The association between sequential changes in serum antineuronal antibodies and neuropsychiatric systemic lupus erythematosus

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Summary: To determine the significance of changes in serum antineuronal antibody levels in systemic lupus erythematosus, 9 patients who had a rise and 11 patients who had a fall in neuronal antibody titre over a mean duration of 2.1 years (range 0.25–5.2) were identified. These changes were examined in the light of concurrent changes in other serological variables, overall disease activity, neuropsychiatric disease and neuropsychological tests.

Changes in antineuronal antibodies were frequently associated with concurrent changes in anti-DNA antibodies and overall disease activity. When neuropsychiatric disease or cognitive dysfunction were present, their course showed a close correlation with changes in antineuronal antibody levels. The results support the association between antineuronal antibodies and neuropsychiatric-systemic lupus erythematosus, but suggest that their measurement will provide useful information of disease status in only a subpopulation of patients.

Introduction

Neuropsychiatric (NP) manifestations of systemic lupus erythematosus (SLE) contribute substantially to the morbidity and mortality of the disease. They occur in approximately 50% of patients[1-5] and are said to account for up to 19% of deaths.[2,6] The features of NP disease vary from overt neurological dysfunction such as psychosis, organic brain syndrome and seizure disorders[7,8] to more subtle subclinical defects of neurocognitive function demonstrable by detailed neuropsychological analysis.[4,5,9] There are no specific features of the disease and physicians caring for such patients must therefore rely on their clinical judgement, supported by non-specific laboratory investigations such as cerebrospinal fluid (CSF) examination, electroencephalography, computerized tomographic, radionuclide and other forms of brain scanning[7,8] in establishing a diagnosis.

Antineuronal or brain cross-reactive lymphocyte antibodies, which may demonstrate binding to either surface or intracellular antigens, have been associated with NP-SLE in a subset of patients.[10-19] A pathogenic role for such antibodies is supported by their temporal relationship with NP events,[11,13,15,16] their presence in both CSF and serum in relation to NP manifestations or cognitive dysfunction[14,15,17] and their detection in neuronal tissues of SLE patients succumbing to NP disease.[12,18] The aim of the present study was to examine the association between changes in serum antibody levels to surface neuronal antigens and NP-SLE, and to determine if this information would assist in the management of some patients with NP disease. Patients who demonstrated a substantial rise or fall in serum IgG antineuronal antibody levels were identified. Changes in antibody levels were examined in the light of other serological variables, overall disease activity and NP manifestations.

Materials and methods

Patients

Patients who fulfilled the 1982 American Rheumatism Association criteria for SLE[20] attending the Lupus Clinics at McMaster University Teaching Hospitals

were considered for inclusion in the study. The majority of these patients are seen at regular intervals when a full clinical assessment is carried out in addition to routine laboratory investigations. The latter include peripheral blood haemoglobin levels, total white cell and differential counts, platelet count, urinalysis, immunoglobulin levels, antinuclear antibody (ANA) using rat liver as substrate, DNA antibodies measured by the Farr assay[21] (normal range 20%) and total C3 and C4 levels. In addition, serum antineuronal antibody levels are routinely measured. For the present study those patients who showed a substantial rise or fall in antineuronal antibody levels, as defined below, were identified from a group of 135 patients. A retrospective chart review was then undertaken to obtain relevant clinical and serological data.

Overall SLE disease activity was quantified using the Lupus Activity Criteria Count (LACC),[22] which has a range of 0 to 7, and a score of 2 or more signifies active SLE. NP manifestations of SLE were defined according to the criteria of How et al.[13] In all cases NP manifestations could not be attributed to any cause such as uraemia, hypertension, infection or corticosteroids, other than the disease process itself.

Neuropsychological tests

The initial neuropsychological evaluation was based on a comprehensive set of psychological tests administered over a 3-hour period. These tests assessed a wide range of cognitive functions including attention, psychomotor speed, memory and problem solving. The assessment procedures and interpretative criteria are described in detail elsewhere.[4,5] Subsequent evaluations were based on either a repeat of the initial full test battery or an abbreviated battery which was administered over a one hour period. Each test score was converted to a standard (Z) score using the mean and standard deviation for that test score in a group of normal controls. A summary Z-score was derived from the test scores from each assessment. The summary score is the mean of 17 standard scores, which were available from both the abbreviated and the full test battery. Thus, the summary Z-scores from the initial and follow-up evaluations are based on comparable data sets. Although neuropsychological evaluation is carried out routinely on all SLE patients attending our clinic, only five of the patients in the present study had paired data available around the time of serum sampling.

Antineuronal antibodies

Human neuroblastoma cell lines were used as the source of neuronal antigens: SK-N-Mc, SK-N-SH (J. Fogh, Sloan-Kettering Cancer Research Institute,

New York, NY), NMB-7 and IMR-6 (R. Kennet, Department of Genetics, University of Pennsylvania, Philadelphia, PA). IgG antineuronal antibodies were measured by a mixed haemadsorption assay as previously described.[13] All antibody testing was carried out by one of us (SB) without knowledge of the patient's clinical status. In the present study a significant rise in antineuronal antibody levels was defined by the presence of either of the following criteria: a change from undetectable levels to positive reactivity at a titre of 1:40, or a three-fold or greater rise in titre, either of which was observed with two or more neuroblastoma cell lines over a minimum period separating the serum sampling of 3 months. Likewise, a significant fall in antineuronal antibody levels was defined by a change from positive reactivity at a titre of 1:40 to undetectable levels or a three-fold or greater fall in titre, either of which was observed with two or more neuroblastoma cell lines over a minimum period separating the serum sampling of 3 months.

Statistical analysis

Differences between paired data were analysed using the Wilcoxon signed rank test.

Results

Antineuronal antibodies and other serological variables

Nineteen patients were identified who had substantial changes in antineuronal antibody levels over a mean duration of 2.1 years (range 0.25–5.2) (Table I). Nine patients (group A) had a rise in antineuronal antibody levels, one of whom had two rises on separate occasions. Eleven patients (group B) had a fall in antineuronal antibody levels. One patient had a rise and fall in antibody levels on two separate occasions and was included in both groups. The changes in antineuronal antibody levels, total immunoglobulins (IgM, IgG, IgA), ANA, anti-DNA antibodies, C3 and C4 levels over the study period are shown in Table II. In group A there was no sigificant change in total immunoglobulin levels, ANA titres or total C3 and C4. In contrast, group B patients showed a significant fall in IgG ($P < 0.01$) and a significant rise in C3 ($P < 0.02$) and C4 ($P < 0.01$) levels. Antibodies to DNA were detected in 8 patients in group A and 9 patients in group B. In individual patients, changes in anti-DNA antibodies frequently reflected those seen in antineuronal antibodies, although overall changes in anti-DNA antibodies only achieved statistical significance in group B ($P < 0.02$). In group A, three patients, including the patient with two separate rises in antineuronal antibodies, did not manifest a rise in anti-DNA antibodies. Conversely, in group B, one

Table I SLE patients with a rise (group A) or fall (group B) in serum antineuronal antibodies

		Total	Group A	Group B
Number of patients		19	9†	11†
Mean age and range (years)*		34.5 (16–56)	36.7 (22–56)	32.2 (16–54)
Female : Male		14 : 5	7 : 2	8 : 3
Disease duration (years):*	mean	5.5	5.2	6.8
	range	0.08–25	0.3–13	0.08–25
Duration between				
assessments (years):	mean	2.1	1.8	2.2
	range	0.25–5.2	0.25–3.4	0.06–5.2

*At time of first assessment in study.
†One patient included in both groups (see text).

Table II Change in serological variables in patients with a rise (group A) or fall (group B) in serum antineuronal antibodies

	Group A Assessment		Group B Assessment	
	First	Second	First	Second
Anti-IMR-6†	20 (0–160)	40 (0–2560)	80 (0–2560)	0 (0–80)
Anti-NMB-7†	20 (0–320)	160 (0–2560)	80 (0–5120)	0 (0–40)
Anti-SK-N-Mc†	0 (0–1280)	80 (0–5120)	160 (0–40960)	0 (0–320)
Anti-SK-N-SH†	40 (0–640)	960 (40–5120)	80 (0–5120)	0 (0–80)
IgM (g/l)	1.44 (0.3–3.9)	1.16 (0.4–4.7)	1.61 (0.2–3.7)	0.66 (0.2–1.9)
IgG (g/l)	13.75 (6.4–21.8)	13.56 (10.2–22.2)	12.00 (8.9–38.3)	9.23 (4.9–30.7)**
IgGa (g/l)	2.31 (1.3–6.0)	2.01 (1.2–6.6)	2.10 (1.0–7.3)	1.73 (0.2–2.7)
ANA†	1500 (16–4096)	768 (32–8000)	500 (32–4000)	128 (8–2048)
Anti-DNA (%)	28 (0–82)	63 (0–91)	38 (0–99)	0 (0–77)*
C3 (g/l)	0.94 (0.6–1.4)	0.7 (0.3–1.5)	0.6 (0.3–1.0)	0.9 (0.4–1.5)*
C4 (g/l)	0.09 (0.02–0.19)	0.11 (0.04–0.28)	0.11 (0.02–0.12)	0.14 (0.08–0.24)**

Results expressed as median and range.
†Values for anti-IMR-6, NMB-7, SK-N-Mc, SK-N-SH and ANA are the reciprocal of the titre.
*$P < 0.02$; **$P < 0.01$.

patient had a selective fall in antineuronal antibodies with no corresponding fall in anti-DNA antibodies over the same period.

General SLE disease activity

In group A there was no overall change in LACC score between assessments [median and range: 1 (0–2) to 1 (1–5)]. Two patients developed active SLE over the study period. In group B the overall LACC score fell between assessments [median and range: 2 (0–3) to 1 (0–2)]. In five patients SLE became inactive over the study period. Exclusion of neurological manifestations when generating LACC scores did not substantially alter these values.

NP-SLE and neuropsychological tests

In group A, four patients had clinical criteria for a diagnosis of NP-SLE at follow-up compared to one patient at the initial assessment. Two of these patients were considered to have 'focal' NP disease, that is, lesions which could be attributed to a specific localized lesion, while the remaining two patients had 'diffuse' NP disease, that is manifestations which could not be attributed to a specific localized lesion. In group B, NP-SLE was identified in four patients. Of these, two patients showed clinical improvement over the study period, one patient had a full resolution of NP manifestations and another patient had evidence of NP-SLE at the follow-up assessment only, when

antineuronal antibodies were not detected. One of these patients had 'focal' NP disease, two patients had 'diffuse' disease and the remaining patient had manifestations attributed to both 'diffuse' and 'focal' disease.

Results of neuropsychological tests were available in five patients (Figure 1). One patient in group A showed a major deterioration over the study period and three patients in group B showed a major improvement. The remaining patient in group B showed a change which was within the normal variability seen on repeat testing in normal subjects (Z score = 0.46). Of these five patients, two did not have overt clinical evidence of NP-SLE. In two patients the changes in neuropsychological tests concurred with the clinical impression of changes in NP-SLE and the remaining patient showed discordant changes.

Drug therapy

In group A six patients were receiving prednisone at the initial assessment (median dose: 10 mg), compared to four patients at follow-up (median dose: 6.3 mg). In contrast, in group B, six patients were receiving prednisone at the initial assessment (median dose: 12.5 mg), compared to seven patients at follow-up (median dose: 10 mg) and two other patients were receiving antimalarials. Furthermore, four patients in group B had been treated with high oral doses of corticosteroids and two with cyclophosphamide between assessments. The indications for more aggres-

sive drug therapy varied but usually included major neurological or renal manifestations of SLE.

Discussion

The management of NP-SLE is hindered by a lack of understanding of its pathogenesis and the absence of a specific laboratory test for diagnosis or assessment of disease activity. Antineuronal antibodies have been associated with NP-SLE in some patients and may play a role in its pathogenesis. However, in cross-sectional studies,[11,13] the lack of diagnostic specificity of antineuronal antibodies has limited their applicability in the clinical management of SLE patients. The present study was undertaken to examine the association between changes in antineuronal antibodies and NP manifestations of SLE, and to determine if serial measurements contribute information in addition to changes in other serological variables and general disease activity, which would assist in the management of some patients with NP-SLE. The results suggest that, in general, serum levels of antineuronal antibodies, as measured in the present study, change concurrently with other serological variables and overall disease activity. However, in some cases, selective changes in antineuronal antibodies may occur. In particular, changes in NP status or in neuropsychological testing corresponded with changes in antineuronal antibody levels. These results support the association between antineuronal antibodies and NP-SLE but suggest that their measurement will provide useful information of disease status in only a subpopulation of patients.

A variety of assays have been established to measure antibodies to surface neuronal antigens.[10,11,13] In the present study we used a panel of four human neuroblastoma cell lines in a mixed haemadsorption assay which has previously been shown to be sensitive to non-HLA, non-ABO determinants on the surface of neuroblastoma, but not glioblastoma cells.[13] Using this assay we have detected circulating antineuronal antibodies in 42% of patients with NP-SLE, 14% of SLE patients without NP disease and in 12% of controls.[13] The latter included patients with rheumatoid arthritis, patients with psychiatric disease not associated with SLE, patients with other auto-immune and connective tissue diseases and healthy subjects.[13] Although there are obvious differences between neuroblastoma cells and neurones in normal brain tissue, nevertheless, there is evidence that some surface antigens are shared by both cell types.[19,23] In the present study, the criteria for a significant change in the quantity of antineuronal antibodies were compiled to ensure that such changes represented meaningful alterations in antibody levels and not merely intrinsic variation in the assay.
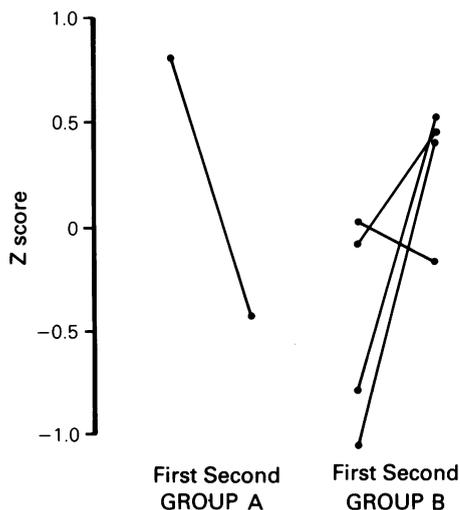

Figure 1   Change in neuropsychological tests (summary Z scores) between first and second assessments in patients showing a substantial rise (group A) or fall (group B) in antineuronal antibodies.

The selectivity of the rise or fall in antineuronal antibodies was examined by comparing them to concurrent changes in other serological variables. A rise in antineuronal antibodies was more likely than a fall to represent a selective change. The disparity in the ability of the antineuronal antibody response to distinguish between active NP-SLE and quiescent NP-SLE in the two groups is most likely related to the difference in drug therapy. Patients in group A had a slight reduction in their maintenance therapy over the study period, while in group B aggressive immunosuppressive therapy was introduced. Therefore, clinical and serological changes in group A most likely reflect underlying disease activity while those in group B probably reflect the effects of non-specific immunosuppression.

There is a lack of consensus concerning the degree of involvement of the central nervous system in SLE and its various manifestations. Overt features of NP-SLE have been recognized by many investigators,[1-3,7,8] while we,[4,5] and others,[9] have also reported more subtle abnormalities, which are frequently subclinical, and not attributable to chronic disease or psychological distress, detected by detailed neuropsychological testing. In the present study we attempted to examine changes in both overt and subtle NP disease by using a modified clinical classification of NP-SLE culled from previous classifications,[13] and results of neuropsychological tests which were available in a limited number of patients. Using the former classification, 8 patients had evidence of NP-SLE over the study period. Clinical improvement or deterioration in NP-SLE occurred concurrently with a fall or rise in antineuronal antibodies respectively in 6 of these patients. Furthermore, significant changes in sequential neuropsychological testing in 4 patients showed 100% concordance with changes in antineuronal antibody levels. Therefore, there was a consistent correlation between changes in NP disease and neuropsychological testing and concurrent changes in antineuronal antibody levels, although a similar correlation was also frequently seen with anti-DNA antibody levels.

It is generally agreed that antineuronal antibodies do not represent the only potential pathogenic mechanism in NP-SLE.[1,7,8,12] The evidence implicating their role in the pathogenesis of the disease is still largely circumstantial. It is likely that in some patients with SLE the presence of such antibodies, as detected by current assays, may reflect the presence of polyclonal activation, cross-reactive autoantibodies or a secondary response to neuronal damage. Therefore, in an attempt to improve the diagnostic specificity and to further our understanding of antineuronal antibodies in NP-SLE, efforts have been made to identify the antigenic specificities of these antibodies. In a recent study, Bonfa *et al.*[16] reported the presence of anti-ribosomal P protein antibodies in 18 of 20 patients with lupus psychosis, as defined by a behavioural disturbance lasting 2 weeks and requiring hospitalization and psychotropic medications. Overall, this antibody is present in 12% of SLE patients, 50% of whom have psychosis by this definition. The target antigen of the anti-P antibody is intra-cellular in origin and its reactivity is not restricted to neuronal cells. Our antineuronal antibody assay reflects binding to surface neuronal antigens, among which at least one high molecular weight antigen (97K) is unique to neuronal cells and precipitated only by SLE sera.[24] Further work is needed to determine the clinical and pathogenic significance of this finding.

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