Clin. exp. Immunol. (1982) **47**, 275–282.

A study of humoral and cell-mediated immune response following typhoid vaccination in human volunteers

PREMAVATHY RAJAGOPALAN, R. KUMAR & A. N. MALAVIYA *Clinical Immunology Section, Department of Medicine, and Department of Microbiology, All-India Institute of Medical Sciences, New Delhi, 110029, India*

(Accepted for publication 8 May 1981)

SUMMARY

The specific antibody response to O, H and Vi antigens, levels of IgG, IgM, IgA and C3, presence of soluble immune complexes, *in vitro* tests of cellular immunity and subsets of lymphocytes in the peripheral blood were investigated in human volunteers after a single dose of 0·1 ml intradermal typhoid vaccination. The results indicated that typhoid vaccination induced antibody formation, slightly increased IgA levels and led to a decrease in C3 which was probably due to immune complex formation. There was also a relative increase in circulating Fc-IgG receptor-bearing lymphocytes and T-gamma cells after vaccination. In those subjects who showed specific cellular immunity before vaccination, a transient depression in lymphocyte transformation and a negative leucocyte migration inhibition test with typhoid baccilli occurred post-vaccination. These findings raise doubts over the advantages of giving typhoid vaccine during an epidemic.

INTRODUCTION

The exact mechanism of protective immunity in typhoid fever is not known. Earlier work from our laboratory indicated that the recovery in typhoid could be related to the development of cell-mediated immune response (CMIR) rather than antibodies (Kumar *et al.*, 1974; Sarma *et al.*, 1977). Furthermore, the vaccine was found to induce transient suppression of CMIR to Salmonella antigen in individuals who were otherwise naturally positive prior to vaccination (Nath *et al.*, 1977). Therefore, in the present work an attempt was made to define the possible cause of this post-vaccination anergy. As circulating immune complexes (CIC) and suppressor T subsets of lymphocytes probably cause perturbances of CMIR (WHO, 1977), they were investigated in the study subjects. The presence of CIC seems to be a strong stimulus for the formation of rheumatoid factor (RF) (Zubler & Lambert, 1977) and which has previously been noted in some patients with typhoid fever (Sarma, Malaviya & Kumar, 1978; Rajagopalan, Kumar & Malaviya, 1981). Our patients were therefore also screened for RF.

MATERIALS AND METHODS

Subjects. The subjects for this study comprised 15 normal healthy volunteers from amongst the staff members and residents of the hospital campus and nine normal volunteers from outside the campus. A careful history was taken from all subjects to exclude those who had had typhoid fever or

Correspondence: Dr Ramesh Kumar, Department of Microbiology, All-India Institute of Medical Sciences, New Delhi 110029, India.

0009-9104/82/0200-0275$02.00

TAB vaccination during the preceding 5 years. Their immunological status was investigated before vaccination as well as 3 and 8 weeks after vaccination. In this protocol, the subjects acted as their own controls.

Dosage and route of administration of the vaccine. The typhoid vaccine was obtained from the Central Research Institute, Kasauli, Himachal Pradesh, India. It contained 10^9 heat-killed (0·5% phenol-preserved) *Salmonella typhi* (Strain Ty-2) per ml. Volunteers were given only one injection of 0·1 ml typhoid vaccine intradermally on the volar surface of the arm. In our earlier study, this dose and route of administration were shown to induce an adequate antibody response (Nath *et al.*, 1977).

Humoral immune responses. Immunoglobulins, C3, O, H and Vi antibodies, rheumatoid factor and circulating immune complexes were studied. The details of the techniques have been described in a recent communication (Rajagopalan, Kumar & Malaviya, 1981). Circulating immune complexes were detected by two more additional techniques, besides the latex agglutination inhibition technique of Lurhuma *et al.* (1976). These two techniques were polyethylene glycol precipitation and anticomplementary activity.

Latex agglutination inhibition test. The technique of Lurhuma *et al.* (1976) was used. In brief, it consisted of using the serum of a seropositive rheumatoid arthritis patient with known titre of rheumatoid factor as an indicator. This was mixed with the serum to be tested for CIC and then mixed with the IgG-coated particles (latex reagent). Allowances were made for dilution factors. Inhibition of the rheumatoid arthritis serum's (indicator) capacity to cause agglutination of IgG-coated latex particles by the sera under test was considered as indicative of the presence of CIC. Aggregated human IgG was used as a positive control.

Polyethylene glycol precipitation (PEG). The technique of Haskova *et al.*, 1978) was used. Two millilitres of borate buffer (pH 8·4, 0·1 M) and 2 ml of buffered polyethylene glycol 6000 solution (4·166%, Sigma Chemicals, USA) were separately added to 0·22 ml of test serum stored at −20°C and prediluted with borate buffer at 1 : 3 to obtain a final concentration of 3·75% PEG and 1 : 30 of serum. After standard mixing in a vortex mixer and a 60-min incubation at room temperature, the difference in the light absorbance of the two samples (serum in borate buffer and serum in PEG) at a wavelength of 450 nm using 1-cm quartz cuvettes was measured photometrically (Carl–Zeiss, Germany). Heat-aggregated human IgG and borate buffer were used as positive and negative controls respectively.

Anticomplementary activity. Anticomplementary activity of the serum was assayed by the laboratory branch complement fixation test (LBCF Procedure, 1965) and by the technique of Verrier-Jones & Cumming (1977). Sheep red blood cells (SRBC) and hyperimmune sera raised in rabbits against SRBC were used as the haemolytic system. The optimal sensitizing dose of the haemolysin and the 50% haemolytic dose of the guinea-pig complement were predetermined.

Serum stored at −20°C was inactivated at 53°C for 30 min. Two-fold dilutions of the sera were made in microtitre plates in 25 μl of buffer and 50 μl of complement containing 5 CH50 units were added to each well. Control wells contained buffer alone with 5, 2·5 and 1·25 CH50 units of the complement. Aggregated human IgG was used as a positive control. The plates were sealed with transparent non-toxic tapes and kept at 4°C overnight. Sensitized cells were prepared by mixing thoroughly 3% SRBC with the optimal sensitizing does of haemolysin and incubating at 37°C for 30 min. In the meantime, the plates were placed at 37°C. After 15 min, 50 μl sensitized SRBC were added to each well and the plates were incubated at 37°C for 1 hr. Colour standards (representing 0–100% lysis) were prepared and distributed in separate wells. The plates were centrifuged and the results were interpreted as follows. Serum which lysed all the red cells (100%) was scored as negative and, where no lysis occurred (0%), as a positive indicator of anticomplementary activity. The intermediary reactions, as compared with colour standards, were scored as 25, 50 or 75%. Fifty percent lysis was taken as the end-point. The reciprocal of the highest dilution giving a 50% lysis was taken as the titre of the anticomplementary activity of the serum.

Cell-mediated immune responses. The *in vitro* parameters for cell-mediated immunity were studied as follows: (i) leucocyte migration inhibition test using specific antigen *S. typhi*, (ii) lymphocyte blast transformation using phytohaemagglutinin (PHA) as well as specific antigen *S. typhi*, and (III) subpopulations of lymphocytes (i.e. T, B, Fc-IgG receptor-bearing lymphocytes,

and subsets of T lymphocytes—Tμ and Tγ). The techniques used for the enumeration of lymphocyte subpopulations have been described previously (Gupta *et al.*, 1979).

Leucocyte migration inhibition test (LMIT). The technique of Federlin *et al.* (1971) was used with minor modifications. The antigen used was an ultrasonicated culture of *S. typhi* which had been heat-killed at 56°C for 1 hr. The dose–response was determined and the optimal non-toxic dose was found to be 3 mg/ml. The exact details of LMIT using *S. typhi* antigen have been described previously (Nath *et al.*, 1977).

Lymphocyte blast transformation. The whole blood technique of Waller & MacLennan (1977) was followed with minor modifications. Heparinized whole blood was taken and cells washed free of plasma and resuspended in RPMI 1640 (GIBCO, USA). The volume was adjusted so that 1 ml blood contained 1×10^6 lymphocytes. Macrocultures were set up using inactivated hepatitis B surface antigen-negative 20% pooled AB serum, in triplicate with each tube containing $0{\cdot}5 \times 10^6$ lymphocytes.

The lymphocyte response to *S. typhi* antigen was determined using normal controls who were *S. typhi*-positive and -negative in the LMI test. The prepraration of *S. typhi* antigen has been described previously (Nath *et al.*, 1977). For determining the lymphocyte response to PHA, normal controls irrespective of their LMI status to *S. typhi* antigen were used. PHA-P was obtained from DIFCO (Detroit, USA).

Preliminary studies were done with antigen doses from 0·1 to 1·0 mg/ml and the cultures were incubated for 96, 120 and 144 hr. Similarly, the optimum dose of PHA-P (DIFCO) was determined using amounts varying from 10 to 100 μg/ml for 48 and 72 hr in culture. One microcurie of ^{3}H-thymidine (sp.act. 9·8 mCi/mmol, Batch No. 26, BARC, Bombay, India) was added 24 hr before termination of cultures. After cell harvesting and TCA precipitation, the results (c.p.m.) were read in a beta liquid scintillation counter (Beckman, USA). The stimulation index was calculated by dividing the mean triplicate counts per minute (c.p.m.) in the presence of antigen or mitogen by the mean of triplicate c.p.m. in the absence of antigen or mitogen respectively. Maximum ^{3}H-thymidine uptake by the cells was obtained with 0·5 mg/ml of *S. typhi* antigen and 40 μg/ml of PHA. The stimulation response peaked at 120 hr for the antigen and 72 hr for the mitogen. These concentrations and timings were therefore used in further assays.

Statistical analysis and presentation of results. The titres of O, H and Vi antibodies and anticomplimentary activity are represented in natural logarithmic values. The serum immunoglobulins G, M and A and C3 levels are represented as geometric mean values. The results of lymphocyte transformation expressed as c.p.m. have been transformed for evaluating the statistical test of significance. The actual c.p.m. are given in Table 3. A one-way analysis of variance for unequal group sizes was applied to test for significance. The values are represented as mean $\pm$ standard error.

RESULTS

Twenty-four subjects were investigated for humoral and cell-mediated immune responses before vaccination. Of these, only 20 subjects could be investigated at 3 weeks post-vaccination and 16 subjects at 8 weeks post-vaccination.

Immunoglobulins and C3

The immunoglobulins G, M, A and serum complement C3 levels before and after typhoid vaccination are presented in Table 1. The IgA levels were significantly elevated 8 weeks after vaccination in comparison to pre-vaccination and 3 weeks post-vaccination levels ($P < 0{\cdot}05$). Serum C3 levels were significantly decreased 3 weeks following vaccination when compared to pre-vaccination levels ($P < 0{\cdot}05$). The C3 levels before vaccination and 8 weeks after vaccination were comparable. The levels of IgM and IgG remained unchanged after vaccination.

Antibody response

The mean initial titres for O and H antibodies were 3·83 and 4·41 respectively. Three weeks after

Table 1. Immunoglobulins and C3 levels and antibodies before and after typhoid vaccination in human volunteers

	Pre- vaccination ($n=24$)	3 weeks post-vaccination ($n=20$)	7 weeks post vaccination ($n=16$)
IgG	2·08±0·03	2·05±0·04	2·18±0·06
IgM	2·23±0·06	2·06±0·06	2·15±0·07
IgA	2·26±0·05	2·31±0·08	2·61±0·16*
C3	2·20±0·06	1·97±0·03†	2·18±0·04
O	3·83±0·20	5·35±0·24‡	5·68±0·24‡
H	4·41±0·25	6·05±0·30§	6·50±0·04§
Vi	2·54±0·30	2·25±0·31	2·90±0·34

Results expressed as mean±s.e.

* Significantly increased ($P<0·05$) as compared to pre- and 3 weeks post-vaccination levels.

† Significantly decreased ($P<0·05$) in comparison to pre- and 8 weeks post-vaccination levels.

‡ Significantly increased ($P<0·01$) in comparison to pre-vaccination levels.

§ Significantly increased ($P<0·001$) in comparison to pre-vaccination levels.

Table 2. Rheumatoid factor (RF) and immune complexes (IC) before and after typhoid vaccination in human volunteers

	Pre-vaccination ($n=24$)		3 weeks post-vaccination ($n=20$)		8 weeks post-vaccination ($n=16$)	
	Pos	Neg	Pos	Neg	Pos	Neg
RF	2	22	2	18	3	13
IC-latex inhibition	2	22	3	17	3	13
PEG Mean±s.e.	0·03±0·004		0·04±0·004		0·04±0·005	
ACA Mean±s.e.	2·05±0·58		7·14±0·33*		5·20±0·44†	

Figures for RF and IC-latex inhibition show numbers of subjects with positive or negative tests. See Materials and Methods for explanation of figures for PEG and ACA tests.

$P<0·01$; significantly increased in comparison to pre-vaccination levels.

† $P<0·05$; significantly decreased in comparison to 3 weeks post-vaccination levels, but remained significantly increased in comparison to pre-vaccination levels.

vaccination the respective mean titres were 5·35 and 6·05 ($P < 0·01$ for O and $P < 0·001$ for H). None of the values at 8 weeks after vaccination were statistically significant in comparison to values at 3 weeks after vaccination. The post-vaccination of Vi titres were comparable to the pre-vaccination Vi titres (Table 1).

Circulating immune complexes and rheumatoid factor (RF)

The presence of immune complexes and rheumatoid factor before and after vaccination is shown in Table 2.

Anticomplementary activity (AGA). Significant levels of anticomplementary activity were detectable in the sera 3 weeks post-vaccination as compared to pre-vaccination titres ($P < 0·01$). After 8 weeks of vaccination, AGA fell towards baseline yet remained significantly increased in comparison to pre-vaccination titres ($P < 0·05$).

PEG precipitation. CIC detected by this technique did not show any significant difference in the sera, before and after typhoid vaccination ($P > 0·05$).

Latex agglutination inhibition technique. CIC detected by this technique also did not show any significant difference in the pre- and post-vaccination sera ($P > 0·05$).

Rheumatoid factor (RF). There was no significant difference in the number of subjects with positive RF before and after vaccination.

Leucocyte migration inhibition test (LMIT)

The mean LMI index in the subjects studied before and after typhoid vaccination is given in Table 3. It was found to be significantly increased in subjects investigated at 3 weeks post-vaccination in comparison to pre-vaccination and 8 weeks post-vaccination migration index values ($P < 0·05$). The LMI index was significantly decreased 8 weeks after vaccination ($P < 0·01$) but was comparable to the pre-vaccination levels.

Of the 24 subjects investigated before vaccination, 15 were LMIT-positive (LMI index < 0·76). Three weeks after vaccination, 14 of the 15 pre-LMIT-positive subjects were investigated for LMIT. It was observed that all but one became LMIT-negative (LMI index > 0·76). Only 12 of them could be tested 8 weeks after vaccination and all of them reverted to a positive LMI state. The results presented in Fig. 1 were found to be statistically significant (chi-square, $P < 0·01$).

Table 3. Leucocyte migration inhibition (LMI) and blast transformation before and after typhoid vaccination in human volunteers

		Blast transformation			
		Stimulation index		c.p.m./10^6 cells	
	LMI index	PHA	*S. typhi*	PHA	*S. typhi*
Pre-vaccination ($n = 24$)	0·73 ± 0·04	92 ± 2‡	13 ± 2‡	54,667 ± 16,293‡	8,564 ± 1,653‡
3 weeks post-vaccination ($n = 20$)	0·90 ± 0·04*	1·04 ± 19	4 ± 1†	34,429 ± 6,808	1,389 ± 458‡
8 weeks post-vaccination ($n = 16$)	0·62 ± 0·07	67 ± 11	10 ± 3	28,691 ± 6,383	3,209 ± 1,376

Results expressed as mean ± s.e.

* Significantly increased in comparison to pre-vaccination and 8 weeks post-vaccination levels ($P < 0·05$)

† Significantly decreased at 3 weeks post-vaccination in comparison to pre-vaccination and 8 weeks post-vaccination levels ($P < 0·01$).

‡ $n = 20$.

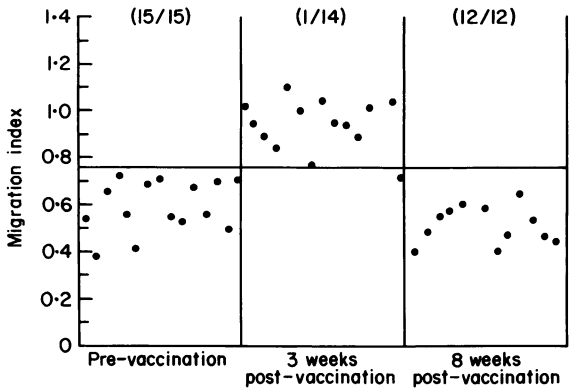

Fig. 1. Longitudinal study of leucocyte migration inhibition test in pre-vaccination LMIT-positive subjects. Migration index = mean area of leucocyte migration in the presence of antigen/mean area of leucocyte migration in the absence of antigen. Figures in parentheses denote the number of positive LMIT subjects over the total number of subjects investigated for LMIT at that particular period.

Lymphocyte blast transformation

The blast transformation response to PHA and *S. typhi* antigen is summarized in Table 3. A significantly depressed response to *S. typhi* antigen was observed 3 weeks after vaccination in comparison to pre-vaccination and 8 weeks post-vaccination response ($P < 0{\cdot}01$). The transformation response to PHA remained unaltered.

Lymphocyte subpopulations

The proportion of lymphocyte subpopulations in the peripheral blood of subjects before and after typhoid vaccination is shown in Table 4. T and null lymphocyte subsets were comparable before and after vaccination. However, absolute numbers of B cells were significantly increased 8 weeks

Table 4. Proportion of lymphocyte subpopulations in the peripheral blood before and after typhoid vaccination in human volunteers

Subject	T	B	Null cells	Tμ	Tγ	Tu/Tγ	Fc-IgG
Pre-vaccination ($n=23$)	75±1	12±1	12±2	56±1‡	14±1	4±0·3	16±1
3 weeks Post-vaccination ($n=20$)	75±2	11±0	14±2	37±2*	28±1*	1±œ*	29±2*§
8 weeks post-vaccination ($n=16$)	73±9	14±1	14±3	50±2†	18±0·6†	3±0·1†	19±1†

Results expressed as mean±s.e.

* $P < 0{\cdot}001$; significant increase of Fc receptor and Tγ cells, decrease of Tμ cells and Tμ/Tγ ratio at 3 weeks post-vaccination as compared to pre- and 8 weeks post-vaccination.

† $P < 0{\cdot}001$; Tμ cells and Tμ/Tγ ratio significantly increased and Tγ cells significantly decreased in comparison to 3 weeks post-vaccination levels but non-comparable to pre-vaccination levels.

‡ $n=22$.

§ $n=19$.

after vaccination in comparison to pre- and 3 weeks post-vaccination levels ($P < 0{\cdot}05$—not shown in Table 4). Tγ cells were significantly elevated 3 weeks after vaccination ($P < 0{\cdot}001$) and decreased significantly at 8 weeks post-vaccination but the pre-vaccination levels were not attained ($P < 0{\cdot}01$). Tμ cells showed the reverse pattern. They were decreased ($P < 0{\cdot}001$) 3 weeks after vaccination and increased at 8 weeks post-vaccination—though not comparable to the pre-vaccination levels ($P < 0{\cdot}01$). These changes in Tμ and Tγ subsets of T cells led to a significant decrease in the Tμ/Tγ ratio at 3 weeks post-vaccination ($P < 0{\cdot}001$). The Tμ/Tγ ratio though increased at 8 weeks after vaccination, was still not comparable to the pre-vaccination ratio ($P < 0{\cdot}01$). Fc-IgG receptor-bearing cells were elevated at 3 weeks after vaccination ($P < 0{\cdot}001$) and reverted to pre-vaccination levels after 8 weeks.

DISCUSSION

The present paper reports our findings on some parameters of the immune response following a single 0·1 ml dose of typhoid vaccine given intracutaneously in volunteers.

No appreciable difference was observed in the IgG or IgM levels following vaccination. However, IgA levels were found to be significantly higher 8 weeks after vaccination than the pre-vaccination level. The significance of this increase is uncertain, but it is known that systemic immunization with killed cholera vaccine affords definite protection (Tomasi, 1972) and that mucosa may be the source of specific serum IgA (Tomasi & Gray, 1972).

Specific antibodies appeared within 3 weeks of immunization and the levels plateaued between 3 to 8 weeks. However, the *in vitro* tests for CMIR did not become positive during that period. Furthermore, the volunteers showing positive *in vitro* tests for CMIR prior to immunization gave negative tests 3 weeks after the vaccination. However, they had reverted to the positive state by 8 weeks. This confirms our earlier observation that the typhoid vaccine induces transient depression of specific CMIR in the immediate post-vaccination period (Nath *et al.*, 1977). Since the CMIR may be relevant in protection, vaccination may increase susceptibility to disease in the immediate post-vaccination period. This could be responsible for the provocative typhoid after vaccination described in the older literature (Paul, 1953). It also implies that typhoid vaccine should be avoided during epidemics.

The anticomplementary activity and low C3 levels in the serum after 3 weeks of typhoid vaccination suggest the presence of circulating immune complexes and C3 consumption. However, the other less sensitive tests for the detection of circulating immune complexes (viz. PEG precipitation and latex inhibition) remained negative after vaccination. It is known that anticomplementary activity of the serum is one of the most sensitive indicators for immune complexes (Verrier-Jones & Cumming, 1977). However, as the other tests for CIC remained negative, the possibility of anticomplementary activity being due to some other factors must also be considered. It is known that RF, bacterial contamination or aggregation of immunoglobulins due to denaturation may be responsible for a positive anticomplementary test (Verrier-Jones & Cumming, 1977). In the present study, care was taken to avoid these problems.

There was a increase in Fc-receptor-bearing lymphocytes and Tγ cells with a relative decrease in Tμ cells 3 weeks after vaccination. These alterations started to revert to normal 8 weeks after vaccination. The Fc receptor-bearing lymphocytes represent a heterogeneous population of cells and the significance of an increase in their number is not clear. It is known that T-suppressor and T-helper activity is contained within the population of T cells identified as Tγ and Tμ respectively (Moretta *et al.*, 1977). Thus an increase in Tγ and a decrease in Tμ cells at 3 weeks after vaccination may lead to suppression of immune responses.

Thus both circulating immune complexes and increase in T-suppressor activity may be responsible for the post-vaccination depression of cellular immunity observed in our subjects. A similar finding has recently been reported by Gefford & Orbach-Arbouys (1976) and Watson & Collins (1980) in mice injected with large numbers of mycobacteria. However, another possibility for the apparent depressed CMIR to *S. typhi* is that the sensitized lymphocytes in the circulation are trapped in the lymphoid organs where the immune reaction is taken place. Sequestration of such

lymphocytes may lead to reduced population of such cells in the peripheral blood leading to an erroneous impression of depressed CMIR.

It is interesting that the abnormalities we have found after typhoid vaccination mimic our previously reported findings in typhoid fever (Rajagopalan *et al.*, 1981). It is tempting to suggest that some antigens of *S. typhi* have evolved because they lead to a suppression of specific immunity and help the organism survive in man.

This work was supported financially by a grant from the Department of Science and Technology, Government of India. The authors are very grateful to the Bio-Statistics Department for excellent co-operation in analysing the data. The invaluable help of Dr Vinod Raina, Senior Resident, Department of Medicine, in carrying out the investigations on the volunteers for this study is gratefully acknowledged.

REFERENCES

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

GEFFORD, M. & ORBACH-ARBOUYS, S. (1976) Enhancement of T-suppressor activity in mice by high doses of BCG. *Cancer Immunol. Immunother.* **1**, 41.

GUPTA, S., MALAVIYA, A.N., RAJAGOPALAN, P. & GOOD, R.A. (1979) Subpopulations of human T-lymphocytes. IX. Imbalance of T-cell subpopulations in patients with progressive systemic sclerosis. *Clin. exp. Immunol.* **36**, 342.

HASKOVA, V., KASLIK, J. RIHA, I., MATL, I. & REVENSKY, J. (1978) Simple method of circulating immune complex detection in human sera by polyethylene glycol precipitation. *Z. Immunitätsforsch.* **154**, 399.

KUMAR, R., MALAVIYA, A.N., MURTHY, R.G.S., VENKATARAMAN, M. & MOHAPATRA, L.N. (1974) Immunological study of typhoid; immunoglobulins, C3, antibodies and leukocyte migration inhibition in patients with typhoid fever and TAB-vaccinated individuals. *Infect. Immun.* **10**, 1219.

LABORATORY BRANCH COMPLEMENT FIXATION TEST PROCEDURE (1965) *Public Health Monogr. Ser.* No. 74. Government Printing House, Washington, DC.

LURHUMA, A.C., CAMBIASO, C.L., MASSON, P.L. & HEREMANS, J.F. (1976) Detection of circulating antigen–antibody complexes by their inhibitory effect on the agglutination of IgG-coated particles by rheumatoid factor or C1q. *Clin. exp. Immunol.* **25**, 212.

MORETTA, L., WEBB, S.R., GROSSI, C.E., LYDYARD, P.M. & COOPER, M.D. (1977) Functional analysis of two human T-cell-bearing receptors for IgM (T_M) or IgG (T_G). *J. exp. Med.* **146**, 184.

NATH, T.R., MALAVIYA, A.N., KUMAR, R., BALAKRISHNAN, K. & SINGH, B.P. (1977) A study of the efficacy of typhoid vaccine in inducing humoral and cell-mediated immune responses in human volunteers. *Clin. exp. Immunol.* **30**, 38.

PAUL, H. (1953) Immunity and immunization. In *Encyclopaedia of Medical Practice* (ed. by Lord Horder). Vol. 7, p. 36. St Louis.

RAJAGOPALAN, P., KUMAR, R. & MALAVIYA, A.N. (1981) Immunological studies in typhoid fever. I. Immunoglobulins, C3, antibodies, rehumatoid factor and circulating immune complexes in patients with typhoid fever. *Clin. exp. Immunol.* **44**, 68.

SARMA, V.N.B., MALAVIYA, 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.

SARMA, V.N.B., MALAVIYA, A.N. & KUMAR, R. (1978) Immunological studies in typhoid: immunoglobulins, C3, lymphocyte subpopulation and rheumatoid factor. *Indian J. Med. Res.* **67**, 903.

TOMASI, T.B. (1972) Secretory immunoglobulins. *N. Engl. J. Med.* **287**, 500.

TOMASI, T.B. & GRAY, H.M. (1972) Structure and function of immunoglobulin A. In *Progress in Allergy* (ed. by P. Kallos, Helsinborg, B. H. Waksman and A. de. Weck) Vol. 18. S. Krager, Basel.

VERRIER-JONES, J. & CUMMING, N. (1977) Tests for detecting circulating immune complexes. *Techniques in Clinical Immunology* (ed. by R. A. Thompson). Blackwell, Oxford.

WALLER, C.A. & MACLENNAN, I.C.M. (1977) Analysis of lymphocytes in blood and tissues. In *Techniques in Clinical Immunology* (ed. by R. A. Thompson), p. 170. Blackwell, Oxford.

WATSON, S.R. & COLLINS, F.M. (1980) Development of suppressor T-cells in mice heavily infected with mycobacteria. *Immunology*, **39**, 367.

WHO (1977) Report of a scientific group. The role of immune complexes in disease. *WHO Tech. Rep. Ser.* No. 606.

ZUBLER, R.H. & LAMBERT, P.H. (1977) Immune complexes in clinical investigation. In *Recent Advances in Clinical Immunology* (ed. by R. A. Thompson), Vol. 1. p. 125. Churchill Livingstone, Edinburgh, New York.