Simple, Speedy, Sensitive, and Specific Serodiagnosis of Pertussis by Using a Particle Agglutination Test

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We developed a particle agglutination test (KPA) with poly(γ-methyl L-glutamate) as the solid particle for measurement of pertussis toxin (PT) antibody. In this study, KPA was assessed as a means of serodiagnosing pertussis, and the results were compared with those of indirect enzyme-linked immunosorbent assay (indirect ELISA) and the microagglutination test. First, four serum samples were collected from each of 21 healthy children: before and 4 weeks after receiving three primary doses of acellular pertussis vaccines and before and 4 weeks after receiving a booster dose. In all 21 vaccinees, a significant rise in PT antibody titers was observed by KPA after each vaccination, and among all 84 serum samples collected, an excellent correlation was demonstrated between the values obtained by indirect ELISA and those obtained by KPA ($r = 0.92$). Second, paired serum samples were collected at intervals of approximately 2 weeks from 51 patients with culture-confirmed pertussis. A significant increase in titer (fourfold or more) was observed in 39 (76%) patients by KPA, 34 (67%) patients by indirect ELISA, and 23 (45%) patients by the microagglutination test. In acute- and convalescent-phase sera collected from 20 nonpertussis patients, there were no changes in titers by KPA. The KPA procedure was as simple as that of the microagglutination test, and the reaction time was only 2 h (or overnight). In this study, KPA was demonstrated to be a simple, speedy, sensitive, and specific serodiagnostic method for pertussis.

Procedures currently available for the serodiagnosis of pertussis include the microagglutination test with whole bacteria (7), indirect enzyme-linked immunosorbent assay (indirect ELISA) with purified antigens (2, 4, 8, 10), and Chinese hamster ovary (CHO) cell assay (3). Four pertussis-specific antigens are currently available; these are pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin, and fimbriae 2 and 3, among which PT is the most important antigen against *Bordetella pertussis* infection (1, 9). Antibody against PT can be measured by ELISA and the CHO cell assay. Among these antibodies, immunoglobulin G (IgG) antibodies against PT detected by ELISA is the most useful for the serodiagnosis of pertussis (2, 4). Although ELISA with these purified antigens has been successfully applied to the diagnosis of pertussis, the requirements for special equipment and techniques, as well as difficulties with technical reproducibility, limit its availability (8). We developed a particle agglutination test (KPA) with poly(γ-methyl L-glutamate) (PMLG) for measurement of the PT antibody. The KPA procedure is as simple as that of the microagglutination test, and the reaction time is only 2 h (or overnight, if desired). In this study, KPA was assessed as a means of serodiagnosing pertussis and was compared with the indirect ELISA and the microagglutination test.

MATERIALS AND METHODS

Production and sensitization of particles. By using a suspension and evaporation method, porous spherical particles were produced from PMLG (6). Because the application of particles is diameter dependent, particles with diameters of 3 to 4 μm were used as artificial carriers in the KPA for immunoassay. Partially 3-aminoethylated poly(L-glutamic acid), which was obtained by allowing PMLG to react with ethylenediamine, was used as a hydrophilic polyamino acid. The partially aminated poly(L-glutamic acid) was mixed with gum arabic aqueous solution, and Reactive Blue solution was then added to the coacervates and the mixture was stirred at 37°C. Following centrifugation, the stabilized spherical particles were prepared as described previously (5). After the particles had been immobilized with glutaraldehyde, PT (Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) at 2 μg/ml and tannic acid were mixed and applied at 37°C. After washing with phosphate-buffered saline solution, the PT-sensitized particles were stored in phosphate-buffered saline.

KPA. Twenty-five microliters of diluted sample was placed in each U-bottom well of a microtiter plate in a series of successive twofold dilutions. A 25-μl quantity of the dispersion of the PT-sensitized particles was added to each well and the mixtures were allowed to stand for 2 h at room temperature after being shaken for 3 min. The particle agglutination patterns were interpreted with the naked eye, and the antibody titer was expressed as the highest dilution yielding complete agglutination.

Specimens. First, 21 healthy children ages 2 years or younger were immunized with three primary doses of acellular diphtheria and tetanus toxoids and pertussis vaccine (DTP) and a booster dose. Four serum samples were collected from each child at the following times: before the first DTP vaccination and before receiving the booster and 4 weeks after receiving the primary series and 4 weeks after receiving the booster. Second, paired serum specimens were collected from 51 patients, including 26 children ages 0 to 1 year with culture-confirmed pertussis. The first serum samples were obtained from each patient on mean day 14.1 ± 5.3 (14.1 days following the onset of disease), and the second serum samples were obtained from each patient on mean day 30.8 ± 8.0. In addition, paired serum specimens were collected at intervals of approximately 2 weeks from 20 nonpertussis patients with cough, including 3 patients with *Mycoplasma pneumoniae* infection, 3 patients with respiratory syncytial virus infection, and 2 patients with parainfluenza virus type 3 infection.

Indirect ELISA and microagglutination test. IgG antibodies against PT and FHA were measured by indirect ELISA, essentially following the procedure described by Meade et al. (8). Purified PT or FHA (Chemo-Sero-Therapeutic Research Institute) was used as a coating antigen. A parallel-line bioassay method with U.S. Reference Pertussis Antiserum Lot 3 was used to calculate the units. The agglutinin titer for serotype 1.3 (Kitasato Institute, Tokyo, Japan), the

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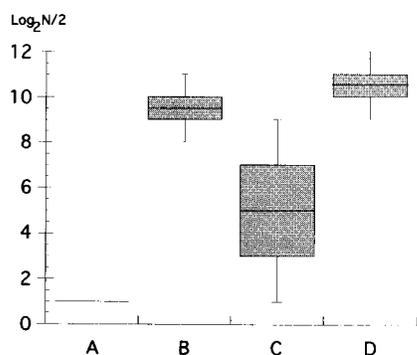
FIG. 1. Change in anti-PT antibody responses in 21 children immunized with acellular DTP, as measured by KPA. A, B, C, and D correspond to before vaccination, 4 weeks after receiving the primary series, before receiving the booster, and 4 weeks after receiving the booster, respectively. Each box encloses 50% of the data, with the median indicated by the line inside the box. The lines extending from the top and bottom of each box denote the maximum and minimum for each variable.

strain most prevalent in Japan (11), was measured by the microagglutination test essentially by the same method described by Manclark et al. (7). At least fourfold increases in the titers of PT IgG by ELISA and FHA IgG by ELISA or the microagglutination test in paired serum specimens were taken as serological confirmation of pertussis.

Statistics. The chi-square and Fisher's exact probability tests were used to evaluate the statistical significance of differences between proportions.

RESULTS

Significant anti-PT antibody responses were observed in all 21 vaccinated individuals after receiving three primary doses and one booster by KPA as well as by the indirect ELISA (Fig. 1). An excellent correlation was demonstrated between values obtained by the indirect ELISA and those obtained by KPA for the 84 serum samples collected from vaccinees ($r = 0.92$) (Fig. 2). The reproducibility of KPA, assessed with both high- and low-titer sera, was consistently twofold or less. There was no agglutination with unsensitized particles against any of the sera tested.

Among paired serum specimens obtained from 51 patients with culture-confirmed pertussis, a significant antibody increase was observed in 39 (76%) by KPA, 34 (67%) by PT IgG ELISA, 25 (49%) by FHA IgG ELISA, 23 (45%) by microagglutination, and 42 (82%) by at least one of the four methods. KPA was more sensitive than FHA IgG ELISA ($P < 0.01$) and microagglutination ($P < 0.01$). Among the 51 patients with pertussis, 26 were children ages 0 to 1 year. Among the paired serum specimens obtained from these 26 young children, a significant titer increase was observed in 22 (85%) by KPA, 19 (73%) by PT IgG ELISA, 14 (54%) by FHA IgG ELISA, 11 (42%) by microagglutination, and 24 (92%) by at least one of the four methods. In young children, KPA was also more sensitive than FHA IgG ELISA ($P < 0.05$) and microagglutination ($P < 0.01$). No titer changes were observed in paired serum specimens obtained from 20 nonpertussis patients with cough.

In 35 of the 51 first serum samples obtained approximately 2 weeks after the onset of disease, PT antibody was not detected (below fourfold) by KPA. In all of the 51 second serum samples obtained approximately 1 month after disease onset, PT antibody was detected by KPA and the median PT antibody titer was 256-fold.

DISCUSSION

Since the most sensitive and specific assay for the serodiagnosis of pertussis is measurement of the anti-PT IgG antibody titer by ELISA, the anti-PT antibody titer measured by KPA was compared with that measured by ELISA in vaccinated children. PT antibody responses following vaccination were clearly demonstrated by KPA, and a significant correlation was observed between the results of PT IgG ELISA and those of KPA.

Although the indirect ELISA is very useful in serodiagnosis, it has a few drawbacks, such as requirements for special equipment and techniques, a long reaction time, and difficulties with technical reproducibility (7). In contrast, the KPA procedure is as simple as that of the microagglutination test, no special equipment is needed, and the reaction time is only 2 h. Furthermore, the excellent specificity and reproducibility of KPA were confirmed in this study.

As for the kinetics of PT antibody in pertussis patients measured by KPA, the PT antibody titer started to increase at 2 weeks after the onset of disease, when a paroxysmal stage of pertussis begins. All patients with pertussis had detectable PT antibody 1 month after disease onset, which is in the middle of the paroxysmal stage. The PT antibody titer reached a plateau between 1 and 2 months after disease onset, although the titer was not fully analyzed.

The sensitivity of KPA is slightly better than that of the PT IgG ELISA. The increase in sensitivity is, in part, attributable to the agglutinating antibody, including both IgG and IgM antibodies. The sensitivity of KPA was the highest among the four procedures tested and was equivalent to the total sensitivity of the four tests that were performed, i.e., the PT IgG and FHA IgG ELISAs, the microagglutination test, and KPA, even for young children.

The three situations in which serodiagnosis has been used are epidemiological studies, vaccine trials, and patient management. Because KPA is a simple and speedy diagnostic method, it is most applicable to patient management. Because pertussis is a life threatening disease for infants and its clinical manifestations are atypical, the good sensitivity of the test for young patients and its specificity for PT antibody rather than antibodies against other respiratory pathogens favor KPA for patient management. Because an accurate and speedy diagnosis is necessary for patient management, both antigen detection and serology are required. Detection of the organism by culture or PCR is rapid and specific, but not complete. Serodiag-
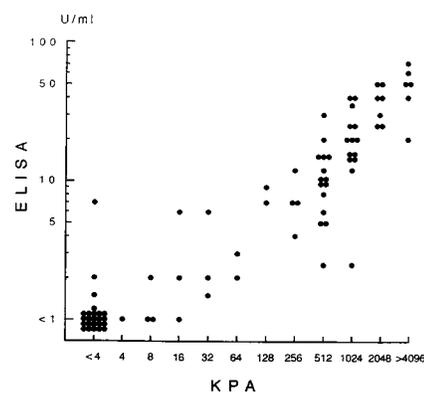

FIG. 2. Correlation coefficients between values obtained by KPA and those obtained by the indirect ELISA for 84 serum specimens collected from 21 children immunized with acellular DTP ($r = 0.92$).

nosis of pertussis by KPA with paired serum specimens is simple, speedy, specific, and sensitive enough to increase the accuracy of diagnosis.

In this study, KPA clearly demonstrated PT antibody responses induced by vaccination, indicating that KPA would facilitate assessments of the prevalence of PT antibody in a large population (unpublished data). In addition, because the same particle can be used to detect diphtheria and tetanus antibodies, it is applicable to epidemiological surveys.

KPA is a simpler and more rapid diagnostic procedure than ELISA, requiring neither special equipment nor special techniques. In this study, KPA was demonstrated to be as sensitive as PT IgG titer determination by ELISA, particularly for infants, and to be specific for the serodiagnosis of pertussis. We advocate the widespread application of KPA for the diagnosis of pertussis.

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