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A Hypothesis on the Biological Role of ABH, Lewis and P Blood Group Determinant Structures in Glycosphingolipids and Glycoproteins

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A hypothesis is presented that glycosphingolipids of circulating erythrocytes are membrane-packing substances providing for an energetically cheap carbohydrate protective coat at the cell surface. The glycosphingolipids should cover the membrane surface not occupied by functional glycoproteins. This role is envisaged for the globo series of glycosphingolipids which are P^k and P antigens of human blood. Glycosphingolipids of the neolacto series terminated with non-informative A,B,H, Lewis, P_1 antigenic structures as well as with sialic acid residues should serve the same purpose. These carbohydrate structures may be also used for conferring biological inertness on otherwise functionally active carbohydrate structures and provide protection for circulatory and membrane glycoproteins from proteolysis, denaturation and recognition of potentially antigenic sites of protein moieties by the immunosurveillance system of the body. At the external body surface the same carbohydrate structures may protect cells from the action of pathogenic microorganisms and other environmental factors. The roles of the above mentioned carbohydrate sequences on glycosphingolipids and glycoproteins in the development, tumorigenesis and evolution of blood group polymorphism are discussed.

1. Introduction

ABO blood groups were discovered 81 years ago. More than half a century later the chemical structures and biosynthetic pathways of ABH immunodeterminant groups on secretory glycoproteins (GPs) were elucidated [1, 2] and subsequently identified on erythrocyte membrane glycosphingolipids (GSLs) [3, 4] and GPs [5]. These structures (and also the related Lewis antigens) turned out to be di- to pentasaccharide sequences loca-

Abbreviations: GP, glycoprotein; GSL, glycosphingolipid; GC, glycoconjugate.

ted at terminal non-reducing ends of saccharide chains of glycoconjugates (GCs). Meanwhile, some 21 additional blood group systems including 170 antigens have been described [6, 7]. Some of these antigens were investigated by chemical methods and also found to be GCs. Thus P_1, P, and P^k antigens belonging to the formerly delineated P blood group system [6] were identified as GSLs [8] while I [9] and Cad antigens are both GPs and GSLs [10, 11]. In all these antigens the blood group specificity depends on the structure of the carbohydrate moieties of GCs. Yet in spite of all the progress in the field and a considerable amount of time and effort the blood groups have not as yet been assigned a function. Exactly the same applies to most GSLs which in spite of their early discovery (in 1874) are a class of compounds without a definite biological function. I would like to propose a function for at least some of the GSLs and blood group substances in that they provide an inert, non-informative carbohydrate coat at the cell surface. My argumentation will be speculative and therefore open to criticism. Nevertheless, in view of the limited understanding of the biological role of the cell surface carbohydrates and neutral GSLs and of blood group substances in particular, putting my ideas to print might be useful at least for the sake of discussion.

2. A Possible Role for the Cell Surface Carbohydrate

The basic role of the cell membrane carbohydrate has been subject to numerous hypotheses. Among others it has been proposed that they may prevent cell fusion [5] or lock membrane GPs in position, i.e. preventing them from diffusing back to the cytoplasm [12]. A more specific function claimed for membrane carbohydrates is to provide recognition sites for animal lectins [13], glycosyltransferases [14] and various aggregation as well as adhesive proteins some of which are developmentally regulated [15, 16]. Cell surface carbohydrates of mammals and presumably other animals are also used as recognition molecules for pathogenic microorganisms [17], toxins [18] and viruses [19]. Conversely, the immune surveillance system of mammals recognizes carbohydrate structures of foreign cells [20] and pathogenic microorganisms. The recognition function of carbohydrate structures is substantiated by the intracellular sorting mechanism of lysosomal GP hydrolases which is dependent on sugar-phosphate markers [21].

Studies on the role of carbohydrates in blood plasma GPs allow the generalization that they confer upon the protein moiety water solubility and resistance to denaturation and proteolysis [22]. Carbohydrates may also mask potentially antigenic sites of polypeptide chains, thus allowing for greater evolutionary flexibility. Yet they may not be indispensable for the biological function of GPs as seems to be the case for immunoglobulins [22]. Conclusions drawn from the studies on blood plasma GPs should be probably also valid for those present at the cell membranes. I would favour the concept that the basic function of carbohydrates in GPs and at the surface of lipid membranes has been that of protection. An informative role of the carbohydrates may have developed later in evolution. The carbohydrates on the cell membrane are present partly as GPs and partly as GSLs. Unlike the former which are also widely distributed in body fluids and secretions the latter are largely restricted to the cell membranes.

3. Glycosphingolipids versus Glycoproteins

GSLs are composed solely of glycan and ceramide. The ceramide portion is inserted into the outer leaflet of the membrane bilayer whereas the glycan projects into the cell environment. Thus the compounds may be specifically designed to contribute to the carbohydrate-rich layer at the membrane surface. The best characterized membrane GPs are the integral GPs of human erythrocytes. The carbohydrate chains of these GPs are asymmetrically distributed and attached only to that portion of the peptide which extends beyond and outside the membrane boundary. The inner portions of the peptide chains pierce through the membrane and may provide anchorage for the contractile proteins and other cytoskeleton elements inside the cells [23]. Thus some integral membrane GPs of erythrocytes are instrumental in maintaining cell shape. In addition they may provide specific channels for transport of hydrophilic solutes across the cell membrane. I assume that all membrane GPs should have some function apart from that they contribute to cell surface carbohydrates, even if it is unknown at present. I assume further that GSLs have primarily evolved as inexpensive carbohydrate-containing membrane packing material. Biosynthesis of the ceramide is energetically cheap ($\sim$ 20 kcal/mole) and requires only ubiquitous fatty acids and serine [4]. The ceramide thus obtained allows dependable attachment of a glycan to the membrane. In contrast, the biosynthesis of proteins as membrane glycan carriers is energetically expensive and requires 29.2 kcal per single peptide bond. Therefore GPs of the membranes should perhaps be synthesized for specific functions. Membrane packing properties of GSLs are exemplified by the structure of myelin. This consists of multiple layers of densely packed lipid membranes and contains galactosylceramides as the main carbohydrate constituent at a molar proportion of 1:2 with respect to phospholipids [24]. I do not rule out specific functions of GSLs but at least for some of them like those present at the surface of erythrocytes I would envisage primarily the role of largely inert membrane packing carbohydrate-containing substances. In general I would expect that such inert GSLs would occupy membrane space not covered by specific GPs and peripheral proteins.

4. The Circulating Erythrocyte

The main function of the erythrocyte is to carry and exchange oxygen for carbon dioxide throughout the body. During its life span of 120 days the erythrocyte is subjected to numerous filtrations within the reticulo-endothelial system. This is a strenuous exercise since the erythrocyte squeezes through narrow slits between endothelial cells with much change of its shape. Moreover, it comes in close contact with cells endowed with phagocytic activity. Yet it escapes unharmed, providing its elasticity and carbohydrate coat are maintained [23]. Thus we can regard its surface carbohydrate components as largely inert at least with respect to the environment within the cardiovascular system. This conclusion probably applies to other cells of the body, and substratum molecules.

GSLs of erythrocytes belong to the globo and neolacto series (see Tables 1 and 2). The globo series is predominant and represents $\sim$ 75% of all GSLs and more than 20% of the total surface carbohydrate. GSLs of the neolacto series are highly diversified with respect to complexity, terminal structures and branching pattern (Table 2). Yet their core

Table 1. Structures of GSLs of the globo series and some other GSLs referred to in the text.

Galactosylceramide[a]	Galβ1-1Cer[b]
Lactosylceramide	Galβ1-4Glcβ1-1Cer
Sialosyllactosylceramide, G_{M3}-ganglioside	Neu5Acα2-3Galβ1-4Glcβ1-1Cer
Globotriosylceramide, P^k antigen	Galα1-4Galβ1-4Glcβ1-1Cer
Globotetraosylceramide, globoside, P antigen	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer
Globopentaosylceramide, Forssman antigen[a]	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer
G_{M2}-ganglioside[a]	GalNAcβ1-4[Neu5Acα2-3]Galβ1-4Glcβ1-1Cer
G_{D2}-ganglioside[a]	GalNAcβ1-4[Neu5Acα2-8Neu5Acα2-3]Galβ1-4Glcβ1-1Cer
G_{M1}-ganglioside[a]	Galβ1-3GalNAcβ1-4[Neu5Acα2-3]Galβ1-4Glcβ1-1Cer

[a] not present in human erythrocytes.
[b] Abbreviations: Gal, D-galactose; Glc, D-glucose; GalNAc, *N*-acetyl-D-galactosamine; Neu5Ac, *N*-acetylneuraminic acid; Cer, ceramide.

structures are made on a simple plan of alternating β(1-3)-substituted residues of galactose and β(1-4)-substituted residues of *N*-acetylglucosamine. Side chains, if present, are always initiated by *N*-acetylglucosamine residues attached by β(1-6)-glycosidic linkages to internal galactopyranosyl residues (see Table 2). The most complex GSLs of erythrocytes are multibranched polyglycosylceramides which on the average contain ~ 30 glycosyl residues, but minor fractions have been described with as many as 59 sugar units [25]. Similar types of carbohydrate structures were found on erythrocyte glycoproteins, including band 3 and band 4.5 [5]. Glycophorin A contains 15 short *O*-glycosidically- linked tetrasaccharides with the structure Neu5Acα2-3Galβ1-3[Neu5Acα2-6]GalNAc-Ser/Thr and a single *N*-glycan of a bi-antennary type terminated with Neu5Acα2-6Galβ-1-4GlcNAc... sequences [5]. I assume that most of the α-linked carbohydrate substituents at the membrane surface (see Table 3) as well as the terminal β-*N*-acetylgalactosamine residues of globoside are largely inert, i.e. are not involved in significant interactions with other cells and ligands, discounting allo-antibodies and exogenous lectins or enzymes of pathogenic microorganisms. An exception is probably structure 5 of Table 3 (see also Section 6) which is not present in erythrocytes. Treatment of erythrocytes with sialidase results in the exposure of terminal β-galactopyranosyl residues of GCs to galactose-binding lectins of hepatocytes, macrophages and to serum autoantibodies, leading to the rapid clearance of erythrocytes from the circulation [19]. Thus sialic acids mask β-galactopyranosyl residues [19].

Treatment of blood-group B human erythrocytes with α-galactosidase converts them to blood-group O cells which can be transfused to blood recipients of any blood group [26]. In contrast, rabbit erythrocytes, in which the blood group B determinant structure is of a different type i.e. Galα1-3Galβ1-4GlcNAc... (not based upon the H determinant), require only a single treatment with α-galactosidase to be removed from the circulation [27]. For this reason I would consider Neu5Acα2-3Gal... and Fucα1-2Gal... structures as singly locked, but blood group A and B active structures as doubly locked since the exposure of the β-galactopyranosyl group requires the sequential action of two glycohydrolases. The blood group B determinant of the rabbit would be on the other hand singly locked. A potentially multiply-locked molecule is a recently described A_1-active GSL of

Table 2. Common core structures of neolacto GSLs of human erythrocytes. The core structures are substituted at terminal galactose residues to a large extent with Neu5Acα(2-3), and depending on ABO blood group status with GalNAcα1-3[Fucα1-2] , Galα1-3[Fucα1-2] and Fucα(1-2) structures (see also Table 3).

1	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer[a]
2	[Galβ1-4GlcNAcβ1-3]$_n$Galβ1-4Glcβ1-1Cer[b]
3	Galβ1-4GlcNAcβ1-3[Galβ1-4GlcNAcβ1-6]Galβ1-4Glcβ1-1Cer[c]

[a] Abbreviations: Fuc, L-fucose; GlcNAc, *N*-acetyl-D-glucosamine. Other abbreviations as in Table 1.
[b] n=2 or 3. Core structures with n=3 have been described only recently [76].
[c] A fourth type of core structure is based on this structure but contains a repeating Galβ1-4GlcNAcβ1-3 structure substituted at C-6 of every second galactose residue (polyglycosylceramides).

erythrocytes (see Table 3). Globoside should be a doubly locked molecule since it contains two inert structures at the non-reducing end, i.e. GalNAcβ1-3Gal α(1-4)-linked to lactosylceramide. The latter GSL is not exposed at the membrane surface [28].

Therefore I consider globoside, globotriosylceramide and G_{M3}-ganglioside as good candidates for largely inert membrane-packing GSLs. On the other hand ABH and P_1 blood group determinants as well as Neu5Acα(2-3/6)-linked sialic acid would constitute "inactivating caps" or masks for most carbohydate structures including the lacto, or type 1 series. The latter are therefore candidates for some kind of regulation at the cell membrane level although little evidence for this type of regulating activity is available at present. Sialic acid caps are of course distinguished from those containing neutral sugars through their charge-conferring and cation-binding properties [18, 19]. This important biological function of sialic acid will not, however, be discussed. It should be only mentioned that monosialogangliosides bind much less Ca^{2+} than di- and trisialogangliosides [18]. The potential biological significance of locked molecules is further discussed in Section 7.

It should be pointed out that terminal β-galactopyranosyl residues are also present in erythrocyte membrane GCs, but presumably their density at the erythrocyte surface is too low for any appreciably interaction with macrophages and autoantibodies. Other GSLs not present in erythrocytes may have a specific function, and good candidates for this role are gangliosides of the ganglio series [18]. Recently a regulatory role of G_{M1}-ganglioside in the interaction of epidermal growth factor and platelet-derived growth factor with membrane receptors has been proposed [29]. Not all gangliosides may be, however, biologically active since erythrocytes of mice and rats contain gangliosides of the ganglio series [18]. A very variable GSL composition of erythrocytes from different mammalian species may reflect adaptation to environment (see Section 7).

The significance of a particular combination in human erythrocytes of GSLs of the globo and neolacto series terminated with inert carbohydrate sequences cannot be presently explained. This combination possibly contributes to the long life span of human erythrocytes. A general similarity of the function of GSLs of the globo and neolacto series is, however, suggested by the fact that in erythrocytes of the rare p phenotype the former are missing and are replaced to a large extent by the latter [8] without any adverse effect on erythrocyte viability.

Table 3. Some non-reducing terminal structures capped with α-linked sugars in GSLs as well as *O*- and *N*-linked (complex) GPs.

	Structure	Blood group activity
1	Fucα1-2Galβ1-3/4GlcNAc...[a]	H
2	GalNAcα1-3[Fucα1-2]Galβ1-3/4GlcNAc...	A
3	Galα1-3[Fucα1-2]Galβ1-3/4GlcNAc...	B
4	GalNAcα1-3[Fucα1-2]Galβ1-3GalNAcα1-3[Fucα1-2]Galβ1-4GlcNAc...[b]	A_1
5	Galβ1-4[Fucα1-3]GlcNAc...	-
6	Galβ1-3[Fucα1-4]GlcNAc...	Le^a
7	Fucα1-2Galβ1-3[Fucα1-4]GlcNAc...	Le^b
8	GalNAcα1-3[Fucα1-2]Galβ1-3[Fucα1-4]GlcNAc...	ALe^b
9	Galα1-3[Fucα1-2]Galβ1-3[Fucα1-4]GlcNAc...	BLe^b
10	Neu5Acα2-3Galβ1-4GlcNAc...	-
11	Neu5Acα2-6Galβ1-4GlcNAc...	-
12	Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAc...	-
13	Neu5Acα2-6Galβ1-4[Fucα1-3]GlcNAc...	-
14	Neu5Acα2-3Galβ1-3[Fucα1-4]GlcNAc...[c]	-
15	Galα1-4Galβ1-4GlcNAc...	P_1

[a] In erythrocytes ABH sequences terminate the type 2 chain (neolacto) structure i.e. Galβ1-4GlcNAc...; in GPs of secretions, GSLs of blood plasma and cells lining the digestive tract ABH sequences terminate in addition type 1 (lacto) structure i.e. Galβ1-3GlcNAc... In type 1 and 2 chains fucose may be added also to position 4 and 3 of *N*-acetylglucosaminyl residues (these structures are not present in erythrocytes). The former are the structural basis for Le^a and Le^b blood group determinants [see 1-5, 13].

[b] Found so far on erythrocyte GSLs [76].

[c] A common antigen of colon cancer [79] but present also in normal tissues [see 20].

5. Supportive Evidence

Any good hypothesis should be subject to experimental verification. If my views are correct and GSLs such as those of the globo and neolacto series terminated with largely inert structures simply fill in the membrane space unoccupied by functional GPs and proteins, then GSL biosynthesis may be correlated to GP biosynthesis. There should also be an inverse relationship between membrane space occupied by functional GPs (in my view probably all GPs), functional GSLs (gangliosides?) and inert GSLs. This relationship should be somehow reflected during maturation and differentiation and be amenable to experimental proof. In fact some supportive evidence in favour of the hypothesis is already available.

5.1 GSL Content of Different Cells and Tissues

From the literature it can be generalized that many differentiated cells and tissues that are highly responsive to external stimulation do not have appreciable amounts of globo and neolacto GSLs terminated with inert structures. Thus, these GSLs are only very minor components of the nervous tissue [24] and of white cells [30]. In neutrophils, ABH immunodominant structures are lacking and so are globo series GSLs [31]. Lymphocytes contain globo GSLs but the major GSL species is lactosylceramide [32]. It should be

pointed out that in nucleated cells inert GSLs may be present in the internal membranes [33, 34]. On the other hand, inert carbohydrate structures like ABH determinants are abundant in epithelia of blood vessels and those covering the external body surfaces like skin and the digestive and urinary tracts [35]. It may be significant that apart from erythrocytes, globo series GSLs are present in high concentrations in the kidney [24] although information on their localization in different types of kidney cells is not available.I would predict that these are cells which come into direct contact with urine.

It would be interesting to determine the exact GSL content of En(a-) erythrocytes which totally lack glycophorin A [36]. Individuals with this phenotype are apparently healthy. This is not, however, evidence for the lack of biological function of glycophorin A. For example nobody denies the biological function of serum albumin although the protein is not present in individuals with a rare genetical abnormality (analbuminemia) without any ill effect on health [37]. Concentrations of other serum proteins are increased in these people and the oncotic pressure of blood is maintained.

5.2 Biosynthesis of GSLs and GPs During the Cell Cycle

GSLs are synthesized throughout the cell cycle but a very significant accumulation of the globo series of GSLs takes place during mitosis and the G1 phase [38, 39]. In contrast, a peak of GP biosynthesis occurs late in the S phase. Other studies emphasized accumulation of globo GSLs during the G1 phase [40]. Thus the biosynthesis of GPs precedes that of GSLs (in the S phase of the cycle hydrolysis of GSLs prevails over biosynthesis). In erythrocytes from patients with congenital dyserythropoietic anaemia type II, accumulation of most membrane GSLs occurs [41, 42]. Erythroblasts of these patients exhibit a prolongation of the G1 phase of the cell cycle [43].

5.3 A Possible Role of Inert Carbohydrate Structures During Development and Tumourigenesis

Irrespective of different developmental strategies (for references see [44-46]) a common feature of embryogenesis in all vertebrates is cell migration. It is evident already during gastrulation that the majority of later embryonic primordia are formed by migration of a certain cell type into a particular locus rather than by cell multiplication at that locus. Once the cells settle in their new locations they gradually differentiate into mature and functional types and develop cell-specific affinity. The molecular basis of this affinity has been the subject of numerous investigations. Various cell adhesion molecules (CAM), defined by their ability to enhance cellular aggregation (cognins), block the aggregation (ligatins) or block antibody-induced disruption of cellular adhesion, have been described [15, 47]. Many of these molecules appear to be integral membrane GPs and some persist into adult life though their molecular forms may be changed. In some of the reactions a role of carbohydrate structures or carbohydrate-binding lectins has been implicated [13, 48].

Mouse teratoma cells which are of embryonal origin may also maintain contact through specific carbohydrate-binding substances claimed to be animal lectins [49, 50], galactosyltransferase [14] or a galactose-dependent adhesion factor [51]. In addition, the embryonal cells presumably display characteristic interactions with substratum molecules. Considering that there are so many little-understood interactions between embryonal

cells during development, I think that it is as important for a developing cell not to respond to a factor addressed to some other cell type or substratum molecule as it is to answer a self-addressed message. Therefore a developing cell may contain a limited number of homing receptors, whereas other surface components should remain relatively inert. This should be particularly important during the migratory period when cells often have to pass through layers of other cells committed to a different development programme. Once the cells settle in their surrounding they presumably develop new membrane structures designed for function and membrane space allocated for inert GSLs may diminish.

Evidence for this sequence of events comes from the work of Szulman who examined the expression of ABH and Lewis antigens in human embryos employing the fluorescent antibody technique [35, 52]. The main conclusion of Szulman's work was that human embryos examined from the fifth post-fertilization week express ABH antigens on the cell surfaces of the endothelium and the epithelia of most early organs and organ rudiments with the exception of the central nervous system, the liver, and the adrenal glands. Towards the end of the first trimester of pregnancy an orderly recession of the antigens is observed which coincides with the morphological and functional maturation of the organs. ABH antigens stay for life in the stratified epithelia of skin, mouth, pharynx, oesophagus, urinary bladder, exocervix and vagina, simple epithelia of the urethers, Wolfian duct, and in the endothelium of the cardiovascular system. Szulman pointed out that the developing liver and the central nervous system might have gone through the antigen-positive stage during earlier periods that escaped investigation. Otherwise other inert GSLs might have been present at the surface of early nervous and liver cells. The obvious candidates for nerve cells would be some species of gangliosides.

It is much more difficult to predict the role of inert carbohydrate structures in the very early stage of embryogenesis, prior to gastrulation. There is evidence that, at least in mammals, these embryonal cells may be totipotent and their fate depends very much on spatial position within the embryo [53]. Yet in view of the very basic role I envisage for inert GSLs as membrane-packing materials, it is not surprising that globoside is already present at the 2-4 cell stage of the mouse embryo [54]. In mouse embryonal carcinoma [54] and human teratoma cells [55] the globoside and related GSLs are present as major components. Human teratoma cells contain in addition minor amounts of globotetraosylceramides substituted at the *N*-acetylgalactosaminyl residue with Galβ1-3, Neu5Acα2-3Galβ1-3 and Fucα1-2Galβ1-3 sequences [55]. All these structures are reactive to a different extent with anti-SSEA-3 monoclonal antibody raised against the 4-8 cell stage mouse embryo [56], but present also in the zygote [57].

Mouse and human teratoma cells contain highly branched, protein-bound glycans similar to those present in human erythrocytes, but containing fucose attached by an α(1-3)-linkage to *N*-acetylglucosamine [58]. The sequence seems to be the immunodeterminant structure for the SSEA-1 antigen of the mouse embryo [59, 60] which is expressed maximally at the morula stage. Presumably because of the presence of these highly branched glycans, the morula reacts with anti-I antibodies [61, 62] specific for the Galβ1-4GlcNAcβ1-3[Galβ1-4GlcNAcβ1-6]Galβ1-4GlcNAc... structure [9]. Later on, the first differentiated cells of the primitive endoderm show reactivity with anti-i antibodies recognizing the linear Galβ1-4GlcNAcβ1-3 structure [9].

Thus the development of the mouse embryo reverses to some extent the sequence of events during human erythropoiesis which proceeds from the linear i-active to the branched I-active glycans, with globoside added at the final steps of erythrocyte maturation [63]. This should not be surprising; the erythrocyte, with its largely inert surface characteristics, differentiates through a series of steps from a highly responsive stem cell which gives rise to all cellular elements of blood; presumably according to the signals it receives. Thus the surface of erythrocyte precursors should be largely occupied by functional receptors with less room for inert GSLs like globoside. On the other hand the zygote and its immediate daughter cells develop largely in their own time and may acquire specific receptors later during embryogenesis.

Tumour cells show some similarities to embryonal cells with respect to isoenzyme patterns [64], antigens at the cell surface [65] and the type of protein they may secrete [22]. As a rule the GC composition and the structure of tumour cells are changed to a variable extent in comparison with normal cells from which they originated. It has been suggested that GSL profiles of tumour cells may reflect their immaturity due to the arrest of the development programme, or dedifferentiation [57]. At the same time GCs at the surface of tumour cells may be subjected to an environmental pressure by the tumour host. For example, two human melanoma gangliosides, G_{M2} and G_{D2}, which are present in fetal or embryonal nervous tissue but not adult nervous tissue elicited an immune response of the tumour host [66]. Evidence is also available that the ganglioside composition of human melanoma cells may be subject to a change within a few days after transplantation to a foreign host (nude mouse) or transferring into *in vitro* conditions [67]. For these two reasons, i.e. immaturiy and environmental pressure, I expect that tumour cells would express in general largely inert carbohydrate structures in GSLs and GPs. This seems to be the case and the majority of monoclonal antibodies raised against human tumours detect the structures which I consider inert i.e. ABH and Lewis antigens, a so called sialylated Le^a (Neu5Acα2-3Galβ1-3[Fucα1-4]GlcNAc-R) or GSLs of the globo series [20].

I am not deterred by a loss of A and B antigens from tumour tissues as reported in some instances [57]. The loss may result from elevations of other glycosyltransferases like α3/4-fucosyltransferases [68] or sialyltransferases [69], which may compete with *A*, *B* or *H* gene-dependent glycosyltransferases for substrate molecules. It is also probable that in general more of the membrane space on tumour cells is occupied by inert GSLs due to a loss of some functional GPs. An exception to this would be cells differentiating towards general inertness of surface like those of erythroid lineage. If my ideas are correct then the gangliosides of early embryonal nerve cells and those of melanomas (presumably very similar) would be relatively inert with respect to cells committed to other differentiation programmes. This would not necessarily apply to immunocompetent cells of adult humans (in embryonal cells the immunosurveillance system is largely unoperational).

6. Possible Modulatory Activity of Inert Carbohydrate Structures

It has already been mentioned in Section 4 that inert carbohydrate structures may in theory modulate the biological activity of functional molecules. Evidence for this type of regulation is only circumstantial. In embryonal cells at the morula stage and in eryth-

rocytes, highly branched glycans with I activity are present at the cell surfaces (see Section 5.3). The glycans of embryonal cells carry Galβ1-4[Fucα1-3]GlcNAc... structures and there is evidence that these may be involved in cellular adhesion [70, 71]. Significantly, such terminal structures are present in neutrophils [31], specifically designed for interactions with other types of cells. In chronic myelogenous leukemia cells these structures are sialylated at terminal galactopyranosyl residues [72]. In erythrocytes, highly branched glycans carry ABH determinant structures. Thus, addition of ABH or Neu5Ac inactivating caps to lactosaminoglycans may confer biological neutrality.

All cells during mitosis are probably least reactive to external signals as exemplified by their reduced permeability to small molecules [73]. This coincides with a high expression of H antigen as demonstrated in human HeLa cells [74] and mouse fibroblasts [75]. Evidence is available that after stimulation of murine mast cells, globoside is brought to the cell surface from internal secretory granules [33]. In my view the inert globoside may replace a specific antibody-antigen complex and provides for a refractory period following the stimulation. A modulatory activity of sialic acid and its acetyl groups for molecules with biological activity [19], as well as its masking role in highly metastatic cancer cells [77] have been proposed [19].

7. Blood Group Polymorphism

It is a common understanding that the mucus barrier of the digestive tract offers protection against environmental factors, including pathogenic microorganisms. Presumably the pathogens have first to hydrolyse the carbohydrate moiety of mucus in order to penetrate it. If the organism is not endowed with an endoglycosidase a sequential hydrolysis of carbohydrate structures of mucus probably has to be carried out. Thus the advantage of locked molecules synthesized by the action of ABH and Lewis blood group gene-dependent glycosyltransferases becomes obvious. Bacterial and protozoan enzymes decomposing sequentially ABH and Lewis structures have been described [1]. It is quite possible that the type 1 chain of GCs has evolved in the digestive tract so as to increase the structural heterogeneity of the glycans and thus make them more resistant to enzymic hydrolysis. On the other hand some pathogenic microorganisms have developed recognition systems for the protective carbohydrate coat at the cell surface. This seems to be the case for viruses and some protozoa which bind to sialic acid residues of GCs [19] as well as for some *Escherichia coli* strains recognizing globo series GSLs [17].

Thus blood group polymorphism may have developed in part due to a very diversified environmental pressure of pathogenic microorganisms. It is also possible that the structure and composition of cell surface carbohydrate may have been influenced by eating habits. For example many animals like rodents, birds and herbivorae eat seeds which may contain carbohydrate-binding and often toxic lectins [78] (humans eat seeds as well, but mostly after cooking). Locked molecules may offer some evolutionary advantage, irrespective of the action of pathogenic microorganisms, at least for the circulating erythrocytes. It may be so that during numerous filtrations of the erythrocytes through the reticulo-endothelial system their carbohydrate coat wears down due to a slow action of glycosidases, or even mechanical factors. For example, hydrolysis of one terminal glycosyl unit from a doubly locked molecule would not necessarily expose its β-galactosyl residue to the action of the galactose-binding lectin of macrophages or hepatocytes.

A vast array of carbohydrate structures available for protection of the cell membranes and proteins as well as for conferring biological inertness upon otherwise functional carbohydrates may be of considerable evolutionary advantage.

8. Conclusions

Many carbohydrate sequences which have been studied extensively in the past are relegated in this paper to biologically inert structures and some GSLs even to simple membrane-packing substances. This does not mean, however, that they are biologically unimportant. In computer language the 0 sign is as important as the 1 sign. There is ample evidence that cells communicate with the environment and other cells through functional receptors at the cell membrane and some of these are carbohydrate in nature. At least in my mind a minimum of "blank" space should be left between the functional regions of the membrane. This space may be larger in cells which are not highly responsive to external stimulation. For example this article would be very difficult to read without any blank space between words and lines. The role of inert carbohydrate structures at the cell membrane would be to provide for the blank space and possibly delete a receptor function during certain periods of cell life. In cells lining the external body surface the inert carbohydrate coat at the cell membrane and the mucus barrier may be essential for protection against environmental factors.

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