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Canine distemper virus neutralization activity is low in human serum and it is sensitive to an amino acid substitution in the hemagglutinin protein

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ABSTRACT

Serum was analyzed from 146 healthy adult volunteers in eastern Africa to evaluate measles virus (MV) and canine distemper virus (CDV) neutralizing antibody (nAb) prevalence and potency. MV plaque reduction neutralization test (PRNT) results indicated that all sera were positive for MV nAbs. Furthermore, the 50% neutralizing dose (ND50) for the majority of sera corresponded to antibody titers induced by MV vaccination. CDV nAbs titers were low and generally were detected in sera with high MV nAb titers. A mutant CDV was generated that was less sensitive to neutralization by human serum. The mutant virus genome had 10 nucleotide substitutions, which coded for single amino acid substitutions in the fusion (F) and hemagglutinin (H) glycoproteins and two substitutions in the large polymerase (L) protein. The H substitution occurred in a conserved region involved in receptor interactions among morbilliviruses, implying that this region is a target for cross-reactive neutralizing antibodies.

Introduction

Canine distemper virus (CDV) is a member of the *Morbillivirus* genus, which also includes measles virus (MV), rinderpest virus (RPV), peste des petits ruminants virus and morbilliviruses that infect aquatic mammals (Blixenkrone-Moller, 1993; Di Guardo et al., 2005). These related viruses generally each have a restricted natural host range. For example MV infects humans, RPV infects cattle and other even-toed ungulates, and CDV infects a variety of carnivorous animals. However, CDV infection has been observed in monkey colonies indicating that its host range can extend to primates (Qiu et al., 2011; Sakai et al., 2013a), but so far, there is no conclusive evidence linking CDV to human disease in spite of its speculative association to illness of unknown etiology (Rima and Duprex, 2006). Lab-adapted CDV has been injected into humans without causing symptoms of infection suggesting that humans are not a permissive host for the virus (Hoekenga et al., 1960), which is consistent with recent studies showing that mutations facilitating both entry and replication are needed for CDV to efficiently adapt to human cells (Otsuki et al., 2013; Sakai et al., 2013b). Prevalent MV immunity induced by universal vaccination or natural infections might also play a role in preventing CDV from crossing the human barrier (de Vries et al., 2014). Despite considerable characterization of antigenic and immunological relationships between CDV and MV

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(Haile et al., 1982; Orvell and Norrby, 1974, 1980; Stephenson and ter Meulen, 1979), CDV neutralizing antibodies (nAbs) in humans have not been extensively investigated.

Morbilliviruses are attractive candidates for development of replication-competent vectors because modified live vaccines (e.g. MV, CDV, and RPV) have proven to be very safe and efficacious (Buczkowski et al., 2014), and promising preclinical results have been generated with a number of experimental vectors (Brandler et al., 2007; Brandler and Tangy, 2008; Despres et al., 2005; Gauvrit et al., 2008; Guerbois et al., 2009; Miest and Cattaneo, 2014; Wang et al., 2012). Morbilliviruses seem particularly relevant for development of replication-competent AIDS vaccine vectors since this genera of viruses replicates in lymphoid tissues like HIV (Draper and Heeney, 2010; Koff et al., 2013; Parks et al., 2013). Pre-existing MV immunity may interfere with use of MV vectors, and unlike other viral vector systems in which rare serotype viruses can be used as vector alternatives (Mingozzi et al., 2013; Santra et al., 2009), MV has just one serotype. Thus, CDV has been considered as a MV alternative to minimize the effect of widespread anti-MV antibodies (Miest et al., 2011; Zhang et al., 2013b). Because antibodies specific to MV do cross-react with CDV (Appel et al., 1984; de Vries et al., 2014; Rima, 1983; Taylor et al., 1991), it is important to evaluate the prevalence and potency of CDV neutralizing activity in humans.

In this study, 146 serum samples collected from healthy adults in three eastern Africa countries were surveyed for both MV and CDV nAbs. We found that MV nAbs were prevalent in these samples while the frequency of samples with significant CDV nAb titers was low. Moreover, when CDV neutralizing activity was detected, it correlated with high anti-MV titers. We also used human anti-serum to derive an in vitro escape mutant CDV strain with increased resistance to neutralization. Genomic sequence analysis of the resistant strain revealed an amino acid substitution in a conserved region of the MV and CDV hemagglutinin (H) proteins that may help identify the domain recognized by cross-reactive nAbs and aide in future design H variants that are less sensitive to the effect of anti-vector immunity.

Results

MV nAbs in African serum samples

Serum was collected from 146 healthy adult male and female volunteers between 19 and 50 years of age (Table 1). The volunteers were participants in vaccine trial preparedness cohorts (Kamali et al., 2014) enrolled at 5 clinical research centers (CRCs) supported by IAVI in Kenya (Kilifi and Nairobi), Rwanda (Kigali), and Uganda (Masaka and Entebbe).

The threshold for MV nAb positivity was defined as average ND50 titer of naïve macaque serum plus 3 times standard deviation. When the PRNT was performed with the naïve macaque serum control, the threshold was calculated as 8.63. All serum samples from African volunteers were positive for MV nAbs since their titers were above this threshold (Fig. 1) and the ND50 values ranged from 16.0 to 6270. For comparison, serum analyzed from a monkey vaccinated with an MV vaccine had an ND50 value of 1446 and earlier studies in college students indicated that ND50 titers below 120 do not prevent measles (Chen et al., 1990). Overall, 77.4% of the African serum MV ND50 values fell between 120 and 2000, which is approximately equivalent to 300–5000 milli-International Units (mIU) of WHO international standard MV antibodies (Cohen et al., 2007). The 300–5000 mIU range is consistent with MV nAb levels induced by routine vaccination (Hussain et al., 2013; Leuridan et al., 2010), suggesting that these volunteers probably were vaccinated, although an MV vaccination history was not available to confirm this. Thirteen percent of the samples exhibited MV ND50 titers above 2000, which was indicative of a stronger response than typically observed after vaccination suggesting that some volunteers had contracted measles at some point (Leuridan et al., 2010). No significant difference in ND50 titers was observed across gender or age groups. Overall antibody titers were similar among the regions except for Nairobi where volunteers exhibited significantly lower titers than other regions ($p=0.01$).

Table 1
Demographic characteristics of volunteers involved in the study ($n=146$). CRC: Clinical Research Center.

CRC	Total	Volunteer sex				Volunteer age			
		Male		Female					
		N	%	*N*	%	Median	Mean	Min	Max
Kigali	30	14	46.7	16	53.3	27.5	30.2	20	50
Masaka	30	20	66.7	10	33.3	36.5	36.7	23	48
Kilifi	26	13	50.0	13	50.0	32.5	32.2	20	46
Nairobi	30	17	56.7	13	43.3	26.5	29.0	20	41
Entebbe	30	15	50.0	15	50.0	30.5	30.1	19	45
Total	146	79	54.1	67	45.9	32.0	31.6	19	50

Generally low CDV nAbs titers in African serum samples and their correlation with the magnitude of MV nAbs

When the CDV PRNT was performed with naïve ferret serum, the average CDV ND50 titer plus 3 times standard deviation was determined to be 29.29, which we employed as our threshold for positivity. Based on this, approximately 33.6% of the African volunteer serum samples were negative. ND50 titers in 46.6% of the volunteers were between 29.3 and 120 (Fig. 2) and the remaining 19.8% had CDV ND50 titers above 120 but below 800. For comparison, serum from a ferret recently vaccinated with live-attenuated CDV was 33,551. Similar to MV nAbs, CDV ND50 titers were not significantly different across gender. With the exception of the volunteers from the Masaka CRC where higher CDV nAbs ($p<0.001$) were detected, no significant difference was observed for the other geographical regions. Although the CDV nAb titers overall were low, positive CDV nAb values generally correlated with higher magnitude MV titers (Fig. 3A, Spearman's $\rho=0.61$, $p<0.001$). This relationship persisted when the correlation analyses were performed using nAb data that were stratified by

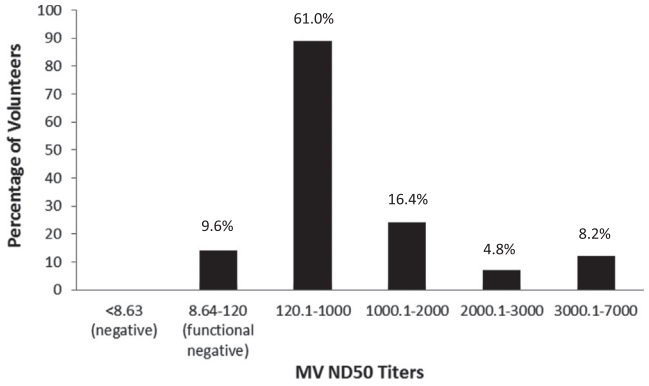

Fig. 1. Distribution of MV nAb titers. Negative threshold was defined as the average nAb titer of unvaccinated monkey serum plus 3 × standard deviation. Functional negative threshold was ND50 titer 120 since MV ND50 titers lower than that do not prevent measles (Chen et al., 1990). For the 146 volunteers, 90.4% had MV titers higher than 120 and majority of the titers were in ranges of 120.1–1000 (61.0%) and 1000–2000 (16.4%), which correspond to MV antibodies induced by vaccinations (Hussain et al., 2013; Leuridan et al., 2010).