Short Communication

Received: January 12, 1990

Accepted after revision: January 16, 1991

Int Arch Allergy Appl Immunol 1991;95:282-284

Interleukin-4 Gene Expression in Human Peripheral Blood Mononuclear Cells

A.	Akio	Mori
K.	Kazuhiko	Yamamoto
M.	Makoto	Dohi
M.	Matsunobu	Suko
H.	Hirokazu	Okudaira

Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Japan

Abstract

Interleukin-4 (IL-4) mRNA was detected in normal human peripheral blood mononuclear cells (PBMC) stimulated with concanavalin A by Northern blot analysis. The signal was undetectable in PBMC before the stimulation, but became detectable 3 hrs after the stimulation and reached a maximum in 3–6 h and disappeared gradually thereafter. Immunosuppressive drugs such as ciclosporin, hydrocortisone and prednisolone inhibited the IL-4 mRNA expression dose dependently. Interferon- γ did not show any inhibitory effect on IL-4 gene expression.

Correspondence to: Dr. Hirokazu Okudaira, Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Tokyo 113 (Japan)

To date, accumulating investigations have revealed that IgE synthesis is highly dependent upon T cells [1]. Recently, the essential role of interleukin-4 (IL-4), a T cell lymphokine, in the formation of IgE has been widely accepted [2, 3]. For example, normal human B cells produce IgE when incubated with IL-4 in the presence of T cells [4]. Some T cell clones secreting IL-4 potentiate IgE production by B cells [5, 6]. Therefore, it is important and useful to establish an experimental system to detect IL-4 gene expression by human T cells and then examine the effects of various pharmacological agents which may modulate IL-4 gene expression. Thus, experiments were carried out to investigate the responsiveness of human T cells to produce IL-4 by detecting IL-4 gene transcripts in human peripheral blood mononuclear cells (PBMC). PBMC were isolated by Ficoll-Hypaque density gradient centrifugation. The isolated PBMC were washed and suspended in culture media (RPMI-1640 supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin) at a density of 106/ml. PBMC were then stimulated with concanavalin A (Con A) in the presence or absence of immunosuppressive drugs such as ciclosporin (CS) or glucocorticoids. In other experiments, recombinant human interferon- γ (IFN- γ), kindly provided by Kyowa Hakko Inc. (Tokyo, Japan) was added to the culture. Total cellular RNA was extracted from these cells using guanidinium thiocyanate lysis and cesium chloride density gradient centrifugation. 20 μ g of RNA were electrophoresed, blotted onto a nylon membrane and hybridized with ³²P-labeled human IL-4 probes (kindly supplied by Dr. K. Arai, DNAX, USA) [7]. The membranes were then exposed to Fuji RX X-ray film at -70 °C using a screen intensifier. To confirm that equal amounts of RNA were loaded on each lane, the gels before the

blotting were stained with ethidium bromide and visualized under UV light. Furthermore, nylon membranes were usually rehybridized with an α -tubulin cDNA probe.

RNA from control or Con A (2 μ g/ml) stimulated PBMC were used for the first experiment.

Figure 1A shows that human IL-4 mRNA was not detected by Northern blot in PBMC before the stimulation. Maximal IL-4 gene expression was observed after 6 h stimulation and IL-4 mRNA disappeared thereafter. In a follow-up study, the expression of IL-4 mRNA in different experimental conditions was examined. Figure 1B shows that IL-4 mRNA became detectable after 3 h and reached a peak in 3–6 h when stimulated with 8 μ g/ml of Con A. The time course observed in this experiment was similar to that of IL-2 mRNA [8]. It was also observed that the IL-4 mRNA expression is dose dependent. Although precise studies were not conducted, stimulation with PHA or PWM also induced IL-4 mRNA (data not shown).

Figure 2A shows that 10^{-7} , 10^{-6} and 10^{-5} M of hydro-

Human Interleukin-4 Gene Expression

283

5 6
12 3 4 5 6 7

ÊÊ

φ

B

Fig. 1. Kinetics of IL-4 mRNA expressed in PBMC stimulated with Con A. A Lanes 1–6: RNA from PBMC stimulated with Con A (2 μ g/ml) for 0, 6, 12, 24, 48 and 72 h, respectively. B Lanes 1–3: RNA from PBMC stimulated with Con A (8 μ g/ml) for 3, 6 and 9 h. Lane 4: RNA from PBMC stimulated with Con A (8 μ g/ml) for 6 h in the presence of CS (1 μ g/ml). Lane 5: RNA from PBMC stimulated with Con A (8 μ g/ml) for 6 h in the presence of hydro-cortisone (HC) (10^{-5} M). Lane 6: RNA from PBMC stimulated with Con A (4 μ g/ml) for 6 h. Lane 7: RNA from PBMC stimulated with Con A (16 μ g/ml) for 6 h.

12 3 4 5 6

\

•

Fig. 2. Pharmacological modulation of IL-4 gene expression. PBMC were stimulated with Con A (8 μ g/ml) for 6 h in the presence of various agents. 20 μ g RNA for each lane were electrophoresed, blotted and hybridized with human IL-4 probes. A Lane 1: Control. Lanes 2–4:

Hydrocortisone (HC) 10^{-7} , 10^{-6} and 10^{-5} M. Lanes 5 and 6: CS (0.01 or 0.1 μ g/ml). B Lane 1: Control. Lanes 2–4: prednisolone (PSL) 10^{-7} , 10^{-6} and 10^{-5} M. Lanes 5 and 6: CS 0.1 or 1.0 μ g/ml. Lane 7: IFN- γ (100 U/ml).

cortisone significantly inhibited IL-4 gene expression in PBMC stimulated with Con A (8 μ g/ml) for 6 h. 0.1 μ g/ml of CS also inhibited the IL-4 gene expression in the same experiment. Figure

2B shows that 10^{-7} , 10^{-6} and 10^{-5} M of prednisolone inhibited the IL-4 gene expression. $1.0 \mu\text{g/ml}$ of CS, but not $0.1 \mu\text{g/ml}$, inhibited the IL-4 gene expression in this experiment. The results are in agreement with an observation reported by Laing and Weiss [9] that CS inhibited IL-4 gene expression in PBMC stimulated with OKT-3 or OKT-3 plus PMA. It was reported that 10^{-6} M of hydrocortisone consistently inhibited IgE synthesis in vitro [10]. CS also inhibited the IgE antibody response in mice [11,12]. IFN- γ (100 U/ml) did not affect IL-4 gene expression. Based on the present experimental data and observations reported by others, it may be reasonable to speculate that the suppressive effect of glucocorticoids and CS on IgE synthesis might be attributed to their inhibitory activity on IL-4 production from T cells.

References

- Ishizaka K: Cellular events in the IgE antibody response. *Adv Immunol* 1976;23:1–75.
- Finkelman FD, Katona IM, Urban JF Jr, Snapper CM, Ohara J, Paul WE: Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. *Proc Natl Acad Sci USA* 1986;83:9675–9678.
- Finkelman FD, Katona IM, Urban JF, Holmes J Jr, Ohara J, Tung AS, Sample JV, Paul WE: IL-4 is required to generate and sustain in vivo IgE responses. *J Immunol* 1988;141:2335–2341.
- Pene J, Rousset F, Briere F, Chretien J, Bonnefoy J, Spits H, Yokota A, Arai N, Arai K, Banchereau J, Vries JE: IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons and prostaglandins. *Proc Natl Acad Sci USA* 1988;85:6880–6884.
- Pene J, Rousset F, Briere F, Chretien I, Paliard X, Banchereau J, Spits H, Vries JE: 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* 1988;141:1218–1224.
- Vercelli D, Tabara HH, Arai K, Geha RS: Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD 3 complex and MHC class II antigens. *J Exp Med* 1989;169:1295–1307.
- Yokota T, Otsuka T, Mosmann T, Banchereau J, DeFrance T, Balanchard D, Vries ED, Lee F, Arai K: Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that express B-cell and T-cell-stimulating activities. *Proc Natl Acad Sci USA* 1986;83: 5894.
- Granelli-Piperono A, Anreus L, Steinman RM: Lymphokine and nonlymphokine mRNA levels in stimulated human T cells. *J Exp Med* 1986;163:922–937.
- 284
- Mori/Yamamoto/Dohi/Suko/Okudaira

9 Laing T, Weiss A: Evidence for IL-2 independent proliferation in human T cells. *J Immunol* 1988;140:1056–1062.

Ricci M, Del Prete GF, Vercelli D, Maggi E, Macchia D, Par-ronchi P, Rossi O, Romagnani S: Immunologic and pharma-cologic regulation of human IgE synthesis in vitro; in Reed CE (ed): *Proc XII Int Congr Allergol Clin Immunol*. St.Louis, Mosby, 1985, pp 31–35.

Okudaira H, Sakurai Y, Terada K, Ogita T, Miyamoto T: Cy-closporin A-induced suppression of ongoing IgE antibody formation in the mouse. *Int Arch Allergy Appl Immunol* 1986; 79:164–168.

Carriere V, Auriault C, Dessaint JP, Capron A: Differential effect of cyclosporin A (CyA) on IgE response: Role of CyA-induced suppressor cells. *Immunol Lett* 1987;16:145–149.

Coffman RL, Carty J: A T cell activity that enhances poly-clonal IgE production and its inhibition by interferon- γ . J Immunol 1986;136:949–954.

14 Finkelman FD

Katona IM

Mosmann TR

Coffman RL: IFN-regulates the isotypes of Ig secreted during in vivo humoral immune responses. J Immunol 1988;140:1022–1027.