

Complement Seals a Virus to Block Infection

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In this issue of *Cell Host & Microbe*, [Bottermann et al. \(2019\)](#) reveal that complement component C4 inhibits adenovirus by inactivating the virus capsid through mechanisms requiring antibody engagement, but not late-acting complement pathways. This antiviral function likely broadly impacts non-enveloped viruses and may help illuminate the process of virus disassembly.

Antibodies are a critical component of antiviral immunity. They can directly neutralize viral infection by blocking cell binding, interfering with membrane penetration or fusion, or inhibiting uncoating; however, additional antibody-dependent functions that limit viral infection or spread are the subject of increasing investigation. These include antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis as well as a mechanism whereby an intracellular antibody receptor, TRIM21, mediates proteasome-dependent degradation of antibody-bound viruses that enter the cytoplasm ([Foss et al., 2015](#)). The TRIM21-dependent mechanism was previously discovered by the James Lab ([Mallery et al., 2010](#)), whose latest investigations described in this issue of *Cell Host & Microbe* ([Bottermann et al., 2019](#)) shed new light on an established antibody-dependent antiviral process, the activation of the classical complement cascade.

In the classical pathway, C1q binds to antibodies that were previously bound to a virus. Subsequent activation of the entire C1q,r,s complex (C1) leads to cleavage of C4 into C4a and C4b. Although important for downstream C3-dependent processes, generation of C4b also exposes a highly reactive thioester that can react with hydroxyl or amino groups on the viral capsid that are in close proximity to the antibody. It is this function of C4b that is critical for the capsid coating/entry blocking mechanism described by Bottermann and colleagues ([Figure 1](#)). Although an important role for C3-dependent complement functions in antiviral immunity has been appreciated for many years, direct blockade of non-enveloped viral infection by C4b

opsonization has not been previously reported.

The discovery of this C4b-mediated neutralization pathway resulted from efforts to identify new antibody-dependent neutralization mechanisms for human adenovirus (HAdV). The authors were investigating the effects of mutations that prevent complement C1q binding on the activity of a well-characterized adenovirus neutralizing antibody (nAb). The nAb binds to an epitope on the major capsid protein, hexon, and functions in part through TRIM21 interactions ([Mallery et al., 2010](#)). Unlike the native nAb, mutant nAbs were unable to block HAdV infection in TRIM21 knockout cells. Intriguingly, the activity of the native nAb in TRIM21 knockout (KO) cells was dependent on the presence of both C1 and C4, but not C2 or C3. These results were extended to anti-HAdV antibodies present in normal human serum. Moreover, C1 and C4 could be provided either in serum or as recombinant proteins. Thus, the authors established TRIM21-independent antibody-mediated neutralization that requires activation of the classical complement pathway and is independent of C3-mediated functions.

The authors then investigated the mechanism of the infection block in TRIM21 KO cells. A series of assays established that the complement cascade was activated in the presence of HAdV, nAb, and purified C1 and C4 and that HAdV binding to the cell was not blocked. Rather, there was a modest ~3-fold increase in the amount of virus that was bound. Similarly, the kinetics of internalization of the virus from the cell surface were unaffected. In contrast, capsid uncoating and release of an internal capsid protein (VI) that is required for

HAdV to escape the endosome and traffic to the nucleus were blocked ([Figure 1](#)). A combination of nAb, C1, and C4 was both necessary and sufficient for these effects. Finally, the TRIM21-dependent and C4-dependent mechanisms were unraveled *in vivo* by examining HAdV-mediated luciferase transduction of the liver. In wild-type mice injected with native nAb, transduction was reduced at least 1,000-fold. TRIM21 accounted for a ~60-fold reduction in transduction, while complement imposed a ~20-fold reduction. And in the absence of both C1q binding and TRIM21, the nAb had almost no effect. Thus, the additive effect of TRIM21 and this newly described Ab- and complement-dependent mechanism appear to account for all the nAb activity both *in vitro* and *in vivo*.

C4b antiviral function is highly reminiscent of the antiviral activity of α -defensins, a class of innate immune host defense peptides with potent antiviral properties ([Smith and Nemerow, 2008](#)). Like C4b, α -defensin binding to HAdV stabilizes the capsid, blocks disassembly (uncoating), restricts release of the membrane-lytic protein VI, and prevents HAdV from escaping the endosome. This antiviral mechanism of α -defensins is largely conserved across multiple families of non-enveloped viruses ([Holly et al., 2017](#)), while the C4b-dependent antiviral pathway has not yet been examined for other non-enveloped viruses. There are HAdVs and mouse AdVs that are resistant to the effects of α -defensins ([Holly et al., 2017](#); [Wilson et al., 2017](#)). It is tempting to speculate that although viruses can evade the activity of directly neutralizing antibodies that must bind to specific capsid features to mediate their functions, C4b-dependent functions that require



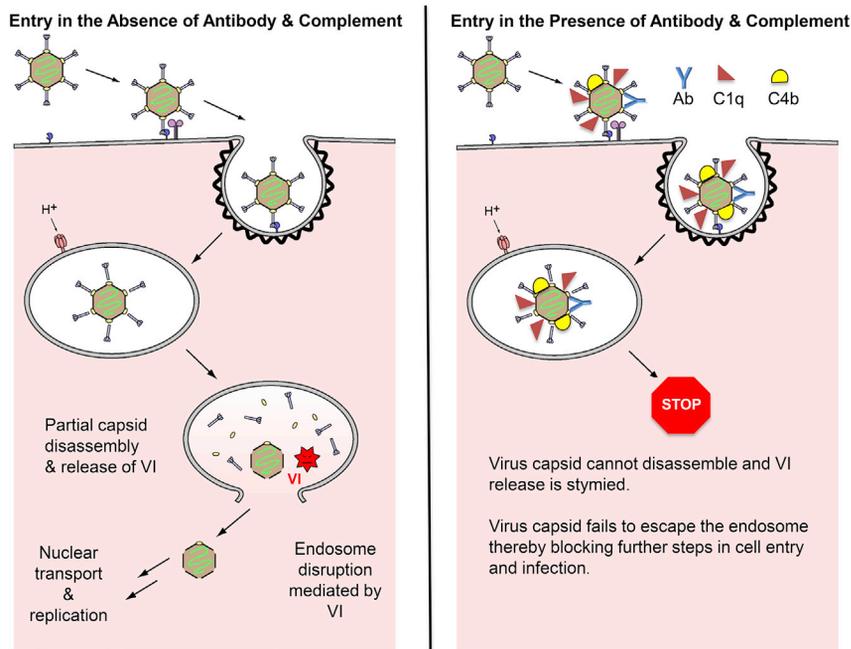


Figure 1. Schematic Representation of Adenovirus Cell Entry in the Absence of Immune Mediators (Left) or in the Presence of Antibody and Complement (Right)

Antibody and complement-mediated virus neutralization is achieved by stabilization of the adenoviral capsid that prevents endosome rupture as revealed by the studies of Bottermann and colleagues.

non-selective binding would be harder to evade. In this regard, it is unlikely that TRIM21- and C4b-dependent mechanisms contribute to the diversification of HAdV serotypes. Rather, they provide a general layer of broad protection that is consistent with their classification as innate immune effectors despite their reliance on components of adaptive immunity for their activity.

This mechanism has implications both for immunity to pathogenic infections and for the use of HAdV vectors for gene therapy or vaccines. Opsonization leading to a block in infection could alter antigen presentation, making the virus more immunogenic. We have proposed this effect for α -defensins, although it remains to be proven (Gounder et al., 2016). To explore this idea, Bottermann and colleagues assessed T cell responses to HAdV in mice in the presence or absence of nAb and as a function of C1q binding. Rather than enhancing immunogenicity, the nAb significantly attenuated the development of T cell responses. In addition, coating the virus with a Fab (this study) or single chain Fv trimer (Schmid et al., 2018) derived from this antibody has

been shown to reduce antibody binding leading to increased transduction, reduced antibody responses, and the potential for administration of HAdV-derived viral vectors. Thus, in the likely scenario that this mechanism contributes to the neutralization of other non-enveloped viruses, similar approaches could be used to improve their efficacy for gene therapy.

One open question in regard to the effect of C4b on uncoating and infection is the extent to which the capsid must be modified to have an inhibitory effect. The thioester bond is highly reactive, so it is likely that C4b would be deposited in close proximity to the nAb epitope on hexon, which has been identified for the monoclonal 9C12 nAb used in these studies (Myers et al., 2013; Schmid et al., 2018; Varghese et al., 2004). Although ELISA data indicate that C4 is associated with the capsid, a covalent modification of one or more outer capsid proteins has not been formally demonstrated. Nor has the number of C4b molecules bound to the capsid been estimated. Because polyclonal serum can also mediate C4b-dependent neutralization, the location of the hexon-specific

9C12 epitope is not likely to be critical. However, whether complement activation by antibodies specific for other capsid proteins, such as penton base or fiber, also leads to C4-dependent neutralization remains to be determined. Thus, understanding the structural basis for how C4b blocks uncoating will be an exciting avenue for further investigation that might provide insight into the normal processes of HAdV uncoating and may also inform the neutralization mechanisms of site-specific nAbs and of α -defensins.

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