Editorial

Ebola's exploits: debilitating host immune responses through interferon inhibiting domains

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The recent Ebola virus disease (EVD) outbreak in West Africa was unprecedented in human suffering (over 28,000 reported cases, more than 11,000 deaths), timespan [2014–2016], and geographic scope (epidemic spread in Guinea, Liberia, and Sierra Leone with exportation of cases to multiple continents) (1). Ebola virus (EBOV), an etiologic agent of acute hemorrhagic fever, usually results in high case fatality rates. At least part of the virulence of EBOV is attributed to its tropism for cells of the innate immune system: monocytes, macrophages, and immature dendritic cells (2,3). Upon infection, EBOV actively dampens intrinsic antiviral immune defenses, causing systemic immunosuppression, lymphocyte apoptosis, and lymphopenia. In fatal cases, these hallmarks of EVD and the accompanying innate immune disarmament allow unfettered viral replication within the host, resulting in a highly lethal disease with extreme pathogenic characteristics. The 2014 EBOV outbreak renewed interest in characterizing EBOV immunoevasion strategies, primarily occurring through viral antagonism of type I interferons (IFN-I), which may be critical components of vaccine and antiviral therapeutic applications for controlling future EBOV outbreaks.

To this end, a December 2016 study published in PLOS Pathogens by Lubaki *et al.* explored the impact of EBOV interferon inhibitory domains (IIDs) on adaptive immune responses (4). Previous work demonstrated that inhibition of dendritic cell maturation is modulated through IIDs within the VP35 and VP24 viral proteins (5). It was also shown that creating loss of function mutations in EBOV IIDs promotes maturation of dendritic cells accompanied by the secretion of cytokines and chemokines (6). However, the specific molecular mechanisms mediated by VP35 and VP24 to diminish anti-EBOV adaptive immune responses were less well understood. In their recent article, Lubaki *et al.* used human PBMCs and a panel of recombinant EBOV viruses, containing point mutations disabling VP24 and/or VP35, to systematically interrogate the effect of EBOV IIDs on T cells, B cells, and NK cells *in vitro* following EBOV infection (4). Lubaki *et al.* demonstrated inhibiting DC maturation results in an inability to stimulate T cells, thereby failing to activate and propagate adaptive immune responses. Through these studies, the group found that EBOV mediates global immunosuppression via IIDs that not only actively limit DC maturation and function, but also drive immune dysregulation of innate-dependent and cellular-dependent immunity.

As adaptive immune cells, such as T cells, have been shown to be resistant to EBOV infection, the authors hypothesized that the IID-mediated effects are associated with interactions between adaptive immune cells and cells that are susceptible to infections, specifically, DCs. In order to further interrogate this hypothesis, the authors used PBMCs from CMV seropositive patients and two *in vitro* co-culture systems. Under the first co-culture conditions, DCs were infected with a panel of recombinant EBOV and cultured with CMV-peptide primed PBMC. The panel of viruses included wildtype EBOV, EBOV carrying the mutation R312A in the VP35 IID or mutation K142A in the VP24 IID, or both mutations (VP24/VP35). A modification of this system was used to include a CMV-specific expanded

population of CD137$^+$ T cells as responders. The authors addressed the effect of EBOV IIDs on T cell proliferation, activation, and phenotype through flow cytometry and intracellular cytokine staining assays. The results affirm previous work in the field, demonstrating that EBOV infection of DCs limits T cell proliferation and cytokine production (2,3). Novel to these studies, the authors show that EBOV suppression of T cells by DCs is mediated in large part by the VP35 IID. Specifically, VP35 seems to play a major role in suppression of Th1 responses, as infection of DCs with EBOV carrying mutations in the VP35 IID resulted in robust IFNγ/IL-2/TNFα polyfunctional cytokine production by CD4+ T cells while suppressing Th2 cytokines, IL-13 and IL-5. The VP24 IID mutant virus also enhanced Th1 cytokines, in addition to Th2 cytokines, IL-13, IL-4, and IL-5. Analysis of cytokines and chemokines from co-culture supernatants of virus-infected DCs and PBMCs indicated a global increase in cytokines and chemokines upon mutation of VP35 and VP24 IIDs. Moreover, the addition of supernatant from EBOV mutant R312A infected DCs resulted in IFNγ production by naive CD4+ T cells. These results indicate that soluble factors released from the DCs upon infection with EBOV play a role in inhibiting T cell responses.

In order to elucidate a mechanism by which EBOV IIDs limit adaptive cellular-mediated responses, Lubaki *et al.* examined the formation of immunological synapses in co-cultures. Previous work has demonstrated that infection of DCs with EBOV results in aberrant maturation. Therefore, cell-surface molecules that facilitate immunological synapse formation are likely not present during EBOV infection. Consequently, Lubaki *et al.* report diminished immunological synapse formation upon EBOV infection. When VP35 IID is disabled in EBOV, however, immunological synapse formation ensues and correlates with enhanced phosphorylation of T cell signaling molecules critical in T cell activation and proliferation. Many studies have shown the role of VP35 on suppression of intracellular viral RNA sensing and induction of IFN-I pathway. Accordingly, both a lack of DC maturation in addition to impaired IFN I signaling may play a role in suppression of T cell responses. However, IFNAR subunit 2 blockade and exogenous IFN α/β addition played a limited role in limiting DC maturation or hindering T cell activation or IFNγ secretion. The authors explain that other cytokines, such as TNFα, may also be contributing to EBOV-mediated maturation effects on DCs.

In addition to T cell responses, the authors also examined the effect of EBOV IIDs on B cells and NKs, albeit to a lesser extent than T cells. Infection of PBMCs with EBOV VP35 IID mutant resulted in a significant increase in the percent of class-switched and post-class switched memory B cells and plasma cells. This indicates that EBOV infection hinders the generation of memory B cell class-switching and differentiation into plasma cells. Studies with EBOV infected PBMCs also indicated that VP35 IID may play a role in suppressing cytotoxicity and inducing cell death in NK cells. This data supports previous publications demonstrating enhanced NK cytotoxicity upon treatment of both mice and human PBMCs with EBOV virus-like particles, which lack VP35 and VP24 (7,8). The specific role of IIDs on modulating these anti-viral immune responses warrant more study.

The magnitude and severity of the 2014 West African Ebola outbreak prompted the acceleration of development of vaccines and therapeutics to prevent and control infection in an epidemic setting. One of the many challenges of developing effective EBOV therapeutics can be attributed to multiple cellular targets of Ebola viral proteins, VP24 and VP35, which cause widespread immune suppression and allow for rampant viral replication. EBOV immune evasion has effects that reach beyond IFN-I suppression, through interactions of the IIDs with viral sensing machinery in the innate immune cells, i.e., DCs as well as via indirect mechanisms such as altering secreted factors and essential surface molecules on adaptive immune cells, i.e., T cells. Additional studies focusing on the molecular and cellular mechanisms by which the EBOV IID interacts with B cells and NK cells are warranted, as well as, a more in depth examination of CD8+ T cell responses, which are impacted during EVD. To this end dysregulation of both innate and adaptive responses described in the study of Lubaki *et al.* should be a focus of *in vivo* studies of EBOV infection that complement these *in vitro* studies while incorporating the intricate crosstalk that likely occurs between immune cells and other infected non-immune cells. Additionally, *in vivo* animal models would allow the interrogation of the effects of IIDs on formation of memory responses essential for durable protection against EBOV.

The data presented by Lubaki *et al.* may aid to the development of rationale treatment strategies. Based on the report by Lubaki *et al.*, it may be valuable to use vaccination in a prophylactic setting in order to mount strong immunological memory responses prior to infection. This would be valuable for first-response workers or individuals anticipated to deploy to a region where EBOV is endemic.

However, in an infection setting, EBOV viral proteins actively quell the cellular machinery required to form productive vaccine-induced immunity. Therefore, targeting EBOV IIDs, specifically in VP35, may be a more effective therapeutic option that also has the potential to boost anti-viral immunity. Further studies probing the effect of EBOV on cell signaling programs linking innate and adaptive immune cells have the potential to aid in the development of novel treatments, as key protective immune pathway targets may have yet to be discovered.

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