

IgG subclass and light chain distribution of anticardiolipin and anti-DNA antibodies in systemic lupus erythematosus

A E GHARAVI,¹ E N HARRIS,² M D LOCKSHIN,¹ G R V HUGHES,² AND K B ELKON¹

From ¹The Hospital for Special Surgery, Cornell University Medical Center, New York, NY; and ²St Thomas's Hospital, London, UK

SUMMARY The IgG subclass and light chain distribution of anticardiolipin and anti-DNA antibodies were determined in serum samples from patients with systemic lupus erythematosus. With an enzyme linked immunosorbent assay (ELISA) and mouse monoclonal antibodies to individual subclasses, significant differences in the distributions of IgG2, IgG3, and IgG4 subclasses were observed between anticardiolipin and anti-DNA antibodies. Whereas anti-DNA antibodies were predominantly IgG1 and IgG3, all subclasses of anticardiolipin were detected with a prevalence ranging from 34% (IgG3) to 57% (IgG1). Clinical complications were found slightly more frequently (83%) in patients with sera containing the non or weak complement fixing subclasses (IgG2 and IgG4) than in patients with sera containing complement fixing (IgG1 and IgG3) subclasses (62%). Light chain analysis by ELISA showed a trend towards use of κ chains for anti-DNA and λ chains for anticardiolipin antibodies. These findings further emphasise the differences between anti-DNA and anticardiolipin antibodies in terms of their origins and potential mechanisms for producing tissue injury.

Key words: antiphospholipid antibodies, antinucleic acid antibodies, thrombosis, fetal loss, thrombocytopenia.

Anticardiolipin antibodies are found in a variety of clinical conditions, including infections,¹⁻³ autoimmune,⁴⁻⁷ lymphoproliferative,⁸ and drug induced diseases.⁹⁻¹⁰ In autoimmune disorders these antibodies persist and are associated with clinical complications such as thrombosis, fetal loss, and thrombocytopenia.^{7, 11-13} Although anticardiolipin antibodies of all three major isotypes are produced in systemic lupus erythematosus (SLE), the IgG class is predominantly associated with clinical complications.^{14, 15}

The IgG subclass distribution of antibodies is of interest because of the relation between certain subclasses and effector function. For example, IgG1 and IgG3 efficiently fix complement and bind to most Fc receptors.^{16, 17} IgG2 and IgG4, on the other hand, have few known effector functions attribu-

table to the constant region of their heavy chains.^{16, 17} In addition, the distribution of IgG subclasses may provide information about the nature of the inducing stimulus and the T cell control of the antibody response. Most foreign proteins elicit predominantly IgG1 and IgG3 antibody responses,^{18, 19} whereas many bacterial polysaccharides elicit a predominantly IgG2 subclass response, which is not regulated by T cells.²⁰⁻²² We, therefore, evaluated the IgG subclass distribution of anticardiolipin antibodies and compared the results with the subclass distribution of anti-double-stranded DNA (anti-dsDNA) antibodies in patients with SLE.

Patients and methods

PATIENTS

Serum samples from 60 patients with SLE and SLE-like autoimmune disorders were used in the study. Thirty five samples had high levels of anticardiolipin

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Correspondence to Dr A E Gharavi, Hospital for Special Surgery, Cornell University Medical Center, 535 East 70th St, New York, NY 10021, USA.

antibodies, and 29 had high titres of anti-dsDNA antibodies. Only four serum samples had moderate to high titres of both antibodies, and these four were included in both groups. The clinical features of these patients have been described elsewhere.^{7 13 14} Serum samples from 12 healthy laboratory and hospital personnel were used as normal controls.

MATERIALS

Cardiolipin (CL), calf thymus DNA, poly-L-lysine, bovine serum albumin (BSA), and alkaline phosphatase conjugates of goat antimouse IgG, anti-human IgG, antihuman light chain kappa (κ), and antihuman light chain lambda (λ) were purchased from Sigma (Sigma Chemical Co, St. Louis, MO). Mouse monoclonal antibodies²³ against human IgG1 (HP 6001), IgG2 (HP 6014), IgG3 (HP 6050), and IgG4 (HP 6020) were kindly provided by Dr Charles Reimer, CDC, Atlanta, GA. Purified IgG subclasses were donated by Dr F Skvaril, WHO, Berne, Switzerland.

ELISA FOR IgG ANTICARDIOLIPIN AND ANTI-dsDNA ANTIBODIES

The anticardiolipin solid phase assay was performed as described in detail previously.¹⁴ The anti-dsDNA ELISA was a minor modification of a previously described radioimmunoassay for dsDNA.²⁴ Plates were serially coated with poly-L-lysine 50 μ g/ml in phosphate buffered saline (PBS) for two hours, followed by calf thymus DNA 100 μ g/ml overnight at 4°C. DNA was digested with S1-nuclease 100 units/ml in 0.02 M sodium acetate, 0.05 M NaCl, 0.1 mM ZnCl₂, 5% glycerol pH 4.6 for one hour at 37°C. Plates were then washed and blocked with 10% adult bovine serum (ABS) in PBS. Wells were incubated with patient serum, followed by anti-human IgG conjugate. For both assays optical density (OD) values greater than three standard deviations above the mean of a group of 12 normals were considered positive.

IgG SUBCLASS ANALYSIS

Plates coated either with cardiolipin or dsDNA were incubated with test sera for three hours as described above. Mouse monoclonal IgG antibodies to human IgG subclasses G1, G2, G3, or G4 at the optimal

dilution in 10% ABS/PBS were added and incubated for 90 minutes at room temperature. After washing, alkaline phosphatase conjugated goat antimouse IgG 1/1000 in 10% ABS was added and incubated for one hour. Patient serum samples were diluted 1:100 for IgG1 and IgG2 and 1:10 for IgG3 and IgG4. The optimal working dilutions for monoclonal anti-subclasses were determined by a quantitative ELISA with purified myeloma IgG subclasses as antigens. Briefly, plates were coated with affinity purified goat antihuman γ chain (2.5 μ g/ml overnight) and blocked with PBS/1% BSA. Wells were incubated with serial dilutions from 200 ng/ml to 3 ng/ml of purified myeloma IgG subclasses in PBS/1% BSA. Chequerboard dilutions of monoclonal anti-subclass reagents (1/500 to 1/500 000) were added and incubated for 90 minutes, followed by a one hour incubation with antimouse IgG conjugate (1:1000 in 10% ABS/PBS). The optimal working dilutions, which gave similar OD for equal quantities of different subclasses, were 1:1000 for IgG1 and IgG2 and 1:10 000 for IgG3 and IgG4.

LIGHT CHAIN TYPE

Light chain distribution of anticardiolipin and anti-dsDNA was studied using alkaline phosphatase conjugated light chain specific antisera. Anti- κ and anti- λ sera were calibrated so that at the working dilutions chosen the normal light chain ratio of κ : λ (2:1) produced an equal OD reading. To achieve this chequerboard titrations of serial dilutions of pooled normal human IgG were coated onto microtitre wells and incubated with serial dilutions of anti- κ or anti- λ serum. The dilutions chosen were 1/1000 (anti- κ) and 1/1500 (anti- λ). Only serum samples with antibody levels equal or greater than three standard deviations above the mean of the normal were compared.

STATISTICAL ANALYSIS

The number of patients positive for subclass specific anti-DNA and anticardiolipin antibodies were compared by χ^2 analysis.

Results

IgG SUBCLASS

Fig. 1 and Table 1 give the IgG subclass distribution

Table 1 IgG subclass distribution of anticardiolipin (aCL) and anti-dsDNA antibodies. Values are numbers positive (percentages)

Antibody	Number of serum samples	IgG1	IgG2	IgG3	IgG4
Anti-CL	35	20 (57)	15 (43)	12 (34)	18 (51)
Anti-DNA	29	22 (76)	3 (10)*	25 (86)**	0 (0)**

*p v aCL<0.005; **p v aCL<0.0005.

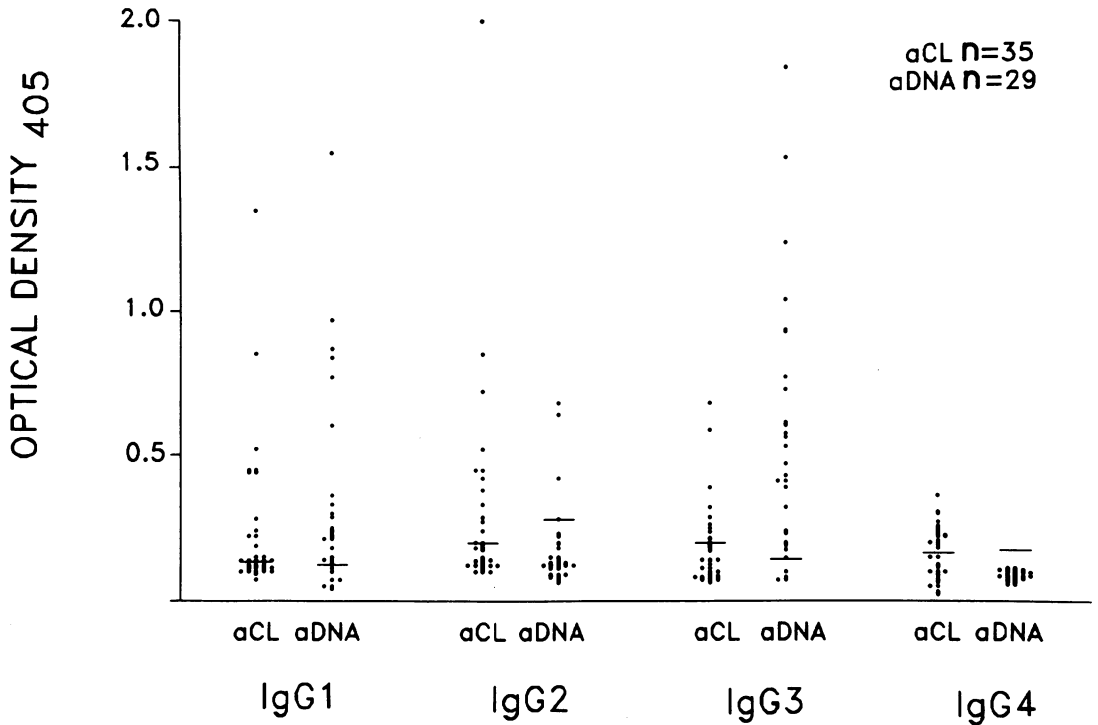


Fig. 1 IgG subclasses of anticardiolipin antibodies in serum samples from 35 patients, and anti-dsDNA in samples from 29 patients measured by ELISA using mouse monoclonal subclass specific antibodies. Horizontal lines indicate three standard deviations above the mean of a group of 12 normals. aCL=anticardiolipin; aDNA=anti-dsDNA.

of anticardiolipin antibodies from 35 patients. There was an approximately equal distribution of subclass autoantibodies. In contrast, the subclass distribution of anti-dsDNA antibodies comprised predominantly IgG1 and IgG3 (Fig. 1 and Table 1). The differences between the IgG2 ($p < 0.005$), IgG3 and IgG4 ($p < 0.0005$) profiles of anticardiolipin and anti-DNA antibodies were highly significant. As only four of the samples tested had moderate to high titre of both anticardiolipin and anti-dsDNA antibodies in the same serum, subclass distribution of both antibodies could not be compared in a significant number of patients.

LIGHT CHAIN TYPE

Fig. 2 shows the relation between κ and λ containing anticardiolipin and anti-dsDNA antibodies. Whereas the κ/λ ratio for anti-dsDNA was greater than normal in 76% of the samples, 65% of sera showed a reversed κ/λ ratio for the anticardiolipin antibodies.

CLINICAL CORRELATIONS

Twenty two of the 35 patients with anticardiolipin antibodies have been studied in detail at the

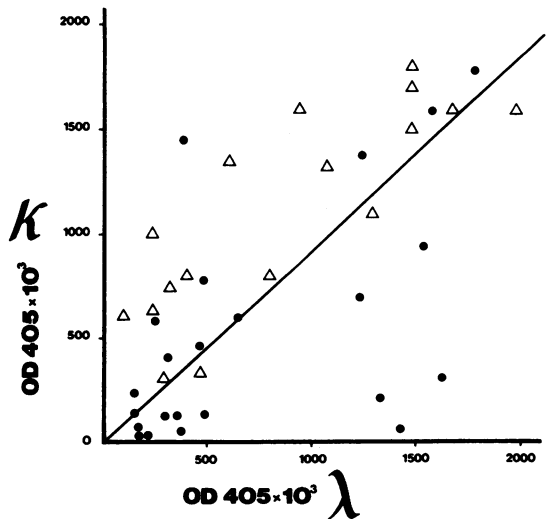


Fig. 2 Correlation between kappa (κ) and lambda (λ) light chains to anti-CL and anti-dsDNA antibodies detected by ELISA. ●=anti-CL, Δ =anti-dsDNA.

Table 2 *IgG subclasses and clinical complications in 22 patients with anticardiolipin antibodies*

<i>IgG anti-CL subclasses</i>	<i>Number of patients</i>	<i>Number of patients with complications</i>	<i>Thrombosis</i>	<i>Fetal loss</i>	<i>Thrombocytopenia</i>
Complement fixing					
IgG1, IgG3	4	2	2	1	0
IgG1, IgG2, IgG3, IgG4	12	8	5	4	4
Subtotal	16	10	7	5	4
Non-complement fixing					
IgG2, IgG4	6	5	3	2	2
Total	22	15	10	7	6

Hospital for Special Surgery, New York. Table 2 shows the occurrence of one of the associated clinical complications, namely thrombosis, fetal loss, or thrombocytopenia. Most (8/10) patients with complications had both complement fixing (IgG1 and IgG3) and weak/non-complement fixing (IgG2 and IgG4) antibodies. Of the six patients with only IgG2 or IgG4 anticardiolipin antibodies, or both, five had complications.

Discussion

We have previously shown that patients with SLE and related diseases produce anticardiolipin antibodies of all major isotypes, but that IgG antibodies are predominantly associated with specific clinical complications.^{14 15} In the present study we determined which IgG subclass antibodies are expressed and whether the subclass and light chain distributions are similar in the anticardiolipin and anti-DNA populations of antibodies. The results show considerable differences between the two antibody populations, with expression of all IgG subclasses and a predominance of λ light chains in anticardiolipin antibodies compared with a predominance of IgG1, IgG3, and κ light chains in anti-dsDNA antibodies. Differences in the patterns of subclass and light chain expression provide further evidence that although some cross reactive (polyspecific) antibodies may occur in SLE,²⁵ for the most part, the anticardiolipin and anti-dsDNA antibody populations are discrete.²⁶

Anti-DNA antibodies have been shown by others to comprise IgG1 and IgG3 subclasses,²⁷⁻³⁰ which are capable of efficient complement fixation.^{16 17} It has been suggested that complement activation by anti-DNA antibodies is related to clinical nephritis in SLE.^{31 32} In contrast, the mechanism(s) of tissue injury associated with anticardiolipin antibodies is unknown. IgM is highly efficient at fixing complement,³³ yet patients with the exclusive presence of IgM anticardiolipin antibodies do not manifest

clinical complications.^{14 15} It therefore seems unlikely that the complement cascade is intimately involved in the clinical complications associated with this antibody. Similarly, the detection of only IgG2 and IgG4 anticardiolipin antibodies, subclasses that are weak or inefficient in complement fixation,^{16 17} in one third of patients with complications emphasises that if these antibodies are directly pathogenic they may cause tissue injury by complement independent mechanisms. Such mechanisms could include binding to platelets or endothelial cells with subsequent mediator release and clotting. Also, as IgG2 and IgG4 do not bind to placental Fc receptors,³⁴ and are therefore poorly transmitted to the fetus, the disproportionately low anticardiolipin antibody levels observed in cord blood³⁵ may be explained by lack of transfer of these subclasses.

The identification of autoantibody subclasses has been controversial owing to the lack of monospecific high affinity antibodies and difficulties in purifying antigens to homogeneity. The recent availability of high affinity, thoroughly evaluated monoclonal antibodies²³ as used in the present study, should rapidly resolve these controversies. IgG1 and IgG3 subclasses predominate in the responses to dsDNA and certain protein antigens,²⁶⁻³¹ but prominent IgG2 subclass responses to U1 RNP,²⁶ islet cell antigens,³⁶ and the ribosomal P proteins^{37 38} have also recently been described. These findings indicate that the subclass distribution of various autoantibodies is not uniform and is not necessarily T cell regulated in the same way as are subclass responses to foreign protein antigens.³⁹ Although the results of the present study are not strictly quantitative as anti-IgG3 and IgG4 subclass antibodies were measured at low serum dilutions, detection of all four subclasses with anticardiolipin activity suggests that extensive class switching is occurring at the B cell level. Whether the switch is antigen driven and T cell controlled remains to be determined.

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