

A study of anti-poly(ADP-ribose) antibodies and an anti-DNA antibody idio type and other immunological abnormalities in lupus family members

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SUMMARY The genetic background of systemic lupus erythematosus (SLE) has been re-examined in a study of the serum of 31 lupus patients and 80 asymptomatic first degree relatives by measuring a common, cross reacting anti-DNA antibody idio type designated 134, antibodies to poly(ADP-ribose), serum C3, circulating immune complexes, and antinuclear antibodies (ANA). Over 30% of the relatives had raised 134 and anti-poly(ADP-ribose) levels, and 9% had ANA titres > 1/20. In contrast, only one relative had a low serum C3 level. These results confirm that immunogenetic abnormalities associated with the production of autoantibodies and particular idiotypes must exist amongst lupus relatives as well as the patients. The production of autoantibodies, however, is not necessarily matched to the clinical expression of SLE.

Key words: SLE, SLE immunogenetics, circulating immune complexes.

The precise aetiology of systemic lupus erythematosus (SLE) is unknown, but the pathogenesis is clearly dependent upon a complex interaction of trigger factors and hormonal influences, set against a background of genetic predisposition.

It has been shown that the prevalence of SLE varies between ethnic groups. A Hawaiian study reported prevalence rates ranging from 5.8 per 100 000 among Caucasians to 24.1 per 100 000 among ethnic Chinese,¹ and other reports show lupus to be most common in black women.²

An individual's potential to develop lupus is dependent upon both inherited and environmental factors.³ Estes and Christian reported a positive family history in SLE in 4% of cases.⁴ The concord-

ance rate of SLE in monozygotic twins, however, is 65%.⁵

Inherited complement deficiency states are also associated with SLE, notably a deficiency of C2 and the terminal sequence (C5-C9).⁶ A recent study showed that over 80% of Caucasian lupus patients had silent or null alleles of C4A or C4B (and in one case C2) compared with only 40% of a matched normal group.⁷ HLA studies have most consistently shown associations with A1, B8, DR2, and DR3,⁸ and the frequency of the Gm allotype 1, 17; 5, 6, 13 was shown to be substantially raised in black American lupus patients.⁹

The sera of asymptomatic relatives of lupus patients have been shown to contain autoantibodies¹⁰ and raised circulating immune complex levels.¹¹ Further, hypergammaglobulinaemia,¹² decreased T suppressor cell function,¹³ and lymphocytotoxic antibodies¹⁴ have also been found in some studies.

Accepted for publication 9 December 1985.

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In a previous study we detected the presence of two common cross reacting anti-DNA antibody idiotypes, designated 16/6 and 32/15, in lupus patients and their relatives.¹⁵ Raised 16/6 and 32/15 serum idiotype levels were found in 24% and 7% respectively of the relatives. The presence of these idiotypes, which were originally identified on hybridoma derived human monoclonal anti-DNA antibodies from different patients, implies that they are the products of genes present throughout the population.

In this study we have re-examined the sera of a large number of lupus patients and their relatives, looking for a variety of immunological abnormalities in order to re-evaluate the importance of the genetic component in SLE. Specifically, we have looked at antibodies against poly(adenosine diphosphate-ribose), which are found in SLE patients as frequently, if not more frequently, than antibodies against double stranded DNA.^{16 17} Anti-poly(ADP-ribose) antibodies were first discovered in the sera of SLE patients by Kanai *et al.*¹⁸ and these antibody levels have been shown to correlate well with disease activity.¹⁹

We have also looked for the presence of another anti-DNA antibody, idiotype 134. This idiotype differs from the idiotypes previously examined in that the antibody on which it was first identified binds strongly to double stranded DNA (dsDNA) in an enzyme linked immunosorbent assay (ELISA).¹⁹ In contrast, 16/6 and 32/15 bind to single stranded DNA (ssDNA) but show little binding to dsDNA.¹⁹

We have also examined the sera for circulating immune complex levels, serum C3 levels, and the presence of antinuclear antibodies by immunofluorescence on a HEp₂ cell line.

Patients and methods

PATIENTS

Thirty one patients with SLE from 30 different families were studied. Twenty nine of these patients were female, and all patients, male and female, fulfilled four or more of the American Rheumatism Association's revised criteria for the classification of SLE.²⁰ Sera from 80 of their healthy first degree relatives, 49 female and 31 male, were also included in the study. The age range of the relatives was 12–80 years (mean \pm SD 45 \pm 19). (The age was not recorded in a few cases). The 40 clinically healthy controls (28 female and 12 male) were healthy volunteers, blood bank donors, or people with soft tissue injuries only, who attended a rheumatology clinic. The age range of the controls was 23–87 years (mean age \pm SD 43.8 \pm 19). These controls were used to establish a normal range in the anti-poly (ADP-

ribose) antibody assay and the 134 antibody assay. Over 100 healthy individuals were used to establish the range of C3 and immune complexes.

ANTI-POLY(ADP-RIBOSE) ANTIBODY ELISA

A 96 well polystyrene ELISA plate (Immulon 2, Dynatech) was precoated with 50 μ l of poly-L-lysine (Sigma) (50 μ g/ml in distilled water) and incubated for one hour at 37°C. Each well was then washed three times with phosphate buffered saline (PBS) (Flow) before the addition of 50 μ l of poly(ADP-ribose) (10 μ g/ml) in PBS. The poly(ADP-ribose) was prepared as previously described.²¹ After incubation overnight at 4°C the plates were washed three times with PBS. They were blocked with 100 μ l of 2% casein (BDH) in PBS to prevent non-specific binding, and after one hour's incubation at 37°C were washed three times with PBS-0.1% Tween (PBS-T) (Sigma). Test serum samples were diluted 1/50 in PBS-T and 50 μ l aliquots were added to the wells in duplicate. A known high positive serum in quadruplicate and sera from eight healthy individuals were included on each plate.

After incubation for two hours at 4°C the plates were washed three times with PBS-T, and 50 μ l of alkaline phosphatase, conjugated goat antihuman polyvalent immunoglobulin (Sigma) diluted 1/350 in PBS-T/0.5% casein was then added.

Incubation was carried out at 37°C for one hour, and after washing six times with PBS-T, 50 μ l of substrate, *p*-nitrophenyl phosphate disodium in 50 mM carbonate buffer, pH 9.5, containing 2 mM Mg²⁺, was added to the plate.

The reaction was allowed to proceed for one hour at 37°C, and the resulting colour change was recorded at an absorbance of 410 nm with a Dynatech MR600 ELISA reader. The values obtained were expressed as a percentage of the known high positive value.

134 ANTIBODY IDIOTYPE ELISA

The human monoclonal anti-DNA antibody 134 was produced as described elsewhere.²² Idiotype 134 bound to both dsDNA and ssDNA, though more strongly to the former. The polyclonal, rabbit anti-134 was derived by a previously described method.²³

The test serum samples were diluted 1/1000 in borate buffer, and 100 μ l aliquots were incubated in duplicate in the wells of a 96 well polystyrene ELISA plate (Immulon 2, Dynatech) overnight at 4°C. A known high positive serum (in quadruplicate) and sera from eight normal healthy controls were included on each plate.

The plates were washed three times in PBS (Flow) containing 1% Tween (Sigma) and three times in PBS, and after a two hour incubation at room temperature with 100 μ l of rabbit anti-134

idiotype at a dilution of 1/10 000 they were washed again (as above) and incubated overnight at room temperature with 100 μ l of alkaline phosphatase, conjugated goat antirabbit immunoglobulin (Sigma) diluted 1/350 in PBS-T. After the same washing procedure 100 μ l of substrate, *p*-nitrophenyl phosphate disodium in 50 mM carbonate buffer, pH 9.5, containing 2 mM Mg²⁺, was added to the plate.

The reaction was allowed to proceed for one hour at 37°C, and the resultant colour change was recorded at an absorbance of 410 nm with a Dynatech MR600 ELISA reader. The values obtained were expressed as a percentage of the known high positive value.

DNA ANTIBODY MEASUREMENT BY ELISA

The majority of the sera with raised 134 idiotype levels were also tested by an ELISA method for DNA binding by a modification of a previously described method.¹⁵ In brief: 96 well polystyrene plates (Immulon, Dynatech) were precoated with 50 μ l of poly-L-lysine (Sigma) and incubated for one hour at 37°C. After washing with phosphate buffered saline the plates were coated with dsDNA 10 μ g/ml or ssDNA 5 μ g/ml. The dsDNA was prepared by treating calf thymus DNA (Sigma) with S1 nuclease (Sigma) for 30 minutes at 37°C. The ssDNA was prepared by boiling calf thymus DNA for 10 minutes and cooling on ice for 15 minutes. The plates were treated with 2% casein (BDH) in PBS to prevent non-specific binding, and after washing 50 μ l test serum samples diluted 1/200 in PBS-T were added in duplicate. A known high positive serum and sera from eight healthy individuals were included on each plate. The ELISA assay then proceeded as described previously,¹⁵ and the values obtained were expressed as a percentage of the known high positive value. The upper limits of normal on the 40 healthy controls were set at IgM ssDNA antibodies 10%; IgG ssDNA antibodies 20%; IgM dsDNA antibodies 7.5%; IgG dsDNA antibodies 12%. Each figure is greater than the mean plus three standard deviations.

Serum C3 was measured by radial immunodiffusion as described elsewhere.²⁴ Circulating immune complexes were measured by polyethylene glycol precipitation using the method of Creighton *et al*.²⁵ as modified by Poulton and colleagues.²⁶ The presence of antinuclear antibodies (ANA) was tested with serial dilutions of serum on a human epithelial cell line (HEP₂). Fluorescence at a titre > 1/20 was considered to be positive. The five highest 134 idiotype binding sera in the patient and relative groups were tested for the presence of IgG, IgA, and IgM rheumatoid factor by a standard solid phase radioimmunoassay.

Results

Serum levels of anti-poly(ADP-ribose) antibodies were expressed as a percentage of the value for a known high positive individual, and the values in the 40 normal persons gave a mean of 16.5 (SD 7.4). The upper limit of normal was set at 32% (> mean +2SD). Only one of the controls (2.5%) exceeded this. In contrast, 18 of the lupus patients (58%) and 35 (44%) of the relatives were found to have raised levels of anti-poly(ADP-ribose) antibodies (see Fig. 1). When the median group values were compared there were significant differences between both patient and relative groups and the normal population ($p < 0.0001$, Mann-Whitney U test). The difference between patient and relative groups did not reach significance.

Serum levels of the 134 idiotype in the 40 healthy controls gave a mean percentage value (compared with the value for the known high positive individual) of 10.5% \pm 10%. Fourteen of the 31 patients (45%) and 24 of the 80 relatives (30%) had 134 idiotype levels in excess of the upper limit of normal also set at 32% (> mean +2SD). Only one of the controls exceeded this value (Fig. 2) A comparison of the median group values of the three groups again

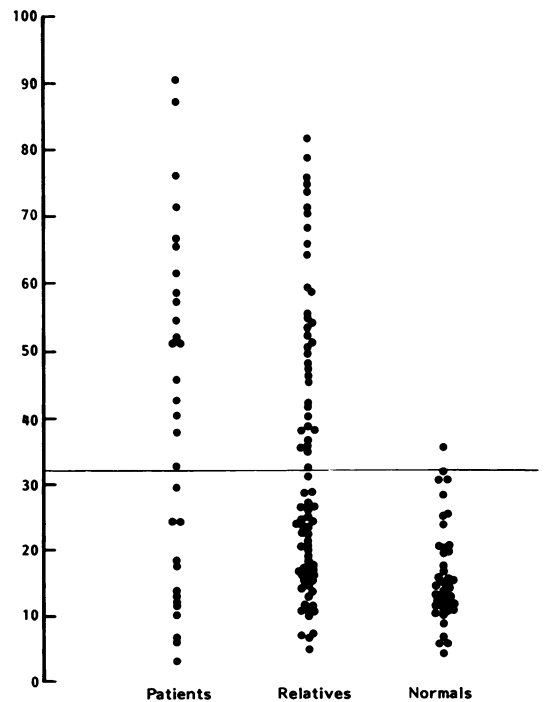


Fig. 1 Serum poly(ADP-ribose) levels in patients, relatives, and normals.

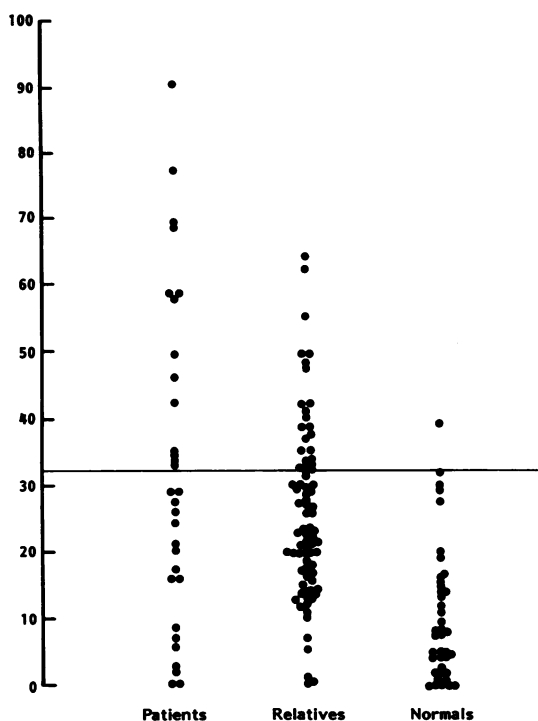


Fig. 2 Serum 134 idiotype levels in patients, relatives, and normals.

showed a statistically significant difference between both patient and relative groups and the normals ($p < 0.0001$, Mann-Whitney U test), but no difference between the patient and relative groups. The relatives with high 134 idiotype levels were confined to 10 families. In six of these families the index case also had a raised 134 idiotype level. One family was notable as the index case and five relatives had high levels. In two other families the index case and three relatives had raised 134 idiotype levels.

Of the 11 patients and 20 relatives with high 134 idiotype levels who were tested for anti-DNA antibodies, nine of the patients, but only five of the relatives, had raised levels of IgG/IgM antibodies to ss/dsDNA. The difference was statistically significant ($p < 0.01$, χ^2 analysis).

Circulating immune complex levels were raised in 13 (46%) of the lupus patients but in only seven (10%) of the relatives (see Fig. 3). Only two lupus patients (both with very active disease) and one relative had low serum C3 levels. A positive ANA test with a titre $> 1/20$ was found in 75% of the patients and 9% of the relatives. When the sera in the patient and relative groups with the highest

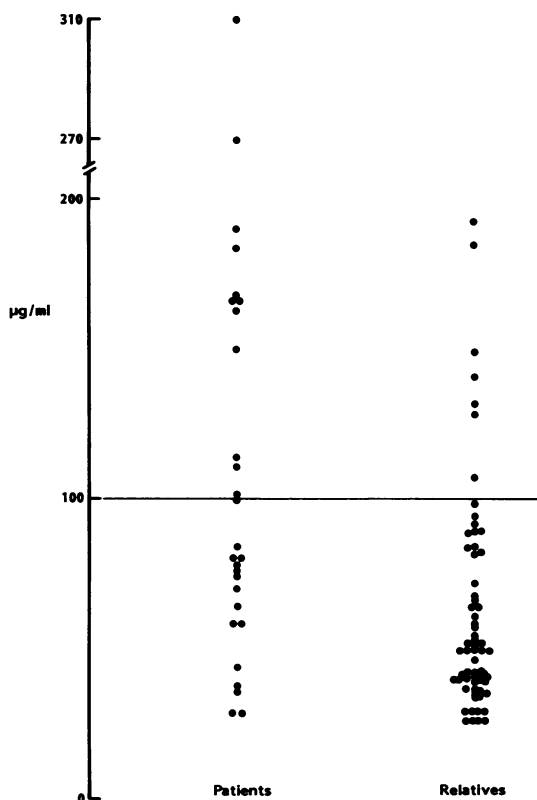


Fig. 3 Serum circulating immune complex levels in patients and relatives.

idiotype levels were tested for rheumatoid factor only one patient was found to have a moderately raised IgA level (data not shown).

Discussion

This study confirms that family members of lupus patients may express autoantibodies and idiotypes identified on autoantibodies without having the disease.

Previous reports have shown that anti-poly(ADP-ribose) antibodies show relative disease specificity and are rarely found in normal individuals.^{16 17} These studies used radioimmunoassays. In this study, using an ELISA, we have confirmed that healthy individuals do not express anti-poly(ADP-ribose) antibodies, though they are found in many SLE patients. More than 40% of healthy family members, however, also had raised levels.

In our previous report we examined idiotypes first identified on hybridoma derived monoclonal anti-ssDNA antibodies and showed that these idiotypes

(16/6, 32/15) were frequently found on antibodies in the serum of SLE patients and, less often, in their asymptomatic relatives.¹⁵ These original results are extended by the present report which describes the idiotype 134. The 134 idiotype was originally identified with an ELISA on a hybridoma derived monoclonal antibody binding strongly to dsDNA, though a subsequent report of a radioimmunoassay showed strong binding to ssDNA.²⁷ We have found the 134 idiotype in 45% of SLE patients and 30% of their asymptomatic relatives. These results are in concordance with a recent report which showed that the anti-DNA idiotype 3I, found in 7/8 SLE patients, was also found in 15/19 relatives.²⁸ (These values are slightly higher than the values for our idiotype.)

The presence of serum autoantibodies with the 134 idiotype in healthy relatives implies that it is not simply a pathogenic marker of SLE. The idiotype is clearly not confined to anti-dsDNA antibodies, but it is well recognised that idiotypes may be shared by antibody molecules with different antigen binding properties.²⁹⁻³⁰ Precisely which antibodies bear the 134 idiotype remains uncertain, though our results would indicate that they are not on rheumatoid factors. This idiotype, however, does mark a group of individuals, perhaps those in whom the idiotype is found on dsDNA antibodies more likely to have the disease, as shown by its presence in 45% of SLE patients. The expression of common idiotypes in SLE patients and their healthy family relatives may imply not so much a propensity to develop the disease as the inheritance of germ line immunoglobulin genes encoding for these relatively public idiotypes.³¹ An alternative explanation is that the presence of the idiotype represents a dominant immunoglobulin gene rearrangement both in the patients and their relatives. Clonal analysis of SLE B lymphocytes for dominant immunoglobulin gene rearrangements at the DNA level could be used to confirm this.³²

It would be interesting to look at the 134 idiotype and anti-poly(ADP-ribose) antibody levels in patients suffering from inflammatory diseases, in which anti-DNA or anti-poly(ADP-ribose) antibodies are present due to an infection such as malaria or leprosy, and compare their idiotype and anti-poly(ADP-ribose) antibody levels with those of their uninfected first degree relatives. If both relatives and patients were 134 positive then the hypothesis of restricted V regions in SLE might not be accurate, but if the relatives were 134 negative (and the patients 134 positive) this might suggest that the expression of idiotype V genes is related not just to the germ line but to some other factor peculiar to the stimulus for anti-DNA antibodies.

Additional experiments, such as assaying the pokeweed mitogen stimulated peripheral blood mononuclear cell supernatants in seronegative relatives or normals, would give some clue as to the origin of this idiotype.

Approaches similar to those employed in tracing the idiotype through the family could be used to trace the development of anti-poly(ADP-ribose) antibodies. This might give some clue as to the stimulus for their production. A vertical inheritance through one parent to one of many children, for example, would provide evidence against an infectious aetiology.

In this study very few patients or relatives had low C3 levels. This may reflect the fact that many of the patients, who were attending outpatient clinics, had restricted disease activity. In addition, the bias in our departments is towards patients with rheumatological or haematological disease, or both, rather than towards those with renal involvement, which is more commonly associated with low C3 levels.

Forty six per cent of the SLE patients had raised circulating immune complex (CIC) levels, but only 10% of relatives had values above the normal limit. This is in contrast with the work of Elkon *et al*, who showed that 26% of relatives had raised CIC levels.¹¹ This may reflect the different CIC assays used.

Antinuclear antibodies (ANA) as previously reviewed by Walport *et al*⁸ have been found to occur in anything from 4 to 60% of lupus relatives. Our findings show that only 9% of relatives had antinuclear antibodies, which is at the lower end of the range and a little lower than the 17% we noted in our previous study.¹⁵ One puzzling result was the low percentage of ANA positive relatives who had anti-poly(ADP-ribose) antibodies, since the nucleus of epithelial cells (HEp₂) does contain poly(ADP-ribose).³³ The explanation may relate to the sensitivities of the test systems used.

The above observations taken together with the other reports cited confirm that healthy family members of SLE patients often have serological abnormalities suggestive of the disease, but without any clinical evidence of involvement. This re-emphasises that although the genetic component of lupus is obviously important, it is not the only arbiter of disease expression.

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