Development of a Cocktail of Recombinant-Expressed Human Rabies Virus–Neutralizing Monoclonal Antibodies for Postexposure Prophylaxis of Rabies

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To provide a cost-effective and safe replacement for human rabies immunoglobulin (HRIG), we used DNA recombinant technology to express 3 human rabies virus–neutralizing human monoclonal antibodies (huMAbs) in a rhabdovirus vector (RhV). Infection of either baby hamster kidney cells or CHO cells, with the resulting RhV-huMAb recombinant viruses, yielded high-level production (≥40 µg/mL/48 h) of RhV recombinant-expressed huMAbs (rhuMAbs) that differ in both isotype and epitope-recognition specificity. A cocktail of these rhuMAbs neutralizes several fixed and street wild-type rabies viruses (RVs). Mice and hamsters treated only once with this rhuMAb cocktail after infection with a lethal dose of RV were protected. In the mouse models, the postexposure prophylaxis (PEP) efficacy obtained with the rhuMAb cocktail was comparable to that obtained with HRIG, a finding strongly suggesting that rhuMAbs should be given serious consideration for use in future PEP of humans.

In cases where antibody can provide immediate immunity, passive immunization has been widely used for the therapy of infectious diseases [1], including rabies. According to World Health Organization (WHO) guidelines, category 3 exposures to rabies, which are defined as either single or multiple transdermal bites or contamination of mucous membranes with saliva of a rabid animal, require rabies postexposure prophylaxis (PEP) [2]. Rabies PEP includes administration of both vaccine and anti-rabies immunoglobulin (RIG). At present, RIG for PEP is prepared from the serum samples of either rabies virus–immune humans (human RIG [HRIG]) or rabies virus–immune horses (equine RIG [ERIG]). Because of the potential adverse effects (e.g., anaphylactic shock) associated with the use of ERIG, HRIG is used only in the United States, and the people who have been exposed to rabid animals (~39,000 persons annually) receive HRIG in addition to rabies virus (RV) vaccine [2]. In developing countries, however, <1% of all PEP includes administration of RIG, because HRIG and ERIG either are not available in sufficient quantities or, as is especially the case for HRIG, are too expensive [3]. Furthermore, some animal-protection groups condemn the rearing of animals for serum production [3]. In addition, the possibility of contamination of HRIG by known or unknown pathogens is a concern of the regulatory authorities. The limited worldwide supply of cost-effective and safe RIG could be overcome by the production of human RV-neutralizing monoclonal antibodies (MAbs), by use of high-yield expression systems (e.g., those employing transfected cells) in conjunction with bioreactor technology.

Administration of RV-neutralizing mouse or human monoclonal antibodies (MAbs) has been shown to be efficient in PEP of rodents [4–6], a finding suggesting that therapy with MAbs is likely to be effective in PEP of humans. To attain the level of safety, as well as to replicate the protective activities, of HRIG, a cocktail consisting of several human MAbs must be prepared. The huMAbs selected for this cocktail should (1) be of IgG isotypes, (2) neutralize all RV strains and, as recommended by WHO, other lyssaviruses as well, and (3) to prevent the escape of neutralization-resistant variants, differ in their epitope-recognition specificities [3]. We selected 3 hybridomas—JA, JB, and J57—that secrete the huMAbs designated as “SOJA,” “SOJB,” and “SO57,” respectively. Details of both the generation and the analysis of these huMAbs have been reported elsewhere [5, 7]. All 3 huMAbs neutralize a wide spectrum of RVs and related viruses, however, whereas (1) huMAb SO57 neutralizes only fixed (e.g., Pitman-Moore, challenge-virus standard [CVS], and Evelyn-Rokitnicki-Abelseth) and street (e.g., dog RV 4 [DRV-4] and silver-haired bat rabies virus 18 [SHBRV-18]) RV strains [5], (2) huMAb SOJB neutralizes European bat virus 2 (EBV-2), and (3) huMAb SOJA neutralizes EBV-2, Lagos bat virus, and Mokola viruses [7, 8]. This indicates that the 3 huMAbs recognize different epitopes.
Immunoglobulin heavy chain (Ig H) and immunoglobulin light chain (Ig L) mRNAs were isolated from the 3 hybridomas, and a rhabdovirus vector (RhV) was used to express the antibodies, to high levels, in BSR (a subclone of baby hamster kidney cells) or CHO cells. To obtain the complete nucleic-acid sequences of the Ig H and Ig L mRNAs, total RNA was isolated from each hybridoma cell line by use of the Tri-Reagent protocol (Sigma) and the RNeasy RNA extraction kit (Qiagen), according to the manufacturers’ recommendations. The Ig L and Ig H cDNA fragments were amplified by use of the Rapid Amplification of cDNA Ends kit (GIBCO-BRL) and gene-specific primers (GSPs) corresponding to the 3′ ends of the constant-region Ig H and Ig L genes. In brief, 2.5 µg of total RNA was reverse transcribed by use of a ThermoScript reverse transcriptase (Life Technologies) and a GSP, for 60 min at 55°C. The mRNA was then degraded by RNase H, and the cDNA was purified by use of GlassMAX spin cartridges (GIBCO-BRL). After dC-tailing of the cDNA, by terminal deoxynucleotidyltransferase and dCTP, the cDNA was PCR-amplified by use of the Abridged Anchor Primer (GIBCO-BRL) and a second GSP, to obtain a nested product. The PCR products were reamplified by use of a third GSP, were purified by gel electrophoresis, and were sequenced. As a final step, sequences were analyzed by both DNASTAR software (DNAsstar) and National Center for Biotechnology Information Web-site tools (http://www.ncbi.nlm.nih.gov/blast/).

To clone full-length Ig H and Ig L cDNAs, 1 µg of total RNA from each hybridoma was reverse transcribed by use of 10 pmol of oligo dT primer, 200 units of Superscript II, and 20 units of RNase inhibitor, in a 40-µl reaction, with First Strand Buffer, 200 µM dNTP, and 10 mM dithiothreitol. A 5-µl portion of the reverse-transcription reaction was then PCR-amplified by use of the Expand High Fidelity PCR System (Roche) and 10 pmol of primers complementary to the 5′ end and the 3′ end of the Ig L and Ig H cDNAs. The GenBank accession numbers for the cloned Ig cDNAs are as follows: SOJA Ig L, AY172961; SOJA Ig H, 172959; SOJB Ig L, AY172962; SOJB Ig H, AY172958; SO57 Ig L, AY172960; and SO57 Ig H, AY172957. For gene assembly, JA, JB, and J57 Ig H cDNAs were reamplified by use of a primer corresponding to the 5′ end of the cDNAs (JAHF, 5′-AACCGTACGATGGAGTTTGGGGCTGAGCTGGCTT-3′; JBHF, 5′-AACCGTAGATGGACACACTTTGCTCCACGCTCCT-3′; and J57HF, 5′-AAACGTACGACCATGGACCTGGAGCTGGTTC-3′) and heavy chain reverse primer (5′-TGGTACGATGGAGTTTGGGGCTGAGCTGGCTT-3′), which is complementary to the 3′ end of each Ig H cDNA and to a linker sequence consisting of rabies-virus transcription stop/start signal [9]. The Ig L cDNAs were reamplified by use of (1) light chain forward primers including a linker region and regions specific to the 5′ end of the different light-chain cDNAs (5′-GGTAAATGAGTCATGAAAAAAACTAACACCCCTAGC-NNNNNNNNNNNNNNNNNNNNNNNNN′), where N is a 5′ end of the light chain of cDNAs) and (2) a primer for the 3′ end of cDNAs (JALR, 5′-AAAGCTAGCCTAACACTCTCCCCTGGTGTGAAGCTC-3′; JBLR, 5′-AAGCTAGCCTAAACATCTCTCGTTGGAGCT-3′; and J57LR, 5′-AAATCTAGCTACACATGCCTGGGAGCGGCAAC-3′). The Ig H and Ig L cDNAs were then linked by PCR, and the resulting Ig H + linker + Ig L cDNA was digested by BsiWI and Nhel and was inserted into the corresponding sites of plasmid pSPBN. Three different Ig H + Ig L cDNAs were inserted, which resulted in plasmids pSPBN-SOJA, pSPBN-SOJB, and pSPBN-SO57.

The recombinant viruses generated from these plasmids were rescued as described elsewhere [10] and were used to infect either BSR cells or CHO cells, at an MOI of 0.1. After infection, the cells were incubated with Cellgro-FREE culture medium (Mediatech), at 34°C. After 3 days of incubation, tissue-culture supernatants were collected and were subjected to UV irradiation to inactivate the virus. Table 1 shows the amounts of the different recombinant-expressed huMabs (rhuMabs) that were secreted into the tissue-culture supernatant. The individual rhuMabs...
were then purified by Protein A (Pharmacia) chromatography [9] and, after calibration to the same protein content, were tested for their capacity to neutralize fixed (e.g., CVS-N2c and CVS-11) and street (e.g., SHBRV-18 and DRV-4) RV strains. The 3 rhuMabs exhibit qualitative and quantitative differences in their capacities to neutralize the different RVs [table 1]. Furthermore, the relative ratio of the virus-neutralization antibody (VNA) titers for the RV strains, obtained by a cocktail consisting of equal molar concentrations of each of the 3 rhuMabs, was similar to that obtained with HRIG, except for the anti–SHBRV-18 HRIG VNA titer, which was slightly higher. To determine the protective activity of the rhuMAb cocktail in vivo, groups of mice were intranasally infected with 10 LD$_{50}$ of CVS-N2c RV [10] and, 1 h later, were treated intraperitoneally with the indicated concentrations of antibody. The mice were observed for 5 weeks, for the appearance of clinical signs of rabies. IU, international unit.

Table 2. Postexposure prophylaxis of mice, with human rabies immunoglobulin (HRIG) and human anti-rabies recombinant-expressed monoclonal antibody (rhuMAB) cocktail.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>HRIG</th>
<th>rhuMAB cocktail$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 IU/kg</td>
<td>9/10</td>
<td>8/10</td>
</tr>
<tr>
<td>10 IU/kg</td>
<td>6/10</td>
<td>7/10</td>
</tr>
<tr>
<td>5 IU/kg</td>
<td>6/10</td>
<td>4/10</td>
</tr>
<tr>
<td>2.5 IU/kg</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>ED$_{50}$ IU/kg</td>
<td>3.38</td>
<td>4.47</td>
</tr>
</tbody>
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NOTE. Unless otherwise indicated, data are no. of mice that survived rabies virus (RV) infection. Groups of 10 female Swiss Webster mice were intranasally infected with 10 LD$_{50}$ of CVS-N2c RV [10] and, 1 h later, were treated intraperitoneally with the indicated concentrations of antibody. The mice were observed for 5 weeks, for the appearance of clinical signs of rabies. IU, international unit.

Once an individual has been exposed to rabies virus, the disease can readily be prevented by appropriate treatment, which includes both passive and active immunization [2]. The passive administration of antibody is believed to be important in neutralizing the virus at the site of entry, as well as in interfering with spread of the virus to the CNS [11]. These 2 actions likely allow time for the development of active immunity to the virus, immunity that ultimately results in the clearance of the virus. With advances in the technology for production of huMabs, it may be unnecessary to consider the use of mouse Mabs in humans; this might be a beneficial development, since mouse Mabs’ immunogenicity in humans—in whom mouse Mabs’ half-life could be limited, possibly leading to either serum sickness or anaphylactic shock—may prove problematic. Moreover, the interactions between huMAB and Fc receptors are likely to be more efficacious than would be those between comparable mouse reagents.

The RhV expression system has several unique features that make it exceptionally suitable for the production of huMabs: (1) since RhV is not cytopathic, infected cells can continuously produce MAb s for a long time (>2 weeks), a situation that results in cost-efficient MAb production; (2) a variety of mammalian cell cultures are susceptible to infection with RhV, and, therefore, cell lines that already have been approved for vaccine or antibody production (e.g., African-derived green monkey kidney cells and CHO cells) can be used for MAb production by RhV; and (3) RhV can easily be destroyed by use of either UV radiation or chemicals (e.g., nonionic detergents or ethanol) that do not alter the activity of the antibody. Moreover, RhV contains a vesicularstomatitisvirus glycoprotein (G) protein gene (instead of the RV G gene) that carries the major determinants responsible for the pathogenicity of RV [14] and that therefore presents limited biosafety concerns.

Using the RhV expression system, we have expressed 3 huMabs, with different specificities, that are suitable for rabies PEP. When combined in a cocktail, these antibodies are as effective in a mouse model of rabies PEP as is conventional HRIG made from pooled rabies immune–human serum. In contrast to HRIG, the huMAB cocktail is unlikely to be contaminated by known or unknown human pathogens, and it can be produced with no limitation or variation in efficacy. We propose that such a reagent should replace the RIGs currently used in the rabies PEP of humans, which includes passive im-

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munization with anti-rabies antibodies and active immunization with RV vaccine.

References