Review Article

Structural and functional analysis of a new cytokine, ML-1 (interleukin-17F)

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ABSTRACT

Recent large-scale sequencing of the human genome has facilitated novel gene discovery. A novel gene, ML-1 (interleukin (IL)-17F), was identified from a human expressed sequence tag (EST) sequence, a genomic DNA clone and T cell cDNA sequences. It was found that ML-1 shares significant sequence identity and functional similarity with the gene encoding IL-17A, but a distinct functional property for ML-1 has been demonstrated. While limited information is currently available, recent functional studies have suggested that ML-1 (IL-17F) is a multifunctional protein with a wide tissue expression pattern. In particular, the potent proinflammatory action of ML-1 (IL-17F) identified thus far, together with its association with asthma, suggests that ML-1 (IL-17F) may have a significant role in the regulation of airway inflammatory processes.

Key words: airway inflammation cytokine network interleukin-17 family, mitogen-activated protein kinase.

INTRODUCTION

The homeostasis and expression of various inflammatory diseases involve highly interactive cytokine and chemokine networks. It is believed that dysregulation of the cytokine network contributes to the expression of inflammatory airway diseases, such as bronchial asthma, chronic obstructive pulmonary disease (COPD) and bacterial pneumonia. Understanding of the complexity of the cytokine network, together with identification of relevant novel genes, would help to uncover the molecular mechanisms of inflammatory diseases. The availability of human genome sequences and bioinformatics tools has facilitated the gene discovery processes and the newly identified gene can be integrated in the pursuit of the molecular mechanisms leading to various diseases.

Interleukin (IL)-17A is a CD4+ T cell-derived cytokine and human IL-17A exists as glycosylated 20–30 kDa homodimers. Interleukin-17A was initially recognized for its similarity to a sequence belonging to Herpesvirus saimiri, but it had little relatedness to any other known cytokines or other mammalian proteins.1,2 Recent cloning and sequencing studies have demonstrated a family of IL-17A-related genes with potential proinflammatory functions.3–6 Recently, we and others have independently discovered a novel cytokine, ML-17 (or IL-17F8,9), belonging to the IL-17 gene family, but its function and signaling pathways remain to be defined. As part of our ongoing molecular genetic studies of complex diseases, the coding region sequence of ML-1 was initially identified from a human genomic DNA clone by the use of bioinformatics tools and sequencing. In the present review, the structural and functional characteristics of this novel cytokine gene and the initial evidence for its involvement in airway inflammatory disease are summarized.

STRUCTURAL ANALYSIS OF ML-1 (IL-17F) GENE AND PROTEIN

As part of a positional cloning study of a region on chromosome 6p12 for susceptibility gene discovery for an inherited disease, the coding region sequence of ML-1 (IL-17F) with homology to human IL-17A was identified from a genomic DNA clone using a GenScan prediction program (http://www.genes.mit.edu/GENSCAN.html). Reverse transcription–polymerase chain
reaction (RT-PCR) and sequencing analysis of human allergen-specific T cells confirmed the predicted sequence and the splicing sites between the exons. A 947 bp full-length cDNA was obtained using both 5’- and 3’-rapid amplification of cDNA ends (RACE), demonstrating a transcription start site 346 bp upstream of the start codon and a poly(A) sequence 271 bp 3’ to the stop codon. Basic local alignment search tool (BLAST) analysis shows a significant degree of homology between the second exon of the ML-1 gene and the third exon of IL-17A. It is of interest to note that the IL-17 gene is localized on the same genomic DNA clone approximately 50 kb telomeric to the ML-1 gene and that both genes are in a tail-to-tail orientation, suggesting a potential gene duplication event.

Alignment of the predicted amino acid sequence of ML-1 with sequences of IL-17A and the other members of the IL-17 family reveals the highest overall amino acid sequence homology (70%) between ML-1 and IL-17A, whereas there is only 20% amino acid identity between ML-1 and the other family members. The alignment shows several conserved amino acids, including a tryptophan and four cysteine residues. The four cysteines in the C-terminal half of the proteins have been shown to form a cystine knot in the crystal structure. This cystine knot is similar to a common structural motif seen in growth factors, such as bone morphogenetic proteins, transforming growth factor (TGF)-β, nerve growth factor and platelet-derived growth factor. In addition, IL-17F is believed to form disulfide-bonded homodimers, whereas other members of the IL-17 family are expressed as tightly associated dimers.

**ML-1 (IL-17F) GENE EXPRESSION**

While the expression of ML-1 (IL-17F) is seen in various cell types and human tissues, IL-17F, like IL-17A, is produced primarily in activated T-cells. Interleukin-17B, IL-17C, IL-17D and IL-17E are expressed in a wide variety of tissues. Interestingly, ML-1 expression in liver, lung, ovary and fetal liver is unique when compared with IL-17A. In addition, although the expression of ML-1 was not detected in unstimulated cells, with the exception of cord blood-derived mast cells, increased ML-1 expression was clearly evident in different cell types after activation. Those cell types included ragweed allergen-stimulated peripheral blood mononuclear cells (PBMC), allergen-specific T cell clones with different cytokine phenotypes (Th0, Th1 and Th2), activated basophils and activated mast cells. In addition, expression of ML-1 was found in activated CD4+ T cells, but not CD8+ and monocytes. In contrast, IL-17A gene expression was found in only activated CD4+ T cells and PBMC.

Analyses of gene expression in bronchoalveolar lavage (BAL) cells from asthmatic subjects challenged with allergen or saline control shows that whereas no detectable expression of ML-1 was seen in the BAL from saline-challenged sites, ML-1 gene expression was clearly demonstrated in BAL from allergen-challenged sites of all four study subjects. In contrast, no IL-17A transcripts were detected in the BAL of subjects challenged with allergen. These findings suggest the distribution of ML-1 is much wider than that of IL-17A, suggesting that ML-1 has more biological functions.

**FUNCTION OF ML-1 (IL-17F) IN VITRO**

Human ML-1 has pleiotrophic biological activities (see Table 1 for a summary of currently known biological activity). It is able to induce IL-6 and IL-8 in normal human bronchial epithelial cells (NHBE) and human umbilical vein endothelial cells (HUVEC) in a dose- and time-dependent manner. ML-1 and IL-17A show similar potency in the induction of IL-6 and IL-8, suggesting that ML-1 is also involved in neutrophil recruitment into the airway. In addition, IL-17F induces IL-8 in fibroblast. The level of IL-8 is increased in inflammatory airway diseases, such as bronchial asthma, COPD and cystic fibrosis. Neutrophil recruitment into the airway is regulated through the chemokine network. There is a strong correlation between the level of IL-8 and the increase in neutrophil numbers. Administration of anti-IL-8 monoclonal antibody results in a reduction of neutrophil chemotaxis in patients with COPD and cystic fibrosis. In addition, bronchial epithelial cells and vascular endothelial cells are important cell sources of IL-8 and may thereby control neutrophil influx in the airway. Therefore, IL-8 release in these cell types may play a crucial role in modulating neutrophil-associated airway inflammation.

Although IL-17A and ML-1 induce IL-6 and IL-8 expression, IL-17A fails to induce intercellular adhesion molecule-1 (ICAM-1) expression in NHBE. In comparison, ML-1 markedly induced ICAM-1 expression. It has been shown that ICAM-1 expression is increased in bronchial asthma. In particular, a high level of ICAM-1
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expression, along with inflammatory cell infiltration, has been demonstrated in bronchial biopsies from both stable asthmatics and subjects after allergen challenge.\textsuperscript{13,14} Moreover, allergen challenge upregulated ICAM-1 expression in airway epithelium, correlating with eosinophil infiltration. It is of interest to note that ML-1 induces C-X-C chemokines, but not C-C chemokines, such as eotaxin and RANTES,\textsuperscript{7} which are potent chemotactants for eosinophils, suggesting a selective role of ML-1 in neutrophil recruitment and activation. These findings suggest that ML-1 may be involved in the expression of airway inflammation by, at least in part, facilitating leukocyte recruitment and activation via the induction of IL-6, IL-8 and ICAM-1. Moreover, in our recent unpublished study, an additive effect on the induction of IL-6 and IL-8 has been observed when cells are treated with ML-1 (IL-17F) in combination with either Th2 cytokines (IL-4 and IL-13) or Th1 cytokine (interferon (IFN)-\gamma; M Kawaguchi et al., unpubl. obs., 1999).

In other studies, IL-17F has been shown to be able to induce the expression of TGF-\beta, monocyte chemotactant protein (MCP)-1 and IL-2 in HUVEC and inhibits endothelial cell angiogenesis, but has no effect on hematopoiesis.\textsuperscript{8} These findings suggest that IL-17F would play a possible role in cancer immunotherapy by inhibiting the generation of tumor vascular supply. Interleukin-17F also induces significant cartilage matrix synthesis, inhibits new cartilage matrix synthesis in a dose-dependent manner and IL-6 production in articular cartilage suggests that IL-17F may be able to promote skeletal tissue destruction.\textsuperscript{9}

ML-1 (IL-17)-INDUCED SIGNALING

The bronchial epithelial cells and endothelial cells are exposed to numerous environmental stimuli that can activate intracellular signaling cascades, leading to altered gene expression and to a wide range of responses, such as cytokine expression, proliferation and apoptosis. The mitogen-activated protein kinase (MAPK) family of protein kinases is likely to be central to these processes because it is known to regulate intracellular signal transduction in response to many agonists, including growth factors, cytokines, hormones, oxidants and environmental stress factors.\textsuperscript{15} Mitogen-activated protein kinase signaling pathways are highly conserved and control a diverse range of cellular functions in organisms, ranging from yeast to humans. In mammals, particularly, MAPK signaling pathways have been implicated in the control
of cell proliferation, differentiation and apoptosis. Of the five MAPK cascades identified to date, three have been well characterized. These comprise: (i) the extracellular regulated kinases (ERK) 1 and 2 (also known as MAPK 1/2); (ii) the c-Jun NH2-terminal kinases (JNK) 1, 2 and 3 (also known as stress-activated protein kinases (SAPK): SAPK1α, SAPK1β and SAPK1c); and (iii) the p38 MAPK α, β, γ and δ isoforms (also known as SAPK2α, SAPK2b, SAPK3/ERK6 and SAPK4). More recent additions include ERK3, ERK4 and ERK5/big mitogen-activated kinase (BMK)/SAPK5. These are grouped according to their sequence homology, specificity towards upstream activators and different responses to stimuli.

ML-1-induced phosphorylation of ERK1/2 reached a maximum at 20 and 10 min in NHBE and HUVEC, respectively, and returned to baseline levels by 60 min. In contrast, no activation of p38 and JNK kinases was seen at any time point, even after a 4 h stimulation of cells with ML-1. In addition, preincubation of cells with the MEK inhibitor PD98059 diminished the activation of ERK1/2 in ML-1 treated HUVEC, whereas pretreatment of cells with MeSO did not affect the phosphorylation of ERK1/2. We next asked whether the activation of ERK1/2 was necessary for the stimulation of IL-6 and IL-8 production in cell types. PD98059 inhibited, in a dose-dependent manner, the production of both IL-6 and IL-8, whereas pretreatment of cells with MeSO did not affect cytokine protein release in both NHBE and HUVEC. Decreased levels of gene expression for both IL-6 and IL-8 were noted in PD98059-treated, but not in vehicle-treated, cells, suggesting that the inhibitory effect was at the level of transcription. This inhibitory effect of PD98059 was further confirmed by the use of an additional inhibitor, U0126.

Mitogen-activated protein kinases are known to play a central role in airway epithelial activation in response to various stimuli, such as tumor necrosis factor-α, IL-1, diesel exhaust particles, and influenza virus infection. In addition, MAPK, including ERK1/2, are involved in cytokine signaling in HUVEC. The involvement of ERK kinase, but not p38 or JNK, in ML-1-induced IL-6 and IL-8 production was demonstrated using primary bronchial epithelial cells and endothelial cells. Interestingly, previous data showed that ERK1/2, but not p38 or JNK, may play an important role in cytokine release in NHBE, although all three members of the MAPK family are involved in cytokine expression. Indeed, IL-17A also activates only ERK1/2 kinase in

**Function of ML-1 (IL-17F) in vivo**

In addition to eosinophils, it has been reported that neutrophil accumulation into the airway is also a feature of bronchial asthma. After allergen challenge, neutrophil infiltration was seen in the airways of patients with severe asthma. The number of CD4+ T cells is also increased in parallel with the number of neutrophils in the airway of patients with COPD and bronchiectasis. Furthermore, specific inhibition of CD4+ T cells, either with a CD4 antibody or an IL-2 receptor antibody, prevents allergen-induced recruitment of both eosinophils and neutrophils in animal models. However, little is known about how CD4+ T cells induce neutrophil accumulation into the airway. Based on our current and previous data, it is suggested that ML-1 is the key regulator for T cell-mediated bronchial neutrophilia. Indeed, using the adenoviral gene transfer strategy, it has been suggested that the transduced IL-17F expression leads to an increased number of BAL neutrophils.
Of interest is the finding that lung tissues from transduced mice show substantial increases in the levels of inflammatory cytokines and chemokines, including IL-6, IFN-γ, inflammatory protein 10 and monokine induced by IFN-γ. When combined with available in vitro data, a working hypothesis can be formulated to suggest the potential role of ML-1 (IL-17F) in the expression of pulmonary inflammation (Fig. 1).

**CONCLUSION**

ML-1 (IL-17F) is expressed in a variety of human tissues and in activated CD4+ T cells, mast cells, basophils and PBMC. ML-1 is able to induce several cytokines, chemokines and adhesion molecules from various cell types. While the ML-1 receptor remains unknown, the Raf1-MEK-ERK1/2 is a central signal pathway of ML-1 (M Kawaguchi et al., unpubl. obs., 2002). These data suggest a potential role for ML-1 (IL-17F) in the pathogenesis of inflammatory processes, such as COPD, bronchial asthma and bacterial pneumonia. Further work is clearly needed to define the role of ML-1 (IL-17F), and analyses of ML-1 (IL-17F) transgenic and/or deficient mice would provide a crucial step towards this aim.

**REFERENCES**


