Glycoconjugate Journal 17, 887–893, 2000

Role of linkage specific 9-O-acetylated sialoglycoconjugates in activation of the alternate complement pathway in mammalian erythrocytes

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Substitution of the -OH group at C-9 of sialic acid by an O-acetyl ester has been suggested to modify various biological phenomena that are regulated by sialic acids. Amongst them, enhancement of erythrocyte lysis by 9-O-acetylated sialic acid determinants through modulation of the alternate pathway of complement has been extensively studied on murine erythrocytes [1]. A variable expression of linkage specific 9-O-acetylated sialoglycoconjugates as defined by the lectinogenic epitope of Achatinin-H namely 9-O-acetylated sialic acid $\alpha 2 \rightarrow$ 6Gal NAc was identified on rabbit, guinea pig, hamster, rat, mouse and human erythrocytes. This differential expression of linkage specific 9-O-acetylated sialoglycoconjugates strongly correlated with the susceptibility of mammalian erythrocytes to lysis by the alternate pathway of complement. Additionally, low levels of antibodies directed against O-acetylated sialic acids in these mammalian species suggested that these constitutively present determinants have low immunogenicity. Taken together, our results indicate that complement mediated hemolysis depends not simply upon the extent of surface 9-O-acetylated sialic acids present but more importantly upon the specific linkage.

Keywords: **9-O-acetylated sialic acids, erythrocytes, Achatinin-H, alternate complement pathway, hemolysis**

Introduction

Sialic acids comprise a family of 40 naturally occurring derivatives of neuraminic acid, which are commonly observed as terminal residues of vertebrate oligosaccharides. Amongst their diverse multitude, the commonest modification is O-acetylation at position C-7/8/9 leading to a family of 7/8/or 9-O-acetylated sialoglycoconjugates or O-AcSGs [2,3]. Since O-acetyl esters from C-7 and C-8 positions are known to migrate to C-9 position even under physiological conditions, O-acetylation at C-9 position is generally considered as the commonest biologically occurring modification [4,5]. Further structural diversity amongst these O-AcSGs is generated depending upon their respective linkages to the underlying sugar chain, subterminal sugars as well as the glycoconjugates (glycoprotein or glycolipid) to which they are attached [6].

Owing to their terminal position, 9-O-AcSGs are reported to play a major regulatory role in a multitude of cellular and molecular interactions [7,8]. They are considered as both an ontogenic and oncogenic antigen as they have been identified as having a role to play in both tissue development and serving as an important antigenic determinant associated with melanomas [9], childhood acute lymphoblastic leukemia [10,11] and colon carcinomas [12,13]. These 9-O-AcSGs can also mask binding sites, notably of sialic acid binding lectins like Siglec 1 (sialoadhesin, CD169), a macrophage adhesion molecule and Siglec 2 (CD22), a mature B cell surface glycoprotein [14,15]. It can also block the recognition of sialic acids by hemagglutinins of Influenza A and B viruses while conversely serve as a receptor determinant for Influenza C [16, 17]. Molecular dynamic studies have confirmed that linkage of 9-O-AcSA to subterminal galactose residues provides flexibility to the oligosaccharide chain [18] reflected in its wide regulation of a number of cellular and molecular interactions.

Qualitative mapping of cell surface 9-O-AcSGs can be achieved using probes with narrow binding specificity towards 9-O-AcSGs that unfortunately to date are still limited. They include Achatinin-H, a lectin derived from the hemolymph of the African giant snail *Achatina fulica* [19,20]. This lectin was affinity-purified using bovine submaxillary mucin (BSM),

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known to contain a high percentage of 9-O-AcSGs in a specific $\alpha 2 \rightarrow 6$ linkage with a defined subterminal sugar namely GalNAc of the underlying oligosaccharide chain [21]. Accordingly, the lectinogenic epitope of Achatinin-H may be stated as being 9-O-AcSA $\alpha 2 \rightarrow 6$ GalNAc [20].

Sialic acids have been identified as critical determinants of erythrocyte survival. By virtue of their binding to factor H, they prevent formation of the C3b3b complex that in turn prevents activation of the alternate pathway of complement [22]. To achieve this negative regulatory function, the exocyclic side chain of sialic acid is vital as studies on murine erythrocytes have shown that replacement of sialic acid by its bulky 9-O-acetylated derivative prevented binding of sialic acid to factor H. This resulted in activation of the alternate complement pathway and subsequent hemolysis. In an elegant study by Varki and Kornfeld [1], a positive correlation has been reported between the % 9-O-AcSA content of erythrocytes of various murine species and their degree of complement mediated hemolysis. Accordingly, this study was undertaken to examine whether such a similar correlation exists amongst other mammalian erythrocytes that constitutively express 9-O-AcSGs. Utilizing the narrow binding specificity of Achatinin-H, we have non-invasively analyzed (i) the status of cell surface $\alpha 2 \rightarrow 6$ linked 9-O-AcSGs on erythrocytes of different mammalian species (ii) whether a correlation exists between the degree of alternate complement mediated hemolysis and these linkage specific 9-O-AcSGs that are constitutively present on mammalian erythrocytes and finally (iii) the immunogenicity of this newly identified glycotope.

Materials and methods

Heparinised blood was collected from normal donors and adult animals namely New Zealand Albino rabbits, Albino guinea pigs, Syrian golden hamsters, Sprague Dawley rats and BALB/c mice, erythrocytes were harvested while the plasma was separated and stored at -20°C.

Preparation of bovine submaxillary mucin (BSM)

BSM was prepared according to the method of Murphy and Gottschalk [23] and modified as previously described [24]. Estimation of % of 9-O-acetylated sialic acid derivatives present in BSM was measured flurimetrically according to the method of Shukla and Schauer [25] and modified as described by Sharma *et al*. [26]. Accordingly, we calculated the % of sialic acid present in BSM that is O-acetylated, as 22.5%.

Purification of Achatinin-H

Achatinin-H was affinity-purified from the hemolymph of the *Achatina fulica* snail using BSM coupled to Sepharose4B [20]. Its specificity towards 9-O-Acetylated sialoglycoconjugates (9-O-AcSGs) was determined by several biochemical approaches [27–29].

Estimation of (9)8-O-acetylsialic acid on erythrocyte membranes

Erythrocyte ghosts were prepared and their cell surface (9)8-O-AcSA content flurimetrically quantitated using the method of Shukla and Schauer [25]. The relative flurescence intensity [(max (excitation = 410 nm), (max (emission = 510 nm)] of each sample was measured against reagent blanks. The percentage of 9-O-AcSA was determined by subtracting the amount of unsubstituted sialic acids from that obtained following de-O-acetylation.

Hemagglutination assay (HA) and hemagglutination inhibition assay

Blood samples from various mammalian species were collected in heparin, the buffy coat was removed and erythrocytes were washed four times with saline. HA was performed as per the method of Sarkar *et al*. [30] using serially diluted Achatinin-H (25 µl of 50 µg/ml). The reciprocal of the highest dilution of Achatinin-H that produced visible agglutination was taken as the hemagglutination titre expressed in hemagglutination units. Specificity of binding of mammalian erythrocytes towards 9-O-AcSGs was confirmed using a fixed concentration of Achatinin-H (HU = 16 units) in the presence of BSM and de-O-acetylated BSM.

Cell lysis assay

Human serum was collected from normal healthy donors and served as the source of complement. Blood was collected from different animals in phosphate buffered saline containing 0.1% gelatin and 0.04 M EDTA (GPB-EDTA). After an initial centrifugation for 10 min at 2000 rpm at 25°C, to remove the buffy coat, the erythrocytes were then washed four times in phosphate buffered saline containing 0.1% gelatin (GPB) with the final wash being in GPB containing 2 mM $Mg^{++}Cl_2$ and 8 mM EGTA (GPB-Mg-EGTA). The cell concentration was then adjusted to 1×10^8 cells/ml.

Activation of alternate complement pathway was assessed by quantitating the percent lysis of mammalian erythrocytes in the presence of serially diluted fresh human serum [22]. To prevent any contribution by the classical complement pathway, serum was diluted with GPB-Mg^{++}-EGTA. Erythrocytes (1×10^7/0.1 ml) were added to 250 µl of GPB-Mg^{++}-EGTA containing increasing amounts of serum and incubated at 37°C for 45 min with mild agitation. The reaction was terminated by the addition of cold saline (1.25 ml) and the percentage lysis of erythrocytes was measured against a reagent blank (0%) at 412 nm. The degree of lysis was compared with that obtained by addition of cold distilled water which represented 100% lysis.

Estimation of antibodies against O-Acetylated sialic acids (O-AcSA)

Microtitre plates were coated with BSM prepared as described above (10 µg/ml, 100 µl/well) in 0.02 M phosphate buffer, pH

7.4 overnight at 4°C. Following three washes with phosphate buffered saline (PBS) containing 0.1% Tween-20 (PBS-T), the wells were then blocked with 2% BSA for 2 h at 25°C. Mammalian sera, at different dilutions were incubated overnight at 4°C and its binding to BSM colorimetrically measured using horse radish peroxidase (HRP) conjugated Protein A (1:15 000, Zymed Labs, San Francisco, USA) and azino-bis thio-sulfonic acid (ABTS) as the substrate on an ELISA reader at 405 nm [24,31].

Results

Flurimetric determination of unsubstituted and 9(8)-O-acetylated sialic acids in mammalian erythrocytes

Flurimetric quantitation of the % of sialic acid that is 9(8)-O-acetylated on mammalian erythrocytes indicated minor variations ranging from 20–25% for rabbit, guinea pig and hamster. The content was relatively higher on rat and murine erythrocytes being 40 and 60% respectively while notably, human erythrocytes had the least amount of 9-O-AcSA being 5% (Table 1).

Variation in hemagglutination titre amongst mammalian species using Achatinin-H

The narrow binding specificity of Achatinin-H, a 9-O-acetyl sialic acid binding lectin was confirmed by hemagglutination inhibition assay using several inhibitors containing variable amounts of 9-O-acetyl sialic acid (Table 2).

Despite a comparable % 9-O-AcSA content on rabbit, guinea pig and hamster erythrocytes, their agglutination profile using Achatinin-H showed wide variations in their hemagglutination units (HU) (Table 1). Rabbit erythrocytes had a 32 fold higher HU being 1024 as compared to guinea pig and hamster who showed a comparable HU of 32. Notably, murine erythrocytes that have the highest content of % 9-O-AcSA showed no agglutination with Achatinin-H (Table 1). No hemagglutination was observed with human erythrocytes which contain negligible amounts of OAcSA.

To reconfirm that the binding of Achatinin-H with mammalian erythrocytes may be attributed to the presence of cell surface O-AcSGs, we carried out a hemagglutination inhibition assay. As the lectin binding was strongly inhibited by BSM known to contain 22.5% O-AcSA and not by de-O-acetylated BSM (Table 3), it reconfirmed the presence of O-acetylated sialoglycans on the surface of mammalian erythrocytes.

Altered activation of alternate complement pathway amongst mammalian species

A variable degree of erythrocyte lysis was induced in mammalian species that progressively increased with increasing amounts of normal human serum (Figure 1). The maximal degree of hemolysis was induced in rabbit erythrocytes (95.5%); guinea pig and mouse showed comparable levels of hemolysis whereas rat and hamster showed baseline levels.

Correlation of degree of complement mediated hemolysis with linkage specific 9-O-AcSA and total cell surface 9-O-AcSA

In order to correlate the degree of complement mediated hemolysis induced by 50 µl of normal human serum we compared it with the degree of Achatinin-H induced hemagglutination. A strong positive correlation was observed between the extent of complement mediated hemolysis of mammalian erythrocytes and the presence of linkage specific 9-O-AcSAs specifically recognized by Achatinin-H ($r = 0.90$). In contrast, when the % complement mediated hemolysis was correlated with the total surface 9-O-AcSA content present on mammalian erythrocytes as measured flurimetrically, it was comparatively poor ($r = 0.22$).

Table 1. Comparison of total and linkage specific 9-O-acetylated sialic acids present on mammalian erythrocytes with % of hemolysis induced by alternate complement pathway

*Mammalian species	#% 9-O-AcSA	@HU of Achatinin-H	$% Hemolysis
Rabbit	20	1024	70.0
Guinea Pig	22	32	23.0
Hamster	25	32	3.0
Rat	40	16	4.0
Human	5	0	ND
Mouse	60	0	30.0

*Blood was collected in heparin.
#Total percent of 9-O-Acetylated sialic acid present on erythrocyte membranes was flurimetrically estimated as described in Materials and methods.
@HU is defined as the reciprocal of the highest dilution of Achatinin-H that produced visible agglutination.
$The percent hemolysis induced by the alternate complement pathway using 50 µl of normal human serum.
ND: Not done as human serum was used as a source of complement.

Table 2. Binding specificity of Achatinin-H by hemagglutination inhibition assay [11]

Saccharides/Sialoglycoproteins	*Types/Nature of terminal linkages*	*I_{50}
(i) Monosaccharides	Neu 5 Ac	30.48
	Neu 5,9 Ac_2	1.30
	Neu 4,5 Ac_2	NI[a]
(ii) Disaccharides	α-Neu 5, Ac-(2 $\rightarrow$ 6)-GalNAc-ol	NI[b]
(iii) Sialoglycoproteins		
BSM	α-Neu5,9Ac_2-(2 $\rightarrow$ 6)-β-DGalNAc-	0.0002
SSM	α-Neu5Ac-(2 $\rightarrow$ 6)-β-DGalNAc-	NI[b]

I_{50}: The minimal concentration of the monosaccharide required for 50% inhibition of 16 Hemagglutination units of Achatinin-H; NI[a]: Not inhibited upto a concentration of 100 mM; BSM: Bovine submaxillary mucin; SSM: Sheep submaxillary mucin; NI[b]: 350 fold less inhibitory than BSM on the basis of 9-O-acetyl sialic Acid.

Table 3. Hemagglutination inhibition of mammalian erythrocytes

	*Inhibitory conc. (μM)			
Sugar	*Rabbit*	*Guinea pig*	*Rat*	*Hamster*
BSM	0.003	0.2	0.2	0.4
De-O-acetylated BSM	NI	NI	NI	NI

*On the basis of concentration of O-acetylated sialic acid present in bovine submaxillary mucin (BSM).
Erythrocyte suspension (2%) in saline is used for hemagglutination inhibition assay as described in Materials and methods.

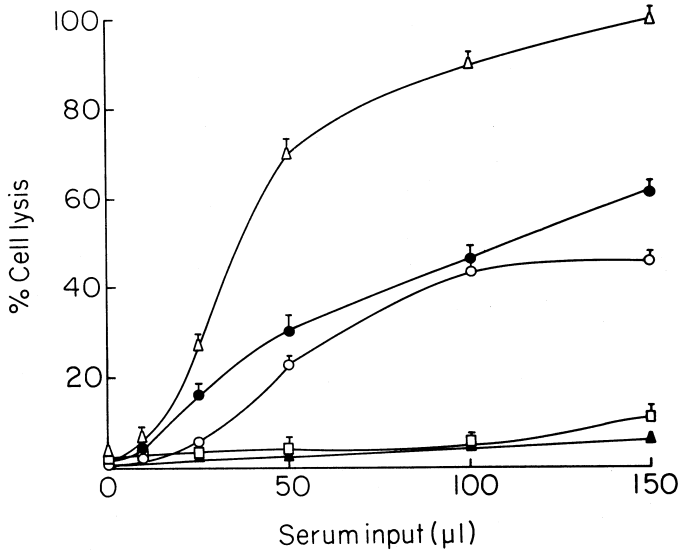

Figure 1. Alternate complement mediated hemolysis of mammalian erythrocytes: Cell lysis assay of different mammalian erythrocytes was carried out using increasing amounts of fresh human serum as the source of complement as described in Materials and methods. Mammalian species included rabbit (△—△); guinea pig (●---●); mouse (○---○); rat (□---□) and hamster (▲- - -▲). Each point is the average of triplicate determinations.

Baseline levels of antibodies directed against O-AcSA

To identify whether these 9-O-AcSGs, constitutively present on mammalian erythrocytes, show immunogenicity we estimated the levels of antibodies against O-AcSA by an ELISA using BSM as the coating antigen, based on the long standing information that BSM contains a high % of O-AcSA [21]. Sera from mammals e.g. rabbit, guinea pig and human consistently showed low levels of O-AcSA specific antibodies (Figure 2). The level of anti-O-AcSA was even lower in hamster and mouse (data not shown). The presence of anti O-AcSA in rats was not measured as it does not bind to HRP-Protein A that was used as the second antibody. These antibodies were specifically directed against O-AcSA as antibody binding decreased significantly following replacement of the coating antigen BSM with de-O-acetylated BSM (Figure 2).

Discussion

Study of the cell biology and biochemistry of 9-O-acetylated sialic acids remains limited, as accurate quantification of these O-acetylated sialoglycans requires their previous release from glycosidic linkages by either enzymatic or chemical hydrolysis.

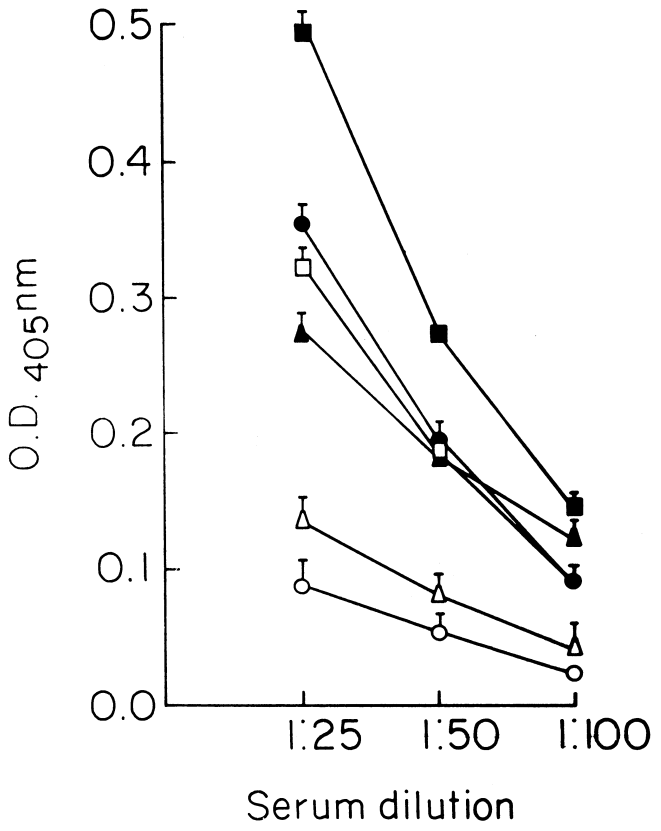

Figure 2. Measurement of antibodies using BSM as the coating antigen: BSM (filled symbols) and de-O-acetylated BSM (open symbols) were used as the coating antigen, sera from rabbit (▲,△) guinea pig (●,○) and human (■,□) were diluted 1:25, 1:50 and 1:100 and assayed as described in Materials and methods. Each point is the average of duplicate determinations.

The currently available methods are not totally satisfactory as potential pitfalls during analysis include incomplete release of sialic acids, de O-acetylation and spontaneous migration of O-acetyl groups [32]. Amongst the currently available tools that can detect these alkali labile O-AcSGs preferably bypassing their previous liberation, the commonest is Influenza-C-virus [16,17] or its recombinant soluble form with the Fc portion of human IgG (CHE-Fc) [33]. They have a broad specificity as they can detect 9-O-AcSGs irrespective of their linkage and the subterminal sugar. Another lectin available from the Californian coastal crab *Cancer antennarius* binds to O-acetylated sialic acids both at C-4 and C-9 positions [34] and is similar to the lectin purified from the human placenta [35]. However, in terms of identifying linkage specific O-acetylated determinants, till today, the most effective probe would be the snail lectin Achatinin-H whose glycotope has been defined as 9-O-AcSA $\alpha 2 \rightarrow 6$ to subterminal GalNAc [20].

Although mammalian erythrocytes have an abundance of surface sialic acids, the distribution of its O-acetylated derivative is less, the maximum being 60% in mouse while humans showed no measurable O-acetylated sialic acid (Table 1). This high amount of 9-O-AcSA on murine erythrocytes has been quantitated by gas liquid chromatography-mass spectrometry as accounting for over 50% of the sialic acid [36] but the underlying linkage was not established. Another approach has been the flurimetric quantitation of O-AcSA that measures the total % of sialic acid that is 9(8)-O-acetylated irrespective of the linkage or the subterminal sugar (Table 1). However, the total % 9-O-AcSA present on the mammalian erythrocytes did not corroborate with hemagglutination titres obtained with the lectin Achatinin-H (Table 1). Despite comparable amounts of total surface 9-O-AcSAs on rabbit, guinea pig and hamster erythrocytes, there was wide variation in the degree of Achatinin-H induced hemagglutination (Table 1). The most notable difference was in murine erythrocytes whose % 9-O-AcSA was flurimetrically estimated to be 60% yet there was no agglutination with Achatinin-H. The specificity of binding of Achatinin-H has previously been defined as 9-O-AcSA $\alpha 2 \rightarrow 6$ GalNAc (Table 2). As binding of Achatinin-H to mammalian erythrocytes could be inhibited by BSM but not by de-O-acetylated BSM, it reconfirmed that Achatinin-H was binding to cell surface O-AcSAs present in an $\alpha 2 \rightarrow 6$ linkage to subterminal GalNAc (Table 3). Taken together, our results suggest the presence of variable amounts of linkage specific O-AcSAs on mammalian erythrocytes of which only a certain proportion are in the linkage recognized by Achatinin-H. Thus although 9-O-AcSA determinants constitute 50% of sialic acid present on murine erythrocytes [36] they are not in the linkage identified by Achatinin-H. It may be envisaged that these 9-O-AcSAs are possibly present in a $\alpha 2 \rightarrow 3$ or $\alpha 2 \rightarrow 8$ linkage or are cryptic, or the subterminal sugars may be different, though this needs to be established.

The 9-O-AcSAs present on the surface of murine erythrocytes and murine erythroleukemia cells have been reported to contribute towards their susceptibility towards lysis by activation of the alternate pathway of complement [1,37]. It may therefore be extrapolated that erythrocytes having a higher amount of 9-O-AcSA would have a higher degree of hemolysis and vice-versa. However, the case is not so simple, as we observed that the degree of complement mediated hemolysis showed a poor correlation with total 9-O-AcSA present on mammalian erythrocytes (Figure 1, Table 1, $r = 0.22$). Instead, a better correlation was obtained between the degree of Achatinin-H induced hemagglutination and the degree of complement mediated hemolysis ($r = 0.90$). Taken together, our results strongly suggest that the degree of complement mediated hemolysis observed on mammalian erythrocytes depends not simply upon the total amount of 9-O-AcSA but more importantly, on the linkage specific 9-O-AcSA determinants. Accordingly, it maybe postulated that in mammalian erythrocytes, those having 9-OAcSA in an $\alpha 2 \rightarrow 6$ linkage with GalNAc are more susceptible to complement mediated hemolysis. In previous studies, we have reported an increased presence of the glycotope recognized by Achatinin-H on human erythrocytes from patients with Visceral Leishmaniasis as compared to normal erythrocytes [26]. Interestingly, these

diseased erythrocytes showed a significantly higher degree of complement mediated hemolysis as compared to erythrocytes from normal healthy donors thus reconfirming that linkage specific 9-O-AcSGs play a contributory role in complement mediated hemolysis [38]. It may be noted that anemia is a common manifestation in this disease and has been attributed to bone marrow infiltration, hypersplenism and auto immune hemolysis [39]. It would be interesting to study whether the enhanced presence of 9-O-acetylated sialoglycans with this specific glycotype on these diseased erythrocytes also plays a contributory role towards anemia.

Sialic acids have been identified as playing a critical role in controlling the life span of erythrocytes, as the senescence or aging of erythrocytes has been shown to be associated with a progressive decline of surface sialylation [40]. It has been reported that rat erythrocytes having surface terminal sialic acids, are poorly bound to macrophages while sialidase treatment resulted in their enhanced binding to macrophages [41]. It can therefore be envisaged that in aging erythrocytes, the decrease in sialic acid leads to their increased binding to macrophages and their eventual sequestration and clearance. In fact, anemia associated with lead poisoning has been shown to be associated with a concomitant decrease in sialic acid content and consequent shortening of erythrocyte survival [42]. It would be interesting to study whether this decrease in sialic acid content observed in aging or diseased erythrocytes is associated with a concomitant increase in 9-O-AcSA in a linkage specific manner resulting in their enhanced complement mediated hemolysis.

In previous studies we have reported high titres of IgG antibodies directed against O-acetylated derivatives of sialic acids (O-AcSA) in patients of childhood acute lymphoblastic leukemia (ALL) [31] and Visceral Leishmaniasis (VL) of both human and canine origin [24,43]. Western blotting of membranes derived from peripheral blood mononuclear cells (PBMC) of childhood ALL patients confirmed antibody binding to O-AcSGs corresponding to 144, 135, 120, 90 and 36 kDa whereas binding to PBMC from normal individuals corresponded to 144 and 36 kDa [31]. The low anti O-AcSA levels in normal individuals indicates that constitutively present 9-O-AcSGs have poorer immunogenicity which is greatly enhanced in certain diseased conditions [24,31,43,44]. To assess the immunogenicity of similar constitutively present 9-O-AcSGs in mammalian species included in this study, we measured the anti O-AcSA levels. In all mammalian sera studied, the anti O-AcSA levels remained low; their specificity towards O-AcSA was confirmed by a decrease in binding following substitution of the coating antigen BSM by its de-O-acetylated form (Figure. 2). The prognostic potential of this biomarker in assessment of antimonial effectiveness in VL patients has been established [24,43]. Accordingly, we propose that assessment of chemotherapeutic effectiveness and monitoring of the disease status in animal models may well be achieved; such studies are underway.

Acknowledgements

This work was supported by the Department of Biotechnology and Science & Technology, Govt. of India. V. Sharma was a Senior Research fellow of the University Grants Commission, India. G. Sen was a Senior Research fellow of the Council of Scientific and Industrial Research and Ch Anil Kumar is a recipient of Lavanya Prova Bose Trust fellowship. We thank Mr. Asish Mallick for his excellent technical assistance.

References

1 Varki A, Kornfeld S, *J Exp Med* **152**, 532–44 (1980).
2 Sinha D, Chatterjee M, Mandal C, *TIGG* **12**, 1–17 (2000).
3 Schauer R, *Adv Carbohydr Chem Biochem* **40**, 131–234 (1982).
4 Varki A, *Glycobiology* **2**, 25–40 (1992).
5 Varki A, *FASEB J* **11**, 248–55 (1997).
6 Schauer R, In *Carbohydrates in Chemistry and Biology-Part II*, Wiley-VCH, Weinheim, 2000, pp. 227–43.
7 Kelm S, Schauer R, *Int Rev Cytol* **175**, 137–240 (1997).
8 Traving C, Schauer R, *Cell Mol Life Sci* **54**, 1330–49 (1998).
9 Rabindranath MH, Paulson JC, Irie RF, *J Biol Chem* **263**, 2079–86 (1988).
10 Mandal C, Sinha D, Sharma V, Bhattacharya DK, *Ind J Biochem Biophys* **34**, 82–6 (1997).
11 Sinha D, Mandal C, Bhattacharya DK, *Leukemia* **13**, 119–25 (1999).
12 Corfield AP, Wagner SA, O'Donn LJ, Durdey P, Mountford RA, Clamp JR, *Glycoconjugate J* **10**, 72–81 (1993).
13 Hutchins JT, Reading C, Giarazzi R, Hoaglund J, Jessup JM, *Cancer Res* **48**, 483–9 (1988).
14 Sjoberg ER, Powell LD, Klein A, Varki A, *J Cell Biol* **126**, 549–62 (1994).
15 Kelm S, Schauer R, Manuguerra JC, Gross HJ, Crocker PR, *Glycoconjugate J* **11**, 576–85 (1994).
16 Rogers GN, Herrler G, Paulson JC, Klenk HD, *J Biol Chem* **261**, 5947–51 (1986).
17 Muchmore E, Varki A, *Science* **236**, 1293–95 (1987).
18 Siebert H-C, von der Lieth C-W, Dong X, Reuter G, Schauer R, Gabius H-J, Vliegenthart JFG, *Glycobiology* **6**, 561–72 (1996).
19 Mandal C, Basu S, *Biochem Biophys Res Commun* **148**, 795–801 (1987).
20 Sen G, Mandal C, *Carbohydr Res* **268**, 115–25 (1995).
21 Reuter G, Pfeil R, Stoll S, Schauer R, Kamerling JP, Versluis C, Vliegenthart JFG, *Eur J Biochem* **134**, 139–43 (1983).
22 Nydegger UW, Fearon DT, Austen KF, *J Immunol* **120**, 1404–8 (1978).
23 Murphy WH, Gottschalk A, *Biochimica et Biophysica Acta* **52**, 349–60 (1961).
24 Chatterjee M, Sharma V, Mandal C, Sundar S, Sen S, *Glycoconjugate J* **15**, 1139–45 (1998).
25 Shukla AK, Schauer R, *Hoppe-Seylers' Physiol Chem* **363**, 255–62 (1982).
26 Sharma V, Chatterjee M, Mandal C, Basu D, Sen S, *Am J Trop Med Hyg* **58**, 551–4 (1998).
27 Mandal C, Basu S, Mandal C, *Biochem J* **257**, 65–71 (1989).
28 Basu S, Mandal C, Allen AK, *Biochem J* **254**, 195–202 (1988).
29 Mandal C, Mandal C, *Experentia* **46**, 433–41 (1990).

30 Sarkar M, Bachhawat BK, Mandal C, *Arch Biochim Biophys* **233**, 286–9 (1984).
31 Pal S, Chatterjee M, Bhattacharyya DK, Bandhyopadhyay S, Mandal C, *Glycobiology* **10**, 539–49 (2000).
32 Varki A, Diaz S, *Anal Biochem* **137**, 236–47 (1984).
33 Zimmer G, Reuter G, Schauer R, *Eur J Biochem* **204**, 209–15 (1992).
34 Ravindranath MH, Higa H, Cooper EL, Paulson J, *J Biol Chem* **260**, 8850–6 (1985).
35 Ahmed H, Gabius HJ, *J Biol Chem* **264**, 18 673–8 (1989).
36 Reuter G, Vliegenthart JFG, Wember M, Schauer R, Howard RJ, *Biochem Biophys Res Comm* **94**, 567–72 (1980).
37 Shi WX, Chammas R, Varki NM, Powell L, Varki A, *J Biol Chem* **271**, 31526–32 (1996).
38 Chatterjee M, Ph.D. Thesis. Jadavpur University, Calcutta, India (2000).
39 Herwaldt BL. In *Harrison's Principles of Internal Medicine*, 14th edition, Fauci S, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL (eds), New York, McGraw Hill Publishers, 1998, pp. 1191.
40 Bratosin D, Mazurier J, Debray H, Lecocq M, Boilly B, Alonso C, Moisei M, Motas C, *Glycoconjugate J* **12**, 258–67 (1995).
41 Kichne K, Schauer R, *Biol Chem Hoppe Seyler* **373**, 1117–23 (1992).
42 Terayama K, Muratsugu M, *Br J Haematol* **66**, 565–70 (1987).
43 Chatterjee M, Baneth G, Jaffe CL, Sharma V, Mandal C, *Vet Immunol Immunopathol* **70**, 55–65 (1999).
44 Mandal C, Chatterjee M, Sinha D, *Br J Haematol* **110**, 801–12 (2000).

Received 5 June 2000, revised 14 February 2001, accepted 12 March 2001