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ORIGINAL ARTICLE

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Antineutrophil cytoplasmic antibodies in patients with systemic lupus erythematosus: prevalence, antigen specificity, and clinical associations

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Abstract Fifty-five patients with systemic lupus erythematosus (SLE) were examined for antineutrophil cytoplasmic antibodies (ANCA) by indirect immunofluorescence (IIF). Enzyme-linked immunosorbent assay (ELISA) for ANCA against myeloperoxidase (MPO), lactoferrin (LF), proteinase 3 (PR3), elastase (HLE), and bactericidal/permeability-increasing protein (BPI) was performed. The prevalence of ANCA by IIF was 29.1% (16/55 patients). MPO-ANCA were found in 10.9% (6/55), LF-ANCA in 18.2% (10/55), PR3-ANCA in 12.7% (7/55), BPI-ANCA in 23.6% (13/55), and HLE-ANCA in 1.8% (1/55). The levels of BPI-, LF-, and PR3-ANCA correlated with disease activity. A significant association between serositis and the presence of BPI-, LF-, and PR3-ANCA was observed, and PR3-ANCA were found to be associated with arthritis as well. Our results demonstrate that ANCA of various specificities occur in SLE, and BPI appears to be an important target antigen.

Keywords Antineutrophil cytoplasmic antibodies · Systemic lupus erythematosus

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Introduction

Antineutrophil cytoplasmic antibodies (ANCA) are a system of autoantibodies targeting a variety of cytoplasmic constituents of neutrophils and monocytes. There are two main types of ANCA as determined by indirect immunofluorescence assay (IIF): C-ANCA that produce a cytoplasmic staining pattern and P-ANCA, producing a perinuclear staining pattern on ethanol-fixed neutrophils [1, 2]. Most C-ANCA react with proteinase 3 (PR3), whereas P-ANCA react with myeloperoxidase (MPO) but also with lactoferrin (LF), elastase (HLE), and other myeloid proteins [3, 4, 5]. C-ANCA with specificity to PR3 are considered important serological markers for Wegener's granulomatosis (WG) [6]. P-ANCA with specificity to MPO have an established association with other necrotising small vessel vasculitides such as microscopic polyangiitis (MPA) and pauci-immune crescentic glomerulonephritis [4, 7]. In addition, a C-ANCA (atypical) pattern that produces homogeneous granular cytoplasmic staining of neutrophils without central accentuation was identified, but its corresponding antigen specificities are usually unknown [8].

The presence of circulating ANCA in serum is not confined to patients with systemic small vessel vasculitides. It can also be found in connective tissue diseases such as systemic lupus erythematosus (SLE) [9], but this occurrence has not received the same attention as in primary systemic vasculitides due to the presence of a wide array of autoantibodies, some of which are closely associated with specific clinical manifestations and disease activity. Vasculitis and glomerulonephritis are characteristic manifestations of SLE, which frequently appears in the differential diagnosis of primary systemic small vessel vasculitides. This fact necessitates the examination and characterisation of ANCA in SLE in order to establish their diagnostic value and the effective use of ANCA testing in clinical practice.

Recently, a number of articles concerning ANCA prevalence in SLE appeared. ANCA directed against

various neutrophil cytoplasmic constituents have been demonstrated by antigen-specific assays, but the antigens responsible for ANCA reactivity in SLE have still not been fully determined and the role of ANCA in SLE remains unclear. Moreover, reports are controversial concerning their clinical relevance [10, 11, 12, 13, 14, 15, 16].

The objectives of this study were to evaluate the prevalence of ANCA and their target antigens in Bulgarian patients with SLE, to correlate the presence of ANCA with disease activity, and to determine the possible association of ANCA with some of the clinical manifestations of SLE.

Patients and methods

Patients

From January 1996 to July 2000, serum samples were collected from 55 consecutive SLE patients (two male, 53 female, mean age 36.5 years, range 14–68 years). The samples included in this study were obtained for diagnostic purposes and routine testing from outpatients and inpatients of the Thracian University Hospital Department of Internal Medicine in Stara Zagora, Bulgaria. All patients fulfilled the 1982 revised criteria of the American Rheumatism Association for the diagnosis of SLE [17] and were enrolled in the study independently of disease status and medication. Patients with drug-induced lupus were excluded. Medical records of the study subjects were thoroughly reviewed retrospectively and all clinical and laboratory data relevant to SLE were registered. For each patient, the organ system involvement and disease activity in SLE at the time of the sample collection were assessed by a rheumatologist (M.D.) using the SLE disease activity index (SLEDAI) [18]. Any value above 0 was considered as indicating active disease, which was diagnosed in all 55 patients (median SLEDAI score 8, range 1–33). The frequencies of clinical manifestations as determined by SLEDAI were: central nervous system 5.5%, vascular 21.9%, renal 21.9%, musculoskeletal 52.7%, serosal 32.8%, dermal 72.7%, constitutional 41.8%, and hematologic 56.4%.

Controls

Twenty blood donors and 20 patients with reactive arthritis served as controls (four male, 36 female, mean age 32.6 years, range 11–63 years). All serum samples were stored frozen at –20°C until assayed.

Indirect immunofluorescence assay

Indirect immunofluorescence assay (IIF) for ANCA was performed by a standard method delineated at the First International Workshop on ANCA, Copenhagen 1988 [19]. Briefly, human peripheral blood neutrophils were deposited on glass slides by cytospin, fixed in 96% ethanol at 4°C for 5 min, and air-dried. The slides were incubated with patient sera in a dilution of 1:20 in phosphate-buffered saline (PBS); titration of the sera was not performed. Antibody binding was detected with fluorescein isothiocyanate (FITC)-labelled sheep antihuman IgG (Binding Site, Birmingham, UK). The slides were examined with an epifluorescence microscope at ×400 magnification by two independent investigators. Positive and negative control sera were included for all assays. Fluorescence patterns of ANCA were classified as cytoplasmic (C-ANCA), perinuclear (P-ANCA), or C-ANCA (atypical) [20]. All samples with perinuclear or combined perinuclear and nuclear staining patterns were additionally tested on formaldehyde-fixed granulocytes (Binding Site) for differentiation of P-ANCA from antinuclear antibodies (ANA). If there was no cytoplasmic fluorescence on formaldehyde-fixed granulocytes, the result was considered negative.

Antinuclear antibodies were detected on human epithelioma type 2 (HEp-2) cells using a commercial kit system (Binding Site). Serum samples positive for ANA at the screening dilution of 1:40 were subsequently tested at twofold dilution to determine a titre. The fluorescence patterns were assessed and recorded as homogeneous, rim, speckled, nucleolar, or centromeric.

ELISA for ANCA specificities

Commercial quantitative ELISA kits (Binding Site) were used for detection of anti-PR3, anti-BPI, and anti-MPO. Serum dilution of 1:50 was used in all assays. The test samples were run in duplicates along with six standards (3.1 U/ml, 6.2 U/ml, 12.5 U/ml, 25 U/ml, 50 U/ml, and 100 U/ml). Peroxidase-labelled antihuman IgG was used as a second antibody. The reaction was visualised with 3,3′,5,5′ tetramethylbenzidine (TMB) as a substrate and read at 450 nm. Following the manufacturer's instructions, values exceeding 3.5 U/ml for anti-PR3, 15 U/ml for anti-BPI, and 9 U/ml for anti-MPO were regarded as positive.

The use of ELISA for detecting anti-HLE and anti-LF has been previously described [21]. Briefly, ELISA microtitre plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with elastase from human leukocytes (Sigma, St. Louis, Mo., USA) and lactoferrin purified from human milk (Sigma) at concentrations of 1 μg/ml and 3 μg/ml, respectively, in 0.05 M carbonate buffer (pH 9.6). After washing with PBS containing 0.05% polysorbate (Tween) 20 (PBS/T20), the wells were incubated with blocking buffer (1% bovine serum albumin in PBS) for 1 h at room temperature. Test and control sera were applied to duplicate wells at a dilution of 1:50 in dilution buffer (1% bovine serum albumin in PBS/T20) for 1 h at room temperature. Bound antibodies were detected by incubation for 1 h at room temperature with alkaline phosphatase-conjugated goat antihuman IgG (γ-chain specific) antibody (Sigma) diluted to 1:20,000 in dilution buffer. After a final wash, the substrate p-nitrophenyl phosphate at a concentration of 1 mg/ml in 0.05 M carbonate buffer (pH 9.8) containing 1.0 mM $MgCl_2$ was added and incubated for 30 min at room temperature. The optical density (OD) was measured at 405 nm. Results were expressed as OD index (ODI), which is the ratio of OD in the patient serum to the mean of normal control sera. The positive cutoff values (2.41 ODI for anti-HLE and 2.00 ODI for anti-LF) were determined as the mean value of 40 normal sera ± 3 SD.

Statistics

To establish the statistical significance of the observed differences, Fisher's exact test (frequencies) and the nonparametric Mann-Whitney U test (means) were used. Optical density values are expressed as mean $\pm$ SD. Possible correlations between levels of autoantibodies and SLEDAI score were examined using Spearman's correlation test. For all statistical tests, $P < 0.05$ was considered significant.

Results

The results of ANCA testing by IIF and antigen-specific ELISA in SLE patients are summarised in Table 1.

Prevalence of ANCA by IIF

Perinuclear/nuclear staining patterns on ethanol-fixed granulocytes occurred in 41 of 55 patient sera, and one

Table 1 Prevalence of ANCA in 55 SLE patients. Numbers in parentheses are percentages. *ELISA + ve* ELISA-positive, *ELISA–ve* ELISA-. negative. See text for abbreviations in tables

All patients	ANCA-IIF	ELISA + ve					ELISA–ve	*N* sera reactive with one antigen
		Anti-MPO	Anti-PR3	Anti-BPI	Anti-LF	Anti-HLE		
	–	6 (10.9)	7 (12.7)	13 (23.6)	10 (18.2)	1 (1.8)	32 (58.2)	8
IIF + ve	16 (29.1)	5	2	5	3	1	7	4
IIF–ve	39 (70.9)	1	5	8	7	0	25	4

patient's serum produced atypical cytoplasmic staining. Forty of 41 cases with perinuclear/nuclear staining pattern were also positive for ANA by IIF on HEp-2 cells and were additionally examined on formaldehyde-fixed granulocytes. In 15/40 sera, the perinuclear/nuclear staining pattern shifted to a cytoplasmic pattern, while the remaining 25 became negative.

After correction for nuclear/perinuclear staining of neutrophils due to the presence of antinuclear antibodies, 16/55 (29.1%) patients with SLE were identified as positive for ANCA by IIF. In 15/55 (27.3%) cases, a P-ANCA pattern was found, and 1/55 (1.8%) had a C-ANCA (atypical) pattern.

All 40 healthy control sera tested produced a negative reaction on ethanol-fixed and formaldehyde-fixed granulocytes.

Prevalence of ANCA subspecificities by ELISA

IgG-ANCA as measured by antigen-specific ELISA were found in 23/55 (41.8%) patients with SLE, of whom six (10.9%) had MPO-ANCA, seven (12.7%) had PR3-ANCA, 13 (23.6%) had BPI-ANCA, ten (18.2%) had LF-ANCA, and one (1.8%) had borderline HLE-ANCA. Reactivity to more than one antigen was observed in 8/55 sera (14.5%) (Table 1).

All control sera were ANCA-negative by ELISA. The ELISA values of controls and SLE patients are shown on Fig. 1.

Comparison of ANCA obtained by IIF and ELISA

There were discrepancies between the results for ANCA as determined by IIF and ELISA in 21/55 (38.2%) cases.

Nine out of 15 P-ANCA-positive sera showed reactivity to at least one of the specific ELISAs; five had MPO-ANCA, five had BPI-ANCA, three had LF-ANCA, two had PR3-ANCA, and one had HLE-ANCA. However, six of 15 P-ANCA-positive sera and one C-ANCA (atypical)-positive serum were negative by ELISA.

A significant association was found between ANCA-IIF positivity and MPO-ANCA ($P = 0.006$, Fisher's exact test). No correlation was found between ANCA-IIF positivity and PR3-, BPI-, LF-, or HLE-ANCA.

The IIF data of ELISA-positive SLE patients are shown on Table 2. Fourteen out of 23 (60.9%) ELISA-positive sera were IIF-negative and nine out of 23 (39.1%) were positive.

Clinical associations of ANCA-IIF

No statistically significant difference was found to exist between SLEDAI of IIF-ANCA-positive and -negative patients (11.6 ± 7.756 vs 10.1 ± 7.53, respectively, $P = 0.42$, Mann Whitney U test). No association was found between IIF-ANCA and any of the clinical manifestations at the time of assessment.

Clinical associations of ANCA of defined specificity

Statistically significant differences were found between the SLEDAI of BPI-ANCA-positive and -negative patients (13.6 ± 7.06 vs 9.6 ± 7.52, respectively, $P = 0.031$), PR3-ANCA-positive and -negative patients (16.1 ± 6.2 vs 9.7 ± 7.4, $P = 0.009$), and LF-ANCA-positive and negative patients (16.2 ± 8.5 vs 9.2 ± 6.8, $P = 0.014$), as determined by Mann Whitney U test. Moreover, we found a significant correlation between SLEDAI and the levels of BPI-ANCA ($r = 0.56$, $P < 0.01$), PR3-ANCA ($r = 0.43$, $P < 0.01$), and LF-ANCA ($r = 0.34$, $P = 0.01$) by Spearman's correlation test as well.

Anti-PR3 antibodies were found to be associated with arthritis ($P = 0.02$) and serositis ($P = 0.031$) (Fisher's exact test). Anti-LF and anti-BPI antibodies were also associated with serositis ($P = 0.009$ and $P = 0.006$, respectively, Fisher's exact test).

No association was found between any ANCA subspecificities and the presence of vasculitis or renal disease at the time of assessment.

Discussion

In the present study, we tested the prevalence, antigen specificities, and clinical relevance of ANCA in 55 Bulgarian patients with SLE.

As detected by IIF technique using ethanol- and formaldehyde-fixed neutrophils as substrate, ANCA were present in 29.1% of SLE patients, a percentage similar to that found by Schnabel et al. [9]. Almost all of the positive samples produced a P-ANCA staining pattern, while the cytoplasmic pattern was seen in only one

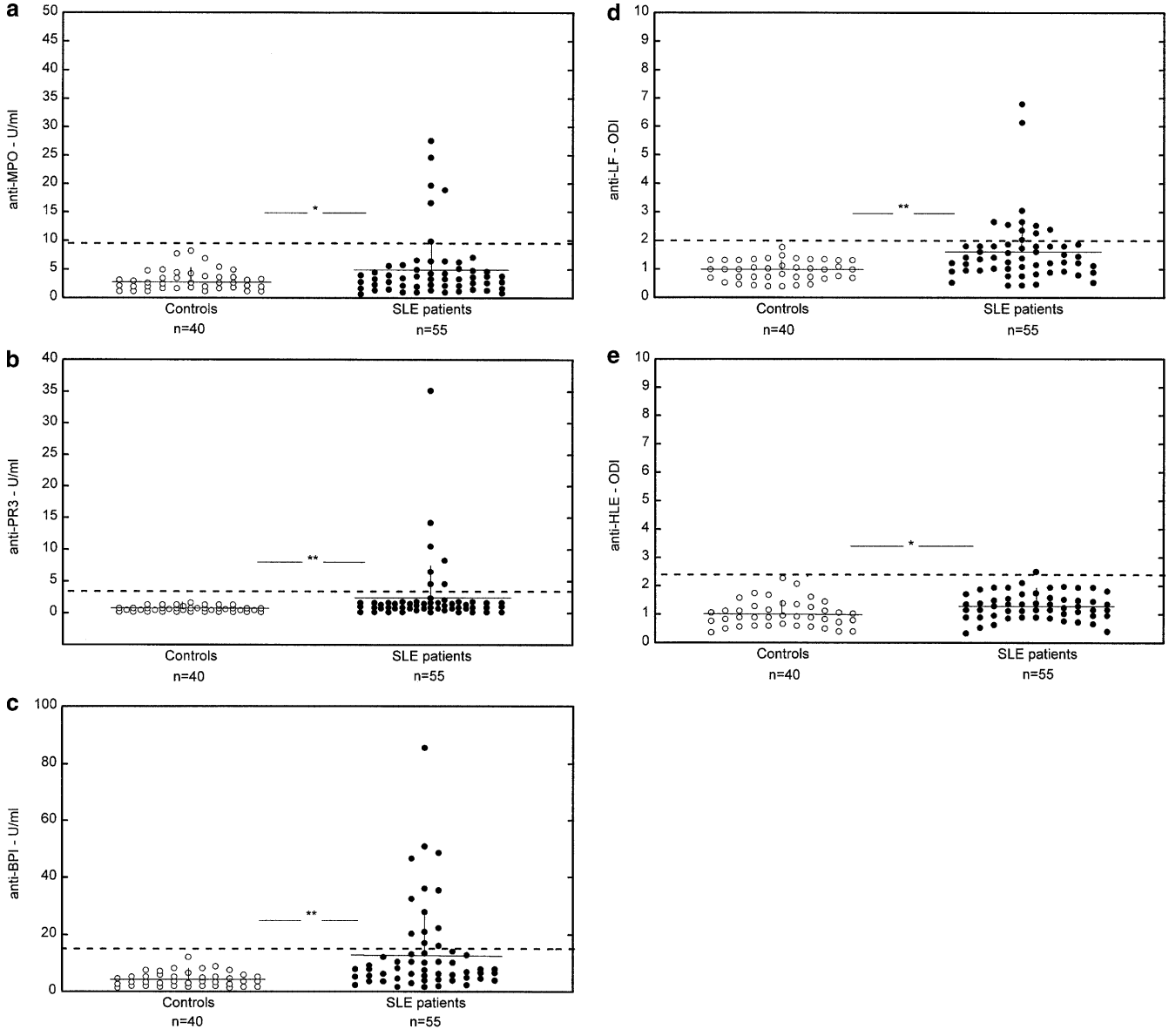

Fig. 1a–e Autoantibodies to MPO (**a**), PR3 (**b**), BPI (**c**), LF (**d**), and HLE (**e**) as determined by ELISA in controls and SLE patients. The results are expressed as U/ml or OD index (ODI). The *horizontal hatched line* represents the cutoff level of the kits and 3 SD above the mean of normal controls in in-house ELISA. Values above this line were regarded as positive. *Bars* indicate mean ± SD

serum. In all but one case, P-ANCA were associated with the presence of antinuclear antibodies.

Our ELISA results indicate that 41.8% of SLE patients show reactivity to several neutrophil cytoplasmic antigens, and more than one antibody specificity is often found in the same serum (Table 1). Anti-BPI (13 sera) was the most frequently found antibody and anti-LF (ten sera) the second most common. Antibodies to PR3 (seven sera), MPO (six sera), and HLE (one serum) were also demonstrated at low-to-medium levels. However, ANCA target antigens were not identified in about 44% of IIF ANCA-positive sera.

Our study shows the inherent difficulties of using IIF technique to determine ANCA specificities in SLE patients. The demonstration of ANCA by IIF on ethanol-fixed granulocytes in SLE is quite difficult because of the simultaneous presence of ANA in the majority of sera. Our results confirm that antinuclear antibodies, especially those with a homogeneous fluorescence aspect, produce a nuclear/perinuclear staining of neutrophils which is indistinguishable from that of P-ANCA [22]. On the other hand, ANA usually present in high titres, so they can "overshadow" the more weakly positive ANCA. Additional testing on formaldehyde-fixed granulocytes is always necessary to differentiate true P-ANCA from ANA [9]. However, some authors doubt the usefulness of IIF on formaldehyde-fixed neutrophils and do not recommend it for routine use because formalin-acetone fixation leads to increased autofluorescence, difficulties in visualising weak positive reactions, and loss of antigen reactivity to autoantibodies [23].

Table 2 Relationships between ELISA and IIF results in the sera of SLE patients positive for various ANCA specificities. IIF used ethanol-fixed (*EF*) and formalin-fixed (*FF*) granulocytes as substrates for detecting ANCA and HEp-2 cells for the detection of ANA, respectively. *N* nuclear, *P* perinuclear, *P*/*N* perinuclear/nuclear, + positive, – negative

	ELISA					IIF		
Patient	Anti-MPO	Anti-PR3	Anti-BPI	Anti-LF	Anti-HLE	EF granulocytes	FF granulocytes	HEp-2
ANCA-IIF-positive (n = 9)								
1	–	–	+	–	–	P/N	Cytoplasmic	Fine speckled
2	–	–	+	+	–	P/N	Cytoplasmic	Homogeneous
3	+	–	–	–	–	P/N	Cytoplasmic	Homogeneous, speckled
4	–	–	–	+	–	P	Cytoplasmic	Homogeneous
5	–	–	–	+	–	P	Cytoplasmic	Homogeneous
6	+	+	+	–	–	P/N	Cytoplasmic	Homogeneous
7	+	–	+	–	+	P/N	Cytoplasmic	Homogeneous
8	+	–	–	–	–	P/N	Cytoplasmic	Homogeneous
9	+	+	+	–	–	P/N	Cytoplasmic	Homogeneous, nucleolar
ANCA-IIF-negative (n = 14)								
10	–	–	+	–	–	Negative	Negative	Negative
11	–	–	–	+	–	P/N	Negative	Fine speckled
12	–	+	+	+	–	P/N	Negative	Homogeneous
13	–	+	+	–	–	P/N	Negative	Homogeneous
14	+	+	+	+	–	P/N	Negative	Homogeneous
15	–	–	+	–	–	Negative	Negative	Negative
16	–	–	+	–	–	P/N	Negative	Homogeneous
17	–	–	+	–	–	P/N	Negative	Fine speckled
18	–	–	–	+	–	P/N	Negative	Speckled
19	–	–	–	+	–	P/N	Negative	Speckled
20	–	–	–	+	–	P	Negative	Homogeneous
21	–	+	–	+	–	P/N	Negative	Fine speckled
22	–	+	–	–	–	P/N	Negative	Homogeneous, speckled
23	–	–	+	–	–	Negative	Negative	Negative

Because 61% of ELISA-positive sera in our survey yielded negative reactions on formaldehyde-fixed granulocytes, we are inclined to agree with the latter viewpoint.

In this study, we showed a discrepancy between the results for ANCA as determined by IIF and ELISA in 38.2% of cases which can be attributed mainly to anti-BPI antibodies that were present in all three IIF-negative sera on ethanol-fixed neutrophils. Zhao et al. also found the lack of correlation between ELISA and IIF results concerning BPI [24]. Jones et al. showed that BPI is very susceptible to degradation by PR3 and HLE [25]. This finding explains the possibility that BPI is subjected to protease cleavage during ethanol fixation of neutrophils. The discrepant data between IIF and ELISA for ANCA detection are frequently reported in autoimmune conditions different from ANCA-associated vasculitides. Positive ELISA results for defined ANCA specificities such as anti-LF, anti-BPI, and other neutrophil target antigens in the presence of negative findings by IIF have been reported in patients with inflammatory bowel diseases [26], primary sclerosing cholangitis [27], autoimmune liver disease [28, 29], and SLE as well [11]. We think that the discrepancy we found between IIF findings and ELISA reactivity may be related to antigen instability in ethanol-fixated neutrophils [25], low serum levels of specific ANCA, and different sensitivities of the two methods. On the other hand, we found that some of the IIF-positive sera were negative when tested in ELISA. Thus we can hypothesise that these autoantibodies may be directed against antigenic targets not screened for in this study.

We also found that, except for P-ANCA and MPO-ANCA, the IIF patterns did not correspond to a defined antigen specificity. Furthermore, PR3-ANCA were detected in seven SLE sera, producing perinuclear fluorescence on ethanol-fixed granulocytes. It is well known that PR3-ANCA from patients with WG typically produce cytoplasmic fluorescence, but some studies report that sera with P-ANCA can be directed against PR3 [30, 31, 32]. An explanation for the perinuclear fluorescence associated with antibodies to PR3 in our SLE sera is that PR3-ANCA in these patients occurred at low levels together with high titres of ANA. Another explanation for such a staining pattern is that antibodies to MPO, BPI, and LF, usually producing perinuclear fluorescence, coexisted with anti-PR3 in the same serum.

In general, the weak correlation between IIF findings and ELISA reactivity may be ascribed to the coexistence of multiple ANCA specificities in SLE sera and the presence of antinuclear antibodies. Thus, on the basis of our data, it appears that ELISA to screen for ANCA in SLE is more reliable than IIF, which is the standard screening technique for ANCA detection in the systemic vasculitides.

In contrast to the systemic vasculitides, in which ANCA are directed against either PR3 or MPO, antibodies to several antigens are found in SLE. ANCA with

specificities to MPO, HLE, LF, lysozyme, and cathepsin G have been detected in SLE sera, but in most studies these reactivities were reported in small percentages and without a predominating antigen [10, 11, 12, 13, 14, 15, 16]. A recent study reported a high prevalence (62.1%) of ANCA against cathepsin G [33], but this has not been confirmed by other studies. The frequencies of antibodies against MPO, LF, and HLE in SLE patients established by us do not differ considerably from those reported by other authors [14, 16]. Surprisingly, we found anti-PR3 antibodies in 12.7% of SLE patients, whereas the frequencies reported by other authors vary between 0% and 1.7% [13, 14, 16, 34]. Only one study reported reactivity with purified PR3 in six out of 44 samples from patients with active lupus nephritis [35], comparable to our results. In the present study, we used commercially available kits for PR3-ANCA and the group of controls included both healthy blood donors and patients with reactive arthritis. These facts, together with our experience in studying PR3-ANCA in patients with various inflammatory disorders, excludes the possibility of false positive results due to low cutoff values. It is more likely that cross-reactivity is involved, because in 6/7 PR3-positive patients, PR3-ANCA were present in low titres and in combination with other ANCA specificities. If truly positive PR3-ANCA can be detected in SLE, the question arises as to the utility of ANCA findings in clinical diagnosis of idiopathic small-vessel vasculitides. In our opinion, low-titre PR3-ANCA and MPO-ANCA coexisting with antinuclear antibodies more probably indicate the presence of collagen vascular diseases than primary systemic vasculitides. Despite the established high specificity of PR3-ANCA for WG [36, 37], positive PR3-ANCA results should always be interpreted in the context of the whole range of laboratory, radiology, and histology tests.

In contrast to the low prevalence of BPI-ANCA in SLE sera found by Zhao et al. [33], we observed antibodies against BPI in about 24% and with comparatively high titres. Antibodies to this antigen were originally detected in ANCA-positive sera from patients with vasculitides who were negative on ELISA for PR3- and MPO-ANCA [24]. Subsequently, BPI-ANCA were described in 37% of patients with ulcerative colitis, 23% with Crohn's disease, 36% with primary sclerosing cholangitis, and with cystic fibrosis [38, 39]. In the last condition, BPI-ANCA are very common in patients with *Pseudomonas* infection [39]. It has been supposed that antibodies to BPI are somehow related to the chronic lung colonisation with *Pseudomonas aeruginosa* [39]. In our study, no patients with BPI-ANCA showed clinical or laboratory evidence of concomitant infection (data not shown). Recently Cooper et al., studying the clinical association of BPI-ANCA, reported that BPI represents a major target of ANCA in a number of rheumatological diseases [40]. In their study, low-titre BPI-ANCA were present in 17% of SLE patients, a result quite similar to ours. As the presence of anti-BPI antibodies in SLE patients was examined in few studies, further investigation is necessary to establish the frequency of BPI-ANCA in SLE.

In general, the reported frequencies of ANCA subspecificities in patients with SLE vary. This may be related to differences in assay techniques, patient selection, disease heterogeneity, genetic background, and the limited selection of ANCA antigens used. However, the characteristic variability in symptoms and antibody profile between individual SLE patients should not be neglected. We suggest that the variations in ANCA prevalence in various patient populations reflect disease heterogeneity rather than ethnic differences.

The second part of this study addressed the association of ANCA with clinical features of SLE. Conflicting results have been reported concerning clinical associations of ANCA in SLE. Several studies failed to find an association of ANCA subspecificities with any clinical manifestation in SLE [12, 13], while others did find such links. An association between anti-HLE antibodies and neurological manifestations in SLE patients was found by Nassberger et al. [10]. They and Cambridge et al. established an association between anti-MPO antibodies and drug-induced SLE [10, 41]. High anti-LF reactivity was found in SLE patients with crescentic glomerulonephritis by Lee et al. [11] as well as significantly increased expression of antibodies against cathepsin G (CG) in patients with active renal lesions [30]. An increased prevalence of ANCA of various specificities, in particular anti-LF, anti-MPO, and anti-CG, was reported by Spronk et al. in patients with pericarditis, arthritis, and longer disease duration [14]. Galeazzi et al. demonstrate the correlation between IIF-ANCA and serositis, arthritis, livedo reticularis, and thrombosis as well as of LF-ANCA and serositis and livedo reticularis in a study of 566 SLE patients from 11 European centres [16]. Nishiya et al. report a correlation of ANCA to lupus activity [15] which is supported by other authors [11, 42].

As measured by SLEDAI, we found that SLE patients with antibodies to BPI, PR3, and LF exhibited significantly more active disease than those without these antibodies. Moreover, levels of antibodies to BPI, PR3, and LF correlated significantly with SLEDAI scores. We also investigated the relationships between ANCA and various clinical manifestations as defined by the SLEDAI index and found associations between BPI-ANCA, PR3-ANCA, LF-ANCA, and some of the most common clinical manifestations in SLE such as arthritis and serositis. Namely, antibodies to LF, BPI, and PR3 were found more frequently in patients with serositis, while PR3-ANCA were also found to be associated with arthritis. Our results concerning the association of ANCA with serositis and arthritis are in agreement with those of Galeazzi et al. Unfortunately, in that huge European survey of 566 SLE patients, the authors did not study the correlation between disease activity and ANCA. The discrepant data concerning clinical associations of ANCA may result from the different classification criteria, different scoring systems for assessing disease activity, and disease heterogeneity.

The biological significance of ANCA in SLE is unknown. They could result from polyclonal B cell activation or be generated as a consequence of chronic antigenic stimulation with neutrophil granular proteins in the course of an inflammatory reaction. The simultaneous occurrence of antibodies to more than one antigen supports the notion of disturbance of the immune regulation causing the appearance of ANCA in SLE. Whether antibodies to neutrophil cytoplasmic constituents in SLE are epiphenomena of immune inflammation or are linked in some way to the pathogenic mechanisms maintaining or even enhancing the inflammatory process is still unclear.

In conclusion, our results demonstrate that ANCA with specificities to MPO, LF, PR3, HLE, and BPI occur in SLE and that the last named is a common ANCA target antigen in SLE patients. Antibodies to various antigenic specificities are frequently present in the same serum sample. BPI-ANCA, LF-ANCA, and PR3-ANCA correlated with disease activity and were related to particular clinical features. The presence of ANCA may be an additional serological characteristic of some clinical manifestations in SLE. Further longitudinal studies are required to evaluate the relationships of certain ANCA specificities to disease activity and expression.

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