

The Expression of IL-4 and Interferon-gamma Genes in Peripheral Blood Mononuclear Cells of Patients with Severe Atopic Dermatitis : Evaluation in Proportion to Serum IgE Levels

Chul Jong Park, M.D., Mun Gan Rhyu*, M.D., Jin Woo Kim, M.D.,
Chung Won Kim, M.D., and Jong Yuk Yi, M.D.

Department of Dermatology and Microbiology, College of Medicine,
The Catholic University of Korea, Seoul, Korea*

Background : It is not yet clear whether the abnormal cytokine production in relation to serum IgE levels in atopic dermatitis (AD) is associated with the amount of mRNA of cytokine gene.

Objective : Our purpose was to delineate the effect of reciprocal correlation in the level of mRNA between interleukin-4 (IL-4) and interferon-gamma (IFN- γ) in severe AD.

Methods : We examined 15 cases including 5 AD patients with high serum IgE (>2,000 kU / liter), 5 AD patients with low serum IgE (<100 kU / liter), and 5 healthy controls. Using semi quantitative reverse transcription-polymerase chain reaction, IL-4 and IFN- γ gene expressions in peripheral mononuclear cells (PBMC) were examined.

Results : 1) IL-4 gene expression in spontaneous PBMC was higher in AD patient groups than in control group, significantly higher only in AD patient group with high serum IgE level ($p < 0.05$).

2) IFN- γ gene expression in spontaneous PBMC showed increased tendency in AD patient groups than in control group without statistical significance. 3) IL-4 and IFN- γ gene expressions in stimulated PBMC were not different among all three groups.

Conclusion : In light of our results, high and low IgE subgroups in AD can exist and AD may not be characterized by the shift in the reciprocal relationship between IL-4 and IFN- γ when T cells are stimulated under antigen presenting cell-independent conditions.

(Ann Dermatol 13(2) 76~81, 2001).

Key Words : Atopic dermatitis, IFN- γ , IgE, IL-4

Although the pathogenesis of atopic dermatitis (AD) is unknown, genetic susceptibility, environmental factors such as allergens, and immune dysfunction

contribute to its development. Since high levels of serum total IgE and allergen-specific IgE to environmental and food allergens have been demonstrated in most AD patients¹⁻⁵ and about 80% of AD patients show positive immediate skin test to allergens⁶, it is thought that major immunologic abnormality in AD is serum IgE elevation.

Recent studies have demonstrated that two T cell - derived lymphokines, IL-4 and IFN- γ , play a crucial role in the induction of IgE synthesis⁷⁻¹⁰. IL-4 enhances the production of IgE and induces the expression of the low affinity IgE Fc receptor (CD23) on B cells, while IFN- γ inhibits IgE synthesis and CD23 expression^{11,12}. Indeed, peripheral blood mononuclear cells (PBMC) of AD

Received June 1, 2000.

Accepted for publicaion November 9, 2000.

Reprint request to : Jong Yuk Yi, M.D., Department of Dermatology, College of Medicine, The Catholic University of Korea, Ui Jong Bu St. Mary's Hospital 65-1, Kum Oh Dong, Ui Jong Bu City, Kyung Gi Do, 480-130, Korea

Tel. 031-820-3124, Fax. 031-846-4799

*This study was supported by 'The Catholic Medical Center Research Grants for Special Project 1996' .

Table 1. Profile of study population

Group	Subjects	Age (years)	Sex	Prick test	Total IgE (kU / l)	Severity score	Atopic state
HIGH	1	26	F	+	3,220	5	AD*
HIGH	2	14	F	+	5,020	5	AD,AR**
HIGH	3	14	F	+	2,783	5	AD
HIGH	4	25	F	+	3,150	5	AD
HIGH	5	22	M	+	8,850	5	AD,AR
LOW	1	21	F	-	70	5	AD
LOW	2	32	F	+	47.2	5	AD
LOW	3	13	M	-	27.4	5	AD
LOW	4	23	F	-	50.4	5	AD
LOW	5	20	F	-	55	5	AD
CONTROL	1	26	F	-	57.7	-	-
CONTROL	2	33	F	-	34.4	-	-
CONTROL	3	26	M	-	71.3	-	-
CONTROL	4	32	F	-	30.1	-	-
CONTROL	5	30	F	-	58.2	-	-

AD*: atopic dermatitis

AR**: allergic rhinitis

patients show a decreased level of IFN- γ and an increased level of IL-4¹³⁻¹⁵. Since these findings indicate that an immunologic abnormality in AD is imbalance between IL-4 and IFN- γ , IFN- γ has been used in the treatment of AD patients¹⁶.

AD patients with elevated levels of serum IgE have been studied in most investigations to date.

Nevertheless, about 20% of all AD cases did not show elevated levels of serum IgE¹⁷. Recently, Kagi et al.¹⁸ reported the concept of basic immunologic differences between AD with elevated levels (> 200 kU / liter) of IgE, positive radioallergosorbent (RAST) test, positive immediate type skin reactions toward environmental allergens and a positive history for IgE-mediated allergies, and nonatopic form of AD (NAD) with normal levels of serum IgE, negative RAST test, negative prick test, and a negative history of atopy. They demonstrated AD patients with elevated levels of both IL-4 and IL-5 and NAD patients with elevated levels of IL-5 only.

However, it is not yet clear whether the abnormal cytokine production in relation to serum IgE levels is associated with the amount of mRNA of cytokine gene. To answer this question, we tried semi-quantitative reverse transcription - polymerase chain reaction (semi-quantitative RT-PCR) to determine IL-4 and IFN- γ mRNA in PBMC from AD patients with high or low levels of serum IgE before or after stimulation of phorbol

12-myristate 13-acetate / calcium ionophore A23187 (PMA / CA).

SUBJECTS AND METHODS

Patient Selection

A total of ten patients with severe AD including five with high levels (> 2,000 kU / liter) of serum IgE (high serum IgE group), and five with low levels (< 100 kU / liter) of serum IgE (low serum IgE group) were selected in the present study. Five healthy control subjects, who had a negative history of atopic diseases (AD, allergic bronchial asthma, allergic rhinitis) were studied for comparative purposes. None had received oral steroid therapy for at least 1 month before testing. The mean age of high serum IgE group was 20.2 years (range, 14 to 26 years), low serum IgE group, 21.8 years (range, 13 to 32 years), and healthy controls, 26.2 years (range, 18 to 32 years). Patient profiles are represented in Table 1. All AD patients fulfilled the diagnostic criteria of Hanifin and Rajka¹⁹, and were in the category of severe AD²⁰.

RNA Extraction

PBMC were obtained from heparinized blood by density gradient centrifugation over Ficoll-Hypaque²¹. 2×10^6 cells / ml were cultured in RPMI 1640 medium (Life Technologies, Inc., Grand Island, NY, U. S. A.)

Fig. 1. Expression of IL-4 and interferon-gamma (IFN- γ) genes in spontaneous peripheral blood mononuclear cells was evaluated by semi-quantitative reverse transcription-polymerase chain reaction in 10 atopic dermatitis patients with high serum IgE (HIGH, $n = 5$) and low serum IgE (LOW, $n = 5$), and 5 normal healthy controls. Results were expressed as means \pm SD.

* $p < 0.05$ compared with 'LOW'

Fig. 2. PCR-assisted mRNA amplifications for IL-4, interferon-gamma (IFN- γ), and β -actin in spontaneous peripheral blood mononuclear cells from 10 atopic dermatitis patients with high serum IgE (HIGH, $n = 5$) and low serum IgE (LOW, $n = 5$), and 5 normal healthy controls.

supplemented with 10 % FCS, 2 mM L-glutamine, 100 U / ml penicillin and 100 μ g / ml streptomycin at 37 $^{\circ}$ C in a humidified atmosphere with 5% CO₂. PMA 50 ng / ml in combination with CA 2.0 μ M (Sigma Chemical Co., St. Louis, MO, U. S. A.) was used as a stimulant. Cultures were incubated with medium alone or stimulated with PMA / CA for 3 hr, 6 hr, 12 hr or 24 hr. Cells were isolated by brief centrifugation and then stored at -70 $^{\circ}$ C until further processing. RNA extraction was performed using acid guanidinium thiocyanate-phenol-chloroform extraction²².

Reverse Transcription

The total RNA mixture, which was composed of 1 μ l random hexamer primer (Promega, Madison, WI, U. S. A.), 1 μ l oligo (dT)₁₅ primer (Promega), 6 μ l total

Fig. 3. Expression of IL-4 and interferon-gamma (IFN- γ) genes in peripheral blood mononuclear cells stimulated during 3 hr (IL-4) or 6 hr (IFN- γ) by phorbol 12 - myristate 13 - acetate / calcium ionophore A23187 was evaluated by semiquantitative reverse transcription - polymerase chain reaction in 10 atopic dermatitis patients with high serum IgE (HIGH, $n = 5$) and low serum IgE (LOW, $n = 5$), and 5 normal healthy controls. Results were expressed as means \pm SD.

Fig. 4. Representative profiles of the kinetics of IL-4 and interferon-gamma (IFN- γ) gene expression in atopic dermatitis patients with high serum IgE (HIGH) and low serum IgE (LOW), and normal healthy controls. PCR-assisted mRNA amplifications for IL-4, IFN- γ , and β -actin in peripheral blood mononuclear cells were examined at 3 hr, 6 hr, 12 hr, and 24 hr after stimulation with phorbol 12 - myristate 13 - acetate / calcium ionophore A23107.

RNA solution, 5.5 μ l DEPC-DW, was denaturated for 2 min at 72 $^{\circ}$ C and cooled on ice. cDNA was synthesized for 1 hr at 42 $^{\circ}$ C using denaturated RNA mixture, 4 μ l 5 x reaction buffer (Life Technologies, Inc.), 1 μ l dNTP (10 mM mixture of dATP, dCTP, dGTP, and dTTP, Boehringer Mannheim, Germany), 0.5 μ l RNAase inhibitor (Promega), and 1 μ l SuperscriptaseTM II (Life Technologies, Inc.), and heated for 5 min at 95 $^{\circ}$ C. The cDNA preparations were stored at -20 $^{\circ}$ C until further processing.

Polymerase Chain Reaction (PCR)

Nucleotide sequences for oligonucleotide 5' and 3'

primers, respectively, were as follows: β -actin, GTGGG GCGCC CCAGG CACCA and CTCCT TAATG TCACG CACGA TTTC; IL-4, CTTCC CCCTC TGTTT TTCCT and TTCCT GTCGA GCCGT TTCAG; and IFN- γ , AGTTA TATCT TGGCT TTTCA and ACCGA ATAAT TAGTC AGCTT²³. cDNA for PCR was diluted 20-fold for β -actin and 2-fold for IL-4 and IFN- γ . Template mixtures contained 2 μ l diluted cDNA, 1 μ l upstream primer (20 pM), 1 μ l downstream primer (20 pM), and 1 μ l DW for PCR of spontaneous PBMC, and 1 μ l cDNA, 1 μ l upstream primer, 1 μ l downstream primer, and 2 μ l DW for PCR of stimulated PBMC. After 30 μ l mineral oil was added, template mixtures were incubated for 2 min at 100°C and then reaction mixtures, which contained 1 μ l dNTP (1.25 mM mixture of dATP, dCTP, dGTP, and dTTP, Boehringer Mannheim, Germany), 1 μ l 10x thermophilic reaction buffer, 0.6 μ l MgCl₂ (25 mM), 2.3 μ l DW, and 1 μ l Taq DNA polymerase (Promega, Madison, WI, U. S. A.) were added at 94°C. The cDNA amplification was carried out in a Ericomp thermal cycler (Ericomp Co., San Diego, CA, U. S. A.). Denaturation was performed 94°C 1 min; annealing, 60°C 1 min for β -actin, 58°C 1 min for IL-4, 55°C 1 min for IFN- γ ; extension, 72°C 1 min. PCR cycles were 30 for β -actin, 37 for IL-4, and 33 for IFN- γ .

Upon PCR amplification of serial dilutions of sample cDNA, a concomitant decrease in PCR product was observed. PCR amplification of a negative control (a sample of buffer alone) did not yield PCR product, confirming the absence of extraneous cDNA or PCR product contaminating the samples. These primer sequences were confirmed to amplify specific cDNA by Southern blot analysis.

PCR products were resolved in 2% agarose gels and visualized by ethidium bromide staining (0.5 μ g / ml) in an Eagle Eye™ II still video system (Stratagene, La Jolla, CA, U.S.A.). Densitometric analysis of gel images by dynamic integration method for spontaneous PBMC and by integration method for stimulated PBMC was performed using Eagle Sight™ 3.0 Image Capture and Analysis Software (Stratagene).

Statistics

Kinetics of stimulated PBMC was compared by cytokine (IL-4 or IFN- γ) mRNA / β -actin mRNA (%) by repeated measure ANOVA test. The gene

expression of spontaneous or stimulated PBMC was compared by cytokine (IL-4 or IFN- γ) mRNA / β -actin mRNA (%) by Kruskal-Wallis test in the comparison among the three groups and by Wilcoxon rank sum test in the comparison between the two groups. The results were expressed by mean \pm SD, $p < 0.05$ was considered significant.

RESULTS

Expression of IL-4 gene

1) Expression of IL-4 gene in spontaneous PBMC

IL-4 mRNA / β -actin mRNA (% , mean \pm SD) in spontaneous PBMC was 73.7 ± 63.3 in high serum IgE group, 63.0 ± 39.4 in low serum IgE group, and 32.9 ± 7.9 in healthy control group. IL-4 gene expression in spontaneous PBMC was higher in AD patient groups than in healthy control group but statistically higher only in high serum IgE group than in healthy control group ($p < 0.05$, Fig. 1 & 2).

2) Expression of IL-4 gene in stimulated PBMC

When PBMC were stimulated by PMA / CA for 3 hr, 6 hr, 12 hr, or 24 hr, IL-4 gene expression was first detected at 3 hr after stimulation in vitro and persisted until 6 hr, and slowly decreased in all three groups (data not shown).

IL-4 mRNA / β -actin mRNA in 3hr-stimulated PBMC was 98.9 ± 20.7 in high serum IgE group, 93.7 ± 35.2 in low serum IgE group, and 86.4 ± 20.1 in healthy control group. IL-4 gene expression in stimulated PBMC was not different among all three groups (Fig. 3 & 4).

Expression of IFN- γ gene

1) Expression of IFN- γ gene in spontaneous PBMC

IFN- γ mRNA / β -actin mRNA in spontaneous PBMC was 72.9 ± 24.3 in high serum IgE group, 58.1 ± 23.5 in low serum IgE group, and 41.4 ± 23.9 in healthy control group. IFN- γ gene expression in spontaneous PBMC showed increased tendency in patient groups with AD than at healthy control group without statistical significance (Fig. 1 & 2).

2) Expression of IFN- γ gene in stimulated PBMC

When PBMC were stimulated by PMA / CA for 3 hr,

6 hr, 12 hr, or 24 hr, IFN- γ gene expression was first detected at 3 hr and persisted until 24 hr in all three groups (data not shown).

IFN- γ mRNA / β -actin mRNA in 6 hr-stimulated PBMC was 113.2 ± 5.0 in high serum IgE group, 118.7 ± 12.3 in low serum IgE group, and 117.1 ± 22.3 in healthy control group. IFN- γ gene expression in stimulated PBMC was not different among all three groups (Fig. 3 & 4).

DISCUSSION

IL-4 gene expression in spontaneous PBMC was higher in AD patient groups than in healthy control group, indicating that T lymphocytes in AD patients have been activated in vivo. In relation to serum IgE levels, IL-4 gene expression was higher in high serum IgE group than in healthy control group and not different between low serum IgE group and healthy control group. On the other hand, no difference of IL-4 gene expression was detectable between high and low serum IgE group. These obscure results appeared to be due to a wide range of cytokine mRNA levels evaluated with RT-PCR among individuals of a given group. Therefore, it is likely that the difference of IL-4 gene expression between high and low serum IgE group may be detected if the number of each group member is increased to exclude the heterogeneity in the patterns of immune responsiveness in AD patients²⁴.

Tang and Kemp²⁵ reported that spontaneous expression of IL-4 gene was detected in four of eight patients with severe AD, but no spontaneous expression of IL-4 gene was found in healthy controls.

In the previous reports examining IL-4 gene expression in PBMC or T cell cultures from healthy controls and neonates by Northern analysis, transcripts have not been detected in vivo²⁶⁻²⁸. But in this study, IL-4 gene was expressed in healthy controls. It is speculated that it can depend on the difference of the method of the experiment.

Kagi et al.¹⁸ reported that spontaneous PBMC from AD patients with elevated IgE levels, positive RAST test, positive immediate type skin reactions and a positive history for IgE-mediated allergies, demonstrated elevated levels for IL-4 and IL-5. But nonatopic form of AD (NAD) with normal serum IgE levels, negative RAST test, negative prick test, and a negative history of atopy, displayed elevated levels of IL-5 only. Although

low serum IgE group in this study may be different from NAD in the report of Kagi et al. in respect of the results of prick test and RAST test, spontaneous PBMC of low serum IgE group expressed IL-4 gene detectable by RT-PCR. To find abnormal immunologic defects in the signal pathway leading to cytokine production, further evaluation at the protein level is needed to follow the various stimulations either dependent or independent of antigen presenting cell.

IFN- γ gene expression in spontaneous PBMC showed increased tendency in AD patient groups than in healthy control group without statistical significance and not different between AD patient groups. In the previous reports²⁹, no spontaneous expression of IFN- γ gene was found in healthy controls. In this study, however, IFN- γ gene was expressed in healthy controls. The difference may depend on the method to test cytokine gene expression.

IL-4 or IFN- γ gene expression in 3 hr or 6 hr - stimulated PBMC, respectively, was not different among all three groups. No difference of IL-4 and IFN- γ gene expressions in PBMC stimulated with PMA / CA suggests that AD is not characterized by the shift in the reciprocal relationship between IL-4 and IFN- γ when T cells are stimulated under antigen presenting cell-independent conditions. It is thought that further evaluation by antigen presenting cell-dependent stimulants such as phytohemagglutinin or concanavalin A, would be necessary.

REFERENCES

1. Hanifin JM: Atopic dermatitis. *J Am Acad Dermatol* 6:1-13, 1982.
2. Leung DYM, Rhodes AR, Geha RS, et al.: atopic dermatitis (atopic eczema) In : Fitzpatrick TB, Eisen AZ, Wolff K, et al. (eds): *Dermatology in general medicine*. McGraw-Hill, New York, 1993, pp1543-1564.
3. Sampson HA: The role of food allergy and mediator release in atopic dermatitis. *J Allergy Clin Immunol* 81:635-645, 1988.
4. Wuthrich B: Serum IgE in atopic dermatitis. *Clin Allergy* 8:241-248, 1978.
5. Wuthrich B: Atopic dermatitis flare provoked by inhalant allergens. *Dermatologica* 178:51-53, 1989.
6. Rajka G: Prurigo Besnier (atopic dermatitis) with special reference to the role of allergic factors. II. The evaluation of the results of skin reactions. *Acta Derm Venereol (Stockh)* 41:1-39, 1961.
7. Miyajima A, Miyatake S, Schreurs J, et al.: Coordinate

- regulation of immune and inflammatory response by T cell derived lymphokines. *FASEB J* 2:2462-2473, 1988.
8. Leung DYM, Geha RS: Regulation of the human IgE antibody response. *Int Rev Immunol* 2:75-91, 1987.
 9. Pene J, Rousset F, Briere, et al.: IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons and prostaglandin E2. *Proc Natl Acad Sci USA* 85: 6880-6884, 1988.
 10. Del Prete G, Maggi E, Parronchi P, et al.: IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 140:4193-4198, 1988.
 11. Pene J, Rousset F, Briere F, et al.: IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN- γ . *J Immunol* 141:1218-1224, 1988.
 12. Defrance T, Aubry JP, Rousset F, et al.: Human recombinant IL-4 induces Fc receptors (CD23) on normal B lymphocytes. *J Exp Med* 165:1459-1467, 1987.
 13. Jujo K, Renz H, Abe J, et al.: Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. *J Allergy Clin Immunol* 90:323-331, 1992.
 14. Reinhold U, Wehrmann W, Kukel S, Kreysel HW: Evidence that defective interferon-gamma production in atopic dermatitis patients is due to intrinsic abnormalities. *Clin Exp Immunol* 79:374-379, 1990.
 15. Tang M, Kemp A, Varigos G: IL-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 92:120-124, 1993.
 16. Boguniewicz M: Recombinant gamma interferon in treatment of patients with atopic dermatitis and elevated IgE levels. *Am J Med* 88:365-370, 1990.
 17. Juhlin L, Johansson SGO, Bennich H: Immunoglobulin E in dermatoses : levels in atopic dermatitis and urticaria. *Arch Dermatol* 100:12-16, 1969.
 18. Kagi MK, Wuthrich B, Montano E, et al.: Differential cytokine profiles in peripheral blood lymphocyte supernatants and skin biopsies from patients with different forms of atopic dermatitis, psoriasis and normal individuals. *Int Arch Allergy Immunol* 103:332-340, 1994.
 19. Hanifin JM, Rajka G: Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh) supp* 192:44-47, 1980.
 20. Jakob T, Hermann K, Ring J: Eosinophilic cationic protein in atopic eczema. *Acta Dermatol Res* 283:5-6, 1991.
 21. Boyum A: Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 97:77-89, 1968.
 22. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry* 162:156-159, 1987.
 23. Yamamura M, Ueymura K, Deans RJ, et al.: Defining protective response to pathogens : cytokine profiles in leprosy lesions. *Science* 254:277-279, 1991.
 24. Simson MR, Cooper KD, Norris RB, et al.: Antigen presenting cell-independent cytokine and spontaneous in vitro IgE production in patients with atopic dermatitis: Increased interferon- γ production and lack of effects of in vivo low-dose interferon- γ treatment. *J Allergy Clin Immunol* 96:84-91, 1995.
 25. Tang MLK, Kemp AS: Spontaneous expression of IL-4 mRNA in lymphocytes from children with atopic dermatitis. *Clin Exp Immunol* 97:491-498, 1994.
 26. Gauchat JF, Gauchat D, de Weck AL, Stadler BM: Cytokine mRNA levels in antigen-stimulated peripheral blood mononuclear cells. *Eur J Immunol* 19:1079-1085, 1989.
 27. Lewis DB, Yu CC, Meyer J, et al.: Cellular and molecular mechanisms for reduced interleukin 4 and interferon- γ production by neonatal T cells. *J Clin Invest* 87:194-202, 1991.
 28. Waalen K, Sioud M, Natvig JB, Forre O: Spontaneous in vivo gene transcription of interleukin-2, interleukin-3, interleukin-4 interleukin-6, interferon-gamma, interleukin-2 receptor (CD25) and proto-oncogene c-myc by rheumatoid synovial T lymphocytes. *Scand J Immunol* 36:865-873, 1992.
 29. Tang MLK, Varigos G, Kemp AS: Reduced interferon-gamma (IFN- γ) secretion with increased IFN- γ mRNA expression in atopic dermatitis : evidence for a post-transcriptional defect. *Clin Exp Immunol* 97:483-490, 1994.