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ANTINEUTROPHIL CYTOPLASMIC AUTOANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Antineutrophil cytoplasmic autoantibodies (ANCA) were first described in patients with necrotizing glomerulonephritis. The original observation passed unnoticed until an association was made between ANCA and active Wegener's granulomatosis. Since then, tremendous progress has been made in elucidating the association between ANCA subtypes and clinicopathologic syndromes, and the potential pathologic role of ANCA in vascular inflammation. The gold standard method for ANCA detection is indirect immunofluorescence (IIF) microscopy of ethanol-fixed cells. By this technique two general patterns of ANCA, a cytoplasmic pattern (c-ANCA) and a perinuclear pattern (p-ANCA) can be identifed. Recently some reports have been published concerning the prevalence, subtypes, role and specificity of ANCA. In this study we searched for ANCA prevalence, subtypes, and its relationship with disease activity in SLE patients by IIF technique. Our results showed that all normal subjects were ANCA negative, while at least 50% of patients were ANCA positive (majority c-ANCA type). Statistical analysis revealed that there is no correlation between the presence of ANCA and disease activity ($p<0.01$).

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INTRODUCTION

Autoantibodies to neutrophils have been known to exist for a long time. As early as 1964, a granulocyte-specific antinuclear factor was reported.[1] Later, Wiik and Munthe in 1972 described a standardized technique for granulocyte specific antinuclear antibodies (GS-ANA)[2]. More than ten years ago, anti-neutrophil cytoplasmic autoantibodies (ANCA) were first described in patients with necrotizing glomerulonephritis.[3] Two different ANCA subtypes have been recognized and the disease specificity of ANCA subtypes was described by Wieslander.[4] The gold standard method for ANCA detection is indirect immunofluorescence (IIF) technique on ethanol-fixed cells.[5] By this technique two general patterns [cytoplasmic (c-ANCA) and perinuclear (p-ANCA)] may be observed. c-ANCA mainly reacts with a serine proteinase called proteinase-3 (PR3), and p-ANCA mainly reacts with myeloperoxidase.[4,6] ANCA has been found in patients with a wide spectrum of pauci-immune necrotizing vascular inflammation[7,8] and is a serologic marker for this wide range of vascular inflammatory diseases.[9] There are some reports about the presence, prevalence and subtypes of ANCA in SLE.[10,11] The correlation between ANCA, disease activity studied by some investigators.[12,13] According to these studies up to 69% of SLE patients were ANCA positive, mainly of the p-ANCA subtype.[14,15] Their findings indicated that there is no correlation between ANCA presence, disease activity

and organ involvement. In this study we tried to clarify the prevalence and subtypes of ANCA and its correlation with disease activity in SLE patients which were referred to the SLE research unit of Shariati Hospital, Tehran.

MATERIALS AND METHODS

Patients

34 SLE patients (33 female and 1 male) from different provinces referred to the SLE research unit of the Rheumatology Research Center of Shariati Hospital, Tehran, and 51 healthy adult volunteers were selected. The subjects' sera were screened for antineutrophil cytoplasmic antibody (ANCA) and antinuclear antibody (ANA) by indirect immunofluorescence technique (IIF). The presence of ANCA in undiluted serum and ANA titers over 1:40 were considered as positive. Based on the SLE research unit's protocol, disease stages and severity were scored as 0, 1, 2 or 3 for remission, mild, moderate or active, and severe states, respectively.

Indirect immunofluorescence test

ANCA was detected using the indirect immunofluorescence method as delineated during the first ANCA workshop.[16] Briefly, granulocytes were isolated from heparinized blood of healthy subjects using dextran sedimentation. The remaining erythrocytes were removed by osmotic shock lysis. The granulocytes were cytocentrifuged onto glass slides, air-dired, and fixed in ethanol for 14 min at 4°C. The slides were subsequently stored at 70°C. Slides were washed for 15 min in PBS just before use, and were incubated with patient's sera for 30 min in a humidified chamber at room temperature. After three washes in PBS, the slides were incubated with FITC-labelled goat anti-human IgG. Positive sera were tested at dilutions of 1:10, 1:20, 1:40 and so on to establish the end titer of ANCA. ANCA-positive sera were also screened for the presence of ANA on guinea pig kidney cells in an indirect immunofluorescent test.

Statistical analysis

The McNemar test was used to compare ANCA and ANA for the diagnosis of SLE. Chi-square test was used to clarify the relation between the presence of ANCA and disease acitvity.

RESULTS

ANCA was detected by IIF on ethanol fixed granulocytes in the sera of 17 SLE patients (50%), but were not detected in the control group. ANA was screened by IIF on frozen sections of guinea pig kidney tissue. 44% of patients (15:34) were ANA positive and all normal subjects were negative.

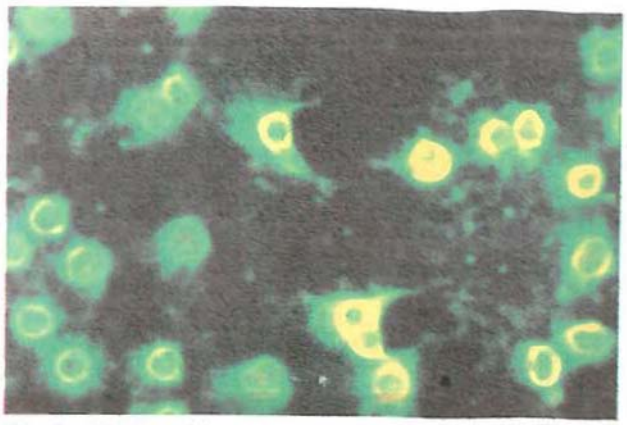

Fig. 1. ANCA-positive neutrophils. The cytoplasm of the cells show fluorescence, while the nuclei lack this activity.

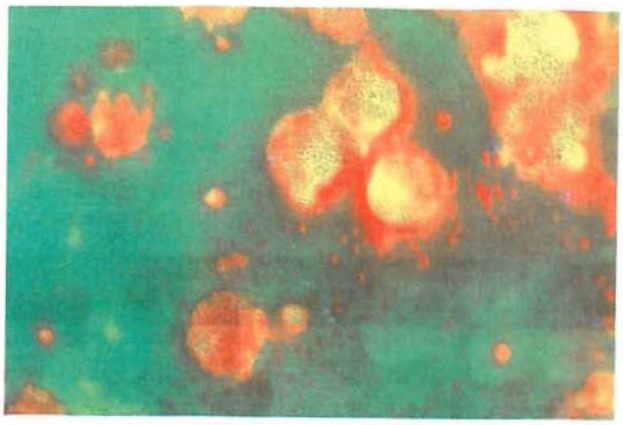

Fig. 2. ANCA-negative neutrophils. The nucleus and the cytoplasm of these cells do not show fluorescence. The yellow appearance is due to background autofluorescence.

In all ANCA positive cases (except one), the ANA titer was higher than the ANCA titer. Statistical analysis showed that there was no correlation between ANCA level and disease acitivity. The majority of ANCA positive SLE patients had a c-ANCA type pattern. In this pattern the cytoplasm of neutrophils had fluorescent staining, but their nuclei showed no fluorescent activity (Figs. 1,2).

The McNemar test revealed that there was no significant difference between ANCA and ANA tests in SLE diagnosis ($p \leq 0.05$). Chi-square test indicated that there was no correlation between disease severity and the ANCA titer.

DISCUSSION

ANCA his been detected in vascular inflammatory disorders such as Wegener's granulomatosis, polyarteritis nodosa and systemic lupus erythematosus.[10,11,16] In this study the prevalence and subtypes of ANCA in SLE were determined, and results showed that 50% of SLE cases were

ANCA positive. Different results have been reported concerning the prevalence of ANCA in SLE. In the Rheumatologic Clinic of Lubeck University in Germany, 157 SLE patients were studied, in which 40 cases (25%) had ANCA.[10] The prevalence and antigen specificity of ANCA in the sera of 23 children with active SLE was studied and 69% of cases were shown to be positive.[13] Our findings showed that 16 patients (95%) had a c-ANCA pattern, while other results have shown that ANCA in SLE is mainly of the p-ANCA subtype.[11,15] ELISA has been used for detecting the antigen specificity of ANCA. Different reports have indicated that the target antigens in p- and c-ANCA are myeloperoxidase and proteinase-3, respectively.[4] But in this study definition of antigen specificity of ANCA was not the aim. Results also showed that as supported by other reports,[13] there is no correlation between disease activity and the presence of ANCA. 44% of patients (15 cases) were ANA positive. All ANCA positive cases (except one) had higher ANA titers that ANCA. This study suggests that there is no significant difference between ANCA and ANA tests in the diagnosis of SLE. It is supposed that ANCA as a pathogenic factor could amplify the PMN response to proinflammatory cytokines, resulting in a disturbance of the conventionally known course of the inflammatory process.[17] Therefore, ANCA may be important in SLE classification, diagnosis, treatment, follow up, and prognosis. However, further research must be done to reveal the role of ANCA in SLE activity and pathogenesis.

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