Reply to Crispin et al.: Molecular model that accounts for the biological and physical properties of sialylated Fc

We recently published a model (1) that proposes a molecular basis for the anti-inflammatory properties of sialylated IgG Fc (sFc), resulting from its observed shift in receptor specificity from FcγRs to dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)/CD23, a consequence of changes to the structure of the CH2 domain that we observe upon sialylation. However, Crispin et al. (2) report a crystal structure of a sFc that resembles that of an earlier reported structure, 1H3Y (3), which carries a nonsialylated Fc. The basis for this discrepancy likely results from the fact that both crystal structures of sialylated and nonsialylated Fcs have been obtained under similar but nonphysiological conditions (>2 M sodium chloride, pH <4.5) that may alter or even prevent the noncovalent interactions between the carbohydrate and protein moieties. Moreover, both crystals are of the same space group with identical cell constants, indicating that the same crystal contacts are present. It has been previously described (3) that the aforementioned structure of the nonsialylated Fc obtained from high-salt conditions varies significantly from that of other Fcs crystallized under more physiological conditions and in different space groups (e.g., PBD ID code 1FC1). As a consequence, no significant structural reorientations were observed by Crispin, et al. (2).

Structural results obtained by X-ray analysis represent one of the many conformational states present in solution. Crystallography, however, cannot determine if the identified state represents the dominant form, especially for flexible molecules such as Fcs. For example, despite the fact that most crystal structures depict the nonglycosylated Fc in a closed conformation, nonglycosylated Fc fragments shift between "open" and "closed" conformations in solution, indicating that Fc fragments may sample multiple conformations without passing significant energy barriers (4). Therefore, Fc conformation within a crystal is strongly influenced by the crystallization conditions and the space group.

According to our model, the addition of sialic acid residues to the Fc-glycan results in its reorientation, allowing a more closed conformation and leading to the accessibility of the DC-SIGN/CD23 binding sites. We believe this interpretation is consistent with our biophysical data, which shows that sialylation destabilizes the CH2 domain and exposes hydrophobic residues in a manner analogous to the inherent instability of the Cε3 domain of IgE, which confers its ability to bind CD23. We not only detected changes in secondary structure by circular dichroism, but the increase in accessible hydrophobic surface area as revealed by chemical denaturation and anilinonaphthalene sulfonates fluorescence also suggests changes in protein tertiary structure. We also believe this interpretation is consistent with solution-state NMR analysis of the Fc glycan, which finds that both glycan arms can be bound to protein or exposed to solvent with sialylation restricting motion of the 1,6-arm and increasing mobility of the 1,3-arm (5). Furthermore, this conformational change would also account for the reduction in FcγR binding observed upon Fc sialylation (6). Thus, we believe that the high-salt crystal structure of sFc reported by Crispin, et al. (2) is not representative of the conformational changes we observe under physiological conditions in solution, and thus cannot explain the switch in binding specificity and biological activity of IgG upon sialylation.

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1 Sondermann P, Pincetic A, Maamary J, Lammens K, Ravetch JV (2013) General mechanism for modulating immunoglobulin effector function. *Proc Natl Acad Sci USA* 110(24):9868–9872.
2 Crispin M, Yu X, Bowden TA (2013) Crystal structure of sialylated IgG Fc: Implications for the mechanism of intravenous immunoglobulin therapy. *Proc Natl Acad Sci USA* 110:E3544–E3546.
3 Krapp S, Mimura Y, Jefferis R, Huber R, Sondermann P (2003) Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. *J Mol Biol* 325(5):979–989.
4 Borrok MJ, Jung ST, Kang TH, Monzingo AF, Georgiou G (2012) Revisiting the role of glycosylation in the structure of human IgG Fc. *ACS Chem Biol* 7(9):1596–1602.
5 Barb AW, et al. (2012) NMR characterization of immunoglobulin G Fc glycan motion on enzymatic sialylation. *Biochemistry* 51(22):4618–4626.
6 Kaneko Y, Nimmerjahn F, Ravetch JV (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 313(5787):670–673.

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The authors declare no conflict of interest.

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