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Antigen-specific antibody Fc-glycosylation enhances humoral immunity via the recruitment of complement

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Abstract

HIV-specific broadly neutralizing antibodies (bNAbs) confer protection following passive immunization, but the immunological mechanisms that drive their development following HIV infection are poorly understood. Structural features of bNAbs indicate that they originate from extensive germinal center selection, which relies on persistent germinal center activity. However, why a fraction of infected individuals are able to successfully drive more effective affinity maturation is unclear. Delivery of antigens in the form of antibody-immune complexes (IC), that bind to complement receptors (CRs) or Fc-receptors (FcRs) on follicular dendritic cells (FDCs), represents an effective mechanism for antigen delivery to the GC. Thus we sought to define whether IC-FcR or CR interactions differ among subjects who develop broadly neutralizing antibody responses to HIV. Enhanced Fc-effector functions and FcR/CR interactions, via altered Fc-glycosylation profiles, were observed among subjects with neutralizing antibody responses to HIV compared to subjects without neutralizing antibody activity. Moreover, both polyclonal Neutralizer ICs and monoclonal-IC mimics of Neutralizer antibodies induced higher antibody titers, higher-avidity antibodies, and expanded germinal center B cell reactions following immunization of mice, via accelerated antigen deposition within B cell follicles in a complement dependent manner. Thus, these data point to a direct role for altered Fc-profile/complement interactions in shaping the maturation of the humoral immune response, providing insights on how GC activity may be enhanced to drive affinity maturation in next generation vaccine approaches.

Conclusion

While efforts towards vaccine design to induce broadly neutralizing antibodies have resulted in limited success in driving weak tier 2 heterologous immunity, understanding the immunological features that allow some individuals to develop broad cross-tier neutralizing breadth may accelerate the path to the induction of these unusual, but protective, responses. Here, we observed that individuals who developed bNAbs possessed HIV-specific antibodies with enhanced CR and FcγR binding, selectively skewed to induce phagocytosis and complement deposition, via enhanced HIV-specific antibody Fc-domain sialylation. Given the importance of Fc binding for antigen deposition in the GC and our emerging appreciation for enhanced and persistent GC activation among individuals with neutralizing antibodies, we speculated that the enhanced Fc functionality could actively contribute to the evolution of HIV-specific neutralization breadth. Remarkably, ICs generated with either antibodies from individuals with these enhanced Fc functions or with highly sialylated monoclonal antibodies induced both higher antibody titers and more avid antibodies following IC immunization via recruitment of complement. Thus, these data suggest that the Fc domain of the antibody may play a critical role in the formation of the GC reaction, catalyzing the persistent affinity maturation of B cells that may be required to drive the unusual B cell selection associated with the development of bNAbs against HIV.

Fig.1: Selective Fc functional profile differences exist among Neutralizers

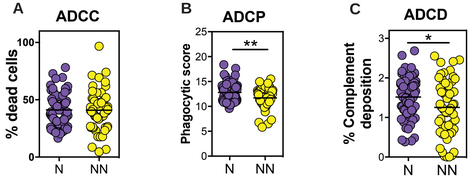


Fig.1: Unique Fc functional profiling of HIV specific antibodies from Neutralizers. Serum antibodies from Neutralizers (N=purple) and Non-Neutralizers (NN=yellow) were evaluated for their ability to drive NK-dependent ADCC against gp120-pulsed CD4+ cells (A), monocyte directed phagocytosis of gp120-functionalized fluorescent beads (B), or complement deposition (C3b) on the surface of gp120-pulsed CD4+ target cells (C). An unpaired t-test was used for statistical analysis: *p<0.05, **p<0.01.

References: Burton DR and Mascola JR Nat Immunol 2015; Borrow P and Moody MA, Immunol Rev. 2017; Mascola JR and Haynes BF, Immunol Rev 2013; Dugast AS et al., Clin Infect Dis 2017; Carroll MC et al., Immunity 2012; Batista FD et al., Nat Rev Immunol 2009; Heesters BA et al., Immunity 2013; Wang T et al., Cell 2015; Chung AW et al., Cell 2015.

Fig.2: Enhanced Fc-receptor and complement binding by HIV-specific antibodies in Neutralizers.

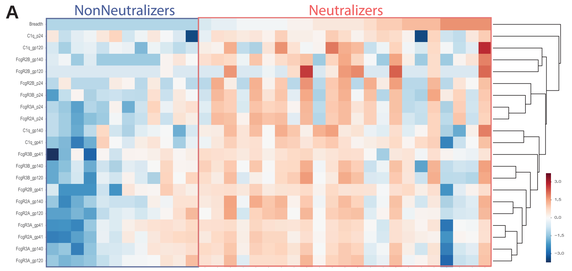


Fig.2: Enhanced Fc-receptor and complement binding by HIV-specific antibodies in Neutralizers. (A) The heatmap depicts the binding capacity (MFI) of gp140-, gp120-, gp41-, p24-specific serum antibodies to FcγRIIA, FcγRIIB, FcγRIIIA, FcγRIIIB and C1q protein. Each row represents a specific binding except for row 1 where the HIV neutralization breadth is reported. Each column represent one subject, either a Neutralizer (purple) or a non-Neutralizer (yellow). The scale bar represents the Z-normalized scores. (B) The dot plots report the univariate binding of gp120-specific antibodies from each group to FcγRIIA, FcγRIIB, FcγRIIIA, FcγRIIIB and C1q. (C) The dot plot shows the evolution of gp120-specific FcγRIIa binding antibodies in the first year following infection in a group of participants caught in acute HIV infection half of which go on to develop bNAbs (purple) or not (yellow). Each dot represents one subject, with Neutralizers (N) in violet and Non-Neutralizers (NN) in yellow. An unpaired t-test was used for statistical analysis to compare groups, whereas an Anova with a post-hoc Tukey test was used to compare between groups across timepoints. *p<0.05, **p<0.01.

Fig.4: Unique antigen-specific antibody glycosylation and elevated IgG1 are enriched among Neutralizers

Fig. 4. Sialylated IgG1 gp120-specific antibodies are increased in Neutralizers. (A) The Radar plot describes the relative differences in gp120-specific IgG1, IgG2, IgG3 and IgG4 antibody titers in sera from Neutralizers (N, red) or Non-Neutralizers (NN, blue). (B) The dot plot shows the gp120-specific IgG1 antibody titer differences. (C) The radar plot describes the relative distribution of gp120-specific IgG antibody glycan levels in sera from Neutralizers (N, red) or Non-Neutralizers (NN, blue). (D) The dot plot depicts the percentage of sialylated gp120-specific IgG antibodies among the groups. An unpaired t-test was used for statistical analysis: *p<0.05. (E) The dot plot depicts the PLSDA analysis using all Fc-profile data, including gp120-specific antibody titers, glycan profiles, binding to Fc-receptors and complement proteins, ADCP, ADCD and ADCC, to separate the 2 groups. (F) Variable Importance in Projection (VIP) scores rank the minimal antibody features that are able to separate the Neutralizers (red bars) and non-Neutralizers (blue bar) Fc-profiles.

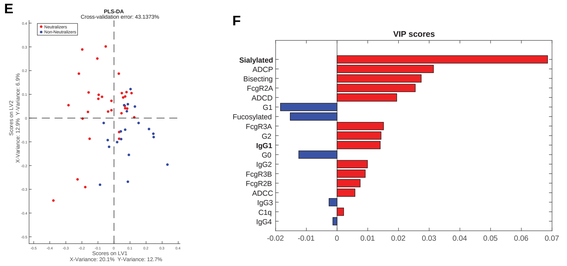


Fig.3: HIV-specific antibodies from Neutralizers promote germinal center reactions

Fig.3. Neutralizer-immune complexes drive higher avidity antibodies and expanded germinal center B cells. BALB/c mice were immunized twice, 3 weeks apart, with alum-adjuvanted gp120 immune complexes (ICs-alum) generated with serum antibodies from Neutralizers (N) or Non-Neutralizers (NN). Control groups received alum-adjuvanted gp120 or adjuvant alone. Ten days after the last immunization we measured the titers of gp120-specific IgG antibodies (A), the titers of high-avidity gp120-specific IgG antibodies (B) in sera, and the percentage of germinal center B cells (C), defined as live CD3-CD19+CD38-CD95+ cells, in draining lymph nodes of immune mice. A two-sided Wilcoxon rank sum test was use to compare responses induced by Neutralizer (N) or Non-Neutralizer (NN) immunized animals. *p<0.05

Fig.5: HIV antibody glycoforms modulate IC-driven innate and adaptive immunity via recruitment of complement

Fig. 5. Sialylated antibody-immune complexes promote innate and adaptive immunity. (A) The dot plot depicts the antibody titers (left) or avid antibody titers (right) 10 days after the second immunization in Balb/c mice receiving alum alone (green), alum-adjuvanted gp120 (blue), agalactosylated antibodies (G0-Immune Complexes) with alum (aqua), non-sialylated galactosylated antibodies (G1/G2 NS-ICs) with alum (lavender), or sialylated galactosylated antibodies (G1/G2 S-ICs) with alum (pink). (B) The bar graph depicts the number of fluorescently labeled ICs on draining lymphnode non-cognate B cells (gp120+B cells), macrophages (gp120+SSM), or FDCs (gp120+FDCs) after 1h and 3h post injection. (C) The confocal image depicts the level of IC deposition after 1h in a B cell follicle from a mouse immunized with a non-sialylated IC (left) or a sialylated IC (right). (D) The bar graph shows the number (left) and area occupied (right) of gp120+FDCs in a draining lymphnode. (E) The confocal image depicts IC deposition in a B cell follical 3h after immunization, stained for the antigen (blue), macrophages (green), and GL-7: to detect the germinal center area (red). (F) The line graphs show the role of FcγR2b and complement in glycan-modified IC deposition on a non-cognate B cell, Raji, cells. (G) The dot plot shows the role of complement in the glycan-optimized IC immunization using C57/Black wild type (wt, n=1–4) or C1q-/- knockout (C1q-/-, n=1–4) mice. Both, gp120-specific IgG antibody titers (left) and high-avidity gp120-specific antibody titers (right) are depicted following immunization with alum (white), gp12-alum (blue), non-sialylated galactosylated antibodies (NS-ICs, yellow), or sialylated galactosylated antibodies (S-ICs, pink). One-way ANOVA followed by Tukey's post-test was used for statistical analyses: *p<0.05, **p<0.01, ***p<0.001.

