	Moderate
E054	F435H-7	George/Poncelet/Laurent/Sampol	100	K-562; not GPI on 293 or HUVEC; not found on JY	100	Moderate
E056	F39E-10	George/Poncelet/Laurent/Sampol	100	K-562; not GPI on 293 or HUVEC; not found on JY	100	Moderate
Orphan mAb						
A049	3F4	Loertscher	33–35	JY, 293, K-562	100	High
E057	C4A9.2.3	Klickstein/Springer		HUVEC; not found on JY, K-562, or 293	100	High
NK28	H5B2	Thompson		K-562; not GPI on JY or 293	100	Moderate
NK29	H5D1	Thompson		K-562; not GPI on JY or 293	100	Moderate
T010	4dD8	Hogg		JY; not found on K-562 or 293	90	High

*Cell line in which mAb stained the normal cell clone but not the GPI-anchor-deficient clone.
†% of staining of normal cell clone absent on GPI-anchor-deficient clone.
‡High confidence indicates that the GPI anchor was observed on two different cell types or by both PI-PLC-treated and GPI-anchor-deficient cells or that the normal cells stained brightly with the indicated mAb while staining was absent on the mutant cell clone or upon PI-PLC treatment. Moderate confidence indicates that the GPI-anchor-deficient K-562 clone, 5K2.5, did not stain with the indicated mAb and there are no other data supporting GPI anchoring.

Table 3 CDw75 and CDw76 subgroups identified by analysis of GPI-anchor-deficient cells

Workshop mAb				% of loss of staining on GPI-anchor-deficient clone†
Code	Clone name	Donor	Cell line*	
CDw75				
Group 1				
CDw75.3	LN1	Epstein	K-562; not GPI on JY; negative on 293	90
CDw75.1	OKB4	Rao	K-562; not GPI on JY; negative on 293	90
Group 2				
B010	HH1	Funderud	K-562; not GPI on JY or 293	90
CDw76				
Group 1				
CD76.1	CRIS-4	Vilella	K-562; not GPI on JY or 293	90
CD76.2	HD66	Dörken/Moldenhauer	K-562; not GPI on JY or 293	90
B025	FB21	Nozawa/Abe/Wakasa	K-562; not GPI on JY or 293	90
B056	HB-6	Tedder/Cooper	K-562; not GPI on JY or 293	90
B012	EBU-65	Gramatzki	K-562; not GPI on JY or 293	90
Group 2				
B027	J3-89	Pesando	K-562, JY; not GPI on 293	90, 50
Group 3				
B055	HB-4	Tedder/Cooper	K-562; not GPI on JY or 293‡	95
B057	8H7	Tedder/Engel	K-562; not GPI on JY or 293‡	95

*Cell line in which mAb stained the normal cell clone but not the GPI-anchor-deficient clone.
†% of staining of normal cell clone absent on GPI-anchor-deficient clone.
‡Staining of 293 cells was diminished 50% by PI-PLC treatment, but the GPI-anchor-deficient cell clone expressed normal amounts of the antigen.

characteristic broad pattern. The CDw75 mAb could be divided into two groups on the basis of their reactivity with 293 cells. There were eight mAb, including the reference mAb, that clustered as CDw76 [Shaw *et al.*, BD1.3]. Comparison of reactivity on the three different GPI-anchor-deficient cell clones allowed subdivision of the CDw76 mAb into three groups (Table 3). CDw76 group 1 mAb reacted with GPI-anchor-deficient JY and 293 cells, but not with GPI-anchor-deficient K-562 cells. CDw76 group 2 mAb reacted with GPI-anchor-deficient 293 cells, but not or only partially with GPI-anchor-deficient JY and not with GPI-anchor-deficient K-562 cells. Furthermore, this mAb stained 293 cells much more brightly than mAb in the other groups. These findings indicate either that the CDw76 determinant(s) are found on GPI-anchored antigens or that the carbohydrate CDw75 and CDw76 antigens share, in part, a common biosynthetic pathway with the GPI anchor structure. Furthermore, there is heterogeneity within the CDw75 and CDw76 clusters.

Acknowledgements

This work was supported by NIH grants CA31798, CA31799, and HL48675 (TAS) and a Merck–AFCR award (LBK).

References

1. Cross, G. A. M. *Ann. Rev. Cell Biol.* **6**, 1 (1990).
2. Kodukula, K., Gerber, L. D., Amthauer, R., Brink, S., and Udenfriend, S. *J. Cell Biol.* **120**, 657 (1993).
3. Coyne, K. E., Crisci, A., and Lublin, D. M. *J. biol. Chem.* **268**, 6689 (1993).
4. Caras, I. W., Weddell, G. N., and Williams, S. R. *J. Cell Biol.* **108**, 1387 (1989).
5. Miyata, T., Takeda, J., Iida, Y., Yamada, N., Inoue, N., Takahashi, M., Maeda, K., Kitani, T., and Kinoshita, T. *Science* **259**, 1318 (1993).
6. Hollander, N., Selvaraj, P., and Springer, T. A. *J. Immunol.* **141**, 4283 (1988).
7. Bast, B. J., Zhou, L. J., Freeman, G. K., Colley, K. J., Ernst, T. J., Munro, J. M., and Tedder, T. F. *J. Cell Biol.* **116**, 423 (1992).