

Specific versus cooperative regulatory mechanisms of the cytokine genes that are clustered on the same chromosome

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The genes for IL-3, IL-4, IL-5, IL-9, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are known to be clustered on human chromosome 5q and on mouse chromosome 11. IL-2 and interferon gamma (IFN- γ) genes are located on separate chromosomes. It is well known that upon stimulation by antigen presentation, T_{H1} and T_{H2} subsets of T helper cells start to transcribe distinct sets of cytokine genes. Thus mechanisms should exist that transmit extracellular signals into the nucleus, thereby coordinately turning on transcriptional machinery in cell type-specific manners. Several different mechanisms exist in which specific as well as coordinated expression of cytokines are regulated at the transcriptional level. These include (1) regulation by proximal cis-elements, to which specific transcription factors bind, (2) regulation by distal cis-elements, such as enhancers or locus controlling elements, especially those located several kilobases away from the target gene, and (3) enhancement of transcription by viral trans-activators in a pathologic state. In this article, we review the recent studies on the above issues, with particular emphasis on our own results that support the presence of different modes of control mechanisms. We also discuss the possible approaches to the thorough understanding of the coordinated and specific regulation of cytokines. (J Allergy Clin Immunol 1996;98:S174-82.)

Key words: Genes for IL-2, IL-3, IL-4, IL-5, IL-13, and GM-CSF, nuclear factor of activated T cells (NF-AT), NF- κ B, T helper cells, enhancer, coordinated expression, transcriptional regulation

The human chromosome 5q, and the mouse chromosome 11 that has synteny with human 5q, harbors the genes for hemopoietic and immunoregulatory growth factors including IL-3, IL-4, IL-5, IL-9, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF), whereas the genes for IL-2 and interferon gamma (IFN- γ) are located separately on human chromosomes 4q and 12q, respectively.¹ The former group of genes has been mapped to a region of several hundred kilobases long. Of these, IL-3 and GM-CSF, as well as IL-13 and IL-4, are arranged particularly closely to each other on the human and mouse chromosomes; IL-3 and GM-CSF are 10 kilobases and 13 kilobases apart on the human and mouse

Abbreviations used

CAT:	chloramphenicol acetyltransferase
CsA:	cyclosporin A
NF-AT:	nuclear factor of activated T cells
PKC:	protein kinase C
PMA:	phorbol 12-myristate 13-acetate
T_H :	T helper cell

chromosomes, respectively, and IL-13 and IL-4 are 12 kilobases apart on both the human and mouse chromosomes (Fig. 1).^{2,3} The close proximity of these genes and their conserved genomic structures suggest that they have evolved by gene duplication. On the basis of the cytokines produced, T helper cells are classified into two subsets, T_{H1} and T_{H2} .^{4,5} Cytokine genes are transcribed in an inducible manner by stimulation through the T cell receptor, which can be mimicked by treatment with phorbol-12-myristate-13-acetate (PMA), an activator of protein kinase C (PKC) and calcium ionophore (A23187).^{6,7} T_{H1} cells produce IL-2, IL-3, GM-CSF, and IFN- γ , whereas T_{H2} cells

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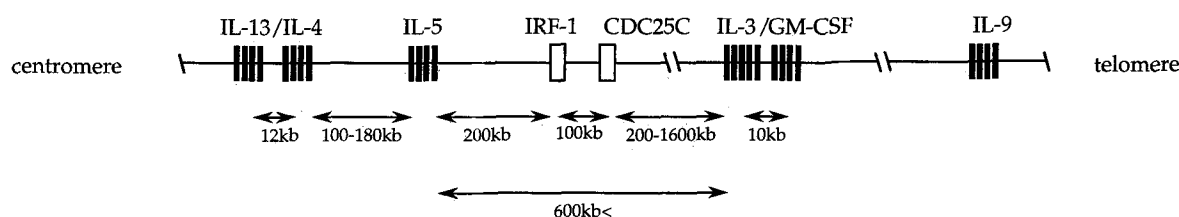


FIG. 1. Map of the cytokine gene cluster on human chromosome 5 (near segment q31) or the syntenic region on mouse chromosome 11. The relative locations of IL-13, IL-4, IL-5, IL-3, GM-CSF, and IL-9 genes are shown. Distances are not accurate and the orientation of each gene is unknown (except that IL-3 and GM-CSF are 10 kilobases apart, IL-13 and IL-4 are 12 kilobases apart, and IL-3 and GM-CSF, and IL-4 and IL-13 are transcribed in the same orientation, respectively). The human IL-4 and IL-5 genes are 110 to 180 kilobases apart from one another and are at least 600 kilobases from the IL-3 and GM-CSF genes.²⁵

produce IL-3, IL-4, IL-5, IL-13, and GM-CSF. T_{H1} cytokines activate cytotoxic and inflammatory functions and induce delayed-type hypersensitivity reactions. T_{H2} cytokines encourage antibody production, particularly IgE responses, and also enhance eosinophil proliferation and function. Accordingly, T_{H2} cytokines are commonly found in association with strong antibody and allergic responses. An interesting feature of T cell subsets is that the characteristic cytokine products of T_{H1} and T_{H2} cells are mutually inhibitory for the differentiation and effector functions of the reciprocal phenotype. This cross-regulation may partly explain the strong biases toward T_{H1} or T_{H2} responses during many infections in mice and humans. In several cases, alteration of these patterns by cytokine or anticytokine reagents reverses host resistance or susceptibility to infection. Thus the patterns of cytokine expression are important in mediating resistance to several infectious agents.⁸ The aim of our study is to reveal the intracellular machineries that transmit the activation signal to the nucleus and confer the coordinated as well as cell-type specific transcription of the cytokine genes.

Different mechanisms can regulate the expression of cytokine genes at the transcriptional level. First, the expression can be regulated by proximal *cis*-elements that usually are operationally defined by deletion or mutation analyses of the 5' upstream region. Specific transcription factors bind to the *cis*-elements and enhance transcription initiation. In most cases, the elements determined in this way map within the flanking region that is conserved over species. In this report, we briefly summarize such elements in each of the cytokine gene promoters. Second, regulation can be conferred by distal *cis*-elements such as enhancers or locus controlling elements. These types of ele-

ments are located in *cis*, but usually several kilobases or even several hundred kilobases away from the gene, and can activate transcription of multiple genes. Because of their remoteness from the proximal elements, they are not easily detected by using conventional promoter analyses but by using other methods that allow us to scan much longer stretches of the genome. One such analysis is called the nuclease hypersensitivity assay.⁹ It assumes that particular regions in the chromosome active in terms of transcriptional or other types of regulation are free of histones and thus hypersensitive to nucleases. Digestion of the nucleus with varying amounts of nucleases such as DNaseI, micrococcal nuclease, restriction enzymes, or alkylating reagents can identify such hypersensitive elements. Indeed, this assay has revealed a number of important regulatory regions in different biological systems. Here we describe a study that identified an intergenic enhancer element that confers coordinated expression of IL-3 and GM-CSF upon T cell activation, and also our approach to the IL-4/IL-13 locus. Finally, cytokine gene expression can be enhanced by viral *trans*-activators. Although this type of regulation is expected to occur in pathologic situations, knowing how these activators use the cellular machinery may help us to understand the physiologic regulation of the cytokine genes. In this study, we show that the Tax protein, a *trans*-activator encoded by human T cell leukemia virus (HTLV)-I, enhances the expression of the IL-3 gene.

RESULTS AND DISCUSSION

Regulation by gene-proximal elements

Cis-acting elements in the cytokine gene promoters are summarized in Fig. 2. In the IL-2 promoter, the binding sites for two nuclear factors of activated T cells (NF-ATs), an nuclear factor κ B

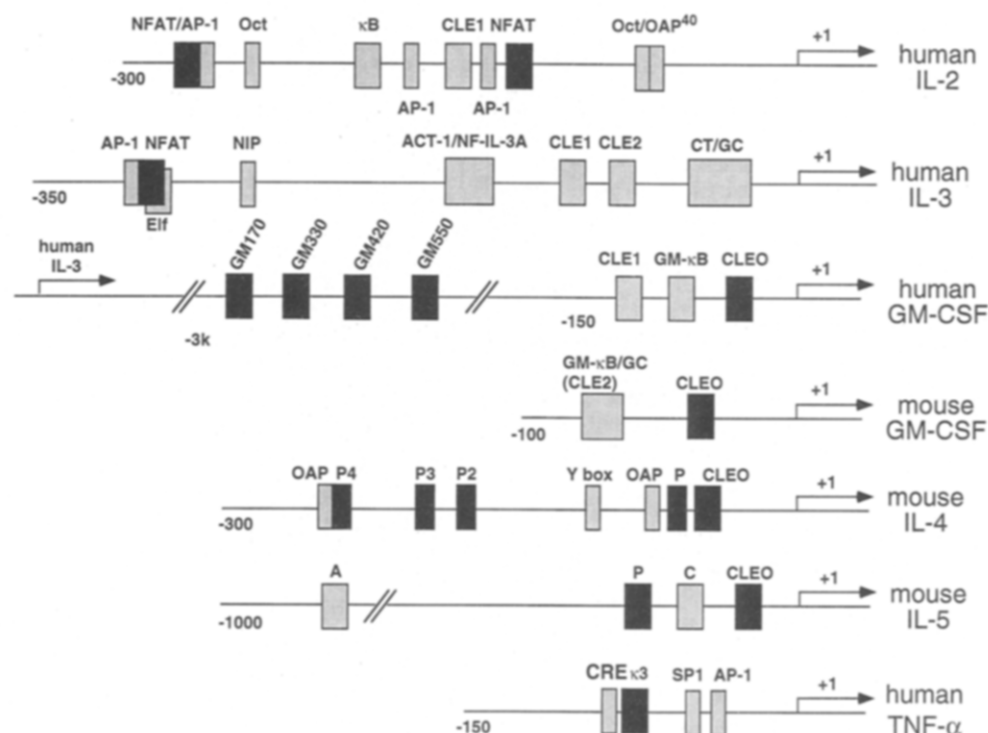


FIG. 2. *cis*-acting DNA elements required for cytokine gene expression. Sequences that bind NF-AT family proteins are shown in black boxes.

site, the proximal activator protein 1, and the proximal Oct elements are required for maximal activity.¹⁰ The cytoplasmic component of the NF-AT protein translocates into the nucleus upon calcium mobilization. This translocation is thought to be mediated by a protein phosphatase, calcineurin, which is cyclosporin A (CsA)-sensitive. The NF-AT protein heterodimerizes with AP-1 family proteins that are newly synthesized according to PKC/Ras pathway activation, and bind to the two NF-AT sites. Homodimers of p50 NF- κ B proteins (NF- κ B1), which are transcriptionally inactive, bind to the NF- κ B site in resting T cells. On stimulation by phorbol 12-myristate 13-acetate (PMA) and calcium ionophore, the amount of homodimer decreases and the amount of the heterodimer of p50 and p65 (RelA), which is capable of activating transcription, increases. AP-1-like factors bind to the AP-1 site by activation of the PKC/Ras pathway. A complex of Oct1 and OAP⁴⁰ is known to bind to the proximal Oct site in response to T cell activation. This induction requires both activation of PKC and a calcium signal.

In the human IL-3 promoter, AP-1, NIP, and ACT-1/NF-IL3A binding sites as well as GC/CT-rich regions have functionally been defined in

Jurkat cells and MLA144 cells.¹¹⁻¹³ AP-1 and NIP are bound by AP-1 family proteins and an uncharacterized negative factor, respectively. A binding site for Elf-1, an Ets family protein, is located 6 bases apart from the AP-1 site. The region recognized by Elf-1 shows a sequence similarity to the NF-AT binding site, so it may be possible that members of the NF-AT family bind to this region.¹⁴ Oct1 binds to the ACT-1/NF-IL3A sequence constitutively, whereas OAP⁴⁰ inducibly binds to this sequence.¹⁵ The functional significance of conserved lymphokine element (CLE) 1 and CLE2 in this promoter is unknown.

Two sequences in the mouse GM-CSF promoter were shown to be required for its activation in Jurkat cells. NF-GM2, a factor indistinguishable from NF- κ B, binds to the GM- κ B/GC region,¹⁶ and NF-AT in association with AP-1 binds to the CLE0 sequence.¹⁷ Initial experiments studying the effect of CLE1 on the induction of transcription upon stimulation through the T cell receptor (TCR) were, if anything, modest. It has become apparent, however, that CLE1 is a target of signals generated not from the TCR but from the accessory molecule CD28, which delivers a costimulatory signal.¹⁸

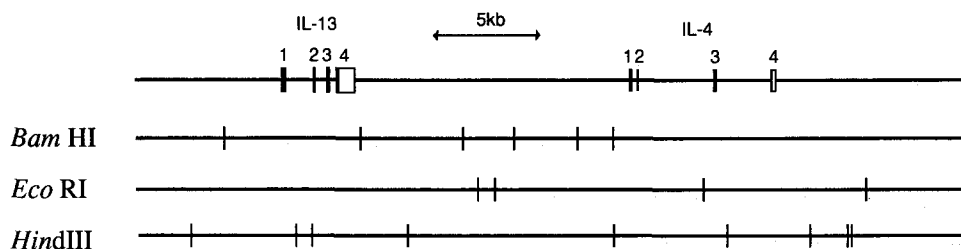


FIG. 3. Physical map of the mouse IL-13/IL-4 gene region. A 40 kilobase-cosmid clone #129 containing mouse IL-13 and IL-4 genes was analyzed using restriction mapping. The map was verified by Southern hybridization of the genomic DNA.

The P element of the human IL-4 promoter was first defined as a stimulation response element in Jurkat cells and has been shown to bind NF-AT.¹⁹ A sequence similar to the P element also has been found in the murine IL-4 promoter. The NF-AT binding capacities of CLE0, P2, P3, and P4 elements that have homology with the P element were implicated by using sequence comparison, and it was recently shown that they actually bind NF-AT-related complexes in EL-4 cells.²⁰

IL-5A, IL-5P, IL-5C (GATA), and IL5CLE0 in the mouse IL-5 promoter have been shown to respond to PMA and cyclic adenosine monophosphate (cAMP) signals in EL-4 cells.²¹ Of these four elements, IL-5P binds a protein complex that shares various properties with NF-AT.

It is notable that common nuclear factors are used for multiple cytokine gene transcription; NF-AT for IL-2, GM-CSF, IL-4, IL-5, and possibly IL-3; octamer binding proteins for IL-2 and IL-3; NF- κ B for IL-2 and GM-CSF; and AP-1 (in most cases in combination with NF-AT) for IL-2, IL-3, GM-CSF, IL-4, and IL-5. The involvement of common factors may partly explain the coordinated transcription of the cytokine genes. We should note, however, that most of these analyses have been done using Jurkat or EL-4 cells, which are transformed cell lines and cannot be classified into either T_{H1} or T_{H2} subtypes. The transcription factors stated above are expressed in both T_{H1} and T_{H2} cells, and with our current knowledge, we cannot explain the cell type specificity of cytokine gene expression by differential usage of transcription factors. In this respect, it is interesting that the p65 subunit of NF- κ B, which heterodimerizes with p50 and activates transcription, is translocated to the nucleus in T_{H1} but not in T_{H2} cells (Tsuruta L, unpublished observation, 1996).²² This is consistent with our finding that optimal transcription from the IL-2 and GM-CSF promoters, which are

preferentially expressed in T_{H1} cells, require NF- κ B sites. If similar examples of differential translocation are found for other transcription factors, they possibly would provide a general working hypothesis for the differential expression of cytokine genes.

Regulation by distal *cis*-elements

Intergenic enhancer of the human IL-3 and GM-CSF genes. IL-3 and GM-CSF are expressed from both T_{H1} and T_{H2} cells and are transcribed concomitantly on T cell activation. Because the genes for these cytokines are closely located on the chromosome, a common regulatory region, besides promoter elements, has been speculated to reside in the genome and coordinately activate transcription of the two genes. Cockerill et al.²³ provided evidence for an additional regulatory element that possibly functions to coordinate the transcription of the human IL-3 and GM-CSF genes. The researchers detected a PMA/A23187-inducible DNaseI hypersensitive site in the intergenic region of the human IL-3 and GM-CSF genes (Fig. 2). T cell activation induced the CsA-sensitive hypersensitivity before the transcription of these genes. When this region was inserted upstream of the human IL-3 or GM-CSF promoters, it led to a 40-fold and 8-fold augmentation of their promoter activities, respectively. Interestingly, this region with an enhancer activity contained three NF-AT binding sites and an AP-1 binding site. Thus the actions of AP-1 and NF-AT do not appear to be restricted to 5' flanking sequences close to promoters. A 400 bp element, 76% identical to this sequence, also was identified in the mouse IL-3/GM-CSF intergenic region and showed similar functional properties.²⁴ However, since the mouse GM-CSF promoter was significantly more active than the human GM-CSF promoter, the activity elevated in the presence of the mouse GM-CSF enhancer was not as prominent as that in the human case. The

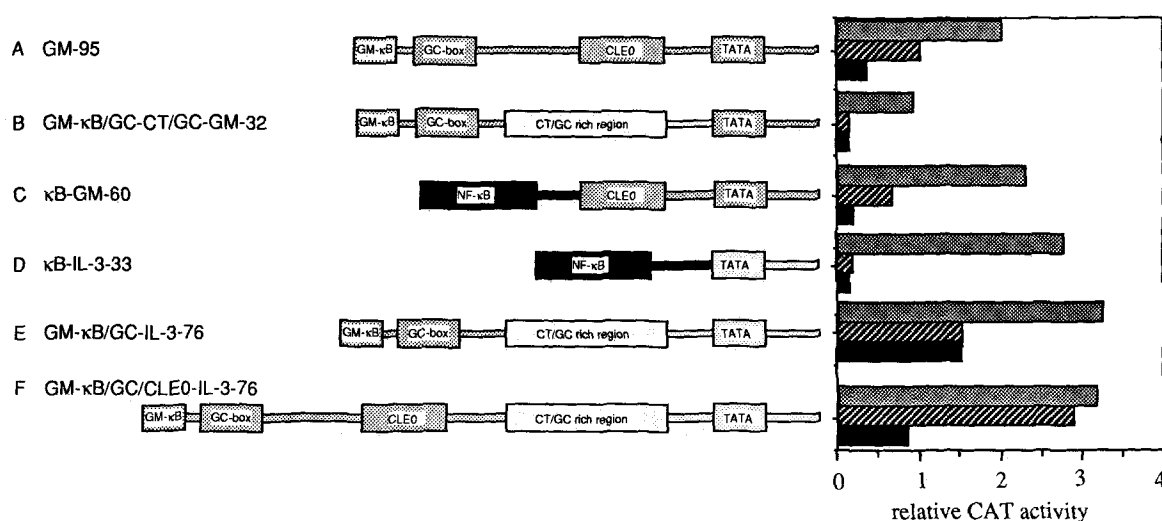


FIG. 4. Transcription activity of plasmids carrying various chimeric promoter units. The GM-κB/GC-box and CLE0 are sequences from the GM-CSF promoter. The CT/GC-rich region is from the IL-3 promoter. The NF-κB sequence is from the immunoglobulin enhancer. Each chimeric promoter linked to the chloramphenicol acetyltransferase (CAT) gene was transfected to Jurkat cells by the DEAE-dextran method.²⁶ Cells were stimulated with 20 ng/ml of PMA and 0.5 mM of A23187 40 hours after transfection, and cultured for an additional 8 hours. Transcriptional activity was determined by measuring CAT activity; bars represent unstimulated (solid), A23187/PMA-treated (hatched), and Tax-cotransfected (stippled) cells, respectively. Transfections were repeated at least three times. Each of the CAT activities was normalized to that of GM-95 stimulated with PMA/A23187, and the average is shown.

results described by Cockerill et al. are examples of how a common *cis*-regulatory element may contribute to the coordinated expression of T cell-derived cytokines, providing a framework for the coregulatory mechanisms of other closely located cytokine genes.

The mouse genes for IL-13 and IL-4 are located within 12 kilobases on the chromosome; are there any intergenic enhancers? IL-13 and IL-4 have been mapped within 50 kilobases on the mouse chromosome.²⁵ To know their relative orientations and accurate distances from one another, we constructed a physical map encompassing the IL-13/IL-4 locus. Restriction mapping, long range polymerase chain reaction (PCR), and Southern hybridization revealed that the mouse IL-13 and IL-4 were arranged in a head-to-tail orientation and were 12 kilobases apart on the genome (Fig. 3). These properties and the exon-intron structure of the two genes were similar to those of human genes. Because both IL-13 and IL-4 were transcribed in the T_H2 subset of helper T cells, the existence of a coregulatory element similar to that of the IL-3/GM-CSF locus is plausible. We currently are testing for the presence of DNaseI hypersensitive sites within this region.

Activation of cytokine genes by viral trans-activators

*Trans-activation of the GM-CSF promoter by Tax takes place through the GM-κB/GC-box, an NF-κB binding site, and does not require CLE0, an NF-AT binding site.*²⁶ We previously showed that activation of the GM-CSF promoter by either Tax or PMA/A23187 takes place through the GM-κB/GC-box.¹⁶ Because NF-GM2, a protein complex closely related to NF-κB, binds to the GM-κB/GC-box, we questioned whether a common pathway involving NF-κB family proteins existed in Tax-dependent and PMA/A23187-dependent activation mechanisms. To answer this question, we constructed various chimeric cytokine gene promoters and measured the transcriptional activity in response to PMA/A23187 and to exogenously introduced Tax protein (Fig. 4).

When we replaced the GM-κB/GC-box with the NF-κB site of the Igκ chain enhancer, the pattern of the responses to PMA/A23187 or to Tax was essentially the same as that of the wild type promoter (Figs. 4A and C). The GM-κB/GC-box and the NF-κB binding site required the presence of CLE0 to activate transcription in response to PMA/A23187 (Figs. 4A-F). These results indicated

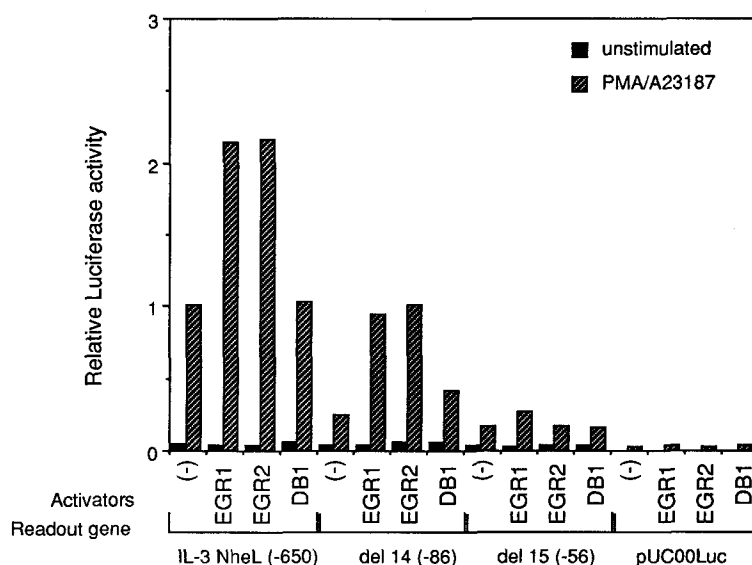


FIG. 5. Effect of EGR1, EGR2, and DB1 on the IL-3 promoter activity. Plasmids containing the 5' upstream region of the IL-3 gene, IL-3 NheL (from position -650), del 14 (position -86), del 15 (position -56) were cotransfected to Jurkat cells with vector alone (-), or with the expression plasmid for DB1, EGR1, or EGR2. Cells were stimulated at 40 hours, and harvested at 48 hours after transfection. IL-3 NheL and del 14 but not del 15 contain the CT/GC-rich region.

that the GM- κ B/GC-box and the NF- κ B binding motif were functionally interchangeable for the inducible activation of the GM-CSF promoter in T cells, and that the GM- κ B/GC-box or the NF- κ B binding motif in combination with CLE0 constituted a functional unit responding to PMA/A23187.

Our next question was whether *trans*-activation of the GM-CSF promoter by Tax also required the CLE0 element. As shown in Figures 4B and E, the GM- κ B/GC-box without CLE0 did not respond to PMA/A23187 but was activated by Tax. Likewise, a single copy of the NF- κ B binding site, which did not respond to PMA/A23187, responded well to cotransfected Tax (Fig. 4D). The response to Tax was not affected by the presence of CLE0 (Figs. 4C and F). The difference in sequence requirements between Tax and PMA/A232187 stimuli was further demonstrated by introducing mutations to CLE0 (data not shown). Thus we concluded that although PMA/calcium signals required both GM- κ B/GC and CLE0 elements, Tax-mediated *trans*-activation depended solely on the GM- κ B/GC-box. Previous reports have shown that Tax directly interacts with NF- κ B.^{27,28} Thus these results suggested that Tax operated as a bridge between the upstream enhancer binding protein, NF-GM2, and general transcription factors, and bypassed the requirement of NF-AT, the CLE0 binding protein. This hypothesis is consistent with our observation that *trans*-activation by Tax is CsA-insensitive (note that transcriptional activity driven

by NF- κ B is CsA-insensitive, and that of NF-AT is CsA-sensitive).

*cDNA cloning of transcription factors that bind to the CT/GC-rich region of the IL-3 promoter.*²⁹ We concluded that the GM- κ B/GC region was a Tax-response element in the GM-CSF promoter.²⁶ In our previous studies, we also showed that the CT/GC-rich region of the IL-3 promoter responded to Tax.¹³ This sequence also was required to support basal transcriptional activity of the IL-3 promoter. Because this sequence was not homologous to any of the reported Tax responsive elements, we tried to identify the factor that mediated Tax activation through this element.²⁹ By Southwestern screening of phage libraries, we obtained three clones containing zinc finger motifs, EGR1, EGR2, and DB1. DB1 turned out to be a novel clone and constitutively bound to the CT/GC-rich region, whereas EGR1 bound in a PMA/A23187-inducible manner. The function of each clone on IL-3 transcription was tested using cotransfection assays (Fig. 5). Cotransfection of the expression vectors of EGR1 and EGR2 cDNA with IL-3 promoter-luciferase plasmids IL-3 NheL and del 14 which contained the CT/GC-rich region, enhanced IL-3 transcription in the presence of PMA/A23187 stimulation. Because EGR1 bound to the probe for the CT/GC-rich region in an inducible manner, we assumed that EGR1 took part in the inducible expression of the IL-3 gene. Because the

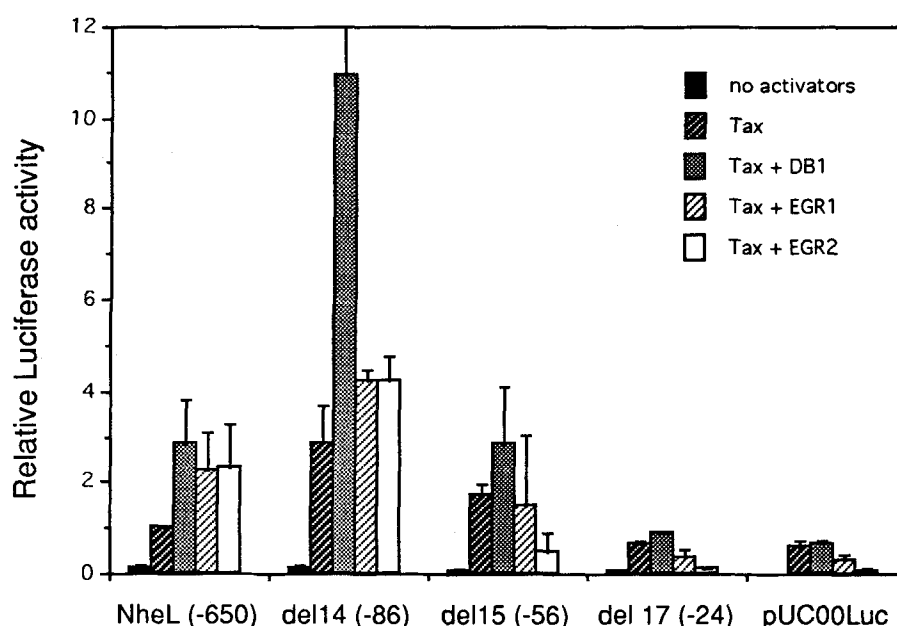


FIG. 6. Cotransfection effect of Tax with EGR1, EGR2, and DB1 on the IL-3 promoter. Tax and the expression vector of EGR1, EGR2, or DB1 were cotransfected to Jurkat cells with IL-3 reporter plasmids. Cells were stimulated at 40 hours and harvested at 48 hours after transfection.

only two major bands detected in electrophoretic mobility shift assay (EMSA) contained DB1 and EGR1, respectively, EGR2 may not be a major member required for the binding to the CT/GC-rich region.

Cotransfection of the DB1 expression vector augmented the Tax effect on IL-3 del 14, and EGR1 and EGR2 had a smaller effect on this reporter plasmid (Fig. 6). No evidence exists that Tax binds to DNA; instead, an association of DNA-binding proteins with Tax has been reported.^{27, 30-32} Although it remains to be determined whether DB1 directly interacts with Tax to activate the transcriptional machinery, our result suggests that DB1 does mediate the Tax effect in *trans*-activating the IL-3 gene.

This type of analysis using viral *trans*-activators may help us to classify the transcription factors according to whether they cooperate with the *trans*-activators, and may help us understand the mechanism by which transcription factors are recruited to transcribe cytokine genes in a physiologic state.

FUTURE PERSPECTIVES

Coupling of signal transduction machinery and transcription factors

A common feature revealed from the promoter analyses of the cytokine genes is that all the functional protein binding sites must be occupied to give rise to full activation of the promoters.⁶ The

effect of each of the binding sequences is synergistic rather than additive; mutation or deletion of most of the sequences leads to a substantial reduction in the activity of the promoter. Thus a highly cooperative interaction must occur among the various binding proteins. One implication of the requirement for full occupancy is that signals that activate only one of the enhancer elements will not give rise to the transcription of cytokine genes. This may account, in part, for the requirement of both the PKC/Ras pathway and the calcium signals for T cell activation. Thus activation of transcription factors such as AP-1 or NF- κ B, which assume DNA binding and transcriptional activation with only PMA induction, does not activate IL-2 or GM-CSF transcription. Likewise, nuclear entry of NF-AT by calcium flux alone does not activate IL-2 transcription. Both stimuli, however, lead to the activation of each of the essential transcription factors and presumably initiate IL-2 gene transcription.

In this respect, it is interesting to note that we observed a significant difference in signal requirements for the expression of cytokine genes.^{33, 34} On the basis of signal requirement patterns, T_{H1} (D1.1) and T_{H2} (D10) cytokines were classified into three groups. Group 1 contained GM-CSF and IL-2, which strictly require both the PKC and calcium signals. Group 2 consisted of IL-3, IFN- γ ,

and IL-4, which are inducible by activation of the calcium pathway alone. Group 3 had IL-5, IL-6, and IL-10, which are inducible by activation of the PKC pathway alone. Of the promoters of these cytokines, GM-CSF and IL-2 had NF-AT sequences that required AP-1 for their binding activities, whereas NF-AT could bind to the IL-4 promoter without AP-1.^{17,35} This observation was consistent with the signal requirement of each of the cytokine genes. It appeared that divergence of the binding sequence may have provided different ways of interaction among the transcription factors, and hence a different signal requirement or a different function of the same factors. Thus one issue to be studied is the difference between the related *cis*-acting elements that had been considered to be the same transcription factor binding sites.

Mechanism of differential expression of cytokine genes in T_{H1} and T_{H2} cells

Although many candidate transcription factors that coordinately regulate the cytokine genes have been identified, we have come to a dilemma in that all of the factors tested thus far are present both in T_{H1} and T_{H2} cells, and currently no link exists between the mechanism of gene activation and differential expression in T cell subtypes. NF-AT, AP-1, and NF- κ B are expressed even in non-T cells. Because most of the analyses have been done using transformed cell lines such as Jurkat, EL-4, or MLA144, which may not necessarily reflect the physiologic state, we may need to reevaluate the regulatory region in other T_{H1}/T_{H2} cell lines or primary cultured T cells to solve this paradox. Possible points of differential regulation of transcription factors may be alternative splicing, modification, such as phosphorylation and processing, subcellular localization, and stability, or presence of inhibitors. An additional factor that we should take into account is that most of the transcription factors comprise a family. The AP-1, NF-AT, and NF- κ B families each are known to consist of at least several members. Combined usage of the family members would increase the variety of signal requirements and endow specificity of transcriptional activation.

Another possible mode of regulation is by the differences of intracellular machinery that are highly restricted by prior developmental events, making the outcome of the common T cell activation signal more diverse than would be expected. The preconditioning of cells can take place at two levels. First, it may occur at the level of the signal

transduction machinery. Some reports support the possibility that T_{H1} and T_{H2} cells have different signaling molecules; T_{H1} cells lack the second chain of the IFN- γ receptor and hence are insensitive to IFN- γ ,^{36,37} whereas cells committed to the T_{H2} lineage lose the IL-12 signaling machinery.³⁸ Thus although no evidence currently exists, signal transduction machinery downstream of the T cell receptor may differ between T_{H1} and T_{H2} cells. The second possibility is at the level of the chromatin structure. In this case, a genetic locus might be prepared, perhaps by remodeling chromatin, to respond to a common set of transcription factors. Such preconditioning may include displacement of histones, change of nucleosome positioning, methylation, bending or twisting of DNA, and may occur by the time the cell has become committed to T cells or to T_{H1} or T_{H2} cells. This type of regulation may determine whether the locus is ready to transcribe the gene when it encounters the same set of transcription factors. We currently are testing for such possibilities, together with the analyses of transcription factors. Such combined consideration of multiple factors that could influence transcription may solve the problem of tissue-specificity and provide us with the whole view of cytokine gene regulation.

We thank Gerard Zurawski (DNAX Research Institute, Calif.) for providing us with the cosmid clone 129; Yasushi Nakagawa and Naoko Arai for critical reading of the manuscript; and Esteban Masuda and Lisako Tsuruta for their help in preparation of the manuscript.

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