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Neutralizing the Threat: Pan-Ebolavirus Antibodies Close the Loop

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Abstract

The glycoprotein (GP) of ebolaviruses participates in a critical membrane fusion process to establish infection of a cell and therefore, represents an important target of both vaccines and antivirals. The latest reports on pan-ebolavirus monoclonal antibodies in small animal models may offer promising outcomes and insight into how best to target the GP in vaccine and antiviral discovery.

History of Ebolavirus Polyclonal Immunotherapy

The ebolaviruses cause severe and often fatal hemorrhagic fever in humans and nonhuman primates (NHPs). The *Ebolavirus* genus is divided into five separate species: *Zaire ebolavirus* (EBOV), *Sudan ebolavirus* (SUDV), *Bundibugyo ebolavirus* (BDBV), *Tai Forest ebolavirus* (TAFV) (also known as *Côte d'Ivoire ebolavirus* or *Ivory Coast ebolavirus*), and *Reston ebolavirus* (RESTV). The EBOV epidemic in West Africa that spanned late 2013 to early 2016 brought to light the need for effective, licensed antivirals and vaccines to respond to and/or prevent future outbreaks of this scale. Due to the almost nonexistent stockpiles of experimental antivirals at the peak of the epidemic, the World Health Organization (WHO) released guidance in the affected West African countries on the use of EBOV-convalescent whole blood or plasma, typically referred to as immunotherapy, due to the antibodies in these matrices. These represented a 'local' resource to potentially contain the outbreak; similar limited use was enacted in a number of cases in the 1976 and 1995 EBOV outbreaks. Clinical trials using convalescent blood-based products were initiated in Sierra Leone, Liberia, and Guinea, where the results of one of the trials showed no benefit of convalescent plasma; similar results have been observed in NHP studies, suggesting that putative protection with this resource does not constitute an effective immunotherapy against EBOV [1]. Although these and other data have suggested that efficacious passive immunotherapy against EBOV was not achievable, the field continued to pursue this therapy from various angles, resulting in the first report of a successful passive immunotherapy (polyclonal or monoclonal) against EBOV in NHPs [2]. Specifically, in 2012, the use of purified polyclonal

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antibodies from the sera of vaccinated NHPs against the EBOV GP (treatment initiated 2 days post-challenge with EBOV) resulted in 100% survival from EBOV disease [2].

Emergence of EBOV Monoclonal Immunotherapy

Before the 2012 polyclonal antibody report, the monoclonal antibody (mAb) KZ52, which binds to and neutralizes the EBOV GP, was identified from an EBOV survivor [3]. KZ52 was observed to neutralize EBOV infection in cell cultures and in a guinea pig-adapted EBOV model but unfortunately was not efficacious in a NHP model of EBOV infection [3]. The KZ52 mAb immunotherapy ‘failure’ in the ‘gold-standard’ NHP model was discouraging; however, as polyclonal immunotherapy continued to be examined, so was mAb immunotherapy. In 2012 two different laboratories identified mAb combinations directed against the EBOV GP that were efficacious against EBOV challenge in the NHP model. These mAb combinations were designated ZMab (2G4, 4G7, and 1H3) [4] and MB-003 (6D8, 13F6, and 13C6) [5]. Through subsequent collaborations, three of the mAbs (2G4, 4G7, and 13F6) were combined to form ZMapp, which completely protected NHPs from lethal EBOV challenge even when administered as late as 5 days following virus exposure [6]. While impressive, this cocktail is specific only for the EBOV GP and not the GPs of other ebolavirus species. However, these successful NHP studies led to a clearer picture of the sites on the EBOV GP that were vulnerable to mAbs and that might also confer protection in humans [7]. The anti-EBOV GP mAbs discussed to this point are not an exhaustive list, but represent the state of the art in anti-EBOV GP mAbs at a time when polyclonal or mAb immunotherapy against EBOV was only an afterthought, especially when considering immunotherapies against other ebolavirus species (Figure 1).

More Than One Ebolavirus Threat

From late 2013 through 2016, the world was concerned about media reports of the EBOV outbreak turned epidemic occurring in West Africa. One point that may not have been apparent from these reports is that this epidemic was caused by only one of a number of different species of ebolavirus. Of the five known ebolavirus species, EBOV, SUDV, and BDBV have resulted in lethal outbreaks in humans. Before 2013, the vast majority of ebolavirus outbreaks occurred in Central Africa. Of particular interest, outbreaks of EBOV, SUDV, and BDBV occurred in the Democratic Republic of Congo (DRC) or on the borders of the DRC in South Sudan and Uganda. This represents an area where these three ebolavirus species are known to be endemic. However, as witnessed in the West African EBOV epidemic, where there had been no previous reports of EBOV, multiple ebolavirus species might be lurking in areas that have not yet seen an outbreak, consequently presenting a public health concern. With this in mind, research on ebolaviruses has progressed to the ultimate goal of designing or discovering cross-protective vaccines or antivirals that are efficacious against, at a minimum, EBOV, SUDV, and BDBV – a feat that has been referred to as searching for a mythical ‘rainbow unicorn’.

Myth Busted?

The ebolavirus species diverge by 32% to 41% at the nucleotide and amino acid level and in the GP. These differences are evident on GP surfaces where antibodies can easily bind; this has hampered cross-protection efforts over the years, particularly because the location of antibody-binding sites, which have proved to be protective targets in NHP models, are masked within the GP structure [8]. Nevertheless, two separate reports published recently in *Cell* identified antibodies that could cross-bind to various ebolaviruses; namely, the EBOV, SUDV, and BDBV GPs. These mAbs bound to a greater extent to the basic form of the GP than to the fully mature GP (once the protein has undergone multistep proteolytic cleavage events required for ebolavirus entry into host cells) [9,10]. This form of GP, referred to as cleaved GP (GP_{CL}), lacks the mask over the critical fusion loop of the GP. The fusion loop is required for insertion of the GP into the host-cell endosomal membrane, causing lipids to mix between this membrane and the viral membrane and resulting in a fusion pore and the subsequent release of the viral genome into the cytoplasm [9,10]. GP_{CL}, which lacks the mask, greatly exposes the loop, thus providing access for high-affinity binding in the nano- to picomolar range, and enables clamping of the mAbs ADI-15878/ADI-15742 [9] and CA45 [10]. Using an *in vitro* liposome fusion assay [10] or a cell-based assay to image fusion events based on a recombinant *Vesicular stomatitis virus* ebolavirus GP pseudo-type [9], the studies found that these antibodies prevented the entry of ebolaviruses through neutralization of fusion, blocking the fusion loop from inserting into the target membrane. While there is divergence among the GPs of the different ebolavirus species, amino acid locations that were mostly conserved within the structure of the fusion loop region were shared for the EBOV GP (CA45: R64, Y517, H549, N550; ADI-15878/ADI-15742: E157, G528, L529, A530, W531, Q560, N563, E564, Q567).

Such conserved residues were demonstrated to contribute to the binding of these antibodies to multiple ebolavirus GPs, which in turn proved to be antibody-targeted sites, resulting in the broadly protective nature of these mAbs in small animal models of EBOV (mouse and guinea pig), SUDV (mouse), and BDBV (ferret) [9,10]. These were the first studies to reveal that single antibodies recognizing the EBOV, SUDV, and BDBV GPs were protective against challenge with all three ebolaviruses in animal models, a feat once considered impossible due to the previous knowledge that these antibody-binding sites are heterogeneous areas on the GP. With this in mind, one can now envisage antibody cocktails that will be at the ready, and presumably efficacious, regardless of the ebolavirus species triggering the outbreak. While optimism should be the response to these reports, a need for caution should be heeded, as the potential for antibody-escape mutants exists; the development/discovery of more of these antibodies will hopefully hamper the emergence of escape mutants against cocktails of similar pan-ebolavirus antibodies. The next steps will be to show the efficacy of these – and structurally similar – mAbs in NHPs and for each virus. Remarkably, these studies have identified mAbs that are broadly cross-protective against the medically important ebolavirus species, and they have also laid the foundation for potential vaccine design strategies given that conserved sites of vulnerability in the ebolavirus GP have been characterized; these may constitute *avant-garde*, promising tools for the ebolavirus field.

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Figure 1.

Ebolavirus Glycoprotein (GP) and Neutralizing Antibodies.

Top panel: Cartoon of an ebolavirus GP trimer showing the putative mucin-like domains that theoretically block the more conserved (amino acid) receptor binding sites, rendering binding to the fusion loop region of the GP less accessible. After proteolytic cleavage (GP_{CL}), these sites become accessible during entry into the host cell; the monoclonal antibodies (mAbs) CA45 and ADI-15878/15742 both bind the GPs depicted but also bind to GP_{CL} with higher affinity. Bottom panel: mAb-binding sites and antibody-binding angles for KZ52 (blue), 2G4 (purple), 4G7 (yellow), 13C6 (green), CA45 (orange), and ADI-15878/15742 (rainbow). Note that KZ52, 2G4, 4G7, CA45, and ADI-15878/15742 all bind in areas in close proximity to one another; however, the regions where CA45 and ADI-15878/15742 bind are conserved at the amino acid level between EBOV, SUDV, and

BDBV, leading to improved affinity and therapeutic efficacy against all three GPs in small animal models (EBOV: mouse, guinea pig; SUDV: mouse, guinea pig; BDBV: ferret).

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