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Humoral and cell-mediated immune responses in chronic typhoid carriers

S. K. DHAM* & R. A. THOMPSON *Department of Immunology, East Birmingham Hospital, Birmingham, UK*

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SUMMARY

Humoral and cell-mediated immune responses to *Salmonella typhi* were studied in 10 chronic typhoid carriers, and in healthy controls. Carriers showed impaired cellular reactivity to *S. typhi* antigens in the leucocyte migration inhibition test (LMI). Carriers did not show a generalized depression of cell-mediated immunity in that delayed hypersensitivity skin test responses to recall antigens, peripheral blood T cell numbers, and lymphocyte transformation responses to mitogens were normal. Lymphocyte transformation in the presence of *S. typhi* antigen occurred to a greater extent than normal in four of six subjects tested and suggested the possibility of dissociated defects of cellular immunity. Carriers showed normal humoral immunity, as judged by antibodies to the flagellar and somatic antigens of *S. typhi* and *S. paratyphi* and to Vi antigens of *S. typhi.* The results suggest that the carrier state may be the consequence of a specific defect in cell-mediated immune responses to *S. typhi.*

INTRODUCTION

The asymptomatic excretion of pathogenic Salmonella organisms is a considerable public health hazard. About 3% of patients recovering from acute typhoid fever continue to harbour the organisms and excrete typhoid bacilli in the stools for periods in excess of 1 year and are termed chronic typhoid carriers (Huckstep, 1962). Some individuals are found to be carriers in the absence of a history of an acute illness. Up to 10^{10} organisms per gram of the faeces may be excreted (Merselis *et al.*, 1964) and this constitutes an important reservoir of infection. The mechanism of the chronic carrier state remains unclear. It is believed that scarred avascular areas of the gall bladder are colonized, which shelter the organisms from blood borne anti-microbial action and host defence. However, since gall bladder disease is not invariable in carriers, this is unlikely to be the full explanation. Little information is available concerning the immune responses, particularly the cell-mediated immune responses in these subjects, although cellular and humoral immunity is known to develop during acute typhoid fever (Dham & Thompson, 1982).

The present study describes an assessment of humoral and cell-mediated immune responses in chronic typhoid carriers and appropriate controls.

MATERIALS AND METHODS

Typhoid carriers studied. Ten chronic typhoid carriers who were under surveillance by the

* Present address: Armed Forces Medical College, Pune, India.

Correspondence: R.A.Thompson, Regional Department of Immunology, East Birmingham Hospital, Birmingham B9 5St, UK.

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Environmental Health Department, Birmingham, comprised the material for the present study. All were excreting typhoid bacilli in their stools. Six were excreting *S. typhi*, two *S. paratyphi A* and two *S. paratyphi B*. There were six females and four males with an average age of 35·5 years ±20·18 years (mean ±s.e.m.). The mean duration of the carrier state was 6·3 years (range 2–12 years). Three were Caucasians, six were Asians and one was a Negro. All subjects were asymptomatic and in good health with no evidence of any overt clinical abnormality. Only one subject gave a history of having suffered with typhoid fever in the past. The organisms were found in their stools during routine examination for public health purposes or for the investigation of symptoms which were unrelated or had resolved.

Controls. The controls consisted of 10 healthy Asians and 10 healthy Caucasians. There were four males and six females in each control group, and their mean age was 33 years. None of the control subjects gave a history suggestive of typhoid infection in the past, or had received typhoid vaccination in the preceding 2 years.

Serological tests. The antibodies to O, H and Vi antigens of *S. typhi* were determined by the standard Widal agglutination test, using suspensions of organisms obtained from the Central Public Health Laboratory at Colindale, London.

Immunoglobulin levels. The serum immunoglobulins (IgG, IgM and IgA) were measured by the single radial diffusion technique (Mancini, Carbonara & Hermans, 1965). IgA in whole mouth saliva, collected into a clean Universal container over a 30 min period, was also measured by the single radial diffusion technique using specific anti-α chain antiserum and a standard containing a known IgA concentration.

Assessment of cellular immunity

T cells. The enumeration of T cells was by the standard sheep red blood cell rosetting technique, (Stjernsward *et al.*, 1972).

Leucocyte migration inhibition (LMI). Specific cell-mediated immunity to *S. typhi* was assessed by the method of leucocyte migration inhibition (Federline *et al.*, 1971) using a heat-killed and sonicated *S. typhi* suspension as described previously (Dham & Thompson, 1982).

In vitro *lymphocyte transformation.* The response of peripheral blood lymphocytes was assessed by their ability to incorporate ^{3}H-thymidine in tissue culture in the presence of purified phytohaemagglutinin (PHA; Wellcome Reagents), pokeweed mitogen (Difco), PPD (0·5 mg/ml), *Candida albicans* extract (5 mg/ml) and the same concentrations of the *S. typhi* antigens used in the LMI test. Cultures were set up in quadruplicate, using the whole blood method (Ling & MacLennan, 1981) and the results expressed as the ratio of the radioactive counts in the test cultures divided by the counts in control cultures with medium alone.

Delayed hypersensitivity skin test. Pre-existing cellular immunity was assessed by delayed hypersensitivity reactions to recall antigens. The subjects were injected intradermally with 0·1 ml each of streptokinase/streptodornase (10 units and 2·5 units respectively), PPD (10 units), *C. albicans* protein extract 0·5% (Bencard), and 1% trichophyton extract (Bencard). Isotonic saline was used as control. The response was assessed by measuring the induration at the site of injection after 48 hr. A positive response was defined as an induration of 5 mm and above.

RESULTS

T cells

The mean (±s.d.) percentage of spontaneous E rosettes among carriers was 66·5 (±7·30) as against a mean of 71·1 (±3·21) in the control group. This difference was not statistically significant.

Leucocyte migration inhibition test

A positive LMI test to *S. typhi* antigen was obtained in only three out of 10 typhoid carriers (Fig. 1). Only one subject showed positive responses to all three concentrations of the antigen. Among the controls, two subjects (one each from the Asian and Caucasian group) showed a positive LMI test at the highest antigen concentration only (50×10^{6} organism/ml). There was no statistically significant

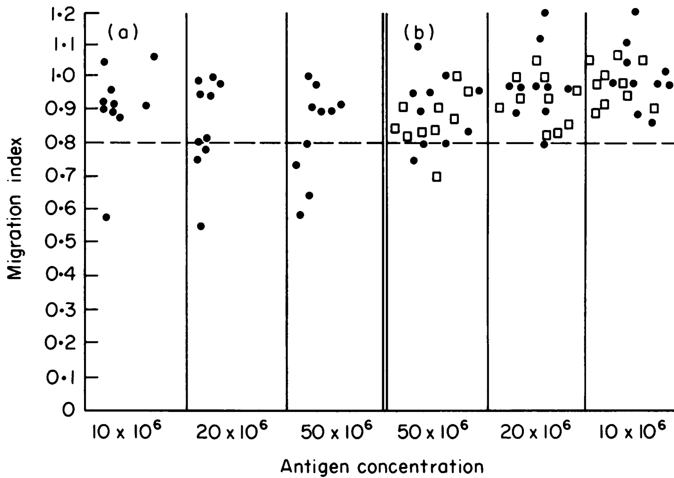

Fig. 1. The leucocyte migration indices in typhoid carriers, compared to Asian and Caucasian controls. (a) = typhoid carriers ($n = 10$); (b) controls ($n = 20$). (● = Asians; □ = Caucasians).

difference in the responses between the carriers and controls for each antigen concentration (Table 1).

Lymphocyte transformation

The results of *in vitro* lymphocyte transformation responses to mitogens and antigens are shown in Table 2. The *S. typhi* antigen preparation caused maximum stimulation at the highest antigen concentration (50×10^6 orgs./ml). At this concentration it resulted in weak stimulation in the six healthy unvaccinated controls and more pronounced responses in three of the six carriers who could be evaluated by this method. The responses of the typhoid carriers to mitogens were generally satisfactory, and apart from a poor PWM response in one subject, were similar to those of the healthy controls. One typhoid carrier showed weak *in vitro* lymphocyte transformation to PPD and Candida as well as to the *S. typhi* antigen.

There was some evidence of dissociation of cell-mediated immunity functions in that three subjects GS, AK, SH, (Table 2) with a moderate or good response to *S. typhi* antigens by *in vitro* transformation, were negative in the LMI test. However, the fourth individual (SK) with a good *S.*

Table 1. Leucocyte migration inhibition (LMI) index of typhoid carriers, Asian and caucasian controls in presence of *S. typhi* antigen

	LMI index (mean ±s.d.)		
Antigen concentration per well	Typhoid carriers	Asian controls	Caucaisn controls
50×10^6 Bacteria/ml	0·81±0·13 ($n = 10$)	0·93±0·14 ($n = 10$)	0·87±0·08 ($n = 10$)
20×10^6 Bacteria/ml	0·84±0·14 ($n = 10$)	0·95±0·12 ($n = 10$)	0·93±0·06 ($n = 10$)
10×10^6 Bacteria/ml	0·90±0·15 ($n = 10$)	0·98±0·09 ($n = 10$)	0·97±0·02 ($n = 10$)

There were no statistically significant differences ($P > 0·05$) between the carriers and either control group at any antigen concentration.

Table 2. *In vitro* lymphocyte transformation in typhoid carriers and controls

Subject	PHA (2 μg/ml)	Pokeweed mitogen (0·1%)	PPD (0·5 μg/ml)	*Candida albicans* (0·5%)	*S. typhi* (50 × 10⁶/ml)
SK	13	11·5	24	23	24
GS	12	170	67	47	14
AK	13	86	18	2·5	28
SH	49	1·5	3	1·6	4
KK	20	24	20	1·9	1·2
SB	18	26	1·2	1·4	1·1
Controls ($n=6$)	6–29	15–66	47–77	0·9–3·6	1·2–3·8

Expressed as stimulation index = ratio of ^{3}H-thymidine incorporation in cultures stimulated by antigen/mitogen relative to the cultures from the same subject without antigen.

typhi lymphocyte transformation response, showed a positive LMI test, and the two subjects with poor lymphocyte transformation to *S. typhi* antigens were also negative in the LMI test.

Skin tests

The results of delayed hypersensitivity reactions to PPD, streptokinase/streptodornase, Candida and trichophyton antigens are shown in Table 3. They indicated normal cellular responses to recall antigens.

Serum antibodies

The titres of O and H antibodies were found to be significantly higher ($P<0·01$) in chronic carriers in comparison to both Asian and Caucasian controls (Table 4). All *S. typhi* carriers had positive Vi antibody titres in the range 1/20–1/80, whereas all of the eight controls tested were negative (< 1/4).

Immunoglobulins

Table 4 also shows the means and standard deviations of the serum immunoglobulin levels (IgG, IgA and IgM) in chronic typhoid carriers and controls. Statistically significant differences in the levels of IgG ($P<0·01$) and IgA ($P<0·05$) were found in carriers only when compared with Caucasian controls.

The meaning of such differences was difficult to evaluate because of the significant difference in IgG levels ($P<0·01$) between Asian and Caucasian controls, and the varied ethnic origin of the carriers. However, there was no evidence of an immunoglobulin class preponderance or deficiency in these individuals.

Table 3. Delayed hypersensitivity reactions in chronic typhoid carriers and healthy controls (mean ± s.d. area of induration in mm)—subjects giving positive response shown in parentheses

	Strepto-kinase (10 units)/Strepto-dornase (2·5 units)	PPD (10 units)	Candida (0·5%)	Tricophyton (1%)
Chronic typhoid carriers ($n=10$)	9·5 ± 3·4 (10)	9·05 ± 1·35 (9)	5·5 ± 1·35 (7)	6 ± 1·34 (7)
Healthy controls ($n=8$)	12·0 ± 5·2 (8)	7·1 ± 1·1 (8)	5·5 ± 1·0 (6)	4·2 ± 1·0 (2)

Table 4. Serum immunoglobulin levels and agglutinin titres of typhoid carriers and controls

	Serum immunoglobulin levels (g/l)			Agglutinin titres*	
Subjects	IgG	IgA	IgM	Anti-H	Anti-O
Typhoid carriers ($n = 10$)	17·72±3·98	2·29±0·66	2·01±1·20	4·76±1·29	4·48±1·10
Asian controls ($n = 10$)	18·12±2·4	2·85±1·07	1·36±0·40	3·0±0·85	3·5±0·67
Caucasian controls ($n = 10$)	11·81±3·2	3·19±1·06	1·68±0·68	3·08±0·63	3·22±0·56

* Mean (±s.d.) titres expressed as log 10; sera negative at initial dilution of 1/25 assumed to have a value of 1/12.

Secretory IgA levels in the saliva of carriers were significantly raised ($P < 0{\cdot}001$) with a mean value of 0·61 g/l ±0·18 ($n = 10$) as against the mean in the control group of 0·08 g/l ±0·05 ($n = 10$).

DISCUSSION

Patients with acute typhoid fever develop cell-mediated immunity as demonstrated by the leucocyte migration inhibition test, which lasts for periods up to a year after recovery when they are no longer excreting the organisms, (Dham & Thompson, 1982). The importance of cell-mediated immune responses in recovery from typhoid infection in experimental animals (Ushiba, 1965, Makaness, Bladen & Collins 1966) and in humans (Sarma *et al.*, 1977) has been enphasized. There are very few previous studies on cell-mediated immune responses in chronic typhoid carriers. However, Lerenman *et al.* (1976), reproduced a typhoid carrier state in rabbits by injecting typhoid bacilli into the femoral bone marrow. In rabbits whose cell-mediated immunity was depressed by prior corticosteroid treatment, there was a prolonged persistence of typhoid bacilli in the bone marrow, with a much greater excretion of organisms in the faeces.

The present work indicates impaired cellular immunity to the antigens of heat killed *S. typhi* in chronic typhoid carriers. In fact, there were not statistically significant differences between the carriers and controls in the LMI tests. Carriers did not show evidence of a generalized depression of cell-mediated immunity in that peripheral blood T cells were present in normal numbers, lymphocyte transformation responses to mitogens were generally satisfactory, and delayed hypersensitivity skin tests to recall antigens were also normal. It was not considered ethical to attempt to demonstrate delayed hypersensitivity by a cutaneous reaction to the crude *S. typhi* antigen preparation.

The *S. typhi* antigen caused significant lymphocyte transformation in four of six carriers who were tested. While this, coupled with poor leucocyte migration inhibition, may indicate a dissociated cellular immune defect in some typhoid carriers, the crude antigen preparation may also have had some non-specific mitogenic effects (Peavy *et al.*, 1973).

As reported by others (Ledingham & Arkwright, 1912) typhoid carriers showed good antibody responses. Both O and H antibodies were significantly increased compared with controls and significant Vi antibody titres were detected in all *S. typhi* carriers, compatible with the persistance of live organisms in the host. The lack of protection afforded by these antibodies is well documented (Hornick *et al.*, 1970, Brodie, 1977).

Serum IgG levels were significantly raised in carriers compared with Caucasian controls but not with Asian controls. High serum IgA levels have been reported in carriers by other workers, (Lashin *et al.*, 1976; Bilibin *et al.*, 1976), but were not found in this study. Bilibin *et al.* (1976) also reported

lower levels of IgM in carriers and studies by Chernokhvostova *et al.* (1968) revealed a selective impairment in IgM antibody production. This was not apparent in this study.

The significant increase in salivary IgA levels possibly reflects greater antigenic challenge of the secretory immune system in carriers. However, mixed mouth saliva derives a significant proportion of its immunoglobulin content from crevicular fluid. It is likely to be increased in conditions of poor oral hygiene and this point was not evaluated at the time of the study. Nevertheless, assessment of specific local antibody activity *to S. typhi* antigens would be important in understanding the mechanism of the carrier state.

The role of the gall bladder and of chronic gall bladder disease in providing a haven for such organisms is also interesting. Three of the subjects in this study were children without evident gall bladder disease. The recently described role of the bile as the means of elimination of secretory IgA complexed antigens (Hall & Andrew, 1980) may be more significant in this context, and it is likely that further investigation of this aspect will be rewarding.

The results of the present study suggest a specific defect in cell-mediated immune responsiveness *to S. typhi.* The nature of the defect is clearly complex, but it bears some similarity to the immune defect shown in chronic mucocutaneous candidiasis (Lehner, Witton & Ivanhi, 1972). This could be responsible for the persistance and multiplication of typhoid bacilli, since the organisms are facultative intracellular parasites. In the absence of the macrophage enhancing activity of specifically sensitized lymphocytes, organisms may be able to shelter within such cells, safe from the humoral mechanisms of antibody and complement.

Treatment to terminate the carrier state should, in addition to the more conventional chemotherapeutic measures, also be aimed at enhancing specific cell-mediated immunity. A report of the preliminary results of such a treatment regime is in preparation.

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REFERENCES

BILIBIN, A.F., KHUNDANOV, L.L., POGORESKAYA, L.V. & LASIN, V.YA, (1976) Quantitative determination of specific immunoglobulins in sera of typhoid carriers. *Zh.Microbiol.Epidemiol.* **53,** 42.

BRODIE, J. (1977) Antibodies and the Aberdeen typhoid outbreak of 1964. *J. Hyg.* **79,** 161.

CHERNOKHVOSTOVA, K.I., LUXEMBURG, V.S., NATALIA, A. & GALINA, G. (1969) Study on the production of IgG, IgA and IgM antibodies to somatic antigens of salmonella typhi in humans. *Clin. exp. Immunol.* **4,** 407.

DHAM, S.K. & THOMPSON, R.A. (1982) Studies of Cellular and humoral immunity in Typhoid fever and TAB vaccinated subjects. *Clin. exp. Immunol.* **48,** 389.

FEDERLIN, K.R., MAINE, N., RUSSELL, A.S. & DUMONDE, D.C. (1971) A micro-method for peripheral leucocyte migration in tuberculin sensitivity. *J. clin. Pathol.* **24,** 533.

HALL, J.G. & ANDREW, E. (1980) Biliglobulin: a new look at IgA. *Immunol. Tod.* **1,** 100.

HORNICK, R.B., GRIESMAN, S.E., WOODWARD, T.E., DUPONT, H.L., DAWKINS, Z.T. & SNYDEN, M.J. (1970) Typhoid fever. Pathogenesis and immunological control (2 parts) *N. Engl. J. Med.* **283,** 691.

HUCKSTEP, R.L. Ed. (1962) *Typhoid Fever and other Salmonella infections.* E. & S Livingston Ltd., Edinburgh and London.

LASHIN, V.YA., MARKOVA, K.A., KHUNDANOV, L.L. & POGORELSKAYA, L.V. (1976) Immunoglobulin levels (IgA, IgG and IgM) in various forms of typhoid. *Zh.Mikrobiol. Epidemol.Immunobiol.* **53,** 75.

LEDINGHAM, J.C.G. & ARKWRIGHT, J.A. (1912) The carrier problem in infectious diseases. Arnold, London.

LEHNER, T., WILTON, J.M.A. & IVANHI, L. (1972) Immunodeficiencies in chronic mucocutaneous candidiasis. *Immunology,* **22,** 775.

LERENMAN, M.YA., NEVSKY, M.V., VEDENSKAYS, V.A., OSTER, N.R. ABIDOV, A.A., NAZHMETIOINOV, A.M. & MERZLYAKOVA, N.M. (1976) Participation of thymus in the mechanisms of bacterial discharge in carriers of typhoid bacteria. *Zh.Mikrobiol.Epidemol.Immunobiol.* **12,** 99.

LING, N.R. & MACLENNAN, I.C.M. (1981) Analysis of lymphocytes and tissues. In *Techniques in Clinical Immunology* (ed. by R.A. Thompson) 2 edn. p. 222. Blackwell Scientific Publications, Oxford.

MAKANESS, G.M., BLANDEN, R.M. & COLLINS, F.M. (1966) Host parasite relations in mouse typhoid. *J. exp. Med.* **124,** 573.

MANCINI, G., CARBONARA, O. & HERMANS, J.R. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochem.* **2,** 235.

MERSELIS, J.G., KAYE, D., CONNOLLY, C.S. & HOOK, E.W. (1964) The typhoid carrier state. Quantitative bacteriology and preliminary observations on therapy *E. Afr. Med. J.* **41,** 319.

PEAVY, D.L., SHANDS, J.W., ALTER, W.H. & SMITH R.T. (1973) Mitogenicity of bacterial endotoxins. Characterisation of mitogenic principles. *J. Immunol.* **111,** 352.

SARMA, B.V.N., MALAVIGA, A.N., KUMAR, R., GHAI, O.P. & BAKHTARY, M.M. (1977) Development of immune responses during typhoid fever in man. *Clin. exp. Immunol.* **28,** 35.

STJERNSWARD, J., JONDAL, M., VANKY, F., WIGZELL, H. & SEALY, R. (1972) Lymphopenia and changes in distribution of human B and T lymphocytes in peripheral blood induced by irradiation for Mammary Carcinoma. *Lancet,* **i,** 1352.

USHIBA, D. (1965) Two types of immunity in experimental typhoid 'cellular immunity' and 'humoral immunity' *Keio. J. Med.* **14,** 45.