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Review Letter

Novel structurally distinct family of leucocyte surface glycoproteins including CD9, CD37, CD53 and CD63

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Several of the recently described leucocyte surface (glyco)-proteins with significant amino acid sequence similarity (human CD9, CD37, CD53, CD63, TAPA-1, CO-029 and R2 and several homologues of other species) are distinguished by the polypeptide chain apparently four times crossing the membrane. Although the biological role of none of these molecules is known, their structure, associations with other membrane components and the effects of specific monoclonal antibodies suggest that they may constitute a family of ion channels or other transport molecules.

Leucocyte surface glycoprotein; CD9; CD37; CD53; CD63; Integral membrane protein

Surface molecules of leucocytes, the cells of the immune system, are currently the subject of intense research because of their great theoretical and practical importance. So far more than 100 of structurally well-characterized human leucocyte surface proteins, glycoproteins and glycolipids have been described and many of them have been assigned biological functions (for review see [1]). Recent rapid progress in this field is mainly due to the use of monoclonal antibodies (mAbs) and modern cDNA cloning methods but also the result of the regular organization of International Workshops on Human Leucocyte Differentiation Antigens. The Workshop conferences define 'novel' leucocyte surface molecules ('antigens') on the basis of broad comparitive mAb-based studies and assign them official names of the CD-system (CD stands for 'cluster of differentiation' and denotes originally a cluster of monoclonal antibodies that were found during these workshop studies to react similarly in various tests, ultimately therefore recognizing a common cell-surface antigen). So far there are CD1 to CD78, some of them actually being groups of several closely related molecular species (e.g. CD1a, b, c, or CD45RA, RB, RC, RO). Several tens of other well-defined molecules are still waiting for their CD-names. It seems likely that many if not most other molecules expressed on the surface of various types of leucocytes are yet to be discovered.

Several functionally distinct groups of these molecules can be clearly delineated such as antigen-specific receptors, cytokine receptors, several types of adhesion molecules, complement receptors and complement-regulatory proteins, Fc-receptors, surface enzymes, transport proteins, etc. Individual members of these functional groups are often also structurally related and thus constitute structural families such as the immunoglobulins, cytokine receptors, complement receptors, integrins and membrane lectins [1].

Recently, several of the newly cloned surface (glyco)proteins have been found to be structurally closely related and thus constituting a novel family (Tables I and II). A characteristic feature of all these molecules is the polypeptide chain containing four hydrophobic, presumably membrane-spanning segments and a single major presumably extracellular and usually *N*-glycosylated loop (Fig. 1). It seems very likely that both the NH_2- and COOH-termini are localized intracellularly [4]. The highest degree of sequence similarity between the members of this new family is observed within the putative transmembrane regions. Among other highly conserved residues are six cysteine residues (positions 71, 80, 144, 145, 161 and 170 in CD53) and some of the amino acids flanking them (Fig. 1). It is not known how many of these (and other) cysteines are involved in cystine bridges, presumably stabilizing the structure of these molecules. Several short sequence motifs appear to be strikingly conserved

71 80 145 170

such as the GCXGXXXEXXC, CCG, or EGC (numbering taken from CD53). These highly conserved sequences could perhaps give some clues about the

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Table I

Properties of the CD9/CD37/CD53/CD63 family members

	Expression	Mol.wt. (kDa)	Polypeptide chain length (amino acid residues)	Comments
CD9 [2] (human)	thrombocytes, pre-B cells	24	227	
CD37 [3,4] (human)	B cells (strongly) other leucocytes (weakly)	40-50	281	intracellularly present in vesicle-like structures [3]; the original sequence [4] was revised and the C-terminus corrected [5]
CD53 [6,7] (human)	leucocytes	32-40	219	CD53 appears to be the best pan-leucocyte marker
CD63/ME491 [8,9] (human)	very broad intracellularly	30-60	228	primarily localized in lysosomal membranes [21]; cell surface expression can be induced by various types of activation and on some tumour cells
TAPA-1 [10,11] [human]	broad	26	236	mAb has reversible antiproliferative effects [10]; associated with several other components including Leu13 [11]; probably not glycosylated
R2 [12] (human)	mRNA strongly upregulated in activated cells	protein undescribed	267	cDNA cloned and sequenced; protein product so far undescribed
CO-029 [13] (human)	some carcinoma cell lines	27-34	237	
rat CD37 [5]		protein undescribed	281	cDNA cloned and sequenced; protein product so far undescribed
OX-44 [4,14] (rat)	leucocytes (except for $CD4^+8^+$ (thymocytes)	40-50	219	rat CD53 homologue
Sm23 [15] (*Schisostoma mansoni*)		23	218	probably not glycosylated
S5.7 (human)	broad			so far unpublished paper by I. Stamenkovic and G. Rovera cited in [7]

Table II

Degree of sequence similarity between the members of the family[a]

	CD37	CD53	CD63 (ME491)	R2	TAPA-1	CO-029	rat CD37	OX-44 (rat CD53)	Sm23
CD29	20	24	25	24	45	36	22	26	21
CD37		33	22	34	21	22	80	33	20
CD53			31	33	27	26	34	81	28
CD63				25	20	26	22	34	36
R2					23	28	34	32	25
TAPA-1						33	20	27	20
CO-029							22	25	26
rat CD37								34	21
OX-44									28

[a] Given in % of amino acid residues that are identical at the corresponding positions in the sequence of the two compared molecules. The match was optimized by introducing appropriate deletions into the compared sequences; the positions with a deletion in one of the partners were not taken into account. The molecules are actually much more similar, as many of the non-identities are conservative replacements.

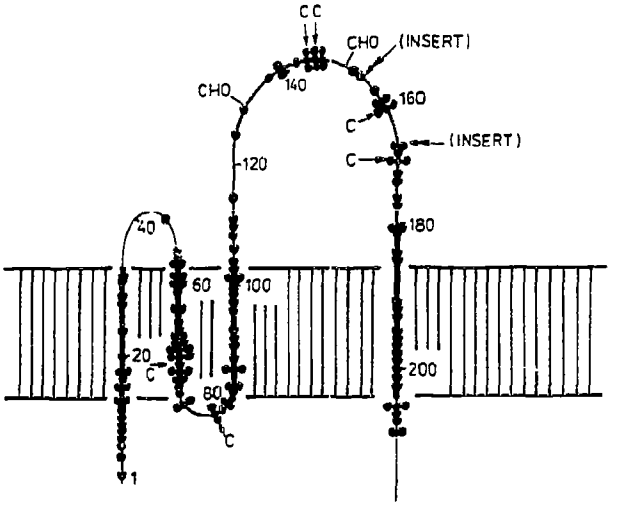

Fig. 1. Schematic representation of the CD53 polypeptide chain and degree of sequence similarity to other members of the family. Thick line represents the hydrophobic, presumably membrane-spanning regions. The membrane and the orientation of the polypeptide chain therein is also schematically shown. Both polypeptide chain ends are presumably located intracellularly, while the major *N*-glycosylated loop is extracellular. The positions in which four or five (●), six or seven (●●) and all eight (●●●) fully sequenced family members (CD9, CD37, CD53, CD63, TAPA-1, R2, CO-029 and Sm23) are identical; rat CD37 and OX-44 (rat CD53) were excluded because of their close similarity to the human homologues. Arrows indicate the positions of conserved cysteines, double arrows indicate approximate positions of major sites where in some of the family members additional stretches of amino acids are inserted. The sites of *N*-glycosylation are indicated (CHO).

functionally important regions of these molecules. On the other hand, there are relatively large differences in the sizes of the putative major extracellular loops (approx. 125 amino acids in CD37 vs approx. 80 residues in CD9, CD53, TAPA-1 and Sm23). Most of these molecules are heavily *N*-glycosylated in the putative extracellular loop, while TAPA-1 and Sm23 seem to lack any carbohydrate moiety [10,15]. At least CD9 and TAPA-1 have been shown to be covalently modified by acylation [10,16].

Several other membrane proteins exist whose organization is roughly similar, i.e. their polypeptide chains presumably cross the membrane four times. One of them is the β-subunit of the high-affinity IgE receptor [17]. The others are several neuronal amino acid receptors (but in this case both ends of the polypeptide chain are localized extracellularly [18]). None of these proteins share any significant sequence similarity to the CD9/CD37/CD53/CD63 family.

Individual members of this family widely differ in their expression (Table I): while CD9 and CD37 are present in very specific cell types only, CD53 is expressed in all leucocytes (but not in other cells) and CD63 (ME491) is a ubiquitous molecule. R2 seems to be remarkably up-regulated in activated T lymphocytes [12].

Biological functions of these molecules are unknown, but functional effects of some specific mAbs may provide clues in this respect. MAbs may either mimic the effects of natural ligands of putative receptors, block them or induce conformational changes modifying e.g. functions of transport molecules. However, the results of such experiments must always be judged with some caution as crosslinking of cell surface molecules with bivalent or multivalent antibodies may in some cases induce artificial effects unrelated to their true biological role. An ant-CD37 mAb has been shown to modulate activation of B lymphocytes [19]. Addition of an mAb against CD53 to monocytes followed by crosslinking with polyclonal antibodies against mouse immunoglobulin elicits strong oxidative burst response in these cells (V. Bažil and V. Hořejší, unpublished). An mAb against the TAPA-1 antigen strongly reversibly inhibits proliferation of some lymphoma cell lines [10]. MAbs against the CD9 antigen induce homotypic adhesion (aggregation) of pre-B cell lines [20] and thrombocytes [21] and calcium influx in these cells [22]. The mAb-induced aggregation of thrombocytes is dependent on co-crosslinking of CD9 and thrombocyte Fc γ-receptor II (CDw32) and is associated with full activation of these cells [23].

Very interesting is the ME491 antigen which has been recently found to be identical to CD63 [24]. ME491/CD63 is one of the major components of lysosome membranes; it was suggested that due to its extremely heavy glycosylation it may be highly resistant to lysosomal enzymes and thus may be one of the glycoproteins protecting and stabilizing the lysosomal membrane [24] (interestingly, the CD37 antigen is also strongly expressed in so far uncharacterized intracellular vesicle-like structures [3]). Expression of this molecule on the cell surface may be a consequence of the fusion of lysosomal membranes with the plasma membrane in some activated cells. However, other observations may indicate that the cell surface ME491/CD63 molecules do have a functional role: the JW7 mAb markedly inhibited phorbolester-induced monocyte adherence to serum-coated surfaces and aggregation of T and B lymphocytes and U937 cells [25]. The ME491 antibody was reported to be internalized after binding to the surface of melanoma and carcinoma cells; inside the cells it was bound to a 55 kDa chromatin protein and markedly inhibited transcription of ribosomal RNA genes [26]. The relationship of the chromatin protein to the ME491 antigen is unclear. Nuclear uptake of the antibody was stimulated after preincubation of cells with puromycin or actinomycin D [26]. It is quite unclear whether and how this remarkable phenomenon is related to the biological function of ME491/CD63. In addition, an mAb against rat homologue of CD63/ME491 inhibited IgE-mediated histamine release from basophilic leukaemia cells [27].

The existence of a member of this family, the Sm23 antigen, in the phylogenetically very distant invertebrate species *Schisostoma mansoni* may indicate that these proteins are evolutionarily very old and serve some essential function(s). On the other hand, the antigenic similarity between the parasite molecule and multiple leucocyte surface molecules might play an as yet undetermined role in combating the host immune system [12].

Some of the above described effects initiated by the binding of antibodies to these cell surface molecules may be related to the reported non-covalent associations of some of them with other cell surface components: the CD9 antigen may be associated on the thrombocyte surface with the major adhesive molecule of these cells, the integrin gp IIb/IIIa [28]. It was even claimed that CD9 has a kinase activity [29]. The TAPA-1 protein is associated on some cell lines with several other molecules, including the 16 kDa Leu-13 antigen of so far unknown function [11]. Especially interesting is the recent report on apparent association of rat homologue of the ME491 (CD63) antigen with the high affinity IgE receptor [27].

The structure of this family of membrane (glyco)proteins (polypeptide chain multiply crossing the membrane) is reminiscent of many well known ion channels and other transport molecules [30–32] and also of some receptors [18,33,34]. The observed functional effects of some mAbs against these molecules seem to be compatible with a similar role. With respect to the reported associations with other cell surface components it is tempting to suggest that these molecules could serve as receptor-linked ion channels. Future experiments are needed to test such a hypothesis.

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