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REVIEW ARTICLE

Interaction of adenovirus with antibodies, complement, and coagulation factors

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Adenovirus (AdV) is one of the most widely used vectors for gene therapy and vaccine studies due to its excellent transduction efficiency, capacity for large transgenes, and high levels of gene expression. When administered intravascularly, the fate of AdV vectors is heavily influenced by interactions with host plasma proteins. Some plasma proteins can neutralize AdV, but AdV can also specifically bind plasma proteins that protect against neutralization and preserve activity. This review summarizes the plasma proteins that interact with AdV, including antibodies, complement, and vitamin K-dependent coagulation factors. We will also review the complex interactions of these plasma proteins with each other and with cellular proteins, as well as strategies for developing better AdV vectors that evade or manipulate plasma proteins.

Keywords: adenovirus; complement; natural antibody; gene therapy; immunoglobulin G; immunoglobulin M; tripartite motif-containing protein 21; vitamin K-dependent coagulation factors

Successful clinical use of Adenovirus (AdV) vectors depends on the ability of viral particles (also referred to as virions) to reach target tissues while evading the host's antiviral defense systems. The biodistribution and activity of AdV gene therapy vectors are dramatically influenced by opsonizing proteins in the host's blood, including complement, antibodies, and coagulation factors [1–3]. Many of these opsonins are innate defense proteins that can bind AdV virions, even if the host has never been exposed to AdV. Opsonins such as antibodies and complement impair AdV virions, limiting the ability of AdV-based vaccines to induce immune responses or blocking the ability of AdV gene therapy vectors to transduce target tissues. In contrast, certain AdVs selectively bind coagulation factors that protect virions from complement-mediated neutralization, suggesting that AdV virions can actively manipulate opsonization as a defense against assault by host proteins [4,5].

Human AdVs are classified into seven species, ranging from A through G. AdV type 5 (AdV-5) is a species C AdV and is the most used type of AdV vector for gene therapy and vaccine applications. The tropism of AdV-5 vectors is heavily influenced by blood proteins. In rodents and nonhuman primates, intravascular administration of AdV-5 vectors results in selective transduction of hepatocytes [6–9]. Interestingly, coagulation factors bind to AdV-5 virions and facilitate transduction of hepatocytes [10], illustrating how certain blood proteins can expand the tropism of AdV vectors. In contrast, anti-AdV immunoglobulin G (IgG) antibodies have a powerful negative influence on the ability of AdV to transduce hepatocytes [11], which shows how blood proteins can interfere with AdV-based gene therapy.

Much of what is known about the interaction of AdV with blood proteins has been learned from *in vitro* studies. Neutralization assays are often used to

Abbreviations
AdV, adenovirus; CR1, complement receptor 1; FIX, factor IX; FVII, factor VII; FX, factor X; GLA, γ-carboxyl glutamic acid; HSPG, heparan sulfate proteoglycans; HVR, hypervariable region; IgG, immunoglobulin G; IgM, immunoglobulin M; KC, Kupffer cell; MAV, mouse adenovirus; TRIM21, tripartite motif-containing protein 21.

study the impact of antibodies and complement on AdV. Neutralization assays involve mixing serum or plasma together with AdV and then measuring the ability of the virus to transduce or infect cells. In animal models, however, AdV virions behave in much more complex ways that do not always align in a straightforward manner with *in vitro* mechanisms. As this review will show, *in vitro* studies do not always accurately predict the fate of AdV vectors *in vivo*. Even *in vivo* studies in mice or other animal models do not always adequately predict the behavior of AdV in humans. Often, a combination of experimental approaches using purified proteins, serum, and animal models is required to fully understand the complex impact of blood proteins on AdV.

Antibodies and their interactions with AdV

Natural IgM and antigen-specific IgG

A subset of B cells innately secretes immunoglobulin M (IgM), with no need for prior antigen exposure [12]. These antibodies —known as natural IgM—can be found in newborn babies [13] and germ-free mice [14]. The pentameric (sometimes hexameric) structure of IgM is comprised of 10–12 flexible antigen-binding regions, and these multiple antigen-binding regions facilitate multivalent interactions with antigens [15]. The antigen-binding regions of natural IgM antibodies are polyreactive, meaning that they can interact weakly with many different antigens [16]. Although each individual antigen-binding region of IgM may bind to a particular antigen with only low affinity, the highly repetitive surfaces of viruses and bacteria allow IgM to form multivalent interactions, which increases binding avidity. This combination of multivalency and polyreactivity makes natural IgM an important component of the innate immune system for recognizing microbes and allows IgM to function as the first line of defense against invading pathogens before antigen-specific IgG come into play [17]. Accordingly, the serum of naïve mice contains polyreactive IgM antibodies that can bind AdV [18,19].

In the weeks following infection with AdV or exposure to AdV vectors, the adaptive immune system produces high-affinity anti-AdV IgG antibodies. The surface of AdV capsids consists of three exposed proteins that are accessible to antibodies: hexon, fiber, and penton. The icosahedral AdV capsid contains 720 hexon proteins organized in 240 homotrimers, and the 12 vertices of the capsid contain pentameric penton proteins and trimeric fiber proteins (Fig. 1). Antibodies against these three capsid proteins can have very different effects on AdV, as discussed in more detail below.

Due to immunity induced by childhood AdV infections, anti-AdV IgG antibodies are common in the adult human population. Approximately, 30–50% of American adults have anti-AdV-5 antibodies, while the AdV-5 seropositivity rate can exceed 90% in other parts of the world [20–23]. AdV-5-specific IgG antibodies have been shown to suppress the activity of AdV-5 vectors in both preclinical studies and clinical trials, reducing the efficiency of gene transfer and blocking any subsequent readministration of AdV-5 vectors [20,24]. As a result, investigators are increasingly turning to vectors constructed from AdVs that have lower levels of pre-existing seropositivity, including rare human AdVs and animal AdVs [2,23].

Mechanisms of neutralization by antibodies

Adenovirus follows an intricately choreographed series of steps when infecting cells, and antibodies can disrupt this process at several different stages. AdV binds to cell-surface receptors *via* fiber, and internalization of AdV is facilitated by penton [25]. Fiber starts dissociating from virions at the cell surface, and this process continues as virions enter early endosomes [26]. AdV capsids then release a membrane-disrupting protein that allows endosomal escape [27]. After escaping from endosomes, the partially disassembled capsid and the encapsidated viral genome are trafficked to the nucleus [28].

Mouse and human natural IgM have no direct neutralizing activity for AdV *in vitro* [5,29]. In contrast, anti-AdV IgG can interfere with several steps of the cell entry process, depending on which capsid protein the antibodies bind. Anti-fiber antibodies neutralize AdV mainly by aggregating virions and by inhibiting the ability of virions to bind to receptors (Fig. 1) [30,31]. Anti-penton antibodies are not very effective at blocking AdV entry on their own, reducing it by only about 50% [30,31]**.** However, penton- and fiber-specific antibodies can work synergistically to neutralize AdV [32].

IgG antibodies against hexon are highly effective at blocking AdV infection, and AdV-5 can be neutralized by as few as 1.4 anti-hexon IgG antibodies per capsid [31,33]. Recent studies have shown that anti-hexon IgG antibodies primarily neutralize AdV-5 in the cytoplasm by triggering a novel pathway that leads to destruction of capsids [34]. AdV–IgG complexes in the cytosol are recognized by tripartite motif-containing

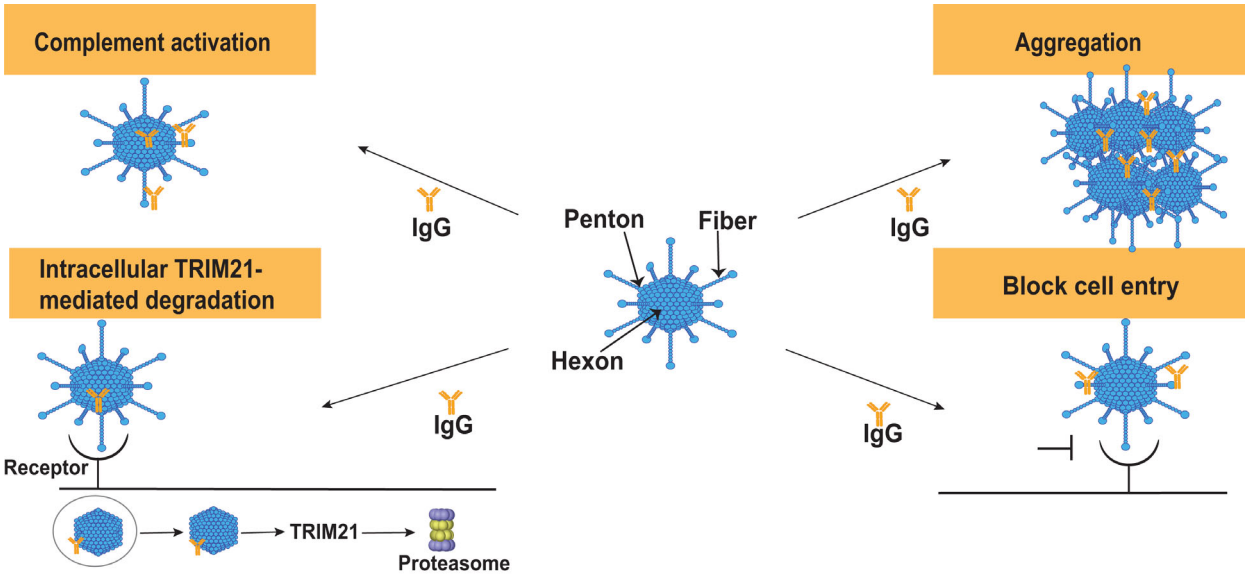

Fig. 1. Mechanisms of AdV neutralization by IgG. Binding of IgG to fiber neutralizes AdV by inhibiting receptor binding or aggregating virions. If transported into the cell, hexon-bound IgG is recognized by TRIM21, which leads to TRIM21-mediated degradation of cytosolic AdV-IgG complexes by the proteasome. IgG binding to AdV can also initiate complement activation, resulting in inhibition of receptor binding and intracellular neutralization.

protein 21 (TRIM21), which is a widely expressed intracellular Fc receptor with ubiquitin ligase activity [35]. The interaction between IgG and TRIM21 is highly conserved, and murine and human TRIM21 can bind to IgG from multiple mammalian species [36]. TRIM21 binds the Fc domain of antibodies and catalyzes the formation of ubiquitin chains [37]. This ubiquitination targets the antibody-bound capsid for unfolding by AAA-adenosine triphosphatase valosin-containing protein, followed by proteolysis by the proteasome (Fig. 1) [38,39]. In mice, TRIM21 mediates the inhibitory effect of IgG on AdV-5-based gene therapy vectors and vaccines [39].

In addition to directing the degradation of antibody-bound pathogens, TRIM21 acts as a positive immune regulator in mice by increasing the expression of inflammatory response markers and innate immune proteins [39]. Interestingly, TRIM21 itself is upregulated by type I interferon [35]. Thus, internalization of IgG-opsonized AdV not only leads to destruction of the virion, but also activates a proinflammatory feedback loop.

Impact of antibodies on AdV *in vivo*

It is difficult to study the pathogenesis of human AdV infection in mice because AdVs are highly species-specific. However, mouse adenovirus 1 (MAV-1) can be used as a model system to study AdV pathogenesis in mice. Natural IgM on its own is insufficient to control MAV infection, but early T-cell-independent IgM antibodies play a vital role in reducing MAV-1 titers [40]. TRIM21 is critical for effective antibody-mediated control of MAV-1. Following infection with MAV-1, TRIM21-deficient mice show higher viral loads and increased mortality rates when compared with wild-type mice [41]. Passive transfer of immune serum containing anti-MAV-1 IgG improves the ability of wild-type mice to survive MAV-1, but TRIM21-deficient mice are poorly protected by immune serum [41]. These studies demonstrate that both IgM and IgG contribute to the control of AdV infection, and that TRIM21 helps to mediate the antiviral effects of IgG *in vivo*.

In mice, AdV gene therapy vectors are inhibited in different ways by IgM and IgG antibodies. Anti-AdV IgG antibodies are able to neutralize virions, and therefore they directly suppress the ability of intravenously administered AdV vectors to transduce the liver in mouse models [42,43]. In contrast, natural IgM has little direct AdV-neutralizing activity [5]. Instead, natural IgM can enhance the clearance of AdV by liver macrophages known as Kupffer cells (KCs) [18], which reduces the amount of virions available to transduce hepatocytes. IgM-deficient mice show increased hepatocyte transduction by AdV-5 vectors when compared with wild-type mice [19,44,45]. Moreover, the concentration of IgM in

different strains of mice inversely correlates with the ability of intravenously administered AdV vectors to transduce hepatocytes. For example, BALB/c mice have high levels of IgM and low hepatocyte transduction, C57BL/6 mice have low levels of IgM and high hepatocyte transduction, and hybrid crosses between these two mouse strains have intermediate levels of IgM and hepatocyte transduction [19]. These effects of natural IgM are mainly mediated by the ability of IgM to enhance clearance of AdV by macrophages [19]. In certain situations, however, natural IgM can have a more direct antiviral effect by activating complement [5], as discussed in more depth below.

Anti-AdV IgG antibodies also inhibit the effectiveness of AdV-based vaccines. These antibodies have been shown to reduce or block the effectiveness of AdV-5-based vaccines in mice [46–49] and rhesus macaques [50]. In vaccine clinical trials, patients with pre-existing anti-AdV-5 IgGs show reduced immune responses against the Ebola and HIV-1 antigens encoded in AdV-5-based vectors [51,52]. Similar to *in vitro* findings, antibodies against different capsid proteins have different effects. In mice, anti-hexon antibodies reduce the response to AdV-5 vaccines more effectively than anti-fiber antibodies [20]. Thus, anti-AdV IgG antibodies markedly inhibit responses to AdV-vectored vaccines, regardless of whether the antibodies are due to prior infection with AdV or previous exposure to AdV vectors.

Complement

The complement system

The complement system is a proteolytic cascade comprised of both soluble and membrane-bound proteins that work together to detect and label pathogens for destruction (Fig. 2). The complement cascade can proceed along three pathways: classical, lectin, and alternative. The classical pathway is activated by antibodies, the lectin pathway is activated by pathogen-associated carbohydrates, and the alternative pathway is spontaneously activated after contact with lipids and carbohydrates found on self and non-self macromolecules [53,54].

Both IgG and IgM activate complement *via* the classical pathway. After binding to an antigen, IgM undergoes a conformational change that allows complement protein C1q to attach to IgM [55]. IgM is a strong activator of complement; in fact, a single IgM pentamer can initiate the classical pathway [56]. In contrast, IgG-mediated complement activation only occurs when C1q interacts with multiple clustered IgG that are bound to antigen [55].

Intravenous injection of AdV vectors in mice strongly activates the complement system, as evidenced by the appearance of cleaved C3 in the blood [57]. This complement activation involves both the classical and alternative pathways and is caused primarily by AdV-induced tissue damage, rather than direct virion-mediated complement activation [57]. As discussed in

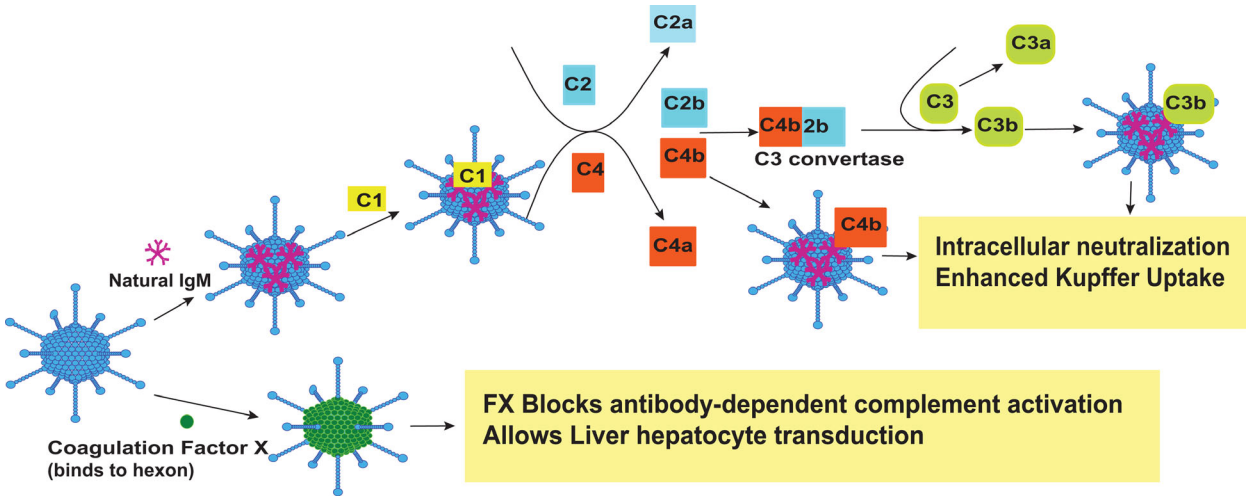

Fig. 2. Activation of the classical complement cascade by IgM. IgM binding to AdV recruits and activates the C1 complex. C1 cleaves C4 and C2, which combine to form the C3 convertase C4b2b (formerly referred to as C4b2a). The C3 convertase cleaves C3 into C3a and C3b. Both C4b and C3b can covalently bind to AdV. Complement proteins enhance uptake by KCs and neutralize the virus intracellularly. AdV-5 hexon can bind FX, which prevents both complement activation and neutralization by complement, thereby preserving the activity of AdV-5 both *in vitro* and *in vivo*.

more depth below, activation of complement by AdV virions is mainly antibody-dependent and is therefore mediated by the classical pathway [5,57,58].

Mechanisms of AdV inhibition by complement

After binding to antibody, activated C1 cleaves complement proteins C2 and C4 (Fig. 2). Two of the resulting cleavage products form the C3 convertase C4b2b (formerly C4b2a). This C3 convertase cleaves C3 into C3a and C3b [59]. Importantly, C3b and C4b both contain highly reactive thioesters that can covalently bind to amino acids or carbohydrates on the target antigen [60]. As a result, antibody-dependent activation of complement by AdV leads to covalent linkage of C4b and C3b on the surface of virions [5,61,62].

Opsonization by complement not only inhibits the ability of AdV to bind cells [5] but covalently attached C3b and C4b can also inhibit AdV intracellularly [61,62]. Studies with human complement show that C3b-opsonized AdV is degraded by the proteasome in a TRIM21-independent manner, and intracellular C3b also activates proinflammatory signaling pathways [61]. In contrast, delivery of C4b-bound AdV into the nucleus is inhibited at the stage of capsid disassembly and endosomal escape [62]. Virions that have activated the classical pathway will likely have both C3b and C4b attached to their capsids, and thus all of these antiviral pathways may be triggered simultaneously.

Studies in mice have shown that the impact of complement on AdV vectors is more complex than the impact of complement *in vitro* and involves a greater number of potential mechanisms. Complement-opsonized AdV virions can be recognized by complement receptors on cells, including erythrocytes [63], neutrophils [64], and KCs [65]. For example, human erythrocytes express complement receptor 1 (CR1), which can bind C3b-opsonized AdV-5 and thereby alters vector clearance and biodistribution [63]. Interestingly, murine erythrocytes do not express CR1, highlighting the importance of choosing appropriate model systems to study AdV. The complement receptor CRIg plays a role in the clearance of AdV-5 from the circulation by KCs in mice [65], although the contribution of complement to AdV clearance is not as prominent as the role of natural antibodies [18].

Vitamin K-dependent coagulation factors

Interaction of coagulation factors with hexon

The plasma proteins factor VII (FVII), factor IX (FIX), and factor X (FX) are vitamin K-dependent coagulation factors that interact with AdV-5 [10,66]. These coagulation factors are synthesized in the liver and circulate in the plasma as inactive zymogens. Vitamin K-dependent coagulation factors share a common structure that includes a γ-carboxyl glutamic acid (GLA) domain at the N terminus and a serine protease domain at the C terminus [67].

The GLA domains of FVII, FIX, and FX bind to AdV-5 with affinity up to 1 nM [66]. Cryoelectron microscopy shows that the GLA domain binds to a central cavity on the outer surface of AdV-5 hexon trimer—one GLA domain binds to one hexon trimer, meaning that each capsid has the capacity to bind 240 coagulation factor proteins [66,68,69]. The AdV hexon sequence includes seven outward-facing hypervariable regions (HVRs) that vary significantly in sequence and length among AdV types [70]. Site-directed mutagenesis has demonstrated that the FX GLA domain interacts with HVR5 and HVR7, which form the inner walls of the hexon trimer cavity [68,71]. Other AdVs bind coagulation factors with varying affinity, with a significant number of AdV types failing to bind coagulation factors to any detectable extent. In addition to AdV-5, species C AdV-2 and species B AdV-16 bind strongly to FX. However, species A (AdV-12), species B (AdV-3 and AdV-21), species D (AdV-26 and AdV-48), species E (AdV-4), and species F (AdV-41) bind FX with micromolar affinity or do not bind at all [66,69,72]. Species A AdV-31 binds to FIX, but not FX [72]. In addition, the species origin of coagulation factors affects their ability to bind AdV. For example, AdV-5 binds human FX and mouse FX with similar affinity, but AdV-2 binds human FX with 10-fold lower affinity than it binds mouse FX [66]. These data illustrate that coagulation factors exhibit marked differences in interactions with diverse AdV types.

When AdV-5 vectors are injected intravenously in mice, they efficiently transduce hepatocytes. This hepatotropism is critically dependent on the ability of AdV-5 hexon to bind FX. Hepatocyte transduction by AdV-5 vectors can be abolished by depleting or blocking vitamin K-dependent coagulation factors, or by mutating AdV-5 hexon so that FX no longer binds [10,66,69,71,73]. In contrast, non-FX binding AdVs, such as AdV-48 and AdV-26, show no hepatocyte tropism [4]. Furthermore, if the hexon HVRs of AdV-5 are replaced with those from AdV-48, the resulting hexon-chimeric vector no longer binds FX and no longer transduces hepatocytes, demonstrating that the hepatotropism of AdV-5 depends on hexon, rather than fiber or penton [69]. Similar to mice, liver transduction with AdV-5 vectors also depends on FX in rats and nonhuman primates [8,69].

How does FX enhance the hepatotropism of AdV-5 vectors? Conversion of the FX zymogen to the active form (FXa) is not required, and the serine protease activity of FXa plays no role in enhancing the tropism of AdV-5 [5,10,69]. However, the FX serine protease domain has a positively charged exosite, and this exosite acts as a bridge that allows FX-coated AdV-5 to bind to negatively charged heparan sulfate proteoglycans (HSPGs) on the cell surface [10,69,74]. Blocking the interaction of FX and HSPG prevents FX from enhancing AdV-5 transduction of cells, and FX has no effect on transduction of cells that lack cell-surface expression of HSPGs [10,69].

While FX clearly acts as a bridge to HSPGs *in vitro*, the mechanism for enhancement of liver transduction by FX is not nearly so straightforward when vectors are administered intravascularly. In fact, genetically modified mice that lack heparan sulfate on hepatocytes show completely normal hepatocyte transduction by AdV-5 vectors, contrary to the bridging hypothesis [75]. As described below, FX enhancement of liver transduction is mediated by a novel mechanism through which FX protects AdV-5 from attack by the classical complement system.

FX protects AdV-5 from complement

Although wild-type mice require FX for liver transduction by AdV-5 vectors, it was discovered that antibody-deficient mice have no such requirement for FX [5]. The lack of natural IgM is the key difference in these antibody-deficient mice: in fact, administration of purified natural IgM to antibody-deficient mice is sufficient to make AdV-5 liver transduction dependent on FX. Of note, FX is also dispensable for liver transduction in mice that lack complement proteins C1q or C4 [5]. Collectively, these findings show that FX is required for AdV-5 liver transduction only in mice that possess both natural IgM and an intact classical complement pathway.

In vitro experiments with mouse serum provided a mechanistic explanation for these unusual observations in mice: FX protects AdV-5 from being neutralized by blood proteins [5]. AdV-5 is normally stable when incubated in mouse serum but, when FX is blocked, AdV-5 strongly activates complement and becomes neutralized *via* a mechanism that requires both natural IgM and the classical complement pathway [5]. In some circumstances, AdV-5 may also activate complement independently of antibodies, *via* the alternative pathway [76,77]. Following complement activation, C3b and C4b become covalently attached to AdV-5, and this opsonization by complement can both interfere with virus binding to cells [5] and induce intracellular neutralization [61,62]. Thus, the primary reason that FX is required for AdV-5 transduction of liver is that FX protects AdV-5 from attack by complement, not because FX acts as a bridge receptor to HSPGs.

While FX protects AdV-5 from complement, this mechanism is not widespread among other AdV types. AdV-35 and AdV-50 can bind to FX but are not neutralized by mouse serum, even when FX is blocked [4]. AdV-26 and AdV-48 are also resistant to neutralization by mouse serum, even though they are completely unable to bind FX [4]. Susceptibility or resistance to neutralization by complement is conferred by hexon HVRs [4], but the mechanism for how HVRs affect complement activation is unknown, and this will be an interesting topic for further investigation.

The mouse is the most common preclinical model for AdV gene therapy studies, and it is therefore important to understand the potential differences between mice and humans regarding the impact of FX on AdV-5 vectors. An initial study of the role of FX in human sera found that FX protects AdV-5 from human complement (similar to the role of FX in mouse serum), but only about half of the human donors had neutralizing activity for AdV-5 in the absence of FX, suggesting that there is variability in the human population [78]. A more recent study, however, found a significant protective effect of FX in human sera, and all donors had significant neutralizing activity for AdV-5 in the absence of FX [29]. Complement proteins are very labile, and complement activity can be affected by collection and storage conditions. Therefore, it is possible that variability among human sera may be more related to differences in complement preservation in serum samples, as opposed to actual variability among humans. In spite of this variability, both studies agreed that FX plays a role in protecting AdV-5 from neutralization by complement in human serum [29,78].

AdV-48 has no ability to bind either mouse or human FX [29,69], and therefore one might assume that AdV-48 would behave similarly in mouse and human serum. As already mentioned, AdV-48 is completely resistant to neutralization by mouse serum, regardless of whether or not FX is present [4]. Interestingly, however, human serum neutralizes a chimeric AdV-5 vector with AdV-48 hexon in a complement-dependent manner [29]. The reason for this prominent difference in neutralization between mouse and human serum is not clear, but this finding suggests caution when extrapolating data from mouse models to humans, at least for vectors with hexon from AdV-48.

Improving AdV vectors

Using low-prevalence AdVs

Due to the high prevalence of IgG antibodies against AdV-5 in humans, investigators are increasingly turning to AdV vectors derived from rare human AdVs or animal AdVs. For example, vaccines and oncolytic vectors based on human AdVs that have relatively low seroprevalence—including types 11, 26, and 35—are functional in recipients who have pre-existing anti-AdV-5 antibodies [46,79,80]. Another potential advantage of using non-AdV-5 types is that human AdVs utilize several different receptors and exhibit varied tissue tropism, which can be advantageous when trying to target gene therapy vectors to nonhepatic sites. For example, species B AdVs show strong tropism for respiratory and ocular tissues [25].

A variety of animal AdVs have been isolated and used to construct vectors, including AdVs from pigs [81], dogs [82], cattle [83], and nonhuman primates [84]. Humans typically have low levels of neutralizing antibodies against nonhuman AdVs [81,84], but in certain populations antibody frequencies may be elevated due to contact with animals or cross-reactivity between human and animal AdVs [85]. Nonhuman AdVs from several species are being tested as vaccines and gene delivery vectors. In vaccine studies, chimpanzee AdV vectors can induce an immune response comparable to AdV-5 even in the presence of anti-AdV-5 antibodies [86,87]. An Ebola vaccine based on chimpanzee AdV-3 has been evaluated in clinical trials [88].

Rational modification of hexon

Hexon is a central focus of efforts to improve AdV vectors, in large part because of the role that it plays in interacting with blood proteins. As already discussed, hexon is the most important target for anti-AdV antibodies and contains the binding site for coagulation factors. Strategies to modify hexon include point mutations that block FX binding [71], swapping all or most of the hexon with hexon from another AdV type [89,90], swapping hexon HVRs [91,92], and

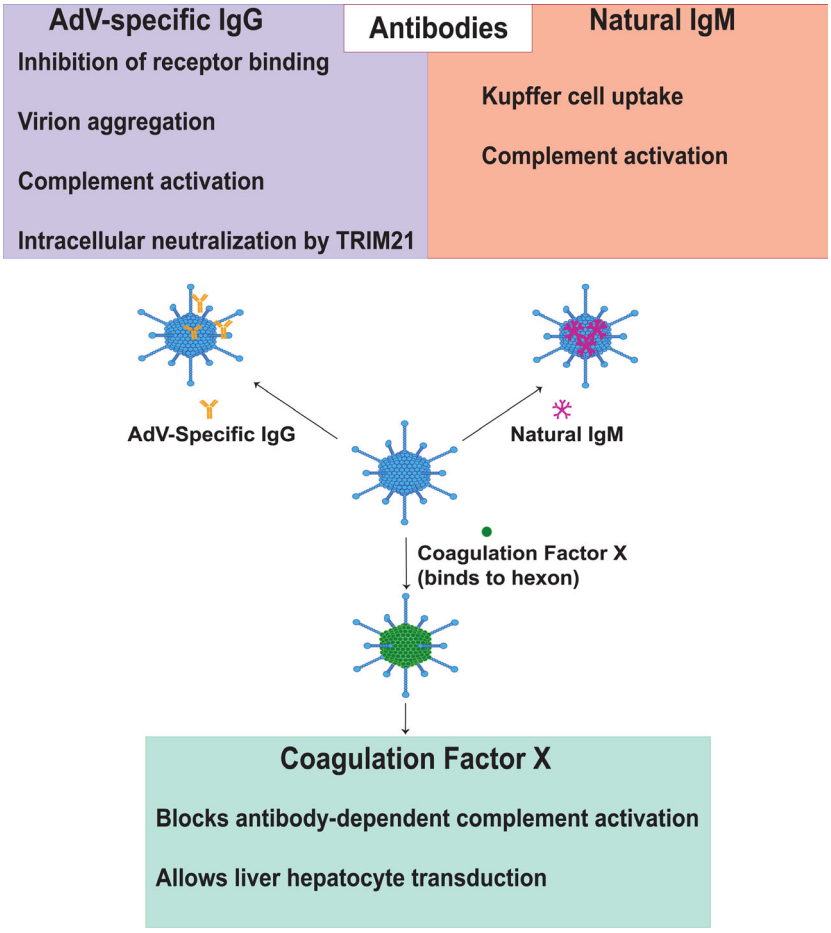

Fig. 3. Consequences of the interaction between plasma proteins and AdV. AdV-specific IgG inhibits binding of virions to cell-surface receptors, aggregates virions, activates complement, and neutralizes AdV intracellularly *via* TRIM21. Natural IgM can enhance KCs uptake and activate complement. Coagulation FX protects AdV-5 from antibody-dependent complement activation.

covalently linking polymers to hexon [93,94]. In mice, AdV vectors with modified hexon can exhibit altered properties, such as reduced liver transduction [69,92,95], increased ability to evade anti-AdV-5 antibodies [89,91] or reduced KC uptake [44]. For example, an AdV-5 vector with hexon from AdV-6 exhibits improved biodistribution in mouse models [44].

Despite the benefits of hexon modification, significant technical challenges remain. Hexon swapping is usually more successful when using closely related AdV types than when using distantly related AdV types [96]. Hexon HVR swaps often fail to produce viable virus [97], presumably because of problems with protein folding. In addition, chimeric vectors can show altered toxicity profiles in mice [98]. Nevertheless, the availability of an increasing number of successful hexon modifications creates a flexible toolkit that allows gene therapy and vaccine researchers to design vectors with highly desirable properties.

Conclusions

Blood proteins play a central role in controlling AdV infections and in influencing AdV vectors and, consequently, considerable effort has been invested in defining the blood proteins that bind to AdV. These studies have unveiled the complex interaction of AdV with coagulation factors, antibodies, and complement (Fig. 3). For AdV gene therapy vectors, it has become evident that these host proteins control vector biodistribution and tissue tropism in surprising and complex ways.

In vitro and *in vivo* studies with AdV have revealed novel pathways for intracellular neutralization and novel protection mechanisms involving coagulation factors. Animal models remain essential for studying AdV gene therapies and vaccines, because *in vitro* systems cannot fully capture the complex interplay between AdV, blood proteins, and host cells. As described in this review, determining relevance of animal studies to humans requires careful examination of the similarities and differences between animal models and humans. For example, TRIM21 is a highly conserved protein that mediates much of the neutralizing effect of anti-AdV-5 IgG in human cells and, in mice, TRIM21 is required for IgG to inhibit both human AdV vectors and mouse AdV infection [39,41]. Although the role of TRIM21 has not been directly demonstrated in humans *in vivo*, these results suggest that TRIM21 is very likely to play a key role in control of AdV infection in humans.

As another example, AdV-5 vectors bind mouse FX and human FX equally well, and FX protects AdV-5 vectors from neutralization by both mouse and human complement [29,78]. Because the efficiency of intravascularly administered AdV-5 vectors depends on FX in mice, rats, and nonhuman primates, it is reasonable to predict that intravascular administration of AdV-5 vectors in humans would also depend on FX. In contrast, vectors based on AdV-48 are neutralized by human serum but not by mouse serum [29], suggesting caution when interpreting mouse studies with AdV-48 vectors.

Future studies should take into account the potential impact of differences between blood proteins in animal models and humans. Although mouse and human blood proteins frequently interact with AdV vectors in similar ways, this is not always the case. In addition, the field is rapidly moving beyond AdV-5 vectors, and much more work is needed to understand whether findings valid for AdV-5 can be extrapolated to other AdV types. Finally, the key role of hexon in interacting with blood proteins calls for a more efficient and predictable engineering of hexon.

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