Title
Sugar and spice: Fc glycosylation in antibody-mediated transplant rejection

Journal
Cell Reports Medicine, 3(11)

ISSN
2666-3791

Author
Valenzuela, Nicole M

Publication Date
2022-11-01

DOI
10.1016/j.xcrm.2022.100809

Peer reviewed

Cell Reports Medicine

CellPress
OPEN ACCESS

Preview

Sugar and spice: Fc glycosylation in antibody-mediated transplant rejection

Nicole M. Valenzuela[1,*]
[1]Department of Pathology and Laboratory Medicine, University of California, Los Angeles, CA, USA
*Correspondence: npyburn@mednet.ucla.edu
https://doi.org/10.1016/j.xcrm.2022.100809

Bharadwaj et al.[1] demonstrate that anti-donor HLA antibodies display low levels of Fc fucosylation. This signature was associated with potent provocation of NK cell effector functions and was discriminative for active antibody-mediated rejection among patients with donor specific HLA antibodies.

Transplantation is a life-saving treatment for patients with end-stage organ failure. This year, the United Network for Organ Sharing (UNOS) reported its one millionth transplant, and currently, more than 350,000 people are living with an organ allograft in the United States alone. However, organ transplant longevity does not match recipient life expectancy, with median graft survival ranging from 5 to 15 years. Alloimmunity results from recognition of genetically mismatched proteins in the donor as foreign by the recipient's adaptive immune system. The most potent target of alloimmunity is the highly polymorphic human leukocyte antigen (HLA) system. Although transplant recipients are maintained on lifelong, systemic anti-T cell immunosuppression, antibody-mediated graft damage remains one of the most stubbornly important challenges to long-term transplant success. In particular, the presence of anti-donor HLA antibodies (DSA) represents a significant independent risk factor for acute rejection, chronic occlusive vasculopathy, and graft loss across transplanted organ types.

Decades of research have demonstrated that anti-HLA antibodies bind to donor vascular cells, and activate the classical complement system, induce vascular inflammatory signaling, and promote infiltration of recipient immune cells into the transplanted organ. Nevertheless, the simple presence of DSA is not a binary determinant of whether a patient will experience rejection or graft failure. Many groups have thus surmised that additional characteristics of DSA influence its pathogenicity.[2] Features such as immunoglobulin isotype and subclass, titer/quantity, and Fc glycosylation are important determinants of antibody effector function. The four human immunoglobulin G (IgG) subclasses have discrete affinities for different families of Fc gamma receptors (FcγRs) borne by innate immune cells such as NK cells, monocytes and neutrophils.[3] IgG subclasses also exhibit unequal potency in activating the classical complement cascade. Consequently, there is high interest in the transplant field to understand if the relative abundance of IgG subclasses among anti-donor HLA antibodies is better correlated with risk of rejection than presence of HLA donor specific antibody alone.

Similarly, differential glycosylation of the Fc region of IgG controls engagement of FcγRs and the classical complement initiator C1q. This variance dynamically regulates an antibody's capacity to activate effector functions like cell-mediated cytotoxicity and leukocyte adherence. For example, IgG glycovariants lacking fucose residues elicit greater natural killer (NK) cell antibody-dependent cytotoxicity,[4] while galactosylation augments and sialylation inhibits complement activation.[5] The majority of serum IgG is fucosylated, and investigators have not found substantial fluctuations in overall abundance of fucosylated IgG during inflammation (comprehensively reviewed in Seeling et al.[6] and Oosterhoff et al.[7]). Nonetheless, fucosylation is reduced among platelet and RhD-specific antibodies causing fetal thrombocytopenia and hemophilia,[8] suggesting that variation in this moiety may rather occur at the level of antigen-specific immunoglobulin (excellent recent review in Oosterhoff et al.[7]). On the other hand, changes in global IgG sialylation or galactosylation are associated with autoimmune disease activity.[6] Thus, in theory, alterations in anti-HLA antibody subclass and glycosylation may likewise represent one factor explaining disparate outcomes in transplant recipients with donor-specific antibodies.

Recent efforts to characterize HLA antibodies have used serological subtyping approaches to describe the pattern of antibody subclasses in transplant recipients and their associated risk, although this technique has important limitations (reviewed in Valenzuela and Schaub[2]). Analysis of antibody glycosylation has proven more complex. In 2019, Barba et al. investigated the sialylation status of donor specific antibodies using a lectin purification approach but did not find that high or low sialyl content of IgG was associated with transplant outcome or functional complement-dependent cell cytotoxicity.[9] A follow-up study used mass spectrometry to profile anti-HLA antibody subclass, and then focused on the glycosylation of IgG3.[10] They determined that high sialylation of IgG3 *de novo* DSA was protective from acute antibody-mediated rejection (OR 0.7, $p = 0.002$), while another glycan moiety, bisected GlcNAc, was associated with increased risk (OR 1.23, $p = 0.014$).

In the report in this issue,[1] the Ackerman lab applied sophisticated glycopeptidomics assays to simultaneously analyze the subclass and glycosylation status of anti-donor HLA antibodies purified from transplant recipient serum. They then employed complementary *in vitro* approaches to interrogate the

functional interaction of differentially glycosylated HLA antibodies with FcγRs. Their findings show that anti-donor HLA antibodies exhibit reduced fucosylation compared with total IgG and that specific IgG subclasses display unique patterns of glycosylation. They go on to demonstrate that antibodies lacking fucose moieties trigger enhanced NK cell-mediated cytotoxicity, attributable to prolonged binding to FcγRIIIa and greater FcγRIIIa-induced intracellular signaling. Lastly, the authors find that, among patients with donor-specific antibodies, the degree of fucosylation was lower in those experiencing acute antibody-mediated rejection.

These proof-of-principle data provide evidence that antibody subclass and glycosylation coordinate effector functions, at least *in vitro*, that are relevant to transplant rejection. The data further suggest that examining these dynamic characteristics in a curated, longitudinal population of clinical transplant recipients may discriminate of ''pathogenic'' DSA or have predictive value for rejection risk stratification.

Many new questions arise from these findings. Herein, the authors focused on antibodies directed against one HLA antigen (HLA-A2) based on its high allele frequency in the donor population. Future studies confirming these findings across different HLA genotypes, particularly those directed against the HLA-DQ molecule, will be of high interest to the field. Further, NK cells are one of several FcγR-bearing leukocytes implicated in transplant rejection. The importance of subclass and glycosylation for interaction with monocytes and neutrophils will be an intriguing follow-up study. Similarly, complement activation is a critical pathogenic phenomenon exacerbating inflammation and tissue damage, which has hitherto been defined in *in vivo* assays measuring binding of C1q protein to HLA antibodies. Drilling down on the molecular features of HLA antibodies that more or less potently engage these systems is an exciting new avenue for the field of transplantation, with strong functional relevance to non-transplant contexts such as autoimmune vasculitis.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Bharadwaj, P.S., Sweta, Pongracz, T., Concetta, C., Sharma, S., Le Moine, Alain, de Haan, Noortje, Murakami, N., et al. (2022). Afucosylation of HLA-specific IgG1 as a potential predictor of antibody pathogenicity in kidney transplantation. Cell Reports Medicine, in press.
2. Valenzuela, N.M., and Schaub, S. (2018). The Biology of IgG subclasses and their clinical relevance to transplantation. Transplantation *102* (*Suppl 1*), S7–S13. https://doi.org/10.1097/tp.0000000000001816.
3. Nimmerjahn, F., and Ravetch, J.V. (2005). Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science *310*, 1510–1512. https://doi.org/10.1126/science.1118948.
4. Shinkawa, T., Nakamura, K., Yamane, N., Shoji-Hosaka, E., Kanda, Y., Sakurada, M., Uchida, K., Anazawa, H., Satoh, M., Yamasaki, M., et al. (2003). The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J. Biol. Chem. *278*, 3466–3473. https://doi.org/10.1074/jbc.m210665200.
5. van Osch, T.L.J., Nouta, J., Derksen, N.I.L., van Mierlo, G., van der Schoot, C.E., Wuhrer, M., Rispens, T., and Vidarsson, G. (2021). Fc galactosylation promotes Hexamerization of human IgG1, Leading to enhanced classical complement activation. J. Immunol. *207*, 1545–1554. https://doi.org/10.4049/jimmunol.2100399.
6. Seeling, M., Bruckner, C., and Nimmerjahn, F. (2017). Differential antibody glycosylation in autoimmunity: sweet biomarker or modulator of disease activity? Nat. Rev. Rheumatol. *13*, 621–630. https://doi.org/10.1038/nrrheum.2017.146.
7. Oosterhoff, J.J., Larsen, M.D., van der Schoot, C.E., and Vidarsson, G. (2022). Afucosylated IgG responses in humans - structural clues to the regulation of humoral immunity. Trends Immunol. *43*, 800–814. https://doi.org/10.1016/j.it.2022.08.001.
8. Kapur, R., Kustiawan, I., Vestrheim, A., Koeleman, C.A.M., Visser, R., Einarsdottir, H.K., Porcelijn, L., Jackson, D., Kumpel, B., Deelder, A.M., et al. (2014). A prominent lack of IgG1-Fc fucosylation of platelet alloantibodies in pregnancy. Blood *123*, 471–480. https://doi.org/10.1182/blood-2013-09-527978.
9. Barba, T., Harb, J., Ducreux, S., Koenig, A., Mathias, V., Rabeyrin, M., Pouliquen, E., Sicard, A., Chartoire, D., Dugast, E., et al. (2019). Highly variable sialylation status of donor-specific antibodies does not Impact humoral rejection outcomes. Front. Immunol. *10*, 513. https://doi.org/10.3389/fimmu.2019.00513.
10. Pernin, V., Bec, N., Beyze, A., Bourgeois, A., Szwarc, I., Champion, C., Chauvin, A., Rene, C., Mourad, G., Merville, P., et al. (2022). IgG3 donor-specific antibodies with a proinflammatory glycosylation profile may be associated with the risk of antibody-mediated rejection after kidney transplantation. Am. J. Transplant. *22*, 865–875. https://doi.org/10.1111/ajt.16904.