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The serum polymeric IgA antibody response to typhoid vaccination; its relationship to the intestinal IgA response

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SUMMARY

The relationship between the IgA antibody response in serum (total and polymeric IgA) and intestinal secretions was examined in volunteers subjected to oral and parenteral typhoid vaccination. After oral vaccination (three doses of 10^{11} live Ty21a vaccine given at 48-hr intervals), serum pIgA antibody to typhoid lipopolysaccharide (LPS) was detected in seven of the 14 subjects ($46{\cdot}4 \pm 59$ U/100 μl, mean $\pm$ SD). However, all 14 showed a significant intestinal IgA response (993 ± 2516 and 9349 ± 6754 U/mg pre- and post-vaccine; $t = 5{\cdot}25$, $P = 0{\cdot}0002$). The level of pIgA antibody declined rapidly, whereas intestinal IgA antibody levels remained elevated. Serum pIgA antibody was also found after parenteral immunization (two doses of 5×10^8 heat-killed bacteria given 14 days apart to six subjects), but an intestinal IgA antibody response was detected in these individuals only after a subsequent course of the oral vaccine given 1 month after initial parenteral immunization. Changes in serum pIgA antibody followed those of total serum IgA antibody rather than those of intestinal antibody. The results indicate that a serum pIgA response can be induced by an antigenic stimulus delivered either orally or parenterally, whereas an intestinal IgA response is induced only by a local antigen stimulus. The regulation of serum pIgA and intestinal IgA appear to be independent.

INTRODUCTION

Immunoglobulin A, the predominant immunoglobulin in the mucosal secretions of humans (Tomasi *et al.*, 1965), also constitutes a significant proportion (6–16%) of the circulating immunoglobulin (Conley & Delacroix, 1987). In humans the relationship between IgA in serum and secretions is not well understood. There have been several studies demonstrating that recovery from mucosal infections parallels the secretory IgA antibody response better than the serum antibody response (Tomasi & Bienenstock, 1968; Ogra, 1985).

In humans, 98% of the IgA entering the intestinal lumen is produced locally within the intestinal lamina propria (Jonard *et al.*, 1984). Most of this IgA is polymeric IgA (Tomasi *et al.*, 1965; Heremans, 1974). The glycoprotein secretory component (SC) binds this polymeric IgA (pIgA) and translocates it across the intestinal epithelial cells into the intestinal lumen, where it exists as secretory IgA, a polymeric IgA bound to SC (Brown, Isobe & Nakane, 1976; Brandtzaeg, 1978; Nagura, Nakane & Brown, 1979). In contrast the major source of IgA in the vascular compartment in humans is the bone marrow (Hijmans, Shuit & Hulsing Hesselink, 1971), with approximately 85% of serum IgA being monomeric (Heremans, 1974; Delacroix *et al.*, 1983; Newkirk *et al.*, 1983). The source of the monomeric IgA is the bone marrow (Radl *et al.*, 1974; Kutteh, Prince & Mestecky, 1982). Controversy surrounds the origins of the small fraction of human serum IgA that is polymeric. It has been suggested that the pIgA originates at mucosal surfaces (Heremans, 1974; André *et al.*, 1980). Others dispute this (Conley & Delacroix, 1987) as pIgA antibody responses in serum can be generated by parenteral immunization with tetanus toxoid in primed individuals (Mascart-Lemone *et al.*, 1987). pIgA may originate from sites other than mucosal surfaces. For example, Kutteh *et al.* (1982) have demonstrated that tonsillar, lymph node and peripheral blood cells produce approximately equal proportions of monomeric and pIgA.

In this paper we address the uncertainty regarding the origins of serum pIgA in humans by using typhoid vaccination as a stimulus to the immune system. We have previously demonstrated that the live oral typhoid vaccine Ty21a (Germanier & Fürer, 1975) reliably generates a measurable immune response in intestinal fluid and serum (Bartholomeusz *et al.*, 1986; Forrest, 1988). The effect of oral vaccination with Ty21a was compared with that of parenteral immunization with conventional heat-killed typhoid vaccine. Antigen-specific pIgA in serum was measured by radioimmunoassay (Bartholomeusz

Abbreviations: BSA, bovine serum albumin, ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; pIgA, polymeric IgA; RIA, radioimmunoassay; SC, secretory component.

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et al., 1989). Intestinal IgA antibody and total IgA antibody in serum were measured by enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Subjects

Twenty healthy volunteers (mean age 25 years; range 19 to 52) took part in these studies. None had a history of typhoid fever and none had previously been immunized against the disease. They gave written informed consent to the study which conformed to the ethical standards of the Human Ethics Committee of the Royal Adelaide Hospital and University of Adelaide and the Helsinki declaration of 1975.

Vaccine

The oral Ty21a vaccine was prepared by Dr G. Boehm of Enterovax Research Pty. Ltd, Adelaide, in the form of lyophilized oral doses each comprising 10^{11} viable organisms. Volunteers reported for the vaccine after an 8-hr fast. After drinking 50 ml of 2% sodium bicarbonate to neutralize stomach acid, they ingested the vaccine resuspended in 50 ml of 0·9% sodium chloride.

The parenteral vaccine was the one commercially available (Commonwealth Serum Laboratories, Melbourne) in Australia. Each dose consisted of 5×10^8 smooth *Salmonella typhi* organisms that had been heat-killed and was administered in 0·5 ml as a subcutaneous injection.

Study design

There were three groups of volunteers. Groups 1 and 2 received oral Ty21a according to an identical schedule, but in Group 2 the intestinal antibody response was followed to 6 weeks to better define the kinetics of the immune response, which was followed to 3 weeks in Group 1.

Group 1. The seven subjects in Group 1 each received three doses of oral vaccine 48 hr apart. Samples of intestinal fluid were obtained before vaccination and 7, 14 and 21 days after the first dose. Samples of blood were obtained every 3 to 4 days after vaccination.

Group 2. The seven subjects in this group also received three doses of oral Ty21a 48 hr apart. Samples of intestinal fluid were obtained before vaccination and on Days 15, 29 and 43 after vaccination. Blood was obtained on Days 0, 8, 12, 15, 29 and 43.

Group 3. These six subjects were initially vaccinated by subcutaneous injection with $0{\cdot}5 \times 10^9$ of heat-killed *Salmonella typhi* on Day 0 and Day 14. Three doses of Ty21a were administered orally on Days 33, 35 and 38. Each dose contained 1×10^{11} organisms.

Intestinal fluid was obtained from these volunteers before vaccination and on Days 31 (3 weeks after the second parenteral vaccination and just before the commencement of oral vaccination) and 52 (3 weeks after the commencement of oral vaccination).

Samples

Intestinal fluid was obtained from the upper jejunum with an 'ANPRO AN20 Andersen' Tungsten Weighted Sump Tube (HW Andersen Products, Oyster Bay, NY). Fluoroscopy was used to position the tube. Samples were centifuged at 4000 ***g*** at 4° and stored in aliquots at −70° until the assays were performed. Serum samples were stored in aliquots at −20°.

Assays

Polyvinyl microtitre plates containing 96-wells (Costar Data Packaging Corp, Cambridge, MA) were used for all the assays.

The plates were coated overnight at 4° with 5 μg/ml *Salmonella typhi* Ty2 LPS (Sigma, Catalogue No. 6386, St Louis, MO) that had been linked to methylated bovine serum albumin (5 μg/ml in 0·1 M sodium carbonate/bicarbonate coating buffer, pH 9·6; 100 μl per well). The wells were blocked with 150-μl aliquots of 0·5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 45 min at room temperature before washing with PBS containing 0·05% Tween 20.

ELISA for IgA antibody in intestinal fluid and serum

Antibody against *S. typhi* LPS was measured in intestinal fluid and serum as described previously (Bartholomeusz *et al.*, 1986) using an IgA-specific antibody coupled to alkaline phosphatase (Kirkegaard and Perry Laboratories, Gaithersberg, MD). Each assay included as a positive control the serum of a convalescent typhoid patient with high antibody titre and as a negative control the serum of a person never exposed to typhoid. The serum antibody results are expressed in antibody units per 100 μl (the volume added to each well). One unit of antibody was defined as the reciprocal dilution calculated to produce an optical density at 405 nm of 0·15 (Bartholomeusz *et al.*, 1986). As the immunoglobulin content of intestinal fluid is variable, the antibody results for intestinal fluid are expressed in terms of units of typhoid specific antibody per milligram of immunoglobulin. Immunoglobulin concentrations were measured by single radial immunodiffusion (Bartholomeusz *et al.*, 1986).

Radioimmunoassay (RIA) for serum pIgA antibody to S. typhi *LPS*

The assay for antigen-specific serum pIgA antibody was performed as previously described (Bartholomeusz *et al.*, 1989). In brief, 0·5-ml aliquots of serum were totally depleted of IgM by affinity chromatography using 2-ml columns containing IgM-specific antibodies coupled to Sepharose 4B. The IgM-free samples were incubated in wells coated with typhoid LPS and bound pIgA antibody was detected, after washing, by adding 10 ng of radiolabelled secretory component to each well (Bartholomeusz *et al.*, 1989). The radioactivity bound to individual wells cut from a plate was measured using an LKB 1282 Compugamma gamma counter. The end-point was defined as the dilution (= 1 unit) giving five times the background binding (the latter being the binding of SC to antigen in the absence of serum or antibody). Results are expressed as units of pIgA antibody per 100 μl.

The removal of IgM by the affinity chromatography step was confirmed by ELISA and single radial immunodiffusion.

Statistical analyses

Statistical analysis of the differences between levels of antibody to typhoid before and after vaccination was performed using the Students' paired *t*-test. The Spearman rank correlation coefficient (r_s) was used to determine the relationship between serum pIgA antibody, intestinal IgA antibody and total serum IgA antibody.

RESULTS

Group 1. Serum pIgA antibody to typhoid LPS was detected in four of the seven subjects after oral immunization. The peak level of serum anti-typhoid pIgA antibody after vaccination was 55·5 ± 70·6 U/100 μl (mean ± SD). The time-course of the serum pIgA response, the intestinal IgA response and the total serum IgA response is illustrated in Fig. 1. All seven subjects had an intestinal response to the vaccine (1856 ± 3454 and 8826 ± 6689 U antibody/mg pre- and post-vaccine, respectively). This difference was statistically significant ($t = 4·086$, $P = 0·0064$). The peak intestinal anti-typhoid IgA antibody was found on Day 14 (three subjects) or Day 21 (four subjects). With the exception of one subject, serum pIgA antibody could not be detected 21 days after vaccination, despite the prevalence of anti-typhoid IgA antibody in intestinal fluid at this time. Although there was a rise in total serum IgA anti-typhoid antibody in all seven subjects (from 43 ± 26 pre-vaccination to 541 ± 535 antibody U/100 μl post-vaccination; mean ± SD), this difference just failed to reach significance, $t = 2·434$, $P = 0·0509$). The pIgA response closely paralleled the total serum IgA response (Fig. 1).

Group 2. Anti-typhoid serum pIgA antibody was detected after oral vaccination in three of the seven subjects (27·3 ± 55 U/100 μl; mean ± SD). All seven subjects exhibited a significant rise in intestinal IgA antibody to typhoid after vaccination. The mean antibody levels pre-vaccination and post-vaccination were 131 ± 223 and 9867 ± 7315 U/mg, respectively. There was a rise in total serum IgA antibody to typhoid after vaccination (76 ± 81, cf. 323 ± 283 U/100 μl). This difference was significant ($t = 2·588$, $P = 0·0413$). The time-course of the antibody response was similar to that illustrated in Fig. 1. Anti-typhoid serum pIgA antibody levels peaked between Days 12 and 15 post-vaccination and could not be detected in serum by Day 29, although anti-typhoid IgA antibody was still present in intestinal fluid collected on Days 29 and 43 (5345 ± 8701 and 5416 ± 7680 U/mg, respectively).

Overall, considering the 14 volunteers vaccinated by the oral route, a significant rise in intestinal IgA antibody and total serum IgA antibody to typhoid was evident after vaccination (intestinal IgA: 993 ± 2516 U/mg pre- ; 9349 ± 6754 U/mg, post- ; $t = 5·25$, $P = 0·0002$; total serum IgA: 59·5 ± 60 U/100 μl pre- ; 432 ± 427 U/100 μl post- ; $t = 3·271$, $P = 0·0061$).

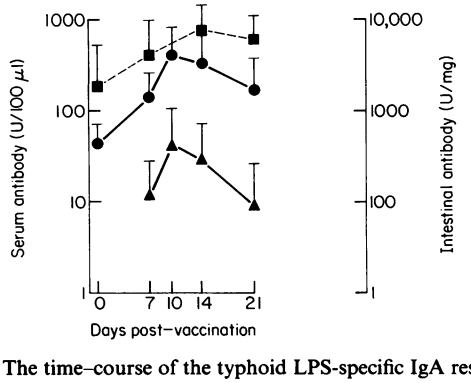

Figure 1. The time-course of the typhoid LPS-specific IgA response to oral typhoid vaccination in seven subjects. Serum pIgA was measured by RIA, total serum IgA was measured by ELISA. Vertical bars represent 1 SD. (■) Intestinal IgA; (●) total serum IgA; (▲) pIgA.

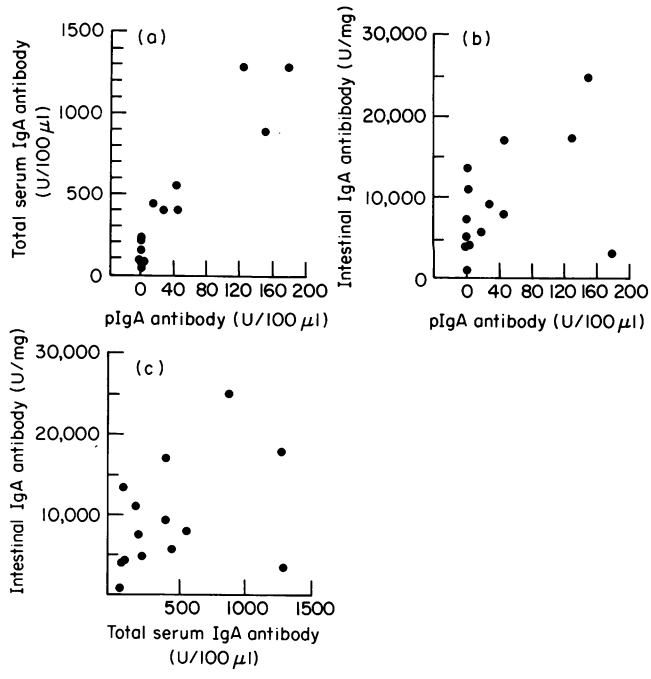

Figure 2. Relationship between serum and intestinal typhoid LPS-specific IgA antibody in the 14 volunteers given the oral vaccine. (a) Correlation between anti-typhoid serum pIgA antibody and total serum anti-typhoid IgA antibody. (b) Correlation between intestinal IgA and serum pIgA anti-typhoid antibody. (c) Correlation between intestinal IgA antibody and total serum IgA antibody to typhoid LPS.

None of the volunteers had pIgA antibody to typhoid detectable before vaccination. Seven developed pIgA antibody to typhoid after oral vaccination (46·4 ± 59 U/100 μl; post-vaccination). This difference was significant ($t = 2·477$, $P = 0·0278$). There was a significant correlation between the level of anti-typhoid serum pIgA antibody and total serum anti-typhoid IgA antibody found in these 14 volunteers after oral vaccination ($r_s = 0·841$, critical value of r_s at the 0·05 level of significance for the one-tailed test = 0·456, Fig. 2a). On the other hand, no correlation was evident between the peak level of intestinal IgA antibody and serum pIgA antibody to typhoid after vaccination ($r_s = 0·227$, critical value at 0·05 level of significance = 0·456, Fig. 2b). Neither was there a correlation between the intestinal IgA antibody level and the total antityphoid serum IgA antibody after vaccination ($r_s = 0·373$ critical value of r_s at 0·05 level of significance = 0·456, Fig. 2c).

Group 3. Three of the six volunteers vaccinated by injection had detectable levels of serum pIgA against *S. typhi* LPS before vaccination (28·3 ± 36·5 antibody U/100 μl). pIgA antibody rose in all six volunteers (130·5 ± 94·6 U/100 μl, Fig. 3a) after the injection ($t = 2·302$, $P = 0·069$) in the paired t-test; $P < 0·05$ in the Wilcoxon signed rank test. The peak level of pIgA antibody to typhoid was seen 10–13 days after injection in five volunteers and on the 10th day after the second injection in the sixth. The level of serum pIgA antibody to typhoid declined by Day 33 (47·8 ± 35·4 U/100 μl). A further rise in anti-typhoid serum pIgA occurred after the oral vaccine was administered with the peak level seen on Day 41 (three subjects) or Day 44 (three subjects) after the injection (or Days 9 and 11 after the first oral dose, respectively). The difference between the peak pIgA antibody levels before and after the oral vaccine was administered

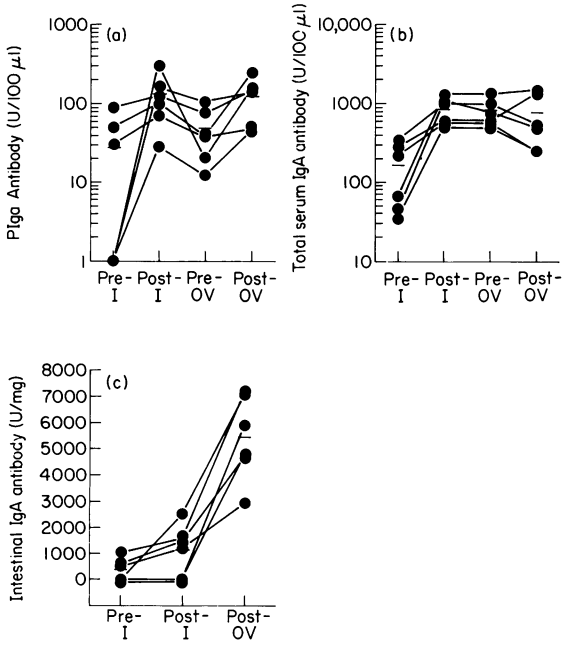

Figure 3. Typhoid LPS-specific IgA antibody response to parenteral vaccination followed by oral vaccine one month later. The group consisted of six volunteers. Horizontal bars indicate means. (a) pIgA antibody. (b) Total serum IgA antibody. (c) Intestinal IgA antibody. Pre-I, pre-injection; Post-I, post-injection; Pre-OV pre-oral vaccine; Post-OV, post-oral vaccine.

(47·8 ± 35·4 and 127·2 ± 73·2 U/100 μl) was significant ($t = 2·644$, $P = 0·0457$).

There was no significant difference in intestinal IgA antibody to typhoid LPS after the parenteral vaccination (344 ± 415 pre- and 1124 ± 975 post- ; U/mg, $t = 2·074$, $P = 0·0927$). However, all six subjects had a rise in intestinal IgA antibody to typhoid after oral immunization (Fig. 3b) from 1124 ± 975 before to 5410 ± 1615 U/mg (mean ± SD) after immunization. This difference was significant ($t = 6·734$, $P = 0·0011$). The mean total serum IgA antibody levels to typhoid before and after the injection were 166 ± 135 and 858 ± 341 U/100 μl (mean ± SD), respectively (Fig. 3c). This difference was significant ($t = 4·32$; $P = 0·0076$). The level of serum IgA antibody hardly declined before the oral vaccine was administered. The levels of antibody before and after oral vaccination were 808 ± 320 and 777 ± 553 U/100 μl, respectively ($t = 0·1696$, $P = 0·8719$).

DISCUSSION

In this study we have shown that a serum pIgA antibody response can be generated by delivery of an antigenic stimulus to the intestine. Volunteers given the attenuated live oral typhoid vaccine developed an early, short-lived serum pIgA antibody to typhoid, peak levels being detected 10–14 days after vaccination. A serum pIgA antibody response was also produced by parenteral immunization with killed typhoid organisms. In contrast, an intestinal immune response was only generated by oral vaccination. Intestinal IgA antibody was demonstrated in all the volunteers given three doses of 10^{11} organisms; findings consistent with our previous report (Bartholomeusz *et al.*, 1986). In the earlier study, intestinal IgA antibody to typhoid was detected as long as 1 year after vaccination (Bartholomeusz *et al.*, 1986). In the present study, anti-typhoid IgA antibody was still present in intestinal fluid 54 days after vaccination, at a time when the serum pIgA antibody had disappeared.

Parenteral typhoid vaccination did not elicit on intestinal typhoid-specific IgA antibody response. This is consistent with previous reports that parenteral immunization with various antigens usually does not stimulate a secretory IgA immune response in external secretions (Heremans, 1974; Ogra *et al.*, 1968). Intestinal IgA antibody to typhoid was detected in these same subjects after they were given the oral vaccine. Three of the volunteers given the parenteral killed organism had detectable levels of serum pIgA antibody to typhoid before they received the vaccine and mounted the greatest response to it. Although they had no recollection of being previously immunized to the organism, these results raise that possibility.

This study confirms previous observations that even when the IgA class of antibody is examined after intestinal infection or immunization, there is no correlation between the intestinal antibody response and serum antibody (LaBrooy *et al.*, 1980; Chau *et al.*, 1981; Jertborn, Svennerholm & Holmgren, 1986). In the present study there was no correlation between the peak level of anti-typhoid IgA antibody in intestinal fluid and the anti-typhoid serum pIgA antibody or total anti-typhoid serum IgA antibody. There was a close relationship between the serum pIgA antibody response and the total serum IgA antibody response. The kinetics of the pIgA response parallelled the serum IgA antibody response after oral immunization. These results also confirm and extend the observations of Mascart-Lemone *et al.* (1987), who demonstrated the occurrence of a short-lived serum polymeric IgA response that paralleled the monomeric IgA response in serum following parenteral vaccination with tetanus toxoid. They did not address the question of the relationship of these serum responses to that seen in the intestine.

This study demonstrates that IgA antibody in serum does not reflect IgA antibody produced locally in the intestine in health. This conclusion does not necessarily apply to different circumstances that may operate in a number of diseases or where there is damage to the intestinal mucosa. The presence of pIgA antibody to gliadin in the serum of patients with coeliac disease ingesting gluten (Mascart-Lemone *et al.*, 1988; Bartholomeusz *et al.*, 1988), the presence of pIgA in skin deposits in dermatitis herpetiformis (Unsworth *et al.*, 1982), which disappear with gluten exclusion, and the deposition of pIgA in the kidneys of patients with IgA nephropathy (Béne, Gaure & Duheille, 1982; Tomino *et al.*, 1982) has led to the suggestion that the pIgA originates from mucosal surfaces in these diseases. Experimental secondary infection with Influenza A virus, a virus that infects mucosal surfaces, resulted in a predominantly pIgA antibody response to the viral haemagglutinin in serum, probably reflecting pIgA antibody derived from secretory surfaces (Brown *et al.*, 1985).

Although the present study suggests strongly that the pIgA antibody detected in the circulation after vaccination, whether oral or parenteral, is not derived from the intestinal mucosa, it does not shed light directly on where it comes from. Kutteh *et al.* (1982) have demonstrated that upto 40% of the IgA secreted by activated lymphnode cells is polymeric. Lymphocytes in peripheral blood produce pIgA both spontaneously and after mitogen stimulation (Kutteh *et al.*, 1980), though these cells may be migrating through the blood stream prior to homing back to the mucosa, having been initially activated by antigen in a mucosal site (McDermott & Bienenstock, 1979; Czerkinsky *et al.*, 1987;

Forrest, 1988). The appearance of antigen-specific pIgA in the circulation and not in intestinal secretions after parenteral vaccination also argues in favour of serum pIgA originating in extra-mucosal sites.

In summary, this study suggests that a serum pIgA response can be produced by delivery of an immune stimulus to either the mucosal or vascular compartments in healthy subjects and that the regulation of the production of pIgA and intestinal IgA are independent. This may change in disease states.

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