Impaired Antibody Response to Group B Streptococcal Type III Capsular Polysaccharide in C3- and Complement Receptor 2-Deficient Mice

Olga Pozdnyakova, Hilde-Kari Guttormsen, Farah N. Lalani, Michael C. Carroll and Dennis L. Kasper

J Immunol 2003; 170:84-90; doi: 10.4049/jimmunol.170.1.84
http://www.jimmunol.org/content/170/1/84

References
This article cites 38 articles, 18 of which you can access for free at:
http://www.jimmunol.org/content/170/1/84.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
Impaired Antibody Response to Group B Streptococcal Type III Capsular Polysaccharide in C3- and Complement Receptor 2-Deficient Mice

Olga Pozdnyakova, 2* Hilde-Kari Guttormsen, 2‡ Farah N. Lalani, ‡ Michael C. Carroll,* and Dennis L. Kasper 3†‡

Group B Streptococcus (GBS) is the foremost bacterial cause of serious neonatal infections. Protective immunity to GBS is mediated by specific Abs to the organism’s capsular polysaccharide Ags. To examine the role of complement in the humoral immune response to type III GBS capsular polysaccharide (III-PS), mice deficient in C3 or in CD21/CD35 (i.e., complement receptors 1 and 2; CR1/CR2) were immunized with III-PS. Mice deficient in C3 or Cr2 had an impaired primary immune response to III-PS. The defective response was characterized by low IgM levels and the lack of an isotype switch from IgM to IgG Ab production. Compared with wild-type mice, C3- and Cr2-deficient mice exhibited decreased uptake of III-PS by follicular dendritic cells within the germinal centers and impaired localization of III-PS to the marginal zone B cells. Complement-dependent uptake of capsular polysaccharide by marginal zone B cells appears necessary for an effective immune response to III-PS. The normal immune response in wild-type mice may require localization of polysaccharide to marginal zone B cells with subsequent transfer of the Ag to follicular dendritic cells. The Journal of Immunology, 2003, 170: 84–90.

Despite advances in antimicrobial therapy, infections with extracellular encapsulated bacteria such as Neisseria meningitidis, Streptococcus pneumoniae, and group B Streptococcus (GBS) remain important clinical problems. GBS is the most common cause of serious infections in newborns and young infants in most of the developed world, with a 5–8% case-fatality ratio in the United States (1). Nine serotypes of GBS have been identified on the basis of distinct capsular polysaccharides. Type III is the serotype most frequently associated with meningitis.

Ab responses in the spleen play an essential role in host defense against encapsulated bacteria (2). Accordingly, patients with anatomic or functional asplenia are highly susceptible to infections with these organisms (3, 4). Protection against neonatal type III GBS infections is associated with the presence of Abs specific for the type III GBS capsular polysaccharide (III-PS). Pure polysaccharides are characterized as thymus-independent type 2 (TI-2) Ags. These Ags can induce a primary immune response without the help of T cells, but the presence of T cell factors enhances the IgG response.

Despite the clinical significance of bacterial polysaccharides, the mechanisms underlying immune responses to polysaccharide Ags have not yet been fully elucidated. It has been suggested that the cells within the splenic marginal zone (MZ) play an important role in responses to TI-2 Ags. The MZ is a specialized compartment containing a subpopulation of B lymphocytes, i.e., MZ B cells, dendritic cells, and MZ macrophages (5). One hypothesis implicates MZ B cells in the induction of humoral responses to TI-2 Ags. MZ B cells are distinguished from follicular B cells by cell surface phenotype (e.g., IgM high, CD21 high, IgD low, CD23 low). Lane et al. (6) reported that the immune response of recovering x-irradiated mice to TI-2 Ags correlated with the reappearance of B cells in the MZ. Furthermore, responsiveness to polysaccharide Ag develops in children at ~2 years of age—a timing that coincides with the maturation of MZ B cells (7, 8). A recent study with Pyk-2-deficient mice lacking MZ B cells showed a reduction in IgM and IgG responses to trinitrophenyl (TNP)-Ficoll (a model TI-2 Ag) as well as abrogated Ag localization to MZ B cells. However, studies of CD19-deficient mice, which also have reduced numbers of MZ B cells, have revealed normal—if not elevated—responses to haptenated Ficoll (9, 10). An alternative hypothesis is that MZ macrophages are essential in early uptake of TI-2 Ags. Macrophages within the MZ take-up and retain carbohydrate macromolecules such as Ficoll (11). However, the importance of macrophage uptake remains unclear because in vivo elimination of MZ macrophages appears not to alter the response to TNP-Ficoll (12).

Binding of Ficoll by MZ B cells in naive wild-type (WT) mice has been shown to be dependent on C3 and on CD21/CD35 (complement receptors (CR) 1 and 2; CR1/CR2) (13), whereas uptake by MZ macrophages takes place independent of C3 (3). Despite this differential complement dependence for Ag uptake by MZ cells, a role for complement in the humoral response to TI-2 Ags remains controversial. Early studies by Pepys (14) showed that
complement depletion with cobra venom factor (CVF) had no effect on the primary response to polyvinylpyrrolidone 360. Studies by Markham et al. (15) demonstrated that complement depletion with CVF abrogated the Ab response to pneumococcal type 14 polysaccharide but did not affect the response to the sialic acid-containing III-PS. In contrast, studies by Pryjma and Humphrey (16) identified a reduced immune response to pneumococcal type 3 polysaccharide in CVF-treated mice. Moreover, Griffioen et al. (17) documented enhanced Ab responses to pneumococcal polysaccharide upon conjugation to C3d. Humans deficient in complement exhibit increased susceptibility to infections by encapsulated bacteria, possibly because of impaired humoral immunity and lack of effector function (18).

Much of our knowledge about humoral immunity to TI-2 Ags comes from studies conducted with nonphysiological Ags. To investigate the role of complement in humoral immunity to a clinically relevant polysaccharide, we immunized mice deficient in C3 or CD21/CD35 with purified III-PS. These mice had impaired IgM and IgG responses to III-PS; the impairment was characterized by a negligible uptake of Ag by follicular dendritic cells (FDCs) and MZ B cells. These observations suggest that humoral responses to a clinically relevant TI-2 Ag-containing sialic acid depend on complement-tagged Ag trapping and processing by MZ B cells.

Materials and Methods

Mice

WT, C3-deficient (C3<sup>−/−</sup>), and CD21/CD35-deficient (Cr2<sup>−/−</sup>) mice were maintained on a C57BL6/129Sv mixed background. T cell-deficient (CD3<sup>−/−</sup>)tg mice and WT controls were maintained on a C3H background. All mice were used at 6–10 wk of age. Mice were housed in a specific pathogen-free barrier animal facility at Harvard Medical School (Boston, MA).

Preparation of III-PS and conjugates

Type III GBS strain M781 was the source of the purified polysaccharide. III-PS was conjugated to human serum albumin (HSA; Sigma-Aldrich, St. Louis, MO) and to biotin hydralazine (Pierce, Rockford, IL) by reductive amination (19). The conjugated polysaccharide retained the specificity of the pure polysaccharide, as shown by ELISA inhibition.

Immunization protocol

On day 0, mice were injected i.p. with 8 μg of III-PS in saline or with saline only. Mice were bled 3 days before and 11 days after immunization. Levels of type III-specific IgM and IgG were quantified by ELISA. To assess III-PS localization in spleens, mice were injected i.v. with 50 μg of biotinylated III-PS at different time points before harvest (15 min, 30 min, 1 h, 2 h, or 16 h). Harvested spleens were either snap-frozen for cryosectioning or mechanically dissociated in preparation for FACS analysis and/or ELISPOT.

Reconstitution with III-PS-specific IgG and IgM

Sera from naive mice and from mice immunized i.p. with three 2-μg doses of III-PS covalently linked to tetanus toxoid (III-PS-TT absorbed to 0.5 mg ALO<sub>2</sub>) were separated into IgG- and IgM-containing fractions over a protein G column using an Immunopure G IgG Purification kit (Pierce) per instructions from the manufacturer. The fractions were desalted over a PD10 column (Amersham Pharmacia Biotech, Piscataway, NJ) and calibrated with PBS (pH 7.4). The fractions from mice immunized contained 5.9 and 4.3 μg/ml of III-PS-specific IgG and IgM, respectively; those fractions from naive mice contained 0.005 and 0.023 μg/ml, respectively. WT and C3<sup>−/−</sup> mice received an i.v. injection of IgG or IgM (200 μg each) from immunized and naive mice 2 h before injection of biotinylated III-PS.

ELISA for III-PS-specific Ab

Levels of III-PS-specific Abs were quantitated by ELISA, as described previously (20). The limits of IgM and IgG Ab detection were 20 and 5 ng/ml, respectively.

ELISPOT assay for III-PS-specific Ab-secreting cells (ASCs)

The frequency of ASCs in spleens was determined by ELISPOT assay. In brief, 24-well plates (Falcon, San Diego, CA) were coated overnight with III-PS conjugated to HSA (III-HSA; 5 μg/ml). Wells were blocked with 1% BSA in PBS, splenocytes diluted in DMEM (Life Technologies, Grand Island, NY) were added (5 × 10<sup>5</sup>, 1 × 10<sup>6</sup>, or 0.5 × 10<sup>6</sup> cells per well), and preparations were incubated for 12–18 h at 37°C. Plates were washed in 0.1% BSA, then coated for 4 h at room temperature with goat anti-mouse IgM or IgG conjugated to alkaline phosphatase (Sigma-Aldrich). Plates were developed with X-phosphate/5-bromo-4-chloro-indolyl-phosphate (BCIP; Boehringer Mannheim, Indianapolis, IN) in 2-amino-2-methyl-1-propanol (AMP; Sigma-Aldrich) agarose solution. Spots were counted with a Leica microscope (Deerfield, IL).

FIGURE 1. The Ab response to III-PS is dependent on C3 and Cr2. Primary IgM (a) and IgG (b) responses to III-PS were significantly lower in mice deficient in C3 or in CD21/CD35 than in WT mice 11 days after immunization. ELISA data from one representative experiment are shown; a total of five experiments were performed. *, Values of <i>p</i> < 0.05. Horizontal bars indicate means. The frequency of IgM ASCs (c) was significantly reduced in the spleens of C3- and C2-deficient mice and in those of saline-injected WT mice. Mice were immunized with 8 μg of III-PS, and splenocytes were collected for an ELISPOT assay on day 16 after immunization.
Immunofluorescence analysis of splenic sections

For immunofluorescence analysis, 7-mm cryosections of spleen were cut, fixed, and stained as described previously (21). At least 10 sections per spleen were analyzed. Biotinylated Ag was detected with fluorescence-labeled streptavidin (streptavidin-Cy-Chrome, BD PharMingen (San Diego, CA); or streptavidin-Alexa 568 Fluor, Molecular Probes (Eugene, OR). The following Abs were used for staining: rat anti-mouse FDC-M1, anti-mouse IgM-Cy5 (clone 341.12), peanut lectin agglutinin (PNA)-FITC (EY Laboratories, San Mateo, CA), anti-mouse CD3-PE (BD PharMingen), and anti-human CD3d-FITC (DAKO, Carpinteria, CA). Pictures were taken with a Bio-Rad MRC confocal microscope (Hercules, CA) using Bio-Rad Radiance 2000 software.

FACS analysis

Single-cell suspensions of splenocytes were stained with rat anti-mouse Abs CD11b-PE, CD24-PE, and CD23-FITC (BD PharMingen). III-PS biotin was detected with streptavidin-allophycocyanin (Molecular Probes). Cells were analyzed with a FACSCalibur (BD Biosciences, Mountain View, CA) flow cytometer.

Statistical analysis

Statistical analysis was performed with Prism software, version 2.0b (GraphPad Software, San Diego, CA). The Student t test for unequal variances was used to compare means, and the χ^2 test was used to compare frequencies for different experimental groups. A two-tailed p value of <0.05 was considered significant.

Results and Discussion

Primary Ab response to III-PS in WT, C3null, and Cr2null mice

To analyze the role of complement in primary Ab responses to III-PS, we immunized WT, C3null, and Cr2null mice i.p. with 8 μg of III-PS and collected serum samples at multiple time points after immunization. III-PS-specific Ig were quantified by ELISA (Fig. 1, a and b). Before day 11 no significant IgM response was observed in either complement-deficient or WT mice (data not shown). However, the day 11 IgM response of immune WT mice to the polysaccharide Ag was significantly greater than that of saline-injected WT mice (6493 ± 2418 ng/ml vs 190 ± 25 ng/ml; p < 0.05). By contrast, the primary IgM response was impaired in the deficient mice. Notably, IgM Ab levels in deficient mice were in a range similar to that measured in saline-injected WT mice (311 ± 43 ng/ml for C3null mice and 80 ± 18 ng/ml for Cr2null mice; Fig. 1a). The IgG isotype switch observed in immunized WT mice (366 ± 173 ng/ml) did not occur in immunized C3null mice (4 ± 0.3 ng/ml), immunized Cr2null mice (5 ± 3 ng/ml), or saline-injected WT mice (6 ± 1 ng/ml) (p < 0.01 for all groups; Fig. 1b).

To further examine the nature of the defective response in deficient mice, we analyzed the frequency of IgM ASCs. Spleens were harvested on day 16 after immunization, and single-cell suspensions were analyzed in an ELISPOT assay (Fig. 1c). Consistent with the low Ab levels, only negligible numbers of IgM ASCs were observed in C3null, Cr2null, and saline-injected WT mice as opposed to immunized WT mice; specific values (per 5 × 10^6) were 56 ± 22 for immunized WT mice, 5 ± 1 for immunized C3null mice, 7 ± 3 for immunized Cr2null mice, and 4 ± 0.5 for saline-injected WT mice (p < 0.01 for all groups). Negligible numbers of IgG ASCs were observed in all four groups (data not shown).

Germinat center (GC) response and complement-mediated retention of III-PS within GCs

Humoral responses are dependent on histologically defined areas within splenic follicles called GCs. Although GC formation is usually described in immune responses to thymus-dependent Ags, recent studies have demonstrated that GCs can also develop in response to TI Ags such as dextran BS12 (22, 23). To investigate GC formation in response to III-PS, we harvested spleens and prepared splenic sections on day 13 after primary immunization. Although the number of splenic follicles did not differ among the four groups, the number of GCs (PNA+ clusters) was significantly lower in immunized Cr2-deficient mice and saline-injected WT mice than in immunized WT mice (Table I). Notably, PNA-positive GCs were observed in C3-deficient mice despite an impaired Ab response and an absence of specific Ag staining in the follicles. The GCs probably represent an ongoing response to environmental Ags.

To examine whether III-PS localization within GCs is dependent on C3 and Cr2, we injected immunized mice with biotinylated III-PS and harvested their spleens 16 h later (Fig. 2 and Table I). Immunofluorescence analysis of splenic sections revealed a high percentage of III-PS-positive GCs in immunized WT mice (47% ± 10%). In contrast, a negligible number of splenic follicles stained positive for the capsular polysaccharide in immunized deficient mice and saline-injected WT mice. As expected, III-PS colocalized with M1+ FDCs within GCs of immunized WT mice (Fig. 2, a and b). However, no III-PS was detected in GCs of immunized deficient mice or in saline-injected WT controls (Fig. 2, c–h, and Table I) despite the presence of a network of FDCs in each group. Localization of III-PS to GCs does not in itself define specificity of GC B cells, it does, however, provide a relative measure of the humoral response to the specific Ag.

To examine the importance of T cells in humoral and GC responses to III-PS, we immunized CD3ε tg mice, which are deficient in T and NK cells, with III-PS (24). CD3ε tg mice had normal IgM responses; mean anti-III-PS IgM levels ± SEM were 1000 ± 0.01 for III-PS-injected mice compared to saline-injected mice.

Table I. III-PS retention within the GCs of mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. Spleen Follicles</th>
<th>No. GCs</th>
<th>No. III-PS+ GCs</th>
<th>No. III-PS+ GCs × 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-C57BL6/129Sv (n = 10)</td>
<td>45.7 ± 3.3</td>
<td>21.7 ± 2.9</td>
<td>10.6 ± 2.6</td>
<td>47.1 ± 10.0</td>
</tr>
<tr>
<td>C3null-C57BL6/129Sv (n = 8)</td>
<td>37.9 ± 2.6</td>
<td>21.8 ± 4.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cr2null-C57BL6/129Sv (n = 6)</td>
<td>50.0 ± 5.3</td>
<td>4.0 ± 1.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>WT-C57BL6/129Sv saline (n = 10)</td>
<td>31.8 ± 2.5</td>
<td>9.5 ± 1.4</td>
<td>0.3 ± 0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>CD3ε tg/C3H (n = 10)</td>
<td>29.8 ± 2.7</td>
<td>0.4 ± 0.3</td>
<td>3.5 ± 1.0</td>
<td>27.8 ± 5.4</td>
</tr>
<tr>
<td>WT/C3H (n = 9)</td>
<td>32.3 ± 1.4</td>
<td>12.6 ± 1.7</td>
<td>3.5 ± 1.0</td>
<td>27.8 ± 5.4</td>
</tr>
</tbody>
</table>

* Immunohistochemical analysis of splenic sections prepared from WT, C3null, and Cr2null mice maintained on a mixed C57BL6/129Sv background and of CD3ε tg and WT mice maintained on a C3H background. Spleens were harvested from immunized and control mice 16 h after i.v. injection of 50 μg of biotinylated III-PS. Sections were analyzed, and the mean ± SEM values are presented. Follicles and GCs were identified by anti-IgM and PNA staining, respectively. Ag was detected by biotin-streptavidin binding. No significant difference in spleen size among mouse strains (as indicated by the total number of spleen follicles) was observed.

* p < 0.01 vs immunized WT mice.
Localization and deposition of III-PS in the MZ

The splenic MZ is an important site in defense against encapsulated bacteria. Detection and uptake of particulate Ags are enhanced by the macrophages lining the MZ sinuses (12). Moreover, the MZ region includes B cells that bear specific receptors for bacterial Ags, including bacterium-specific carbohydrate Ags (26). To investigate which cell types in the MZ of the spleen are responsible for the uptake of III-PS and to determine whether clearance is complement-dependent, we injected biotinylated III-PS i.v. into immune and nonimmune mice of each group and harvested the spleens at various time points. Ag uptake in the MZ was assessed by confocal microscopy and FACS analysis. Analysis of splenic sections from nonimmune mice identified III-PS staining within the MZ of all three groups (Fig. 3, a, d, and g). Ag deposition colocalized with CD11b+ cells in naive mice, a result suggesting uptake by macrophages (data not shown). In immune WT mice, III-PS localized to MZ B cells and FDCs within GCs (Fig. 3b). A significant finding was the lack of Ag staining in splenic sections from immunized C3- and Cr2-deficient mice (Fig. 3, e and h). These results suggest that Ag uptake by MZ B cells is dependent on C3 and CD21/CD35 in immune mice, whereas uptake of III-PS by macrophages in naive mice is complement-independent.

To determine whether III-PS detected on MZ B cells of immune mice correlates with complement deposition, we stained splenic sections with anti-C3d, III-PS-biotin, and anti-IgM (Fig. 3, c, f, and i). As predicted, C3 protein and III-PS colocalized to FDCs within GCs and MZ B cells in immune WT mice (Fig. 3c). By comparison, low levels of C3 staining were evident in the sinuses and blood vessels in spleens from Cr2-deficient mice, but no C3 was detectable within the splenic follicles (Fig. 3i). No C3 protein or III-PS was found in spleens of C3-deficient mice (Fig. 3f).

To further examine colocalization of III-PS and C3 protein on MZ B cells, we prepared suspensions of splenic cells from immunized WT and deficient mice and subjected these cells to FACS analysis (Fig. 3j and Table II). MZ B cells were identified as positive for B220 and CD24 (heat-stable Ag) and negative for CD23. No reduction in the number of MZ B cells was apparent in C3- and Cr2-deficient mice (data not shown). Analysis of MZ B cells harvested at various time points indicated increasing uptake of Ag over the 120-min period after i.v. Ag injection (Fig. 3j and Table II). The frequency of III-PS-positive MZ B cells was significantly greater in WT mice than in deficient mice at each time point examined. Numbers of III-PS-positive MZ B cells were negligible in saline-injected WT mice (data not shown).

The frequency of C3 binding by MZ B cells increased after injection of Ag in WT mice (Table II). However, a background level of C3-positive MZ B cells was observed in saline-injected WT controls. This result suggests that complexes of C3 protein and Ags (probably environmental Ags) are taken up constitutively by MZ B cells. C3 detection was judged to be specific because negligible numbers of C3-positive cells were observed in C3-deficient mice. Uptake of III-PS and C3 is essentially Cr2-dependent, although some C3 and III-PS binding took place in the absence of Cr2. Expression of other complement receptors (e.g., crry/p65) that bind C3b (27) or residual CD21/CD35 (28) may explain the low levels of C3 on Cr2null MZ B cells. The results of splenic cell analysis by confocal microscopy and FACS strongly suggest that III-PS decorated with activated complement products efficiently localizes to FDCs and MZ B cells by binding through CD21/CD35 complement receptors.

An alternative explanation for the lack of III-PS uptake by MZ B cells in C3- and Cr2-deficient mice is the failure of both strains to produce significant levels of III-PS-specific Abs (Fig. 1). To
determine whether Ab alone or both Ab and complement are required for opsonization of III-PS, naive WT and C3 null mice were given an i.v. injection of 1.2 $\mu$g of IgG or 1 $\mu$g of IgM anti-III-PS Abs before receiving an injection of biotinylated III-PS. The levels of III-PS-specific Abs detected in sera at the time of harvest were similar in WT and C3 null mice (data not shown). FACS analysis performed on splenic cells from the two strains revealed uptake of III-PS by MZ B cells from WT mice (4.2 and 4.9% for III-PS-specific IgG and IgM, respectively) but not by those from C3 null mice (0.7 and 0.8%, respectively) 2 h after i.v. Ag injection (Fig. 4). Therefore, uptake of III-PS by MZ B cells requires both specific Abs and C3. Uptake of III-PS by MZ B cells from WT and C3 null mice that were given IgG and IgM fractions from naive sera was <1% (data not shown). Notably, the level of uptake in naive WT mice reconstituted with III-PS specific Abs was lower than that in immunized WT mice (4.2 (IgG naive mice) and 4.9% (IgM naive mice) vs 18.4% (III-PS immunized)). One possible explanation for the differences could be the reduced levels of circulating III-PS-specific Abs in reconstituted WT mice. Alternatively, actively immunized mice developed an ongoing response, whereas passively immunized mice received only one dose of III-PS-specific Abs. Another explanation is the possible clonal expansion of III-PS-specific B cells in the MZ of immune mice. Evidence of clonal expansion of MZ B cells in humans was recently reported (29).

In summary, we have demonstrated that the humoral response to the clinically relevant, sialic acid-containing capsular polysaccharide Ag of GBS type III is complement-dependent. Notably, mice deficient in C3 or CD21/CD35 have impaired responses characterized by low IgM levels and negligible ASC numbers. No isotype switch to IgG Abs takes place in these deficient mice. In naive mice, III-PS is rapidly taken up by macrophages within the splenic MZ in a complement-independent manner. By contrast, in immune mice, III-PS is localized to the surface of MZ B cells by a mechanism that is dependent on both C3 and CD21/CD35. The lack of III-PS bound to MZ B cells in C3- and Cr2-deficient mice correlates with impaired localization of Ag to FDCs within GCs.
Whether MZ B cells are required for transferring III-PS to FDCs is undetermined from these results. One possible pathway is that MZ B cells bind complexes of C3 and Ag and transport them into the follicular zone.

In a recent report, Guinamard et al. (13) observed that the humoral response to TNP-Ficoll was dependent on an intact MZ compartment and the presence of C3 and Cr2. Injection of naive mice with TNP-Ficoll Ag resulted in rapid uptake by MZ B cells in WT mice, but only negligible uptake by MZ B cells in mice deficient in C3 or CD21. In addition, some background binding of C3 to other complement receptors is possible in C2null mice.

In a previous study, Markham et al. (15) reported that transient depletion of C3 by CVF had no significant effect on the day 5 IgM response to III-PS in CFA injected s.c. Moreover, they reported that 5 days following immunization, specific IgM synthesis was not dependent on an intact spleen and that no III-PS specific splenic ASCs were identified. One explanation for the apparent differences between this earlier study and our current results is that depletion of circulating C3 with CVF is not likely to affect local production of C3 by myeloid cells within the lymphoid compartment. Recent studies have demonstrated that local C3 synthesis by myeloid cells within the spleen and peripheral lymph nodes is sufficient to enhance the humoral response to protein Ags irrespective of the C3 levels in the blood (31, 32).

One model that could account for our current observations is that phagocytic cells within the splenic MZ and other secondary lymphoid tissues bind Ag via carbohydrate recognition, such as mannose receptor or mannan binding protein, and transport it into the B cell follicles, as proposed by Martinez-Pomares et al. (33). Alternatively, myeloid dendritic cells bind III-PS and "present" the Ag to B cells within the MZ, leading to plasmablast formation and plasma cell differentiation.

### Table II. III-PS and C3d binding to MZ B cells in immunized mice

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Genotype</th>
<th>III-PS binding</th>
<th>C3 binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No Ag 30 min 120 min</td>
<td>No Ag 30 min 120 min</td>
</tr>
<tr>
<td>1: C57BL6/129Sv</td>
<td>WT</td>
<td>0.3 15.3 72.1</td>
<td>28.9 91.9 89.9</td>
</tr>
<tr>
<td></td>
<td>Cr2null</td>
<td>0.1 0.1 0.3</td>
<td>18.7 25.0 25.9</td>
</tr>
<tr>
<td>2: C57BL6/129Sv</td>
<td>WT</td>
<td>0.3 17.3 67.2</td>
<td>15.5 87.0 81.7</td>
</tr>
<tr>
<td></td>
<td>C3null</td>
<td>0.1 0.2 0.2</td>
<td>0.1 0.1 0.1</td>
</tr>
<tr>
<td></td>
<td>Cr2null</td>
<td>0.3 0.1 0.1</td>
<td>8.3 9.4 16.9</td>
</tr>
<tr>
<td>3: C57BL6/129Sv</td>
<td>WT</td>
<td>0.2 3.5 5.2</td>
<td>21.8 35.8 38.3</td>
</tr>
<tr>
<td></td>
<td>WT</td>
<td>ND 6.1 10.3</td>
<td>33.1 44.2</td>
</tr>
<tr>
<td></td>
<td>C3null</td>
<td>0.1 0.4 0.3</td>
<td>0.1 0.1 0.1</td>
</tr>
<tr>
<td></td>
<td>Cr2null</td>
<td>ND 0.2 0.3</td>
<td>15.6 20.7 16.8</td>
</tr>
<tr>
<td></td>
<td>Cr2null</td>
<td>0.2 0.1 0.2</td>
<td>18.2 14.6</td>
</tr>
<tr>
<td>4: C57BL6</td>
<td>WT</td>
<td>0.4 3.7 9.8</td>
<td>ND ND ND</td>
</tr>
<tr>
<td></td>
<td>C3null</td>
<td>0.2 0.2 0.1</td>
<td>ND ND ND</td>
</tr>
<tr>
<td></td>
<td>Cr2null</td>
<td>0.1 0.2 0.3</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>5: C57BL6</td>
<td>WT</td>
<td>0.3 4.9 12.3</td>
<td>ND ND ND</td>
</tr>
<tr>
<td></td>
<td>C3null</td>
<td>0.2 0.6 0.4</td>
<td>ND ND ND</td>
</tr>
<tr>
<td></td>
<td>Cr2null</td>
<td>0.2 0.2 0.1</td>
<td>ND ND ND</td>
</tr>
</tbody>
</table>

*Data represent the frequency (%) of MZ B cells binding III-PS or C3 based on FACS analysis. Splenic cells were harvested 30 or 120 min after i.v. injection of biotinylated III-PS into immunized WT and C3null or Cr2null mice and analyzed. III-PS and C3 protein were detected on the surface of CD24CD23 MZ B cells. A total of five different experiments were performed.

III-PS binding was dependent on C3 and Cr2. The amount of Ag and C3d bound to MZ B cells increased with time. Differences in Ag and C3 binding between experiments can be explained in part by differences between lots of biotinylated III-PS.
specific IgM release (34–36). In this scenario, the presence of specific Ab overcomes the inhibitory effect of sialic acid and activates classical pathway complement, resulting in C3 attachment and more efficient uptake by MZ B cells. The high frequency with which MZ B cells bind C3-coated complexes of III-PS in WT immune mice suggests an efficient process of Ag localization to the MZ compartment. MZ B cells express higher levels of CD21 than follicular B cells and might be expected to preferentially bind C3d-III-PS complexes. Because we find a high frequency of Ag-C3d-positive B cells, it is unlikely that only III-PS-specific B cells bind C3d complexes (Fig. 3j). However, efficient localization to this compartment would enhance specific interaction with cognate B cells and recent studies have reported clonal expansion of Ag-activated MZ B cells (29). An alternative mechanism for complement enhancement in the current model is that C3d-coated III-PS coligates the CD21/CD19/CD81 coreceptor and B cell receptor on MZ B cells, thereby lowering the threshold for activation of B cells in a manner similar to previous observations using protein Ags (37). Recent work by Cariappa et al. (38) suggests that Cr2− mice have increased numbers of MZ B cells. The results reported herein demonstrate similar MZ B cell numbers in all experimental groups. Although the generation and localization of MZ B cells appear to be independent of CD21/CD35, MZ B cells lacking CD21/CD35 clearly cannot capture complement-coated III-PS.

In conclusion, the data presented in this study show that TI responses to III-PS are complement-dependent. They further suggest that complement-tagged III-PS is trapped via CD21/CD35 on MZ B cells, which, in turn, transport the Ag to FDCs within GCs. The proximity of MZ B cells to marginal sinuses and the high-level expression of CD21/CD35 by these cells appear to emphasize the importance of their function as sentinel cells for recognizing systemic bacterial infections.

Acknowledgments

We thank Robert A. Barrington and Elahna Paul for comments on this manuscript, Barbara G. Reinap for purification and oxidation of the polysaccharide, and Valerie M. Brostrom for fractionation of mouse Abs.

References


