

ILEAL PERFORATION IN TYPHOID: BACTERIOLOGICAL AND IMMUNOLOGICAL FINDINGS

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Abstract. In typhoid perforation patients, *Salmonella typhi* was isolated from blood in 4%, ileal contents in 23%, peritoneal pus in 13% and from mesenteric lymph nodes in 71%. While isolation of *S. typhi* was made from patients with less than 4 days of chloramphenicol therapy, cultures were negative from these sites after 5 days of therapy; however, *S. typhi* appeared to remain viable in the lymph nodes even after such therapy. All isolates of *S. typhi* were sensitive to chloramphenicol.

Significant SAT titers ($0 \geq 1/240$) were obtained in only 7/21 (33%) of patients. The perforated group had lower geometric mean titers (0-1/138; H-1/46), when compared to matched patients with uncomplicated typhoid fever (0-1/476; H-1/148). This difference was significant (0- $p < 0.005$; H- $p < 0.0025$). The two groups (uncomplicated and perforated) showed no significant difference in total serum IgG, IgM and IgA or isohemagglutinin levels, indicating that the apparent hyporeactivity was not due to a generalized humoral immunodeficiency. Mesenteric lymph node histology showed hyporeactivity in both the T cell and B cell zones. These findings are discussed with the suggestion that *S. typhi*-specific host immunological hyporeactivity could be an explanation for these observations and a basis for the pathogenesis of perforation.

Aerobic cultures of the peritoneal pus gave 39 isolates from 25 patients; the predominant isolates were *Escherichia coli* (24) and *Klebsiella pneumoniae* (12). On no occasion was *S. typhi* the predominant isolate. Gentamicin and kanamycin were the only two antibiotics which were consistently effective *in vitro* against the aerobic isolates from peritoneal pus.

INTRODUCTION

The literature on typhoid ileal perforation has been extensively reviewed by Bitar and Tarpley (1985). The bulk of the published literature deals with the clinical features and the controversy over surgical versus conservative management of this complication. The bacteriological aspects have received less study, whereas the immunological and pathogenetic aspects have hardly been investigated, although there has been no lack of speculation on the latter.

This paper deals with the bacteriology and immunology of typhoid perforation. The implications of these results on pathogenesis are discussed with suggestions for rational antibiotic therapy.

MATERIALS AND METHODS

Patients: Thirty-one patients with ileal perforation were investigated bacteriologically and immunologically as detailed below. Perforation was confirmed at laparotomy in all patients. The diagno-

sis of typhoid was established by the isolation of *S. typhi* from the blood or mesenteric lymph nodes.

Bacteriology: Blood/clot cultures were done by standard methods (Cruickshank, 1975). Intestinal contents and peritoneal pus were obtained at laparotomy and were cultured for *S. typhi* in Selenite-F broth. Peritoneal pus was plated on sheep blood agar and incubated aerobically at 37°C. Anaerobic cultures were attempted but in view of the emergency nature of the surgery and the logistical problems encountered in specimen collection and transport, meaningful interpretation was not possible.

Mesenteric lymph nodes removed at laparotomy were minced using sterile scissors and cultured in Selenite-F broth with subculture on MacConkey agar. Bacterial isolates were identified by standard bacteriological methods (Edwards and Ewing, 1972; Cowan, 1974).

Immunology: Serum samples from patients were collected on admission and a second sample was

collected 5 days later, where possible. Standard agglutination tests (SAT) were done as described by Cruickshank (1975) and quantitation of total serum immunoglobulins was done by radial immunodiffusion using class specific anti-human sera (Wellcome Reagents) and WHO standards (lot 67/97). The results were expressed as grams/liter. Isohemagglutinin titers were determined as described by Boorman and Dodd (1961).

The SAT and total Ig results on the sera from patients with typhoid perforation were compared with those obtained on sera from patients with culture positive uncomplicated typhoid fever matched for age, sex, duration of prior chloramphenicol therapy and duration of illness. Past TAB vaccination was not specifically matched for, but on subsequent analysis, the two groups were found to be comparable.

Isohemagglutinin titers of typhoid perforation patients were compared with those in sera from healthy controls, matched for age, sex and blood group.

Mesenteric lymph node histology: Mesenteric lymph nodes removed at laparotomy were fixed in Carnoy's fixative and sections were stained with methyl green-pyronine; immunological reactivity was reported according to Cottier *et al* (1973).

Antibiotic sensitivity tests: Antibiotic sensitivity tests on *S. typhi* and the aerobic isolates from peritoneal pus were done on Mueller Hinton agar by the Kirby-Bauer method (Ericsson and Sherris, 1971) using Neosensitabs (A/S Rosco, Denmark) antibiotic discs. Minimum inhibitory concentrations of chloramphenicol for *S. typhi* isolates were determined (Ericsson and Sherris, 1971) using chloramphenicol obtained from Div. Chimica Industriale, Milano (Batch 7753 5 D0093).

Statistics: Results were analysed using Yate's correction for testing associations, and Student's *t*-test. For statistical analysis of serum titers, log transformation of reciprocal serum dilutions was used.

RESULTS

A bacteriological diagnosis of typhoid was confirmed in 21 of the 31 patients, these patients having yielded *S. typhi* from the blood or mesenteric

lymph nodes and a further 2 patients demonstrated an O antibody titer of \geq than 240 in the SAT. In Sri Lanka, O antibody titers of over 240 have been shown to be highly suggestive of recent typhoid fever (Thevanesam, 1985). In 6 patients the probable diagnosis was based on clinical and laparotomy findings.

The highest rate of isolation of *S. typhi* (71%) was from mesenteric lymph nodes. Although prior chloramphenicol therapy of 5 days or more was associated with the inability to isolate *S. typhi* from blood, ileal contents or peritoneal pus, five out of six such patients gave positive cultures from mesenteric lymph nodes (Table 2).

An 'O' antibody titer of \geq 240 was seen in only 7 of the 21 patients while a titer of \geq 120 was seen in 14. In Sri Lanka where typhoid is endemic, only an O titer of \geq 240 could be considered to be suggestive of recent typhoid (Thevanesam, 1985).

All *S. typhi* isolates were sensitive to chloramphenicol, ampicillin and co-trimoxazole by disc diffusion antibiotic sensitivity tests. The minimum inhibitory concentrations of chloramphenicol on the isolates were \leq 8 μ g/ml by tube dilution antibiotic sensitivity tests.

Investigation of the aerobic flora of the peritoneal pus showed that on no occasion was *S. typhi* the predominant isolate. Even in the four patients from whom *S. typhi* was isolated from peritoneal pus, it was detected only after inoculation into enrichment medium (Selenite-F broth).

Aerobic pus cultures on blood agar yielded a total of 39 isolates from 25 patients. Coliforms predominated accounting for 36 (92%) of the isolates. Of the coliforms, 24 were *E. coli* and 12 were *K. pneumoniae*. In two patients in whom coliforms were the numerically predominant isolate from the peritoneal pus, *S. faecalis* was isolated in lesser number. One patient yielded a pure growth of *S. aureus* from the pus. On no occasion was a 'sterile exudate' seen in the peritoneum.

The antibiotic sensitivity patterns of the coliform peritoneal isolates are shown in Table 3. Only the antibiotics available for clinical use in Sri Lanka at the time of the study (*viz* 1975-1978) were included in sensitivity tests. It is notable that resistance to chloramphenicol, ampicillin and tetracycline was common, in many isolates the resistance having been multiple. The *S. aureus* isolate was

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resistant to penicillin and chloramphenicol, but was sensitive to cloxacillin and gentamicin. The fecal streptococci were sensitive to most antibiotics tested.

The antibiotic sensitivity test results were available to the clinician only in retrospect, due to delays in obtaining the aminoglycoside antibiotic discs. Hence some patients received a post-operative antibiotic regimen that was not effective against the aerobic peritoneal isolates as judged by the *in vitro* tests. In 3 out of 15 patients with typhoid perforation who survived, the aerobic isolates from peritoneal pus were resistant to the antibiotics in the therapeutic regime used, while in 12 patients who survived, the isolates were sensitive. The association between a favorable prognosis and sensitivity of the aerobic isolates to the post-operative antibiotics was statistically significant (DF = 10, $p < 0.05$).

Fig 1, which compares the humoral immune responses in typhoid perforation with those in uncomplicated typhoid fever, shows that patients with typhoid perforation had lower geometric mean titers of both O and H antibodies by the SAT (O - 1/138; H - 1/46) compared with patients with uncomplicated typhoid matched for age, sex, duration of illness and prior antibiotic therapy and comparable in past TAB vaccination history (O - 1/476; H - 1/148). These differences were highly significant statistically (O antibody - $p < 0.005$; H antibody - $p < 0.0025$). However, no patient with typhoid perforation was completely non-reactive to both H and O antigens of *S. typhi*. Humoral immune competence was also assessed by determining total serum IgG, IgM and IgA levels in the two groups but no significant difference was seen (Fig 2).

Whereas all patients with uncomplicated typhoid fever had total serum IgG, IgM and IgA levels within the normal range, two patients with perforation had IgM levels below the normal level. One of them had low serum levels of all three Ig classes (IgG - 3.5 g/l; IgM - 0.44 g/l; IgA - 0.48 g/l). This was a patient with typhoid relapse who subsequently presented with ileal perforation. *S. typhi* isolated from mesenteric lymph nodes of this patient at laparotomy was sensitive to chloramphenicol.

Isohemagglutinin titers in typhoid perforation patients were comparable with titers obtained in healthy controls matched for age, sex and blood

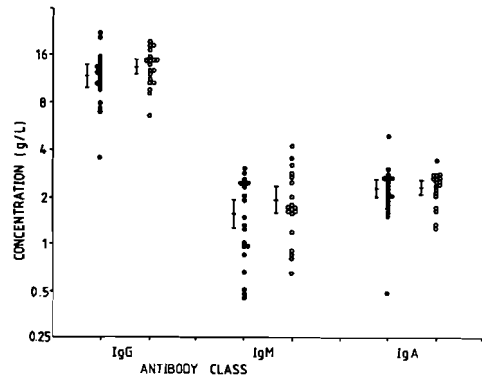


Fig 1—Distribution of 'O' and 'H' anti-typhoid agglutination titers (SAT) in patients with typhoid ileal perforation (●) compared with titers in matched patients with uncomplicated typhoid (○).

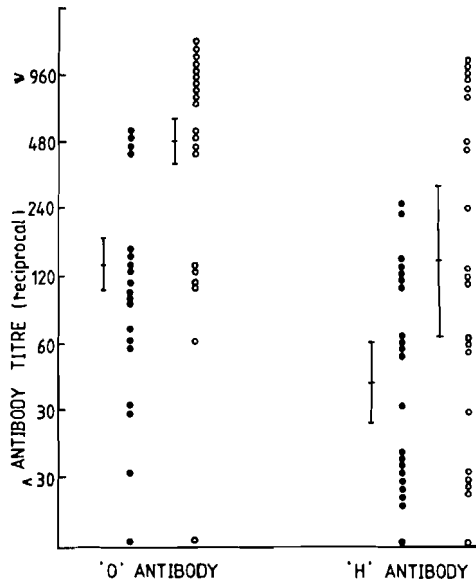


Fig 2—Distribution of class specific total serum IgG, IgM and IgA levels in patients with typhoid perforation (●) compared with levels in matched patients with uncomplicated typhoid (○).

group (data not presented).

Mesenteric lymph node histology of the 15 patients with typhoid perforation so examined showed that only two patients demonstrated evidence of T cell reactivity (extent and lymphoid cellularity of the paracortical zone) and three patients demonstrated active B cell responses (prominent germinal centers with presence of

large lymphoid cells and medullary cords). The other patients showed feeble or no B and T cell reactivity.

DISCUSSION

Mesenteric lymph nodes were the most useful specimen for the isolation of *S. typhi* (Table 1). Prior chloramphenicol treatment had little effect on the isolation rate from mesenteric lymph nodes whereas other sites (ileal contents, blood and peritoneal pus) were with one exception, negative for *S. typhi* when chloramphenicol treatment for more than 48 hours had been given (Table 2). Chloramphenicol resistance of *S. typhi in vitro* was not detected during the present study. Further, 107 strains of *S. typhi* isolated from uncomplicated typhoid fever in the same district during the same period were all sensitive to chloramphenicol *in vitro* (Thevanesam, 1985). Hence the persistence of *S. typhi* in the mesenteric lymph node following chloramphenicol treatment was not due to antibiotic resistance.

In a necropsy study of typhoid, Goodpasture (1937) documented that *S. typhi* in the mesenteric lymph nodes was found predominantly within phagocytes. Experimental studies on *S. typhi* within mouse fibroblast cells in *in vitro* culture have shown that addition of chloramphenicol to the culture medium resulted in cessation of intracellular bacterial multiplication within one hour. However, intracellular bacilli remained viable though dormant for upto 31 days of antibiotic treatment of the cell culture. The bacilli started to multiply when the antibiotic was withdrawn (Showacre *et al*, 1961). Studies on the multiplication of *S. typhimurium* in mouse macrophages in culture showed that addition of cephalothin to the culture medium was ineffective in killing intracellular bacteria. However, when liposome encapsulated antibiotic was used, efficient intracellular killing of intracellular bacilli occurred as a consequence of endocytic uptake of the liposomes which delivered the antibiotic to the cell cytosol (Desiderio and Campbell, 1983). Thus it is conceivable that the high isolation rate of *S. typhi* from mesenteric lymph nodes even after chloramphenicol therapy may have been due to the protection of bacilli from the antibiotic when within the macrophages.

Chloramphenicol resistance of *S. typhi* was not

a problem as judged by *in vitro* sensitivity tests; chloramphenicol and co-trimoxazole remain the drugs of choice in the treatment of *S. typhi*.

The aerobic cultures of the peritoneal pus on non-selective media showed that *S. typhi* is numerically insignificant in comparison with other pathogens, suggesting that once the fecal peritonitis has been initiated following ileal perforation, *S. typhi* plays little further role in the peritonitis. The predominant aerobic organisms were the coliforms in all but one patient who yielded *S. aureus*. Although anaerobic cultures were not done in this study, they (particularly *Bacteroides* spp) are known to be important pathogens in fecal peritonitis. In experimental models of fecal peritonitis in dogs, it has been shown that the early deaths were due to endotoxemia caused by the coliform organisms, whereas the late morbidity (localized intra-abdominal abscess formation) is caused predominantly by the anaerobic organisms (Zaleznik and Kasper, 1982).

The correlation observed between the final prognosis and the sensitivity of the predominant aerobic isolate from the peritoneal pus to the antimicrobials used probably indicates the importance of the aerobic flora of the peritonitis in the outcome of this disease. The trend towards improved prognosis seen after introduction of kanamycin to the post-operative antibiotic regimen (Kumarakulasinghe *et al*, 1978; unpublished data) also supports this contention.

Our results show that in Sri Lanka, chloramphenicol and ampicillin can no longer be relied upon to be active against the aerobic organisms of peritonitis. Gentamicin, the newer aminoglycosides or cephalosporins would now be suitable for this purpose. The use of kanamycin in this study was determined by the antibiotic availability in Sri Lanka at the time of this study (1975-1978) and cannot be recommended if gentamicin is available. Rational chemotherapy of these patients would also require agents active against the anaerobic flora of the peritonitis. Although chloramphenicol has significant activity against many anaerobic bacteria *in vitro*, it must be regarded as an agent of second choice for this purpose because (a) some anaerobic bacteria inactivate chloramphenicol, and (b) resistance of *B. fragilis* to chloramphenicol is documented (Bartlett, 1982). Metronidazole would be the antimicrobial of choice in this situation. Thus, a combination of chloramphenicol (in

areas where chloramphenicol resistant *S. typhi* is uncommon), gentamicin (or an alternative aminoglycoside) and metronidazole would be indicated for patients with typhoid ileal perforation. Although antagonism between chloramphenicol and gentamicin has been reported both *in vitro* and *in vivo* (Sande and Overton, 1973; Strausbaugh *et al.*, 1975), there is no consensus on the significance of these results. It is believed that when this combination of antibiotics is clinically indicated, it should be used (Kagan, 1980).

The pathogenesis of typhoid perforation is poorly understood. The suggestion that ileal perforation is a consequence of neglected and untreated typhoid fever is not borne out by other data (Kumarakulasinghe *et al.*, personal communication) where this complication occurred relatively early in the disease and in spite of treatment with chloramphenicol. Further, the incidence of typhoid perforation has not declined following the introduction of chloramphenicol therapy in the 1950s although the incidence of other complications of typhoid (eg 'toxemia', hemorrhage) has declined (Christie, 1974). Hypotheses advanced to explain the enigma of typhoid perforation are (a) the variation in virulence of *S. typhi* strains (Badoe, 1966), (b) reduced host resistance (Archampong, 1976), (c) hypersensitivity to *S. typhi* (Archampong, 1969) and sequential infections (Butler *et al.*, 1985). There is however little evidence to support any of these hypotheses.

Facts that favor the role of reduced host resistance in the pathogenesis of typhoid perforation are (a) progression to perforation in spite of adequate chloramphenicol therapy and, (b) the marked sex difference which is even more pronounced when the reproductive aged females are considered (Kumarakulasinghe *et al.*, unpublished data). Whereas occupational differences may explain the latter, immune reactivity is known to be influenced by gonadal hormones (Whittingham *et al.*, 1978; Grossman, 1985) and this could be an alternative explanation.

In the present study, the histology of the mesenteric lymph nodes suggested B and T cell hyporeactivity in most patients. The immunoglobulin concentrations and isohemagglutinin titers in the serum were normal making unlikely a generalized humoral immune defect. One patient however, who had a typhoid relapse subsequently

complicated by perforation had low levels of all three Ig classes whereas another patient with perforation had an IgM level marginally below normal. Specific immune responsiveness to *S. typhi* antigens O and H as measured by the SAT appears to be significantly lower in patients with typhoid perforation when compared with a group of patients with uncomplicated typhoid matched for age, sex, duration of illness, prior antibiotic therapy and TAB vaccination status. These findings suggest a specific immunological hyporeactivity to *S. typhi* antigens in patients with typhoid perforation.

These findings may be due to (a) the severe ill-health and hypercatabolic state caused by the perforation, (b) "mopping up" of serum antibody in perforation patients by a heavier bacterial load, (c) host hyporeactivity to *S. typhi* antigens due to environmental, nutritional or genetic causes. Since 74% of patients presented within 3 days of perforation it is doubtful whether the ill-health secondary to perforation could account for the SAT hyporeactivity via decreased Ig production. A hypercatabolic state which might follow peritonitis should affect the total Ig levels as well. Moreover malnutrition was not a feature in the patient with perforation in our series. Therefore a deficient nutritional state could not explain the observed findings.

While the observed humoral hyporeactivity to *S. typhi* antigens cannot be explained as a consequence of the perforation, the T-cell hyporeactivity as reflected in the lymph node histology may be secondary to the 'stress' of the perforation.

"Mopping up" of antibody would necessitate that patients with typhoid perforation harbor a much heavier antigen load of *S. typhi* than patients with uncomplicated typhoid. This is plausible if the peritonitis contained *S. typhi* in large numbers. This was not so although the possibility of "mopping up" cannot be ruled out.

Genetic, environmental and nutritional factors leading to hyporeactivity to *S. typhi* antigens merits consideration; malnutrition however was not a feature in our patients with perforation.

Genetically based hyporeactivity to *S. typhi-murium* antigens have been documented in inbred strains of mice. O'Brien *et al.* (1981) demonstrated an X-linked genetic locus *xid* in mice, which determined the magnitude of the IgG response to *S.*

typhimurium. The higher-responder phenotype correlated with *in vivo* resistance to challenge. Genetic factors in mice can determine the antibody response to *S. typhimurium* O and H antigens, high-responder mice showing a greater than 20 fold higher titer to both antigens when compared to low responder mice (Biozzi *et al*, 1984). The high and low response in this model applied to many different antigens and were shown to be controlled at the step of antigen presentation by the macrophage. However in contrast to the *xid* locus studied by O'Brien, the Biozzi high-responder mice were more susceptible to *in vivo* challenge when compared with low-responders. The low-responders in the latter model had macrophages which catabolized the antigen faster and which were less permissive to the intracellular multiplication of the *Salmonella*. CMI responses were not different in the two mouse lines. Although genetic control of immune responses in humans have been difficult to document, they do probably exist. Whittingham *et al* (1980) demonstrated that the titer of the primary IgG response to *S. adelaide* flagellin in normal humans is related to the Gm allotypes and the HLA-B locus antigens. Clinical observations that are compatible with a genetic predisposition to ileal perforation in typhoid are, a report of two identical twins developing typhoid perforation (Vyas *et al*, 1980) and perforation occurring in two sisters (Vogel *et al*, 1983).

In conclusion, we believe that the finding of lower H and O antibody titers together with the poor T and B cells response seen in the mesenteric lymph node histology is strongly suggestive of an immunological hyporeactivity associated with, and probably etiologically related to, the complication of ileal perforation in typhoid.

Further, the prognosis in the disease is related to adequate and effective antimicrobial therapy of the fecal peritonitis, which is an aspect poorly emphasized in most papers on this subject.

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