Human IgG subclass responses and subclass restriction to *Schistosoma mansoni* egg antigens

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SUMMARY

In areas endemic for schistosomiasis, there is great heterogeneity in antibody isotype responses to parasite antigens amongst infected individuals. At the population level, the isotype composition of antibody responses undergoes dynamic changes which are associated with the age of infected individuals. Here we examine the IgG subclass responses to *Schistosoma mansoni* eggs (soluble egg antigens; SEA) of infected individuals by immunoblot and ELISA. By controlled treatment of SEA-coated ELISA plates and immunoblot nitrocellular strips with sodium periodate, in order to oxidize terminal carbohydrate residues selectively, we were able to relate individuals subjects' isotype responses to the different antigens that they responded to, and to the presence of putative carbohydrate and peptide epitopes on those antigens. IgG2 responses were restricted strictly to sodium periodate-sensitive carbohydrate epitopes and antigens of relatively high molecular weight. These antigens were not usually recognized by other isotypes and, therefore, they were only recognized by individuals who had high levels of IgG2. IgG1 and IgG3 responses were directed against both carbohydrate and peptide epitopes, whereas IgG4 responses were restricted to periodate-resistant epitopes. This suggests that the fall in IgG2 responses, and reciprocal rise in IgG4 antibodies, seen in young children as their intensities of schistosome infection increase, is not the result of isotype switching, and that, if these two subclasses are involved in blocking immunity to schistosomiasis, they are operating independently.

INTRODUCTION

Schistosomiasis is a parasitic helminth infection that affects an estimated 200 million people in tropical regions. Infections are chronic in nature, with adult worms living for several years in the host's blood stream. In areas of Kenya endemic for *Schistosoma mansoni*, it has been found that older individuals in the population are apparently 'immune' to reinfection after treatment, while younger individuals are highly 'susceptible' to reinfection. Susceptibility to reinfection does not appear to be due to a general failure to respond against the schistosome, as

all infected individuals are found to be hyper-responsive to parasite antigens.[1] However, the isotype composition of antibody responses against the parasite does vary greatly between infected individuals. As different human antibody isotypes differ in their biological properties,[2] including their ability to mediate or block killing of schistosomes via a variety of immune effector mechanisms,[3–5] this heterogeneity in isotype response may influence the outcome of infections in different individuals. Indeed, it has been shown that high levels of human IgG2 directed against the parasite egg are associated with subsequent high intensities of reinfection after treatment,[1] whereas high levels of IgE directed against various stages of the parasite life cycle have been significantly correlated with low intensities of reinfection.[6–8]

Statistical comparisons of anti-schistosome isotype responses in human populations have suggested that IgG4 recognizes a group of epitopes that are distinct from those recognized by other human IgG subclasses.[9,10] Cross-sectional surveys of human populations suffering endemic schistosomiasis have shown that there are dynamic changes in the isotype composition of the antibody response to schistosomes with the age of infected patients. Comparisons of the IgG isotype responses in ELISA to carbohydrate antigens and partially deglycosylated glycoproteins have found that the IgG2

anti-carbohydrate responses decline with age, whereas IgG4 levels correlate more closely with the age-related rise and fall in intensity of infection seen in endemic populations.[9] Some responses, such as IgE, increase steadily with age, so that high levels of IgE are generally found in older individuals within the population.[6,7]

Studies of the physicochemical nature of target antigens and the antibodies they elicit in other systems have revealed a restriction in the ability of immunoglobulin subclasses to be induced by certain antigens. Thus, human antibody responses to streptococcal group A carbohydrates are predominantly restricted to the IgG2 subclass, whilst IgG4 antibodies appear only to be able to recognize the protein antigens streptolysin O and deoxyribonuclease B from the same bacterium.[11] Similar subclass restrictions have been noted in helminthic infections other than schistosomiasis. For example, it has been shown using sera from patients infected with filarial worms that the chronic antigenic stimulation cannot overcome the restriction which prevents humans from making IgG4 antibodies to carbohydrate antigens.[12]

The age-dependent changes in isotype response to schistosomes seen in human populations, and the isotype heterogeneity seen between individuals, are of potential importance in schistosomiasis in terms of immunity and immunopathology. In addition, these responses in chronically infected human populations present an opportunity to study the relationship between individual diversity in isotype responses, the different antigens recognized and the physicochemical nature of the epitopes these antigens contain. Here we describe the variation between individuals *S. mansoni*-infected subjects in the overall IgG subclass responses to antigens from the parasite egg, and the patterns of antigens recognized by different subclasses. We also compare the influence of carbohydrate and non-carbohydrate epitopes on the IgG subclass composition of anti-egg responses and the antigens responded to by individual subjects.

MATERIALS AND METHODS

Human sera

Sera were collected from 94 *S. mansoni*-infected subjects, from 5 to 67 years of age, living in the Machakos District of Kenya. Active infections were confirmed by Kato examination for the presence of parasite eggs in the stools of each individual. Normal human sera were from European donors with no history of schistosomiasis.

Parasite antigen

A Puerto Rican isolate of *S. mansoni* was maintained in *Biomphalaria glabrata* snails and TO strain mice (Tuck Ltd, Battleridge, UK). *Schistosoma mansoni* eggs were collected from the livers and intestines of mice. Soluble egg antigen (SEA) refers to the supernatant fluid obtained from eggs that had been homogenized in phosphate-buffered saline (PBS), pH 7·2.[9]

IgG subclass responses to S. mansoni *SEA in ELISA*

The ELISA methods used were essentially those described elsewhere.[13,14] Microtitre plates were coated overnight at room temperature with 0·08 μg SEA/well. Plates were then washed in 0·5% Tween-20 in PBS. For total specific IgG antibodies, IgG1 and IgG4 assays, sera were tested at 1:200; for IgG3 and IgG2 assays sera were at 1:100. Plates were washed after 6 hr incubation in test serum and then mouse anti-human isotype-specific monoclonal antibody (mAb) was added and the plates incubated overnight at room temperature. Mouse mAb anti-human IgG subclasses were the same as used previously.[9] Rabbit anti-human IgG conjugated with horseradish (Dako Ltd, High Wycombe, UK) was used to determine total IgG responses. Anti-human IgG subclass antibodies were added at dilutions previously determined to give maximal binding. Biotinylated rabbit anti-mouse IgG antibody (Amersham International, Aylesbury, UK), followed by biotin–streptavidin–horseradish peroxidase (Amersham) was used to detect the murine antibodies. Plates were developed using orthophenylene diamine. The optical density of each well was determined at a wavelength of 490 nm.

The subclass distribution of IgG-binding carbohydrate and non-carbohydrate SEA epitopes was determined by oxidizing the antigen with sodium periodate.[13] After overnight coating of ELISA plates with SEA, half of the wells were treated with 0·01 M sodium periodate in 0·1 M sodium acetate. The remainder of the wells was incubated with sodium acetate alone. Plates were incubated overnight, in the dark at 4°, washed and sera added. On each plate serum from an individual was added to corresponding wells on both the periodate-treated and untreated halves. Plates were probed as before for the presence of IgG1, IgG2, IgG3 or IgG4. Each plate also contained wells to which the biotinylated lectins concanavalin A, peanut agglutinin, soya bean agglutinin and wheat germ agglutinin were added to determine the extent of periodate oxidation of terminal glycosyl residues. In each case, binding of soya bean agglutinin and wheat germ agglutinin to SEA was reduced by 80–95% after sodium periodate treatment, the binding of concanavalin A was reduced by between 20 and 60%, whilst peanut agglutinin binding was reduced by 5–20%. Each antibody assay was carried out on duplicate or triplicate plates.

Identification of antigens recognized by human anti-SEA IgG isotypes by immunoblotting

The antigens recognized by anti-SEA IgG subclasses in sera from infected individuals were identified by immunoblotting.[7] SEA was separated under reducing conditions by SDS–PAGE on a 8–20% polyacrylamide gel and transferred to nitrocellulose paper (NCP). The NCP was then cut into strips, which were probed overnight with infection sera, and then incubated with anti-isotype-specific antibodies. Infection sera, mouse anti-IgG subclass-specific antibodies and peroxidase-conjugated rabbit anti-mouse IgG serum were used at the same dilutions as in ELISA. The substrate used was 3,3'-diaminobenzidine tetrahydrochloride. When assaying for antibodies recognizing periodate-resistant epitopes, the SEA-coated NCP was washed and cut lengthways into two pieces; both halves were incubated overnight in the dark at 4°, one in 0·1 M sodium acetate containing 0·01 M sodium periodate, the other in 0·1 M sodium acetate alone. Each sheet was washed extensively and cut into strips, which were probed overnight with infection sera. The same sera were used with periodate-treated and untreated strips, which were then probed for the different human IgG subclasses.

Figure 1. Distribution of IgG1 (1), IgG2 (2), IgG3 (3) and IgG4 (4) antibody subclasses in the total anti-SEA IgG response (G) of 12 individuals, labelled A–L. Each bar represents the mean optical density at 490 nm from duplicate or triplicate ELISA wells.

RESULTS

Distribution of IgG anti-SEA subclass responses in sera from infected individuals

The contribution of each IgG subclass to the overall anti-SEA response varied considerably between individuals. To illustrate this we selected individuals with high levels of anti-SEA IgG in ELISA and determined their subclass responses (Fig. 1). Whereas some sera showed generally elevated OD values for all isotypes tested (see particularly individual F), in others, such as A, B, D and H, two subclasses in the total anti-SEA IgG response produced particularly high OD values. In two individuals (J and L), the overall IgG anti-SEA response appeared to be almost exclusively IgG4, whilst in others (A, E and G) this isotype was virtually undetectable. The total IgG response of an individual to SEA may be composed of any combination of the four IgG subclasses.

Diversity of antigens recognized by IgG subclasses

To determine whether differences in the subclass response measured by ELISA were reflected by differences in the recognition of individual antigens, we examined the anti-SEA subclass response patients by immunoblot. This showed that the total IgG response was, in the number and variety of antigens recognized, the 'sum' of the individual subclass responses (Fig. 2), with all antigens recognized by the separate subclasses contributing to the total anti-IgG response. The pattern of antigen recognition was found to vary from subclass to subclass and from individual to individual. In serum B (Fig. 2), for example, the higher molecular weight antigens that were recognized in IgG2 did not bind antibodies of the other subclasses, nor IgG2 in five other sera. In some individuals a group of antigens was recognized by two or more subclasses, whereas in others only a single isotype recognized similarly sized antigens.
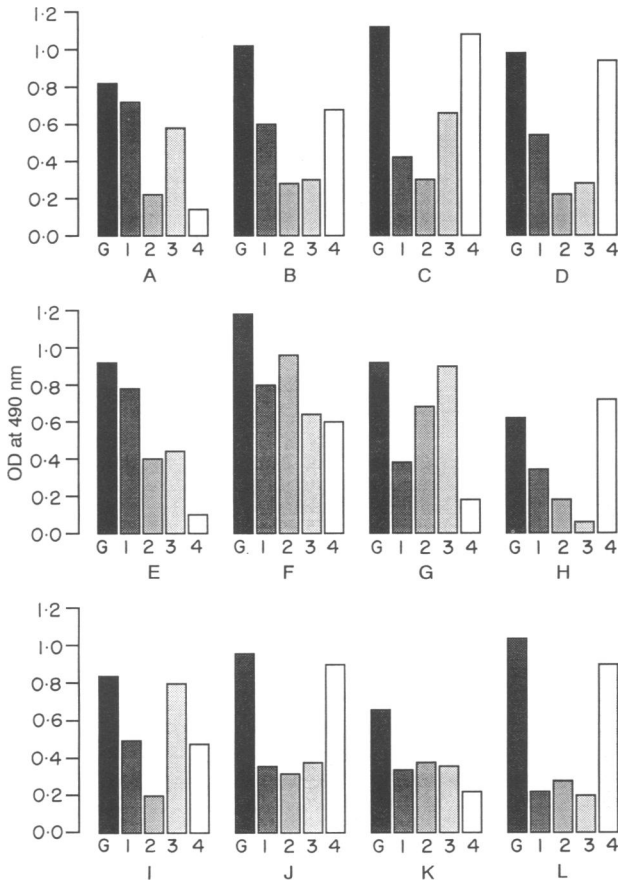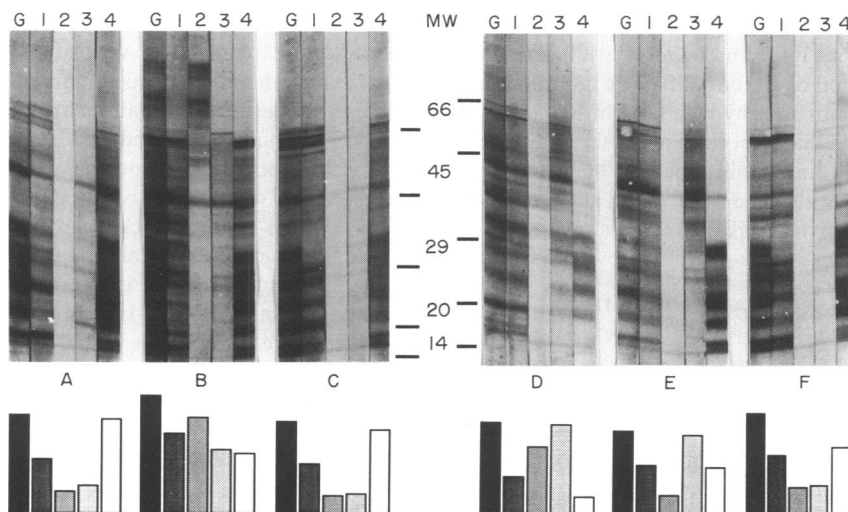

Figure 2. Immunoblot of antigens recognized by IgG antibody subclasses in SEA in sera from six infected patients (A–F). Each set of five strips was probed with murine antibodies to total specific IgG, IgG1, IgG2, IgG3 and IgG4, as shown. Molecular weight markers (× 1000) are indicated. The corresponding IgG subclass responses seen in ELISA are illustrated below each immunoblot.
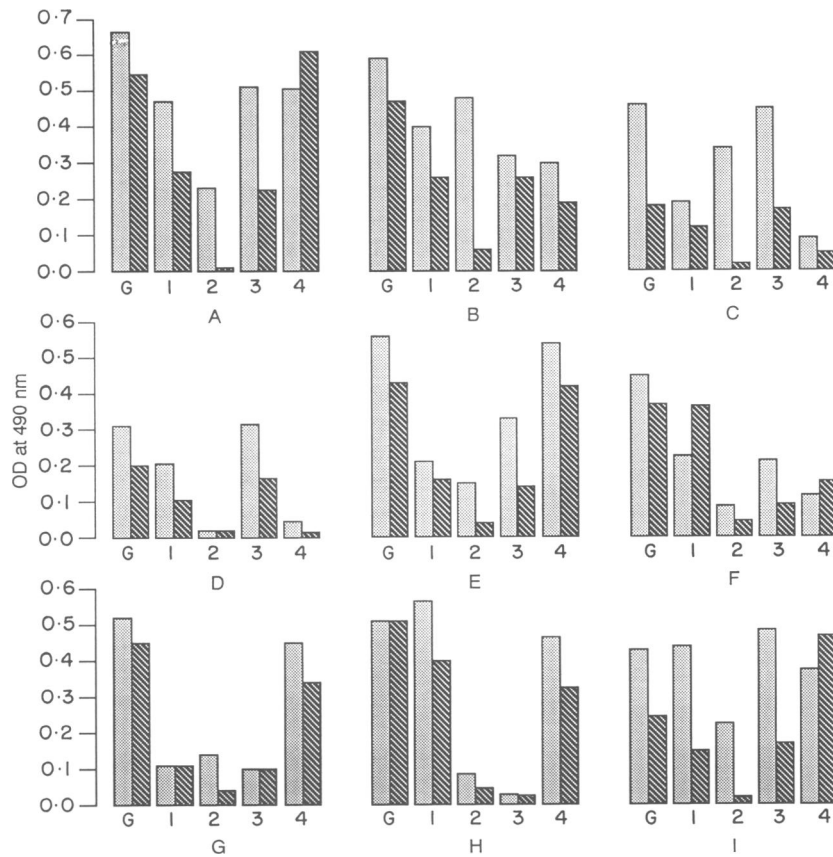
Figure 3. Response of IgG subclass antibodies to both intact and periodate-oxidized SEA (i.e. SEA in which only peptide antigens were accessible) in sera from nine individuals, labelled A–I. The dotted bars show antibodies binding to intact SEA, with the hatched bars showing antibodies binding to periodate-treated SEA. Each bar represents the mean optical density at 490 nm of duplicate or triplicate ELISA wells.

Antibody binding in immunoblots generally conformed with the patterns of subclass distribution observed in ELISA. For example, in sera A, C and F (Fig. 2) the highest OD values in ELISA and the strongest antibody reactions in immunoblot were found with subclasses IgG1 and IgG4, whilst individual B showed a general elevation of all four anti-SEA IgG subclasses by ELISA, which was mirrored by reactivity in immunoblot. The only anomaly seen in this respect was with serum D which, despite having less IgG4 immunoblot activity than the other sera, was still clearly IgG4-reactive with several antigens whilst having very low IgG4 activity in ELISA.

IgG subclass responses to peptide and carbohydrate epitopes in SEA

A number of sera was selected, based on results in ELISA, to show the change in binding of each antibody subclass resulting from sodium periodate oxidation of SEA. These sera varied from showing little diminution in total IgG binding following treatment to almost complete ablation. Figure 3 shows that the affect of total IgG antibody binding of periodate treatment of SEA could be accounted for by the subclass distribution and the ability of the individual subclasses to bind to oxidized antigen. In serum G, for example, for which the total level of IgG binding was largely unaffected by treatment of SEA with

periodate, the IgG4 assay gave a high OD value, with this isotype binding to the intact and oxidized antigens at similar high levels. In other sera in which IgG4 antibodies appeared to be elevated, the binding of this subclass was also unaffected by periodate treatment of SEA.

In sera where there was a marked reduction in binding of IgG antibodies to periodate-treated SEA (see individual C), high OD values were produced by the assays for IgG2 and IgG3, and both of these isotypes were found to bind at reduced levels, if at all, to the oxidized antigen. The presence of carbohydrate-binding subclasses did not necessarily result in an obvious reduction in total IgG antibody binding following periodate treatment of SEA; in sera A and B, for example, there was no reduction in total IgG antibody binding to the treated SEA, despite both these sera having substantial antibody reactivity that was specific for the intact antigen. Generally, however, the overall binding of IgG to periodate-oxidized SEA was dependent on the effect of periodate treatment on the binding of individual IgG subclasses and the contribution of each subclass to the total IgG response to intact SEA.

The profile of antigens recognized by each subclass in immunoblots, with and without sodium periodate treatment, is shown in Fig. 4. IgG4 antibodies (Fig. 4d) mainly bound to similar numbers and sizes of antigens in the intact or oxidized SEA (particularly evident in lanes E, H and I). However, in at
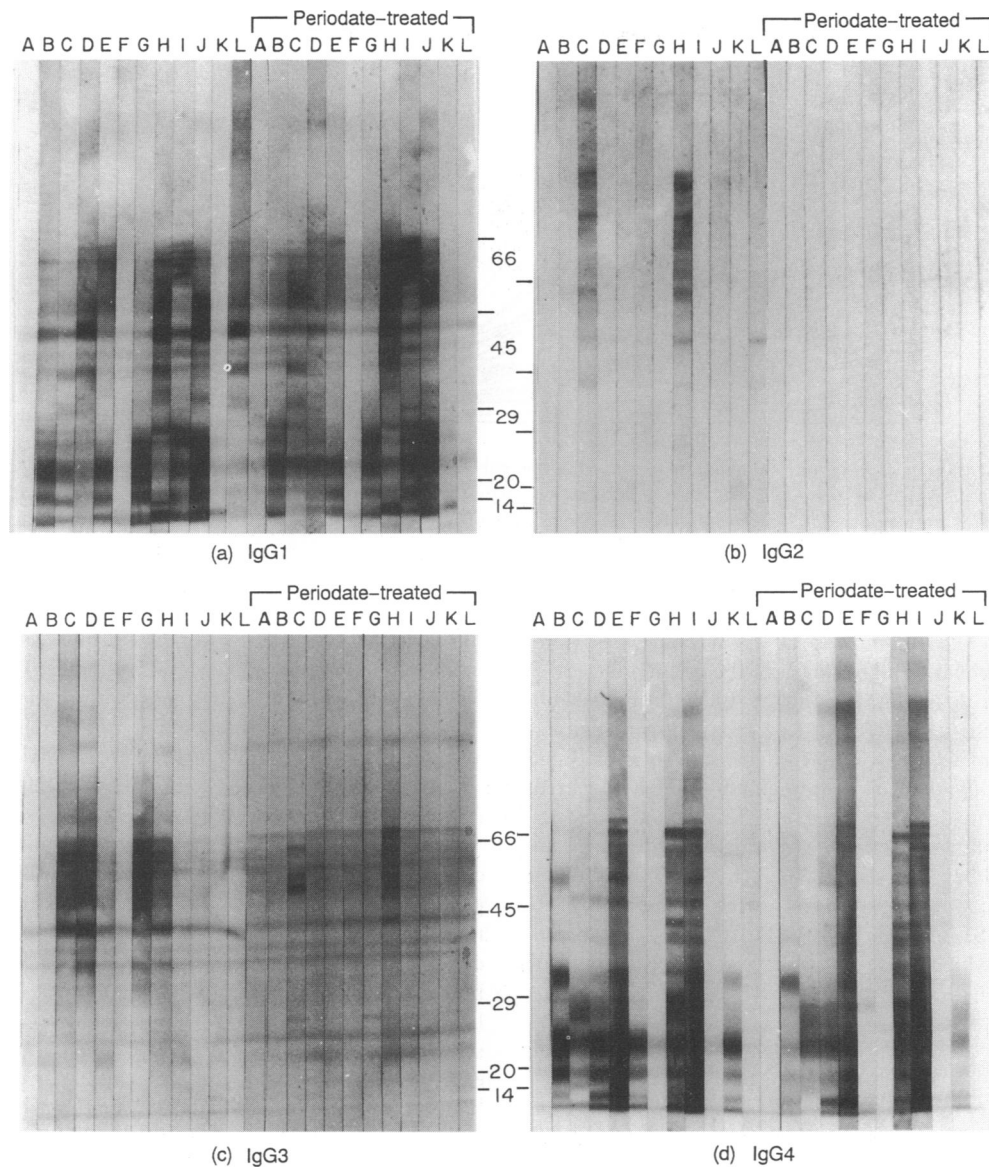
Figure 4. Immunoblots showing the binding of IgG antibody subclasses to carbohydrate (periodate-sensitive) and peptide (periodate-insensitive) epitopes on SEA antigens in sera from 11 individuals infected with *S. mansoni* (lanes B–L). Serum used in lane A was from an uninfected European. In each panel, one half of the immunoblotted SEA, as indicated in the figure, was treated with sodium periodate prior to addition of sera. Molecular weights ($\times 1000$) are indicated.

least one case (lane F), and perhaps two (lane B), there was a reduction in the intensity of IgG4 binding, and possibly in the numbers of antigens recognized. A clear illustration of the inability of IgG2 antibodies to bind to periodate-insensitive (non-carbohydrate) antigens is seen in Fig. 4b, in which antibodies bound to the intact SEA in lanes C, H and L but completely failed to recognize periodate-treated antigens. In Fig. 4c, IgG3 anti-SEA antibodies showed similar reductions of binding (lanes D and G) following periodate treatment of SEA. Unlike IgG2, however, IgG3 antibodies in some sera did bind to the oxidized antigen (lanes C and H). Interestingly, whereas antibody binding to the treated antigen was reduced in lane C, in lane H there was no overall reduction, antibody binding to a 70 000 MW antigen possibly even increasing following periodation of SEA. There were also examples of IgG1 (Fig. 4a) failing

to bind to periodate-treated SEA (lane L), or showing similar patterns and intensity of antigen recognition of intact and oxidized SEA (lanes B, G and K), or increased reactivity, if not number of antigens recognized, after periodation of SEA (lanes H and I).

Restriction in IgG antibody subclass responses to peptide and carbohydrate antigens

By comparing the IgG subclass responses to intact and oxidized SEA for a large number of individuals, it was possible to view the general restriction of some IgG subclass responses to either carbohydrate or peptide (oxidized) antigens (Fig. 5). It was evident from this that both IgG4 and IgG2 antibodies were restricted in their ability to respond to either carbohydrate or
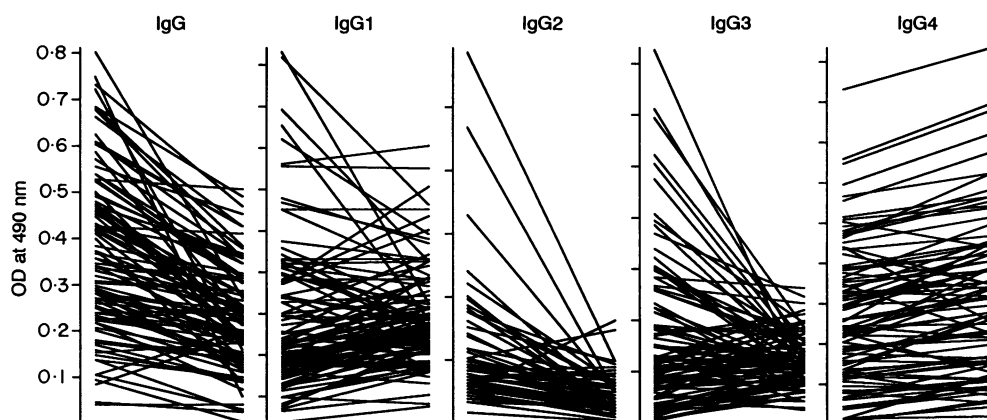
Figure 5. Alteration in levels of antibody binding of anti-SEA IgG subclasses in a cohort of infected individuals following destruction of carbohydrate residues by periodate oxidation. Each line represents the change in mean optical densities in ELISA at 490 nm of individual serum samples added to intact SEA (left-hand side of graphs) and periodate-treated SEA (right-hand side). Optical densities were mean values obtained from duplicate or triplicate wells.

peptide antigens. Thus in most sera, IgG4 antibodies were found to bind at similar or higher levels to the periodate-treated SEA as to the intact antigen. In contrast, IgG2 antibodies did bind to the intact SEA, but not to the oxidized antigen. Neither IgG3 nor IgG1 had such absolute restrictions to their binding. Although sera with high levels of IgG3 binding often appeared to recognize carbohydrate epitopes, exhibiting marked reductions of binding following periodate treatment, there was also a considerable number of sera in which IgG3 antibodies continued to bind at moderate levels after periodation. The IgG1 response to SEA appeared to be fairly evenly distributed between sera in which these antibodies were predominantly recognizing carbohydrate or peptide epitopes.

DISCUSSION

IgG subclasses vary in their ability to mediate immune effector mechanisms directed against schistosomes.[4,5] The overall immune status of an infected individual, therefore, may depend upon the distribution of anti-schistosome subclasses. We investigated the variation between individuals in IgG subclass responses, examining the relationship between quantitative measurements of anti-SEA IgG and the qualitative responses, including the numbers and diversity of antigens recognized and the physicochemical properties of target epitopes for each subclass. Infected individuals exhibited considerable diversity in IgG subclass responses, not just in the presence or absence of a particular isotype, but also in the number and variety of antigens recognized.

Secondary antibody responses are characterized by affinity maturation and isotype switching. There is increasing evidence that isotype switching is controlled strictly by a number of cytokines. In mice IgG1, IgA and IgE production are controlled by interleukin-4 (IL-4), IgG2a by interferon-γ (IFN-γ) and IgG2b by tumour necrosis factor-β (TNF-β).[15–18] Secretion of the thymus-independent antibody IgG3 may be under the control of cytokines (principally IFN-γ).[19] In humans, IgE and IgG4 antibodies appear to be potentiated by IL-4 and IL-13;[20,21] modulation of other immunoglobulin classes and subclasses is not yet clear, but is probably similarly

controlled. By analogy with the murine system it might be predicted that production of IgG2 antibodies, which are elicited by thymus-independent antigens,[11,22] is directed by IFN-γ secreted by non-T-cell sources. Cytokines are not, however, the sole initiators of immunoglobulin subclass switching, having to act in concert with a B-cell activator, an antigen receptor cross-linker or an activated T cell before switching can occur.[23,24]

It has been shown that there is considerable variation in the concentrations of different anti-schistosome IgG subclasses present in the sera of infected individuals,[25,26] perhaps reflecting differences in cytokine expression between individuals during infection. There may be a polarization of the immune response in the T-helper type 2 (Th2) direction in helminth-infected humans.[27] This seems to be the case with schistosome-infected Kenyans, despite continuing low level expression of IFN-γ.[28] Cytokines released by either Th1 or Th2 lymphocyte subsets will inhibit the synthesis of cytokines from the other subset (reviewed in ref. 29), and any resulting polarization of the immune response in either the Th1 or Th2 direction may bias isotype switching towards specific IgG subclasses.

Whilst cytokines are important in isotype switching, other factors also affect the isotype response to an antigen. The ability of an antigen to stimulate a particular antibody response is to some extent dependent on its physicochemical properties. In mice polysaccharide antigens predominantly elicit IgG3 responses in the absence of T-cell help.[30] *Schistosoma mansoni* eggs have been shown to contain thymus-independent (TI) type II antigens, which induce murine IgG3.[31] In humans, the predominant antibody response to polysaccharides and other TI type II antigens is IgG2,[22] which is almost exclusively directed against carbohydrate epitopes. It has also been suggested that IgG1 may exhibit a restrictive carbohydrate-directed response.[32] In contrast to IgG2 (and perhaps IgG1), IgG4 exhibits an antigen-restricted response that is directed against peptide antigens.[12]

Periodate treatment can be used to identify antibody responses directed against carbohydrate-containing epitopes on various glycoconjugates by associating the oxidation of vicinal hydroxyl groups on sugars to dialdehydes[33] with the loss

of antibody recognition.[34,13] The extent of oxidation depends on the conditions used. Mild oxidation has been used to identify antibodies directed against epitopes that incorporate terminal, non-reducing sugars and pyranosidically linked hexoses within oligosaccharide chains in which carbon 3 is unsubstituted.[35] Caution must be taken as prolonged oxidation at higher periodate concentrations will destroy amino acids within polypeptides. In addition, it is possible that oxidation of sugar moieties will affect antibody recognition of conformational peptide epitopes, although the immobilization of antigens on plastic or nitrocellulose prior to periodate treatment should minimize any such conformational changes. The oxidation conditions used in the experiments reported here were established by monitoring the effects of progressive oxidation of schistosome antigens on the binding of lectins.[13] Not all lectin-binding was abolished by the oxidation conditions used in these experiments, as not all carbohydrate epitopes are sensitive to periodate cleavage. For example, linear sugar chains with linkages at carbon 3 should not be sensitive and it has been shown that epitopes containing partially O-acetylated sialic acids are periodate resistant.[36] However, since anti-carbohydrate antibodies are usually directed against non-reducing terminal sugar moieties,[37] periodate oxidation remains a good, simple means to distinguish between the overall anti-carbohydrate and anti-peptide responses to a crude antigen extract.

The differential effect of sodium periodate treatment of SEA on the binding of individual IgG subclasses was shown by the reduced binding of IgG2 to periodate-treated SEA, which contrasted with the lack of effect on IgG4 binding. This is consistent with IgG4 not being elicited in response to polysaccharides[2,12,30,32] and IgG2 being principally directed against polysaccharide antigens.[2,9,22,32,35] IgG1 and IgG3 bound to carbohydrate or peptide epitopes less consistently, suggesting that these subclasses may be elicited in response to either type of epitope. The demonstration that IgG2 and IgG4 subclasses are directed against different types of epitope on different groups of antigens suggests that the fall in IgG2 responses, and reciprocal rise in IgG4 antibodies, seen in young children as their intensities of infection increase[9] are not the result of isotype switching. This also supports the view that if these two subclasses are involved in blocking immunity to schistosomiasis, they are operating independently.[10]

ACKNOWLEDGMENTS

REFERENCES

1. BUTTERWORTH A.E., DUNNE D.W., FULFORD A. *et al.* (1988) Immunity after treatment of human schistosomiasis: cross-reactive IgM and IgG2 anti-carbohydrate antibodies block the expression of immunity. *Biochimie* **70,** 1053.

2. JEFFERIS R. & KUMARARATNE D.S. (1990) Selective IgG subclass deficiency: quantification and clinical relevance. *Clin Exp Immunol* **81,** 357.

3. KHALIFE J., CAPRON M., CAPRON A. *et al.* (1986) Immunity in human schistosomiasis mansoni: regulation of protective immune mechanisms by IgM blocking antibodies. *J Exp Med* **164,** 1626.

4. KHALIFE J., DUNNE D.W., RICHARDSON B.A. *et al.* (1989) Functional role of human IgG subclasses in eosinophil-mediated killing of schistosomula of Schistosoma mansoni. *J Immunol* **142,** 4422.

5. DUNNE D.W., RICHARDSON B.A., JONES F.M., CLARK M., THORNE K.J.I. & BUTTERWORTH A.E. (1993) The use of mouse/human chimeric antibodies to investigate the roles of different antibody isotypes, including IgA2, in the killing of Schistosoma mansoni schistosomula by eosinophils. *Parasite Immunol* **15,** 181.

6. HAGAN P., BLUMENTHAL U.J., DUNN D., SIMPSON A.J.G. & WILKINS H.A. (1991) Human IgE, IgG4 and resistance to reinfection with Schistosoma haematobium. *Nature* **349,** 243.

7. DUNNE D.W., BUTTERWORTH A.E., FULFORD A.J.C. *et al.* (1992) Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* **22,** 1483.

8. RIHET P., DEMEURE C.E., BOURGOIS A., PRATA A. & DESSEIN A.J. (1991) Evidence for an association between human resistance to Schistosoma mansoni and high anti-larval IgE levels. *Eur J Immunol* **21,** 2679.

9. DUNNE D.W., GRABOWSKA A.M., FULFORD A.J.C. *et al.* (1988) Human antibody responses to Schistosoma mansoni: the influence of epitopes shared between different life-cycle stages on the response to the schistosomulum. *Eur J Immunol* **18,** 123.

10. DEMEURE C.E., RIHET P., ABEL L., OUATTARA M., BOURGOIS A. & DESSEIN A.J. (1993) Resistance to Schistosoma mansoni in humans: influence of the IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy. *J Infect Dis* **168,** 1000.

11. SHACKELFORD P.G., NELSON S.J., PALMA A.T. & NAHM M.H. (1988) Human antibodies to group A streptococcal carbohydrate. Ontogeny, subclass restriction, and clonal diversity. *J Immunol* **140,** 3200.

12. LAL R.B., DHAWAN R.R., TARRAND J.J., AYOUB E.M. & OTTESEN E.A. (1991) Lack of IgG4 response to carbohydrate antigens in patients with lymphatic filariasis. *Immunology* **74,** 333.

13. LANGLEY J.G. & DUNNE D.W. (1992) Temporal variation in the carbohydrate and peptide surface epitopes in antibody-dependent, eosinophil mediated killing of Schistosoma mansoni schistosomula. *Parasite Immunol* **14,** 185.

14. DUNNE D.W., BAIN J., LILLYWHITE J. & DOENHOFF M.J. (1984) The stage-, strain- and species-specificity of a Schistosoma mansoni egg antigen fraction (CEF6) with serodiagnostic potential. *Trans Roy Soc Trop Med Hyg* **78,** 460.

15. COFFMAN R.L. & CARTY J. (1986) A T cell activity that enhances polyclonal IgE production and its inhibition by interferon gamma. *J Immunol* **136,** 949.

16. GOODMAN D.J., GAFF C. & GERONDAKIS S. (1993) The IL-4 induced increase in the frequency of resting murine splenic B cells expressing germline Ig heavy γ1 transcripts correlates with subsequent switching to IgG1. *Int Immunol* **5,** 199.

17. SNAPPER C.M. & PAUL W.E. (1987) Interferon-γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* **236,** 944.

18. SNAPPER C.M. & MOND J.J. (1993) Towards a comprehensive view of immunoglobulin class switching. *Immunol Today* **14,** 15.

19. SNAPPER C.M., McINTYRE T.M., MANDER R. *et al.* (1992) Induction of IgG3 secretion by interferon-γ: a model for T cell-independent class switching in response to T cell-independent type 2 antigens. *J Exp Med* **175,** 1367.

20. SPIEGELBERG H.L., O'CONNOR R.D., FALKOFF R.J.M. & BECK L. (1991) Interleukin-4 induced IgE and IgG4 secretion by B cells from atopic dermatitis patients. *Int Arch Allergy Appl Immunol* **94,** 181.

21. PUNNONEN J., AVERSA G., COCKS B.G. *et al.* (1993) Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and

CD23 expression by human B cells. *Proc Natl Acad Sci USA* **90**, 3730.

22. Siber G.R., Schur P.H., Aisenberg A.C., Weltzmann S.A. & Schiffman G. (1980) Correlation between serum IgG2 levels and the antibody response to bacterial polysaccharide antigens. *New Engl J Med* **303**, 178.

23. Esser C. & Radbruch A. (1990) Immunoglobulin class switching: molecular and cellular analysis. *Annu Rev Immunol* **8**, 717.

24. Stavnezer-Nordgren J. & Serlin S. (1986) Specificity of immunoglobulin RNA in single cells of the B lymphocyte lineage with radiolabelled DNA probes. *EMBO J* **5**, 95.

25. Jassim A., Hassan K. & Catty D. (1987) Antibody isotypes in human schistosomiasis mansoni. *Parasite Immunol* **9**, 627.

26. Boctor F.N. & Peter J.B. (1990) IgG subclasses in human chronic schistosomiasis: over production of schistosome-specific and non-specific IgG4. *Clin Exp Immunol* **82**, 574.

27. Mahanty S., Abrams J.S., King C.L., Limage A.P. & Nutman T.B. (1992) Parallel regulation of IL-4 and IL-5 in human helminth infections. *J Immunol* **148**, 3567.

28. Roberts M., Butterworth A.E., Kimani G. *et al.* (1993) Immunity after treatment of human schistosomiasis: association between cellular responses and resistance to reinfection. *Infect Immun* **61**, 4984.

29. Finkelman F.D. & Urban J.F. (1992) Cytokines: making the right choice. *Parasitol Today* **8**, 311.

30. Perlmutter R.M., Nahn M., Stein K.E. *et al.* (1979) Immunoglobulin subclass-specific immunodeficiency in mice with an X-linked B lymphocyte defect. *J Exp Med* **149**, 993.

31. Mazza G., Dunne D.W. & Butterworth A.E. (1990) Antibody isotype responses to the *Schistosoma mansoni* schistosomulum in the CBA/N mouse induced by different stages of the parasite life cycle. *Parasite Immunol* **12**, 529.

32. Scott M.G., Shackelford P.G., Brilles D.E. & Nahm M.H. (1988) Human IgG subclasses and their relation to carbohydrate antigen immunocompetence. *Diag Clin Immunol* **5**, 241.

33. Bobbit J.M. (1956) Periodate oxidation of carbohydrates. *Adv Carbohydrate Chem Biochem* **11**, 1.

34. Woodward M.P., Young W.W. & Bloodgood R.A. (1985) Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. *J Immunol Meth* **78**, 143.

35. Kannagi R. & Hakomori S. (1986) Monoclonal antibodies directed to carbohydrate antigens. In: *Handbook of Experimental Immunology* (ed. D.M. Weir), Vol. 4, 4th edn, p. 117.1. Blackwell Scientific Publications, Oxford.

36. Cheresh D.A., Reisfeld R.A. & Varki A.P. (1984) O-acetylation of disialoganglioside GD3 by human melanoma cells creates a unique antigenic determinant. *Science* **225**, 844.

37. Hakomori S. & Kannagi R. (1983) Glycosphingolipids as tumour-associated and differentiation markers. *J Natl Cancer Inst* **71**, 231.