Heterogeneity of interactions mediated by membrane glycoproteins of lymphocytes

A. N. Barclay and M. H. Brown

MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, U.K.

Introduction

About 150 different proteins and glycoproteins have been characterized on the surface of various leucocyte populations that are not widely expressed on other cell types [1]. These proteins are candidates for mediation of the many functions that depend on interactions at the leucocyte surface. With regard to lymphocytes, the types of functions that can be expected involve receptors for soluble factors such as cytokines, those involved in antigen recognition on T-cells and B-cells and a category of molecules often termed adhesion proteins which are thought to interact with proteins on the surface of other cell types and so modify the specific response through the specific antigen receptors. Other cell surface proteins will be responsible for the interactions that specify the migration of lymphocytes from blood, through the lymphoid tissues and back to blood via the lymph. Analysis of the amino acid sequences of the surface proteins show that many contain regions of sequence similarity to other proteins, that is they belong to different superfamilies. The most common of these is the immunoglobulin superfamily (IgSF), and domains belonging to this superfamily are present in about 40% of leucocyte surface proteins [1]. The importance of the superfamily concept is that domains belonging to a particular superfamily are predicted to be related in evolution, likely to have a similar fold and probably have a similar type of function. There are now many structures for which these concepts have been confirmed. About 20 different superfamilies have been identified, and the most common domain types are often involved in recognition events, e.g. IgSF domains and fibronectin type-III domains. One simple conclusion from this type of analysis is how few enzymes have been recognized at the cell surface (<5% of the sequences), and no enzyme activity has been found with many of the different superfamily types (IgSF, fibronectin type II or III, cytokine receptor, complement control protein, C and S-type lectins, link, etc.) [1].

There are two diametrically opposite approaches to the molecular analysis of lymphocyte surface functions. One is to take a particular function and identify and characterize the proteins responsible for it, e.g. antigen recognition, and the second is to look at the surface proteins and attempt to characterize their functions. Recent advances in the latter approach are described below.

Organization of proteins and glycoproteins at the surface of leucocytes

There is a wide variety of proteins at the cell surface. They vary in size from consisting of only 12 amino acids plus one N-linked glycosylation site in the case of CD52 to very large proteins that appear from electron-microscopy studies on the isolated proteins to be extended linear molecules of up to 50 nm in length for CD43 and CD45 and 80 nm for the complement receptor I [1]. CD43 and CD45 are very abundant and are present on thymocytes at about 100 000 molecules per cell compared with 15 000–25 000 for proteins such as CD2, CD4 and CD8. CD43 and
CD45 are also highly glycosylated, with 50–75% of the extracellular part being carbohydrate. It is clear that large molecules like CD45 and CD43 will have a major impact on cell–cell interactions and may well be the first molecules that interact with other cells. Some evidence for the importance of CD43 comes from the effect of its knock-out on the stickiness of the cells and the greater ease with which they can be stimulated [2]. Another feature that will affect the ease with which interactions occur between cells is the local distribution of the molecule at the cell surface. Thus L-selectin (CD62L) is found only at the tips of microvilli of neutrophils [3]. It is therefore in an ideal position to make the first contact with endothelium and mediate the rolling of neutrophils that precedes stronger adherence, flattening and migration between the endothelium involving integrins and other proteins [4].

**Many interactions at the surface of lymphocytes are transient and involve low-affinity interactions**

One feature of leucocytes that distinguishes them from the cells of most other tissues is the transient nature of their interactions. These occur during the migration of cells through the tissues in specific patterns (see, for example, the patterns of B- and T-cells in lymphoid organs [5]) and in the interactions between say T-cells and antigen-presenting cells. The use of recombinant proteins has enabled the kinetic parameters of these interactions to be studied with new methods using surface plasmon resonance as the detection system in the BLAcore apparatus. The ability to be able to follow weak interactions in real time using unlabelled materials has shown that some of the adhesion interactions have very low affinities (of the order of $K_d = 25 \, \mu M$) and extremely fast dissociation rates (with a half-life of less than a fraction of a second) (reviewed in [6]). The best characterized interactions are those between CD2 and its ligands CD48 in rodents [7] and CD58 in humans [8]. All these proteins contain two IgSF domains.

**Identification of ligands for cell surface proteins**

A key step in the characterization of the lymphocyte cell surface is the identification of ligands for the proteins at the surface. The best characterized groups include the receptors for cytokines, which make up about 10% of the leucocyte surface proteins. In this case the interactions are of high affinity and involve soluble ligands. Another group of proteins related to tumour necrosis factor receptor bind either soluble or membrane-bound proteins, which themselves form a family related to tumour necrosis factor; these also have high affinities. However, there are a very large group of proteins, including the largest superfamily of proteins at the leucocyte surface, the IgSF, for which few ligands have been identified. As discussed above it seems likely that one reason for the difficulty in identifying their ligands is the low affinity of their interactions. In some cases the dissociation rate of the interactions is so fast that the half-life of the monomeric interaction is only a fraction of a second [7,8]. Thus most methods using monomeric proteins will fail to detect this type of interaction because any binding that occurs will be lost during the washing stages necessary in most assays. The alternative is to make the ligand polymeric. There are a number of ways of doing this, including expressing the proteins at high levels on cells and testing for interactions with other cells, as was used for the detection of the interaction of CD4 with MHC class-II antigens [9], the production of chimaeric multimeric proteins, such as chimaeras between the ligand of interest and IgFc regions, and the coupling of proteins to magnetic or fluorescent beads. One method is described below.

**Detection of low-affinity interactions using chimaeric recombinant proteins and fluorescent Covaspheres™**

One of the main tools for looking at cell surface antigens is the production of the extracellular parts of the antigens using recombinant DNA techniques. This allows large amounts of proteins to be produced. In addition, they can be made with antigenic tags, which can be used to recognize the proteins and display them in a multimeric array. Figure 1 shows a number of different chimaeric proteins using Ig Fc regions from IgG or IgM to give bivalent or decavalent proteins respectively or the hinge region of CD8 or domains 3 + 4 of CD4. This latter method was introduced because this CD4d3+4 construct was expressed at very high levels as a recombinant protein. The protein was stable even at high concentration and its structure determined by X-ray crystallography [11]. Several different types of
domain have been made into chimaeras with CD4d3+4 [12]. One advantage of using a monoclonal chimaera with CD4d3+4 is that the protein can be used for kinetic studies and this is illustrated in studies of CD2 and its ligands (see [6] and above and Figure 2).

Figure 3 illustrates the method in which the chimaeric proteins were coupled to fluorescent Covaspheres in order to display the ligand indirectly through antibodies that recognize the CD4d3+4 part. The beads were tested using chimaeras of CD2 with CD4d3+4 [13].
Although this reaction is of extremely low affinity (see above), very strong labelling of cells was obtained with this construct (Figure 3). Immunoglobulin CD48 fusion proteins gave no direct labelling of cells but when coupled to the Covaspheres gave good labelling (not shown but see [13]). A variety of other constructs have been tested and preliminary data indicate that there is a ligand for the MRC OX2 antigen, another 2 IgSF domain protein, on macrophages (S. Preston, M. H. Brown and A. N. Barclay, unpublished work).

Conclusions
The Covsphere assay provides a method of identifying interactions of leucocyte surface proteins, many of which are likely to be of low affinity and may only be detectable using methods giving multimeric displays of protein. The method has also been used in other systems such as the characterization of brain adhesion proteins, for example the L1 protein which contains six IgSF domains and five fibronectin type-III domains [14]. One factor that distinguishes many neural cell surface IgSF proteins is their high degree of amino acid sequence conservation across species. This suggests that there are more constraints on neural cell interactions than in the immune system. Recently, mutants within the IgSF domains of L1 have been shown to be associated with X-linked hydrocephalus and the related MASA (mental retardation, aphasias, shuffling gait and adducted thumbs); these mutant L1 proteins result in altered homophilic adhesion and neurogenic potential [15]. In contrast, polymorphisms are particularly common in lymphocyte cell surface proteins (reviewed in [1]). These were of particular value in the 1970s in the recognition of cell surface proteins as alloantigens using antibodies raised between strains of a species, e.g. CD8 (Ly2,3), CD45 (Ly5) and CD5 (Ly1).

5 Barclay, A. N. (1981) Immunology 42, 593–600
The term metastasis was used first to describe the spread of infectious disease from one part of the body to another [1]. Metastasis results from a multistep process of invasion, occurring within microecosystems in which invaders and host cells are involved in continuous molecular cross-talk [2]. Such microecosystems are found at the sites of primary invasion, entry into the circulation and extravasation. The multistep process of invasion is not unique to cancer cells. Leucocytes, parasites and bacteria also metastasize, and cellular and molecular mechanisms of the multistep invasion process of metastasis show resemblance in all these invaders [3]. Glycoconjugates play a role in several of the crucial molecular cross-talks between invaders and host cells.

In the present paper we discuss selected examples, from our own experience as well as from the literature, asking the question whether or not glycoconjugates are implicated in the molecular cross-talk of invasion by cancer cells, leucocytes and micro-organisms. Most of the examples concern carbohydrate–protein (lectin) interactions. Less attention has been paid, so far, to carbohydrate–carbohydrate interactions [4].

Cancer cells
Cell-surface-bound heparan sulphate proteoglycans are involved in the regulation of cancer cell invasion-associated activities such as cell–cell adhesion, cell–matrix adhesion, motility and proteolysis [5]. Furthermore most of the molecules that participate in these cellular activities are expressed at the cell surface or are secreted glycoproteins. Accordingly, invasion in vitro can be manipulated with some inhibitors of glycoprotein synthesis and processing, although not by other inhibitors [6].

Cell–cell adhesion molecules may suppress as well as promote invasion, depending on the type of microecosystem. They not only mediate attachment of cells to each other but they also have signalling functions. E-Cadherin is a 120 kDa transmembrane glycoprotein that acts as an epithelial organizer and a suppressor of primary invasion in experimental and clinical cancer [7–10]. The anti-invasive as well as other functions of E-cadherin depend on correct linkage to the cytoplasmic α-, β- or γ-catenins, with molecular masses of respectively 102, 97 and 82 kDa, to the 120 kDa cadherin-associated p60α-substrate, p120αS and probably to other proteins also. α-Catenin links the complex to the actin cytoskeleton. The E-cadherin–catenin complex is regulated at various levels. Its structure may be altered through genomic mutation, mRNA instability or post-translational modifications of one of its components [11,12]. E-Cadherin is complex glycosylated but this glycosylation does not seem to play a major role [13]. E-Cadherin is synthesized as a 150 kDa precursor polypeptide that is glycosylated in the endoplasmic reticulum. After addition of the complex carbohydrate groups in the Golgi complex, it is processed to the mature 120 kDa glycoprotein and delivered to the cell surface. Experiments with tunicamycin showed that glycosylation is not required for ef-