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Complement-Dependent Lysis of Influenza A Virus-Infected Cells by Broadly Cross-Reactive Human Monoclonal Antibodies

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We characterized human monoclonal antibodies (MAbs) cloned from influenza virus-infected patients and from influenza vaccine recipients by complement-dependent lysis (CDL) assay. Most MAbs active in CDL were neutralizing, but not all neutralizing MAbs can mediate CDL. Two of the three stalk-specific neutralizing MAbs tested were able to mediate CDL and were more cross-reactive to temporally distant H1N1 strains than the conventional hemagglutination-inhibiting and neutralizing MAbs. One of the stalk-specific MAbs was subtype cross-reactive to H1 and H2 hemagglutinins, suggesting a role for stalk-specific antibodies in protection against influenza illness, especially by a novel viral subtype which can cause pandemics.

Human influenza is a highly contagious acute respiratory illness that is responsible for significant morbidity and excess mortality, especially in the elderly and the very young worldwide. Every year in the United States, on average 5% to 20% of the population acquires influenza, more than 200,000 people are hospitalized for influenza complications, and influenza-related deaths range from 3,000 to 49,000. The elderly, young children, and individuals with certain health conditions are at high risk for serious influenza complications (Centers for Disease Control and Prevention [http://www.cdc.gov/flu/about/disease/index.htm]). Current vaccine approaches depend primarily on the induction of antibodies to the viral surface protein hemagglutinin (HA). Serum hemagglutination inhibition (HAI) titers to the circulating virus of 1:40 or greater are considered subtype specific and bind to the globular head region of the HA, a receptor binding site (14). In 1993, however, HAI titers measured pre- and postvaccination were not distinguishable between subjects who subsequently developed influenza illness and those who did not (12), showing the limitation of the HAI titer as an indicator of protection in this population.

Antibodies inducing HAI and neutralization are generally associated with significant protection against influenza illness (15). In the elderly, however, HAI titers measured pre- and postvaccination were not distinguishable between subjects who subsequently developed influenza illness and those who did not (12), showing the limitation of the HAI titer as an indicator of protection in this population.

Phylogenetic analysis [17] influenza A viruses (1, 2, 20). These stalk region-specific antibodies cannot inhibit hemagglutination (2, 13, 20, 23). The presence of these MAbs indicates that at the clonal level, some neutralizing and hemagglutination-inhibiting antibodies are distinct and their activities are not correlated.

In addition to the neutralization of cell-free virus by antibodies to HA and the interference of virus release from infected cells by antibodies to neuraminidase (NA), influenza virus-specific antibodies bind to infected cells and are able to lyse the virus-infected cells through activation of complement (complement-dependent lysis [CDL]) (16, 21). The complement system plays several roles in response to influenza virus infection. In primary infection with influenza virus, mice deficient in component C3 showed delayed viral clearance and increased viral titers in lungs (9). The addition of complement can enhance the neutralization of influenza virus by antibodies in vitro (5). Complement is also known to enhance influenza virus-specific CD4+ and CD8+ T cell responses and to help maintain long-term memory of influenza viruses in mice (3, 9). Complement, therefore, can link innate and adaptive immunities and is probably important to consider for vaccine development (4).

In this study, we analyzed 13 HA-specific human MAbs molecularly cloned from plasmablasts obtained from patients infected with 2009 pandemic influenza (23) or from recipients of prepandemic seasonal influenza vaccines (24) by CDL assay, which is a modification of a method reported previously (16, 21). Cells from the human lung cancer cell line A549 (type II alveolar epithelial cells) (11) infected with influenza virus were used as targets instead of mouse kidney or embryo cells. All MAbs have the same constant region of human IgG1 subclass (the variable region of an antibody was cloned by reverse transcription [RT]-PCR and recombined with the constant region of IgG1), the most abundant subclass which can activate the classical pathway of the complement system (7, 8). These MAbs were categorized into four different groups based on
TABLE 1. CDL activities of MAbs against target cells infected with 2009 pandemic or seasonal H1N1 influenza A virus strains

<table>
<thead>
<tr>
<th>Mab</th>
<th>Virus specificity</th>
<th>MN/HAI+</th>
<th>MN/HAI-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pandemic H1N1</td>
<td>Seasonal H1N1</td>
</tr>
<tr>
<td>MN+HAI+</td>
<td>Globular head specific</td>
<td>1009-3B06</td>
<td>HIN1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1009-3F01</td>
<td>HIN1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM-4C04</td>
<td>HIN1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TIV-1</td>
<td>HIN1</td>
</tr>
<tr>
<td>MN+HAI+</td>
<td>Stalk specific</td>
<td>1009-3B05</td>
<td>HIN1, HSN1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1009-3E06</td>
<td>HIN1, HSN1</td>
</tr>
<tr>
<td>70-1F02</td>
<td></td>
<td>14.2</td>
<td>39.9</td>
</tr>
<tr>
<td>MN-HAI-</td>
<td></td>
<td>TIV-2</td>
<td>HIN1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000-1B02</td>
<td>HIN1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000-2C02</td>
<td>HIN1</td>
</tr>
<tr>
<td>70-D01</td>
<td></td>
<td>0.2</td>
<td>3.1</td>
</tr>
<tr>
<td>70-5D01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Data are from reference 23 except those for MAbs TIV-1 and TIV-2.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>MAbs were tested at 10 μg/ml in CDL assays. Low-Tox guinea pig complement (Cedarlane Laboratories, Burlington, NC) was added at a final dilution of 1:20. Target cells are A549 cells infected with seasonal [A/Solomon Islands/3/2006 (H1N1)] or 2009 pandemic [A/California/7/2009 (H1N1)] influenza A virus strains at multiplicities of infection of 5 to 10. Percent specific lysis is calculated as (% lysis by antibody + complement) − (% lysis by complement only) (% maximum lysis) − (% lysis by complement only). Maximum lysis was obtained by lysing target cells by Renex 30 detergent (Uniqema, New Castle, DE). All assays were performed in triplicate. Positive lysis results are shown in bold.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>Not neutralizing, but not all neutralizing MAbs can mediate CDL.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>Neutralizing, but not all neutralizing MAbs can mediate CDL.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: Data are from reference 23 except those for MAbs TIV-1 and TIV-2. 
b: MAbs were tested at 10 μg/ml in CDL assays. Low-Tox guinea pig complement (Cedarlane Laboratories, Burlington, NC) was added at a final dilution of 1:20. Target cells are A549 cells infected with seasonal [A/Solomon Islands/3/2006 (H1N1)] or 2009 pandemic [A/California/7/2009 (H1N1)] influenza A virus strains at multiplicities of infection of 5 to 10. Percent specific lysis is calculated as (% lysis by antibody + complement) − (% lysis by complement only) (% maximum lysis) − (% lysis by complement only). Maximum lysis was obtained by lysing target cells by Renex 30 detergent (Uniqema, New Castle, DE). All assays were performed in triplicate. Positive lysis results are shown in bold. 
c: Not neutralized at the highest concentration tested (30 μg/ml). 
d: Bound to H5 HA in ELISA. 
e: Not neutralizing, but not all neutralizing MAbs can mediate CDL. 
f: Neutralizing, but not all neutralizing MAbs can mediate CDL.
was subtype cross-reactive to H2 HA. It is likely that this MAb recognizes an epitope slightly different from those of other stalk-specific MAbs or binds to the same epitope at a different angle.

Recently, Steel et al. reported the generation of new influenza vaccines (a combination of a DNA vaccine and a virus-like particle) based on the stalk region and their immunogenicity in mice (19). Wei et al. reported induction of stalk-
specific antibodies by a prime/boost combination of plasmid DNA plus an adenovirus vector or plasmid DNA plus a seasonal vaccine in mice, ferrets, and monkeys (22). Corti et al. cloned memory B cells producing subtype cross-reactive stalk-specific IgG from four healthy human donors who received a seasonal influenza vaccine (1), while Wrammert et al. cloned human MAbs only from 2009 pandemic influenza virus-infected patients, but not from seasonal influenza vaccine recipients (23). It would be helpful to learn how common these subtype-cross-reactive stalk-specific antibodies are and about their biologic functions in vivo.

Although primary infection of BALB/c mice induced CDL antibodies that were subtype specific (21), human studies conducted after the reemergence of the H1N1 subtype in 1977 found that sera from three young adults, who were unlikely to have previously experienced H1N1 influenza viruses (they were exposed to H2N2 and H3N2 subtypes between 1957 and 1976, but not to the H1N1 subtype), showed low levels of CDL against target cells infected with A/USSR/90/1977 (H1N1) (16). These low levels of CDL to “novel” influenza virus subtypes may be mediated by antibodies cross-reactive to H1 and H2 HAS. A study using mouse MAbs showed that, in addition to anti-HA antibodies, NA- and nucleoprotein-specific MAbs mediated CDL of influenza virus-infected target cells, but matrix protein 1-specific MAbs did not (25).

In conclusion, we have shown that antibodies that bind to the stalk region of the HA molecule can not only neutralize free influenza virus particles but can also eliminate virus-infected cells through CDL. These CDL antibodies are broadly cross-reactive within an HA subtype, and some appear to be subtype cross-reactive, suggesting their role in protection against influenza illness, especially in heterologous protection.

Nucleotide sequence accession numbers. Variable region sequences of all MAbs except for TIV-1 and -2 have been deposited in GenBank under accession numbers HQ689787, HQ689752, HQ689762, HQ689763, HQ689731, HQ689727, HQ689750, HQ689751, HQ689789, HQ689789, HQ689778, HQ689776, HQ689713, HQ689705, HQ689702, HQ689718, HQ689774, HQ689785, HQ689783, HQ689766, HQ689772, and HQ689756.

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REFERENCES


AUTHOR’S CORRECTION

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