ejpmr, 2018,5(9), 268-272

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DIFFERENTIAL EXPRESSION OF ALPHA 2, 6 - LINKED SIALIC ACID RESIDUES ON THE CELL SURFACE OF MOUSE LYMPHOBLASTOID CELL LINES WEHI-231 AND A-20

Priyanka Banerjee*

***Assistant Professor, Dept. of Biotechnology, M. S. Ramaiah College of Arts, Science and Commerce, MSRIT Post Bangalore - 560 054, Karnataka, India.

***Corresponding Author: Dr. Priyanka Banerjee**
Assistant Professor, Dept. of Biotechnology, M. S. Ramaiah College of Arts, Science and Commerce, MSRIT Post Bangalore - 560 054, Karnataka, India.

Article Received on 03/07/2018 **Article Revised on 23/07/2018** **Article Accepted on 12/08/2018**

ABSTRACT

Significant changes in sialylation are known to accompany cancer and autoimmune disease. Therefore, it is important to understand the functional role that sialic acid plays in lymphocyte development so that novel therapeutic strategies to combat these pathologies may be developed. In this study, cell surface alpha-2,6 linked sialic acid on the surface of two lymphoblastic cell lines were studied. Both both WEHI-231 and A-20 cell lines are mature B cell lines from mouse that are known to express sialic acid on their cell surfaces. This is the first study done to analyse cell surface alpha 2,6-linked sialic acid on the cell lines by flow cytometry. An investigation was done to study how cytokine IL-4 afftects the expression of cell surface alpha 2,6-linked sialic acid in these cell lines. Results clearly demonstrated that both A-20 and WEHI-231 cell lines express alpha 2,6- linked sialic acid on their cell surfaces. However, WEHI-231 cells showed a heterogeneity in their cell surface sialic acid expression, which, after stimulation with cytokine IL-4, homogeneously started expressing sialic acid on their cell surface. While a few hypotheses have been made trying to explain this characteristic change in WEHI-231 cell line, further experiments need to be carried out for a better understanding of this behaviour.

KEYWORDS: Sialic acid, mouse lymphoblastoid cell lines.

INTRODUCTION

Mammalian cells are covered by a dense glycocalyx consisting of glycolipids, glycoproteins, and proteoglycans.[1] Many of these glycoconjugates carry glycan chains terminating with sialic acids (Sias), a family of acidic 9-carbon sugars that are prominently expressed in animals of the deuterostome lineage.[2] They contribute to the enormous structural diversity of complex carbohydrates, which are major constituents of proteins and lipids of cell membranes and secreted macromolecules.[3] Sialic acids are prominently positioned, usually at the outer end of these molecules. The diversity of glycan chains is even more increased by the biosynthesis of various kinds of sialic acids[1] used by glycoconjugates.

Sialic acids play multifaceted roles in intracellular functioning within the immune system, mediating or modulating a variety of normal and pathological processes. Sialic acids are involved in a variety of cellular functions, such as cell-cell interactions. There is a distinct dichotomy in the roles that sialic acids play in the mammalian immune system. Sialic acid residues provide a mechanism by which toxins and pathogens, such as cholera toxin[4] and enterovirus 70[5], infiltrate host cells. They serve as components of binding sites for various pathogens and toxins.[6,7] Conversely, sialic acids regulate a diverse number of host immune responses.[7] For example, sialic aid moieties modulate the complement pathway factor via selectin family of sialic acid-binding proteins.[8] In clinical pathology, sialic acid measurements of body fluids are used to predict disease risk. There have been many papers suggesting that measurements of total sialic acids in the serum[8,9] can predict the risk of various diseases.

The importance of ST6Gal I in the immune system was established by the finding that ST6Gal I deficient mice have impaired thymopoiesis and granulopoiesis[10], as well as defective B cell maturation and antibody production.[11] Although the ST6Gal I gene is widely expressed in multiple tissues throughout the body[12], the only major defect observed in the ST6Gal I knockout mice is an impaired humoral immune response. This is demonstrated by reduced levels of circulating IgM, impaired B cell proliferation in response to various activation signals and impaired antibody production in response to both T-dependent and T-independent antigens.[13,14]

The transfer of sialic acid from CMP-Sias onto newly synthesised glycoconjugates passing through eukaryotic Golgi compartments is catalyzed by a family of linkage-specific sialyltransferases.[1] As with most glycosyltransferases, sialyltransferases are type II membrane proteins with complex signals dictating Golgi localization. The sialyltransferase primary structures from many mammalian tissues and from some bacterial species are known, and today there exists a family of at least 15 members creating α2→3, α2→6, α2→8 and α2→9 linkages between Neu5Ac and the accepting sugar.[15] The ST6 Gal I sialyltransferases (ST6 Gal I) transfers sialic acid to galactose via α2, 6- linkage.

α 2,6-linked sialic acids are markedly up-regulated during the development of B cells[16], coinciding with the binding preference of the B cell surface molecule CD22/Siglec-2, which modulates the B cell response to antigen stimulation.[17] Although both WEHI-231 and A-20 are mature B cell lines that are known to express ST6Gal I gene, the A-20 cell line is further ahead in the process of maturation as compared to WEHI-231.[18] In this study, cell surface sialic acid was analysed in the two mouse lymphoblastoid cell lines followed by the effect of IL-4 on the regulation of cell surface sialic acid.

MATERIALS AND METHODS

Cell culture

WEHI-231 and A-20 cell lines were originally purchased from ATCC (American Type Culture Collection). The cells were maintained in RPMI 1640 medium supplemented with 10% heat- inactivated FBS (Fetal Bovine Serum), 5% penicillin- streptomycin and 2µl β-Mercaptoethanol at 37^{o}C in a 5% CO_2 incubator. The cells were subcutured to maintain a cell density of 1 x 10^6 cells/ml.

Stimulation of cell lines with IL-4

Mouse recombinant interleukin-4 (IL-4) was used at 5ng/ml. 2 X 10^5 cells were transferred to fresh RPMI-1640 media. IL-4 was added and the cells were allowed to grow in 10% CO_2 incubator at 37^{o}C for 24 hours.

Cell Staining with Lectin

5 X 10^6 cells were stained with fluorescein isothiocyanate (FITC)-conjugated *Saumbucus nigra* ag-glutinin (SNA), specific for 2, 6- linked sialic acid. Lectins were used at 2µg/ml. The cells were finally fixed by adding 200µl of 3.7% formaldehyde prepared in Hanks's Balanced salt Solution (HBSS).

Flow Cytometric Analysis of Stained Cells using Bioanalyzer

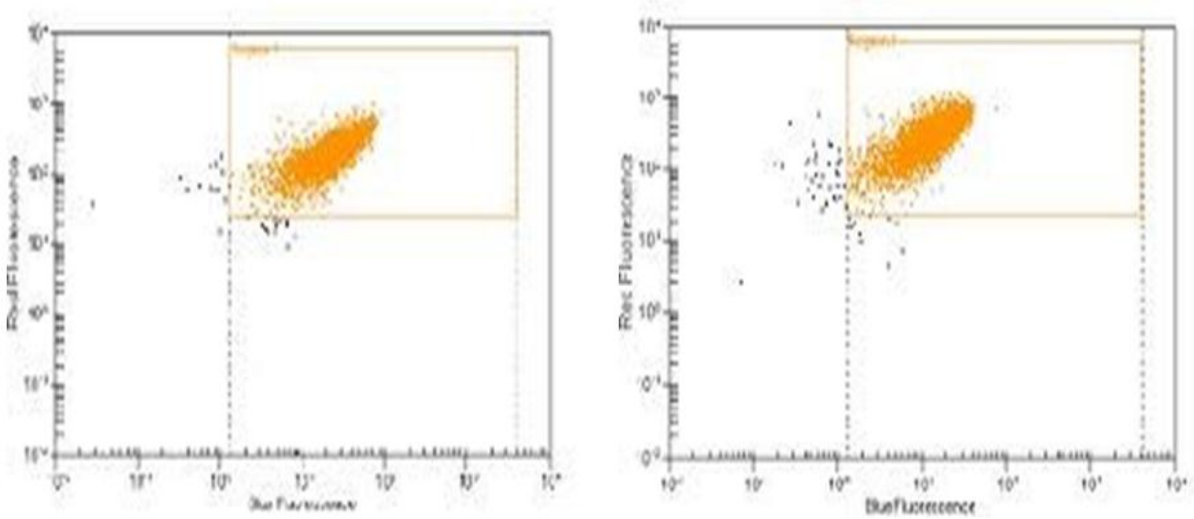
Agilent 2100 bioanalyzer offers on-chip flow cytometry of cell-based fluorescence data. Pre-stained cells were loaded onto a chip and fluorescence intensities in two channels for approximately 750 single cells per sample were measured within 25 minutes.

Stimulation of cell lines with IL-4

Mouse recombinant interleukin-4 (IL-4) was used at 5ng/ml. 2 X 10^5 cells were transferred to fresh RPMI-1640 media. IL-4 was added and the cells were allowed to grow in 10% CO_2 incubator at 37^{o}C for 24 hours.

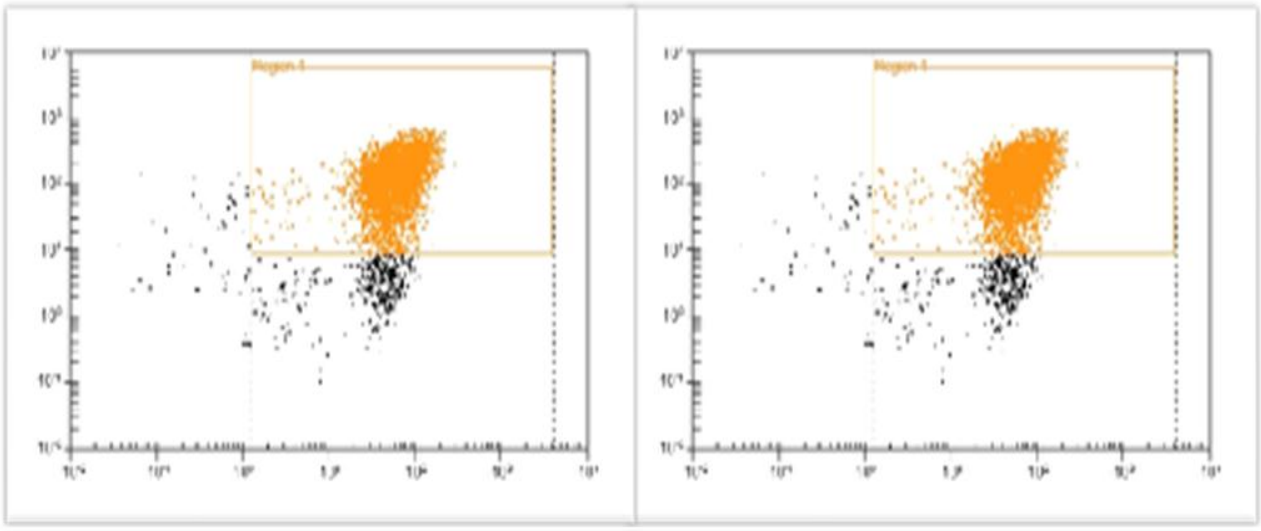
RESULTS AND DISCUSSION

Although both WEHI-231 and A-20 are mature B cell lines that are known to express ST6Gal I gene, the A-20 cell line is further ahead in the process of maturation as compared to WEHI-231.[18] In this set of experiments, A-20 and WEHI-231 cells lines were analysed for sialic acid expression at different time intervals (24hrs and 48hrs).



(a) **(b)**

Fig. 1: A-20 cells stained with SNA at (a) 24 hours, and (b) 48 hours.

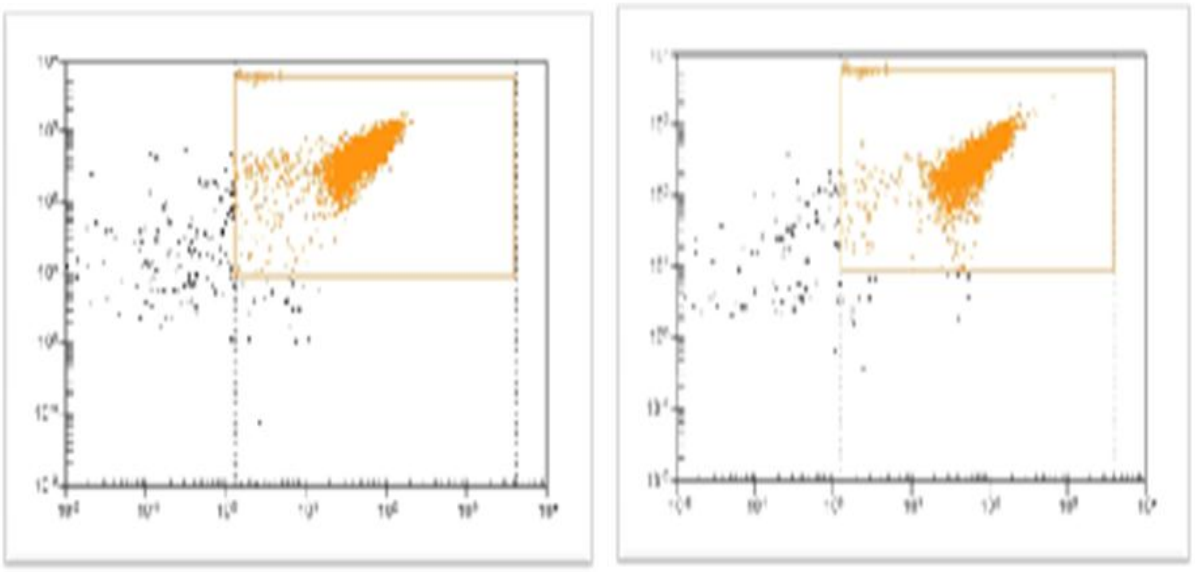


(a) **(b)**

Fig. 2: WEHI-231 cells stained with SNA at (a) 24.

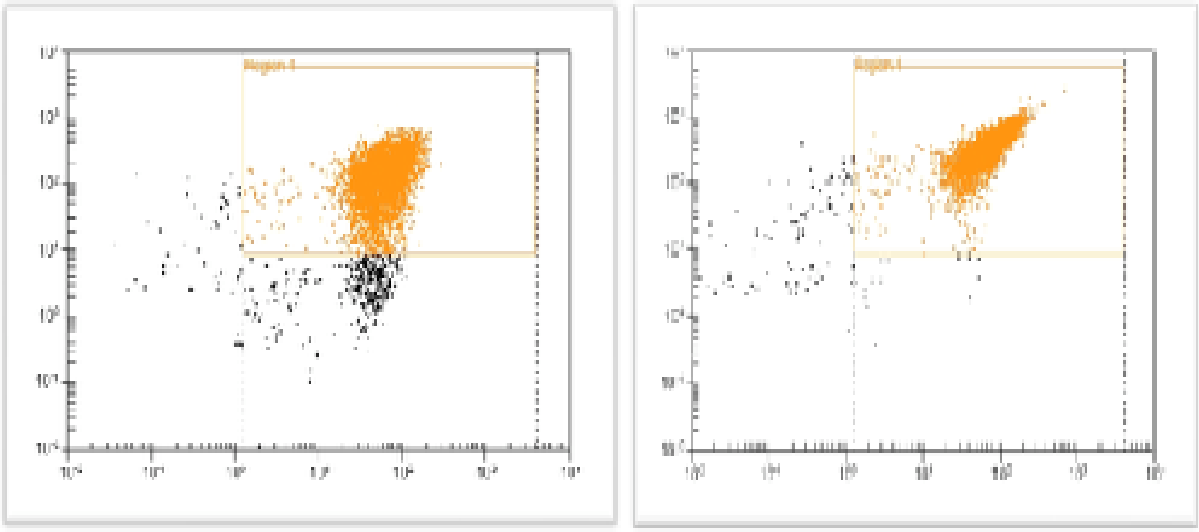
The gated cells in region 1 are viable cells that express sialic acid on their cell surface and have been stained. The cells below the gated area are viable cells that did not take up the stain, probably because they did not express sialic acid on cell surface. The cells that showed high red fluorescence are dead cells.

The cytokine interleukin 4 (IL-4) is known to induce class switching in B cells. In the next set of experiments, both the cell lines (A-20 and WEHI-231) were incubated in cytokine IL-4 (500pg/ml) for 48 hours and stained with fluorescein isocyanate (FITC)-conjugated *Sambucus nigra* (SNA) specific for 2, 6-linked sialic acid. Cell staining was read using Agilent 2100 Bioanalyzer. The results are as shown in the next figures.



(a) **(b)**

Fig. 3: A-20 cells grown in the presence of IL-4 and stained with SNA at (a) 24 hours and (b) 48 hours.



(a) **(b)**

Fig. 4: WEHI-231 cells grown in the presence of IL-4 and stained with SNA at (a) 24 hours and (b) 48 hours.

The A-20 cells recapitulated the results seen in the previous experiment. The cells expressed a high level of cell surface sialic acid and there was only a very minute increase in sialic acid expression when the cells were cultured in the presence of IL-4 (figure 3). In contrast, WEHI-231 cells were found to be relatively low expressers of sialic acid on their surface. But the sialic acid expression was significantly increased when incubated with IL-4. There was a clear upward shift in the cells stained with SNA lectin when exposed to IL-4 in WEHI-231 cells (figure 4).

This study demonstrates that B-lymphoblastoid cell lines WEHI-231 and A-20 both express cell surface sialic acid, and when stimulated with cytokine IL-4, that there is an increased α- 2,6 linked sialic acid on WEHI-231 cells (figure 4). This result is consistent with previous studies that have shown that B-cell maturation is accompanied by a dramatic induction of ST6Gal I expression and consequent display of cell surface α-2,6 sialyl linkage.[19] The A-20 cells showed a very high degree of cell surface sialic acid which when stimulated with cytokine IL-4 did not show much difference in the expression of sialic acid expression. This can be explained by the fact that the A-20 cell line is a mature B cell line.[18] Maturity of B cells in known to induce cell surface sialic acid. Therefore, it can be concluded that the A-20 cells are already expressing maximum cell surface sialic acid expression and stimulation with IL-4 fails to make any significant difference in their further expres- sion.

WEHI-231 cell line however, shows an interesting heterogeneity in its cell surface sialic acid expression in the un-stimulated cells (figure 4 (a) and (b)). There seemed two possible scenarios that might explain this observation. The first and least interesting possibility was that this was a population of cells on a threshold of stimulation and that any particular cell within the population might express differing levels of sialic acid on its surface due to minor fluctuations in growth conditions within the flask. On the other hand, Gottschalk et al.[20] in a review explained that continuous passage of WEHI-231 cells in different laboratories yielded variants that differed greatly in their response to anti-Ig treatment. Given these previous studies, it seemed possible that the ATCC WEHI-231 cell line, which should ideally have been genetically uniform, none-the-less consisted of a collection of heterogeneous clones, at least with respect to expression of cell surface sialic acid.

REFERENCES

1. Varki, A. 1992. Diversity in the sialic acids. Glycobiology, 2: 25.
2. Powell, L. D., Jain, R. K., Matta, K. L., Sabesan, S., and Varki, A. 1995. Characterization of sialoligosaccharide binding by recombinant soluble and naïve cell-associated CD22. J. Biol. Chem., 270: 7523.
3. Varki, A. 2009. Multiple changes in sialic acid biology during human evolution. Glycoconj. J., 26: 231.
4. Richards RL, Moss, J., Avling, C. R., Fishman, P. H., Brady, R. O. 1979. Choleragen (cholera toxin): a bacterial lectin. Proc Natl Acad Sci USA., 76: 1673.
5. Nokhbeh, M. R., Hazra, S., Alexander, D. A., Khan, A., MaAllister, M., Griffith, M, Dimock, K. 2005. Enterovirus 70 binds to different glycoconjugates containing Aplha2, 3-linked sialic acid on different cell lines. Journal of Virology., 79: 7087.
6. Angata, T., Varki, A. 2002. Chemical diversity in the sialic acids and related alpha-keto acids, an evolutionary perspective. Chem. Rev., 102: 439.
7. Varki, A. 2007. Glycan-based interactions involving vertebrate sialic-acid recognizing proteins. Nature., 446: 1023.
8. Pickup, J. C., Mattock MB, Crook MA, Chusney

GD, Burt D, Fitzgerald AP. 1995. Serum sialic acid concentration and coronary heart disease NIDDM. Diabetes care., 18: 1100.
9. Crook, M. A., Earl, K., Morokutti, A., Yip, J., Viberti, G. 1994. Serum sialic acid, a risk factor for cardiovascular disease, is increased in IDDM patients with microalbuminuria and clinical proteinuria. Diabetes care., 17: 305.
10. Marino, J. H., Tan, C., Davis, B., Han, E. S. Hickey, M., Naukam, R., Taylor, A., Miller, K. S., Van De Weile, C., and Teague, T. K. 2008. Disruption of thymopoiesis in ST6Gal I-deficient mice. Glycobiology, 18: 719.
11. Hennet, T., Chui, D., Paulson, J. C., and Marth, J. D. 1998. Immune regulation by ST6gal sialyltransferase. Proc. Natl. Acad. Sci. USA, 95: 4504.
12. Dall'Olio, F. 2001. The sialyl a2, 6 lactosaminyl structure: biosysthesis and functional role. Glycoconjugate J., 17: 669.
13. Cornall, R. J., Cyster, J. G., Hibbs, M. L., Dunn, A. R., Clark, E. A., and Goodnow, C. C. 1998. Polygenic autoimmune traits: Lyn, CD22, and SHP1 are limiting elements of a biochemical pathway regulating B cell signaling and selection. Immunity., 8: 497.
14. Tedder, T. F., Tuscano, J., Sato, S., and Kehrl, J. H. 1997. CD 22- a B lymphocyte specific ad- hesion molecule that regulates antigen receptor signaling. Annual Review Immunology., 15: 481.
15. Schauer, R. 1982. Chemistry, metabolism and biological functions of sialic acids. Adv. Carb. Chem. Biochem., 40: 357.
16. Wuensch, S. A. Huang RY, Ewing J, Liang X, Lau JT. 2000. Murine B cell differentiation is accompanied by programmed expression of multiple novel beta- galactoside α2, 6-sialyltrans- ferase mRNA forms. Glycobiology., 10: 67.
17. Crocker, P. R. 2007. Siglecs and their roles in the immune system. Nat. Rev. Immunol., 7: 255.
18. Kim, K. W., S. W. Kim, K. S. Min, C. H. Kim, Y. C. Lee 1981. Genomic structure of human GM3 synthase gene (hST3GalV) and identification of mRNA isoforms in the 5'- untranslated region. Gene, 273: 163.
19. Wang, X. C., Vertino-Bell, A., Eddy, R. L., Byers, M. G., Shows, T. B., and, Lau, J. T. 1993. Chromosome mapping and organization of the human β-galactoside α-2,6 sialyltransferase gene: differential and cell specific usage of upstream exon sequences in B-lymphoblastoid cells. J. Biol. Chem., 268: 4355.
20. Gottschalk, A. R., and J. Quintáns. 1995. Apoptosis in B lymphocytes: the WEHI-231 perspec- tive. Immunol. Cell Biol., 73: 8–16.