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# Interleukin-13 in autoimmune rheumatic diseases: Relationship with the autoantibody profile

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**Key words**: Interleukin-13, rheumatoid factor, antinuclear antibodies, autoimmune rheumatic diseases.

**ABSTRACT**

**Objective:** *Several cytokines play a role in the production of autoantibodies such as RF and ANA by B-lymphocytes; the role of IL-13 in this process has not been previously studied. We investigated the relationship between the serum concentration of this cytokine and circulating autoantibodies.*

**Methods:** *IL-13 serum levels, as well as RF and ANA, were evaluated in 282 patients with autoimmune rheumatic diseases including RA (n=84), SLE (n=114), SS (n=52) and Scl (n=32).*

**Results:** *Serum levels of IL-13 (pg/ml) were significantly higher in patients with RA ($p < 0.00003$), SLE ($p < 0.03$), SS ($p < 0.0007$), or Scl ($p < 0.025$) compared to controls. IL-13 serum levels correlated with those of RF in RA ($p < 0.00001$), SLE ($p < 0.003$) and Scl ($p < 0.03$). IL-13 levels were higher in RA ($p<0.0003$), SLE ($p<0.005$) and Scl ($p<0.05$) patients with RF than in patients without RF. SS patients with anti-SSA/Ro antibodies had significantly higher IL-13 levels than SS patients without this autoantibody ($p < 0.04$). No statistically significant correlation was found between IL-13 levels and any other antinuclear autoantibody, total immunoglobulin levels or the main clinical features of each disease.*

**Conclusion:** *The evidence of higher IL-13 levels in our RA, SLE, SS and Scl patients confirms that this cytokine is involved in the pathogenesis of autoimmune rheumatic diseases. The relationship of this cytokine with RF in RA, SLE and Scl, as well as with antiSSA/Ro antibody in SS, strengthens the hypothesis that it plays a role in autoantibody production. However, the different autoantibody synthesis by B-cells recognises different pathways depending on the underlying autoimmune disease.*

## Introduction

Interleukin 13 (IL-13) is a protein secreted by activated T cells that inhibits proinflammatory molecule production by activated human monocytes (1) and that modulates B-cell functions *in vitro* (2). IL-13 seems to play a significant role in B-lymphocyte proliferation, differentiation (2-4) and immunoglobulin production (2, 3, 5). The presence of circulating autoantibodies, including rheumatoid factor (RF) and antinuclear antibodies (ANA), represents an important characteristic of autoimmune rheumatic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS) and systemic sclerosis (Scl) (6). It has been proved that RF and ANA production by B-lymphocytes is regulated by the action of different cytokines, including IL-10 (7-10), IL-6 (10-14) and IL-4 (5, 16). The effect of IL-13, if any, on autoantibodies production is unknown.

The aim of this study was to evaluate serum IL-13 levels in patients with autoimmune rheumatic diseases in order to investigate the relationship of this cytokine with the autoantibody synthesis profile.

## Patients and methods

We studied 282 patients with rheumatic diseases including RA (n = 84) classified according to Arnett (17), SLE (n = 114) classified according to the updated ACR criteria (18), SS (n = 52) classified according to European criteria (19), and Scl (n = 32) classified according to ARA criteria (20).

We evaluated the main clinical and laboratory parameters, including the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), immunoglobulins (IgG, IgA, IgM), and the circulating autoantibody profile in all patients. RF determination was performed by the $F_{II}$ Latex test (21); antinuclear (ANA) and anti-DNA antibodies by the standard indirect immunofluorescence technique on HEp2 cells and Crithidia Luciliae respectively; and anti-Sm, anti-RNP, anti-SSA/Ro, anti-SSB/La and anti-Scl70 antibodies by immunodiffusion (22).

The IL-13 measurement has been done on serum samples stored at -70° using an enzyme-linked immunosorbent assay (ELISA) kit (Bender Medsystem, Vienna, Austria). In brief, polystyrene microplate (M29AUS flat-bottom, Sterilin) wells were coated overnight at 4°C with a murine monoclonal antibody (2.5 g/ml diluted in PBS) directed against IL-13. PBS containing Tween

20 (0.05%) was used to wash each well between incubation steps. After incubation (2 hours at room temperature) with 250 l of PBS-Tween 20 (0.05%) containing bovine serum albumin (0.5%), the serum sample and HRP-conjugated murine monoclonal antibody (1:2000) directed against IL-13 were added to the coated wells. After incubation (2 hours at room temperature), the substrate solution (1:2 mixture of $H_20_2$ and tetramethylbenzidine) was added and the reaction was stopped after 15 minutes by 4N sulfuric acid.

Optical density (OD) was read on a Titertek Multiscan microplate reader (Flow Laboratories) at a wavelength of 450 nm. All samples and standards were tested in duplicate. For each set of duplicate standards and samples, the average adsorption values were calculated and expressed as pg/ml using a standard curve. Intra-assay and inter-assay coefficients of variation were 4.1% and 5.0% respectively. The sensitivity limit of this IL-13 assay, defined as the value above the mean plus 3 SD of the dilution medium or serum sample spiked with cytokine and its dilutions, was 2 pg/ml. Although the interference of RF in the IL-13 determination was not significant (23), we spiked a serum characterised by elevated levels of RF and -globulins with different concentrations of IL-13. The average recovery ranged from 79% to 112%, with an overall recovery of 97.7%.

IL-13 serum levels were also determined in 20 healthy subjects (M/F = 4/16; mean age/range: 48.8/ 29-69).

*Statistical analysis*

Categorical variables were analysed by the $^2$ test or Fisher's exact test. Continuous variables were compared using the Mann Whitney test for unpaired samples. The significance of any correlation was determined using Spearman's rank correlation coefficient. P values less than 0.05 were considered statistically significant.

## Results

The main demographic and laboratory parameters of patients admitted to the study are shown in Table I.

The frequency of detectable IL-13 levels (> 1.56 pg/ml) in RA, SLE, SS, Scl patients, was 77.4%, 51.8%, 77.1%, 71.9% respectively.

IL-13 serum levels (pg/ml) were significantly higher in RA (median/ 25th-75th percentile = 20.5/ 2.5-224; p < 0.00003), SLE (median/ 25th-75th percentile = 5.5/ 0-36; p < 0.03), SS (median/ 25th-75th percentile = 34.5/ 0-91; p < 0.0007), and Scl (median/ 25th - 75th percentile = 7.5/ 0-17; p < 0.025) patients with respect to controls (median/25th - 75th percentile = 0/ 0-5.5) (Fig. 1).

IL-13 serum levels correlated with RF in RA (p < 0.00001), SLE (p < 0.003) and Scl (p < 0.03), but not with IgG, IgA or IgM levels (Table II). The comparison of patients with or without RF revealed a significant difference in IL-13 serum levels in patients with RA (median/ 25th-75th percentile = 32/10-256 versus 2/0-11; p < 0.0003) (Fig. 2), as well as in patients with SLE (median/ 25th-75th percentile=19/0-110 versus 0/0-22; p<0.005) and Scl (median/ 25th-75th percentile = 12/7-30 versus 4 / 0-11; p < 0.05).

SS patients with antiSSA/Ro antibodies had higher IL-13 levels (median/ 25th-75th percentile) respect to SS patients without this autoantibody (42/ 5.5-91 versus 0/ 0-63; p<0.04) (Fig. 3).

No statistically significant correlations between serum IL-13 levels and any other autoantibodies, ESR and CRP values were found in this study. In our patients with autoimmune rheumatic diseases, the amount of IL-13 detectable in the serum did not identify any

**Table I**. Main demographic and laboratory features of the patients admitted to the study.

| | RA | SLE | SS | Scl |
|---|---|---|---|---|
| Number | 84 | 114 | 52 | 32 |
| Age (years)* | 55.2 (25-76) | 38.3 (15-70) | 55.2 (26-81) | 50.6 (20-73) |
| Age at onset (years)* | 46.6 (21-74) | 32.2 (10-65) | 48.2 (24-80) | 40.2 (15-71) |
| Sex ( F/M) | 62/22 | 97/17 | 50/2 | 31/1 |
| Disease duration (months)* | 116 (5-605) | 76.9 (1-456) | 81.9 (3-540) | 113.5 (12-276) |
| ESR (mm/hr)** | 45 (30-65) | 30 (16-52) | 34.5 (22-48) | 24 (10-56) |
| CRP (mg/l)** | 24 (12-48) | 6 (6-24) | 6 (6-12) | 3 (0-24) |
| IgG (mg/dl)** | 1500(1210-1865) | 1690 (1280-2110) | 2032 (1370-2450) | 1250 (925-1842) |
| IgA (mg/dl)** | 371 (263-445) | 331 (215-420) | 340 (212-500) | 345 (193-424) |
| IgM (mg/dl)** | 190 (108-225) | 233 (152-299) | 200 (154-270) | 247 (167-298) |
| RF +ve (%) | 75 | 32.4 | 78.8 | 43.7 |
| ANA +ve (%) | 22.6 | 97.4 | 92.3 | 96.8 |
| Anti-dsDNA +ve (%) | 0 | 64.9 | 0 | 0 |
| Anti-SSA/Ro +ve (%) | 0 | 39.5 | 65.4 | 18.7 |
| Anti-SSB/La +ve (%) | 0 | 19.3 | 42.3 | 3.1 |
| Anti-Sm +ve (%) | 0 | 26.3 | 1.9 | 3.1 |
| Anti-RNP +ve (%) | 0 | 23.7 | 4 | 28.1 |
| Anti-Scl70 +ve (%) | 0 | 1.0 | 0 | 37.5 |

* Mean (range); ** Median (25th - 75th percentile)

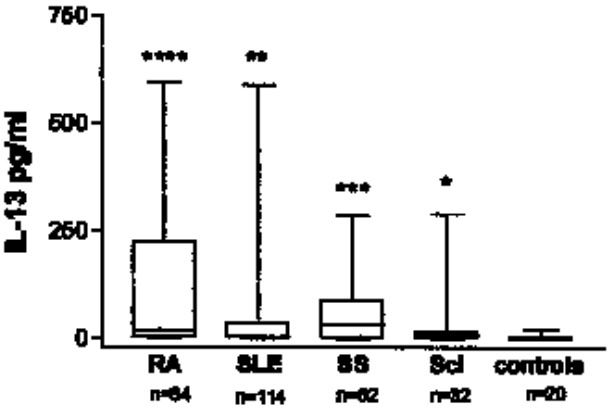


**Fig. 1.** IL-13 levels (box and whiskers plot: median/25th-75th percentile/ range) in patients with RA, SLE, SS, Scl and controls. *p<0.025; **p<0.03; ***p<0.0007; ****p<0.00003 versus controls.

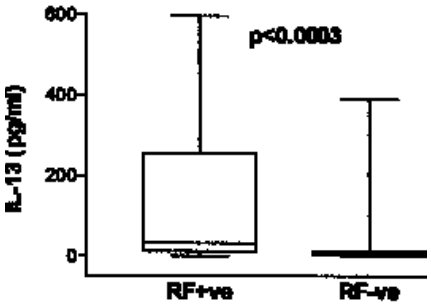


**Fig. 2**. IL-13 levels (box and whiskers plot: median/25th-75th percentile/range) in RA patients with (n = 63) or without (n = 21) RF.

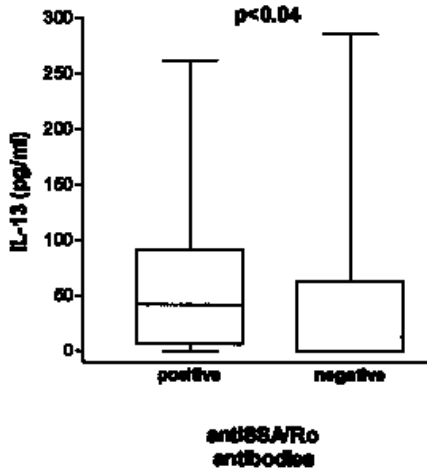


**Fig. 3**. IL-13 levels (box and whiskers plot: median/25th-75th percentile/range) in SS patients with (n = 34) or without (n = 18) antiSSA/Ro antibodies.

particular clinical patient subset when evaluated in terms of disease-related signs and symptoms.

## Discussion

The evidence of elevated IL-13 serum levels in our RA, SLE, SS and Scl patients confirms the involvement of this cytokine in the pathogenesis of autoimmune rheumatic diseases (23-26). Despite *in vitro* evidence of anti-inflammatory properties of IL-13, we did not find any relationship between the measurable amount of this cytokine and clinical or laboratory parameters of disease activity. These results agree with a previous study showing that ESR and CRP did not correlate with levels of IL-13, which were probably insufficient for optimum inhibition of pro-inflammatory cytokines (23). Moreover clinical and laboratory parameters of disease activity depend on the balanced complex action of the cytokine network, whereas IL-13, IL-4 and IL-10 share similar or independent anti-inflammatory effects (3). Nevertheless, the capability of inhibiting pro-inflammatory Th1 cytokines such as IL-12 and IFN , suggests that IL-13 as well as IL-4 and IL-10 could facilitate a Th2 response (3) and therefore modulate the immune response. IL-13 effects on human B cells include surface molecule modulation, the enhancement of proliferation, immunoglobulin production and isotypic switching (IgG4 and IgE) (3). IL-13, when added to a purified B cell culture in the presence of activated CD4+ T-cells, induces a 4- to 10-fold increase in IgM and IgG production (4).

**Table II.** Correlations of IL-13 with RF and immunoglobulin levels in patients with RA, SLE, SS and Scl.

| | RA | SLE | SS | Scl |
|---|---|---|---|---|
| IL-13 vs RF | $r_s = 0.511$; $p < 0.00001$ | $r_s = 0.281$; $p < 0.003$ | $r_s = -0.104$ n.s. | $r_s = 0.382$; $p < 0.03$ |
| IL-13 vs IgG | $r_s = -0.235$ n.s. | $r_s = -0.015$ n.s. | $r_s = -0.069$ n.s. | $r_s = 0.118$ n.s. |
| IL-13 vs IgA | $r_s = 0.129$ n.s. | $r_s = -0.172$ n.s. | $r_s = -0.018$ n.s. | $r_s = -0.088$ rn.s. |
| IL-13 vs IgM | $r_s = 0.105$ n.s. | $r_s = 0.110$ n.s. | $r_s = 0.152$ n.s. | $r_s = 0.264$ n.s. |

Although we did not find any correlation between IL-13 serum levels and total IgG, IgA or IgM, the relationship of this cytokine with RF in RA, SLE and (to a lesser extent) in Scl, strengthens the hypothesis of its role in autoantibody production. The discrepancy between IL-13 effects on RF and total immunoglobulin production is not surprising considering that other cytokines, such as IL-6 and IL-10, show a similar action. It has been reported in fact that IL-10 induces the IgM-RF secretion of peripheral blood mononuclear cells, with no increase in total IgM (8). In addition, in synovial fluid (SF) IL-6 activity significantly correlated with the accumulation of SF IgM-RF (12), as well as with the other class-specific SF RFs, lacking any relationship with the corresponding SF immunoglobulin isotype concentration (11). On the other hand, IL-2 enhances polyclonal IgM but not IgM-RF synthesis by activated human peripheral blood B cells (27). The different effect of these cytokines on antibody production is easily explained by the observation that RF accounts only for a minor fraction of the total immunoglobulins (28).

The lack of correlation between IL-13 levels and RF in SS patients is probably due to the peculiar characteristics of RF in SS with respect to RA and other autoimmune rheumatic diseases. Various idiotypes may be shared by the RFs in patients suffering from different diseases such as RA, cryoglobulinemia, and primary SS (29). Studies of monoclonal antibodies reacting with cross reactive idiotypes (CRI) such as 17.109, PSL2 and PSL3, have identified different patterns of RF idiotypic expression in SS and RA (29, 30). The different patterns of CRI expression strengthened the observation that IgM-RF occurring in SS patients have an idiotypic homogeneity with respect to germline idiotypes compared to IgM-RF from RA patients (31). In SS, RFs are encoded by Vk genes with relatively few somatic mutations, in contrast to the RF in RA patients that are heterogeneous and encoded by several Vk genes that have been somatically diversified (29). This finding was further confirmed by studies on the variable heavy chain (32,33). The IgM-RF idiotypic heterogeneity of RA patients is associated with a lower avidity compared to the IgM-RF of SS or SLE patients (34). The pathogenic role of IL-13 in the immunological abnormalities associated with the autoimmune rheumatic diseases is confirmed by the elevated serum levels of this cytokine in SS patients with anti-SSA/Ro antibody com-

pared to SS patients without this autoantibody. It is worth noting that IL-13 is demonstrable at the lip salivary gland level (25), where lymphocyte infiltrates could partially produce anti-SSA/Ro as well as anti-SSB/La antibodies (35), that may be related to more severe glandular damage (36, 37).

This study suggests that IL-13 could be involved in the pathogenesis of autoimmune rheumatic diseases, with a significant role particularly in RF production. We can conclude that the mechanisms involved in this autoantibody synthesis by B-cells probably recognise different pathways depending on the underlying autoimmune disease.

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