CHAPTER V

DISCUSSION

Typhoid fever is a disease for which medical researchers have long been seeking an effective vaccine. Currently available parenteral vaccines such as heat/phenol inactivated (L) and acetone inactivated (K) are effective, (the K vaccine gave 74-84 % protection in Yugoslavian and Guyanan field trials) (78,80,84); they are not widely used because undesirable side-effects are frequently reported. Bodhidatta et al (113) have recently used a heat/phenol inactivated typhoid vaccine in Thailand and they considered it highly effective in reducing the number of cases of typhoid fever in Bangkok. However, they recorded significant side effects.

The development of a safe and effective oral vaccine has generally been considered the ultimate goal. Such a vaccine is now available for wide-scale public use. The vaccine was developed by Dr. R.Germanier, of the Swiss Serum and Vaccine Institute, and was derived from Salmonella typhi Ty2. The strain Ty 21a, is a stable double mutant of the wild-type Ty 2 strain which lacks the enzyme UDP-gal-4-epimerase. Now, it is commercially available in an enteric-capsule formulation.

Recently, Dr.B.A.D. Stocker (114,115,116) described the development of a strain of salmonella made non-virulent by an aromatic nutritional requirement. Thus this auxotroph is dependent on external supplies of aromatic compounds. One of these aromatic - dependent strains, the stable aro^- derivative of Vi-positive S.typhi ($serC^-$ aro^- phenotype) (116) is a likely candidate for an oral vaccine, and Dr. Stocker now porposes to test, the aro^- his^- pur^- strains, in its Vi-positive and Vi-negative forms, in volunteers, as an oral live S.typhi vaccine.

S.typhi is an intracellular parasite and the role of the immune response in protecting the host from these organisms is mostly by local-cell mediated immunity. Because of the direct anti-bacterial action of the lymphoid cells which are present in high numbers within the intestinal epithelium and the lamina propria, this may be regarded as a major first line of defence against gastro-enteric infection (115). Although detection of such protective immunity cannot be performed directly in human subjects, systemic CMIR can be investigated, and recent studies in man indicate a possible protective role for the systemic CMIR in typhoid fever (51,54). So our studies were undertaken to investigate whether oral typhoid vaccine (Ty 21a) can induce CMIR and the local production sIgA.

Our approach has been to vaccinate orally two groups of volunteers with two types of oral typhoid vaccine,

commercial Vivotif ® and the Thai Red Cross vaccine. The two oral typhoid vaccines are constituted from the same auxotrophic mutant S.typhi Ty 21a, previously shown to be effective by Germanier and co-workers (92,93), and when have undergone controlled field trials in Alexandria, North America, Egypt, Chile and Santiago (97,105). The results showed good efficacy and no adverse reactions were encountured (87,96,106).

In this thesis we have compared the Thai Red Cross and Vivotif ® vaccines for ability to induce immune responses such as secretory IgA and systemic CMIR. We also took advantage of this study to compare the specificity and the sensitivity of urease and alkaline phosphatase conjugates in an ELISA systems. As the urease system end-point is readily measured by eye, it has significant advantages for field and country clinic use, provided its specificity and sensitivity can be shown to be adequate.

Systemic CMIR, serum and secretory IgA response

In our studies we measured specific IgA anti - crude LPS after vaccination with typhoid vaccine Ty 21a, from systemic and secretory sources. It was found that specific IgA from serum, saliva and stool extract, in both groups of vaccinees, did not significantly increase when compared with initial control values. However, IgA obtained from intestinal lavage significantly increased at

1-2 weeks after vaccination when compared with control (Table 5,6,7,8 and Figure 15,16,17,18,19,20,21,22).

The work of Sack and co-workers (103), who measured the IgA in serum, saliva, and in intestinal lavage of pateints who had severe dehydration following infection with V.cholera is relevant here. They found that the levels of IgA in lavage increased after infection but were unable to find a similar increase in serum or saliva.

Further, Banchuin(19) and Sarasombath (101,119,120), demonstrated that after vaccinating human volunteers with Ty 21a vaccine and also in typhoid patients, the systemic CMIR, and IgA in secretory intestinal lavage were increased but the systemic serum antibodies occured in low titer and irregularly. Tagliabue et al (100),demonstrated that following oral vaccination with Ty 21a, 16/17 of the vaccinated subjects acquire the capacity to express specific cellular immunity against S.typhi.

Similarly our results with regard to specific LMI revealed a good response. There is no clear evidence to explain why the oral vaccine which shows a poor stimulation of systemic antibodies should provide a relatively good stimulation of cellular immunity. It is possible that a local CMIR was immediate on initial exposure to the organisms with induced systemic CMIR also. It could be that the oral vaccine does not stimulate systemic antibodies

because the Ty 21a bacteria rapidly autolyse after primary stimulation of lymphocytes at the local lamina propria. Although these stimulated lymphocytes enter to circulation. The absence of a secondary stimulation, the IgA in serum after vaccination with Ty 21a would be expected to occure at only low levels and to be transient.

From the results of Maneerushapisal (119), Srisart (120) and Supapong (50) in the mouse models support our findings that after vaccination with live oral typhoid, the local cell-mediated immunity at lamina propria occur simultaneously with the local IgA response and systemic CMIR. However, Srisart et al (120), were able to show very significant increase of IgA in the serum of mice following oral administraion of a S.typhimurium auxotroph and the increase in serum IgA was show to correlate very well with protection.

With reference to IgA in saliva and stool extract samples, we should point out, that we found it most difficult to standardize conditions.

The first one is the collection of the samples. In saliva, the protein flow rate of an individual were not stable so that the quantitation of the Igs were most variable. Further, the appearance of stool samples were different although we used the castor oil to make formed stools samples but formed stools did not always occur

assessment by the time that we collected our samples, so an accurate assessment of IgA in the stool samples was not possible. The second problem was, that there was insufficient specific IgA anti-crude LPS in saliva and stool samples to optimized the condition of ELISA for measurement of specific IgA anti-crude LPS in the samples. So that, the values of these IgA from saliva and stool are likely to be inaccurate.

On the other hand, both oral typhoid vaccines, Vivotif ® and Thai Red Cross can stimulate the local antibodies at gastrointestinal tract very well. Because these vaccines are given orally, the Ty 21a antigen will contact the lymphoid tissue at GALT so that, the level of IgA in lavage is best to show the appearance of local immunity. From the data in Table 9 , show the geometric mean titer of specific IgA in intestinal lavage after vaccination of two vaccines, the levels of IgA in both groups are increased 1 week though 12 weeks after vaccination except in the 12 th week of Thai Red Cross, the levels of IgA is decreased from 8 th week because of the low levels deteccted in one of volunteers, so that the average values are decreased also.

The average age group of the two groups of volunteers were different (Table 1). The volunteers in Vivotif ® were older than in the Thai Red Cross group. The data in Table 9 , shows that older volunteers can stimulate

immune response similar to these of younger volunteers in Thai Red Cross group. So that, age of volunteers is not important in assessing the immune response.

On the other hand, Cancllieri and Fara (121), found formed stools positive for IgA anti-LPS. In our experiments the levels of IgA extracted from stools did not significantly increase over that of the control. However, as previously stated we found it most difficult to standardise the collection of stool samples and so an accurate quantitative comparison of the IgA in individual samples was not possible.

Over all we can conclude that oral typhoid Ty 21a vaccine at least stimulated the production of intestinal IgA and systemic CMIR and both provide good evidence for an immune response after oral vaccination. On the other hand, levels of serum, saliva and stool extract IgA appear inadequate for this purpose.

The Comparison of Urease and Alkaline phosphatase conjugates in the ELISA.

We compared the activity of urease with alkaline phosphatase (AP) we found that (Table 11 and Table 12), the titre of the sera from our volunteers, when measured by the urease conjugate, was lower by one to two dilutions than when we used AP. The detection of urease conjugate is by a

visual end point (color change from yellow to violet) and one could hardly expect it to have quite the sensitivity of AP which is determined spectophotometrically.

However, we showed that the sensitivity and specificity of urease compared to AP, is 70 % for sensitivity and 100 % for specificity (Table 12).

Chander (122) has compared the activity of the enzyme conjugates, horse-radish peroxidase (HRP) , alkaline phosphatase (AP) and urease in the titration of anti-tetanus horse serum. He found that the activity of these enzyme conjugates to be vary similar. Chander's report does not specify the titer of three enzyme conjugates that he used. However, we think that if we can raise the activity of urease by an improved conjugation technique, it will prove very useful in the determination of many other antigens or antibodies associated with infectious diseases. Further, because the sharpness of the end point, with its ease of visual detection, does not require the use of a spectrophotometer, it can be readily used in field trials and outback clinics or in private hospitals, which have no access to an expensive ELISA reader.

In summary this work has shown;

a) Both oral vaccine, Vivotif ® and Thai Red Cross stimulated sIgA in the gastro-intestinal tract very

well but the levels of specific IgA found in blood, saliva and faeces were disappointing

b) The peripheral LMI-index specific for anti-crude LPS was positive following oral vaccination against typhoid fever with Ty 21a vaccine

c) Ty 21a,Thai Red Cross vaccine stimulated specific sIgA anti-crude LPS in GI and CMIR, at least as well as the commercial, Vivotif ®

d) The enzyme urease conjugate ELISA system may certainly be used for the detection of antigens or antibodies particularly in the less sophisticated field environment