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Immune Response against Lipopolysaccharide and Invasion Plasmid-Coded Antigens of Shigellae in Vietnamese and Swedish Dysenteric Patients

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The peripheral immune responses of adult Vietnamese patients infected with *Shigella dysenteriae* 1 and *Shigella flexneri* 1b and 2a and those of *S. flexneri*-infected Swedish patients were studied against various lipopolysaccharide and invasion plasmid-coded antigens (Ipa-s) and compared with the titers for the corresponding local healthy populations. Both Vietnamese and Swedish patients reacted with significant (P, <0.05) titer increases in the immunoglobulin A and G classes against the homologous lipopolysaccharide antigen. However, significant titer responses against the Ipa-s were seen among only the Swedish patients. We surmise that the weak-to-moderate responses against the Ipa-s in Vietnamese patients are due to the fact that the high level of titers induced by previous infections in the local population could not be considerably increased further by a recent infection.

Bacillary dysentery caused by members of the *Shigella* genus is one of the major causes of diarrhea in developing countries (10). The two major antigenic constituents of the surface of virulent shigellae and those of most of the recent anti-dysentery vaccines (12) are the invasion plasmid-coded protein antigens (Ipa-s) (1, 2, 8, 9) and the lipopolysaccharide (LPS) (7). Animals or humans infected or vaccinated with shigellae react with an immune response against both the LPS and the Ipa-s (3–5, 15–18). However, there are few studies which have simultaneously investigated the responses against both antigens (16, 18). The comparative description of the immune responses after natural infection against these two major antigens could provide valuable data for the evaluation of the antigen delivery and immunogenic stimulation by experimental vaccines. We here report on a study of the differences in natural infection-induced LPS- and Ipa-specific immune responses in a population living in an area of endemicity, such as Vietnam (e.g., where future large-scale vaccine trials are to be conducted) and among individuals in whom the actual shigella infection was probably the only encounter with this pathogen—as in Sweden—and who are usually the subjects for the first human safety and immunogenicity tests during vaccine development.

Formerly, we have extensively studied the LPS-specific responses of Vietnamese and Swedish patients (13). We found that following bacillary dysentery young Vietnamese children and Swedish adults reacted with significant LPS-specific immunoglobulin A (IgA) and IgG responses, while Vietnamese adults reacted with a significant IgG response only (13). Therefore, the Vietnamese and Swedish populations seemed to be particularly suited for comparative investigation of the immune responses against various *Shigella* antigens.

The serum immune responses of adult patients against *Shigella dysenteriae* 1, *Shigella flexneri* Y, and *Salmonella* BO LPS and against the Ipa-s by enzyme immunoassay (EIA) were studied. The preparation of the antigens and the enzyme immunoassay (EIA) techniques have been previously described (11). For the *S. flexneri* LPS-specific enzyme immunoassay, the antigen prepared from serotype Y was used since the response against this antigen is almost identical to those against other, but type 6, *S. flexneri* serotypes (5). Vietnamese patients infected with *S. dysenteriae* 1 (n = 10), *S. flexneri* 1b (n = 9), and *S. flexneri* 2a (n = 12) and Swedish patients infected with *S. flexneri* (n = 9) were included in the study. Vietnamese patients' serum samples were taken 7 days (range, 6 to 10 days) after the onset of the infection and after 30 (range, 27 to 35), 90 (range, 85 to 110), and 180 (range, 175 to 190) days in the follow-up period. In Sweden, the samples were collected during the acute phase of infection (e.g., 7 to 12 days after the patient became ill) and in the convalescence phase (after 30 to 50 days).

The titers in patients' serum samples were compared to those in serum samples from the local healthy population (38 Vietnamese and 22 Swedish healthy adults). The Vietnamese healthy controls had significantly higher serum titers than the Swedish controls (Kolmogorov-Smirnov test; P, <0.05) against all antigens except *Salmonella* BO LPS and the IgG class. The Vietnamese mean IgA and IgM titers against the various LPS antigens exceeded 2.0 to 2.4 times the Swedish mean values, while the IgG titers of the same specificities showed only a 1.5- to 1.9-fold difference (Fig. 1 to 4, Control). In contrast, the Ipa-specific IgG titers were more than 10 times higher in the Vietnamese serum samples than in the Swedish samples (mean ± standard deviation, 710 ± 190 versus 70 ± 70), and the Ipa-specific mean IgA titer was also 3.2 times higher than the Swedish level (130 ± 70 versus 40 ± 30).

In all the four groups of patients, the basic characteristics of the LPS-specific responses were similar (Fig. 1 to 4, panels A to C). The infection was followed by an acute IgA response at day 7 against the *S. flexneri* Y LPS in the *S. flexneri* 1b- and 2a-infected Vietnamese patients (mean IgA titer ± standard deviation, 660 ± 80 and 560 ± 200, respec-

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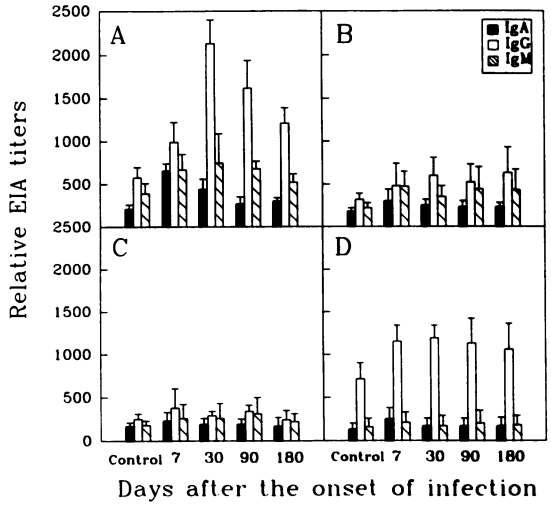

FIG. 1. Serum immune response of *S. flexneri* 1b-infected Vietnamese patients against various antigens. (A) *S. flexneri* Y LPS; (B) *S. dysenteriae* 1 LPS; (C) *Salmonella* BO LPS; (D) Ipa-s. EIA, enzyme immunoassay.

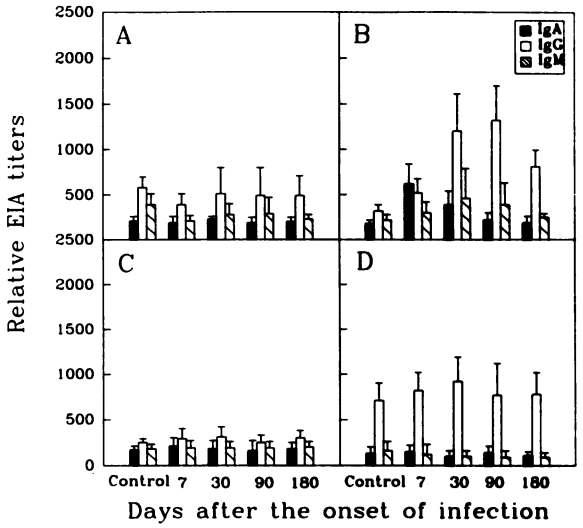

FIG. 3. Serum immune response of *S. dysenteriae* 1-infected Vietnamese patients against various antigens. (A) *S. flexneri* Y LPS; (B) *S. dysenteriae* 1 LPS; (C) *Salmonella* BO LPS; (D) Ipa-s. EIA, enzyme immunoassay.

tively) and in the Swedish patients (590 ± 280) and against the *S. dysenteriae* 1 LPS in the *S. dysenteriae* 1-infected group (620 ± 220). By day 30, the IgA titers had already decreased and, as tested in the Vietnamese patients, by days 90 to 180 were in the range of the local normal level. There was a significant response (P, <0.05) against the LPS antigen in the IgG class, too. This reached its maximum during early convalescence (day 30), represented by mean titers ± standard deviations of 2,130 ± 270, 1,820 ± 440, 1,320 ± 380, and 1,250 ± 230 in Vietnamese groups infected with *S. flexneri* 1b and 2a and *S. dysenteriae* 1 and in the Swedish group, respectively. It should be noted that in the *S. flexneri*-infected Vietnamese patients, the LPS-specific IgG titers were also significantly (P, <0.05) above the level of the local healthy population 6 months after the infection. The IgM titers showed patterns similar to those of IgG, although the actual titers were lower. With the exception of the *S. dysenteriae* 1-specific IgM titers in *S. flexneri* 1b-infected Vietnamese patients, the titer increases against the heterologous LPS antigens never reached the twofold level. The most important difference between the LPS-specific responses of the Vietnamese and Swedish patients was that the maximal responses in the Vietnamese groups were seen in the IgG class in the day 30 samples (magnitudes of responses varied between 3.1 and 3.7). Although showing an extensive (4.2-fold ± 0.8-fold) IgG response as well, the previously uninfected Swedish patients exhibited the maximal responses in the IgA class (6.6-fold ± 3.0-fold increase by day 7), bringing their IgA titers into the range of the Vietnamese

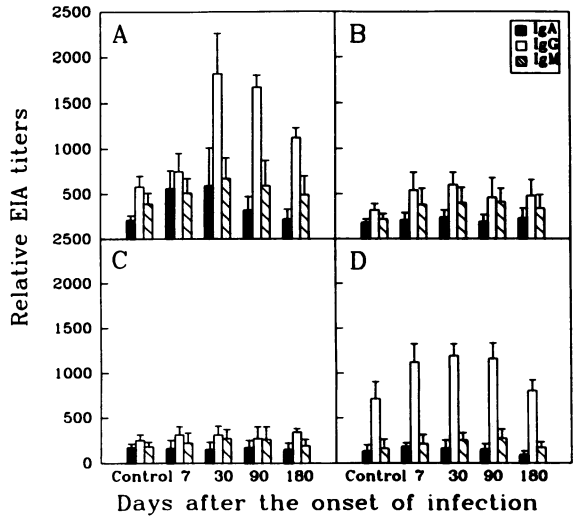

FIG. 2. Serum immune response of *S. flexneri* 2a-infected Vietnamese patients against various antigens. (A) *S. flexneri* Y LPS; (B) *S. dysenteriae* 1 LPS; (C) *Salmonella* BO LPS; (D) Ipa-s. EIA, enzyme immunoassay.

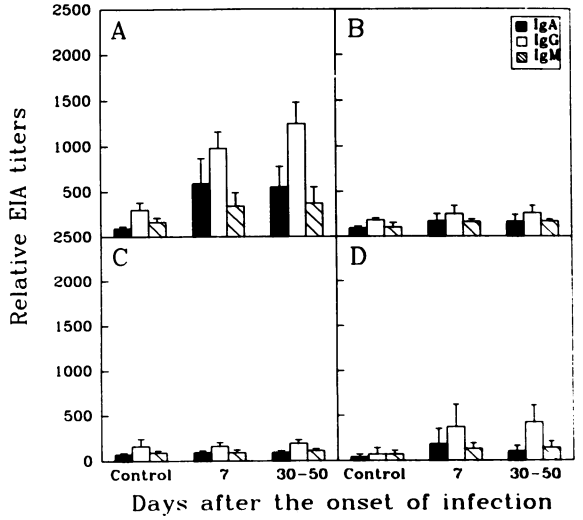

FIG. 4. Serum immune response of *S. flexneri*-infected Swedish patients against various antigens. (A) *S. flexneri* Y LPS; (B) *S. dysenteriae* 1 LPS; (C) *Salmonella* BO LPS; (D) Ipa-s. EIA, enzyme immunoassay.

patients. In this respect, the uninfected Swedish patients showed responses similar to those of *S. sonnei*-infected Israeli military personnel (3).

The differences between the Vietnamese and Swedish patients were more pronounced when their responses against the Ipa-s were studied (Fig. 1 to 4, panels D). In the Vietnamese patients, only moderate Ipa-specific responses were seen—unlike those against the LPS antigen—and in only the IgG class. In contrast, Swedish patients reacted with a strong Ipa-specific response. The absolute value of the Ipa-specific IgA titers (180 ± 170) by day 7 represented a level more than four times higher than that of the normal Swedish population. The Ipa-specific IgG titers also increased rapidly, and in the early convalescence phase exhibited a titer of 420 ± 190, corresponding to an increase of 5.8 ± 2.7. It should be noted however that even after these extensive responses, the Ipa-specific mean IgA titer just slightly exceeded, while the IgG titer did not even approach, the titer for the healthy Vietnamese population (130 ± 70 and 710 ± 190, respectively). In Swedish patients, the relationship between the extent of the LPS- and Ipa-specific responses was also studied. In the individual patients, the levels of only day 7 IgA titers showed a limited correlation (Spearman's rank correlation coefficient, 0.517; data not shown).

On the basis of these data, it is evident that the immune responses against various *Shigella* antigens following natural infection are considerably different in primed and unprimed populations. While Swedish patients with dysentery exhibited extensive responses against both LPS and Ipa, Vietnamese patients showed only moderate Ipa-specific titer increases. It is possible that in Vietnam the infection "selected" those with low titers, presumably those who were relatively unprotected. Comparing the levels of response with the patients' own preinfection titers, one may expect to see more-extensive responses. However, the relatively uniform high titers seen among healthy Vietnamese people in the present study and in former studies (6, 13, 14) indicate that the population is rather evenly primed with a limited number of *Shigella* serotypes (14a). An alternative explanation for the lack of Ipa-specific responses in Vietnamese might be that these titers could be only slightly increased by a recent infection from the high titers of the local population.

The *Shigella* LPS-specific IgG titers of the healthy Vietnamese people were significantly lower than the levels observed in the patients 6 months after the infection. This indicates that the last *S. flexneri* or *S. dysenteriae* 1 infections of the controls occurred more than half a year earlier. In contrast, their Ipa-specific IgG titers were almost as high as the peak values seen in the acute-subacute phase of the infection. This could be the result of recent stimuli with other *Shigella* serotypes. However, the most frequently identified serotypes in Vietnam (13, 14a) would also have caused the elevation of titers against the LPS antigens used in the study. Alternatively, we assume that once the titers had been increased by—formerly, probably repeated—infections, the elevated IgG level against a multitude of antigenic sites on a mixture of proteins (e.g., the Ipa-s) would last longer than against the LPS antigen expressing fewer epitopes. Further studies with highly purified antigens should reveal the differences between the extent of immune responses against the protein and polysaccharide antigens of shigellae.

These data show that monitoring the LPS-specific peripheral immune response can be a sensitive indicator of a recent encounter with *Shigella* bacteria in both previously infected and uninfected populations. However, especially in areas of high endemicity, Ipa-specific serum Ig responses appear to be of moderate use because of the existing high titers in the normal population. This should be kept in mind when evaluating the immunogenicity of antidysentery vaccines in countries with a high prevalence of shigellosis.

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