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Enzyme-Linked Immunosorbent Assay for Detection of Antibodies Against *Streptococcus pneumoniae* Capsular Polysaccharides

HAROLD RUSSELL,* LAURA R. EDWARDS, EDITH W. WORTHAM, AND RICHARD R. FACKLAM

Staphylococcus and Streptococcus Section, Bacteriology Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia 30333

The development of an assay to measure the human immune response to pneumococcal capsular polysaccharides is described.

The licensure of polyvalent pneumococcal vaccines in the United States has created a demand for a relatively simple, nonhazardous assay to measure the immune response in vaccine recipients. The widely accepted radioimmunoassay for measuring pneumococcal antibodies (G. Schiffman and R. Austrian, Fed. Proc. **30**:658, 1971) is an excellent, reliable assay; it is however the only available tool for measuring pneumococcal antibodies in human sera.

Numerous questions concerning the immune response to pneumococcal vaccines have not been answered. What antibody titer or concentration offers protection from pneumococcal disease? Will antibodies against a given antigen type protect individuals from disease caused by a different serological type? Do individuals produce antibodies against all antigen types in the vaccine? Additional means of measuring antibodies in large quantities of sera are needed to answer some of these questions. In this study we show that the enzyme-linked immunosorbent assay (ELISA) can possibly be used to detect an immune response in individuals vaccinated with a polyvalent licensed pneumococcal vaccine. The reagents, antigen, and conjugate are stable for long periods. No radioactive chemicals are used, thus eliminating the potential hazards of the radioimmunoassay

The ELISA sandwich technique was a modification of the method of Engvall and Perlmann (3). Polystyrene tubes were coated with 1-ml portions of 0.05 M carbonate solutions of type-specific immunoglobulin G (5 μg/ml) at 37°C for 3 h. The tubes were stored at 4°C overnight. The tubes were then washed three times with approximately 4 ml of 0.9% NaCl containing 0.05% Tween 20 and coated with 1-ml fractions of purified pneumococcal polysaccharide solutions in phosphate-buffered saline-Tween (5 μg/ml) of homologous type antigen. The 14 purified antigens were kindly supplied by A. F. Woodhour of Merck Sharp & Dohme, West Point, Pa. After incubation at 37°C for 1 h the tubes were washed three times, and 1-ml amounts of the preimmune and postimmune sera from five healthy adult volunteers diluted in 0.1 M phosphate-buffered saline (pH 7.0) were added. The tubes were washed three times again after 1 h of incubation at 37°C, and 1 ml of conjugate, goat anti-human Fab fragment, conjugated to alkaline phosphatase, was added. The tubes were kept at 37°C overnight (16 h). The tubes were washed three times, and 1 ml of 0.025% *p*-nitrophenyl phosphate was added. The enzymatic reaction was stopped after 100 min with 0.1 ml of 1 N NaOH, and the absorbances were read at 400 nm in a spectrophotometer. ELISA results were expressed as the ratio of absorbance of postimmune serum to preimmune serum.

The 14 type-specific antigens were cross-matched with the 14 type-specific serum fractions. All homologous reactions were stronger than the heterologous reactions. Cross-reactions included slight reactions with antigen type 1 and antibody types 2, 3, and 14. The homologous reaction, however, was three times stronger than the heterologous reaction. Antigen types 1, 2, 4, 6A, 7F, 8, 9N, 12F, 18C, 19F, and 25 reacted slightly with antibody type 3, but the homologous reaction was three times stronger than any of the heterologous reactions. Antigens 1, 2, 4, 6A, 8, 9N, 12F, 18C, and 19F reacted slightly with antibody type 14, but the homologous reaction was four times stronger than the highest cross-reacting antigen. Antibody types 1, 4, 6A, 7F, 8, 9N, 12F, 18C, 19F, 23F, and 25 reacted only with their homologous antigens.

Rabbit anti-pneumococcus serum was fractionated on a Sephadex G-200 chromatographic column to obtain the specific immunoglobulin fraction for the first coating of the polystyrene tubes. The active fraction was detected by the capillary precipitin test (6) with purified type-specific antigen. The specific immunoglobulin was usually found in the immunoglobulin G fraction. Any single serum dilution of 1:100 to 1:800 yielded the same ratio of absorbance of

postimmune to preimmune serum. A semilogarithmic plot of optical density values and serum dilution factors showed a linear relationship. We used a dilution of 1:200 in all reported experiments. All assays were done in duplicate. Table 1 represents the absorbance ratio values from five paired sera (pre- and postimmune). Each pair of sera was reacted with each of the 14 capsular antigens present in the commerical vaccine Pneumovax. Results show that the ELISA detected at least a twofold response against all antigens tested in one out of five serum pairs tested. The mean fold increase ranged from 1.5 against antigen type 25 to 6.2 against antigen type 8. Individuals responded differently, however, to most of the antigens. The highest individual response was 11.6-fold against type 3 antigen.

TABLE 1. *Serum anti-capsular antibody response in the ELISA following vaccination of five adult volunteers with pneumococcal vaccine Pneumovax*

Antigen type[a]	Absorbance ratios from[b]:					MFI[c]
	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	
1	1	2.4	1	1.1	2.4	1.6
2	3.2	2.4	1.7	3.8	2.2	2.7
3	1.9	1.8	11.6	1.6	3.8	4.1
4	7	2	2.1	1.8	1.7	2.9
6A (6)	1.5	1.9	1.9	2	0.8	1.6
7F (51)	6.2	3	3.2	1.5	1	3.0
8	8	8.1	6.4	6.1	2.6	6.2
9N (9)	6.4	6.5	3.1	1.9	6.1	4.8
12F (12)	6.2	1.5	1	1	1	2.1
14	4	1.7	1.2	0.9	1.2	1.8
18 (56)	4	4	8.8	1.4	6.5	4.9
19F (19)	3	1.6	2.3	1.6	1	1.9
23F (23)	2	1.8	1.6	5.4	1.8	2.5
25	2.5	1.2	1.5	1.0	1.5	1.5

[a] Nomenclature of the Danish and American (parentheses) pneumococcal typing systems is from references 2 and 5.

[b] Optical density postimmune sera/optical density preimmune sera, from individuals vaccinated with Pneumovax and bled 1 month after vaccination.

[c] MFI, Mean fold increase (average ratio).

Although Berntsson et al. (1) obtained adherence of pneumococcal capsular polysaccharides directly to polystyrene tubes, we were not successful in obtaining reproducible results with direct adherence. One likely reason for the discrepancy is that the tubes were purchased from different companies, and, therefore, the final polystyrene products were probably different.

Results from this preliminary study show that ELISA shows promise as another means of detecting an immune response to pneumococcal vaccine in human sera. Responses to various antigens varied from individual to individual probably because some individuals have higher existing antibody levels than do others. Although the number of sera tested in this study was small, the data show that an immunological response could be demonstrated against each of the 14 antigens in the Pneumovax. Additional studies are in progress to establish the efficacy of the assay and to determine whether the ELISA results correlate with the radioimmunoassay results.

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