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Antibodies to endothelial cells in systemic lupus erythematosus: a potential marker for nephritis and vasculitis

D. P. D'CRUZ, F. A. HOUSSIAU, G. RAMIREZ, E. BAGULEY, J. McCUTCHEON*, J. VIANNA, H.-J. HAGA, G. T. SWANA*, M. A. KHAMASHTA, J. C. TAYLOR*, D. R. DAVIES†, & G. R. V. HUGHES

*The Lupus Arthritis Research Unit, The Rayne Institute, Departments of *Immunology and †Histopathology, St Thomas' Hospital, London, England*

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SUMMARY

Using an ELISA, anti-endothelial cell antibodies (AECA) have been found in sera obtained at the time of renal biopsy in 46 out of 57 patients (81%) with systemic lupus erythematosus (SLE) and nephritis (mean binding index (BI) = 84% ± 52·8) compared with 22 out of 50 SLE patients (44%) without nephritis (mean BI = 45% ± 35·9). Seventy normal human sera had a mean BI of 10% ± 9·8. The highest levels were seen in patients with diffuse proliferative glomerulonephritis (WHO grade IV) and in patients with proteinuria and nephrotic syndrome. When the biopsies were assessed for activity and chronicity scores, AECA were associated with active renal lesions ($P < 0{\cdot}001$). AECA levels correlated with low complement levels but not with anti-DNA antibodies to extractable nuclear antigens (ENA), anti-cardiolipin or anti-neutrophil cytoplasmic antibodies. The presence of AECA conferred a positive predictive value of 0·68 for the presence of nephritis. Twenty-five patients had active vasculitis at the time of assay and the highest AECA values were seen in patients with both nephritis and vasculitis. No correlation was seen with serum immunoglobulin levels and immune complexes did not bind significantly to the endothelial surface. The possible role of these antibodies as a marker in lupus nephritis is discussed.

Keywords endothelium lupus nephritis vasculitis

INTRODUCTION

Antibodies to endothelial cells (AECA) were first described by Lindqvist & Osterland (1971) and Tan *et al.* (1972) using frozen mouse kidney sections as the substrate in immunofluorescence techniques. Since then these antibodies have been described in a variety of connective tissue disease (Shingu & Hurd, 1981; Cohen, Johnson & Hurd, 1983; LeRoux *et al.*, 1986; Baguley & Hughes, 1987; Hashemi, Smith & Izaguirre, 1987; Rosenbaum *et al.*, 1988) and in association with vasculitis (Leung *et al.*, 1986a, 1986b; Brasile *et al.*, 1989; Heurkens *et al.*, 1989; Ferraro *et al.*, 1990). Work in our laboratory and those of several other authors has shown that binding of AECA to endothelial cells is mediated by the $F(ab')_2$ portion and not the Fc portion (Hashemi *et al.*, 1987; Rosenbaum *et al.*, 1988; Baguley & Hughes, 1989; Heurkens *et al.*, 1989). In addition it has also been shown that some AECA activity can be absorbed out of sera using fibroblasts and monocytes, suggesting that these cells also may have similar epitopes that bind AECA (Rosenbaum *et al.*, 1988; Heurkens *et al.*, 1989).

Correspondence: Dr D. P. D'Cruz, The Lupus Arthritis Research Unit, The Rayne Institute, St Thomas' Hospital, London SE1 7EH.

AECA have also been described in IgA nephropathy (Yap *et al.*, 1988) and in addition, interest has been directed at the presence of AECA in acute and chronic renal transplant rejection where endothelial damage occurs at an early stage in the rejected organ (Cerilli *et al.*, 1977; Paul, Claas & van Es, 1979; Claas *et al.*, 1980). It has been further suggested that AECA may have a role in the development of glomerulonephritis (Matsuo *et al.*, 1987).

The aim of this paper is to examine the prevalence of antibodies directed against human umbilical vein endothelial cells in the sera of patients with lupus and to determine whether these antibodies may be a marker for disease pattern and activity with particular reference to nephritis and vasculitis.

MATERIALS AND METHODS

Lupus nephritis patients

Fifty-seven consecutive patients with lupus nephritis (53 women, four men) with a mean age of 32·8 ± 9·5 years from the Connective Tissue Disease Clinic at St Thomas' Hospital were studied retrospectively. Forty-eight had one or more renal biopsies performed as part of their assessment and the renal histology was classified according to the WHO criteria (Churg &

Sobin, 1982). A further nine patients had lupus nephritis confirmed clinically by the presence of persistent proteinuria on dipstick testing, 24-h protein excretion of greater than 1 g/day, an active urine sediment and/or renal impairment but did not, for various reasons, have a biopsy (anti-coagulation, abnormal clotting function, refused consent). All these patients fulfilled the American Rheumatism Association's (ARA) criteria for the classification of systemic lupus erythematosus (SLE) (Tan *et al.*, 1982). These patients had had SLE for a mean duration of $71{\cdot}2 \pm 64{\cdot}1$ months and developed nephritis $36{\cdot}0 \pm 68{\cdot}6$ months prior to biopsy. Sera from these patients were obtained at the time of renal biopsy in all but two patients where the sera were obtained within 6 weeks of biopsy and stored at $-70°C$. Serum was obtained prior to the commencement of specific therapy for nephritis in all patients.

The nephritis patients were designated into four clinical groups according to their serum albumin (g/*l*), 24-h protein excretion rates (g/day), and serum creatinine (mmol/*l*) at the time of biopsy: Proteinuria: albumin > 30, 24 h protein < 3·0, creatinine < 150; proteinuria + renal failure: albumin > 30, 24 h protein < 3·0, creatinine > 150; nephrotic syndrome: albumin < 30, 24 h protein > 3·0, creatinine < 150; and nephrotic syndrome + renal failure: albumin < 30, 24 h protein > 3·0, creatinine > 150.

Data on treatment at the time of assay was available for 54 patients and of these 24 were taking oral prednisolone (mean dose 28 ± 21 mg/day), 20 were taking both prednisolone and azathioprine (mean dose of prednisolone 28 ± 20 mg/day, mean dose of azathioprine 115 ± 33 mg/day) and 10 were taking no treatment.

Lupus control patients

Fifty patients, all female, with SLE but without clinical evidence of nephritis (consistently normal renal function and negative urinalysis for proteinuria by dip tests) were selected at random from the connective tissue disease clinic for comparison with the nephritis patients. These patients had a mean age of $37{\cdot}8 \pm 10{\cdot}5$ years and had a mean disease duration of $113{\cdot}9 \pm 87{\cdot}1$ months. All these patients also fulfilled the ARA criteria for the classification of SLE. Sera were stored at $-70°C$ until analysed.

Data on treatment were available for 47 patients and of these 23 were taking prednisolone (mean dose 11 ± 7 mg/day), 11 were taking prednisolone and azathioprine (mean dose of prednisolone 10 ± 8 mg/day, mean dose of azathioprine 96 ± 42 mg/day) and 13 were taking no treatment.

Non-lupus disease controls

Ten sera from patients with glomerulonephritis associated with other diseases (eight Wegener's granulomatosis, one IgA nephropathy and one systemic vasculitis) were assayed for AECA. All these patients had active nephritis at the time of assay.

Normal controls

Seventy normal human sera obtained from the Blood Transfusion Service were assayed for AECA.

Vasculitis

The records of all the patients were studied to ascertain whether there was clinical evidence of cutaneous or digital vasculitis at the time serum was obtained for assay. Digital vasculitis was considered to be present if the patient had nail fold or pulp infarcts, vasculitic ulcers and/or splinter haemorrhages. In all cases the vasculitis had been documented personally by one or more of us (D.P.D'C., E.B. and G.R.V.H.)

Routine investigations

Renal function studies including creatinine clearance and protein excretion were performed using standard laboratory methods. Anti-DNA antibodies were assayed using a radioimmunoassay kit (Amersham International, Amersham, UK). Complement studies were performed using radial immunodiffusion according to the method of Harrison & Lachmann (1986). All these investigations related to the time of biopsy and AECA assay in the nephritis patients and in the SLE controls to the time serum was assayed for AECA.

Assay for antibodies to endothelial cells

Human endothelial cell culture. Human umbilical vein endothelial cells were obtained by collagenase digestion (Sigma Chemical Co., Poole, UK) of cords as previously described (Jaffe *et al.*, 1973) with minor modifications of our own. Cells from each vein were grown to confluence in T25 flasks (Nunculon) coated with 1% gelatin (Sigma) in RPMI 1640 (Sigma). The medium was supplemented with glutamine (300 mg/*l*), 20% heat inactivated fetal calf serum (FCS) (GIBCO), 100 U/ml penicillin and streptomycin (Flow) and HEPES buffer (25 mM) (GIBCO). Neither endothelial cell growth factor nor heparin was used in these studies. All cultures were incubated at 37°C in 5% CO_2. The cells were fed at 2-day intervals and morphology was confirmed by phase contrast light microscopy showing the typical cobblestone monolayer appearance of endothelial cells. Immunofluorescence with factor VIII antigen was positive in greater than 95% of cells. Non-confluent flasks and those with fibroblasts present were discarded. Cells at first passage were removed from the flasks using 0·25% trypsin/EDTA (Sigma) and transferred to the inner wells of sterile 96-well microtitre plates pre-coated with gelatin. Confluence was achieved within 48 h with 1×10^6 cells per plate. All plates were used within 4 days in the ELISA and were not fixed before use. In addition, the cells were used in the resting state and stimulation with cytokines was not used.

Anti-endothelial cell antibody ELISA. Confluent plates were checked before use in the ELISA with phase contrast microscopy and at all stages to ensure confluency was maintained in each well throughout the procedure. All incubation stages were at 37°C in 5% CO_2. Non-specific binding sites were blocked for 1 h with 180 μl/well of adult bovine serum (ABS), (RPMI 1640 supplemented as above but with 10% FCS and 10% ABS (Imperial)). The plate was then washed twice with HBSS (GIBCO) and test and reference sera were added at a dilution of 1/20 to wells in triplicate. Each plate had at least one positive and four negative reference sera and these same sera were used in all the experiments.

After a 3-h incubation, the wells were carefully washed three times with HBSS, and 120 μl/well of goat anti-human alkaline-phosphatase-linked polyvalent (IgG, IgM and IgA) immunoglobulin conjugate (Sigma) was added at a dilution of 1/500 of ABS. After a 2-h incubation, the plate was washed three times with HBSS and 50 μl/well paranitrophenylphosphate substrate (1 mg/ml) in diethanolamine buffer (pH 9·8) were added. Plates were then incubated and read after 180 min when the optical

density (OD) of the standard reference serum was >0·800. The binding index (BI) for each specimen was defined as:

$$BI\ (\%) = \frac{\text{OD mean test sera} - \text{OD mean of negative reference sera}}{\text{OD mean positive reference sera} - \text{OD mean of negative reference sera}} \times 100$$

A positive sample was defined as a BI of greater than the mean+3 s.d. of 70 normal sera. In this case the value was 40% (mean 10%, s.d.=9·8). The isotypes of the positive sera were determined by repeating the positive sera in the ELISA using IgG, IgM and IgA alkaline-phosphatase-linked conjugates.

The ELISA was reproducible with coefficients of variation within plates of 5% and between plates of 11%.

AECA cytotoxicity assay

A ^{51}Cr release assay was used to access whether the AECA-positive sera displayed potential cytotoxicity. Endothelial cells were cultured in 96-well titre plates as above and confluent plates were labelled for 18 h with ^{51}Cr (100 μl of culture medium containing 1 μCi of ^{51}Cr per well). Following removal of the excess ^{51}Cr and washing three times with ABS (150 μl/well), the cells were incubated with 10% ABS for 1 h. After two further washes, 100 μl of patient or control sera diluted 1/4 was added to four adjacent wells. After a 90 min incubation at 37°C in 5% CO_2 the cells were washed three times and young rabbit complement was added (100 μl per well at a 1/4 dilution in ABS). Maximal release was obtained by adding a lytic buffer containing a detergent (2% NP40, BDH Chemicals, Poole, UK) to four control wells and spontaneous release obtained by adding culture medium alone to four further control wells.

After a 120-min incubation the plates were centrifuged at 1000 *g* for 5 min and 100 μl of supernatant were carefully removed and the released radioactivity was measured in an LKB 1282 Compugamma universal gamma counter. The specific release (SR) for each specimen was calculated as follows:

$$SR = \frac{\text{Experimental} - \text{Spontaneous release}}{\text{Maximum} - \text{Spontaneous release}} \times 100$$

Each plate included at least one positive and two negative control sera, and 25 normal control sera from the Blood Transfusion Service were assayed to obtain a normal range for SR.

Anti-neutrophil cytoplasmic antibodies (ANCA)

Antibodies against neutrophil cytoplasm were detected by standard indirect immunofluorescence as described by Wiik (1989).

Anti-cardiolipin antibodies

Anti-cardiolipin antibodies were determined according to the method of Gharavi *et al.* (1987) using a standardized ELISA technique and the results expressed according to Harris *et al.* (1987).

All the antibody assays were performed on the patients and controls in a single blind fashion, the sera having been previously aliquoted in numbered vials independently. The code was opened only when all assays had been completed.

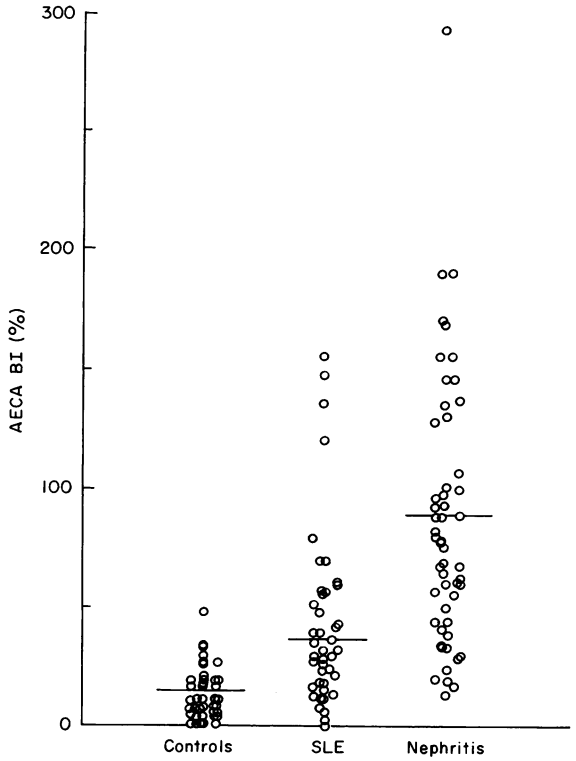

Fig. 1. AECA BIs in the normal controls, SLE controls and patients with SLE and nephritis.

Immune complexes

Immune complexes were assayed by the PEG precipitation of the complexes to a final concentration of PEG of 2% using a modification of the method of Poulton *et al.* (1978). The precipitated immune complexes were then quantified by single radial immunodiffusion IgG (Behring Diagnostics, Summerville, NJ). Values above 4·9 mg IgG/dl of serum were considered to be positive.

Statistical analysis

Statistical analysis was carried out in conjunction with the statistics department at this hospital using analyses of variance, unpaired Student's *t*-tests, Kendall's correlation coefficients and the χ^2 tests. Positive predictive values were also calculated.

RESULTS

AECA and lupus nephritis

The normal control sera had a mean BI = 10% ± 9·8. The SLE control sera had a significantly higher mean BI = 45% ± 35·9 ($P < 0·001$ *versus* normal controls). The lupus nephritis sera had the highest mean BI = 84% ± 52·8 ($P < 0·001$ *versus* SLE controls, $P < 0·001$ *versus* normal controls). These results are shown in Fig. 1. Of the lupus nephritis sera 46 out of 57 (81%) were defined as positive for AECA whereas 22 out of 50 (44%) of the SLE controls were positive and this was significantly different ($\chi^2 = 15·57$, $P < 0·001$).

When the results were analysed according to the WHO classification of lupus nephritis, the commonest pathological class was diffuse proliferative glomerulonephritis—WHO grade IV. The highest AECA BIs were seen in these patients and this

Table 1. Correlation between AECA and WHO grade

WHO grade	*n*	AECA$^+$ (BI > 40%)	AECA BI % (mean ± s.d.)	DNA antibodies (normal < 7 U/ml)
II	3	1*	50 ± 49·1	7 ± 4
III	9	6*	83 ± 56·7	35 ± 39
IV	27	24*	92 ± 56·5	76 ± 27
V	6	4*	69 ± 30·2	22 ± 32
VI	3	1*	30 ± 19·1	5 ± 3

* $\chi^2 = 9{\cdot}5$; $0{\cdot}05 < P < 0{\cdot}02$.

Table 2. Correlation of complement levels with WHO grade

WHO grade	*n*	CH100 (%) (60–140)	C ALT (%) (50–125)	C3 (mg/dl) (55–120)	C4 (mg/dl) (20–50)
II	2	105	192	72	11 ± 1
III	8	88 ± 24*	90 ± 29	87 ± 39	17 ± 9
IV	23	58 ± 30*	79 ± 28	70 ± 39	16 ± 13
V	6	97 ± 11*	90 ± 18	101 ± 16	23 ± 24
VI	2	—	—	94 ± 36	49 ± 21

*Student's *t*-test: CH 100 levels: grade IV *versus* grade III: $P < 0{\cdot}02$; grade IV *versus* grade V: $P < 0{\cdot}004$; CH 100, total haemolytic complement pathway levels; C ALT, alternate pathway levels.

was significantly different from the AECA levels in the other histological classes (Table 1). A similar pattern was seen with anti-DNA antibodies. All complement component levels were reduced in the WHO grade IV group although only the total haemolytic complement (CH100) values were significantly reduced when compared with those patients with focal proliferative glomerulonephritis (WHO grade III) and membranous glomerulonephritis (WHO grade V) (Table 2).

Twenty-eight biopsies were further assessed according to the Austin score for activity and chronicity (Austin *et al.*, 1984). In these biopsies the mean activity index was 8·0 ± 5·5, the mean chronicity index was 4·75 ± 2·4 and the mean AECA BI was 93% ± 61·3. High AECA levels in the nephritis sera were associated with high activity indices in the renal biopsies ($\chi^2 = 52{\cdot}4$, $P < 0{\cdot}001$).

When the results were analysed according to the clinical degree of renal involvement, significantly more patients with proteinuria and nephrotic syndrome had AECA reactivity than those patients who also had renal failure (Table 3). No such correlation with anti-DNA antibodies was seen in these groups.

Follow-up data were available in 16 lupus nephritis patients and showed that AECA levels declined following treatment from 108% ± 57·3 at the time of biopsy to 49% ± 26·8 after 14 ± 6·3 months of follow-up. Figure 2 shows a typical example of the pattern of AECA levels following treatment.

Treatment of nephritis in these patients was with three i.v. pulses of cyclophosphamide 500 mg, with Mesna, given at weekly intervals, followed by oral azathioprine (2 mg/kg per day) in seven patients, three pulses of cyclophosphamide

Table 3. Correlation between AECA and clinical group

Clinical group	*n*	AECA$^+$ (BI > 40%)	AECA BI % (mean ± s.d.)
Proteinuria	18	14*	94 ± 67·8
Nephrotic syndrome	19	19*	91 ± 37·5
Proteinuria + renal failure	5	3*	61 ± 49·8
Nephrotic syndrome + renal failure	12	9*	78 ± 52·2

* $\chi^2 = 8{\cdot}92$, $0{\cdot}02 < P < 0{\cdot}05$.

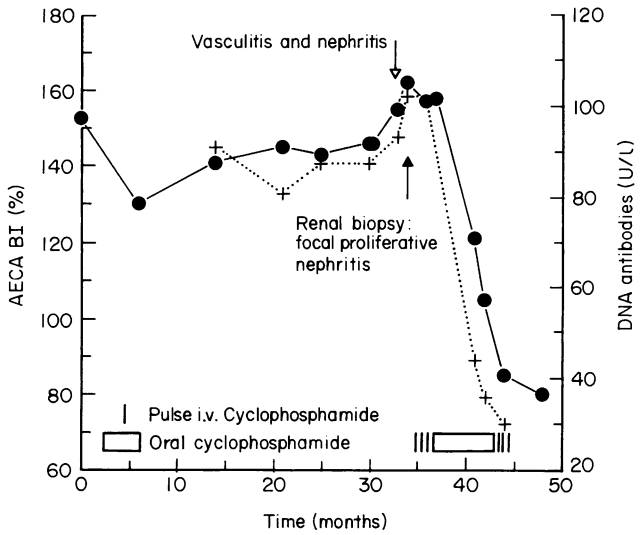

Fig. 2. Serological profile of a patient with SLE who developed vasculitis and nephritis. The AECA (——) and DNA (.....) levels rise with the onset of vasculitis and nephritis and fall rapidly following treatment with pulse and oral cyclophosphamide. (All serial aliquots were assayed for AECA on one ELISA plate.)

followed by oral cyclophosphamide (2 mg/kg per day) in three patients and oral azathioprine (2 mg/kg per day) in six. All patients received oral prednisolone, 30–40 mg daily, reducing to 7·5–10 mg daily within the first 8 weeks.

All the normal controls and 8/10 non-lupus disease controls were negative for AECA. The presence of AECA in the serum of SLE patients thus had a positive predictive value of 0·68 for the presence of nephritis. The assay had an overall sensitivity of 81% and a specificity of 82% for the detection of AECA in these patients.

ANCA, anti-cardiolipin antibodies and ENA

There was no relationship in these lupus patients between the presence of ANCA or anti-cardiolipin antibodies and either nephritis or vasculitis. None of the lupus patients with nephritis or vasculitis was positive for ANCA and only two of the SLE controls were weakly positive.

Five nephritis patients and six control patients were positive for anti-cardiolipin antibodies and this was not significantly different.

No correlation was seen between anti-ENA antibodies and either AECA or nephritis.

AECA and complement levels

In the lupus nephritis patients, there were low but significant correlations between the levels of AECA and total haemolytic complement CH100 ($r = -0{\cdot}278$, $P < 0{\cdot}008$), alternate pathway levels ($r = -0{\cdot}234$, $P < 0{\cdot}02$), low complement C3 ($r = -0{\cdot}205$, $P < 0{\cdot}02$) and low complement C4 ($r = -0{\cdot}218$, $P < 0{\cdot}018$).

In the SLE control group there was no correlation between AECA and any of the complement components.

Isotypes of AECA

In 33 nephritis patients AECA of the IgG isotype were present and in 33 of the IgM isotype, while eight had IgA antibodies. Sixteen sera had both IgG and IgM isotypes present whereas only five had all three isotypes present. No correlation could be found between any particular isotype and reduced complement levels although the numbers involved are small. There was no relation between the isotype of AECA in the sera of 18 patients with nephritis and the pattern of glomerular deposition of immunoglobulins IgG, IgM or IgA as detected by immunofluorescence in their biopsies.

AECA, anti-nuclear antibodies and anti-DNA antibodies

In the nephritis patients, no correlation between AECA BIs and DNA binding levels by radioimmunoassay was seen but there was a weak correlation between AECA BIs and anti-DNA antibodies detected by immunofluorescence with *Crithidia luciliae* ($r = 0{\cdot}2$, $P < 0{\cdot}01$) and with anti-nuclear antibodies ($r = 0{\cdot}305$, $P < 0{\cdot}02$). There were no such correlations in the SLE control patients.

AECA cytotoxicity assay results

The mean $\pm$ standard deviation specific release for the 25 normal controls was $8 \pm 8{\cdot}6$, giving a value of 34% (mean + 3 s.d.) above which a serum was considered to have potential cytotoxicity.

Thirty sera from the lupus nephritis patients, all of which were positive for AECA, were assayed. Only two patients were considered weakly positive with values of 36% and 39% respectively compared with a value of 80% for the positive control.

Circulating immune complexes

There was a correlation between AECA and the levels of circulating immune complexes ($r = 0{\cdot}519$, $P < 0{\cdot}002$). In order to investigate whether immune complexes were being deposited on the endothelial surface and accounting for the anti-endothelial reactivity in the ELISA we conducted the following experiment. Ten sera that were strongly positive for AECA and 10 AECA-negative sera were aliquoted. All sera were negative for rheumatoid factor. Immune complexes were precipitated by the addition of polyethylene glycol to a final concentration of 2%. Each serum and its supernatant following PEG precipitation was analysed on the same ELISA plate for AECA. The precipitated immune complexes were resuspended and quantified using radial immunodiffusion.

The mean BI of the AECA positive sera was $123{\cdot}3\% \pm 71$ compared with $116{\cdot}3\% \pm 89$ in the supernatant following PEG precipitation of immune complexes. This difference was not significant. The mean immune complex levels for the AECA-positive and AECA-negative sera were 9·6 and 1·99 mg IgG/dl respectively. The mean BIs for the AECA-negative sera before and after precipitation were $11{\cdot}3\% \pm 10{\cdot}3$ and $6{\cdot}7\% \pm 9{\cdot}9$ ($P > 0{\cdot}5$) respectively. Thus there was no significant fall in the mean AECA BI despite removal of immune complexes from the sera.

Serum immunoglobulins

In order to assess whether there was any correlation between AECA and serum immunoglobulins, the same 20 sera used above were assayed for IgG, IgM and IgA levels. In each case there was no correlation between the levels of any immunoglobulin isotype and AECA levels (data not shown).

Clinical correlations

Age and duration of disease. There was no correlation between AECA and the age of the patient or the duration of SLE. In the nephritis patients, no relationship could be found between AECA and the duration of nephritis.

Vasculitis. Twenty-five patients had active cutaneous and/or digital vasculitis including nail fold and pulp infarcts at the time of assay for AECA. The lupus control patients (without vasculitis) ($n = 40$) had a mean $BI = 40\% \pm 33{\cdot}3$ ($P < 0{\cdot}001$ *versus* normal control sera); the vasculitis patients ($n = 10$) had a mean $BI = 64\% \pm 41{\cdot}6$ ($P < 0{\cdot}001$ *versus* normal control sera, $P < 0{\cdot}063$ *versus* SLE controls) and the patients with both vasculitis and nephritis ($n = 15$) had a mean $BI = 109\% \pm 63{\cdot}7$ ($P < 0{\cdot}001$ *versus* normal control sera, $P < 0{\cdot}001$ *versus* SLE controls, $P < 0{\cdot}03$ *versus* SLE vasculitis). Thus those patients with lupus nephritis and vasculitis had significantly higher levels of AECA than either the SLE controls or the patients with SLE and vasculitis.

When only those lupus control and nephritis patients who did not have vasculitis were considered, the strong correlation of AECA with lupus nephritis was still present: lupus nephritis (without vasculitis) mean $BI = 75\% \pm 45{\cdot}9$ *versus* lupus controls (without vasculitis) mean $BI = 40\% \pm 33{\cdot}3$: $t = 3{\cdot}4$, $P < 0{\cdot}001$.

Serological disease indices. Table 4 shows the levels of the various serological parameters measured in this study. In general terms, therefore, those lupus patients with increased disease activity manifested clinically by the presence of vasculitis, nephritis or both had higher levels of AECA and anti-DNA antibodies together with lower levels of complement.

DISCUSSION

The endothelial monolayer lining the human vasculature plays a pivotal role in the traffic of cells, proteins and immunoglobulins between the blood and the rest of the body. As such it has a major role in the regulation of inflammatory and immune reactions. The endothelial cells in the glomerular capillaries are fenestrated and differ in morphology from most other vascular endothelial cells. Two barrier systems are present, one size dependent and the other charge dependent. Alterations in the structure, composition or charge of the endothelial cells or other components of the capillary wall may result in the loss of proteins through the glomerulus.

Antibodies to cultured human endothelial cells have been described in vasculitic disorders such as SLE, rheumatoid vasculitis and other disorders such as mixed connective tissue disease and scleroderma (Baguley & Hughes, 1989). The pathogenesis of the vascular injury in these diseases is unclear

Table 4. Correlation with serological disease activity markers

Group	AECA (BI %)	DNA (U/ml)	CH100 (%)	C ALT (%)	C3 (mg/dl)	C4 (mg/dl)	ESR (mm/h)
SLE controls ($n=40$)	41±33	22±31	85±24	94±21	95±38	24±13	33±21
SLE vasculitis ($n=10$)	64±42	22±31	85±15	92±15	75±27	30±23	33±21
SLE nephritis ($n=42$)	75±46	42±40	79±26	94±38	83±34	21±16	60±41
Nephritis+vasculitis ($n=15$)	109±64	73±36	57±32	79±21	68±48	15±10	55±35

but it is possible that immunological injury to endothelial cells may be important. Yap *et al.* (1988) described anti-vascular endothelial cell antibodies predominantly of the IgA class in the sera of 32% of their patients with IgA nephropathy, compared with 9% of patients with primary glomerulonephritis. Other authors have described antibodies against the endothelial–monocyte antigen system developing in patients following renal transplantation who suffer acute or chronic rejection. The antibodies were detected in both sera and eluates from rejected grafts, suggesting a pathogenetic role in these patients (Claas *et al.*, 1980; Paul *et al.*, 1979).

Evidence that the glomerular endothelium may be important in the development of some forms of glomerulonephritis has been derived from experimental work in animals. Matsuda *et al.* (1988) immunized guinea pigs with plasma membrane products derived from cultured rat brain endothelium and cultured human umbilical vein endothelium. AECA and immune complexes were then detected in the immunized animals' sera and they went on to develop clinically and histologically documented nephritis. They postulated that these antibodies reacted with a surface antigen on the glomerular endothelium, resulting in the shedding of immune complexes from the cell surface and accumulation in the subendothelial region as electron dense deposits. Subendothelial deposits are common in lupus nephritis.

In addition, Matsuo *et al.* (1987) injected rabbits with labelled antibodies directed against endothelial membrane-bound angiotensin-converting enzyme. The labelled antibodies were detected on the surface of glomerular endothelial cells and a mild, transient but consistently reproducible nephrotoxic glomerulonephritis developed which was documented histologically.

Our data demonstrate that patients with lupus nephritis have significantly elevated levels of antibodies against human umbilical vein endothelial cells and appear to correlate with reduced total haemolytic and alternate pathway complement levels and low C3 and C4 levels. The lack of a direct correlation with anti-DNA antibodies suggests that these antibodies are either a distinct population of antibodies or a small subset of anti-DNA antibodies that cross-react with endothelial cell antigenic epitopes. In clinical terms, these antibodies may be useful as a marker for activity in SLE patients with nephritis and further evaluation of these patients continues.

Our results confirm previous findings of a lack of correlation between AECA and DNA antibodies and the lack of a correlation between AECA and ENA antibodies. It is interesting to note that all the lupus vasculitis sera were negative for ANCA. This confirms the view that ANCA is specific for the systemic vasculitis of Wegener's granulomatosis rather than the vasculitis of SLE and also suggests that ANCA are not associated with lupus nephritis. Similarly, we found no correlation between anti-cardiolipin antibodies and the development of lupus nephritis.

The immune complex data confirm extensive previous data that immune complexes do not bind significantly to unstimulated endothelial cells (Hashemi *et al.*, 1987; Yap *et al.*, 1988; Heurkens *et al.*, 1989). This is supported by work that suggests that Fc receptors that could bind immune complexes are only expressed after cytokine stimulation or cell injury (Ryan, Schultz & Ryan, 1981; Cines *et al.*, 1982). The lack of a correlation with serum immunoglobulin levels supports the evidence that AECA bind specifically to certain surface epitopes on endothelial cells and are not deposited in a non-specific fashion.

The cytotoxicity data confirm previous work (Rosenbaum *et al.*, 1988; Baguley & Hughes, 1989) that AECA generally do not display potential cytotoxicity, at least when unstimulated endothelium is used. Whether there is a role for antibody-dependent cellular cytotoxicity in the pathogenesis of lupus nephritis remains to be seen.

The association between AECA and the more aggressive diffuse proliferative lupus nephritis (WHO grade IV) is interesting, although the relatively small numbers of patients in the other classes does not allow for valid conclusions to be made. These data are supported by the strong association of AECA with active lesions on the renal biopsy.

Whether these antibodies have a role in the development of lupus nephritis in a similar fashion to the experimental nephritis models or whether they are merely a response to glomerular damage by another process remains to be defined. Another consideration is that glomerular endothelial cells differ significantly in morphology from umbilical vein endothelial cells and may also differ in their surface glycoprotein characteristics. This raises the possibility that antibodies in lupus sera to umbilical vein endothelial cells may bind different epitopes on glomerular endothelial cells, with differing consequences. We are currently investigating the antigenic specificities of AECA in patients with lupus nephritis and vasculitis.

Our data also confirm previous reports that lupus patients with vasculitis have significantly higher levels of AECA than control patients (Cines *et al.*, 1984; Baguley *et al.*, 1987; Rosenbaum *et al.*, 1988). Once again the pathogenesis of the

vasculitis and its relationship to these antibodies remain unclear. Prospective studies are underway to investigate whether the control lupus patients with these antibodies are more likely to develop either nephritis or vasculitis or both.

It is interesting to speculate on a common pathogenetic mechanism where AECA bind to common epitopes shared between glomerular, microvascular and larger vessel endothelial cells. The initiation of complement consumption with the presence of membrane attack complexes on the endothelial surface could then result in endothelial damage. Additionally, cell-mediated immunity involving T cells, neutrophils and cells of the monocyte–macrophage system may be involved, resulting in the development of vasculitis, nephritis or both.

Thus, we have demonstrated that AECA are significantly elevated in patients with lupus nephritis and are correlated with reduced complement levels but not with anti-DNA antibodies. There is a correlation with diffuse proliferative lupus nephritis and especially with the more active lesions on the renal biopsy, with clinical evidence of proteinuria in excess of 1 g/day and also with nephrotic syndrome.

We suggest that AECA may have a potential role as a marker in lupus patients who develop nephritis or vasculitis.

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