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Oligoclonality of Serum Immunoglobulin G Antibody Responses to *Streptococcus pneumoniae* Capsular Polysaccharide Serotypes 6B, 14, and 23F

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Serum antibodies (Abs) specific for the capsular polysaccharides of *Streptococcus pneumoniae* provide protection against invasive pneumococcal disease. Previous studies indicate that Abs to pneumococcal polysaccharide (PPS) serotypes 1 and 6B have limited clonal diversity. To determine if restricted diversity was a feature common to other PPS specificities, we examined the light (L)-chain expression and isoelectric heterogeneity of type 6B, 14, and 23F Abs elicited in 15 adults following PPS vaccination. At the population level, both PPS-6B and PPS-14 Abs expressed κ and λ chains, although 6B Abs more frequently expressed λ chains lambda and 14 Abs more frequently expressed κ chains. In individual sera, Abs were generally skewed towards either κ or λ expression. 23F-specific Abs had predominantly κ chains. Isoelectric focusing analyses showed that sera contained one or at most a few immunoglobulin G Ab spectrotypes to all three respective capsular serotypes, a result indicative of oligoclonality. A sequence analysis of a purified PPS-14-specific Ab having a single spectrotype gave uniform amino-terminal sequences for both the heavy chain (V_HIII subgroup) and the L chain (κIII-A27 V region). From these results we conclude that within individual adults, serum Ab responses to PPS serotypes 6B, 14, and 23F derive from a small number of dominant B-cell clones, and consequently variable-region expression is probably individually limited as well. Oligoclonality appears to be a general characteristic of human PPS-specific Ab repertoires, and we suggest that this property could lead to individual differences in Ab fine specificity and/or functional activity against encapsulated pneumococci.

Streptococcus pneumoniae is an encapsulated pathogen causing pneumonia, meningitis, bacteremia, and acute otitis media (2, 8). Approximately 90 different pneumococcal capsular serotypes have been identified worldwide, but only a subset of these are responsible for the majority of disease. For example, it has been estimated that less than 20 serotypes account for 90% of the ~40,000 deaths caused per year in the United States by pneumococcal infection in adults (8, 52). As with other encapsulated pathogens, susceptibility to developing invasive pneumococcal disease is increased among the young, the elderly, and the immunocompromised.

Complement-dependent opsonophagocytosis mediated by antibodies (Abs) reactive with the capsular polysaccharides (PS) provides a principal means of protection against invasive pneumococcal disease (2, 12, 40). Accordingly, pneumococcal vaccine development has focused upon the induction of protective PS serotype-specific Ab responses (8, 23, 52, 60). The pneumococcal vaccine currently licensed in the United States consists of a mixture of 23 purified capsular PS. While estimates of efficacy vary, this vaccine appears to be 60 to 80% effective in preventing invasive disease in the elderly and other high-risk populations (13, 64). However, as with Ab responses to other PS antigens, children under 2 years of age respond poorly if at all to most pneumococcal PS (PPS) components of the vaccine, and their ability to produce Abs may not mature fully until 4 or 5 years of age (8, 11, 52, 60). Since pneumococci are the major cause of bacteremia and acute otitis media in young children, this population represents a major target for pneumococcal vaccination. Because of the success of *Haemophilus influenzae* type b (Hib) pediatric vaccines (17), multivalent protein-conjugated PPS vaccines are presently being developed and evaluated for safety and immunogenicity in various populations including the young and the elderly (4, 26, 27, 45, 63, 65).

The immune response to PPS antigens has been a subject of long-standing interest (7). This interest stems in part from the practical need to evaluate Ab responses to vaccination. In addition, PPS antigens serve as useful antigenic probes to study the genetic determinants and somatic forces dictating the expression of human Ab repertoires (31). Recent reports indicate that within individuals the Ab repertoire to some PPS antigens may be of markedly limited heterogeneity. Konradsen and colleagues used isoelectric focusing (IEF) to demonstrate that 1 to 3 distinct anti-PPS type 1 Ab clones were present in individual sera following pneumococcal vaccination (29). Park and colleagues showed a similar pattern with PPS-6B Abs, and in addition they identified several candidate light (L)-chain variable (V)-region genes encoding anti-6B Abs (44). Studies of the Hib PS repertoire have shown that Ab fine specificity, avidity, and protective capability can correlate with the expression of particular clones and V regions (20, 31, 33, 41, 57). Moreover, different Hib PS vaccines elicit Ab populations with distinctive patterns of V region expression and disparate functional capabilities (20, 33, 41, 56). Ab responses to PPS anti-

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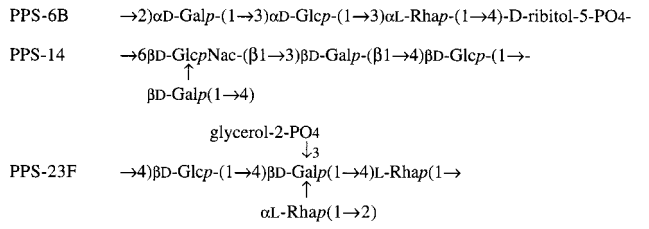

FIG. 1. Pneumococcal capsular polysaccharide unit structures. References are as follows: PPS-6B, references 28 and 49; PPS-14, reference 30; PPS-23F, reference 51.

gens may show similar properties. Therefore, defining the diversity and V-region usage of pneumococcal Ab repertoires is relevant to efforts aimed at developing reliable surrogates of protective immunity and understanding differences between individuals and/or populations in their abilities to respond to different PPS vaccines (39). In this study we sought to extend the previous studies of type 1 and 6B Abs to determine if oligoclonality was a feature common to other PPS Abs. Abs specific for PPS types 6B, 14, and 23F (Danish nomenclature), elicited in adults following immunization with polyvalent pneumococcal vaccine, were analyzed for L-chain expression and isoelectric heterogeneity. We chose to focus upon these three serotypes because the related organisms are significant pathogens, particularly in children, and because the PPS differ in chemical structure and immunogenicity. Type 14 is relatively immunogenic, whereas types 6B and 23F are of more limited immunogenicity. Their monomer unit structures are shown in Fig. 1.

MATERIALS AND METHODS

Human subjects and vaccinations. Fifteen healthy adults, ranging in age from 18 to 43 years (mean age = 28.7 years), received a single intramuscular injection of polyvalent pneumococcal vaccine (PNU-IMUNE 23, Wyeth-Lederle). Blood was taken immediately prior to vaccination and 30 days following vaccination. For long-term storage sera were frozen at −80°C, and for short-term storage, they were sterilized by passage through a 0.22-μ-pore-size filter and kept at 4°C. Pre- and postvaccination sera were evaluated for immunoglobulin G (IgG) anti-PPS Ab by enzyme-linked immunosorbent assay (ELISA) (see below). Geometric mean prevaccination serum IgG Ab levels (95% confidence intervals), in micrograms per milliliter, were 2.17 (0.95 to 1.99), 1.04 (0.35 to 3.11), and 0.81 (0.39 to 1.68) for PPS-6B, -14, and -23F, respectively. The geometric mean postvaccination serum IgG Ab levels (95% confidence intervals), in micrograms per milliliter, were 7.89 (4.78 to 13.0), 18.0 (7.5 to 43.0), and 6.22 (3.20 to 12.0) for PPS-6B, -14, and -23F, respectively. Ten, 13, and 13 of 15 subjects responded with ≥twofold rises in serum IgG Ab titer for PPS-6B, -14, and -23F, respectively.

PPS ELISA. ELISA was used to determine IgG and L-chain levels of anti-PPS Abs (45, 47). Nunc microtiter wells were coated for 5 h at 37°C with purified PPS-6B, -14, and -23F (obtained from the American Type Culture Collection [ATCC], Rockville, Md.) diluted in phosphate-buffered saline (PBS). Coating concentrations were 10, 1.0, and 10 μg/ml for PPS-6B, -14, and -23F, respectively. Wells were then blocked for 30 min at room temperature with PBS–0.05% Tween 20 and washed five times with PBS-Tween. Serum samples, diluted in PBS-Tween containing 10 μg of purified cell wall PS (C-PS; Statens Seruminstitut, Copenhagen, Denmark) per ml, were added to wells overnight at 4°C. Wells were washed five times with PBS-Tween, followed by addition of alkaline phosphatase-conjugated Abs specific for either human IgG (HP6043) or human κ or λ L chains (Biosource Inc., Camarillo, Calif.). After incubation at 37°C for 3 h, wells were washed and *p*-nitrophenyl phosphate in pH 9 diethanolamine buffer was added. Absorbance at 405 nm was determined after ~30 min. Anti-PPS IgG levels were calculated from a standard curve generated with reference serum 89-SF obtained from Carl Frasch, Center for Biologics Evaluation and Research, Rockville, Md. Serum 89-SF was assigned IgG Ab levels of 16.9, 27.8, and 8.10 μg/ml for PPS-6B, -14, and -23F, respectively, as previously reported (47).

The L-chain assays were calibrated with standards whose concentrations of κ or λ type-specific Abs were assigned in the following manner. Thirty-day postvaccination sera initially were screened in the PPS ELISA for κ or λ activities against PPS-6B, -14, and -23F, and individual sera showing a preponderance of either κ or λ Abs were identified. For PPS-14, serum from donor 13 gave high κ activity and serum from donor 1 gave high λ activity. Serum 13 was adsorbed with agarose to which an anti-human λ monoclonal Ab (MAb) had been coupled (HP6054; ATCC), and serum 1 was adsorbed with agarose to which two anti-human κ MAbs (HP6053 and 141-PF11; ATCC) had been coupled. Following adsorption, PPS-14 binding of the sera was analyzed by radioantigen binding assay (RABA; see below) for total Ab level and by ELISA for κ and λ expression. Ninety percent or more of the ELISA activity was of the appropriate L-chain type. Serum 13 was assigned a κ anti-PPS-14 value of 118 μg/ml, and serum 1 was assigned a λ anti-PPS-14 value of 131 μg/ml based on the corrected RABA values, i.e., 0.9 × the total Ab concentration by RABA. The same procedures were used to prepare the PPS-6B and PPS-23F standards, except that affinity-purified Abs rather than whole sera were used for the PPS-6B and PPS-23F λ standards. For PPS-6B, the κ standard was serum 10, assigned an anti-PPS-6B value of 5.2 μg/ml, and the λ standard was purified Ab from serum 6, assigned an anti-PPS-6B value of 11.3 μg/ml. For PPS-23F, the κ standard was serum 4, assigned an anti-PPS-23F value of 17.1 μg/ml, and the λ standard was purified Ab from serum 6, assigned an anti-PPS-23F value of 1.1 μg/ml. Concentrations of κ and λ anti-PPS Abs in test sera were calculated by comparison to the respective standard curves. Values used for generating κ/λ ratios are the means of several independent assays.

Radioiodination of PPS. PPSs were coupled with tyramine as follows. To 2.0 mg of PPS dissolved in 0.4 ml of 0.2 M $NaHCO_3$ was added 4 μl of cyanogen bromide dissolved in dimethylformamide at a concentration of 100 mg/ml. The solution was stirred for 6 min on ice. Sixty microliters of tyramine-HCl (10 mg of 0.2 M $NaHCO_3$ per ml) was added, and the mixture was incubated overnight at 4°C with stirring. The tyraminated PPS was dialyzed against water and then PBS and was stored in small aliquots at −70°C. The tyraminated PPS were labelled with ^{125}I by using chloramine T. Specific activities were 3×10^7 to 5×10^7 cpm/μg for PPS-6B and -14 and 8×10^7 to 9×10^7 cpm/μg for PPS-23F.

RABA. Total amounts of Ab to PPS were measured by a RABA similar to a previously described method (42). The diluent consisted of PBS–10% fetal calf serum–10 μg of C-PS/ml–0.1% sodium azide. Fifty microliters of ^{125}I-PPS containing ~250,000 cpm was mixed with 50 μl of diluted serum. The mixtures were incubated for 2 h at 37°C and overnight at 4°C. One hundred microliters of 100% saturated ammonium sulfate was added, and the mixtures were incubated for 4 h at 4°C. The precipitates were harvested by centrifugation (16,000 × *g*), washed once with 50% saturated ammonium sulfate, and counted in a dry-well gamma counter. Ab levels in test sera were calculated from a standard curve generated with reference serum 89-SF, which was assigned total specific Ab levels of 24.3, 37.0, and 11.9 μg/ml for PPS-6B, -14, and -23F, respectively, as previously reported (47). In this assay the observed binding was specific for the respective iodinated PPS as no inhibition of binding was observed when heterologous PPS was present in great excess (100 μg/ml), whereas homologous PPS completely inhibited binding at 0.1 μg/ml. Furthermore, the presence of C-PS at a fixed concentration of 10 μg/ml was sufficient to quench C-PS-specific Abs in sera, as the addition of excess C-PS (100 μg/ml) gave no inhibition of ^{125}I-PPS binding.

Analytical IEF. Sera were focused in 0.8-mm-thick 5% polyacrylamide gels containing ampholytes (pH 3.5 to 9.5 or 5 to 8) (Pharmacia Biotech, Inc., Piscataway, NJ), as described previously (34, 35). Gels were calibrated with commercial pI standards (Pharmacia Biotech). Following electrophoresis, samples were transferred electrically to a polyvinyldifluoride (PVDF) membrane (Novex, San Diego, Calif.) in 0.7% acetic acid. Blots were incubated overnight at 4°C in BLOTTO (1% nonfat dry milk–0.1% bovine serum albumin–0.1% sodium azide–PBS) containing 10 μg of C-PS/ml. The blots were reacted with ^{125}I-PPS (~5×10^5 cpm/ml in BLOTTO with C-PS) for 3 h at room temperature with agitation and then washed several times over 1 h with PBS–0.1% Tween 20. Blots were exposed to X-ray film (Hyperfilm; Amersham International, Buckinghamshire, England) at −70°C for various times. The lower limit of detection of PPS-specific antibodies was ~200 ng for PPS-6B and PPS-14 and 100 ng for PPS-23F.

Preparation of immunoabsorbents. PS were initially modified by adding 0.75 ml of cyanogen bromide (500 mg/ml of dimethylformamide) to 15 mg of PS (2.0 mg/ml of water) on ice. The reaction proceeded for 10 min, and pH was maintained at 9.0 to 9.5 by the addition of NaOH. Then, 7.5 ml of 0.2 M $NaHCO_3$ was added, and the solution was mixed with 7.5 ml of amino-hexyl-agarose (Sigma Chemical Co., St. Louis, Mo.) that had been previously washed with 0.2 M $NaHCO_3$. The mixture was gently rotated overnight at 4°C. The agarose was washed with Tris-buffered saline and was rotated at room temperature in Tris-buffered saline for 4 h. The agarose was washed alternately with 0.1 M $NaHCO_3$–0.25 M NaCl and with 0.1 M acetic acid–0.25 M NaCl. Agarose slurries (~2 mg of PS/ml of swollen gel) were stored at 4°C in PBS–0.1% sodium azide.

Affinity purification of anti-PPS Ab. Thirty-day postvaccination sera were heat inactivated at 56°C for 30 min. Sera were first adsorbed with C-PS agarose to remove anti-C-PS Abs. Sera were mixed with PPS-agarose for 2 h at room temperature with gentle rotation. The agarose was washed extensively with 0.5 M NaCl–0.2 M phosphate buffer, pH 7.2, until the absorbance at 280 nm in the eluate was less than 0.05. Bound Ab was eluted with ice-cold 3.5 M NaSCN, pH 7.2, followed by immediate dialysis against cold PBS. The Ab was centrifuged at 10,000 × *g* and concentrated by using a stirred cell with a PM-10 membrane (Amicon Corp., Beverly, Mass.).

Isolation and amino acid sequence analysis of monoclonotypic anti-PPS-14 Ab. Affinity-purified anti-PPS-14 Ab from subject 13 was subjected to preparative IEF. The sample, which consisted of 1.0 ml of anti-PPS-14 Ab having 950 μg of Ab as determined by RABA, was mixed with 50 ml of water containing 1%

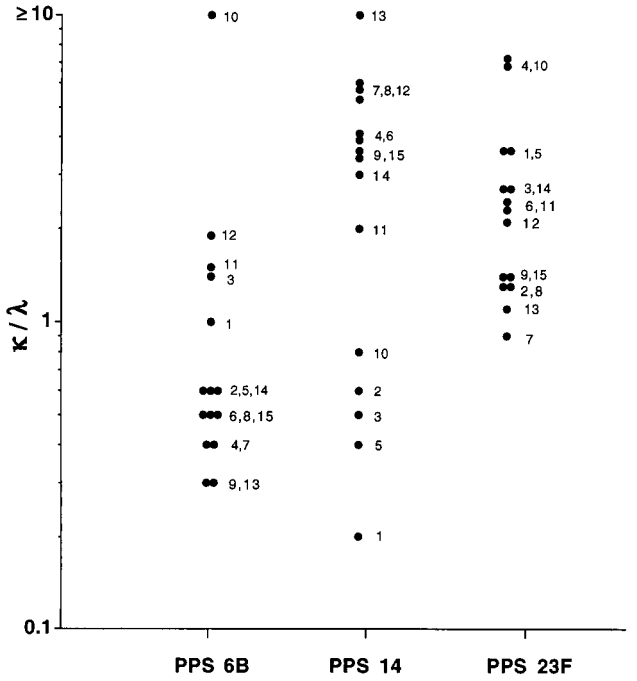

FIG. 2. L-chain expression by PPS-specific Abs. Each solid circle represents the κ/λ ratio of an individual 30-day postvaccination serum sample. Serum donor numbers are shown. The levels of κ and λ Abs were determined by ELISA using calibrated standards as described in Materials and Methods.

ampholytes (pH 3.5 to 9.5; Pharmacia Biotech). The sample was loaded into a Rotofor apparatus (Bio-Rad, Richmond, Calif.) and focused at 11 W for ~5 h. Individual fractions spanning pH values of 4 to 10 were assayed for anti-PPS-14 activity by RABA. A single peak of Ab activity that extended from pH 7.5 to 8.1 was identified. These fractions were pooled, dialyzed against PBS, and then concentrated with a centrifugal filter (Biomax 10; Millipore Corp., Bedford, Mass.). The donor 13 anti-PPS-14 Ab was typed as IgG2, κIII as determined in the PPS-14 ELISA using secondary MAbs specific for the human IgG subclasses and for the κIII subgroup.

Purified anti-PPS-14 Ab was separated into heavy (H) and L chains by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 12% Tris-glycine gel (Novex) under reducing conditions. H and L chains were transferred electrically onto a PVDF membrane (Novex) in 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid–10% methanol, pH 11. The membrane was stained with Coomassie blue, washed in 50% methanol, rinsed in water, and dried. H- and L-chain bands were excised and sent to the Protein Structure Laboratory, University of California, Davis, for amino acid analysis. H and L chains were sequenced directly from the PVDF membrane on ABI 477A sequencer (Applied Biosystems, Foster City, Calif.) by using an ABI 120 online high-pressure liquid chromatography system. Data were collected and analyzed with ABI 610 software.

RESULTS

L-chain expression of PPS-specific Abs was analyzed by an ELISA with calibrated reference Abs as described in Materials and Methods. Figure 2 shows the serum κ/λ ratios of anti-PPS Abs in individual postvaccination sera. κ and λ chains were present among both type 6B and type 14 Abs. However, 6B Abs were skewed towards λ expression; Ten of 15 subjects had κ/λ ratios ≤0.6. PPS-14-specific Abs more frequently showed κ expression, as 10 of 15 subjects had κ/λ ratios ≥ 2.0. Type 23F Abs were uniformly biased towards κ expression. The κ/λ distributions indicate that the majority of sera contained either a predominance of κ or λ Abs of a particular PPS specificity as shown by the tendency of the values to polarize away from equivalent expression (κ/λ = 1.0). This trend is most apparent with type 14 Abs where 11 of 15 subjects had κ/λ ratios either exceeding 2.0 or less than 0.5. Also, it is interesting to note that within a single serum κ Abs can predominate one PPS specificity whereas λ Abs predominate a different PPS specificity (see, for example, sera 1, 5, 9, and 13).

The heterogeneity of PPS-specific Abs was evaluated by IEF. Since most sera contain Abs reactive with C-PS and since purified PPS contain C-PS, it is necessary to quench the C-PS Abs to permit specific detection of serotype-specific IEF clonotypes (29, 44). Preincubation of IEF blots with C-PS (10 μg/ml) in BLOTTO diluent prior to and during exposure to iodinated PPS was sufficient to eliminate detection of C-PS Abs. The specificity of this method was demonstrated by the complete inhibition of the respective ^{125}I-PPS binding by homologous PPS and by the lack of inhibition by heterologous PPS (data not shown).

Because of differential glycosylation and amidation, a single MAb will give an IEF spectrotype consisting of several isoforms with pIs that can range from 0.5 to 1.0 pH units. Although this charge heterogeneity complicates precise estimation of Ab clonality by IEF, MAbs can be used to calibrate IEF spectrotypic patterns. Since hybridoma-derived human MAbs specific for PPS were unavailable, we purified an IgG_2 PPS-14-specific Ab from a single postvaccination serum by affinity chromatography and preparative IEF. This Ab was isolated from subject 13, who produced a κ-predominated anti-PPS-14 Ab after vaccination (Fig. 2). The purified Ab was analyzed by IEF, and N-terminal amino acid sequences were obtained by sequential Edman degradation of the isolated H and L chains. The results are shown in Fig. 3. The purified Ab gave an IEF spectrotype, as detected by ^{125}I-PPS-14 staining (panel A, lane 2) and by silver (protein) staining (panel B, lane 2), that was coincident with the PPS-14 spectrotype present in the unfractionated serum (panel A, lane 1). Shown for comparison is the IEF spectrotype given by human anti-Hib PS MAb CA4 (35) as detected by silver staining (panel B, lane 3) and ^{125}I-Hib PS (panel C, lane 3). Amino acid sequence analysis of the serum 13 anti-PPS-14 Ab gave uniform and unambiguous sequences for both the H and L chains. The sequences were compared to those in human V gene data banks, and the H chain was identified as a member of the V_HIII subgroup. Since the V_HIII family is large and since many members of this subgroup have identical sequences in framework 1, we were unable to assign a specific candidate V_H region. The sequence of the V_L region was identical to that of the translation product of the A27 gene, a member of the κIII subgroup. Framework 1 of human κ chains is sufficiently unique to make this assignment definitive.

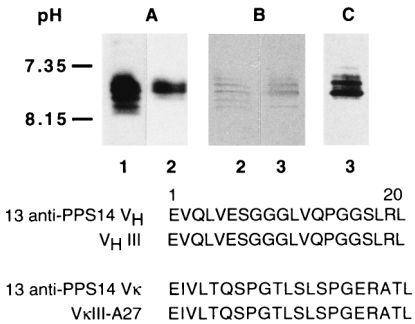

FIG. 3. IEF and N-terminal amino acid sequence analysis of purified serum anti-PPS-14 Ab from subject 13. Samples: lane 1, donor 13 postvaccination serum; lane 2, purified anti-PPS-14 Ab isolated from donor 13 serum; lane 3, purified anti-Hib PS MAb CA4. PPS binding was detected with either ^{125}I-PPS-14 (A) or ^{125}I-Hib PS (C) treatment followed by autoradiography, and protein was detected by silver staining (B). N-terminal amino acid sequences of H and L chains of purified donor 13 anti-PPS-14 Abs were determined by sequential Edman analysis and are compared to the translated sequences of a V_HIII germ line sequence and the κIII-A27 gene.

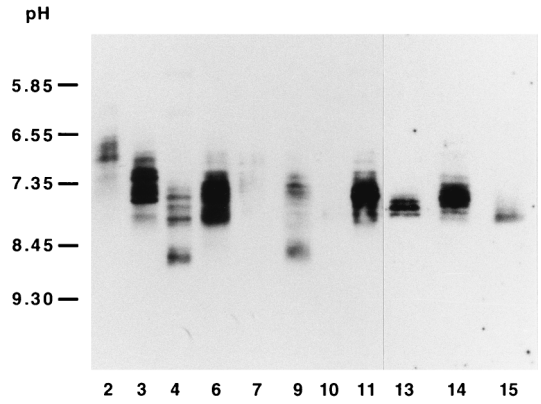

FIG. 4. Anti-PPS 6B IEF spectrotypes present in individual postvaccination sera. Lane numbers identify serum donors.

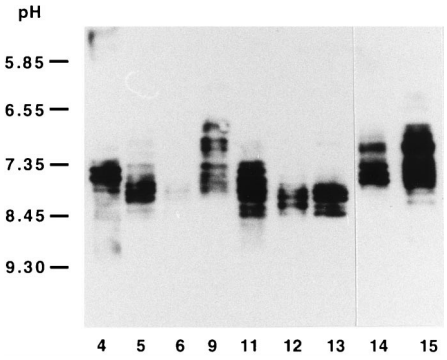

FIG. 5. Anti-PPS-14 IEF spectrotypes present in individual postvaccination sera. Lane numbers identify serum donors.

Serological analysis using a MAb specific for the human κIII subgroup confirmed that the serum 13 anti-PPS-14 Ab expressed a κIII V region (data not shown).

This analysis demonstrates that the spectrotype pattern of the purified serum 13 anti PPS-14 Ab resembles that given by a MAb, and therefore it can be used to calibrate the IEF analyses shown below. The results in Fig. 3 also show the similarity between the spectrotypes of the serum 13 anti-PPS-14 Ab and the CA4 Ab, although they have different PS specificities and are independently derived. These two Abs are related to one another with respect to V region and constant (C) region usage. Both use G2 and kappa C regions, and both use a subgroup III V_H region paired with the A27 V_L region (35). While these Abs undoubtedly differ in V-region amino acid sequences and in the extent of posttranslational modifications, these differences apparently are not sufficient to confer charge disparities distinguishable by IEF, at least with this level of resolution. Therefore, similarities in pI and isoform banding patterns cannot necessarily serve as a criterion for clonal relatedness.

We analyzed the isoelectric heterogeneity of PPS-6B, PPS-14, and PPS-23F IgG Abs in sera from adults immunized with polyvalent pneumococcal vaccine. This analysis is restricted to anti-PPS Abs of the IgG class because higher-molecular-weight polymeric IgM and IgA Abs do not enter the IEF acrylamide gel (53). Also, some sera had to be excluded from the study because of insufficient levels of serotype-specific Ab to be detected by this IEF method. The most striking pattern apparent from the IEF results is the limited Ab complexity (Fig. 4, 5, and 6). For PPS-6B, subjects 2, 13, 14, and 15 had a single spectrotype encompassing a relatively narrow pH range (Fig. 4). While subjects 3, 6, 9, and 11 had more complex 6B spectrotypes, their Abs nonetheless appear to be the products of no more than two or possibly three clones. The same pattern was evident for PPS-14 IgG Abs (Fig. 5). Spectrotypes of subjects 4, 5, 12, and 14 are consistent with marked oligoclonality, compared to the serum 13 monoclonotypic calibrator. Subjects 9, 11, and 15 gave more complex spectrotypes, but again the number of clonotypes probably does not exceed three. PPS-23F Abs also appear to be uniformly restricted in heterogeneity (Fig. 6). Subjects 1, 5, 6, 9, 10, and 14 all had spectrotypes resembling that given by a MAb. Several sera gave lightly stained bands with disparate pIs (2, 3, 4, and 13), but even within these sera the clonal complexity is quite limited.

DISCUSSION

The results reported here demonstrate the restricted clonal heterogeneity of individual IgG Ab responses to pneumococcal serotypes 6B, 14, and 23F. Based upon IEF analyses calibrated with a purified monoclonotypic anti-PPS-14 Ab, we estimate that one or at most a few Ab clones dominate individual PPS-specific Ab repertoires. Sera generally showed a preponderance of either κ or λ L chains among these PPS specificities, and this skewed distribution provides further evidence for clonal dominance.

Our findings parallel those of previous studies examining the heterogeneity of PPS-1 and PPS-6B Abs. In a study of mono- and dizygotic twins, Konradsen et al. showed that Abs to PPS-1 gave IEF patterns consistent with the expression of 1 to 3 clones in individuals (29). Six different spectrotypes were identified from a sample of 84 subjects, and, interestingly, identical twins did not necessarily express the same spectrotype. Park and colleagues reported that most individuals produced only 1 or 2 PPS-6B-specific spectrotypes following pneumococcal vaccination and that 6B Abs were biased toward λ expression (44). Our results agree with and extend these findings. Thus, to date, human Ab responses to a total of four PPS have been examined, and all show a consistent pattern of oligoclonality. Ab responses of restricted heterogeneity are well known in murine models (10, 16, 19, 22), and it is becoming increasingly apparent that this phenomenon can occur frequently in human populations. The Ab response to Hib PS has been particularly well characterized, and several experimental approaches including idiotypic studies (20, 31, 32, 34), V-region sequence analyses

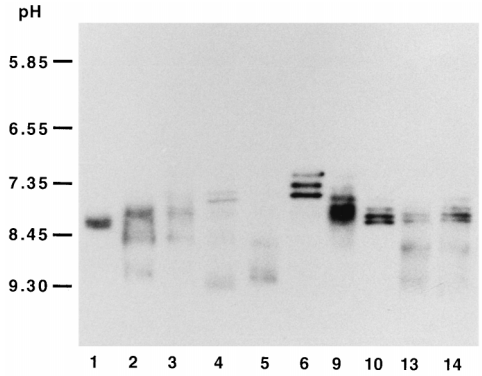

FIG. 6. Anti-PPS-23F IEF spectrotypes present in individual postvaccination sera. Lane numbers identify serum donors.

(14, 57–59), molecular cloning of peripheral blood B-cell populations (9), and IEF (24, 25) have documented the restricted nature of individually expressed repertoires. In addition, IEF studies have demonstrated the oligoclonality of human Ab responses to streptococcal group A carbohydrate (50, 61) and diphtheria toxoid fragment A (37).

It is important to note that the dominance of an individual's serum repertoire by one or a few PPS-specific clones does not exclude the existence of other clones which, although constituting a minor component of the serum pool at the time of sampling, could nonetheless play an important role in host defense. For example, following vaccination with PPS antigens a significant fraction of the specific B cells that transiently appear in the peripheral circulation are of the IgA isotype (36). Our analysis focused upon IgG Abs present in serum 30 days after vaccination, and therefore we have not assessed the clonal representation of IgA Abs, a population that could mediate protection against pneumococci, particularly at mucosal surfaces. Furthermore, studies of the human Hib PS response (58) and of murine Ab responses (6, 46) emphasize the plasticity of the repertoire such that minor clones are able to assume predominance under conditions where normally dominant clones are either absent or silenced.

A direct consequence of oligoclonality in individual PPS responses is the expression of a limited number of V domains. This restriction is apparent in the PPS-14 response of subject 13 where the serum was dominated by an IgG2, κ Ab expressing a V_HIII and an A27 V domain. A similar pattern has been observed with PPS-6B Abs (44). While IEF analyses do not permit a precise estimate of clonality and V-region usage since a single spectrotype could contain more than one B-cell product, we think it is likely that a small set of V regions will dominate individual Ab responses to PPS antigens.

One explanation for restricted V-region usage is that since PS antigens are polymers of a repeated carbohydrate unit, they possess relatively few antigenic determinants, and therefore they evoke a limited number of complementary combining sites. While Ab responses to some carbohydrate epitopes may be intrinsically genetically restricted (19), considerable evidence indicates that anti-carbohydrate Ab responses also can be quite diverse (1, 10, 18, 54, 66). Even in situations where individual repertoires are markedly oligoclonal, the genetic potential for diversity can be high. This is illustrated by the Hib PS repertoire which at the population level is encoded by three or four V_H gene segments and by 12 V_L genes, but at the individual level the response is oligoclonal and predominated by particular V_H-V_L combinations (14, 31, 34, 59).

Although anti-PPS Abs within individuals appear to use a limited number of V regions, several lines of evidence suggest that the population repertoire may be diverse. Our L-chain analyses show that both κ and λ V regions contribute to PPS-6B and PPS-14-specificities, and a previous study demonstrated the expression of κI, κII and κIII V regions by PPS-6B Abs (44). Furthermore, the differences between individual spectrotype patterns of a given PPS specificity are consistent with structural diversity. Together, these results indicate that at the population level PPS-6B and PPS-14 V-region repertoires may be complex. 23F-specific Abs in contrast are biased toward κ expression, a finding suggesting that V_L diversity may be more restricted than in the 6B and 14 populations.

If we assume that PPS-specific V-region repertoires are potentially diverse at the population level, then the question arises as to why individually expressed repertoires are so clonally limited. Clonal representation is likely a dynamic process where competition could exist among a diverse B-cell precursor pool. Properties such as enhanced affinity or larger precursor frequency could confer a selective advantage sufficient to establish clonal dominance. This could apply to the memory compartment as well, where an initially favored clone has expanded and possibly affinity matured such that it effectively outcompetes any new entries into the repertoire. The stability of clonal expression has been demonstrated in the Hib PS response where the same dominant serum spectrotype may persist over 2 years (25). Natural exposure to pneumococci or to cross-reactive antigens probably drives maturation of the repertoire, and the quality of these stimuli may bias clonal expression. Different molecular forms of an antigenic determinant can shape the expressed repertoire such that particular clones or V regions are selectively stimulated (15, 20, 33, 56). Other influences that might play an important role in influencing the representation of V domains include deletion or modification of PPS-specific clones having cross-reactivity with self antigens, temporal differences in the pattern of V-gene assembly, and differences in the rate of establishment of the generative PPS-specific B-cell pool. Furthermore, differences in the contents of germ line V genes between individuals (and/or ethnic groups) could in principle limit the number of germ line combinations encoding PPS Ab specificities. Examples of inherited polymorphisms that may influence the potential repertoire range from differences in the number of V_H (55) and V_L genes (43) to more subtle changes involving a small number of base changes that convert nonfunctional genes with minor defects into functional genes (5, 48).

The properties of oligoclonality and restricted V-region usage in PPS Ab responses are likely to have functional implications. Ab uniformity increases as a response approaches monoclonality, and consequently there is a greater likelihood that individual sera will differ with respect to antigenic fine specificity, avidity, or biological activity against pneumococci. Studies of the Hib response have demonstrated correlations between V-region usage and Ab functional activity (14, 33, 41), and some evidence suggests that this phenomenon might apply to PPS Abs. Fine-specificity differences have been observed among 6B Abs in their cross-reactivities with PPS-6A (44), and this reactivity pattern might correlate with usage of particular V regions. In addition, Shaw and colleagues described two human MAbs to PPS-3 that were encoded by different V-region genes; despite having similar affinities, these Abs showed marked differences in fine specificity, opsonophagocytosis, and mouse protection activities against type 3 pneumococci (62). One could imagine that even when an individual's anti-PPS response is derived from a single precursor B cell, Ab quality could change dramatically during the course of clonal maturation. A striking example of this phenomenon has been described for the murine response to the capsular PS of *Cryptococcus neoformans* where Abs derived from progeny of a single B cell differed sufficiently in V-region somatic mutations such that they reacted with distinct epitopes of the *Cryptococcus* capsule and also differed in protective efficacy (38). Oligoclonality and its corollary of skewed functional activity are likely to be relevant to the serological evaluation of Ab responses to different PPS vaccines and the development of reliable in vitro correlates of protective immunity (3, 21). A major challenge for future studies will be to elucidate the mechanisms of oligoclonality and to determine the extent to which limited repertoire size may impact responsiveness to PPS antigens (39) and immunity to pneumococcal infection.

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