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The neutralizing capacity of antibodies elicited by parainfluenza virus infection of African Green Monkeys is dependent on complement

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ABSTRACT

The African Green Monkey (AGM) model was used to analyze the role of complement in neutralization of parainfluenza virus. Parainfluenza virus 5 (PIV5) and human parainfluenza virus type 2 were effectively neutralized in vitro by naïve AGM sera, but neutralizing capacity was lost by heat-inactivation. The mechanism of neutralization involved formation of massive aggregates, with no evidence of virion lysis. Following inoculation of the respiratory tract with a PIV5 vector expressing HIV gp160, AGM produced high levels of serum and tracheal antibodies against gp120 and the viral F and HN proteins. However, in the absence of complement these anti-PIV5 antibodies had very poor neutralizing capacity. Virions showed extensive deposition of IgG and C1q with post- but not pre-immune sera. These results highlight the importance of complement in the initial antibody response to parainfluenza viruses, with implications for understanding infant immune responses and design of vaccine strategies for these pediatric pathogens.

Introduction

The complement system is an important component of the innate immune response to viruses. Complement (C') antiviral functions include a large number of activities, including recognition of viruses and virus-infected cells, direct neutralization of virus infectivity, recruitment and stimulation of leukocytes at sites of infection, phagocytosis by immune cells, and activation of antiviral T and B cells (Blue et al., 2004; Gasque, 2004; Kemper and Atkinson, 2007). Likewise, viruses employ mechanisms to limit C' functions (e.g., Blue et al., 2004; Johnson et al. 2012). The balance between C' effectiveness and virus inhibition of C' can have important implications for viral pathogenesis and dissemination (Delgado and Polack, 2004; Morrison et al., 2007, Stoermer and Morrison, 2011). C' can also directly impact adaptive immunity (Carroll, 2004; Kemper and Atkinson, 2007) and can influence the quality of anti-viral antibody responses (Pierson et al., 2008). The overall goal of the work described here was to determine the contribution of C' to the neutralizing capacity of antibodies elicited by respiratory tract infection of nonhuman primates with parainfluenza virus.

The C' proteolytic cascade can be initiated through three main pathways: the classical pathway, lectin pathway and alternative pathway (Gasque, 2004; Roozendaal and Carroll, 2006). Activation of the classical pathway typically involves binding of the C1q component to virus-antibody complexes. Human Immunodeficiency Virus (HIV; Ebenbichler et al., 1991) and vesicular stomatitis virus (VSV; Beebe and Cooper, 1981) are known to activate the classical pathway. The lectin pathway is activated through recognition of carbohydrate signatures on viral glycoproteins by the cellular mannan-binding lectin (MBL). This is an important pathway in the pathogenesis of Ross River Virus (Gunn et al., 2012) and in the opsonization of influenza virus (Hartshorn et al., 1993). Compared to activation of the classical and lectin pathways, the signals that activate the alternative pathway are less well understood, but they are thought to involve recognition of foreign surfaces by an antibody-independent mechanism (Gasque, 2004; Pangburn et al., 1981).

Parainfluenza virus 5 (PIV5), human parainfluenza virus 2 (HPIV2) and mumps virus (MuV) are closely-related negative strand RNA viruses belonging to the rubulavirus genus of the paramyxovirus family (Lamb and Parks, 2013; Parks et al. 2011). Prior work has shown that the rubulavirus attachment protein (Hemagglutinin-Neuraminidase; HN) and the fusion protein (F) can both contribute to activation of the alternative pathway (McSharry et al., 1981; Hirsch et al., 1986; Johnson et al., 2008; 2013). For PIV5 and MuV, the extent of alternative pathway activation is directly related to the loss of sialic acid on particles

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due to the presence of neuraminidase activity in the HN protein (McSharry et al., 1981; Hirsch et al., 1986). Furthermore, the rubulavirus F protein can dictate which arm of the C' pathway is activated. This was evident by our recent finding that a single point mutation in the ectodomain of the PIV5 F protein led to increased fusion activity, but also led to enhanced binding of IgG contained in normal human sera (NHS) and a subsequent shift in C' activation from the alternative to the classical pathway (Johnson et al., 2013).

Once activated, C' components are capable of direct neutralization of viruses, through mechanisms that can include aggregation or virion lysis (Blue et al., 2004; Stoermer and Morrison, 2011). In addition, C' can enhance the neutralizing capacity of antibodies (Mehlhop et al., 2009). For HPIV2, our prior results demonstrated very high levels of neutralizing antibody in NHS (Johnson et al., 2008), making the contribution of C' to neutralization difficult to analyze. In addition, repeated exposure to parainfluenza virus as infants (Karron and Collins, 2013) and the use of adult NHS in neutralization assays makes it difficult to determine the role of C' in the antibody function following the very first exposure at an early age to human parainfluenza virus infection. By contrast, we have previously shown in reconstitution experiments that PIV5 is neutralized through pathways that are highly dependent on the alternative C' pathway (Johnson et al., 2008). These mechanisms are either independent of antibody or involved antibodies in NHS that are only highly effective when coupled with C'.

Given the importance of understanding the initial immune response to parainfluenza virus infections, we have examined the role of C' in a primary PIV5 respiratory tract infection of African Green Monkeys (AGM), an increasingly important model system for understanding primate immunology (Messaoudi et al., 2011). The animals used in this study were part of a multigenerational, pedigreed, and genotyped Vervet Research Colony (VRC) at the Wake Forest University Primate Center (described in Jasinska et al., 2012). A powerful aspect of using this AGM system lies in the ability to measure the effect of C' on parainfluenza virus immunity in both naïve and vaccinated animals with immune systems that are closely related to that of humans. We show that respiratory tract infection of AGM with PIV5 elicits high levels of antibodies to viral antigens. However, these antibodies have poor inherent neutralizing capacity and are highly dependent on C' pathways. Our results have implications for including C' pathways as a major contributing factor in analysis of the potency of immune responses to parainfluenza virus infections and vaccine vectors.

Results

Normal monkey serum has potent neutralizing capacity against PIV5 and HPIV2

To determine the ability of normal African Green monkey serum (NAGS) to neutralize PIV5 in vitro, serum samples were collected from 20 AGM and used in neutralization assays with 100 PFU of PIV5-GFP. After 1 h at 37 °C, remaining infectivity was determined by plaque assay. As shown in Fig. 1A for representative sera from eight individual monkeys, a 1/20 dilution of NAGS reduced PIV5 infectivity to undetectable levels. In contrast, HI AGM serum was ineffective in neutralization. Titration experiments (Fig. 1B) showed effective neutralization of PIV5 by at least a 1:160 dilution of NAGS, but HI serum had no neutralizing capacity. Similar results were seen with neutralization of HPIV2 by NAGS (Fig. 1C). These results indicate that in the absence of C', NAGS from the WFU VRC have low capacity to neutralize these two parainfluenza viruses.

C'-mediated neutralization with NAGS could occur by lysis of virion particles (e.g. as seen with VSV; Johnson et al., 2012) or by formation of large aggregates (e.g., PIV5; Johnson et al., 2008). To determine the mechanism of PIV5 neutralization by NAGS, purified virus was incubated in solution at 37 °C alone or with a 1:20 dilution of NAGS before applying to a grid for analysis by electron microscopy. As shown in Fig. 1D, treatment of PIV5 particles with NAGS resulted in the formation of massive aggregates (note scale bar in panels), with little evidence of virus lysis. As a control, treatment of vesicular stomatitis virus with human sera resulted in virion lysis as evident by the detection of nucleocapsid structures (Fig. 1D). Thus, like human sera (Johnson et al. 2008), the potent C'-dependent neutralization of PIV5 by NAGS is through a mechanism involving aggregate formation.

Antibodies do not contribute to C'-mediated neutralization of PIV5 by naïve NAGS

To determine if the animals used in this study had detectable levels of anti-PIV5 antibodies, mock infected or PIV5-infected A549 cells were analyzed for cell surface staining by NAGS. As shown in Fig. 2A, the positive control polyclonal anti-PIV5 mouse serum gave strong cell surface staining on PIV5 infected cells. As shown for one representative animal (Fig. 2A; animal #1484), the NAGS from animals used in this study showed no significant staining on PIV5-infected cells compared to mock infected controls. Using western blotting of lysates from mock infected and PIV5-infected cells, sera from a few animals showed very low level reactivity against N protein (star, Fig. 2B). However, similar to the results from the immunofluorescence analysis, there was no detectable signal for reactivity against the viral glycoproteins.

To directly test the role of antibodies in PIV5 neutralization by naïve animals, NAGS was depleted of IgG by treatment with Protein G-sepharose or with sepharose alone as a control and then tested for their capacity to neutralize PIV5-GFP in vitro. As shown in Fig. 2C, a 1:20 dilution of NAGS was equally effective in neutralizing PIV5-GFP when left untreated or following treatment with Protein G-sepharose or control sepharose lacking Protein G. Together, these data support the proposal that the animals used in this study lack substantial levels of antibodies to the PIV5 glycoproteins which could contribute to neutralization, and that the C'-mediated neutralization shown above in Fig. 1 is through an antibody-independent mechanism.

A recombinant PIV5 expressing HIV gp160 elicits strong serum antibody titers to PIV5 antigens and gp120

To determine the ability of a PIV5 vector to elicit antibody responses to viral and vector-encoded antigens in AGM, PIV5 was engineered to express the HIV IIIB gp160 protein as an additional gene between HN and L (Fig. 3A). Cells infected with the PIV5-gp160 vector expressed high levels of gp160 as detected by western blotting (Fig. 3B). Analysis of multi-step growth in tissue culture cells showed that the PIV5-gp160 virus grew slightly slower than the control PIV5-GFP (Fig. 3C), but this difference was less apparent in analysis of single step growth (high moi).

To determine the capacity of PIV5-gp160 to elicit antibody responses, four adult 3 year old AGM were innoculated (in two separate experiments using two animals each) by both the intranasal and intratracheal routes with 10^8 PFU of purified virus. Tracheal washes were obtained on d4 and d8, and serum was collected on d7 and d14 post inoculation. Throughout the time-course of the experiment, animals showed no signs of overt illness, as evidenced by the lack of significant rise in temperature, no loss of cognitive function and the absence of labored breathing (data