

In vitro release of soluble CD23 by human lymphocytes in ragweed-sensitive versus nonatopic patients following stimulation with ragweed antigen E

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Background: Soluble CD23 is the proteolytic cleavage product of the low affinity receptor (FcERII). Functions of CD23 and its soluble products may include upregulation of IgE production and stimulation of B lymphocyte growth.

Methods: Soluble CD23 was quantitated in supernatants of lymphocytes from nine ragweed-sensitive and eight nonatopic subjects stimulated in vitro by antigen E (amb A1), crude ragweed extract, and pokeweed mitogen (PWM).

Results: Although PWM stimulation produced no significant difference between groups, sCD23 release was significantly elevated in the cells of nonatopic patients stimulated with antigen E and crude ragweed extract. ($P < .05$)

Conclusions: This finding supports the concept of separate pathways of activation by antigen and mitogen for sCD23 release and suggests ragweed-sensitive and nonatopic patients have fundamental differences in the response of sCD23 release to ragweed antigen stimulation.

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INTRODUCTION

Soluble CD23 (sCD23) is the proteolytic cleavage product of the low affinity IgE receptor (FcERII) with molecular weights ranging between 14 and 37 kD. CD23 has substantial homology with several animal lectins and is found on a wide variety of cell types including lymphocytes, monocytes, and eosinophils. CD23 is considered both a receptor and a cytokine. Functions of CD23 and its soluble products may include upregulation of IgE production and stimulation of B lymphocyte growth. The mechanisms of action of sCD23 have not been fully elucidated. Recent hypotheses implicate possible feedback mechanisms whereby IgE can decrease its own synthesis by inhibiting the release of cer-

tain IgE potentiating factors including sCD23 and IL-4, which play a major role in both IgE synthesis and B cell proliferation.^{1–6}

Mean serum levels of sCD23 have been shown to be elevated in a variety of disease states including atopy as well as chronic lymphocytic leukemia, rheumatoid arthritis, autoimmune thyroiditis, myasthenia gravis, chronic uremia, and helminth infections.^{7–9}

Lymphocyte proliferative response to purified ragweed antigen in vitro has been studied in the past in ragweed-sensitive patients versus nonatopic controls.^{10–12} Tritiated thymidine uptake was used to measure DNA synthetic response to stimulation by antigen E (amb A1), the major short ragweed pollen antigen. Although both nonatopic controls and ragweed-sensitive subjects had marked lymphocyte proliferative response to antigen E, there was no statistically significant difference between the two populations. Given this finding, Buckley et al suggested that the results could be re-

lated to the mitogenic properties of ragweed antigen.¹² It has been shown that increased sCD23 is produced in vitro with certain mitogens.^{13–15}

Because sCD23 has been reported to upregulate IgE production, we hypothesized that differential responses in sCD23 release might occur between atopics and nonatopics. We conjectured that ragweed-sensitive patients would release greater amounts of sCD23 when stimulated with crude ragweed extract or antigen E (AgE). Accordingly, we measured in vitro the amount of supernatant sCD23 release from peripheral blood lymphocytes in ragweed-sensitive and nonatopic individuals when stimulated by the following: pokeweed mitogen, crude ragweed extract, and antigen E.

Analysis of such data could clarify whether separate pathways of sCD23 release exist for antigens versus mitogens in atopics and nonatopics and whether the effect of antigen in such a system is mitogenic.

SUBJECTS

The study group consisted of nine ragweed-sensitive and eight nonatopic subjects (Table 1) recruited from the R. A. Cooke Institute of Allergy Clinic and employees of St. Luke's-Roosevelt Hospital Center. Subjects were entered into the study after informed consent, approved by the Institutional Review Board (IRB). Prior to entry into the study, all subjects completed a symptom questionnaire and a panel of skin prick tests to six common inhalant allergens was administered. Subjects were excluded if they were receiving

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Table 1. Patient Demographics

	Ragweed-Sensitive	Nonatopic
Mean age, yr (range)	38.9 (25–61)	32.8 (30–50)
Male	6	4
Female	3	4
Number of observations	11	9

or had received immunotherapy. The allergens used for skin testing were *D. farinae*, ragweed, grass mix, Eastern tree mix, Hormodendrum, and standardized cat. Histamine and diluent were used as positive and negative controls, respectively. Subjects selected for the study all had histories of atopic symptoms during the ragweed season and positive ragweed skin tests. Nonatopic subjects were asymptomatic and had negative skin tests with reactive histamine controls.

MATERIALS AND METHODS

Peripheral blood samples were collected in heparinized tubes and diluted with Hank's balanced salt solution. Lymphocytes were separated using Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation, then washed three times and resuspended in 10% human serum. Lymphocytes were cultured in triplicate in flat bottom micotiter plates (Flow Laboratories, McLean, VA), (2×10^5 lymphocytes/well) and incubated in the medium with antigen or mitogen. The medium contained 0.2 mL Eagle's minimal essential medium (GIBCO, Grand Island, NY) supplemented with 10% heat inactivated human serum, 0.25 mmol of HEPES buffer, 2 mmol of L-glutamine + 100 units of penicillin/mL and 100 μ g of streptomycin/mL (GIBCO). Supernatants were pooled after humidified incubation in 5% CO₂ at 37.5 °C. Based on preliminary studies, two concentrations of 85% pure antigen E (17.5 and 8.75 μ g/mL) were used (provided by Dr. David Klapper, Department of Microbiology, University of North Carolina, Chapel Hill, NC). Two concentrations of crude ragweed extract (.75 and 0.375 μ g/mL) were also used (Greer Laboratories, Lenoir, NC). Pokeweed

mitogen (GIBCO), 1:100 vol/vol was used as a control.

To determine the length of incubation and the concentration of antigen and mitogen that would provide the maximal stimulation, cells were incubated and analyzed at 5, 9, 12, and 15 days. We used the following concentrations: PWM: 1:50, 1:100, 1:200; AgE: 17.5, 8.75, 4.5, 1.75 μ g/mL; and ragweed extract: 6, 3, 1.5, 0.75, 0.375 μ g/mL.

Supernatant sCD23 concentrations were measured on day 12 of the incubation period using an ELISA technique (T Cell Diagnostics, Cambridge, MA). The detection limit was approximately 15 units/mL. Spontaneous release of sCD23 by unstimulated cells was subtracted from the baseline in the final data analysis. Supernatant sCD23 concentrations are expressed as units/mL.

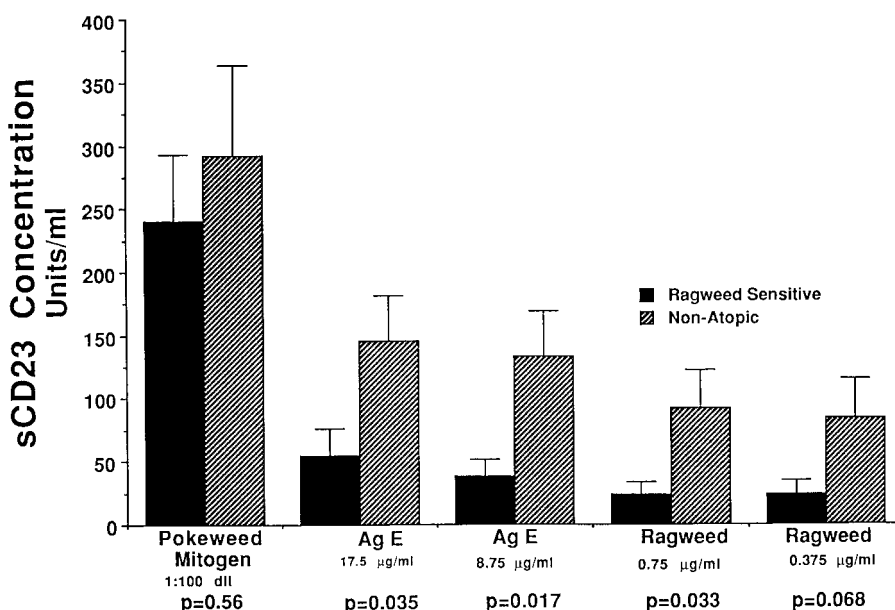


Figure 1. Mean concentration (+ standard deviation) of supernatant sCD23 at day 12 after incubation with PWM and ragweed antigen.

Statistical Analysis

The student's *t* test was used and a two-tailed *P* < .05 was considered statistically significant. Means are expressed as $\pm$ standard deviation.

RESULTS

At day 12 of pokeweed mitogen stimulation, no differences in sCD23 concentration were appreciated between ragweed sensitive (239.78 ± 176.98) and nonatopic (291.38 ± 201.93) subjects (*P* = .56) (Fig 1); however, pokeweed mitogen elicited a higher sCD23 concentration than antigen E and crude ragweed extract stimulation.

Both the ragweed-sensitive and nonatopic patients produced sCD23 above background levels at day 12 in response to Ag E and crude ragweed extract stimulation. Significantly higher concentrations of sCD23 in the nonatopic group compared with the ragweed-sensitive group were observed for both concentrations of AgE (Fig 1). At an AgE concentration of 17.5 μ g/mL, the nonatopic subjects showed a mean sCD23 of 145.56 ± 107.54 versus the ragweed-sensitive 55.27 ± 68.88 (*P* = .035). At 8.75 μ g/mL of AgE, the nonatopic subjects showed a mean sCD23 concentration

of 132.67 ± 108.27 as opposed to the ragweed-sensitive subjects whose mean concentration was 37.72 ± 45.95 ($P = .017$).

When incubated with crude ragweed extract at a concentration of $0.75 \mu\text{g/mL}$, the cells of nonatopic subjects produced significantly more sCD23 (91.67 ± 91.56) than the ragweed-sensitive (23.91 ± 30.47) group ($P = .033$). Differences in concentrations of sCD23 using the lowest concentration of crude ragweed extract ($0.375 \mu\text{g/mL}$) did not reach statistical significance between the two groups studied ($P = .068$) but showed a similar trend toward significance.

DISCUSSION

Soluble CD23 is the proteolytic cleavage product of the low affinity IgE receptor (Fc ϵ RII). Understanding the mechanism of action of sCD23 may elucidate why atopics and nonatopics have differential response to allergens despite widely overlapping serum IgE antibody levels. Although pokeweed mitogen stimulation produced no significant difference in supernatant sCD23, there was a statistically significant difference between atopic and nonatopic subjects with both concentrations of antigen E and one concentration of $0.75 \mu\text{g/mL}$ crude ragweed extract with the $0.375 \mu\text{g/mL}$ concentration showing a similar trend. Thus, antigen stimulation showed a response different from that of a mitogen. Contrary to expectation, it was the nonatopic group that produced higher levels of supernatant sCD23 than the ragweed-sensitive group.

A study by Buckley¹² assessed DNA synthetic response by measuring ^3H thymidine uptake of peripheral blood lymphocytes after stimulation by antigen E. Buckley's study compared proliferative responses between normal newborn, agammaglobulinemic, and ragweed-sensitive subjects. Although no mitogen control was used, the DNA synthetic response was not significantly different between the ragweed-sensitive subjects and the nonatopic controls. It should be noted that eight of the 19 subjects had received immu-

notherapy, whereas in our study, none of the ragweed-sensitive group had received immunotherapy. In addition, Buckley's study assessed cell activation as evidenced by ^3H thymidine uptake. No measurement of sCD23 was undertaken. The present study as well as Buckley's did not control for the possible influence of seasonal variation.

Weisberg et al¹¹ studied ^3H thymidine uptake of peripheral blood lymphocytes from nonatopic individuals only. Cells were incubated in vitro with pollen extracts (including ragweed) as well as phytohemagglutinin (PHA). Weisberg found a more marked response with PHA compared with ragweed antigen. Marked proliferative responses occurred in response to antigens in subjects who had never been previously exposed. Both of these findings were consistent with ours in relation to nonatopic subjects.

Monteseirin et al¹³ compared in vitro supernatant sCD23 release from peripheral blood lymphocytes of olive pollen-sensitive asthmatic patients and nonatopic controls. Peripheral blood lymphocytes were stimulated in vitro with PHA, varying concentrations of olea (olive pollen antigen) and *D. pteronyssinus*, an antigen to which the patients were not sensitive. Both PHA stimulation and olea stimulation produced significantly higher concentrations of sCD23 in the olive-sensitive asthmatic patients compared with the nonatopic subjects. No differences were found with *D. pteronyssinus*.

These results are in contrast to our findings of higher concentrations of sCD23 in nonatopics. Monteseirin's study suggests that sCD23 release is antigen specific and not simply a mitogenic property of the antigen since there was no response to *D. pteronyssinus* antigen.

It should be pointed out that in Monteseirin's study the number of patients and the sensitivity of the assay for sCD23 is not given. In addition, the production of sCD23 was not measured beyond day 7 and was found to peak at day 4. This is in contrast to our study where peak levels were obtained

at day 12; no statistical analysis of results with pollen stimulation was provided.

One hypothesis that could explain why the nonatopics in our study had a greater release of sCD23 in response to antigen E is suggested by Gordon et al¹⁷ who found that IgE binding of CD23 on the cell surface protects the molecule from proteolytic cleavage and therefore decreases the release of sCD23. This is consistent with the hypothesis of feedback inhibition by IgE of IgE potentiating factors. Measurement of ragweed-specific IgE and total IgE in both serum and supernatants of study subjects would help clarify this issue.

Several investigators have studied mitogen and cytokine effects on peripheral blood lymphocytes with respect to IgE synthesis, CD23 expression, and sCD23 release as well as the role of IL-4 and gamma IFN in this process.¹⁴⁻¹⁸ In particular, in vitro studies by Kicza et al¹⁶ from atopic donors demonstrated differences in CD23 expression depending on whether peripheral blood lymphocytes were stimulated prior to or after separation into their components (B cells, T cells, monocytes). B cells and monocytes revealed a strong increase in CD23 expression when the cells were separated prior to stimulation compared with data when the cells were stimulated unseparated. A marked increase in sCD23 release was noted in the supernatants of unseparated peripheral blood lymphocytes stimulated with IL-4. Separated peripheral blood demonstrated marked increases in sCD23 in B cells and monocyte supernatants but not in T cells.

Statistical analysis of Kicza's data was confined to comparisons of triplicate results in individual subjects. The methods of statistical analysis were not given. Interestingly, this paper reported that similar results were found with cells of nonatopic donors, the percentage of CD23 expression and sCD23 release after cytokine stimulation being much lower compared with the results obtained for atopic donor cells. The data however, for the nona-

topic donors were not shown. Although Kiczka's findings in nonatopic subjects conflict with ours, his results are difficult to verify.

We are currently conducting a followup study using the same patient groups, antigens, and mitogens but stimulating the cells in culture with IL-4. IL-4 upregulates the synthesis of IgE via the mechanism of class switching of immunoglobulin isotype. Our current results suggest that IgE has a feedback inhibitory effect on CD23 function.

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