Clin. exp. Immunol. (1985) **60**, 329–338.

Family distribution of anti-F(ab′)$_2$ antibodies in relatives of patients with systemic lupus erythematosus

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(Accepted for publication 18 December 1984)

SUMMARY

Recently we reported an inverse relationship between the levels of anti-F(ab′)$_2$ antibodies and disease activity in systemic lupus erythematosus (SLE). The present study focused on anti-F(ab′)$_2$ antibodies in unaffected relatives of SLE patients. Sixty sera from first degree family members from 11 SLE families and 49 sera from 8 control families were studied. Percentage of SLE family members with anti-DNA antibodies (15%) was higher than control family sera (8%, $P < 0{\cdot}05$). Anti-F(ab′)$_2$ antibodies were measured using ELISA assays. The SLE family sera had higher amounts of anti-F(ab′)$_2$ antibodies than the normal control family group ($P = 0{\cdot}0051$). In an effort to determine if anti-F(ab′)$_2$ antibodies found in high titres in the sera of some SLE family members had specificity for the F(ab′)$_2$ fragment of anti-DNA antibodies of the SLE relative patients, DNA–anti-DNA inhibition experiments were performed using anti-F(ab′)$_2$ prepared from the relative in parallel with anti-F(ab′)$_2$ prepared from normal controls with equivalent high titres of serum anti-F(ab′)$_2$. Inhibition exhibited by anti-F(ab′)$_2$ of first degree relatives was higher than that obtained from control normal donors ($P < 0{\cdot}02$). Such differences in inhibition were not recorded using a control tetanus toxoid–anti-tetanus toxoid assay. In direct binding ELISA experiments, peroxidase-conjugated anti-F(ab′)$_2$ antibodies from the same first degree relative showed high relative specificity against purified anti-DNA antibodies of his SLE proband when compared to those obtained against different anti-DNA antibodies isolated from unrelated SLE patients ($P < 0{\cdot}001$). Such a substantial difference was not observed in parallel experiments using peroxidase conjugated anti-F(ab′)$_2$ antibodies from normal controls unrelated to SLE subjects.

Keywords anti-F(ab′)$_2$ antibodies systemic lupus erythematosus SLE idiotypes

INTRODUCTION

One of the most perplexing questions related to understanding of the pathogenesis of systemic lupus erythematosus (SLE) is the intriguing problem of immunological abnormalities which occur in unaffected family members of SLE patients. During recent years, many investigators have correlated immunological aberrations in the families of SLE patients to the broad spectrum of defective immune mechanisms that could be involved in the pathogenesis of the disease (Morteo *et al.*, 1961; Ansell & Lawrence, 1963; Leonhardt, 1964; Arnett & Shulman, 1976; Allen *et al.*, 1978).

Defects in humoral immunity, such as C4 activation, hypergammaglobulinaemia and autoantibodies directed against a wide variety of self antigens such as nucleic acids, nuclear proteins, lymphocyte surface antigens or thyroglobulin, have been exhaustively described in the sera of asymptomatic SLE relatives (Holman & Deicher, 1960; Galo & Forde, 1966; Larsen, 1972;

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Solheim & Larsen, 1972; Searles *et al.*, 1977). In addition cellular defects such as abnormal suppressor T cell function, polyclonal B cell activation or defective macrophage activity have also been reported in asymptomatic SLE relatives (Miller & Schwartz, 1979). Familial studies involving autoantibodies have implicated environmental factors in the expression of the disease (DeHoratius & Messner 1975; DeHoratius *et al.*, 1975). The increased frequency of HLA, DR2 and DR3 genetic loci in SLE families suggests the involvement of hereditary factors in the development of the disease (Nies *et al.*, 1976; Reinertsen *et al.*, 1978). However, none of the studies of immunological defects performed both in SLE patients and their asymptomatic relatives explains the enigma that often under the same environmental, genetic and immunological conditions, SLE patients manifest the disease but their relatives do not.

Recently, considerable interest has been directed at the definition of the function of anti-F(ab′)$_2$ antibodies in SLE (Nasu *et al.*, 1980; Heimer, Wolf & Abruzzo, 1982). Anti-F(ab′)$_2$ antibodies may occur naturally in normal donors at low levels (Nasu *et al.*, 1980); however, they are present at higher titre in the sera of rheumatoid arthritis (RA) and SLE patients (Heimer, Wolfe & Abruzzo, 1982; Birdsall, Lidsky, & Rossen, 1983). It is possible that these anti-F(ab′)$_2$ antibodies may function in the control of some autoimmune diseases. In studies of SLE we have recently presented evidence which suggests that levels of anti-F(ab′)$_2$ vary inversely with disease activity (Silvestris *et al.*, 1983; Silvestris *et al.*, 1984). Indeed, in studies reported by Nasu *et al.* (1982) the anti-F(ab′)$_2$ preparations from SLE sera appear to contain large amounts of combining specificities which are directed against idiotypic or unknown allotypic determinants of autoantibody molecules, such as affinity purified autologous anti-DNA antibodies. We have recently confirmed the results originally reported by Nasu *et al.* (1982) and have reported a correlation of high titre of anti-F(ab′)$_2$ antibodies in the sera of SLE patients in remission and conversely low levels of anti-DNA antibodies (Silvestris *et al.*, 1984). It seems possible that clinical improvement of SLE may be strictly related to the activation of a self-regulating anti-idiotypic system, namely the idiotypic network originally proposed by Jerne (1974). Data supporting such a hypothesis have been presented by Abdou (Abdou *et al.*, 1981; Reissman & Abdou, 1983). However, at the present time very little information is available regarding the expression of anti-F(ab′)$_2$ antibodies in relatives of SLE patients. Since genetic factors are probably involved in the development of SLE, it seems possible that relatives of SLE patients, although manifesting many demonstrable immunological abnormalities, remain disease free by successful activation of anti-idiotype immunoregulatory systems. This activation may thus effectively preserve unaffected relatives from development of clinically apparent autoimmune disease.

Evidence emerging from the present study indicates an interesting difference in activation of the anti-idiotypic immunoregulatory system in a number of relatives of SLE patients when compared to a similar group of normal unaffected control families.

MATERIALS AND METHODS

SLE patients and their relatives. Sera from 11 SLE patients and from 60 of their asymptomatic relatives were examined. All SLE patients satisfied the recent ARA criteria for the definite diagnosis of SLE (Tan *et al.*, 1982). Sixty relatives, 8–73 years of age, were studied. All relatives' sera were derived from the 11 SLE patients' families. All family members were clinically healthy and unaffected by any autoimmune disease. All relatives studied were first degree and included parents, siblings or children of SLE patients.

Normal controls. Normal sera from 49 donors collected as control family groups from eight families were studied as controls. The age of control family members was similar to that of SLE relatives.

Anti-ssDNA antibody assay. The previously described ELISA method for the detection of anti-DNA antibodies was performed (Kavai *et al.*, 1982; Eaton, Schneider & Schur, 1983) with slight modifications. Briefly, after activation of the microtitre polyvinylchloride plates (Dynatech Lab., Alexandria, Virginia, USA) with poly-L-lysine (Sigma, St Louis, Missouri, USA) diluted at 50 μg/ml for 45 min at room temperature, 50 μl of heat-denaturated calf thymus DNA (Sigma) at a

concentration of 1 μg/ml in 5% calf serum (CS), was placed in each well of the plate. After overnight incubation at 4°C, the plates were washed three times with 5% CS in phosphate-buffered saline and then incubated for 1 h at room temperature with the same solution in order to block any subsequent, non-specific binding. Sera diluted 1/40 in 5% CS were incubated for 3 h at room temperature and after washing the plates five times with the same diluting solution, 50 μl of goat peroxidase conjugated anti-human immunoglobulin antiserum (Tago Labs, Burlingame, California, USA) diluted 1/1500 in identical 5% CS solution, were added to each well. After incubation for 1 h at room temperature, the plates were washed and the ABTS (2·2 azimo-di-[3-ethyl-benzthiazoline sulphonate]) staining solution (Boehringer Mannheim Biochemicals, Indianapolis, Indiana, USA) was added to the wells. Plates were read 20 min later at 405 nm in a 'Micro-ELISA Spectrophotometer' (Dynatech, Alexandria, Virginia, USA). Each sample determination was performed in triplicate. The absorbance of each determination was evaluated on a standard titration curve obtained in the same assay by correlating the optical density of 12 known amounts of affinity purified anti-DNA antibodies. The arithmetic mean of the three determinations for each serum was considered the relative amount in μg/ml of anti-DNA antibodies. Specificity of the ELISA assay for ssDNA was confirmed using inhibition with ssDNA in parallel with other nucleotides and unrelated antigens.

Anti-F(ab')$_2$ antibody assay. The enzyme linked immunosorbent ELISA assay for the detection and the measurement of the anti-F(ab')$_2$ antibodies was performed in the same way as previously described (Silvestris *et al.*, 1984). Briefly, test serum samples diluted 1/40 were added to the plates previously activated by F(ab')$_2$ derived from pepsin digested normal IgG. After 5 h incubation at room temperature, the plates were washed three times with 5% CS and subsequently goat peroxidase-conjugated, γ-chain specific, anti-human Fc fragment antiserum (Cappel Labs, Cochranville, Pennsylvania, USA), previously absorbed with normal F(ab')$_2$ and diluted at 1/3,000 in 5% CS was added and the test completed in the same way as the anti-DNA antibody assay. Also in this case each determination was done in triplicate and the relative values were estimated on a standard titration curve, performed in each test with known concentrations of affinity purified anti-F(ab')$_2$ antibodies, in order to obtain the amount of anti-F(ab')$_2$ antibodies present in the sera as a value expressed in μg/ml. Therefore, the values obtained in this way were not absolute amounts of anti-F(ab')$_2$ antibodies in the sera, but were determined relative to the known concentrations of protein used as controls in the microtitration curve.

Isolation of anti-DNA antibodies. Anti-DNA antibodies were isolated from plasma derived from different SLE patients with active disease. The method developed by Arndt-Jovin *et al.* (1975) using calf thymus DNA conjugated to Sepharose 4B was utilized with slight modifications. Heat denaturated and homogenized calf thymus DNA was coupled to Sepharose 4B. Anti-DNA antibodies from different plasma samples were absorbed by affinity column chromatography using neutral buffer and then eluted with acidic buffer (glycine-HCl 0·1 M, pH 1·8). All eluted fractions with optical density higher than 0·05 λ were collected and concentrated approximately 100-fold by negative pressure ultrafiltration. High levels of anti-DNA activity were demonstrated in these eluted fractions in subsequent ELISA tests. DNAase digestion of these fractions, using DNAase coupled to sepharose 4B (10 mg DNAase per 20 ml of Sepharose), was performed to ensure that no free DNA was present in column eluates. Subsequent assays of these digests revealed no detectable DNA.

Purification of anti-F(ab')$_2$ antibodies. F(ab')$_2$ fragment derived from normal human pepsin digested IgG was coupled to Sepharose 4B in order to purify anti-F(ab')$_2$ antibodies from the sera of different SLE patients and from the sera of several unaffected family members. Each affinity column of F(ab')$_2$ Sepharose contained approximately 5 mg of protein per ml of gel. In subsequent ELISA tests these affinity-purified preparations showed high reactivity against F(ab')$_2$ from normal pooled IgG.

Immunoglobulins and their fragments. Normal IgG purified from Cohn fraction II (Sigma) by DEAE-cellulose chromatography using 0·015 M phosphate buffer pH 6·3, was the source of F(ab')$_2$. Nisonoff's method using 2% pepsin digestion of whole IgG in 0·1 M pH 4·1 acetate buffer was followed to obtain human F(ab')$_2$ fragment (Nisonoff *et al.*, 1960). Pepsin-digested IgG was passed through a Sephadex G-200 column in neutral buffer. Immunodiffusion analysis of the first filtration peak showed no residual reactivity using anti-Fc antiserum.

Inhibition tests. Inhibition tests of the DNA–anti-DNA binding of isolated anti-DNA antibodies obtained from SLE patient (PC) were performed using affinity purified anti-F(ab′)$_2$ antibodies derived from the same patient, from some of her relatives (father and brother), and from three different subjects with high serum titre of anti-F(ab′)$_2$ antibodies. These three different subjects were derived from the normal control families group.

All the purified anti-F(ab′)$_2$ antibodies were diluted to the same concentration (200 μg/ml) in 5% CS in PBS and incubated overnight at 4°C with 30 μg/ml of isolated anti-DNA antibodies. The incubation was performed in glass tubes in order to avoid any possible non-specific binding to the plastic and the tubes were finally left 1 h at room temperature before completing the test in a microELISA–anti-DNA assay. The same solution containing a known amount of isolated anti-DNA antibody (30 μg/ml) was used as standard. The residual activity against the DNA after the incubation gave the relative degree of inhibition produced by the anti-F(ab′)$_2$ in antibodies tested in the anti-DNA antibody assay system. Each sample was tested in triplicate.

In order to verify the relative specificity in the DNA anti-DNA system, additional control experiments using an unrelated human antigen–antibody assay (tetanus toxoid–purified anti-tetanus toxoid) were performed. Briefly, the isolated anti-F(ab′)$_2$ antibodies were incubated under the same conditions (200 μg/ml) with purified anti-tetanus toxoid (TT) (30 μg/ml), previously isolated from an immunized normal donor by affinity chromatography. In the same way as already outlined in the DNA–anti-DNA system, the residual activity against TT was measured after incubation of purified anti-TT with the same individual affinity isolated anti-F(ab′)$_2$ antibodies which had been tested in the other (DNA–anti-DNA) system. A microELISA anti-TT assay was utilized. Relative degree of inhibition induced by the anti-F(ab′)$_2$ antibodies was measured in the TT–anti-TT sysem. Each experiment was done in triplicate.

The percentage of inhibition was evaluated in the following way:

$$\frac{\text{OD of the standard} - \text{OD of the standard incubated with anti-F(ab')}_2}{\text{OD of the standard}} \times 100$$

Direct binding experiments. Additional experiments were performed in order to verify the reactions of selected individual affinity purified anti-F(ab′)$_2$ antibodies with certain isolated anti-DNA antibodies from individual patients. Normal IgG and affinity-purified anti-TT specific IgG were also used as control substrates.

Briefly, purified anti-F(ab′)$_2$ obtained from the SLE patient's brother who showed a high degree of inhibition using his anti-F(ab′)$_2$ in his own SLE sister's isolated DNA–anti-DNA system was conjugated with horseradish peroxidase (HRPO) by the method of Nakane & Kawaoi (1974). A microtitration plate activated by six different individual affinity isolated anti-DNA antibodies, all at the same concentration (50 μg/ml), was washed three times with 5% CS and then each well was incubated with 50 μl of anti-F(ab′)$_2$ antibodies previously conjugated with HRPO and used at the final concentration of 50 μg/ml. After 2 h incubation at room temperature, the ABTS peroxidase staining solution was added to the wells and 30 min later, the plates were read in the microELISA reader. As control in this test, we used isolated anti-F(ab′)$_2$ antibodies previously conjugated with HRPO obtained from a member of a normal control family. Both the HRPO anti-F(ab′)$_2$ from the SLE patient's brother and from the normal control were tested under identical conditions and were tested against each substrate.

Because inhibition results using isolated anti-F(ab′)$_2$ antibodies from close family members showed apparent preferential specificity for F(ab′)$_2$ determinants of anti-DNA antibodies isolated from the lupus proband within the same family, it seemed possible that the anti-F(ab′)$_2$ antibodies we were measuring by the ELISA assay might show some sort of specificity for genetically determined antigenic markers on F(ab′)$_2$ molecules uniquely expressed within individual families. Accordingly all SLE family sera and control family sera were typed for a number of genetically determined Gm γ-globulin markers including Gm(a), Gm(b), Gm(g), Gm(f) and Gm(z). In addition, typing for Km1 ad Km3 was also performed. Reagents for Gm(z) and Gm(f) typing were kindly provided by Dr Stephen D. Litwin (Guthrie Research Institute, Sayre, Pennsylvania, USA) and Dr William J. Yount (University of North Carolina, Chapel Hill, North Carolina, USA). In

addition, Gm typing reagents, reference standards and controls were generously furnished by Dr Moses S. Shanfield (Genetic Testing Institute, Atlanta, Georgia, USA). Typing for Gm(f) and Gm(z) as well as for the Km systems was essential since these genetic markers are all located on the F(ab')$_2$ portions of the IgG molecule.

Statistical analysis. Analysis of variances were performed by using χ^2 test, Mann–Whitney test and Student *t*-test.

RESULTS

The first set of experiments was performed to clarify the question as to whether there was a difference in the titre of anti-DNA antibodies between SLE patient's relatives and normal control families. Because of the extreme sensitivity of the ELISA assay system, an arbitrary designation was made for lower limit of positivity which included tests showing anti-DNA of 5 μg/ml or higher in any test-serum sample. The percentage of positive sera was higher in the SLE patients' relatives group (15%) than in the controls (8%) ($P=0{\cdot}048$).

As previously noted (Silvestris *et al.*, 1984), a characteristic difference in titres of anti-F(ab')$_2$ antibodies emerged when SLE patients' family members and normal control family members were compared. Sixty sera of SLE patients' unaffected family members contained higher amounts of anti-F(ab')$_2$ antibodies than the normal control family groups ($P=0{\cdot}0051$). Of great interest was the finding in four of 11 SLE families of marked elevations in anti-F(ab')$_2$ antibody activity among several close relatives. Similar isolated elevations of anti-F(ab')$_2$ were noted in only two of eight control families. Examples of these patterns are shown in Fig. 1.

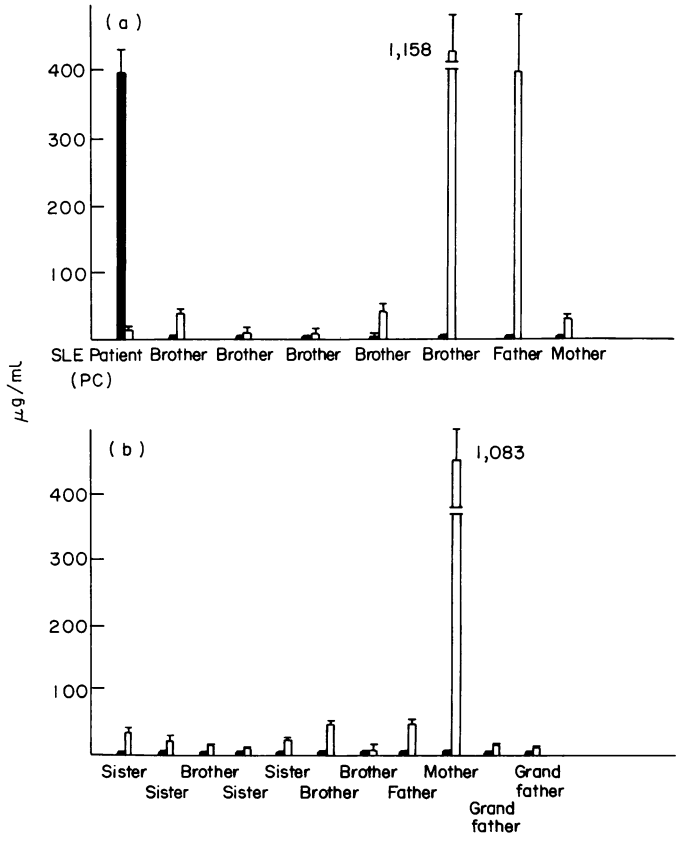

Fig. 1. Comparative patterns of anti-DNA antibodies (■) and conversely anti-F(ab')$_2$ antibodies (□) detected by ELISA methods in (a) a SLE patient's family and in (b) a control family.

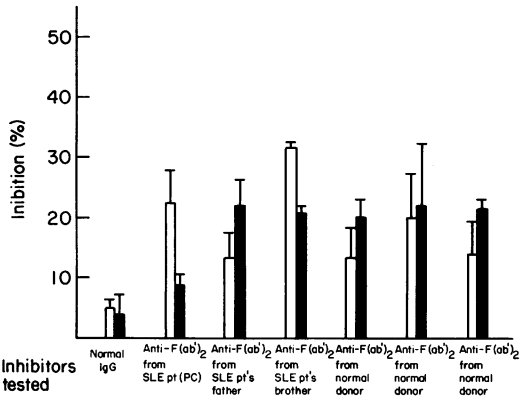

Fig. 2. Inhibitions of anti-DNA (□) and anti-TT (■) antibodies obtained with various preparations of affinity-purified anti-F(ab′)$_2$ antibodies are shown. The results are expressed as percentage inhibition in the two systems. The inhibition (31·4%) induced in the DNA–anti-DNA system by purified anti-F(ab′)$_2$ from the SLE (PC) patient's brother is the highest and different from normal controls ($P < 0{\cdot}02$). In the TT–anti-TT system the anti-F(ab′)$_2$ from the same subject inhibits binding in similar fashion to that of normal controls ($P > 0{\cdot}1$). The difference in inhibition in the two systems mediated by purified anti-F(ab′)$_2$ from the brother of the SLE patient (PC) is still significant ($P < 0{\cdot}001$). The inhibitions induced with normal IgG in both the systems (4·9%–3·7%) were considered as baseline inhibition of the standards in the two test assays.

The next set of experiments addressed the question of whether the anti-F(ab′)$_2$ antibodies found at higher concentration in the sera of some close family members of selected SLE patients had any peculiar specificity for the F(ab′)$_2$ fragment of the anti-DNA antibodies of their SLE relative. Inhibition experiments were performed using anti-F(ab′)$_2$ purified from the same patient and in parallel with those obtained from first degree family members (father and brother), as well as those from normal controls as inhibitors of the affinity purified anti-DNA antibody obtained from the SLE patient PC. As can be seen in Fig. 2, the highest degree of inhibition (31·4%) was mediated by the anti-F(ab′)$_2$ isolated from the patient's own brother. This inhibition induced by the anti-F(ab′)$_2$ of the brother of patient PC was considerably higher than that of the patient herself (22·8%) and was significantly different from the inhibition induced by anti-F(ab′)$_2$ derived from normal controls ($P < 0{\cdot}02$). In the parallel TT–anti-TT inhibition test, predominant inhibition by F(ab′)$_2$ from the patient's brother was not seen. Indeed, in the TT–anti-TT inhibition test all the purified anti-F(ab′)$_2$ antibodies from the family members of SLE patient PC and from the normal controls showed a very similar degree of inhibition without signifiant variation ($P > 0{\cdot}1$). It is also important to point out that some inhibition by isolated anti-F(ab′)$_2$ antibodies occurred in the TT–anti-TT system but that the SLE patients' relatives consistently showed greater inhibition for DNA–anti-DNA systems of their lupus relative. Besides the data shown in Fig. 2, similar findings were observed in two other SLE families. It should be emphasized that the anti-F(ab′)$_2$ from the normal donors were isolated from those normal control family members showing highest titres of anti-F(ab′)$_2$ as shown in Fig. 1. Moreover, as can be seen in Fig. 2, the difference in inhibitions by the anti-F(ab′)$_2$ of patient PC's brother when the DNA–anti-DNA system was compared to the TT–anti-TT system was still significant ($P < 0{\cdot}001$). That is, anti-F(ab′)$_2$ from patient PC's brother showed highly significant inhibition in the PC anti-DNA system but no significant difference from other controls when tested in the TT–anti-TT assay. Moreover, the inhibition detected with normal pooled IgG in both DNA–anti-DNA and TT–anti-TT systems (4·9%–3·7%) was considered as baseline inhibition of the standards in the two parallel test assays.

Finally, a last set of experiments was performed to verify the specificity of anti-F(ab′)$_2$ antibodies obtained from the brother of SLE patient PC using six different IgG anti-DNA antibodies isolated from six different SLE patients. All of these isolated antibodies showed equivalent anti-DNA activity (OD) by ELISA assay. In this series of experiments the direct method

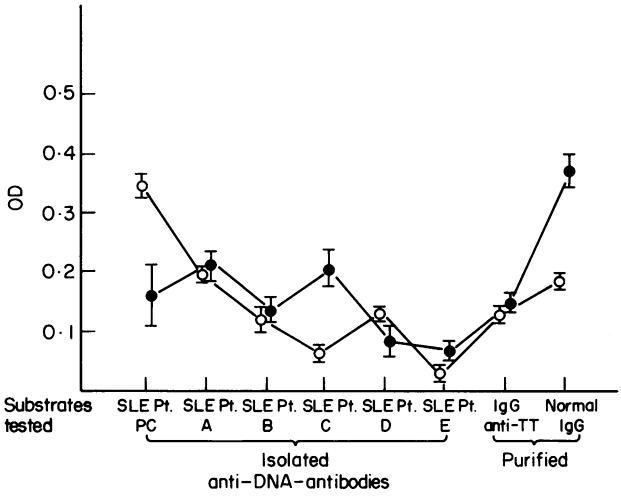

Fig. 3. Specificity of different affinity purified peroxidase conjugated anti-F(ab′)$_2$ antibodies against the same substrates. Results are expressed as OD, mean ±s.d. The reactivity of the SLE patient's brother was significantly greater against his sister (PC) affinity-isolated anti-DNA antibody than against anti-DNA from unrelated SLE patients ($P < 0{\cdot}001$). This specificity of the HRPO anti-F(ab′)$_2$ antibodies of the brother of SLE patient (PC) against the anti-DNA-antibody of the sister is highly significant when compared to a normal control (●) ($t = 6{\cdot}72$, $P < 0{\cdot}005$).

(HRPO–anti-F(ab′)$_2$ on different substrates) was used in order to avoid any hypothetical interactions which could have been involved in the DNA–anti-DNA inhibition tests. As shown in Fig. 3, the highest activity from the HRPO–anti-F(ab′)$_2$ antibodies of the SLE patient's brother was directed against his sister's (PC) isolated anti-DNA antibody substrate while the specificities against other substrates were considerably lower and not substantial ($P < 0{\cdot}001$ compared with all the other substrates). Control experiments performed by using HRPO–anti-F(ab′)$_2$ from a control family member with anti-DNA from SLE patient PC showed entirely different patterns of inhibition when compared to the anti-F(ab′)$_2$ activity of the patient's brother ($P < 0{\cdot}005$) (left portion of Fig. 3). The activities against other substrates with the exception of the reaction with SLE patient C, were quite similar to those of the brother's HRPO–anti-F(ab′)$_2$ antibodies. Also of great interest was the difference in specificity shown for the two different HRPO–anti-F(ab′)$_2$ antibodies using normal IgG as substrate. In converse fashion to results noted with isolated anti-DNA from SLE patient PC, the brother's anti-F(ab′)$_2$ showed low activity with normal IgG whereas normal control anti-F(ab′)$_2$ showed high levels of reaction. These comparative parallel experiments indicated clearcut differences in specificity of the anti-F(ab′)$_2$ preparations tested.

Gm and Km typing

All sera from both control and SLE family members were typed for Gm(a), (b), (g), (f), (z) and Km specificities. In no instance could any of the anti-F(ab′)$_2$ antibody reactions either in SLE patient's or family members' sera be directly related to anti-Gm(f) anti-Gm(z) or anti-Km specificities.

DISCUSSION

The results presented in the present study represent an extension of our previous report on the changes in serum anti-F(ab′)$_2$ antibody activity during the clinical course of disease in SLE (Silvestris *et al.*, 1983, 1984a). It was previously noted that levels of anti-F(ab′)$_2$ were often markedly decreased during active disease, whereas patients with quiescent or relatively inactive SLE most often showed elevated levels of serum anti-F(ab′)$_2$ antibodies. It would appear that anti-F(ab′)$_2$

antibodies in general may represent a pool of antibody molecules which serve to regulate a number of both normal and abnormal immune responses. Thus, low but detectable levels of anti-F(ab′)$_2$ are frequently seen in normal subjects (Heimer, Wolfe & Abruzzo, 1982; Nasu *et al.*, 1982). Moreover, when affinity-isolated anti-F(ab′)$_2$ antibodies from normal subjects, SLE relatives or SLE patients themselves are studied for their ability to inhibit control antigen–antibody reactions (such as tetanus toxoid reacting with affinity-purified anti-tetanus toxoid antibody) some baseline level of inhibition was always recorded. This is clearly apparent from results presented in Fig. 2. By contrast, if whole IgG such as that derived from pooled normal human immunoglobulins of Cohn Fr. II was tested at the same protein concentrations, then very little significant inhibition was recorded. The apparent relative specificity for DNA–anti-DNA systems documented in the studies reported here is not absolute since significant inhibition of control TT–anti-TT assays was also demonstrated. These findings probably relate to the fact that only a fraction of the anti-F(ab′)$_2$ antibodies in SLE or SLE relatives' sera bear clearcut relative anti-idiotypic specificity. Thus, the inhibition or direct binding results using anti-F(ab′)$_2$ antibodies originally isolated from affinity columns of F(ab′)$_2$ from whole pooled IgG offer a rough but imperfect marker for true anti-idiotypic activity. From the data presented in the present study, it would therefore appear that anti-F(ab′)$_2$ antibodies in general may be part of a broad spectrum of factors which are potentially capable of so-called anti-idiotypic regulation or control. That the same group of antibodies isolated from sera by adsorption to and elution from isolubilized F(ab′)$_2$ of pooled normal human IgG may also react in an inhibitory way with antigen–antibody systems not directly relevant to autoimmune disorders such as the tetanus toxoid–anti-tetanus toxoid system merely indicates that this general class of factors can indeed be considered to represent a pool of specificities. This is also true of the studies reported here concerning close family members of SLE patients.

The most interesting findings reported here have to do with the apparent familial distribution of high titre anti-F(ab′)$_2$ antibodies among many close SLE relatives. This was significantly more common among the SLE relatives than among members of the eight control families studied. Most important of all was the apparent relative specificities of isolated anti-F(ab′)$_2$ antibodies for DNA–anti-DNA antigen antibody systems of lupus patients within the individual SLE families. This unique specificity for significant intra-family reactivity is clearly demonstrated within such related SLE groups as shown in Figs 2 and 3 as well as in other similar intra-familial studies (data not shown). Particularly striking was the demonstration of intra-family specificity using both affinity-isolated anti-F(ab′)$_2$ antibodies as inhibitors in the proband SLE patient's DNA–anti-DNA antibody reaction in parallel with separate individual specificities using direct combining reactions with isolated anti-F(ab′)$_2$ linked to HRPO instead of by inhibition techniques (Fig. 3).

Because of the distribution of apparent anti-SLE patient reactivity of anti-F(ab′)$_2$ antibodies in SLE families, initially it seemed highly likely that we were dealing with some kind of anti-F(ab′)$_2$ specificity which might be related to genetically controlled antigenic F(ab′)$_2$ determinants. The only known genetic gamma globulin markers located on F(ab′)$_2$ fragments relate to the Gm(f), Gm(z), and Inv or Km systems. Accordingly, extensive Gm phenotype testing was performed not only on all 60 SLE family sera studied but also on control family members. In no single instance could the anti-F(ab′)$_2$ specificities described here be directly correlated with Gm or Km typing either within the family members showing strong anti-F(ab′)$_2$ reactivity or within the Gm, Km phenotype of the individual SLE probands. Despite these negative findings it seems possible that specificity against antigenic determinants similar to those genetically controlled Gm antigens already described in the conventional Gm(z) or Gm(f) test assays may be present but perhaps the proper markers or assay systems have yet to be worked out.

The results reported here raise the question of actual immunochemical specificity of the anti-F(ab′)$_2$ antibodies studied. Previous reports on anti-γ-globulins reacting with determinants on pepsin-digested IgG (Osterland, Harboe & Kunkel, 1963) indicated that such factors reacted wth relatively buried determinants uncovered by pepsin digestion. Later Harboe, Rau & Aho, (1965) observed that the anti-γ-globulin activity of these so-called pepsin agglutinators could be absorbed using human IgG antibody–antigen precipitates. Nasu *et al.* (1980) found that anti-F(ab′)$_2$ antibodies isolated by techniques similar to those we have utilized reacted with both F(ab′)$_2$ fragments of IgG as well as whole IgG. Our own studies of isolated anti-F(ab′)$_2$ antibodies from

SLE sera studied in the present report indicate very little overlap with pepsin agglutinators (Silvestris, Williams & Searles, 1984) since only three of ten isolated anti-F(ab')$_2$ antibodies produced agglutination of Rh positive erythrocytes coated with pepsin digested anti-Rh antibody Ripley. Moreover, additional studies using antibody fragments of affinity purified human IgG anti-tetanus antibody have indicated that anti-F(ab')$_2$ antibodies react with Fv determinants which retain active combining site activity (Silvestris, Williams & Searles, 1984).

The studies recorded here of significant familial distribution within SLE relatives of anti-F(ab')$_2$ antibody showing preferential reactivity for autoantibody reactions defined by DNA–anti-DNA test systems derived from the SLE proband within the same family thus represent additional useful information which can now be considered in parallel with all the other studies of immunological abnormalities within SLE families. Since the idiotypic-like specificity of anti-F(ab')$_2$ antibodies for the SLE antigen antibody system derived from the family lupus proband involved blocking reactions for DNA–anti-DNA, it is conceivable that high titres of anti-F(ab')$_2$ in these family members reflect exposure to the same stimulus or agent as was originally involved in producing SLE in the affected proband. In the case of these non-affected relatives the presence of high titre anti-F(ab')$_2$ may merely serve as a marker for disease agent exposure or even continued disease agent persistence. Further studies related to these latter points are currently in progress. The present study represents the first demonstration of differences in an immunoregulatory anti-idiotype network in unaffected family members of SLE patients. It seems possible that successful activation of the anti-idiotypic mechanism in these relatives may be under some sort of genetic control. The nature of this control if present and whether linked to DR, HLA or complement allotypes remains to be elucidated.

Supported in part by Grants AMAI 13690, CA 24873 and AMAI 13814 from the National Institutes of Health and in part by a Center Grant from the Arthritis Foundation. We are indebted to Dr S. Kanig for helping to collect patient samples and to Mrs Kay Adam for expert secretarial assistance.

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