Central Role of Complement in Passive Protection by Human IgG1 and IgG2 Anti-pneumococcal Antibodies in Mice


http://www.jimmunol.org/content/170/12/6158

References This article cites 56 articles, 16 of which you can access for free at: http://www.jimmunol.org/content/170/12/6158.full#ref-list-1

Subscription Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Central Role of Complement in Passive Protection by Human IgG1 and IgG2 Anti-pneumococcal Antibodies in Mice

Eirikur Saeland,* Gestur Vidarsson,** Jeanette H. W. Leusen,* Evert van Garderen,§ Moon H. Nahm,¶ Henriette Vile-Weekhout,∥ Vanessa Walraven,† Annette M. Stemerding,* J. Sjef Verbeek,‡ Ger T. Rijkers,‡ Wietske Kuis,‡ Elisabeth A. M. Sanders,† and Jan G. J. van de Winkel2‡∥

Streptococcus pneumoniae (pneumococcus) is a leading cause of community-acquired pneumonia, bacteremia, and meningitis (1, 2). Capsular polysaccharides of pneumococci are considered the most important Ags conferring immunity, and have been shown to elicit serotype-specific Ab responses. Opsonization of pneumococci facilitates phagocytosis and induces host protection (3, 4). More than 90 different capsular serotypes exist, and 23 of the most important causes of disease are included in the current polysaccharide vaccine. The vaccine is efficacious in immunocompetent adults (5), but is not recommended for use in infants (6). The newly developed multivalent polysaccharide protein conjugate vaccines are protective against invasive disease in this age group (7, 8). Adults primarily make IgG2 Ab after polysaccharide vaccination, while infants and children make IgG1 (9–11).

Phagocytes express several receptor classes that facilitate phagocytosis. These include receptors for the Fc part of Ig (FcR) and complement receptors (CR), which interact with C fragments on pathogen surfaces (12). Leukocyte IgG receptors (FcγR) in both mice and humans are divided in three classes: FcγRI, II, and III. Murine FcγRI (mFcγRI) I and III are composed of a unique ligand-binding ɑ-chain and the promiscuous signaling γ-chain. mFcγRII is a single molecule with intrinsic inhibitory signaling capacity, and is widely expressed on leukocytes (13). C represents an important part of the innate immune system, because it induces destruction of Gram-negative bacteria and facilitates bacterial uptake by leukocytes. Currently, three C pathways are recognized. The classical pathway is initiated by Ab:Ag complexes and requires complement components C1, C4, and C2. The alternative pathway (AP) is initiated by C3b coated on surfaces of pathogens and requires factors B and D. Finally, the lectin pathway is triggered by mannan-binding lectin bound to pathogen surfaces (14). Activation of any of the C pathways leads to production and deposition of the opsonins C3b and iC3b. Neutrophils are equipped with CR 1 and 3, respectively, which interact with these fragments and facilitate uptake (12, 15).

FcR are crucial for Ab-mediated pneumococcal phagocytosis in humans (16, 17), which may be further augmented by CR (3, 18). In the absence of Ab, C may be deposited on pneumococcal surfaces, although this may be serotype dependent (18–21). C-mediated phagocytosis of pneumococcus is hampered by the pneumococcal capsule, as it prevents CR to reach C fragments bound on the capsular polysaccharide. This prevents CR to reach C fragments bound on the capsular polysaccharide, allowing interaction with the capsular polysaccharide.
CR (22), but does not result in lysis of pneumococci (23). C has been demonstrated to play an important role in protection in animal models of pneumococcal bacteremia, both in the presence (24, 25) and absence of specific Abs (26, 27).

Human IgG1 (hlgG1) and hlgG2 display intrinsic functional differences. Both subclasses are potent C activators and interact with specific FcγR subclases (3, 28). The aim of this study was to address the relative contribution of FcγR and C in a mouse model of Ab-mediated protection against pneumococcal pneumonia and bacteremia. For this purpose, we generated serogroup 6-specific rhlgG1 and rhlgG2 Ab containing identical V regions. This enabled comparison of the contribution of Ab-mediated effector functions in host protection. We show hlgG1 to efficiently interact with mouse leukocyte FcγRII and FcγRIII, whereas IgG2 does not. Nevertheless, protection against S. pneumoniae serotype 6A by hlgG was shown to be mediated primarily by C.

Materials and Methods

Recombinant Ab

The V regions of the polysaccharide serotype 6 (PS6)-specific human Dob1 hybridoma (29) were amplified using V gene-specific oligonucleotide primers (accession numbers AF211205 and AF211206) cloned and expressed as y1 or y2 H chains, together with C L chains. The L chain V region was expressed with the Cκ region and the H chain V region with y1 or y2 C regions, as described in detail (30, 31). The resulting Ab s were renamed Gdb1. H and L chain-transfected baby hamster kidney cells secreted Ab, which were purified using protein A chromatography. Isolated IgG preparations were ultrafiltrated at 89,000 × g to remove protein complexes; supernatants were snap frozen in liquid nitrogen and stored in small (20-μl) aliquots at −80°C.

Concentration of Ag-specific IgG Ab was measured in a pneumococcal polysaccharide (PS)-specific ELISA (32). ELISA plates (Maxisorp, Nunc, Denmark) were coated by overnight incubation with 10 μg/ml PS6B (American Type Culture Collection (ATCC), Manassas, VA) in PBS at 4°C. The international standard 89-SP, with known IgG titer to PS6B, was diluted 1/50 and adsorbed with 10 μg/ml cell wall polysaccharide (Statens Serum Institut, Copenhagen, Denmark) for 30 min. The adsorbed standard and IgG Ab were incubated in parallel in eight 2-fold dilutions for 2 h in polysaccharide-coated ELISA plates. Bound IgG was detected with HRP-labeled goat anti-hIgG (Jackson ImmunoResearch Laboratories, West Grove, PA), anti-hlgG1 HRP (Southern Biotechnology (SBA), Birmingham, AL), or anti-hlgG2 HRP (SBA), and reactions were developed with ABTS (Roche, Mannheim, Germany). To confirm the results of the IgG ELISA, FITC-labeled mouse anti-human κ L chain (SBA) was used, followed by incubation with anti-FITC HRP (Amersham, Arlington Heights, IL). Absorbance was measured at an OD of 415 nm.

Both recombinant Abs bound to PS6B in ELISA with similar activity and were of the appropriate molecular size (as determined by SDS-PAGE under reduced and nonreduced conditions). Anti-hlgG1 conjugate did not react with the rhlgG2 Ab in ELISA, and vice versa, confirming that the Ab s were of the corresponding subclasses.

Mice

Wild-type C57BL/6 mice were from Harlan (Horst, The Netherlands). C2 factor B (FB)−/− (33) and C1q−/− mice (34) (both F1 C57BL/6) were kindly provided by M. Botto (Imperial College, London, U.K.), and FcRγ chain−/− mice were provided by T. Saito (Chiba University Graduate School of Medicine, Chiba, Japan) (35). Mice deficient in FcγR II, and IIIc-chains were in a C57BL/6 × Ola129 × BALB/c background (36), and control mice used for them were F1 Ola129 × C57BL/6. Mice were matched for age and gender in each experiment, and both 8- to 12-week-old males and females were used. They were bred at the Transgenic Mouse Facility of the Central Animal Laboratory at Utrecht University. Experiments were performed in the Infection Facility, according to institutional and national guidelines.

Effector cells

White blood cells were isolated from mice injected s.c. at day −3 with 15 μg pegylated G-CSF (Amgen, Thousand Oaks, CA) to increase numbers of polymorphonuclear cells (PMN). Pegylated G-CSF has a longer half-life in vivo than the nonpegylated form, and only one injection is needed to steeply increase the numbers of circulating PMN. Numbers of PMN reach a maximum at day 3, corresponding to ~50% of the total number of leucocytes (37). Whole blood was collected in heparinized containers by orbital puncture; RBCs were lysed with lysis buffer (containing 0.83% ammonium chloride, 0.1% potassium bicarbonate, and 0.0037% sodium EDTA); and remaining cells were washed once in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% FCS.

The FcR-negative IIA1.6 murine B cell line (38) was cultured in RPMI 1640 medium + 10% FCS. Cells were stably transfected with mFcyRII cDNA, and cultured with neonycin (G418; Life Technologies) or mFcyRIII cDNA, and cultured with methotrexate (Pharmachemie, Haarlem, The Netherlands). FcγR-expressing cells were selected using MACS beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Expression of mFcyRII was quantitated by mAb 2.4G2 recognizing both mFcyRII and III (BD Pharmingen, San Diego, CA) (39).

IgG1 and IgG2 dimer binding to FcγR

Interaction of IgG dimers with mFcyRII was analyzed, as described (40). In brief, purified paraproteins of IgG1 and IgG2 subclasses (CLB, Amsterdam, The Netherlands) were incubated in equimolar ratios with F(ab′)2 of mouse anti-human κ L chain mAb 3K5 for 2 days at 4°C. Two-fold dilutions of dimers were incubated with mouse cells or mFcyRII transfectants in RPMI 1640 medium + FCS at 4°C for 30 min under rotation. After washing twice, PE-conjugated F(ab′)2 of goat anti-hlgG (SBA) were added and cells were analyzed by flow cytometry.

Bacteria

S. pneumoniae serotype 6A (ATCC) was stored in batches in tryptose broth containing 20% glycerol at −80°C until use. On the day of an infection experiment, pneumococci were cultured to mid log phase in Todd Hewitt broth containing 10% FCS at 37°C with 5% CO2 centrifuged at 2200 × g for 15 min, and resuspended in PBS. Inoculum densities were confirmed by making serial dilutions, and plated on blood agar to express CFU after overnight incubation at 37°C.

Phagocytosis

Opsonophagocytosis assays were performed, as described (41). In brief, heat-killed FITC-labeled, S. pneumoniae serotype 6B were preopsonized with Ab, under shaking conditions at 37°C for 30 min. After washing, pneumococci were incubated with effector cells at a ratio of 40:1 in RPMI 1640 medium + 10% FCS for 45 min at 4°C. Cells were washed three times and split over two tubes for incubation at either 4°C or 37°C for 20 min. PE-conjugated goat F(ab′)2 of anti-hlgG (SBA) were added after washing, and the cells were analyzed by flow cytometry. PMN were gated on the basis of forward and sideward scatter properties. In phagocytosis experiments using IIA1.6 transfectants, viable cells were gated. FITC and PE fluorescence intensities of cells maintained at 4°C served as control for bacterial binding. The decrease in PE fluorescence of FITC-positive cells after incubation at 37°C reflected internalization of pneumococci. Phagocytosis rates were calculated according to the following formula: ([ΔPE/PEi] × F), where F is the total geometric mean fluorescence of PMN maintained at 4°C. PEi the fluorescence of FITC-positive PMN maintained at 4°C, and ΔPE the difference in PE intensity of FITC-positive PMN at 4°C and 37°C (41).

Complement assays

Heat-killed pneumococci (107 CFU/ml) were incubated at 37°C for 15 min with hlgG1 or hlgG2 in serial dilutions in PBS in the presence of 10% serum from wild-type C57BL/6, C1q−/− or C2Fb−/− mice (42). Heat-inactivated FCS was used as control serum. The reactions were stopped by adding ice-cold PBS containing 1% BSA and 5 mM EDTA. C3c deposition was detected by incubation of pneumococci with rabbit anti-human C3c FITC (cross-reactive with murine C3c; DAKO, Glostrup, Denmark) before quantification by FACS.

The effect of C on the viability of pneumococci was analyzed by incubating live pneumococci in the presence or absence of serially diluted recombinant Abs and 10 or 20% murine serum in RPMI at 37°C for 30 min. Next, aliquots were plated on blood agar and incubated overnight. Bacterial viability was assessed by counting of CFU.

Pneumococcal infection model

The pneumococcal infection model has been described before (43, 44). In brief, mice were passively immunized i.p. with 200 μl of recombinant Ab (diluted in PBS), 3 h before challenge. Mice were anesthetized with sodium pentobarbital (50 mg/kg) and challenged intranasally (i.n.) with 5 × 106 CFU pneumococci in 50 μl PBS. Twenty-four hours after challenge, blood was taken from tail veins, serially diluted, and plated on Columbia agar containing 5% sheep blood (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). Plates were incubated overnight at 37°C, and pneumococcal colonies were counted. Survival of mice was monitored in parallel. We
have previously shown that this pneumococcal strain causes maximum levels of bacteremia 20–24 h after challenge (43). Lungs and spleens were aseptically removed 24 h after challenge for histological analysis. Lungs were inflated with 10% buffered Formalin via the trachea to allow analysis of the tissue by microscopy.

In some experiments, C57BL/6 mice were treated with cobra venom factor (CVF), purified from Naja haje egyptian cobra venom (Sigma- Aldrich, St. Louis, MO), as described (45). This type of CVF selectively activates C3 and not C5. Mice were injected twice i.p. with an 8-h interval, a day before they were challenged with pneumococci (46). The amount of CVF given eliminated C activity, as measured by CH50 assay (47) for at least 2 consecutive days (data not shown).

In one experiment, wild-type mice were injected with 400 μg of the anti-Ly-6 Ab Gr-1 (BD PharMingen) i.p. on days −3 and −1. This treatment resulted in PMN depletion for at least 2 days (the duration of the experiment), as determined by Gr-1 staining and FACS analyses.

**Histopathological analysis of tissues**

Lungs and spleens were fixed in 10% buffered Formalin and embedded in paraffin. Sections (4 μm) were stained with H&E before microscopic examination of the tissues.

**Statistical analysis**

Statistical differences between groups were analyzed by nonparametric Kruskal-Wallis tests, corrected for multiple comparisons by Dunn’s tests. Kaplan-Meyer tests were performed on survival data. Statistical differences between groups were analyzed by nonparametric Statistical analysis.

**Results**

**Interaction of hIgG1 and hIgG2 with mFcR and complement**

We first analyzed the interaction of hIgG1 and hIgG2 dimers with mFcR, using IIA1.6 cells, stably transfected with either mFcRII or mFcRIII. These two classes of FcR are low affinity receptors and only bind IgG complexes (48). The level of surface-expressed FcR, as determined by binding of mAb 2.4G2, was similar for FcγRII and FcγRIII. hIgG1 dimers bound IIA1.6 cells transfected with both mFcγRII and mFcγRIII in a concentration-dependent manner, whereas IgG2 dimers bound to a much lesser extent. Only IgG1 dimers bound effectively to mouse PMN (see Fig. 1).

To address the functional consequences of the observed difference in interaction between hIgG1 and hIgG2 and mFcR, we generated recombinant PS6-specific Abs (see Materials and Methods). First, we studied the activity of the newly generated human anti-PS6 Ab in opsonophagocytosis. Wild-type mouse PMN phagocytosed hIgG1-opsonized 6B pneumococci (Fig. 2). Recently, we demonstrated FcγRII- or FcγRIII-transfected IIA1.6 cells (B). Phagocytic indices were calculated, as described in Materials and Methods. In A, the experiment was performed three times, and means ± SDs are shown. In B, the experiment was performed three times, yielding similar results.

**Ab-MEDIATED PROTECTION TO PNEUMOCOCCUS**

**Additional Figure captions**

**FIGURE 1.** Interaction of hlgG1 and hlgG2 isotypes with mFcγR. Dimers of IgG1 (thin lines) and IgG2 (broken lines) were incubated with mouse PMN (A) IIA1.6 cells transfected with mFcγRII (B) or mFcγRIII (C). IIA1.6 cells transfected with human FcγRIIA-H131 (D) served as a positive control. Thick lines mark the negative control cells (incubated with secondary Ab alone). This experiment was performed three times, yielding essentially identical results.

**FIGURE 2.** In vitro opsonophagocytosis of pneumococci induced by hlgG1 and hlgG2. Heat-killed, FITC-labeled S. pneumoniae serotype 6B were preopsonized with different concentrations of IgG1 or IgG2. Opsonized pneumococci were allowed to attach to effector cells at 4°C. Next, samples were divided in two aliquots; one was further incubated at 4°C, the other at 37°C. Pneumococci on the surface of cells were detected by PE-labeled F(ab’), of anti-IgG. The effector cells evaluated were: wild-type PMN or FcγRII- or FcγRIII-transfected IIA1.6 cells (B). Phagocytic indices were calculated, as described in Materials and Methods. In A, the experiment was performed three times, and means ± SDs are shown. In B, the experiment was performed three times, yielding similar results.

**FIGURE 3.** Murine complement deposition on pneumococci induced by hlgG1 and hlgG2. Heat-killed pneumococci were incubated with 1 μg/ml hlgG1 or hlgG2 in the presence of 10% pooled murine serum from wild-type mice (WT C) or C1q−/− mice (C1q−/− C) at 37°C for 15 min. C3c deposition was detected by incubation with rabbit anti-C3c FITC and flow cytometry. Thick line represents IgG1 + WT C; thin lines, IgG2 + WT C; dotted line, IgG1 + C1q−/− C; and solid histogram, WT C in the absence of IgG.
hlgG1 and hlgG2 anti-PS6 protect mice against invasive pneumococcal infection

Next, we compared the protective capacity of hlgG1 and hlgG2 against *S. pneumoniae* in vivo using a pneumonia and bacteremia model. C57BL/6 mice were passively immunized with different concentrations of hlgG 3 h before challenge with *S. pneumoniae* serotype 6A. Bacteremia was determined 24 h after challenge (A and C), and survival was monitored for 14 days (B and D). Data are pooled from four independent experiments, with PBS- and Ab-treated wild-type mice in each experiment. Wild-type, C2/FB−/− mice, and FcR triple-deleted mice were passively immunized with hlgG1 or hlgG2. Lower concentrations were not protective (Fig. 4). Protection failed in 3 of 23 mice receiving >0.2 μg (which may be explained by interindividual differences or technical error). These data indicated hlgG1 and hlgG2 to exhibit similar protective capacity in this mouse model.

In vivo protection by human anti-PS6 Abs is dependent on complement

Considering that both hlgG subclasses were protective, and only hlgG1 bound mFcγR, the contribution of FcγR in conferring Ab-induced protection was unclear. To assess whether FcγR contributed to Ab-mediated protection in vivo, we used mice lacking FcγR. FcγR γ-chain−/− mice, lacking expression of the activatory FcγRI and III (35), and mice deficient for all three FcγR classes (FcγR triple−/−) (36) were passively immunized with hlgG1 or hlgG2 before challenge with serotype 6A. At limiting amounts of either subclass (0.1 μg of hlgG1, and 0.2 μg of hlgG2), mice were protected against bacteremia and death, as were wild-type control mice (Fig. 5).

These data indicated that other factors might play a role in protection by hlgG1 and hlgG2 anti-pneumococcal Ab. The relevance of C for protection was assessed in mice deficient in complement receptors.
component C2 and FB (C2/FB \(^{-/-}\)) and in C1q (C1q \(^{-/-}\)). Furthermore, wild-type mice were treated with cobra venom factor (CVF) to deplete C3 (45). C1q \(^{-/-}\) mice, C2/FB \(^{-/-}\) mice, and CVF-treated mice almost all developed bacteremia after passive immunization with 0.2 \(\mu\)g of the hlgG1 or hlgG2 subclasses (Fig. 6). This indicated C to be important for Ab-mediated protection. However, passively immunized wild-type mice (D) had normal distribution and normal relative proportion of red and white pulp areas. Splenic hyperemia was observed in passively immunized C2/FB \(^{-/-}\) mice (E) and, to a lesser extent, in C1q \(^{-/-}\) mice. Each group consisted of five mice. Lungs from a C1q \(^{-/-}\) mouse (G) showing infiltration of perivascular/peribronchiolar spaces by PMN.

**FIGURE 7.** Histopathology of lungs and spleens after challenge with S. pneumoniae. Representative sections of lungs and spleens from mice 24 h after i.n. challenge (H&E staining; all panels \(\times 10\) magnification). Spleen sections from PBS-treated wild-type (A), C2/FB \(^{-/-}\) (B), and C1q \(^{-/-}\) (C) mice show similar hyperemia of red pulp areas. Splenic thrombi (marked by arrows) were only observed in wild-type and C1q \(^{-/-}\) spleens (see text for explanation). Spleens from passively immunized wild-type mice (D) had normal distribution and normal relative proportion of red and white pulp areas. Splenic hyperemia was observed in passively immunized C2/FB \(^{-/-}\) mice (E) and, to a lesser extent, in C1q \(^{-/-}\) mice. (F) Each group consisted of five mice. Lungs from a C1q \(^{-/-}\) mouse (G) showing infiltration of perivascular/peribronchiolar spaces by PMN.

Pneumococcal serotype 6A initiates severe disease due to its bacteremic properties. Spleens from PBS-treated mice were hyperemic on cut surface and significantly enlarged. Microscopical examination of splenic sections of these mice confirmed hyperemia of red pulp areas, and multiple (micro)thrombi were observed in either red pulp or the larger splenic veins of wild-type (all 5 mice) and C1q \(^{-/-}\) mice (all 5 mice). Interestingly, no clear thrombi were observed in spleens from C2/FB \(^{-/-}\) mice (all 5 mice), despite high numbers of pneumococci in blood (Fig. 7, A–C). Pneumococci were clearly visible by microscopical examination in C2/FB \(^{-/-}\) spleens (in all five mice), while wild-type or C1q \(^{-/-}\) spleens had either no (4 of 5 in each group) or very few detectable pneumococci (1 of 5 mice in each group). Passive immunization completely prevented development of splenic pathology by pneumococci in wild-type mice: spleens appeared to be normal and no pneumococci were detected (Fig. 7D). Passively immunized C1q \(^{-/-}\) mice had either normal (2 of 5), or moderate (2 of 5), to relatively strong (1 of 5) hyperemic spleens, with no signs of thrombosis or the presence of pneumococci (Fig. 7F). Spleens of passively immunized C2/FB \(^{-/-}\) mice were hyperemic (Fig. 7E), but pneumococci could not be detected microscopically.

**Discussion**

In this study, we evaluated the relative contribution of FcγR and C in hlgG1- and hlgG2-mediated protection against invasive pneumococcal infection. Pneumococcus is an extracellular bacterial
pathogen and an important cause of respiratory tract disease and bacterial sepsis in humans. Abs directed against the polysaccharide capsule of the pneumococcus mediate phagocytosis and protect against disease (4).

We generated rhlgG1 and rhlgG2 Ab, containing identical V regions, directed against pneumococcal serogroup 6. The lgG molecules were shown to contain the expected molecular characteristics. Both recombinant Ab bound comparably to PS6B (ELISA). The central aim of the present study was to assess protection by these human subclasses in a mouse model of pneumonia and bacteremia. As little is known about the interaction of lgG subclasses with the mouse immune system, we first studied the interaction of these lgG subclasses with mFcR and their capacity to activate mouse complement. We observed lgG1 Ab to interact more efficiently with mFcγRII and III than lgG2. Only lgG1 was shown to induce FcγRIII (CD16)-mediated pneumococcal phagocytosis by PMN in vitro. A contribution of FcγRI to pneumococcal phagocytosis can be excluded because mouse PMN do not express this receptor (36). Phagocytic activity of macrophages, which bear all three FcγR, was not examined. Although C was not present in our phagocytosis assays, opsonization by lgG1 and lgG2 resulted in C3c deposition on the pneumococcal surfaces. No C3c deposition was observed in the presence of serum from C1q−/− and C2/FB−/− mice. As expected, C deposition did not cause pneumococcal lysis (23).

The induction of local pulmonary inflammatory responses is crucial for protection against S. pneumoniae in murine models (49–51). We hypothesized that FcR-bearing phagocytes interacting with Ab-opsimized pneumococci would be important effector cells for these inflammatory reactions. However, both lgG1 and lgG2 proved similarly protective against bacteremia and death caused by S. pneumoniae serotype 6A. Furthermore, both lgG subclasses protected FcR γ-chain−/− and FcR triple−/− mice, indicating FcγR not to be essential for Ab-induced protection. In contrast, mice deficient for C factors C1q or C2/FB and mice treated with CVF were not protected against bacteremia and death upon passive immunization with recombinant Ab. This is in agreement with previous reports demonstrating the importance of complement in Ab-mediated protection against pneumococcal disease (26, 27). Furthermore, in agreement with our results, serum-derived lgG1 and lgG2 Abs against capsular polysaccharides of Hemophilus influenzae have been shown to exhibit similar functional activity (52).

Interestingly, a reduced pneumococcal burden was observed in passively immunized C1q−/− mice compared with PBS-treated mice, suggesting the importance of the AP for protection. However, passively immunized C1q−/−, C2/FB−/−, and CVF-treated mice all showed significantly reduced survival rates, compared with passively immunized wild-type mice, and C1q−/− mice displayed lower levels of bacteremia than C2/FB−/− mice. Activation of the classical pathway by Abs is initiated by C1q, but may require intact AP function for amplification of C opsonization. Recently, Xu et al. (42) generated mice deficient for factor D, the enzyme responsible for FB cleavage during formation of C3bBb, the C3 convertase of the AP. C3 deposition on the surface of IgM-coated nonencapsulated pneumococci was much slower in serum from factor D−/− mice than wild-type mice, indicating the importance of the AP for efficient opsonization initiated by the classical pathway.

The observed differences between wild-type and C−/− mice were evaluated in histopathological analyses of both lungs and spleens. Unlike other pneumococcal strains, which cause extensive pneumonia in mice during experimental infection (49, 53), pneumococcal serotype 6A causes moderate PMN infiltration into perivascular/peribronchiolar spaces. It cannot be excluded that inflammatory properties of C affected this infiltration at earlier time points.

Pneumococcal bacteremia was shown to cause extensive morphological changes of spleens. Spleens of all PBS-treated mice showed strong hyperemia, and several (micro)thrombi were observed in spleens from wild-type and C1q−/− mice (Fig. 7). Notably, no thrombi were observed in spleens from C2/FB−/− mice, indicating components of the AP to be crucial for this process. The C system is known to interact with the coagulation system (54, 55). Different C factors have been implicated in this interaction, including C3 convertase of the AP, C3bBb, which can cleave prothrombin into active thrombin, an essential factor for platelet activation (54, 56). Degranulation of activated platelets may amplify the coagulation response, and eventually cause formation of thrombi.

Passive immunization with lgG1 prevented the development of significant pathology in spleens from wild-type mice. Spleens from passively immunized C1q−/− mice were only mildly hyperemic, in contrast to those from C2/FB−/− mice. Interestingly, administration of lgG1 Abs significantly reduced the number of pneumococcal CFU in spleens of C2/FB−/− mice, suggesting an alternative mechanism for Ab-mediated pneumococcal clearance that warrants further investigation.

Our present work demonstrates a crucial role for complement in protection against pneumococcal serotype 6A pneumonia and bacteremia by PS-specific lgG1 and lgG2 Abs. Serotype 6A was shown to cause severe bacteremia, splenic thrombosis, and death. lgG Abs provided efficient protection, in the presence of intact classical and possibly alternative C pathways. Activation of the AP was shown to contribute to thrombotic complications in spleens. The present findings clarify the mechanism of lgG Ab-induced protection against invasive pneumococcal disease.

Acknowledgments

We are indebted to Dr. Marina Botto (Imperial College, London, U.K.) for providing the C2/FB−/− and C1q−/− mice, and Dr. Takashi Saito (Chiba University Graduate School of Medicine, Chiba, Japan) for providing the FcR γ-chain−/− mice. We, furthermore, thank Piet Aerts and Dr. Hans van Dijk (University Medical Center Utrecht) for helpful discussions and providing materials for CH50 assays. Ingrid van den Brink, Marleen Voorhorst (Genmab), and Jantine Bakema (Department of Immunology, University Medical Center Utrecht) are acknowledged for excellent technical assistance, and Dr. Ludo van der Pol for advice on the IgG dimer-binding assays and for critically reading the manuscript.

References


23. Frank, M. M. 1992. The mechanism by which microorganisms avoid complement
s defense against
18. Winkelstein, J. A. 1981. The role of complement in the host defense against

31. Boel, E., S. Verlaan, M. J. Poppelier, N. A. Westerdaal, J. A. van Strijp, and
14. Fridman, W. H., C. Bonnerot, M. Daeron, S. Amigorena, J. L. Teillaud, and
13. Fridman, W. H., C. Bonnerot, M. Daeron, S. Amigorena, J. L. Teillaud, and