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Enhancement of neutralizing activity of influenza virus-specific antibodies by serum components

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Abstract

The role of serum components in enhancing virus neutralizing (VN) activity of influenza virus A/PR/8/34 hemagglutinin (HA)-specific MAbs in vitro was investigated. The degree of enhancement depended on the MAb's fine specificity and heavy chain isotype and on type of serum. Greatest enhancement (>100-fold) was seen with sera from immunodeficient mice that lacked serum immunoglobulin. At least two serum components were involved: C1q and a heat-resistant factor. C1q was mandatory for enhancement, and other components of the complement system were not required. C1q appeared to operate by improving MAb-mediated inhibition of virus attachment to host cells and was most effective with MAbs that inhibited virus attachment poorly on their own. The heat-resistant factor enhanced VN activity only in the presence of C1q and appeared to operate by enhancing VN activity at a post-attachment stage.

Keywords: Virus neutralization; Influenza A virus; C1q; Complement; Ab-mediated protection

Introduction

Antibodies (Abs) play important roles in protection against and recovery from influenza virus infection (reviewed in Gerhard, 2001). They may contribute to the control of the infection by reaction with (i) infectious virus particles and thereby impair their infectivity and/or (ii) infected host cells and thereby limit the yield of virus progeny. Abs may mediate protective activities autonomously or in conjunction with other host components such as complement, Fc-receptor (FcR)-expressing cells and lectins.

In a previous study, we compared four influenza virus HA-specific MAbs of G2a isotype for VN activity in vitro and prophylactic activity in a passive transfer system in vivo (Mozdzanowska et al., 1997). Although VN activity in vitro and Ab-mediated prophylaxis in vivo appear to measure similar Ab-mediated activities, we found that the four MAbs exhibited surprisingly large discrepancies in these activities. For instance, one MAb directed to the antigenic region Cb, which is located membrane-proximal of the sialic acid (SA) binding site of HA (Fig. 1) (Caton et al., 1982), exhibited VN activity in vitro at 2.5 μg/ml (Ab concentration at which 50% of MDCK cell cultures are protected from infection by 100 $TCID_{50}$ of virus) and prophylactic activity in vivo at 8 μg/ml (serum Ab concentration at which 50% of SCID mice are protected from intranasal infection by 200 $TCID_{50}$) (Mozdzanowska et al., 1997, and unpublished observations). By contrast, another MAb of the same isotype but directed to the antigenic region Sb of HA, which is located membrane-distal of the SA binding site (Fig. 1) (Caton et al., 1982), displayed 2500-fold greater VN activity in vitro (0.001 μg/ml) but only eight-fold greater prophylactic activity (1 μg/ml serum). Thus, in relation to their VN activity in vitro, these MAbs differed by ~300-fold in prophylactic activity in vivo. This suggested that components/conditions in vivo differentially affected their prophylactic activity, e.g., by inhibiting Sb-specific or enhancing Cb-specific MAbs. The latter appeared more likely in view of the finding that performance of the VN assay in vitro in the presence of 1.6% non-inactivated serum resulted in preferential enhancement of the VN activity of Cb-specific MAbs (Mozdzanowska et al., 1997). The general relation between Ab fine specificity and differential enhancement of their hemagglutination inhibition (HI) activity by serum

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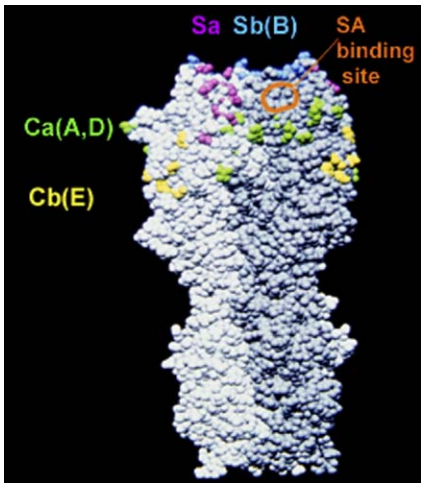

Fig. 1. Location of antigenic regions on HA relative to the sialic acid (SA) binding site. The 3D structure of the HA timer of H3 subtype HA (Wilson et al., 1981) is shown in side view, with the left monomer in lighter and the right monomer in darker blue. The viral membrane is to be located at the bottom edge of the figure. The location of amino acid replacements in escape mutants used for identification of antigenic regions Sa (magenta), Sb (blue), Ca (green) and Cb (yellow) of PR8 are superimposed onto the H3 structure. The designation of the corresponding sites in the H3 model (Wiley et al., 1981) is indicated in brackets. The brown circle shows the approximate location of the SA binding site on one of the monomers.

components was subsequently confirmed with a large panel of HA-specific MAbs (Feng et al., 2002).

Here, we describe further studies on the enhancement of Ab-mediated VN activity by serum components. We found that at least two serum components were involved: C1q, the binding moiety of the first complement component, and a still undefined heat-resistant factor, whose enhancing activity was dependent on C1q. A high concentration of this factor in sera of mice that lacked serum immunoglobulin appeared to account for their elevated VN enhancing activity. C1q appeared to enhance VN activity at the pre-attachment stage and was effective mainly with MAbs that inhibited virus attachment poorly on their own. By contrast, the heat-resistant factor appeared to enhance VN activity at a post-attachment stage and was similarly effective with MAbs of high or low autonomous VN activity.

Results

Measurement of virus neutralization enhancement (VNE)

VNE was assessed in the MDCK cell assay by measuring Ab-mediated VN activity in the absence and presence of 0.5% non-inactivated naive mouse serum (NMS). Briefly, dilutions of MAb were incubated in parallel for 60 min at 37 °C with 100 $TCID_{50}$ of PR8 virus in medium with or without 0.5% non-inactivated NMS. Replicate samples were then transferred to MDCK cell microcultures and incubated for another 60 min at 37 °C to allow initiation of infection by residual infectious virus. The inocula were then replaced by serum-free medium and the cultures tested for productive infection after 3–4 days of incubation. In the absence of MAb, 0.5% NMS had usually no measurable effect on virus infectivity as shown by titration of the virus inoculum in medium with or without NMS (Fig. 2A). Nevertheless, this control was performed in each assay to identify and exclude occasional serum batches that displayed significant VN activity on their own. By contrast, when HA-specific MAbs were tested in the presence and absence of 0.5% NMS, their VN activity (defined as Ab concentration at which 50% of microcultures were protected from infection) was often greatly enhanced in the presence of serum. The difference in VN activity in the presence versus absence of NMS was defined as VNE and is 92 in the example shown in Fig. 2B. VNE decreased with decreasing concentration of NMS and became insignificant below 0.05% of NMS (Fig. 2C).

SCID sera mediate stronger VNE than BALB/c sera

We noticed that SCID NMS mediated 10–20 times stronger VNE than BALB/c NMS against the HA(Cb)-specific MAb H35-C12. To determine whether this was a peculiarity of H35-

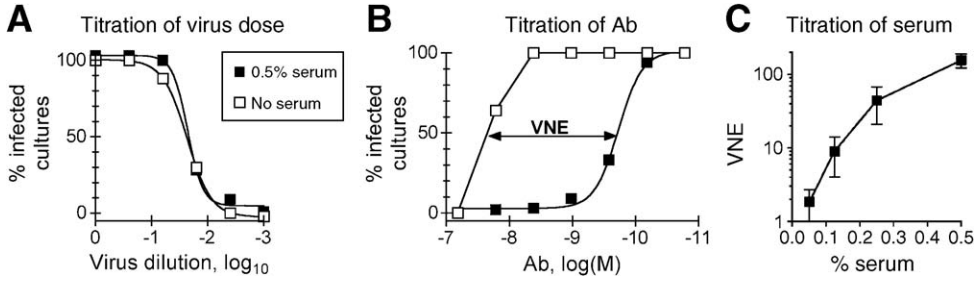

Fig. 2. Measurement of VNE. An example of a typical VN assay is shown. (A) Dilutions of the virus challenge dose (~2000 $TCID_{50}$/ml) were incubated for 1 h at 37 °C in medium without (open symbols) or with 0.5% non-inactivated SCID serum (both without MAb). Replicate samples (6 × 50 μl) of each virus dilution were then transferred to washed MDCK cell microcultures and incubated for 1 h at 37 °C. The inoculum was replaced by culture medium (ISC-CM, 0.1% BSA, 1 μg/ml trypsin) and tested after 3–4 days of incubation for evidence of virus replication. (B) Dilutions of MAb H35-C12 were incubated for 1 h at 37 °C with PR8 (~2000 $TCID_{50}$/ml) in the absence or presence of non-inactivated SCID serum. Replicate samples (12 × 50 μl) of each Ab-virus mixture were transferred to washed MDCK cell microcultures and incubated for 1 h at 37 °C before replacement of the inoculum with culture medium as above. After 3–4 days of incubation, cultures were tested for evidence of virus replication and the MAb concentration required for protecting 50% of the cultures determined by the method of Reed and Munch (1938). The percentage of cumulative non-infected cultures at each MAb dilution is shown. The difference in MAb-mediated VN activity in the absence versus presence of NMS measures VNE and is indicated by the two-sided arrow. (C) VNE was determined as above with SCID serum at the indicated concentrations. Each symbol shows the mean and SEM of 3–5 independent determinations.