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Neutralization capacity of measles virus H protein specific IgG determines the balance between antibody-enhanced infectivity and protection in microglial cells

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ABSTRACT

Neutralizing antibodies directed against measles virus (MV) surface glycoproteins prevent viral attachment and entry through the natural receptors. H protein specific IgG can enhance MV infectivity in macrophages *via* Fcγ receptor (FcγR)-dependent mechanism. H-specific IgM, anti-F antibodies and complement cascade activation are protective against antibody-mediated enhancement of MV infection. However, protective role of anti-H IgG against antibody-enhanced infection is not well understood. Here we designed a set of experiments to test the protective effect of H-specific IgG against FcγR-mediated infection in microglial cells. Microglial cells are also potential target of the antibody-mediated enhancement and spread of MV infection in the central nervous system. A partially neutralizing IgG monoclonal antibody (MAb) CL55, specific for MV H protein, at 10 μg/ml enhanced MV infection in mouse microglial cells by 13–14-fold. Infection-enhancing antibody concentrations induced large multinucleated syncytia formation 48–72 h post-inoculation. We generated anti-H IgG MAb 20H6 with a strong neutralization capacity >1:80,000 at 1 mg/ml concentration in MV plaque-reduction neutralization assay. In contrast to the partially protective MAb CL55, enhancement of MV infectivity by MAb 20H6 required dilutions below the 1:120 serum titer considered protective against measles infection in humans. At a concentration of 10 μg/ml MAb 20H6 exhibited a dominant protective effect and prevented MAb CL55-mediated enhancement of MV infection and virus-mediated fusion. These results indicate that neutralization capacity of the H-specific IgG determines the balance between antibody enhancement and protection against MV infection in microglial cells.

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1. Introduction

Measles is considered the most contagious human infectious disease with millions of cases and more than 150,000 deaths reported annually (Centers for Disease Control and Prevention (CDC), 2012). Measles virus (MV) is a lymphotropic virus with activated lymphocytes, dendritic cells and monocyte/macrophages being the main infection target (Griffin, 2001). Massive infection of immune cells results in transient immune suppression and complications by secondary bacterial infections. Replication in respiratory epithelial cells is an important part of the virus life cycle and epidemic spread. MV is a paramyxovirus with lipoprotein envelope and negative RNA genome, consisting of six genes encoding eight viral proteins (Griffin, 2001). The two MV surface glycoproteins – hemagglutin (H) and fusion (F) protein are responsible for binding to viral receptors on the surface of host cells and subsequent entry by cell membrane fusion. Natural infection or vaccination with the live attenuated MV strains induces life-long immunity against re-infection. Introduction of the live MV vaccine and massive immunization program have drastically reduced measles morbidity and mortality. Vaccination is not 100% efficient in measles prevention, however. Genetic polymorphism in HLA, cytokine receptors and anti-viral effector proteins are some of the individual factors contributing to the poor response and low antibody titers (Dhiman et al., 2007; Haralambieva et al., 2011b; Ovsyannikova et al., 2004). Identification of strongly protective B-cell-restricted H and F epitopes is critical in the development of more efficient, highly immunogenic vaccines against measles. Humoral immune response is complex and includes protective envelope glycoprotein specific antibodies and non-protective antibodies directed to the other

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MV proteins (Griffin, 2001). H and F specific antibodies prevent viral attachment and fusion. Neutralization capacity of these antibodies depends on their epitope specificity, affinity and isotype.

Viruses can utilize antibodies or complement components for attachment and entry *via* Fcγ receptors (FcγRs) or complement receptors (Halstead et al., 2010; Huisman et al., 2009; Robinson et al., 1990; Takada and Kawaoka, 2003). Antibody-enhanced infectivity is responsible for severe forms of dengue virus infection – hemorrhagic fever and shock syndrome with high fatality rate (Dejnirattisai et al., 2010). Antibody and complement increased infection has been observed for HIV, flavi- and filoviruses (Dowd and Pierson, 2011; June et al., 1991; Meyer et al., 2008; Takada et al., 2003; Takeda et al., 1988). In addition, ligation of Fcγ receptor (FcγR) by immune complexes triggers signaling mechanisms that facilitate pathogen propagation by modulation of the innate anti-viral response (Halstead et al., 2010; Suhrbier and La Linn, 2003). Formation of the virus-antibody immune complexes requires previous sensitization to the pathogen antigens and IgG response. Pre-existing IgG can retarget and increase viral replication in macrophages and mature dendritic cells.

Previously, we have reported the existence of an antibody-mediated mechanism of enhanced MV infectivity (Iankov et al., 2006). Partially neutralizing IgG monoclonal antibody (MAb) against the H protein significantly increased MV infection in macrophageal cells. Human and mouse polyclonal anti-MV antibodies were also infection-enhancing at higher dilutions. In contrast, anti-F antibodies and complement did not increase viral infectivity and were protective preventing anti-H antibody-mediated enhancement of MV infection. H-specific IgM and classical complement activation also prevented IgG-mediated infection enhancement. Passive immunization of animals with serum in corresponding to antibody-enhancing infectivity concentrations increased viral replication and reporter gene expression following intraperitoneal (i.p.) administration of MV (Iankov et al., 2006). These data suggest that H-specific IgG-mediated entry can facilitate MV replication at certain anatomical locations in the presence of strong anti-viral immunity, possibly being invoked in the immunopathology of atypical measles. However, the effect of strongly neutralizing H-specific antibodies of IgG isotype on antibody-mediated enhancement of MV infection has not been investigated.

Here, we demonstrate that partially neutralizing IgG antibodies specific for MV hemagglutinin can enhance not only individual cell infection but also can trigger cell-to cell fusion and giant syncytia formation in central nervous system (CNS) microglial cells. Antibodies with strong neutralization activity against infection in epithelial cells at relevant protective levels prevent the antibody enhancement and syncytia formation induced by non-neutralizing IgG. However, at high subprotective dilutions they can also trigger infection in FcγR expressing cells suggesting that neutralization capacity of anti-H IgG determines its ability to increase MV infectivity.

2. Materials and methods

2.1. Cell lines, plasmids, MV strains and culture conditions

African green monkey Vero cell line, mouse J774A.1 macrophageal cells and human embryonic kidney 293 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD). Mouse microglial cell lines N13 (Righi et al., 1989) and BV2 (Blasi et al., 1990) were kindly provided by Dr. J.P. Godbout, Ohio State University, Columbus, OH (Wynne et al., 2010). The cells were maintained in DMEM (from ATCC or HyClone) supplemented with 10% fetal bovine serum (FBS) and antibiotics (Invitrogen). Hybridoma clone CL55 expressing IgG2b MAb against MV H protein (Giraudon and Wild, 1985) was kindly provided by T.F. Wild, Institut Pasteur de Lyon, France.

MV expressing green fluorescent protein (MV-GFP) (Duprex et al., 1999) and recently characterized MV expressing *Helicobacter pylori* NAP protein (MV-s-NAP) (Iankov et al., 2011) were amplified on Vero cells. Viral stocks were prepared using repeated freezing-thawing procedure and virus titer was determined in both plaque-forming units (PFU) or tissue culture infectious doses 50% ($TCID_{50}$) per ml (Iankov et al., 2011). MV encoding human sodium iodide symporter (MV-NIS) (Dingli et al., 2004) was purified as previously described (Langfield et al., 2011).

Plasmids PCG-H and PCG-F encoding the H or F protein (Cathomen et al., 1995; Leonard et al., 2008) of MV were kindly provided by Dr. R. Cattaneo, Mayo Clinic, Rochester, MN. PCG-H(Edm) and PCG-H(wt-323) encode H protein from Edmonston vaccine strain or wild type MV strain IC-323 respectively.

2.2. Production of MV neutralizing monoclonal antibodies (MAbs)

Hybridomas were generated after immunization of MV infection permissive interferon type I receptor knockout and human CD46 transgenic (Ifnarko-CD46Ge) mice (Mrkic et al., 1998; Iankov et al., 2011). The animals were immunized with 10^6 $TCID_{50}$ of live MV-s-NAP by an i.p. route. Spleen cells were collected and fused with myeloma line Sp2/0-Ag14 (ATCC) as described previously (Köhler and Milstein, 1975; Campbell, 1991). Hybridoma culture supernatants were tested by immunoblotting, virus neutralization (VN) and antigen-mediated ELISA. Hybridomas producing MAbs against MV antigens were cloned from a single cell and grown in DMEM (ATCC) supplemented with 10% FBS, antibiotics (Invitrogen) and 2 ng/ml recombinant IL-6 (Novus Biologicals). MAb isotype was determined using an IsoStrip Monoclonal Antibody Isotyping kit (Santa Cruz Biotechnology).

2.3. MAb characterization, purification and conjugation

MAb reactivity against MV antigens was characterized by VN test, ELISA and flow cytometry. Highly neutralizing H protein specific MAb 20H6 (IgG2a isotype) was selected for further characterization. Neutralization capacity of the clone was determined by VN test. Hybridoma 20H6 cells were cultured in serum-free medium (Invitrogen) supplemented with IL-6 and MAb was purified on Protein G column (Pierce). Protein concentration was determined using a BCA kit (Pierce). Purified antibody was conjugated to horse-radish peroxidase (HRP) or biotin using Lightning-Link conjugation kits (Innova Biosciences, UK). MAb CL55 was purified as described previously (Iankov et al., 2006).

2.4. Virus neutralization (VN) test

Neutralizing titer against MV of MAbs was measured by plaque-reduction microneutralization assay and plaque reduction neutralization titer 50% (PNT_{50}) was calculated as described previously (Haralambieva et al., 2008). *In vitro* MV neutralization capacity of MAbs was determine based on the antibody concentration required for 50% neutralization of the viral particles (defined as 1 PNT_{50} activity).

2.5. Human serum antibodies

Serum samples used in the study was collected from AB(+) blood group healthy donors following Institutional Review Board approval and their protective titers against measles have been analyzed and reported previously (Iankov et al., 2010). VN activity

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