

# Immunoglobulin E and systemic lupus erythematosus

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## Abstract

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by intense polyclonal production of autoantibodies and circulating immune complexes. Some reports have associated SLE with a Th2 immune response and allergy. In the present study 21 female patients with SLE were investigated for total IgE and IgE antibodies to dust house aeroallergens by an automated enzyme-linked fluorescent assay, and were also evaluated for antinuclear IgE autoantibodies by a modified indirect immunofluorescence test using HEp-2 cells as antigen substrate. Additionally, immunocapture ELISA was used to investigate serum anti-IgE IgG autoantibodies. Serum IgE above 150 IU/ml, ranging from 152 to 609 IU/ml (median = 394 IU IgE/ml), was observed in 7 of 21 SLE patients (33%), 5 of them presenting proteinuria, urinary cellular casts and augmented production of anti-dsDNA antibodies. While only 2 of 21 SLE patients (9.5%) were positive for IgE antibodies to aeroallergens, all 10 patients with respiratory allergy (100%) from the atopic control group (3 males and 7 females), had these immunoglobulins. SLE patients and healthy controls presented similar anti-IgE IgG autoantibody titers ( $X = 0.37 \pm 0.20$  and  $0.34 \pm 0.18$ , respectively), differing from atopic controls ( $0.94 \pm 0.26$ ). Antinuclear IgE autoantibodies were detected in 17 of 21 (81%) sera from SLE patients, predominating the fine speckled pattern of fluorescence, that was also observed in IgG-ANA. Concluding, SLE patients can present increased IgE levels and antinuclear IgE autoantibodies without specific clinical signs of allergy or production of anti-allergen IgE antibodies, excluding a possible association between SLE and allergy.

### Key words

- Systemic lupus erythematosus
- Antinuclear IgE antibody
- Allergy
- IgG anti-IgE
- IgE

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by intense polyclonal activation of B lymphocytes and vigorous production of autoantibodies. This disease is associated with patient race and socioeconomic status and courses with chronic systemic inflammation,

mediated mainly by deposits of immune complexes in different organs and systems, and high production of autoantibodies with specificity for different nuclear antigens. Some antinuclear autoantibodies are associated with clinical aspects of SLE, such as anti-dsDNA antibodies, which show a close relationship

with impairment of renal function, and also anti-SSA/Ro and anti-SSB/La antibodies, which are more frequently detected in patients with cutaneous manifestations and photosensitivity (1,2).

SLE is characterized by a Th2 immune response, presenting significant interleukin 10 (IL-10) production (1). Additionally, some reports have shown increased IgE production in this disease and have associated SLE with allergy (3-8). In addition, the occurrence of anti-IgE IgG autoantibodies has been demonstrated in SLE patients with articular involvement, lymphadenopathy and anti-DNA antibodies (9). The human Th2 immune response is mainly observed in atopy and in some parasitic diseases, being characterized by significant production of IL-4, IL-10 and IgE antibodies. An increased serum IgE concentration has been associated with SLE activity and lupus nephritis (4,6,10,11). However, the occurrence of antinuclear IgE autoantibodies in SLE patients has not been appropriately investigated. To date, there are only two isolated reports showing IgE antinuclear autoantibodies in SLE patients: one using rat liver tissue as antigen substrate, which does not have important extractable nuclear antigens such as SSA/Ro, and the other obtained with granulocyte nuclei (12,13).

The aim of the present study was to evaluate the IgE immune response in Brazilian SLE patients by determining the serum concentration of this immunoglobulin, the occurrence of specific IgE antibodies to aeroallergens and nuclear antigens, and also the presence of anti-IgE IgG autoantibodies in patient sera.

Serum samples from 21 women (15 to 60 years old), previously classified as SLE patients using the revised criteria of the American College of Rheumatology (14) were evaluated. Patient sera were obtained before therapy and examined for SLE laboratory criteria (IgG-ANA, anti-dsDNA autoantibodies, urinalysis, and antiphospholipid anti-

bodies). However, a few patients were already using corticosteroids. All patients were seropositive for IgG-ANA by conventional indirect immunofluorescent test (IFAT) using HEp-2 cells as antigen substrate. Seventeen presented antinuclear antibody titers of 1/160 and more. In addition, 19 of 21 (86%) patients had serum anti-dsDNA autoantibodies, presenting a 95% confidence interval from 311 to 862 IU antibody/ml, as determined by indirect ELISA with 50 IU antibody/ml as the cut-off (Hemagen Diagnostics Inc., Columbia, MD, USA). All SLE patients were negative for intestinal parasitic infection by helminths or protozoa, as assessed by serial exams of three stool samples. Eleven of 21 subjects reported sporadic and nonspecific episodes of mild adverse drug reactions characterized mainly by dermatological manifestations like erythema, and 8 of 21 had laboratory findings of renal disorders such as hematuria, cellular casts and proteinuria.

Sera from 10 atopic patients, 3 males and 7 females, with a clinical diagnosis of respiratory allergy and presenting both positive skin prick tests and IgE antibody reactivity to common aeroallergens *in vitro* were included in the immunoassay to evaluate serum anti-IgE IgG autoantibodies. In addition, sera from 20 healthy individuals, 8 males and 12 females ranging in age from 15 to 60 years, were used as negative control in this assay and also in the IFAT to investigate antinuclear IgE antibodies. These subjects did not have a clinical history of atopy or specific IgE antibodies to aeroallergens.

Serum IgE concentration was determined by enzyme-linked fluorescent assay (ELFA) using the Vidas 30 Immunoanalyzer from bioMérieux (Rio de Janeiro, RJ, Brazil). The International Reference Value of 150 IU of IgE/ml was used in the evaluation of the results.

IgE antibodies to a panel of aeroallergens consisting of proteins from pollens, mites, molds, dog and cat epithelia, and also from

cockroaches were investigated by ELFA using the Stallertest immunoassay from the bioMérieux and a Vidas 30 Immunoanalyzer. The cut-off of 0.70 relative fluorescence value (RFV) of this test was used.

IgE immunocapture ELISA using polystyrene microplates containing wells coated with 500 ng of goat IgG anti-human IgE and a goat IgG anti-human IgG peroxidase conjugate from the same source was used to detect anti-IgE IgG autoantibodies (15). This assay detects IgG autoantibodies that recognize IgE epitopes, which are exposed after the capture of this immunoglobulin by goat IgG anti-human IgE adsorbed to the solid phase. Briefly, 100  $\mu$ l of sera diluted 1:2 in phosphate buffered-saline (PBS), pH 7.4, containing 1% bovine serum albumin and 0.05% Tween 20 was incubated for 1 h at room temperature in the wells to capture IgE. The wells were washed with PBS and the immune interaction of anti-IgE autoantibodies with IgE epitopes was detected after incubation with 100  $\mu$ l of the diluted conjugate under the same conditions. The wells were washed again and the reactions were developed with hydrogen peroxide plus 3,3',5,5'-tetramethylbenzidine (TMB) for 30 min as usual, stopped with 2 N HCl and determined at 450-600 nm using a Diamedix BP-12 (Miami, FL, USA) microassay apparatus.

IgE antinuclear antibodies were investigated by an IFAT carried out with fixed epithelial HEp-2 cell slides purchased from a commercial source (DTS, Rio de Janeiro, RJ, Brazil). In this IFAT, sera were first depleted of IgG by 1/5 dilution in a purified solution of anti-human IgG sheep IgG (RF Absorbent™, DADE-Behring, Marburg, Germany) as recommended for IFAT. After centrifugation to sediment IgG immune complexes, 25  $\mu$ l of the IgG-depleted supernatants was incubated with HEp-2 cell smears for 18 h at 4°C in a humid chamber to carry out the primary immune reaction. The slides were washed three times with PBS (150 mM

NaCl plus 10 mM sodium phosphate, pH 7.2) and the cell smears submitted to a second incubation with 25  $\mu$ l of properly diluted goat IgG anti-human IgE FITC conjugate (Sigma, St. Louis, MO, USA) in PBS containing 0.01% Evans blue, for 2 h at 37°C. After a third wash with PBS, the cell nuclei were examined at 400X magnification under an Olympus BHF (Shibuya, Tokyo, Japan) fluorescence microscope after the addition of mounting medium (90% glycerol in 100 mM carbonate buffer, pH 9.0). The results of the reactions were reported as positive or negative, and the pattern of fluorescence was described as advised for IgG antinuclear antibodies (16).

The Prism PC program (version 1.00; GraphPad Software Inc., 1994) and the chi-square, Kruskal-Wallis and Mann-Whitney tests were used for statistic analysis.

Increased serum IgE concentration above the International Reference Value of 150 IU/ml was observed in 7 of 21 (33%) SLE patients. In this group, IgE levels ranged from 152 to 609 IU/ml (median = 394 IU IgE/ml) and laboratory urinary findings of renal disorders such as persistent proteinuria, hematuria and cellular casts was documented in 5 of the 7 positive patients. IgE was increased in the renal group (ranged from 152 to 582 IU/ml, median = 190 IU) when compared with the patients without renal disease, who presented a range from 10 to 609 and a median of 61 IU/ml ( $\chi^2 = 0.05$ , significant level). The serum concentration of anti-dsDNA antibodies was also augmented in the renal SLE group, that presented a median of 777 IU antibodies/ml as compared to 175 IU displayed by SLE patients without renal disorders ( $P = 0.01$ , Mann-Whitney test; Figure 1).

Specific IgE antibodies to aeroallergens were observed in 2 of 21 (9.5%) SLE patients, one of whom presented a renal disorder. The serum level of anti-IgE IgG autoantibodies in the SLE group ( $X = 0.37 \pm 0.20$ , mean  $\pm$  SD) was similar to that of healthy

controls ( $X = 0.34 \pm 0.18$ ), but a higher titer of anti-IgE IgG autoantibodies of  $0.94 \pm 0.26$  was observed in the atopic group ( $P < 0.05$ , Kruskal-Wallis test; Table 1).

Antinuclear IgE autoantibodies were detected by IFAT in 17 of 21 (81%) sera from SLE patients, with 6 of them presenting renal disorders. A fine speckled pattern of nuclear fluorescence was observed in sera from 15 patients, while the other two reacted with antigens of the nuclear membrane. There was no correlation between high serum IgE concentration and positive IgE-ANA. However, positive IgE-ANA was more frequent in sera presenting an IgG-ANA titer of 160 or more.

We demonstrate here that Brazilian SLE

patients can present increased serum IgE concentration, as previously reported for American and Hungarian SLE patients (4,5, 11). This mild elevation of serum IgE was not associated with intestinal parasitic diseases or serological findings of common respiratory allergy, as demonstrated by the negative results of 19 of 21 SLE patients evaluated by specific IgE immunoassay for usual aeroallergens. On the other hand, the 2 SLE patients having IgE antibodies to aeroallergens did not report any history of adverse drug reactions. These findings differed from those of previous studies reporting evidence of atopy in SLE patients, obtained indirectly through application of allergy-directed questionnaires (3,4,10). Moreover, a recent and controlled study evaluating the immune response of SLE patients to skin prick tests with aeroallergens also showed a complete lack of association between systemic lupus erythematosus and allergy (17).

A possible pathogenic role of IgE antibodies in lupus nephritis has been claimed by some research groups that observed an increased serum concentration of this immunoglobulin in renal SLE patients (4,10). Additionally, IgE was detected in glomerular deposits of immune complexes by immunofluorescence assays of kidney biopsies from renal SLE subjects (18,19). In the present study, 5 of 8 SLE patients with laboratory findings of renal disorders such as proteinuria, hematuria and urinary cellular casts had high serum IgE concentrations and 6 of them had antinuclear IgE autoantibodies. However, there was no association between renal disorders and antinuclear IgE seropositivity when compared with the SLE group without renal involvement. SLE renal patients produced more anti-dsDNA antibodies than SLE subjects without renal disorders, confirming a close relationship between the level of these antibodies and renal manifestations of SLE (20).

Anti-IgE autoantibody concentration was similar in both SLE and healthy control

Figure 1. Titers of anti-dsDNA autoantibodies in systemic lupus erythematosus (SLE) patients without renal involvement (N = 12) and with renal involvement (Renal SLE, N = 7). A median of 175 IU antibody/ml with 25 and 75% of 55 and 503 IU antibody/ml, respectively, was observed for the SLE group, whereas a median of 777 IU antibody/ml, and 25 and 75% of 323 and 1433 were observed in the SLE patients with renal involvement. The maximum and minimum titers are indicated by bars.

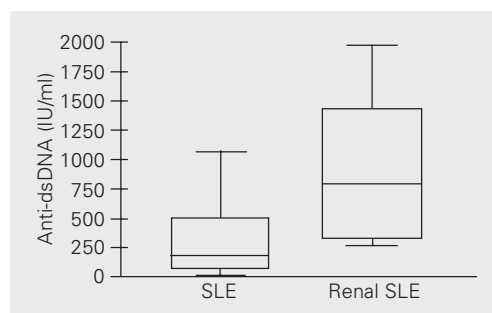


Table 1. Serological findings in SLE patients evaluated for IgE immune response.

Immune evaluation	Clinical group		
	SLE (N = 21)	Atopy (N = 10)	Healthy (N = 20)
Total IgE (IU/ml)	394	ND	<150*
Anti-allergen IgE antibody	2/21 (9.5%)	10/10 (100%)	0/20 (0%)
Anti-IgE IgG autoantibody	$0.37 \pm 0.20$	$0.94 \pm 0.26$	$0.34 \pm 0.18$
Antinuclear IgG autoantibody	21/21 (100%)	ND	0/20 (0%)
Antinuclear IgE autoantibody	17/21 (81%)	ND	0/20 (0%)

\*International Reference Value for adolescent and adult subjects. IgE concentration (reported as median) and anti-allergen IgE antibodies were determined by enzyme-linked fluorescent assay using the automated analyzer Vidas 30 from bioMérieux. Antinuclear IgG and IgE autoantibodies were investigated by indirect immunofluorescent test using HEP-2 cells as nuclear antigen substrate. Anti-IgE IgG autoantibodies were detected using an immunocapture ELISA, being reported as mean  $\pm$  SD. The frequencies of antinuclear IgG and IgE autoantibodies and anti-allergen IgE antibodies are represented by percent positivity. ND = not determined.

groups, differing from the atopic one, that presented elevated levels of these antibodies. These results show that the production of anti-IgE IgG autoantibodies is apparently unaffected by the intense polyclonal activation of B lymphocytes observed in SLE.

We have demonstrated for the first time that antinuclear IgE autoantibodies can be frequently demonstrated in SLE patients, with the predominance of a fine speckled pattern of fluorescence in both IgE and IgG-ANA suggesting that both isotypes of autoanti-

bodies recognize common extractable nuclear antigens. Apparently, the production of IgE antinuclear autoantibodies in SLE is controlled by specific autoimmune recognition and is not affected by polyclonal activation of B-lymphocytes. Such conclusion is supported in this study by a consistent lack of production of anti-allergen IgE antibodies in SLE patients and by the occurrence of IgE-ANA in individuals having normal and low serum IgE concentrations.

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