Effect of immunotherapy on the production of IgE regulatory soluble factors. The response of normal lymphocytes

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SUMMARY

IgE synthesis is controlled not only by antigen-specific mechanism but also by other factors which selectively affect the IgE isotype. Several groups demonstrated that factors released by lymphocytes were involved in this regulation. Our previous investigations were also directed in this field. Immunotherapy improves clinical symptoms in allergic respiratory diseases and produces cellular and humoral changes that affect IgE production. So we decided to investigate if immunotherapy had any influence in the production of soluble factors. Bidirectional mixed cultures were performed with lymphocytes from healthy controls and allergic patients with different time periods of IT, and the supernatants obtained from chromatography were tested to determine their effect on IgE synthesis of normal lymphocytes.

IT time periods exerted influence on the production of enhancing factors. Suppressor factors derived from allergic patients had no effect on IgE synthesis of normal lymphocytes.

Key words: IgE; Soluble factors; Regulation IgE synthesis; Immunotherapy.

INTRODUCTION

About a quarter of a century has passed since Ishizaka and his coworkers identified the IgE antibody. Although the precise mechanism of IgE regulation remains obscure, progress in modern immunology has led to more information about this sophisticated mechanism. It is a current concept that lymphocytes play an important role in the regulation of IgE synthesis (10). Recent interest has been focussed on lymphocyte products (lymphokines) (3).

IgE synthesis turned out to be controlled not only by antigen-specific mechanism but also by other factors which selectively affect the IgE isotype (7).

Several groups have demonstrated in humans (2, 9, 18, 20, 21) as well as in animals (8, 12) that soluble factors released in the supernatants of lymphocytes in culture are involved in the isotype-specific regulation of IgE synthesis, some are IgE binding factors, and others are not (10, 11, 27).

Our previous investigations were also directed towards this field (22, 23, 24). As a first step, we isolated an Enhancing Factor with a molecular weight of 15 kdaltons and a Suppressor Factor with 30 kdaltons from supernatants obtained from mixed lymphocyte cultures of each of these three groups of people: 1) normal healthy controls, 2) pollen sensitive patients, and 3) atopic patients with serum IgE higher than 1,000 IU/ml.

Soluble factors derived from these 3 groups were tested for their enhancing and suppressor activities on IgE production of lymphocytes of healthy controls (23) and allergic patients (24). The Enhancing Factor derived from the atopic patient group significantly enhanced IgE synthesis of normal lymphocytes but Enhancing Factor derived from the pollen sensitive group, which were collected irrespective of being treated with immunotherapy revealed an inverse effect.

Immunotherapy improves clinical symptoms in allergic respiratory diseases when it is correctly indicated and applied. Many hypotheses have been proposed (17) about the mechanism of immunotherapy action: the induction of suppressor T cells (16), the decrease in sensitivity and reactivity of mast cells (6) and basophils (13, 14, 15), the feed back mechanism (25) and the formation of blocking antibodies (3).

Immunotherapy is generally regarded as being more deeply involved in the antigen-specific mechanism of IgE regulation compared to the IgE isotype-specific mechanism.

But it is also the fact that immunotherapy (IT) produces humoral and cellular changes which affect not only clinical symptoms but also IgE production (1, 4). We therefore decided, as the following step in our research, to study if there was any modification of these soluble factors released by lymphocytes derived from patients who had been treated with this kind of therapy. This present study was carried out to search for some approach to the obscure mechanism of immunotherapy with a stock of soluble IgE regulator factors. It was the aim of our present investigation to demonstrate an interaction between the antigen-specific mechanism of immunotherapy and the isotype-specific mechanism governing IgE synthesis.
MATERIALS

1. Preparation for Isolation of the Soluble Factors

With previous consent, 40 ml of heparinized blood samples were obtained from 62 controls and 77 allergic patients. The blood samples were obtained from healthy adults with no history of allergic disease and normal levels of serum IgE. Allergic patients were diagnosed on the basis of clinical history of allergic respiratory disease, laboratory and cutaneous tests. They had high levels of serum IgE (mean total serum IgE > 1,000 IU/mL). We classified allergic patients into 4 groups according to their time period of immunotherapy (IT). The isolation of soluble factors (to be described later) performed separately from each of the following 5 groups: (G indicates the group of patients)

G0: healthy controls (n = 62)
G1: allergic patients without IT (n = 21)
G2: allergic patients with IT < 1 year (n = 22)
G3: allergic patients with IT > 1 year (n = 15)
G4: allergic patients with IT > 2 years (n = 19)

Among the allergic groups, there were no significant differences in total serum IgE.

We must comment that IT was strictly selected according to clinical indications, using aqueous extracts and its known methodology of administration. Clinical improvement was observed in all cases.

2. Preparation for Study of Biological Activities of Soluble Factors

In order to isolate the soluble factors for their biological activity, lymphocytes from peripheral blood were collected from healthy normal volunteers with normal serum IgE and of no history of allergic disease.

METHODS

1. Isolation of soluble factors

These experiments were carried out following the same procedures as in our previous study (22). Briefly, lymphocytes of peripheral blood samples from individuals were isolated using the method of Böyum. Then bidirectional mixed lymphocyte culture was performed between individuals of the same group at a concentration of 2 × 10^6 cells/ml in complete RPMI. Supernatants were collected after incubation for 7 days at 37°C in 5% CO₂ and then were processed through precipita-

2. Study of Biological Activities of Soluble Factors

The factors were tested to determine their effects on IgE synthesis of normal lymphocytes.

First, lymphocytes were isolated (Böyum) from peripheral blood samples of normal healthy controls. Then they were incubated at a concentration of 1 × 10^6 cells/ml with and without the fractions (eluted fraction enriched in suppressor factors and eluted fraction enriched in enhancing factors) derived from G0, G1, G2, G3, G4 and medium at various concentrations (C.5, 5.0, 10, 20, and 50 µg/ml).

After 7 days' incubation, supernatants were collected for IgE measurement by radioimmunoassay modified for low levels (19, 22, 26).

To simplify our results, we used a ratio which indicated the patient's response rate of each individual.

The ratio was calculated as follows:

\[ \text{modified IgE synthesis with soluble factors} \]

\[ \% \text{IgE} = \frac{\text{spontaneous IgE synthesis without soluble factors}}{100} \]

Enhancement is suggested by a % IgE greater than 100.

3. Statistical analysis

The statistical analysis of the results was performed by Student's t-test for paired data and analysis of variance for paired data.

RESULTS

1. Effect of Enhancing Factor on in vitro IgE synthesis

The modifications exerted on in vitro IgE syntheti-

cism of isolated lymphocytes from 6 normal controls af-

ter the addition of enhancing factors (eluted fractions) derived from supernatants of mixed lymphocyte cul-

tiones from five studied groups (G0, G1, G2, G3, G4) and from the medium, at a concentration of 10 and 20 µg/ml, are shown in Fig. 1.

Enhancing Factor (EF) from normal controls (EFG0) had little or no enhancing effect on IgE synthesis of normal lymphocytes. Twenty percent of EFG0 exerted a statistical positive effect (117.9%, p < 0.05), but neither nor 50% of EFG0 did. There was some dispersion observed in the data about enhancing effect of EFG0.

EF derived from allergic patients without immunotherapy (EFG1) showed significant stimulatory effects on IgE synthesis of normal lymphocytes (10% of EFG1: 151.8%, p < 0.05 and 20% of EFG1: 200.9%, p < 0.01).

EF from allergic patients with IT shorter than one year (EFG2) also showed remarkable enhancing effects (10% of EFG2: 149.8%, p < 0.01 and 20% of EFG2: 207.9%, p < 0.001).

It is noteworthy that EF derived from allergic patients with IT longer than one year (EFG3) showed the high-
est peak of enhancing effect (10% of EFG3: 190.6%, p < 0.01 and 20% of EFG3: 317.8%, p < 0.01).

The effect of EF derived from allergic patients with IT longer than two years (EFG4), also exerted remarkable enhancing effects (10% of EFG4: 213.9%, p < 0.05 and 20% of EFG4: 280.2%, p < 0.01).

The eluted fraction from culture medium (EF-Medication) did not have any effect on the IgE synthesis of normal lymphocytes. The results in pg/ml are shown in Table I.

2. Dose-response effect of EF

We tested each enhancing factor at a concentration of 2.5, 5.0, 10, 200 and 500 µg/ml to know which concentra-
tion could be effective. It should be noted that only EF derived from the supernatant of allergic patients'
3. Effect of Suppressor Factor on In vitro IgG Synthesis of normal lymphocytes

Each suppressor factor from lymphocyte supernatants and medium (SF-Medium, SF-G0, SF-G1, SF-G2, SF-G3 and SF-G4) was tested for its inhibitory effect on IgG synthesis of normal lymphocytes. Following our method, isolated lymphocytes of six healthy normal controls were incubated with free or each SF, and then IgG synthesis was measured. None of the SF had any significant effect on IgG synthesis of normal lymphocytes (Fig. 3, Table II).

Although there was no statistical significance, SF derived from controls (SF-G0) showed maximal modifications (10% of SF-G0: 90.7%, n.s. 8% and 20% of SF-G0: 88.1%, n.s.). Data are not shown but SF-G0, at a concentration of 50%, remarkably suppressed IgG synthesis of normal lymphocytes. Percentage of modified IgG synthesis reached less than 70% of spontaneous IgG synthesis.

4. Effect of Enhancing and Suppressor Factors

Fig. 4 gives the outcomes of biological activities of both enhancing and suppressor factors.

As mentioned before, EF from allergic patients (EF-G1, EF-G2, EF-G3 and EF-G4) obviously enhanced IgG synthesis of normal lymphocytes, although EF derived from controls (EF-G0) did not. As described, there were less differences in the effect between SF and EF derived from supernatant of the controls (SF-G0 and EF-G0), but in other groups (allergic groups), the effects of enhancing factors were clearly predominant over those of suppressor factors.

DISCUSSION

Ishizaka (8), Katz (10) and other investigators have demonstrated the existence of soluble factors which regulate IgG synthesis. We recently also isolated (22, 23, 25) two factors of molecular weight of 30 and 15 kilodaltons which had an enhancing and a suppressor activity on IgG synthesis regulation.

Our interest was drawn to reconfirm our previous results and study if EF modified these suppressor and enhancing factors and their activity, and therefore could have a possible mode of action of EF.

The enhancing factor derived from allergic patients' lymphocytes has a significant stimulatory effect on IgG synthesis of normal lymphocytes. Their enhancing effects are dose-dependent. It is noticeable that EF derived from allergic patients with IT > 1 year. G3. EF derived from allergic patients with IT > 1 year. G4. EF derived from allergic patients with IT > 2 years.

REGULATORY FACTORS AND IMMUNOTHERAPY

The balance between activities of SF and EF regulates IgG synthesis. Loss of this equilibrium will cause a change in the clinical picture. Finally, we would like to again emphasize that the immunotherapy time periods surely influence the production of enhancing factors. Further studies would be required to see if a long period of immunotherapy normalizes the production of enhancing factors. As our study was directed towards the effect of suppressor factors on lymphocytes of controls we had advantages in the evaluation of enhancing factors, but we could hardly make enough estimation of the suppressor factor activity. The only thing we can comment is that SF derived from allergic patients (SF-G1, SF-G2, SF-G3 and SF-G4) at any concentration, has no effect on IgG synthesis of normal lymphocytes, and that SF derived from controls (SF-G0) seems to have some effect. These results are in accordance with the clinical experience that allergic patients generally have high levels of serum IgE, while normal controls have low levels.

It would be necessary to test the same soluble factors as we used in this present study for their effect on allergic lymphocytes. This would then inform us of any differences in reactivity to our soluble factors between these allergic lymphocytes and normal lymphocytes, and therefore, of the effect of suppressor factors.

RESUMEN

Es bien conocido que los linfocitos juegan un importante papel en la regulación de la síntesis de IgE. Recientemente, el interés se está focalizando en el estudio de las sustancias liberadas por dichos linfocitos. Varios grupos han demostrado en sobrenadantes de cultivos linfocitarios tanto humanos como de animales la existencia de factores solubles relacionados con la regulación isotipo-específica de la síntesis de IgE. Nuestras investigaciones previas se dirigieron también en ese sentido y así pudimos aislar dos fracciones cromatográficas de 30 y 15 kilodaltons respectivamente que estaban presentes en individuos sanos y pacientes atópicos que provocaron modificaciones en dicha síntesis.

Sabemos que la immunoterapia correctamente indicada y administrada proporciona tanto una mejora clínica en la mayoría de los pacientes como una clara modificación de los niveles plasmáticos de IgE, pero su mecanismo de acción permanece todavía oscuro a pesar de las numerosas hipótesis propuestas. Se decidió, continuando nuestra línea de investigación, estudiar el efecto del tiempo de immunoterapia sobre la producción de dichos factores solubles.

Se realizaron cultivos mixtos linfocitarios bidireccio- nales entre linfocitos asilados de individuos sanos (G0) y pacientes atópicos con diferentes tiempos de immunoterapia (G1, G2, G3, G4), siendo procesados posteriormente los sobrenadantes obtenidos en ord-
rentes concentraciones (10 y 20% V/V) en su capacidad para modificar la síntesis de IgE. in vitro sobre los linfoides asitados de sangre periférica de 6 individuos sanos. La producción de IgE se cuantificó por radiorituninosayo modificado para bajas niveles. El análisis matemático de los datos se realizó por el test de la T de Student para datos pareados.

Las primeras fracciones cromatográficas o "factor supresor" (SF) inducidas al cultivo de linfocitos control no suprimieron la síntesis de IgE de un modo significativo estadísticamente (fig. 3, tabla II), aunque el SF procedente de controles sanos mostró el máximo porcentaje de supresión (90.7 y 89.1% al 10 y 20% respectivamente) con respecto a la producción basal (100%). Sin embargo, puesto que en este resultado se deben al efecto de los SF sobre linfocitos normales (con bajas niveles de IgE) pensamos que no ha sido posible una correcta estimación de los mismos. Sería conveniente testarlos sobre linfocitos de pacientes alérgicos con altos niveles de IgE.

Tras el estímulo con las dos fracciones cromatográficas o "factor estimulador" (EF) hubo grandes variaciones en la síntesis de IgE de los linfocitos de individuos normales. Este efecto estimulador fue de diferente magnitud, dependiendo del tiempo de inmunoterapia, a que estuvieran sometidos los pacientes alérgicos, de modo que se observa un aumento y caída en la producción de EF con un pico máximo a la altura de 1-2 años de inmunoterapia (fig. 5). El EF derivado de individuos normales no tuvo efecto estimulador.

A la vista de los resultados obtenidos, nos inclinamos a pensar que el tiempo de inmunoterapia ejerce influencia sobre la producción de EF. Se debería realizar estudios ulteriores para averiguar si un largo período de inmunoterapia llega a normalizar dichos patrones.

Palabras clave: IgE. Factores solubles. Regulación de la síntesis de IgE. Inmunoterapia.

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Lymphocyte response to IgE regulatory factors in allergic patients during the course of immunotherapy

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SUMMARY

In previous studies we demonstrated isotype-specific but antigen non-restricted soluble factors produced by human lymphocytes of allergic patients and normal controls. In a subsequent investigation we isolated these soluble factors from allergic patients classified in various groups according to their immunotherapy (IT) time periods and from controls, and we tested them on lymphocytes of healthy controls. Then, we decided to amplify the study to include the effects on allergic patients’ lymphocytes. Bidirectional mixed cultures were grown with lymphocytes from healthy controls and allergic patients with different IT time periods and supernatants obtained from chromatography were tested on lymphocytes of twenty-one allergic patients. None of the enhancing factors showed statistical significant effects on IgE synthesis of allergic lymphocytes. There clearly existed a change in the production of suppressor factors during the course of IT, but the long time period of IT did not increase this production.

Key words: IgE. Soluble Factors. Regulation of IgE synthesis. Immunotherapy.

INTRODUCTION

In previous studies based on other investigations (1, 3, 4, 5, 6), we demonstrated isotype-specific but antigen non-restricted soluble factors produced by human lymphocytes of allergic patients and normal controls (8, 9, 10). Enhancing soluble factors (ESFs) derived from lymphocytes of pollen sensitive patients, which were collected irrespective of being treated with immunotherapy, revealed to have no enhancing effect on IgE synthesis of normal and allergic lymphocytes. We suspected that a modification of soluble factors by immunotherapy should exist.

A subsequent investigation was performed because of the aforementioned motive (11). We isolated soluble factors from allergic patients who had been classified in various groups according to their immunotherapy time periods and from controls. The results were as follows: EF derived from allergic patients showed significant stimulatory effects on IgE synthesis of normal lymphocytes. We have demonstrated that immunotherapy time periods had some influence on the enhancing factor production with a “rise and fall” in EF production during the course of IT. EF from allergic patients enhanced IgE synthesis of normal lymphocytes in a dose-dependent relation. None of the suppressor factors showed a statistically significant suppressor activity on IgE synthesis of lymphocytes from normal controls. But SF derived from controls seemed to have more suppressor effects than SF from allergic patients. Our latest study gave only a rough estimation of the suppressor factor activity, because of the low responsiveness of normal lymphocytes to suppressor factors. Therefore, we consider it would be worth studying whether there are any differences in the lymphocytes’ response to soluble factors between lymphocytes derived from normal controls and allergic patients. In this study, we tested the same soluble factors as that used in our last study, for their activities on IgE synthesis of lymphocytes of allergic patients.

MATERIAL AND METHODS

1. Isolation of Soluble Factors

With the purpose of isolating soluble factors, heparinized blood samples were obtained from 62 non-allergic healthy controls and 77 allergic patients. Controls were defined as healthy adults with no history of allergic disease and normal levels of serum IgE. Allergic patients were diagnosed on the basis of a clinical history of allergic respiratory diseases, and positive cutaneous and in vitro tests. They had high IgE levels of serum IgE (mean total serum IgE > 1,000 IU/ml). We classified allergic patients into 4 groups according to their IT time periods:

Isolation of soluble factors was performed separately in each of the following 5 groups (G indicates group of patients):

- G0: healthy controls (n = 62)
- G1: allergic patients without IT (n = 21)
- G2: allergic patients with IT < 1 year (n = 22)
- G3: allergic patients with IT > 1 year (n = 15)
- G4: allergic patients with IT > 2 years (n = 19)
There were no significant differences in total serum IgE in the allergic groups. The isolation of soluble factors was performed according to our previous study (11). Briefly, lymphocytes of human peripheral blood were isolated, based on Blyum's method, and (mean serum IgE = 1,000 IU/ml).

Lymphocytes were isolated from peripheral blood samples of allergic patients (Blyum). Then they were incubated at a concentration of 1 x 10^7 cells/ml with and without the fractions (eluent fractions enriched in suppressor factor and eluted fractions enriched in enhancing factor) derived from G0, G1, G2, G3 and G4 at a concentration of 20% v/v.

After 7 days' incubation, supernatants were collected for IgE measurement by radioimmunosay method at low levels (2, 7, 11, 12). To simplify our results, we used a ratio which indicated the lymphocytes' response rate of each individual.

The ratio was calculated as follows:

\[ \text{IgE%/modified IgG synthesis with soluble factors} \times 100 \]

Enhancement was suggested by an IgE% greater than 100 and suppression by an IgE% less than 100. The bidirectional lymphocyte culture was obtained from individuals of the same study group at a concentration of 2 x 10^7 cells/ml in complete RPMI. After incubation for seven days at 37°C in 5% CO₂, supernatants were collected and processed through precipitation with ammonium sulphate saturated to 50%, prolonging dialysis against PBS, and concentration against polyethylene glycol (PEG).

The materials were then passed through the Concanavalin A sepharose column (1.5 x 7.5 cm, Pharmacia). The eluent fraction was collected first, then the second fraction (the eluted one) was obtained through competition with alpha-methyl glucopyranoside at 50% with PBS. The protein concentration of both fractions was measured (Biorad) and they were adjusted to 1,000 mg/ml 100 ml in eluent fractions and 200 mg/ml 100 ml in eluted fractions (11).

2. Study of Biological Activity of Soluble Factors

The isolated factors were tested to determine their effects on IgE synthesis of allergic patients.

For this purpose, peripheral blood samples were collected from 21 patients allergic to Dermatophagoides driescius or Phleum pratensis during the pollen season. Patients were diagnosed on the basis of clinical history of allergic respiratory diseases, positive cutaneous and - in vitro - tests.

3. Statistical analysis

The statistical analysis of the results was performed by a two-tailed Student t test for paired data and analysis of variance for paired data.

**RESULTS**

1. Effect of Suppressor Factors (SF) on IgE synthesis of lymphocytes of allergic patients

Figure 1 and Table I show the modifications exerted on in vitro IgE synthesis in the allergic patients studied.

SF derived from controls (SGF0) showed a maximum suppressor effect on IgE synthesis of lymphocytes from allergic patients (82.3%, p < 0.001). SF from allergic patients without immunotherapy (SGF1) had no significant suppressor effect on IgE synthesis of lymphocytes from allergic patients (93.6%). SF from allergic patients with IT < 1 year (SGF2) showed a remarkable suppressor effect (87.1%, p < 0.01). SF from allergic patients with IT > 1 year (SGF3) also showed an inhibitory effect on IgE synthesis of lymphocytes from allergic patients (91.3%, p < 0.05), but less suppressor effect than the former group (SGF2). But SF derived from allergic patients with IT > 2 years (SGF4) had no suppressor activity (94.7%).

There were statistical differences in the percentage of IgE synthesis (modified IgG synthesis/spontaneous IgG synthesis) between SGF0 and SGF1, SGF2 and SGF1, SGF3 and SGF0, and SGF2 (Fig. 1).

2. Effect of Enhancing Factors (EF) on IgE synthesis of lymphocytes of allergic patients

Figure 2 and Table I show activities of 5 kinds of enhancing factors (EF0, EF1, EF2, EF3 and EF4) on in vitro IgE synthesis of lymphocytes of 21 allergic patients.

EF derived from normal controls (EF0) had no enhancing effect on IgE synthesis of allergic patients (95.6%). EF from allergic patients without IT > 2 years (EF3) showed an enhancing effect (112.8%). EF from allergic patients with IT > 1 year (EF4) also showed a positive effect (108.7%), but less than EF3.

It is noteworthy that the data of effects were not significant.

There were statistical differences in IgE% between EF0 and EF1, EF2, EF3 (Fig. 1). Enhancing and suppressor activities are compared in figure 3.

3. Comparison between effect of Suppressor Factors (SF) and Enhancing Factors (EF)

Enhancing and suppressor activities are compared in figure 3. With regards to soluble factors derived from controls (SGF0) and from allergic patients with IT < 1 year (G2), the effects of SF were clearly predominant over those of EF, but particular to those from allergic patients without IT (G1), with IT > 1 year (G3), and with IT > 2 years (G4), no possible discrimination could be found between SF and EF.

**DISCUSSION**

We demonstrated the effects of our soluble factors (SF and EF) on IgE synthesis of normal lymphocytes in the previous study reported. In the present study, 21 allergic patients were again included in the sample to test soluble factors for their activity on lymphocytes of allergic patients.

It is noticeable that the suppressor factor derived from normal controls (SGF0) exerted an inhibitory effect on IgE synthesis of lymphocytes of allergic patients. The effect of SGF0 is greater than any other suppressor factor derived from allergic groups (SGF1, SGF2, SGF3 and SGF4).

Among suppressor factors derived from allergic groups, those from allergic patients with immunotherapy shorter than one year (SGF2 and SGF3) showed inhibitory effects on IgE synthesis of lymphocytes of allergic patients. SGF0 suppressor factor derived from allergic patients without immunotherapy and with immunotherapy longer than two years (SGF1 and SGF4) do not. It appears that induction of suppressor factors occurs initially during the short period of immunotherapy and then disappears after the long period. There clearly exists a change in the production of suppressor factors during the course of IT. This result suggests that the immunotherapy time periods exert influence on the lymphocytes' production of suppressor factors.

But it is surprising that IT does not increase the production of SF; it does not seem to serve the purpose of
IT. So SF does not give enough explanation to immunologic improvements (decrease of both specific and total IgE) of allergic patients during and after IT.

Meanwhile, none of the enhancing factors show statistically significant effects on IgE synthesis of allergic lymphocytes. But it is noteworthy that there is a "rise and fall" in EF production by allergic groups (EFG1, EF2, EF3, and EF4) during the course of IT. Interestingly, this tendency is nearly the same as the response of normal lymphocytes shown in our last study (11). Fig. 4 shows the comparison in the lymphocytes' response to EF between allergic patients and normal controls.

Considering this result not only from normal lymphocytes but also from allergic lymphocytes, we are convinced that the duration of IT affects EF production.

We must again mention that none of the enhancing factors can have any statistically significant effect on IgE synthesis of allergic lymphocytes to exogenous enhancing factors (see fig. 4). For the moment, we do not precisely know the reason why allergic patients' lymphocytes respond to these enhancing factors, only to a certain extent.

In the previous study (11) SFG0 had no effects on IgE synthesis of normal lymphocytes, but this soluble factor proved to have a significant suppressor effect on allergic lymphocytes in this present study. These results have revealed how the difference in SF responsiveness between normal and allergic lymphocytes (see fig. 5).

With special references to lymphocytes of normal control (G3), it seems that these lymphocytes produce SF rather than EF.

Allergic lymphocytes appear to respond to SF derived from five groups (G0, G1, G2, G3 and G4) rather than to EF, while normal lymphocytes respond to EF rather than SF. The different characteristics of these lymphocytes turn out to be available for the evaluation of both SF and EF effect.

Therefore, we had obtained useful information on an outline of lymphocyte production of EF and SF during IT. But this information was limited to the responsive-ness of lymphocytes of normal healthy individuals, so an interest arises to observe the changes in the responsive-ness of lymphocytes, which have been treated with IT, to these soluble factors. This approach will enable us to promote a further understanding of immunologic mechanisms not only of IgE regulation but also of IT.

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