THE DETERMINATION OF ANTI -*TOXOPLASMA GONDII* ANTIBODIES IN DIFFERENT IgG SUBCLASSES OF HUMAN SERA BY THE ENZYMELINKED IMMUNOSORBENT ASSAY (ELISA).

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INTRODUCTION

In the humoral response to *T. gondii*, the major class of immunoglobulins produced in humans is IgG (Krahenbuhl and Remington, 1982). In normal human serum, IgG consists of 4 subclasses, IgG1, IgG2, IgG3 and IgG4, in the proportions, 65%, 23%, 8% and 4% respectively (Roitt, 1988). Various studies have shown that certain antigens elicit IgG production restricted to only some of the 4 subclasses (Spiegelberg, 1974; Ottesen *et al.*, 1985).

The IgG subclass response to viral and bacterial infections has been extensively studied. The IgG2 subclass reactivity in humans was observed to be restricted to polysaccharide antigen (Siber *et al.*, 1980; Barrett *et al.*, 1986) while anti-protein antibodies were usually of the other IgG subclasses, especially IgG1 and IgG3 (Persson *et al.*, 1988). Epstein-Barr and rubella viruses induced a predominant IgG3 response (Walker *et al.*, 1983; Bird *et al.*, 1984). IgG1 and IgG3 were most frequently found in antibodies to hepatitis B surface antigen (Persson *et al.*, 1988). Streptococcal M-associated protein elicited a predominant IgG3 response (Mortimer and Widdowson, 1979) whereas IgG1 and IgG3 were exclusively found in the primary stage of *Treponema pallidum* infection (Van der Sluis *et al.*, 1986). Patients with severe acne produced antibodies in the IgG2 and IgG3 subclasses (Holland *et al.*, 1986). Although antibody activity to tetanus toxoid was found in all 4 subclasses, IgG1 was predominant (Bird *et al.*, 1984; van der Giessen *et al.*, 1976).

IgG subclass activity is also varied in responses to parasitic infections. *Plasmodium falciparum* infection preferentially triggered IgG1 and IgG3 in Liberians, while IgG1 and IgG2 were produced in Swedes (Wahlgren *et al.*, 1983). Salimanou, *et al.*, (1982) found that Nigerians with malaria had high levels of IgG1. Infection with *Leishmania* resulted in elevated levels of IgG1 and IgG3 in sera from Sudanese patients (El Amin *et al.*, 1986). Similarly, patients suffering from Chagas' disease produced IgG antibodies that were mostly of the IgG1 and IgG3 subclasses (Scott and Goss-Sampson, 1984). *Schistosoma* infection produced high levels of IgG4 and modestly high levels of IgG3 in Egyptian

patients (Iskander *et al.*, 1981). A similar increase of IgG4 subclass activity was also observed in human filariasis (Ottesen *et al.*, 1985; Hussain & Ottesen, 1986).

The present study was undertaken to determine, by enzyme-linked immunosorbent assay (ELISA), the predominant IgG subclasses in the IgG response to toxoplasmosis.

MATERIALS AND METHODS

Antigen

Toxoplasma gondii antigen was prepared from tachyzoites obtained from peritoneal cavities of outbred Swiss albino mice infected 4 days earlier with the RH strain. Parasites were harvested in sterile saline, washed 5 times, resuspended in sterile saline and filtered through 3 μm polycarbonate membranes (Nucleopore Corp. Pleasanton, USA). The tachyzoites were then washed twice, suspended in sterile saline and stored at -70 ° C overnight. The parasite suspension was thawed and sonicated in an ice-bath at 14 microns amplitude (Soniprep 150, MSE, USA) 10 × 1 minute. The sonicate was centrifuged at 10,000 *g* for 30 minutes at 4 ° C and the supernatant was stored at -70 ° C and used as soluble antigen. Protein content of the antigen was estimated by the Bio-Rad dye-binding assay (Bio-Rad Laboratories, Richmond, California) with bovine serum albumin (BSA) as standard.

Sera

Sera of adult patients clinically suspected of toxoplasmosis were obtained from various clinics and hospitals in Singapore. Control sera were donated by several staff and students in the Department of Microbiology, National University of Singapore.

ELISA for anti-*T. gondii* IgG antibodies

The ELISA performed was a modification of the micro-ELISA developed by Voller, *et al.*, (1976). The concentrations of antigen and antisera in the ELISA were determined by checkerboard titrations. The antigen was diluted in carbonate buffer (pH 9.6) and incubated overnight at 4 ° C in wells of polyvinyl chloride (PVC) microtitre plates at a concentration of 4 ug per 100 μl per well. Plates were then washed 4 times for 1 min. each with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T20). Wells were postcoated with 100 μl 1% BSA in PBS for 1 1/2 h at 37 ° C. Plates were washed as described. Sera were diluted 1:200 in PBS-T20 with 0.5% BSA and incubated for 2 h at 37 ° C. The plates were again washed as before and 100 μl horse radish peroxidase (HRP)-conjugated rabbit antibodies to human IgG (Dakopatts, Denmark) diluted 1:5,000 in PBS-T20 with 0.5% BSA were added to each well and incubated as above. Washing was performed as described before. Enzyme substrate solution (40 mg orthophenylenediamine per 100 ml citrate buffer, 40 μl 30% H_2O_2 per 100 ml buffer) was added to each well (200 μl/well) and incubated at room temperature. The reaction was terminated by addition of 50 μl/well 50% H_2SO_4. Absorbance (or optical density) at 490 nm was determined with a micro-ElISA reader (Dynateach Laboratories, USA).

ELISA for IgG subclasses

The IgG subclass activity was determined by ELISA which was essentially similar to that described above. Monoclonal antibodies against human IgG1, 2, 3 or 4 (Unipath, England) diluted 1:1,000 and HRP-labelled rabbit antibodies against mouse antibodies (Dakopatts, Denmark) diluted 1:3,000 were sequentially added instead of HRP-labelled

rabbit antibodies to human IgG.

RESULTS

One hundred and seventeen sera were tested for anti-*T. gondii* IgG antibodies and 44 were found to be IgG-positive and 73 IgG-negative. The activity of each IgG-subclass in the IgG-positive sera was compared with that of IgG-negative sera using Student's *t* test.

IgG1 activity was elevated in positive sera and was significantly different from that of negative sera ($P < 0.05$). The results are shown in Fig. 1a and 2a. The range of absorbance (or optical density, OD) values of IgG-positive sera in the IgG1-ELISA was wide, from 0.020 to 1.250 ($\bar{x} = 0.375$, SD = 0.323, CV = 86.1%). The IgG-negative sera had OD values less than 0.100 in the IgG1-ELISA ($\bar{x} = 0.037$, SD = 0.020, CV = 54.1%). The OD values of the IgG-positive sera in the IgG1-ELISA correlated favourably (Fig. 2a) with those of the IgG-ELISA ($r = 0.714$). Virtually no correlation was obtained with the IgG-negative sera ($r = -0.005$).

IgG2 activity was not evident as there was no significant difference ($P > 0.05$) between the 2 groups of sera (Fig. 1b). The OD values obtained were below 0.200. There was no correlation ($r = 0.245$ for IgG-positive sera, $r = 0.152$ for IgG-negative sera) with the OD values of the IgG-ELISA (Fig. 2b).

The level of IgG3 in IgG-positive sera was low ($\bar{x} = 0.372$, SD = 0.052, CV = 14.0%) but differed significantly ($P < 0.05$) from that of IgG-negative sera ($\bar{x} = 0.327$, SD = 0.030, CV = 9.2%). The results are shown in Fig. 1c. There was, however, poor correlation ($r = 0.235$ for IgG-positive sera, $r = -0.003$ for IgG-negative sera) with the OD values of the IgG-ELISA (Fig. 2c).

Similarly, as shown in Fig. 1d, IgG4 activity was low in positive sera ($\bar{x} = 0.085$, SD = 0.049, CV = 57.6%) but differed significantly ($P < 0.05$) from that of negative sera ($\bar{x} = 0.071$, SD = 0.022, CV = 31.0%. In addition, there was weak correlation ($r = 0.549$) of the OD values of IgG-positive sera in the IgG4- and IgG-ELISA (Fig. 2d). No correlation ($r = -0.005$) was obtained with the IgG-negative sera.

DISCUSSION

The present study showed that IgG1 is the dominant IgG subclass involved in the humoral response to *T. gondii* infection in humans. IgG3 and IgG4 may be produced at low but significant levels while IgG2 production was not apparent. The results may reflect the preferential recognition of protein antigens of *T. gondii* by IgG antibodies, as IgG1, IgG3 and, to a smaller extent, IgG4 preferentially react with protein antigens. Mineo, *et al.*, (1980) reported that IgG antibodies reacted primarily with protein antigens of *T. gondii*. However, others (Sharma *et al.*, 1983; Naot *et al.*, 1983) have found that IgG reacted with both protein and polysaccharide antigens of the parasites.

In humans, IgG1 and 3 avidly bind complement and the classical complement pathway activation has been demonstrated as the activator system for antibodies to *T. gondii* (Schreiber and Feldman, 1980). IgG1 and 3 also bind to mononuclear cells (Alexander, 1980) and activated macrophages and monocytes have been implicated as the major effector cell population in cell-mediated immunity (CMI) to *T. gondii* (Wilson and Remington, 1980). Phagocytosis is promoted when the cells bind with the opsonized microorganisms through specialized receptors for IgG (IgG1 and 3 in humans)

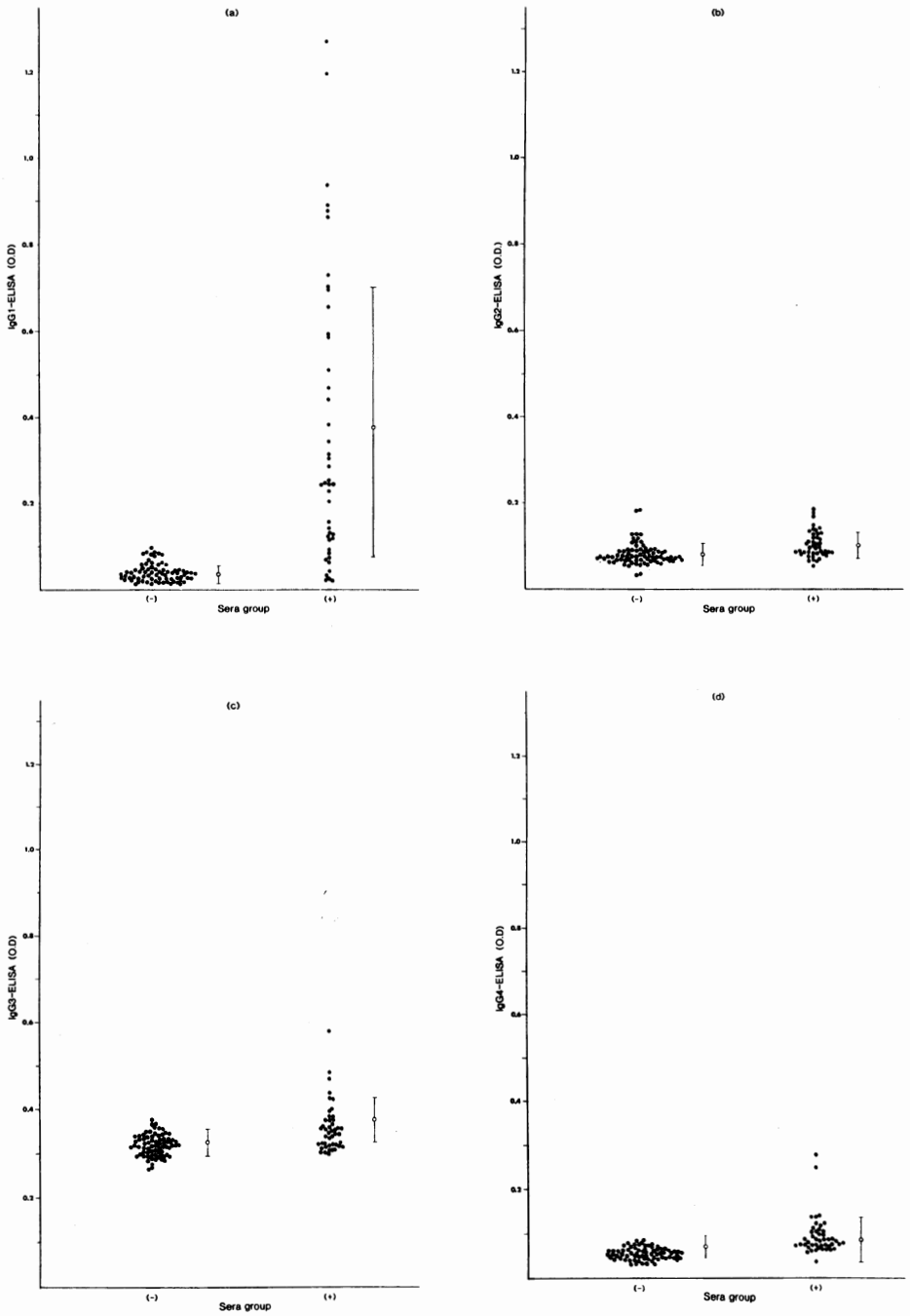

Fig. 1–Anti-*T. gondii* antibodies in different IgG subclasses determined by ELISA.
(a) : IgG1; (b) : IgG2; (c) : IgG3; (d) : IgG4
(−) : sera negative by IgG-ELISA for anti-*T. gondii* IgG antibodies; (+) : sera positive by IgG-ELISA for anti-*T. gondii* IgG antibodies.

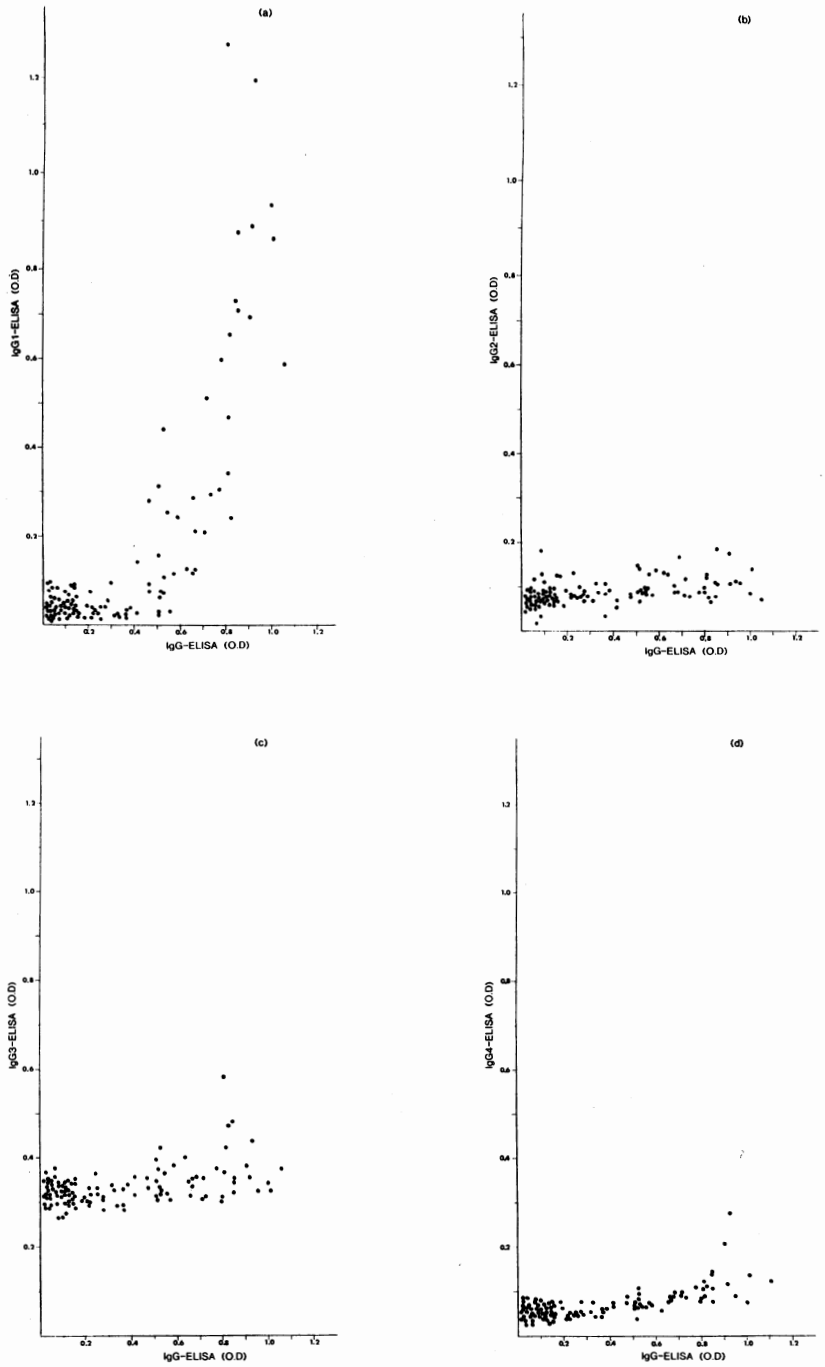

Fig. 2–Correlation of absorbance values (OD) of ELISA for each IgG subclass with the absorbance values of ELISA for IgG antibodies to *T. gondii*. The cut-off OD for the IgG-ELISA is 0.400.

and complement (C3b in humans). Multivalent binding of IgG to Fc receptors can induce phagocytosis. In addition, IgG augments the opsonising effects of C3b. Normal macrophages can kill phagocytosed *Toxoplasma* if it is coated with antibody (Wilson and Remington, 1980).

The possible role of IgG4, however, is unclear. Relatively few conditions have been described in which IgG4 is predominant. There appears to be an association between increased IgG4 and allergic-type reactions (Ottesen *et al.*, 1985; Creticos and Norman, 1987; Shakib *et al.*, 1977; van der Giessen *et al.*, 1976). There also appears to be many parallels between IgE and IgG4 responses. IgE and IgG4 levels were elevated in human filariasis (Hussain and Ottesen, 1986; Ottesen *et al.*, 1985) and schistosomiasis (Iskander *et al.*, 1981). It has been suggested that IgG4 antibodies become important when antigenic exposure is chronic (Aalberse *et al.*, 1983). It is also possible that IgG4 antibodies serve as 'blocking antibody' (van der Giessen *et al.*, 1976; Hussain and Ottesen 1986; Ottesen *et al.*, 1985) to modulate IgE-mediated allergy reactivity. Involvement of IgG4 in immediate-type hypersensitivity has been suggested (Hussain & Ottesen, 1986; Shakib *et al.*, 1977). However, the role of IgG4 in immunity to toxoplasmosis is unclear. Toxoplasmosis is not known to trigger IgE production, which is a marked feature of the immune response to allergens and helminthic infections like schistosomiasis (Roitt, 1988). In addition, Type IV (delayed type) and not the immediate-type hypersensitivity is encountered in *Toxoplasma* infection (Hughes, 1985; WHO scientific group report, 1972; Remington and Desmonts, 1983).

Cell-mediated immunity (CMI) has also been demonstrated to play a major role in host defence against the parasite in the mouse model with activated macrophages as the major effector cells involved (Anderson and Remington, 1974). In addition, there is evidence that pretreatment of the parasite with specific antibody alone prepared the tachyzoites for intracellular destruction by normal mouse macrophages (Anderson *et al.*, 1976). Moreover, specific antibody and activated macropahges also appeared to act together to provide significant protection against infection (Eisenhauer *et al.*, 1988). An interesting feature in a study by Handman and Remington (1980) on mouse antibody responses to *Toxoplasma* antigens, was the presence of high levels of the IgG2 subclass. The IgG3 subclass was present at low levels. IgG1 and IgG4 levels were not elevated although IgG1 is the major subclass in normal mouse serum. In mice, IgG2 and IgG3 activate complement and bind to macrophages (Krahenbuhl and Remington, 1982) which possess Fc receptors for IgG2 and complement receptor for C3b (Roitt *et al.*, 1985).

The present study showed that IgG1 and, to a lesser extent perhaps, IgG3 and IgG4 were produced in the IgG response to *T. gondii* infection. IgG1 and IgG3 enhance phagocytosis of parasites by promoting binding of the mononuclear cells to the parasite and complement activation. The possible role of IgG4, which was produced at low levels, is unclear.

SUMMARY

The level of activity of the 4 IgG subclasses in toxoplasmosis were determined in an ELISA employing commercially available monoclonal antibodies to the 4 subclasses. Forty-four sera positive by IgG-ELISA and 73 negative sera were tested for IgG subclass

activity. IgG1 was found to be the predominant subclass while IgG3 and IgG4 were probably produced at low but significant levels in sera which were positive for IgG antibodies. The significance of the results were discussed.

REFERENCES

AALBERSE, R.C., VAN DER GAAG, R. and VAN LEEUWEN, J., (1983). Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J. Immunol., 130* : 722.

ALEXANDER, M.D., (1980). Specificity of Fc receptors on human monocytes for IgG1 and IgG3. *Int. Archs. Allergy appl. Immun., 62* : 99.

ANDERSON, S.E., JR., BAUTISTA, S.C. and REMINGTON, J.S., (1976). Specific antibody-dependent killing of *Toxoplasma gondii* by normal macrophages. *Clin. exp. Immunol., 26* : 375.

ANDERSON, S.E., JR. and REMINGTON, J.S., (1974). Effect of normal and activated human macrophages on *Toxoplasma gondii, J. exp. Med., 139* : 1154.

BARRETT, D.J. and AYOUB, E.M., (1986). IgG2 subclass restriction of antibody to pneumococcal polysacharrides. *Clin. exp. Immunol. 63* : 127.

BIRD, P., LOWE, J., STOKES, R.P. BIRD, A.G., LING, N.R. and JEFFERIES, R., (1984). The separation of human serum IgG into subclass fractions by immunoaffinity chromatography and assessment of specific antibody activity. *J. Immunol. Methods. 71* : 97.

CRETICOS P.S. and NORMAN, P.S., (1987). Immunotherapy with allergens. *JAMA 258* : 2874.

EISENHAUER, P., MACK, D.G. and MCLEOD, R., (1988). Prevention of peroral and congenital acquisition of *Toxoplasma gondii* by antibody and activated macrophages. *Infect. Immun. 56* : 83.

EL AMIN, E.M., WRIGHT, E.P. and VLUG, A., (1986). Characterisation of humoral immune response in Sudanese Leishmaniasis : specific antibody detected by class- and subclass-specific reagents. *Clin exp. Immunol 64* : 14.

HANDMAN, E. and REMINGTON, J.S., (1980). Antibody response to *Toxoplasma* antigens in mice infected with strains of different virulence. *Infect. Immun. 29* : 215.

HOLLAND, D.B., INGHAM, E., GOWLAND, G. and CUNLIFFE, W.J., (1986). IgG subclasses in acne vulgaris. *Brit. J. Dermatol. 114* : 349.

HUGHES, H.P.A., (1985). Toxoplasmosis : the need for improved diagnostic techniques and accurate risk assessment. *Current Topics Microbiol. Immunol. 120* : 105.

HUSSAIN, R. and OTTESEN, E.A., (1986). IgE responses in human filariasis. IV Parallel antigen recognition by IgE and IgG4 subclass antibodies. *J. Immunol. 136* : 1859.

ISKANDER, R., DAS, P.K. and AALBERSE, R.C., (1981). IgG4 antibodies in Egyptian patients with schistosomiasis. *Int. Archs. Allergy appl. immun. 66* : 200.

KRAHENBUHL, J.L. and REMINGTON, J.S., (1982). The Immunology of Toxoplasma and Toxoplasmosis. *In* : Immunology of Parasitic Infection. Cohen S. and Warren, K.S., (eds.) Blackwell Scientific Publications, London.

MINEO, J.R., CAMARGO, M.E. and FERREIRA, A.W.,(1980). Enzyme-linked immunosorbent assay for antibodies to *Toxoplasma gondii* polysaccharides in human toxoplasmosis. *Infect. Immun. 27* : 283.

MORTIMER, G.E. and WIDDOWSON, J.P., (1979). Predominance of immunoglobulin G subclasses 3 among the complement-fixing antibodies to streptococcal M-associated protein. *Clin. exp. Immunol. 37* : 247.

NAOT, Y., GUPTILL, D.R., MULLENAX, J. and REMINGTON, J.S., (1983). Characterization of *Toxoplasma gondii* antigens that react with human immunoglobulin M. and immunoglobulin G antibodies. *Infect. Immun. 41* : 331.

OTTESEN, E.A., SKVARIL, F., TRIPATHY, S.P., POINDEXTER, R.W. and HUSSAIN, R., (1985). Prominence of IgG4 in the IgG antibody response to human filariasis. *J. Immunol. 134* : 2707.

PERSSON, M.A.A., *et al.*, (1988). IgG subclass-associated affinity differences of specific antibodies in humans. *J. Immunol. 140* : 3875.

REMINGTON, J.S. and DESMONTS, G., (1983). Toxoplasmosis. *In* : Infectious diseases of the fetus and newborn infant. Remington J.S. and O'Klein, J., (eds.) W.B. Saunders Company.

ROITT, I.M., (1985). Essential Immunology, PG Publishing Pte. Ltd. Singapore.

ROITT, I.M., (1988). Essential Immunology. Blackwell Scientific Publications. London.

SALIMANOU, L.S., WILLIAMS, A.I.O. and OSUNKAYA, B.O.,(1982). IgG subclass levels in malaria-infected Nigerians. *Vox Sang. 42* : 248.

SCHREIBER, R.D. and FELDMAN, H.A., (1980). Identification of the activator system for antibody to *Toxoplasma* as the classical complement pathway. *J. Infect. Dis. 141* : 366.

SCOTT.M.T.and GOSS-SAMPSON, M.,(1984). Restricted IgG profiles in *T.cruzi* infected mice and Chagas' disease patients. *Clin. exp. Immunol. 58* : 372.

SHAKIB, F., *et al.*, (1977). Elevated serum IgE and IgG4 in patients with atopic dermatitis. *Br. J. Dermatol. 97* : 59.

SHARMA, S.D., *et al.*, (1983). Western blot analysis of the antigens of *Toxoplasma gondii* recognised by human IgM and IgG antibodies. *J. Immunol. 131* : 977.

SIBER, G.R. *et al.*, (1980). Correlation between serum IgG2 concentrations and the antibody response to bacterial polysaccharide antigens. *N. Eng. J. Med. 303* : 178.

SPEIGELBERG, H.L., (1974). Biological activities of immunoglobulins of different classess and subclasses. *Adv. Immunol. 19* : 259.

VAN DER GIESSON, M. *et al.*,(1976). Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. *Int. Archs. Allergy appl. Immun. 50* : 625.

VAN DER GIESSON, M. and GROENEBOER-KEMPERS, O., (1976). The subclasses of human IgG antibodies against tetanus oxoid. *Clin. exp. Immunol. 25* : 117.

VAN DER SLUIS, J.J., VAN REEDE, E.C. and BOER, M., (1986). Immunoglobulin G subclasses of flourescent anti-*Treponema pallidum* antibodies : evidence of sequential development of specific

anti-*T.pallidum* immunoglobulin G responses in patients with early syphilis. *J. Clin. Microbiol. 24* : 418.

WAHLGREN, M., *et al.*, (1983). characterisation of the humoral immune response in *Plasmodium falciparum* malaris. II IgG subclass levels of anti-*P.falciparum* antibodies in different sera. *Clin. exp. Immunol. 54* : 135.

WALKER, L., JOHNSON, G.D. and MACLENNAN, I.C.M., (1983). The IgG subclass responses of human lymphocytes to B-cell activators. *Immunol. 50* : 269.

WHO Scientific Group on Cell-mediated immunity and resistance to infection. September, 1972 : 6-64.

WILSON, C.B. and REMINGTON, J.S.,(1980). What can be done to prevent congenital toxoplasmosis? *Am. J. Obstet. Gynecol. 138* : 357.