Immuno\-globulin G (IgG) antibodies function, in part, through ligation of cell-surface Fc receptors such as Fc\-γRIIIA (also known as CD16A). IgG glycosylation is known to impact antibody function, but the role of Fc\-γRIIIA glycans, if any, is unclear. Patel et al. now reveal that these glycans do impact protein conformation and IgG affinity and display cell-specific glycosylation patterns, leading to a potential model in which the affinity and possibly function of Fc receptors is dictated by the cell type and its surface glycome.

IgG, the most prevalent antibody class, functions in a number of ways, including direct binding and neutralization of target antigens, complement activation and fixation, and cellular activation through binding surface receptors that associate through the IgG “fragment, crystallizable” (Fc) domain (1). IgG molecules are well-known as the desired outcome of immunizations, because they are affinity-matured and are typically a hallmark of memory, and they are the template for all antibody-based “biologic” drugs.

Since the development of monoclonal antibody (mAb) technology in 1975 (2), which earned Drs. Köhler and Milstein the Nobel Prize in 1984, the uses of antibodies in the laboratory and clinical setting has expanded exponentially. Rituximab (Rituxan), an anti-CD20 mAb that is used to treat B-cell lym\-phoma patients, exemplifies the current use of antibodies to treat cancer (2). It has been known for some time that the Fc domain of IgG can interact with the Fcγ receptors on cell surfaces. These receptors are cell-specific, with receptors from primary human natural killer (NK) cells displaying marked differences from recombinant forms. These results suggest that FcγRs-mediated antibody effects could be tuned by an individual cell’s glycome.

In humans, there are seven FcγR molecules. IgG binding to FcγR glycans results in a conformation change and a 12-fold increase in FcγR-mediated antibody function and affinity. However, the potential role for glycosylation of partner FcγRs has been largely overlooked. A new report by Patel et al. (6) refocuses the spotlight on these receptors, reporting that the glycans present on one IgG receptor, FcγRIIIA (i.e. CD16A), impact conformation and the binding affinity for IgG. Moreover, they show that FcγRIIIA glycoforms are cell-specific, with receptors from primary human natural killer (NK) cells displaying marked differences from recombinant forms. These results suggest that FcγRs-mediated antibody effects could be tuned by an individual cell’s glycome.

To gain insights into FcγRIIIA N-glycans, Patel et al. (6) first had to optimize a method to purify the receptor from primary human cells at sufficient quantities for analysis. They used a two-step process, first enriching blood samples for NK cells using leukocyte reduction filters and then employing immunoprecipitation to isolate mature FcγRIIIA-N. With material in hand, the authors compared the FcγRIIIA N-glycans from primary isolated NK cells from human donors with those from HEK293 cells expressing either full-length or the soluble ectodomain of FcγRIIIA. They found that the native NK cell FcγRIIIA glycan distribution was distinctly different, containing less processed N-glycan forms, including high mannose and hybrid N-glycans, whereas the recombinant proteins from HEK293 cells were nearly completely highly branched complex-type N-glycans (Fig. 1). Perhaps, more importantly, the apparent unnatural saturation of FcγRIIIA with complex N-glycans resulted in a conformation change and a 12-fold reduction in IgG-binding affinity.

These results raise a number of immediate questions at the basic science level. First, Patel et al. (6) clearly demonstrated...
The final and broadest implication arising from this study is the recognition that cell-specific surface glycosylation can drive changes in receptor affinity and therefore the downstream cellular responses. It has been known for many years that the surface glycome of any given cell is driven by many factors, including the underlying metabolism, nutrient availability, expression pattern of the glycosyltransferases, glycosidases, and nucleotide–sugar transporters, rate of protein synthesis, and others. This study reveals that Fc receptor ligand affinity and possibly function may be tuned in a cell-specific fashion. This makes intuitive sense because it imbues the cell with increased flexibility to regulate its response to specific signals in a context-dependent fashion, while enabling a single receptor to play cell- and tissue-specific roles in responding to a stimulus.

The differences in receptor affinity driven by the glycome could lead to significant problems in translating in vitro, ex vivo, and animal studies to the clinic. Even when the drug target is essentially identical in sequence between animals and humans, the glycans may be substantially different. This might result in changes in drug efficacy, dosing, and other parameters when the glycans are not appropriately considered. It is therefore important to remember that nearly every secreted and integral membrane protein is glycosylated, and, as it has been said before, ignore glycans at your own peril.

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**EDITORS’ PICK HIGHLIGHT:** IgG receptor glycosylation

**Figure 1.** Together with the γ2 chain, FcγRIIIA recognizes and binds to the Fc domain of IgG to initiate cellular activation signals. Patel et al. (6) have discovered that the glycans on FcγRIIIA from primary and uncultured NK cells from human donors carry a much higher proportion of high mannose and hybrid N-glycans than recombinant FcγRIIIA expressed by HEK293 cells in culture. HEK293 cells preferentially placed complex-type N-glycans on the receptor, which cause a different conformational change from the NK-mediated glycans and a 12-fold reduction in binding affinity (Kd) for IgG. These findings suggest that FcγR-mediated cell signaling and overall response to IgG stimulation may depend on the cell-surface glycome.

Differences in conformation and affinity, but does this actually yield a differential response in vivo? Second, what are the contributions of FcγR glycosylation on the other Fc receptors? Finally, are the high mannose and hybrid N-glycans on FcγRIIIA unique to NK cells?

More broadly, these findings hold significance for the research community in three major ways. First, on a practical level, the isolation of a relatively rare cell type from human blood samples and the subsequent success at purification of FcγRIIIA in quantities high enough to enable glycan analysis is quite noteworthy. Obtaining adequate material for glycobiology has long been a roadblock in the field, and this study demonstrates the feasibility of native-sourced glycoprotein structure–function studies.

Second, the discovery that binding to IgG is influenced by FcγR N-glycosylation is significant. The influence of Fc receptor glycosylation on affinity could—like the counterpart discovery of IgG glycosylation influencing function—hold profound importance for biologic drug efficacy. This is especially important since IgG prevention of cancer is strongly associated with FcγR affinity (8), and many disease states are associated with changes in the glycome (9), which could alter IgG-induced signaling. In this way, it may turn out that biologic drug efficacy is influenced by the disease state of the individual, the corresponding glycome, and the target tissue, cell, or molecule.