Ebola from Bedside to Bench

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A longitudinal study by Davis et al. followed the evolution of antibody responses in four survivors of the 2014 Ebola outbreak treated in the United States and provides insight into the emergence of neutralizing antibodies long after convalescence.

Zaire ebolavirus (EBOV) is a negative-sense single-stranded RNA virus that is the etiologic agent of the highly lethal Ebola virus disease. Between 2014 and 2016, the largest EBOV outbreak ever documented took place in West Africa, where over 28,000 individuals were infected and more than 11,000 died. In late 2014, four EBOV-infected patients were treated at the Emory University Hospital, all of whom survived the infection. These events provided the unique opportunity to follow the development and maturation of antibody responses to the virus for over two years (Figure 1A). In this issue of *Cell*, Davis et al. (2019) report on their analysis of this progression in extraordinary detail.

In response to viral infections, antibodies undergo an intricate process of adaptation to the viral antigens known as affinity maturation. B cells responding to the virus proliferate intensively to form germinal centers (GCs), temporary structures in secondary lymphoid organs where B cell clones randomly edit their immunoglobulin genes through somatic hypermutation (SHM). In Darwinian-like fashion, B cells bearing somatic mutations that increase affinity for antigen leave more progeny, leading to enrichment for higher-affinity somatic variants across the population (Mesin et al., 2016). Sporadic differentiation of GC B cells into antibody-secreting plasma cells throughout this evolutionary process underlies the improvement in the affinity of serum antibodies over time.

The extent to which affinity maturation is required for the generation of protective antibodies varies from virus to virus. In influenza, for example, low-affinity antibodies emerging early after infection have been reported to be protective (Miyauchi et al., 2016), even though the generation of broadly neutralizing antibodies cross-reactive to antigenically drifted viral strains may require further maturation (Corti and Lanzavecchia, 2013). By contrast, in dramatic cases such as HIV, it may take several years of continuous evolution before antibodies with hundreds of somatic mutations emerge that are capable of neutralizing broadly across viral strains (Burton and Hangartner, 2016). Understanding how the relationship between affinity maturation and protection plays out for each particular virus can instruct the development of vaccines and immunotherapies.

In the case of EBOV, although multiple neutralizing antibodies targeting the glycoprotein (GP) on the viral surface have previously been isolated from infected patients and shown to protect animals from viral challenge (Saphire et al., 2018), little is known about the kinetics of the emergence of these antibodies after infection and the range of epitope specificities that allow neutralizing activity. Davis et al. show that, whereas antibody titers to EBOV are detectable quickly after infection, neutralizing antibodies in plasma arise only gradually, peaking at about 1 year after infection (Figure 1B). Strikingly, EBOV-specific IgG_4 antibody titers appear to rise abruptly around this same time, suggesting that the B cell response in these patients continues to actively evolve, even at these very late time points.

Sequencing of the Ig genes of single EBOV GP-binding circulating B cells allowed the authors to identify B cell lineages involved in the EBOV response. These lineages were then traced back in time using RNA samples extracted from bulk blood mononuclear cells from earlier time points, allowing for a longitudinal analysis of their evolution by somatic mutation. Gradual increases in the mutational content of GP-binding B cells in three out of four patients reinforced the notion that the B cell response to EBOV remains active for many months after viremia is cleared. Future studies should be able to determine whether this is related to the long-term persistence of EBOV in sanctuary sites such as the eye, testes, and brain.

Next, in a formidable series of experiments, the authors cloned and produced over 300 monoclonal antibodies (mAbs) based on the heavy- and light-chain V-region BCR sequences obtained from GP-binding B cells and mapped their targeted epitopes at various levels of resolution. Using a stable cell line displaying native or thermolysin-cleaved GP (this cleavage, which mimics what occurs naturally to GP upon viral exposure to endosomal cathepsins, removes the apical glycan cap and mucin-like domains of the protein), the authors found that the vast majority of antibodies were reactive to cleaved GP only. This indicates that the region of the protein exposed by the protease is strongly immunodominant. Strikingly, however, all of the clones capable of neutralizing EBOV *in vitro* were found among the small fraction of clones capable of binding native GP (Figure 1C). This bias was confirmed by detailed epitope mapping of the neutralizing antibodies using an arsenal of techniques, including competitive ELISAS, protein truncations, viral escape mutants, cell-surface display of a mutant GP library, and scanning against overlapping linear peptides. Binding sites were identified in the glycan cap, GP1 core, GP1/2 interface, fusion loop, stalk (HR2), and membrane-proximal extracellular region (MPER) of GP, all of which are exposed in native GP. This strong negative

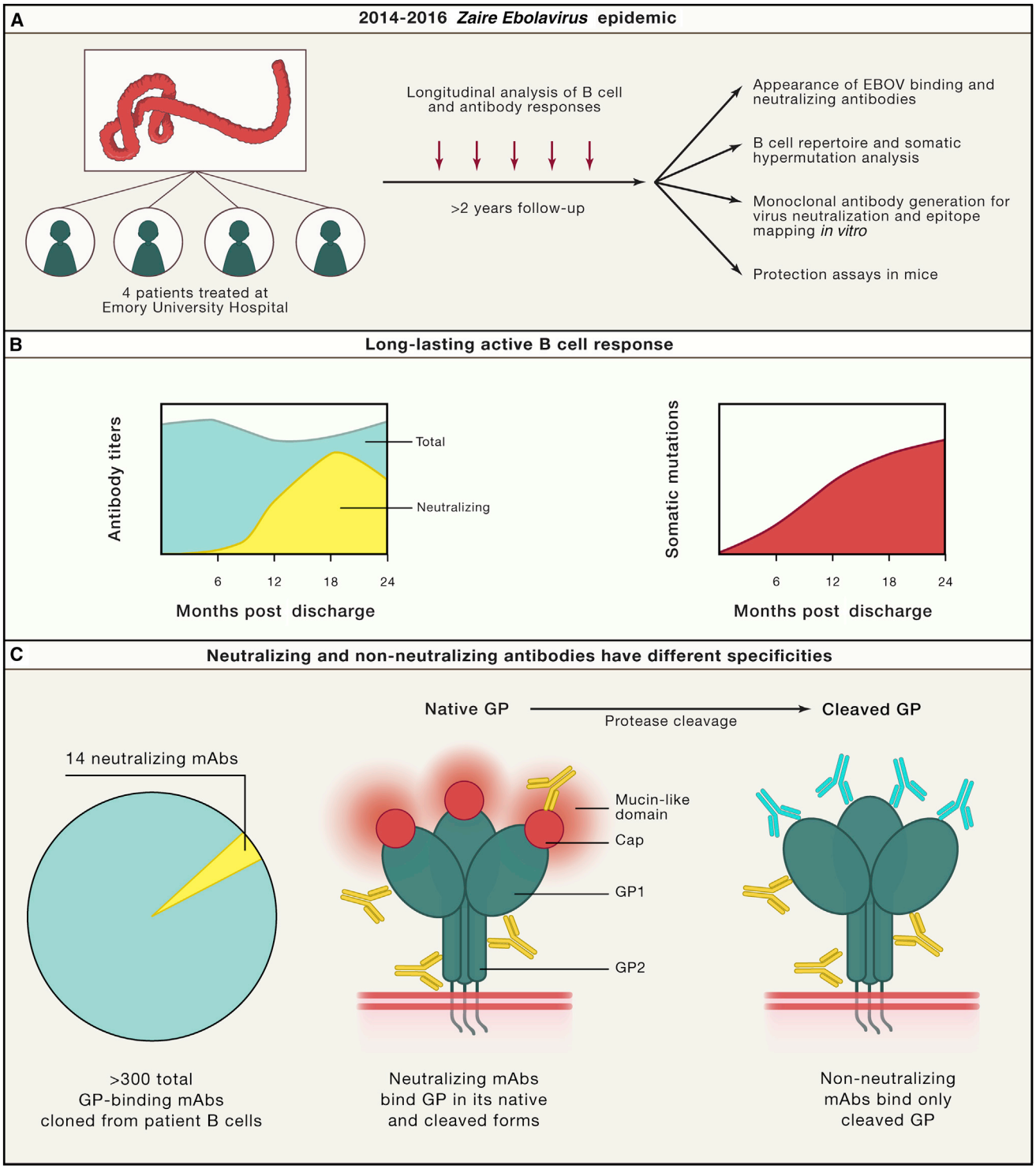

Figure 1. Longitudinal Analysis of Antibody Responses in Ebola Virus Disease Survivors

(A) During the 2014–16 West African EBOV epidemic, four patients were treated at the Emory University Hospital, allowing for a detailed follow up of their antibody responses.

(B) Patients showed evidence of a long-lasting active B cell response, with progressive somatic hypermutation and late appearance of neutralizing antibodies at >1 year post infection.

(C) Cloning of hundreds of monoclonal antibodies from memory B cells that bound EBOV glycoprotein (GP) showed that ability to bind the native form of GP expressed on a cell surface typifies the small minority of monoclonals that have neutralizing activity.

association between immunodominance and neutralization suggests that cleaved GP produced during infection may serve as a decoy, swaying the antibody response away from key neutralizing epitopes through clonal competition.

Previous studies have shown that the performance of EBOV-specific mAbs in neutralization assays does not perfectly forecast their protective capacity *in vivo* (Saphire et al., 2018). For example, the antibody cocktail MB-003, consisting of mAbs targeting GP glycan cap and mucin-like domains, is a poor neutralizer *in vitro* but strongly protects *in vivo* (Olinger et al., 2012; Wilson et al., 2000). Conversely, mAb KZ52, targeting the fusion loop of GP, performs well in neutralization assays but is not protective *in vivo* (Maruyama et al., 1999; Oswald et al., 2007). Davis et al. show that several of their neutralizing mAbs were also able to protect mice from EBOV challenge, as was one non-neutralizing mAb that showed exceptionally strong binding to native GP *in vitro*. Interestingly, mAbs conferring *in vivo* protection were cloned from both early and late time points, suggesting that they may have played a role in controlling the primary infection.

The work by Davis et al. also raises a number of questions for future studies. One is the relationship between affinity maturation and neutralizing activity. While neutralization arises late, after EBOV-specific lineages have accumulated substantial somatic mutation, it is not clear that the mutations themselves are required for efficient neutralization, a notion that is supported by the identification of potent antibodies also at early time points. Future studies where the most effective antibodies are progressively reverted to their unmutated configurations should shed light on when in their evolution neutralizing activity actually emerged. A related but distinct finding is that the epitopes targeted by serum antibody appear to change over time from the early focus on immunodominant epitopes present only on cleaved GP to a later spread toward neutralizing epitopes present on both native and cleaved forms. How competition or cooperation between B cell clones with different specificities leads to this shift over time should provide an interesting case study on the rules governing B cell clonal dynamics.

Valuable lessons can be learned from in-depth studies of small cohorts of patients. The findings in Davis et al. point to key parameters of the antibody response—such as the preference of neutralizing antibodies to bind to non-cleaved, cell surface-displayed GP—that can now be investigated in larger cohorts of infected or vaccinated individuals, informing the design of vaccines and immunotherapies. Their findings are also relevant to our basic understanding of the process through which neutralizing antibodies arise in the course of viral infection, and these lessons may prove useful in contexts well beyond Ebola virus disease.

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