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Identification of a Novel Cytokine, ML-1, and Its Expression in Subjects with Asthma

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A novel gene, designated ML-1, was identified from a human genomic DNA clone and human T cell cDNA sequences. The second exon of ML-1 gene shares significant sequence identity with the gene encoding IL-17 (IL-17). ML-1 gene expression was up-regulated in activated PBMCs, CD4+ T cells, allergen-specific Th0, Th1, and Th2 clones, activated basophils, and mast cells. Increased expression of the ML-1 gene, but not IL-17, was seen following allergen challenge in four asthmatic subjects, suggesting its role in allergic inflammatory responses. ML-1 from transiently transfected COS-7 cells was able to induce gene expression and protein production for IL-6 and IL-8 (at 10 ng/ml of ML-1: for IL-6, 599.6 ± 19.1 pg/ml; for IL-8, 1724.2 ± 132.9 pg/ml; and at 100 ng/ml of ML-1: for IL-6, 1005.3 ± 55.6 pg/ml; for IL-8, 4371.4 ± 280.5 pg/ml; p < 0.05 for both doses vs baseline) in primary bronchial epithelial (PBE) cells. Furthermore, increased expression of ICAM-1 was found in ML-1-stimulated PBE cells (mean fluorescence intensity (MFI) = 31.42 ± 4.39 vs baseline, MFI = 12.26 ± 1.77, p < 0.05), a functional feature distinct from IL-17 (MFI = 11.07 ± 1.22). This effect was not inhibited by a saturating amount of IL-17. These findings demonstrate that ML-1 is a novel cytokine with a distinct function, and suggest a different receptor for ML-1 on PBE cells. The Journal of Immunology, 2001, 167: 4430–4435.

The maintenance of normal physiology and the expression of various inflammatory diseases involve highly interactive molecular and cellular networks. Dysregulation of the cytokine network is an important contributing factor in allergic inflammatory diseases, such as asthma. Understanding of this intricate network, along with characterization of relevant novel genes, would aid in discovering the molecular basis of inflammatory diseases. Recent advances in human genome sequencing and the use of bioinformatics tools have facilitated the process for gene discovery and identifying its functional correlate, particularly for those genes with cell type-specific expression patterns. The newly discovered gene can be integrated in the search for the molecular basis of various diseases.

Recent cloning and sequencing studies have demonstrated a family of IL-17-related genes with potential proinflammatory functions. Human IL-17 is a T cell-derived, homodimeric protein and exhibits pleiotropic biological activities (1–3). IL-17 is able to stimulate the production of IL-6, IL-8, G-CSF, stem cell factor, and PGE2 from various cell types, such as fibroblasts, keratinocytes, and renal epithelial cells (3–7). Although the expression of IL-17 is restricted to activated T cells, the IL-17R is widely expressed. In addition, elevated IL-17 mRNA expression has been found in mononuclear cells from patients with multiple sclerosis (8), in patients with rheumatoid arthritis (9), and in patients with systemic lupus erythematosus (10), suggesting a role for IL-17 in the initiation or maintenance of inflammatory responses. In the airway, IL-17 induces expression of the C-X-C chemokines, IL-8 and macrophage inflammatory protein 2, which selectively recruit neutrophils into the airway (11). Moreover, IL-17 synergizes, but not by itself, with IFN-γ in the induction of ICAM-1 expression on epithelial cells (12); the induction of ICAM-1 is associated with airway inflammation seen in bronchial asthma (13).

As part of our ongoing molecular genetic studies of complex diseases, a potential coding region sequence with sequence homology with IL-17 was initially identified from a human genomic DNA clone by the use of bioinformatics tools and sequencing. We describe herein functional characterization of this novel cytokine gene and demonstrate its expression in an inflammatory disease.

Materials and Methods

Full-length cDNA sequence of the ML-1 gene

To obtain a full-length cDNA sequence, both 5′- and 3′-RACE were performed using cDNAs from activated ragweed-allergen-specific T cells as templates. For 3′-RACE, cDNAs were amplified using poly(dT) and a predicted exon sequence primer, 5′-GGCATCATCATAATGAAAACCAG-3′. The PCR products were run on 1% low-melting agarose gel, purified using a GeneClean kit (Qiogene, Carlsbad, CA), and subjected to a nested PCR using an internal sequence primer, 5′-TTCATGTACGTTAGC-3′. After PCR, the products were cloned and sequenced. For 5′-RACE, cDNAs were first tailed with poly(dA) oligonucleotides using TdT enzyme, purified using Sephadex G25 spin columns, and subjected to nested PCRs using poly(dT), a coding region sequence (5′-TACCCAGCACCTTCTCCAAC-3′) and an internal sequence (5′-AAGAAACAGGAGCATCTTTGG-3′) primer. The PCR products were then cloned and sequenced.
Analysis of tissue distribution, RNA isolation, and cellular expression of ML-1

Tissue distribution data for IL-17 and ML-1 were acquired using Rapid-Scan gene expression panels for human tissues ( OriGene Technologies, Rockville, MD) according to the manufacturer’s instructions with 5 mM magnetum and ML-1 and IL-17-specific primer pairs. The sequences of primers for ML-1 were as follows: forward, 5’-GGGATCATCATGAA AACCAG-3’; reverse, 5’-TCCACCAGCTTCCTTCAAC-3’. The sequences of primers for IL-17 were: forward, 5’-ACTCTGGAGAAGCT CATTG-3’; reverse, 5’-GGCCACATGGTGACACATTG-3’. PCR products were visualized by ethidium bromide-containing gel and photographed. Tissues were graded on a 0-4 grading system based on visualization of bands at the concentrations of cDNA provided by the manufacturer (+ + + + = product obtained with >1 pg/ml; + + + = product obtained with >10 pg/ml; + + = product obtained with >100 pg/ml; + = product obtained with >1000 pg/ml; 0 = no product obtained with 1000 pg/ml). Appropriate normalization of cDNA provided by the manufacturer was confirmed by PCR amplification for the constitutive marker gene, β-actin.

PBMCs were isolated from the blood of allergic subjects. Peripheral blood CD4+ CD8+ T cells, and monocytes were isolated (>90% purity) from a normal healthy individual using RosetteSep kits from StemCell Technology (Vancouver, Canada) according to the manufacturer’s instructions. Human allergen-specific T cell clones were generated by limiting dilution cloning and subcloning from two tonic subjects, followed by biweekly stimulation of T cells with ragweed allergen extract or purified Amb a 1 (a major ragweed allergen) along with irradiated autologous PBMCs as APCs as described previously (17). The cytokine profiles of T cell clones were determined as described previously (17). Basophils were isolated and purified to homogeneity (>98% purity) following double Percoll density centrifugation and negative selection using a mixture of mAbs (CD2, CD3, CD14, CD16, CD24, CD34, CD36, CD45RA, CD56, glycoprotein) and magnetic colloidal beads (StemCell Technologies) as described previously (18). Basophils were activated by stimulation with anti-IgE Abs as described previously (18). Human mast cells were generated from umbilical cord blood as described previously (19). Briefly, the mononuclear cell fraction was obtained by centrifugation and suspended in modified DMEM containing 10 μg/ml insulin, 10 μg/ml transferrin, 10 μM 2-ME, 25 mM HEPES, 2.6 ng/ml NaSeO4, 5% FCS, human recombinant stem cell factor at 80 ng/ml (Amgen, Thousand Oaks, CA), human rIL-6 at 50 ng/ml (Amgen), and PGE1 (Sigma, St. Louis, MO) at 300 nM. Cells were used when conformity in morphology and shape of the cells is reached. Purity of mast cells in the preparations was always >95%. Mast cells were activated using myeloma IgE alone (1 μg/ml) or a combination of myeloma IgE (1 μg/ml) and human anti-IgE Ig (1 μg/ml) kindly provided by Dr. R. Hamilton, Johns Hopkins University, Baltimore, MD). Mast cells were sensitized overnight with myeloma IgE at 37°C before challenge with anti-IgE. ML-1 gene expression was also assayed from bronchoalveolar lavage (BAL)4 cells of four asthmatic patients challenged with either allergen (ragweed, 100 protein nitrogen units) or saline control as described previously (20). The BAL cells were collected 19 h after challenge. Total RNA was isolated from 1) ragweed-activated PMBCs (5 × 106 cells; 6 h after stimulation); 2) peripheral blood CD4+ (1 × 106 cells); CD8+ T cells (5 × 106 cells), and monocytes (1 × 106 cells); all three cell types were stimulated with PMA (100 ng/ml)/ionomycin (2 μg/ml) for 4 h; 3) cloned T cells (2 × 106 cells; 6 h after ragweed allergen stimulation); 4) basophils (2 × 106 cells; 4 h after stimulation); and 5) mast cells (2 × 106 cells; 6 h after stimulation) using RNAzol B according to the manufacturer’s instructions (Tel-Test, Friendswood, TX). CDNAs were synthesized from 500 ng of total RNA in the absence of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Sigma), oligo(dT) primer, and reaction buffer at 42°C for 90 min, followed by PCR. Each cDNA sample was amplified (30 cycles of 1 min at 95°C, 1 min at 52°C and 1 min at 72°C) using ML-1 and IL-17 sequence-specific primers (see above). The sequences of primers for a housekeeping gene, G3PDH, were as follows: forward, 5’-ACCACAGTGCA TGGCATAC-3’; reverse, 5’-TCCACCACCGTGTGCTGA-3’. The expected size for the PCR product is 220 bp, for the IL-17 is 462 bp, and for G3PDH is 450 bp. Recombinant ML-1-His fusion proteins

The coding sequence of ML-1 was amplified by PCR and subcloned into the BamHI and XhoI sites of pcDNA 3.1 (Invitrogen, Carlsbad, CA) to generate a C-terminal fusion gene with the His and c-Myc tags. The vector pcDNA 3.1 was transfected into COS-7 cells by an Effecten reactive (Qiagen, Chatsworth, CA) according to the manufacturer’s instructions. Two days after transfection, the supernatants were concentrated over Centricon-10 columns (Amicon, Beverly, MA) and subjected to affinity purification by Ni-NTA agarose beads (Qiagen) for His-tagged proteins. To examine the protein expression, SDS-PAGE analysis was performed on the affinity-purified recombinant proteins under a reducing condition, followed by Western blot analysis using anti-His mAb (Santa Cruz Biotechnology, Santa Cruz, CA).

Analysis of ML-1-induced gene expression

Primary bronchial epithelial (PBE) cells were purchased from Clonetics (San Diego, CA). PBE cells were cultured according to the manufacturer’s instructions. The cells were treated with IL-17 (10 or 100 ng/ml), or ML-1 (10 or 100 ng/ml), or a control His-tagged protein (Positope, 10 or 100 ng/ml; Invitrogen). The affinity-purified control His protein was dissolved in the same buffer as ML-1. Total RNA was extracted using RNeasy (Qiagen) from 1 × 106 cells 4 h after stimulation or exchange of medium. The protocol for cDNA synthesis was the same as described above. For PCR, the sequences of PCR primers were based on the human IL-8 6 min sequence. The sequences of PCR primers for IL-8 were: forward, 5’-TCTGCAAGCTCTGTTGGAAG-3’; reverse, 5’-TAAATTTCTGTGTGGCGA C3’; IL-6: forward, 5’-ATGAACTCTCTTCTCACAAGGGC-3’; reverse, 5’-GAAGAAGCCCTAGGGTGGACTG-3’. Also, gene expression for two chemokines, eotaxin and RANTES, was also analyzed. The primers for eotaxin and RANTES as follows: forward, 5’-AGAGGAGAACTCAGGATGAC-3’, reverse, 5’-AGGATTCACGGACGGATCG-3’, reverse, 5’-ACTCAGTGATGCTACTG-3’, c-CAACCTGGGAGGACTCC-3’. The amplification reaction was performed for 25 cycles with denaturation at 94°C for 45 s, annealing at 54°C for 45 s, and extension at 72°C for 45 s. PCR products were detected by ethidium bromide staining and normalized by the intensity of an amplified housekeeping gene, G3PDH (see above). The expected size for IL-8 was 154 bp and for IL-6, 628 bp. IL-6 and IL-8 protein levels in the collected supernatants were determined with a commercially available ELISA kit (BioSource International, Camarillo, CA) according to the manufacturer’s instruction. Analysis of ICAM-1 surface expression by flow cytometry

PBE cells were treated with IL-17 (100 ng/ml), ML-1 (100 ng/ml), or a control His protein (Positope, 100 ng/ml) for 48 h. Cells were harvested following treatment with 0.25% trypsin-0.02% EDTA at 37°C for 5 min and suspended in PBS containing 2% FBS and 0.02% sodium azide. Briefly, 106 cells were incubated with a mouse anti-human ICAM-1 mAb (R&D Systems, Minneapolis, MN) on ice for 30 min. After three washes with PBS, the cells were incubated with FITC-conjugated goat anti-mouse IgG (Bio-Rad, Hercules, CA) on ice for 30 min. After three additional washes with PBS, the cells were resuspended in PBS and immediately analyzed with a FACSflow cytometer (BD Biosciences, Mountain View, CA). In control samples, staining was performed using isotype-matched control Abs. The mean fluorescence intensity (MFI) was expressed as mean ± SD (n = 5) by subtracting the mean background fluorescence.

Effect of IL-17 on ML-1-induced ICAM-1 gene expression

To assess whether ML-1 interacts with IL-17R, competitive inhibition experiments were performed using the level of ICAM-1 gene expression as a functional readout, since IL-17 by itself is unable to induce ICAM-1 expression. PBE cells were treated with either medium, ML-1 (100 ng/ml) alone, or in combination with varying doses (10–2000 ng/ml) of IL-17 (R&D Systems for 4 h, and total RNAs were extracted from each sample as described above. RT-PCR was performed using the primers based on the human ICAM-1 cDNA sequence. The sequences of primers for ICAM-1 were forward, 5’-CAGAGAGTTATGACCCACAG-3’, reverse, 5’-GCCCTGTCGTAAGAT-3’. The amplification was performed for 25 cycles with denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s. PCR products were detected by ethidium bromide staining and normalized by the intensity of an amplified housekeeping gene, G3PDH (see above). The expected size for ICAM-1 was 196 bp.

Data analysis

All data are expressed as mean ± SD. Differences between the groups were analyzed by a one-way ANOVA analysis with the Scheffe F test by use of the StatView program (SAS Institute, Cary, NC) on an Apple computer (Apple Computer, Cupertino, CA). Differences were considered to be significant at p < 0.05.
Results

Full-length cDNA sequence of the ML-1 gene

As part of a positional cloning study of a region on chromosome 6p for susceptibility gene discovery for an inherited disease, a potential coding region sequence with homology to human IL-17 was identified from a genomic DNA clone, PAC108C2* (Sanger Center Database, http://www.sanger.ac.uk), using a GenScan prediction program. The predicted expressed sequence with a centromeric-telomeric orientation was composed of 2 exons of 221 and 238 bp, respectively. RT-PCR and sequencing analysis of activated, human allergen-specific T cell clones confirmed the predicted sequence and the splicing sites between the exons. However, the open reading frame utilizes a start codon 129 bp 3' to the predicted start site, encompassing a 92-bp segment in the first exon. A full-length cDNA 947 bp in length was obtained using both 5' and 3'-RACE, revealing a transcription start site 346 bp upstream of the start codon and a poly(A) sequence 271 bp 3' to the stop codon.

Homology searches using the BLAST program shows a significant degree of homology between the second exon of the ML-1 gene and the third exon of IL-17. Alignment of the predicted amino acid sequence of ML-1 with sequences of IL-17 and the other members of the IL-17 family shows that while there is the highest overall amino acid sequence homology (70%) between ML-1 and IL-17, there is only 20% amino acid identity between ML-1 and the three other family members (Fig. 1). The alignment shows several conserved amino acids including a tryptophan residue and four cysteines in the C-terminal half of the proteins.

Expression of the ML-1 gene

The expression of ML-1 in various cell types and human tissues was examined using PCR and the Rapid-Scan gene expression panels for human tissues. Table I shows the tissue expression of ML-1 and IL-17. ML-1 was strongly expressed in liver, lung, spleen, placenta, adrenal gland, ovary, and fetal liver. Interestingly, ML-1 expression in liver, lung, ovary, and fetal liver was unique when compared with IL-17 (Table I). In addition, although the expression of ML-1 was not detected in unstimulated cells, with the exception of mast cells (Fig. 2A), increased ML-1 expression was clearly evident in six different cell types after activation (Fig. 2B). Those cell types included ragweed allergen-stimulated PBMCs, ragweed allergen-specific T cell clones with different cytokine phenotypes (Th0 (clone 12), Th1 (clone 2B7), and Th2 (clone 2D2)), activated basophils, and activated mast cells (Fig. 2B). Also, expression of ML-1 was found in activated peripheral blood CD4+ T cells, but not CD8+ and monocytes (Fig. 2C). In contrast, IL-17 gene expression was found in only activated peripheral blood CD4+ T cells, Th0 clones, and PBMCs (Fig. 2).

This unique expression pattern of the ML-1 gene in activated Th2 cells, a cell type known to be associated with allergic asthma, prompted us to determine the in vivo relevance of ML-1 gene expression. We performed analyses of gene expression in BAL cells from asthmatic subjects challenged with allergen or saline control. Fig. 2C shows representative data demonstrating that while no detectable expression of ML-1 was seen in the BALs from saline-challenged sites, ML-1 gene expression was clearly demonstrated in the BALs from allergen-challenged sites of all four study subjects. In contrast, no IL-17 transcripts were detected in the BALs of subjects challenged with allergen (Fig. 2D).

Table I. Tissue expression of ML-1 and IL-17a

<table>
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<tr>
<th>Tissue</th>
<th>IL-17</th>
<th>ML-1</th>
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<tbody>
<tr>
<td>Spleen</td>
<td>+</td>
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<tr>
<td>Liver</td>
<td>0</td>
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</tr>
<tr>
<td>Lung</td>
<td>0</td>
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<tr>
<td>Small intestine</td>
<td>+</td>
<td>0</td>
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<tr>
<td>Stomach</td>
<td>+</td>
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<tr>
<td>Testis</td>
<td>+</td>
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<tr>
<td>Placenta</td>
<td>0</td>
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<tr>
<td>Adrenal gland</td>
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<tr>
<td>Ovary</td>
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<td>Prostate</td>
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<td>Skin</td>
<td>0</td>
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<tr>
<td>Fetal liver</td>
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a Grade 0, brain, heart, kidney, colon, muscle, salivary gland, thyroid gland, pancreas, bone marrow, fetal brain.

FIGURE 1. Amino acid sequence alignment of ML-1, IL-17, IL-17C, and IL-17B. The residues identical to the ML-1 sequence are colored, and conserved cysteine residues are indicated by arrows. The GenBank accession number for the ML-1 cDNA sequence is AF332389.

FIGURE 2. Expression patterns of the ML-1 gene. RT-PCR analysis of ML-1 and IL-17 expression in unstimulated (A) and activated (B) cell types as indicated. Expression of ML-1 and IL-17 genes in resting or activated peripheral blood CD4+, CD8+ T cells, and monocytes (C). Expression of ML-1 and IL-17 genes at sites of airway inflammation (D). Detection of gene expression in BAL cells from four asthmatic patients (BAL #638, #646, #688, and #1081) challenged with allergen (Ag) and saline control (NS). Amplification of G3PDH was performed as a positive control.
Induction of IL-6, IL-8, and ICAM-1 expression in PBE cells

To assess the biological functions of ML-1, His-tagged ML-1 protein was expressed in COS-7 cells and affinity purified (Fig. 3A). The supernatants from COS-7 cells transfected with a gene construct containing the full-length ML-1 sequence and a tag sequence encoding poly(His) peptide. ML-1-His fusion proteins were purified by His affinity column, run on 8–16% SDS-PAGE under reducing conditions, and analyzed by Western blot using anti-His mAb. Lane 1, A mock-transfected sample; lane 2, purified protein from an ML-1-transfected sample. The location of a protein size marker, 19.5 kDa, is indicated. Effect of ML-1 and IL-17 on IL-8 (B) and IL-6 (C) gene expression. PBE cells were treated with medium alone, rIL-17 (100 ng/ml), purified His-tagged protein (Positope, 100 ng/ml), or ML-1 (100 ng/ml) as indicated. The cells were harvested 4 h after stimulation, and total RNA was extracted and subjected to RT-PCR using IL-8, IL-6, and G3PDH primers. The relative level of IL-8 expression is normalized by the level of G3PDH. Effect of ML-1 and IL-17 on IL-8 (D) and IL-6 (E) protein production. PBE cells were treated as indicated, and the supernatants were harvested 48 h after stimulation. The experiment was conducted five times. *, p < 0.05. Analysis of gene expression for eotaxin and RANTES (RAN) in PBE cells in the presence (+) or absence (−) of 100 ng/ml ML-1 (F).

Induction of IL-6, IL-8, and ICAM-1 expression in PBE cells

It has been previously shown that IL-17 alone is not able to induce ICAM-1 expression on airway epithelial cells (12), but synergizes with IFN-γ for the induction of ICAM-1 expression on epithelial cells. Up-regulation of ICAM-1 expression is associated with IL-6, 599.6 ± 19.1 and 1005.3 ± 55.6 pg/ml, respectively. No increase of IL-6 and IL-8 expression was seen in cells treated with either His-tag control protein or medium control. Furthermore, ML-1 did not induce the gene expression for two CC chemokines, eotaxin and RANTES (Fig. 3F).

It has been previously shown that IL-17 alone is not able to induce ICAM-1 expression on airway epithelial cells (12), but synergizes with IFN-γ for the induction of ICAM-1 expression on epithelial cells.

FIGURE 3. Expression of ML-1 protein in COS-7 cells (A). The supernatants from COS-7 cells transfected with a gene construct containing the full-length ML-1 sequence and a tag sequence encoding poly(His) peptide. ML-1-His fusion proteins were purified by His affinity column, run on 8–16% SDS-PAGE under reducing conditions, and analyzed by Western blot using anti-His mAb. Lane 1, A mock-transfected sample; lane 2, purified protein from an ML-1-transfected sample. The location of a protein size marker, 19.5 kDa, is indicated. Effect of ML-1 and IL-17 on IL-8 (B) and IL-6 (C) gene expression. PBE cells were treated with medium alone, rIL-17 (100 ng/ml), purified His-tagged protein (Positope, 100 ng/ml), or ML-1 (100 ng/ml) as indicated. The cells were harvested 4 h after stimulation, and total RNA was extracted and subjected to RT-PCR using IL-8, IL-6, and G3PDH primers. The relative level of IL-8 expression is normalized by the level of G3PDH. Effect of ML-1 and IL-17 on IL-8 (D) and IL-6 (E) protein production. PBE cells were treated as indicated, and the supernatants were harvested 48 h after stimulation. The experiment was conducted five times. *, p < 0.05. Analysis of gene expression for eotaxin and RANTES (RAN) in PBE cells in the presence (+) or absence (−) of 100 ng/ml ML-1 (F).

FIGURE 4. Effect of ML-1 on ICAM-1 expression on PBE cells. A, Flow cytometry analysis. PBE cells were treated for 48 h under different conditions: medium only, cells treated with rIL-17 (100 ng/ml), cells treated with purified His-tagged protein (Positope, 100 ng/ml), or cells treated with ML-1 (100 ng/ml) as indicated, and the MFI of ICAM-1 surface expression was measured. The experiment was conducted five times. *, p < 0.05. B, ML-1-induced ICAM-1 gene expression. PBE cells were treated with either medium, ML-1 (100 ng/ml) alone, or in combination with different doses of IL-17 as indicated. The cells were harvested 4 h after stimulation, and total RNA was extracted and subjected to RT-PCR. The relative level of ICAM-1 expression is normalized by the level of G3PDH. The experiment was conducted three times, and the results shown are from a representative experiment. *, p < 0.05.
with airway inflammation seen in bronchial asthma (13). To examine whether ML-1 is able to induce ICAM-1 expression on PBE cells, surface expression of ICAM-1 on PBE cells was measured and the effects of ML-1 vs IL-17 were compared. Fig. 4A demonstrates that ICAM-1 was constitutively, but weakly, expressed on PBE cells (MFI = 12.26 ± 1.77) and IL-17 (100 ng/ml) alone did not affect ICAM-1 surface expression at 48 h (MFI = 11.07 ± 1.22). The supernatants of COS-7 cells alone or in combination with IL-17 did not induce ICAM-1 expression (data not shown). In contrast, ML-1 (100 ng/ml) significantly induced ICAM-1 expression at 48 h (MFI = 31.42 ± 4.39) (p < 0.05) when compared with those seen in cells stimulated with IL-17 and a His-tagged protein (MFI = 12.99 ± 2.09). Furthermore, similar patterns of differential effects on ICAM-1 gene expression were also observed (data not shown). These findings suggest a distinct function of ML-1, despite the fact that IL-17 and ML-1 share a significant degree of sequence homology. Furthermore, the functional disparity between IL-17 and ML-1 in the induction of ICAM-1 expression suggests differential interaction with cellular receptors. To assess whether ML-1 interacts with IL-17R, competitive inhibition experiments were performed by measuring the level of ML-1-induced ICAM-1 gene expression in the presence of varying concentrations of IL-17. As shown in Fig. 4B, ML-1 at 100 ng/ml markedly induced ICAM-1 expression, and the level of gene expression normalized by the level of G3PDH expression was not markedly induced ICAM-1 expression. It has been shown that ICAM-1 expression is increased in airway diseases, such as bronchial asthma (13). In particular, a high level of ICAM-1 expression, along with inflammatory cell infiltration, has been demonstrated in bronchial biopsies from both stable asthmatics and subjects after allergen challenge (13, 26). Moreover, allergen challenge up-regulated ICAM-1 expression in airway epithelium, correlating with eosinophil infiltration. Increased expression of ML-1, but not IL-17, is also seen in patients with asthma following allergen challenge. Moreover, expression of ML-1 is detected in activated Th2 cells, basophils, and mast cells, three important cell types involved in allergic responses. These findings suggest, therefore, 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-8 and ICAM-1 in airway epithelium. Further investigation is necessary to clarify the mechanism(s) by which ML-1 is involved in normal physiology, asthma, and other inflammatory diseases.

Discussion

We report herein the identification and characterization of a new cytokine with a distinct expression pattern and function when compared with other members of the IL-17 gene family. Although members of the IL-17 gene family are classified based on amino acid sequence similarity, the genes are selectively expressed in different tissues and are dispersed in the genome (1, 14, 15). Although IL-17 expression is restricted to activated PBMCs and activated Th0 cells, but not in activated Th2 cells (Fig. 2B; Refs. 1–3 and 21), ML-1 is expressed in various cell types and tissues. Its distribution is much wider than that of IL-17, suggesting ML-1 has more biological functions. In contrast to ML-1 and IL-17, two other members of the IL-17 family, IL-17B and IL-17C, were not detected in activated CD4+ T cells (14, 15). Moreover, among the IL-17 family members, ML-1 shows the highest homology with human IL-17. It is noted, however, that contrary to a previous report, the IL-17 gene is localized on the same genomic DNA clone (GenBank accession no. AL391221) ~50 kb telemeric to ML-1 sequence and both genes are in a tail-to-tail orientation (data not shown), suggesting a potential gene duplication event.

Two other members of the IL-17 gene family, IL-17B and IL-17C, each share ~27% amino acid identity with IL-17 (14, 15). IL-17B mRNA is expressed in adult pancreas, small intestine, and stomach, whereas IL-17C mRNA is not detected in the same set of adult tissues. Interestingly, no expression of IL-17B or IL-17C mRNA is found in activated T cells (15). Both IL-17B and IL-17C stimulate the release of TNF and IL-1 from a monocytic cell line, THP-1. However, IL-17B and IL-17C are not able to stimulate IL-6 production from human fibroblasts and do not bind to the human IL-17R. Furthermore, a newly discovered member of the IL-17 gene family, IL-17E, has been shown to induce activation of NF-κB and IL-8 via a distinct receptor (16). Taken together, these results and other published studies suggest the existence of a family of IL-17-related cytokines differing in their patterns of tissue/cell expression and in their potential roles in immunological responses.

ML-1 and IL-17 showed the same potency in the induction of IL-6 and IL-8, suggesting that ML-1 is involved in neutrophil recruitment into the airway such as IL-17 (11). The level of IL-8 is increased in inflammatory airway diseases, such as bronchial asthma, chronic obstructive pulmonary disease, and cystic fibrosis (22–24). There is a strong correlation between the level of IL-8 and the increase in neutrophil numbers (22). An anti-IL-8 mAb inhibited neutrophil chemotaxis in patients with chronic obstructive pulmonary disease or cystic fibrosis (24). In addition, bronchial epithelial cells are an important cell source of IL-8 and may thereby control neutrophil influx in the airway (25). Therefore, IL-8 release in bronchial epithelial cells may play a crucial role in modulating neutrophil-associated airway inflammation.

Although IL-17 and ML-1 induce IL-8 expression from PBE cells, IL-17 fails to induce ICAM-1 expression in human bronchial epithelial cells (Fig. 4A and Ref. 12). By comparison, ML-1 markedly induced ICAM-1 expression. It has been shown that ICAM-1 expression is induced in airway diseases, such as bronchial asthma (13). In particular, a high level of ICAM-1 expression, along with inflammatory cell infiltration, has been demonstrated in bronchial biopsies from both stable asthmatics and subjects after allergen challenge (13, 26). Moreover, allergen challenge up-regulated ICAM-1 expression in airway epithelium, correlating with eosinophil infiltration. Increased expression of ML-1, but not IL-17, is also seen in patients with asthma following allergen challenge. Moreover, expression of ML-1 is detected in activated Th2 cells, basophils, and mast cells, three important cell types involved in allergic responses. These findings suggest, therefore, 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-8 and ICAM-1 in airway epithelium. Further investigation is necessary to clarify the mechanism(s) by which ML-1 is involved in normal physiology, asthma, and other inflammatory diseases.

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References


