

Outstanding Contribution

Viewing AIDS from a glycobiological perspective: potential linkages to the human fetoe embryonic defence system hypothesis

Gary F.Clark^{1,5}, Anne Dell², Howard R.Morris², Manish Patankar¹, Sergio Oehninger³ and Markku Seppälä⁴

¹Department of Biochemistry, Eastern Virginia Medical School, 700 Olney Road, Norfolk, VA 23501–1980, ²Department of Biochemistry, Imperial College, London, SW7 2AZ, UK, ³Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA 23501, USA, and ⁴Department of Obstetrics and Gynaecology, University Central Hospital, Helsinki, FIN-00290, Finland

⁵To whom correspondence should be addressed

The primary molecular changes that lead to development of acquired immunodeficiency syndrome (AIDS) are very poorly understood, as are the mechanisms underlying the protection of the developing human from the maternal immune response. Recent data suggests that the human immunodeficiency virus (HIV) may be using the glycosylation system of the T lymphocytes to acquire glycans for its glycoproteins that enable it to disrupt carbohydrate dependent immune cell interactions or induce aberrant immune reactions. Consistent with this hypothesis, gp120 from HIV infected human H9 lymphoblastoid cells expresses biantennary *N*-linked glycans with a bisecting GlcNAc sequence on 11% of their total oligosaccharides. This specific carbohydrate sequence has recently been shown to protect K562 erythroleukemic cells from natural killer (NK) cell responses when presented on the cell surface. We have recently demonstrated that bisecting biantennary type *N*-linked glycans are also expressed on the human zona pellucida (ZP); previous lectin binding studies indicate that it is also expressed on human spermatozoa. Thus both the human gametes and HIV produced by H9 cells carry this same protective carbohydrate epitope on their outer surfaces. Human α -fetoprotein expressed in the developing human also carries the bisecting GlcNAc sequence, indicating that it may be suppressing the emerging fetal immune response by using its carbohydrate sequence as a functional group. We have suggested that the developing human and the gametes are also protected by soluble immunosuppressive glycoproteins found in the amniotic fluid and seminal plasma known as glycode lin-A (GdA) and glycode lin-S (GdS) respectively. Structural analysis of their *N*-linked oligosaccharides combined with other functional studies suggest that GdA and GdS employ their very unusual carbohydrate sequences as functional groups that enable them to manifest their immunosuppressive activities. GdA and GdS are significant components of our recently proposed model for the protection of the developing human and gametes designated the human fetoe embryonic defence system hypothesis. A striking relationship now emerging is that the same unusual carbohydrate sequences associated with these immunosuppressive glycode lins are also specifically expressed on intravascular helminthic parasites, *Helicobacter pylori*, human tumour cells, and HIV infected T lymphocytes. The information presented in this review suggests that two new corollaries should be added to our recently proposed defence system hypothesis: (i) mimicry or acquisition of glycans that are used in this protective system by pathogens or tumour cells may enable them to either subvert or misdirect the human immune response, thereby greatly increasing their pathogenicity; and (ii) expression of glycoconjugates used in this system by normal cells and tissues outside the reproductive system may protect them from immune responses, especially in those cases where major histocompatibility recognition is either absent or minimal. A better understanding of this hypothesis and its corollaries may enable us to address the molecular mechanisms underlying not only AIDS but also a host of other very serious pathological conditions in the human.

Key words: fetomaternal tolerance/glycode lins/glycoconjugates/HIV/immunosuppression

Introduction

The protection of the developing human from the maternal immune response is a vital function in reproduction (Hegde, 1991). Similarly, defending human spermatozoa and eggs from

potential immune responses would also be beneficial for the propagation of the species. We have recently proposed that the developing human and the gametes are protected by a system of soluble and cell surface associated glycoconjugates that are localized to the male and female reproductive systems

(Clark *et al.*, 1996). We have referred to this model as the human foetoembryonic defence system hypothesis. Thus both the human reproductive system and human immunodeficiency virus (HIV) induce immunosuppression in the human, the first in a spatially and temporally regulated manner, the other in a disseminated fashion that induces a systemic loss of immune response.

It has previously been suggested that HIV could disrupt the carbohydrate dependent adhesion/signalling system required by the immune system to manifest its biological activities (Adachi *et al.*, 1988; Kashiwagi *et al.*, 1994; Velupillai and Harn, 1994). However, to our knowledge, a coherent paradigm detailing how acquired immunodeficiency syndrome (AIDS) could be caused by aberrant glycosylation processes induced by HIV infection has not yet been presented. Also, there has been no suggestion that HIV is using the same system that protects the developing human and the gametes from the immune response to manifest its pathological activities. We will outline some new lines of evidence relating to the immunosuppressive glycodefins (Dell *et al.*, 1995; Koistinen *et al.*, 1996; Morris *et al.*, 1996) that complement relevant older data supporting the concept that AIDS is the result of carbohydrate dependent pathological effects evoked by HIV infection. Moreover, it is now apparent that not only HIV but many other pathogens may integrate themselves into this system to protect themselves and promote their infectivity and pathological effects.

Glycosylation of HIV glycoproteins: the development of diversity

Complex *N*-linked glycans are assembled in a multienzyme process involving the action of specific glycosidases and glycosyltransferases in both the endoplasmic reticulum and Golgi apparatus (Kornfeld and Kornfeld, 1985; Dennis, 1991). The potential types of glycan sequences that can be synthesized by any specific cell type will be determined in part genetically by the cell specific expression of these enzymes. However, the type of glycan that will be attached to any particular protein will be dependent upon the interaction of these glycosidases and glycosyltransferases with their protein substrates (Helenius, 1994). Therefore the three-dimensional structure of a protein and the location of its specific glycosylation sites will also be crucial factors for determining the structure of the glycans attached to a glycoprotein. Logically, the specificity of this combination could provide a mechanism for cell specific functionality in certain cases. An understanding of these factors is essential for comprehending how aberrant glycosylation processes could play a key role in the development of AIDS.

HIV is unique because of its ability to preferentially infect T lymphocytes (Barre-Sinoussi *et al.*, 1983). Thus, at the outset, the virus has access to the set of specific T cell lineage glycosylation enzymes. These enzymes presumably enable the T cell to synthesize oligosaccharides that normally act as 'functional groups' for T cell glycoproteins involved in carbohydrate dependent immune cell interactions.

It has been established that some lectin-like proteins involved in the immune or inflammatory response need only

the expression of an appropriate carbohydrate sequence for binding. For example, E-selectin will bind directly to glycolipids with sialyl Lewis^x sequences at their terminal ends (Phillips *et al.*, 1990; Larkin *et al.*, 1992). Other carbohydrate specific interactions require that both the oligosaccharide sequence and the protein component must be recognized for specific binding to occur. A model for this type of interaction is the observation that high affinity binding of CD22 to a glycoprotein requires not only terminal α 2-6 linked sialic acid but also an appropriate protein structure (Powell and Varki, 1994; Hanasaki *et al.*, 1995; Powell *et al.*, 1995). Thus many glycoproteins and mucins that express terminal α 2-6 linked sialic acid will not bind to CD22 (Hanasaki *et al.*, 1995).

If a viral protein can appropriately mimic the structure of the naturally-occurring T cell protein used in the immune response, then it may also be able to interact with the same glycosyltransferases as the T cell proteins, enabling it to acquire the same glycans. If appropriate glycan expression alone is sufficient to enable a viral glycoprotein to inhibit or evoke a particular immune or inflammatory response, then simple acquisition of the glycan may be sufficient to cause a pathological response. However, if both the protein and the carbohydrate sequence together are necessary to obtain the appropriate determinant necessary to interfere with a crucial immune response (e.g. an HIV glycoprotein that could bind to CD22), then both the protein and the carbohydrate sequence must be close enough in structure to fit the binding site of the complementary immune receptor glycoprotein.

In this model of HIV action, the key is to generate an HIV glycoprotein(s) that can block carbohydrate dependent interactions that occur in the immune system or induce aberrant immune responses. It is significant that there are several reports detailing the structural similarity between the protein components of HIV glycoproteins and native T cell glycoproteins (Golding *et al.*, 1988; 1989; Imberti *et al.*, 1991; Dalglish *et al.*, 1992; Levy, 1993). Thus it is not unreasonable to postulate that viral glycoproteins could also acquire complementary functional glycans by mimicking the protein structure of T cell glycoproteins.

Upon initial infection, progeny virus may not need to express a glycoprotein or combination of proteins that can potentially block glycoconjugate dependent interactions in the immune system. The primary goal of the virus in its early infective stage is to establish itself in cell types that allow it to be protected and propagate. In addition, immediate destruction of the immune system would be counterproductive because there would be insufficient time for transmission of the virus to another host. Because of its high rate of mutation, productively infected cells produce mutant viruses that would either be destroyed or propagate depending on their ability to infect other cells and evade or misdirect the immune response. Mutant HIV proteins with slightly different three dimensional structure will be generated over time that will differentially interact with the glycosylation machinery thus enabling them to acquire different glycans. The virus would therefore select the appropriate composite glycoprotein(s) that could defeat the immune system by combining its high mutational rate with the T cell lineage glycosylation system.

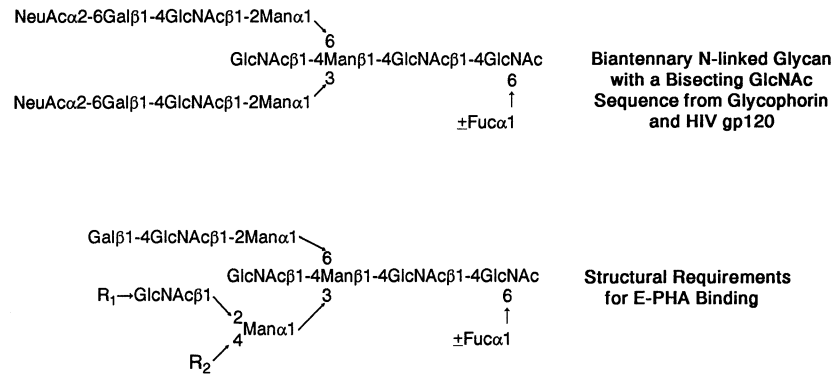


Figure 1. Bisecting *N*-linked glycans referred to in the text. R_1 and R_2 refer to either attached hydrogen or sugars as described previously (Yamashita *et al.*, 1983). Two absolute requirements for E-PHA binding are firstly the presence of a GlcNAc linked $\beta 1\text{-4}$ to the β -linked mannose of the trimannosyl core sequence (the 'bisecting' GlcNAc); and secondly, a single *N*-acetylglucosamine sequence with an accessible terminal galactose attached via *C*-2 of the $\alpha 1\text{-6}$ linked mannose of the core.

What would be the predicted effects of such manoeuvring by HIV? First, viral isotypes carrying the 'right' sequences could suppress immune responses directed against them. Secondly, the variant viruses produced by this mechanism could interact with the carbohydrate receptors of different cells, enabling them to infect a variety of different immune and non-immune cell types. Finally, soluble or cell surface associated viral glycoproteins carrying the appropriate carbohydrate chains could inhibit normal immune function or induce aberrant immune response. Eventually, a variant virus or a set of variant viruses would arise that could overwhelm the immune system, leading to the development of AIDS.

HIV and the human gametes: potential carriers of immunosuppressive carbohydrate sequences

Recent evidence indicates that surface expression of a specific glycan sequence induces the suppression of a restricted cell-mediated response. The insertion of glycophorin into the surface membranes of K562 erythroleukemic cells renders them much more resistant to human natural killer (NK) cells (El-Ouagari *et al.*, 1995). However, *N*-glycanase treatment of glycophorin prior to its insertion renders it inactive as an immunosuppressive glycoprotein in this experimental system. The carbohydrate sequence responsible for this effect is a biantennary *N*-linked glycan carrying the bisecting GlcNAc sequence (Figure 1) (El-Ouagari *et al.*, 1995). This type of glycan sequence is specifically recognized by the lectin known as erythroagglutinating phytohemagglutinin (E-PHA) (Cummings and Kornfeld, 1982; Yamashita *et al.*, 1983; Mizuochi *et al.*, 1990). E-PHA will only bind to bi- or triantennary glycans carrying the bisecting GlcNAc sequence (Figure 1).

Additional evidence supporting E-PHA binding glycans in the suppression of NK cell mediated response has been reported more recently. Stable transfection of K562 cells with the enzyme that catalyses the synthesis of the bisecting GlcNAc sequence (GlcNAc transferase III) yields transformants that bind E-PHA and are completely resistant to NK cell-mediated lysis (Yoshimura *et al.*, 1996). In addition, another study indicates that infection of human NK cells with HIV renders them incapable of responding to K562 cell targets (Robinson

et al., 1988). Thus glycoproteins carrying this sequence may be capable of blocking NK cell activity when expressed on the cell surface.

There is evidence indicating that HIV may also acquire biantennary glycans with the bisecting GlcNAc sequence. Infection of human H9 lymphoblastoid cells with HIV yields viral gp120 that bears a substantial proportion of glycans that specifically bind to E-PHA (Mizuochi *et al.*, 1990). Analysis of the *N*-linked glycans associated with gp120 indicates that ~11% of these oligosaccharides carry the bisecting GlcNAc on their biantennary glycans (Figure 1) (Mizuochi *et al.*, 1990).

We have recently reported that E-PHA binds to the surface of the human zona pellucida (ZP) in a carbohydrate-specific manner (Patankar *et al.*, 1996). The plasma membranes of human spermatozoa also bind E-PHA in a carbohydrate dependent manner (Cross and Overstreet, 1987). This result suggests that the ZP of the human egg that surrounds the embryo before hatching is coated with an immunosuppressive carbohydrate sequence that may protect it from NK cells, the most prevalent immune cell type in the uterus that is responsible for the majority of its innate immune response. Spermatozoa which must enter the uterus to fertilize the egg may also be protected from such cell-mediated responses by expressing bisecting *N*-linked glycans on their surfaces. It also suggests a rather significant potential convergence: that human gametes and HIV may carry the same immunosuppressive carbohydrate sequence *in vivo*.

α -Fetoprotein (AFP) is a glycoprotein associated with the yolk sac and fetal liver that has been implicated in mediating immunosuppression of the fetal and perhaps maternal responses (Tomasi, 1978). Suppression of the emerging fetal immune response during pregnancy is also likely to be crucial for maintaining tolerance of the developing human. Data collected some time ago indicates that AFP is a carrier of bisecting *N*-linked glycans that react specifically with E-PHA (Tsuchida *et al.*, 1984). Therefore AFP may be blocking NK cell function and perhaps other immune functions within the uterus. It is significant that the level of bisecting GlcNAc sequence on AFP peaks around 16 weeks and then declines slowly so that at birth no bisecting GlcNAc sequence are detected (Ishiguro, 1991). Therefore AFP is likely to be another soluble component

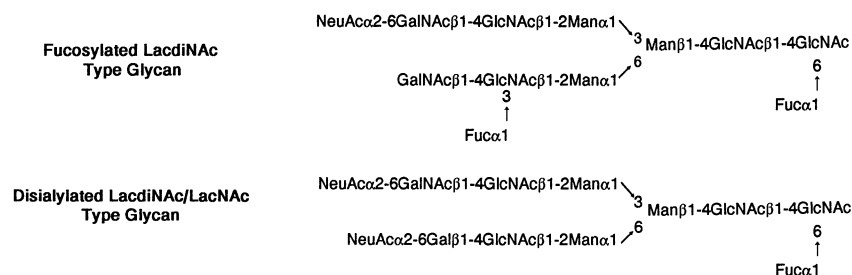


Figure 2. Unusual *N*-linked glycans associated with glycodelin-A.

of the human fetoe embryonic defense system that suppresses fetal immune responses primarily by using its carbohydrate chains as functional groups.

Glycans other than the bisecting *N*-linked glycans may play a role in mediating immunosuppression. Analysis of gp120 from H9 cells indicates that >80% of their oligosaccharides are high mannose-type glycans (Mizuochi *et al.*, 1990). Mannose receptor proteins expressed primarily on macrophages play an essential role in the protection against bacterial pathogens (Ezekowitz and Stahl, 1988). There is evidence that HIV infection impairs alveolar macrophage mannose receptor function against *Pneumocystis carinii* (Koziel *et al.*, 1993). Whether this immunosuppressive effect on mannose receptor function by HIV or its glycoproteins is direct or indirect remains to be determined.

Natural forms of immunosuppression mediated by glycoconjugates

Recent studies indicate that a highly localized form of immunosuppression is mediated via glycoconjugate expression during the process of human reproduction. Glycodelin-A (GdA) is a major endometrial glycoprotein induced during pregnancy that manifests several immunosuppressive effects *in vitro* (Bolton *et al.*, 1987; Pockley *et al.*, 1988; Pockley and Bolton, 1990; Okamoto *et al.*, 1991). GdA expresses very unusual fucosylated lacdiNAc sequences (GalNAcβ1-4[Fucα1-3]GlcNAc) on the terminal ends of some of its *N*-linked glycans (Figure 2) (Dell *et al.*, 1995). Oligosaccharides terminated with this sequence have been shown to potently block selectin-mediated adhesion of neutrophils to inflamed endothelium (Grinnell *et al.*, 1994). GdA also carries oligosaccharides with α2-6 linked sialic acid at their terminal ends (NeuAcα2-6Gal(NAc)β1-4GlcNAc) (Figure 2), potential ligands for CD22, a human B cell receptor glycoprotein (Powell and Varki, 1994; Hanasaki *et al.*, 1995; Powell *et al.*, 1995). CD22 is thought to interact with CD45, the leukocyte specific receptor-linked phosphotyrosine phosphatase involved in T cell activation, via its α2-6 linked sialic acid terminals (Stamenkovic *et al.*, 1991; Sato *et al.*, 1993; Sgroi *et al.*, 1995). These findings suggest that GdA may utilize its carbohydrate sequences as 'functional groups' that enable it to manifest its immunosuppressive activities.

A seminal plasma analogue of GdA designated glycodelin-S (GdS) has recently been characterized by us and shown to express major biantennary *N*-linked glycans terminated with unusual Lewis^y (Le^y) and Lewis^x (Le^x) sequences (Koistinen *et al.*, 1996; Morris *et al.*, 1996) (Figure 3). GdS also

exclusively carries high mannose type *N*-linked oligosaccharides at its first glycosylation site (Asn-25) (Morris *et al.*, 1996). The protein components of GdA and GdS are very similar based on *N*-terminal sequencing, mass spectrometric analysis of tryptic peptide fragments, size and charge after deglycosylation, and immunological cross reactivity (Koistinen *et al.*, 1996). However, unlike GdA, GdS does not block human sperm-zona pellucida binding (Morris *et al.*, 1996) but has previously been implicated as an immunosuppressive component of human seminal plasma (Bolton *et al.*, 1987). Studies are now underway in our laboratories to determine exactly how GdS manifests its immunosuppressive effect.

This potential type of localized induction of tolerance need not be limited to the reproductive system. It is more likely a poorly understood general phenomenon that also acts as a protective tissue specific counterbalance to the so called 'danger hypothesis' (Matzinger, 1994; Pennesi, 1996) which suggests that signals from pathogenic organisms or viruses are necessary to trigger immune response. For example, it is now apparent that glycophorin, a major erythrocyte glycoprotein, carries a carbohydrate sequence that could protect erythrocytes from NK cell mediated cytotoxicity (El-Ouagari *et al.*, 1995). The recent demonstration that immunoreactive forms of glycodelin are also expressed in megakaryocytes and the ovary suggests the possibility that this glycoprotein may also be protecting cells and tissues at other sites (Morrow *et al.*, 1994; Kamarainen *et al.*, 1996). This type of localized carbohydrate mediated suppression may be very critical when the expression of major histocompatibility antigens is either absent or low.

The concept that tolerance or immunosuppression can be induced by soluble and cell surface associated glycoconjugates is significant because the next logical extrapolation is that viruses, bacteria and parasitological organisms that can mimic or acquire one or more components of this system logically would be able to circumvent or misdirect the immune response. Strong evidence exists that precisely this type of subterfuge is being manifested not only by HIV but by several other types of pathogens.

Mimicry of oligosaccharide ligands found on glycodelin-A and glycodelin-S by pathogenic organisms

Schistosomiasis is a parasitological disease that in its chronic form leads to immunosuppression (Allen and Maizels, 1996). The specific deficit involves a substantial diminution of Th-1 (cell-mediated) responses and enhancement of Th-2 (humoral)

with the Lewis^x determinants on intravascular cells. Since neutrophils abundantly express Le^x sequences, this response was proposed to be responsible for the neutropenia observed in patients infected with this parasite (Van Dam *et al.*, 1994).

In schistosome infected mice, Le^x based neoglycoproteins specifically induce B cell proliferation and greatly increase the secretion of interleukin 10 (IL-10) and prostaglandin E₂ (Velupillai and Harn, 1994). IL-10 is thought to negatively influence Th-1 (cell mediated) responses, causing a shift to Th-2 (humoral) responses (de Waal-Malefyt *et al.*, 1991; Fiorentino *et al.*, 1991). These intravascular parasites probably also possess protective mechanisms that inactivate humoral responses, making them virtually invulnerable to all forms of immune response.

Another very significant pathogen is the bacterium *Helicobacter pylori*, an organism that has now been unequivocally shown to induce gastric ulcers in humans (Warren, 1983; Marshall, 1983, 1995). Recent studies indicate that terminal Le^x and Le^y sequences are also expressed on the lipopolysaccharides produced by this organism (Figure 4) (Aspinall *et al.*, 1996a, 1996b). There is evidence that the antibody responses directed against this bacterium, possibly against the Le^x determinant, may play a role in the development of an autoimmune reaction that contributes to the disease process (Aspinall *et al.*, 1996a).

We find it significant that the same terminal carbohydrate sequences found on GdA (fucosylated LacdiNAc) and GdS (Le^y/Le^x) are also associated with helminthic and bacterial pathogens. It is very likely that such glycans are expressed to either subvert or misdirect the human immune response. The same situation is likely directly applicable to HIV infection leading to AIDS.

Induction of Le^y antigen expression in HIV-infected T cells

Le^y active biantennary glycans are expressed on ~53% of all GdS monomers in either its monovalent (Le^y/Le^x active) or divalent form (di-Le^y) (Morris *et al.*, 1996) (Figure 3). The percentage of CD8⁺ lymphocytes expressing the Le^y epitope increases from 3–5% in uninfected lymphocytes to 20–25% in patients with AIDS (Adachi *et al.*, 1988). CD4⁺ cells also undergo similar changes in the expression of Le^y following HIV infection (Kashiwagi *et al.*, 1994). This increase in the percentage of Le^y positive cells is directly correlated with the severity of clinical immunosuppression manifested by HIV infected patients (Kashiwagi *et al.*, 1994). Elevated Le^y expression is likely a direct result of HIV infection, since the percentage of human H9 lymphoblastoid cells reactive with anti-Le^y specific antibody increases from 12% under normal circumstances to 97% after exposure to HIV (Kashiwagi *et al.*, 1994). Another interesting correlation here is that Le^y is induced on many malignant tumour cell types (Hiraishi *et al.*, 1993). The Le^y epitope has also been associated with malignant cells undergoing apoptosis, a common process observed in HIV infected CD4⁺ lymphocytes (Hiraishi *et al.*, 1993).

Le^y sequences were not detected on HIV or HIV glycoproteins in patients or in H9 cell culture (Kashiwagi *et al.*, 1994).

Therefore it is likely that HIV affects Le^y expression by one or more of the following mechanisms. HIV could induce an increase in the specific glycosyltransferases necessary for Le^y synthesis or alternately increases the expression of the normal glycoprotein carriers of the Le^y epitope. Another possibility is that HIV infected Le^y reactive cells are more resistant to the cytotoxic effects by immune effector cells, leading to an overall increase in their relative percentage in the circulation during the progression to AIDS.

The precise role of the Le^y determinant in the human immune response is not yet clearly understood. However, Velupillai and Harn (1994) have demonstrated that Le^y based neoglycoproteins induce IL-10 production by B cell enriched spleen cells from normal but not schistosome infected mice. Alternately, or in addition, the Le^y sequence, when expressed on an appropriate protein carrier, may act as a functional group enabling that glycoprotein to induce aberrant immune responses or mediate signals that protect tumour or HIV infected cells from the immune response. It will be absolutely essential to determine how these unusual carbohydrate sequences attached to GdS and to T cell glycoproteins influence immune function.

Another very significant factor to consider here is the precise manner in which the Le^y epitope is presented on human glycoproteins. GdS exists as a dimer in solution, so the great majority of GdS molecules present multiple Le^x and Le^y terminals just as the neoglycoproteins in in-vitro systems do (Velupillai and Harn, 1994). Moreover, the ligands are presented on *N*-linked oligosaccharides rather than *O*-linked oligosaccharides, allowing very close spatial alignment of divalent Le^y, divalent Lewis^x or mixed Le^x/Le^y epitopes (Morris *et al.*, 1996) (Figure 3). Of course, the protein component of GdS may also play a vital role in any potential immunosuppressive interaction involving this glycoprotein.

The dual effects of azidothymidine

Indirect evidence also suggests that AIDS could be caused by induction of aberrant glycosylation. 3'-Azidothymidine (AZT, zidovudine) is a drug that has been shown to temporarily alleviate symptoms in AIDS patients (Fischl *et al.*, 1987). On the other hand, AZT also blocks mitogen stimulated human lymphocyte proliferation *in vitro*, suggesting that it could have immunosuppressive effects (Heagy *et al.*, 1991). AZT and its metabolites are relatively poor inhibitors of DNA polymerases at the levels found in human cells and tissues (Hall *et al.*, 1994). However, recent studies indicate that the major metabolic form of AZT (monophosphorylated AZT) is a powerful inhibitor of pyrimidine nucleotide sugar transport at physiological concentrations, making it a potent inhibitor of glycosylation (Hall *et al.*, 1994; Yan *et al.*, 1995).

If the pathological effects of HIV infection are caused by aberrant glycan expression, then AZT therapy should be positively synergistic in the short term (blocking both viral replication and glycosylation). However, because glycosylation is ultimately necessary for immune and inflammatory responses, the long-term effect of high dose AZT treatment should be both cytotoxic and immunosuppressive, as Duesberg (1988) asserts. The resulting clinical observations indicating a

relatively brief period of efficacy for high dose AZT treatment thus indirectly support a potential glycobiological cause for AIDS.

Conclusions

The information that we have presented here suggests that pathogenic organisms are synthesizing or acquiring specific carbohydrate sequences to deflect or subvert the human immune response. This mimicry or acquisition by such pathogens infers that the immune system uses specific carbohydrate dependent interactions for discrete recognition and signalling events in the reproductive system and perhaps elsewhere in human tissues. Our studies also suggest that the immune and reproductive systems exchange information via such processes in the human (Okamoto *et al.*, 1991; Oehninger *et al.*, 1995; Clark *et al.*, 1996). It is likely that many pathogens mimic or acquire human type glycoconjugates to efficiently integrate themselves into the host's recognition system used to protect human gametes and all stages of human development *in utero* from the immune response. It would be very logical for pathogenic organisms, viruses and tumour cells to employ this strategy, because continuation of any species absolutely relies upon reproduction.

As the collective data suggests, HIV may not only induce aberrant glycosylation of normal T lymphocyte glycoproteins but may also acquire glycans for its glycoproteins thus enabling them to interfere with immune related functions. Therefore HIV may be one of many different pathogens that employs a type of 'glycobiological subterfuge' to suppress the human immune response.

The adaptive and trophic capabilities of HIV are quite remarkable (Cheng-Meyer *et al.*, 1991; Weiss, 1993) inferring a very significant degree of plasticity. Our studies related to the expression of the glycodefins in the human reproductive system and immune effector cells indicate that functional plasticity can also result from differential glycosylation (Dell *et al.*, 1995; Clark *et al.*, 1996; Koistinen *et al.*, 1996; Morris *et al.*, 1996). Similarly, the glycosylation system in T lymphocytes is complex and presumably capable of conferring specific immune binding activities on glycoproteins when they have the appropriate protein structure.

It is likely that HIV is utilizing its host cell's glycosylation system synergistically with its mutational ability to potentiate its immunosuppressive activities and to develop trophic capabilities that greatly increase its ability to infect other cell types in the human. The predicted consequences of this combination would likely be circumvention of the immune response, promotion of viral propagation, expansion of infection into many cell and tissue types, and ultimately the development of AIDS in HIV infected individuals.

In summary, we present two new corollaries to our recently established human foetoembryonic defence system hypothesis: (i) mimicry or acquisition of glycans that are used in this protective system by pathogens or tumour cells may enable them to either subvert or misdirect the human immune response; thus greatly increasing their pathogenicity; and (ii) expression of glycans used in this system by normal tissues and cells

outside the reproductive system may protect them from immune responses, especially in those cases where major histocompatibility recognition is either absent or minimal.

This paradigm is presented not to exclude or denigrate other potential models explaining how HIV related pathology is induced. Absolute evidence supporting this hypothesis as the sole mechanism for AIDS induced pathology is not available at this time. However, a more complete understanding of the system that protects the developing human and the gametes from potential immune responses may enable us to address the molecular basis underlying not only AIDS but also a host of other pathological conditions in the human.

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References

- Adachi, M., Hayami, M., Kashiwagi, N. *et al.* (1988) Expression of Le^y antigen in human immunodeficiency virus-infected human T cell lines and in peripheral lymphocytes of patients with acquired immune deficiency syndrome (AIDS) and AIDS related complex (ARC). *J. Exp. Med.*, **167**, 323–331.
- Allen, J.E. and Maizels, R.M. (1996) Immunology of human helminth infection. *Int. Arch. Allergy Immunol.*, **109**, 3–10.
- Aspinall, G.O., Monteiro, M.A., Pang, H. *et al.* (1996a) Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC 11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. *Biochemistry*, **35**, 2489–2497.
- Aspinall, G.O. and Monteiro, M.A. (1996b) Lipopolysaccharides of *Helicobacter pylori* strains P466 and M019: structures of the O antigen and core oligosaccharide regions. *Biochemistry*, **35**, 2498–2504.
- Barre-Sinoussi, F., Chermann, J.C., Rey, F. *et al.* (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*, **220**, 868–871.
- Bolton, A.E., Pockley, A.G., Clough, K.J. *et al.* (1987) Identification of placental protein 14 as an immunosuppressive factor in human reproduction. *Lancet*, **i**, 593–595.
- Cheng-Meyer, C., Seto, D., and Levy, J.A. (1991) Altered host range of HIV-1 after passage through various human cell types. *Virology*, **181**, 288–294.
- Clark, G.F., Oehninger, S., Patankar, M.S. *et al.* (1996) A role for glycoconjugates in human development: the human foeto-embryonic defence system hypothesis. *Hum. Reprod.*, **11**, 467–473.
- Cross, N. L. and Overstreet, J. (1987) Glycoconjugates of the human sperm surface: distribution and alterations that accompany capacitation *in vitro*. *Gamete Res.*, **16**, 23–35.
- Cummings, R.C. and Kornfeld, S. (1982) Characterization of the structural determinants required for the high affinity interaction of asparagine-linked oligosaccharides with immobilized *Phaseolus vulgaris* leucoagglutinating and erythroagglutinating lectins. *J. Biol. Chem.*, **257**, 11230–11234.
- Dalgleish, A.G., Wilson, S., Gompels, M. *et al.* (1992) T-cell receptor variable gene products and early HIV-1 infection. *Lancet*, **339**, 824–828.
- de Waal-Malefyt, R., Haanen, J., Spits, H. *et al.* (1991) Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J. Exp. Med.*, **174**, 915–924.
- Dell, A., Morris, H.R., Easton, R.L. *et al.* (1995) Structural analysis of the

- oligosaccharides derived from glycodelin, a human glycoprotein with potent immunosuppressive and contraceptive activities. *J. Biol. Chem.*, **270**, 24116–24126.
- Dennis, J.W. (1991) N-linked oligosaccharide processing and tumor cell biology. *Semin. Cancer Biol.*, **2**, 411–420.
- Duesberg, P. (1988) HIV is not the cause of AIDS. *Science*, **241**, 514–517.
- El-Ouagari, K., Teissie, J. and Benoist, H. (1995) Glycophorin A protects K562 cells from natural killer cell attack. Role of oligosaccharides. *J. Biol. Chem.*, **270**, 26970–26975.
- Ezekowitz, R.A. and Stahl, P.D. (1988) The structure and function of vertebrate mannose lectin-like proteins. *J. Cell Sci.*, **9** (Suppl.), 121–133.
- Fiorentino, D.F., Zlotnik, A., Vieira, P. *et al.* (1991) IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J. Immunol.*, **146**, 3444–3451.
- Fischl, M.A., Richman, D.D., Grieco, M.H. *et al.* (1987) The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N. Engl. J. Med.*, **317**, 185–191.
- Golding, H., Robey, F.A., Gates, F.T. III, *et al.* (1988) Identification of homologous regions in human immunodeficiency virus I gp41 and human MHC class II beta 1 domain. I. Monoclonal antibodies against the gp41-derived peptide and patients' sera react with native HLA class II antigens, suggesting a role for autoimmunity in the pathogenesis of acquired immune deficiency syndrome. *J. Exp. Med.*, **167**, 914–923.
- Golding, H., Shearer, G.M., Hillman, K. *et al.* (1989) Common epitope in human immunodeficiency virus (HIV) I-GP41 and HLA class II elicits immunosuppressive autoantibodies capable of contributing to immune dysfunction in HIV I-infected individuals. *J. Clin. Invest.*, **83**, 1430–1435.
- Grinnell, B.W., Hermann, R.B. and Yan, B.S. (1994) Human protein C inhibits selectin-mediated cell adhesion: role of a unique fucosylated sequence. *Glycobiology*, **4**, 221–225.
- Hall, E.T., Yan, J.P., Melancon, P. and Kuchta, R.D. (1994) 3'-Azido-3'-deoxythymidine potentially inhibits protein glycosylation. A novel mechanism for AZT cytotoxicity. *J. Biol. Chem.*, **269**, 14355–14388.
- Hanasaki, K., Varki, A. and Powell, L. D. (1995) Binding of human plasma sialoglycoproteins by the B cell-specific lectin CD22. Selective recognition of immunoglobulin M and haptoglobin. *J. Biol. Chem.*, **270**, 7543–7550.
- Heagy, W., Crumpacker, C., Lopez, P.A. and Finberg, R.W. (1991) Inhibition of immune functions by antiviral drugs. *J. Clin. Invest.*, **87**, 1916–1924.
- Hegde, U.C. (1991) Immunomodulation of the mother during pregnancy. *Med. Hypoth.*, **35**, 159–164.
- Helenius, A. (1994) How N-linked oligosaccharides affect glycoprotein folding in the endoplasmic reticulum. *Mol. Biol. Cell*, **5**, 253–265.
- Hiraishi, K., Suzuki, K., Hakomori, S.-I. and Adachi, M. (1993) Le^x antigen expression is correlated with apoptosis (programmed cell death). *Glycobiology*, **3**, 381–390.
- Imberti, L., Sottini, A., Bettinardi, A. *et al.* (1991) Selective depletion in HIV infection of T cells that bear specific T cell receptor V beta sequences. *Science*, **254**, 860–862.
- Ishiguro, T. (1991) Microheterogeneity of α -fetoprotein in the amniotic fluid-developmental changes in the molecular structure of carbohydrate chain. *Nippon Sanka Fujinka Gakkai Zasshi*, **43**, 51–56.
- Kamarainen, M., Leivo, I., Koistinen, R. *et al.* (1996) Normal human ovary and ovarian tumors express glycodelin, a glycoprotein with immunosuppressive and contraceptive properties. Evidence from immunohistochemical staining and *in situ* hybridization. *Am. J. Pathol.*, **148**, 1435–1443.
- Kang, S., Cummings, R.D. and McCall, J.W. (1993) Characterization of the N-linked oligosaccharides in glycoproteins synthesized by microfilariae of *Dirofilaria immitis*. *J. Parasitol.*, **79**, 815–828.
- Kashiwagi, N., Gill, M.J., Adachi, M. *et al.* (1994) Lymphocyte membrane modifications induced by HIV infection. *Tohoku J. Exp. Med.*, **173**, 115–131.
- Ko, A.I., Drager, U.C., and Harn, D.A. (1990) A *Schistosoma mansoni* epitope recognized by a protective monoclonal antibody is identical to the stage-specific embryonic antigen 1. *Proc. Natl. Acad. Sci. USA*, **87**, 4159–4163.
- Koistinen, H., Koistinen, R., Dell, A. *et al.* (1996) Glycodelin from seminal plasma is a differentially glycosylated form of contraceptive glycodelin-A. *Mol. Hum. Reprod.*, **2**, 759–766.
- Kornfeld, S. and Kornfeld, R. (1985) Assembly of asparagine-linked oligosaccharides. *Ann. Rev. Biochem.*, **54**, 631–634.
- Koziel, H., Kruskal, B.A., Ezekowitz, R.A. and Rose, R.M. (1993) HIV impairs alveolar macrophage mannose receptor function against *Pneumocystis carinii*. *Chest*, **103** (Suppl. 2), 111S–112S.
- Larkin, M., Ahern, T.J., Stoll, M.S. *et al.* (1992) Spectrum of sialylated and nonsialylated fuco-oligosaccharides bound by the endothelial-leukocyte adhesion molecule E-selectin. Dependence of the carbohydrate binding activity on E-selectin density. *J. Biol. Chem.*, **267**, 13661–13668.
- Levy, J.A. (1993) Pathogenesis of human immunodeficiency virus infection. *Microbiol. Rev.*, **57**, 183–289.
- Marshall, B.J. (1983) Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*, **i**, 1273–1275.
- Marshall, B.J. (1995) *Helicobacter pylori* in peptic ulcer: have Koch's postulates been fulfilled? *Ann. Med.*, **27**, 565–568.
- Matzinger, P. (1994) Tolerance, danger, and the extended family. *Ann. Rev. Immunol.*, **12**, 991–1045.
- Mizuochi, T., Matthews, T.J., Kato, M. *et al.* (1990) Diversity of the oligosaccharide structures on the envelope glycoprotein gp120 of human immunodeficiency virus 1 from the lymphoblastoid cell line H9. *J. Biol. Chem.*, **265**, 8519–8524.
- Morris, H.R., Dell, A., Easton, R.L. *et al.* (1996) Gender specific glycosylation of human glycodelin dictates its contraceptive activity. *J. Biol. Chem.*, **271**, 32159–32167.
- Morrow, D.M., Getty, R.R., Ilan, J. *et al.* (1994) Hematopoietic placental protein 14 (PP14): a second polypeptide isoform in a leukemic cell of the megakaryocytic lineage. *Am. J. Pathol.*, **145**, 1485–1495.
- Nakagomi, H., Pisa, P., Pisa, E.K. *et al.* (1995) Lack of interleukin-2 (IL-2) expression and selective expression of IL-10 mRNA in human renal cell carcinoma. *Int. J. Cancer*, **63**, 366–371.
- Oehninger, S., Coddington, C. C., Hodgen, G.D. and Seppälä, M. (1995) Factors affecting fertilization: endometrial placental protein 14 reduces the capacity of human sperm to bind to the human zona pellucida. *Fertil. Steril.*, **63**, 377–383.
- Okamoto, N. A., Uchida, A., Takamura, K. *et al.* (1991) Suppression by human placental protein 14 of natural killer cell activity. *Am. J. Reprod. Immunol.*, **26**, 137–142.
- Patankar, M.S., Ozgur, K., Oehninger, S. *et al.* (1996) Expression of immunosuppressive carbohydrate sequences on the surface of human gametes. *Glycobiology* (in press).
- Pearce, E.J., Caspar, P., Grzych, J.M. *et al.* (1991) Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *J. Exp. Med.*, **173**, 159–166.
- Pennesi, E. (1996) Teetering on the brink of danger. *Science*, **271**, 1665–1667.
- Phillips, M.L., Nudelman, E., Gaeta, F.C. *et al.* (1990) ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lewis^x. *Science*, **250**, 1130–1132.
- Pisa, P., Halapi, E., Pisa, E.K. *et al.* (1992) Selective expression of interleukin 10, interferon gamma, and granulocyte-macrophage colony-stimulating factor in ovarian cancer biopsies. *Proc. Natl. Acad. Sci. USA*, **89**, 7708–7712.
- Pockley, A.G. and Bolton, A. (1990) The effect of human placental protein 14 (PP14) on the production of interleukin-1 from mitogenically stimulated mononuclear cell cultures. *Immunology*, **69**, 277–281.
- Pockley, A.G., Mowles, E. A., Stoker, R.J. *et al.* (1988) Suppression of *in vitro* lymphocyte reactivity to phytohemagglutinin by placental protein 14. *J. Reprod. Immunol.*, **13**, 31–39.
- Powell, L. and Varki, A. (1994) The oligosaccharide binding specificities of CD22b, a sialic acid-specific lectin of B cells. *J. Biol. Chem.*, **269**, 10628–10636.
- Powell, L.D., Jain, R.K., Matta, K.L. *et al.* (1995) Characterization of sialyloligosaccharide binding by recombinant soluble and native cell-associated CD22. Evidence for a minimal structural recognition motif and the potential importance of multisite binding. *J. Biol. Chem.*, **270**, 7523–7532.
- Reiner, S.L., Wang, Z.E., Hatam, F. *et al.* (1993) TH1 and TH2 cell antigen receptors in experimental leishmaniasis. *Science*, **259**, 1457–1460.
- Robinson, W.E. Jr., Mitchell, W.M., Chambers, W.H. *et al.* (1988) Natural killer cell infection and inactivation *in vitro* by the human immunodeficiency virus. *Hum. Pathol.*, **19**, 535–540.
- Rustin, G.J., van-der-Burg, M.E. and Berek, J.S. (1993) Advanced ovarian cancer. Tumour markers. *Ann. Oncol.*, **4** (Suppl. 4), 71–77.
- Sato, T., Furukawa, K., Autero, M. *et al.* (1993) Structural study of the sugar chains of human leukocyte common antigen CD45. *Biochemistry*, **32**, 12694–12704.
- Sgroi, D., Koretzky, G.A., and Stamenkovic, I. (1995) Regulation of CD45 engagement by the B-cell receptor CD22. *Proc. Natl. Acad. Sci. USA*, **92**, 4026–30.
- Shearer, G.M. and Clerici, M. (1992) T helper cell immune dysfunction in asymptomatic, HIV-1-seropositive individuals: the role of TH1-TH2 cross-regulation. *Chem. Immunol.*, **54**, 21–43.

- Sher, A., Gazzinelli, R.T., Oswald, I.P. *et al.* (1992) Role of T-cell derived cytokines in the downregulation of immune responses in parasitic and retroviral infection. *Immunol. Rev.*, **127**, 183–204.
- Srivatsan, J., Smith, D.F. and Cummings, R.D. (1992) *Schistosoma mansoni* synthesizes novel biantennary Asn-linked oligosaccharides containing terminal β -linked *N*-acetylgalactosamine. *Glycobiology*, **2**, 445–452.
- Stamenkovic, I., Sgroi, D., Aruffo, A. *et al.* (1991) The B lymphocyte adhesion molecule CD22 interacts with leukocyte common antigen CD45RO on T cells and α 2–6 sialyltransferase, CD75, on B cells *Cell*, **66**, 1133–1144.
- Tomasi, T.B. (1978) Suppressive factors in amniotic fluid and newborn serum. Is α -fetoprotein involved? *Cell Immunol.*, **37**, 459–466.
- Tsuchida, Y., Yamashita, K., K., Kobata, A. *et al.* (1984) Structure of the sugar chain of α -fetoprotein purified from a human yolk sac tumor and its reactivity with concanavalin A. *Tumour Biol.*, **5**, 33–40.
- Van Dam, G.J., Bergwerff, A.A., Thomas-Oates, J.E. *et al.* (1994) The immunologically reactive *O*-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke *Schistosoma mansoni* have Lewis^x as repeating unit. *Eur. J. Biochem.*, **225**, 467–482.
- Velupillai, P. and Harn, P. A. (1994) Oligosaccharide-specific induction of interleukin 10 production by B220⁺ cells from schistosome-infected mice: a mechanism for regulation of CD4⁺ T-cell subsets. *Proc. Natl. Acad. Sci. USA*, **91**, 18–22.
- Warren, J.R. (1983) Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*, **i**, 1273.
- Weiss, R.A. (1993) How does HIV cause AIDS? *Science*, **260**, 1273–1279.
- Yan, J.-P., Ilsley, D.D., Fröhlick, C. *et al.* (1995) Azidothymidine (zidovudine) inhibits glycosylation and dramatically alters glycosphingolipid synthesis in whole cells at clinically relevant concentrations. *J. Biol. Chem.*, **270**, 22836–22841.
- Yamashita, K., Hitoi, A., and Kobata, A. (1983) Structural determinants of *Phasoleus vulgaris* erythroagglutinating lectin for oligosaccharides. *J. Biol. Chem.*, **258**, 14753–14755.
- Yoshimura, M., Ihara, Y., Ohnishi, A. *et al.* (1996) Bisecting *N*-acetylglucosamine on K562 cells suppresses natural killer cell cytotoxicity and promotes spleen colonization. *Cancer Res.*, **56**, 412–418.

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