Effective Combination Therapy for Invasive Pneumococcal Pneumonia with Ampicillin and Intravenous Immunoglobulins in a Mouse Model

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Intranasal immunotherapy for Streptococcus pneumoniae invasive pneumonia with polyvalent immunoglobulins (IVIG) was effective in mice against pneumonia but failed to prevent bacteremia. The combination of subcurative doses of IVIG and of ampicillin was fully protective. Such an approach, successfully applied in the preantibiotic era, offers new perspectives for modern therapies.

New therapeutic strategies against pneumococcal diseases are needed due to the multiple resistance to antibiotics of certain strains (8, 15). In the preantibiotic era, antibody-based immunotherapy was effectively used, when pneumococcal infections were treated by serotherapy or combined serum plus chemotherapy (6). Polyvalent human immunoglobulins (IVIG) contain a variety of antimicrobial immunoglobulin G (IgG) antibodies (3, 9, 14, 18), including antibodies to Streptococcus pneumoniae (12). Experimental pneumonia in leukopenic mice, induced by a serotype 14 S. pneumoniae strain which was avirulent for immunocompetent mice, can be cured by the intranasal administration of IVIG (23). However, the virulence of S. pneumoniae depends not only on the immune status of the host (5, 7, 13) but also on the capsular type of the strain (4). In this study, we evaluated the efficacy of IVIG and a combination therapy with IVIG and ampicillin against a serotype 3 S. pneumoniae strain that is virulent for immunocompetent mice.

Female, 6-week-old BALB/c mice (Charles River Laboratories, Saint Aubin-les-Elbeuf, France) were challenged intranasally, as previously described (23), with S. pneumoniae Pn4241 (2). Inocula were prepared from a 6-h subculture in brain heart infusion broth (Difco, Detroit, Mich.) at 37°C, reaching 109 CFU/ml and diluted in phosphate-buffered saline (PBS; Sigma, Saint Quentin-Fallavier, France) to a desired density according to the A590 and CFU counts on blood-agar (Biome`rieux, Marcy l’Etoile, France). Experimental pneumonia in leukopenic mice, induced by a serotype 14 S. pneumoniae strain which was avirulent for immunocompetent mice, can be cured by the intranasal administration of IVIG (23). However, the virulence of S. pneumoniae depends not only on the immune status of the host (5, 7, 13) but also on the capsular type of the strain (4). In this study, we evaluated the efficacy of IVIG and a combination therapy with IVIG and ampicillin against a serotype 3 S. pneumoniae strain that is virulent for immunocompetent mice.

Female, 6-week-old BALB/c mice (Charles River Laboratories, Saint Aubin-les-Elbeuf, France) were challenged intranasally, as previously described (23), with S. pneumoniae Pn4241 (2). Inocula were prepared from a 6-h subculture in brain heart infusion broth (Difco, Detroit, Mich.) at 37°C, reaching 10⁹ CFU/ml and diluted in phosphate-buffered saline (PBS; Sigma, Saint Quentin-Fallavier, France) to a desired density according to the A590 and CFU counts on blood-agar (Biome`rieux, Marcy l’Etoile, France). Statistical analysis of CFU counts in blood and lung homogenates in groups of five mice were performed by using the Student Fisher t test. Lethality for mice was scored each day for 15 days. The mean 50% lethal dose (LD50) of S. pneumoniae Pn4241 for intranasally infected mice was 5 × 10⁴. IVIG (Tégeline [lot 50060432] from the Laboratoire du Fractionnement et des Biotechnologies, Les Ulis, France) was used at the dose of 50 mg/kg throughout the study because this was the highest protective dose tolerated intranasally by the mice. Antibodies to S. pneumoniae, either preab-
ampicillin-susceptible *S. pneumoniae* strain Pn4241 (MIC of 0.016 mg/liter as determined by E-test [AB-Biodisk, Solna, Sweden]). Subcurative doses of ampicillin (200 μg/kg) and of IVIG (10 mg/kg) were selected from preliminary experiments in which mice challenged with $10^9$ or $10^8$ CFU were treated either with ampicillin at 0, 100, 200, or 1,000 μg/kg subcutaneously in a volume of 200 μl at 3 h after infection or intranasally with IVIG at 0, 5, 10, or 50 μg/kg given 24 h before infection because these were the highest doses inducing >10-fold transient reduction in CFU pulmonary counts at 24 h, followed by a regrowth at 48 h, thus mimicking a treatment failure. The efficacy of combined therapy was compared to that of single therapy with ampicillin given at 3 h after challenge (in mice treated intranasally with PBS 24 h before) or with IVIG given 24 h before (and PBS given subcutaneously 3 h after) the challenge. Controls received 50 μl of PBS intranasally 24 h before the challenge and 200 μl subcutaneously at 3 h after the challenge. CFU counts at 48 h in the groups given single treatments were lower but not significantly different from those of the controls ($P > 0.1$), whereas in the group given both treatments the CFU counts in the lungs and blood were below the threshold of detection (Fig. 2). The survival data were consistent with these results: 9 of 10 mice given the combined treatment survived versus 2 and 3 of the 10 mice in the ampicillin and IVIG single-treatment groups, respectively.

Recent advances in immunology have led to renewed interest in passive immunotherapy against infectious agents for which there is no effective treatment, such as with most viruses and multiple-antibiotic-resistant bacteria (6, 18, 27, 28, 29). Topical immunotherapy is a promising approach for epithelial infections (27, 29), particularly for pulmonary infections (22, 23, 24). In this study, intranasal administration of IVIG was effective against pneumonia induced by various lethal pneumococcal inocula of 10 to 1,000 times the LD$_{50}$ but did not significantly neutralize bacteremia, which is the major threat in pneumococcal infections (13, 20), probably due to the short lifetime of IVIG in the lungs after intranasal administration. Indeed, *S. pneumoniae* Pn4241 antibody titer in IVIG was low, but an ELISA does not assess antibacterial efficacy which involves not only antibody binding to surface epitopes but also complement activation and opsonophagocytosis (16, 25). Specific *S. pneumoniae* capsule-type antibody would have been more effective, but the variety of capsular types of *S. pneumoniae* (>85) requires a polyvalent immunotherapeutic approach such as that developed for vaccines. The development of combinatorial antibody library technology may be the way forward for polyvalent passive immunotherapy against pathogens with antigenic diversity (28).

The combination therapy with IVIG and ampicillin which we tested in a way similar to that applied 60 years ago with immune serum and sulfapyridine (21) and utilized by other investigators more recently (11) was effective for curing invasive pneumonia. Combining antibody-based immunotherapy with chemotherapy may make it possible to achieve effective antibacterial therapy with standard doses of antibiotics for strains with diminished susceptibility, thereby reducing the risk of selection of more resistant variants (8, 15).

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