CONCISE REPORT

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. (Ann Rheum Dis 1998;57:566–570)

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 al7). 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 GN2 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, 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/Middlessex) 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) antibodies and cold agglutinins, 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.2 We
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 category, B is the next most active, and then the others are inactive. The BILAG score is computed after the clinical features of the patients with SLE were recorded in clinic and the laboratory tests were recorded. This second, smaller group of patients 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.

### Table 1 Logistic regression analysis of 9G4 idiotype level (+/−) with disease variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unitvariate OR</th>
<th>p Value</th>
<th>CI</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></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></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>0.004</td>
<td></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, 3.64)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>1.63</td>
<td>0.235</td>
<td>(0.73, 3.64)</td>
</tr>
<tr>
<td>CVS/Respiratory</td>
<td>3.33</td>
<td>0.037</td>
<td>(1.08, 10.29)</td>
</tr>
<tr>
<td>Renal</td>
<td>2.42</td>
<td>0.047</td>
<td>(1.01, 5.07)</td>
</tr>
<tr>
<td>Haematological</td>
<td>2.30</td>
<td>0.040</td>
<td>(1.04, 5.07)</td>
</tr>
</tbody>
</table>

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

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 utilise 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%.

### Table 3 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...
as positive and values less than this regarded as negative. 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.

Results
CROSS SECTIONAL SURVEY
At least one blood sample was available for all of the 190 patients included in this part of the study. Among the patients 96% had a history of general features involvement; 78% mucocutaneous; 96% musculoskeletal; 51% cardiorespiratory; 24% renal; 25% CNS; 39% vasculitis, and 85% haematological. Seventy five patients (39.8%) were found to have a 9G4 Id level of at least 22.5% (which is greater than three standard deviations above the mean level of controls as reported elsewhere) and were thus regarded as 9G4 Id positive. Complete BILAG data were available on 170 of these patients and the logistic regression analysis shown in table 1 is based on these patients. Similarly, serum C3 and DNA antibody levels were available from bleeds taken on the same day on 171 patients. Both univariate and multivariate results are shown.

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 idiotype 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 idiotype 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 idiotype 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,
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. Thus 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 haematological system, and the renal system. In a previous serial study of black female lupus patients we reported a strong correlation between anti-dsDNA and both renal and cardiovascular/respiratory disease. We do 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 evident 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 autoantibodies. The expression of this idiotope on anti-MPO antibodies in serum samples from vasculitis 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.

Table 3

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total 9G4 (% inhibition)</th>
<th>9G4+ve anti-DNA (E&lt;sub&gt;60&lt;/sub&gt; 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.

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.

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 autoantibodies. The expression of this idiotope on anti-MPO antibodies in serum samples from vasculitis 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 specificities other than DNA. At present these remain to be determined.


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 and F Stevenson

Ann Rheum Dis 1998 57: 566-570
doi: 10.1136/ard.57.9.566

Updated information and services can be found at:
http://ard.bmj.com/content/57/9/566

These include:

References
This article cites 14 articles, 4 of which you can access for free at:
http://ard.bmj.com/content/57/9/566#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Connective tissue disease (4253)
Immunology (including allergy) (5144)
Systemic lupus erythematosus (571)
Epidemiology (1376)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/