Glycoconjugate Journal (1996) **13:** 567–573

Constitutively hyposialylated human T-lymphocyte clones in the Tn-syndrome: binding characteristics of plant and animal lectins

KRISTINA MRKOCI[1], SØRGE KELM[2], PAUL R. CROCKER[3], ROLAND SCHAUER[2] and ERIC G. BERGER[1]*

[1]*Institute of Physiology, University of Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland*
[2]*Biochemisches Institut, University of Kiel, 24098 Kiel, Germany*
[3]*Imperial Cancer Research Fund Laboratories, Institute of Molecular Medicine, University of Oxford, Oxford OX3 9DU,UK*

Received 18 October 1995

Previously, β1,3-galactosyltransferase-deficient (Tn+) and normal (TF+) T-lymphocyte clones have been established from a patient suffering from Tn-syndrome [Thurnher *et al.* (1992) *Eur J Immunol* **22**: 1835–42]. Tn+ T lymphocytes express only Tn antigen (GalNAcα1-O-R) while other O-glycan structures such as sialosyl-Tn (Neu5Acα2,6GalNAcα1-O-R) or TF (Galβ1-3GalNAcα1-O-R) antigens are absent from these cells as shown by flow cytometry using specific mABs for TF and sialosyl-Tn antigen, respectively. Normal T lymphocytes express the TF antigen and derivatives thereof. The surface glycans of Tn+ and TF+ cells were then analysed by flow cytometry using the following sialic acid-binding lectins: *Amaranthus caudatus* (ACA), *Maackia amurensis* (MAA), *Limax flavus* (LFA), *Sambucus nigra* (SNA) and *Triticum vulgare* (WGA). Equal and weak binding of MAA and SNA to both TF+ and Tn+ cells was found. WGA, LFA and ACA bound more strongly to TF+ cells than to Tn+ cells. Binding of ACA to TF+ cells was enhanced after sialidase treatment. To investigate the possible biological consequences of hyposialylation, binding of three sialic acid-dependent adhesion molecules to Tn+ and TF+ cells was estimated using radiolabelled Fc-chimeras of sialoadhesin (Sn), myelin-associated glycoprotein (MAG) and CD22. Equal and strong binding of human CD22 to both TF+ and Tn+ cells was found. Whereas binding of Sn and MAG to TF+ cells was strong (100%), binding to Tn+ cells amounted only to 33% (Sn) and 19% (MAG). These results indicate that the *in vivo* interactions of T lymphocytes in the Tn syndrome with CD22 are not likely to be affected, whereas adhesion mediated by Sn or MAG could be strongly reduced.

Keywords: human T-lymphocytes, Tn syndrome, plant lectin, sialoadhesin, myelin-associated glycoprotein, CD22

Introduction

Cell surface glycans are increasingly being recognized as mediators of biospecific cell adhesion and recognition (for review, cf [1]. The difficulties in establishing the structural basis of the carbohydrate ligands in protein-carbohydrate interactions is well exemplified by the intense but not yet conclusive search for the nature of the selectin ligands [2]. One way to characterize the structural basis of carbohydrate adhesion ligands on the cell surface is to investigate the consequences of specific glycosylation deficiencies on the binding of various cell adhesion molecules which have been shown to mediate cell adhesion through protein-carbohydrate interactions. The prerequisites for this approach to be of relevance are the knowledge of how the biosynthetic pathways are affected by the glycosylation defect and its long term stability.

Previously, we have described a stable glycosylation defect in blood cells of a patient (R.R.) suffering from 'permanent mixed field polyagglutinability' or Tn-syndrome [3, 4], for review see [5]. A stable subpopulation of cells of all haemopoietic lineages is affected by this syndrome [6–8], in which O-glycan-specific β1,3-galactosyltransferase (GT) is repressed [9]. Due to this defect, the Tn antigen (GalNAcα1-O-Ser/Thr) is

*To whom correspondence should be addressed.

expressed instead of the Thomsen-Friedenreich (TF)-antigen (Galβ1-3GalNAcα1-O-Ser/Thr). The biosynthetic pathway is summarized in Fig. 1. The Tn-antigen is the first carbohydrate structure formed in the biosynthesis of O-glycans. GalNAc normally is substituted by Gal at the C_3 and eventually by Neu5Ac at the C_6 position. Lack of substituting GT is expected to lead to abrogation of O-glycan-associated sialic acid unless core GalNAc is directly sialylated to form the sialosyl-Tn epitope.

Tn+ and TF+ T-lymphocyte clones were established from a patient with Tn-syndrome [11]. In order to use these cells for binding studies with sialic acid dependent adhesion molecules, we first characterized the extent of the changes in cell surface sialic acid due to GT deficiency as they may be predicted from the scheme shown in Fig. 1 [10]. Thereafter, to infer on the possible biological consequences of this glycosylation defect, binding of these cells to the three mammalian sialic acid-binding adhesion molecules of the Sialoadhesin family [2] was investigated. These adhesion molecules, sialoadhesin (Sn), CD22, and myelin-associated glycoprotein (MAG) have recently been shown to belong to a subgroup of the immunoglobulin superfamily, which recognize specific, sialylated glycans as carbohydrate ligands mediating lectin-like interactions in cell adhesion and recognition processes [12]. Their specificities have been established using *in vitro* binding assays and binding to desialylated erythrocytes resialylated by specific sialyltransferases. Briefly, Sn binds equally to NeuAcα2,3Galβ1,3(4)GlcNAc or NeuAcα2,3Galβ1,3GalNAc, MAG recognizes NeuAcα2,3Galβ1,3GalNAc and CD22 binds specifically to NeuAcα2,6Galβ1,4GlcNAc [12].

Materials and methods

1. Reagents

Sodium metaperiodate, sodium *m*-arsenite, 2-thiobarbituric acid, bovine serum albumin (BSA), and *N*-acetylneuraminic acid from bovine milk (Neu5Ac) were purchased from Fluka (Buchs, Switzerland), dimethyl sulfoxide, formaldehyde from Merck (Darmstadt, Germany), butylated hydroxytoluene from Sigma (St Louis, USA), and fluorescein isothiocyanate (FITC) from Fluka. Phytohaemagglutinin (PHA) was obtained from Wellcome (Beckenham, UK), and *Vibrio cholerae* sialidase (VCS) from Boehringer (Mannheim, Germany).

2. Antibodies

IE3 (IgG2a anti-Tn) [11], HH8 (IgM anti-TF) [13] and TKH2(IgG1 anti-sialosyl Tn) [14] mAb have been described previously. FITC-conjugated goat $F(ab)_2$ anti-mouse Ig was from Dakopatts (Glostrup, DK), FITC-

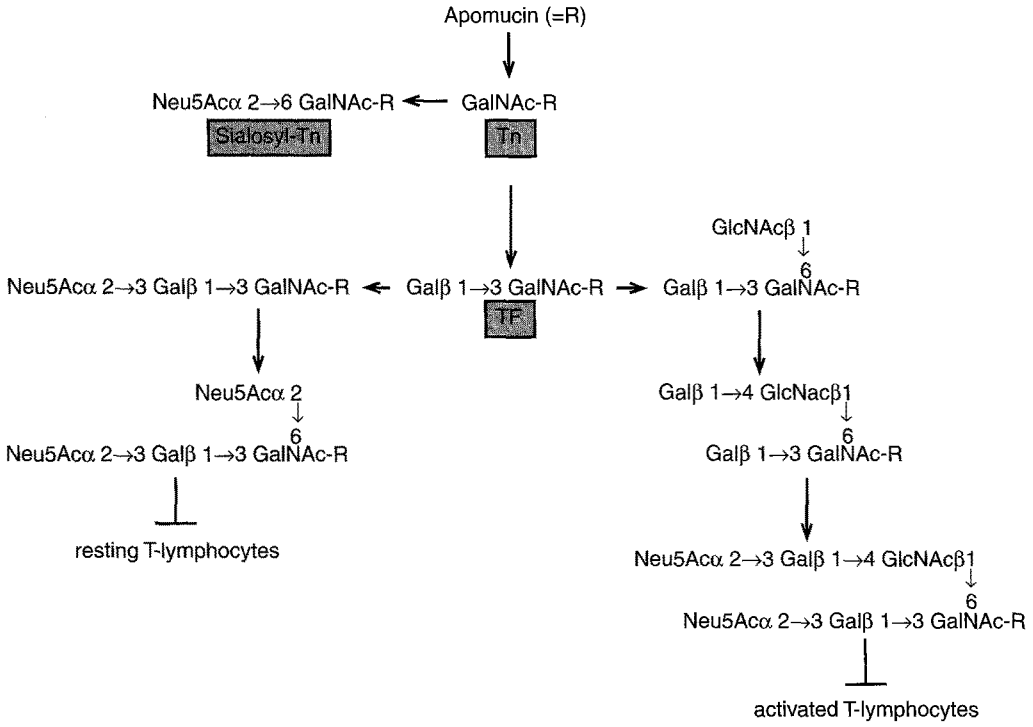

Figure 1. Biosynthetic pathways and structures of TF-, Tn- and sialosyl-Tn antigens. The main membrane component on leukocytes carrying the structures shown is leukosialin (CD43). Activation of lymphocytes leads to branching as shown on the right side (for review see [10]).

conjugated goat anti-mouse IgM from Southern Biotechnology (Birmingham, AL, USA).

3. Lectins

The FITC conjugates of the lectins, from *Amaranthus caudatus* (ACA), *Maackia amurensis* (MAA), *Triticum vulgare* (WGA), and *Sambucus nigra* (SNA) were purchased from EY Laboratories (San Mateo, CA, USA). *Limax flavus* agglutinin (LFA) was from Calbiochem (San Diego, USA).

4. Binding assays with Fc-proteins

The construction and production of Fc-chimeras of Sn, MAG and murine CD22 containing the N-terminal 3 or 4 (Sn) domains fused to the Fc and hinge region of human IgG have been described previously [12]. The plasmid for the Fc-protein containing the N-terminal 3 domains of human CD22 was a kind gift of Dr Stamenkovic [15]. Fc-proteins were labelled with ^{125}I. 5×10^4 glutardialdehyde-fixed cells were incubated with 100 fmol ^{125}I-labelled Fc-proteins complexed with anti-human IgG antibodies at 4 °C overnight and bound radioactivity was estimated as described previously [12].

5. Culture media

Iscove's modified Dulbecco's medium (IMDM) was supplemented with 1% (vol/vol) of a 100 × mixture of nonessential amino acids, 50 μg ml^{-1} gentamycin, 60 μg ml^{-1} anti-PPLO agent, final concentrations resp., 1 mM sodium pyruvate, and 10% (vol/vol) heat-inactivated (56 °C, 60 min) fetal calf serum (FCS) (IMDM-FCS). (All of these products were obtained from Gibco Grand Island, NY, USA.) For the cultivation of T-cell clones, IMDM-FCS was supplemented with 1000 U ml^{-1} rec. IL-2 (IMDM-FCS-IL2), kindly provided by Dr A. Lanzavecchia, Basel Institute for Immunology.

6. T-cell lines and culture

The generation and characterization of the Tn+/CD4+ and TF+/CD4+ T-cell clones used in this study have been described [11]. For restimulation, $2–4 \times 10^5$ T-cells and 10^6 antigen-presenting cells (irradiated allogeneic peripheral blood mononuclear cells) were incubated in 2 ml of IMDM-FCS-IL2 and 1 μg ml^{-1} PHA. T-cells were expanded and maintained in culture by periodic restimulation (2–3 weeks). The Jurkat cell line was kindly provided by Dr de la Hera, Basel Institute of Immunology. A clone was established by limiting dilution and was cultured in IMDM-FCS at a density of $0.2–0.6 \times 10^6$ cells per ml. The K562 cell line (human erythroleukemic cells) was obtained from ATCC and cultured as described in [16].

7. FITC-conjugation of Limax flavus agglutinin

FITC-conjugation was carried out by adding 20 μg FITC to 1 mg LFA in 200 μl 0.5 M bicarbonate buffer (pH 9.5) in the presence of 50 mM Neu5Ac in order to protect the sugar-binding sites. After overnight incubation at 4 °C in the dark, the conjugated lectin was separated from free fluorochrome by extensive dialysis against PBS.

8. Sialidase treatment

For desialylation, 10^6 cells in 100 μl PBS, pH 7.4, containing 0.5% (wt/vol) BSA were treated with 2 mU VCS for 60 min at 37 °C. Cells were washed twice with PBS containing 1% (vol/vol) FCS.

9. Flow cytometry

T-cells were stained at 10^6 cells per 100 μl with a subagglutinating concentration of the FITC-conjugated lectins in PBS:2% (wt/vol) BSA for 30 min at 4 °C. The cells were routinely checked for the expression of the Tn+ or TF+ phenotype by using mAb 1E3 or HH8, respectively. Expression of sialosyl-Tn was assessed with mAb TKH2. Before staining with the HH8 antibody, the cells were treated with VCS [11]. Controls were treated the same way except that the primary antibody was omitted. After each incubation, the cells were washed twice with PBS:1% (vol/vol) FCS. Cells were fixed in PBS:2% (vol/vol) formaldehyde:0.1% (wt/vol) BSA and stored at 4 °C in the dark until analysis. Possible interference by antigen-presenting cells was excluded by gating the lymphocyte population. Cells were analysed for immunofluorescence on an EPICS Profile flow cytometer, collecting data for 10^4 cells for each histogram.

10. Colorimetric determination of cell surface sialic acid

For the quantification of sialic acids of cells a new modification of the periodic acid-thiobarbituric acid assay described in [17] and [18] was developed. Cells were washed in cold PBS and aliquots of 10^6 cells were collected by centrifugation. Mild acid hydrolysis was performed to release glycosidically bound sialic acid from the cell. For hydrolysis, the cell pellet was resuspended in 100 ml H_2O, 200 ml 0.75 M H_2SO_4 and 3 μl of 1% (wt/vol) butylated hydroxytoluene and incubated for 15 min at 95 °C. 200 μl of 1.1 M NaOH was added, the sample was kept at room temperature for 1 h and the cells were pelleted. To a 400 μl aliquot of the supernatant, 50 μl 0.1 M sodium metaperiodate in 125 mM H_2SO_4 was added and incubated for 20 min at 30 °C. The reaction was terminated by adding 100 μl 6% (wt/vol) sodium *m*-arsenite in 0.5 M HCl, followed by the addition of 250 μl 2% (wt/vol) thiobarbituric acid, adjusted to pH 8–9 with NaOH. After incubation for 30 min at 95 °C, 500 μl of dimethyl sulfoxide was added and absorbance at 552 nm was measured. The sialic acid content was determined by absorption at 552 nm after calibration with Neu5Ac acid under test conditions in the range of 0.1–3.0 μg per 100 μl (corresponding to 0.32–9.6 nM).

Results and discussion

Surface sialic acid content of Tn+ cells and absence of the sialosyl-Tn epitope

We have previously shown by lectin flow cytometry that Tn+ T-lymphocytes are hyposialylated [11]. To quantitatively determine and confirm the extent of hyposialylation on Tn+ T-lymphocytes as compared with their normally sialylated counterpart cells, we established a modification of the periodic acid-thiobarbituric acid assay with chemical cleavage of sialic acid [18] for lymphoid cells using Jurkat cells as a model cell line [19]. The modification was optimized for lymphocyte samples of 10^6 cells and for the range of sialic acid determination between 0.1–3.0 mg. Dimethyl sulphoxide was used as the chromophore stabilizer and colour intensifier [17]. Lipid peroxidation and formation of malondialdehyde, which can interfere during photometric determination of the sialic acid chromophore, was avoided by addition of butylated hydroxytoluene during acid release. The determinations of sialic acid on the different cell types showed a 40% reduction of the sialic acid content of Tn+ T-lymphocytes compared to TF+ T-lymphocytes (Table 1). Jurkat cells showed a 23% higher sialic acid content with respect to TF+ T-lymphocytes.

In previous reports, co-expression of the Tn antigen with the sialosyl-Tn antigen has been described in red cells of Tn patients [20]. Since sialidase treatment of Tn+ lymphocytes did not increase binding of anti-Tn mAB [11] we assumed that sialosyl-Tn was not expressed on Tn cells. To ascertain this we investigated the expression of sialosyl-Tn using the anti-sialosyl-Tn mAb TKH2 for flow cytometry of Tn+ T-lymphocytes and the cell line K562, which is known to express the sialosyl-Tn structure [16]. As shown in Fig. 2, K562 cells clearly bind the mAb TKH2 while Tn+ T-lymphocytes show no reactivity to TKH2. We can thus conclude that Tn+ T-lymphocytes do not form sialosyl-Tn as defined by the specificity of this antibody.

Table 1. Sialic acid content of Tn+, TF+ T-lymphocyte clones and Jurkat cells. The mean value and standard deviation was calculated from three independent sialic acid determinations.

Cells	*Sialic acid (mg per 10^6 cells)*
Tn+	0.81 ± 0.05
TF+	1.35 ± 0.23
Jurkat	1.78 ± 0.20

Binding of sialic acid-dependent plant and invertebrate lectins to Tn+ cells

To establish the nature of hyposialylation on Tn+ T-lymphocytes, flow cytometric measurements with various fluoresceinated plant and invertebrate lectins were performed. Table 2 lists their known specificities, the subagglutinating concentrations used and the mean fluorescence intensities of the flow cytometric measurements including controls with sialidase treatment prior to staining.

SNA has a high specificity for Neu5Acα2-6Gal/GalNAc [21] but does not bind to Galβ1-3(Neu5Acα2-6)GalNAc due to a steric effect of the galactose linked to GalNAc. Therefore, SNA is not able to react with the 2,6-sialylated TF-antigen [21, 22]. As shown on Table 2, binding of SNA to both Tn+ and TF+ T-lymphocytes, was equally weak, and decreased further after sialidase treatment. This observation is compatible with absence of sialosyl-Tn epitopes on Tn+ cells and that SNA binds mainly to Neu5Acα2-6Gal on N-glycans of Tn+ cells.

MAA has a binding specificity for the trisaccharide Neu5Acα2-3Galβ1-4GlcNAc/Glc [22]. The binding intensity of MAA was equal and very low in Tn+- and TF+ T-lymphocytes as well as in the sialidase-treated controls.

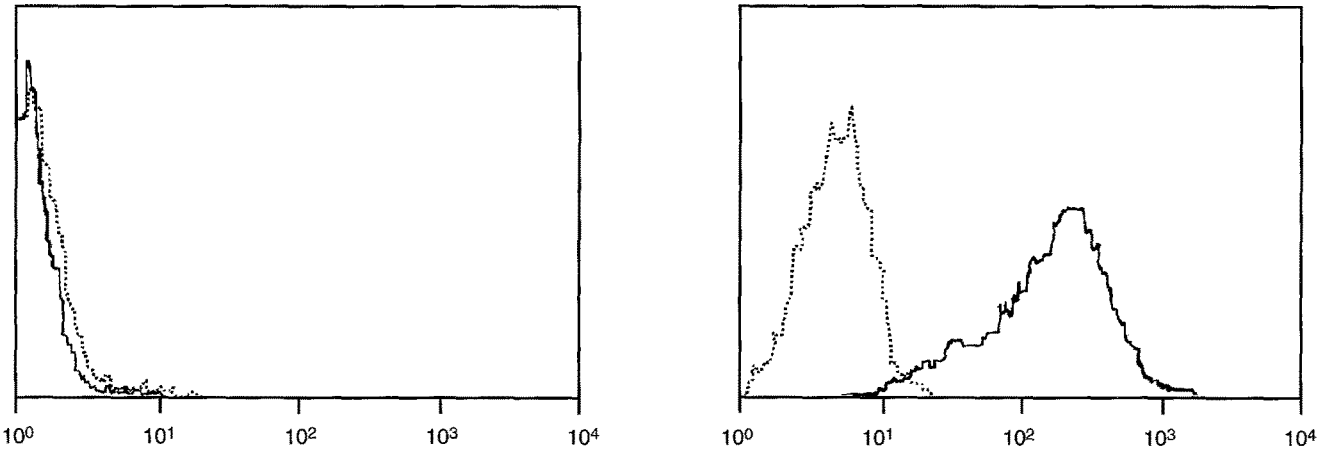

Figure 2. Binding of mAB THK2 to Tn+ cells (left panel) and K562 cells (right panel). K562 cells or Tn+ cells, respectively, were incubated with undiluted mAB THK2 [14] followed by second antibody (solid lines) or with second antibody only (dotted lines).

Table 2. Mean fluorescence intensities (MFI) of flow cytometric analysis of Tn+ and TF+ T-lymphocyte clones with FITC-labelled plant lectins. The MFI indicates the amount of bound FITC-labelled lectin per cell. Controls of cells pretreated with Vibrio cholerae sialidase (VCS) are included. All MFI figures relate to 100% of cells analysed (single populations).

				Mean fluorescence intensity			
				Tn		*TF*	
Lectin	*Binding specificity*	*Reference*	*Concentration* ($\mu g\,ml^{-1}$)	*+VCS*	*−VCS*	*+VCS*	*−VCS*
Sambucus nigra (SNA)	Neu5Acα2,6Gal/GalNac	21	10	0.227	0.677	0.258	0.707
Maackia amurensis (MAA)	Neu5Acα2,3Galβ1,4GlcNAc/Glc	22,23	5	0.446	0.407	0.478	0.456
Amaranthus caudatus (ACA)	Galβ1,3GalNAc Neu5Ac-Galβ1,3GalNAc	25	2	0.162	0.149	3.445	0.775
Triticum vulgare (WGA)	GlcNAc-, Neu5Ac	26	2	8.191	7.215	4.280	11.400
Limax flavus	Neu5Ac-	22,27	2	0.394	2.509	0.430	4.157

Lack of reactivity to MAA before and after sialidase treatment indicates that there is no or little α2,3-linked sialic acid on N-glycans on Tn+ or TF+ T-lymphocytes. The biosynthetic pathway of the Tn-antigen shown on Fig. 1 does not lead to structures that could act as ligands for MAA. Binding sites for MAA can only be formed on O-glycans along the pathway shown for activated T lymphocytes requiring at least the TF-antigen as precursor [24]. Our results may indicate that at the time of analysis our TF+ cells exhibit the glycosylation pattern of resting T lymphocytes.

ACA has an extended carbohydrate binding site which is specific for Galβ1-3GalNAcα1-O-R (the TF-antigen) and for its sialylated derivatives [25]. According to Table 2, little binding to Tn+ T-lymphocytes and the VCS treated controls was observed, whereas weak binding to TF+ T-lymphocytes was observed, which was strongly enhanced after sialidase treatment. These findings are in full agreement with the specificity of ACA as defined by neoglycoproteins [25] showing that sialylation of TF-antigen reduced the binding of ACA.

WGA binds to sialic acid and to *N*-acetylglucosamine residues [26]. Binding of this lectin to Tn+ T-lymphocytes increased slightly after sialidase treatment, indicating predominant binding to GlcNAc rather than to Neu5Ac, which is compatible with a lower sialic acid density on Tn+ cells. In contrast, the mean fluorescence intensity of WGA binding to native TF+ cells was higher and reduced by over 60% after sialidase treatment, indicating that in this case WGA binding is mainly sialic acid-dependent. This supports the notion of a higher sialic acid density on TF+ over Tn+ T-lymphocytes.

The fluorescence of Tn+ T-lymphocytes labelled with LFA was 40% lower than of TF+ T-lymphocytes (Table 2). Sialidase treatment reduced LFA binding to both cell types to equally low levels. LFA binds to sialic acid in all of its modifications and linkages [27]. Thus, this result is compatible with the periodic-acid-thiobarbituric acid measurement of cell surface sialic acid of these T-lymphocyte clones. The extensive reduction of LFA binding indicates that the bulk of sialic acid occurs on O-glycans. These data are compatible with the data published previously [11] and further confirm the stability of the Tn+ phenotype.

Binding of Sialoadhesins

Having established the extent and nature of hyposialylation on Tn+ T-lymphocyte clones, we then investigated how the altered glycosylation of lymphocytes from the Tn-syndrome affects the binding of adhesion molecules recognizing specific sialylated glycans. Therefore, quantitative binding assays with three members of the sialoadhesin family [12], CD22, sialoadhesin (Sn) and myelin associated glycoprotein (MAG), were performed. As shown in Fig. 3, binding of human or murine CD22 was only slightly decreased in Tn+ cells compared to TF+ lymphocytes as already observed for binding of SNA whose specificity is similar. The reason for this slight reduction of CD22 binding to Tn+ T-lymphocytes is unclear since the characterized hyposialylation of O-glycans on Tn+ T-lymphocytes does not concern N-glycans. Binding of MAG and Sn to Tn+ T-lymphocytes was strongly reduced, in accordance with the hyposialylation state of these cells. The highest level of binding to both cell types was observed with human CD22 (12–15 fmol bound), whereas binding of the murine homologue was much lower (3 fmol). This is in agreement with the low affinity of the latter protein toward Neu5Ac compared to Neu5Gc [28], a sialic acid not found on human cells under normal conditions [29]. In control experiments with murine lymphocytes which contain

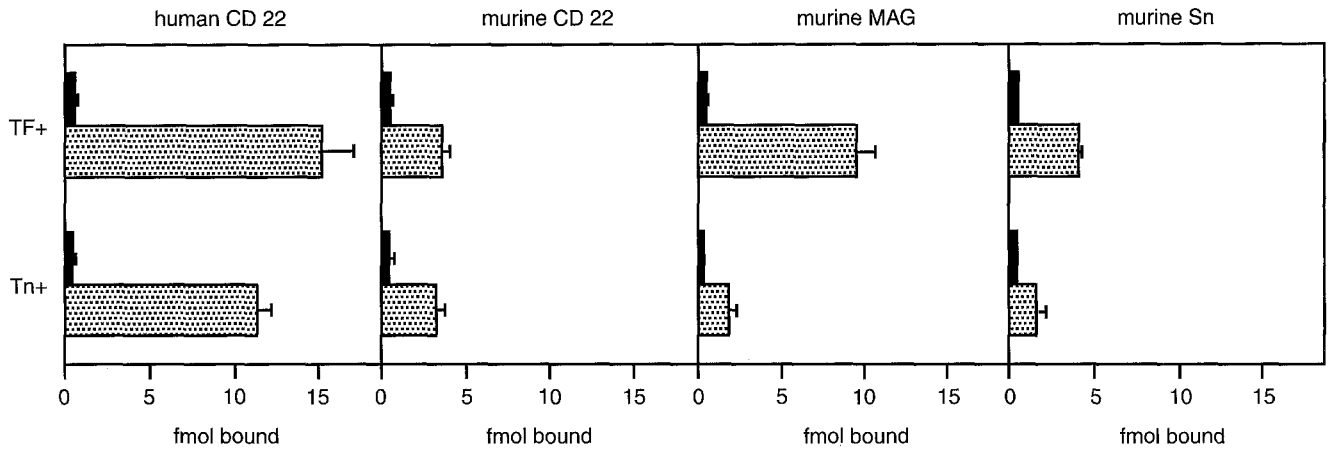

Figure 3. Binding of sialoadhesins to Tn+ and TF+ cells. Binding of ^{125}I-Fc-chimeras of the adhesion molecules to 5×10^4 cells was estimated as described under Material and methods. Black bars represent binding to VCS treated cells. Data shown are mean values of triplicates from one out of five experiments. For technical details see Materials and methods.

Neu5Gc and Neu5Ac, binding of both the human and the murine CD22 were similar (data not shown), demonstrating that these protein preparations were able to bind to cells to a similar extent. The overall lower binding of MAG (10 fmol) and Sn (4 fmol) to the TF+ cells is in agreement with the low binding of these adhesion molecules to lymphocytes [12] and reflects a low content of α2,3-linked Neu5Ac.

In vivo, interactions of lymphocytes with CD22 [15] or Sn [30] could be of physiological relevance. In contrast, MAG is exclusively found on myelinating cells and therefore MAG would only be expected to interact with T cells under pathological conditions like demyelinating diseases such as multiple sclerosis. Since cytotoxic T-lymphocytes appear to be involved in demyelinating disease, Tn+ T-lymphocytes may serve as useful controls for studies aimed at delineating MAG binding to T cells by distinguishing non-specific, carbohydrate-mediated interaction of T-lymphocytes with myelin from immune recognition of myelin epitopes [31, 32]. Our data confirm that human CD22 binds strongly to T cells of both the Tn+ and the TF+ phenotype suggesting that cellular interactions involving CD22 are not altered in the Tn+ syndrome. In contrast, interactions of T cells expressing Sn or MAG would be greatly reduced. Since Sn-expressing macrophages may interact with some T cell populations [30], reduced binding of Sn to Tn+ cells could be of biological relevance. The puzzle, why only a few percent of peripheral blood mononuclear cells [6, 11] exhibited the Tn+ phenotype in all patients suffering from Tn+ syndrome [33] cannot be solved on the basis of these results. One possible explanation would be a shorter half-life of Tn+ cells *in vivo* or an efficient removal of these cells during maturation. It remains an interesting question whether the altered O-glycans of Tn+ cells play a critical role for their turnover. Further work will be needed to elucidate the causes for the low percentage of peripheral Tn+ lymphocytes as opposed to Tn+ erythrocytes.

Acknowledgements

We thank Professor H. Eppenberger for his continuous interest and Professor H. Clausen for providing the monoclonal antibodies used in this work.

This work was supported in part by grant No. 3100-40749.94 of the Swiss National Foundation to E.G.B. and by Mizutani Foundation to SK and PRC.

References

1. Varki A (1993) *Glycobiology* **3**: 97–130.
2. Varki A (1992) *Curr Opin Cell Biol* **4**: 257–66.
3. Berger EG, Kozdrowski I (1978) *FEBS Lett* **93**: 105–8.
4. Cartron JP, Andreu G, Cartron J, Bird GW, Salmon C, Gerbal A (1978) *Eur J Biochem* **92**: 11–19.
5. Berger EG, Dinter A, Thurnher M (1994) *Trends Glycosci Glycotechnol* **6**: 51–63.
6. Brouet JC, Vainchenker W, Blanchard D, Testa U, Cartron JP (1983) *Eur J Immunol* **13**: 350–52.
7. Vainchenker W, Testa U, Deschamps JP, Henri A, Titeux M, Breton-Gorius J, Rochant H, Lee D, Cartron JP (1982) *J Clin Invest* **69**: 1081–91.
8. Cartron JP, Nurden AT (1979) *Nature* **282**: 621–23.
9. Thurnher M, Rusconi S, Berger EG (1993) *J Clin Invest* **91**: 2103–10.
10. Fukuda M (1994) In *Molecular Glycobiology* (Fukuda M, Hindsgaul O, eds) pp. 1–52. Oxford: IRL Press at Oxford University Press.
11. Thurnher M, Clausen H, Fierz W, Lanzavecchia A, Berger EG (1992) *Eur J Immunol* **22**: 1835–42.

12. Kelm S, Pelz A, Schauer R, Filbin MT, Tang S, de Bellard ME, Schnaar RL, Mahoney JA, Hartnell A, Bradfield P, Crocker PR (1994) *Curr Biol* **4**: 965–72.
13. Clausen H, Stroud M, Parker J, Springer GF, Hakomori S (1988) *Mol Immunol* **25**: 199–204.
14. Kjeldsen T, Clausen H, Hirohashi S, Ogawa T, Iijima H, Hakomori S (1988) *Cancer Res* **48**: 2214–20.
15. Stamenkovic I, Sgroi D, Aruffo A, Sy MS, Anderson T (1991) *Cell* **66**: 1133–44.
16. Muroi K, Suda T, Nakamura M, Okada S, Nojiri H, Amemiya Y, Miura Y, Hakomori S (1994) *Blood* **183**: 84-91.
17. Skoza L, Mohos S (1976) *Biochem J* **159**: 457–62.
18. Holzhauser R, Faillard H (1988) *Carbohydrate Research* **183**: 89–95.
19. Piller V, Piller F, Fukuda M (1990) *J Biol Chem* **265**: 9264–71.
20. Kjeldsen T, Hakomori S, Springer GF, Desai P, Harris T, Clausen H (1989) *Vox Sang* **57**: 81–87.
21. Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Pe B, Peumans WJ (1987) *J Biol Chem* **262**: 1596–601.
22. Knibbs RN, Goldstein IJ, Ratcliffe RM, Shibuya N (1991) *J Biol Chem* **266**: 83–88.
23. Kaku H, Mori Y, Goldstein IJ, Shibuya N (1993) *J Biol Chem* **268**: 13237–41.
24. Piller F, Piller V, Fox RI, Fukuda M (1988) *J Biol Chem* **263**: 15146–50.
25. Rinderle SJ, Goldstein IJ, Matta KL, Ratcliffe MR (1989) *J Biol Chem* **264**: 16123–31.
26. Nagata Y, Burger MM (1974) *J Biol Chem* **249**: 3116–22.
27. Miller R (1987) *Methods Enzymol* **138**: 527–36.
28. Kelm S, Schauer R, Manguerra JC, Gross HJ, Crocker PR (1994) *Glycoconjugate J* **11**: 576–85.
29. Schauer R (1985) *Trends Biochem Sci* **10**: 357–60.
30. Van den Berg TK, Breve JJP, Damoiseaux JGMC, Dopp EA, Kelm S, Crocker PR, Dijkstra CD, Kral G (1992) *J Exp Med* **176**: 647–55.
31. Zhang Y, Burger D, Saruhan G, Jeannet M, Steck AJ (1993) *Neurology* **43**: 403–7.
32. Tsuchida T, Parker KC, Turner RV, McFarland HF, Coligan JE, Biddison WE (1994) *Proc Natl Acad Sci* **91**: 10859–63.
33. Berger EG, Thurnher M, Dinter A (1994) *Transfus Clin Biol* **2**: 103–8.