Chapter 2

The immune response to pneumococcal conjugate vaccine does not depend on the presence of complement

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Abstract

The antibody response to polysaccharides is evoked when polysaccharides bind complement factor C3d, and these polysaccharide-C3d complexes subsequently localize on splenic marginal zone B cells strongly expressing CD21 (complement receptor 2). Infants and children under the age of two years express only low levels of CD21 on their marginal zone B cells, and consequently do not adequately respond to polysaccharides. However, polysaccharide-protein conjugate vaccines are able to induce antibodies at this young age. Conjugate vaccines apparently overcome the necessity for CD21-C3d interaction for an anti-polysaccharide antibody response.

We demonstrate in a rat model that splenic localization of pneumococcal polysaccharides indeed is complement-dependent, and that the antibody response to pneumococcal polysaccharides is impaired after complement-depletion. We show that pneumococcal conjugates are able to initiate antibody responses without depending on the presence of complement. Furthermore, polysaccharide conjugates do not specifically localize on splenic marginal zone B cells. Thus, the induction of anti-polysaccharide antibodies by conjugate vaccines apparently can occur independently of CD21-C3d interaction. These basic findings may explain their effectiveness in young children, and may open the way for application of conjugated vaccines in patient groups.

Introduction

Antibodies directed to capsular polysaccharides are essential in the defense against infections with encapsulated bacteria such as *Streptococcus pneumoniae.* In infants and young children, the antibody response to polysaccharides is inadequate, resulting in increased incidence of disease caused by encapsulated bacteria.

Polysaccharide molecules are made up of a number of repeating epitopes. As a consequence, soluble polysaccharides have the capacity to cross-link antigen receptors on the surface of B cells. Furthermore, polysaccharides can activate the complement system via the alternative pathway, without the need for anti-polysaccharide antibodies [1]. Complement fragment C3d, that is generated during this process, is bound by the polysaccharide molecules to form polysaccharide-complement complexes [2]. These polysaccharide-C3d complexes are better *in vitro* immunogens than native polysaccharides [3]. They have the ability to bind to B cells via interaction of C3d with CD21 (complement receptor 2, CR2) [4]. The co-ligation of CD21 and antigen receptors may account for the fact that the antibody response to polysaccharides (so-called T cell independent type 2 (TI-2) antigens [5]) can occur in the absence of a functional thymus, although T cells can augment antibody production [6-8].

Splenic marginal zone B cells express the CD21 surface molecule in particularly high density, and blood flow in the marginal zone is low. These two characteristics enable polysaccharide-complement complexes to localize in the marginal zone by interaction of the complement fragment C3d with CR2 on B cells [9-12], providing maximum opportunity for induction of a humoral immune response [13;14]. The marginal zone of the spleen is thus a major site of initiation of the anti-polysaccharide antibody response.

Infants and young children do not yet express high levels of CD21 on their splenic marginal zone B cells, which explains their inability to respond to polysaccharide antigens [10]. Covalent conjugation of the polysaccharides to protein carriers overcomes this anti-polysaccharide unresponsiveness in early life [15;16]. This suggests that conjugates might initiate the anti-polysaccharide antibody response in a way that is not dependent on polysaccharide-C3d complex localization on splenic marginal zone B cells by C3d-CD21 interaction.

The importance of marginal zone B cells, complement receptors, and complement in the antibody response to a synthetic TI-2 antigen is shown in recent studies in C3$^{-/-}$, CRI/II$^{-/-}$, and Pyk2$^{-/-}$ mice [17]. No such data exist for the antibody response to natural TI-2 antigens like bacterial capsular polysaccharides, nor is it known whether presence of complement and localization on marginal zone B cells is important for induction of antibody responses to polysaccharides conjugated to proteins.

We studied the effect of complement depletion on the antibody response to pneumococcal polysaccharide- and conjugate-vaccines in a previously described rat model, in which the *in vitro* complement dependency of pneumococcal polysaccharide localization on splenic marginal zone B cells was already shown [13].

Experimental Procedures

Animals, immunization, blood drawing and tissue preparation

Young adult male Wistar rats (Harlan, Horst, the Netherlands), of approximately 200 g, were housed under standard laboratory conditions on a 12 h light-dark cycle. They were fed standard laboratory rat food (Hope Farms, Inc., Woerden, the Netherlands) and tap water ad libitum. The rats were immunized intravenously (i.v.) in the tail vein with either one of the vaccines described below.

For serological studies, five rats per group were used. 1, 3, 6, 8, 14 and 21 days after intravenous immunization, blood was obtained from the tail vein, and allowed to clot at 0ºC. All rats were individually marked, so antibody titers could be analyzed separately for each rat, and thus followed over time.

For pneumococcal polysaccharide localization studies and antigen detection in serum, at least three rats per group were used. Rats were sacrificed at 15 minutes, 7 hours, and 5 days after vaccination (the timepoints were based on previous experience [13]). Blood was drawn by cardiac puncture and allowed to clot at 0ºC. Tissue blocks of spleen and liver were immediately frozen by immersion in liquid 2-methylbutane (cooled in a freezer to -80ºC). The tissue blocks were stored in a freezer at -80ºC until sectioned.

In vivo complement inactivation.

Decomplementation was performed by i.v. injection of 1200 U of CVF, isolated as described [18] in 200 µl of NaCl 0.9%. CVF-treatment was given 24 and 18 hours before immunization and, when prolonged complement depletion was required -for serological studies- also 1, 3, 6 and 8 days after immunization.

Vaccines

For serological studies, the following vaccines were used:

1) A heptavalent PCV (Prevnar®; Wyeth Lederle Vaccines and Pediatrics, Rochester, NY) including polysaccharides of pneumococcal serotypes 4, 6B, 9V, 14, 19F and 23F and an oligosaccharide of serotype 18C, conjugated to CRM_{197} carrier protein. Each dose PCV contains 2 µg polysaccharides of serotypes 4, 9V, 14, 18C, 19F and 23F, and 4 µg of serotype 6B in 0.5 mL aluminum phosphate reconstitution fluid.

2) As unconjugated equivalent of this vaccine, pneumococcal polysaccharide serotypes 4, 6B, 9V, 14, 18C, 19F and 23F (PPS, American Type Culture Collection, Rockville, MD) were used. Each dose contained 20 µg of each of the pneumococcal polysaccharides type 4, 6B, 9V, 14, 18C, 19F and 23F in 0.5 mL saline.

For pneumococcal polysaccharide localization studies we used:

3) heptavalent PCV

4) an equivalent dose (2 µg per serotype) of the corresponding unconjugated polysaccharide serotypes 4, 6B, 9V, 14, 18C, 19F and 23F

5) a ten-fold higher dose of one pneumococcal polysaccharide serotype (20 µg of PS19F)

Measurement of serum complement activity

Efficiency of decomplementation was assessed by a hemolytic assay of the classical complement pathway employing sensitized sheep erythrocytes. Clotting of serum was allowed to take place at 0°C to minimize complement activation. Inactivation of complement was performed by heating rat serum at 56°C for 30 min. 1:10 dilutions of the rat sera were incubated with sensitized sheep erythrocytes for 30 mins at 37°C. The OD at 405 nm of the supernatant was used as a measure of complement activity. To correct for possible hemolysed serum (resulting from the blooddrawing procedure) OD values resulting from incubation of heat-inactivated serum with sensitized sheep erythrocytes were subtracted.

Immunohistochemical detection of *in vivo*-administered PPS and PCV.

Sections (4 µm) of spleen and liver tissue were prepared, air dried for 20 min, fixed for 10 min in acetone (100%), air dried, washed in PBS (pH 7.4) for 5 min, incubated with a type-specific polyclonal rabbit anti-PPS antibody (State Serum Institute, Copenhagen, Denmark) in PBS. Endogeneous peroxidase activity was blocked with 0.075% (vol/vol) H_2O_2. Sections were next incubated with peroxidase-conjugated swine anti-rabbit immunoglobulin (SAR[PER], Dakopatts, Glostrup, Denmark). Peroxidase activity was visualized by using 3-amino-9-ethylcarbazole plus H_2O_2 as a reagent. Finally, sections were counterstained in Mayer's hematoxylin and embedded in Kaiser's glycerin-gelatin.

Detection of *in vivo*-administered PPS and PCV simultaneously with immunohistochemical characterization of splenic cells.

For the simultaneous detection of PPS and immunohistochemical characterization of splenic cells, sections were incubated with a type-specific polyclonal rabbit anti-PPS antibody (State Serum Institute, Copenhagen, Denmark) in PBS and stained with SAR[PER] as described above. The sections were next incubated with a given monoclonal antibody (MoAb, Table 1) and subsequently with an alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Dakopatts). Alkaline phosphatase activity was visualized with naphthol AS-MX phosphate as a substrate (Sigma Chemical Co., St. Louis, MO) and fast blue BB as a chromogen; peroxidase activity was visualized by the 3-amino-9-ethylcarbazole reaction. Finally, sections were embedded in Kaiser's glycerin-gelatin.

The following MoAbs were used for immunohistochemistry: ED1 and ED3 [19](Serotec Ltd, Oxford, UK); T cell receptor (TCR) (Pharmingen, San Diego, CA, USA) and CD45R [20;21], IgM, IgD, and HIS57 [22] (Table 1).

MoAb	Clone	Reactivity	Reference
IgM	HIS40	Immunoglobulin M, B cells	[22]
IgD	MaRD3	Immunoglobulin D, B cells	[22]
CD68	ED1	Macrophages	[19]
ED3	ED3	Marginal metallophilic macrophages	[19]
TCR	R73	αβ T cell receptor	[27]
HIS57	HIS57	Marginal zone B cells	[22]
CD45R	HIS24	B cells, leukocyte common antigen	[20;21]

Table 1: Reactivity of monoclonal antibodies (MoAb) used.

Detection of pneumococcal polysaccharide serotype 19F in rat serum

10µL of rat serum samples were spotted onto nitrocellulose filters (BioRad Trans-Blot, 0.45 µm) and dried at 37°C. Pre-incubation with blocking buffer (0.1 M Tris HCl, pH 7.5; 2.5% milk powder; and Tween-20 0.05% vol/vol in 0.9% NaCl) for 30 min at room temperature was followed by incubation with rabbit anti-PPS19F (State Serum Institute, Copenhagen, Denmark) 1:10,000 in blocking buffer for 90 min at room temperature. After washing (0.1 M Tris HCl, pH 7.5; Tween-20 0.05% vol/vol in 0.9% NaCl), the nitrocellulose filters were incubated with peroxidase-labelled donkey anti-rabbit antibodies (Amersham NA934) 1:10,000 in blocking buffer for 60 min at room temperature. After treatment with ECL^{TM} (Amersham Pharmacia) for 1 min, a photofilm was developed for 1 min. 1:10 dilutions of PPS 19F (ATCC), starting concentration 10 µg/ml) in 0.9% NaCl were used as a standard. This assay has a sensitivity of detecting 0.1 µg/ml polysaccharide.

Anti-pneumococcal antibody determination by ELISA.

IgG- and IgM- antibodies against three (PS6B, PS19F and PS14) of the seven capsular polysaccharide serotypes present in both vaccines were measured by ELISA in pre- and post-immunization sera.

All serum samples were preincubated overnight with excess free common cell wall polysaccharide (CPS) to remove anti-CPS antibodies [23;24]. Microtiter plates (Greiner Labortechnik, Langerthal, Germany) were coated with pneumococcal capsular polysaccharides (ATCC, 10 µg/ml in saline solution) at 37°C, overnight. Subsequently, plates were washed with phosphate buffered saline (PBS), Tween-20 0.05% vol/vol and incubated with serial dilutions of serum samples in PBS, 0.05% Tween-20, 1% bovine serum albumin (BSA, vol/vol). After washing (PBS-0.05 % Tween-20), the plates were incubated for two hours (37°C) with peroxidase labelled goat anti-rat IgM (Santa Cruz Biotechnology, Inc.) or goat anti-rat IgG antibodies (Southern Biotechnology Associates, Birmingham, AL). After washing and incubation with enzyme substrate for 20 min at room temperature, absorbance was read at 450 nm on a Milennia ELISA reader (Flow Laboratories, Irvine, CA). The antibody concentrations in the serum samples were calculated by comparison with a rat hyperimmune serum pool generated from sera of both PCV and PPS vaccinated rats; this hyperimmune serum pool was included in every ELISA-run as a standard. The antibody concentrations of the different serotypes in this pool were assigned 100 U/ml (100%) for each serotype.

Serum samples obtained pre-vaccination and at the different time points post-vaccination from individual rats were analyzed simultaneously.

Statistical Analysis

Anti-pneumococcal antibody concentrations from rats of the different vaccine groups (having received either the non-conjugated PPS vaccine or the 7-valent PCV, with or without CVF treatment, 5 rats per group) were analyzed and compared for statistically significant differences by repeated measurement analysis using the General Linear Model procedure of the statistical package SPSS version 9.0 (SPSS Inc., Chicago, IL).

OD values as measure of serum-complement activity were compared for significant differences between treated and untreated groups of rats at different time-points after CVF-treatment by using the Mann-Whitney U test. P-values <0.05 were considered significant.

Results

Complement depletion by cobra venom factor

In vivo complement depletion was induced by cobra venom factor (CVF) administration. The efficiency of complement depletion was assessed by a hemolytic complement assay.
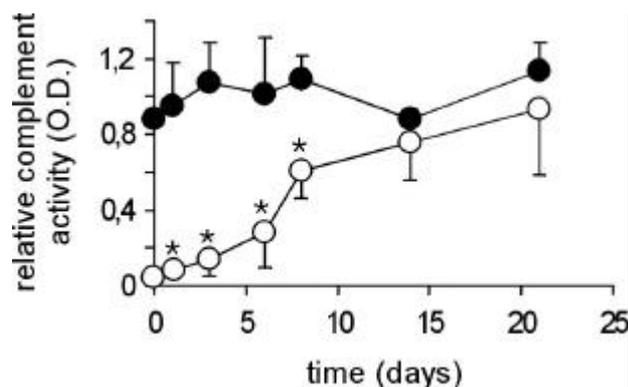

Figure 1. Complement activity in rat serum

Rats were treated with 1200 U cobra venom factor (CVF) at T= -24 h, -18 h, and T= 1, 3, 6, and 8 days. Degree of lysis of sensitized sheep erythrocytes by rat serum, expressed as OD, was used as a measure of complement-activity.
Open symbols: CVF-treated animals; closed symbols: non-treated animals. Each data-point represents the mean ± SD of five animals. Statistically significant differences are indicated (*).

The relative complement activity in serum decreased immediately after the first two injections with CVF. By a regimen of alternate day CVF injections, significantly lower complement activity as compared with control rats could be maintained for approximately 8 days. Despite ongoing treatment with CVF, complement activity in serum from CVF-treated rats was completely recovered after 14 days (Figure 1). CVF-treatment did not affect the overall histological architecture of the spleen, nor did it alter the distribution of lymphocyte and macrophage subpopulations in the spleen.

Splenic localization of pneumococcal polysaccharides

Control rats and CVF-treated rats were i.v. immunized with a mixture of serotype 4, 6B, 9V, 14, 18C, 19F and 23F pneumococcal polysaccharides (PPS). The *in vivo* splenic localization of pneumococcal polysaccharides in control rats was similar as described earlier [13]. Fifteen minutes after vaccination, PS19F was detected in the splenic marginal zone (Figure 2a). This marginal zone localization decreased in time and was followed by increased localization in the primary follicle corona (Figure 2b) at 7 hours after injection. After 5 days, polysaccharides were predominantly localized in the border of the lymphocyte corona and the germinal centers (Figure 2c).
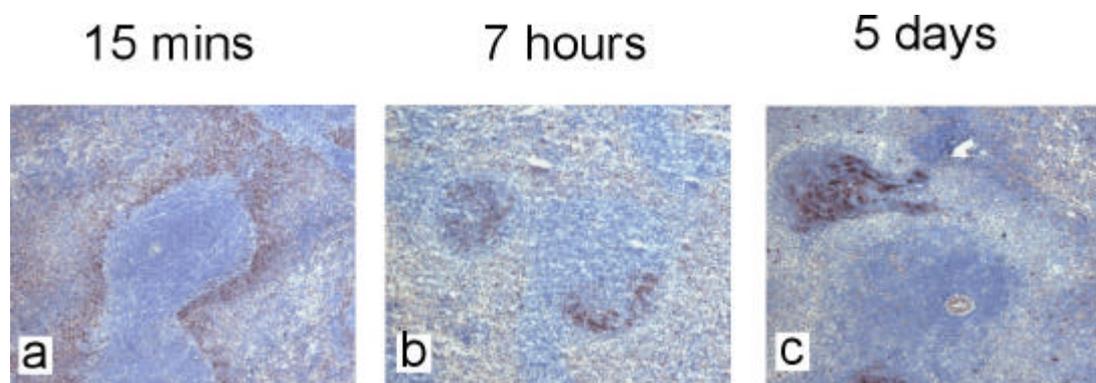

Figure 2.
Splenic localization of pneumococcal polysaccharide 19F after pneumococcal polysaccharide vaccination

2 µg of the pneumococcal polysaccharides (PPS) 4, 6B, 9V, 14, 18C, 19F and 23F were injected i.v. in control rats (panels a-c). Spleens were removed 15 minutes (a), 7 hours (b) and 5 days (c) after i.v. injection. Peumococcal polysaccharides were detected immunohistochemically on 4 µm cryostat sections, using serotype 19F-specific polyclonal rabbit antiserum followed by a peroxidase reaction (brown). Original magnification 10x.

For pneumococcal polysaccharides of serotypes 4, 6B, 9V and 23F, a similar localization pattern was observed, while PS14 did not localize in the spleen (data not shown). In

complement-depleted rats, PPS were undetectable in the spleen at all three time-points. We therefore repeated the localization experiments with a ten-fold higher dose (20 µg) PS19F (Figure 3). Even with this dose, no splenic localization was observed after CVF-treatment (Figure 3c).
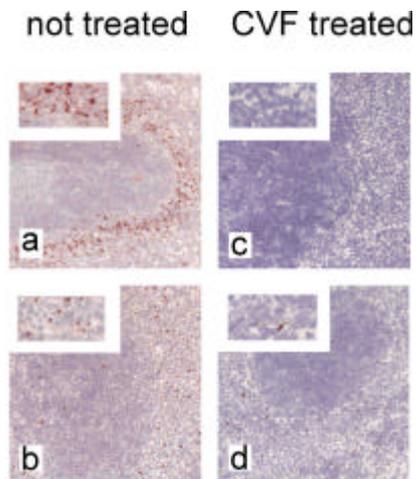

Figure 3.(left hand side)
Complement dependency of splenic localization of pneumococcal polysaccharide 19F after pneumococcal polyssacharide and conjugate vaccination

20 µg of PS 19F was injected i.v. in control (panel a) and in CVF-treated (panel c) rats. A heptavalent pneumococcal conjugate vaccine (PCV, containing 2 µg of the pneumococcal serotypes 4, 9V, 14, 18C, 19F and 23F and 4 µg of serotype 6B) was injected i.v. in control rats (panel b) and in CVF-treated rats (panel d). Spleens were removed 15 minutes after i.v. injection. Peumococcal polysaccharide was detected immunohistochemically on 4 µm cryostat sections, using serotype 19F-specific polyclonal rabbit antiserum followed by a peroxidase reaction (brown). Insets show transition from marginal zone (upper part) to germinal center (lower part). Original magnification 10x; inset 25x.
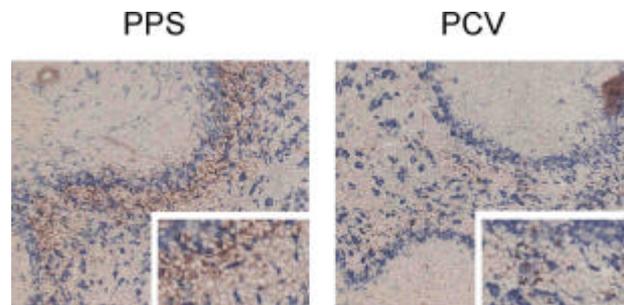
Figure 4. (right hand side)
Splenic localization of pneumococcal polysaccharide 19F after PPS and PCV vaccination, with simultaneous immunohistochemical characterization of splenic cells

15 minutes after i.v. injection of PPS (a) or PCV (b), pneumococcal polysaccharides were detected immunohistochemically, using serotype 19F-specific polyclonal rabbit anti-pneumococcal polysaccharide antibodies followed by a peroxidase reaction (brown). Splenic marginal zone macrophages are stained blue by MoAb ED3. Insets show the marginal zone. 25x original magnification, inset 40x.

In complement-sufficient rats, staining for the different polysaccharide serotypes 15 minutes after injection of pneumococcal conjugate vaccine showed a particulate polysaccharide staining pattern in the outer marginal zone (Figure 3b). After 7 hours, polysaccharides were detected in the corona; no polysaccharide was detectable after 5 days (data not shown). In complement-depleted animals, 15 minutes after PCV injection a same localization pattern (Figure 3d) of polysaccharide 19F was seen as in untreated animals, although the staining was less pronounced. In the splenic marginal zone, polysaccharides localized in association with

marginal zone B cells (Figure 4a). PCV localized partly in proximity of ED3-positive cells (Figure 4b).

Detection of pneumococcal polysaccharide 19F in rat serum

Because in decomplemented rats i.v. injected PPS does not localize in the spleen nor in the liver (data not shown), we investigated whether complement depletion resulted in prolonged circulation of PPS in blood. In serum of three complement-depleted rats, the concentration of circulating PS19F was approximately 5 times higher than in the three control rats at T=7 hours (Figure 5), indicating prolonged circulation after complement-depletion. The presence of CVF itself did not interfere with the binding of PS19F to nitrocellulose, nor did it interfere with detection by the anti-pneumococcal antiserum used (data not shown).
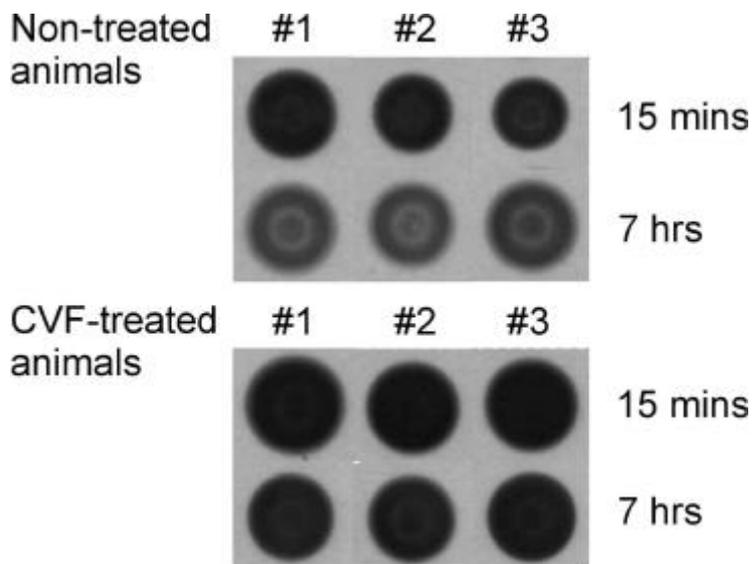

Figure 5. Detection of pneumococcal polysaccharide19F in serum

10 µl of serum drawn 15 minutes or 7 hours after i.v PPS vaccination from 3 rats treated with CVF (lower panel) and from 3 control rats (upper panel) was spotted on nitrocellulose filter. PS19F was detected using serotype 19F-specific polyclonal rabbit antiserum. After treatment with ECL™ (Amersham Pharmacia) for 1 min, a photofilm was developed for 1 min.

Anti-pneumococcal polysaccharide antibody responses.

On the base of localization data obtained earlier in this rat model (partly described in [13]), IgM and IgG antibodies against three (PS6B, PS19F and PS14) of the seven capsular polysaccharide serotypes present in both vaccines were determined.
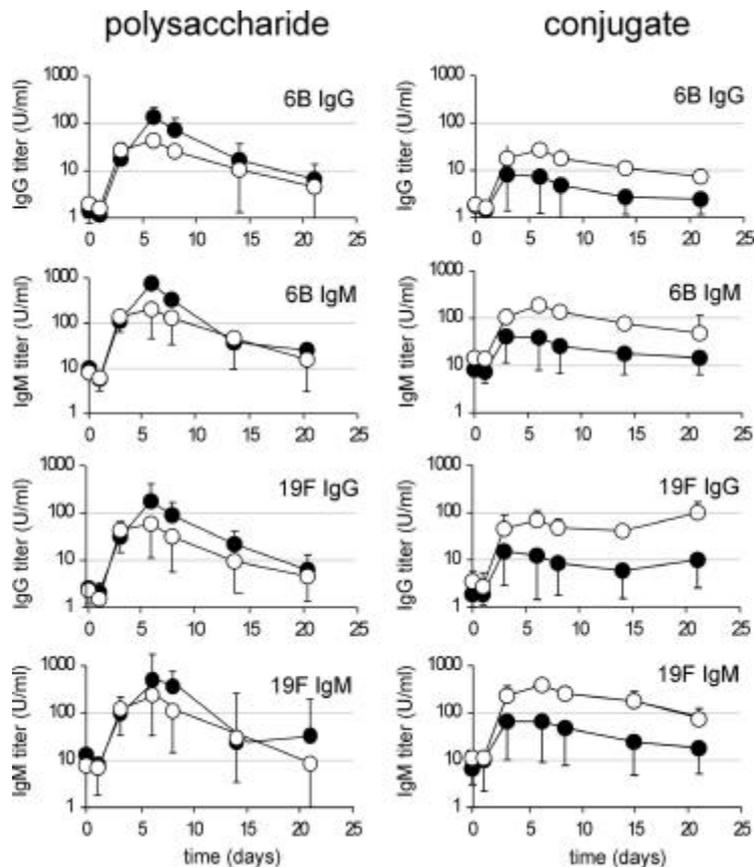
Figure 6. Serotype specific IgG and IgM anti-polysaccharide antibody response

Sera from rats immunized with PPS (lefthand panel) or PCV (righthand panel) were drawn at T= 0, 1, 3, 6, 8, 14, and 21 days after i.v. vaccination. IgG and IgM antibody concentrations against PS 6B and PS 19F were measured by ELISA. Antibody titers are expressed in U/ml. A rat hyperimmune serum pool used as a standard was assigned 100 U/ml for each serotype. Open symbols: CVF-treated animals; closed symbols: non-treated animals. Each data point represents the mean $x/\div$ SEM of 5 animals.

Immunization of control rats with PCV resulted in rises in IgG and IgM antibody titers for serotypes 6B and 19F, with an early peak at 4-6 days, followed by a slow decrease in antibody titers. Conjugating the poorly immunogenic PS14 to the protein carrier did not result in enhancement of immunogenicity in these control rats (data not shown).

In complement-sufficient rats, maximum antibody titers for IgG- and IgM-anti-6B and PS19F were reached 6 days after vaccination with 20 µg PPS (Figure 6). These titers dropped quickly, and were only slightly above baseline level 21 days after vaccination. After complement-depletion, antibody concentrations were significantly lower (p=0.043). Anti-PS14 antibody titers were not detectable after PPS vaccination (data not shown).

In contrast to the decreased response to polysaccharides after complement-depletion, immunization with PCV after CVF-treatment resulted in hígher IgG and IgM antibody titers than those obtained in control rats; and IgG and IgM anti-PS 14 antibodies were now detectable. Especially IgG anti-PS19F antibodies persisted longer after decomplementation. These differences did not reach significance.

Discussion

The initiation of the antibody response to polysaccharides is thought to be dependent on polysaccharide-C3d-complex localization on marginal zone B cells by interaction with complement receptor 2 (CD21). This model is based on the indirect argument that marginal zone B cells acquire high levels of CD21 only relatively late during ontogeny: a time frame that coincides with the acquisition of responsiveness to polysaccharides [10]. Direct evidence comes from the recent findings of C3 and CRI/II dependent localization of a synthetic TI-2 antigen, TNP-Ficoll, on murine marginal zone B cells [17].

In this study, we showed that antibody responses to pneumococcal polysaccharide-protein conjugates can be induced after complement-depletion. This finding is compatible with the observed immunogenicity of conjugate vaccines in infants and young children. Apparently no complement-dependent localization on marginal zone B cells is required for the initiation of an anti-polysaccharide antibody response by conjugated polysaccharides. This hypothesis is supported by our observations that conjugated polysaccharides localize in the splenic marginal zone without obvious relation to marginal zone B cells. A similar localization also is observed after complement-depletion, suggesting that it indeed is complement-independent.

The localization of unconjugated polysaccharides described in this study is consistent with data reported earlier [13]. At an early time point after immunization, specific localization is seen in the splenic marginal zone, in a staining pattern consistent with surface localization on marginal zone B cells. This supports the importance of the marginal zone B cell subset in the initial response to TI-2 antigens. In time, marginal zone localization decreases, concurrent with an increasing concentration first in the outer follicular mantle and later in a dendritic pattern in the complete follicle and follicle corona. Subsequently, in the center of the follicles, transfer of polysaccharides to the surface of follicular dendritic cells (FDC) coincides with the appearance of small germinal centers.

It has been previously shown [13], that *in vitro* localization of pneumococcal polysaccharides in the rat splenic marginal zone and follicle is complement-dependent. We are now able to extend this *in vitro* finding to the *in vivo* situation: in CVF-treated rats, i.e. in the absence of complement, no splenic localization of polysaccharides is observed. This *in vivo* finding extends the arguments derived from human *in vitro* studies [25] in favour of the current hypothesis that the initiation of an anti-polysaccharide antibody response is dependent on the presence of complement (presumably C3d) in combination with the presence of a functionally intact spleen. When these requirements are fulfilled, polysaccharide-complement complexes are formed and localize at the surface of the marginal zone B cells by interaction with the complement C3d receptor.

Several lines of evidence point towards a major role for marginal zone B cells in the immune response against TI-2 antigens [9;10]. Our data strongly indicate that the splenic marginal zone plays a major role in the immune response against native polysaccharides, but that the response to conjugated polysaccharides is not dependent on this specific B cell compartment. Consequently, an adequate response to conjugates may even be independent of the presence of a functioning spleen.

All pneumococcal polysaccharide serotypes tested localized clearly in the splenic marginal zone, except PS14. In humans, the localization patterns of all pneumococcal serotypes including serotype 14 are very similar [25]. PS14 is the only neutrally charged pneumococcal capsular polysaccharide, which may lead to trapping in extrasplenic tissue because of effective interaction with glycosyl receptors in rat [26]. It is tempting to correlate this aberrant pattern of PS14 localization with poor antibody responses to this serotype in this animal species.

We speculate that in the absence of complement, the initiation of the anti-polysaccharide antibody response is impaired because of insufficient formation of polysaccharide-C3d complexes. Consequently decreased interaction with complement receptors on splenic marginal zone B cells and thus less polysaccharide localization in the marginal zone leads to prolonged circulation of polysaccharides in blood. The higher levels of PS19F circulating in the bloodstream after CVF treatment are compatible with this hypothesis. The fact that the antibody response is not inhibited completely after CVF-treatment may be due to incomplete inactivation of complement. The interaction of C3d with the complement receptor is so

efficient that even low levels of complement still present after CVF treatment could be sufficient to induce a humoral immune response, even though splenic polysaccharide localization is no longer visible.

In the absence of complement, the antibody response to conjugated polysaccharide tends to be higher than in the presence of it. We speculate that this is due to the fact that complement components other than C3d (possibly C3b) play a role in the binding of the protein portion of the conjugate to receptors in e.g. the liver, resulting in lower levels of conjugate being available for a specific humoral immune response.

In conclusion, both our data on antigen localization as well as antibody levels show that the immune response to conjugated polysaccharides is distinctly different from that to native polysaccharides. Splenic localization of polysaccharides, and consequently the initiation of an anti-polysaccharide antibody response, is complement-dependent. The initiation of an anti-polysaccharide antibody response is impaired in absence of complement (this study, [17]) and/or B cells with adequate expression of CD21 [10;17], reflecting this reduced splenic localization. Coupling of polysaccharide to a protein carrier overcomes this C3d-CD21 dependency. These basic findings help to understand the success of conjugated vaccines in groups that are traditionally at risk for infections with encapsulated bacteria, especially infants and young children. This may open the way for application of conjugated vaccines in patient groups with inadequate anti-polysaccharide responses because of either decreased levels of complement or impaired splenic function.

Acknowledgements.

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Haemophilus influenzae type b conjugate vaccines: a systematic review of schedule related. RCTs. (Subject to change). Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland. 1.1 Objectives. The objectives of the systematic review were to collect evidence on Haemophilus influenzae type b (Hib) conjugate vaccine schedules, to summarize the available data and to identify gaps in evidence that might shape future research in this area. 1.2 Review methods. Â All doses are Hib conjugate vaccine unless otherwise noted. Protective effects of Hib conjugate vaccine against clinical disease are reported as vaccine efficacy (VE). October 1st 2012. Not for circulation or citation. Haemophilus influenzae type b (Hib) occurs worldwide although rates are very low in Canada where the incidence is highest in infants less than one year of age and children one to four years of age. Hib can cause bacterial meningitis and other serious invasive infections in young children. In addition to the primary series at 2, 4 and 6 months of age, receipt of a dose of Hib vaccine at or after 12 months of age is critical for sustained protection. Â Hib-containing vaccine is recommended for routine immunization of infants and children 2 to 59 months of age (up to the fifth birthday). Â Act-HIBÂ® (Haemophilus influenzae type b conjugate vaccine [tetanus protein conjugate]), Sanofi Pasteur SA (manufacturer), Sanofi Pasteur Ltd. (distributor). (Hib). Haemophilus influenzae type b (Hib). H. influenzae on a blood agar plate. Haemophilus influenzae is a Gram-negative coccobacillus that commonly infects the upper respiratory track of children through the transfer of nasal secretions. Whereas non-encapsulated strains of the bacillus are relatively benign, strains with a polysaccharide capsule or coat cause a more serious disease. The polysaccharide, is the primary factor associated with virulence. Of the 6 capsular types of H influenzae, type b (Hib) is responsible for more than 90% of systemic infections. This organism causes primarily pneumon...