Published in final edited form as:

Nat Chem Biol. 2014 January ; 10(1): 7–8. doi:10.1038/nchembio.1415.

Glyco-engineering ‘Super-self’

Matthew S Macauley and James C Paulson

Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA USA

James C Paulson: jpaulson@scripps.edu

Abstract

Altered glycosylation of cancer cells confers phenotypic changes that promote spread and evasion of immune responses. A novel method for engineering cell surface glycans is providing insights into these mechanisms.

Cancer cells exhibit changes in glycosylation that contribute to cancer progression, metastasis, and evasion of immune responses¹. One of the most striking changes exhibited by some cancer cells is increased expression of sialic acids on glycans of cell surface glycoproteins and glycolipids. Sialic acids are ligands for members of the sialic acid binding immunoglobulin-like lectin (siglec) family that are differentially expressed on white blood cells of the immune system². Many of the siglecs are inhibitory co-receptors that are thought to aid immune cells in ‘self/non-self’ discrimination through engagement with sialic acid containing glycans on the contacted cell^{2,3}. This results in recruitment of the siglec to the site of cell contact and suppression of activating receptors on the leukocyte that interact with their cognate ligands on the opposing cell. In this regard, natural killer (NK) cells are known to exhibit killing of some cancer cells that are effectively recognized as ‘non-self’, while other cancer cells are resistant to NK cell killing. However, the mechanisms by which some cancer cells evade killing by NK cells are not well understood. In this issue, Hudek *et al.* employ a novel approach to investigate the possibility that cells expressing high levels of sialic acid are able to suppress NK cell killing by recruitment of the inhibitory receptor Siglec-7⁴. They find that cells engineered to contain synthetic lipid-linked polymers displaying sialic acid ligands of Siglec-7 results in recruitment of the siglec to the site of cell contact and suppression of NK cell activation.

Natural Killer (NK) cells play a critical role in the immune system by destroying cells considered ‘non-self’. To aid in ‘self/non-self’ discrimination, NK cells express a variety of inhibitory co-receptors that recognize ‘self’ ligands that can suppress their activation⁵. Thus, if an NK cell contacts a cell with sufficient number of ‘self’ ligands, these inhibitory receptors will act singly or in combination to suppress activation and cell killing. Conversely, cells that are devoid of ligands for these receptors are ‘missing self’ and are susceptible to killing by NK cells if they contain ligands recognized by activating receptors.

Among the inhibitory receptors of NK cells is Siglec-7, which can be recruited to the site of contact between NK cells and target cells containing its ligands, resulting in modulation of NK cell activation^{2,3,5,6}. Recognizing that cancer cells with high levels of sialic acid may escape NK cell killing through suppression by Siglec-7, Hudak *et al.*⁴ set out to determine if increasing the ligand density for this co-receptor would be sufficient to suppress cell killing. To do so, they employed a technique, previously developed by the Bertozzi group, to engineer cells with desired glycan sequences by inserting chemically-defined glycan polymers attached to lipids which can spontaneously insert into the membrane of a living cell⁷. They initially selected Jurkat cells, a cancer cell line that is susceptible to killing by NK cells and documented to be hyposialylated relative to normal lymphocytes⁸. Using the sialic acid decorated glycan polymers, Jurkat cells were transformed from a state of ‘missing

self' in which they strongly activated NK cells, to a state of 'super-self' where NK cell activation is strongly inhibited (Fig. 1A). The mechanism of NK cell suppression involves redistribution of Siglec-7 to the immunological synapse, resulting in its phosphorylation and recruitment of the phosphatase Shp-1, which is known to blunt signaling by activating receptors. These effects were mediated by Siglec-7 interactions with the glycopolymers as evidenced by abrogation of inhibition by an anti-Siglec-7 blocking antibody. Natural sialoside ligands were also found to support this inhibitory mechanism since enzymatic removal of endogenous sialic acid on several different cell lines increased their ability to activate NK cells. The implications from this work are that natural Siglec-7 ligands are used by NK cells to suppress activation toward 'self', and that hypersialylation of cancer cells may provide 'enhanced self' to exploit a natural inhibitory pathway for immune evasion (Fig. 1B).

The study by Hudak *et al.* supports the view that a primary function of the siglecs are to aid immune cells in 'self/non-self' recognition². In this context, recruitment of siglecs to an immunological synapse by ligands expressed on other cells may be a mechanism shared by other inhibitory siglecs to suppress inadvertent activation of immune cells against 'self'. Another example involves the B cell siglecs, CD22 and Siglec-G/10, where ligands presented on an artificial membrane with both ligands and antigen, results in dramatic dampening of cell activation and apoptosis of a B cell that recognizes the antigen⁹. This is in keeping with the current view that B cell siglecs play a role in enforcing tolerance to self-antigens to prevent autoimmunity^{9,10}. The method developed by Bertozzi and coworkers for manipulating cellular glycosylation by insertion of chemically defined lipid-linked glycopolymers into cell membranes will likely find broad utility in the functional investigation of other members the siglec family, as well as other families of glycan binding proteins.

References

1. Fuster MM, Esko JD. Nature Reviews Cancer. 2005; 5:526–542.
2. Paulson JC, Macauley MS, Kawasaki N. Ann N Y Acad Sci. 2012; 1253:37–48. [PubMed: 22288608]
3. Crocker PR, Paulson JC, Varki A. Nat Rev Immunol. 2007; 7:255–66. [PubMed: 17380156]
4. Hudak JE, Canham SM, Bertozzi CR. Nature Chemical Biology. 2013:xxx–yyy.
5. Kumar V, McNERNEY ME. Nat Rev Immunol. 2005; 5:363–74. [PubMed: 15841099]
6. Nicoll G, et al. Eur J Immunol. 2003; 33:1642–8. [PubMed: 12778482]
7. Rabuka D, Forstner MB, Groves JT, Bertozzi CR. Journal of the American Chemical Society. 2008; 130:5947–5953. [PubMed: 18402449]
8. Praz F, Lesavre P. J Immunol. 1983; 131:1396–9. [PubMed: 6604099]
9. Macauley MS, et al. J Clin Invest. 2013; 123:3074–83. [PubMed: 23722906]
10. Jellusova J, Nitschke L. Front Immunol. 2011; 2:96. [PubMed: 22566885]

Figure 1. Sialic acid mediated recruitment of Siglec-7 inhibits NK cell activation

(a) Cells with low levels of sialic acid (hyposialylated) are 'missing self', resulting in strong activation of the NK cells (*left*). Glyco-engineering target cells with sialic acid polymers can transform them into 'super self', where the high density of ligands efficiently recruits Siglec-7 to suppress NK cell activation (*right*). (b) Endogenous levels of sialic acids comprise 'self' markers on normal cells, which may engage Siglec-7 to aid in 'self' recognition and suppress unwanted activation of NK cells (*left*). Many cancer cells have higher levels of sialic acid (hypersialylated), which could recruit more Siglec-7 and provide a strong inhibitory signal to the NK cells and help in immune evasion.