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Leveraging neutrophils to control HIV via IgA

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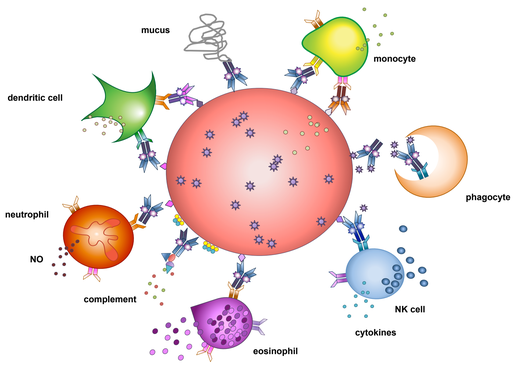
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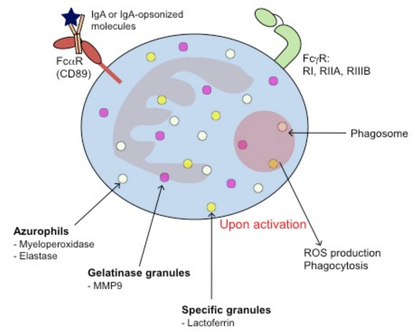
Introduction and Method

Neutrophils are the most abundant white blood cell and are rapid responders to infections. Neutrophils are also the most potent mediators of Fc-effector functions and in addition to phagocytosis, neutrophils degranulate and can kill through the formation of extracellular traps. However, little is known about the role of neutrophils in antiviral control of HIV. Thus, here we aimed to define the role of neutrophil-recruiting antibodies in antiviral control of HIV.



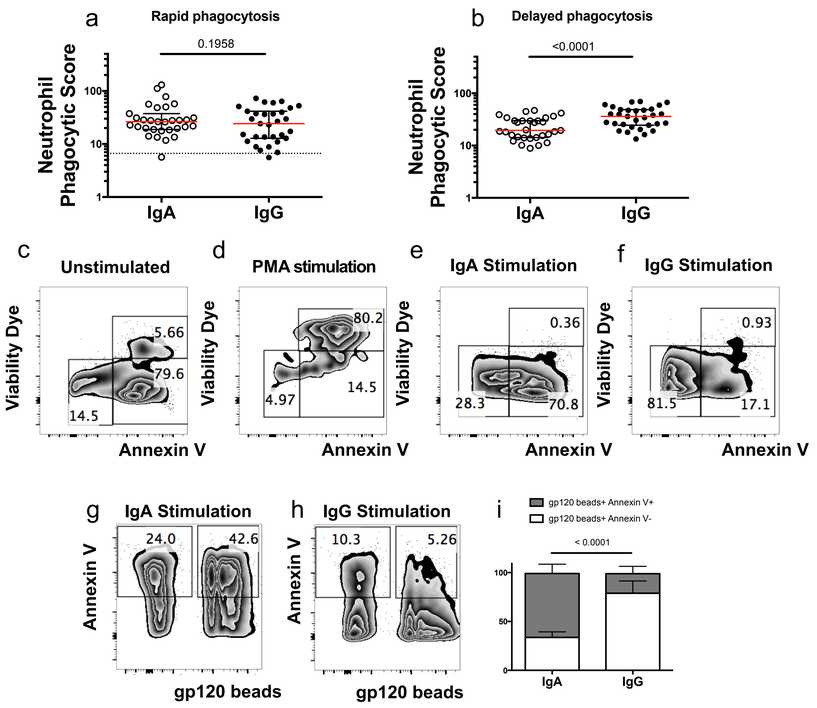
Because neutrophils express both an IgA and IgG Fc-receptor, both IgA and IgG were purified from HIV infected patient serum and compared to healthy controls. The functional activity of these antibodies were compared for phagocytic, degranulation, NET formation, and release of elastase activity, along with other degranulation markers. Further, we looked at the impact of the same IgA specific functions in RV144 subjects, where IgA was considered a correlate of risk.

Neutrophils maintain activity through functions beyond phagocytosis such as, cytokine secretion and ROS production during degranulation. The release of cytokines and degranulation markers is therefore imperative since these can trigger other responses during an infection and are a direct measure of neutrophil activation. The order of release of granular content is the opposite to the order they are formed and can be measured by the upregulation or release of markers like Myeloperoxidase (MPO) and Elastase, Lactoferrin and MMP-9, all neutrophil markers triggered by different signals during an immune response.



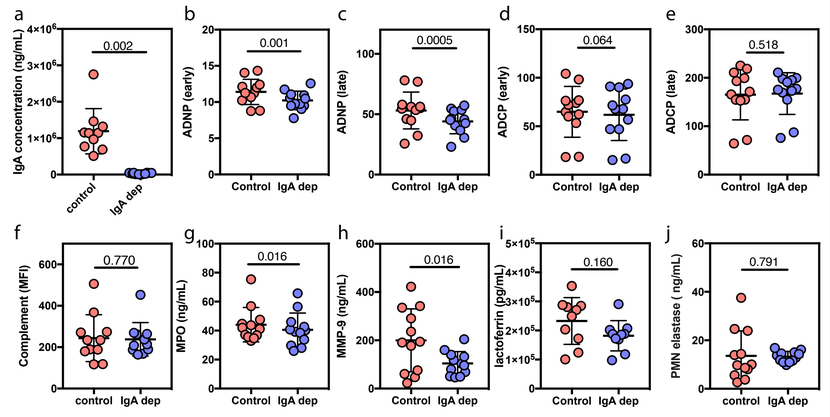
Gp120-specific IgA drives neutrophil phagocytosis and killing.

Figure 2. The ability of gp120-directed IgA and IgG antibodies from HIV-infected donors to drive antibody dependent neutrophil phagocytosis was assessed at the early time point (rapid phagocytosis), 40 minutes post-stimulation, on (a) blood neutrophils and (b) cervical neutrophils as well as at the later time point (delayed phagocytosis), 24 hours post-stimulation (c). Dotted line indicates neutrophil response to gp120-beads (no antibodies). Lack of stimulation (d), FcαR stimulation (f) but not FcγR stimulation (g) induced an apoptotic phenotype in neutrophils. PMA stimulation (positive control) induced complete death of neutrophils (e). The majority of neutrophils that phagocytosed gp120-coated beads via FcαR (h) display an apoptotic phenotype, in contrast to neutrophils that phagocytosed gp120-coated beads via FcγR (i). Quantification of live and apoptotic neutrophils post IgA- and IgG-stimulation is illustrated in (j).



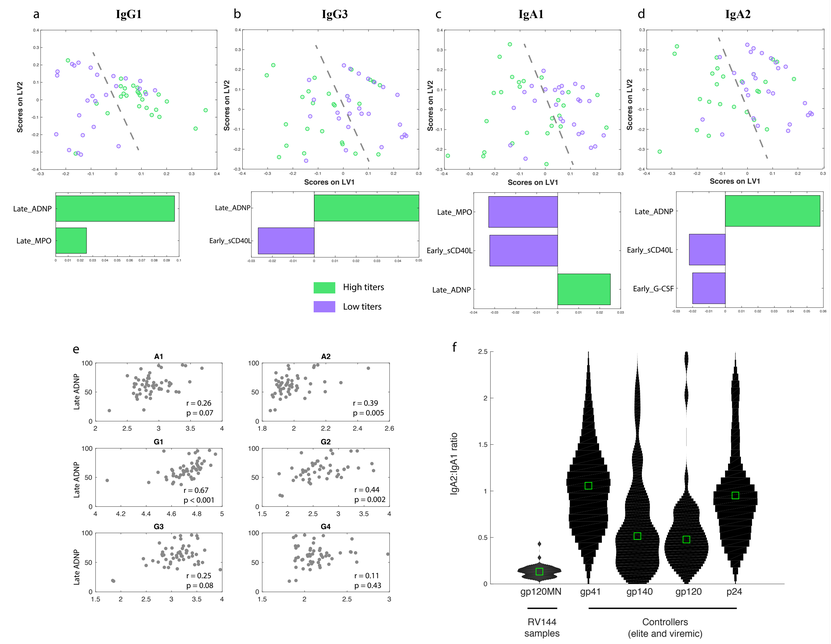
Depletion of total IgA from RV144 patients impacts gp120-specific ADNP, but has no impact on other functions.

Figure 4. Decrease in ADNP (b,c) but not ADCP (d,e) or ADCD (f) with IgA depletion (a) in RV144 patients, corresponds decrease in MPO (g) and MMP-9 (h). Lactoferrin (i) and PMN elastase (j) stay unchanged suggesting that the IgA directs neutrophil phagocytosis through a specific pathway. Levels of MPO, MMP-9, Lactoferrin and PMN released Elastase were measured by ELISA using the supernatant from late phagocytosis.



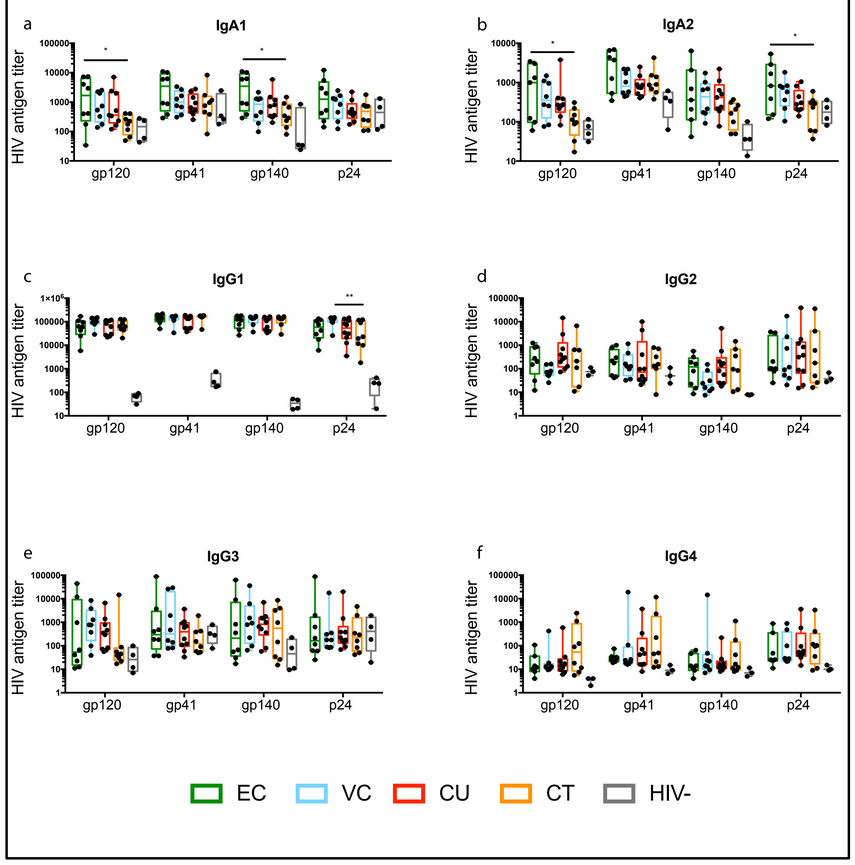
High gp120-IgA1 and IgA2 titers correlate strongly with ADNP. IgA2 is important for proper IgA function.

Figure 5. Individual multivariate models for IgG and IgA subclasses based on functions, cytokines, activation and degranulation markers. Separation of high (green) vs. low (purple) titers, shows Late ADNP correlates highly with (a) IgG1, (b) IgG3, (c) IgA1 and (d) IgA2, but IgA1 the least. (e) ADNP correlates highest with IgA2 and IgG1 even in RV144. (f) Balance of IgA2:IgA1 is important for IgA. For controllers, the A2:A1 ratio is significantly higher than RV144 samples across all 4 antigens they have A2:A1 ratio around 1.



HIV-Specific IgA1 and IgA2 are elevated in Elite and Viremic controllers across multiple antigens.

Figure 1. HIV-specific IgA1 (a), IgA2 (b), IgG1 (c), IgG2 (d), IgG3 (e) and IgG4 (f) plasma titers in HIV-infected patients within different clinical subgroups: elite controllers (EC, green), viremic controllers (VC, blue), chronic untreated (CU, red), chronic treated (CT, orange) and HIV-uninfected controls (HIV-, grey).



IgA drives formation of NETs and promotes degranulation upon HIV infection.

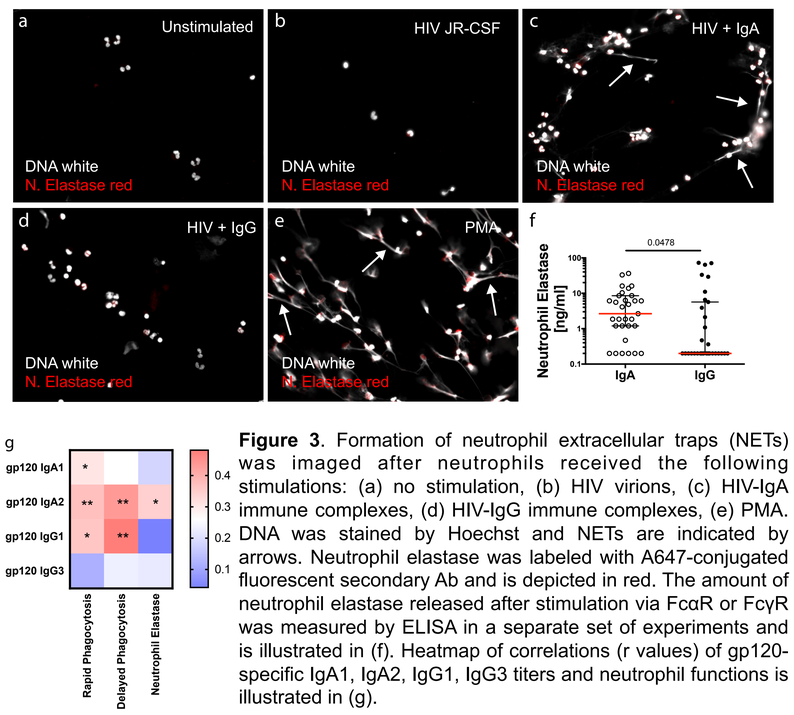


Figure 3. Formation of neutrophil extracellular traps (NETs) was imaged after neutrophils received the following stimulations: (a) no stimulation, (b) HIV virions, (c) HIV-IgA immune complexes, (d) HIV-IgG immune complexes, (e) PMA. DNA was stained by Hoechst and NETs are indicated by arrows. Neutrophil elastase was labeled with A647-conjugated fluorescent secondary Ab and is depicted in red. The amount of neutrophil elastase released after stimulation via FcαR or FcγR was measured by ELISA in a separate set of experiments and is illustrated in (f). Heatmap of correlations (r values) of gp120-specific IgA1, IgA2, IgG1, IgG3 titers and neutrophil functions is illustrated in (g).

Conclusions

Sera from elite controllers are enriched in HIV-specific IgA1 and IgA2 antibodies (Figure 1). In contrast, no major differences were observed in the distribution of IgG subclasses across HIV+ subjects in different clinical groups.

HIV-specific IgA is able to efficiently drive neutrophil phagocytosis, induce cell death over a 24 hour period (Figure 2) and subsequently induce NETs and robust neutrophil elastase release (Figure 3).

The IgA2 subclass was more potent in driving strong neutrophil responses compared to IgA1 antibodies. In addition, IgA2 is the only subclass that correlated with degranulation and NET formation.

To understand the discrepancy between beneficial association of HIV-specific IgAs in spontaneous controllers and increased risk of infection in RV144-HIV vaccine trail recipients, we identified that IgA antibodies in both, controllers and vaccines, activate neutrophils in the same manner. Depletion of total IgA from RV144 patients gave rise to a decrease in gp120-specific ADNP, but did not have an impact on other functions (Figure 4). Furthermore, as seen in controllers, depletion of IgA also decreased abundance of MPO and MMP-9, suggesting that degranulation of neutrophils was an IgA driven phenomenon, specifically IgA2.

Abundance of IgA2, the stronger neutrophil activator, was diminished in RV144-recipients (Figure 5), which was not the case in controllers, highlighting the importance of IgA2 for function.

Acknowledgments

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