A Human Monoclonal Immunoglobulin M Reduces Bacteremia and Inflammation in a Mouse Model of Systemic Pneumococcal Infection

Kevin Fabrizio, Abraham Groner, Marianne Boes, and Liise-anne Pirofski

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461; Department of Pathology, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115; and Division of Infectious Diseases, Department of Medicine, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York

Received 9 October 2006/Returned for modification 13 November 2006/Accepted 5 February 2007

Antibody-based approaches to pneumococcal disease may hold promise for immunocompromised patients in whom vaccines are less immunogenic and/or in the context of antimicrobial resistance. Antibody-mediated protection against experimental pneumococcal pneumonia has been shown to depend on immunoregulation, but the relationship between antibody and protection against pneumococcal sepsis and immunoregulation has not been examined. Similarly, the requirement for B and T cells for antibody efficacy is not known. In this study, we determined the efficacy of the human pneumococcal capsular polysaccharide serotype 3-specific antibody, A7 (immunoglobulin M [IgM]), in secretory IgM (sIgM)−/−, CD4−/−, CD8−/−, μMT−/−, and SCID mice and investigated its effect on cytokine and chemokine expression in sera and spleens from mice with intact cellular immunity. A7 is known to be protective against systemic infection with serotype 3 and to require complement for efficacy. Compared to that of an isotype control antibody, A7 administration prolonged the survival of mice of each immunodeficient strain and was associated with a significant reduction in CFU in blood, lung, and spleen samples and a significantly reduced level of keratinocyte-derived chemokine (KC), interleukin-6 (IL-6), and macrophage inflammatory protein-2 (MIP-2) expression in normal and sIgM−/− mice. Studies with mice treated with penicillin revealed similar reductions in CFU and similar levels of IL-6, KC, or MIP-2 expression in A7- and penicillin-treated mice. These findings demonstrate that natural IgM and B and T cells are dispensable for A7-mediated protection against experimental pneumococcal sepsis and suggest that the efficacy of antibody-mediated protection depends on immunomodulation. Taken together, our data extend the association between antibody-mediated protection and immunomodulation to protection against systemic pneumococcal infection and to a clinically important serotype often responsible for pneumococcal sepsis.

Streptococcus pneumoniae, the most common etiologic agent of community-acquired pneumonia in the United States, is the cause of over 40% of all infectious disease-related deaths (46). Pneumococcal infection is marked by a profound host inflammatory response characterized by high levels of chemokines and cytokines and bacteria in blood and tissues (2, 16, 27). In light of the host damage elicited by the immune response to pneumococcal infection, agents that modulate the inflammatory response may hold promise for reducing the morbidity and mortality associated with pneumococcal disease (15).

The first antimicrobial therapy for pneumococcal infections was sera consisting of serotype-specific antibodies (7, 24). This modality, serum therapy, was used until the late 1930s but was abandoned due to the toxicity of heterologous sera in humans and the introduction of antibiotics (7). However, the late-20th-century appearance of antibiotic-resistant strains and the increased number of immunocompromised patients at risk for pneumococcal disease (7) have led to the search for new approaches to treat and prevent pneumococcal disease. Vaccination with pneumococcal capsular polysaccharide (PPS)-based vaccines is effective in preventing invasive pneumococcal disease in individuals considered to be at low to moderate risk (22, 31, 54). PPS vaccination is recommended for adults over the age of 65 and those with underlying conditions that predispose to pneumococcal disease (29); however, PPS vaccines are poorly immunogenic in adults who are at high risk for pneumococcal disease, such as elderly and immunocompromised patients, including those with human immunodeficiency virus infection (41). A PPS-protein conjugate vaccine is effective against invasive disease in infants and young children (34).

PPS vaccines are thought to prevent pneumococcal disease by inducing the production of opsonic, type-specific immunoglobulin G (IgG) (43). However, a human PPS-specific IgM monoclonal antibody (mAb), D11, that is highly protective against lethal systemic infection (56) and pneumonia (8, 9) with serotype 8 pneumococci in mice does not promote opsonic killing in vitro (9). Interestingly, the efficacy of D11 against pneumonia is associated with immunoregulation (8). It is not known if the association between antibody efficacy and immunoregulation is limited to pneumonia or infection with serotype 8 or if it extends to other manifestations of pneumococcal disease and/or other serotypes. The challenge of treating infection with antibiotic-resistant pneumococcal strains and of managing pneumococcal disease in immunocompromised patients has
brought the prospect of immunomodulation as antimicrobial therapy to the forefront (40). As such, extending the observation that antibody efficacy is associated with immunoregulation to additional disease models and pneumococcal serotypes is an important step towards translating the concept of immunomodulation as an approach to antimicrobial therapy to the bedside. In this study, we investigated whether the efficacy of a protective human mAb to serotype 3 pneumococcal capsular polysaccharide that depends on an intact complement pathway to mediate protection also depends on B or T cells and determined the effect of the mAb on the cytokine-chemokine response to systemic infection with serotype 3. Our results showed that B and T cells were dispensable for antibody efficacy and that antibody-mediated protection was associated with immunomodulation.

MATERIALS AND METHODS

Bacteria. *S. pneumoniae* serotype 3 strain 6303 (American Type Culture Collection, Manassas, VA) was grown in tryptic soy broth (TSB; Difco Laboratories, Sparks, MD) to mid-log phase in 5% CO2 at 37°C, frozen in TSB in 10% glycerol, and stored at −80°C until it was used as described previously (13, 45). Prior to infection, pneumococci were rapidly thawed, placed on ice, and diluted in TSB to the desired concentration. To confirm the desired concentration, diluted pneumococci were plated onto a Trypticase agar plate containing 5% sheep’s blood (Becton Dickinson, Franklin Lakes, NJ) and incubated overnight at 5% CO2 at 37°C.

Antibodies. mAb A7 [IgM(e)] is a mAb derived from XenoMouse mice (23) that was previously shown to bind PPS3 and protect mice from death after systemic pneumococcal challenge (13). A7 was purified by affinity chromatography using anti-human IgM-coated beads (Sigma-Aldrich, St. Louis, MO). A human myeloma IgM (Calbiochem, San Diego, CA) was used as a negative control.

Mice. To determine the role of B and T cells in mAb-mediated protection, CD4 T-cell-deficient (CD4−/−), CD8 T-cell-deficient (CD8−/−), B-cell-deficient (μMT−/−), and SCID mice were used, with C57BL/6 mice as a wild-type background. The generation of SCID mice was determined as described previously for another human IgM mAb to PPS (9, 38).

To determine the ability of A7 to mediate protection also depends on B or T cells and determined the effect of the mAb on the cytokine-chemokine response to systemic infection with serotype 3. Our results showed that B and T cells were dispensable for antibody efficacy and that antibody-mediated protection was associated with immunomodulation.

Mouse infection model and mouse protection studies. A7 was previously shown to protect mice against death from intraperitoneal (i.p.) infection with serotype 3 pneumococci (13). The same model was used in this study: 10 μg/100 μl of A7, human myeloma isotype control IgM, or phosphate-buffered saline (PBS) was administered i.p. 1 h prior to i.p. infection with 30 CFU of serotype 3 pneumococci. Survival was monitored for 14 days. The 50% lethal dose (LD50) for each mouse strain was determined by Reed and Muench LD50 calculations (data not shown). For C57BL/6 mice, the LD50 was 2.3 × 104 CFU. In IgM−/−, μMT−/−, CD4−/−, CD8−/−, and SCID mice, 100% lethality with 10 CFU was observed (data not shown).

Evaluation of blood, lung, and spleen bacterial burdens. In experiments separate from the survival studies, mice were infected as described above, bled from the retro-orbital sinus 18 h postinfection, and killed by cervical dislocation, after which their lungs and spleens were removed aseptically, washed in PBS, and homogenized in Hanks’ balanced salt solution (Mediatech, Herndon, VA). The time period between infection and bleeding was selected after pilot studies revealed that it was associated with the appearance of cytokines in the sera of surviving mice (data not shown). To compare IgM- and antibiopsied mice, penicillin (PCN; penicillin G potassium salt; Sigma-Aldrich) was administered in a separate group of experiments. The PCN dosage and administration schedule were adapted from those of Knudsen et al. (28) with some modifications: 10 mg of PCN/kg of body weight was administered subcutaneously in the neck region 1 h prior to i.p. infection with 30 CFU of serotype 3 pneumococci, followed by two more PCN injections once every 6 h. Mice were sacrificed either 6 or 18 h postinfection. Tissue samples were serially diluted in TSB, plated onto TSB plates with 5% sheep’s blood (Becton Dickinson), and incubated for 24 h at 5% CO2 at 37°C; CFU in the samples were counted the following day. The lowest limit of detection was 20 CFU/ml, and results were reported as such if no bacteria were detected in undiluted samples. Care was exercised to avoid endotoxin contamination by using autoclaved materials and thoroughly rinsing instruments with 70% ethanol between each dissection.

Determination of levels of cytokines in spleen tissue and sera. Levels of keratinocyte-derived chemokine (KC) and interleukin-6 (IL-6) in sera and levels of KC, IL-6, and macrophage inflammatory protein-2 (MIP-2) in spleen tissue were determined by enzyme-linked immunosorbent assay (ELISA) using samples from A7-, myeloma IgM-, and PBS-treated mice collected 18 h after infection. Blood was allowed to clot on ice for 1 h, after which serum was separated by centrifugation for 30 min at 3,000 × g at 4°C and stored at −20°C until use. Spleens were homogenized in 1 ml of Hank’s balanced solution (Mediatech) and centrifuged for 30 min at 2,000 × g at 4°C. Supernatants were collected and stored at −20°C until use. ELISA kits (R&D Systems, Minneapolis, MN) were used according to the manufacturer’s protocol as previously described (8, 9).

White blood cell count. Whole blood was diluted 1:20 in Turk’s solution (1% glacial acetic acid and 0.01% gentian violet in distilled H2O) as described previously (47). Viable cells were then scored with a hemocytometer to determine the total white blood cell count. For the differential count, white blood was diluted 1:20 in 10% EDTA and smeared onto a slide. Monocytes, lymphocytes, and neutrophils were scored based on morphology by light microscopy until a total of 100 cells was reached.

Opsonophagocytic killing assay. The ability of A7 to mediate the opsonophagocytic killing (opsonophagocytosis) of type 3 pneumococci by murine macrophage-like cells was determined as described previously (9, 38). Briefly, opsonophagocytic killing assays were performed with a total volume of 100 μl; 2 × 104 CFU of serotype 3 pneumococci were coincubated at room temperature for 30 min with a 1-, 10-, or 50-μg/ml solution of A7 or control IgM. 3774.1 murine macrophages (106 cells/well) and 10% (by volume) murine serum were added to each well (Sigma-Aldrich) were added, and the mixtures were incubated for 1 h at 37°C. Dilutions of the cultures were spread onto TSB plates with 5% sheep’s blood (Becton Dickinson) and incubated at 37°C overnight in 5% CO2, and CFU were counted the following day. A separate experiment was done with heat-killed complement by incubating the complement source at 65°C for 30 min. The numbers of CFU were normalized as percentages of the number of CFU in the control (pneumococci plus macrophages or polymorphonuclear leukocytes [PMNs] plus complement). To determine whether the ability of A7 to promote opsonic killing was influenced by the species mismatch in this protocol, we determined the ability of A7 to promote pneumococcal killing by human PMNs as described previously for another human IgM mAb to PPS (9).

Statistical analysis. The numbers of CFU in blood, spleen, and lung lysates and those in samples after opsonophagocytic killing assays and the levels of cytokines and chemokines were compared by performing an unpaired t test or, if the data were not normally distributed, the Mann-Whitney test. A one-sample t test was used to compare CFU data represented by the lowest limit of detection to the data from the appropriate controls. For those cytokines that were not detected, the lowest limit of detection of the assay (according to the manufacturer’s directions) was used in a one-sample t test for comparison with the results for the other treatment groups. Grubbs’ test was used to detect outliers. Mouse survival data were analyzed statistically by using the Kaplan-Meier log rank survival test. All statistical analyses were performed using Prism (v.4.02 for Windows; GraphPad Software, San Diego, CA). A P value of <0.05 was used for statistical significance.

RESULTS

Mouse protection experiments. Infection with *S. pneumoniae* ATCC 6303 serotype 3 resulted in almost 100% lethality in all strains of mice (Fig. 1). The administration of A7 significantly prolonged survival compared to that of control IgM-treated mice of all strains (P values: 0.007, C57BL/6 mice; 0.0001, CD4−/− mice; 0.030, CD8−/− mice; 0.007, μMT−/− mice; 0.003, SCID mice; 0.001, C57BL/6 × SV129 mice; 0.003, IgM−/− mice; Kaplan-Meier log rank survival test) and PBS-
treated mice (P values: 0.001, C57BL/6 mice; 0.0001, CD4/+/H11002/C57BL/6 mice; 0.0002, CD8/+/H11002/CD8+/+/H11002 mice; 0.0001, H11002/SCID mice; 0.0009, C57BL/6/H11003/SCID mice; 0.0009, sIgM/+/H11002/+/sIgM+/+/H11002 mice; Kaplan-Meier log rank survival test) (Fig. 1).

**CFU in blood and tissue samples.** Bacteria were detected in the blood of both PBS- and IgM-treated mice 18 h postinfection. There were no bacteria detectable in the blood of A7-treated sIgM/+/+/H11002 mice or C57BL/6/H11003/SCID mice (Fig. 2A). The lowest limit of detection in this assay was 20 CFU/ml. Lung and spleen homogenates from A7-treated mice also had significantly fewer bacteria than those from PBS- and IgM-treated mice (Fig. 2B and C). Numbers of CFU in samples from mice that received 3 or 30 CFU were comparable 18 h postinfection (data not shown).

**Serum and splenic cytokine protein levels.** Serum IL-6 and KC protein levels and splenic IL-6, KC, and MIP-2 levels were assessed by ELISA. The levels of IL-6 and KC in the sera and those of IL-6, KC, and MIP-2 in the spleen tissue from A7-treated sIgM/−/− mice and wild-type mice were significantly lower than those in samples from control mice of both strains (Fig. 3 and 4). The levels of IL-6 in spleen tissue differed significantly between PBS-treated C57BL/6/×Sv129 and sIgM/−/− mice (Fig. 4A).

**Total white blood cell count and differential count.** The total leukocyte count was determined by enumerating Turk’s solution-stained cells. Treatment with A7 significantly increased the numbers of total leukocytes and lymphocytes in sIgM/−/− mice compared to those in PBS- and IgM-treated controls and...
increased the numbers in C57BL/6 × Sv129 mice compared to those in PBS-treated controls (Fig. 5A and B). There was no significant difference in numbers of monocytes and neutrophils among the treatment groups and mouse strains (Fig. 5C and D).

**Opsonophagocytic killing assays.** The ability of A7 to promote the killing of type 3 pneumococci by murine macrophage-like cells was evaluated in vitro under the same conditions in two separate experiments. In both experiments, there was no significant difference in numbers of CFU under A7 and IgM treatment conditions when either 10 or 1 μg of mAb was used (Fig. 6A). A7 also did not promote killing by human PMNs (Fig. 6B). Results with heat-inactivated complement sources were similar (data not shown). Larger amounts of A7 could not be used, as they resulted in clumping, which was observed with 50 μg of A7 (data not shown).

**Penicillin studies.** To compare the activity of A7 to that of an antibiotic used to treat pneumococcal disease, mice were treated with PCN 1 h prior to i.p. pneumococcal infection in studies similar to those described by Knudsen et al. (28). The initial dose of PCN was followed by two more doses 6 h apart. There were no bacteria detectable at 18 h postinfection in the blood, lungs, or spleens of mice that received either A7 or PCN (Fig. 7). The lowest limit of detection in this assay was 20 CFU/ml. There were no statistically significant differences in the levels of the cytokines measured between mice receiving A7 and those receiving PCN. However, for each cytokine measured, there was a trend towards a higher level in PCN-treated mice, with the difference in IL-6 levels in sera and spleen tissue almost reaching significance at P of 0.06 (Fig. 8). CFU and cytokine data obtained 6 h postinfection were similar to those obtained 18 h postinfection, but blood, lung, and spleen samples from mice receiving PCN had almost 1-log-higher numbers of CFU than those from A7-treated mice, although the difference was not statistically significant (data not shown).

**DISCUSSION**

The studies reported herein were undertaken to determine whether A7-mediated protection against systemic pneumococcal infection involved immunomodulation or depended on cellular subsets of acquired immunity. Although our data show that A7-mediated protection did not require B or T cells or natural IgM, the fact that the LD_{50}s for the strains we used were lower for the immunodeficient mice than the wild-type strains demonstrates that these immune system components...
are important for innate resistance to systemic pneumococcal challenge. The dependence of antibody immunity upon T lymphocytes has been recognized for antibodies to other pathogens, including Cryptococcus neoformans, herpes simplex virus, and Francisella tularensis (10). For West Nile virus infection, antibody-mediated protection was shown to depend on B and T cells (17). The passive administration of specific IgM was protective against West Nile virus in sIgM−/− mice, although

FIG. 5. Total white blood cell counts and differential counts after i.p. infection with serotype 3 pneumococci. Total leukocytes (A), lymphocytes (B), monocytes (C), and neutrophils (D) were enumerated 18 h after i.p. infection with serotype 3 pneumococci. Black bars represent C57BL/6 × Sv129 mice, while white bars represent sIgM−/− mice. Each bar represents the mean for the designated group; the error bars show the standard errors of the means. *, P of <0.05 between groups of sIgM−/− mice; #, P of <0.05 for comparison to PBS-treated C57BL/6 × Sv129 mice; unpaired t test. n, 3 to 9 mice per group.

FIG. 6. Opsonophagocytic activity of A7 against serotype 3 pneumococci. The quantities of CFU as percentages of the numbers of CFU in the controls were determined after opsonophagocytic killing by murine macrophages (A) and human PMNs (B). Results shown are the combination of results from two separate experiments conducted in duplicate. Each bar represents the mean for the designated group; the error bars show the standard errors of the mean. *, P of <0.05 between groups receiving the designated treatments; unpaired t test. P, pneumococcus; mac, macrophages; comp, complement.
this strain of mice had enhanced susceptibility to infection (18). Natural IgM also enhanced the innate immune response to pneumococcal pneumonia in a model using a different serotype (6). Similarly, our data show that although the susceptibility of sIgM−/− mice was greater than that of controls, natural IgM is not required for protection when specific IgM is administered. The importance of CD4 T lymphocytes for innate resistance to pneumococcal pneumonia and intranasal colonization (25, 35) has been demonstrated by other groups. Our finding that CD4 T lymphocytes are dispensable for specific antibody-mediated protection against systemic pneumococcal infection suggests that antibody-based therapies may hold promise for treating pneumococcal sepsis in patients with such deficiencies, notably those with human immunodeficiency virus infection.

Cytokine responses in wild-type as well as sIgM−/− mice were assessed in order to enable us to determine whether natural IgM influences the cytokine response in the context of antibody therapy. The use of sIgM−/− mice permitted us to study the effect of acquired antibody immunity in the absence of the complexities introduced by naturally occurring immunity and to ascertain whether the efficacy of acquired antibody immunity requires the presence of natural antibody. We did not identify significant differences in A7 efficacy or the accompanying cytokine response to pneumococcal infection between sIgM−/− and wild-type mice. We sought to establish a difference by employing a smaller inoculum for infection (3 CFU), but this inoculum was as lethal as the larger one (data not shown). Mouse availability precluded studies to examine the effect of T- and B-cell deficiency on the inflammatory response; however, this is a goal of ongoing studies.

In this study, we focused on examining the expression of IL-6, because IL-6 is often a marker of sepsis (21), and that of MIP-2 and KC, because they are PMN-derived chemokines that are often elevated in pneumococcal pneumonia (16). Pneumolysin, a pneumococcal virulence factor (42), induces the secretion of IL-6 and MIP-2, a human IL-8 homolog (55). Mice deficient in IL-6 are more susceptible to pneumococcal pneumonia, having higher numbers of CFU, myeloperoxidase activity, and tumor necrosis factor levels, suggesting that IL-6 may be required for the clearance of the bacteria (52). Our data show that A7 administration was associated with a marked decrease in levels of IL-6 and KC in sera and levels of IL-6, KC, and MIP-2 in spleen tissue. A7 also led to a reduction in the bacterial loads in blood, spleen, and lung samples. Taken together, these observations suggest that although IL-6 may be required for innate resistance to pneumococci (52), specific antibody may preclude the need for the inflammatory properties of IL-6 in host defense. A limitation of our studies is that they do not discriminate between direct immunomodulatory activity and indirect activity as a result of bacterial clearance. However, the marked trend towards a lower level of IL-6

FIG. 7. CFU and total leukocytes after i.p. infection with serotype 3 pneumococci. CFU in blood (A), lungs (B), and spleens (C) and total leukocytes (D) were enumerated 18 h after i.p. infection with serotype 3 pneumococci. Each point represents the value for a single mouse, while the horizontal lines represent the means for the designated groups. CFU data represent the lowest level of detection in two separate experiments (40 or 100 CFU). n, 8 mice per group.
expression in A7-treated mice than in PCN-treated mice in the context of comparable numbers of CFU (18 h after infection) suggests that there may be a component of direct immunomodulation by A7 and/or that it does not induce inflammatory mediator release. This possibility is supported by evidence for the antibiotic-induced activation of Toll-like receptor-mediated mediator release (36, 37) and deserves further investigation.

The opsonophagocytic activity of PPS-specific antibody has been proposed as a surrogate for PPS vaccine efficacy in humans (5, 30, 44, 51). However, in our study, A7 did not promote opsonophagocytosis in mouse or human cells in vitro. Hence, the efficacy of A7 would not have been predicted by determining its in vitro opsonophagocytic efficacy. A similar finding was reported for another human IgM mAb, D11, which was protective against both systemic and pulmonary infection with serotype 8 in mice (8, 56). D11 was also associated with decreased IL-6 and MIP-2 levels and functioned as an immunomodulator in a pneumonia model of serotype 8 infection (8). The immunomodulatory activity of D11 in systemic infection was not determined. Hence, our studies with A7 provide proof of principle and extend to another disease model and serotype the association between the efficacy of a nonopsonic antibody and a reduction in proinflammatory cytokine and chemokine expression. A7 efficacy and immunomodulation were associated with controlling the bacterial burden; however, the mechanism by which this control is mediated is unknown. Available knowledge does not provide an explanation for how A7 or other nonopsonic antibodies mediate bacterial clearance. It is possible that they promote killing in vivo, and this possibility requires further investigation. Both A7 and D11 require complement for efficacy against pneumococcal infection (13, 56). Since neither promotes effector cell killing in vitro, their dependence on complement may in part involve other complement-mediated functions, such as the generation of C5a or opsonization without phagocytosis and killing. The role of C5a in A7-mediated protection deserves investigation but was beyond the scope of this study. CD4 T lymphocytes and macrophages contribute to pneumococcal clearance (19, 53), and our data show that naïve CD4-deficient mice were more susceptible to infection than wild-type mice. Interestingly, we also found that the numbers of peripheral lymphocytes increased without a change in numbers of PMNs in A7-treated mice. Since pneumococcal infection generally induces PMN leukocytosis (26, 49), our findings suggest that antibody-mediated immunoregulation may enhance cellular immunity to effect clearance (10, 11).

We compared the ability of A7 to induce bacterial clearance and affect proinflammatory expression to that of PCN, the gold standard for therapy for pneumococcal infection. PCN-treated mice had almost 1 log more CFU in the blood and in lung and spleen tissue than A7-treated mice 6 h postinfection, with a trend, albeit nonsignificant, towards higher cytokine levels. Since numbers of CFU were similar 18 h after infection, one dose of A7 had the same effect as three doses of PCN. This result may be due to the short half-life of PCN in the serum (12 min in mice) versus that of IgM (12 h in mice) and/or the observation that PCN is ineffective during the bacterial lag
phase (14, 20, 48). The longer half-life of circulating IgM may allow for a more sustained response. The higher rate of bacterial clearance by A7 in our study is not unexpected. In the preantibiotic era, patients were reported to experience rapid sterilization of the blood and improvement in their symptoms and fever following one dose of antipneumococcal serum, even though the serum was administered after the onset of infection (24). A comparable pace of improvement was often not observed following antibiotic therapy (1). In our model, both A7 and PCN were administered before infection, but historically, therapeutic antibodies were always administered after the onset of clinical disease after being validated in models in which they were given before infection (the pneumococcal serum potency model) (7, 12, 24).

The data reported herein show that the efficacy of a nonopsonic human IgM to serotype 3 pneumococcal capsular polysaccharide against a lethal systemic infection with serotype 3 is associated with bacterial clearance and a reduction in the expression of proinflammatory mediators. Our data extend the association of antibody efficacy and immunomodulation previously described for a pneumonia model of serotype 8 infection (8) to a different disease model and a different serotype. Serotype 3 is an important cause of adult pneumococcal disease that is not represented in the PPS-based conjugate vaccine presently used for children (32, 33). Hence, antibody-based approaches to treatment of serotype 3 infections may be needed as an adjunct to present therapies in light of the continuing problem of pneumococcal drug resistance and the phenomenon of serotype replacement in PPS-vaccinated children (39, 50). The treatment of resistant pneumococcal strains is an area of critical need. Our findings that a single dose of antibody can induce rapid bacterial clearance and reduce levels of the proinflammatory mediators that are responsible for many of the symptoms of pneumococcal disease suggest that mAb-based therapies may hold promise for the treatment of pneumococcal infection. Although a resistant serotype 3 strain was not available for our studies, we expect that A7 would be effective against such a strain since the antibody is specific for capsular polysaccharide, which is not involved in drug resistance. In fact, mAb therapies have been proposed as an approach to the treatment of infections with drug-resistant microbes (40). We hope to validate our findings with drug-resistant strains in the future. The comparable efficacies of A7 and PCN in our model suggest that mAb-based therapies may have the potential to be dose sparing and provide adjunctive beneficial immunomodulation. Finally, our finding that natural IgM and T and B cells were dispensable for A7-mediated protection suggests that mAb-based therapies may hold promise for treating patients with immune system impairment. At present, our goal is to fully characterize the mechanism by which A7 functions as an immunomodulator with antimicrobial effects.

ACKNOWLEDGMENT

This work was supported by grants from the National Institutes of Health (ROI AI045459 and ROI0144374) to L.P.

REFERENCES

1. Austriam, R., and J. Gold. 1964. Pneumococcal bacteremia with especial reference to bacteremic pneumococcal pneumonia. Ann. Intern. Med. 60:759–776.

2. Blue, C. E., G. K. Paterson, A. R. Kerr, M. Berge, J. P. Claverys, and T. J. Mitchell. 2003. ZmpB, a novel virulence factor of Streptococcus pneumoniae that induces tumor necrosis factor alpha production in the respiratory tract. Infect. Immun. 71:4924–4929.

3. Boes, M., C. Esau, M. F. Fischer, T. Schmidt, M. Carroll, and J. Chen. 1998. Enhanced B-1 cell development, but impaired IgG antibody responses in mice deficient in secreted IgM. J. Immunol. 160:4776–4787.

4. Boes, M., A. P. Prodeus, T. Schmidt, M. Carroll, and J. Chen. 1998. A critical role of natural immunoglobulin M in immediate defense against systemic bacterial infection. J. Exp. Med. 188:2381–2386.

5. Bogarta, D., M. Shijuyit, R. De Groot, and P. W. Hermans. 2004. Multiplex opsonophagocytosis assay (MOPA): a useful tool for the monitoring of the efficacy of pneumococcal conjugate vaccine. Vaccine. 22:6494–6500.

6. Brown, J. S., T. Russell, S. M. Gilliland, D. W. Holdon, J. C. Paton, M. R. Ehrenstein, M. J. Walport, and M. Botto. 2002. The classical pathway is the dominant complement pathway required for innate immunity to Streptococcus pneumoniae infection in mice. Proc. Natl. Acad. Sci. USA 99:16969–16974.

7. Buchwald, U. K., and L. Pirofski. 2003. Immune therapy for infectious diseases at the dawn of the 21st century: the past, present and future role of antibody therapy, therapeutic vaccination and biological response modifiers. Curr. Pharm. Des. 9:945–968.

8. Burns, T., M. Abadi, and L. Pirofski. 2005. Modulation of the lung inflammatory response to serotype 8 pneumococcus infection by a human monoclonal immunoglobulin M to serotype 8 capsular polysaccharide. Infect. Immun. 73:4530–4538.

9. Burns, T., Z. Zhong, M. Steinitz, and L. Pirofski. 2003. Modulation of polymorphonuclear cell interleukin-8 secretion by human monoclonal antibodies to type 8 pneumococcal capsular polysaccharide. Infect. Immun. 71:6171–6173.

10. Casadevall, A., and L. Pirofski. 2003. Antibody mediated regulation of cellular immunity and the inflammatory response. Trends Immunol. 24:374–378.

11. Casadevall, A., and L. A. Pirofski. 2006. A reappraisal of humoral immunity based on mechanisms of antibody-mediated protection against intracellular pathogens. Adv. Immunol. 91:1–44.

12. Casadevall, A., and M. D. Scharff. 1995. Return to the past: the case for antibody-based therapies in infectious diseases. Clin. Infect. Dis. 21:155–161.

13. Chang, Q., Z. Zhong, A. Lees, M. Pekna, and L. Pirofski. 2002. Structure-function relationships for human antibodies to pneumococcal capsular polysaccharide from transgenic mice with human immunoglobulin loci. Infect. Immun. 70:4977–4982.

14. Charan, S., and R. M. Zinkernagel. 1986. Antibody mediated suppression of secondary IgM response in nude mice against vesicular stomatitis virus. J. Immunol. 136:3057–3061.

15. Committee on New Directions in the Study of Antimicrobial Therapeutics: Immunomodulations. 2006. Treating infectious diseases in the microbial world: report of two workshops on novel antimicrobial therapies. National Academies Press, Washington, DC.

16. Dallaire, F., N. Ouellet, Y. Bergeron, V. Turmel, M. C. Gauthier, M. Simard, and M. G. Bergeron. 2001. Microbiological and inflammatory factors associated with the development of pneumococcal pneumonia. J. Infect. Dis. 184:292–300.

17. Diamond, M. S., B. Shrestha, A. Marri, D. Mahan, and M. Engle. 2003. B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. J. Virol. 77:2578–2586.

18. Diamond, M. S., E. M. Stiati, L. D. Friend, S. Higgs, B. Shrestha, and M. Engle. 2003. A critical role for induced IgM in the protection against West Nile virus infection. J. Exp. Med. 198:1853–1862.

19. Dockrell, D. H., H. M. Marriott, L. R. Prince, V. C. Ridger, P. G. Ince, P. G. Hellewell, and M. K. Whyte. 2003. Alveolar macrophage apoptosis contributes to pneumococcal clearance in a resolving model of pulmonary infection. J. Immunol. 171:5380–5388.

20. Frimodt-Moller, N., O. Sebbesen, and V. Frolund Thomsen. 1983. The pneumococcus and the mouse protection test: importance of the lag phase in vivo. Chemotherapy 29:128–134.

21. Gaini, S., O. G. Koldkjaer, C. Pedersen, and S. S. Pedersen. 2006. Procalcitonin, lipopolysaccharide-binding protein, interleukin-6 and C-reactive protein in community-acquired infections and sepsis: a prospective study. Crit. Care 10:R53.

22. Giebink, G. S., J. E. Foker, Y. Kim, and G. Schifman. 1980. Serum antibody and opsonic responses to vaccination with pneumococcal capsular polysaccharide in normal and splenectomized children. J. Infect. Dis. 141:404–412.

23. Green, L. L. 1999. Antibody engineering via genetic engineering of the mouse: Xenomouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies. J. Immunol. Methods 231:11–23.

24. Heffron, R. 1939. Pneumonia, with special reference to pneumococcus lobar pneumonia, p. 805–921. Harvard University Press, Cambridge, MA.

25. Kadin, A., W. Coward, M. J. Colston, C. R. Hewitt, and P. W. Andrew. 2004. CD4-T-lymphocyte interactions with pneumolysin and pneumococci suggest a crucial protective role in the host response to pneumococcal infection. Infect. Immun. 72:2689–2697.

26. Kadin, A., W. Coward, M. J. Colston, C. R. Hewitt, and P. W. Andrew. 2004. CD4-T-lymphocyte interactions with pneumolysin and pneumococci suggest a crucial protective role in the host response to pneumococcal infection. Infect. Immun. 72:2689–2697.

27. Kadin, A., W. Coward, M. J. Colston, C. R. Hewitt, and P. W. Andrew. 2004. CD4-T-lymphocyte interactions with pneumolysin and pneumococci suggest a crucial protective role in the host response to pneumococcal infection. Infect. Immun. 72:2689–2697.
26. Kemp, K., H. Bruunsgaard, P. Skinhøj, and P. B. Klarlund. 2002. Pneumococcal infections in humans are associated with increased apoptosis and trafficking of type 1 cytokine-producing T cells. Infect. Immun. 70:5019–5024.

27. Kirby, A. C., L. G. Raynes, and P. M. Kaye. 2005. The role played by tumor necrosis factor during localized and systemic infection with Streptococcus pneumoniae. J. Infect. Dis. 191:1538–1547.

28. Knudsen, J. D., L. Odenholt, H. Erlendsdottir, M. Gottfredsson, O. Cars, N. Frimodt-Møller, F. Espersen, K. G. Kristinsson, and S. Gudmundsson. 2003. Selection of resistant Streptococcus pneumoniae during penicillin treatment in vitro and in three animal models. Antimicrob. Agents Chemother. 47:2499–2506.

29. Kolb, K. S., I. Smithson, B. Rahbeger, S. Khuder, and M. A. Westerink. 2005. Immune response to pneumococcal polysaccharides 4 and 14 in elderly and young adults: analysis of the variable heavy chain repertoire. Infect. Immun. 73:7465–7476.

30. Kroon, F. P., J. T. van Dissel, E. Ravensbergen, P. H. Sibbing, and R. van Furth. 2000. Enhanced antibody response to pneumococcal polysaccharide vaccine after prior immunization with conjugate pneumococcal vaccine in HIV-infected adults. Vaccine 19:886–894.

31. Lakshman, R., C. Murdoch, G. Race, R. Lumley, T. Shaw, and A. Finn. 2003. Pneumococcal nasopharyngeal carriage in children following heptavalent pneumococcal conjugate vaccination in infancy. Arch. Dis. Child. 88:211–214.

32. Lesca, C. A., R. Lynfield, R. Daquila, P. Plisov, R. Facklam, M. M. Farley, L. H. Harrison, W. Schaffer, A. Reingold, N. M. Bennett, J. Hadler, P. R. Cieslak, and C. G. Whitney. 2005. Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. JAMA 294:2043–2051.

33. Lien, T. A., G. T. Bay, S. B. Black, J. C. Butler, J. O. Klein, R. F. Breiman, M. A. Miller, and H. R. Shinefield. 2000. Projected cost-effectiveness of pneumococcal conjugate vaccination of healthy infants and young children. JAMA 283:1460–1468.

34. Malley, R., K. Trzcinski, A. Srivastava, C. M. Thompson, P. W. Anderson, and M. Lipsitch. 2005. CD4+ T cells mediate antibody-independent acquired immunity to pneumococcal colonization. Proc. Natl. Acad. Sci. USA 102:4848–4853.

35. Moore, L. J., A. C. Pridmore, S. K. Dower, and R. C. Read. 2003. Penicillin enhances the toll-like receptor-2-mediated proinflammatory activity of Streptococcus pneumoniae. J. Infect. Dis. 188:1040–1048.

36. Moore, L. J., A. C. Pridmore, S. K. Dower, and R. C. Read. 2004. The glycopeptide vancomycin does not enhance toll-like receptor 2 (TLR2) activation by Streptococcus pneumoniae. J. Antimicrob. Chemother. 54:76–78.

37. Namh, M. H., J. V. Olander, and M. Magyaraki. 1997. Identification of cross-reactive antibodies with low opsonophagocytic activity for Streptococcus pneumoniae. J. Infect. Dis. 176:698–703.

38. Naunheimer, E. L., and W. R. Bishai. 2004. Antibiotic resistance in Streptococcus pneumoniae: what does the future hold? Clin. Infect. Dis. 38(Suppl. 4):S363–S371.

39. Pirofski, L., and A. Casaclang. 2006. Immunomodulators as an antimicrobial tool. Curr. Opin. Microbiol. 9:489–495.

40. Pirofski, L., and A. Casaclang. 1998. The use of licensed vaccines for active immunization of the immunocompromised host. Clin. Microbiol. Rev. 11:1–26.

41. Rijneveld, A. W., G. P. van den Doolstelstein, S. Florquin, T. J. Standfost, P. Speelman, M. Van Alphen, and T. van der Poll. 2012. Roles of interleukin-6 and macrophage inflammatory protein-2 in pneumolysin-induced lung inflammation in mice. J. Infect. Dis. 185:123–126.

42. Robbins, J. B., R. Schneerson, and S. C. Szu. 1995. Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J. Infect. Dis. 171:1387–1398.

43. Romero-Steiner, S., C. Frasch, N. Conception, D. Goldblatt, H. Kayhty, M. Vakevaliant, C. Lafarrierre, D. Wauters, M. H. Nahm, F. Schinsky, B. D. Pilkaytis, and G. M. Carbone. 2003. Multilaboratory evaluation of a viability assay for measurement of opsonophagocytic antibodies specific to the capsular polysaccharides of Streptococcus pneumoniae. Clin. Diagn. Lab. Immunol. 10:1019–1024.

44. Russell, N., J. R. Corvalan, M. L. Gallo, C. G. Davis, and L. Pirofski. 2000. Production of protective human anti-pneumococcal antibodies by transgenic mice with human immunoglobulin loci. Infect. Immun. 68:1820–1826.

45. Schmick, B., S. Huber, K. Moog, J. Zahntan, A. C. Hoke, B. Opitz, S. Hammerschmidt, T. J. Mitchell, M. Kracht, S. Rosseau, N. Suttorp, and S. H. Hinnisztel. 2006. Pneumococci induced TLR- and Rac1-dependent NF-kappaB recruitment to the IL-8 promoter in lung epithelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 290:L730–L737.

46. Stephens-Romero, S. D., A. J. Mednick, and M. Feldmesser. 2005. The pathogenesis of fatal outcome in murine pulmonary aspergillosis depends on the neutrophil depletion strategy. Infect. Immun. 73:114–125.

47. Sullivan, J. B. 1987. Past, present, and future immunotherapy of snake venom poisoning. Ann. Emerg. Med. 16:938–944.

48. Takahashi, K., K. Tateda, T. Matsumoto, Y. Iizawa, M. Nakao, and K. Yamaguchi. 1997. Role of tumor necrosis factor alpha in pathogenesis of pneumococcal pneumonia in mice. Infect. Immun. 65:257–260.

49. Tomasz, A. 1999. New faces of an old pathogen: emergence and spread of multidrug-resistant Streptococcus pneumoniae. Am. J. Med. 107(1A):S58–S62.

50. Usinger, W. R., and A. H. Lucas. 1999. Avidity as a determinant of the protective efficacy of human antibodies to pneumococcal capsular polysaccharides. Infect. Immun. 67:2366–2370.

51. van der Poll, T., C. V. Kogos, X. Guirao, W. A. Buurman, M. Kopf, and S. F. Lowry. 1997. Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. J. Infect. Dis. 176:439–444.

52. van Rossum, A. M., E. S. Lysenko, and J. N. Weiser. 2005. Host and bacterial factors contributing to the clearance of colonization by Streptococcus pneumoniae in a murine model. Infect. Immun. 73:7718–7726.

53. Vernachio, L., E. J. Neufeld, K. MacDonald, S. Kurth, S. Murakami, C. Hohne, M. King, and D. Molrine. 1987. Paste, present, and future immunotherapy of snake venom poisoning. Ann. Emerg. Med. 16:938–944.

54. van de Poll, T., C. V. Kogos, X. Guirao, W. A. Buurman, M. Kopf, and S. F. Lowry. 1997. Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. J. Infect. Dis. 176:439–444.

55. Vercarleso, L., J. E. Neufeld, K. MacDonald, S. Kurth, M. Nakao, and K. Yamaguchi. 1997. Role of tumor necrosis factor alpha in pathogenesis of pneumococcal pneumonia in mice. Infect. Immun. 65:257–260.

56. Tomasz, A. 1999. New faces of an old pathogen: emergence and spread of multidrug-resistant Streptococcus pneumoniae. Am. J. Med. 107(1A):S58–S62.

57. Usinger, W. R., and A. H. Lucas. 1999. Avidity as a determinant of the protective efficacy of human antibodies to pneumococcal capsular polysaccharides. Infect. Immun. 67:2366–2370.