

Autoantibodies against neutrophil cytoplasm components in systemic lupus erythematosus and in hydralazine-induced lupus

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SUMMARY

Anti-neutrophil cytoplasm antibody (ANCA) has been shown to be no marker of systemic lupus erythematosus (SLE) including lupus nephritis or of progressive systemic sclerosis (PSS). Antibodies against myeloperoxidase (anti-MPO) and elastase, two granulocyte lysosomal enzymes, were found in patients with SLE but not in those with PSS, except for one patient who had anti-MPO. Anti-MPO was present in 21% of patients with SLE, and at low concentrations in about 80% of these cases. Anti-elastase was found in four patients with SLE. In another group of six patients with a SLE-like syndrome induced by anti-hypertensive treatment with the anti-hypertensive hydralazine, anti-MPO antibodies occurred in all six, and anti-elastase antibodies in five. Monitored during a 2-year follow-up period, anti-MPO antibodies were found to persist, whereas anti-elastase antibodies were rapidly eliminated, after withdrawal of the drug.

Keywords anti-neutrophil cytoplasm antibodies anti-myeloperoxidase anti-elastase hydralazine systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is characterized by a number of immunological abnormalities, including B cell hyperactivity and the presence of autoantibodies (Tan, 1982; Martinez-Corden, Alcocer-Varela & Alarcon-Segovia, 1986). The presence of some of the autoantibodies may be related to certain clinical manifestations; anti-DNA is associated with severe disease (Tron & Bach, 1977), anti-cardiolipin with arterial thrombosis (Harris *et al.*, 1983; Sturfelt *et al.*, 1987), and anti-histone antibodies with drug-induced lupus (Hardin & Thomas, 1983).

Recently, a new class of autoantibodies has been described, which react with cytoplasmic components of neutrophil granulocytes. The first of these to be reported was anti-neutrophil cytoplasmic antibody (ANCA), found in patients with Wegener's granulomatosis (van der Woude *et al.*, 1985), and later in patients with other vasculitic conditions (Savage *et al.*, 1987; Feehally *et al.*, 1987; Nässberger *et al.*, 1989b).

Anti-myeloperoxidase antibodies (Anti-MPO) were recently reported in patients presenting with idiopathic necrotizing and crescentic glomerulonephritis (Falk & Jennette, 1988). In addition, circulating antibodies against elastase have been found in a few SLE patients including some who had been on hydralazine treatment (Nässberger *et al.*, 1989a).

Autoantibodies to neutrophil cytoplasm components have gained importance as diagnostic tools, and may also be used to obtain information about pathogenetic mechanisms and disease activity. Both vasculitis and glomerulonephritis are characteristic manifestations of SLE. The main purpose of the present study was to evaluate the frequency and to some extent the significance of neutrophil cytoplasm autoantibodies in SLE generally, and in some patients with hydralazine-induced SLE.

PATIENTS AND METHODS

SLE patients

The 96 patients investigated took part in a prospective SLE control programme at the Department of Rheumatology, University Hospital, Lund, Sweden, during the period 1981–1988. Eighty samples constituted the initial samples from patients retrieved from a defined population (Jonsson, Nived & Sturfelt, 1989). Sixteen additional samples were obtained from 16 patients with severe SLE and with kidney involvement. Samples were obtained during periods of active disease, before treatment was initiated. All patients fulfilled at least four ARA revised criteria for the diagnosis of SLE (Tan *et al.*, 1982).

Patients with hydralazine-induced SLE

Six patients (five women, one man) who at onset of SLE had been on hydralazine treatment for 1–11 years (median 3 years;

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median dose 75 mg/day). These patients fulfilled at least four ARA revised criteria for the diagnosis of SLE (Tan *et al.*, 1982).

Patients with progressive systemic sclerosis (PSS)

The 76 patients with PSS fulfilled the revised criteria for the diagnosis of this condition (Masi *et al.*, 1980).

Serum samples

Samples were collected at admission and stored at -80°C until analysis. Consecutive samples were available from five of the six hydralazine-treated patients. Twenty-one serum samples from healthy blood donors were used as negative controls in the anti-MPO and anti-elastase ELISA.

Indirect immunofluorescence for detection of ANCA

The general procedure was that outlined by us in a previous study (Nässberger *et al.*, 1989b). Briefly, granulocytes were isolated as described by Böyum (1968) on Lymphoprep (Nyggaard, Oslo, Norway). Patient sera were applied at a dilution of 1/10. Detection was carried out by fluorescein-conjugated rabbit anti-human IgG (Fab')₂ fragment (Dakopatts Glostrup, Denmark). Granulocyte nuclear fluorescence was monitored in the course of the investigation for ANCA in sera diluted 1/10.

Detection of ANCA by ELISA

All sera were tested for ANCA with a commercial ELISA kit (Biocarb, Lund, Sweden); sera and conjugate were diluted as recommended by the manufacturer.

ELISA for detection of anti-MPO and anti-elastase antibodies

Human MPO was purified according to Matheson, Wong & Travis (1981) and Olsson, Olofsson & Odeberg (1972), with slight modifications and using buffy coats. Purified elastase was kindly supplied by Dr A. Heubner, Merck, Darmstadt, FRG. Microtitre plates (Dynatech) were coated with 1 μg of MPO or of elastase in 0.1 ml of sodium carbonate buffer 0.05 mol/l, pH 9.6, per well for 18 h at room temperature. Blocking was then performed at 4°C with 1% gelatin overnight. Serum samples (0.1 ml) at a dilution of 1/400 in phosphate-buffered saline (PBS) containing 0.5% Tween 20 were applied in duplicate to antigen-coated wells and to a well coated with gelatin alone. After being left for 2 h at room temperature the plates were washed six times with the buffer. The plates were developed with preparation of F(ab')₂ rabbit anti-IgG (Dakopatts) conjugated according to Voller, Bidwell & Bartlett (1976) with alkaline phosphatase from bovine intestine type VII (Sigma, St Louis, MO). The conjugate was applied at a dilution of 1/1000 for 2 h at room temperature. The plates were washed again six times before addition of substrate *p*-nitrophenylphosphate (Sigma). Absorbance was recorded after 30 min at 405 nm in a Multiskan spectrophotometer. For each sample, the absorbance value was corrected for background by subtraction with the absorbance in the well coated with gelatin alone. In order to compensate for possible inter-assay variation, a positive control serum yielding an absorption value of 1.0 was always included, as well as a negative control serum. Absorbance values below 0.155 for MPO and below 0.1 for elastase (3 s.d. above the mean value) was regarded as negative. Each plate included a positive and a negative control serum.

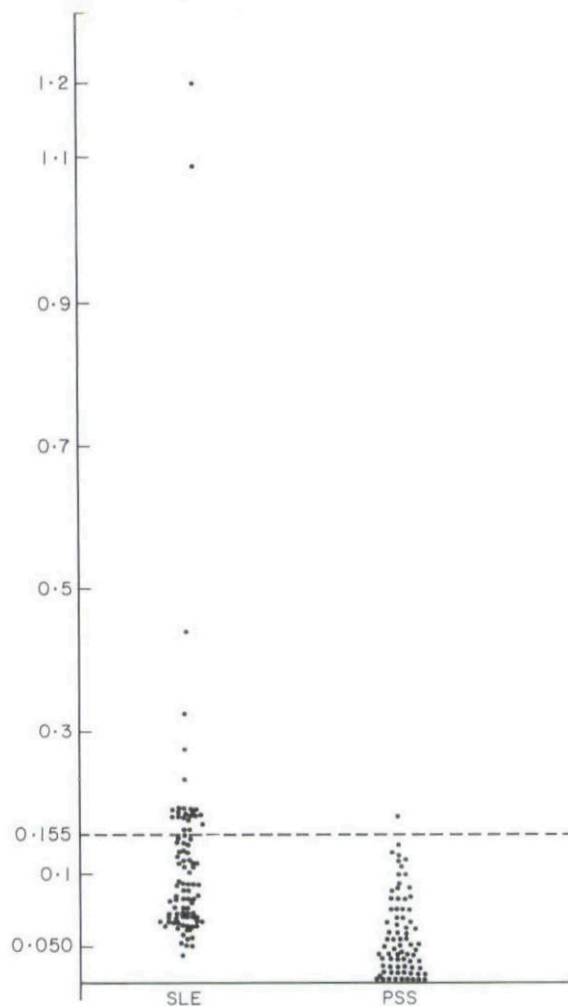


Fig. 1. Distribution of anti-myeloperoxidase antibody concentrations in sera from patients with genuine systemic lupus erythematosus (SLE) and progressive systemic sclerosis (PSS). Values below the dashed line (0.155) are negative.

RESULTS

Detection of ANCA by indirect immunofluorescence and ELISA

None of the SLE or PSS patients exhibited circulating ANCA as judged by indirect immunofluorescence or by ELISA. Ninety-three per cent of the SLE patients and 65% of the PSS patients showed fluorescence patterns restricted to neutrophil nuclei in the assay for detection of ANCA by indirect immunofluorescence.

Circulating anti-MPO

In control sera from healthy adults values were below 0.155. Circulating anti-MPO were found in 21% of the patients with genuine SLE, 80% of these 21% had low levels of anti-MPO (Fig. 1). All six patients treated with hydralazine had circulating antibodies against MPO, although the level was low in one case (0.34 corrected absorbance values). In the other five patients the concentration of the circulating antibodies remained high even 24 months after withdrawal of the drug (Fig. 2), the time period of follow up. There was no correlation between the concentration of anti-MPO antibody and clinical features, or any of the

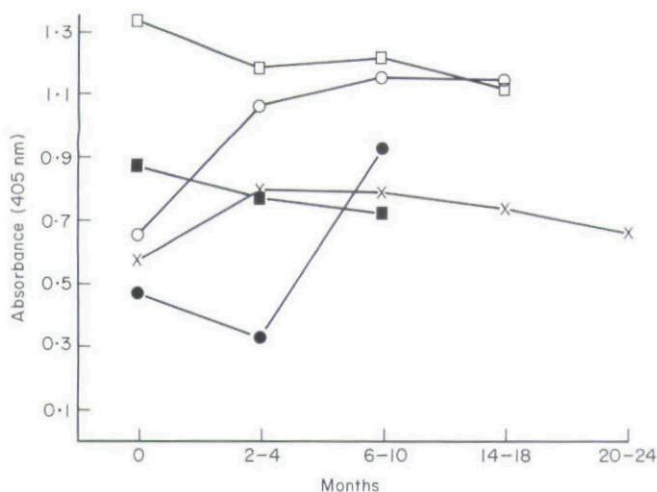


Fig. 2. The kinetic pattern of anti-myeloperoxidase antibodies in patients with hydralazine-induced SLE-like syndrome. Time-point 0 indicates withdrawal of the drug. Absorbance values below 0.155 are negative.

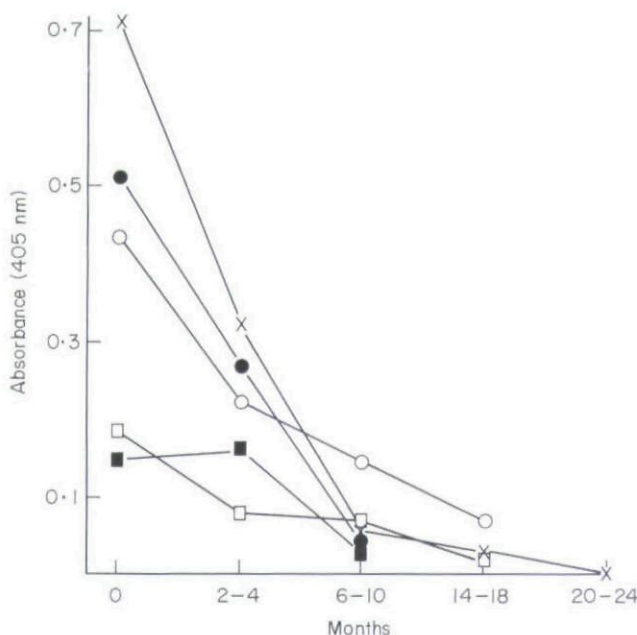


Fig. 3. The kinetic pattern of anti-elastase antibodies in patients with hydralazine-induced SLE-like syndrome. Time-point 0 indicates withdrawal of the drug. Absorbance values below 0.1 are negative.

laboratory variables. One patient with PSS had circulating anti-MPO at low concentration.

Antibodies against elastase

We found circulating anti-elastase antibodies in nine SLE patients, five from the hydralazine-treated group, and four from the genuine SLE group. In the patients with hydralazine-induced SLE, anti-elastase antibody concentrations were initially high, declined rapidly after drug withdrawal (Fig. 3) and disappeared completely within 6 months in four of the five cases. The patient

with the low level of circulating anti-MPO lacked anti-elastase. Of the four patients with genuine SLE, three had central nervous system involvement of various degrees. There was no difference between SLE patients with and without nephritis with regard to anti-MPO or anti-elastase antibodies. Occurrence of anti-elastase and anti-MPO antibodies did not distinguish between the presence or absence of renal involvement in the patients.

DISCUSSION

Our findings clearly demonstrate the absence of ANCA in patients with SLE including those presenting with active kidney involvement. This is in contrast to results reported by Falk & Jennette (1988) who found ANCA in five of 11 patients with lupus nephritis; however, they did not clearly differentiate between ANCA and anti-MPO antibodies. Thus, on the basis of the present findings obtained by investigation with two different techniques, classic ANCA (van der Woude *et al.*, 1985) would not seem to be a marker of SLE. ANCA were also absent in the PSS group, but as the presence of ANCA has been associated with systemic vasculitic disorders, their absence in patients with PSS was to be expected, since the vascular disorders in conjunction with this condition are non-inflammatory. By contrast, circulating anti-MPO and anti-elastase antibodies were found in patients with SLE. In the PSS group only one patient had circulating anti-MPO antibodies, and then only at low concentration, and in complete absence of anti-elastase.

Of the four patients with anti-elastase antibodies in the genuine SLE group, three had CNS involvement, as reported earlier (Nässberger *et al.*, 1989a); however this finding needs further verification.

Another noteworthy finding was that of a subgroup consisting of six patients with circulating antibodies against MPO and elastase. They had all been receiving hydralazine, which is known to cause a SLE-like syndrome (Cameron & Ramsay, 1984) cutaneous vasculitis (Bernstein, Egerton-Vernon & Webster, 1980) and isolated kidney involvement (Björk *et al.*, 1983). Hydralazine has been reported to give rise to this syndrome in 3–20% of cases (Koch-Weser, 1976; Bing *et al.*, 1980). It is possible that the combined presence of anti-MPO and anti-elastase is fairly diagnostic of hydralazine-induced disease.

It is difficult to give a clear and acceptable explanation of the occurrence of anti-MPO and anti-elastase, or of the difference kinetic patterns between these two autoantibodies. The persistence of anti-MPO in drug-induced SLE is in accord with what has been found for anti-nuclear antibodies, which also seem to remain for a long time, often years after withdrawal of the drug, although their titres decline (Cameron & Ramsay, 1984). However, the concentrations of anti-elastase antibodies declined rapidly. Another type of antibody of similar appearance and with rapid elimination is lymphocytotoxic antibody found to occur in cases of a lupus-like syndrome induced by procainamide (Blustein, Redelman & Zvaifler, 1981).

It seems almost certain that ANCA are of no importance in SLE or PSS, neither from diagnostic nor pathogenetic points of view. Presence of ANCA is an indicator of vasculitic disease of an origin other than SLE. Although a large proportion of the SLE patients manifested circulating anti-MPO antibodies at low levels, we were unable to correlate either high or low levels of anti-MPO with other immunological variables or to clinical data. The importance of anti-MPO in SLE remains to be further

elucidated. This study shows, however, that anti-MPO and anti-elastase antibodies may become valuable as diagnostic markers in hydralazine-induced SLE. The importance of these auto-antibodies for the underlying pathogenetic mechanisms remains unclear and needs further investigations. Moreover, it is not known at present whether these antibodies occur in patients who develop SLE-like disease due to drugs other than hydralazine (Hess, 1988).

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REFERENCES

- BERNSTEIN, R.M., EGERTON-VERNON, J. & WEBSTER, J. (1980) Hydralazine-induced cutaneous vasculitis. *Br. med. J.* **280**, 156.
- BING, R.F., RUSSELL, G.J., THURSTON, H. & SWALES, J.D. (1980) Hydralazine in hypertension: is there a safe dose? *Br. med. J.* **281**, 353.
- BJÖRK, S., WESTBERG, G., SVALANDER, C. & MULEC, H. (1983) Rapidly progressive glomerulonephritis after hydralazine. *Lancet*, **ii**, 42.
- BLUESTEIN, H.G., REDELMAN, D. & ZVAIFLER, N.J. (1981) Procainamide—lymphocyte reactions. A possible explanation for drug-induced autoimmunity. *Arthritis Rheum.* **24**, 1019.
- BÖYUM, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Invest.* **977** (Suppl.), 21.
- CAMERON, H.A. & RAMSAY, L.E. (1984) The lupus syndrome induced by hydralazine a common complication with low dose treatment. *Br. med. J.* **289**, 410.
- 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.
- FEEHALLY, J., WHEELER, D.C., WALLS, J., JONES, S., LOCKWOOD, C.M. & SAVAGE, C.O.S. (1987) A case of microscopic polyarteritis associated with antineutrophil cytoplasmic antibodies. *Clin. Nephrol.* **17**, 214.
- HARDIN, J.A. & THOMAS, J.O. (1983) Antibodies to histones in systemic lupus erythematosus; localization of prominent autoantigens on histones H1 and H2B. *Proc. Natl. Acad. Sci. USA*, **80**, 7410.
- HARRIS, E.N., GHARAVI, A.E., BOEY, M.L., POTEI, B.M., MACKWORTH-YOUNG, C.G., LOIZON, S.V. & HUGHES G.R.V. (1983) Anticardiolipin antibodies; detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet*, **ii**, 1211.
- HESS, E. (1988) Drug-induced related lupus. *N. Engl. J. Med.* **318**, 1460.
- JONSSON, H., NIVED, O. & STURFELT, G. (1989) Outcome of systemic lupus erythematosus: a prospective study of patients from a defined population. *Medicine*, **68**, 141.
- KOCH-WESER, J. (1976) Drug therapy: hydralazine. *N. Engl. J. Med.* **295**, 320.
- MARTINEZ-CORDENO, E., ALCOCER-VARELA, J. & ALARCON-SEGOVIA, D. (1986) Stimulating and differentiation factor for human B lymphocytes in systemic lupus erythematosus. *Clin. exp. Immunol.* **65**, 598.
- MASI, A.T., MEDSGER, T.A., RODMAN, G.P., ALTMAN, R.D., D'ANGELO, W.A., FRIES, J.H., LEROY, C., KIRSCHNER, A.B., MACKENZIE, H., MCSHANE, D.J., MYERS, A.R. & SHARP, G.C. (1980) Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum.* **23**, 581.
- MATHESON, N.R., WONG, P.S. & TRAVIS, J. (1981) Isolation and properties of human neutrophil myeloperoxidase. *Biochemistry*, **20**, 325.
- NÄSSBERGER, L., JONSSON, H., SJÖHOLM, A.G., STURFELT, G. & HEUBNER, A. (1989a) Circulating anti-elastase in systemic lupus erythematosus. *Lancet*, **i**, 509.
- NÄSSBERGER, L., SJÖHOLM, A.G., BYGREN, P., THYSELL, H., HOJER-MADSEN, M. & RASMUSSEN, N. (1989b) Circulating antineutrophil cytoplasm antibodies in patients with rapidly progressive glomerulonephritis and extracapillary proliferation. *J. intern. Med.* **225**, 191.
- OLSSON, I., OLOFSSON, T. & ODEBERG, H. (1972) Myeloperoxidase-mediated iodination of granulocytes. *Scand. J. Haematol.* **9**, 483.
- PERRY, H.M. (1973) Late toxicity to hydralazine resembling systemic lupus erythematosus or rheumatoid arthritis. *Am. J. Med.* **54**, 58.
- PERRY, H.M., TAN, E.M., CARMODY, S. & SAKAMOTO, A. (1970) Relationship of acetyl transferase activity to antinuclear antibodies and toxic symptoms in hypertensive patients treated with hydralazine. *J. Lab. clin. Med.* **76**, 114.
- 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 vasculitides. *Lancet*, **i**, 1389.
- STURFELT, G., NIVED, O., NÖRBERG, R., THORSTENSSON, R. & KROOK, K. (1987) Anticardiolipin antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **30**, 1.
- TAN, E.M. (1982) Autoantibodies to nuclear antigen. *Adv. Immunol.* **33**, 167.
- TAN, E.M., COHEN, A.S., FRIES, J., MASI, A.T., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G., TALAL, N. & WINCHESTER, R. (1982) The revised 1982 criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271.
- TRON, F. & BACH, J.F. (1977) Relationships between antibodies to native DNA and glomerulonephritis in systemic lupus erythematosus. *Clin. exp. Immunol.* **28**, 426.
- VOLLER, A., BIDWELL, D.E. & BARTLETT, A. (1976) Enzyme immunoassays in diagnostic medicine. *Bull. WHO*, **53**, 55.
- VAN DER WOUDE, F.J., LOBATO, S., PERMIN, J., VAN DER GIESSEN, RASMUSSEN, N., WIK, A., VAN ES, L.A. & VAN DER HEM, K. (1985) Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet*, **i**, 425.

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