

A reduction in allergen-induced FcεR2/CD23 expression on peripheral B cells correlates with successful hyposensitization in grass pollinosis

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Background: The cellular basis for the mechanism of specific hyposensitization is still unclear.

Objective: We prospectively studied the effect of immunotherapy on allergen-induced proliferation and FcεR2/CD23 expression of lymphocytes.

Methods: Mononuclear cells prepared from the peripheral blood of 22 patients with grass pollen (GP) allergy before, during, and after a preseasonal immunotherapy period with GP were stimulated with GP or control antigens. Tritiated thymidine uptake and percentage of CD23⁺ B cells were determined daily during days 6 to 8 and compared with lymphocyte responsiveness of 11 only symptomatically treated atopic patients and 14 nonatopic individuals.

Results: GP-induced lymphocyte proliferative response of both hyposensitized and symptomatically treated GP-allergic patients decreased markedly before the pollen season and rose again after seasonal allergen exposure, whereas a long-lived decrease in GP-induced FcεR2/CD23⁺ B cells was only observed in GP-treated patients. Alterations in FcεR2/CD23 expression were closely related to changes in symptoms and medication requirement during the following pollen season. In contrast, immunotherapy had no effect on FcεR2/CD23 expression of B cells without stimulation or on B cells cultured in the presence of control antigens.

Conclusion: Because FcεR2/CD23 expression on B cells is antagonistically regulated by the cytokines interleukin-4 and interferon-γ, the decrease of allergen-induced FcεR2/CD23⁺ B cells indicates an altered cytokine secretion pattern of the allergen-specific T lymphocytes with a predominance of interferon-γ. (*J ALLERGY CLIN IMMUNOL* 1995;95:77-87.)

Key words: Hyposensitization, desensitization-immunologic, immunotherapy, CD23, lymphocyte stimulation, grass pollen, hayfever

Although hyposensitization has been successfully applied in the treatment of IgE-mediated disorders for more than 80 years, little is known about its working mechanisms. Various immuno-

Abbreviations used

BP:	Birch pollen
GP:	Grass pollen
IFN-γ:	Interferon-γ
IL:	Interleukin
MNCs:	Mononuclear cells
TT:	Tetanus toxoid

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logic changes are observed during hyposensitization that may in part be responsible for relief of allergic symptoms. They include an increase in allergen-specific IgG antibodies¹ and moderate impairment in the synthesis of allergen-specific IgE,² development of anti-idiotypic antibodies,³ reduced basophil reactivity and sensitivity to allergens,¹ reduced allergen-stimulated lymphocyte

proliferation and lymphokine production,^{4,5} or the generation of allergen-specific suppressor T cells.⁶ However, each of these changes may not be seen in every patient.^{7,8} Only the increase in IgG antibodies has some correlation with clinical outcome in groups of pollen- and venom-treated patients.^{9,10} Therefore a widely held concept is that allergen-specific IgG, when present in excess in mucosal secretions or serum, could compete with IgE on the surface of mediator-releasing cells for binding of the specific allergen, thus blocking the initial triggering event of the allergic symptoms.¹¹

Our recent understanding of IgE synthesis, however, attracts a particular attention to the effects of hyposensitization on the cellular level of IgE-mediated reactions. Interleukin (IL)-4¹² effects and T cell-B cell interactions¹³ seem to be essential for the induction of human IgE synthesis. Furthermore, the low-affinity receptor for the Fc fragment of IgE (FcεR2/CD23) is a molecule intimately involved in the regulation of IgE response.¹⁴ Soluble products of FcεR2/CD23, known as IgE-binding factors, were reported to augment the proliferation and IgE synthesis of peripheral blood mononuclear cells stimulated with suboptimal concentrations of IL-4.¹⁵ Moreover, binding of monoclonal antibodies directed against FcεR2/CD23 selectively inhibits IgE synthesis by purified B cells,¹⁶ whereas interaction of CD23 with CD21 leads to increased IgE production.¹⁷ The critical involvement in the IgE response is further substantiated by the observation that both FcεR2/CD23 expression and IgE production of B cells are enhanced by IL-4.^{12,18} Accordingly, the percentage of FcεR2/CD23⁺ B lymphocytes is enhanced in the blood of atopic patients with elevated serum IgE levels.¹⁹ Furthermore, interferon-γ (IFN-γ), as the major antagonist of IgE production,¹² inhibits the expression of FcεR2/CD23 on IL-4-stimulated B cells.²⁰

Recently, we have shown that the FcεR2/CD23 molecule is transiently expressed on a subpopulation of T lymphocytes from patients with bee venom allergy stimulated with the specific bee venom allergen phospholipase A₂²¹ and is clearly diminished after rush immunotherapy with bee venom.^{22,23} The aim of this investigation was to determine whether specific hyposensitization has similar effects on allergen-induced FcεR2/CD23 expression on B cells. Changes in allergen-induced FcεR2/CD23 expression on B cells during immunotherapy might reflect isotype-specific alterations in the allergen-specific immune response.

METHODS

Study groups

Forty-seven subjects (25 male and 22 female), ranging in age from 12 to 62 years, were selected according to clinical history, prick test reactivity (Bencard, Smith Kline Beecham, Neuss, Germany), and allergen-specific IgE serum levels. Twelve (8 male and 4 female) patients who were allergic to grass pollen were beginning the first course of hyposensitization treatment (age range, 12 to 40 years; mean age, 26 years). Ten (5 male and 5 female) GP-allergic patients were beginning the second or third year of specific hyposensitization treatment (age range, 19 to 54 years; mean age, 31 years). Eleven (7 male and 4 female) GP-allergic patients without prior hyposensitization had been treated only symptomatically (age range, 20 to 62 years; mean age, 32 years). Fourteen (5 male and 9 female) healthy, nonatopic volunteers served as a control group (age range, 22 to 60 years; mean age, 33 years).

Within the group of patients beginning the first year of hyposensitization, eight were also allergic to birch pollen (BP) allergens. Three of them underwent an additional hyposensitization with BP extract.

Treatment

Patients receiving specific hyposensitization were given biweekly injections of a tyrosine-absorbed, glutaraldehyde-treated GP extract (TA Tyrosin-Allergoid Gräserpollen; SmithKline Beecham, Neuss, Germany) in increasing concentrations until a maintenance dose of 2000 Noon Units was reached. Subcutaneous administration of allergen started at the beginning of January 1990 and continued through the preseason in April of the same year. The maximum GP flight in Germany starts at the end of May and continues to the beginning of July. In the period between November 1989 and January 1990, three patients underwent an additional treatment with a BP extract (TA Tyrosin-Allergoid Birkenpollen, SmithKline Beecham).

Blood samples

Blood samples were taken before (January 1990) and 2 months after initiation of therapy (March 1990). A postseasonal value was also obtained in July 1990. Blood from untreated control subjects was drawn at the same time (Fig. 1).

Cell culture

Mononuclear cells (MNCs) isolated from heparinized venous blood by centrifugation in Ficoll-Hypaque (Apotheke der Städtischen Krankenhäuser, Munich, Germany) were cultured in RPMI 1640 growth medium (Gibco Europe, Karlsruhe, Germany) supplemented with 10% heat-inactivated human AB serum (Rotes Kreuz, Munich, Germany), 1% antibiotic-antimycotic solution, 1% sodium pyruvate, and 1% L-glutamine (all from Gibco Europe). Stimulation was performed in 96-well flat-bottom microtiter plates (Greiner, Munich, Germany) with a lyophilized timothy GP extract, a

Experimental protocol

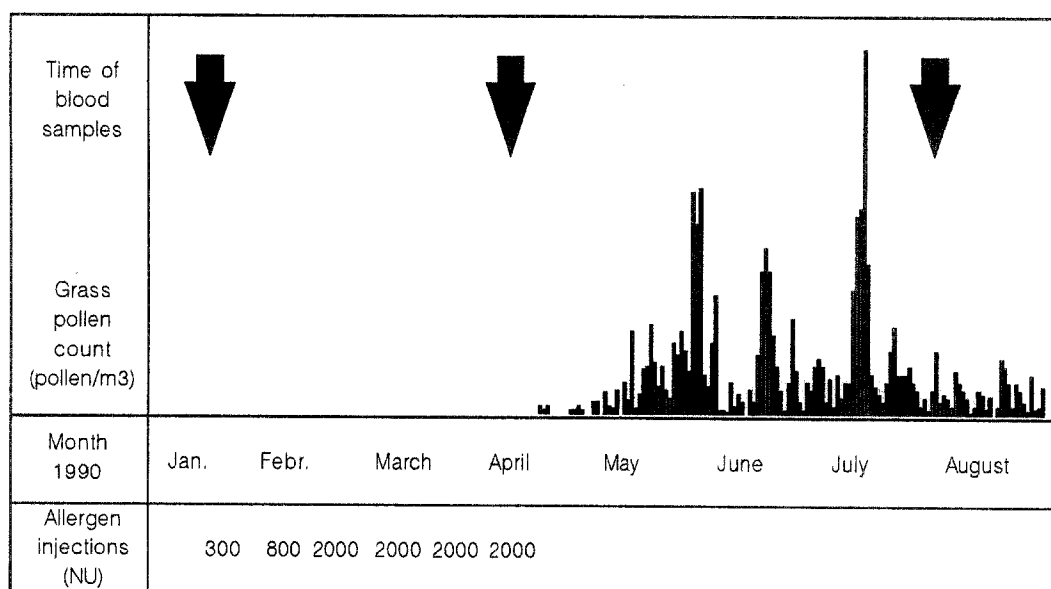


FIG. 1. Experimental protocol from January to August 1990. **Top panel** compares time of blood samples (arrows) with grass pollen flight counted from February through September 1990. **Bottom panel** records hyposensitization by demonstrating Noon Units (NU) of applied allergen.

lyophilized BP extract (both from Scherax, Hamburg, Germany), and tetanus toxoid (TT) (kind gift of Behringwerke AG, Marburg, Germany) at various concentrations. All antigens were free of additives.

Lymphocyte proliferation assay

Lymphocyte proliferation was determined by tritiated thymidine uptake on various days of stimulation. Triplicates with 2×10^5 cells were pulsed with $2 \mu\text{Ci} = 74 \text{ kBq}$ of tritiated thymidine/well (Amersham Buchler, Braunschweig, Germany) for 6 hours. The cultures were harvested with a semiautomatic harvester (Skatron AS, Lier, Norway) onto glass-fiber filters. The radioactivity was counted in a Packard liquid scintillation counter (Packard Instrument Company, Meriden, Conn.).

Determination of FcεR2/CD23-bearing B lymphocytes

To determine the percentage of FcεR2/CD23⁺ B cells, 5×10^5 unstimulated or stimulated MNCs were preincubated with heat-aggregated human IgG (100 $\mu\text{g/ml}$) to block Fcγ-receptors and were stained with a B cell-specific, fluorescein-labeled CD20 monoclonal antibody (B1-FITC; Coulter, Krefeld, Germany). Subsequently, the cells were rosetted with ox erythrocytes that had been coated with the FcεR2/CD23-specific monoclonal antibody M-L25/2E5 by the chromium chloride methods as described by Wilhelm et al.²⁴ The number of FcεR2/CD23-bearing B cells was determined by evaluating rosette formation of fluorescinated B cells with a Leitz Orthoplan microscope (Leitz, Wetzlar, Germany)

with Ploem incident ultraviolet illumination. At least 200 B cells were counted in each sample.

Measurement of pollen counts and IgE antibodies

Pollen grains were counted 3 days weekly from February through September in the Munich Dermatology Department by use of a Burkard trap (Burkhard, London, U.K.). Total IgE and specific IgE serum levels were measured by paper radioimmunosorbent test (PRIST) and RAST with commercially available kits (both from Pharmacia, Uppsala, Sweden).^{25, 26} All samples were tested in a single assay.

Statistical analysis

In each group, a mean and standard deviation were calculated for the examined parameters before, during, and after treatment. Wilcoxon signed-rank sum test for matched pairs was used to compare time-related variations. Variations among the four groups of subjects were analyzed by the nonparametric Mann-Whitney U test. Spearman's correlation coefficient (r_s) was used to compare alteration of allergen-induced proliferation or FcεR2/CD23 expression of lymphocytes with symptom-medication scores.

Symptom-medication scores

A clinical disease score was established retrospectively with a questionnaire, taking into account the severity of allergic symptoms and medication requirement during

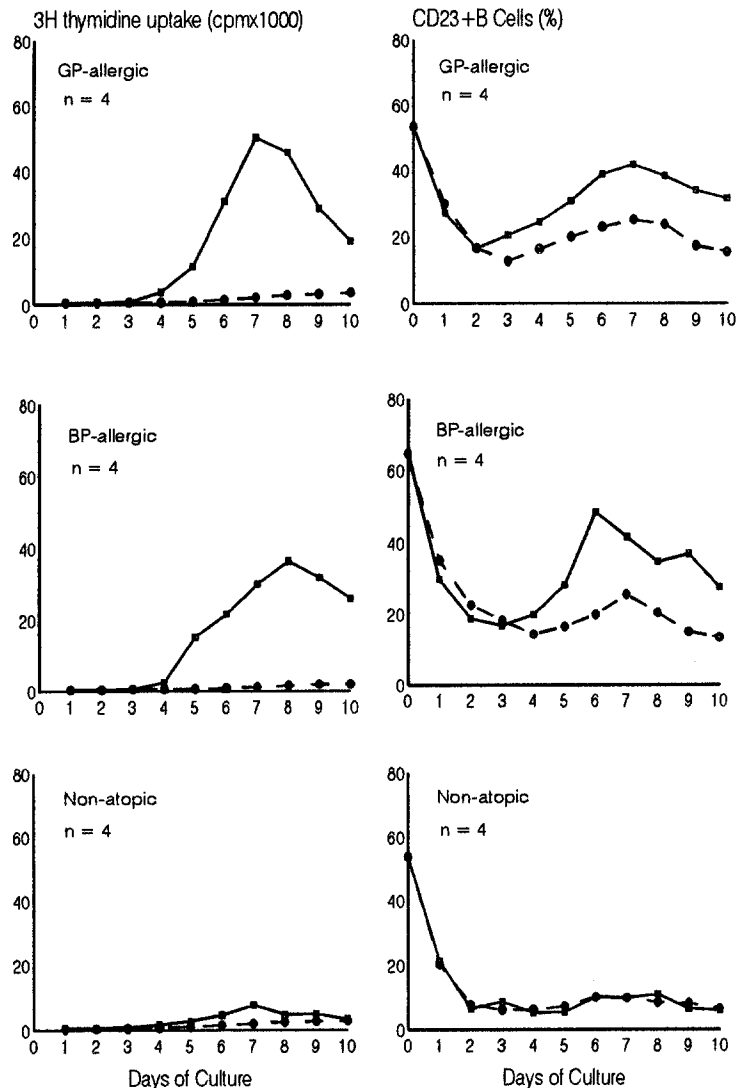


FIG. 2. Time course of allergen-induced lymphocyte proliferation (*left*) and CD23 expression on B cells (*right*). Data presented are the arithmetic means of daily results of MNCs from four GP-allergic (*above*), four BP-allergic (*middle*), and four nonatopic individuals (*below*) between 1 and 10 days after stimulation with 50 μ g/ml GP or BP extract (*solid lines*) or without stimulation (*broken lines*).

the pollen season from May 1990 to July 1990. The results were scored from 0 (no clinical effect, worsening of symptoms, or increase in medication requirement) to 3 (maximum improvement of symptoms or reduction in medication requirement).

RESULTS

Time-course and dose-response studies of the allergen-induced proliferation and Fc ϵ R2/CD23 expression

To determine the kinetics of allergen-induced proliferation and Fc ϵ R2/CD23 expression on B

cells, MNCs from untreated, GP-allergic or BP-allergic patients or nonatopic donors were stimulated with GP or BP extract in a concentration of 5, 50, or 200 μ g/ml. Fc ϵ R2/CD23 expression and tritiated thymidine uptake were determined daily over a period of 10 days.

Most nonatopic donors demonstrated only a marginal response to allergen stimulation, whereas in allergic patients both allergen-induced proliferation and Fc ϵ R2/CD23 expression showed a maximum between day 6 and day 8 (Fig. 2). No response or a low response was recorded if a

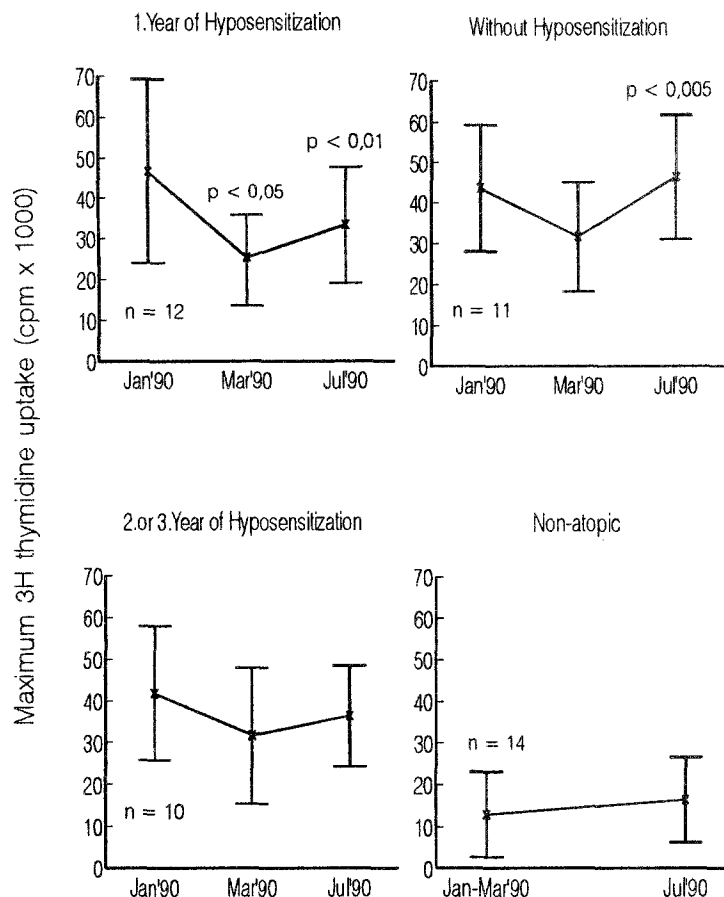


FIG. 3. Effect of hyposensitization with a GP extract on GP-induced lymphocyte proliferation. Data presented are the arithmetic means of maximum results between 6 and 8 days after stimulation of MNCs from 12 GP-treated patients in the first year of hyposensitization (*left above*), 11 only symptomatically treated patients (*right above*), 10 GP-treated patients in the second or third year of hyposensitization (*left below*), and 14 nonatopic donors (*right below*). Standard deviations are indicated by vertical lines.

culture time less than 6 days or more than 8 days was used. FcεR2/CD23 expression on freshly isolated B cells varied between 31.1% and 70.7% ($56.7\% \pm 14.3\%$). During the first 3 days of incubation, nearly one half of the initially FcεR2/CD23-bearing B cells lost the surface glycoprotein. However, FcεR2/CD23 reexpression was observed only on B cells from atopic individuals between the sixth and eighth day of stimulation with the specific allergen ($46.2\% \pm 10.0\%$ with GP stimulation and $60.4\% \pm 6.7\%$ with B stimulation) (Fig. 2). Of the antigen concentrations tested, 50 μg/ml pollen extract elicited a maximum proliferation ($54,300 \pm 17,500$ cpm with GP stimulation and $37,000 \pm 15,800$ cpm with BP stimulation) and FcεR2/CD23 expression on B cells ($46.2\% \pm 10.0\%$ with GP stimulation and $60.4\% \pm 6.7\%$ with BP stimulation).

Effect of hyposensitization on GP-induced proliferation of MNCs

Subsequently, the effect of GP hyposensitization on GP-induced lymphocyte proliferation of MNCs was analyzed. The results from 22 patients who were treated with GP extract were compared with those from 11 allergic patients who were receiving only symptomatic treatment and with those from 14 healthy, nonatopic individuals. MNCs obtained before (January 1990), at the end of (March 1990), and after hyposensitization following the first pollen season (July 1990) were stimulated with 50 μg/ml GP extract, and proliferation was determined on days 6 to 8.

In both hyposensitized and only symptomatically treated patients, the proliferative response to GP decreased markedly before the pollen season and increased again with seasonal allergen exposure.

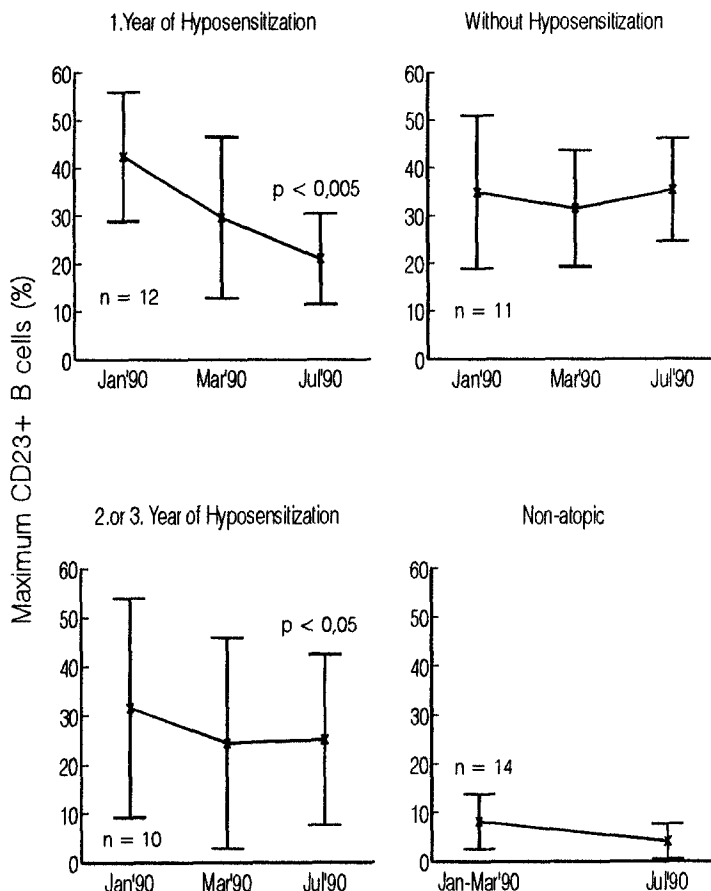


FIG. 4. Effect of hyposensitization with a GP extract on GP-induced CD23 expression on B cells. Data presented are the arithmetic means of maximum results between 6 and 8 days after stimulation of MNCs from 12 GP-treated patients in the first year or hyposensitization (*left above*), 11 only symptomatically treated patients (*right above*), 10 GP-treated patients in the second or third year of hyposensitization (*left below*), and 14 nonatopic donors (*right below*). Standard deviations are indicated by vertical lines.

Although this increase was less prominent in the patients receiving hyposensitization, who had lower proliferation rates after the pollen season ($32,300 \pm 14,800$ cpm) as compared with the pretreatment values ($44,500 \pm 20,100$ cpm) ($\alpha = 0.01$) (Fig. 3), there was no significant difference between GP-induced proliferation of lymphocytes from GP-allergic patients beginning the second or third year ($39,500 \pm 16,400$ cpm) and GP-allergic patients beginning the first course of hyposensitization treatment ($44,500 \pm 20,100$ cpm) or the pharmacotherapeutically treated group ($41,800 \pm 15,500$ cpm). Lymphocyte proliferation rates of nonatopic individuals ($11,200 \pm 10,400$ cpm) were significantly lower than those of atopic patients ($42,100 \pm 17,100$ cpm) and were not markedly changed.

Effect of hyposensitization on GP-induced FcεR2/CD23 expression on B cells

In a similar manner, the effect of GP hyposensitization on GP-induced FcεR2/CD23 expression was analyzed. For this purpose, B lymphocytes from 22 patients treated with GP extract, 11 allergic patients receiving only symptomatic treatment, and 14 healthy, nonatopic individuals were analyzed regarding FcεR2/CD23 expression. In contrast to its effect on proliferation of MNCs, hyposensitization resulted in a persistent decrease in GP-induced FcεR2/CD23 expression on B cells from GP-treated patients undergoing the first hyposensitization course. It decreased from $42.4\% \pm 13.5\%$ to $20.9\% \pm 10.0\%$ ($\alpha = 0.005$) (Fig. 4), whereas in only symptomatically treated patients no marked difference was found between FcεR2/

CD23 expression in January 1990 ($34.9\% \pm 16.0\%$) and July 1990 ($35.1\% \pm 11.4\%$). GP-induced FcεR2/CD23 expression of B cells from GP-allergic patients beginning the second or third year of hyposensitization ($31.6\% \pm 22.4\%$) and nonatopic individuals ($8.2\% \pm 5.6\%$) was significantly lower than that from atopic patients ($40.3\% \pm 14.9\%$). GP-induced FcεR2/CD23 expression on B cells did not significantly correlate with GP-induced lymphocyte proliferation of MNCs either before ($r_s = 0.1607$) or after hyposensitization ($r_s = 0.0390$).

Effect of hyposensitization on BP-induced proliferation and FcεR2/CD23 expression of MNCs

As a control, MNCs from the same patients were cultured in the presence of 50 μg/ml lyophilized BP extract and were examined for changes in BP-induced FcεR2/CD23 expression and tritiated thymidine uptake. Eight of 12 patients beginning the first course of hyposensitization treatment were also allergic to BP. Three of them underwent an additional treatment with a BP extract, whereas five were only treated with GP. Seven of 11 GP-allergic patients without hyposensitization were also allergic to BP. As observed during stimulation with GP, lymphocytes from BP-allergic patients had significantly higher BP-induced tritiated thymidine uptake ($32,100 \pm 12,700$ cpm) and FcεR2/CD23 expression rates ($38.0\% \pm 14.3\%$) than atopic but not BP-allergic patients ($10,300 \pm 5300$ cpm or $13.9\% \pm 8.9\%$) or nonatopic individuals (8000 ± 9800 cpm or $5.4\% \pm 5.9\%$) (Fig. 5, data of tritiated thymidine uptake not shown). Only B cells from those patients who underwent additional hyposensitization with a BP extract showed a decrease in BP-induced lymphocyte proliferation (from $38,100 \pm 11,000$ cpm to $26,700 \pm 15,300$ cpm) and FcεR2/CD23 expression (from $42.7\% \pm 15.3\%$ to $25.9\% \pm 11.0\%$). In patients receiving only GP therapy or symptomatic treatment, BP-induced lymphocyte proliferation and FcεR2/CD23 expression on B lymphocytes remained unchanged (Fig. 6, data of tritiated thymidine uptake not shown).

Effect of hyposensitization on TT-induced proliferation and FcεR2/CD23 expression of MNCs

To further substantiate the selectivity of the hyposensitization effects, MNCs from the same patients were analyzed after stimulation with 50 μg/ml purified TT. No significant changes of the TT-induced lymphocyte proliferation or FcεR2/CD23 expression on B cells were observed. The

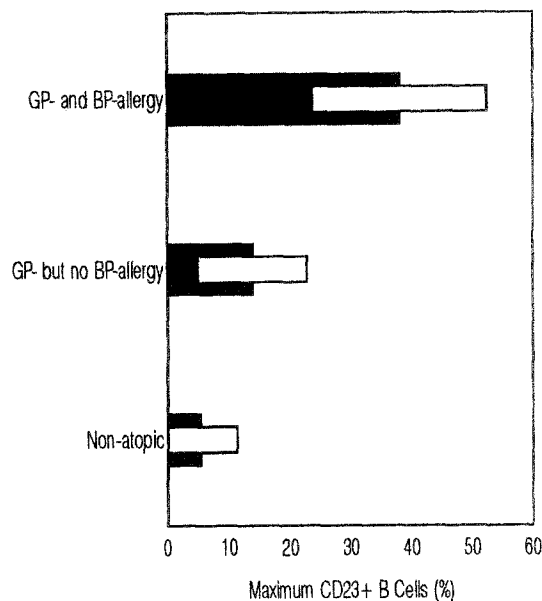


FIG. 5. Allergen specificity of BP-induced CD23 expression on B cells. Data presented are the arithmetic means (black horizontal bars) of maximum results between 6 and 8 days after stimulation of MNCs from 15 GP- and BP-allergic patients (above), eight GP-allergic but not BP-allergic patients (middle), and 14 nonatopic donors (below). Standard deviations are indicated by white horizontal bars.

percentage of FcεR2/CD23⁺ B cells from patients allergic to GP ($28.7\% \pm 9.4\%$) was similar to that of B cells from nonatopic individuals ($31.5\% \pm 19.9\%$). There was also no statistically significant difference in the mean proliferative response of TT-stimulated lymphocyte cultures between the atopic ($31,700 \pm 24,500$ cpm) and nonatopic groups ($34,000 \pm 22,000$ cpm).

Effect of hyposensitization on FcεR2/CD23 expression on B cells without stimulation

Lymphocytes from the same patients were also analyzed with regard to spontaneous FcεR2/CD23 expression on day 0. FcεR2/CD23 expression on freshly isolated B cells from 33 GP-allergic patients ($62.0\% \pm 13.6\%$) was enhanced in comparison with 14 nonatopic individuals ($50.8\% \pm 12.3\%$). In the 23 atopic patients who had not received hyposensitization until January 1990, FcεR2/CD23 expression on freshly isolated B cells correlated weakly with total IgE levels ($r_s = 0.3394$). However, neither spontaneous FcεR2/CD23 expression ($62.9\% \pm 15.2\%$ vs $58.3\% \pm 9.7\%$) nor total IgE level (192.6 ± 214.1 U/ml vs 191.9 ± 183.2 U/ml) was changed over time in pollen-treated patients.

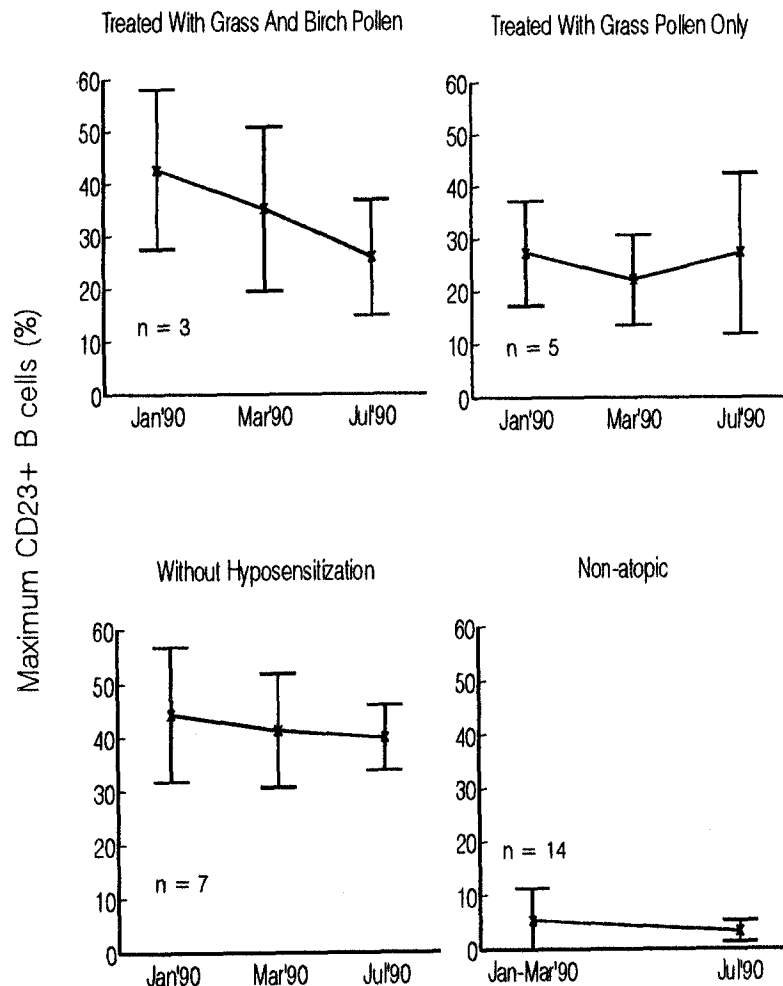


FIG. 6. Effect of hyposensitization with a GP extract on BP-induced CD23 expression on B cells. Data presented are the arithmetic means of maximum results between 6 and 8 days after stimulation of MNCs from three GP- and BP-treated patients (*left above*), five BP-allergic but only GP-treated patients (*right above*), seven BP-allergic but only symptomatically treated patients (*left below*), and 14 nonatopic donors (*right below*). Standard deviations are indicated by vertical lines.

Correlation between GP RAST and GP-induced lymphocyte proliferation or FcεR2/CD23 expression

When RAST values and proliferative responses or the GP-induced FcεR2/CD23 expression on lymphocytes from 23 subjects were plotted in a scattergram, there was no significant correlation between the parameters ($r_s = 0.1954$ or $r_s = 0.2443$). However, mean values of both proliferative response ($42,100 \pm 17,100$ cpm) and FcεR2/CD23 expression ($40.3\% \pm 14.9\%$) on lymphocytes from GP-allergic patients stimulated with GP significantly exceeded lymphocyte reactivity of nonatopic subjects ($11,200 \pm 10,400$ cpm or $8.2\% \pm 5.6\%$) (data not shown).

Correlation between GP-induced lymphocyte proliferation or FcεR2/CD23 expression and symptom-medication scores

Although there was no significant correlation between GP-induced lymphocyte proliferation and symptom-medication scores ($r_s = 0.0701$), changes in the severity of allergic symptoms and medication requirement during the following pollen season were related closely to fluctuations in the proportion of FcεR2/CD23⁺ B cells ($r_s = 0.7506$) (Fig. 7). This means that after the following pollen season, significantly lower percentages of B cells from patients with improvement of symptoms or reduced medication requirement expressed FcεR2/

CD23 molecules than B cells from patients with no clinical effect, worsening of symptoms, or increase in medication requirement.

DISCUSSION

As recently demonstrated, hyposensitization has pleiotropic effects on the immune mechanisms involved in IgE-mediated reactions.^{7,8} The presented data show that hyposensitization with GP extract reduces both allergen-induced lymphocyte FcεR2/CD23 expression and proliferation. In contrast to the lymphoproliferative response, which was largely reconstituted during and after the following pollen season, allergen-induced FcεR2/CD23 expression remained at low levels. This feature was characteristic only for hyposensitized patients, whereas in only symptomatically treated patients FcεR2/CD23 expression was unchanged. A similar lymphocyte proliferation pattern over time with a decrease during hyposensitization, followed by an increase during and after the pollen season, has already been observed by other groups.^{27,28} It suggests that the differences in the proliferative responses reflect distinct migration patterns of the allergen-specific T lymphocytes, depending on allergen exposure. Artificial or natural pollen exposure initially attracts T lymphocytes from the circulation to the site of antigen presentation. At a later time the booster effect of this immunization leads to a subsequent reappearance of allergen-specific T lymphocytes in the circulation.²⁹ The decrease in allergen-specific lymphocyte reactivity in both hyposensitized and symptomatically treated patients can furthermore be explained by the lack of allergen exposure during the winter months. Consequently, tolerization of the allergen-specific T-cell population is not likely to be a working mechanism of hyposensitization. This is additionally supported by the increase of allergen-specific IgG observed during the first weeks of hyposensitization,¹ which depends on T-cell help and increases during long-term hyposensitization.

A major effect of hyposensitization was found with regard to allergen-induced FcεR2/CD23 expression. In spite of a comparable proliferative response of hyposensitized and symptomatically treated patients, allergen-induced FcεR2/CD23 expression was only lost on B cells from hyposensitized patients. CD23 on B cells is predominantly regulated by two T-cell products: its expression is induced by IL-4 and inhibited by IFN-γ.^{18,20} These antagonistic activities of IL-4 and IFN-γ are also reflected at the T-cell level in the newly described

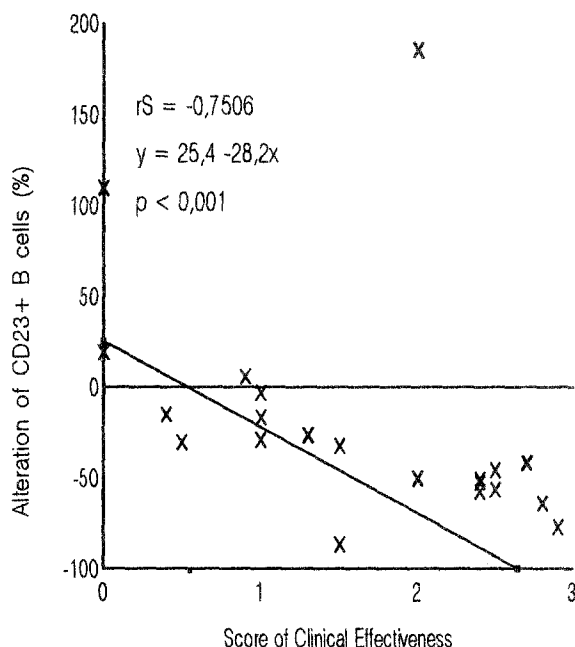


FIG. 7. Alteration in GP-induced CD23 expression on B cells plotted against clinical effectiveness of hyposensitization in 22 GP-treated patients in the first, second, or third year of hyposensitization. The Score of Clinical Effectiveness for each patient represents the arithmetic mean of symptom score and medication requirement during the following pollen season. The solid indicates the statistical regression line. r_s = Spearman's rank correlation coefficient.

T helper cell subsets.^{30,31} T_{H1} cells secrete predominantly IL-2 and IFN-γ, but not IL-4 or IL-5, and thus initiate an IgG-dominated immune response, which is accompanied by an IFN-γ-induced Fcγ-receptor expression on a variety of cell types. On the contrary, T_{H2} cells, by producing predominantly IL-4 and IL-5 but not IL-2 or IFN-γ, promote IgE production and expression of Fc receptors for this cytophilic immunoglobulin.³¹ Accordingly, the loss of CD23 on B cells during allergen-specific stimulation is representative of a change in T-cell function with a decrease in IL-4 or an increase in IFN-γ production.

The reduced CD23 expression in culture after specific allergen stimulation must not be confused with CD23 expression on freshly isolated circulating B cells. As recently reported,³² CD23 expression on resting B cells was hardly affected by hyposensitization or natural pollen exposure. The high numbers of circulating CD23⁺ versus CD23⁻ B lymphocytes in both allergic and nonallergic subjects emphasizes the high sensitivity of our direct antibody rosetting technique, which has a

unique capability of discriminating even cells with weak antigen expression.^{24, 33} The lack of changes in CD23 expression on circulating B lymphocytes stresses the need to analyze CD23 expression during allergen-specific stimulation. Only by this approach were we able to find allergen-specific effects on cellular immune responses induced by hyposensitization. These changes, which are consistent with a shift from a T_{H2} pattern toward a T_{H1} pattern of cytokine secretion, are reflected by the changes in allergen-specific serum immunoglobulin levels: IgG levels increase during hyposensitization,¹ whereas IgE levels decrease.² Our data thus suggest that hyposensitization as a prolonged immunization may act by changing the cytokine secretion patterns of allergen-specific T cells from a T_{H2} to a T_{H1} response. Moreover, the close correlation between reduction in FcεR2/CD23⁺ B cells and improvement of clinical symptoms or medication requirement, respectively, during hyposensitization shows FcεR2/CD23 expression as a possible sensitive parameter, which may be used in immunologic monitoring of hyposensitization and perhaps even in patient selection for hyposensitization.

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REFERENCES

- Lichtenstein LM, Norman PS, Winkenwerden WL, Osler AG. In vitro studies of human ragweed allergy: changes in cellular and humoral activity associated with specific desensitization. *J Clin Invest* 1966;45:1126-36.
- Levy DA, Osler AG. Studies on the mechanism of hypersensitivity phenomena. XVI. In vitro assays of reaginic activity in human sera: effect of therapeutic immunization on seasonal titer changes. *J Immunol* 1967;99:1068-77.
- Castracane JM, Rocklin RE. Detection of human autoantibodies (Ab2). II. Generation of Ab2 in atopic patients undergoing allergen immunotherapy. *Int Arch Allergy Appl Immunol* 1988;86:295-302.
- Evans R, Pence H, Kaplan H, Rocklin RE. The effect of immunotherapy on humoral and cellular responses in ragweed hay fever. *J Clin Invest* 1976;57:1378-85.
- Hsieh K. Altered IL-2-production and responsiveness after hyposensitization to house dust. *J ALLERGY CLIN IMMUNOL* 1985;76:188-95.
- Rocklin RE, Sheffer AL, Greineder DK, Melmon KL. Generation of antigen-specific suppressor cells during allergy desensitization. *N Engl J Med* 1980;22:1213-9.
- Norman PS. Immunotherapy for nasal allergy. *J ALLERGY CLIN IMMUNOL* 1988;81:992-6.
- Rocklin RE. Immune mechanisms in allergen-specific immunotherapy. *Clin Immunol Immunopathol* 1989;53:S119-S131.
- Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect venom hypersensitivity. *N Engl J Med* 1978;299:157-61.
- Starr MS, Weinstock M. Studies of pollen allergy. III. The relationship between blocking antibody levels and symptomatic relief following hyposensitization with alpyral in hayfever subjects. *Int Arch Allergy Appl Immunol* 1970;38:514-21.
- Platts-Mills TAE, Van Maur RK, Ishizaka K, Norman PS, Lichtenstein LM. IgA and IgG anti-ragweed antibodies in nasal secretions: quantitative measurements of antibodies and correlation with inhibition of histamine release. *J Clin Invest* 1976;57:1041-50.
- Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. *J Immunol* 1986;136:949-54.
- Vercelli D, Jabara HH, Arai K, Geha RS. Induction of human IgE synthesis requires IL-4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. *J Exp Med* 1989;169:1295-1307.
- Rieber EP, Pirron U, Endres N, Prinz JC. Human FcεR2/CD23 in the regulation of the allergic immune response. In: Ring J, Braun-Falco O, Przybilla B, eds. *New trends in allergy III*. Berlin: Springer, 1991:82-91.
- Pène J, Rousset F, Brière F. Interleukin 5 enhances interleukin 4-induced IgE production by normal human B cells. The role of CD23 antigen. *Eur J Immunol* 1988;18:929-35.
- Bonnefoy JY, Shields J, Mermod JJ. Inhibition of human interleukin 4-induced IgE synthesis by a subset of anti-CD23/Fc epsilon RII monoclonal antibodies. *Eur J Immunol* 1990;20:139-44.
- Aubry JP, Pochon S, Graber P, Jansen KU, Bonnefoy JY. CD21 is a ligand for CD23 and regulates IgE production. *Nature* 1992;358:505-7.
- Defrance T, Aubry JP, Rousset F, et al. Human recombinant interleukin 4 induces Fcε receptors (CD23) on normal human B lymphocytes. *J Exp Med* 1987;165:1459-67.
- Spiegelberg HL. The expression of Fcε receptors on lymphocytes of allergic patients. *Int Rev Immunol* 1987;2:63-75.
- Galizzi J-P, Cabrillat H, Rousset F, Ménétrier C, De Vries JE, Banchereau J. IFN-γ and prostaglandin E2 inhibit IL-4 induced expression of FcεR2/CD23 on B lymphocytes through different mechanisms without altering binding of IL-4 to its receptor. *J Immunol* 1988;141:1982-8.
- Prinz JC, Endres N, Rank G, Ring J, Rieber EP. Expression of Fcε receptors on activated human T lymphocytes. *Eur J Immunol* 1987;17:757-61.
- Prinz JC, Bieber T, Ring J, Rieber EP. Einfluß der Schnellhyposensibilisierung mit Bienengift auf die Allergen-induzierte Lymphozyten-Proliferation und FcεR2/CD23-Expression auf T-Lymphozyten. *Allergologie* 1990;13:35-8.
- Prinz JC, Rieber EP, Ring J. Loss of FcεR2/CD23-expression on T and B lymphocytes during rush hyposensitization. In: Ring J, Braun-Falco O, Przybilla B, eds. *New trends in allergy III*. Berlin: Springer, 1991:105-8.
- Wilhelm M, Pechumer H, Rank G, Koop E, Riethmüller G, Rieber EP. Direct monoclonal antibody rosetting. An effective method for weak antigen detection and large scale separation of human mononuclear cells. *J Immunol Methods* 1986;90:89-96.
- Gleich GJ, Averbek AK, Swedlund HA. Measurement of IgE in normal and allergic serum by radioimmunoassay. *J Lab Clin Med* 1971;77:690-8.
- Gleich GJ, Jakob GL, Yunginger JW, Henderson LL. Measurement of the absolute levels of IgE antibodies in

- patients with ragweed hay fever. *J ALLERGY CLIN IMMUNOL* 1977;60:188-98.
27. Broman P, Möller E. Lymphocyte transformation by grass pollen allergens: a study of atopic patients receiving immunotherapy. *Allergy* 1988;43:321-31.
 28. Eisenbrey AB, Agarwal MK, Offord KP, Adolphson CR, Yunginger JW, Gleich GJ. Seasonal variation of in vitro lymphocyte proliferative response to ragweed antigen E. *J ALLERGY CLIN IMMUNOL* 1985;75:84-91.
 29. Ford CE. Traffic of lymphoid cells in the body. In: Wolstenholme GE, Porter R, eds. *The thymus. Experimental and clinical studies.* CIBA foundation symposium. London: Churchill, 1966:131-52.
 30. Mosmann TR, Cherwinski H, Bond M, Giedlin A, Coffman RL. Two types of murine helper T-cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-57.
 31. Romagnani S. Human T_{H1} and T_{H2} subsets: regulation of differentiation and role in protection and immunopathology. *Int Arch Allergy Immunol* 1992;98:279-85.
 32. Corominas M, Mestre M, Bas J, Verdaguer J, Valls A, Romeu A, Buendia E. CD23 expression on B-lymphocytes and its modulation by cytokines in allergic patients. *Clin Exp Allergy* 1993;23:612-17.
 33. Prinz JC, Rieber EP. Fc-receptors for IgE on human lymphocytes. Detection with a rosetting assay using a recombinant human/mouse IgE antibody and characterization with monoclonal antibodies. *Hybridoma* 1987;6:1-16.