

Signals and nuclear factors that regulate the expression of interleukin-4 and interleukin-5 genes in helper T cells

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Mouse thymoma line EL-4 cells produce cytokines such as interleukin (IL)-2, IL-3, IL-4, IL-10, and granulocyte-macrophage colony-stimulating factor in response to phorbol 12-myristate 13-acetate (PMA). EL-4 cells also produce low levels of IL-5 when stimulated by PMA alone; however, cAMP greatly augments PMA-dependent IL-5 production. A transient transfection assay revealed that two signals, PMA and cAMP, are required for optimal activation of the IL-5 promoter. In contrast, cAMP almost completely inhibited the PMA-dependent activation of the endogenous IL-2 gene, as well as the transfected IL-2 promoter. These results indicate that the IL-5 gene is positively regulated by cAMP in a manner opposite to that for the IL-2 gene. One of the nuclear factors (NFs) that regulates the response of the IL-5 promoter to cAMP and PMA has properties similar to NF for activated T cell. The P sequence of the IL-4 gene, defined as a responsive element for PMA and calcium ionophore (A23187), shares sequence similarity with the NF- κ B and the NF-activated T cell binding sites. We attempted to determine whether NF(P), a nuclear factor specific for the P sequence, is related to NF- κ B and nuclear factor for activated T cell (NF-AT). In electrophoretic mobility shift assays both NF- κ B (P65 or P65/P50 heterodimer) and NF-AT bound to the P sequence. However, sequence specificity of NF-AT was more similar to that of NF(P), and only a small amount of P65 was detected in NF(P). These results indicate that a component or components of NF-AT have the potential to reconstitute NF(P), whereas NF- κ B alone does not account for NF(P) in Jurkat crude extract. Taken together, these results suggest that NF-AT-like factors are involved in the regulation of IL-4 and IL-5 genes. (J ALLERGY CLIN IMMUNOL 1994;94:594-604.)

Key words: Nuclear factors, IL-4 gene, IL-5 gene, helper T cells, signal transduction

Helper T cells play an important role in immune and inflammatory response through the action of cytokines they elaborate. Mouse helper T-cell clones can be divided into distinct subtypes (T_{H1} and T_{H2}) on the basis of their pattern of cytokine production,¹ and evidence has indicated that human helper T cells also show a distinct pattern of cytokine production.² T_{H1} cells, by producing interleukin (IL)-2 and interferon- γ , promote cellular immunity, including delayed-type hypersensitivity; whereas T_{H2} cells, by producing

Abbreviations used

ATL:	Adult T-cell leukemia
β t ₂ cAMP:	N ⁶ ,O ² -dibutyryl cAMP
CAT:	Chloramphenicol acetyltransferase
CLE:	Conserved lymphokine element
EMSA:	Electrophoretic mobility shift assay
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL:	Interleukin
NF:	Nuclear factor
NF-AT:	Nuclear factor of activated T cell
PGE ₂ :	Prostaglandin E ₂
PKA:	Protein kinase A
PKC:	Protein kinase C

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IL-4, IL-5, and IL-10, regulate humoral immunity.³ T_{H1} -specific and T_{H2} -specific cytokines regulate the differentiation of naive T cells. IL-4 directs the response toward T_{H2} phenotype, whereas interferon- γ induces differentiation to-

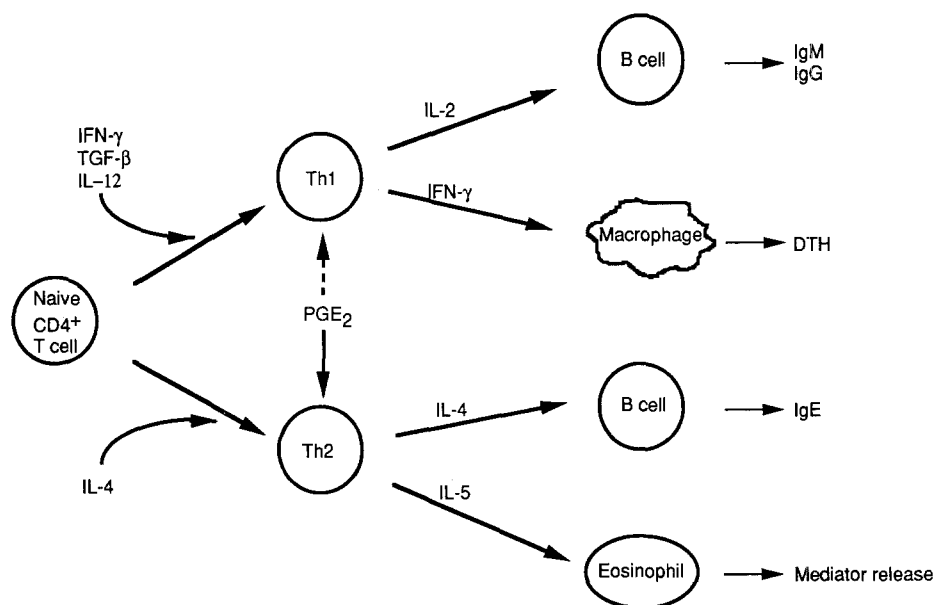


FIG. 1. The T_{H1} and T_{H2} subsets and their effector functions. Cell-mediated and humoral immunity are mediated by distinct helper T cell subsets. PGE_2 may alter the balance of T_{H1} and T_{H2} responses. *IFN- α* , Interferon- α ; *TGF- β* , transforming growth factor β .

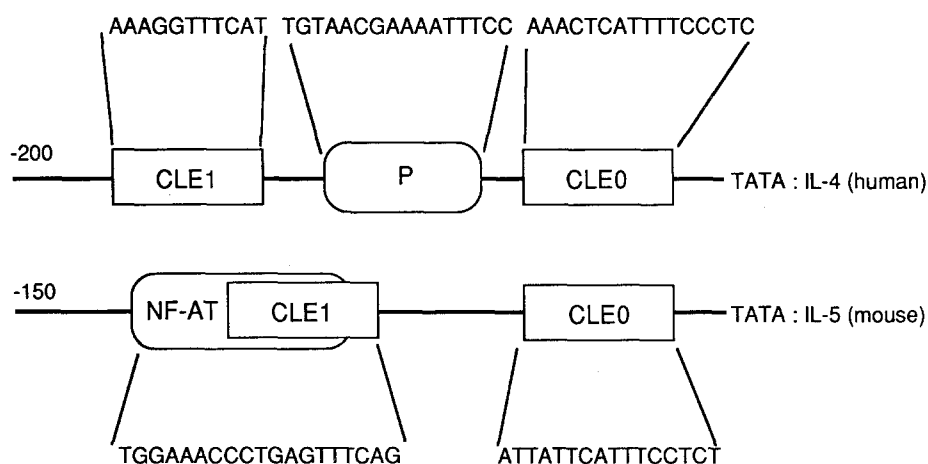


FIG. 2. Comparison of *cis*-acting DNA elements of IL-4 and IL-5 genes. Within the promoter regions, IL-4 and IL-5 genes share common sequence motifs. Coordinated and specific expression of the two genes in T_{H2} cells may be regulated through these elements.

ward T_{H1} phenotype (Fig. 1). Molecules such as prostaglandin E_2 (PGE_2) produced by macrophages or mast cells, also affect the nature of T-cell responses on a T_{H1} -specific or T_{H2} -specific manner.⁴ Thus the immune system seems to have a capacity to develop a T_H subset that is effective in coping with particular types of antigen or pathogen. IL-4 and IL-5, by regulating IgE production and growth and differentiation of mast cells and eosinophils, play a key role in the T_{H2}

response prominent in helminth infection and allergy.

The IL-4 and IL-5 genes are closely located on human chromosome 5 and mouse chromosome 11, within the cluster of the IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF),^{5, 6} and IL-13 genes.⁷ Within 5' flanking regions, the IL-4 and IL-5 genes are associated with short stretches of conserved sequence motifs, designated conserved lymphokine element (CLE) 0 and CLE1,

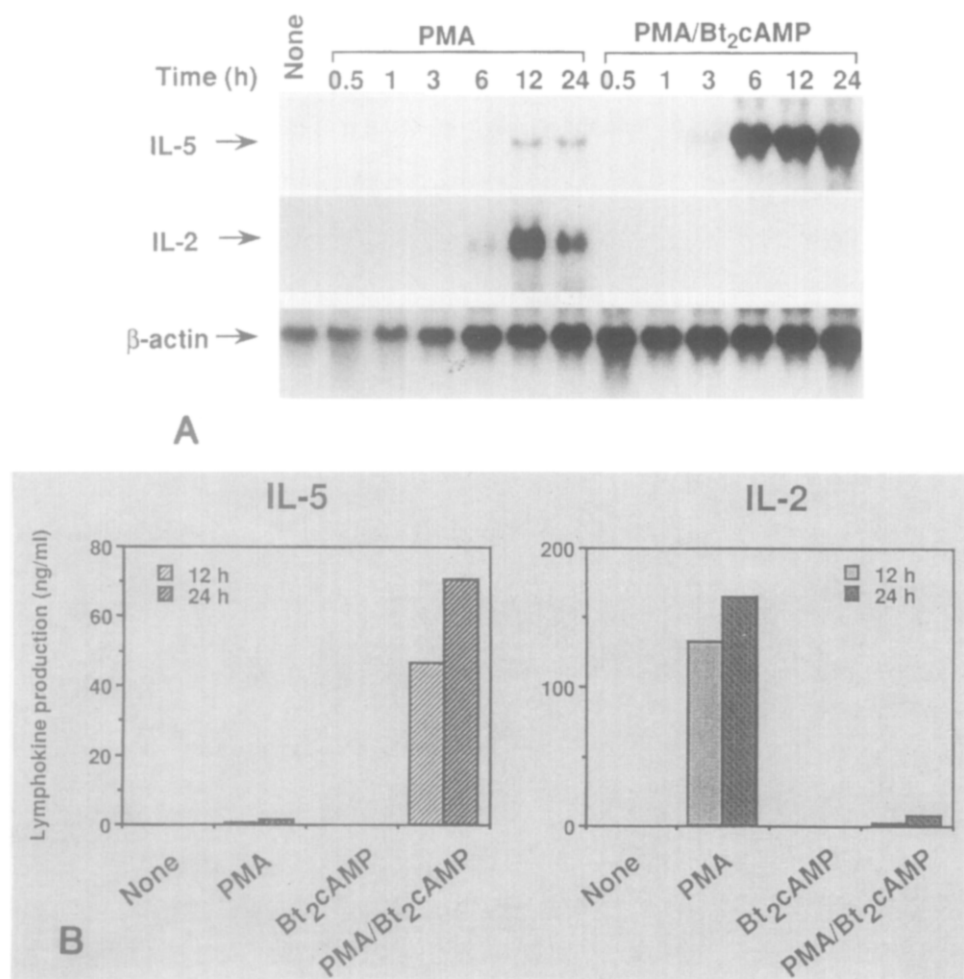


FIG. 3. Effects of cAMP on the production of IL-5 and IL-2 in EL-4 cells. **A**, Northern blot analysis of RNA. EL-4 cells were stimulated with PMA and β_2 cAMP, as indicated. At various times after stimulation, total cellular RNA was extracted, and Northern blot analysis of RNA was made with probes specific for IL-2, IL-5, and β -actin cDNA. **B**, Protein levels of IL-2 and IL-5 in the supernatant of EL-4 cells. Protein levels of IL-2 and IL-5 secreted in the culture supernatants of EL-4 cells used in (**A**) at 12 hours and 24 hours were measured by ELISA.

which are also found in the 5' upstream regions of the IL-3 and GM-CSF genes (Fig. 2). Coordinated and specific expression of IL-4 and IL-5 genes in T_{H2} cells suggest that these genes are activated by a common mechanism. Studies on the mechanisms of IL-4 and IL-5 gene expression are important to account for the T_{H2}-specific cytokine profiles in allergic disease.

EXPERIMENTS

IL-5-producing cells and conditions for production

IL-5 is produced primarily by T cells in response to antigen stimulation. Mast cells also produce IL-5 in response to cross-linking of Fcε

R1 or calcium ionophores.⁸ In mouse T-cell lines, T_{H2} clones, such as D120 cells and CDC35 cells, produced IL-5 mRNA when they were stimulated with phorbol 12-myristate, 13-acetate (PMA) and calcium ionophore (A23187) or anti-CD3 antibody. Cl.Ly1⁺2⁻/9 helper T cell and thymoma line EL-4 also produced IL-5 mRNA after stimulation with concanavalin A and PMA, respectively. Among human T-cell clones examined, the CD4⁺ T-cell clone SP-B21 (T_{H0} type) produced IL-5 mRNA on stimulation with either PMA and A23187 or anti-CD3 antibody.⁹ Adult T-cell leukemia (ATL)-derived T-cell line, ATL16 cells expressed IL-5 mRNA constitutively, whereas Jurkat cells failed to express IL-5 mRNA with any

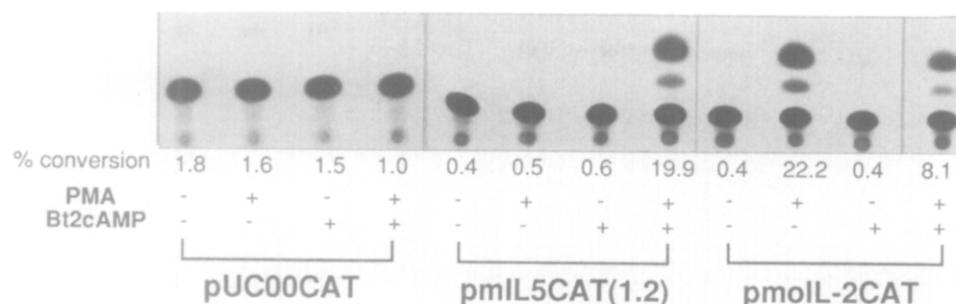


FIG. 4. Effects of cAMP on activities of IL-5 and IL-2 promoters. EL-4 cells were transiently transfected with the IL-5 or IL-2 promoter–chloramphenicol acetyltransferase (CAT) gene fusion construct [pmIL5CAT(1.2) and pmIL-2CAT, respectively] and control plasmid pUC00CAT. Cells were then stimulated with compounds as indicated, and after 24 hours, CAT activity was measured. (From Lee HJ, et al. *J Immunol* 1993;151:6135-42. Copyright 1993, The Journal of Immunology.)

combination of the compounds, judging from reverse transcriptase–polymerase chain reaction analysis. IL-2¹⁰ or IL-4¹¹ was also shown to enhance the production of IL-5 in T cells.

Involvement of cAMP signal in the production of IL-5

Cytokine genes such as IL-2, IL-3, IL-4, IL-5, and GM-CSF are coordinately expressed during T-cell activation. The recognition by the antigen specific T-cell receptor of an antigen associated with the major histocompatibility complex on antigen-presenting cells triggers a series of biochemical events, including elevation of the intracellular calcium ion concentration and activation of protein kinase C (PKC). These processes can be mimicked in part by PMA and A23187.¹² Many, if not all, cytokine genes are induced by PMA/A23187; however, detailed studies revealed that the signals for optimal induction of the cytokine genes are not exactly the same for each gene. Several lines of evidence have indicated that T_{H1} and T_{H2} cells use different signal transduction pathways, resulting in the characteristic pattern of cytokine production. For example, PGE₂, which elevates the intracellular cAMP level, inhibits the production of cytokines from T_{H1} cells but not from T_{H2} cells.¹³ This is an indication that the cAMP level of T_{H2} cells is higher than that of T_{H1} cells.¹⁴ On the basis of these observations, we examined the possibility that cAMP is involved in the regulation of the IL-5 gene expression in EL-4 cells. Unlike T_{H1} or T_{H2} cells, EL-4 cells produce IL-2 (T_{H1}-specific cytokine), as well as IL-4, IL-5, IL-10 (T_{H2}-specific cytokines), GM-CSF, and IL-3 when stimulated by PMA alone.¹⁵ Northern blot analysis revealed that EL-4 cells, which express no

detectable levels of IL-5 mRNA in the absence of stimuli, produced a low level of IL-5 mRNA in response to PMA stimulation. Exposure of EL-4 cells to both PMA and β_2 cAMP substantially increased IL-5 mRNA levels (Fig. 3, A). ELISA revealed that the levels of IL-5 protein in the culture supernatants correlated well with those of IL-5 mRNA (Fig. 2, B).

In marked contrast to IL-5, β_2 cAMP strongly inhibited the accumulation of IL-2 mRNA and protein induced by PMA (Fig. 2, A and B). Because cAMP has differential effects on T_{H1} and T_{H2} cells, it is possible that in certain situations, mediators (e.g., PGE₂), which elevate the cAMP level or activate the protein kinase A (PKA) pathway, modulate T_{H1} and T_{H2} responses in vivo. These mediators may be produced by antigen-presenting cells, mast cells, eosinophils, or other inflammatory cells. The recent report that human immunodeficiency virus proteins activate the PKA pathway¹⁶ is also in keeping with this hypothesis because human immunodeficiency virus infection results in the dominance of the T_{H2}-type response over the T_{H1}-type response.¹⁷ It is interesting that IL-2 (T_{H1}-specific cytokine) and IL-5 (T_{H2}-specific cytokine) are differentially regulated by cAMP in the same cells. Recently, several groups have reported that an increase in cAMP concentration in some cell types, such as certain strains of fibroblasts and fat cells, is inhibitory to the signal transduction from Ras to MAP kinase. If a similar mechanism also operates in T cells, resulting in the inhibition of the activation of IL-2 gene by cAMP, this would indicate that the signaling pathways leading to the activation of IL-2 (or T_{H1}-specific cytokines) and IL-5 (or T_{H2}-specific cytokines) genes are different.

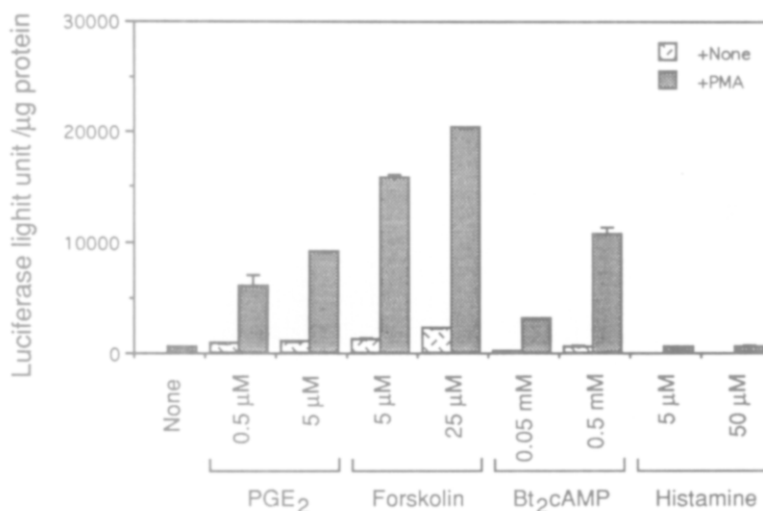


FIG. 5. Effects of cAMP agonist on the IL-5 promoter activity. EL-4 cells were transfected with the IL-5 promoter-luciferase gene fusion construct [pML5Luc(1.2)]. Twenty-four hours after transfection, cells were incubated with the indicated compounds in the presence or absence of PMA for 16 hours, and luciferase activity was measured.

The enhancement of IL-5 production by cAMP in other types of mouse or human T cells was also observed.^{13, 18} The enhanced IL-5 production in response to cAMP may account for some eosinophilia, because IL-5 stimulates growth and differentiation of eosinophils. Most notably, eosinophilia is observed in parasitic infections and allergy-related diseases.¹⁹ Detailed examination of the nature of mediators released in these diseases may provide a clue to our understanding of the significance of the cAMP signal in IL-5 production in pathologic situations. In this respect, it is interesting that in schistosomiasis *mansoni* infection vasoactive intestinal polypeptide, which activates adenylate cyclase, causes a release of IL-5 from activated T cells.²⁰ In vivo, the role of cAMP as a modulator of T_{H1} and T_{H2} responses and the significance of the cAMP signal in the activation of IL-5 gene remain to be determined.

Activation of the IL-5 promoter requires both cAMP and PMA in EL-4 cells

We previously characterized the regulatory regions of the GM-CSF,²¹⁻²³ IL-3,²⁴ and IL-4²⁵ genes, which mediate the response to T-cell activation signals or to human T-cell leukemia virus-I Tax activation. However, the regulatory region of the IL-5 gene remained undefined because neither PMA/A23187 nor Tax activated the IL-5 promoter²⁶ in various T cells. The EL-4 system described above provided an opportunity to establish transient transfection assay to define the

regulatory regions of the IL-5 promoter. Consistent with the results observed with the endogenous IL-5 gene, exposure of EL-4 cells to β_2 cAMP and PMA dramatically enhanced the activity of the transfected IL-5 promoter, which contains the region spanning from nucleotide position -1200 to +33 relative to the transcription initiation sites (Figs. 4 and 5). In contrast to IL-5, the transfected IL-2 promoter was strongly activated by PMA alone, whereas it was inhibited by addition of β_2 cAMP.

cAMP acts on the IL-5 promoter via the PKA pathway

PGE₂ or forskolin, which elevates the intracellular cAMP level, in combination with PMA, stimulated the IL-5 promoter (Fig. 5). When EL-4 cells were cotransfected with the expression plasmid containing cDNA encoding the catalytic subunit of PKA and the IL-5 promoter-luciferase gene fusion construct, PKA augmented PMA-dependent activation of the IL-5 promoter. This result indicated that the activation of the IL-5 promoter by cAMP is mediated by PKA (Fig. 6). The action of β_2 cAMP on the IL-2 promoter was also mimicked on cotransfection of the PKA cDNA.

Structure of the IL-5 gene and possible mechanism of cAMP action

The IL-5 gene is composed of 4 exons and 3 introns. The mouse and human IL-5 genes show a

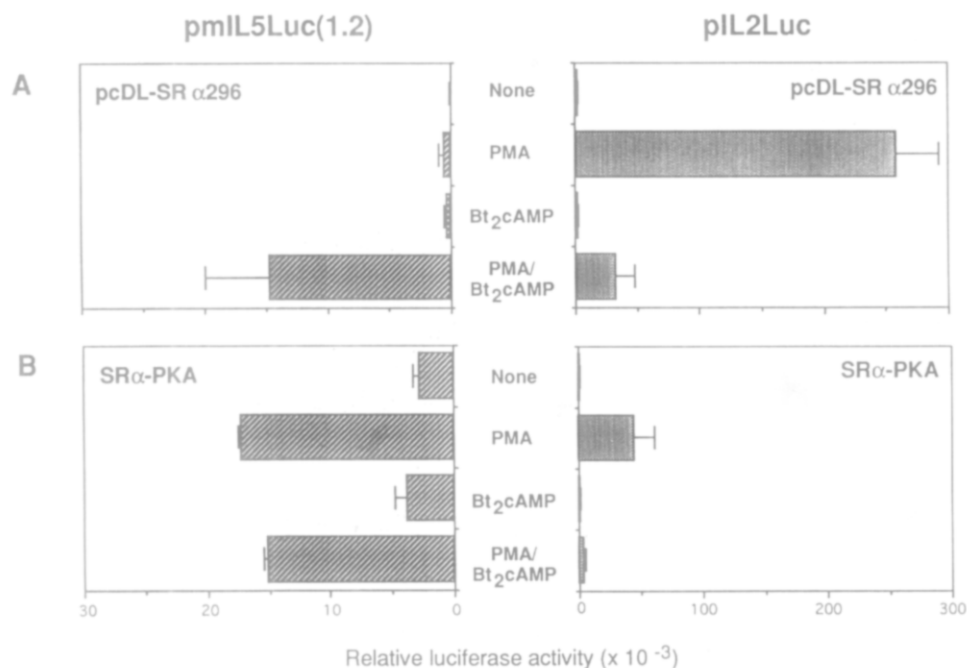


FIG. 6. Effects of cAMP and PKA on IL-5 and IL-2 promoter activities. The IL-5 or IL-2 promoter-luciferase gene fusion construct [pIL5Luc(1.2) and pIL2Luc, respectively] were cotransfected with either an expression plasmid pcDL-SR α 296 containing no insert (**A**) or SR α -PKA containing cDNA encoding the catalytic subunit of PKA (**B**). (From Lee HJ, et al. J Immunol 1993;151: 6135-42. Copyright 1993, The Journal of Immunology.)

high degree of homology (77%) in their 5' flanking regions at nucleotide positions -150 to $+1$,^{27, 28} and within this region, the IL-5 gene is associated with CLE0 and CLE1 (Fig. 2). It has been speculated that this region may be important for regulating the expression of the IL-5 gene in response to the T-cell activation signal.

Many genes that respond to the cAMP signal contain a cAMP response element²⁹ or AP-2³⁰ site in their 5' upstream regions. Deletion and mutational analyses indicate that several *cis*-elements of the 5' flanking region of the IL-5 promoter are required for the activation of the IL-5 promoter in response to PMA and cAMP signals (manuscript in preparation). However, we found no cAMP response element homologous sequences in these regions of the IL-5 promoter. Therefore it is possible that the action of cAMP is indirect; that is, cAMP may regulate the expression of a gene encoding a transactivator required for the activation of IL-5 promoter, because exposure of EL-4 cells to the protein synthesis inhibitor, cycloheximide, completely blocked the activation of the IL-5 gene (Lee et al. Unpublished data). Such a target gene may have a cAMP response element motif in its promoter region. Alternatively, cAMP exerts its effect by modifying (probably through protein phosphorylation by PKA) the components

of the PMA-dependent signal transduction pathway. Mutation analyses of the 5' flanking region of the IL-5 promoter and assays for DNA binding proteins indicate that both mechanisms are involved in the activation of the IL-5 promoter. In an electrophoretic mobility shift assay (EMSA), several nuclear factors that interact with the regulatory sequences of the IL-5 promoter were detected (manuscript in preparation). One of them shares the sequence homology with the nuclear factor of activated T cell (NF-AT) binding site (Fig. 2) and has properties similar to NF-AT. Further analyses of the IL-5 promoter, including definition of the *cis*-acting regulatory sequences and characterization of DNA binding proteins, is required to elucidate the precise mechanism of activation of the IL-5 gene by cAMP and PMA.

Binding of NF(P) to the P sequence of the IL-4 promoter was competitively inhibited by the Ig κ B oligonucleotide

The P sequence of the IL-4 promoter (positions -79 to -69 from the transcription initiation site) was defined as a responsive element for PMA and calcium ionophore (A23187) in Jurkat T cells.²⁵ Nuclear factor(s) specifically binding to the P sequence [termed NF(P)] were identified, and NF(P) binding to the P sequence correlates with

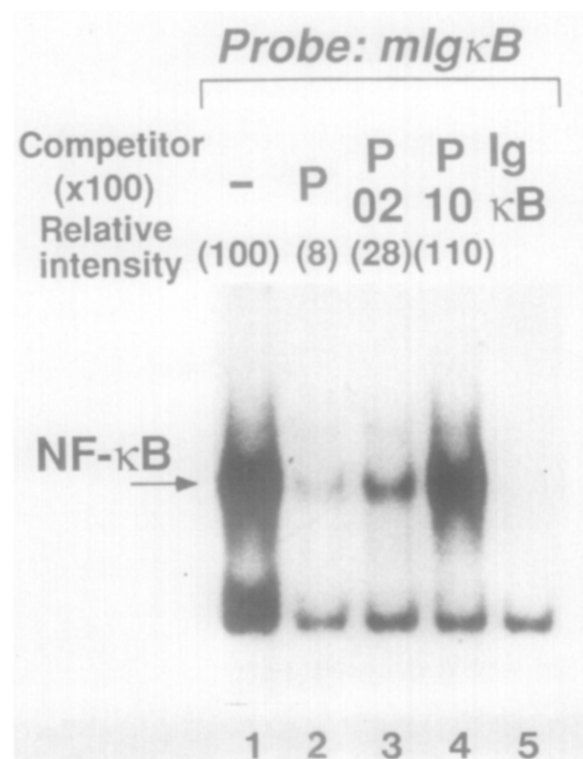


FIG. 7. Competitive inhibition of NF- κ B binding by the P sequence. EMSAs were performed as described previously.³¹ Unlabeled oligonucleotides (100-fold) were used as competitors.

PMA/A23187-mediated transcription activation through the P sequence in Jurkat T cells²⁵: for example, the P02 mutation in the P sequence did not affect NF(P) binding or transcription activation, whereas the P10 mutation abolished both.^{25, 31} The 3' half site of the P sequence (5'-CGAAAATTTCC-3') shares sequence homology with the NF- κ B binding site in the mouse immunoglobulin κ light chain gene enhancer (5'-GGGACTTTCC-3') (the Ig κ B sequence). NF- κ B, a P65/P50 heterodimer, is thought to control transcription of genes for various immunoregulatory molecules.³² To determine whether NF- κ B binds to the P sequence, we performed EMSAs with nuclear extract from PMA/A23187-stimulated Jurkat cells. The unlabeled Ig κ B oligonucleotide competitively inhibited binding of NF(P) to the P sequence probe, suggesting a possible binding of NF- κ B to the P sequence.³¹ Interestingly, NF- κ B binding was also inhibited by the unlabeled P sequence and the P02 sequence, but not by the P10 sequence (Fig. 7, lanes 2 to 4). This sequence specificity was identical to that of NF(P) binding to the P sequence, as described above.²⁵

These data also suggest possible binding of NF- κ B (or related factors) to the P sequence.

This 3' half site of the NF- κ B binding site (5'-TTTCC-3') has been shown to interact more preferentially with the P65 subunit of NF- κ B than the P50 subunit.³³ The homologous motif in the P sequence (5'-CGAAAATTTCC-3') is shown to be essential for NF(P) binding, as well as for PMA/A23187 responsiveness.^{25, 31} Compared with the NF- κ B binding to the Ig κ B sequence NF(P) binding to the P sequence appeared to be more sensitive to the competitive inhibition by the Ig κ B3C sequence (data not shown). The latter has a mutation in the 5' half (i.e., the third base) of the Ig κ B sequence, which is the mutation known to disrupt the binding of P50 subunit.³⁴ Taken together, these results imply that the P65 subunit, rather than the P50 subunit, of NF- κ B may be involved in NF(P) binding.

P65 subunit of NF- κ B, prepared by use of baculovirus expression system, binds to the P sequence

On the basis of the above observations, direct binding of the P65 subunit of NF- κ B to the P sequence was examined by EMSAs with P65 prepared by using a baculovirus expression system (rP65).³⁵ rP65 by itself generated two retarded bands with the P sequence as a probe (Fig. 8, A, lane 1). The Ig κ B sequence also generated two retarded bands with apparently the same mobility as the complex in lane 1 (Fig. 8, A, lane 2), indicating that rP65 binds to the P sequence, as well as to the Ig κ B sequence.

The binding of rP65 to the P sequence (Fig. 8, B, lane 1) was competitively inhibited by the unlabeled P sequence (Fig. 8, B, lane 2) and by the P02 sequence (Fig. 8, B, lane 3). Unlike NF(P) binding in Jurkat nuclear extract,³¹ binding of rP65 to the P sequence was significantly inhibited by the P10 sequence (Fig. 8, B, lane 4). Therefore requirements for rP65 binding to critical bases of the P sequence are not as strict as those for NF(P) binding. Similar results were obtained with nuclear extracts prepared from COS7 cells transiently transfected with an expression plasmid for P65 (unpublished data). These results indicate that P65 alone is insufficient to reconstitute the sequence specificity of NF(P) binding observed in Jurkat crude nuclear extract.^{25, 31} On the other hand, unlike the binding of rP65 to the P sequence (Fig. 8, B), purified NF- κ B (P65/P50 heterodimer) bound to the P sequence specifically, because P65/P50 bound to the P02 sequence but

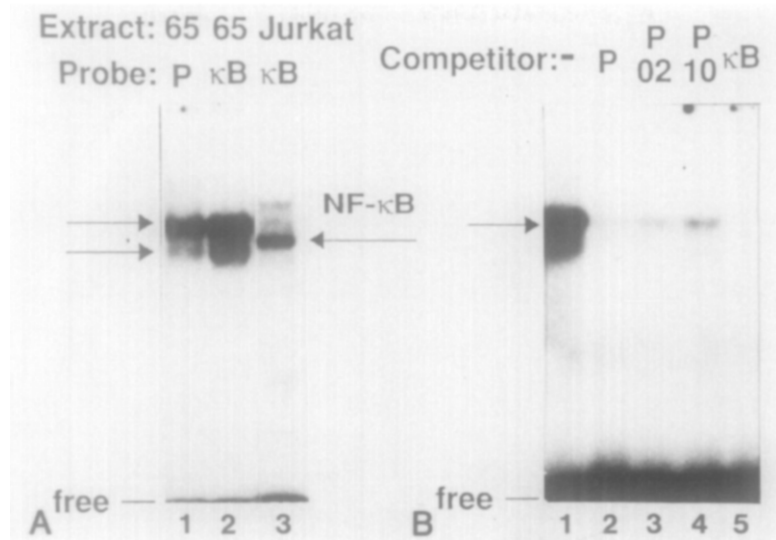


FIG. 8. Binding to the P sequence of recombinant P65 prepared by a baculovirus expression system (rP65). (rP65 was kindly provided by Dr. Garry Nolan.) EMSAs were performed as described previously.^{31, 36} **A**, Binding of rP65. **B**, Competitive inhibition of rP65 binding to the P sequence. Unlabeled oligonucleotides (20-fold excess) were used as competitors.

not to the P10 sequence.³¹ However, P65/P50 was found to bind to the P sequence more weakly than to the IgκB sequence.³¹

Cotransfection of NF-κB cDNA activates transcription from P sequence-driven promoter in Jurkat cells

To determine whether the binding of P65 (Fig. 8) or P65/P50 heterodimer³¹ of NF-κB leads to transcriptional activation through the P sequence in Jurkat cells, expression plasmids [pME(65), pME(105), and pME(Rel)] for three members of NF-κB family subunits (P65, P105 [the precursor of P50], and Rel, respectively) were cotransfected in various combinations with luciferase reporter plasmids, which have the wild type or the mutated P sequences. Forty-two hours after transfection, cell extract was prepared from each transfectant, and luciferase assays were performed. pGMTATA(3P), pGMTATA(3P02), and pGMTATA(3P10) are luciferase reporter plasmids driven by chimeric promoters composed of three copies of the P, P02, and P10 sequences, respectively, linked to the mGM30 fragment (the fragment spanning positions -30 to +27 of the mouse GM-CSF gene).^{22, 23} pmGMLuc-30 has only the mGM30 fragment linked to the luciferase gene and a negative control for the other reporters. pME18S (vector) was used as a negative control for the expression plasmids. As shown in

Fig. 9, cotransfection of pME(65) resulted in activation of transcription either from pGMTATA(3P) or pGMTATA(3P02) but not from pGMTATA(3P10). This sequence specificity correlates well with binding specificity of NF(P)³¹ (Fig. 7) or P65/P50³¹ but not with the specificity of rP65 binding to the P sequence (Fig. 8). Recently, P65 has been shown to interact with AP1 to synergistically activate transcription either from the NF-κB binding site or from the AP1 binding site.³⁶ The Rel homology domains (the DNA binding domain) of P65 and P50 have been shown to interact with the basic leucine zipper domain of AP1³⁶ and NF-IL6, a member of the C/EBP family,³⁷ respectively. It is possible that, in cotransfection of pME(65), P65 activates transcription through the P sequence by interacting with factors that have leucine zipper domains, such as AP1 or C/EBP family proteins.

The activation by P65 is specific because cotransfection of neither pME18S, nor pME(105), nor pME(Rel) (Fig. 9) activated transcription from pGMTATA(3P). The combination of P65/P105 or P65/Rel (Fig. 9) activated transcription either from pGMTATA(3P) or pGMTATA(3P02) but not from pGMTATA(3P10). This sequence specificity is identical to that of activation by pME(65) cotransfection. Activation by cotransfection of P65/P105 (the precursor of P50) or P65/P50 was far weaker than that by P65 alone (Fig. 9).³¹ It is likely

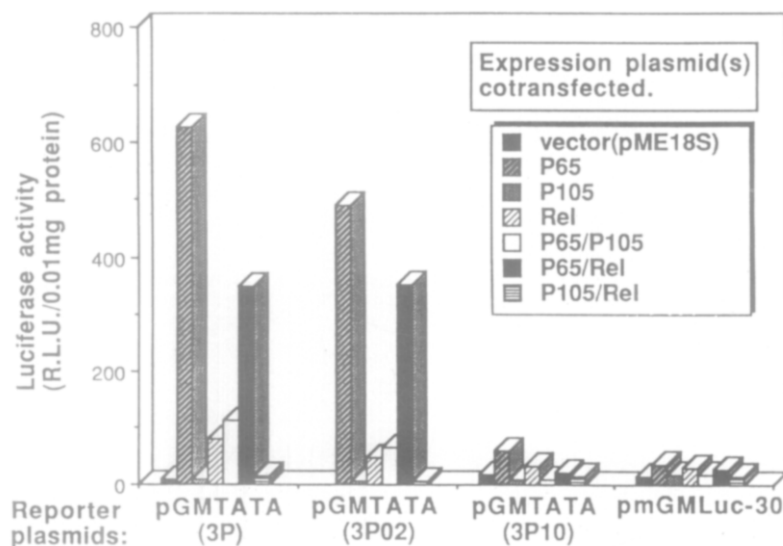


FIG. 9. Contransfection of expression plasmids of NF- κ B activates transcription through the P sequence. Transient transfection into Jurkat cells was performed with the diethylaminoethyl cellulose-dextran method as described by Matsuda et al.³¹ The construction of expression plasmids for NF- κ B has been described.³¹ pGMTATA(3P), pGMTATA(3P02), and pGMTATA(3P10) were constructed by inserting the 3P, 3P02, and 3P10 oligonucleotides,³¹ respectively, into the unique *Bgl* II site of pmGMLuc-30.^{22, 23}

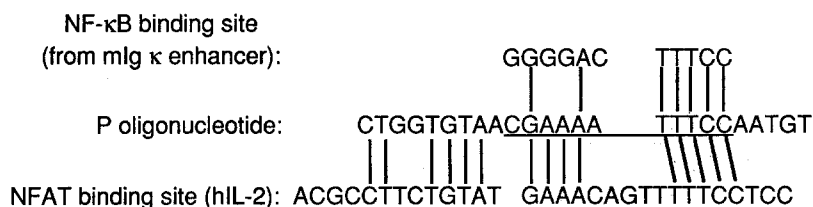


FIG. 10. Sequence comparison of the P sequence, the NF- κ B binding site, and the NF-AT binding site. The P sequence is underlined. (Modified from Matsuda I, et al. Biochem Biophys Res Comm 1994;199:439-46, by permission.)

that in P65/P50 cotransfection, the inhibitory P50 homodimer is formed in addition to the active P65/P50 heterodimer; whereas in cotransfection of P65 alone, all endogenous P50 sequences are complexed with P65.

Contribution of NF- κ B to transcription activation of the IL-4 gene through the P sequence in T cells

Taken together, the results showed that NF- κ B (P65 or P65/P50 heterodimer) has the potential to bind to the P sequences (Figs. 7 and 8)³¹ and activate transcription through the P sequence when their expression plasmids are cotransfected (Fig. 9). However, the results of EMSAs with anti-P65 antibody revealed that only a little

amount of P65 was detected in NF(P),³¹ indicating that P65 and P65/P50 (NF- κ B) was well are not major DNA-binding components of NF(P). Therefore although NF- κ B interacts with the P sequence, NF- κ B failed to account for the properties of NF(P). Some factors, other than NF- κ B, may contribute to the IL-4 gene expression in T cells.

Component of NF-AT also interacts with the P sequence

NF-AT is a nuclear factor complex composed of AP1 and non-AP1 components^{38, 39} and is thought to be essential for T-cell-specific expression of the IL-2 gene.¹² The increase in intracellular calcium concentration during T-cell activa-

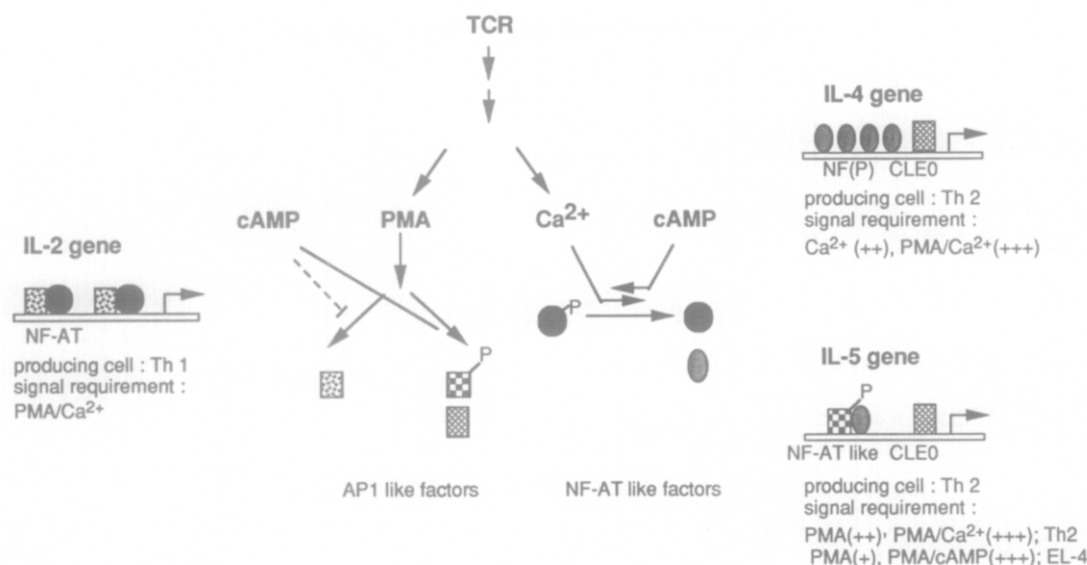


FIG. 11. Model for coordinated and differential expression of cytokine genes by NF-AT-like factors. NF-AT is composed of AP1 and non-AP1 components, which are regulated by PMA and Ca²⁺ signals, respectively. Because NF-AT-like sequences are found in the regulatory regions of various cytokine genes, they are coordinately regulated through NF-AT. The optimal signal for induction is specific to each gene. It is likely that different sets of the NF-AT complex are formed by the combinations of different NF-AT-like factors and their target sequences. TCR, T-cell receptor.

tion is accompanied by nuclear translocation of non-AP1 components of NF-AT, which is blocked by immunosuppressants such as cyclosporine or FK506.⁴⁰ We noticed that the P sequence shares sequence homology with the NF-AT binding site in the human IL-2 promoter (Fig. 10). Recently, using affinity-purified NF-AT complex, we found that components of NF-AT also interact with the P sequence specifically in the absence of AP1.³¹ Purified NF-AT appeared to contain an activity (or activities) capable of reconstituting the NF(P) binding similar to that observed in crude Jurkat nuclear extract.³¹

SUMMARY AND IMPLICATIONS

In summary, the P sequence interacts with NF- κ B, as well as with components of NF-AT. However, NF(P) has properties more similar to those of components of NF-AT.³¹ Because NF-AT is assumed to be T-cell-specific and because at least four P sequence-like elements are found in the 5' upstream region of the mouse IL-4 gene,⁴¹ T-cell-specific expression of the IL-4 gene may be controlled by NF-AT binding to the P sequence. Potential NF-AT binding sites are also observed in enhancer-promoter regions of other T-cell-derived cytokine genes as well. These results strongly suggest that coordinated expression of

T-cell-derived cytokine genes, including IL-4 and IL-5, is mediated by NF-AT (Fig. 11).

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