P.p1 pseudovirions could also be neutralized by sera

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0px; font: 10. 0px Times} Complement proteins are present in the blood and invarious tissues in uncleaved, inactivated forms. Complementaction can be initiated by direct recognition of a microbialinvader by C1q (a component of C1) in the classical pathway, or by recognition of cleaved C3b proteins in the alternativepathway . Humananti-sera neutralize hantavirus pseudovirions at titers comparable to PRNTassaysTo determine if pseudovirionscould also be neutralized by sera from ANDV HPS convalescent patients, wetested 60 sera samples (20 ANDV sera, 40 non-immune sera) for neutralizingactivity against ANDV and VSV-G pseudovirions. ANDV positive sera werepreviously screened for virus neutralization titers by PRNT on Vero E6 cells (Hjelle); negative serawere screened by ELISA (Martinez). Pseudovirions were pre-incubated with 2-fold dilutions of sera prior to infectingVero E6 cells and IC20 values calculated from the resultingneutralization curves.

Representative sera titrations are shown in Fig. 3 and their corresponding IC80 and PRNTvalues reported in Table 1. Wecompleted full titrations in triplicate for all sera.

At the highest sera concentration (1: 50dilution) non-immune sera were not able to neutralize ANDV pseudovirions, whileall ANDV sera were able to completely neutralize infection. ANDV sera Pseudovirion neutralization IC80titers ranged from 1: 400 to 1: 6400. The respective geometric mean titer(GMTs) was 3.

2492. In contrast, PRNT80 titers ranged from 1: 100 to1: 3200, with a GMT of 2. 978. These data show that neutralizing antibodies canbe detected in immune human sera using this pseudovirions system at titerscomparable to those used in PRNT assays.

Glycoprotein-specific antibodies neutralize pseudovirion infectionTo verifythat infectivity was facilitated by ANDV glycoproteins and not by residual VSVG protein, pseudovirions were pretreated with either ANDV HPS convalescentpatient antisera (immune sera) or with a neutralizing VSV G-specific antibody(I14). Infection of Vero E6 cells was reduced by more than 80% (IC80) when ANDVpseudovirions were pre-treated with immune sera at 1: 50 fold dilution, whereas VSV pseudovirions were notaffected (Fig. 2). In contrast, VSV but not ANDV pseudovirions were neutralizedafter pre-treatment with the anti-VSV G monoclonal antibody (I14, 1: 50).

Thus, pseudovirion infection was mediated by the appropriate glycoproteins. ResultsPseudovirion productionAndes (ANDV)pseudovirions were prepared utilizing a vesicular stomatitis virus (VSV) vectorexpressing the Renilla luciferase (rLuc) gene (Ray et al., 2010). VSVpseudovirions with the VSV G gene provided in trans were used to transduce HEK293T cells that had been transfected with plasmids expressing ANDV M segment. After transduction, cells were incubated for 12-24h at 37 °C andpseudovirion-containing media was collected, filtered, and the pH adjusted to8.

0. (Meda et al., 2012) Titration of pseudovirionsTo titrateANDV or VSV G pseudovirions expressing luciferase, we infected Vero E6 cellswith serial dilutions and quantified infection by relative light units (RLUs). Basically to

confirm that rLuc pseudovirion infection yielded signal in thelinear range, cells were infected with 2-fold dilutions of pseudovirions stocksfor 24 h before cell lysis and measurement by luminometer (Fig. 1A and 1B). Subsequent experiments were done at pseudovirion dilutions that resulted inRLUs between 2×106 and 4×106 in Vero E6 cells (1: 16), well within the linear range of the detection.