Asian Pacific Journal of Allergy and Immunology (1992) 10 : 81-83

SPECIAL ARTICLES

Report from an International Symposium on Typhoid Fever

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The First Asia-Pacific Symposium on Typhoid Fever was held in Kuala Lumpur, Malaysia from October 1-3, 1991. Since the International Workshop on Typhoid Fever held in November, 1984, in Washington, DC, [1] an international scientific meeting dedicated only to typhoid fever had not been held. More than 110 participants from 14 countries participated in the Symposium, sponsored by the Ministries of Science, Technology and Environment, and Health, Malaysia, and the International Development Research Centre, Canada. Topics discussed included epidemiology and public health, genetic variation and molecular biology, clinical features and therapy, pathogenesis and virulence, immunology, laboratory diagnosis, and vaccines. A final Panel Discussion was convened to discuss future perspectives and directions for research. The meeting was unique in that it brought together a diverse group of people (clinicians, epidemiologists, clinical microbiologists, research scientists) to discuss a problem of common interest. Although the meeting was somewhat regional in its perspective, many of the issues discussed are of global relevance. All participants agreed that the scope and format of the meeting was useful and stimulating and a Second Symposium in the series is planned for 1994 in Thailand. The highlights of the Symposium are discussed in this report and selected papers from the Symposium will be published as a volume entitled "Typhoid Fever : Strategies for the 90's". [2]

Epidemiology and public health

CS Chee (Malaysia) described the epidemiology of typhoid in Malaysia and noted that lifestyle appears to be an important factor in the continued endemicity of the disease in the country. In particular, the increasingly popular practice in urban areas of buying pre-cooked foods at night markets from hawkers and vendors, who do not practice sufficient hygienic measures, is suspected to be an important factor. Epidemics also appear to be associated with dry spells and occasions where a lot of food is served without proper care and sanitation, eg during food festivals. The identification and management of carriers remains an important problem. Muhd. Amir (Malaysia) reported that chloramphenicol resistance, among *S. typhi* strains isolated in Malaysia does not seem to be a major problem. Related to this, CL Koh (Malaysia) reported that only 8.5% of strains isolated over a 7-year period harboured plasmids; half of these plasmid-bearing strains were resistant to chloramphenicol and tetracycline. P Sudarmono (Indonesia) described the continuing seriousness of typhoid fever in Indonesia, which has the highest incidence in the Southeast Asian region, if not the highest incidence in the world with a rate varying between 400-800/100,000 population; the highest risk group in the population are schoolchildren between the ages of 3-19. It is estimated that about 600,000-1,300,000 cases and 20,000 deaths occur each year due

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to the presence of more severe disease. Hospital admission for typhoid ranked second only to gastroenteritis and up to 43% of febrile cases in certain areas were due to typhoid. YM Cheong (Malaysia) presented data on the Vi phage types of *S. typhi* isolated in Malaysia between 1980-1989. The commonest phage types were untypable Vi strains (22%) followed by E1 (19%), D1 (16%) and A (13%). Discussions also reiterated the fact that Vi phage typing was not readily available and the service was not easy to set up. National centres in the region cannot provide assistance to neighbouring countries due to the heavy workload.

Genetic variation and molecular biology

M Altwegg (Switzerland) reviewed the various approaches for typing *S. typhi* strains and concluded that, so far, only phage typing and ribotyping have been effective in subtyping *S. typhi* strains. It was felt that ribotyping, based on rRNA gene restriction patterns, may be more representative of the genotype of a strain and, therefore, more useful in studies related to strain-specific virulence and molecular epidemiology. As mentioned above, phage typing may not be readily available and in Malaysia, at least, a significant proportion of isolates were untypable. KL Thong (Malaysia) presented results on the subtyping of *S. typhi* using pulsed-field gel elctrophoresis (PFGE). This appeared to be a useful technique in that 28 different isolates could be categorized into eight different groups based on their PFGE fragment profiles. PFGE is convenient to perform as it does not involve Southern transfers and hybridization with labeled probes and the variations seen are over the entire genome. The groupings of isolates by PFGE showed good agreement with ribotyping. PFGE also allowed an estimate to be made of the size of the *S. typhi* chromosome, approximately 4.5 megabasepairs.

E. Calva (Mexico) reviewed the molecular structure and functions of *S. typhi* outer membrane proteins (OMPs) and their genes, focusing on the pore-forming porins OmpC, OmpF, and PhoE. It was found that the *S. typhi* OmpC gene is expressed at high levels both at low and high osmolarity and that expression may be regulated by the OmpR regulon. OmpC showed 79% homology with the *E. coli* protein and the variable regions were mostly in hydrophilic, probably exposed regions. In a related study, JL Puente (Mexico) investigated genetic variation of the OmpC gene among *Salmonella* spp., by hybridization studies with oligonucleotide probes complementary to gene segments that code for the variable regions of OmpC. The topology of the OmpC protein was also studied by site-directed mutagenesis of a hydrophilic variable region, to produce a chimeric protein containing an 18 amino acid insert corresponding to a rotavirus capsid VP4 protein neutralizing epitope. In studying the regulation of expression of *S. typhi* porins, G Mora (Chile) reported that OmpC production is induced under anaerobic conditions. This finding implies that OmpC is probably the only porin expressed during infection *in vivo* and that it is an important antigen in the host immune response during typhoid.

The cloning of other genes from *S. typhi* was also described. T Sukosol (Thailand) described the cloning of a gene from *S. typhi* which codes for a 52kDa protein that appears to be specific for *S. typhi;* this protein may be useful in diagnostic assays (see below). T Ezaki (Japan) described the molecular cloning and characterization of the *viaB* gene which is required for capsular Vi antigen production. E Calva (Mexico) also reported the cloning and partial sequencing of an LT-like enterotoxin gene from *S. typhi.*

Clinical features and therapy

YH Lim (Malaysia) described the clinical features of typhoid fever in Malaysia as seen during the 1987 outbreak on Penang island. The most common symptoms observed were persistent fever (> 1 week), headache, diarrhoea, malaise, anorexia, rigors and cough. P Kaur (Malaysia) reported some differences in the clinical picture among children, notably the increased incidence of vomiting and splenomegaly. In contrast to the relatively mild clinical picture in Malaysia, N Punjabi (Indonesia) reported that severe typhoid fever, in which patients present with abnormal levels of consciousness or shock, still constitute a significant proportion of typhoid cases in Indonesia with significant mortality. A decade of study of severe typhoid fever in Indonesia has shown that high dose dexamethasone consistently reduces the case fatality rate in severe typhoid from 42-44% to 16-20% The treatment regime, however, is still too costly to be used more extensively. With regards to the complications associated with typhoid fever, RHH Nelwan (Indonesia) reported several cases of pancreatic disturbances. This is believed to be the first report of pancreatic complications in typhoid and it was suggested that this complication be officially known as *typhoid pancreatitis.*

With regards to therapy, it seemed clear that chloramphenicol (CM) is still the drug of choice. However, from a study described by Y Hassan (Malaysia), the levels of CM in serum following oral administration can vary significantly, thus suggesting that monitoring of drug levels may be desirable to ensure that therapeutic levels are reached. There was also another report from WA Ariffin (Malaysia) reporting the failure of aztreonam, a monobactam antibiotic, to treat typhoid fever. Several reports also highlighted the successful treatment of typhoid with shorter treatment

regimes, using newer quinolone drugs such as ciproflaxin (EH Monteiro, Singapore) and ofloxacin (M Velmonte, Philippines). However, the cost of these drugs are still prohibitive and further study of dosage schedules is needed. An additional disadvantage of the quinolone drugs is that they are not recommended for children below 11 years of age. Nevertheless, these investigations should be encouraged since these newer medications can reduce the duration of illness and hospitalization, thus they could be cost-effective for groups of patients who can afford them. They can also serve as alternative drugs in cases of CM resistance or in situations where CM could not be used, eg in patients with very low leucocyte counts.

Pathogenesis and virulence

KY Leung (Canada) in his Plenary Lecture reviewed work on the molecular factors related to the virulence of *Salmonella* spp. in eukaryotic cells. These factors enable Salmonellae to invade, survive and replicate within eukaryotic cells, and are essential for successful infection. Invasins appear to be responsible for adhesion and initiation of bacterial entry into host cells and the subsequent triggering of a signal involving protein tyrosine kinase which results in internalization of bacteria. This signal exchange results in extensive aggregations of the host cell cytoskeleton (involving actin filaments) around invading bacteria. Once internalized within vacuoles, Salmonellae appears to be able to survive a low free Fe^{++} and Mg^{++} milieu. The ability to survive intracellularly was related to a gene locus identified from *S. cholerasuis,* which also enabled *E. coli* to survive inside the vacuole of epithelial cells. The identification and characterization of genes that function in the survival and replication of *Salmonella* spp. promises to be an exciting and important area of future research with implications for vaccine design.

HS Hsu (USA) described studies into the pathogenesis of typhoid in a mouse model and concluded that an extracellular mode of replication in host tissues appears to be prominent. T Arai (Japan) described studies indicating that the host selective species-specific virulence of *Salmonella* spp. for certain host cells, appear to be related to differences in the recognition of complement receptors on macrophages. W Noraini (Malaysia) presented data which indicated that arachidonic acid metabolites were released by human and mouse monocytes/macrophages exposed to heat-killed *S. typhi.* In the case of mouse macrophages, these metabolites were found to be mainly prostaglandins and leukotrienes. These results are consistent with the hypothesis of Hoffman (1) that arachidonic acid metabolites may be important mediators in the pathogenesis of typhoid.

Immunology

S Sarasombath (Thailand) reviewed the nature of the host immune response in typhoid fever and emphasized that, at various times during infection, there is development of cell-mediated immunity and systemic and intestinal antibody responses to a multitude of antigenic components of *S. typhi.* Among these antigenic determinants, the porins of outer membrane proteins (OMPs) are of particular interest. It also appears that the cell-mediated immune response plays the more important role in protective immunity. In support of this idea, T Arai (Japan) described experimental studies in which purified porins were able to induce protective immunity and where protection correlated with DTH responses, IL-2 and IFN-γ production but not with antibody titres. To further underscore the interest in the role of the OMPs, J Paniagua (Mexico) reported that OMPs, with molecular weights ranging from 38 to 40 kDa (corresponding to the porins), can induce protection to *S. typhi* in a mouse model; and that both humoral and cellular immune responses against *S. typhi* porins are regulated by the H-2 haplotype and by the non-H-2 *lps* and *ity* genes. LH Gam (Malaysia) attempted to study the reactivity of typhoid sera to short peptides synthesized on polyethylene pins [3] based on the amino acid sequences of putatively exposed, hydrophilic regions of OmpC published previously. [4] No significant binding was detected to any specific peptide, perhaps suggesting that antibodies to linear epitopes of OmpC were not present in sera from typhoid patients. In relation to this, a suggestion was made (E Calva, Mexico) that OMPs might be recognized mainly as complex antigens in association with lipopolysaccharide (LPS).

Laboratory diagnosis

PY Chau (Hongkong) reviewed the current status of the laboratory diagnosis of typhoid fever. Blood culture and the Widal test, despite its many deficiencies, continue to be the major methods of laboratory diagnosis. In developing countries, especially, the Widal test is still widely used. It was once again emphasized that there were many variations being used to perform the Widal test and it would be difficult to compare titres obtained in different laboratories. These two methods do not meet the requirements for an ideal test in terms of being sensitive, specific (except for blood culture), rapid and inexpensive. In the quest for such an ideal test, many new approaches are being explored, including detection of antibodies to other *S. typhi* antigens, detection of antigens directly from clinical specimens and the use of DNA probes to detect *S. typhi*-specific sequences.

A Verdugo-Rodriguez (Mexico), for example, reported the development of an indirect ELISA to detect antibodies to *S. typhi* outer membrane protein (OMP) preparations. This test showed that typhoid sera (col-

lected in the first week of illness) gave a positive:negative absorbance ratio of approximately 2.6 when analysed against a panel of negative sera. Discussions also highlighted the observation that various OMPs of different molecular weights were being detected by various laboratories and that technical differences in the preparation of OMPs (eg heat treatment of OMPs, contamination with flagellin, etc) could account for such differences.

The Symposium also heard reports of a 50-52 kDa protein(s) which appear(s) to be specific for *S. typhi*. A Ismail (Malaysia) reported that antibodies to a 50 kDa protein (IgM and IgG) could be detected using a dot enzyme immunosorbent assay (EIA) within 3-4 hours of receipt of specimen. Limited trials of this EIA assay in Malaysia, reported by KH Ong and KE Choo, indicated that it was highly specific, sensitive, rapid and required only a single specimen. T Sukosol (Thailand) reported the production of a monoclonal antibody to a 52 kDa protein from *S. typhi* which showed no cross-reactivity with proteins from 13 other bacteria causing enteric fever and enteric fever-like illnesses. The gene for this protein has been cloned and the protein expressed. Further evaluation of the value of these proteins as diagnostic antigens, perhaps involving a WHO Multicentre Trial, may eventually allow it to replace the Widal test.

In another attempt to utilize newer approaches in laboratory diagnosis, L David (Malaysia) has used the polymerase chain reaction (PCR) for detecting *S. typhi* DNA, with primers flanking a ribosomal RNA gene. It was found that 1 pg of *S. typhi* DNA was detectable by PCR, but attempts to detect such DNA directly from clinical specimens have so far been unsuccessful. P Sudarmono (Indonesia) reported that a DNA probe for the Vi antigen gene, tested in Indonesia, proved to be highly specific for *S. typhi* with an efficacy higher than 80%, but the test required pre-amplification of bacterial cells in the clinical specimens before testing.

Vaccines

D Rowley (Australia) reviewed the results of typhoid vaccine trials carried out during the last 90 years. It was emphasized that the protective antigens of *S. typhi* are not fully described, and that antigens other than lipopolysaccharide and Vi may also be protective, for example the OMPs. Of the currently used formulations, the oral Ty21a and the parenteral Vi vaccine with similar efficacy seemed to be promoted for use at present. The whole cell, inactivated killed vaccine is still widely used, particularly in developing countries. Variability in efficacy of the Ty21a formulation, however, is still a problem and it is not clear what this is due to. It is also clear that the basis of attenuation is still poorly understood and ignorance of this fact may have dire consequences.

C Simanjuntak (Indonesia) summarized the results of typhoid vaccine trials in Indonesia. In a large scale study involving 22,000 individuals, the efficacy of the oral typhoid vaccine, Ty21a, was found to be only 42% for the enteric coated capsules and 53% for the liquid formulation. This reduced efficacy could be due to many factors, the most important of which is thought to be the very high attack rates for typhoid in Indonesia. It was concluded that, although Ty21a could only prevent disease in about 50% of recipients, it is still of considerable public health value as in Indonesia, if it is given to all individuals between 13-19 years old, it would eliminate at least 250,000 cases of typhoid annually. The vaccine is also easily administered, well-tolerated and has fewer side effects than parenteral, killed typhoid vaccines. Interestingly, about 11% of the vaccine recipients proved to be non-responders. There may be several explanations for this, including genetic control of responsiveness and an incomplete complement of the necessary antigens in the formulation used. The side effects and immunogenicity of the parenteral Vi-capsular polysaccharide vaccine was also evaluated. The vaccine produced fewer side effects (when compared to pneumococcal vaccine) and seroconversion was observed in 73-92% of vaccine recipients. On the basis of his studies with experimental typhoid, T Arai (Japan) suggested that purified porins from *S. typhi* should be considered as possible candidates for safe and effective vaccines.

Conclusions - final panel discussion

The final Panel Discussion (Panel Members : E Calva, D Rowley, N Punjabi, PY Chau, KY Leung) was convened to highlight the more important issues raised during the Symposium and provide perspectives on future directions for research.

It was emphasized during the discussion that typhoid, like most enteric diseases, was mainly a disease of underprivileged people, living under conditions of poor hygiene, sanitation and education and may take a long time to eradicate completely. It is also clear that an increase in the amount of travel and the habit of eating out in the developing countries has also resulted in increased number of cases in the middle or higher income groups of patients. In view of the different clinical severities of disease, it was urged that clinicians compare the incidence and clinical features of typhoid and the results of treatment protocols in different parts of the world where mortality rates apparently differ. The newer techniques of molecular biology may also prove valuable in better defining the molecular epidemiology of *S. typhi* strains.

It was also clear during the Symposium that there was a great

deal of interest in the outer membrane proteins (OMPs) as potential antigens for both diagnostic assays and vaccines. However, more work needs to be done in further confirming which OMPs may be important antigens and whether the ones described by various studies were similar or even identical. With regards to laboratory diagnosis, the search continues for the one simple, reliable and inexpensive diagnostic test and it was clear that some promising new tests are being developed which could significantly improve the quality of diagnostic testing in the near future. However, the methodology for such tests needs to be standardized and the tests themselves required to be evaluated more extensively. In relation to this, the exchange of test reagents, antigens and clinical specimens between different laboratories is very important. It was also emphasized that a close collaboration between clinicians, epidemiologists, and basic science researchers is crucial towards the effective application of laboratory diagnostic methods in typhoid.

The promise and potential of molecular biology in solving many of the problems inherent in typhoid fever research was also clearly illustrated, in areas such as the definition and characterization of virulence factors, molecular epidemiology, laboratory diagnosis and vaccine development. It seems conceivable that the relevant protective antigens against typhoid could eventually be incorporated into foods such as vegetables and yoghurt. A point was also made that, although a great deal of work was being done in the development of new typhoid vaccines,[5] it is imperative that human trials with such vaccines be given a high priority. It was felt that there was often a reluctance to carry out such trials due to the well-known risks.

In summary, many challenges and problems lie ahead; but new and novel aproaches lend promise that at least some solutions might be found shortly to these problems. Together with its importance to human health, the interaction of the typhoid bacillus with host cells continues to be a very interesting and unique biological problem, worthy of continued, intensive investigation.

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