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Rapid Detection of the Poly-γ-D-Glutamic Acid Capsular Antigen of *Bacillus anthracis* by Latex Agglutination

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Abstract

Latex agglutination has been used to detect capsular polysaccharides from a variety of bacteria in body fluids. A latex agglutination assay was constructed for detection of the poly-γ-D-glutamic acid (γDPGA) capsular polypeptide of *Bacillus anthracis* in serum from animal models of pulmonary anthrax. The assay was able to detect γDPGA in serum from infected animals at concentrations of 100–200 ng/ml.

Latex agglutination immunoassay for capsular antigen has been used as an aid for diagnosis of infection by encapsulated bacteria such as Group B *Streptococcus*, *Haemophilus influenza* type b, *Streptococcus pneumonia* and *Neisseria meningitidis* and the encapsulated yeast *Cryptococcus neoformans* (1,4). In each instance, the target antigen has been a capsular polysaccharide. *Bacillus anthracis* is the etiological agent of anthrax. Interestingly, the *B. anthracis* capsule is a polypeptide composed of poly-γ-D-glutamic acid (γDPGA) (2,3). In a murine model of pulmonary anthrax serum γDPGA levels were determined to exceed 500 μg/ml during the latter stage of infection (6). Diagnosis of anthrax is problematic because identification by culture of blood or other clinical samples can take several days for a definitive identification. During that time, the clinical disease may progress to the point where successful treatment with antibiotics is very difficult (5). In a previous study, we reported that immunoassay by ELISA for γDPGA in serum can be an effective surrogate for bacterial culture in early diagnosis of pulmonary anthrax (6). However, identification of antigenemia by ELISA can take several hours and requires careful quality control for the reagents. The goal of the present study was to evaluate the extent to which a latex agglutination assay can serve as a substitute for a quantitative ELISA for detection of γDPGA in a manner similar to latex agglutination assays for capsular polysaccharides.

A quantitative ELISA for detection of γDPGA was constructed using the γDPGA mAb F26G3 in the solid capture phase and HRPO-labeled mAb F26G3 as the indicator as described (6). A

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carbodiimide kit was used to covalently link 0.982 μm diameter carboxylated latex microparticles (Polysciences, Inc.) to mAb F26G3 according to the manufacturer's directions.

An initial experiment evaluated the extent to which mAb-coated latex beads were agglutinated by purified γ_DPGA. γ_DPGA was purified from culture supernatant fluid of *B. anthracis* (Pasteur) as described (6). The agglutination assay was done by pipetting 20 μl of sample containing γ_DPGA diluted in phosphate buffered saline (PBS) or negative controls (PBS alone or uninfected samples) onto a Directigen Meningitis Combo Test Card (Becton Dickinson). mAb F26G3-conjugated latex beads (10 μl) were added to the sample, mixed and spread into a larger circle with a diameter of approximately 12 mm. The test card was then rocked in a circular motion for 2 min. As shown in Fig. 1A, the strongest reaction occurred at 1000 ng/ml; this was assigned an agglutination score of 6 (the concentration of PGA is listed below each reaction and the agglutination score is listed to the right of each reaction in Fig 1A). Agglutination begins to accumulate at the outer edge of the sample at 500 ng/ml (score of 5). Between γ_DPGA concentrations of 250 and 31 ng/ml, the agglutination reaction that forms at the edge of the sample progressively diminishes (score 4−1). No agglutination was evident at 16 ng/ml. This concentration series was tested at least three times with identical results. The ability of four other individuals to independently reproduce the agglutination reactions of the concentration series is shown in Fig. 1B. The observed scores closely tracked the expected agglutination scores shown in Fig. 1A.

We next evaluated the γ_DPGA agglutination assay with serum from murine and non-human primate models of pulmonary anthrax. BALB/c mice were infected by the pulmonary route with spores of *B. anthracis* as described (6). Also, sera from cynomolgus monkeys that were infected via the pulmonary route with *B. anthracis* spores were also used. The sera were collected at various times after challenge and evaluated by the quantitative ELISA and by the latex agglutination immunoassay. We have previously shown that the concentration of γ_DPGA in serum (determined by ELISA) with spleen CFU (6). Interestingly, pulmonary anthrax is asynchronous in both animal models due to the fact that dissemination of *B. anthracis* from the lung is complex (8). As a consequence, plotting antigen concentration as a function of time after challenge does not accurately reflect the extent of disseminated disease in individual animals. Instead, the results are plotted as the latex agglutination score *vs*. the serum γ_DPGA concentration as determined by quantitative ELISA for serum samples taken at various times after infection. The results (Fig. 2) showed that γ_DPGA concentrations as determined by latex agglutination closely tracked results from the quantitative ELISA, with detection of serum γ_DPGA occurring at 100–200 ng γ_DPGA per ml serum. Detection of γ_DPGA at concentrations below 100 ng per ml in serum was variable; there is a slight loss of sensitivity when compared to γ_DPGA diluted in PBS.

Our previous studies in mice and an addition study in rabbits determined that injected γ_DPGA is degraded in vivo and shed into the urine (9,10). As a consequence, this study evaluated the ability of the latex agglutination assay to detect degraded γ_DPGA in urine. BALB/c mice were injected via the intravenous route with 500 μg γ_DPGA, and urine was collected at various times after treatment. Concentrations of γ_DPGA in the urine were determined by the quantitative ELISA, and agglutination of the latex beads was assessed as described in Fig. 1. As shown in Figure 3, urine samples collected after 1 and 2 h showed γ_DPGA concentrations of 9,400 and 5,000 ng/ml, respectively. However, the agglutination score was only 4. A similar loss of sensitivity was observed with the remaining urine samples. Compared to testing the urine samples by ELISA, the sensitivity of the latex agglutination assay decreased in each sample by a minimum of 15-fold. By ELISA six of the urine samples had γ_DPGA concentrations of between 800–1200 ng/ml; in serum or PBS these concentrations would produce an agglutination rank of five or six but in urine they produced a rank of two. In a previous report we determined that the ELISA has shown a decrease in sensitivity in detecting short γ_DPGA

25-mers like those found in urine (10). Therefore, the loss in sensitivity of the latex agglutination assay is probably underestimated. There was no loss in sensitivity of the latex agglutination assay when naïve BALB/c urine was spiked with purified native γ_DPGA, ruling out the possibility of an inhibitor in the urine (data not shown).

In an effort to assess the impact of molecular size of γ_DPGA on the sensitivity of the latex agglutination assay, latex agglutination scores were evaluated for various concentrations of native purified γ_DPGA as well as synthetic 25-mer (3.5 kD) and 10-mer (1.3 kD) γ_DPGA. Synthetic γ_DPGA was prepared as described (7). The results (Fig. 4) showed a substantial loss of sensitivity of the latex agglutination assay for detection of the 25-mer compared with native γ_DPGA. The latex agglutination assay had little ability to detect the 10-mer. These results suggest that the presence of partially degraded γ_DPGA in urine accounts for the decreased sensitivity of the latex agglutination assay in γ_DPGA-treated mice.

Our results indicate that purified γ_DPGA and γ_DPGA in serum from murine and non-human primate models of pulmonary anthrax is readily detected by a latex agglutination immunoassay constructed with a mAb that is specific for γ_DPGA. In contrast, latex agglutination is less effective in detection of smaller molecular weight fragments of γ_DPGA that might be found in urine.

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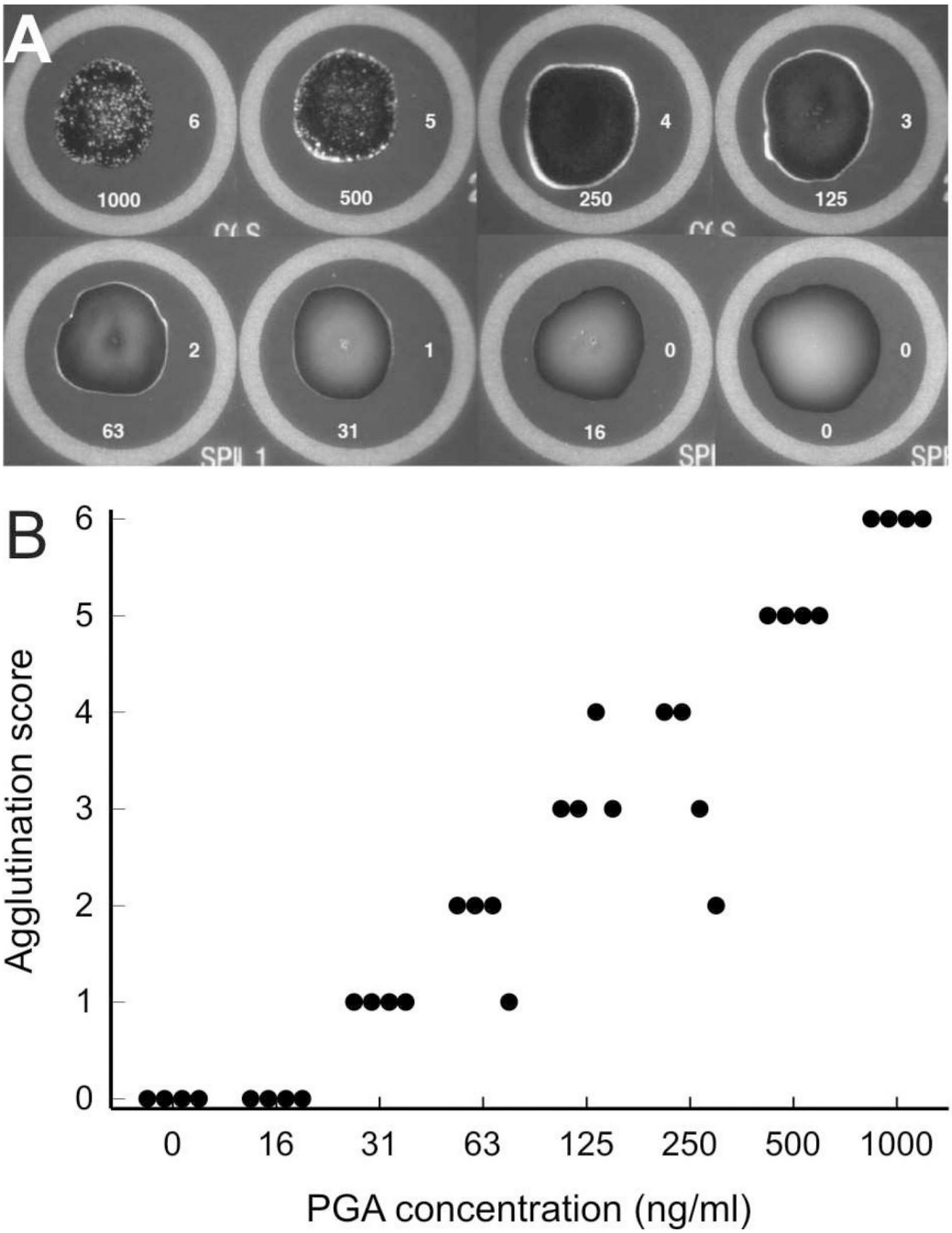

Fig. 1.

Panel A – Agglutination of latex beads coated with γDPGA MAb F26G3 by purified γDPGA. The concentration of γDPGA (ng/ml) is indicated at the bottom of each reaction mixture. The agglutination score assigned to each reaction is indicated at the right of each reaction mixture. Panel B – Agglutination reactions were scored in a blind fashion by four individuals. Scoring was based on the images shown in Panel A. Results of scoring for γDPGA at various concentrations by individual readers is shown for each γDPGA concentration.

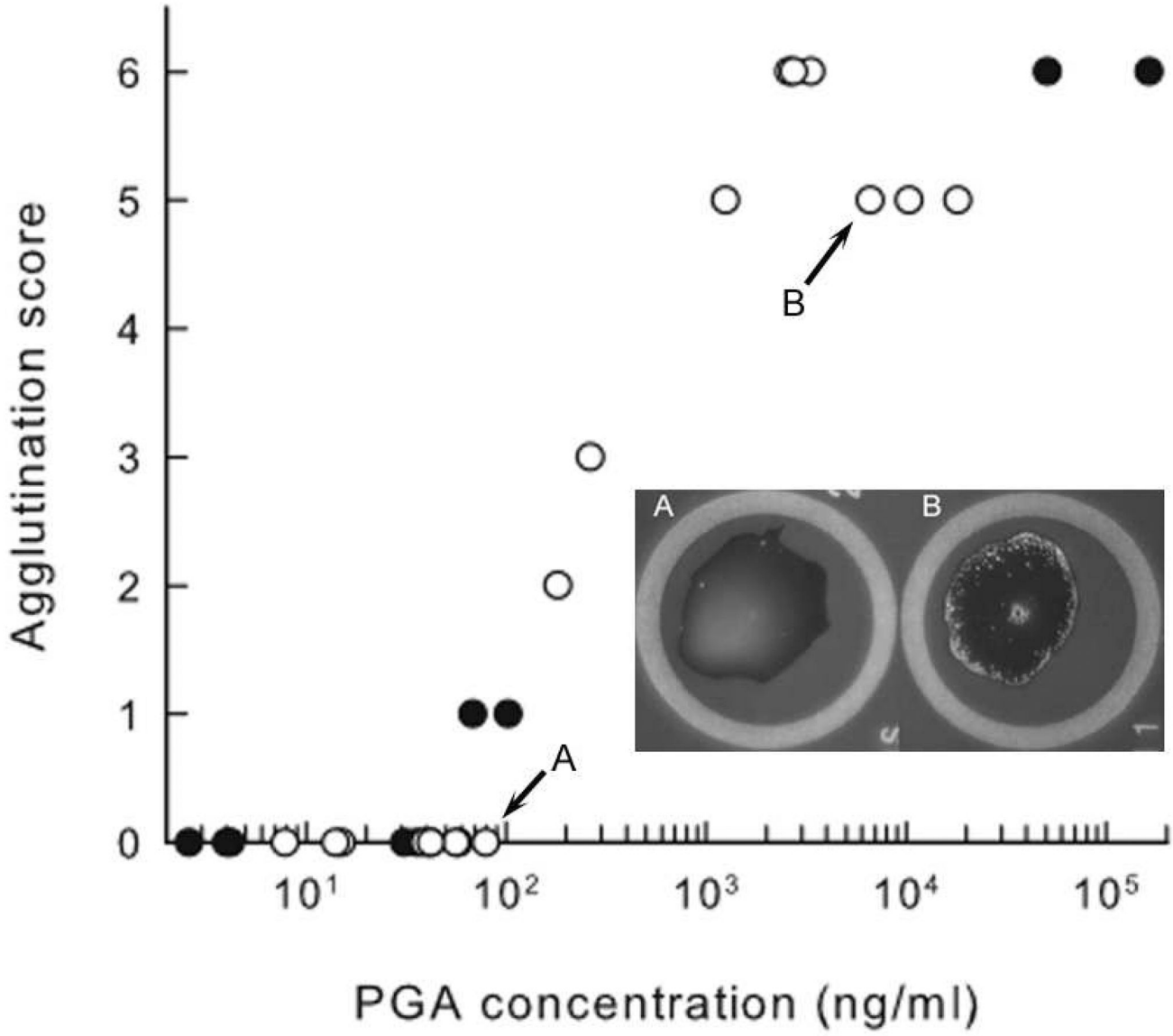

Fig. 2.

Detection of γ_DPGA by quantitative antigen capture ELISA (X-axis) and by latex agglutination (Y-axis) in serum from murine (●) and non-human primate (○) models of pulmonary anthrax. Mice and cynomolgus monkeys were challenged by the pulmonary route with lethal doses of *B. anthracis* spores. Blood was collected at various times after challenge and assayed for γ_DPGA. Each data point represents a single serum sample that was analyzed by both the ELISA (PGA concentration) and by latex agglutination (Agglutination score). Results from the latex agglutination immunoassay of undiluted sera are reported as the agglutination score as described in Fig. 1A. Two agglutination reactions are shown that correspond to the samples labeled A and B.

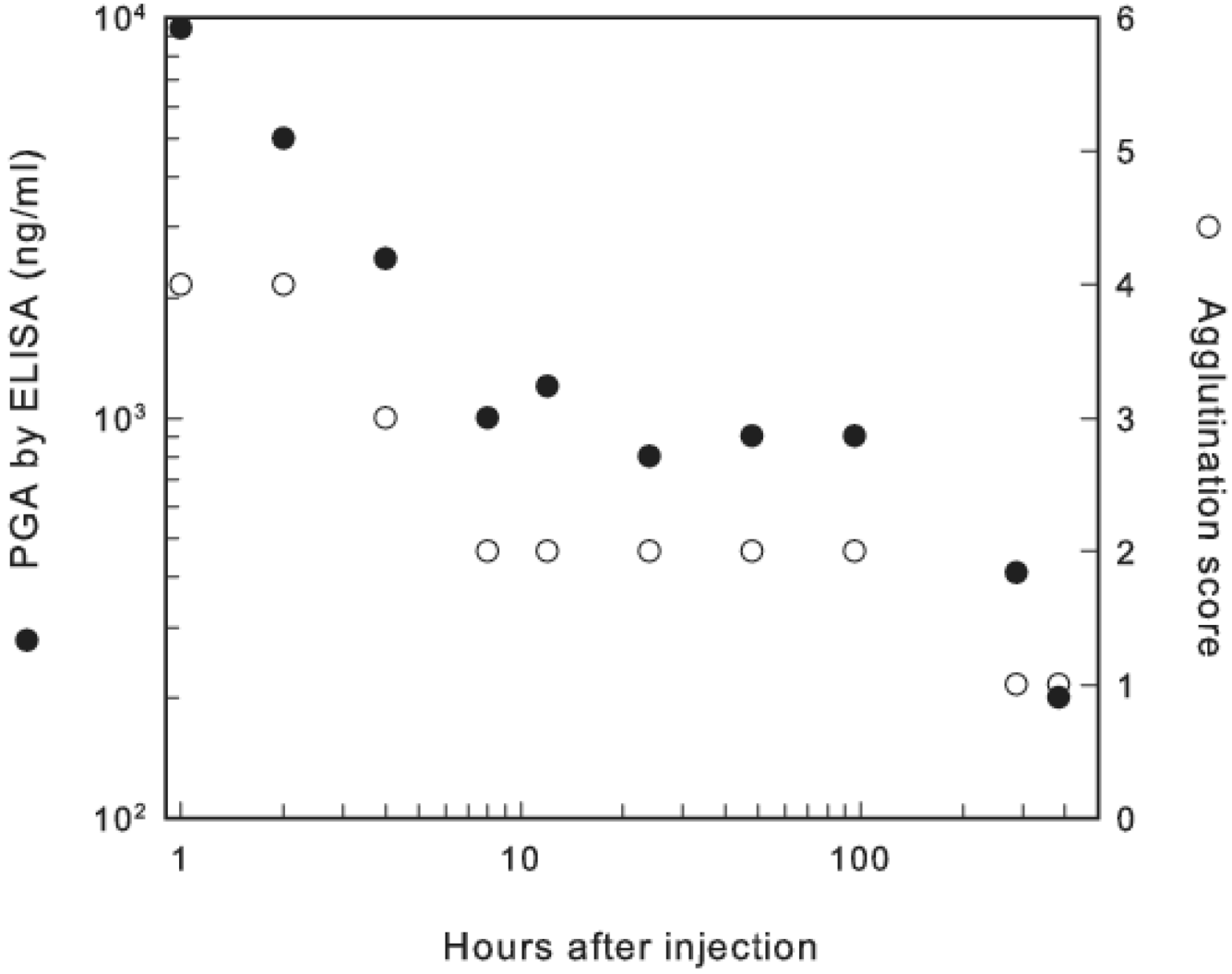

Fig. 3.

Detection of γ_DPGA in urine of mice that were injected by the intravenous route with 500 μg γ_DPGA. Urine was collected at various times after treatment and analyzed by the quantitative ELISA (Y-axis, left - •) and by latex agglutination (Y-axis axis, right - ◦). Latex agglutination scores were determined with undiluted samples as described in Fig. 1A.

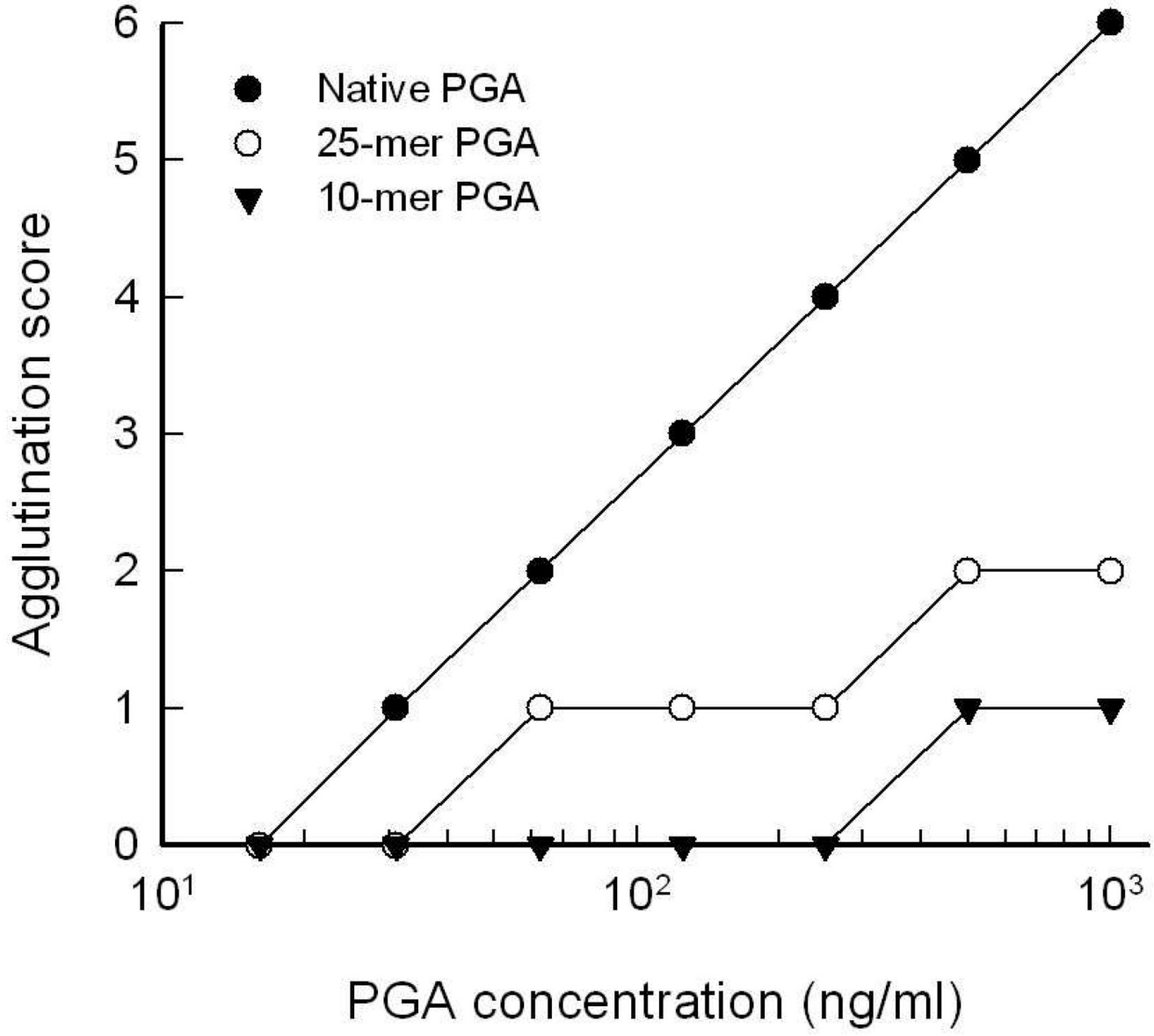

Fig. 4.

Agglutination of latex beads coated with γDPGA MAb F26G3 by various concentrations of native γDPGA and synthetic γDPGA of 10 or 25 amino acids. Concentrations of γDPGA were determined from the dry weight of each antigen. Latex agglutination was scored as described in Fig. 1A.