Analysis of Human Interleukin-5 Gene Transcription by T Cell Clones and Hybridomas

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Introduction

Interleukin (IL)-5 is a T-cell-derived cytokine originally defined by its biological activity to promote differentiation of B cells [1] and eosinophils [2]. The essential role of activated T cells and T cell cytokines, including IL-5, has been widely established in the eosinophilic inflammation of bronchial asthma [3] and atopic dermatitis [4]. We have reported that enhanced IL-5 production by T cells is observed in both atopic and non-atopic asthmatic patients, and control of IL-5 production may be a reasonable approach to manage allergic disorders such as asthma and dermatitis [5]. To elucidate the molecular mechanisms of IL-5 gene transcription, we performed transfection analysis using a luciferase reporter gene construct in human T cell clones and hybridomas established from allergic asthmatic patients.

Results and Discussion

IL-5 Synthesis Is Regulated at the Transcriptional Level

IL-5-producing T cell clone YA5 and IL-5-non-producing T cell clone YA10 were transfected simultaneously with pIL-5Luc which contained the human IL-5 promoter (-511 to +4 relative to the transcription initiation site) connected to a luciferase gene, and pCMV-β-gal. YA5 clearly transcribed pIL-5Luc upon stimulation with phorbol 12-myristate 13-acetate (PAM) + ionomycin (IOM) or IL-2 (1,000 U/ ml). In contrast, YA10 did not transcribe pIL-

5Luc. The transfection efficiency determined by β -galactosidase activity in the cell lysates was almost comparable between each transfection (A574/ μ g protein: 0.462 in YA5 vs. 0.470 in YA10).

IL-2 synthesis is known to be regulated mainly by transcriptional control [6]. Our present findings that pIL-5Luc was effectively transcribed by IL-5-producing clones, but not by IL-5-non-producing clones, suggest that transcriptional control is also important in IL-5 synthesis. Those cells unable to transcribe pIL-5Luc may lack some transcription factors expressed in IL-5-producing T cell clones, or may express some repressor factors which actively interfere with IL-5 gene transcription.

IL-5 Promoter Activity in IL-5-Gene-Expressing and Non-Expressing Transformed T Cell Lines
To analyse the human IL-5 promoter more extensively, T cell clones YA5 and YA10 were fused with a transformed human T cell line (BUC). Resulting hybridomas were tested

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for IL-5 gene expression. IL-5 mRNA was clearly induced upon stimulation with PMA + IOM in S-1 and S-10 cells derived from the YA5 clone, but not in S-4 cells derived from T cell clone YA10, BUC or Jurkat cells. An approximately 250-fold induction of luciferase activity was induced in S-1 cells transfected with pIL-5Luc when stimulated with PMA + IOM. In contrast, S-4 cells or BUC cells showed only 30-fold induction, which was almost comparable to that observed when S-1, S-4 or BUC cells were transfected with pGL2 basic vector which contained no promoter segments. Jurkat cells, known to produce IL-2 and IL-4 but not IL-5 upon stimulation, did not induce any luciferase activity upon stimulation when transfected with pIL-5Luc.

Nonetheless, Jurkat cells did transcribe pIL-2Luc which contained human IL-2 promoter (-480 to +2) upon PMA + IOM or immobilized anti-CD3 + soluble anti-CD28 stimulation. The findings that IL-4-gene-expressing hybridomas did transcribe pIL-5Luc when stimulated with PMA + IOM, but IL-5-gene-non-expressing hybridomas or Jurkat cells did not reflect the transcriptional regulation observed in T cell clones. These hybridomas would be useful tools to delineate potential transcription factors essential for IL-5 gene transcription (tentatively NF-IL-5). Future elucidation of NF-IL-5 and its regulation would facilitate the understanding of allergic inflammation and the development of a potential novel therapeutic modality.

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