

# Glycophosphatidylinositol-Anchored Membrane Proteins as Coreceptors in T-Cell Activation\*

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Successful destruction of tumours or virally infected cells by the immune system is thought to be dependent on induction of an effective T-cell response against endogenous antigens of the target cell, presented in association with major histocompatibility complex (MHC) antigens. The response of T lymphocytes confronted with this type of foreign antigen involves a complex array of molecular interactions between the T cell and stimulator cell, many of which occur at the cell membrane. Whilst the specificity of the response is determined by interaction of the antigen receptor complex on the T cell (TcR/CD3) with processed antigenic fragments presented by class I or class II (MHC) molecules, its magnitude is determined by a number of additional coreceptor/ligand interactions [1]. Originally, these additional molecules were loosely classified as either adhesion molecules or signaling structures. Nowadays, however, this classification has become more diffuse as several "accessory" molecules appear to have dual functions [2], and a large volume of literature is now devoted to their identification and functional characterisation.

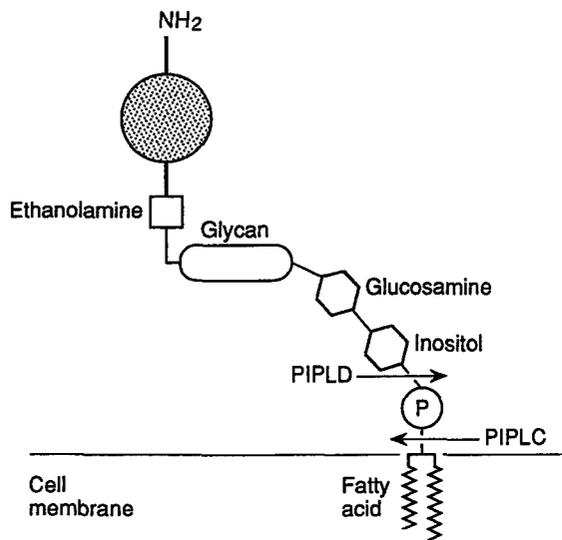
Monoclonal antibodies generated by immunising rats with mouse or human lymphocytes have been important tools for studying coreceptors and their ligands. Panels of hybridomas screened

for their ability to induce proliferation of T lymphocytes routinely contain antibodies reactive with components of the TcR/CD3 complex, but there are clearly a number of other molecules on T cells which have signal-transducing function, including CD2, CD28 and the interleukin receptors [3]. Although the *in vitro* mitogenic effects of these antibodies suggest the existence of alternative, antigen-independent, routes of T-cell activation, it is more likely that, *in vivo*, the function of these additional signaling molecules is to modify the effects of signals delivered by the TcR/CD3 complex.

Curiously, many hybridomas which induce proliferation of T cells bind to cell surface proteins which are linked to the cell membrane through glycophosphatidylinositol (GPI) anchors. The prototype GPI-linked signaling molecule is Thy-1, a differentiation antigen and a marker of the T-cell lineage in mice [4]. In other mammals Thy-1 homologues are found mainly on cells of the nervous system. Antibodies directed against Thy-1 cause proliferation of mouse T lymphocytes, in most cases giving optimal responses in the presence of costimulators such as phorbol myristate acetate (PMA), and anti-immunoglobulin antibodies [5]. Other GPI-linked signaling molecules, such as Ly-6 and 5'-nucleotidase are apparently unrelated to Thy-1 or to each other at the protein level, suggesting that the anchor is critical for signal-transducing capability [6]. A series of transgenic mouse strains carrying either transmembrane or GPI-linked derivatives of Qa-2 and H-2D<sup>b</sup> MHC class I molecules were constructed to test this hypothesis. The

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**Fig. 1.** Composition of a typical GPI membrane anchor. GPI anchors are found in yeast and in all higher eukaryotes so far examined. In some species, GPI appears to be the most common method of membrane anchoring. The anchor core is highly conserved and anchors from trypanosomes and mammalian cells differ only in their carbohydrate side chains and in the composition of the lipids. GPI anchors can be cleaved by phospholipases C and D at the positions shown (arrows), causing removal of the lipids and generation of water-soluble protein molecules

results show that GPI-linked class I polypeptides are effective signaling structures, but that the corresponding transmembrane forms are not [7]. As observed for Thy-1, optimal cell proliferation requires PMA and anti-immunoglobulin antibodies, indicating that these molecules cannot function independently to induce cell division.

Structural analysis of GPI anchors in diverse eukaryotes has provided few clues to the physiological function of this type of membrane anchor (Fig. 1). GPI-anchored molecules, however, possess a number of physicochemical characteristics not shared by other signal-transducing molecules which may be relevant to their function in T-cell responses [8]. Firstly, the majority of GPI anchors are sensitive to cleavage by phospholipases C and D. Treatment of cells with phospholipase C releases GPI-linked protein molecules

from cell membranes in water-soluble form. Phospholipases specific for the phosphatidylinositol linkage (PIPLC and PIPLD) have been isolated both from bacteria and from eukaryotic cells. Enzymes of similar specificity may operate *in vivo* to prevent or to interrupt cell signaling events by detaching GPI-linked signaling molecules or ligands from the responding cell. In this context it is interesting that some populations of T lymphocytes, in particular those which have been previously activated by antigen or mitogens, possess GPI anchors which are resistant to PIPLC [9]. We have also found that a number of cloned antigen-specific mouse T-lymphocyte cell lines have PIPLC-resistant anchors (Robinson and Spencer, unpublished). If release of GPI-linked proteins by PIPLC-like enzymes has a limiting effect on T-cell proliferative responses, it is possible that cells with resistant anchors may have a selective growth advantage. It is therefore of interest to elucidate the molecular basis for this resistance.

A second property of GPI-linked molecules, and one which distinguishes them from other signaling molecules, is the absence of a direct interaction with cytoplasmic components. The fact that the fatty acid anchoring moiety of GPI is similar in size and composition to other membrane phospholipids indicates that it associates with only the outer face of the lipid bilayer. One manifestation of this is that the lateral mobility of GPI-linked molecules within the lipid bilayer is generally substantially greater than that of transmembrane molecules [10]. It also poses a dilemma in explaining how the binding of antibodies to GPI-linked proteins is able to induce intracellular biochemical changes. Two mechanisms have been proposed to resolve this paradox. Firstly, GPI-linked molecules may interact with additional, presumably transmembrane, proteins which transmit signals to the cytoplasm of the cell. Such hypothetical transducer molecules are likely to associate with structural motifs on the anchor, thus enabling a whole

range of structurally unrelated protein molecules to couple to a single signal transduction pathway [6]. Secondly, binding of antibodies or ligands may induce internalisation and subsequent degradation of the anchor, releasing potentially stimulatory fragments [11]. The main evidence for the first hypothesis is that, although a high proportion of molecules of any one GPI-linked species exhibits relatively high lateral mobility in membranes, there is invariably a proportion of low mobility [10]. This suggests that mobility can be restricted by interactions with other membrane components which are in turn associated with cytoskeletal elements. The main argument for the second hypothesis is that antibodies specific for GPI-linked proteins which are stimulatory in soluble form do not function when immobilised to plastic plates [12]. Immobilisation may thus prevent signaling by blocking internalisation of immune complexes. Antibodies against GPI-linked molecules, immobilised together with submitogenic amounts of TcR/CD3-specific antibodies, have been found to be highly mitogenic for T cells, suggesting that this may closely mimic a physiological situation in which the GPI pathway can amplify an antigen-specific stimulus [12].

Future research in this area must concentrate not only on the mechanism of GPI-mediated signaling, but on determining the role played by this group of molecules in cellular immunity. This will require identification of the ligands for GPI-linked molecules. Only one example of the natural ligand for a GPI-anchored protein is known to date. This is CD2, which binds specifically to the GPI-linked lymphocyte function-associated antigen LFA-3 [13]. CD2 is itself a signaling molecule on human T lymphocytes and a homologue is also known in mice. One approach towards identifying new ligands is to determine their distribution using water-soluble, PIPLC-cleaved, receptor molecules as probes to stain tissue sections. This strategy could be adapted for cloning the corresponding genes by

screening cDNA expression libraries produced using episomally replicating vector systems [14]. Until more information is available on ligands for GPI-linked proteins it may be possible to investigate physiological responses using antibodies against GPI-linked molecules attached to the surface of stimulator cells. This can be achieved using antibodies derivatized with esters of palmitic acid [15]. The fatty acid chains can insert into membranes of the stimulatory cell and thus present the antibody molecules to T cells in a form closely resembling a normal membrane protein. This type of "surrogate" ligand more closely resembles a physiological situation than does the present approach of adding free soluble antibodies to cell cultures. There are examples of proteins which naturally use fatty acids, directly attached to the polypeptide backbone, as a means of anchoring in membranes (p56<sup>lck</sup>) [16].

In summary, GPI-linked T-lymphocyte surface proteins are potent signal transducing molecules and are likely to be influential in controlling cellular immune responses. This represents the first clear physiological role for this group of proteins on mammalian cells and points towards a much wider application of this signaling pathway in biological systems.

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