Passive and Active Immunotherapy for Experimental Pneumococcal Pneumonia by Polyvalent Human Immunoglobulin or F(ab')2 Fragments Administered Intranasally

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Experimental pneumococcal pneumonia in leukopenic BALB/c mice enabled evaluation of passive immunotherapy with human polyvalent intravenous immune globulin (IVIG) given intravenously or intranasally and with F(ab')2 fragments administered intranasally. For intravenous and intranasal IVIG, the respective effective doses were <5 but >0.5 mg/kg and <250 but >2.5 µg/kg. For F(ab')2 fragments, the effective dose was <500 but >2.5 µg/kg. Assessment of the acquired immune responses of passively protected mice and convalescing controls 3 weeks after primary infection showed that antibody responses to whole bacteria were serotype-specific in all mice. Mice protected with IVIG and F(ab')2 fragments had more antibodies to pneumolysin than did controls. In addition, treated mice acquired greater resistance to reinfection than untreated survivors. Thus, local passive immunotherapy may be an effective means of treating pneumococcal pneumonia and may promote acquired resistance to reinfection.

Pneumococcal pneumonia continues to be a major infectious disease because of increasing numbers of drug-resistant strains [1–4]. Streptococcus pneumoniae is often isolated from immunocompromised persons, including those infected with the human immunodeficiency virus [5–7]. Among new therapies, use of intravenous immune globulin (IVIG) has aroused increasing interest [8–10]. Passive therapy with IVIG is now used for immunologic disorders and infections [8–11]. IVIG is generally administered intravenously; however, the efficacy of local administration of polyclonal or monoclonal antibodies directed against bacteria or viruses has been demonstrated [12–18]. IVIG contains a variety of antibodies directed against multiple infectious agents, including common serotypes of S. pneumoniae [19, 20]. We reported previously that IVIG and its F(ab')2 fragments can protect against experimental Staphylococcus aureus pneumonia [16]. In the present study, we evaluated the efficacy of IVIG and its F(ab')2 fragments against pneumococcal pneumonia in a mouse model and evaluated the subsequent acquired immunity conferred by intravenous or intranasal administration of IVIG or its F(ab')2 fragments administered intranasally.

Materials and Methods

Bacterial strain. S. pneumoniae Pn 40 serotype 14 was a gift of T. Horaud (Institut Pasteur). Virulent derivatives, selected by mouse passage (intranasal challenge and recovery from lung homogenates at 24 h), were stored at −80°C in 30% glycerol and subcultured in nutrient broth for pneumococci (Diagnostica Pasteur, Marnes-la-Coquette, France) and on trypticase soy agar supplemented with 5% horse blood (bioMérieux, Marcy l’Étoile, France). For experimental infections, 10 mL of broth was inoculated with 500 µL of the stock suspension at 10⁹ colony-forming units (cfu)/mL and incubated overnight at 37°C. A subculture was made in broth until the bacterial density reached ~2 × 10⁶ cfu/mL log phase bacteria.

Mice. Four-week-old female BALB/c mice (Centre d’Élevage R. Janvier, le Genest St. Isles, France), maintained under controlled environmental conditions in a biosafety containment facility, were immunosuppressed by intravenous injection of 150 and 75 mg/kg cyclophosphamide (Sigma, St. Quentin-Fallavier, France) 4 and 1 days, respectively, before intranasal challenge. White blood cells were counted in 3 mice per time point by automatic hemocytometer (CCVI; Becton Dickinson, Le Pont de Claix, France) from 200-µL blood samples obtained by retroorbital puncture and collected in heparin (Liquemine Roche, Neuilly sur Seine, France). Percentages of mononuclear or polymorphonuclear cells were determined by microscopic examination of blood smears after Giemsa staining. Treatment with cyclophosphamide reproducibly induced intense leukopenia: Leukocytes (±SD) fell from 7000 ± 266 to 1986 ± 130/µL and returned to normal levels on day 9, but leukocytosis occurred by day 10 (9000 ± 867).

Experimental infection. The experimental procedure has been described in detail [16]. In brief, ether-anesthetized mice were given 50 µL of the bacterial inoculum intranasally. Pulmonary pneumococcal clearance was measured at selected times, and cfu were counted in lung homogenates (lungs were dissected from the main bronchia, diluted in sterile PBS [Sigma], and plated on blood agar). Bacterial counts from lung homogenates were determined for 5 mice and expressed in log₁₀ cfu/mL as mean ± SE. Statistical significance was determined by Student’s t test.

Passive immunotherapy with IVIG and its F(ab')2 fragments. IVIG (50 mg/mL; lot 50.00505; Biotransfusion, Les Ulis, France)
and its F(ab')2 fragments (30 mg/mL) were obtained by pepsin digestion and affinity purification by *S. aureus* protein A–Sepharose chromatography (Laboratoire Français du Fractionnement et des Biotechnologies, Les Ulis, France). They were diluted extemporaneously in PBS at selected doses for administration to mice. IVIG is 99% IgG (70% IgG1, 20% IgG2, 8% IgG3, and <2% IgG4) and thus contains normal proportions of plasma IgG subclasses. IVIG and F(ab')2 fragments were checked for purity and homogeneity by SDS-PAGE before use. A human monoclonal IgG1 anti-human red blood group antigen rhesus D (RhD) [21] (gift of J. Bartholeyns, Laboratoire Français du Fractionnement et des Biotechnologies) was used as irrelevant human IgG control. Passive immunotherapy was given 3 h after infection in mice anesthetized with sodium pentobarbital (Sanofi Santé Animale, Libourne, France). IVIG was administered intravenously in a 500-μL volume or intranasally in a 50-μL volume.

The Pn 40 antibody titer was evaluated in IVIG and F(ab')2 fragments by ELISA as described previously [16] by coating wells with 100 μL of formalin-killed bacteria (10⁷ cfu/well) in 0.1 M carbonate buffer, pH 9.6, for 1 h at room temperature, and overnight at 4°C. Wells were saturated with 3% PBS–bovine serum albumin and 0.05% Tween 20 for 2 h at room temperature. IVIG or F(ab')2 fragments were incubated for 1 h at room temperature before being washed. Attached antibodies were revealed using peroxidase-labeled rabbit anti-human IgG (Immunotech, Marseille-Luminy, France).

Sera from immunized mice were tested by the same ELISA, except that the antibodies were revealed by goat anti-mouse IgG+IgM (Immunotech). *S. aureus* 184-15 [16] was used as negative control antigen; *S. pneumoniae* 9852 (serotype 23F; gift of P. Geslin, National Reference Center for Pneumococci, Centre Hospitalier Intercommunal de Créteil, France) was used as serotype control. Mouse antibody titers are shown as the last log₁₀ dilution that gave an absorbance >0.1 above nonimmune mouse serum. Absorption of antibodies to cell wall *S. pneumoniae* polysaccharide (CWPS; Statens Seruminstitut, Copenhagen) was done as previously described [22, 23] by mixing a 10 μg/mL solution of CWPS with antibodies diluted 0.5 mg/mL and incubating the mixture overnight at 4°C before use in the ELISA. This procedure allowed the absorption of 95.7% ± 2.3% of anti-CWPS antibodies (mean of 3 experiments).

Anti-human IgG (H+L) in mouse sera was checked by ELISA with donkey anti-Fc and goat-anti-F(ab')2 antibodies (both Immunotech).

Antipneumolysin activity was evaluated by incubating 50 μL of serial 2-fold dilutions of mouse sera or IVIG or F(ab')2 fragments with CWPS for 15 min at 37°C. Then 25 μL of 3% packed, washed group O human erythrocytes was added. The mixture was incubated 60 min at 37°C and left to sediment for 60 min at room temperature as described by Shumway et al. [25].

We used erythrocytes without autolysate as the negative control and autolysate without antibodies as the positive control. The pneumolysin antibody titer was determined by end-point dilution (Log₁₀) of mouse sera, IVIG, or F(ab')2 (the two last preparations were tested beginning at concentrations of 12.5 and 15 mg/mL, respectively).

The in vitro antipneumococcal activity of IVIG was assayed by mixing 100 μL of an 18-h growth of *S. pneumoniae* Pn 40 with 0, 1, 10, 100, and 1000 μg/mL IVIG in 10 mL of broth and incubating the mixture for 10 h at 37°C. Bacterial density was measured every hour at A₆₅₀ after flasks were vigorously shaken. After 10 h, the resultant growth and the control series were Gram’s stained and examined by oil-immersion microscopy.

### Acquired immunity to *S. pneumoniae*.

Nonimmunosuppressed BALB/c mice were resistant to intranasal challenge with Pn 40. Even with inocula of up to 10⁶ cfu/mouse, bacteria were cleared from lungs in <96 h. In contrast, mice challenged after treatment with cyclophosphamide were susceptible to *S. pneumoniae* Pn 40 intranasal challenge in a dose-dependent manner. As few as 10³ cfu/mL induced a bacterial infection with an in vivo growth rate of ~1 log₁₀/24 h. However, the nature of the bacterial inoculum strongly affected the virulence of Pn 40. Thus, washed bacteria were rapidly cleared, but nonwashed bacteria multiplied in the lungs for >3 days. A major enzymatic virulence factor appeared to be released during in vitro growth that was necessary for *S. pneumoniae* Pn 40 to colonize the mouse respiratory tract. Because several groups of researchers identified this factor as pneumolysin [2, 24–26], we prepared challenge inocula from cultures in broth without washing bacterial cells. Under these experimental conditions, the mean LD₅₀ at 96 h was ~10⁵ cfu/mouse (bacterial counts reached 10⁷ cfu/mL of lung homogenate in lethally infected mice).

We checked IVIG and its F(ab')2 fragments for antibodies to *S. pneumoniae* Pn 40 by ELISA before and after absorption with CWPS. Two-fold dilutions (from 25 to 0.012 μg/well) were tested in triplicate; allowable reproducible results were <5% variation in three experiments. Parallel curves were drawn from mean ELISA data for IVIG, CWPS-absorbed IVIG, and F(ab')2. Titters for F(ab')2 were twice those of IVIG. Absorption of IVIG with CWPS reduced the antibody titer to *S. pneumoniae* Pn 40 in IVIG by 73% ± 5%. Thus, the IVIG preparation tested had the common *S. pneumoniae* antibody characteristics of other IVIG preparations [19, 20–23] and was mostly directed to nonserotype-specific polysaccharide antigens.

During the in vitro culture of Pn 40 in the presence of different IVIG concentrations, we did not observe any growth inhibition. On the contrary, at a dose of 1 mg/mL, IVIG enhanced bacterial growth after 6 h of incubation. Thus, at 16 h, the absorbance at 650 nm was three times higher than in control medium (data not shown).
In experiments designed to check the efficacy of IVIG injected at 500, 250, 100, 50, and 10 \( \mu \)g/mouse (table 1), the effective intravenous dose was 50 \( \mu \)g/mouse. Higher doses (500 and 250 \( \mu \)g) of IVIG were apparently less effective than intravenous injection of PBS, which seemed to accelerate bacterial clearance by day 3, although cfu counts varied widely for individual animals and within experiments, making results not statistically significant. The beneficial role of intravenous injection of 250 \( \mu \)L of PBS in reducing proliferation of \( S. pneumoniae \) in the lungs might have been due to rehydration, since infected mice had a weight loss of \( \sim \)10% at day 3. The elevated bacterial burden in the lungs of mice injected with 500 or 250 \( \mu \)g of IVIG was intriguing, because IVIG was dissolved in PBS and administered in the same volume (250 \( \mu \)L) as PBS alone. Perhaps high doses of antibodies may have led to efficient opsonization (as shown by others [20, 27]) but not to efficient intraphagocytic bacterial killing.

When we treated infected mice intranasally (instead of intravenously) with IVIG or with F(ab')\(_2\) fragments at doses of 50, 10, 5, and 1 \( \mu \)g/mouse, we observed that 1 \( \mu \)g of IVIG or 1–5 \( \mu \)g of F(ab')\(_2\) effectively and reproducibly protected mice against \( S. pneumoniae \) infection (table 2). Higher doses were ineffective, and one control experiment with 1 and 0.5 \( \mu \)g of IVIG indicated that 1 \( \mu \)g was the lowest effective dose (data not shown). Thus, as reported for local passive immunotherapy of \( S. aureus \) pneumonia [16], the effective dose range was narrow: <5 but \( >0.5 \) \( \mu \)g/mouse for IVIG and <10 but \( >0.5 \) \( \mu \)g/mouse for F(ab')\(_2\) fragments. To assess the need for specific anti-pneumococcal antibody in IVIG, a series of experiments was designed to compare the efficacies of intranasal administration of the irrelevant control anti-RhD human monoclonal IgG1 to that of IVIG. Results (shown in table 3) suggest that protection with IVIG was not due to a nonspecific effect of IgG and that the irrelevant monoclonal IgG1, which can block FcγRI-directed phagocytosis [21], did not influence the anti–\( S. pneumoniae \) neutralizing efficacy of IVIG. This confirms that the specific antibacterial effect was Fc-independent.

All mice treated with protective doses of IVIG intravenously (50 and 100 \( \mu \)g/mouse) or intranasally (1 \( \mu \)g) or with F(ab')\(_2\) fragments intranasally (1 and 5 \( \mu \)g/mouse) survived >4 weeks. No human antibodies persisted in mouse sera 3 weeks after intranasal administration (data not shown). Anti-human antibodies were detected only in mice treated with IVIG. However, these antibodies were directed only against the Fc fragment of the immunoglobulin and not against F(ab')\(_2\). No anti-human immunoglobulins were detected in mice treated intranasally with IVIG or F(ab')\(_2\) fragments.
Table 3. Specific protective effects of intranasal administration of intravenous immune globulin (IVIG) against *Streptococcus pneumoniae* Pn 40.

<table>
<thead>
<tr>
<th>Experiment, treatment</th>
<th>3 h</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 (inoculum 6.58 log₁₀ cfu/mouse)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVIG (1 µg)</td>
<td>5.79 ± 0.17</td>
<td>5.55 ± 0.50</td>
<td>3.69 ± 0.87*</td>
<td></td>
</tr>
<tr>
<td>Control (anti-RhD) human monoclonal IgG1 (1 µg)</td>
<td>6.60 ± 0.06</td>
<td>6.98 ± 0.41</td>
<td>&gt;8</td>
<td></td>
</tr>
<tr>
<td>PBS-treated controls</td>
<td>6.60 ± 0.02</td>
<td>6.25 ± 0.10</td>
<td>6.85 ± 0.33</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Experiments 2, 3 (inocula 4.62 and 4.67 log₁₀ cfu/mouse, respectively)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVIG (1 µg)</td>
<td>4.94 ± 0.38</td>
<td>5.17 ± 0.80</td>
<td>2.67 ± 0.41*</td>
<td></td>
</tr>
<tr>
<td>Control (anti-RhD) human monoclonal IgG1 (1 µg)</td>
<td>4.73 ± 0.52</td>
<td>7.03 ± 0.17</td>
<td>7.83 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>IVIG (1 µg) mixed with control (anti-RhD) human monoclonal IgG1 (1 µg)</td>
<td>4.09 ± 0.38</td>
<td>4.50 ± 0.50</td>
<td>2.62 ± 0.93*</td>
<td></td>
</tr>
<tr>
<td>PBS-treated controls</td>
<td>4.59 ± 0.12</td>
<td>NT</td>
<td>6.98 ± 0.15</td>
<td>7.95 ± 0.77</td>
</tr>
</tbody>
</table>

NOTE. NT, not tested. RhD, rhesus D.

* Significantly lower than PBS-treated controls (P < .01).

<sup>1</sup> Mean ± SE of both experiments.

ELISAs of serum samples of groups of 10 convalescing mice for antibodies to *S. pneumoniae* Pn 40 showed that all mice treated with IVIG or the F(ab')₂ fragments and survivors in control groups treated intravenously or intranasally with PBS (2 or 3 survivors of 10 in 8 experiments) had developed equivalent antibody titers specific for Pn 40 (mean, 2.2 ± 0.5). No cross-reaction with *S. aureus* or *S. pneumoniae* 23F was detected. The failure of these sera to recognize CWPS by ELISA suggested that anti-*S. pneumoniae* Pn 40 serologic response was serotype specific.

Antipneumolysin activity was tested in duplicate in individual sera from groups of 5 mice that survived sublethal intranasal challenge with *S. pneumoniae* (inoculum ~5.5 log₁₀ cfu/mouse). This activity was compared with the corresponding activity in nonimmune mouse serum, hyperimmune serum from nonimmunosuppressed BALB/c mice immunized subcutaneously with live *S. pneumoniae* Pn 40 (by weekly injection of 6.3 log₁₀ cfu in 100 µL in the neck for 3 weeks and bleeding at week 4), IVIG, and F(ab')₂ fragments. As shown in table 4, all sera from mice either convalescing from *S. pneumoniae* intranasal challenge or hyperimmunized with whole bacterial antigen had significant titers for antibodies that inhibit the hemolytic properties of pneumolysin. The partial inhibition of pneumolysin activity in nonimmune mouse serum can be attributed to nonantibody plasma components [28]. IVIG and its F(ab')₂ fragments, which are pure immunoglobulin molecules, did not inhibit pneumolysin.

One month after intranasal pneumococcal challenge (5.66 log₁₀ cfu/mouse) followed by passive immunotherapy, mice were tested for acquired resistance to Pn 40 by intranasal reinfection. Naive mice (8 weeks old [the same as treated mice]; maintained in the same conditions) were used as controls. Table 5 shows lung bacterial counts after pneumococcal reinfection. Control PBS-treated survivors had acquired resistance to *S. pneumoniae*, but bacterial clearance was faster and more effective in mice that had been passively protected by intravenous or intranasal administration of IVIG or intranasal administration of F(ab')₂. Subsequently, 1 month after the initial challenge, it was clear that passive immunotherapy with intravenous or intranasal IVIG or with intranasal F(ab')₂ fragments not only passively protected mice against pneumococcal pneumonia but also resulted in active immunization and acquired resistance to reinfection.

Table 4. Antipneumolysin titers in mouse sera and intravenous immune globulin (IVIG) or F(ab')₂ fragments.

<table>
<thead>
<tr>
<th>Convalescing mice treated with</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous IVIG (50 µg)</td>
<td>3.00 ± 0.32*</td>
</tr>
<tr>
<td>Intranasal IVIG (1 µg)</td>
<td>3.07 ± 0.24*</td>
</tr>
<tr>
<td>Intranasal F(ab')₂ (1 µg)</td>
<td>3.60 ± 0.91*</td>
</tr>
<tr>
<td>Intranasal PBS</td>
<td>2.28 ± 0.13†</td>
</tr>
<tr>
<td>Nonimmune mouse serum</td>
<td>1.65 ± 0.12</td>
</tr>
<tr>
<td>Hyperimmune mouse serum</td>
<td>2.85 ± 0.12</td>
</tr>
<tr>
<td>IVIG</td>
<td>&lt;1</td>
</tr>
<tr>
<td>F(ab')₂</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

NOTE. Titters are log₁₀ end-point dilutions inhibiting hemolysis.

* Significantly higher than serum titers of convalescing mice treated with intranasal PBS (P < .01).

† Significantly different from nonimmune serum (P < .01).

Discussion

The results of the present study indicate that in immunosuppressed mice local passive immunotherapy using intranasally administered IVIG or F(ab')₂ fragments can replace local anti-
bodies as effectively as intravenous IVIG for clearance of *S. pneumoniae*. In our pneumococcal pneumonia model, our results were similar to those observed in the staphylococcal pneumonia model [16]. This was probably because IVIG prepared from pools of thousands of plasma samples from immunocompetent adult donors contains polyvalent antibodies to a wide variety of commensal or opportunistic microorganisms [10, 20]. The mechanisms of passive protection involved are immunologic- and Fc-independent, since F(ab')₂ fragments were as effective as whole immunoglobulin molecules. This indicates that in pneumococcal pneumonia, inhibition of the infectious process by local delivery of antibodies does not necessarily require opsonization and phagocytosis. Inhibition of colonization and bacterial agglutination may be sufficient to clear extracellular bacteria from epithelial surfaces.

Topical administration of antibodies to microorganisms colonizing the epithelial tissues may be of therapeutic interest because the results of the present and of previous studies [13–18] suggest that effective topical doses are lower than effective intravenous doses. Furthermore, local administration results in immediate delivery of the antibody to the infection site, bypassing the reticuloendothelial system, whereas intravenously injected IVIG may lead to discrepant results [8–10, 29, 30], probably because of unknown IVIG pharmacokinetic parameters and targeting to various anatomic infectious foci. As observed in the staphylococcal pneumonia model [16], an unsolved problem is the very narrow effective dose range for IVIG whether administered intravenously or intranasally. This could be mostly due to the fact that IVIG is polyclonal and thus contains protective, nonprotective, and infection-enhancing antibodies [31].

One mechanism may overlap the effects of enhanced phagocytosis through Fc-FcγR interactions but without intracellular bacterial killing and inhibition of bacterial adherence to cellular receptors. In our study, cyclophosphamide-treated mice infected with *S. pneumoniae* Pn 40 remained leukopenic for 4 days (day 5 after the last cyclophosphamide injection) as did uninfected cyclophosphamide-treated controls (data not shown). Therefore, phagocytic effectors were restricted to resident pulmonary macrophages: No polymorphonuclear leukocytes could be recruited to the infectious foci, and the apparent protective effect observed with intravenous injection of IVIG at doses of 50 and 100 μg but not at 250 and 500 μg/mouse (table 1) may in fact correspond to the resulting difference between effects of nonbactericidal opsonophagocytosis and effective nonopsonizing neutralization by antibodies reaching the bronchoalveolar surfaces. Curiously, a similar narrow dose range was observed after intranasal administration of IVIG and with F(ab')₂ (table 2), which are not known to interact with any phagocytic cell receptor. Further studies on phagocytosis of *S. pneumoniae* after sensitization with IVIG and F(ab')₂ fragments are underway to attempt to explain this narrow dose range.

After passive immunotherapies were tested on mice that had been protected with IVIG, F(ab')₂, or PBS, the mice were checked for acquired immunity. By ELISA, all sera of convalescing mice 3 weeks after primary infection responded specifically to *S. pneumoniae* Pn 40 but not to CWPS, with no detectable differences between groups of mice. This suggests that acute intranasal infection promoted the production of serotype-specific antibodies. Also, an antipneumolysin response was elicited by active infection or immunization that was not detected in IVIG (table 4). Antipneumolysin titers were significantly higher in mice treated with IVIG or F(ab')₂ fragments than in PBS-treated controls. By comparing the acquired resistance to reinfection with *S. pneumoniae* Pn 40 in different groups of convalescing mice (table 5), we observed that groups passively protected with IVIG or its F(ab')₂ fragments also had more efficient acquired protective immunity to reinfection than did PBS-treated controls. Since we did not detect any human IgG or F(ab')₂ fragments in mouse sera 3 weeks after primary infection and passive immunotherapy, we conclude that resistance of mice to reinfection was due to their own immune responses.

Some studies have shown that major antipneumococcal specificities in commercial IVIG are mostly directed against CWPS and that variations in antisertype specificities depend on the manufacturer [19, 20]. Immunized adults express high levels of antibodies to CWPS, but these antibodies cannot prevent the evolution of a slight pulmonary infection to severe pneumonia or bacteremia [22]. Experimental assays of protection against *S. pneumoniae* pneumonia have demonstrated the efficacy of anti-pneumolysin or anti-neuraminidase antibodies [32]. We hypothesize that the immune response of passively treated mice may be predominantly directed against antigens (e.g., pneumolysin, neuraminidase, or other non-CWPS antigens) that play an important part in the pathogenesis of pneumococcal infections [26, 27, 32]. Another hypothesis, based on the fact that CWPS induces a T-independent, poorly protective immune response [33, 34], postulates that pneumococcal anti-

### Table 5. Acquired resistance to *Streptococcus pneumoniae* Pn 40 intranasal challenge of convalescing mice at day 30 after intranasal infection with *S. pneumoniae* Pn 40 and passive immunotherapy.

<table>
<thead>
<tr>
<th>Convalescing mice treated with</th>
<th>6 h</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous PBS</td>
<td>4.65 ± 0.68</td>
<td>5.75 ± 0.26</td>
<td>4.80 ± 0.43</td>
</tr>
<tr>
<td>Intravenous IVIG (50 μg)</td>
<td>4.84 ± 0.23</td>
<td>4.60 ± 0.64</td>
<td>2.65 ± 0.58</td>
</tr>
<tr>
<td>Intranasal PBS</td>
<td>4.82 ± 0.52</td>
<td>6.50 ± 0.28</td>
<td>5.75 ± 0.38</td>
</tr>
<tr>
<td>Intranasal IVIG (1 μg)</td>
<td>4.35 ± 0.18</td>
<td>4.30 ± 0.72</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Intranasal F(ab')₂ (1 μg)</td>
<td>5.05 ± 0.28</td>
<td>2.45 ± 0.70</td>
<td>2.0 ± 0.20</td>
</tr>
<tr>
<td>Naive controls</td>
<td>5.90 ± 0.12</td>
<td>7.18 ± 0.14</td>
<td>7.80 ± 0.17</td>
</tr>
</tbody>
</table>

NOTE. IVIG, intravenous immune globulin.

* Mean of 2 experiments with inocula of 5.65 and 5.73 log₅ cfu/mouse.

<sup>1</sup> Log₅ was lower limit of cfu counts.
gens that bind to IVIG or F(ab')2 and act as protein carriers could induce a T-dependent immune response that may evoke a more adequate antibody response and a memory response [35]. The mechanisms involved in both hypotheses can promote very efficient vaccination. In any case, the present results show that topical administration of polyvalent immunoglobulins may provide safe and effective passive protection in immunocompromised hosts and can even confer acquired immunoprotection.

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