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Signal transduction in leucocytes via GPI-anchored proteins: an experimental artefact or an aspect of immunoreceptor function?

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Abstract

Membrane proteins anchored in the membrane via a glycolipid glycosylphosphatidylinositol (GPI) as well as some glycolipids are able to transduce signals and induce diverse functional responses in cells upon their cross-linking via antibodies or natural ligands. In some cases this signaling capacity seems to be due to associations of these molecules with specific transmembrane proteins. GPI-anchored proteins are components of membrane microdomains enriched in glycosphingolipids and cholesterol and devoid of most transmembrane proteins. These membrane specializations are relatively resistant to solubilization in solutions of some mild detergents at low temperatures. These 'GPI-microdomains' contain also cytoplasmic signaling molecules such as Src-family protein tyrosine kinases and trimeric G-proteins. Thus, at least some signaling elicited upon cross-linking of GPI-anchored proteins and glycolipids may be due to perturbation of the signaling molecules associated with these microdomains. It is suggested that these specialized areas of the membrane rich in signaling molecules interact with immunoreceptors (TCR, BCR, Fc receptors) cross-linked upon their interactions with ligands and importantly contribute to initiation of proximal phases of their signaling pathways.

Keywords: Signaling; Glycosyl phosphatidylinositol; Immunoreceptors; Glycolipids; Kinases

1. Introduction

Most leucocyte surface proteins are transmembrane molecules—they possess an extracellular ligand-binding domain, one or several transmembrane stretches of approximately 20 hydrophobic amino acids and an intracellular part of variable length. Signaling via these molecules, e.g. cytokine receptors or adhesion molecules, is known to be mediated mostly via cytoplasmic molecules such as protein tyrosine kinases or trimeric G-proteins associated with their intracellular domains. However, several leucocyte surface glycoproteins are anchored in the outer leaflet of the membrane via a glycolipid (glycosylphosphatidylinositol; GPI) covalently attached to the C-terminal amino acid of the polypeptide chain [1]. Somewhat paradoxically, ligation of these molecules with antibodies or natural ligands can also elicit signals resulting in various cellular responses in spite of the fact that they possess no intracellular domains and therefore have no direct access to cytoplasmic signaling molecules [2,3]. Similarly, marked cellular responses can be elicited also by cross-linking of membrane glycolipids by antibodies or cholera toxin [4–6].

These GPI-anchored leucocyte surface proteins include the lipopolysaccharide receptor of myeloid cells CD14 [7], granulocyte low affinity IgG receptor CD16B (FcγRIIIb) [8], adhesion molecules CD58 (LFA-3) [9], CD48 [10], CD90 (Thy-1; the adhesion function re-

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mains somewhat unclear) [11,12], Ly-6 proteins [13], CD24 (heat stable antigen; HSA) [14,15] and CD52 (adhesion function unclear) [16], complement-protecting proteins CD55 (decay accelerating factor; DAF) [17] and CD59 [18], ectoenzymes CD73 (5′-nucleotidase) [19], CD157 (BST-1; NAD-glycohydrolase) [20], RT6 (arginine-ADP-ribosyl transferase) [21] and alkaline phosphatase [22], a protease receptor CD87 (urokinase plasminogen activator receptor; uPA-R) [23] and a non-classical MHC class I protein Qa-2 [24]. All these molecules were shown to be able to transduce either activating, inhibitory or even apoptotic signals upon ligation with natural ligands or suitable antibodies. Actually, activation of murine T-cells by cross-linking of their surface Thy-1 molecules with some antibodies resembled so closely the effects elicited by ligation of TCR that this molecule was for some time thought to be possibly a component of the murine TCR complex [25].

Examples of the functional effects elicited by ligation of leucocyte surface GPI-anchored molecules are listed in Table 1. It should be noted that most of these effects were observed after ligation of the GPI-anchored proteins by unnatural ligands – antibodies. Cellular responses elicited by natural ligands were observed in the case of CD14 (after binding of LPS), CD16B (after binding of IgG-containing immune complexes) and CD87 (after binding of uPA).

Notably, in several cases (MHC class I H-2D [101], Qa-2 [101], Ly-6A/E [109]) it could be demonstrated that the signaling capacity of a GPI-anchored protein was dependent on the presence of the GPI-anchor—transmembrane versions of these proteins were unable to signal. In several other instances, such as CD73 [63], Ly-6E [110], CD14 [31] transmembrane versions were able to signal as well.

2. Signalling due to association with transmembrane receptors

The most obvious possibility to explain the surprising signaling capacity of GPI-anchored proteins is their association with putative transmembrane proteins that can signal via conventional mechanisms. Well-defined associations between GPI-anchored and transmembrane proteins were indeed described in several cases: the ciliary neurotrophic factor (CNTF) receptor is a GPI-anchored protein which binds to a conventional transmembrane subunit, gp130 (CD130), the signaling component of, e.g. the IL-6 receptor [111]. Similarly, the GPI-anchored glial cell-derived neutrophic factor (GDNF) receptor-α associates upon the ligand (GDNF) binding to a transmembrane receptor, the receptor tyrosine kinase Ret [112]. Ret is actually a receptor for a composite ligand, the GDNF-GDNF-Rα complex. The GPI-anchor of the GDNF-Rα seems to be essentially dispensable—even soluble GDNF-Rα bound to GDNF activates Ret. Ret can interact and be activated in a very similar way with an alternative ligand neurturin bound to a GPI-anchored receptor [113].

At least three functionally important immunocyte receptors, CD14, CD16B and CD87 (uPA-R) form noncovalent complexes with complement receptor type 3 (CR3), a heterodimeric transmembrane β2-integrin (CD11b/CD18) [114–117,68]. These complexes are based on interactions of the lectin site in the CR3 molecule with a carbohydrate moiety (perhaps the glycan of the GPI-anchor) of these GPI-anchored proteins [117]. It is possible that signaling capacity of CD14, CD16B and CD87 is in this way at least in part dependent on poorly defined interactions of intracellular domains of the CR3 chains with cytoplasmic signaling molecules. A controversy exists concerning signaling induced by LPS, for which CD14 is the major receptor [27]. It is very likely that CD14 (or the complex of CD14 with LPS) binds to another, probably transmembrane protein, that is responsible for the signaling [118,119]. It is not clear whether this elusive 'CD14/LPS receptor' is, at least in myeloid cells, identical to CR3 [117].

3. Signaling based on residence of GPI-anchored proteins in specific membrane microdomains

A characteristic feature of the GPI-anchored proteins is their poor solubility in solutions of certain types of detergents (e.g. Triton X-100, Nonidet P-40, deoxycholate) at low temperatures [120,124–126]; for review see Refs. [127–129]. This contrasts with good solubility of most transmembrane proteins under the same conditions. Thus, if cell membranes are solubilized on ice in buffers containing, e.g. 1% Triton X-100, at least a fraction of GPI-anchored proteins can be more or less easily sedimented by centrifugation. The detergent-resistant particles rich in GPI-anchored proteins are of low buoyant density as demonstrated by density gradient ultracentrifugation [121,125,126]. These 'GPI-complexes' obtained from most cell types are rich in glycosphingolipids and cholesterol but devoid of most transmembrane proteins; alternative names used by different authors for these structures are 'glycosphingolipid-cholesterol rafts' or 'detergent-insoluble glycolipid-enriched domains' (DIGs) [129]. In a first approximation, they appear to correspond to membrane microdomains of distinct composition different from the rest of the membrane that is rich mainly in glycerophospholipids and transmembrane proteins. In polarized epithelial cells a considerable part of the apical (luminal) side of the cell membrane is composed

Table 1
Signaling via GPI-anchored leucocyte surface molecules

Molecule	Responding cells	Agonists and conditions used	Type of response observed	References
CD14 (LPS receptor)	Human monocytes	Soluble mAb	Oxidative burst, IL-1 secretion	[26]
	Human monocytes	Soluble mAb $F(ab')_2$, Fab	Homotypic adhesion (LFA-1 dependent)	[28]
	Human monocytes	Soluble mAb	Suppression of T-cell proliferation (by the treated monocytes)	[30]
	Human monocytes and neutrophils	Soluble mAb $F(ab')_2$,	Increase of cytoplasmic Ca^{2+}, oxidative burst	[29]
	Human monocytes	LPS	TNF secretion	[27]
	Human monocytes	LPS	NF-κB activation, tyrosine phosphorylation	[31]
	Human monocytes and macrophages	LPS	Tyrosine phosphorylation	[32,33]
	Mouse cells transfected with human CD14; macrophages	Bacterial peptidoglycan	Activation of NF-κB	[34]
CD16B (FcγRIIIb)	Human neutrophils	Cross-linking with mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}	[35–37]
	Human neutrophils	Cross-linking with mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}, oxidative burst	[41]
	Human neutrophils	Aggregated $F(ab')_2$ fragments of mAbs; immunocomplexes	Increase of cytoplasmic Ca^{2+}, oxidative burst	[40]
	Human neutrophils	Erythrocytes coated with CD16 mAb	Induction of phagocytosis	[38]
	Human neutrophils	Immobilized CD16 mAbs in combination with CD32 and CD11b mAbs	Oxidative burst, tyrosine phosphorylation of CD32 (FcγRII)	[39]
CD24 (HSA, J11d)	Human B-cells and neutrophils	Cross-linking with mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}, oxidative burst	[42]
	Human B-CLL cells	Cross-linking with mAb and secondary Ab	Tyrosine phosphorylation	[43]
	Human neutrophils (primed with FMLP)	Cross-linking with mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}, oxidative burst	[41]
	Mouse T-cells	Soluble mAb (+suboptimal conc. of CD3/CD28 mAbs)	Proliferation	[44]
	Mouse pre-B-cells	Soluble mAb or cross-linking by mAb and secondary Ab	Apoptosis	[45]
	Mouse resting B-cells	Cross-linking by mAb and secondary Ab	Inhibition of proliferation induced by anti-CD40 and IL-4	[45]
	Mouse monocytic cell line	Cross-linking by mAb and secondary Ab	Homotypic adhesion, tyrosine phosphorylation	[46]
	Mouse B lymphoblasts	Soluble mAb	Inhibition of aggregation, increase of cytoplasmic Ca^{2+}	[47]
CD48	Human neutrophils	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}	[37]
	Human T-cell line Jurkat	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}	[49]
	Human T-cells	Cross-linking by mAb and secondary Ab	Tyrosine phosphorylation	[43]
	Human B-cells	Soluble mAb (+anti-CD40, IL-4, IL-10)	Cell aggregation, proliferation, Ig secretion	[48]
	Human B-cell line Ramos	Soluble mAb (+IL-4)	Expression of activation antigens (CD23)	[48]
	Rat B-cells	Soluble mAbs	Homotypic aggregation	[50]
CD52	Human T-cells	Cross-linking by mAb and secondary Ab (+PMA); unique soluble CD52 mAb	Proliferation, cytokine production	[51]
	Human monocytes	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}, oxidative burst	[41]

Table 1 (*Continued*)

Molecule	Responding cells	Agonists and conditions used	Type of response observed	References
CD55 (DAF)	Human T-cells	Soluble mAb (+PMA); enhanced by crosslinking by secondary Ab	Proliferation	[52]
	Human T-cells	Cross-linking by mAb and secondary Ab	Tyrosine phosphorylation	[43,54]
	Human monocytes	Soluble mAb	Oxidative burst, enhanced phagocytosis	[53]
	Human monocytes and neutrophils	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}, oxidative burst	[37,41]
CD58 (LFA-3)	Human thymic epithelial cells	Soluble mAb or Fab	IL-1 production	[55]
	Human B-cells	Soluble mAb and IL-4	Isotype switch to IgE	[56]
	Human neutrophils	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}	[37]
CD59	Human T-cells	Cross-linking by mAb and secondary Ab (+PMA)	Increase of cytoplasmic Ca^{2+}, inositol phosphate production, IL-2 secretion, proliferation	[57]
	Human T-cells	Cross-linking by mAb and secondary Ab	Tyrosine phosphorylation	[43]
	Human T-cell line Jurkat, T-cells, thymocytes	Cross-linking by mAb and secondary Ab (synergy with PMA)	Tyrosine phosphorylation, IL-2 synthesis	[58]
	Human neutrophils	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}, oxidative burst, tyrosine phosphorylation	[37,41]
	Human T-cell line Jurkat	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}	[49]
CD66b (former CD67), CD66c	Human neutrophils	Soluble mAb	Adhesion to endothelial cells	[59]
	Human neutrophils (primed with FMLP)	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}, oxidative burst	[37,41]
CD73	Human T-cells	Polyclonal or monoclonal Abs (+PMA and monocytes)	Proliferation, IL-2R and IL-2 expression	[60]
	Human T-cells	Immobilized mAbs; strong enhancement by co-activation via CD2 and CD3	Increase of cytoplasmic Ca^{2+}, proliferation	[61]
	Human T-cell line Jurkat transfected with CD73	Soluble mAb and PMA	IL-2 secretion	[62]
	Human lymphocytes	Soluble mAb	Tyrosine phosphorylation	[63]
CD87 (uPA-R)	Human monocytes	uPA	Tyrosine phosphorylation	[64,67]
	Human epidermal cell line	uPA	DAG production	[65]
	Cell lines transfected with human CD87	uPA	Induction of migration	[66]
	Human monocytes	uPA	Differentiation to macrophages, expression of lysosomal proteases	[69]
	Human neutrophils	uPA	Increase of cytoplasmic Ca^{2+}, oxidative burst (after FMLP priming)	[68]
CD90 (Thy-1)	Mouse T-cells	Cross-linking by polyclonal Ab and secondary Ab (+PMA)	Proliferation	[70]
	Mouse T-cells	Soluble mAb	Proliferation, IL-2 production	[25]
	Mouse T_C cells	Soluble mAb	Production of cytokines	[71]
	Mouse T-cells	Cross-linking by mAb and secondary Ab (+PMA) or mixture of 2 soluble mAbs	Increase of cytoplasmic Ca^{2+}, proliferation	[72]
	Mouse T-cells, thymocytes and various transfectants	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}	[73]
	Mouse T-cells	Immobilized mAb to Thy-1 and CD3	Inhibition of activation via CD3	[74]
	Mouse thymocytes	Immobilized mAb	Apoptosis	[75]
	Mouse thymocytes	Cross-linking by mAb and secondary Ab	Apoptosis	[76]

Table 1 (*Continued*)

Molecule	Responding cells	Agonists and conditions used	Type of response observed	References
	Mouse T-cells, T-cell lymphoma	Soluble (self-aggregating rat IgG2c) mAb	Proliferation	[77]
	Mouse T-cell clone	Soluble mAb	Unresponsiveness to stimulation via TCR, down-modulation of TCR expression	[78,79]
	Human T-cell line Jurkat and transfectants	Cross-linking by mAb and secondary Ab	Influx of Ca^{2+} , production of phosphoinositides	[80]
	Mouse T-cells	Cross-linking by mAb and secondary Ab	Proliferation, IL-2, IFN-γ, IL-4 production	[81]
	Rat basophilic leukaemia cells	Soluble mAb	Increase of cytoplasmic Ca^{2+}, tyrosine phosphorylation, degranulation	[82–84]
	Human activated endothelial cells	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}	[85]
CD157 (BST-1, Mo5)	Human myeloid cell lines, transfectants	Soluble polyclonal Abs	Tyrosine phosphorylation of p130; growth inhibition (in transfectants)	[86]
Ly-6A/E	Mouse T-cells	Soluble mAb	Inhibition of proliferation induced by ConA, IL-2 or alloantigen	[87,88]
	Mouse T-cells	Soluble mAb (+IL-1 or accessory cells)	Proliferation; some mAbs-inhibition of antigen-driven activation	[89]
	Mouse thymocytes	Soluble mAb (+PMA)	Proliferation	[90]
	Mouse CTL	Soluble mAb	Proliferation, cytokine release; inhibition of IL-2-driven proliferation	[91]
	(Probably) T-cells, NK cells	MAb administered in vivo	Immunotherapy of tumours	[92]
	Mouse T-cells	Soluble mAb	Inhibition of IL-2 production elicited via TCR/CD3 stimulation	[93]
	Mouse B-cells	Soluble mAb (+IFN-γ, IL-4)	Proliferation, increased expression of Ly-6A/E	[94]
	Mouse B-cells	Cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+}, no effect on phosphoinositide turnover	[95]
	Human T-cell line Jurkat transfected with Ly-6	Soluble mAb (immobilized mAb inactive)	IL-2 secretion	[96]
	Mouse T-cell line EL-4	Soluble mAb	Induction of transcription factors NF-κB, AP-1, NF-AT	[97]
	Mouse thymic and bone marrow stromal cells	Cross-linking by mAb and secondary Ab	GM-CSF production	[98]
Ly-6C	Mouse $CD8^+$ T-cells	Cross-linking by mAb and secondary Ab	Expresssion of adhesion molecules, homotypic aggregation	[99]
Sheep Ly-6	Sheep T-cells	Cross-linking by mAb and secondary Ab or immobilized mAb; effects augmented by PMA	IL-2 secretion; inhibition of PHA responses; augmentation of allogeneic stimulation	[100]
Qa-2	Mouse T-cells	Cross-linking by mAb and secondary Ab	Proliferation	[101]
	Mouse $CD4^+$ T-cells	Cross-linking by mAb and secondary Ab (+PMA)	Proliferation	[102]
	Mouse $CD4^+$ T-cells	Immobilized mAb (+suboptimal conc. of immobilized anti-CD3)	Proliferation, increase of cytoplasmic Ca^{2+}	[103]
	Mouse spleen cells	Suitable pairs of soluble mAbs or cross-linking by mAb and secondary Ab (potentiated by PMA)	Proliferation, increase of cytoplasmic Ca^{2+}, IL-2R and IL-2 expression	[104]
gpTAA	Guinea pig T-cells	Cross-linking by mAb and secondary Ab	Proliferation	[105]
gp42	Rat NK-like cell line	Soluble mAb or cross-linking by mAb and secondary Ab	Increase of cytoplasmic Ca^{2+} and inositol phosphates	[106]
RT6	Rat T-cells	Polyclonal alloantisera	Proliferation	[107]
PrP	Human lymphocytes and cell lines	Polyclonal Abs	Suppression of mitogen-induced activation	[108]

mAb, monoclonal antibody; CLL, chronic lymphoblastoid leukaemia; CTL, cytotoxic T-lymphocytes; DAF, decay accelerating factor; FMLP, N-formyl-methionyl-leucyl-phenylalanine; GM CSF, granulocyte-monocyte colony stimulating factor; HSA, heat stable antigen; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; PHA, phytohemagglutinin; PMA, phorbol myristylacetate; PrP, prion protein; R, receptor; TCR, T-cell receptor; TNF, tumour necrosis factor; uPA, urokinase plasminogen activator.

of these glycosphingolipid-rich domains while basolateral part of the membrane is largely devoid of them [121,130,131]. It is still a matter of debate how exactly corresponds the composition of the GPI-complexes obtained after detergent solubilization to the native membrane microdomains; it is conceivable that the conditions used for solubilization (low temperature, detergent) may remove some components originally present in them in vivo or that originally rather small microdomains may coalesce artificially into larger patches. Nevertheless, model experiments employing artificial liposomes [132,133] and incorporation of fluorescently labeled GPI-proteins into native cell membranes [134] indicate that the GPI microdomains are a reality and not detergent artefacts. Moreover, small membrane fragments very similar to the detergent-resistant GPI-complexes can be obtained by purely mechanical membrane disintegration in the absence of any detergents [135,136]. The existence of such distinct 'floating islets' of distinct lipid composition in the membrane is plausible also from the physico-chemical point of view: a number of studies have demonstrated that certain lipids, such as glycosphingolipids and cholesterol, can form separate phases in the presence of other lipids, distinguished by higher orderedness and lower fluidity [137,138]. This mutual affinity between cholesterol and glycosphingolipids depends primarily on the length (18–26 carbon atoms) and saturated nature of the fatty acid residues in the latter. The glycolipid moiety of the GPI-anchored proteins has structural features similar to those of the glycosphingolipids and this may be the reason why they are accumulating in joint microdomains: the GPI-proteins are effectively a sort of glycolipid.

A point of essential importance is that these GPI-microdomains are enriched in well established signaling molecules such as Src family PTK's and trimeric G-proteins as well as other so far unidentified proteins, some of which are substrates of the kinases [120,122,123,125,126]. These cytoplasmic proteins are anchored in the cytoplasmic leaf of the microdomains also via covalently attached fatty acid residues (or other long aliphatic chains). Src family kinases possess myristic acid residues bound to their N-termini, as well as a second palmitic acid residue bound via a thioester bond to a cysteine residue close to the N-terminus [139,140]. Similarly, subunits of G-proteins are covalently modified by prenylation and palmitylation [141]. These posttranslational modifications are necessary for incorporation of these proteins into the specialized membrane microdomains.

Thus, GPI-anchored proteins, as well as certain glycolipids are components of membrane specializations rich in PTK's and G-proteins; these signaling molecules can be readily demonstrated in the immunoprecipitates obtained from detergent lysates of cells or cell membranes by means of antibodies to GPI-anchored proteins or glycolipids [43,68,120–123,142–144]. The existence of the GPI-microdomains containing such important signaling molecules seems to provide a very plausible explanation for the striking signaling capacity of both GPI-proteins and glycolipids: redistribution of these molecules within the external membrane leaflet of the microdomains may induce a redistribution of the PTK's on the opposite cytoplasmic leaflet. An important factor in this process seems to be the length of the fatty acid residues attached to the GPI-proteins and glycolipids, which may interdigitate with the aliphatic residues attached to the kinases. This model of signaling via the GPI-proteins nicely explains the results of experiments demonstrating the essential importance of their GPI-anchors and lack of signaling through transmembrane versions of these proteins [101,109]. In those cases where transmembrane version did signal [31,63,110], alternative mechanisms are more likely, such as direct association with other transmembrane proteins. The importance of the cholesterol-rich membrane domains in signaling via the GPI-anchored proteins is suggested also by the fact that cholesterol depletion interferes with T-cell activation via at least two different GPI-anchored proteins [49]. Incorporation of exogenously added glycolipids into cell membranes can lead to activation in a T-cell line; this could presumably be due to disturbance of the GPI-domains accompanied by redistribution of the cytoplasmic kinases [145].

When an exogenous fluorescently labelled GPI-anchored protein (CD59) was incorporated into the cells in vitro, it was initially distributed homogeneously on the cell surface, but after 2–3 h incubation the exogenous fluorescent CD59 acquired a punctuate distribution and concomitantly with this transition it acquired signaling capacity upon antibody crosslinking [134]. This appears to be a strong argument in favour of the signaling mechanisms based on residence of the GPI-proteins in the specific membrane microdomains.

Cross-linking of GPI- anchored proteins leading to redistribution and activation of the cytoplasmic kinases may be in some important mechanistic aspects considerably different from the activating cross-linking of other, more conventional receptors, because, plastic-immobilized mAbs to Ly-6 are not effective in triggering the activating process [96]; in contrast, plastic-immobilized mAbs to CD3 or TCR are very efficient in induction of activation.

Some specific transmembrane proteins do occur in the GPI-microdomains and it is possible that they play a role in physically linking the purely extracellular components (GPI proteins and glycolipids) with the cytoplasmic ones (e.g. Src family kinases) and especially in redistribution and activation of the kinases induced by antibodies to GPI-proteins or glycolipids. In most

cases only a relatively small fraction of these specific transmembrane proteins is present in these domains; it is not known what is the difference between the fractions present in them and excluded from them. A likely possibility is a difference in a hydrophobic posttranslational modification such as palmitylation. The transmembrane proteins demonstrated in the leucocyte GPI-domains include CD36 in platelets [146], CD4 and CD8 in T-lymphocytes [147], CD44 in various cell types [126,148–150], β2-integrins in myeloid cells [68], CD26 in lymphocytes [126], influenza virus hemagglutinin and neuraminidase in epithelial cells [151,152] and CD10 in some lymphoid cell lines (P. Angelisová et al; unpublished). Interesting components of these membrane domains are the extremely hydrophobic proteins (proteolipids) of the MAL family [153,154]. Most of these proteins are known to be capable of transmembrane signaling upon antibody cross-linking; it is likely that their presence in these specialized membrane domains is important in this respect.

At present it is not known whether the presence of some critical transmembrane proteins is indispensable for structural integrity of the GPI-domains and for their association with some of the cytoplasmic components, such as kinases. The results of the reconstitution experiments with lipid mixtures indicate that the major factor responsible for the existence of these membrane domains is the proper lipid composition [132,133]. In this respect it should be mentioned that even the GPI-anchored proteins are dispensable: the microdomains of very similar properties, containing glycolipids, Src-family PTKs and some of their cytoplasmic substrates, can be demonstrated in several types of mutant cell lines defective in GPI-synthesis (T. Cinek, T. Brdička, and J. Černý, unpublished). Thus the very name 'GPI-microdomains' suggested previously by us may not be the most appropriate one.

4. Other possible mechanisms of signaling via GPI-anchored proteins

An alternative theoretical possibility to the so far discussed mechanisms is that upon ligation of the GPI-anchored proteins cleavage by specific phospholipases is initiated yielding biologically active second messengers such as phosphatidic acid and diacylglycerol. Alternatively, protein-free GPIs produced after cleaving-off the protein part of the GPI-anchored proteins or soluble glycans derived from these GPI-anchors may have agonistic properties [155]. Indeed, activation-induced proteolytic shedding of some GPI-anchored proteins induced by various stimuli, including cross-linking by antibodies, was described [156].

5. A more general role of the GPI-microdomains in signaling via immunoreceptors?

A problem with the signaling via GPI-anchored proteins has been that this phenomenon was observed and studied almost exclusively using unnatural, model ligands, i.e. antibodies. Furthermore, signaling via CD14 induced by the natural ligand, LPS, seems to be independent of the associated Src family kinases [157,158]. A question is: do most these molecules make use of their potential signaling capacity in the real life at all? Recently a more interesting possibility started to emerge, namely that the PTK-rich GPI-domains play a role in signaling via crucially important immunoreceptors such as TCR, BCR and Fc receptors. These receptors are known to initiate their signaling by activation of Src-family PTKs which leads to phosphorylation of the ITAM motifs in the receptor-associated subunits (CD3 chains, ζ chain family, CD79 chains, FcεR β chain), followed by association of Syk family PTKs to the phosphorylated ITAMs and phosphorylation of further downstream elements of the signaling cascade (for review see Ref. [159–161]). Curiously, the amounts of the Src family kinases associated with the immunoreceptor complexes in the resting state seem to be very small but markedly increase upon receptor ligation. Concomitantly, the detergent solubility of the immunoreceptors markedly decreases upon their ligation and activation, which has been traditionally interpreted as association with cytoskeleton [162–167]. An alternative and attractive possibility is that upon ligation and cross-linking by natural ligands or antibodies the immunoreceptor complexes become somehow attached to the GPI-microdomains. In this way, the ITAM-containing subunits may get to a close contact with the Src family PTKs abundant in these membrane islets and can be phosphorylated by them. The association with the detergent-insoluble GPI-complexes (rather than association with cytoskeleton) may perhaps explain at least in part the previously described activation-induced immunoreceptor insolubility in detergent solutions and increased stoichiometry of association with the Src-kinases. Results of several recent studies support experimentally this idea: Field et al. [165] observed that after the interaction with a cross-linking antigen, FcεRI saturated with specific IgE became rapidly physically associated with the low-density membrane domains. Fluorescence microscopy demonstrated rapid, activation-induced SH2-dependent translocation of the FcεRI and signaling molecules (Syk, PLC-γ1) to punctuate plasma membrane domains rich in glycosphingolipids [166]. Detergent insolubility of TCR induced by its aggregation was not affected by inhibitors of actin or tubulin polymerization [167], indicating that it is not due to cytoskeleton association but perhaps due to association with the detergent-resistant membrane do-

mains. The idea on close structural and functional relationship between TCR signaling and GPI-anchored proteins (thus, by inference GPI-domains) is in agreement with the results of a number of early and more recent studies: T-cells defective in expression of individual or all GPI-anchored proteins exhibit abnormalities in TCR signaling [168–173]; full activation of murine T-cells by antibodies to Thy-1 and Ly-6 is dependent on expression of TCR or at least the ζ chain of the TCR complex [174–176] (however, partial response detectable by Ca^{2+} mobilization can be observed even in the absence of TCR components [175]); a major protein (p38) phosphorylated on tyrosine immediately after CD3 ligation by activating antibodies is a component of the buoyant GPI-complexes. The major protein (p38) of the GPI-domains rapidly phosphorylated on tyrosine upon CD3 ligation has been recently identified by us [184] as the key transmembrane linker protein LAT [185].

In this respect, the constitutive association of a fraction of CD4 and CD8 with GPI-domains described by us [147] may be of special interest—one may speculate that the communication between TCR and the GPI-domains upon TCR ligation is mediated via this fraction of the co-receptor molecules.

Merging of transmembrane proteins with the GPI-domains following their antibody-induced aggregation may be a more general phenomenon as indicated by the recent report on CD20 [177].

A remodelling of the T-cell surface in the area contacting the APC surface seems to be necessary to remove bulky and negatively charged molecules such as CD43 and CD45 interfering with the intercellular contact [178]. The GPI-domains are devoid of these potentially anti-adhesive molecules and adjoining of TCR to them might thus create better conditions for the T-cell-APC contact. The absence of a small but heavily glycosylated (including sialylation) major component of the murine T-cell GPI-domains, Thy-1, may further contribute to better adhesion between the TCR and APC, which is just what is observed experimentally in the Thy-$1^{-/-}$ mice [169]. On the other hand, the tyrosine phosphatase CD45 regulating positively the activity of Src family kinases [179] may be in contact with the outer 'rim' of the GPI-domains; at least one paper describes chemical cross-linking of Thy-1 to CD45 [180]; a fraction of CD45 is found in the domains obtained by non-detergent disintegration of plasma membranes [135] (however, another paper postulates the absence of CD45 from the GPI-domains as an important factor keeping the kinases in the domains in an inactive state [181]).

6. Problems to be solved

In spite of the progress in elucidation of the structural basis of signaling via GPI-anchored proteins and glycolipids and its possible relationship to signaling through some 'real' receptors, a number of questions remain to be solved:

It will be important to obtain more direct data on the size, composition and properties of the GPI-domains in situ. In this respect, transfectants expressing the components of the domains (GPI-anchored proteins, intracellular signaling proteins, the rare transmembrane proteins present in them) possessing fluorescent tags (green fluorescent protein) will be informative models.

It is not clear what is the heterogeneity of these membrane domains: do some of them contain predominantly some while others different GPI-anchored proteins or intracellular signaling proteins? One could speculate that qualitatively different membrane (micro)domains might specialize in cooperation with different transmembrane receptors. Indications of the heterogeneity do exist but research in this area struggles with inherent methodical problems—separation of potential different subspecies of the domains must rely upon minor differences in their size or density; immunoisolation approaches may be of limited use if different domains differ only quantitatively in the content of their individual components.

Of course, a major challenge is to prove or disprove the attractive idea on the reversible physical and functional communication between the GPI-domains and various transmembrane receptors and to determine what are molecular details of these interactions. Standard biochemical techniques may be of limited use because the associations between the receptors and the GPI-domains seem to be rather weak and sensitive even to mild detergents [165].

Another unclear point concerns the role of specific transmembrane proteins present in the domains and the reasons why only a fraction of, e.g. CD4 or CD44 is found in these domains while the rest is outside of them.

We hope it is not just wishful thinking that the years of sometimes frustrating studies on the GPI-domains are beginning to bring interesting fruits. Perhaps the GPI-domains are just a first example of a more general phenomenon—complex compartmentalization of the cell surface into different types of domains in which relevant sets of molecules co-exist and functionally cooperate [182,183]

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