Clin. exp. Immunol. (1986) **66**, 273–278

IgG-subclass expression of anti-DNA and anti-ribonucleoprotein autoantibodies in human malaria

M. ZOUALI, P. DRUILHE* & A. EYQUEM *Service d'Immunohématologie et d'Immunopathologie, Institut Pasteur, Paris, and *Service de Parasitologie et Médecine Tropicale, Hôpital de la Pitié-Salpêtrière, Paris, France*

(Accepted for publication 26 June 1986)

SUMMARY

To understand further the autoimmune phenomena associated with human malaria, we examined the IgG-subclass expression of antibodies to DNA and to ribonucleoproteins (RNP) in the serum of 99 patients with acute malaria. Of the sera, 22% were positive for single-stranded DNA (ssDNA), 18% for double stranded DNA (dsDNA) and 32% for RNP. Using a set of human IgG-subclass-specific murine monoclonal antibodies, we found that autoantibodies to dsDNA were predominantly expressed in the IgG1 subclass. In contrast, anti-ssDNA antibodies were more evenly distributed among the three other isotypes. Antibodies to RNP were essentially of the IgG1 and IgG2 isotypes. However, there was no correlation between these restricted IgG-subclass expressions of the three autoantibodies and the relative levels of total IgG-subclasses in the sera. These results are discussed in the context of previous findings of isotype expression of these autoantibodies in patients with the autoimmune disease systemic lupus erythematosus.

Keywords malaria autoimmunity human IgG-subclasses anti-DNA antibodies

INTRODUCTION

Human and experimental malaria are usually associated with the presence of a variety of antibodies to self-components, including epitopes of the red blood cell (Zouali *et al.*, 1982), DNA (Quakyi *et al.*, 1979; Kreier & Dilley, 1969; Adu *et al.*, 1982; Daniel-Ribeiro *et al.*, 1984), ribonucleoproteins (RNP) (Greenwood, Herrick & Holborow, 1970; Voller, O'Neil & Humphrey, 1972). The question of whether these autoantibodies play a role in the pathogenesis of this infection remains however open. Recent studies (Adu *et al.*, 1982) suggest that the glomerulonephritis in patients with nephrotic syndrome—which might be of malarial aetiology—may be mediated by DNA-anti-DNA complexes.

Autoantibodies to nuclear components are also produced by patients with rheumatic diseases, particularly systemic lupus erythematous (SLE). Interestingly, these self-reactive antibodies exhibit a marked isotypic restriction. For example, anti-double stranded DNA (dsDNA) IgG-subclass distribution has been repeatedly shown to be predominantly of the IgG1 and IgG3 isotypes (Puritz *et al.*, 1973; Sontheimer & Gilliam, 1978; Zouali, Jefferis & Eyquem, 1984).

Using a set of human IgG-subclass-specific monoclonal antibodies (Lowe *et al.*, 1982), we examined the IgG-subclass distribution of autoantibodies to dsDNA, single stranded DNA (ssDNA) and RNP in sera of malaria patients.

Correspondence: Dr Moncef Zouali, Tufts University, School of Medicine, Department of Biochemistry and Pharmacology, 136 Harrison Avenue, Boston, MA 02111, USA.

MATERIALS AND METHODS

Patient sera. A total of 144 serum samples obtained from 99 patients admitted to 'Hôpital de la Salpêtrière, Paris' during the course of an acute malaria attack, were investigated. Negative controls were drawn from blood donors (Centre de Transfusion Sanguine, Institut Pasteur de Paris). Reference human sera monospecific for dsDNA, RNP, Sm and SS-B antigens were from AF-CDC ANA Reference Laboratory (Centers for Disease Control, Atlanta, GA, USA). All sera were stored at −30°C.

Monoclonal antibodies. Murine monoclonal antibodies to human IgG-subclasses (NL-16: anti-IgG1, GOM-2: anti-IgG2; 2G-4: anti-IgG3 and RJ-4: anti-IgG4) were kindly donated by Dr R. Jefferis (University of Birmingham, UK). The four monoclonal antibodies NL-16, GOM-2, 2G4 and RJ-4 have previously been shown to react only with IgG1, IgG2, IgG3 and IgG4 myeloma proteins respectively by haemagglutination (Lowe *et al.*, 1982) and by ELISA (Zouali, Jefferis & Eyquem, 1984).

ELISA for autoantibodies to ssDNA, dsDNA and RNP. Antibodies to ssDNA, dsDNA and RNP were detected by ELISA-assays using polystyrene microtitre plates (Nunk, Denmark) sensitized with these antigens. To eliminate any contamination of dsDNA-coated plates with putative ssDNA, the wells were previously treated with S1 single stranded-specific nuclease. The procedures have been described in detail elsewhere (Zouali, Jefferis & Eyquem, 1984). The results were expressed as a percentage of the reference positive sera. Only sera giving percentages above 30% were considered positive for the antigen tested.

Analysis of autoantibody IgG-subclass expression. This was performed as described (Zouali, Jefferis & Eyquem, 1984). Briefly, ssDNA, dsDNA, or RNP-coated plates were incubated with a 10^{-3} dilution of test serum for 2 h at 4°C. After washing, and addition of a 10^{-3} dilution of murine anti-human IgG-subclass monoclonal antibody, or $0{\cdot}5 \times 10^{-3}$ dilution of rabbit anti-human IgG (γ chain specific) antiserum, the plates were incubated for 18 h at 4°C, and washed again. Bound antibodies were revealed with either peroxidase-conjugated sheep anti-mouse or sheep anti-rabbit immunoglobulin reagent. After a final incubation the plates were washed and supplemented with 0·1 ml/well of orthophenylene diamine diluted in enzyme substrate buffer. All sera were tested in duplicate. Results reported represent the average of two determinations with the background binding of antibody to wells not coated with antigen substracted.

Evaluation of total IgG-subclass levels. This was performed as described previously (Zouali, Jefferis & Eyquem, 1984). Microtitre plates were coated with goat anti-human IgG F(ab')2 fragments at a concentration of 10 μg/ml. The procedure was then continued as indicated above. When purified human myeloma proteins were used under the conditions of this ELISA, the anti-subclass murine antibodies were found to be specific and equally sensitive. Relative IgG subclass levels were expressed as the yield of o.d. obtained with subclass specific reagents and o.d. obtained with anti-IgG (γ specific) reagent.

RESULTS

Ninety-nine sera from patients with acute malaria were screened for antibodies to ssDNA, dsDNA and RNP. The incidence of positive sera is summarized in Table 1. The mean ssDNA, dsDNA and RNP binding of malaria patients was significantly higher than the mean of healthy individuals. Anti-ssDNA, anti-dsDNA and anti-RNP antibodies were positive in 22%, 18% and 32% of the tested sera, respectively. There was no clear correlation between these autoantibody titres in the studied patients. When these autoantibodies were sequentially studied in several malaria patients, the patterns of change of these autoantibodies with time following the infection did not correlate with clearance of parasitemia. In some patients seroconversion from negative to positive, or from positive to negative titres could be observed. In others, titres increased then decreased, or vice versa (data not shown).

Sera selected from these positive autoantibodies through the ELISA assays were then examined for autoantibody IgG-subclass expression, using monoclonal IgG-subclass-specific antibodies. The

Table 1. Incidence of anti-ssDNA, anti-dsDNA and anti-RNP in *Plasmodium* infected patients and in blood donors

	Anti-ssDNA	Anti-dsDNA	Anti-RNP
Malaria patients	22%	18%	32%
Blood donors	3%	0%	1%

144 serum samples from malaria patients and 100 serum samples from blood donors were tested for binding to ssDNA, dsDNA and RNP as indicated under the Materials and Methods section.

specificity of these reagents under the conditions of the enzyme immunassay has been documented (Zouali, Jefferis & Eyquem, 1984). In tests with dsDNA, as antigen, there was a marked preponderance of IgG1 antibodies. Every individual serum developed greater o.d. with the anti-IgG1 reagent than with any of the other three anti-IgG-subclass monoclonal antibodies. When tests were repeated on ssDNA-coated plates, anti-ssDNA antibodies were more evenly distributed among the three other isotypes (Fig. 1). Intriguing results were obtained when IgG-subclass distribution of anti-RNP was examined. In every case, more reactivity was seen with anti-IgG2 and anti-IgG1 than with the two other monoclonal anti-IgG isotype antibodies (Fig. 1).

The relative total IgG-subclass levels were then determined in the autoantibody positive sera. As depicted in Fig. 2, the levels of total IgG-subclasses of malaria patients were parallel to those of blood donors and there was no apparent correlation between autoantibody IgG-subclass expression and total IgG-subclass levels (Fig. 2).

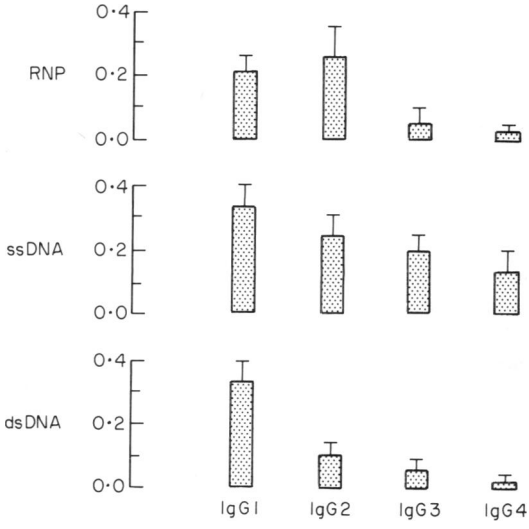

Fig. 1. IgG-subclasses distribution of antibodies to dsDNA, ssDNA and RNP in patients with malaria. Antigen-coated plates were incubated with a 10^{-3} dilution of serum to be tested and then with monoclonal-subclass specific antibody or rabbit anti-IgG antibody. Bound antibodies were developed with sheep anti-mouse or anti-rabbit antibody linked to peroxidase. Shown are the geometric means and standard errors obtained with each serum in duplicate samples.

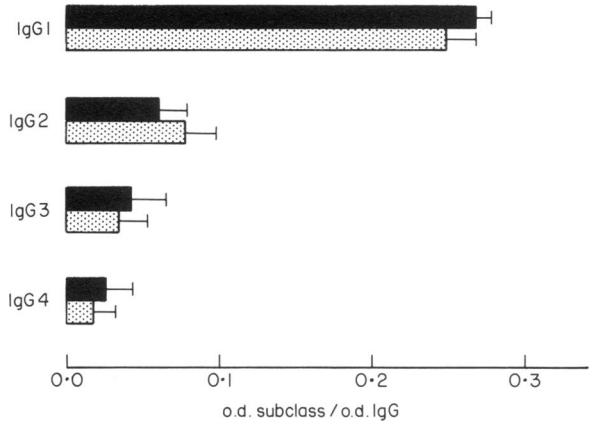

Fig. 2. Distribution of relative IgG-subclass levels in sera from patients with malaria (black bars) compared to sera from normal patients (white bars). The data are represented as geometric means +/− s.e. obtained with individual sera. All tests were performed in duplicate.

DISCUSSION

The results presented in this paper, showing that anti-ssDNA antibodies are present in the serum of patients with malaria, support and extend preliminary findings of Adu *et al.* (1982), Boonpucknavig & Ekapangakul (1984), and Daniel-Ribeiro *et al.* (1984). In contrast to these previous reports, we have found, using an ELISA assay, that malaria sera contain in addition antibodies that react with dsDNA. Antibodies to dsDNA were initially thought to be exquisitely specific to systemic lupus erythematosus. However it became apparent that patients with elevated anti-DNA antibody titres may span a clinical spectrum from no symptoms to severe disease (Rubin & Cait, 1979). There is also evidence that antibodies reactive with ssDNA also occur with great frequency in this disease as initially described by Stollar & Levine (1961). More recently, it has been demonstrated that certain murine (reviewed in Stollar, 1986) as well as human monoclonal anti-DNA auto-antibodies (Zouali, Fine & Eyquem, 1984) cross react with dsDNA and ssDNA. In molecular terms, antibodies to DNA may react with determinants on the bases or determinants on the sugar-phosphate backbone and cross-react with ssDNA, dsDNA and various polynucleotides (reviewed in Stollar, 1986).

Antinuclear antibodies of the speckled immunofluorescence pattern, including antibodies to RNP, occur in rheumatic diseases and are closely associated with a benign systemic syndrome (Sharp *et al.*, 1972) or with mild forms of SLE in which no severe glomerulonephritis is seen. Similarly antibodies showing a speckled pattern of fluorescence on hepatocyte nuclei were described in human malaria (Greenwood *et al.*, 1970; Adu *et al.*, 1982). The data presented herein confirm that in addition to anti-DNA antibodies, malaria sera contain antibodies to RNP. We have previously shown that under the conditions of these immunoassays reference anti-sera monospecific for the antigens Sm and SS-B do not bind the RNP antigen used in this study (Zouali, Jefferis & Eyquem, 1984). However, since we did not probe for antibodies to the Sm antigen, we cannot formerly exclude the possibility that antibodies of this specificity are present in malaria sera. Further studies using additional techniques such as immunoblotting are required to clarify this issue.

The availability of an ELISA suitable for the measurement of antibody levels in subclasses of IgG allowed us to examine the distribution of anti-ssDNA, anti-dsDNA and anti-RNP antibodies in malaria. The results show (i) that anti-ssDNA, anti-dsDNA and anti-RNP antibodies exhibit individual IgG-subclass patterns and (ii) that the levels of total IgG-subclasses are similar in malaria patients and in healthy persons.

The occurrence of IgG-subclass-restricted responses are well documented in mice. For example, while antibodies to proteins, hapten-protein conjugates or sheep red blood cells are mainly IgG1, antibodies to carbonhydrate or hapten-carbohydrate-conjugates are largely IgG3 (Grey, Hirat &

Cohn, 1971). Subsequent studies have revealed that auto-immune-prone strains of mice exhibit IgG-subclass restriction. Most RNA-reactive monoclonal antibodies derived from NZB/W mice were of the IgG2a subclass (Eilat *et al.*, 1982), which also has been found to be the major DNA-binding IgG subclass in NZB/W kidney eluates (Lambert & Dixon, 1968). In the MRL-lpr/lpr strain, antibodies to ssDNA (Izui & Eisenberg, 1980) and antibodies to SM (Eisenberg, Winfield & Cohen, 1982) proved to be mostly IgG2a.

Restricted isotype responses have been repeatedly demonstrated in some auto-immune conditions in man. For example, in SLE patients, the anti-Sm response is markedly restricted to the IgG1 heavy chain isotype (Eisenberg *et al.*, 1985), and antibodies to dsDNA are relatively restricted to IgG1 and IgG3 subclasses whilst antibodies to RNP are essentially restricted to the IgG1 isotype (Zouali, Jefferis & Eyquem, 1984), thus contrasting with the patterns observed in malaria patients. Relevant to this issue is the observation of Wahlgren *et al.* (1983) showing isotype restriction of anti-*Plasmodium falciparum* antibodies.

The ability of the plasma cell to express sequentially certain constant regions, or isotypes, is referred to as isotype switching. Characterization of the mechanisms of isotype expression in antibody response remains one of the central goals of modern immunology. Isotype switching is known to occur at the genetic level through a complex set of DNA and RNA splicing and rearrangement mechanisms (Honjo, 1983). However the events that occur at the cellular level remain unclear. There are reasons to believe that suppressor and/or helper T cells can selectively effect the subclass expression of isotypes and that the nature of the antigen influences IgG subclass expression (Mayumi *et al.*, 1983).

The mechanisms mediating the production of large amounts of auto-antibodies in malaria and the significance of auto-antibodies IgG-subclass expression described in the present paper remain unresolved. An explanation may be that the subclass patterns reflect a dysfunction of the immune response in malaria patients. Another possibility is that IgG-subclasses unbalance results from chronic stimulation of the immune system, for example after release of polyclonal factors by the plasmodium. The fact that human lymphocytes respond distinctly to B-cell activators (Walker, Johnson & MacLennan, 1983) support the further assumption that malaria mitogens may lead to selective expression of isotypes. It is attractive to suggest that different subpopulations of autoreactive B lymphocytes exhibiting individual responsiveness to malaria antigens and/or mitogens, and generating distinct subclasses of autoantibodies are involved. Parasite antigens cross-reacting with self components and/or altered self-components (i.e. by parasite nucleases) could result in the selective stimulation of only some of the auto-reactive B cells and thus to isotype restriction. To understand more fully the mechanisms underlying the selective expression of the isotypes observed, it will be necessary to generate self-epitopes-reactive lymphocyte clones from SLE and malaria patients. This would allow dissection of the processes and identification of the putative T and B cells or factors involved in the pathways leading to distinct isotypic expressions in SLE and in malaria.

We are indebted to Dr R. Jefferis for his generous gift of the anti-human IgG-subclass monoclonal antibodies. We thank Nicole Renaud and Patricia Tortevoye for expert technical assistance.

REFERENCES

ADU, D., WILLIAMS, D.G., QUAKYI, I.A., VOLLER, A., ANIM-ADDO, Y., BRUCE-TAGOE, A.A., JOHNSON, G.D. & HOLBOROW, E.J. (1982) Anti-ssDNA and antinuclear antibodies in human malaria. *Clin. exp. Immunol.* **49,** 310.

BOONPUCKNAVIG, S. & EKAPANGAKUL, G. (1984) Autoantibodies in sera of Thai patients with *Plasmodium falciparum* infection. *Clin. exp. Immunol.* **58,** 77.

DANIEL-RIBEIRO, C.T., DE ROQUEFEUIL, S., DRUILHE, P., MONJOUR, L., HOMBERG, J.C. & GENTILINI, M. (1984) Abnormal anti-single stranded (ss) DNA activity in sera from *Plasmodium falciparum* infected individuals. *Trans. Roy. Soc. Trop. Med. Hyg.* **78,** 742.

EILAT, D., HOCHBERG, M., FISCHEL, R. & LASKOV, R. (1982) Antibodies to RNA from autoimmune NZB/NZW mice recognize a similar antigenic determinant and show a large idiotypic diversity. *Proc. natn. Acad. Sci. USA.* **79,** 3813.

EISENBERG, R.A., WINFIELD, J.B. & COHEN, P.L. (1982) Sub-class restriction of anti-Sm antibodies in MRL mice. *J. Immunol.* **129,** 2146.

EISENBERG, R.A., DYER, K., CRAVEN, S.Y., FULLER,

R.C. & YOUNT, W.J. (1985) Subclass restriction and polyclonality of the systemic lupus erythematosus marker antibody anti-Sm. *J. Clin. Invest.* **75,** 1270.

GREENWOOD, B.M., HERRICK, E.M. & HOLBOROW, E.J. (1970) Speckled antinuclear factor in african sera. *Clin. exp. Immunol.* **7,** 75.

GREY, H.M., HIRAT, J.W. & COHN, M. (1971) A new mouse immunoglobulin: IgG3. *J. Exp. Med.* **133,** 289.

HONJO, T. (1983) Immunoglobulin genes. *Ann. Rev. Immunol.* **1,** 499.

IZUI, S. & EISENBERG, R.A. (1983) Circulating anti-DNA-rheumatoid factor complexes in MRL/1 mice. *Clin. Immunol. Immunopathol.* **15,** 536.

KREIER, J.P. & DILLEY, D.A. (1969) *Plasmodium berghei*: nucleic and agglutinating antibodies in rats. *Exp. Parasit.* **26,** 175.

LAMBERT, P.H. & DIXON, F.J. (1968) Pathogenesis of the glomerulonephritis of NZB/W mice *J. exp. Med.* **127,** 507.

LOWE, J., BIRD, P., HARDIE, D., JEFFERIS, R. & LING, N.R. (1982) Monoclonal antibodies to determinants on human gamma chains: properties of antibodies showing subclass restriction or subclass specificity. *Immunology*, **47,** 329.

MAYUMI, M., KURITANI, T., KUBAGAWA, H. & COOPER, M.D. (1983) IgG-subclass expression by human B lymphocytes and plasma cells: B lymphocytes precommitted to IgG subclass can be preferentially induced by polyclonal mitogens with T cell help. *J. Immunol.* **130,** 671.

PURITZ, E.M., YOUNT, W.J., NEWELI, M. & UTSINGER, P.D. (1973) Immunoglobulin classes and IgG subclasses of human antinuclear antibodies. A correlation of complement fixation and the nephritis of systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **2,** 98.

QUAKYI, I.A., VOLLIR, A., HALL, A.P., JOHNSON, G.D. & HOLBOROW, I.J. (1979) Immunological abnormalities in Caucasians with malaria. *Immunol. Lett.* **1,** 153.

RUBIN, R.L. & CAIT, R.L. (1979) Anti-DNA activity of IgG $F(ab')_2$ from normal human serum. *J. Immunol.* **122,** 1604.

SHARP, G. C., IRVIN, W.S., TAN, E.M., GOULD, R.C. & HOLMAN, H.R. (1972) Mixed connective tissues disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am. J. Med.* **32,** 148.

SONTHEIMER, R.D. & GILLIAM, J.N. (1978) DNA antibody class, subclass and complement fixation in systemic lupus erythematosus with and without nephritis. *Clin. Immunol. Immunopathol.* **10,** 459.

STOLLAR, D. (1986) Antibodies to DNA. *CRC Crit. Rev. Biochem.* **20,** 1.

STOLLAR, D. & LEVINE, J. (1961) Antibodies to denatured deoxyribonucleic acid in a lupus erythematosus serum. *J. Immunol.* **87,** 177.

VOLLER, A., O'NEIL, P. & HUMPHREY, D. (1972) Serological studies in Tanzania. II. Antinuclear factor and malarial indices in populations living at different altitudes. *J. Trop. Med. Hyg.* **75,** 136.

WAHLGREN, M., BERZINS, K., PERLMANN, P. & PERSSON, M. (1983) Characterization of the humoral immune response in *Plasmodium falciparum* malaria. II. IgG-subclass levels of anti-*P. falciparum* antibodies in different sera. *Clin. exp. Immunol.* **53,** 135.

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

ZOUALI, M., DRUIHLE, P., GENTILINI, M. & EYQUEM, A. (1982) High titres of anti-T antibodies and other haemagglutinins in human malaria. *Clin. exp. Immunol.* **50,** 83.

ZOUALI, M., JEFFERIS, R. & EYQUEM, A. (1964) IgG-subclass distribution of autoantibodies to DNA and to nuclear ribonucleoproteins in autoimmune diseases. *Immunology* **51,** 595.

ZOUALI, M., FINE, J.M. & EYQUEM, A. (1984) Anti-DNA autoantibody activity and idiotypic relationships of human monoclonal proteins. *Eur. J. Immunol.* **14,** 1085.