Requirement for Fc Effector Mechanisms in the APOBEC3/Rfv3-Dependent Neutralizing Antibody Response

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Antiretroviral neutralizing antibody (NAb) responses are often evaluated in the absence of Fc-dependent immune effectors. In murine Friend retrovirus infection, APOBEC3/Rfv3 promotes a potent polyclonal NAb response. Here, we show that the APOBEC3/Rfv3-dependent NAb response correlated with virus-specific IgG2 titers and that the in vivo neutralization potency of APOBEC3/Rfv3-resistant antisera was dependent on activating Fcy receptors but not complement. The data strengthen retroviral vaccine strategies aimed at eliciting NAbs that activate specific Fcy receptors.

Neutralizing antibodies (NAbs) are critical for an effective vaccine response (1). However, antibody neutralization is often evaluated in vitro in the absence of immune cells (2). In vivo, the crystallizable fragment of IgG (Fcy) can trigger innate immune effectors to neutralize the pathogen. Cross-linking of Fcy receptors (FcγRs) on the surface of effector leukocytes can induce antibody-dependent cellular phagocytosis (ADCP) of immune complexes or antibody-dependent cell-mediated cytotoxicity (ADCC) of infected cells (3). Fcy also activates the complement cascade leading to the lysis or opsonization of immune complexes (4). Recent studies show that the in vivo activity of HIV-1 broadly neutralizing antibodies (bNAbs) is highly dependent on interactions with FcγRs but not complement (5, 6). However, it remains unclear whether a polyclonal NAb response, such as one elicited by a vaccine, would have similar requirements.

Mouse models of retroviral infection provide a genetically tractable system to investigate the role of Fcy-mediated effector mechanisms. Mice carry three activating FcγRs (i.e., FcγRI, FcγRIII, and FcγRIV), each of which consists of an Fcγ-chain and a signal-transducing common γ-chain. Mice deficient in the common γ-chain (FcerIγ−/−) lack surface expression of the activating FcγRs and produce macrophages and NK cells abrogated in ADCP and ADCC activity, respectively (3, 7). The murine IgG subclasses IgG1, IgG2a, and IgG2b (referred to here as IgG2) exhibit various affinities to each FcγR, resulting in different potencies in vivo. IgG2a and IgG2b exhibit the most potent in autoimmune and tumor models (8). Of note, FcγRIV exclusively binds IgG2 (9), and the in vivo activity of IgG2 is impaired in mice deficient in the α-chain of FcγRIV (Fcγ4−/−) (10).

Friend retrovirus (FV) infection in mice is a well-characterized model to investigate retroviral immunity. FV is a complex of a replication-competent Friend murine leukemia virus (F-MuLV) and a replication-defective spleen focus-forming virus (SFFV) that causes erythroleukemia in adult immunocompetent mice (11). Our group and others showed that mouse APOBEC3 (mA3) encodes Recovery from Friend retrovirus 3 (Rfv3), a gene that influences the recovery from viremia by promoting a stronger NAb response (12). Rfv3-resistant mice such as B6 express about 10-fold more mA3 mRNA than Rfv3-susceptible strains such as BALB/c and A.BY mice (13). As in other viral systems, the data on the antibody neutralization differences between mA3/Rfv3-resistant and -susceptible mice were based on in vitro NAb assays (12, 14, 15). The mA3/Rfv3 paradigm thus provides a potentially useful system to confirm whether in vivo neutralization predicts in vivo neutralization and, through the use of gene knockout (KO) mice, directly test the role of Fc effectors in polyclonal antibody neutralization.

Rfv3 was originally defined in (B6 × A.BY)F1 mice that are highly susceptible to infection with FV containing lactate dehydrogenase-elevating virus (LDV) but eventually recover from viremia and splenomegaly (16). We showed that deletion of B6 mA3 in (B6 × A.BY)F1 mice resulted in weaker NAb responses and abrogated recovery from viremia and disease by 28 days postinfection (dpi) (14, 17). Removal of B6 mA3 in a pure B6 genetic background also resulted in weaker NAb responses (14, 15). However, B6 mice are Fv2 resistant, and thus, splenomegaly does not occur (18). To more closely capture conditions resulting in immune pathology that is countered by mA3/Rfv3, we infected (B6 × A.BY)F1 mice (referred to as mA3+/−/−) and (B6 mA3−/− × A.BY)F1 mice (referred to as mA3−/−/−) with 1,400 spleen focus-forming units (SFFU) of FV/LDV and then harvested and heat-inactivated plasma at 28 dpi (Fig. 1A). Mice (<1 year of age) were handled in accordance with IACUC guidelines [permit B-89709(10)]1E]. As expected, mA3+/−/− antisera had significantly higher NAb titers using a previously described in vitro NAb assay (Fig. 1B) (12, 14, 15). Antisera were pooled at equal volumes, and 3 μl of pooled antisera was coincubated with 140 SFFU of FV in 300 μl for 1 h at 37°C prior to administration in susceptible BALB/c mice. At 7 dpi, the percentages of infected bone marrow cells were evaluated by flow cytometry using a monoclonal antibody (MAb) against the FV glyco-gag protein (Fig. 1A) (19). Compared to control sera from uninfected mice, mA3+/−/− antisera conferred significant protection, whereas mA3−/−/− antisera did not.
Thus, the mA3/Rfv3-dependent NAb response was recapitulated by passive transfer in vivo. We note that the in vivo neutralization potency of mA3/H11001/sanisera at a 1:100 dilution is likely an underestimation in the mice that generated these antibodies, as we were unable to utilize neat plasma due to the limited amounts of plasma that can be obtained from individual mice.

We recently derived and characterized 176 FV-specific MAbs from mA3/H11001/sand mA3/H11002/smice at 21 and 28 dpi (Fig. 2A). We observed a trend for higher levels of IgG2 MAbs in mA3/H11001/smice than in mA3/H11002/smice but not for the other isotypes or IgG subclasses (Fig. 2A). To test if infected mA3/H11001/smice produce higher titers of FV-specific IgG2 antibodies, we quantified virus-specific titers of the different isotypes and IgG subclasses by endpoint titration enzyme-linked immunosorbent assay (ELISA) (19). mA3/H11001/smice produced higher levels of virus-specific IgG2 against native virions than mA3/H11002/smice but similar IgG1, IgG3, and IgM titers (Fig. 2B). Similar results were observed for a different genetic background, (B6 × BALB/c)F1 (Fig. 2C). These data suggest that mA3/Rfv3 resistance is associated with higher IgG2 titers against native virions.
FIG 3 Antisera from Rfv3-resistant mice require Fcγ-mediated effectors to neutralize FV in vivo. (A) Overview of in vivo NAB assay. Pooled antisera from mA3+/s mice at 28 dpi or control antisera from uninfected mA3−/− mice were incubated with 100 SFFU of FV prior to inoculation of various strains of B6 mice. At 7 dpi, splenocytes were harvested and subjected to spleen infectious-center assays. (B) Recipient mice included B6 WT, B6 Fcer1g−/−, B6 C3−/−, and B6 Fgr4−/− mice. Infectious centers per spleen are shown, where each dot represents spleen from one mouse. Bars represent medians, and differences were evaluated by the 2-tailed nonparametric Mann-Whitney U test. **, P < 0.01; *, P < 0.05; ns, not significant.

To test if the in vivo potency of mA3/Rfv3-resistant antisera is dependent on FcγRs or complement, we performed an in vivo neutralization assay in common-γ-chain KO (B6 Fcer1g−/−) (7) and complement component 3 KO (B6 C3−/−) mice (21). Pooled 28-dpi antisera from FV-infected mA3+/s or uninfected mice (3 μl) were incubated with 100 SFFU of FV prior to injection into B6 wild-type (WT), B6 Fcer1g−/−, or B6 C3−/− mice (Fig. 3A). At 7 dpi, splenocytes were harvested and evaluated for infection levels using a spleen infectious-center (IC) assay (13). For each host genetic background, the in vivo neutralization potency of mA3+/s antisera was compared to that of control antisera from uninfected mice. B6 WT mice were significantly protected by the mA3+/s antisera, but B6 Fcer1g−/− mice were not (Fig. 3B). Of note, we detected lower viral loads in the B6 C3−/− mice, consistent with enhanced FV infection of B cells through complement opsonization (22). Interestingly, mA3+/s antisera significantly protected B6 C3−/− mice. These results suggested that FcγR, but not complement, was required for the neutralization activity of mA3/Rfv3-resistant antisera in vivo.

The characterization of several FV-specific MAbs in the early 1980s suggested that IgG2 MAbs may be particularly effective at neutralizing FV in vitro and in vivo (23). However, the contribution of IgG2 antibodies in the in vivo neutralization activity of mA3/Rfv3-resistant antisera remains unclear. To selectively assess the role of FcγR-mediated effectors induced by IgG2 antibodies, we performed passive immunization studies in B6 FcγR4−/− mice (10) and found no significant protection in these mice (Fig. 3B). These data suggested that the in vivo activity of mA3/Rfv3-resistant antisera depends on the interactions between IgG2 antibodies and FcγRIV.

In conclusion, we demonstrate that the mA3/Rfv3-dependent NAB response can be recapitulated by passive transfer in vivo. Notably, the passive protection conferred by anti-FV polyclonal NABs was dependent on activating FcγRs but not complement. These results parallel data obtained for monoclonal bNAbs against HIV-1 (5, 6), suggesting that FcγR-mediated effector mechanisms are evolutionarily conserved processes for IgG-mediated retroviral clearance and play an important role even if multiple B cell epitopes are targeted simultaneously. Of note, our findings seemed at odds with a recent report that concluded that antibody class switching—and, by extension, IgG antibodies—is dispensable for recovery from FV infection (24). However, the first part of this study utilized B6 mice, which are Fv2 resistant and spontaneously recover. Fv2-susceptible (B6 × BALB/c)F1 mice were also studied at 14 dpi, but potent FV IgG NABs do not develop until later (14, 25). We analyzed neutralizing antisera from Fv2-susceptible mice at 28 dpi to directly link our data to immune recovery mediated by mA3/Rfv3.

Interestingly, mA3/Rfv3 resistance was associated with higher FV-specific IgG2 titers. Moreover, in vivo passive protection was dependent on FcγRIV, suggesting a central role for IgG2 antibodies in plasma neutralization. Although uninfected B6 mA3−/− mice do not have defects in antibody class switching (26), mA3 could enhance IgG somatic hypermutation during FV infection (20). Thus, mA3 may directly enhance IgG2 class switching. Alternatively, the increased number of infectious virions in mA3−/− mice (19, 27) may lead to deregulated class switching. Further studies are needed to determine which FcγRIV-expressing cells are required for in vivo antibody neutralization. Overall, our findings support the emerging concept that retroviral vaccines should account not only for direct antibody neutralization but also for the ability to activate specific Fcγ receptors through the induction of the appropriate IgG subclasses.

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