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Immunological studies in typhoid fever

II. CELL-MEDIATED IMMUNE RESPONSES AND LYMPHOCYTE SUBPOPULATIONS IN PATIENTS WITH TYPHOID FEVER

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SUMMARY

The development of the cell-mediated immune response (CMIR) to antigens prepared from *Salmonella typhi* was investigated in patients suffering from typhoid fever and in normal healthy subjects. The leucocyte migration inhibition test, blast transformation of lymphocytes and active rosette-forming cells were used for detecting CMIR. Peripheral blood lymphocytes were analysed for the numbers and proportions of B lymphocytes, T lymphocytes and their subpopulations with receptors for IgM (Tμ) or IgG (Tγ) and cells with Fc receptors for IgG. These parameters were correlated with the duration and the severity of illness. The uncomplicated cases of typhoid fever were found to have an intact CMIR as compared to the complicated cases. The ratio of T lymphocyte subpopulations was grossly imbalanced in typhoid patients, the numbers of T lymphocytes and their subpopulations were further altered in the complicated cases as compared to uncomplicated cases. The present study demonstrates a depressed state of CMIR in complicated patients with typhoid fever. CMIR may thus emerge as the cardinal point for recovery in typhoid fever rather than the specific antibodies. The study further demonstrates that imbalance within the subsets of T lymphocytes may be responsible for the depressed state of CMIR in complicated cases of typhoid fever.

INTRODUCTION

The presence of antibodies during and following typhoid fever has been studied extensively (Olitzki, 1972), although their role in protection is not clear. Some workers have presented evidence to show that antibodies may not be involved in protection (Warren & Hornick, 1979). Earlier work from this laboratory has shown that the cell-mediated immune response in typhoid fever develops almost invariably during the second week of illness in uncomplicated cases while it was often negative in complicated cases (Sarma *et al.*, 1977).

The present paper describes further studies on cell-mediated immune responses in typhoid fever and on lymphocyte subpopulations in the peripheral blood of these patients.

MATERIALS AND METHODS

Subjects. The study was carried out on patients suffering from typhoid fever, diagnosed by a

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positive blood culture for *Salmonella typhi*. The controls included normal healthy volunteers from amongst the staff members and students of this hospital, as well as those living outside the hospital premises. A careful history was taken in all the control subjects to exclude those who had had typhoid fever or TAB vaccine during the preceding 5 years. One hundred and sixteen patients with typhoid fever (74 males and 42 females) and 94 normal controls were studied. The majority of the patients and normal controls have been previously investigated and reported in a previous paper (Rajagopalan, Kumar & Malaviya, 1981a). Their ages ranged from 3 weeks to 40 years. Of the 116 patients investigated, 24 had various complications which are described in the earlier paper (Rajagopalan *et al.*, 1981a).

Leucocyte migration inhibition test (LMIT). The technique of Federlin *et al.* (1971) was used with minor modifications. The details of the technique using *Salmonella typhi* as antigen are described in a recent paper (Rajagopalan *et al.*, 1982).

Blast transformation. The technique of Waller & MacLennan (1977) was followed with minor modifications. The details of the technique have been described in a recent communication (Rajagopalan *et al.*, 1981b).

Active rosette-forming cells (A-RFC). The active E rosette test of Felsberg & Edelman (1977) was followed. In brief, the technique consisted of incubating peripheral blood mononuclear cells, after washing them three times, with or without antigen for 2 hr at 37°C. This was followed by addition of heat-inactivated fetal calf serum and the cells were incubated for a further 1 hr. After 3 hr, the cells were rosetted with sheep red blood cells for 5 min and the A-RFC counted in a haemocytometer. The antigen used for this purpose was the same as that for the LMI test. An optimal concentration of 0·5 mg/ml of the antigen was used for enumerating the A-RFCs.

Subsets of lymphocytes. The details of the technique for enumerating T and B lymphocyte subpopulations and Fc receptor-bearing lymphocytes and subsets of T lymphocytes—Tμ (IgM) and Tγ (IgG)—have been described previously (Gupta *et al.*, 1979).

Statistical analysis. The parametric methods used for analysing the data were one-way analysis of variance, two-by-two and three-by-two Chi-squared analysis and unpaired *t*-test. The non-parametric method used was Wilcoxon's rank test (Wilcoxon, 1945).

RESULTS

The results of the leucocyte migration inhibition (LMI) test in patients with typhoid fever and controls are shown in Table 1. The mean LMI index of typhoid fever patients was less than that of the control subjects ($P < 0{\cdot}01$). Also, many more patients with typhoid fever were LMI-positive (LMI index $< 0{\cdot}76$) as compared to controls ($P < 0{\cdot}01$).

The LMI index according to the duration of illness is shown in Table 2. It is obvious that the lowest LMI index was obtained during the 2nd week of illness.

Table 1. Leucocyte migration inhibition test (LMIT) in patients with typhoid fever and controls

	Leucocyte migration inhibition test		
Subjects	Index (mean ± s.e.)	Positive subjects	Negative subjects
Typhoid ($n = 116$)	0·65 ± 0·02*	84*	32
Control			
Campus ($n = 57$)	0·70 ± 0·02	37	20
Outside			
campus ($n = 26$)	0·79 ± 0·04	10	16

* $P < 0{\cdot}01$ in comparison with controls.

Table 2. Leucocyte migration inhibition in patients with typhoid fever according to the duration of illness

Typhoid fever patients	Leucocyte migration inhibition index (mean ± s.e.)
1st week ($n=5$)	0·75 ± 0·04
2nd week ($n=44$)	0·61 ± 0·03
3rd week ($n=67$)	0·67 ± 0·03

The results of blast transformation are shown in Table 3. There was general agreement between the results of LMI and blast transformation using *S. typhi* antigen. Statistically significant differences were observed in the LMIT-positive subjects as compared to the LMIT-negative subjects with the *S. typhi* antigen ($P<0·001$ in the controls and $P<0·03$ in the typhoid patients). PHA-induced blast transformation did not show any obvious difference between the LMIT-positive and -negative subjects ($P>0·05$).

The active rosette-forming cells also correlated with the LMIT. The A-RFCs were enumerated in 22 typhoid patients and 14 normal controls. Of the 22 patients, 18 were LMIT-positive with a mean A-RFC of 52·67%. The four LMIT-negative patients had a mean A-RFC of 38·25%. The mean A-RFC in the 10 LMIT-positive control subjects was 62·5% while in the four LMIT-negative controls the mean A-RFC was only 24%. The difference between the mean A-RFC in the LMIT-positive and -negative controls and patients was found to be statistically significant ($P<0·05$).

When the patients were classified according to the severity of illness, the patients with complications had a depressed response to *S. typhi* antigen, besides being often LMI-negative (LMI

Table 3. Blast transformation in controls and patients with typhoid fever

	c.p.m./10^6 cells (mean ± s.e.)		Stimulation index (mean ± s.e.)	
Subjects	PHA	*S. typhi*	PHA	*S. typhi*
Controls				
LMI$^+$	$n=12$	$n=15$	$n=12$	$n=15$
	42,590 ± 21,953	12,356 ± 1,975	98·8 ± 29	18·6 ± 2·8
	(25,348)*	(12,273)	(62·4)	(15·7)
LMI$^-$	$n=8$	$n=9$	$n=8$	$n=9$
	59,671 ± 24,603	2,246 ± 1,260	93 ± 30·3	5 ± 2·5
	(34,110)	(389)	(40·7)	(2·0)
P value	>0·05	<0·001	>0·05	<0·001
Typhoid fever				
LMI$^+$	$n=11$	$n=10$	$n=11$	$n=10$
	20,089 ± 6,938	8,594 ± 2,854	40·4 ± 10·5	15·4 ± 5·2
	(9,532)	(5,807)	(44·9)	(12·1)
LMI$^-$	$n=4$	$n=4$	$n=4$	$n=4$
	3,466 ± 2,047	1,218 ± 916	6·9 ± 3·8	2·1 ± 0·9
	(1,692)	(994)	(4·0)	(1·3)
P value	>0·05	<0·03	>0·05	<0·03

* Figures in parentheses indicate the median value.

Table 4. LMIT and blast transformation in patients with typhoid fever: correlation with severity of illness

	Blast transformation					
	c.p.m./10^6 cells (mean ± s.e.)		Stimulation index (mean ± s.e.)		LMIT	
Typhoid patients	PHA	*S. typhi*	PHA	*S. typhi*	Positive	Negative
Uncomplicated	$n=10$ 22,056 ± 7,355 (12,120)*	$n=9$ 9,349 ± 3,031 (5,892)	$n=10$ 42·8 ± 11·2 (45·6)	$n=7$ 17·3 ± 5·4 (13·1)	$n=73$	$n=19$
Complicated	$n=5$ 2,855 ± 1,715 (1,530)	$n=5$ 1,335 ± 1,141 (242)	$n=5$ 8·7 ± 3·8 (4·5)	$n=5$ 1·4 ± 0·2 (1·3)	$n=11$	$n=13$
P value	> 0·05	< 0·05	> 0·05	< 0·05	< 0·01 $\chi^2=9·09$	

* Figures in parentheses indicate the median values.

Table 5. Proportion of lymphocyte subpopulations in patients with typhoid fever and controls

	Percentage lymphocyte subpopulations (mean ± s.e.)						
Subjects	T cells	B cells	Null cells	Fc cells	Tμ cells	Tγ cells	Tμ/Tγ
Controls	$n=43$ 75 ± 1	$n=42$ 12 ± 0·7	$n=42$ 13 ± 1	$n=42$ 17 ± 1	$n=38$ 56 ± 1	$n=42$ 14 ± 1	$n=38$ 4 ± 0·3
Typhoid patients	$n=29$ 63 ± 2	$n=24$ 13 ± 1	$n=24$ 24 ± 3	$n=24$ 27 ± 2	$n=24$ 34 ± 5	$n=29$ 29 ± 1	$n=24$ 1 ± 0·1
P value	< 0·001	n.s.	< 0·001	< 0·001	< 0·001	< 0·001	< 0·001

n.s. = Not significant.

Table 6. Proportion of lymphocyte subpopulations in uncomplicated and complicated typhoid patients

	Percentage lymphocyte subpopulations (mean ± s.e.)						
Subjects	T cells	B cells	Null cells	Fc cells	Tμ cells	Tγ cells	Tμ/Tγ
Uncomplicated patients	$n=22$ 67 ± 2	$n=19$ 13 ± 1·5	$n=19$ 20 ± 2·5	$n=19$ 24 ± 1	$n=17$ 38 ± 3	$n=22$ 28 ± 2	$n=17$ 1·5 ± 0·2
Complicated patients	$n=7$ 50 ± 1·4	$n=5$ 14 ± 2·3	$n=5$ 37 ± 9·5	$n=5$ 38 ± 6	$n=7$ 25 ± 3	$n=7$ 33 ± 2·1	$n=7$ 0·8 ± 0·1
P value	< 0·01	n.s.	< 0·02	< 0·05	< 0·01	< 0·05	< 0·05

n.s. = Not significant.

index > 0·76) in comparison to the uncomplicated cases as shown in Table 4. The differences were statistically significant ($P < 0·05$ for the blast transformation and $P < 0·01$ for LMIT).

The proportion of lymphocyte subsets in the peripheral blood of patients with typhoid fever and controls is shown in Table 5. The percentage of T lymphocytes was reduced ($P < 0·001$) while that of B lymphocytes was similar to that observed in the control subjects. Concomitantly, the null cells were increased in patients as compared to controls ($P < 0·001$). The proportion of cells with Fc receptor for IgG was increased in typhoid fever ($P < 0·001$). Within the T lymphocyte population, the Tμ (IgM) cells were reduced ($P < 0·001$) while Tγ (IgG) cells were increased ($P < 0·001$) in patients in comparison to controls. Thus, the Tμ/Tγ ratio was markedly altered ($P < 0·001$).

Within the typhoid fever cases, the proportion of T and Tμ cells was further reduced ($P < 0·01$) and cells with receptors for Fc-IgG, null cells and Tγ cells were increased ($P < 0·01$) and the Tμ/Tγ ratio was imbalanced ($P < 0·05$) in the complicated cases of typhoid fever in comparison to the uncomplicated cases. There was no difference in B cell numbers between the complicated and uncomplicated cases (Table 6).

DISCUSSION

In this study, the demonstration of specific lymphocyte sensitivity to *S. typhi* during typhoid fever was investigated with three different techniques. Specific lymphocyte sensitivity to *S. typhi* antigen during typhoid fever appeared in the uncomplicated patients during the 2nd week of illness. On the other hand, these tests were usually negative in patients with complications. This finding confirms our earlier observations (Kumar *et al.*, 1974; Sarma *et al.*, 1977). Since specific antibodies were equally distributed between the complicated and uncomplicated cases of typhoid fever (Rajagopalan *et al.*, 1981a) it is reasonable to assume that there might be some direct relationship between complications in typhoid fever and a negative LMI. We therefore studied the mechanism of a negative LMI in complicated cases of typhoid fever. Two factors which might be responsible for a negative LMI were considered:

(a) The presence of immune complexes.
(b) Suppressor T cells.

The first possibility was investigated and reported in the preceding paper where it was shown that immune complexes were seen more often in patients with complicated typhoid fever (Rajagopalan *et al.*, 1981a). The second possibility is reported in this paper.

The patients with typhoid fever had normal proportions of circulating B cells, slightly reduced proportions of T and null cells with increased proportions of IgG-Fc cells. The studies on the subsets of T cells showed significant reductions in the proportion of Tμ cells (a subpopulation of T cells with helper activity), increased proportions of Tγ cells (a subpopulation of T cells with suppressor activity) and a significantly lowered Tμ/Tγ ratio. This suggests, therefore, that there is a reduced number of helper T cells with an increased number of suppressor T cells in typhoid fever. The significantly increased numbers of IgG-Fc cells may also add to the suppression since this subpopulation may represent high-affinity Tγ (suppressor) cells (Moretta *et al.*, 1975).

These aberrations in lymphocyte subpopulations in the peripheral blood of typhoid fever were more pronounced and highly significant in complicated cases of typhoid fever as compared to the uncomplicated cases. The findings of high Tγ (suppressor) lymphocytes associated with complicated typhoid fever suggest that high levels of Tγ cells and circulating immune complexes (as reported earlier by Rajagopalan *et al.*, 1981a) may be responsible for a negative LMI. The simultaneous presence of these two factors is to be expected since it has been reported that immune complexes stimulate the formation of suppressor T cells and other suppressor factors (Perry, Benacerraf & Greene, 1978). However, these changes may not reflect any specific aberration which is unique to typhoid fever. For instance, specifically sensitized lymphocytes may be diverted to antigen in tissues and could give rise to altered lymphocyte populations in the circulation. Such abnormalities may be a general non-specific feature of inflammatory disorders (Huddlestone & Oldstone, 1979; Reinherz & Schlossman, 1980). It should also be noted that after the 2nd week of

illness there is no further increase in antibody titre (Sarma *et al.*, 1977; Rajagopalan *et al.*, 1981a) and further lowering of LMI index (Sarma *et al.*, 1977). This may be due to low T helper/high T suppressor activity at this stage of illness.

If further studies confirm the involvement of circulating immune complexes and suppressor T cells in inducing immunological abnormalities in typhoid fever, then a therapeutic approach to correct these abnormalities could be contemplated. Further functional studies of T suppressor cells in patients with complicated typhoid fever should help resolve this issue.

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