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Autoantibodies to endothelial cells and neutrophil cytoplasmic antigens in systemic vasculitis

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SUMMARY

The interaction of circulating autoantibodies with the endothelium may be an important mechanism in the pathogenesis of systemic vasculitis. In a prospective study, we looked for circulating anti-endothelial cell autoantibodies (AECA) and anti-neutrophil cytoplasm autoantibodies (ANCA) in 80 patients with suspected systemic vasculitis. AECA were measured using an isotype-specific cellular ELISA incorporating human umbilical vein endothelial cells. ANCA activity was determined by indirect immunofluorescence and radioimmunoassay. Sequential studies were performed on sera from four cases with dual positivity, where autoantibody binding was compared with von Willebrand factor (vWF) concentration and disease activity. IgG AECA were significantly higher in the 27 ANCA-positive sera as compared with normal controls ($P=0{\cdot}027$) with IgG ($P=0{\cdot}009$) and IgA ($P=0{\cdot}046$) AECA isotypes correlating with ANCA positivity; in contrast, no differences were found between AECA levels in the ANCA-negative sera and the normal controls. Cross-inhibition studies pointed to the co-existence of two autoantibody populations. An association between autoantibody binding, disease activity and vWF concentration was found for both ANCA and AECA. Some patients with systemic vasculitis have detectable AECA that recognize different epitopes to ANCA and like ANCA, their titre correlates with disease activity and thus they may have a pathogenetic role in these conditions.

Keywords autoantibodies endothelial cells neutrophil cytoplasm systemic vasculitis pathogenesis

INTRODUCTION

Humoral and cellular mechanisms are implicated in the pathogenesis of systemic vasculitis (Nolasco *et al.*, 1987; Falk & Jennette, 1988). Circulating autoantibodies directed against neutrophil cytoplasmic antigens (ANCA) are specific markers for systemic vasculitides such as Wegener's granulomatosis and microscopic polyarteritis (van der Woude *et al.*, 1985; Falk *et al.*, 1989). They have been proposed to have a pathogenetic role in vasculitis due to the correlation of ANCA titre and isotype with disease expression (van der Woude *et al.*, 1985; Jayne *et al.*, 1989a) and the cross-reactivity of ANCA with glomerular and endothelial targets (Abbott *et al.*, 1989); dose-dependent effects of ANCA on neutrophil signal transduction and activation have also been reported (Falk *et al.*, 1989; Lai & Lockwood, 1990). Anti-endothelial cell autoantibodies (AECA) are present in Kawasaki disease (Savage *et al.*, 1989), a childhood ANCA-positive vasculitis, and a pathogenetic role has been demonstrated (Leung *et al.*, 1989). AECA have also been found in systemic lupus erythematosus (SLE) (Cines *et al.*, 1984) with or without renal involvement, rheumatoid arthritis with vasculitis (Heurkens *et al.*, 1989), progressive systemic sclerosis (Holt *et al.*,), overlap syndrome and systemic vasculitis (Bagueley *et al.*, 1987; Bagueley & Hughes, 1988; Brasile *et al.*, 1989; Ferraro *et al.*, 1990).

We sought to determine the occurrence of AECA in an unselected population of 80 patients with suspected systemic vasculitis; and to correlate positivity for AECA with that for ANCA using isotype-specific assays. AECA were detected by the binding of sera to resting human umbilical vein endothelial cells (HUVEC) in a cellular ELISA. In cases positive for both AECA and ANCA, cross-reactivity between the assays was assessed by cross-inhibition assays. Sequential estimations of AECA and ANCA were performed on sera from a group of patients who had both autoantibodies; antibody binding was then compared with von Willebrand factor (vWF) concentration, a marker of endothelial cell injury (Brown *et al.*, 1986) and clinical disease activity.

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PATIENTS AND METHODS

Samples

Sera from 80 patients with suspected systemic vasculitis were studied. They had been referred for ANCA estimation to a reference laboratory, which has been routinely performing ANCA assays since 1986. Control sera were obtained from 48 healthy adults. Sequential samples covering a period of 3–8 months were collected in four patients positive for both AECA and ANCA. All samples were separated into aliquots and stored at −75°C. Clinical diagnosis and the use of immunosuppressive therapy at the time of the assay was determined. ANCA and AECA assays were performed in two centres without knowledge of the clinical diagnosis or the other antibody result.

Patients

Four patients with both autoantibodies were further studied. ANCA and AECA binding was compared to vWF levels and clinical disease activity, assessed on a scale of 0–2 (0, remission; 1, partial remission; and 2, active disease).

Anti-endothelial cell antibody ELISA

HUVEC were isolated and cultured using standard techniques (Jaffe *et al.*, 1973). Second passage HUVEC were seeded at 2×10^4 in 100 μl of RPMI 1640 containing 20% fetal calf serum (FCS) (GIBCO, Paisley, UK), 10 U/ml heparin (Leo Laboratories, Princes Risborough, UK) and 30 μg/ml endothelial growth factor (Sigma, Poole, UK) on sterile polystyrene 96 × 6-mm diameter well plates (Nunc, Roskilde, Denmark) precoated with 100 μl of 1% gelatin (Sigma). After 48 h of culture at 37°C in a humidified 5% CO_2 incubator, the plates were washed with 0·15 M phosphate-buffered saline (PBS), pH 7·4. One-hundred microlitres of 2% bovine serum albumin (BSA) (Sigma) in PBS were added to each well for 1 h and incubated at room temperature to block non-specific binding of immunoglobulins. After three washes in PBS, 100 μl of duplicate or triplicate samples of test serum diluted 1/75 for IgA, 1/300 for IgM and 1/400 for IgG in 2% BSA/PBS were added to each well, and incubated for 1 h at room temperature. After three washes, 100 μl of affinity-purified goat anti-human IgA, IgM or IgG alkaline phosphate conjugate (Tago, Burlingame, CA) diluted 1/500 in 2% BSA/PBS were added to each well for a further 1 h incubation at room temperature. After three washes, 100 μl of *p*-nitrophenyl phosphate (Sigma) at a concentration of 1 mg/ml in 10% v/v diethanolamine buffer, pH 9·8 were added to each well for 1 h at room temperature. The absorbance (OD at 410 nm) was read in a Dynatech ELISA spectrophotometer. The results were expressed as the percentage binding of a standard laboratory positive, for IgG and IgM AECA the sera was from a patient with SLE and for IgA AECA, a patient with IgA nephropathy. A normal range was established from the bank of control sera. The intra-assay coefficient of variation was 6% and the inter-assay coefficient of variation was 10% for all three isotypes.

Anti-neutrophil cytoplasm antibody assay

Indirect immunofluorescence (IIF). This method has been previously described (Lockwood *et al.*, 1987). Briefly, test or control sera diluted 1/8 in PBS were layered onto cytospin normal human neutrophil preparations. The neutrophils were separated from heparinized whole blood on a methyl cellulose/hypaque gradient and fixed in absolute alcohol for 5 min at 4°C. Binding was detected by a FITC-conjugated polyclonal rabbit anti-human immunoglobulin (F200, Dako) diluted 1/32. Incubations were both for 1 h at room temperature and the slides were examined under u.v. microscopy. Two patterns of positive staining are recognized, a granular cytoplasmic pattern (cANCA) which has been associated with binding to serine proteinase 3 (Ludemann, Utecht & Gross, 1990), and a predominantly peri-nuclear staining (pANCA), which has been associated with reactivity to myeloperoxidase (Falk & Jennette, 1988).

Solid-phase radioimmunoassay (RIA). This method is an adaptation of one described before (Savage *et al.*, 1987). Briefly, neutrophils were separated as above, then incubated with 0·2 M sodium acetate, pH 4·2, for 2 h at 4°C. Following sonication the supernatant was separated into aliquots and stored at −80°C. The neutrophil extract was coated at 20 μg/ml onto multititre plates (Dynatech) in PBS. Test and control sera diluted 1/20 in PBS containing 1% gelatin and 0·1% Tween 20 (Sigma) (PBS-G-T), was added in triplicate. Binding was detected by either monoclonal mouse anti-human IgG diluted 1/2000 (Unipath, Bedford, UK), monoclonal mouse anti-human IgA diluted 1/500 (Sigma) or monoclonal mouse anti-human IgM diluted 1/1000 (Sigma); followed by ^{125}I-labelled, affinity-purified polyclonal goat anti-mouse immunoglobulin 2×10^5 ct/100 μl per min (Sigma) (4 μCi/μg). The plates were counted in a Packard Crystal II gammacounter. All incubations were of 1 h at 37°C, dilutions of reagents were in PBS-G-T and plates were washed three times between stages with PBS. Results were expressed as percentage binding of the test sample against a standard laboratory positive for each isotype assay.

Cross-inhibition studies

An endothelial cell extract was prepared using 5×10^6 second passage endothelial cells removed from the culture flask using 0·2% EDTA in Puck's saline, pH 7·4, and washed twice with HBSS. Cells were lysed with a solution containing 50 mM Tris/HCL, 5 mM EDTA, 0·5% NP-40, 1 mM PMSF, pH 7·4. This procedure yielded 0·5–1·0 mg/ml of cell membrane protein. Platelets or lymphocytes from normal controls were also prepared using the same lysing methods.

Sera positive for both ANCA and AECA, diluted 1/200 in 4% BSA/PBS for the AECA assay, or 1/100 in PBS-G-T for the ANCA assay, were then incubated with neutrophil extract at 50 μg/ml, endothelial lysate at 20 μg/ml and PBS alone. Incubations were for 18 h at 4°C or 1 h at 37°C, prior to estimation in the AECA and ANCA assays. Inhibition was determined as the percentage reduction in binding after incubation with the respective cell extract compared with incubation with PBS alone. A reduction in the AECA assay after pre-incubation with the neutrophil extract, or in the ANCA assay after pre-incubation with the endothelial cell lysate would suggest cross-reactivity of one autoantibody species, and subsequent lack of reduction in either assay would point to the co-existence of two autoantibody populations.

vWF assay

This was performed following a previously described procedure (Brown *et al.*, 1986).

Serum immunoglobulin concentrations

Serum IgG, IgM and IgA levels were measured using established ELISA methods.

RESULTS

Anti-neutrophil cytoplasm antibody assay

Of the 80 patient sera, 27 were positive for ANCA by IIF. Of these 27, 16 had IgG ANCA on solid phase RIA, two had IgM ANCA, one had IgA ANCA, four had IgG plus IgA ANCA and three had IgG plus IgM ANCA, and one had no reactivity in the solid-phase assays.

Anti-endothelial cell antibody assay

Normal control sera. The spread of AECA binding was not normally distributed, so parametric statistics were not used. The 95% percentile of AECA for normal controls ($n=48$) was used to establish the normal range for each isotype. Therefore, values of IgG AECA > 20%, IgM > 26% and IgA > 34% were regarded as elevated.

Patient sera. IgG AECA were significantly higher in the 27 ANCA-positive patients as compared with the 48 controls ($P=0{\cdot}027$, Wilcoxon rank sum test) (Fig. 1). In contrast, no statistical difference was found between the 53 ANCA-negative patients and controls ($P=0{\cdot}55$). 30% (eight out of 27) of the ANCA-positive patients had elevated IgG AECA levels compared with 6% (three out of 53) of the ANCA-negative patients or 2% (one out of 48) of the normal controls (Fig. 1).

Of the ANCA-positive patients 19% (five out of 27) had raised IgM AECA levels compared with 9% (five out of 53) of the ANCA-negative patients or 4% (two out of 48) of the controls (Fig. 2). However, there were no significant differences between IgM AECA for ANCA-positive patients ($P=0{\cdot}97$), ANCA-negative patients ($P=0{\cdot}28$) or the normal controls. Similarly, although 30% (eight out of 27) of the ANCA-positive patients had elevated IgA AECA compared with 9% (five out of 53) of the ANCA-negative patients or 4% (two out of 48) of the controls, we found no significant differences in IgA AECA levels when comparing the whole groups of ANCA-positive ($P=0{\cdot}2$) or ANCA-negative patients ($P=0{\cdot}78$) with the controls (Fig. 3).

Some concordance was seen between AECA isotypes in that one patient was positive for all three isotypes, six patients had IgG AECA in association with either IgM AECA or IgA AECA, but none was positive for both IgM and IgA.

Specificity of AECA

There were no statistical associations between AECA levels and serum immunoglobulin concentrations (IgG, $P=0{\cdot}27$; IgM, $P=0{\cdot}25$; and IgA, $P=0{\cdot}06$). Furthermore, AECA of all isotypes could be inhibited from binding to endothelial cells when they were incubated with endothelial cell lysate prior to the application onto our cellular ELISA. Maximum percentage inhibitions obtained for the IgG isotype were 70%, 38%, 36% and 54%; for the IgM isotype 62% and 40%; and for the IgA isotype 85% and 50%.

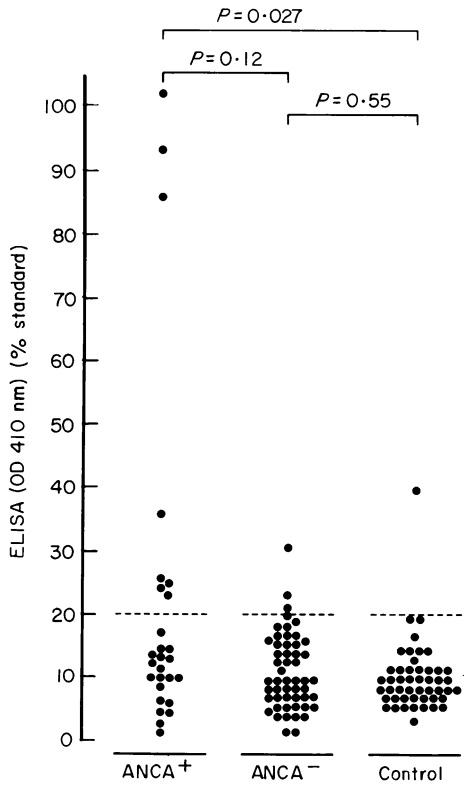

Fig. 1. IgG anti-endothelial cell autoantibodies (AECA) in 27 patients positive for anti-neutrophil cytoplasm autoantibodies (ANCA), 53 ANCA-negative patients and 48 healthy control. The dashed line represents the upper limit of the normal range.

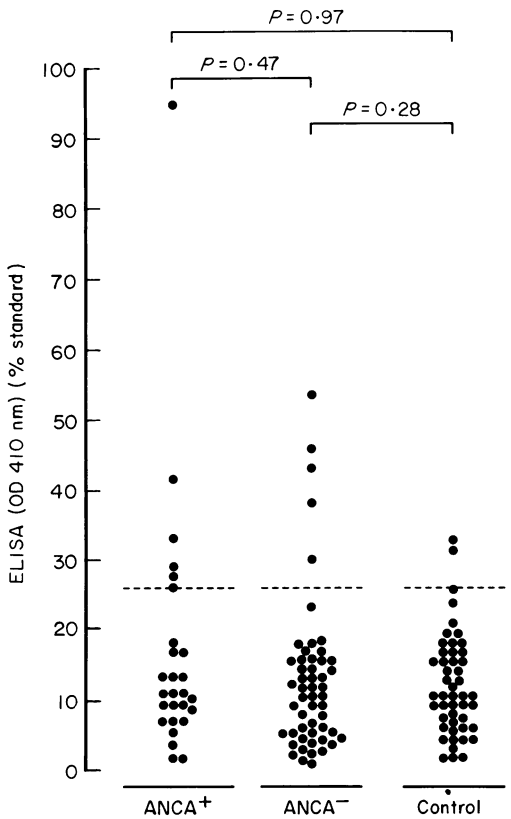

Fig. 2. IgM anti-endothelial cell autoantibodies (AECA) in 27 patients positive for anti-neutrophil cytoplasm autoantibodies (ANCA), 53 ANCA-negative patients and 48 healthy control. The dashed line represents the upper limit of the normal range.

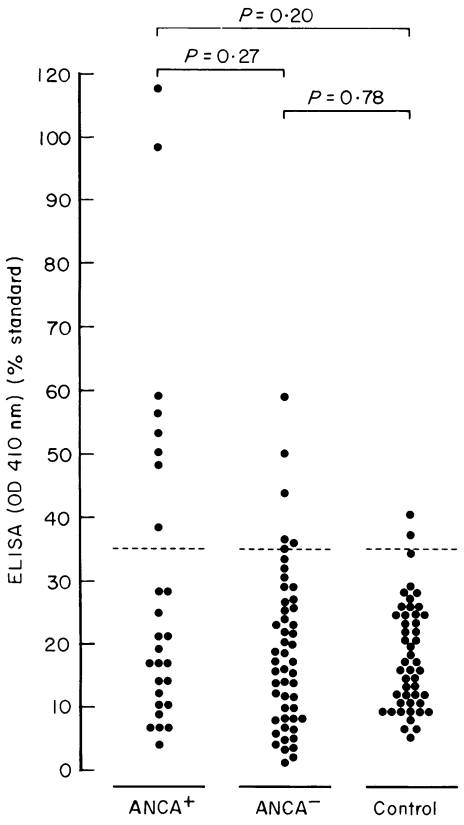

Fig. 3. IgA anti-endothelial cell autoantibodies (AECA) in 27 patients positive for anti-neutrophil cytoplasm autoantibodies (ANCA), 53 ANCA-negative patients and 48 healthy control. The dashed line represents the upper limit of the normal range.

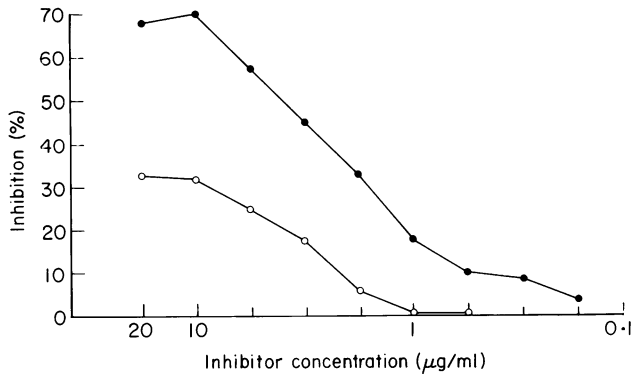

Fig. 4. Inhibition of IgG anti-endothelial cell autoantibodies (AECA) by endothelial cell lysates (●) and neutrophil extract (○) in a patient positive for AECA and anti-neutrophil cytoplasm autoantibodies (ANCA).

Endothelial cells cultured from multiple donors were used in the ELISA assays. Sera from those patients with vasculitis which contained AECA, bound to endothelial cells from all donors. Furthermore, AECA activity could not be adsorbed by prior incubation with platelets or lymphocytes from several donors (data not shown). This suggests therefore that these antibodies were not directed against HLA class I antigens.

Association of AECA with ANCA

ANCA measured by IIF were strongly associated with IgG AECA ($P=0{\cdot}009$, χ^2 with Yates' correction), they were weakly associated with IgA AECA ($P=0{\cdot}046$) and were not associated with IgM AECA ($P=0{\cdot}59$). Similar associations were obtained when ANCA isotype measured by SPRIA was compared with AECA: IgG ANCA to IgG AECA ($P=0{\cdot}028$), IgA ANCA to IgA AECA ($P=0{\cdot}001$) and IgM ANCA to IgM AECA ($P=0{\cdot}13$). Of six patients with Wegener's granulomatosis or microscopic polyarteritis on immunosuppressive therapy negative for ANCA, five were negative for AECA and one had IgM AECA.

Cross-inhibition studies

Inhibition of IgG AECA with neutrophil extract. Of three sera tested, maximum inhibitions of AECA binding of 70%, 38% and 36% were obtained with an endothelial cell lysate and of 30%, 3% and 0%, respectively, for the neutrophil extract (Fig. 4).

Inhibition of IgG ANCA with endothelial lysate. Binding of the three sera was inhibited by the neutrophil extract by 65%, 60% and 55%, and by the endothelial cell lysate by 0%, 0% and 10%, respectively.

Clinical diagnosis and treatment

The clinical diagnoses of the ANCA-positive patients were: Wegener's granulomatosis (14), microscopic polyarteritis (13); of these, none were receiving immunosuppressive therapy. Diagnosis of the ANCA-negative patients were: cutaneous vasculitis (four), Henoch–Schönlein purpura (two), anti-glomerular basement membrane disease (four), SLE (one), idiopathic chronic renal failure (eight), proliferative glomerulonephritis (three), renal transplant rejection (one), polyarteritis nodosa (one), idiopathic rapidly progressive glomerulonephritis (two), retroperitoneal fibrosis one, diabetes with atheroma one, tuberculosis (one), pulmonary eosinophilia (one), polymyositis (one), Wegener's granulomatosis or microscopic polyarteritis on immunosuppressive therapy (six), undetermined vasculitis (three) and unknown (13).

Cases 1 and 4 had Wegener's granulomatosis which was brought under control by prednisolone and cyclophosphamide during the study. Cases 2 and 3 had microscopic polyarteritis, case 2 had a fluctuating level of disease activity and received prednisolone and cyclophosphamide throughout the study period; case 3 was in remission at the start of the study and taking prednisolone alone, but relapsed and cyclophosphamide was added to the therapy. Immunosuppressive treatment was given at standard doses of prednisolone 60 mg/day falling to 10 mg/day after 6 weeks and cyclophosphamide 2 mg/kg per day.

Sequential AECA and ANCA assays

Three patients, 1, 2 and 4, clinically active at the start of the study initially had elevated AECA and ANCA levels. Following immunosuppressive therapy both antibodies fell; however, in two patients AECA and ANCA rose after induction therapy. For patient 2 this occurred at a time of increased disease activity but for patient 1 there was no concurrent clinical relapse (Fig. 5). For patient 3 a relapse of disease activity was clearly reflected by elevations in ANCA and AECA levels.

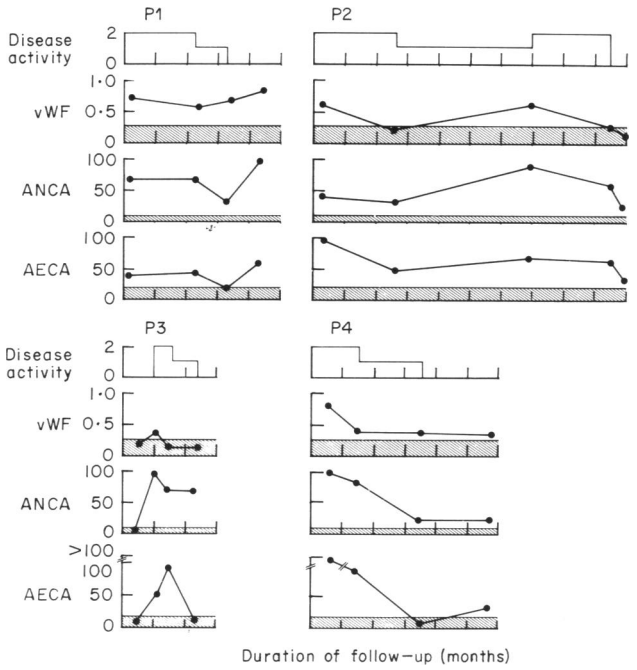

Fig. 5. Sequential anti-neutrophil cytoplasm autoantibodies (ANCA), IgA anti-endothelial cell autoantibodies (AECA) and von Willebrand factor (vWF) in four patients (1–4) with dual positivity for ANCA and AECA. Normal ranges are shadowed.

vWF assay

vWF was elevated in the four cases studied and changes in vWF activity mirrored changes in ANCA, AECA and disease activity (Fig. 5). In patients 1 and 4, vWF levels remained elevated despite clinical remission, but in patients 2 and 3 vWF levels fell into the normal range during the recovery phase (Fig. 5).

DISCUSSION

We report that 30% of the ANCA-positive patients in this study, with either Wegener's granulomatosis or microscopic polyarteritis, had positive IgG AECA, compared with 6% of the ANCA-negative disease controls. This rose to 52% when AECA of all isotypes were included. In a recent report, cytotoxic, complement-fixing autoantibodies to HUVEC were detected using a standard cross-match technique in 86% of 21 patients with confirmed systemic vasculitis (Brasile *et al.*, 1989); a further study (Ferraro *et al.*, 1990) demonstrated the presence of AECA in 50% of patients with Wegener's granulomatosis and microscopic polyarteritis but could not find lytic activity of AECA-positive sera against endothelial cells. The variance in results may be accounted for by methodological differences or by a highly selected patient group. In our study one-third of the patients were already receiving immunosuppression at the time of the sample. We have found that AECA levels reflect clinical disease activity, and we have also shown a prompt fall once treatment is started (Fig. 5).

The correlation of ANCA with IgG AECA is not explained by cross-reactivity between the two assay systems, but appears to be due to two distinct autoantibody populations. Although ANCA have been implicated to have a role in the pathogenesis of vasculitis, there must be other important factors, because there is frequently a discrepancy during the remission phase of vasculitis between the serological relapse of ANCA and the appearance of clinical disease relapse (Jayne *et al.*, 1990). One possible explanation requires both ANCA and cytolytic AECA for injury; as ANCA are capable of activating neutrophils and ANCA also bind to monocytes, they may be capable of producing release of cytokines by monocytes (van der Woude *et al.*, 1985; Falk *et al.*, 1989). In Kawasaki disease, excess monocyte cytokine release is followed by endothelial cell activation and lysis by circulating AECA; recovery is associated with a fall in cytokine release, and not a fall in AECA (Leung *et al.*, 1989).

AECA appear to be markers for disease activity in systemic vasculitis, in a way similar to ANCA. The sequential studies showed a close relation of AECA binding to changes in disease activity for all patients studied. The increase in ANCA and AECA towards the end of the study in patient 1 occurred without clinical relapse; however, at this time the patient was still receiving prednisolone and cyclophosphamide which may have prevented disease expression (Fig. 5). If ANCA and AECA levels do move together, they may be under similar immunoregulatory control; a role for autoanti-idiotypes has been demonstrated in the control of the ANCA response, and it is interesting to speculate that AECA may carry a common idiotype to those on ANCA and thus be subjected to the same regulation (Jayne *et al.*, 1989b). This situation may be similar to the polyclonal activation of autoantibodies induced in the BN rat with mercuric chloride, where the subsequent autoantibody kinetics show two different patterns, reflecting differing regulation; anti-glomerular basement membrane and anti-thyroglobulin antibodies rise and fall at the time of tissue injury; in contrast, anti-DNA and anti-RNA antibodies appear and remain at the same titre (Pusey *et al.*, 1990). Polyclonal activators such as penicillamine and hydralazine also cause systemic vasculitis in man (Banfi, Imbasciati & Guerra, 1983; Mason & Lockwood, 1986).

As sera from patients with Wegener's granulomatosis has already been shown to contain IgG that binds to human cultured glomerular endothelial cells (Abbott *et al.*, 1989), data generated from a HUVEC system may have more general importance. However, the target for AECA needs to be defined before the relevance of AECA to pathogenesis can be determined. An alternative explanation for the presence of AECA is that they are an epiphenomenon, generated by an immune response to hidden endothelial self-antigens exposed after endothelial cell damage by cytotoxic T cells or neutrophils. The correlation we have found of AECA with disease activity as well as the ability of AECA to cause endothelial cell lysis in other systems would argue against this explanation. However, we know little of the autoreactive T cell response in vasculitis, infiltrating T cells are present in vasculitic tissue and they may have a dominant role in these autoimmune disease (Nolasco *et al.*, 1987).

ANCA were the first autoantibodies consistently found in systemic vasculitis and they have rapidly led to advances in the diagnosis, management and classification of these conditions. Although less specific for vasculitis, we have confirmed the presence of AECA in a proportion of vasculitic sera and have demonstrated a dynamic relation to disease activity and release. The high incidence of AECA together with its known potential to cause cytotoxicity indicates that this antibody system could

play a major role in the pathogenesis of vasculitis. The correlation of antibody levels with disease activity would also support this. Further study of the clinical characteristics of AECA-positive and AECA-negative patients is required, but it is possible that AECA, like ANCA, will be important in the classification of vasculitis as well as advancing our understanding of its pathogenesis.

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REFERENCES

ABBOTT, F., JONES S.J., LOCKWOOD, C.M. & REES, A.J. (1989) Autoantibodies to glomerular antigens in patients with Wegener's granulomatosis. *Nephrol. Dial. Transplant.* **4,** 1.

BAGUELEY, E., BROWN, K.A., HASKARD, D., HARRIS, E.N. & HUGHES, G.R.V. (1987) Anti-endothelial antibodies in connective tissue disease. *Br. J. Rheumatol.* 26, A164 (Abstract).

BAGUELEY, E. & HUGHES, G.R.V. (1988) Lytic IgG anti-endothelial cell antibodies in vasculitis. *Lancet*, **ii,** 907 (Letter).

BANFI, G., IMBASCIATI, E. & GUERRA, L. (1983) Extracapillary glomerulonephritis with necrotizing vasculitis in D-penicillamine treatment rheumatoid arthritis. *Nephron*, **33,** 56.

BRASILE, L., KREMER, J.M., CLARKE, J.L. & CERILLI, J. (1989) Identification of an autoantibody to vascular endothelial cell-specific antigens in patients with systemic vasculitis. *Am. J. Med.* **87,** 74.

BROWN, Z., NEILD, G.H., WILLOUGHBY, J.J., SOMIA, N.V. & CAMERON, J.S. (1986) Increased factor VIII as an index of vascular injury in cyclosporin nephrotoxicity. *Transplantation*, **42,** 150.

CINES, D.B., LYSS, A.P., REEBER, M. & DEHORITIAS, R.J. (1984) Presence of complement fixing anti-endothelial cell antibodies in systemic lupus erythematosus. *J. clin. Invest.* **73,** 611.

FALK, R.J. & JENNETTE, J.C, (1988) Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N. Engl. J. Med.* **318,** 1651.

FALK, R.J., TERRELL, R., HUNEYCUTT-CALDER, L. & JENNETTE, J.C. (1989) Anti-neutrophil cytoplasmic autoantibodies (ANCA) stimulate neutrophil activation in vivo. *Kidney Int.* **35,** 346 (Abstract).

FERRARO, G., MERONI, P.L., TINCANI, A., SINICO, A., BARCELLINI, W., RADICE, A., GREGORINI, G., FROLDI, M., BORGHI, M.O. & BALESTRIERI, G. (1990) Anti-endothelial cell antibodies in patients with Wegener's granulomatosis and micropolyarteritis. *Clin. exp. Immunol.* **79,** 47.

HEURKENS, A.H.M., HIEMSTRA, P.S., LAFEBER, G.J.M., DAHA, M.R. & BREEDVELD (1989) Anti-endothelial cell antibodies in patients with rheumatoid arthritis complicated by vasculitis. *Clin. exp. Immunol.* **78,** 7.

HOLT, C.M., LINDSEY, N., MOULT, J., MALIA, R.G., GREAVES, M., HUME, A., NOWELL, N.R. & HUGHES, P. (1989) Antibody-dependent cellular cytotoxicity of vascular endothelium: characterisation and pathogenic associations in systemic sclerosis. *Clin. exp. Immunol.* **78,** 359.

JAFFE, E.A., NACHMAN, R.L., BECKER, C.G. & MINOCK, C.R. (1973) Culture of human endothelial cells derived from umbilical veins. *J. clin. Invest.* **52,** 2745.

JAYNE, D.R.W., JONES, S.J., SEVERN, A., SHAUNAK, S., MURPHY, J. & LOCKWOOD, C.M. (1989a). Severe pulmonary hemorrhage and systemic vasculitis in association with circulating anti-neutrophil cytoplasm antibodies of IgM class only. *Clin. Nephrol.* **32,** 101.

JAYNE, D.R.W., ROSSI, F., KAZATCHKINE, M.D. & LOCKWOOD, C.M. (1989b) Anti-idiotypes against autoantibodies to neutrophil cytoplasmic antigens are present in pooled human immunoglobulin and post-recovery sera. In *7th International Congress of Immunology* P. 273. Gustav Fischer Verlag, Heidelberg.

JAYNE, D.R.W., HEATON, A., EVANS, D.B. & LOCKWOOD, C.M. (1990) Sequential anti-neutrophil cytoplasm antibody titres in the management of systemic vasculitis. *Nephrol. Dial. Transplant.* (In press).

LAI, K.N. & LOCKWOOD, C.M. (1990) Effect of anti-neutrophil cytoplasm autoantibodies on signal transduction in human neutrophils and HL-60 cells. *Kidney Int.* **37,** 442 (Abstract).

LEUNG, D.Y.M., KURT-JONES, E., NEWBURGER, J.W., COTRAN, R.S., BURNS, J.C. & POBER, J.S. (1989) Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. *Lancet*, **i,** 1298.

LOCKWOOD, C.M., BATES, D., JONES, S.J., WHITAKER, K.B., MOSS, D.W. & SAVAGE, C.O.S. (1987) Association of alkaline phosphatase with an autoantigen recognised by circulating anti-neutrophil antibodies in systemic vasculitis. *Lancet*, **i,** 716.

LUDEMANN, J., UTECHT, B. & GROSS, W.L. (1990) Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis recognize an elastinolytic enzyme. *J. exp. Med.* **171,** 357.

MASON, P.D. & LOCKWOOD, C.M. (1986) Rapidly progressive nephritis in patients taking hydralazine. *J. clin. Lab. Immunol.* **20,** 151.

NOLASCO, F., CAMERON, J.S., HARTLEY, B., COELHO, A., HILDRETH, G. & REUBEN, R. (1987) Intraglomerular T cells and monocytes in nephritis: study with monoclonal antibodies. *Kidney Int.* **31,** 1160.

PUSEY, C.D., BOWMAN, C., MORGAN, A., WEETMAN, A.P., HARTLEY, B. & LOCKWOOD, C.M. (1990) Kinetics and pathogenicity of antibodies induced by mercuric chloride in the brown Norway rat. *Clin. exp. Immunol.* **81,** 76.

SAVAGE, C.O.S., WINEARLS, C.G., JONES, S., MARSHALL, P.D. & LOCKWOOD, C.M. (1987) Prospective study of radioimmunoassay for antibodies against neutrophil cytoplasm in diagnosis of systemic vasculitis. *Lancet*, **i,** 1389.

SAVAGE, C.O.S., TIZARD, J., JAYNE, D.R.W., LOCKWOOD, C.M. & DILLON, M.J. (1989) Antineutrophil cytoplasm antibodies in Kawasaki disease. *Arch. Dis. Child.* **64,** 360.

VAN DER WOUDE, F.J., RASMUSSEN, N., LOBATTO, S., WIIK, A., PERMIN, H., VAN ES, L.A., VAN DER GIESSEN, M., VAN DER HEM, G.K. & THE, T.H. (1985) Autoantibodies against neutrophils and monocytes; tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet*, **i,** 425.