Glycoconj J (2013) 30:813–818
DOI 10.1007/s10719-013-9485-7

Stimulation of human peripheral blood mononuclear cells by the sialic acid precursor *N*-propanoylmannosamine

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Received: 26 March 2013 / Revised: 23 May 2013 / Accepted: 17 June 2013 / Published online: 30 June 2013

Abstract *N*-Propanoylmannosamine is an unnatural precursor of sialic acid, which is taken up by a variety of animal cells and metabolized to *N*-propanoylneuraminic acid. In several studies it has been demonstrated that application of unnatural precursors of sialic acids such as *N*-propanoylmannosamine (ManNProp) and homologues interfere with cell differentiation and proliferation of neuronal cells or embryonic stem cells. Since the function of the immune system is known to rely on the presence of sialic acid, we applied ManNProp to human peripheral blood mononuclear cells (PBMC). When culturing those lymphocytes with ManNProp 10 % of the natural sialic acid *N*-acetylneuraminic acid could be replaced by the newly formed *N*-propanoylneuraminic acid. This procedure resulted (a) in a marked stimulation in the rate of proliferation of PBMC, (b) a 10-fold increase of IL-2 production coupled with an up-regulation of its receptor CD25 on the cell surface and (c) a concomitant expression and regulation of the transferrin receptor with cell growth. The stimulation of PBMC by ManNProp might therefore introduce a new approach of immunomodulation.

Keywords *N*-Acetylneuraminic acid · *N*-propanoyl-D-mannosamine · Immunomodulation · CD25 · CD71 · Biochemical engineering · Interleukin-2

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Introduction

Sialic acid is found as the most abundant terminal monosaccharide on the surface of eukaryotic cells. It is of major importance for a multitude of biological mechanisms including cell-cell recognition, cell adhesion and biological stability of glycoconjugates [1, 2]. Sialic acid is also implicated in cell differentiation and maturation *via* distinct signal transduction pathways [3–6]. In the immune system, sialic acid is essential for the function of Siglecs [7] or selectins [8].

The physiological sialic acid in humans is *N*-acetylneuraminic acid, which is synthesized from *N*-acetyl-D-mannosamine (ManNAc). In previous studies we showed that eukaryotic cells metabolize synthetic *N*-propanoyl-D-mannosamine (ManNProp) to *N*-propanoylneuraminic acid, which is incorporated into the cell surface by replacing the physiological *N*-acetylneuraminic acid (for review see Keppler *et al.* [9]) (Fig. 1). This metabolic glycoengineering was further exploited by the groups of Bertozzi [10] and Yarema [11]. Moreover, it could be shown that the application of the unnatural ManNProp influences several biological functions, which are not directly associated with known ligand-receptor relationships, but include transcription of novel genes [5, 6]. First evidence that application of ManNProp stimulates growth or proliferation of cells was concluded from experiments with human diploid lung fibroblasts where ManNProp-treatment resulted in a loss of density-dependent growth control [12]. Furthermore, treatment of neural cell cultures of newborn rats stimulated the proliferation of astrocytes and microglia, and increased the number of oligodendrocyte progenitor cells [3]. In contrast, neurons [13], neuron-like PC12-cells [14] or mouse embryonic stem cells [5] react on application of ManNProp by axonal growth (neurons) or differentiation (stem cells). Moreover, the expression of sialyl Lewis^X is increased after ManNProp treatment of HL60 cells [15].

Over the last years this method using novel sialic acid precursors has been successfully extended by the group of

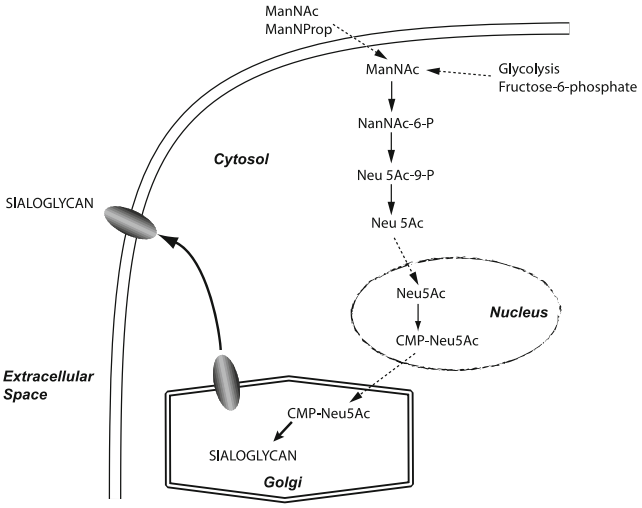

Fig. 1 Biochemical engineering of cell-surface-associated sialic acid

Bertozzi introducing reactive functional groups in the *N*-acyl side chain of *N*-acyl-D-mannosamine leading to new sialic acids in the cell surface with the potency to be linked with photoactive compounds [16] and by the group of Kevin Yarema, which succeeded to introduce butyrate residues to increase the uptake of *N*-acyl-D-mannosamines [17].

Since sialic acid is a major constituent of lymphocyte cell surface components [18], it was reasonable to study specific functions after treatment of lymphocytes with ManNProp.

In this study we show that also peripheral blood mononuclear cells (PBMCs) metabolize ManNProp to *N*-propanoylneuraminic acid. We provide evidences that PBMCs are stimulated by ManNProp resulting in an increased proliferation and expression of activation markers such as CD25 and CD71. Furthermore PBMC secreted IL-2 upon activation with ManNProp.

Material and methods

Cells

Human peripheral blood mononuclear cells (PBMC) were isolated from "*Buffy coat*" as described by Böyum [19]. In brief, cells were centrifuged over a ficoll gradient and resulting PBMC were washed and grown in RPMI supplemented with 10 % fetal calf serum. Adherent monocytes were removed by cultivating cells in Petri dishes over night. Non-adherent cells were further cultured in a density of 5-10×10^6 cells/ml in culture flasks.

Analytical procedures

Protein was determined in 96-well ELISA plates using 200 µl bicinchonic acid protein reagent (Pierce) and a 50 µl sample. Plates were evaluated in a 96-well ELISA reader (Spectra) at 570 nm.

Western blot analysis

Samples were separated on SDS-polyacrylamide gels and transferred to nitrocellulose filters. The blots were blocked with 5 % fat free dry milk powder in PBS, incubated with respective antibodies (p65), washed with PBS, and incubated with the appropriate secondary antibody. Antibodies to mouse immunoglobulins conjugated to HRP were obtained from Dianova. All antibodies were used at 1 µg/ml. After washing proteins were detected by enhanced chemiluminescence (Pierce) according to the manufacturer's instructions and visualized by exposing the blots to a BioRad Imager system for time periods between 10 and 120 s. Band intensity was calculated from micrographs using NIH ImageJ software.

Neuraminic acid precursors

The physiological precursor of neuraminic acid, *N*-acetylmannosamine (ManNAc), was purchased from Sigma. Radiolabelled (^{14}C) GlcNAc and ManNAc (270 mCi/mmol) were purchased from Amersham, Braunschweig. Synthetic derivatives of neuraminic acid precursors with prolonged *N*-acyl-side chains are not commercially available and were synthesized as described [20, 21].

Proliferation assays

PBMC were plated for overnight in a tissue culture bottle to remove macrophages. Cells were then incubated for 72 h in the presence of ManNProp. 10^5 cells were cultured in a 96-well, round-bottom microplate in the presence or absence of amino sugars for 3 days at 37 °C in 5 % CO_2. The cells were pulsed with 0,037 MBq/well of [^{3}H] thymidine during the last 16 h of culture. Incorporation of radioactivity was quantified by liquid scintillation counting.

Quantification of incorporated amino sugars

2×10^6 cells/ml were cultured in 24-well plate in the presence of radioactive-labeled GlcNAc, GlcNProp, ManNAc or ManNProp (10 µCi/well). After 72 h the plasma membrane-bound radioactivity was quantified according to Mans and Novelli [22].

Quantification of incorporated *N*-propanoylneuraminc acids

Cells were maintained for 72 h in the presence of 10 mM ManNProp. Cell pellets (10^7 cells) were lysed by hypotonic shock in distilled water and repeated freezing and thawing (two times). The crude membrane fractions were pelleted by

centrifugation at 4,500× *g* for 20 min (4 °C). The pellets were lyophilized. Glycolipids were extracted using methanol/chloroform (1:2, 1:1, 2:1 (*v/v*)) for 30 min each and centrifugation at 10,000× *g* (30 min, 4 °C). Dried samples were hydrolyzed for 2 h with 200 ml of 2 M acetic acid. The pH values of the hydrolysates were adjusted to 4, and further purification was carried out on a cation exchanger (AG-50 W-X12, H1 form; Bio-Rad, München, Germany). Purified eluted neuraminic acids were fluorescence-labeled according to Hara *et al.* [23]. Labeled neuraminic acids were chromatographed using a reversed phase C18 column (Lichrosorb C18, 5 mm, 250 4 mm; Knauer, Berlin, Germany) with a fluorescence detector (Ginkotek; excitation wavelength, 377 nm; emission wavelength, 448 nm). Eluent A contained distilled water, while eluent B contained acetonitrile/methanol (60:40, *v/v*). The flow rate was 1 ml/min, and separations were carried out using a gradient that first run for 20 min in the isocratic mode with 10 % B; then B was raised to 25 % within 25 min and finally to 50 % within another 15 min. Eluted neuraminic acids were identified by matrix-assisted laser desorption time of flight mass spectrometry and quantified as in Keppler *et al.* [20].

FACS-analysis

10^5 cells were incubated (30 min, 4 °C) with 20 µl of a monoclonal FITC-conjugated anti-CD71 antibody (Immunotech S. A.) or a monoclonal phycoerythrin (PE)-conjugated anti-CD25 antibody (BD Bioscience, Heidelberg, Germany). Cells were washed three times, re-suspended in 500 µl phosphate-buffered saline with 0,1 % NaN_3 and 0,1 % bovine serum albumin and analyzed by flow cytometry. Controls were performed using FITC-conjugated immunoglobulins from identical isotype.

IL-2 quantification

PBMC were cultured in the presence of variable ManNProp concentrations. After 72 h, culture supernatants were collected and IL-2 was quantified by enzyme-linked immunosorbent assay according to the manufacturers instructions (R&D Systems, Wiesbaden, Germany).

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used throughout. When significance was achieved, it was followed by post hoc Bonferroni test. Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad, San Diego, CA, USA) and significance was accepted at $*p<0.05$. We accomplished a minimum of three independent experiments.

Results

ManNProp is metabolized by lymphocytes to *N*-propanoylneuraminic acid

First we studied the uptake and metabolization of ManNProp or related amino sugars such as ManNAc, GlcNAc or GlcNProp in human peripheral blood mononuclear cells (PBMC). Cells were incubated with radioactive-labeled ManNProp and for control with radioactive-labeled ManNAc, GlcNAc or GlcNProp, respectively. Incorporated radioactivity into plasma membranes was quantified after 72 h. Highest incorporation of *N*-propanoylneuraminic acid on the cell membranes was achieved using ManNProp (Fig. 2a). We further compared the metabolization of ManNProp with GlcNProp at different time points (Fig. 2b). Highest incorporation of radioactivity into cell membranes was achieved using ManNProp after 72 h of incubation; longer incubation of PBMC with ManNProp did not result in a further increase of incorporation of radioactivity. We also quantified the incorporated *N*-propanoylneuraminic acid by HPLC analysis and found that after 72 h of incubation with ManNProp 10 % of total membrane bound sialic acids was *N*-propanoylneuraminic acid.

We then analyzed the viability of PBMC in the presence of ManNProp. When cells were cultivated in the presence of variable concentration of ManNProp (0,1–20 mM) and analyzed microscopically no difference in cell viability was observed, as assessed by trypan blue dye exclusion (data not shown).

Biochemical neuraminic acid engineering of lymphocytes stimulates proliferation

Treatment of PBMC with ManNProp stimulated proliferation in a dose-dependent manner (Fig. 3). Cells were incubated for 72 h in the presence or absence of 1,5 or 10 mM ManNProp or for control with the physiological sialic acid precursor ManNAc. Thymidine incorporation as marker for the proliferation rate was quantified. In the presence of ManNProp proliferation was concentration-dependent stimulated, whereas ManNAc had only a minor, but significant, effect on proliferation (Fig. 3).

Treatment of lymphocytes with ManNProp enhances the expression of proliferation markers

When PBMC were cultured in the presence of ManNProp the expression of proliferation markers such as the interleukin-2 receptor (CD25) or the transferrin receptor (CD71) was increased.

Cells were cultured in the presence of 10 mM ManNProp. Expression of CD25 was quantified after 24 h, 48 h, and 72 h. Cytofluorimetric studies revealed a time-dependent

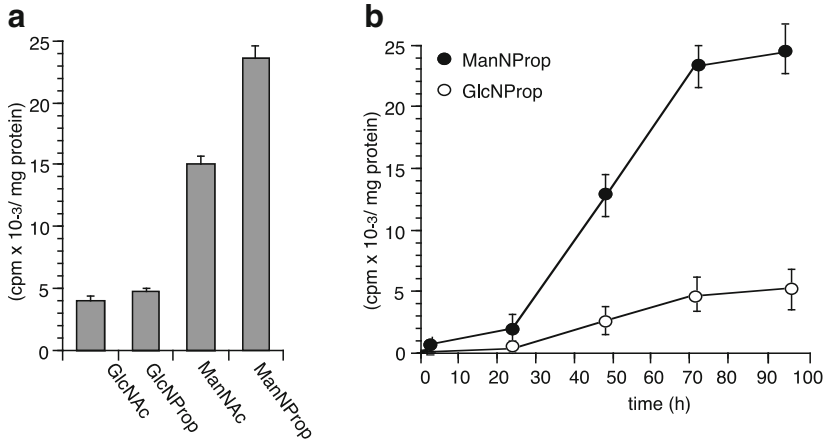

Fig. 2 Incorporation of *N*-propanoylneuraminic acid. **a** Cells were cultured in the presence of radioactive-labeled GlcNAc, GlcNProp, ManNAc or ManNProp. After 72 h the plasma membrane-bound radioactivity was quantified. Bars represent mean values from 3 independent experiments. **b** Time-dependent comparison of the incorporation between GlcNProp and ManNProp. Same conditions were used as in 2A

increase in CD25 expression (Fig. 4). After 72 h of incubation with ManNProp CD25 was detected on 70 % of T-cells. In contrast, lymphocytes grown in the presence of ManNAc did express CD25 only on 14 % of all cells (Fig. 4).

In addition we analyzed the expression of CD71, which is not present on resting blood lymphocytes, after 72 h of incubation with ManNProp. About one third of all cells express CD71 after 72 h, whereas only 4 % do express CD71 when cultured in the presence of ManNAc (Fig. 5). After ManNProp treatment the expression of CD71 on the cell surface is increased and correlates with an enlarged cell size. After 72 h approximately 30 % of the cells cultured in the presence of ManNProp were transformed into lymphoblasts. The majority of them (80 %) do express the T-cell marker CD3.

Since ManNProp stimulated the expression of the CD25, we further asked, whether the secretion of IL-2 itself is up-regulated by ManNProp. Again, PBMC were cultured for 72 h in the presence of various concentrations of ManNProp stimulating the secretion of IL-2 (Fig. 6) considerably in a dose-dependent manner. However, also marginal increase of IL-2 secretion was obtained in the presence of ManNAc (Fig. 6).

Increased expression of CD71 and CD25 was paralleled by an increased expression of the transcription factor NFkB in the nucleus

ManNProp stimulated the expression of the Il-2-receptor (CD25) and it is well established that IL-2 stimulates the activation of NFkB. Therefore, we quantified the presence of NFkB (p65-subunit) in nuclear extracts of ManNProp- or ManNAc-treated PBMCs, respectively. Figure 7a shows one out of three representative Western blot analyses. Under control conditions about 16 % of the NFkB subunit p65 was located in the nuclear fraction of PBMCs. However, after application of ManNAc we found 32 % and after application of ManNProp even 39 % of p65 in the nuclear fraction as compared to untreated PBMC cultures.

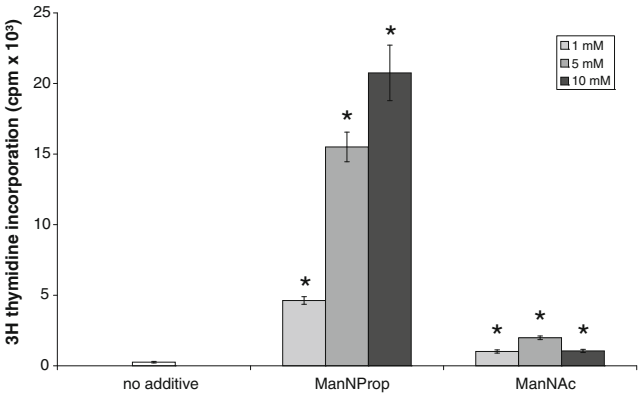

Fig. 3 [^{3}H] Thymidine incorporation in human lymphocytes. ManNProp stimulates the proliferation of PBMC in a dose-dependent manner. Cells were cultured in the absence or presence of various concentrations of ManNProp or ManNAc for 72 h respectively. Bars represent mean values from 3 independent experiments (*$p<0.05$)

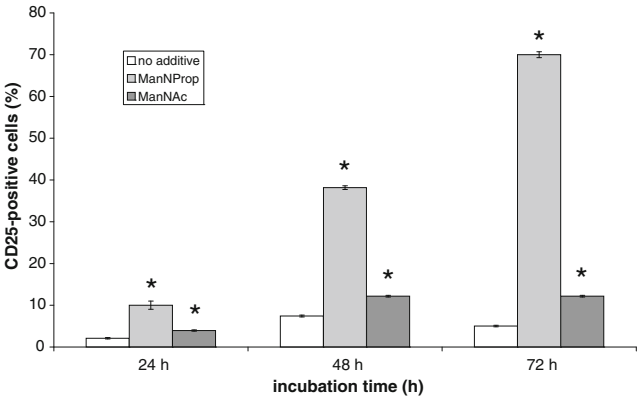

Fig. 4 Time-dependent stimulation of CD25-expression after treatment with ManNProp. PBMC were cultured in the absence or presence 10 mM ManNProp or ManNAc. Expression of CD 25 was analyzed at 24, 48 and 72 h by flow cytometry of CD3-positive cells. Bars represent mean values from two independent experiments (*$p<0.05$)

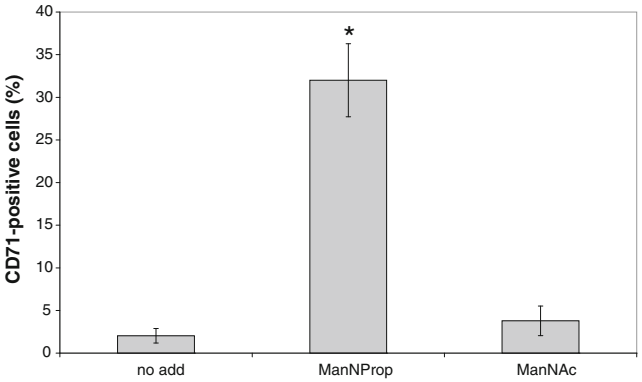

Fig. 5 ManNProp stimulates CD71 expression. PBMC were cultured in the absence (no add) or presence of 10 mM ManNProp or ManNAc for 72 h and analyzed by flow cytometry. Bars represent mean values from two independent experiments (*$p<0.05$)

Discussion

In this study we demonstrated a new tool to stimulate lymphocytes. Lymphocyte activation was achieved by biochemical engineering of the acyl side chain of sialic acids. The first surprising result was that we could measure PBMC -activation after only 10 % incorporation of *N*-propanoyl neuraminic acid. In this point PBMC are different from many other analyzed cell lines, which incorporated up to 85 % of the applied ManNProp to *N*-propanoyl-neuraminic acid [9]. In this context it was also surprising that the incorporation of mannosamines is much better compared to glucosamines. Preliminary data suggest that this depends on the uptake, since cellular uptake of mannosamine is much higher compared to glucosamines (data not shown), a fact we do not understand at the moment. However, our data show that application of ManNProp increases the expression of CD25, CD71 and the secretion of IL-2. Furthermore PBMC return from the G_0-phase back into the cell cycle. The involvement of the activation antigen CD25 in transition

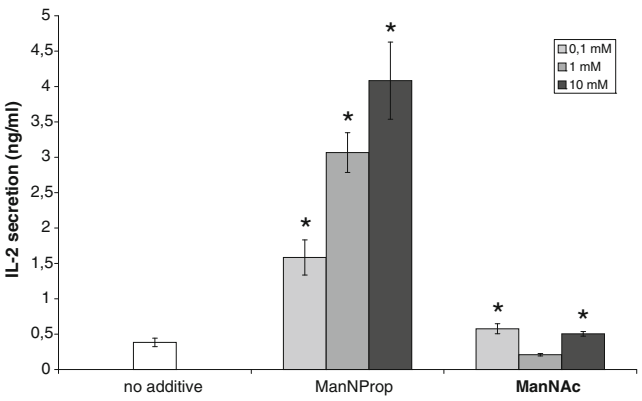

Fig. 6 Dose-dependent stimulation of IL-2-secretion after treatment with ManNProp. PBMC were cultured in the absence or presence of various concentrations of ManNProp or ManNAc for 72 h, respectively. IL-2 from the supernatants was quantified. Bars represent mean values from two independent experiments (*$p<0.05$)

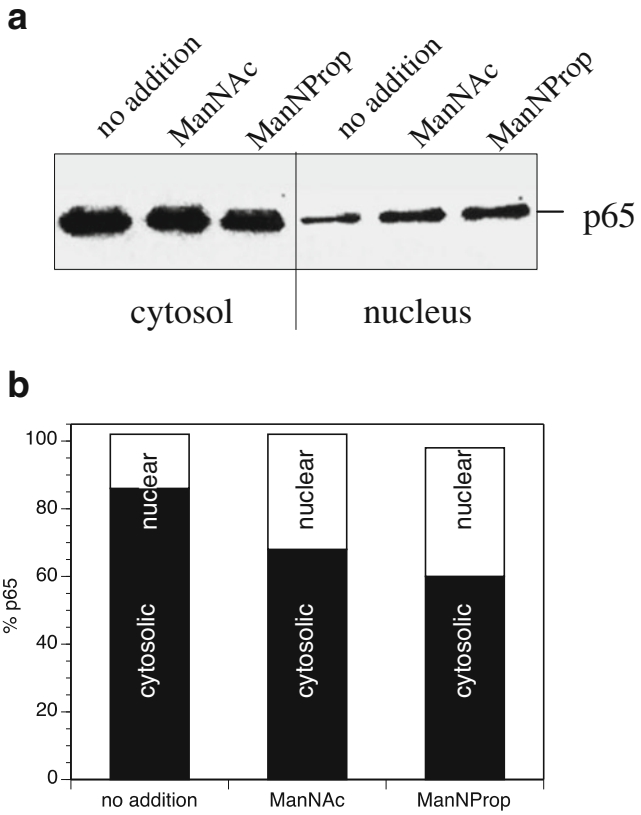

Fig. 7 Western-blot analysis of ManNProp-treated lymphocytes. PBMC were cultured in the presence of 10 mM ManNAc or ManNProp, for 1 h respectively. **a** Cytosolic and nuclear fraction were prepared and subjected to Western-blot analysis of p65. **b** Quantification of the blot shown above. Bars represent total amount of p65. The black lower parts of the bars represent the cytosol fraction, whereas the white upper parts of the bars represent the nuclear fraction

from G_1 to S-phase of activated T-cells has been suggested by several authors [24–26]. Also CD71 is not present on resting blood lymphocytes, but appears in T and B cells after activation [27] and its expression parallels with cell growth. Taken together, the treatment with ManNProp results in an activation of PBMC.

The PBMC stimulation as shown here might be explained by a change of the hydrophobicity on the cell surface induced by ManNProp, which replaces the more hydrophilic acetyl group of sialic acid. This may induce a re-arrangement and/or clustering of the modified receptors. Additionally, these clusters might be stabilized by extending the half live of membrane glycoproteins with receptor function, since it could be shown in engineered PC12-cells that CD66 has a prolonged half live time [28]. This clustering could result in stimulation of intracellular signal transduction. Besides, it should also be taken into account that the unnatural CMP-*N*-propanoylneuraminic acid itself may interact with intracellular proteins of a signal transduction pathway leading to an enhanced cellular response.

In summary, already a minor change in the structure of cell surface sialic acid induced unexpected stimulation of human

PBMC. The stimulation of PBMC using the new means of biochemical engineering the *N*-acyl side chain of sialic acid might open a new means for the search of novel potent immuno-modulators.

Acknowledgment This study has been supported by the Sonderforschungsbereich 366 and the Sonnenfeld Stiftung Berlin.

References

1. Schauer, R.: Achievements and challenges of sialic acid research. Glycoconj. J. **17**, 485–499 (2000)
2. Varki, A.: Sialic acids as ligands in recognition phenomena. FASEB J. **11**, 248–255 (1997)
3. Schmidt, C., Stehling, P., Schnitzer, J., Reutter, W., Horstkorte, R.: Biochemical engineering of neural cell surfaces by the synthetic N-propanoyl-substituted neuraminic acid precursor. J. Biol. Chem. **273**, 19146–19152 (1998)
4. Wang, Z., Sun, Z., Li, A.V., Yarema, K.J.: Roles for UDP-GlcNAc 2-epimerase/ManNAc 6-kinase outside of sialic acid biosynthesis: modulation of sialyltransferase and BiP expression, GM3 and GD3 biosynthesis, proliferation, and apoptosis, and ERK1/2 phosphorylation. J. Biol. Chem. **281**, 27016–27028 (2006)
5. Weidemann, W., Klukas, C., Klein, A., Simm, A., Schreiber, F., Horstkorte, R.: Lessons from GNE-deficient embryonic stem cells: sialic acid biosynthesis is involved in proliferation and gene expression. Glycobiology **20**, 107–117 (2010)
6. Horstkorte, R., Reinke, S., Bauer, C., Reutter, W., Kontou, M.: N-Propionylmannosamine-induced over-expression and secretion of thioredoxin leads to neurite outgrowth of PC12 cells. Biochem. Biophys. Res. Commun. **395**, 296–300 (2010)
7. Crocker, P.R., Paulson, J.C., Varki, A.: Siglecs and their roles in the immune system. Nat. Rev. Immunol. **7**, 255–266 (2007)
8. McEver, R.P., Zhu, C.: Rolling cell adhesion. Annu. Rev. Cell Dev. Biol. **26**, 363–396 (2010)
9. Keppler, O.T., Horstkorte, R., Pawlita, M., Schmidt, C., Reutter, W.: Biochemical engineering of the *N*-acyl side chain of sialic acid: biological implications. Glycobiology **11**, 11R–18R (2001)
10. Mahal, L.K., Bertozzi, C.R.: Engineered cell surfaces: fertile ground for molecular landscaping. Chem. Biol. **4**, 415–422 (1997)
11. Du, J., Meledeo, M.A., Wang, Z., Khanna, H.S., Paruchuri, V.D., Yarema, K.J.: Metabolic glycoengineering: sialic acid and beyond. Glycobiology **19**, 1382–1401 (2009)
12. Wieser, J.R., Heisner, A., Stehling, P., Oesch, F., Reutter, W.: In vivo modulated N-acyl side chain of *N*-acetylneuraminic acid modulates the cell contact-dependent inhibition of growth. FEBS Lett. **395**, 170–173 (1996)
13. Büttner, B., Kannicht, C., Schmidt, C., Löster, K., Reutter, W., Lee, H.Y., Nöhring, S., Horstkorte, R.: Biochemical engineering of cell surface sialic acids stimulates axonal growth. J. Neurosci. **22**, 8869–8875 (2002)
14. Kontou, M., Bauer, C., Reutter, W., Horstkorte, R.: Sialic acid metabolism is involved in the regulation of gene expression during neuronal differentiation of PC12 cells. Glycoconj. J. **25**, 237–244 (2008)
15. Horstkorte, R., Rau, K., Laabs, S., Danker, K., Reutter, W.: Biochemical engineering of the N-acyl side chain of sialic acid leads to increased calcium influx from intracellular compartments and promotes differentiation of HL60 cells. FEBS Lett. **571**, 99–102 (2004)
16. Prescher, J.A., Bertozzi, C.R.: Chemistry in living systems. Nat. Chem. Biol. **1**, 13–21 (2005)
17. Elmouelhi, N., Aich, U., Paruchuri, V.D., Meledeo, M.A., Campbell, C.T., Wang, J.J., Srinivas, R., Khanna, H.S., Yarema, K.J.: Hexosamine template. A platform for modulating gene expression and for sugar-based drug discovery. J. Med. Chem. **52**, 2515–2530 (2009)
18. Whiteheart, S.W., McLenithan, J.C., Hart, G.W.: Surfaces of murine lymphocyte subsets differ in sialylation states and antigen distribution of a major N-linked penultimate saccharide structure. Cell. Immunol. **125**, 337–353 (1990)
19. Boyum, A.: Separation of blood leucocytes, granulocytes and lymphocytes. Tissue Antigens. **4**, 269–274 (1974)
20. Keppler, O.T., Stehling, P., Herrmann, M., Kayser, H., Grunow, D., Reutter, W., Pawlita, M.: Biosynthetic modulation of sialic acid-dependent virus-receptor interactions of two primate polyoma viruses. J. Biol. Chem. **270**, 1308–1314 (1995)
21. Kayser, H., Zeitler, R., Hoppe, B., Reutter, W.: Synthesis of N-acyl-2-amino-2-deoxy-(1-14C)-glucose as precursors for the biosynthesis of novel N-acylneuraminic acids. J. Labelled Comp. Radiopharm **XXXI**, 711–715 (1982)
22. Mans, R.J., Novelli, G.D.: A convenient, rapid and sensitive method for measuring the incorporation of radioactive amino acids into protein. Biochem. Biophys. Res. Commun. **3**, 540–543 (1960)
23. Hara, S., Takemori, Y., Yamaguchi, M., Nakamura, M., Ohkura, Y.: Fluorometric high-performance liquid chromatography of N-acetyl- and N-glycolylneuraminic acids and its application to their microdetermination in human and animal sera, glycoproteins, and glycolipids. Anal. Biochem. **164**, 138–145 (1987)
24. Smith, K.A.: Interleukin-2: inception, impact, and implications. Science **240**, 1169–1176 (1988)
25. Minami, Y., Taniguchi, T.: IL-2 signaling: recruitment and activation of multiple protein tyrosine kinases by the components of the IL-2 receptor. Curr. Opin. Cell Biol. **7**, 156–162 (1995)
26. Kelly, K., Siebenlist, U.: Immediate-early genes induced by antigen receptor stimulation. Curr. Opin. Immunol. **7**, 327–332 (1995)
27. Omary, M.B., Trowbridge, I.S., Battifora, H.A.: Human homologue of murine T200 glycoprotein. J. Exp. Med. **152**, 842–852 (1980)
28. Horstkorte, R., Lee, H.Y., Lucka, L., Danker, K., Mantey, L., Reutter, W.: Biochemical engineering of the side chain of sialic acids increases the biological stability of the highly sialylated cell adhesion molecule CEACAM1. Biochem. Biophys. Res. Commun. **283**, 31–35 (2001)