Archivum Immunologiae et Therapiae Experimentalis, 2001, **49**, 345–351
PL ISSN 0004-069X

Review

Regulation of IL-5 Expression

VIATCHESLAV A. MORDVINOV* and COLIN J. SANDERSON

Curtin University of Technology, Perth, Western Australia

Abstract. Interleukin 5 (IL-5) is a cytokine primarily involved in the pathogenesis of atopic diseases. It specifically controls the production, activation and localization of eosinophils, the major cause of tissue damage in atopic diseases. IL-5 belongs to a gene family shared by IL-3, IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) and is predominantly regulated at the transcriptional level. A variety of stimuli and modulators have been identified as regulating production of IL-5 both *in vivo* and *in vitro*, indicating a highly complex series of control mechanisms. However, a better understanding of the biology of IL-5 and the regulation of its expression is crucial for the development of new therapeutic agents for allergic disease. This review covers the major molecular aspects of IL-5 research.

Key words: interleukin 5; cytokines; allergic; disease.

Introduction

Interleukin 5 (IL-5) is a homodimeric glycoprotein secreted by several cell types, including activated T cells, mast cells and eosinophils[16]. It was originally described in 1985 by SANDERSON et al.[41] as eosinophil differentiation factor (EDF). EDF is identical to the murine B cell growth factor[40]. However, despite the fact that the *in vitro* activity on murine B cells is well characterized, there is little evidence to suggest a biological role for IL-5 in the B cell system *in vivo*. In contrast, the central role of IL-5 in the control of production, activation and differentiation of eosinophils *in vivo* and *in vitro* is well documented[39].

Eosinophils are important to the host in mediating parasite rejection. However, they are also involved in the pathogenesis of a number of allergic diseases, most notably asthma[7]. The presence of eosinophils in the lungs of asthmatics is associated with tissue damage[9] and, in fact, the number of eosinophils has been found to correlate with the severity of the late asthmatic reaction[3]. The critical role of IL-5 in mediating tissue damage through activation of eosinophils has been shown in a mouse asthma model[10].

Eosinophilia is accompanied by the increased expression of IL-5[38]. However, the whole spectrum of hemopoietic factors cannot compensate for the absence of IL-5 activity in the generation of eosinophils in IL-5 knockout mice[10]. Such a close relationship between a gene and a biological effect is unusual in the cytokine field, where pleiotropy and redundancy are more common. The significance of IL-5 in the production of eosinophils suggests a unique and tight control of IL-5 gene expression.

* Correspondence to: Viatcheslav A. Mordvinov, Western Australian Institute for Medical Research, Molecular Immunology Group, Level 5, MRF Building, Rear 50 Murray Street, Perth, Australia 6000, tel.: +618 9224 0354, fax: +618 9224 0360, e-mail: slava@cyllene.uwa.edu.au

Pathways Leading to the Activation of the IL-5 Gene

IL-5 is a member of the cytokine family of inducers of hemopoiesis and immune responses, which is shared by IL-3, IL-4, IL-13 and the granulocyte-macrophage colony-stimulating factor (GM-CSF). The genes encoding these cytokines are located in a cluster on the mouse chromosome 11 and in the syntenic region of the human chromosome 5q31[24, 48], and may be co-expressed in both species[44]. These cytokines are produced by activated lymphocytes which belong to the Th0 and Th2 subsets of mature $CD4^+$ T helper (Th) cells[1, 29]. The peculiarity of IL-5 gene expression is its dependence on *de novo* protein synthesis. The protein synthesis inhibitors CHX and anisomycin completely inhibited IL-5 mRNA synthesis in primary T cells and murine Th2 cell clone D10. G4.1, though they did not inhibit the expression of IL-2, IL-3, IL-4, IL-10 and GM-CSF mRNAs in these cells[30, 44, 47]. Thus, at least one protein critical for the induction of IL-5 gene transcription, but not for the transcription of other lymphokine genes, is newly synthesized in response to T cell stimulation.

The activation of T cells requires the interaction of the T cell receptor (TCR) with antigen in association with the major histocompatibility complex (MHC), which leads to an increase of the intracellular calcium concentration and activation of protein kinase C (PKC)[33]. However, stimulation of the TCR alone is insufficient to fully activate T cells and a second signal provided by antigen-presenting cells (APC) is also required[36]. This co-stimulatory signal is generated by the CD28 ligand[14]. APC-stimulated T cells produce a wide range of lymphokines and IL-5 is often, but not always, co-expressed with cytokines such as IL-4 and IL-13[39]. It is not clear why certain antigens induce IL-5 and others do not, however, this indicates that the gene is individually regulated and control mechanisms for IL-5 expression can be based on the antigen-specific T cell activation.

In vitro, activation of the TCR pathway can be achieved by using phorbol myristate acetate (PMA) in combination with ionomycin. Efficient production of IL-5 requires activation of both the TCR and a second signaling pathway. Anti-CD28 antibody or cyclic adenosine monophosphate (cAMP) in combination with PMA were shown to be necessary for the optimal induction of IL-5 synthesis[17]. PMA/anti-CD28 stimulation activates the expression of a variety of cytokine genes, including IL-2, IL-3, IL-4, IL-10 and GM-CSF[1]. However, cAMP has an inhibitory effect on IL-2, IL-3, IL-10 and GM-CSF[4, 20] and no effect on IL-4[23]. This suggests that IL-5 expression is controlled by at least two independent costimulatory pathways. One of these is the CD28 pathway, which may be regarded as a common pathway for the activation of cytokine genes. The other is the cAMP-dependent pathway which, in the context of lymphokine genes, appears to be unique for the induction of IL-5 expression.

In our work we have used a human T cell line, PER-117[18], which inducibly expresses IL-2, IL-4 and IL-5[19, 27]. As in primary T cells there is no detectable constitutive expression of IL-5, but expression can be induced with PMA and further enhanced by cAMP, calcium ionophore A23187 (CaI) and anti-CD28 antibodies. IL-5 production in PER-117 cells can be inhibited by cyclosporin A (CsA). Cyclosporin A blocks the transcription of most cytokine genes in activated T cells by inhibiting the Ca^{2+}/calmodulin-dependent phosphatase calcinerium. This results in the inhibition of both the DNA-binding and transcriptional activities of the T cell-specific regulatory factor nuclear factor of activated T cells (NFAT)[23], which is essential for the activation of many cytokine genes. Interestingly, the conditions used to stimulate the PER-117 cells determined whether IL-5 production was inhibited by CsA. PMA/CaI-induced IL-5 production in PER-117 is CsA sensitive. However, costimulation of these cells with cAMP activated CsA-insensitive IL-5 production. This observation indicates that NFAT does not play a significant role in PMA/cAMP-activated IL-5 expression. Thus, depending on stimulation conditions, different regulatory factors can play a dominant role in IL-5 transcription.

Transcriptional Control of the IL-5 Gene

The expression of IL-5, like many other cytokines, is regulated at the transcriptional level[39], and various regulatory elements can be identified in the proximal and distal promoter region of the IL-5 gene (Fig. 1). Whilst these promoter elements contribute to the overall transcriptional activity of IL-5, the conserved lymphokine element 0 (CLE0), a *cis*-regulatory element located immediately upstream of the tumor associated transplantation antigens (TATA)-box in the gene promoter, is the on/off switch for IL-5 expression. Studies performed on both murine and human IL-5 regulation demonstrated, through deletion and mutation analysis, that CLE0 is critical to IL-5 expression[39]. A similar, but not identical, element occurs in IL-3, IL-4 and GM-

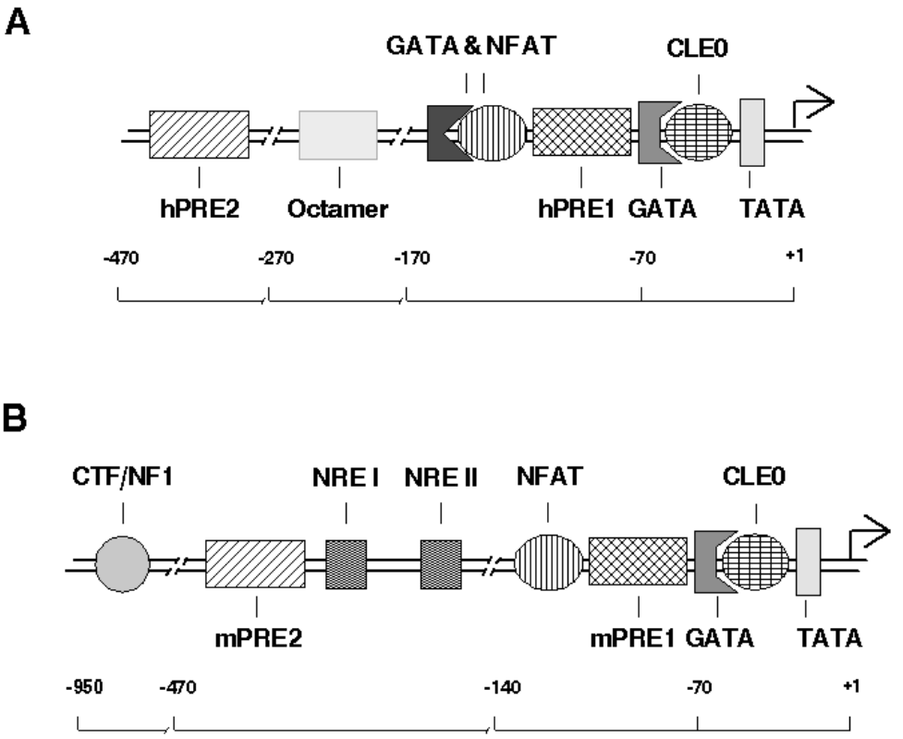

Fig. 1. A diagrammatic representation of the human (A) and murine (B) IL-5 promoter regions. Regulatory elements which have previously been shown to be involved in human and murine IL-5 gene regulation are indicated

-CSF genes, and related sequences also exist in other cytokine genes.

Although the IL-5 CLE0 shares 94% sequence identity with GM-CSF CLE0, the nuclear factors which bind to IL-5 and GM-CSF are not identical. The transcriptional factors AP-1, NFAT and Ets have been shown to bind the GM-CSF CLE0[13, 22], however, AP-1 and octamer transcription factors have been shown to complex with IL-5 CLE0 in activated T cells[16, 39, 46] (SENNA-SALERNO, unpublished data). AP-1 is known to be involved in the activation of many cytokine genes and its role as a positive regulator of CLE0 action is well documented[13, 16]. This transcription factor is a complex composed of proteins belonging to the Jun and Fos families. The human IL-5 CLE0 AP-1 protein complex consists of JunD and Fra-2 nuclear proteins[46], while the mouse CLE0 AP-1 complex contains JunB and FosB[16] (SENNA-SALERNO, unpublished data). Thus, despite of the fact that human and mouse CLE0 sequences are identical, different nuclear proteins bind these elements. This variation may be a result of the species difference or, alternatively, due to the different cell lines used to study IL-5 regulation (PER-117 and EL4). Interestingly, a distinguishing feature of both mouse and human IL-5 CLE0 AP-1 protein complexes is the presence of nuclear factors which can be activated by both PKC and PKA pathways[8, 12, 25, 26]. Indeed, the composition of the IL-5 CLE0 AP-1 complex is stimuli independent. Activation of PER-117 cells and mouse lymphoma cell line EL4 with PMA in combination with anti-CD28, CaI or cAMP resulted in the same AP-1 proteins binding IL-5 CLE0[46] (SENNA-SALERNO, unpublished data). This indicates that different pathways can induce the IL-5-specific AP-1 complex, which is critical for IL-5 transcription.

In addition to AP-1, the ubiquitous transcription factor Oct-1 and the lymphocyte-specific Oct-2 were shown to bind both human[46] and murine IL-5 CLE0 (SENNA-SALERNO, unpublished data). Oct-1 and Oct-2 are constitutively expressed in EL4 cells and bind murine CLE0 in resting and activated cells. However, only Oct-1 binds CLE0 in resting PER-117 cells. Upon PMA/CaI cell activation, both Oct-1 and Oct-2 were shown to complex with this element. In contrast, PMA/cAMP stimulation failed to induce Oct-2-binding. This suggests that the effect of Oct-2 on IL-5 pro-

moter activity may be stimuli dependent. Interestingly, the involvement of Oct factors with CLE0 is unique to the IL-5 gene. Over-expression of Oct-1 or Oct-2 in unstimulated PER-117 and Jurkat cells was shown to markedly enhance human IL-5 promoter activity[46]. This indicates that Oct factors are involved in the positive regulation of the gene and may play a general role in the activation of IL-5 transcription. Indeed, Oct-1, Oct--2A and Oct-2B proteins bind to a perfect octamer motif located in the human IL-5 promoter at position –237 to –244. Mutation of this motif results in a 90% reduction in the human IL-5 promoter activity in EL4 cells[11]. A footprint was seen to cover this Oct-binding site when nuclear extracts from allergen-specific human T cell clones were used in a DNase I assay[5]. However, no footprint was observed with nuclear extracts from PER-117 cells (FOURNIER, unpublished data) and human Th0 T cell clone SP-B21[45]. This suggests that in allergen specific T cells, octamer factors may play an even more essential role in IL-5 transcription than in other T cells.

It is important to note that the –237/–244 Oct-binding site is not conserved in the murine IL-5 gene promoter, so the precise regulation of human and murine IL-5 may be different in some respects. However, the 3' untranslated region of the murine gene contains a positive regulatory element binding Oct-1 and Oct-2 proteins in both EL4 and primary T cells. Deletion of this element from the 9.5-kilobase IL-5 gene construct reduces the transcriptional activity of the construct by more than 3 times[37]. Thus, although distinctions between human and murine promoters exist, octamer factors play an important role in the gene expression of both species.

A GATA element is located immediately upstream of the CLE0 sequence in the human and mouse IL-5 gene. This element has been shown to bind transcription factor GATA-3 and to be critical for the function of the murine promoter[39]. Mutation of the GATA-3 site at –70 in the murine gene totally blocks transcription. Recent results have shown that transcription factor GATA-3 is critical to the development of Th2 cells in the mouse and is expressed by all Th2 cells[32, 49, 50]. GATA-3 is a potential candidate to regulate coordinate expression of IL-4 and IL-5 genes, as it is important in the transcriptional regulation of IL-4[35]. However, the function of the GATA-3 site in the human promoter is unknown and more work is required to clarify the role of this protein in human T cells.

Another GATA element was located in the human promoter flanking the proximal part of the transcriptionally active NFAT binding site, around position –110. The NFAT and GATA elements cooperate in the positive induction of the gene by IgE and antigen in the mouse cell line CPII. Nuclear proteins bind to the NFAT element in an inducible fashion, though binding to the GATA element is constitutive[34]. The murine IL-5 promoter also harbors a NFAT site around position –110, though the GATA motif is not conserved. NFAT, in conjunction with AP-1 proteins, has been found to bind to the human IL-5 NFAT element in the human T cell clone SP-B21[45] and EL4 cells[21]. However, only NFAT factors were observed to bind the human NFAT element in PER-117 cells. Binding of NFAT proteins was totally blocked by CsA and mutation of the element was shown to dramatically reduce PMA/CaI-simulated human IL-5 promoter activity in both PER-117 and primary T cells[6]. However, this site was of little importance for PMA/cAMP-stimulated IL-5 transcription[28]. This correlates well with the insensitivity of PMA/cAMP-induced IL-5 production to CsA and confirms that, depending on stimulation conditions, different regulatory factors can play a dominant role in IL-5 transcription.

Recently, two palindromic regulatory elements, mPRE1-IL-5 (from –79 to –90) and mPRE2-IL-5 (from –459 to –470), have been identified in the murine IL-5 promoter[43]. Mutations of these elements significantly reduce IL-5 promoter activity. This indicates that these elements are essential for enhancing murine IL-5 gene expression. It should be noted that PRE-IL-5 elements are present in all known IL-5 genes (mouse, rat, ovine and human), but not in any other genes in the GenBank database. This suggests the possibility of a role for these elements in the specific regulation of the IL-5 gene. However, human PREs (hPREs) act as negative regulatory elements[28, 42]. Deletion or mutation of hPRE1 (from –78 to –94) leads to a 2.5-fold increase in the promoter activity in both PER-117 and primary T cells. Mutation of hPRE2 (from –447 to –459) increases the promoter activity by 40–60%. This shows once again that the IL-5 regulatory mechanisms employed by mouse and human T cells are not identical.

Human PREs contain overlapping protein binding motifs and bind unique combinations of regulatory factors. Oct-1, Oct-like and YY1 proteins were found to complex with PRE1[28], whereas a combination of YY1 and NFAT was shown to bind hPRE2[42]. Mutation of any protein binding motif within the elements leads to an increase of IL-5 promoter activity, suggesting that all PRE binding proteins are involved in the suppression of IL-5 transcription. Regarding cytokine gene expression, YY1 is known as a repressor protein, but Oct and NFAT factors act as activators of IL-5 expression.

However, a dual function of nuclear proteins such as this is not unusual in the transcription field, where the actual role of the protein may be dependent on the context of the transcription complex.

Two negative regulatory elements, NRE I (from –392 to –431) and NRE II (from –262 to –300), have also been identified in the murine IL-5 gene promoter[44]. Deletion of the regions containing these elements results in a marked increase in inducible promoter activity. However, proteins that may interact with these elements have not yet been characterized.

The final known element present in the murine promoter is located at positions –928 to –940 and is called the CTF/NF1 site. Mutation of this site results in a constitutive activity of IL-5 promoter in EL4 cells. This suggests that the CTF/NF1 site may be important for inducible expression[2].

Conclusion

The unique role of IL-5 in the production, activation and differentiation of eosinophils makes it an attractive target for the generation of new anti-allergic drugs. Therefore, a better understanding of the control mechanisms regulating IL-5 expression is important. IL-5 may be co-expressed with other cytokines such as IL-3, IL-4, IL-13 and GM-CSF, but it is evident that IL-5 expression is also individually regulated. The basis for co-expression and independent IL-5 expression is the ability of the gene to be activated by two different costimulatory pathways. One of these is the CD28 pathway, which may be regarded as a common pathway for the activation of cytokine genes. The other is the cAMP-dependent pathway which, in the context of lymphokine genes, appears to be unique for the induction of IL-5 expression.

The regulation of eosinophilia is highly conserved in mammals. However, with exception of CLE0, the functional organization of mouse and human promoters is distinct. Presumably, different species have found different ways to fine-tune the production of IL-5. This highlights the importance of carrying out experiments in human cells if data are to be applicable to human allergy.

References

1. ABBAS A. K., MURPHY K. M. and SHER A. (1996): Functional diversity of helper T lymphocytes. Nature, **383**, 787–793.
2. BOURKE P. F., VAN LEEUWEN B. H., CAMPBELL H. D. and YOUNG I. G. (1995): Localization of the inducible enhancer in the mouse interleukin-5 gene that is responsive to T cell receptor stimulation. Blood, **85**, 2069–2077.
3. BOUSQUET J., CHANEZ P., LACOSTE J. Y., BARNEON G., GHAVANIAN N., ENANDER I., VENGE P., AHLSTEDT S., SIMONY-LAFONTAINE J., GODARD P. and MICHEL F. B. (1990): Eosinophilic inflammation in asthma. N. Engl. J. Med., **323**, 1033–1039.
4. CHEN D. and ROTHENBERG E. V. (1994): Interleukin 2 transcription factors as molecular targets of cAMP inhibition: delayed inhibition kinetics and combinatorial transcription roles. J. Exp. Med., **179**, 931–942.
5. COUSINS D. J., RICHARDS D., KEMENY D. M., ROMAGNANI S., LEE T. H. and STAYNOV D. Z. (2000): DNase I footprinting of the human interleukin-5 gene promoter. Immunology, **99**, 101–108.
6. DE BOER M. L., MORDVINOV V. A., THOMAS M. A. and SANDERSON C. J. (1999): Role of nuclear factor of activated T cells (NFAT) in the expression of interleukin-5 and other cytokines involved in the regulation of hemopoetic cells. Int. J. Biochem. Cell Biol., **31**, 1221–1236.
7. DE MONCHY J. G., KAUFFMAN H. F., VENGE P., KOETER G. H., JANSEN H. M., SLUITER H. J. and DE VRIES K. (1985): Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am. Rev. Respir. Dis., **131**, 373–376.
8. DEN HERTOG J., DE GROOT R. P., DE LAAT S. W. and KRUIJER W. (1992): EGF-induced jun B-expression in transfected P19 embryonal carcinoma cells expressing EGF-receptors is dependent on Jun D. Nucleic Acids Res., **20**, 125–130.
9. DESREUMAUX P. and CAPRON M. (1996): Eosinophils in allergic reactions. Curr. Opin. Immunol., **8**, 790–795.
10. FOSTER P. S., HOGAN S. P., RAMSAY A. J., MATTHAEI K. I. and YOUNG I. G. (1996): Interleukin-5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. J. Exp. Med., **183**, 195–201.
11. GRUART-GOUILLEUX V., ENGELS P. and SULLIVAN M. (1995): Characterization of the human interleukin-5 gene promoter: involvement of octamer binding sites in the gene promoter activity. Eur. J. Immunol., **25**, 1431–1435.
12. GRUDA M. C., KOVARY K., METZ R. and BRAVO R. (1994): Regulation of Fra-1 and Fra-2 phosphorylation differs during the cell cycle of fibroblasts and phosphorylation *in vitro* by MAP kinase affects DNA binding activity. Oncogene, **9**, 2537–2547.
13. JENKINS F., COCKERILL P. N., BOHMANN D. and SHANNON M. F. (1995): Multiple signals are required for function of the human granulocyte-macrophage colony-stimulating factor gene promoter in T cells. J. Immunol., **155**, 1240–1251.
14. JENKINS M. K. and JOHNSON J. G. (1993): Molecules involved in T-cell costimulation. Curr. Opin. Immunol., **5**, 361–367.
15. KARLEN S., DE BOER M. L., LIPSCOMBE R. J., LUTZ W., MORDVINOV V. A. and SANDERSON C. J. (1998): Biological and molecular characteristics of interleukin-5 and its receptor. Int. Rev. Immunol., **16**, 227–247.
16. KARLEN S., D'ERCOLE M. and SANDERSON C. J. (1996): Two pathways can activate the interleukin-5 gene and induce binding to the conserved lymphokine element 0. Blood, **88**, 211–221.
17. KARLEN S., MORDVINOV V. A. and SANDERSON C. J. (1996): How is expression of the interleukin-5 gene regulated? Immunol. Cell Biol., **74**, 218–223.
18. KEES U. R., FORD J., PRICE P. J., MEYER B. F. and HERRMANN R. P. (1987): PER-117: a new human ALL cell line with an immature thymic phenotype. Leuk. Res., **11**, 489–498.

19. KEES U. R., PERONI S. E., RANFORD P. R. and FORD J. (1994): A new human cell line for the molecular dissection of the requirement for interleukin-1 in the production of interleukin-2 by immature T cells. J. Immunol. Methods, **168**, 1–8.
20. LEE H. J., KOYANO-NAKAGAWA N., NAITO Y., NISHIDA J., ARAI N., ARAI K.-I. and YOKOTA T. (1993): cAMP activates the IL-5 promoter synergistically with phorbol ester through the signaling pathway involving protein kinase A in mouse thymoma line EL-4. J. Immunology, **151**, 6135–6142.
21. LEE H. J., MASUDA E. S., ARAI N., ARAI K. and YOKOTA T. (1995): Definition of *cis*-regulatory elements of the mouse interleukin-5 gene promoter. Involvement of nuclear factor of activated T cells-related factors in interleukin-5 expression. J. Biol. Chem., **270**, 17541–17550.
22. MASUDA E. S., TOKUMITSU H., TSUBOI A., SHLOMAI J., HUNG P., ARAI K.-I. and ARAI N. (1993): The granulocyte-macrophage colony-stimulating factor promoter *cis*-acting element CLE0 mediates induction signals in T cells and is recognised by factors related to AP1 and NFAT. Mol. Cell. Biol., **13**, 7399–7407.
23. MCCAFFREY P. G., LUO C., KERPPOLA T. K., JAIN J., BADALIAN T. M., HO A. H., BURGEON E., LANE W. S., LAMBERT J. N., CURRAN T., VERDINE G. L., RAO A. and HOGAN P. G. (1993): Isolation of the cyclosporin-sensitive T cell transcription factor NFATp. Science, **262**, 750–754.
24. MCKENZIE A. N., LI X., LARGAESPADA D. A., SATO A., KANEDA A., ZURAWSKI S. M., DOYLE E. L., MILATOVICH A., FRANCKE U., COPELAND N. G., JENKINS N. A. and ZURAWSKI G. (1993): Structural comparison and chromosomal localization of the human and mouse IL13 genes. J. Immunol., **150**, 5436–5444.
25. MECHTA F., PIETTE J., HIRAI S. I. and YANIV M. (1989): Stimulation of protein kinase C or protein kinase A mediated signal transduction pathways shows three modes of response among serum inducible genes. New Biol., **1**, 297–304.
26. MILLER C., ZHANG M., HE Y., ZHAO J., PELLETIER J. P., MARTEL-PELLETIER J. and DI BATTISTA J. A. (1998): Transcriptional induction of cyclooxygenase-2 gene by okadaic acid inhibition of phosphatase activity in human chondrocytes: co-stimulation of AP-1 and CRE nuclear binding proteins. J. Cell Biochem., **69**, 392–413.
27. MORDVINOV V. A., PERONI S. E., DE BOER M. L., KEES U. R. and SANDERSON C. J. (1999): A human T-cell line with inducible production of interleukins 5 and 4. A model for studies of gene expression. J. Immunol. Methods, **228**, 163–168.
28. MORDVINOV V. A., SCHWENGER G. T., FOURNIER R., DE BOER M. L., PERONI S. E., SINGH A. D., KARLEN S., HOLLAND J. W. and SANDERSON C. J. (1999): Binding of YY1 and Oct1 to a novel element that downregulates expression of IL-5 in human T cells. J. Allergy Clin. Immunol., **103**, 1125–1135.
29. MOSMANN T. R. and COFFMAN R. L. (1989): TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol., **7**, 145–173.
30. NAORA H. and YOUNG I. G. (1995): Comparison of the mechanisms regulating IL-5, IL-4, and three other lymphokine genes in the Th2 clone D10.G4.1. Exp. Hematol., **23**, 597–602.
31. NOVAK T. J. and ROTHENBERG E. V. (1990): cAMP inhibits induction of interleukin-2 but not of interleukin-4 in T-cells. Proc. Natl. Acad. Sci. USA, **87**, 9353–9357.
32. OUYANG W., RANGANATH S. H., WEINDEL K., BHATTACHARYA D., MURPHY T. L., SHA W. C. and MURPHY K. M. (1998): Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. Immunity, **9**, 745–755.
33. PERLMUTTER R. M., LEVIN S. D., APPLEBY M. W., ANDERSON S. J. and ALBEROLA-ILA J. (1993): Regulation of lymphocyte function by protein phosphorylation. Ann. Rev. Immunol., **11**, 451–499.
34. PRIESCHL E. E., GOUILLEUX-GRUART V., WALKER C., HARRER N. E. and BAUMRUKER T. (1995): A nuclear factor of activated T cell-like transcription factor in mast cells is involved in IL-5 gene regulation after IgE plus antigen stimulation. J. Immunol., **154**, 6112–6119.
35. RANGANATH S., OUYANG W., BHATTARCHARYA D., SHA W. C., GRUPE A., PELTZ G. and MURPHY K. M. (1998): GATA-3-dependent enhancer activity in IL-4 gene regulation. J. Immunol., **161**, 3822–3826.
36. RINCON M. and FLAVELL R. A. (1994): AP-1 transcriptional activity requires both T-cell receptor mediated and co-stimulatory signals in primary T lymphocytes. EMBO J., **13**, 4370–4381.
37. SALERNO M. S., MORDVINOV V. A. and SANDERSON C. J. (2000): Binding of octamer factors to a novel 3'-positive regulatory element in the mouse interleukin-5 gene. J. Biol. Chem., **275**, 4525–4531.
38. SANDERSON C. J. (1992): IL-5, eosinophils and disease. Blood, **79,** 3101–3109.
39. SANDERSON C. J., DE BOER M., SENNA-SALERNO M., SCHWENGER G. M., THOMAS M., MORDVINOV V. A. and KARLEN S. (1999): Regulation of interleukin-5 gene expression. In SANDERSON C. J.: Interleukin-5: from molecule to drug target for asthma. Marcel Dekker Inc., New York, **125**, 267–287.
40. SANDERSON C. J., O'GARRA A., WARREN D. J. and KLAUS G. G. (1986): Eosinophil differentiation factor also has B-cell growth factor activity: proposed name interleukin-4. Proc. Natl. Acad. Sci. USA, **83**, 437–440.
41. SANDERSON C. J., WARREN D. J. and STRATH M. (1985): Identification of a lymphokine that stimulates eosinophil differentiation *in vitro*. J. Exp. Med., **162**, 60–74.
42. SCHWENGER G. T., FOURNIER R., Hall L. M., SANDERSON C. J. and MORDVINOV V. A. (1999): Nuclear factor of activated T cells and YY1 combine to repress IL-5 expression in a human T-cell line. J. Allergy Clin. Immunol., **104**, 820–827.
43. SCHWENGER G. T., MORDVINOV V. A., KARLEN S., D'ERCOLE M. and SANDERSON C. J. (1998): Identification of two novel palindromic regulatory elements in the murine interleukin-5 promoter. Mol. Immunol., **35**, 149–158.
44. STRANICK K. S., PAYVANDI F., ZAMBAS D. N., UMLAND S. P., EGAN R. W. and BILLAH M. M. (1995): Transcription of the murine interleukin-5 gene is regulated by multiple promoter elements. J. Biol. Chem., **270**, 20575–20582.
45. STRANICK K. S., ZAMBAS D. N., USS A. S., EGAN R. W., BILLAH M. M. and UMLAND S. P. (1997): Identification of transcription factor binding sites important in the regulation of the human interleukin-5 gene. J. Biol. Chem., **272**, 16453–16465.
46. THOMAS M. A., MORDVINOV V. A. and SANDERSON C. J. (1999): The activity of the human interleukin-5 conserved lymphokine element 0 is regulated by octamer factors in human cells. Eur. J. Biochem., **265**, 300–307.
47. VAN STRAATEN J. F., DOKTER W. H., STULP B. K. and VELLENGA E. (1994): The regulation of interleukin-5 and interleukin 3 gene expression in human T cells. Cytokine, **6**, 229–234.
48. WILLMAN C. L., SEVER C. E., PALLAVICINI M. G., HARADA H.,

TANAKA N., SLOVAK M. L., YAMAMOTO H., HARADA K., MEEKER T. C., LIST A. F. and TANIGUCHI T. (1993): Deletion of *IRF-1*, mapping to chromosome 5q31.1, in human leukemia and preleukemic myelodysplasia. Science, **259**, 968–971.

49. ZHANG D. H., YANG L., COHN L., PARKYN L., HOMER R., RAY P. and RAY A. (1999): Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. Immunity, **11**, 473–482.

50. ZHENG W. and FLAVELL R. A. (1997): The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell, **89**, 587–596.

Received in May 2000
Accepted in July 2000