Serum lymphocytotoxic antibodies and neurocognitive function in systemic lupus erythematosus

A A Long, S D Denburg, R M Carbotte, D P Singal, J A Denburg

Abstract
The hypothesis that lymphocytotoxic antibodies are associated with neuropsychiatric involvement in systemic lupus erythematosus (NP-SLE) is re-evaluated in this study. In an unselected cohort of 98 women with SLE a cross-sectional study has been performed to analyse associations among standardised clinical, neurological, and neuropsychological assessments and lymphocytotoxic antibodies measured by microcytotoxicity assay. Fifty patients showed objective clinical evidence of continuing or past NP-SLE and 54 patients had cognitive impairment. In accordance with previous observations 44% (24/54) of the cognitively impaired group did not have clinically detectable evidence of NP-SLE. Although lymphocytotoxic antibodies were found to be only marginally more prevalent in those patients with a clinical diagnosis of NP-SLE than in those without (32% + 23%), these antibodies were significantly associated with cognitive impairment ($\chi^2 = 5.42; p < 0.02$). No association was detected between lymphocytotoxic antibodies and either overall systemic disease activity or other organ system involvement, suggesting that the association between lymphocytotoxic antibodies and cognitive dysfunction in SLE is specific.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterised by diverse circulating autoantibodies. The latter may be defined functionally—for example, lymphocytotoxic antibodies, or immunochemically. Associations between central nervous system disease in SLE and neuron reactive or lymphocyte/brain cross-reactive antibodies have been observed in a number of studies, suggesting a possible pathogenic role for these autoantibodies in the neuropsychiatric complications of SLE (NP-SLE). Evidence in support of this idea is the common diffuse clinical presentation of NP-SLE, the virtual absence of histopathologically documentable vasculitis within the brain at necropsy of patients with NP-SLE, and the documentation of an array of shared cell membrane antigens between lymphocytes and neurons (reviewed in ref 13).

Central nervous system involvement in lupus is manifested by a spectrum of neurological and psychiatric disorders, ranging in severity from florid psychosis, hemiparesis, or chorea to mild paraesthesia or ill defined mood alterations.

The neuropsychiatric complications of SLE are typically diagnosed when such heterogeneous neurological or psychiatric features cannot be attributed to other causes. Attempts to classify NP-SLE systematically have been made, but in general the variability of these complications and lack of widely recognised specific diagnostic criteria make both case definition and associations with putative aetiological factors, such as autoantibodies, problematic. We have endeavoured to categorise our patients with SLE systematically according to neuropsychiatric involvement using a classification which is an extension and modification of that proposed by Kassan and Lockshin.

This paper reports a cross-sectional analysis of unselected patients with SLE in which associations between circulating lymphocytotoxic antibodies and clearly defined clinical or subclinical NP-SLE (cognitive impairment) are sought.

Patients and methods
Ninety eight female patients (mean age 35 years range 16–66) fulfilling the 1982 American Rheumatism Association revised criteria for SLE were studied after informed consent. The study population comprised consecutive referrals as either inpatients or outpatients to the lupus clinic at the McMaster University Medical Centre. At the time of the study 62 patients (63%) had active systemic disease as determined by the lupus activity criteria count, and of these, 36 patients were taking steroids.

Each patient underwent a comprehensive clinical evaluation together with laboratory studies to assess systemic disease activity. Laboratory evaluations included haematological evaluation (erythrocyte sedimentation rate, complete blood count, white cell differential count, prothrombin time, partial thromboplastin time), renal evaluation (serum urea, serum creatinine, analysis of urine sediment, 24 hour urine protein measurement, creatinine clearance calculation), and immunological evaluation (antinuclear antibodies, DNA antibodies, rheumatoid factor, quantitative immunoglobulins and serum protein electrophoresis, cryoglobulins, measurement of complement components C3 and C4, antibodies to extractable nuclear antigens, and immune complex measurement). Systemic disease activity was scored according to the lupus activity criteria count, modified to exclude central nervous system disease.
CENTRAL NERVOUS SYSTEM DISEASE ASSESSMENT
In addition to detailed neurological history and physical examination, each patient underwent electroencephalography and brain scan (radioisotope or computed tomography). Clinical criteria for NP-SLE were based on an extension and modification of previous classifications. Briefly, neuropsychiatric manifestations that were not attributable to causes other than SLE itself were divided into 'major' and 'minor' signs or symptoms. Major neurological features included: cerebrovascular event, neuropathy (Peripheral or cranial), movement disorder, transverse myelitis, seizure, organic brain syndrome, and meningitis. Major psychiatric features included: major affective disorder or atypical psychosis. Minor neurological disorders included mood swings and adjustment disorder. Recent consensus studies on the definition of NP-SLE showed that most of the above major categories were agreed upon by a wide variety of specialists (Singer et al., manuscript submitted). The diagnosis of a major neurological or psychiatric disorder was confirmed by a neurologist or psychiatrist. The presence of minor signs or symptoms—for example, adjustment disorder—was determined from the patient's subjective reports or the diagnosis of a psychiatrist. Neuropsychiatric involvement in SLE was diagnosed if the patient fulfilled (a) one major criterion; or (b) one or more minor criteria together with an abnormality in one of the following: electroencephalography, brain scan, cerebrospinal fluid studies, or cerebral angiogram. The patients were classified into one of three groups: group 1: active NP-SLE; group 2: inactive NP-SLE—that is, definite past history of neuropsychiatric lupus, currently resolved, or group 3: never NP-SLE—that is, no past or present evidence of NP-SLE.

NEUropsychological Testing
Neuropsychological testing was performed within two weeks of the clinical evaluation, using a protocol, the administration, analysis, and interpretation of which have been described in detail previously.22 In brief, a battery of well-standardised psychological tests, selected to cover a wide range of cognitive functions—for example, verbal reasoning, verbal memory, visual spatial function—was given to each patient. The test battery included: Wechsler adult intelligence scale—information, comprehension, similarities, digit symbol substitution, picture completion, block design subtests; Wechsler memory scale—with one hour delayed recall of stories, designs, and paired word associates; consonant trigrams; Rey auditory verbal learning test; Rey-Osterreith complex figure drawing—with one hour delayed recall; token test; trailmaking test; Stroop colour word interference test; design fluency test; Benton controlled word association test; animal naming test; finger tapping test; handedness questionnaire. Testing took 2½ to 3 hours to complete.

To identify cognitive impairment the raw scores from each test were converted to standard (Z) scores, with normal controls serving as age matched reference groups for the means and standard deviations needed in these transformations.22 The individual tests were grouped into 17 summary scores or test groupings based on a face-valid analysis of the possible cognitive processes involved. The summary scores for an individual were then compared with a derived estimate of that individual's premorbid function. Any summary score more than two standard deviations below the premorbid level was taken to reflect significant impairment; the individual's test profile was designated impaired if three or more of the 17 summary scores met this criterion.27

MEASUREMENT OF LYMPHOCYTOTOXIC ANTIBODIES
Lymphocytes were separated from freshly drawn venous blood by density gradient centrifugation using Ficoll-Hypaque as described by Boyum28 and suspended in minimum essential medium. Fresh rabbit serum was used as a source of complement. Lymphocytotoxicity was measured by the microdroplet test described by Terasaki and McClelland.29 Positive controls (multiparous serum or commercial antilymphocyte globulin) and negative control wells (normal serum) were included in each microtitre plate. Serum was collected from each patient at the time of clinical evaluation and was tested for lymphocytotoxicity against lymphocytes from 30 panel members selected to cover a wide range of HLA specificities. Reaction of the serum and lymphocytes before the addition of rabbit complement was carried out at 4°C (one hour). Subsequent incubation with complement was carried out at 22°C or 15°C for three hours and cell death was measured by eosin dye exclusion. An individual serum was considered positive if 50% or more cell death occurred in at least 10% (>3 out of 30) of normal panel donor cells. In a further subset of 15 patients, autoreactivity of the lymphocytotoxic antibodies was assessed using the patient's own peripheral blood lymphocytes as target cells. Reaction conditions were as described above and a positive result was recorded if >50% cell death was found. The individual performing the lymphocytotoxic antibody assay was not aware of the results of clinical and psychological evaluations.

Results
ASSESSMENT OF CENTRAL NERVOUS SYSTEM INVOLVEMENT
Twenty six patients had clinically evident active central nervous system disease at the time of study (group 1) (table 1). A further 24 patients had a history of such involvement with subsequent resolution (group 2). Neuropsychiatric

<table>
<thead>
<tr>
<th>Table 1: Characteristics of neuropsychiatric involvement in systemic lupus erythematosus</th>
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<tbody>
<tr>
<td>Clinical (n=98)</td>
</tr>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>Group 2</td>
</tr>
<tr>
<td>Group 3</td>
</tr>
</tbody>
</table>

*Focal NP involvement/diffuse NP involvement (see text).

†Group 1=active NP-SLE; group 2=inactive NP-SLE; group 3=never NP-SLE (see text for details).
Lymphocytotoxins and cognitive function in SLE

Involvement in SLE in these groups covered a range of diagnoses as previously reported, with no patient having meningitis and no patient being categorised on the basis of peripheral neuropathy alone. Clinical manifestations were further categorised as 'diffuse' NP-SLE (n=28)—for example, major psychiatric disorder, organic brain syndrome, or major non-focal neurological disease—or 'focal' NP-SLE (n=22)—for example, transverse myelitis, cerebral vascular event, or isolated cranial neuropathy. Fifty three patients had isotope brain scans, all of which were normal. Of those who had computed tomographic brain scans (n=19), eight were abnormal: cortical atrophy in three; decreased density compatible with brain infarction in three; single area of increased lucency of indeterminate significance in one; features suggestive of subdural haematoma in one. Table 2 shows the relation between the clinical categorisation of NP-SLE and objectively defined cognitive impairment. Patients with clinically defined NP-SLE (groups 1 and 2) were no more likely to show cognitive impairment than those without clinical central nervous system involvement (group 3). This is in accordance with previous data reporting that, in contrast with a control sample of patients with rheumatoid arthritis, there is a high prevalence of cognitive impairment in patients with SLE, irrespective of clinical status or other variables.

LYMPHOCYTOTOXIC ANTIBODIES IN SLE SERUM

Lymphocytotoxic antibodies were present in the serum samples of 27 of the 98 patients. Lymphocytotoxic activity was observed across a range of HLA specificities with lymphocytotoxic sera killing an average of 13 of the 30 lymphocyte preparations (range 4–29). Three lymphocytotoxic sera reacted (>50% cell death) with only four of the panel lymphocyte preparations each, yet an analysis of the HLA phenotypes of these panel members failed to disclose a common antigen for any HLA locus with respect to a given serum (table 3), suggesting that autoantibodies rather than alloantibodies are probably being detected. Serum samples from 15 patients were tested against autologous lymphocytes. Of these, five showed lymphocytotoxic activity when tested against panel lymphocytes as well as cytotoxicity for autologous lymphocytes; of the remaining 10 sera tested, which did not show cytotoxicity against panel lymphocytes, eight were negative for autocytotoxicity (5/5 vs 2/10; \( \chi^2=8.57; p<0.01 \)). Several serum samples showed degrees of cytotoxicity lower than our criteria for positivity, not being reliably differentiated from control sera and thus scored as negative.

CORRELATIONS OF LYMPHOCYTOTOXIC ANTIBODIES

Lymphocytotoxic antibodies were found to be only marginally more prevalent in patients with NP-SLE than in those without neuropsychiatric SLE (16/50 (32%) vs 11/48 (23%)) and this was not statistically significant. Interestingly, when clinical NP-SLE was observed in the presence of lymphocytotoxic antibodies it generally showed a diffuse pattern (12/16 (75%) cases), contrasting with antibody negative patients who showed an equal prevalence of diffuse (16/34 (47%)) and focal (18/34 (53%)) patterns of NP-SLE (\( \chi^2=3.45; p<0.05 \)).

In contrast with the lack of correlation of lymphocytotoxic antibodies with overall clinical NP-SLE, the presence of these antibodies was significantly associated with cognitive impairment (table 4). Seventy four per cent (20/27) of antibody positive patients were cognitively impaired compared with 48% (34/71) of antibody negative patients (p<0.02). A detailed analysis of these neuropsychological data suggests that a particular pattern of visual spatial cognitive deficit is associated with the presence of lymphocytotoxic antibodies.

Table 2: Relation of cognitive impairment to clinical NP-SLE* status

<table>
<thead>
<tr>
<th>Cognitive status</th>
<th>Clinical NP-SLE (groups 1 and 2)</th>
<th>No clinical NP-SLE (group 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired†</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Not impaired</td>
<td>20</td>
<td>24</td>
</tr>
</tbody>
</table>

*NP-SLE=neuropsychiatric involvement in systemic lupus erythematosus.
†Group 1=active NP-SLE; group 2=inactive NP-SLE; group 3=never NP-SLE (see text for details).
‡By criteria previously detailed.23,25
\( \chi^2=1.00; NS \)

Table 4: Serum lymphocytotoxins in relation to cognitive status in systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Lymphocytotoxic antibodies*</th>
<th>Cognitive status</th>
<th>Impaired†</th>
<th>Non-impaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>20</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>34</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

*Microcytotoxicity assay against lymphocytes from 30 panel donors. Present implies that the serum shows >50% cell killing in more than 10% of donors (see text).
†Using criteria described in 'Patients and methods'.
\( \chi^2=5.42; p<0.02 \)

Table 3: HLA phenotypes of lymphocyte preparations killed at >50% by individual lupus serum samples

<table>
<thead>
<tr>
<th>Serum No</th>
<th>Donor No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>A2,26; B35; DR6</td>
</tr>
<tr>
<td>2</td>
<td>A2,28; Bw62, w57; Cw3, 6; Dw4,7</td>
</tr>
<tr>
<td>3</td>
<td>A2,26; B35; Cw6; Dw2,6</td>
</tr>
</tbody>
</table>

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Table 5: Serum lymphocytotoxins in relation to clinical features in systemic lupus erythematosus. Values are shown as number (%) of patients

<table>
<thead>
<tr>
<th></th>
<th>LCA* Present (n=27)</th>
<th>LCA* Absent (n=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active systemic disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>11 (41)</td>
<td>25 (35)</td>
</tr>
<tr>
<td>Joints</td>
<td>20 (74)</td>
<td>43 (61)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>6 (22)</td>
<td>13 (18)</td>
</tr>
<tr>
<td>Seroza</td>
<td>3 (11)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Haematological</td>
<td>10 (37)</td>
<td>26 (37)</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>7 (26)</td>
<td>23 (32)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>9 (33)</td>
<td>23 (32)</td>
</tr>
<tr>
<td>Raynaud's disease</td>
<td>8 (30)</td>
<td>25 (35)</td>
</tr>
</tbody>
</table>

*aLCA=lymphocytotoxic antibodies.
*Lupus activity criteria count score ≥2 (see ref 26), excluding nervous system criterion.
**Past or present involvement determined by clinical and laboratory means with reference to American Rheumatism Association revised criteria where relevant.
NS=not significant.

associations were detected (table 5). Corticosteroid treatment, similarly, did not account for the association between lymphocytotoxic antibodies and cognitive impairment (data not shown).

Seven of the eight patients with computed tomography abnormalities were classified as having either active or inactive NP-SLE and six of these were cognitively impaired. Two of the eight patients had serum lymphocytotoxic antibodies. No significant associations among these variables were found.

Discussion
In an unselected group of female patients with SLE we have used defined criteria to categorise patients systemically according to neuropsychiatric involvement. To describe brain involvement more fully we carried out a detailed neuropsychological evaluation of each patient. This information allowed us to report a specific association between serum lymphocytotoxic antibodies and cognitive dysfunction in SLE, an association which occurs independently of other potential associations with lymphocytotoxic antibodies in SLE, including other organ systems, and treatment of other organ systems, or drug treatment.

Lymphocytotoxic antibodies in SLE sera have been reported repeatedly since 1970 (reviewed in refs 30 and 31). Discrepancies in the prevalence and associations of these antibodies have appeared in published work, due largely to technical problems or inconsistencies in criteria used for the determination of a positive lymphocytotoxic antibody test. Thus, for example, the prevalence of lymphocytotoxic antibodies has been estimated in SLE to be 28–93% (refs 30, 33). These antibodies require incubation at cold (4°C) temperatures for optimal detection, and many reports describe lymphocytotoxic antibodies to sera which kill more than 20% of a given panel lymphocyte preparation in the presence of complement. In our experience, difficulty may arise in reliably distinguishing such minor degrees of cytotoxicity from control results and consequently we used more exacting criteria in the assessment of lymphocytotoxic antibody activity. Also, unless the target lymphocytes killed in the assay show a broad range of HLA specificities it is likely that autoantibodies, related to prior blood transfusion or pregnancies, will be detected and contribute to an apparently high prevalence. With our criteria for lymphocytotoxic antibodies, we recorded a prevalence of 13% in 98 consecutive unselected patients. Examination of SLE specificities and auto-reactivity (table 3) suggests that to a great extent autoantibodies rather than autoantibodies are being detected in this study.

Specific associations between lymphocytotoxic antibodies and central nervous system involvement in SLE have been reported. Such observations have also been inconsistent, however, probably owing not only to differing methods for lymphocytotoxic antibody detection but also to variability in the definition of NP-SLE. Case definition in NP-SLE has proved difficult and until recently there has been no standard of value. Using defined criteria for neuropsychiatric involvement together with systematic neuropsychological categorisation of our patients, we have reported nervous system involvement in approximately 50% of our unselected SLE population, yielding an overall prevalence similar to that of other reported studies (reviewed ref 16). Probably, such an approach could generate information on the actual extent and type of nervous system involvement in SLE. Although we have previously described correlations between serial lymphocytotoxic antibody assessments and overt neuropsychiatric events in a small group of patients with SLE, this does not hold in a larger group of unselected patients whose clinical categorisations have been standardised.

Lymphocytic reactive autoantibodies cross reactive with human brain constituents have been described, and others have shown not only brain/lymphocyte cross reactivity of such antibodies but also a higher incidence of lymphocytotoxic antibodies in NP-SLE. Several potential lymphocyte/brain cross-reactive antigens have now been identified, which may be the targets of SLE autoantibodies and may be involved in access to the brain after blood-brain barrier damage, or as a result of intrathelial immunoglobulin synthesis, or both.

In this study a specific positive association between cognitive dysfunction and serum lymphocytotoxic antibodies was found (table 4). The specificity of this association is emphasised by the finding that it does not seem to be related to either overall systemic disease activity or to other organ system involvement in SLE (table 5). Our findings are of interest as several groups of autoantibodies have been linked with NP-SLE, often with specific patterns of involvement. Serum antineuronal antibodies measured in a mixed haemadsorption assay using cultured neublastoma cells as targets are found chiefly in association with non-focal neuropsychiatric SLE; a novel neuronal antigen is the target of some SLE autoantibodies. Focal disease, in contrast, such as cerebral vascular events or chorea, has been linked to antibodies with phospholipid specificity. Finally, a striking
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A large variety of non-specific cognitive and personality problems have been noted in patients with SLE, and some of these features may be related to the high prevalence of cognitive impairment found in these subjects, particularly those without clinically evident nervous system disease. The observations in this study suggest that neurocognitive evaluation should be included together with neuronal autoantibodies as well as lymphocytotoxic antibodies in the assessment of patients with SLE. In view of the clinical heterogeneity of NP-SLE it is probable that no single mechanism will explain all the features satisfactorily. If lymphocytotoxic antibodies or related autoantibodies are to be shown to be aetiologic factors in NP-SLE, however, prospective studies of unselected groups of patients with standardised clinical categorisations are necessary.

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