

Possible role of tyrosine kinase activity in interleukin 4-induced expression of germ-line C ϵ transcripts in a human Burkitt lymphoma B-cell line, DND39

Koichi Ikizawa, MSc, Keiichi Kajiwaru, BSc, Takehiro Koshio, VMD, and Yuki Yoshi Yanagihara, PhD Sagamihara City, Japan

Despite the recent advances in knowledge of the molecular mechanism by which interleukin-4 (IL-4) induces IgE production, little is known about the signal transduction pathway that leads to this event. This study investigated the signal transduction mechanism responsible for IL-4-induced expression of germ-line C ϵ transcripts with use of a human Burkitt lymphoma B-cell line, DND39, which is known to express germ-line C ϵ transcripts in response to IL-4. On stimulation with IL-4, the generation of inositol triphosphate was observed in the cells. In addition, this generation was associated with activation of phospholipase C- γ 1 (PLC- γ 1). Although herbimycin A, a potent inhibitor of tyrosine kinase, inhibited IL-4-induced activation of PLC- γ 1 and generation of inositol triphosphate, direct phosphorylation of PLC- γ 1 was not determined. Nevertheless, IL-4 stimulation could induce activation of FYN but not LYN kinase, suggesting that additional molecule(s) might link FYN kinase to PLC- γ 1. Interestingly, herbimycin A almost completely inhibited IL-4-induced expression of germ-line C ϵ transcripts when present during the entire culture period. These results indicate that the induction of germ-line C ϵ transcripts in IL-4-stimulated DND39 cells is essentially dependent on the activation of tyrosine kinase, possibly FYN kinase. (J ALLERGY CLIN IMMUNOL 1994;94:620-4.)

Key words: Interleukin-4, germ-line C ϵ transcripts, DND39 cells, phospholipase C- γ 1, tyrosine kinase, FYN kinase, protein kinase C

It has been shown that germ-line transcripts of each C H region except for C δ are expressed before the class switch recombination induced by cytokines.¹⁻⁴ Both IL-4 and IL-13, derived from activated CD4⁺ T cells, can specifically induce the accumulation of germ-line C ϵ transcript.^{3, 5} This unique transcript contains germ-line exon, located upstream of the switch region (S ϵ), which is spliced to the C ϵ coding exons. Because germ-line C ϵ mRNA is a sterile transcript incapable of initiating the synthesis of IgE protein, its precise function remains to be defined. Several studies, however, have shown that the expression of germ-line C ϵ transcripts is essential for the class switch recombination event in which S ϵ region accessibility may be modulated.⁶⁻⁸ Therefore the analysis

Abbreviations used

IL:	Interleukin
IL-4R:	Interleukin-4 receptor
IP ₃ :	Inositol triphosphate
mAb:	Monoclonal antibody
PKC:	Protein kinase C
PLC- γ 1:	Phospholipase C- γ 1
PMA:	Phorbol myristate acetate

of IL-4 signaling pathway leading to the expression of germ-line C ϵ transcripts provides a new insight into the regulation of IgE class switch and IgE production. The aim of this study was to examine the signal transduction mechanism of IL-4-induced germ-line C ϵ transcripts. To this end, we used a human Burkitt lymphoma B-cell line, DND39, which is known to express germ-line C ϵ transcripts in response to IL-4 stimulation.⁸

RESPONSIVENESS OF DND39 CELLS TO IL-4

IL-4 is shown to enhance the expression of CD23, CD40, IgM, HLA-DR, and IL-4R on nor-

From the Clinical Research Center for Allergy, National Sagamihara Hospital, 18-1 Sakuradai, Sagamihara City, Kanagawa, Japan 228.

Supported by a Research Grant from Sandoz Pharmaceuticals. Reprint requests: Yuki Yoshi Yanagihara, PhD, Clinical Research Center for Allergy, National Sagamihara Hospital, 18-1 Sakuradai, Sagamihara City, Kanagawa, Japan 228.

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mal B cells⁹⁻¹¹ and to increase the release of soluble CD23.^{12, 13} On DND39 cells, IL-4 enhanced the expression of CD40, IgM, HLA-DR, and IL-4R, whereas neither the constitutive expression nor the IL-4-induced expression of CD23 and release of soluble CD23 could be observed. With regard to IL-4R, scatchard analysis with the use of ¹²⁵I-labeled IL-4 demonstrated that unstimulated DND39 cells expressed approximately 1500 high-affinity IL-4R per cell ($K_d = 100$ pmol/L) and that its expression was 10 times higher than that of normal B cells. IL-4 stimulation of DND39 cells resulted in a two-fold increase in the number of high-affinity IL-4R without affecting the affinity to IL-4. In addition, IL-4 sufficiently induced germ-line C ϵ transcripts in DND39 cells and in normal B cells. However, DND39 cells had no ability to produce IgE, even when costimulated with IL-4 and anti-CD40 monoclonal antibody (mAb). These observations indicate that the responsiveness of DND39 cells to IL-4 is similar to that of normal B cells except for CD23 expression and IgE production. Therefore DND39 is a useful B-cell line to study the signal transduction mechanism responsible for the induction of human germ-line C ϵ transcripts.

ACTIVATION OF PHOSPHOINOSITIDE TURNOVER BY IL-4

Because it has been reported that IL-4 provokes an immediate and transient elevation of inositol triphosphate (IP₃) and Ca²⁺ levels in human tonsillar B cells,¹⁴ we measured these second messengers in IL-4-stimulated DND39 cells. IL-4 induced the biphasic production of IP₃ in DND39 cells (Fig. 1, A), the initial transient elevation and the later, prolonged production of IP₃. However, IL-4-induced IP₃ production could not cause an elevation of intracellular Ca²⁺ concentration measured fluorometrically with Fura-2 (Fig. 1, B). This may be because of insufficient production of IP₃ at the initial phase, because F(ab')₂ fragments of anti-IgM antibody could induced the marked production of IP₃, which was accompanied by a rapid increase in cytosolic [Ca²⁺]_i (data not shown). Another possibility is that a resting level of Ca²⁺ concentration might be much higher in DND39 cells (160 nmol/L) than in tonsillar B cells, in which 40 nmol/L was found.¹⁴ With regard to the latter occurring, prolonged production of IP₃, IL-4, and anti-IgM showed similar kinetics that may transmit the signal to the next step.

To further investigate the biochemical mechanism of IL-4-induced activation of phosphoi-

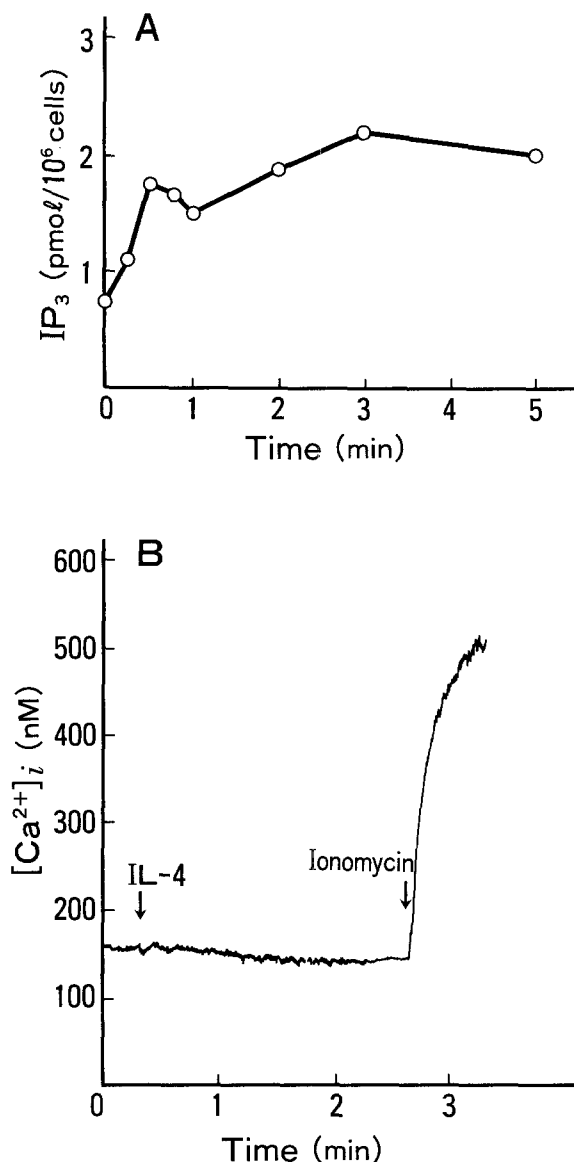


FIG. 1. IL-4 induces IP₃ production but not Ca²⁺ mobilization in DND39 cells. **A**, Cells were stimulated with 250 U/ml IL-4 for indicated times. IP₃ was extracted from cells and measured with an inositol 1,4,5-triphosphate [³H] assay kit. **B**, Cells were loaded with Fura-2 and stimulated with IL-4 or ionomycin. Intracellular Ca²⁺ concentration ([Ca²⁺]_i) was measured in a spectrofluorometer.

noside turnover in DND39 cells, we analyzed the tyrosine phosphorylation of PLC- γ 1. After stimulation with IL-4, the cells were lysed and immunoprecipitated with antiphosphotyrosine mAb. The immunoprecipitates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto a polyvinylidene difluoride membrane, and immunoblotted with anti-PLC- γ 1 mAb. As shown in Fig. 2, A, IL-4 caused a rapid increase in the amount of PLC- γ 1 in antiphospho-

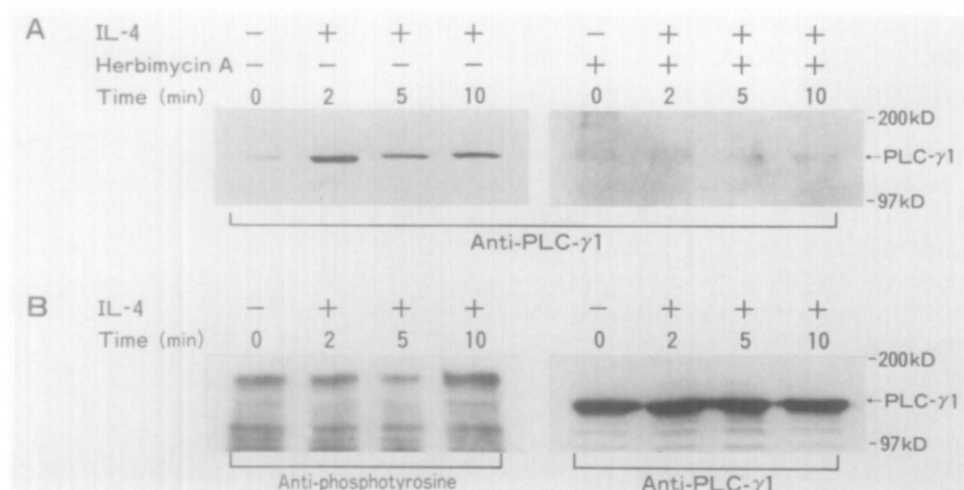


FIG. 2. Immunoblot analysis of PLC- γ 1 in IL-4-stimulated DND39 cells. **A**, Cells were incubated in absence (*left*) or presence (*right*) of 3 μ mol/L in herbimycin A for 16 hours and stimulated with IL-4 for the indicated times. They were lysed with 1% Triton X-100 and immunoprecipitated with antiphosphotyrosine mAb. Immunoprecipitates were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred onto polyvinylidene difluoride membranes, and then probed with anti-PLC- γ 1 mAb. **B**, Cell lysates were immunoprecipitated with anti-PLC- γ 1 mAb. After electrophoresis and blotting the membrane was sequentially probed with antiphosphotyrosine mAb (*left*) and after stripping with anti-PLC- γ 1 mAb (*right*).

tyrosine immunoprecipitates within 2 minutes, and it was still detectable even after 10 minutes. The kinetics were compatible with the prolonged production of IP₃, which occurred at the late phase in IL-4-stimulated DND39 cells, suggesting that PLC- γ 1 contributed to the IL-4-induced IP₃ production. Pretreatment of cells with herbimycin A, a potent inhibitor of tyrosine kinase, not only diminished the PLC- γ 1 content of antiphosphotyrosine immunoprecipitates but also inhibited the IL-4-induced IP₃ production (not shown), indicating the involvement of tyrosine kinase activity in PLC- γ 1 activation. On the other hand, when anti-PLC- γ 1 immunoprecipitates were immunoblotted with antiphosphotyrosine mAb, no significant increase in the phosphotyrosine content of PLC- γ 1 was observed in IL-4-stimulated DND39 cells, whereas an almost equal amount of PLC- γ 1 was detected in each sample (Fig. 2, *B*). These results suggest that IL-4 induces PLC- γ 1 activation in DND39 cells, depending on the association of PLC- γ 1 with some tyrosine phosphorylated molecule(s). Such a manner is inconsistent with the direct tyrosine phosphorylation of PLC- γ 1 induced in NIH 3T3 cells by platelet-derived growth factor.¹⁵ However, it should be noted that the phosphorylated molecule(s), which was immunoprecipitated together with PLC- γ 1, was detected in IL-4-stimulated

DND39 cells. Therefore it is most likely that the association of PLC- γ 1 with some phosphorylated molecule(s) may enhance the catalytic efficiency of PLC- γ 1 by translocating to its membrane substrates. In either case, PLC- γ 1 activation leads to protein kinase C (PKC) activation via the production of diacylglycerol, thereby promoting the expression of germ-line C ϵ transcripts because phorbol myristate acetate (PMA), a strong activator of PKC, has been demonstrated to enhance the IL-4-induced expression of germ-line C ϵ transcripts in DND39 cells.⁸

INVOLVEMENT OF TYROSINE KINASE IN THE INDUCTION OF GERM-LINE C ϵ TRANSCRIPTS

Because IL-4R has no tyrosine kinase domain, non-receptor-type tyrosine kinases of the SVC family are suggested to be associated with IL-4R because an association exists between IL-2R β subunit and LCK.¹⁶ To determine whether IL-4 activates any of the known SVC family tyrosine kinases in DND39 cells, cell lysates were immunoprecipitated with anti-FYN or anti-LYN antibody, and then analyzed by *in vitro* kinase assay. As shown in Fig. 3, unstimulated DND39 cells contained readily detectable amounts of both p59-FYN and p53/56-LYN kinases. Stimulation of the

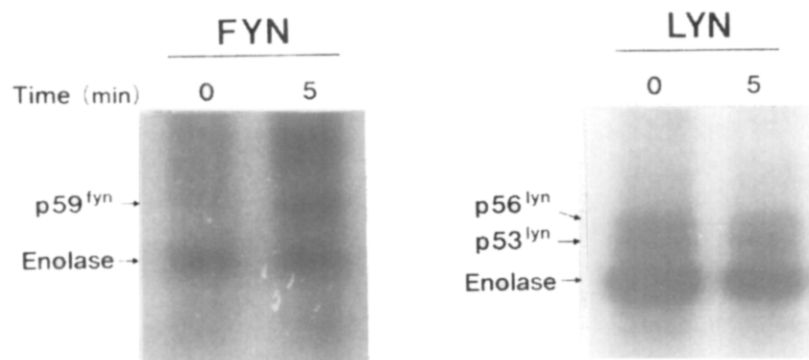


FIG. 3. IL-4 activates FYN but not LYN kinase in DND39 cells. Cell lysates were immunoprecipitated with anti-FYN or anti-LYN antibody. Immunoprecipitates were subjected to in vitro kinase assay with use of enolase as an exogenous substrate. Positions of p59-FYN, p53/p56-LYN, and enolase are indicated by arrows.

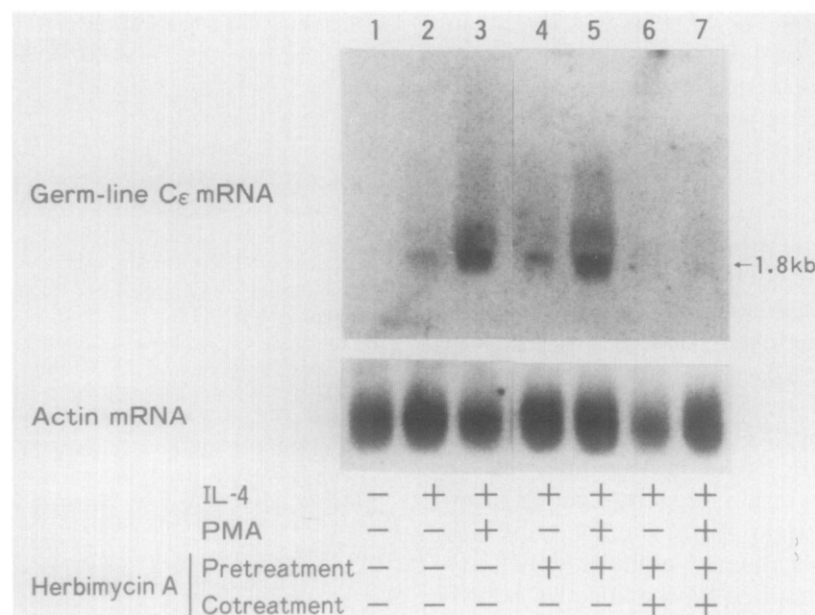


FIG. 4. Effect of herbimycin A on expression of germ-line C_ϵ transcripts in IL-4-stimulated DND39 cells. Cells were pretreated without (lanes 1-3) or with (lanes 4-7) herbimycin A for 16 hours, washed or maintained, and then stimulated with IL-4 alone or IL-4 plus PMA for 48 hours. Total RNA was extracted from cultured cells and subjected to Northern blot analysis with probes complementary to C_ϵ or actin mRNA.

cells with IL-4 could induce augmentation of FYN kinase autophosphorylation and phosphorylation of exogenous substrate enolase, whereas no changes in LYN kinase activity were observed. These observations strongly suggest that activation of FYN kinase plays an important role in the IL-4-induced signal transduction in DND39 cells.

We further examined whether herbimycin A may inhibit induction of germ-line C_ϵ transcripts in DND39 cells. The results are shown in Fig. 4. Stimulation with IL-4 induced the expression of germ-line C_ϵ transcripts (lane 2), and this expression was markedly enhanced in the presence of PMA (lane 3). We used two culture systems to evaluate the suppressive activity of herbimycin A.

In one of the systems, DND39 cells were pretreated with the agent, washed thoroughly, and then cultured with IL-4 alone or IL-4 plus PMA. The IL-4-induced expression of germ-line $C\epsilon$ transcripts was hardly inhibited in the absence (lane 4) and presence (lane 5) of PMA by herbimycin A. It seemed therefore that pretreatment only with the agent would be insufficient for causing its suppressive effect. In the other, herbimycin A remained during the whole culture period. Under this condition, the IL-4-induced expression of germ-line $C\epsilon$ transcripts was almost completely suppressed in the absence (lane 6) and presence (lane 7) of PMA by the inhibitor without affecting the cell viability. The observation that herbimycin A also abolished the enhancement of germ-line $C\epsilon$ transcripts mediated by PMA suggests that coordinated actions of PKC and other molecules may be involved in the downstream events occurring in the tyrosine kinase-dependent pathway. In this regard, IL-4 stimulation is shown to induce the association of phosphatidylinositol 3-kinase and IL-4R in murine myeloid progenitor cells and T cell lines.^{17, 18}

CONCLUSION

Our results demonstrate that activation of tyrosine kinase, probably FYN kinase, is essentially involved in the induction of germ-line $C\epsilon$ transcripts in IL-4-stimulated DND39 cells. Activation of PLC- γ 1 by IL-4 may also provide a co-stimulatory signal via PKC activation. However, direct activation of PKC by PMA could not overcome the inhibitory effect of herbimycin A on IL-4-induced expression of germ-line $C\epsilon$ transcripts. Thus activation of tyrosine kinase by IL-4 may lead to provocation of multiple signal cascades, which are required for the effective expression of germ-line $C\epsilon$ mRNA. More detailed studies are needed to explain the biochemical mechanisms whereby IL-4 induces germ-line $C\epsilon$ transcripts followed by class switching to IgE.

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