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 µg/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 µg 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 μg/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 IB shows that IL-4 mRNA became detectable after 3 h and reached a peak in 3–6 h when stimulated with 8 μg/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-

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

\[ \text{Fig. 2. Pharmacological modulation of IL-4 gene expression. PBMC were stimulated with Con A (8 μg/ml) for 6 h in the presence of various agents. 20 μg RNA for each lane were electrophoresed, blotted and hybridized with human IL-4 probes. A Lane 1: Control. Lanes 2–4: Hydrocortisone (HC) 10^{-7}, 10^{-6} and 10^{-5} M. Lanes 5 and 6: CS (0.01 or 0.1 μg/ml). B Lane 1: Control. Lanes 2–4: prednisolone (PSL) 10^{-7}, 10^{-6} and 10^{-5} M. Lanes 5 and 6: CS 0.1 or 1.0 μg/ml. Lane 7: IFN-γ (100 U/ml).}\]

cortisone significantly inhibited IL-4 gene expression in PBMC stimulated with Con A (8 μg/ml) for 6 h. 0.1 μg/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 µg/ml of CS, but not 0.1 µg/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


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