Correlation of 9G4 idiotope with disease activity in patients with systemic lupus erythematosus

D A Isenberg, C McClure, V Farewell, M Spellerberg, W Williams, G Cambridge, F Stevenson

Abstract

Objective—To compare the levels of the 9G4 idiotope (9G4 Id) in systemic lupus erythematosus (SLE) patients with a detailed disease activity index, the British Isles Lupus Assessment Group (BILAG) index, and serological parameters of disease activity by ds DNA antibody levels and serum C3 concentrations.

Methods—In a cross sectional analysis serum samples from 190 patients with SLE were studied and a further 55 serial bleeds from 14 patients. An enzyme linked immunosorbent assay was used to measure the 9G4 Id, and anti dsDNA and anti-myeloperoxidase (MPO) antibodies. The C3 levels were measured by laser nephelometer.

Results—Seventy six of 190 (40%) of the patients tested had raised 9G4 Id levels. In the cross sectional study 9G4 Id levels were found to correlate with disease activity in the BILAG cardiovascular/respiratory renal, and haematological systems and with global BILAG score (p<0.01). In the serial bleeds 9G4 Id levels correlated with anti-dsDNA antibody and C3 levels, but not with anti-MPO antibodies. No correlations were found with treatment. In six cases the 9G4 Id levels correlated well with global BILAG scores and dsDNA antibody levels. In four cases the BILAG global and 9G4 Id levels alone correlated well.

Conclusions—Raised levels of the 9G4 Id are present in a substantial proportion of serum samples from patients with lupus, correlate with various aspects of disease activity in SLE. The Id is detectable on anti-dsDNA antibodies, though it must also be present on other immunoglobulins whose specificities remain unknown.


Antibodies are usually defined by the antigens to which they bind. Another way of distinguishing antibodies serologically involves an analysis of their idiotypes that may be thought of as phenotypic markers of the variable region genes used to encode the antigen binding (Fab) region of immunoglobulin molecules. These regions encode tertiary structures termed idiotopes. A collection of these idiotopes may together be called an idiotype. Idiotypes may represent amino acid sequences located on light or heavy chains alone or in combination. The sharing of idiotypes by immunoglobulins from different people implies that the genes that encode the idiotypes are also shared.

In the past 12 years a number of idiotypes, recognised by polyclonal reagents and idiotopes, recognised by monoclonal antibodies, on DNA antibodies have been identified (reviewed by Isenberg et al1). Among the 25 or so idiotypes or idiotopes, few can be regarded as specific for systemic lupus erythematosus (SLE) and a limited number have been correlated with disease activity. Among those that have shown correlation with disease activity and are present on immunoglobulins deposited in the renal lesions of tissue from patients are the 16/6,2 GN23 idiotypes and the 9G4 idiotope (Id).4

In our original report demonstrating the presence of the 9G4 Id in 45% of serum samples from patients with SLE,7 we showed that levels of this idiotope fluctuated with disease activity in some patients and the Id was detected in the kidney biopsy specimens of three of 11 patients. However, the global disease activity index (UCH/Middlesex) used in that study was not ideal, never having been tested for validity or reliability.

In this study we have extended our earlier findings. By utilising a disease activity index shown to be validated and reproducible, we have attempted to determine whether 9G4 idiotope (Id) levels reflect that disease activity in a particular organ or system. We have also sought to determine whether the 9G4 Id in the serum samples of lupus patients is present on other antibodies with which it has been associated in other conditions such as anti-myeloperoxidase (MPO) antibodies5 and cold agglutinins,6 and have undertaken further absorption experiments.

Methods

PATIENTS

Serum samples were drawn from 190 patients with SLE who met the revised criteria for the classification of the disease proposed by the American College of Rheumatologists.7 We
Table 1 Logistic regression analysis of 9G4 idiotype level (+/−) with disease variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR</th>
<th>p Value</th>
<th>Multivariate OR</th>
<th>p Value</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>1.20</td>
<td>0.543</td>
<td>1.01</td>
<td>0.987</td>
<td>(0.53, 1.91)</td>
</tr>
<tr>
<td>C3</td>
<td>1.65</td>
<td>0.155</td>
<td>1.46</td>
<td>0.313</td>
<td>(0.70, 3.05)</td>
</tr>
<tr>
<td>Total BILAG score</td>
<td>1.13</td>
<td>0.0004</td>
<td>1.12</td>
<td>0.012</td>
<td>(1.03, 1.22)</td>
</tr>
</tbody>
</table>

OR: odds ratio, CI: 95% confidence intervals.

Table 2 Logistic regression analysis of 9G4 idiotype level with disease activity in different organs/systems represented as BILAG scores

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>p Value</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>0.71</td>
<td>0.609</td>
<td>(0.19, 2.64)</td>
</tr>
<tr>
<td>CNS</td>
<td>4.07</td>
<td>0.115</td>
<td>(0.73, 3.04)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>1.63</td>
<td>0.235</td>
<td>(1.08, 10.29)</td>
</tr>
<tr>
<td>CVS/Respiratory</td>
<td>3.33</td>
<td>0.037</td>
<td>(1.01, 3.79)</td>
</tr>
<tr>
<td>Renal</td>
<td>2.42</td>
<td>0.047</td>
<td>(1.04, 5.07)</td>
</tr>
<tr>
<td>Haematological</td>
<td>2.16</td>
<td>0.0004</td>
<td>(1.03, 1.22)</td>
</tr>
</tbody>
</table>

OR: odds ratio, CI: 95% confidence intervals.

assessed at least one sample from each of the 190 patients as part of a cross sectional survey.

In addition, three or four serum samples (invariably at time interval two to three months apart) from each of 14 patients with SLE were studied to monitor changes in disease activity. In all 55 serial bleeds from these patients were available. This second, smaller group of patients was selected principally because they had had periods of distinct relapse and remission and their serum samples, with major fluctuations in DNA antibody levels, were available.

The disease activity of these patients was assessed using the British Isles Lupus Assessment Group’s (BILAG) disease activity index. This is based upon the physician’s intention to treat principle and divides lupus activity into eight different organs or systems. As described in detail elsewhere, disease activity in each of these organs or systems is divided into an A–E category, in which A represents the most active form of disease and E implies the system has never been active. The system can be converted into a global score whereby A = 9 points, B = 3, C = 1 and D and E = 0. Thus in total a maximum of 72 points could be scored. In this study we have regarded 0–5 points as being relatively inactive, and 6 or more as active. While this level is an arbitrary cut off point, it has been adopted in several studies by the BILAG group. In practice the clinical features of the patients with SLE were recorded in clinic (by several members of the Bloomsbury Rheumatology Unit) and the data entered into an Apple Macintosh computer. A software program written for the BILAG group then works out the score (the few laboratory tests are added later to give the renal and haematological scores). As a precaution all of the BILAG scores were reviewed by one of us (DAI) to ensure that no major omissions or errors had occurred.

Treatment with prednisolone and azathioprine at the time of the bleed was recorded, as were the DNA binding (undertaken by a commercial ELISA kit, upper limit of normal = 100 units, Shield Diagnostics, Dundee) and serum C3 (performed by laser nephelometer in the routine biochemistry laboratory, normal range = 0.75–1.75 g/l).

ANTI-MYELOPEROXIDASE ANTIBODY LEVELS/COLD AGGLUTININS
A random selection of 117 serum samples from the 190 patients with SLE were tested for anti-myeloperoxidase (MPO) antibodies and expression of the 9G4 Id on these autoantibodies as described elsewhere. From a review of the patients’ notes those patients whose samples had been tested for cold agglutinins (by routine laboratory methods) were recorded.

9G4 IDIOTYPE ESTIMATION
As described in detail elsewhere the 9G4 idiotype (9G4 Id) in serum was measured by competitive inhibition ELISA, which utilises a monoclonal rat anti-9G4 idiotype. Briefly, an Id positive IgM was bound to the plate and then incubated with the rat anti-Id, 9G4, which was then detected by HRP-rabbit anti-rat IgG. Serum containing the putative competing Id was added to the rat anti-Id before placing it in the wells, and the reduction in binding of the rat IgG was then measured. A known IgM Id was used to establish a standard inhibition curve and the degree of inhibition was read off this. The ELISA does not distinguish between the Ig classes and has been found to detect both IgM and IgG. For analysis of serum samples, levels are reported as the % inhibition obtained in the ELISA at a serum dilution of 1:30 000. The inter assay variation is < 10%.

Serum samples were not assayed on the same day, but in batches as received. All samples were tested in duplicate. The inclusion of standards ensure reproducibility as indicated above.

Additional samples from 12 patients who had been 9G4 Id+ were selected for further study. In the event five were found to have raised levels and were used to determine whether the 9G4 Id+ immunoglobulins were anti-DNA antibodies.

ANTI-DNA ABS ENCODED BY THE V4–34 HEAVY CHAIN GENE
Anti-ss or dsDNA antibody activities were measured by direct binding ELISAs. The wells were coated with ssDNA or dsDNA at 10 µg/ml in dilutions of serum added: after washing, the MoAb 9G4 coupled to biotin was added and incubated for one hour. This was detected with streptavidin-HRP. A standard curve was established using an Id positive anti-DNA antibody.

STATISTICS
A logistic regression model was used to investigate the relation between the 9G4 idiotype level and the markers reflecting disease activity (global and individual BILAG scores), the DNA binding levels (raised versus normal), and the serum C3 level (low versus normal). As the distribution for the 9G4 idiotype is skewed with a significant mass near zero, a simple binary classification was made, with percentage inhibition values greater than 22.5% regarded...
The total BILAG score was investigated in two ways: firstly, treating it as a continuous variable, and secondly, treating scores greater than 5 as being positive, less than 6 as negative.

A fairly strong relation was found between the 9G4 Id level and the total BILAG score. However, neither DNA nor C3 levels were found to have a significant relation with the 9G4 Id level. Treating the total BILAG scores as being positive (>5) or not, provided a model with comparable fit to the data and an associated odds ratio estimate of 2.17, with a p value of 0.01 and confidence intervals of 1.21, 3.89.

Having established a relation between the total BILAG score and the 9G4 id type level, an exploratory analysis was undertaken to establish the components of the BILAG that were associated. Table 2 shows the multivariate logistic regression of the 9G4 id type level with 7 of the component BILAG scores (regarded as either positive or negative). No patients were positive for vasculitis so this variable was not included in the analysis. The results suggest that the relation between the 9G4 id type level and the total BILAG score found in table 1 is primarily associated with a relation between 9G4 Id level and disease activity in the CVS/respiratory, renal, and haematological systems.

**ANTI-MYELOPEROXIDASE ANTIBODIES/COLD AGGLUTININS**

Of the 117 lupus serum samples tested, 27 had significant binding to MPO in ELISA. (that is, ...
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immunoglobulins. However, a more recent

framework region (FWR) of V4–34 encoded

colleagues located it to positions 23–25 on

has been ascertained. Thus Potter and

one of the relatively few whose structural basis

activity in patients with SLE.

analysis of our original report and focuses on

This study represents a greatly expanded

other immunoglobulins must carry the 9G4 Id.

The implication of this is that in patient 59

9G4 Id+ anti-DNA value of 0.400 in ELISA

had the highest total 9G4 Id level (90%) had a

not however absolute in that patient 59 who

whose total 9G4 Id level was 30% but whose

5G4 Id+ anti-DNA levels. The correlation was

anti-dsDNA and both renal and cardiovascular/respiratory disease. We do not

but not however find a correlation between the 9G4 Id

level with DNA antibodies or C3 in the cross

sectional study, though a close correlation is evi-

dent in some patients in the serial bleeds tested.

The 9G4 Id had been identified on a variety

of autoantibodies including cold agglutinins,

anti-myeloperoxidase antibodies, and anti-

DNA antibodies. In a previous study of serum samples from six patients with lupus, the 9G4 idiotope was detected on 2–19% of serum anti-ssDNA antibodies and 6–17% of dsDNA antibodies. In this study we can confirm the presence of this idiotope on DNA antibodies in the serum samples of some lupus patients. In contrast, we were unable to identify the 9G4 Id on anti-MPO antibodies in serum samples from SLE patients containing these autoanti-

bodies. The expression of this idiotope on anti-

MPO antibodies in serum samples from vascu-

litis patients suggests that different origins may exist for these autoantibodies in SLE and vasculitis. Furthermore, in accordance with an earlier study in bleeds from over 50 patients, we were unable to identify any cold agglutinins, again suggesting that the idiotope is not present on immunoglobulins with this reactivity in patients with lupus. It is evident, however, from the absorption studies that the idiotope is likely to be present on antibodies with specifici-

cies other than DNA. At present these remain to be determined.

Table 3

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total 9G4 (% inhibition)</th>
<th>9G4+ve anti-DNA (E495 at 1/20 dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>40</td>
<td>0.555</td>
</tr>
<tr>
<td>57</td>
<td>30</td>
<td>1.71</td>
</tr>
<tr>
<td>124</td>
<td>24</td>
<td>0.670</td>
</tr>
<tr>
<td>116</td>
<td>27</td>
<td>0.345</td>
</tr>
<tr>
<td>133</td>
<td>22</td>
<td>0.133</td>
</tr>
<tr>
<td>59</td>
<td>90</td>
<td>0.400</td>
</tr>
<tr>
<td>5</td>
<td>&lt;20</td>
<td>ND</td>
</tr>
<tr>
<td>83</td>
<td>22</td>
<td>ND</td>
</tr>
<tr>
<td>90</td>
<td>&lt;20</td>
<td>ND</td>
</tr>
<tr>
<td>155</td>
<td>&lt;20</td>
<td>ND</td>
</tr>
<tr>
<td>67</td>
<td>&lt;20</td>
<td>ND</td>
</tr>
<tr>
<td>154</td>
<td>&lt;20</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not detected.

values of >25% of appropriate positive controls for IgM and/or IgG anti-MPO antibodies). Most samples (22 of 27) contained IgG class anti-MPO antibodies only with two samples containing IgM class only and three both IgM and IgG anti-MPO antibodies. None of the anti-MPO antibodies expressed the 9G4 Id by ELISA (data not shown) and there was no correlation between 9G4 Id levels in serum samples and the presence of anti-MPO antibodies. Fifty two patients had had their serum tested for cold agglutinins but none were positive.

SERIAL BLEED ASSESSMENT

A total of 55 assessments was made on 14 patients. Figure 1 shows the individual results for each patient, comparing global BILAG score, DNA antibody level and 9G4 Id.

Analysis of the individual bleeds show very striking correlation of 9G4 Id levels, DNA antibody levels, and global BILAG score for patients p1, p4, p5, p12, p13, and p14. The 9G4 Id and global BILAG score (but not the DNA antibody levels) appear to correlate well in patients p2, p6, p9, and p11.

Serum samples from 12 patients previously recorded as being positive for the 9G4 Id were selected for further study to assess whether this idiotope was present on anti-DNA antibodies in the patients’ serum. Five of these serum samples had raised 9G4 levels (see table 3) ranging from 27–90%. Generally, as is evident from the table, those patients with raised total 9G4 Id levels were also those who had raised 9G4 Id anti-DNA levels. The correlation was not however absolute in that patient 59 who had the highest total 9G4 Id level (90%) had a 9G4 Id anti-DNA value of 0.400 in ELISA OD units, which contrasts with patient 57 whose total 9G4 Id level was 30% but whose 9G4 Id anti-DNA level was 1.71 OD units. The implication of this is that in patient 59 other immunoglobulins must carry the 9G4 Id.

Discussion

This study represents a greatly expanded analysis of our original report and focuses on the relation between the 9G4 Id and disease activity in patients with SLE.

This idiotope is of particular interest as it is one of the relatively few whose structural basis has been ascertained. Busch Potter and colleagues located it to positions 23–25 on framework region (FWR) 1 of V4–34 encoded immunoglobulins. However, a more recent analysis has indicated that an additional element is required, namely a tryptophan at position 7 in FWR1.

The original UCH/Middlesex global disease activity score, while relatively easy to use, was never adequately validated or shown to be reliable. In contrast the BILAG disease activity score has been shown to be both valid and reliable, and offers the additional advantage of easy comparison of antibody or idiotope levels with disease activity in the numerous organs or systems that lupus can affect. In the cross sectional study that is approximately three times the size of our original study, the percentage of patients found to be 9G4 Id positive at 40% is similar to the 45% found to be positive in our original study. Interestingly the idiotope level was shown to correlate with disease activity in the cardiovascular respiratory system, the haemato-
logical system, and the renal system. In a previous serial study of black female lupus patients we reported a strong correlation be-
tween anti-dsDNA and both renal and cardiovascular/respiratory disease. We do not find however a correlation between the 9G4 Id level with DNA antibodies or C3 in the cross sectional study, though a close correlation is evi-
dent in some patients in the serial bleeds tested.

2 Isenberg DA, Collins C. Detection of cross reactive anti-DNA antibody idiotypes on renal tissue bound immu-


4 Isenberg DA, Spellerberg M, Williams W, Gaffins M, Ste-

5 Longhurst C, Ehrenstein M, Leaker B, Stevenson F, Speller-


