

Spontaneous B-cell IgE production in a patient with remarkable eosinophilia and hyper IgE

Shuji Takashi, MD; Yoshio Okubo, MD; Shiro Horie, MD; Tomoyasu Momose, MD; Akihiro Tsukadaira, MD; Kazunaga Agematsu*, MD; and Morie Sekiguchi, MD

Background: The pathophysiology of eosinophilia and hyper-IgE is not fully elucidated yet.

Objective: To clarify the pathophysiology of a patient with remarkable eosinophilia and hyper IgE, we examined cytokine levels in serum, surface antigens of peripheral blood eosinophils and IgE production in vitro.

Results: Concentrations of tumor necrosis factor- α (TNF- α), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), and granulocyte/macrophage-colony stimulating factor (GM-CSF) in the serum were 21 pg/mL, <15 pg/mL, <15 pg/mL, 8 pg/mL, and <5 pg/mL, respectively. Newly expressed surface antigens CD4, CD25, CD69, and HLA-DR, but not CD54, were observed on peripheral blood eosinophils. Extremely high levels of IgE secretion was found in the patient's mononuclear cells without stimuli; this was not enhanced by IL-4 or IL-4 plus anti-CD40 monoclonal antibody stimulation. Furthermore, highly purified B cells spontaneously produced large amounts of IgE and the production was not enhanced in addition of his T cells.

Conclusion: The eosinophils were activated, and the B cells spontaneously produced IgE independently of T cells or cytokines, suggesting that intrinsic abnormality of B cells leading to dysregulated production of IgE in this disease.

Ann Allergy Asthma Immunol 2000;85:150–155.

INTRODUCTION

The IgE immune response is important because it leads to the development of allergic diseases such as rhinitis and bronchial asthma.¹ The production of immunoglobulin E (IgE) from B cells requires at least two signals: a class switching factor, most commonly interleukin (IL)-4, and the engagement of CD40 molecule on B cells. The combination of IL-4 and anti-CD40 monoclonal antibody (mAb) promotes IgE production in vitro.^{2–4}

Eosinophilia is reported in various diseases such as bronchial asthma, parasite infection and Churg-Strauss syndrome. Mature eosinophils are developed in the presence of interleukin-3 (IL-3), interleukin-5 (IL-5), and gran-

ulocyte/macrophage-colony stimulating factor (GM-CSF).⁵ The functions of eosinophils are survival elongation in vitro,⁶ degranulation,⁷ release of chemical mediator⁵ and cytokine production.⁸ Surface antigens on eosinophils are expressed by various cytokine stimulation⁹ and the newly expressed surface antigens are suggested to be a hallmark of activation.^{10,11}

To clarify the pathophysiology of a patient with high levels of serum IgE and remarkable eosinophilia, we have examined the concentration of cytokines in serum, surface analysis of eosinophils and IgE synthesis in vitro.

CASE REPORT

A 82-year-old man was transferred to our hospital because of edema on the extremities, systemic erythema, eosinophilia, and high levels of serum IgE. He was treated twice for tuberculous pleurisy (16 years old and 26 years old). He had suffered from atopic dermatitis for 5 years and was treated with

steroid ointment. In August 1997, he was admitted to a hospital because of pyrexia. A diagnosis of pulmonary tuberculosis was made based on positive acid-fast stain of the sputum and consolidation shadow of right middle-lower lung fields on chest roentgenogram; the patient was administered the anti-tuberculous drugs isoniazid, rifampicin and ethambutol. Although liver dysfunction occurred (GOT 500 U/L, GPT 487 U/L, LDH 1,092 U/L), it was improved by quitting administration of the anti-tuberculous drugs. The number of peripheral blood eosinophils, however, gradually increased (2,000 to 8,000/ μ L), and systemic erythema worsened in conjunction with the appearance of edema in the lower extremities. On November 19, 1997 he was transferred to our hospital for further examination. His temperature was 37.6°C; several 5 to 10 mm sized superficial lymph nodes were palpable on the bilateral neck, left axilla and bilateral groins. Liver was palpable 3 cm on right midclavicular line. White blood cells of peripheral blood (15,700/ μ L), eosinophils (8,007/ μ L) and LDH (813 U/L) were elevated. Serum IgE and CRP were 25,207 IU/mL and 1.09 mg/dL, respectively. Drug-induced lymphocyte stimulation test for antituberculous drugs was negative. Repeated tests for parasites in the feces were negative. Chest roentgenogram showed consolidation on right middle-lower lung fields with pleural calcification. Biopsy and aspiration of bone marrow showed increased cell number of mature eosinophils without malignant cells and a lymph node biopsy specimen taken from the right inguinal region showed no malignancy. Steroid ointment was

From First Department of Internal Medicine and *Department of Pediatrics, Shinshu University, School of Medicine, Matsumoto, Japan.

Received for publication April 4, 1999.

Accepted for publication in revised form January 20, 2000.

administered; edema on extremities and systemic erythema improved and peripheral eosinophil count gradually decreased (500 to 3,000/ μ L), but the high levels of serum IgE persisted. The patient was discharged on February 11, 1998 with high levels of serum IgE (133,870 IU/mL).

MATERIALS AND METHODS

Antibodies and Reagents

FITC-conjugated mAbs against CD9 (mouse IgG1, Pharmingen, San Diego, USA), CD20 (mouse IgG1, Dakopatts A/S, Denmark), CD25 (mouse IgG1, Dakopatts A/S), CD54 (mouse IgG1, Immunotech SA, Marseille, France), CD69 (mouse IgG1, Becton Dickinson, San Jose, USA), VLA-4 (mouse IgG1, Immunotech SA) and phycoerythrin (PE)-conjugated mAbs against HLA-DR (mouse IgG1, Becton Dickinson), CD4 (mouse IgG1, Becton Dickinson), isotype-matched FITC- or PE-conjugated mouse IgG1 mAbs (Dako patts A/S), purified anti-CD40 mAb (IgG1, Immunotech SA), recombinant human (rh) IL-4 (Genzyme Corp, Boston, USA) were purchased.

Cell Preparation

Peripheral blood mononuclear cells (PBMNCs) from the patient and normal subjects were isolated by Ficoll-Hypaque (Pharmacia, NJ, USA) density gradient centrifugation. Furthermore, erythrocyte rosette-positive (E+) and negative (E-) populations¹² were separated with 5% sheep erythrocytes. After depleting monocytes with silica (IBL, Fujioka, Japan) or adherence to the plastic surface, E- cells were further purified into B cells by positive selection with anti-CD19 mAb-coated immunomagnetic beads (Dyna, Oslo, Norway); anti-CD19 mAb was then removed by use of Detach a bead (Dyna). Ninety-seven percent of B cells were reactive with anti-CD20 mAbs. The B cells thus obtained had some tyrosine phosphorylations, whereas no proliferation and activation were found.

Cytokine and Chemokine Measurements

Peripheral blood (PB) was taken from the patient before steroid treatment. Peripheral blood was kept at room temperature for 30 minutes. Serum for cytokine measurement was obtained by centrifugation at 400 *g* for 10 minutes at 4°C. These samples were kept at -70°C until assay. Cytokine concentration was measured using an ELISA kit. The kits for tumor necrosis factor- α (TNF- α) (Medgenix Diagnostics, Fleurus, Belgium), interleukin-3 (IL-3) (Amersham International plc. Buckinghamshire, UK), interleukin-4 (IL-4) (Amersham International plc.) and interleukin-5 (IL-5) (R&D System, Minneapolis, USA), granulocyte/macrophage colony stimulating factor (GM-CSF) (Amersham International plc.), macrophage/monocyte chemoattractant protein-3 (MCP)-3 (Toray Co, Tokyo, Japan) and regulated on activation, normal T cell expressed and secreted (RANTES) (Amersham International plc.) were employed. The minimum measurable amounts of TNF- α , IL-3, IL-4, IL-5 and GM-CSF are 6 pg/mL, 15 pg/mL, 15 pg/mL, 8 pg/mL, 5 pg/mL, 200 pg/mL and 50 pg/mL, respectively. Each cytokine level of normal control serum was below the minimum measurable sensitivity.

Surface Analysis

To analyze surface antigens of eosinophils, we purified PB eosinophils using an improved immunomagnetic selection procedure.¹³ Briefly, 20 mL heparinized venous peripheral blood was mixed with piperazine-N, N-bis (2-ethane sulfonic acid) (PIPES) buffer (25 mM PIPES, 50 mM NaCl, 5 mM KCl, 25 mM NaOH, 5.4 mM glucose, PH 7.4) at a 1:1 ratio. The diluted blood was overlaid on isotonic Percoll solution (1.082 g/mL) (Sigma) and centrifuged at 1,000 *g* for 30 minutes at 4°C. The mononuclear cell layer was then removed and erythrocytes in the sediment were lysed using two cycles of hypotonic water lysis. Isolated granulocytes were washed twice with PIPES buffer with 1% inactivated fetal

calf serum (FCS) (Life Technologies, Inc, Gaithersburg, USA). The pellet of granulocytes was incubated with anti-CD16 mAb coated immunomagnetic particles (Miltenyi Biotec, Bergish-Gladbach, Germany; 50 μ L for 5×10^7 cells) for 60 minutes at 4°C. Magnetically labeled neutrophils were then depleted by passing the granulocytes through a magnetic cell separation (MACS) column in the field of a permanent magnet (Miltenyi Biotec). This procedure resulted in more than 98% pure eosinophils with less than 2% contamination by neutrophils and without lymphocytes; viability was more than 98%. The purity and viability of eosinophils was established using Randolph staining and trypan blue dye exclusion, respectively. Ten or twenty microlitres of FITC-conjugated mAbs against CD9, CD25, CD54, CD69, VLA-4 and phycoerythrin (PE)-conjugated mAbs against HLA-DR, CD4 and isotype-matched FITC- or PE-conjugated mouse IgG1 mAbs were reacted with PB eosinophils ($5 \times 10^5/50 \mu$ L) for 30 minutes at 4°C. Peripheral blood eosinophils were washed twice with PBS containing 1% inactivated FCS at 300 *g* for 10 minutes. The cells were resuspended in PBS containing 1% inactivated FCS. Gated area by forward light scattering and side light scattering confirmed the presence of eosinophils using anti-CD9 and anti-VLA-4 mAbs by flow cytometry (FACScan, Becton Dickinson). Surface analysis was then performed as reported previously.¹³

Immunoglobulin Assay by ELISA

Peripheral blood mononuclear cells were cultured with 50 ng/mL of IL-4 and 1 μ g/mL of anti-CD40 mAb at a final cell density of 0.125 to 2×10^6 /mL in a volume of 200 μ L/well for 12 to 14 days at 37°C in a humidified atmosphere with 5% CO₂. Highly purified peripheral blood B cells (0.5 to 2×10^5 /mL) were also cultured with or without his E+ cells (50% of purified B cells, 0.25 to 1×10^5 /mL). The cultured supernatants were harvested, and the supernatants and the standard human IgE (Chemicon International

Inc, Temecula, CA, USA) were added to human IgE mAbs (CIA-E-7.12 and CIA-E-4.15, kindly provided by A. Saxon, Division of Clinical Immunology/Allergy, UCLA School of Medicine, Los Angeles, CA) and applied to 96 well flat ELISA plates (Nunc, Roskilde, Denmark). After overnight culture at 4°C, the supernatants were discarded and the wells were washed with 0.05% tween 20 in phosphate buffered saline (PBS); alkaline phosphatase-labeled goat anti-human IgE (Sigma Chemical Co, St. Louis, MO) was then added at a dilution of 1/5,000. After 2 hours incubation at room temperature, color detection was performed using 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer containing *p*-nitrophenyl phosphate (pNPP) (Sigma). Calibration was performed with PBS at standard zero levels. All experiments were performed in duplicate. The minimum measurable amount of IgE is 8 ng/mL.^{14,15}

RESULTS

Cytokine and Chemokine Measurements

To clarify the mechanism of the eosinophilia and extremely high levels of serum IgE, we initially examined serum concentrations of several cytokines which affect eosinophilia or IgE synthesis. The level of TNF- α in serum was 21 pg/mL, while IL-3, IL-4, IL-5, and GM-CSF could not be detected. The levels of MCP-3 and RANTES were 0.8 ng/mL and 28.5 ng/mL, respectively. These levels were within the range of normal subjects (MCP-3: 1.1 ± 0.5 ng/mL; RANTES 27.8 ± 4.6 ng/mL).

Surface Analysis of Eosinophils

To confirm the presence of eosinophils antibodies against CD9 and VLA-4 were used for cytometric analysis. CD9 and VLA-4 were expressed on the cells in the gated area, showing these cells were eosinophils (Fig 1a). It was reported previously that CD4, CD25, HLA-DR, CD54 and CD69 do not express on eosinophils of normal subjects. As shown in Figures 1a and b, the profiles of CD25 (30%), CD69

(13%), CD4 (13%), and HLA-DR (35%), but not CD54 were significantly different from those of isotype matched control, demonstrating them to be newly expressed antigens on the eosinophils. CD25, CD69, CD4, CD54 and HLA-DR on a normal subject's eosinophils were not detected in parallel analysis.

Spontaneous IgE Production

Since the patient's serum contained extremely high levels of IgE, we thoroughly investigated IgE production. As shown in Figure 2, PBMNCs obtained from the patient produced high levels of IgE in a cell number dependent fashion without any stimulation. In contrast, PBMNCs obtained from normal subjects produced low levels of IgE with IL-4 plus anti-CD40 mAb stimulation ($n = 10$), whereas no IgE production (<8 ng/mL) was recognized in medium alone or IL-4 alone (Fig 2). Interestingly, the addition of IL-4 or IL-4 plus anti-CD40 mAb did not enhance IgE secretion compared with medium alone in the patient, indicating spontaneous production of IgE by his PBMNCs. We proceeded to examine IgE production by using highly purified B cells from the patient's peripheral blood. As shown in Figure 3, highly purified B cells produced high levels of IgE in a cell number dependent fashion; this production was not affected by the addition of IL-4 or IL-4 plus anti-CD40 mAb (data not shown). Furthermore, IgE production was not enhanced by the addition of T cells obtained from the patient into purified B cells. Purified B cells obtained from normal subjects showed low levels of IgE production with IL-4 plus CD40 signaling (purified B cells 1×10^4 /well: 10 ± 2 ng/mL; 2×10^4 /well: 16 ± 7 ng/mL; 4×10^4 /well: 21 ± 9 ng/mL, $n = 5$), but no IgE production with medium alone (Fig 3) or IL-4 alone (data not shown). These data demonstrate that B cells from the patient spontaneously produced IgE.

DISCUSSION

Hyper-IgE syndrome is characterized by an immunodeficiency disease which

causes frequent staphylococcal abscesses, persistent eczematoid rashes, and extreme elevations of IgE in serum. Several observations such as erythema, positive acid fast staining, eosinophilia, high level of IgE in serum of the patient were similar to previous reports.^{16,17} Recently, it was reported that hyper-IgE syndrome is an autosomal dominant multisystem disorder,¹⁷ however the patient's family shows no features of the hyper-IgE syndrome.

Eosinophilia is observed in various diseases such as bronchial asthma, helminthic infection,¹⁸ hyper-IgE syndrome^{16,17} and in IL-2 administration.¹⁹ Interleukin-3, IL-5, and GM-CSF are active in stimulating eosinophilopoiesis.⁵ Interleukin-5 transgenic mice have developed massive, life-long eosinophilia,²⁰ suggesting IL-5 would be involved in a candidate mechanism for explaining hypereosinophilia; however, these cytokines were not detected in the patient's serum. The mechanisms of eosinophilia were not clear in this patient, suggesting other eosinophilic factor(s) were involved.

It is reported that numerous surface antigens are constitutively expressed on human eosinophils.⁵ Hypodense eosinophils exhibit various morphologic changes such as cytoplasmic vacuolization, alterations in granules of major basic protein-containing cores or matrix.¹⁸ These morphologic changes are well correlated with activated eosinophils induced by cytokines' (IL-3, IL-5 and GM-CSF) stimulation in vitro. There are several reports concerning newly expressed antigens in a variety of diseases. Expression of CD4 and CD25 on PB eosinophils was reported in hypereosinophilic syndrome (HES), helminth infection, and melanoma or renal cell carcinoma treated with IL-2.^{19,21} CD69 expression on PB and bronchoalveolar lavage fluid (BALF) eosinophils was reported in HES and helminth infection.²² These findings suggest that activated heterogeneous eosinophils are present in various eosinophilic disorders. In this case, CD4, CD25, CD69 and HLA-DR, but not CD54, are expressed on eosinophils.

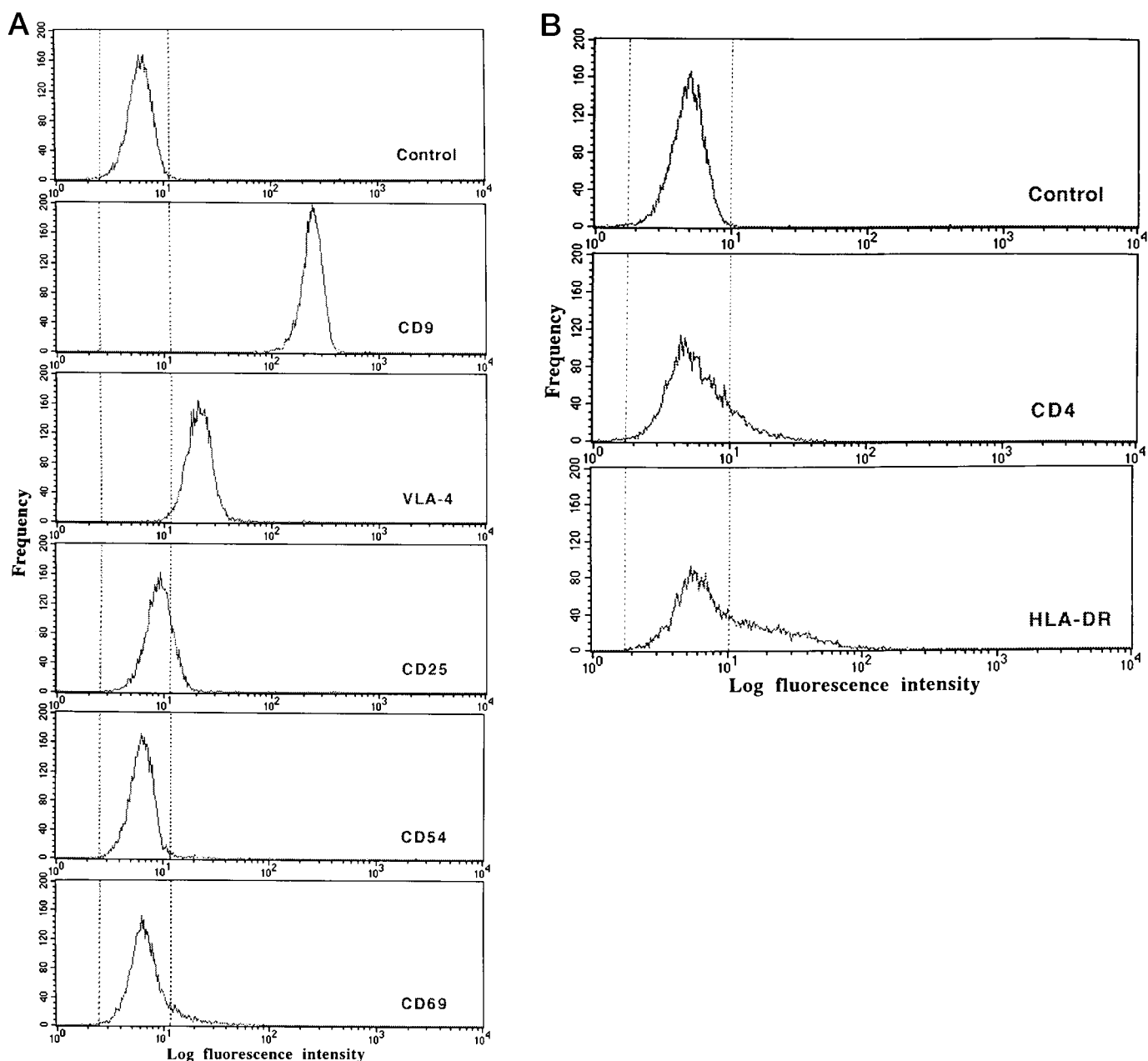


Figure 1. Surface analysis of peripheral blood eosinophils using flow cytometry. Newly expressed surface antigens (CD25 and CD69), but not CD54 could be detected (Fig 1a). Newly expressed surface antigens (CD4 and HLA-DR) could be detected (Fig 1b).

The reason is unclear, however the interaction of cytokines and chemokines may be involved in expression of eosinophil surface antigens.

IgE production of B cells is regulated by cytokines such as IL-4,^{3,23,24} IL-13,^{25,26} and direct contact between B and helper T cells,^{2,24,27,28} in which

CD40/CD154 interaction plays a key role in IgE synthesis.³ Although it is known that IL-4 has a potent function in the production of IgE,^{29,30} IL-4 alone induced germline ϵ transcripts, but not IgE production. Since our patient's B cells produced IgE spontaneously, we investigated germline ϵ transcripts us-

ing the patient's E-cells without stimuli; however, germline ϵ transcripts were not observed (data not shown). There is a possibility that, to our regret, we could not obtain enough B cells. EB virus-transformed B cell lines established from the patient also produced IgE spontaneously at first, but

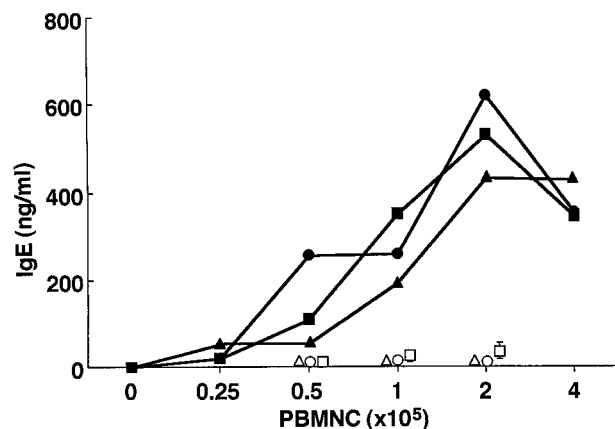


Figure 2. IgE production by peripheral blood mononuclear cells (PBMNCs) of the patient and normal subjects ($n = 10$). Peripheral blood mononuclear cells were cultured with medium ($\blacktriangle$: patient and $\triangle$: normal subjects), IL-4 ($\bullet$: patient and $\circ$: normal subjects), or IL-4 plus anti-CD40 mAb ($\blacksquare$: patient and $\square$: normal subjects) at various concentrations in 96-well round-bottom plates for 12 to 14 days. IgE contents were measured using ELISA. Results are expressed as the mean $\pm$ SE of triplicates, and error bars are sometimes smaller than the plot symbol.

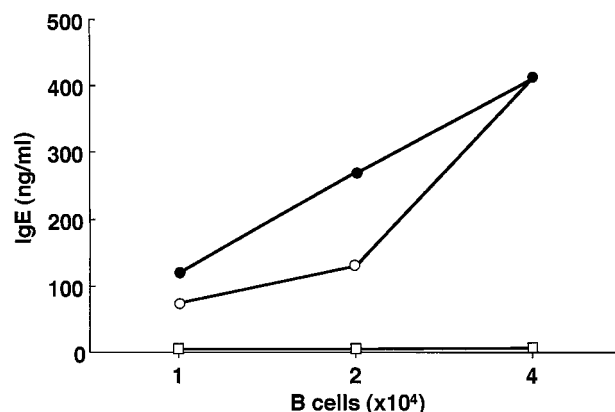


Figure 3. IgE production by purified B cells of the patient and normal subjects ($n = 5$). Highly purified B cells were cultured with medium alone ($\circ$: patient and $\square$: normal subjects) and patient's purified B cells were also cultured with autologous patient's T cells ($\bullet$) at various concentrations in 96-well round-bottom plates for 12 to 14 days. IgE contents were measured using ELISA. Results are expressed as the mean $\pm$ SE of triplicates.

not EB virus-transformed B cell lines obtained from normal volunteers ($n = 5$), whereas the IgE production was gradually decreased and disappeared according to the culture (data not shown). Other immunologic studies of hyper-IgE syndrome have reported little responsiveness of exogenous IL-4 for IgE production.³¹ Claassen et al reported neither PBMNCs nor B cells from the patients with hyper-IgE syndrome spontaneously produce IgE in vitro, little responsiveness of exogenous IL-4 for IgE production and re-

quirement of high concentration of anti-CD40 for IgE production.³¹ Our data presented here are clearly different from the report by Claassen et al in the point of spontaneous IgE production of PBMNCs and B cells and no response of anti-CD40 for IgE production, however, it is similar to the data to be little responsiveness of exogenous IL-4 for IgE production. It is considered that once B cells become committed to IgE synthesis, IL-4 appears to have little role in the regulation of IgE production. Another possibility in low re-

sponsiveness of IL-4 in IgE production is due to variant IL-4 receptor.³² We did not examine the variant IL-4 receptor in the patient. Grimbacher et al reported that IL-4 receptor variant allele was not significantly different from control subjects, suggesting IL-4 receptor gene is not linked to the hyper-IgE syndrome.³²

In conclusion, we report a patient with remarkable eosinophilia and hyper-IgE. Although several cytokines were not detected in the serum, the eosinophils in peripheral blood were activated and the B cells spontaneously produce large amounts of IgE in vitro. Further analyses about the patient are needed to clarify mechanisms of eosinophil activation and IgE production, which will contribute the treatment of allergic diseases.

REFERENCES

1. de Weck AL, Stanworth DR. 1. Anaphylactic hypersensitivity. Introduction: progress in immunology. In: Brent L and Holborow J eds, Amsterdam: North Holland, 1974.
2. Gauchat JF, Lebman DA, Coffman RL, et al. Structure and expression of germline epsilon transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J Exp Med* 1990;172:463-473.
3. Jabara HH, Fu SM, Geha RS, Vercelli D. CD40 and IgE: synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of IgE synthesis by highly purified human B cells. *J Exp Med* 1990;172:1861-1864.
4. Gascan H, Gauchat JF, Aversa G, et al. Anti-CD40 monoclonal antibodies or CD4+ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signaling pathways. *J Immunol* 1991;147:8-13.
5. Weller PF. Human eosinophils. *J Allergy Clin Immunol* 1997;100:283-287.
6. Owen WF Jr, Rothenberg ME, Silberstein DS, et al. Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 1987;166:129-141.
7. Horie S, Kita H. CD11b/CD18(Mac-1) is required for degranulation of human

- eosinophils induced by human recombinant granulocyte-macrophage colony-stimulating factor and platelet-activating factor. *J Immunol* 1994;152:5457–5467.
8. Kita H, Ohnishi T, Okubo Y, et al. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med* 1991;174:745–748.
9. Hansel TT, De Vries JM, Carballido JM, et al. Induction and function of eosinophil intercellular adhesion molecule-1 and HLA-DR. *J Immunol* 1992;149:2130–2136.
10. Hansel TT, Braunstein JB, Walker C, et al. Sputum eosinophils from asthmatics express ICAM-1 and HLA-DR. *Clin Exp Immunol* 1991;86:271–277.
11. Okubo Y, Hossain M, Kai R, et al. Adhesion molecules on eosinophils in acute eosinophilic pneumonia. *Am J Respir Crit Care Med* 1995;151:1259–1262.
12. Agematsu K, Kobata T, Sugita K, et al. Role of CD27 in T cell immune response: analysis by recombinant soluble CD27. *J Immunol* 1994;153:1421–1429.
13. Hossain M, Okubo Y, Horie S, M. Sekiguchi M. Analysis of recombinant human tumour necrosis factor- α -induced CD4 expression on human eosinophils. *Immunology* 1996;88:301–307.
14. Agematsu K, Nagumo H, Shinozaki K, et al. Absence of IgD-CD27+ memory B cell population in X-linked hyper-IgM syndrome. *J Clin Invest* 1998;102:853–860.
15. Nagumo H, Agematsu K, Shinozaki K, et al. CD27/CD70 interaction augments IgE secretion by promoting the differentiation of memory B cells into plasma cells. *J Immunol* 1998;161:6496–6502.
16. Donabedian H, Gallin JI. The hyper-immunoglobulin E recurrent-infection (job's) syndrome A review of the NIH experience and the literature. *Medicine* 1983;62:195–208.
17. Grimbacher B, Holland SM, Gallin JI, et al. Hyper-IgE syndrome with recurrent infections—an autosomal dominant multisystem disorder. *N Engl J Med* 1999;340:692–702.
18. Gleich GJ, Adolphson CR. The eosinophilic leukocyte: structure and function. *Adv Immunol* 1986;39:177–253.
19. Rodgers S, Rees RC, Hancock BW. Changes in the phenotypic characteristics of eosinophils from patients receiving recombinant human interleukin-2 (IL-2) therapy. *Br J Haematol* 1994;86:746–753.
20. Dent LA, Strath M, Mellor AL, Sanderson CJ. Eosinophilia in transgenic mice expressing interleukin 5. *J Exp Med* 1990;172:1425–1431.
21. Riedel D, Brennscheidt U, Kiehntopf M, Brach M, FH. Granulocyte-macrophage colony-stimulating factor and interleukin-3 induce surface expression of interleukin-2 receptor p55-chain and CD4 by human eosinophils. *Immunology* 1990;70:258–261.
22. Nishikawa K, Morii T, Ako H, Hamada K, et al. In vivo expression of CD69 on lung eosinophils in eosinophilic pneumonia: CD69 as a possible activation marker for eosinophils. *J Allergy Clin Immunol* 1992;90:169–174.
23. Claassen JL, Levine AD, Buckley RH. Recombinant human IL-4 induces IgE and IgG synthesis by normal and atopic donor mononuclear cells. Similar dose response, time course, requirement for T cells, and effect of pokeweed mitogen. *J Immunol* 1990;144:2123–2130.
24. Parronchi P, Tiri A, Macchia D, et al. Noncognate contact-dependent B cell activation can promote IL-4-dependent in vitro human IgE synthesis. *J Immunol* 1990;144:2102–2108.
25. McKenzie AN, Culpepper JA, de Waal Malefyt R, et al. Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. *Proc Natl Acad Sci USA* 1993;90:3735–3739.
26. Punnonen J, Aversa G, Cocks BG, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci USA* 1993;90:3730–3734.
27. Vercelli D, Jabara HH, Arai K, Geha RS. Induction of human IgE synthesis requires interleukin 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.
28. DeKruyff RH, Turner T, Abrams JS, et al. Induction of human IgE synthesis by CD4+ T cell clones. Requirement for interleukin 4 and low molecular weight B cell growth factor. *J Exp Med* 1989;170:1477–1493.
29. Snapper CM, Finkelman FD, Paul WE. Regulation of IgG1 and IgE production by interleukin 4. *Immunol Rev* 1988;102:51–75.
30. Banchereau J, De Paoli P, Valle A, et al. Long-term human B cell lines dependent on interleukin-4 and antibody to CD40. *Science* 1991;251:70–72.
31. Claassen JJ, Levine AD, Schiff SE, Buckley RH. Mononuclear cells from patients with hyper IgE syndrome produce little IgE when they are stimulated with recombinant human interleukin-4. *J Allergy Clin Immunol* 1991;88:713–721.
32. Grimbacher B, Holland SM, Puck JM. The interleukin-4 receptor variant Q576R in hyper-IgE syndrome. *N Engl J Med* 1998;338:1073–1074.

Request for reprints should be addressed to:
Yoshio Okubo, MD
First Department of Internal Medicine
Shinshu University School of Medicine
3-1-1 Asahi
Matsumoto 390-8621
Japan