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Specific Immune Response in the Human Respiratory Tract following Oral Immunization with Live Typhoid Vaccine

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Specific antibody responses in the lower respiratory tract of human subjects to orally administered *Salmonella typhi* Ty21a are reported. These responses, predominantly of the immunoglobulin G class, were determined to be a transudate from serum. These results were supported by the similarity in responses to parenteral administration of heat-killed typhoid vaccine. Specific immunoglobulin A antibody was a poor contributor to the respiratory antibody response to either vaccine.

The development of the concept of the common mucosal immune system, whereby the local presentation of an antigen at one mucosal surface can stimulate an immune response at distant mucosal surfaces (6, 8, 9, 11, 16, 17), has led to the examination of oral vaccination as a means of stimulating an effective immune response in the respiratory tract. The live orally administered typhoid vaccine organism, *Salmonella typhi* Ty21a, has been considered as a potential carrier of heterologous protective antigens (3, 5, 13), so the demonstration that *S. typhi* Ty21a is capable of stimulating a respiratory tract immune response following oral administration would permit the subsequent examination of this or similar attenuated organisms as vectors for antigens of respiratory pathogens.

Seventeen healthy nonsmoking adults (five women and twelve men, 18 to 34 years of age), with no previous exposure to *S. typhi* through vaccination or disease, agreed to participate in this study. All subjects provided prior written, informed consent. The study was approved by the Human Ethics Committee of the Royal Adelaide Hospital and the Committee on the Ethics of Human Experimentation of the University of Adelaide.

The subjects were randomly allocated to three study groups; the vaccination and sampling schedules are detailed in Table 1.

For oral vaccination, the organism used was *S. typhi* Ty21a. Each vaccine dose comprised 1.7×10^{11} viable organisms (representing 33% of the total dose) and was administered as previously described (4). This dose is known to consistently stimulate a significant specific intestinal immunoglobulin A (IgA) antibody response without noticeable adverse effects (1, 4). The vaccine was supplied by Enterovax Limited, Salisbury, South Australia, Australia.

The inactivated typhoid vaccine (group C) was the commercial heat-killed preparation (typhoid vaccine; Commonwealth Serum Laboratories, Parkville, Victoria, Australia) in doses of 0.5 ml (5×10^8 organisms).

Serum samples for antibody determination were collected before and every 3 to 4 days after vaccination until day 22. Intestinal (jejunal) fluid samples (groups B and C) were collected and processed as previously described (1, 4, 6) (Table 1).

Saliva was collected from the buccal opening of one of the parotid ducts by using a Curby cap following stimulation with lingual citric acid.

Bronchoalveolar lavage fluid (BAL-F) was collected by the sequential installation of three 50-ml aliquots of sterile buffered saline by fiber-optic bronchoscopy into the right middle lobe, ensuring that the first recovered aliquot was stored and assayed separately from the subsequent two, since this initial sample may contain a disproportionate amount of bronchial airway material. The other two aliquots were pooled, since the BAL-F recovered in these subsequent washes usually represents the alveoli (2, 12). The collected fluid was centrifuged at $800 \times g$ to remove cells and debris and stored at −70°C until required. Sampling timing was selected to enable concurrent comparison of the peak intestinal specific antibody response following oral vaccination (group B) with that in the respiratory tract.

Class-specific anti-*S. typhi* lipopolysaccharide (LPS) antibodies were quantified by a previously described enzyme-linked immunosorbent assay (ELISA) (4). Starting dilutions of serum (1:10), intestinal fluid (1:2), and saliva and BAL (both undiluted) were titrated twofold down the plate in duplicate. A known anti-*S. typhi* LPS antibody high-titer serum sample obtained from a convalescent typhoid patient and a known low-titer serum sample from an unexposed individual were included as positive and negative controls.

A modified single radial immunodiffusion method was used to determine the total class-specific immunoglobulin content of serum and secretions (1, 7).

Serum specific antibody responses are presented as the reciprocal of the final dilution that gave an optical density of 0.15 ELISA absorbance units/0.100 ml, expressed as units of antibody (14). The intestinal fluid, saliva, and BAL-F specific antibody units per 0.100 ml were adjusted for total class-specific immunoglobulin content and expressed as units of specific antibody per milligram of total class-specific immunoglobulin.

The BAL-F results are reported separately for the two collections as X1 and X2, where X represents the immunoglobulin class of interest (e.g., IgA results for sample 1 are referred to as BAL A1 and for sample 2 are BAL A2). If the BAL-F specific antibody was derived from local production in the respiratory tract, then the concentration of antigen-

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TABLE 1. Subject groups, vaccine doses used, and samples obtained

Group	No. of subjects	Vaccine type	Vaccination details			Day(s) postvaccination for:			
			Route	No. of organisms		Dose schedule	Intestinal intubations[a]	BAL sample	Saliva samples[b]
				Total	Viable (% of total)				
A	5	None						0	
B	6	*S. typhi* Ty21a	Oral	5.2×10^{11}	1.7×10^{11} (33)	0, 2, 4	0, 22	23	0, 22
C[c]	6	*S. typhi* Ty2	Subcutaneous	5×10^{8}	0	0, 14	0, 22	23	

[a] All subjects underwent prevaccination intestinal intubation in the week before commencement of vaccination, for convenience are referred to here as day 0. One subject in group B did not undergo intestinal intubation.
[b] Serum samples were collected prevaccination and every 3 to 4 days from commencement of vaccination for 22 days.
[c] Subjects in this group did not ingest sodium hydrogen carbonate solution prevaccination.

specific antibody in the fluid should exceed that from any serum diffusion (15). Therefore, the ratio of the reciprocal of the antibody dilution giving an optical density of 0.15 absorbance units to the total class-specific immunoglobulin concentration should be higher in the BAL-F than in serum.

The statistical significances of the observed results were determined by Student's *t* test (10).

Intestinal response. A fourfold or greater increase in typhoid-specific intestinal IgA antibody was observed in five of five group B volunteers but in only one of six subjects in group C (Fig. 1). Specific intestinal IgG and IgM responses are not reported here, being consistently undetectable in all but exceptional cases.

Serum response. Fourfold or greater increases in typhoid-specific serum IgA were determined in four of five group B subjects and in three of six group C subjects (Fig. 1). Similar significant specific serum antityphoid IgG responses were determined in group B (five of five subjects responding) in contrast to group C (responses only in the same three subjects as for IgA).

The sequential serum anti-*S. typhi* LPS IgA and IgG responses for groups B and C are depicted in Fig. 2. By days 11 and 12, significant differences in antibody response in specific IgA ($P = 0.031$) and IgG ($P = 0.0049$) were apparent. This difference remained present by days 21 and 22 for the specific IgG ($P = 0.0073$) response but not for the IgA response.

Salivary response. Only one of five group B subjects demonstrated a fourfold or greater increase in salivary IgA anti-*S. typhi* LPS antibody postvaccination. The failure of the salivary response to show any correlation with the intestinal specific IgA response confirmed that specific salivary IgA antibody is not a reliable indicator of a mucosal immune response following oral vaccination.

BAL-F response. The individual antityphoid antibody responses for each subject are depicted in Fig. 3. Specific IgA was present in only one of five control subjects (group A), three of six orally vaccinated subjects (group B), and two of six parenterally vaccinated subjects (group C), while specific IgG was present in zero of five controls (group A), one of six parenterally vaccinated subjects (group C), and six of six orally vaccinated subjects (group B).

The antityphoid IgA BAL-F responses of the vaccinated and control subjects were not significantly different.

The specific IgG antityphoid antibody level in BAL G1 of the orally vaccinated group (group B) was significantly elevated above that of the controls (versus group A, $P = 0.0043$) and the parenterally vaccinated group (versus group C, $P = 0.015$).

There was no difference in the total IgA or IgG content of the two separate lavage collections. IgM class antibody was not detectable in the BAL-F of any subject from either the control or vaccinated groups.

Source of specific antibody in BAL-F. The adjusted specific serum IgG level of all vaccinated subjects exceeded the IgG

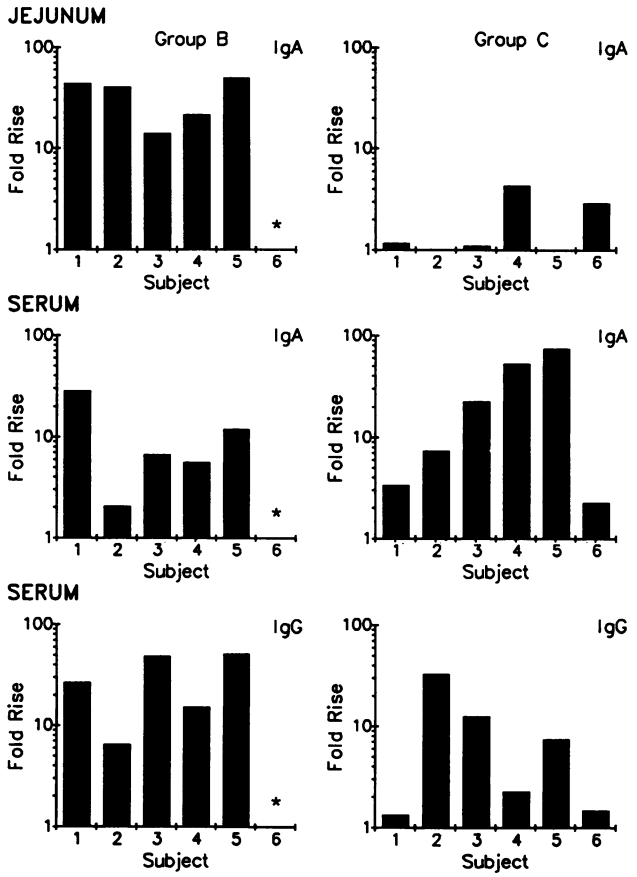

FIG. 1. Individual antityphoid intestinal IgA and serum IgA and IgG immune responses in subjects immunized with either live orally administered or killed parenterally administered typhoid vaccines. The graphs represent the mean rises in postvaccination specific antibody over the prevaccination baseline titers. The data presented for serum are for samples collected on day 12 (group B) and day 11 (group C) postvaccination. *, Sample not obtained. Group B subjects received 1.7×10^{11} live oral *S. typhi* Ty21a administered orally on days 0, 2, and 4. Group C subjects received 5×10^{8} killed *S. typhi* Ty2 administered subcutaneously on days 0 and 14.

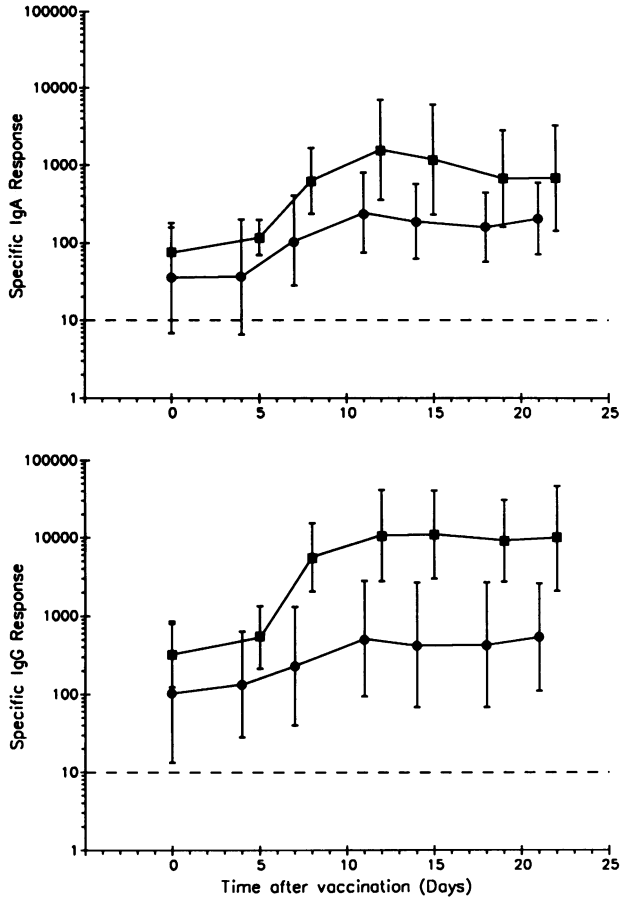

FIG. 2. Serial specific serum IgA and IgG immune responses in experimental groups following immunization with either live orally administered or killed parenterally administered typhoid vaccine. Serum responses are given as geometric means of the serum dilution giving an optical density of 0.15 absorbance units, with 95% confidence intervals. ■, Response following oral immunization with *S. typhi* Ty21a, ●, response following subcutaneous immunization with heat-inactivated typhoid vaccine.

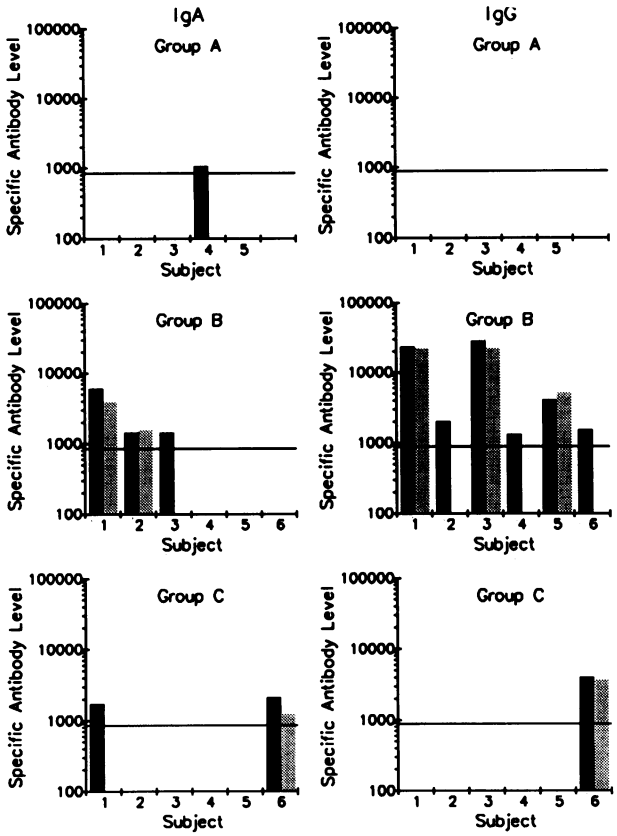

FIG. 3. Individual anti-*S. typhi* LPS IgA and IgG bronchalveolar immune responses. The graphs depict individual specific antibody titers. Solid columns represent specific immune responses in the first 50 ml of BAL for a given antibody class; shaded columns represent that response in the second 100-ml sample of BAL. The absence of either column for a subject indicates that the immune response was below the lower level of detection, indicated by the solid horizontal line. Group A (control) subjects were not vaccinated against typhoid; groups B and C are defined in the legend to Fig. 1.

response in the BAL-F. The day 21 and 22 serum specific IgG levels of all vaccinated subjects (groups B and C) strongly correlated with the BAL-F specific IgG antibody levels (versus BAL-F G1, $r = 0.93$ and $P = 0.0026$; versus BAL-F G2, $r = 0.88$ and $P = 0.0082$).

Therefore, it appears that the specific IgG antityphoid response detected in the lower respiratory tract was due to the transudation of specific IgG from serum and not to local production.

This observation was not apparent with the adjusted IgA levels, although the adjusted specific IgA antibody responses in the BAL-F of three of six subjects also exceeded that in serum, implying a degree of local specific antibody production. This specific IgA antibody probably originated in the upper respiratory tract and represents the technical difficulty associated with preventing upper-airway material from contaminating the BAL of the distal respiratory tract during fiber-optic bronchoscopy. However, this is evidence, albeit weak, of a common mucosal immune system operative after oral vaccination.

In conclusion, we have demonstrated for the first time that an antigen-specific immune response to an orally administered live bacterial vaccine can be induced in the lower respiratory tract of humans.

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