

IL-19 Induced Th2 Cytokines and Was Up-Regulated in Asthma Patients¹

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IL-19 belongs to the IL-10 family, which includes IL-10, IL-19, IL-20, IL-22, melanoma differentiation-associated gene-7 (IL-24), and AK155 (IL-26). IL-10 has been shown to inhibit allergen-induced airway hyperreactivity and inflammation. To determine whether IL-19 was also associated with asthma, we used ELISA to analyze the serum level of IL-19 in patients with asthma and found that their serum IL-19 levels were twice those of healthy controls. Patients with a high level of IL-19 also had high levels of IL-4 and IL-13. In a dust mite-induced murine model of asthma, we found that IL-19 level in asthmatic BALB/cJ mice was also twice that of healthy control mice. IL-19 transcript was also induced in the lungs of asthmatic mice. Electroporation i.m. of the IL-19 gene into healthy mice up-regulated IL-4 and IL-5, but not IL-13. However, IL-19 up-regulated IL-13 in asthmatic mice. In vitro, IL-19 induced IL-4, IL-5, IL-10, and IL-13 production by activated T cells. Activation of T cells was required for induction of IL-13 because IL-19 did not induce IL-13 production on nonstimulated T cells. Taken together, these results demonstrated that IL-19 up-regulates Th2 cytokines on activated T cells and might play an important role in the pathogenesis of asthma. *The Journal of Immunology*, 2004, 173: 6712–6718.

Interleukin-10 was originally described as a cytokine synthesis-inhibitory factor (1). Because IL-10 suppresses the release and function of a number of proinflammatory cytokines, such as IL-1, TNF- α , and IL-6, it is a normal endogenous feedback factor for the control of immune responses and inflammation (1, 2). Autoimmune models of rheumatoid arthritis, thyroiditis, and collagen-induced arthritis and a model of herpetic stromal keratitis all suggest negative regulatory roles for IL-10 in limiting inflammation and immunopathology (2). However, IL-10 is also a stimulatory factor for mast cells, B cells, and thymocytes (3–5), is pleiotropic, and acts on many other cell types, including monocytes, macrophages, T cells, NK cells, neutrophils, endothelial cells, and PBMC (6, 7).

IL-19 belongs to the IL-10 family, which includes IL-19, IL-20, IL-22, MDA-7 (IL-24), and AK155 (IL-26). IL-19 induces IL-6 and TNF- α production in monocytes (8). It also induces cell apoptosis and reactive oxygen species production in monocytes (8). The IL-19 gene is up-regulated by LPS and GM-CSF (9).

Asthma is characterized by airway hyperreactivity to a variety of specific and nonspecific stimuli, by chronic airway hypersecretion, and by increased IgE levels. The pathology in asthma occurs as a consequence of excessive production of IL-4, IL-5, and IL-13 by Th2 cells. IL-4 is critical for Th2 differentiation and IgE Ab switching (10–12). IL-5 is a key factor for eosinophil maturation and egress from bone marrow (13–15). IL-13 contributes to mucus

hypersecretion, induces airway smooth muscle hyperresponsiveness, and activates airway stromal cells to produce chemokines such as eotaxin (16, 17). Although the immunological mechanisms that induces asthma and allergy are relatively well characterized, the specific mechanisms that transpire in vivo to down-regulate Th2 cell-mediated allergic inflammatory response are not yet clear.

T cells secreting IL-10 in the respiratory mucosa can regulate Th2-induced airway hyperreactivity and inflammation, and it has been suggested that IL-10 plays an important inhibitory role in allergic asthma (18). Our aim, therefore, was to determine whether IL-19, a member of IL-10 family, is also associated with the pathogenesis of asthma.

Materials and Methods

Selection of asthmatic patients and preparation of serum

One hundred asthmatic patients (range, 3–12 years old), treated at the pediatric allergy clinic of National Cheng Kung University Hospital and Chi-Mei Hospital, were included in this study. The asthma severity of these patients was classified as severe-persistent, moderate-persistent, or mild-persistent, according to the guidelines of the Global Initiative for Asthma (April 2002). These patients had taken no oral glucocorticosteroids for at least 1 wk before the blood sampling.

Five milliliters of heparinized venous blood were collected from each patient. The blood samples were centrifuged, and the plasma was collected and stored in aliquots at -80°C before analysis. The control serum from healthy age-matched children and adults was similarly prepared.

Expression and purification of IL-19 recombinant protein

A cDNA clone coded for human or mouse IL-19 sequence from leucine to leucine (aa 25–176) was inserted into the expression vector of *Pichia pastoris* (pPICZ- α ; Invitrogen, San Diego, CA). IL-19 was expressed and purified from the culture medium of the yeast cells by affinity chromatography. This protein was used in biological function analysis in vitro and for the generation of polyclonal and mAbs as described below.

Generation of IL-19 mAb

BALB/cJ mice were immunized s.c. weekly for 4 wk with recombinant human IL-19 protein (100 $\mu\text{g}/\text{mouse}$) emulsified with an equal volume of Freund's complete/incomplete adjuvant. Three days before fusion, three mice were boosted by i.v. injection of the Ag without adjuvant. Spleen cells

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(1.2×10^8) from immunized mice were fused with X63-Ag8-6.5.3 myeloma cells (1.5×10^7) in the presence of PEG 4000 (Merck, Whitehouse Station, NJ). After fusion, the cells were distributed into 24-well plates and cultured in hypoxanthine/aminopterin/thymidine medium for 14 days. Via ELISA, culture supernatant was tested for the presence of Ab reacting with human IL-19 (hIL-19).³ For the cloning of the selected hybridoma cell, the limiting dilution was conducted twice. The hybridoma cells were cultured in DMEM (Invitrogen Life Technologies) containing 15% FCS, 1% penicillin/streptomycin, 2% L-glutamine, and 1% adjusted NaHCO_3 solution. The isotype of the selected Ab was IgG as determined by isotyping ELISA. The Ab was purified from ascites using protein A chromatography.

Generation of anti-hIL-19 polyclonal Ab

Human IL-19 polyclonal Ab was generated by injection of hIL-19 recombinant protein into a rabbit, following the standard procedure. Serum samples were collected, and the Ab was purified using protein A chromatography.

Detection of IL-19 protein in human serum

Human serum IL-19 was detected by ELISA, using anti-hIL-19 polyclonal Ab as the capture Ab and anti-hIL-19 mAb as the detecting Ab.

Detection of IL-4, IL-5, IL-10, and IL-13 cytokines in serum and culture medium

The levels of cytokines in serum and culture medium were detected by ELISA, following the manufacturer's protocol (R & D Systems, Minneapolis, MN).

Detection of IL-19 protein in murine sera

Mice were bled from the retro-orbital plexus. The blood was centrifuged at 5000 rpm for 10 min, and serum was collected for an ELISA to detect the murine IL-19 level.

Asthmatic mouse model

The allergen *Dermatophagoides farinae* (Der f; 1 g of lyophilized whole body extract in ether; Allergon, Engelholm, Sweden) (19) was dissolved in pyrogenic-free isotonic saline, filtered through a 0.22- μm pore size filter, and stored at -70°C before use. LPS concentration of the Der f preparations was <0.96 endotoxin U/mg Der f (*Limulus* amoebocyte lysate test, E-Toxate; Sigma-Aldrich, St. Louis, MO). Groups of specific pathogen-free, female, 6- to 8-wk-old BALB/cJ mice (Laboratory Animal Center, National Cheng Kung University, Tainan, Taiwan) were intratracheally inoculated with five doses of Der f (0.5 mg/ml, 50 μl) at 1-wk-intervals according to the method previously described (19). Blood samples were collected via the orbital sinus after the fourth inoculation every other day for 14 days. Control mice were inoculated with saline instead of Der f. The lungs from control mice and asthmatic mice were removed. Total RNA was isolated from the lung for real time PCR analysis.

Electroporation i.m. of IL-19 plasmid

Full-length mouse IL-19 plasmid DNA with a 6-histidine tag at the 3' end was cloned in an expression vector of pcDNA 3.1 (Invitrogen). Mice were anesthetized with 2 ml/kg acepromazine maleate i.p. (Tech America, Elwood, KS). Fifty micrograms of murine IL-19 plasmid DNA was injected, using a 27-gauge needle, into the bilateral quadriceps muscle of each animal. Immediately after injection, transcutaneous electric pulses were applied on each side of the leg with a pair of stainless steel needle electrodes (Genetron, model 508(S); BTX, San Diego, CA) 5 mm long and 0.4 mm in diameter with a 5-mm fixed distance between them. Electrical contact with the leg skin was ensured by shaving each leg. Electrical pulses were delivered with an electrical pulse generator (Electro Square Porator ECM 830; BTX). The shape of the pulse was a square wave; i.e., the voltage remained constant during the pulse. Six pulses of the indicated voltage (100 V or 900 V) were administered to each injection site at a rate of 1 pulse/s, with each pulse 100 μs in duration (20). Electroporation was performed every other day for 8 days on the muscle of alternate tibias, for a total of four administrations per experiment.

Real time PCR analysis of the transcripts of cytokines

To amplify the IL-13 transcripts, real time PCR was performed using the LightCycler-Fast Start DNA Master SYBR Green I kit (Roche, Indianapolis, IN) according to the manufacturer's instructions. Sense (5'-CTTGCT

TGCCTTGGTGGT-3') and antisense (5'-TGGTCTTGTGTGATGTT GCTC-3') primers were used in the real time PCR of the transcripts. A LightCycler was used for real time PCR. cDNA was diluted (1/50) with nuclease-free water, and 2 μl of the solution were used for the LightCycler SYBR-Green mastermix: 0.5 μM primers, 3 mM magnesium chloride, and 2 μl of Master SYBR-Green in nuclease-free water in a final volume of 20 μl . Individual PCR products were analyzed by melting point analysis. Samples were heated from 50°C to 95°C , and the decline in fluorescent signals of each individual sample was assessed. Melting point characteristics differed between individual PCR products. The fluorescence/time-dependent generation of signals was assessed by the manufacturer's software program, and the melting point of each product was matched with its individual melting temperature. GAPDH was used as an internal control gene to normalize for RNA amounts. Real time PCR was analyzed using the comparative C_t method according to the manufacturer's instructions. In brief, sample variation was corrected by subtracting GAPDH C_t values from the C_t values ($=\Delta C_t$) of the obtained cytokines.

In vitro activity of IL-19 on CD4^+ T cells

Single-cell suspensions were prepared from splenocytes depleted of RBCs. CD4^+ T cells were subsequently isolated to $>97\%$ purity with positive selection using anti-CD4 (L3T4) magnetic beads (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instruction. For in vitro differentiation assays, CD4^+ T cells were stimulated for 2 days with 1 $\mu\text{g}/\text{ml}$ plate-bound anti-CD3 and 1 $\mu\text{g}/\text{ml}$ plate-bound anti-CD28 mAbs in the presence of IL-2 (20 U/ml) alone for Th0 cells. IL-2 (20 U/ml), 5 ng/ml IL-12, and 10 $\mu\text{g}/\text{ml}$ anti-IL-4 mAb were added to the T cells for Th1 differentiation, whereas 20 U/ml IL-2, 10 ng/ml IL-4 (10 ng/ml), 1 $\mu\text{g}/\text{ml}$ anti-IL-12 mAb, and 10 $\mu\text{g}/\text{ml}$ anti-IFN- γ mAb were added for Th2 differentiation. All the cytokines and Abs used were purchased from R & D Systems. Three days after primary stimulation, cells were washed and then further cultured in the presence of IL-2 (20 U/ml), IL-12, anti-IL-4 mAb (Th1 polarized) or IL-2 (20 U/ml), IL-4, anti-IL-12 mAb, and anti-IFN- γ mAb (Th2 polarized) for 7 days. Fresh cytokines and Ab were added every 2 days. On day 7, cell culture supernatants were collected for ELISA of Th1 and Th2 cytokines production.

Results

IL-19 level is elevated in asthmatic patients and correlated with an elevated serum IL-4 and IL-13

To explore whether IL-19 was associated with asthma, we compared the average serum level of IL-19 in 100 asthmatic patients with the average levels in 50 healthy adults and 50 healthy age-matched children. A majority of asthmatic patients are younger than 15 years old. Therefore, we chose nonasthmatic adults as well as nonasthmatic children as controls to rule out any age effect. Asthmatic patients had twice the average level of serum IL-19 as controls (Fig. 1).

To see whether a higher IL-19 level in asthmatic patients correlated with Th2 cytokines, we analyzed IL-4 and IL-13 serum

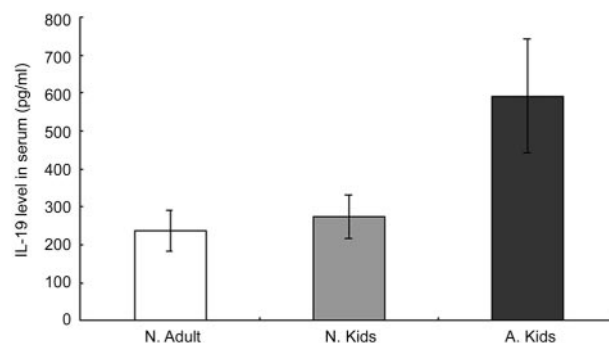


FIGURE 1. Comparison of serum IL-19 in the serum of asthmatic patients and healthy controls. Blood samples were collected from asthma patients (A. Kids; $n = 100$), healthy children (N. Kids; $n = 50$), and healthy adults (N. Adult; $n = 50$). Level of IL-19 in the serum was analyzed by ELISA using anti-hIL-19 polyclonal Ab as the first capture Ab and anti-IL-19 mAb as the second detecting Ab ($p < 0.001$).

³ Abbreviation used in this paper: hIL-19, human IL-19.

levels in the 27 patients with the highest and the 25 patients with the lowest IL-19 levels. IL-4 and IL-13 levels in these 27 patients were >100 times higher than those in healthy controls (Fig. 2). The 25 patients with lower IL-19 also had lower IL-4 and IL-13. Therefore, the IL-19 level correlated with the level of IL-4 and IL-13 in the asthmatic patients.

Overexpression of IL-19 in asthmatic mice

Because IL-19 levels in asthmatic patients were higher than those of healthy controls, we analyzed IL-19 expression in an established murine model of asthma induced by Der f allergen. Serum IL-19 level was twice higher in asthmatic mice than in healthy controls (Fig. 3A). In addition, real time PCR analysis showed that 80% more IL-19 transcript was expressed in the lungs of asthmatic mice than in healthy mice (Fig. 3B).

IL-19 induced IL-4, IL-5, and IL-10 production in healthy mice

Because IL-19 was up-regulated in asthmatic patients and asthmatic mice, and because its level was correlated with those of IL-4 and IL-13, we speculated that IL-19 might up-regulate Th2 cytokines. To test this hypothesis, we injected IL-19 cDNA in the expression vector pcDNA3.1 into mice using i.m. electroporation and then monitored the serum levels of IL-4, IL-5, IL-10, and IL-13. Empty pcDNA3.1 vector was injected into the negative-

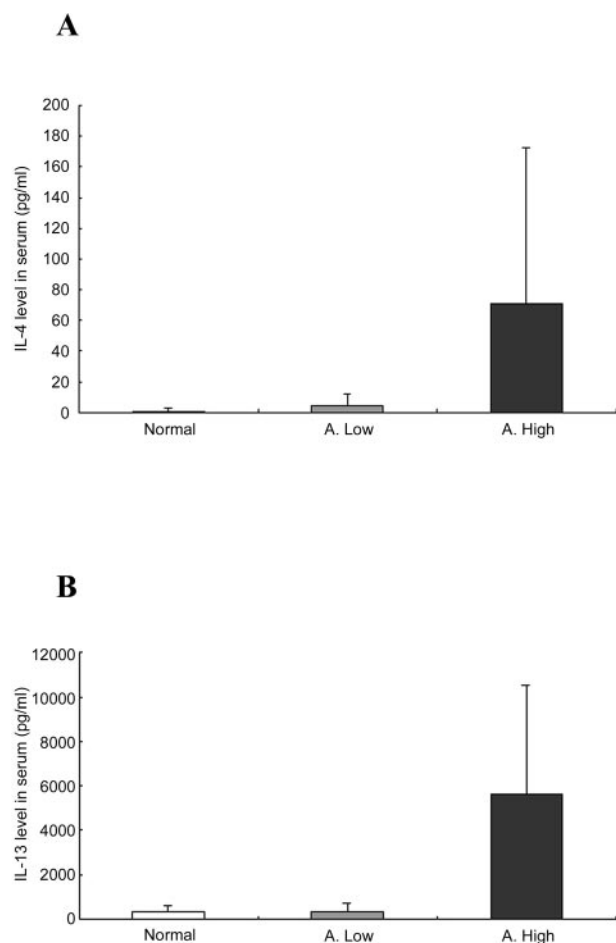


FIGURE 2. IL-4 and IL-13 serum level correlated with IL-19 level in asthmatic patients. Serum from asthma (A) patients with highest IL-19 level ($n = 27$) and lowest IL-19 level ($n = 25$) were collected, and their IL-4 (A) and IL-13 (B) levels were compared with those of healthy individuals using an IL-4 and IL-13 ELISA kit ($p < 0.01$ for IL-4 and $p < 0.001$ for IL-13).

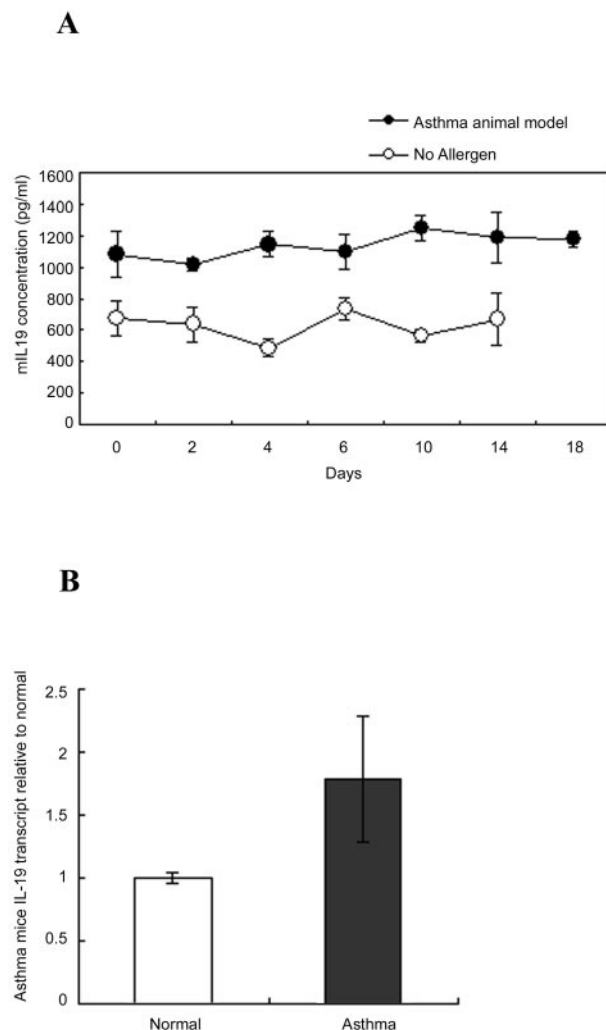


FIGURE 3. Up-regulation of IL-19 in asthmatic mice. An asthmatic animal model was generated by intratracheal inoculation of Der f allergen at 1-wk intervals for 4 wk. Then, blood samples were collected from asthmatic mice ($n = 10$) and normal control mice ($n = 10$) every other day for 14 days via the orbital sinus. The IL-19 level in the serum was analyzed using ELISA. Data are the average of 10 mice (A). Asthmatic mice ($n = 5$) and normal control mice ($n = 5$) were killed, and the lungs were removed for use in IL-19 transcript analysis. RNA was isolated from the lungs. IL-19 transcript was analyzed using real time PCR and expressed as fold of increase of asthmatic mice compared with the normal controls. Data represent the average of five mice in each group (B). mIL-19, Murine IL-19.

control mice. Three days after the first injection, anti-histidine Ab against recombinant IL-19 detected the expression of exogenous IL-19 in the serum of the experimental mice, and the IL-19 level lasted for >14 days (data not shown). IL-19 up-regulated serum levels of IL-4, IL-5, and IL-10 in the experimental mice compared with the negative controls (Fig. 4). Elevated IL-13 levels, however, were not detected in the serum of the experimental mice (data not shown).

IL-19 up-regulates IL-13 transcripts in asthmatic mice

IL-19 induced IL-4, IL-5, and IL-10, but not IL-13, production in healthy mice. In contrast, both asthmatic patients and asthmatic mice had higher levels of IL-19 and IL-13. We therefore speculated that IL-19 might induce IL-13 only in asthmatic patients or mice. To prove this hypothesis, we injected IL-19 cDNA into asthmatic mice using i.m. electroporation. IL-13 levels were analyzed

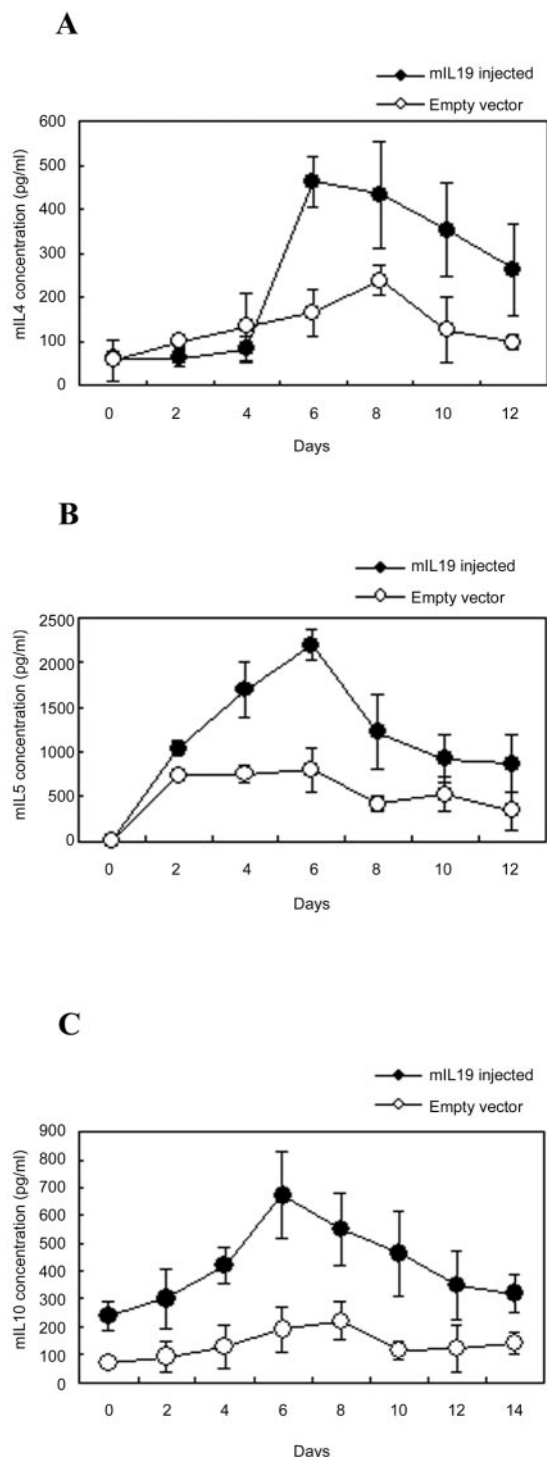


FIGURE 4. IL-19 induced IL-4, IL-5, and IL-10 production in mice ($n = 8$). Plasmid DNA (pcDNA3.1) containing IL-19 cDNA or empty vector (pcDNA3.1 alone) was injected into two groups of normal mice using i.m. electroporation on days 1, 3, 5, and 7. Each group contained eight mice. Blood samples were collected every other day via the retro-orbital plexus. Levels of IL-4 (A), IL-5 (B), and IL-10 were monitored for 14 days using ELISA. Data are the average of eight mice. mIL, Murine IL.

by real time PCR amplification of lung tissue. Compared with levels in empty vector-treated asthmatic mice, IL-13 transcripts increased in the asthmatic mice (Fig. 5). This result demonstrated that IL-19 induced IL-13 only in asthmatic mice and suggested that IL-19 may up-regulate IL-13 only in activated T cells.

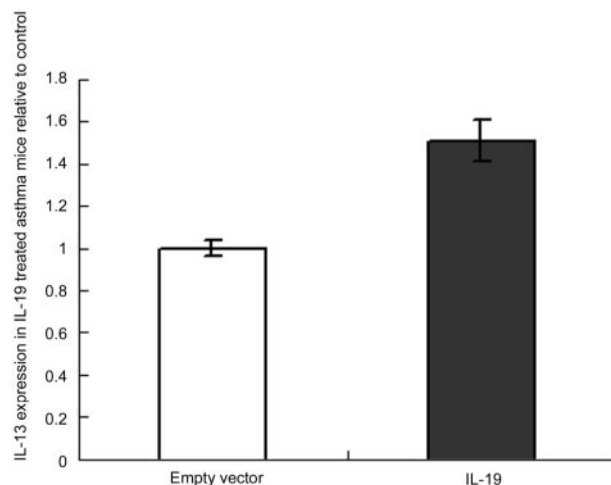


FIGURE 5. IL-13 transcript was induced by IL-19 in asthmatic mice ($n = 7$). An asthmatic animal model was generated by intratracheal inoculation of Der f allergen at 1-wk intervals for 4 wk. Plasmid DNA (pcDNA3.1) containing IL-19 cDNA or empty vector (pcDNA3.1 alone) were injected into asthmatic mice. RNA was purified from the lung. IL-13 transcript was analyzed by reverse transcription-quantitative real time PCR and expressed as a fold increase of IL-19-treated asthmatic mice compared with the control asthmatic mice injected with empty vector. Data represent an average of seven mice.

IL-19 up-regulates IgE production in asthmatic mice

IgE was elevated in the asthmatic mice. To analyze whether IL-19 would up-regulate IgE production, we also used i.m. electroporation to inject IL-19 into asthmatic mice and monitored their serum IgE levels. Compared with serum IgE levels in healthy control mice, serum IgE levels were 2.43 times higher in asthmatic mice untreated with IL-19 but were 3.17 times higher in IL-19-treated asthmatic mice (Fig. 6). IL-19 up-regulated IgE production in asthmatic mice.

IL-19 up-regulates IL-4, IL-5, IL-10, and IL-13 on activated CD4⁺ T cells

To further determine whether IL-19 could induce the production of Th2 cytokines in vitro, we isolated CD4⁺ T cells from the spleens

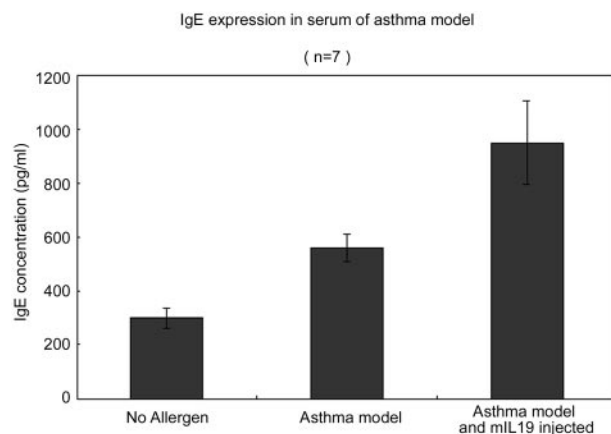


FIGURE 6. IgE was induced by IL-19 in asthmatic mice ($n = 7$). Plasmid DNA (pcDNA3.1) containing IL-19 cDNA was injected into asthmatic mice using i.m. electroporation. Blood samples were collected via the orbital sinus from normal mice ($n = 7$), asthmatic mice ($n = 7$), and IL-19-treated asthmatic mice ($n = 7$). IgE levels in the serum were analyzed using ELISA. Data represent an average of seven mice. mIL, Murine IL.

of mice. The CD4⁺ T cells were incubated on a plate coated with anti-CD3 and anti-CD28 mAbs and stimulated with IL-2 for 2 days. IL-19 was added, and the CD4⁺ T cells were incubated with IL-19 and IL-2 for another 3 or 7 days. The conditioned medium was collected on day 3 or 7, and the secretion of IL-4, IL-5, IL-10, and IL-13 was analyzed by ELISA, which showed that IL-19 up-regulated production of all these cytokines (Fig. 7). In contrast, IFN- γ production was not detected (data not shown).

To further determine whether IL-19 regulated specific cytokine production by Th1 and Th2 cells, we incubated CD4⁺ T cells on a plate coated with anti-CD3 and anti-CD28 for 2 days. IL-2, IL-12, and anti-IL-4 mAb were added to the plate to direct differentiation of Th1 cells. IL-2, IL-4, anti-IL-12 mAb, and anti-IFN- γ mAb were added to direct differentiation of Th2 cells. IL-19 was added to these two groups of Th cells at the same time. Conditioned medium was collected on day 7 from the control and IL-19-treated Th cells. We used ELISA to analyze levels of IL-4, IL-5, IL-10, IL-13, and IFN- γ to monitor their response to IL-19 treatment. IL-19 induced production of IL-4, IL-5, IL-10, and IL-13 in Th2 cells but inhibited production of IFN- γ in Th1 cells (Fig. 8).

IL-19 up-regulated IL-13 transcript on activated Jurkat T cells

IL-19 induced IL-13 production on activated T cells, and the *in vivo* induction of IL-13 occurs only in asthmatic mice. To provide further evidence that this effect occurred directly on activated T cells and was not attributable to any indirect effect of, for example, trace contaminated B cells or monocytes in the primary culture of T cell populations, we treated the activated and nonactivated Jurkat T cells with IL-19 and analyzed IL-13 transcript. Treatment of Jurkat T cells with Con A-PMA increased IL-13 transcript 2-fold (Fig. 9). IL-19 alone did not induce IL-13 transcript. When Jurkat T cells were treated with Con A-PMA followed by IL-19, IL-13 was up-regulated 4-fold. In contrast, if Jurkat T cells were treated with IL-19 followed by Con A-PMA or incubated with IL-19 and stimulant (Con A-PMA) at the same time, induction of IL-13 transcript was similar to that of Con A-PMA alone. These results demonstrated that IL-19 induced IL-13 expression only on activated T cells.

Discussion

IL-4 and IL-13 are two major cytokines produced by Th2 cells, mast cells, and basophils. In addition to their physiological roles, these two cytokines also play major roles in the pathogenesis of asthma. IL-4 is more active in regulating Th2 development, whereas IL-13 is more active in regulating airway hypersensitivity and mucus hypersecretion. The present study demonstrated that elevated IL-19 levels in asthmatic patients were positively correlated with the levels of IL-4 and IL-13. This suggested that IL-19 might play a role in the pathogenesis of asthma. Similarly, we also observed that both serum IL-19 and IL-19 transcript in lung tissue were up-regulated in asthmatic mice. The *i.m.* injection of IL-19 cDNA into mice resulted in the production of IL-4, IL-5, and IL-10, indicating that IL-19 was sufficient to induce Th2 cytokine production *in vivo*. However, IL-19 was unable to induce IL-13 in normal mice. Interestingly, after we injected IL-19 cDNA into asthmatic mice, we found IL-13 transcript up-regulated in the lungs of asthmatic mice and IL-13 protein increased in the serum. We also observed that IgE was induced by IL-19 in asthmatic mice. Induction of IgE could be attributable to the induction of IL-4 and IL-13, which together have induced class switching to IgE in B cells (21–23). Alternatively, IL-19 may directly enhance IgE production by activating B cells. Our preliminary data also

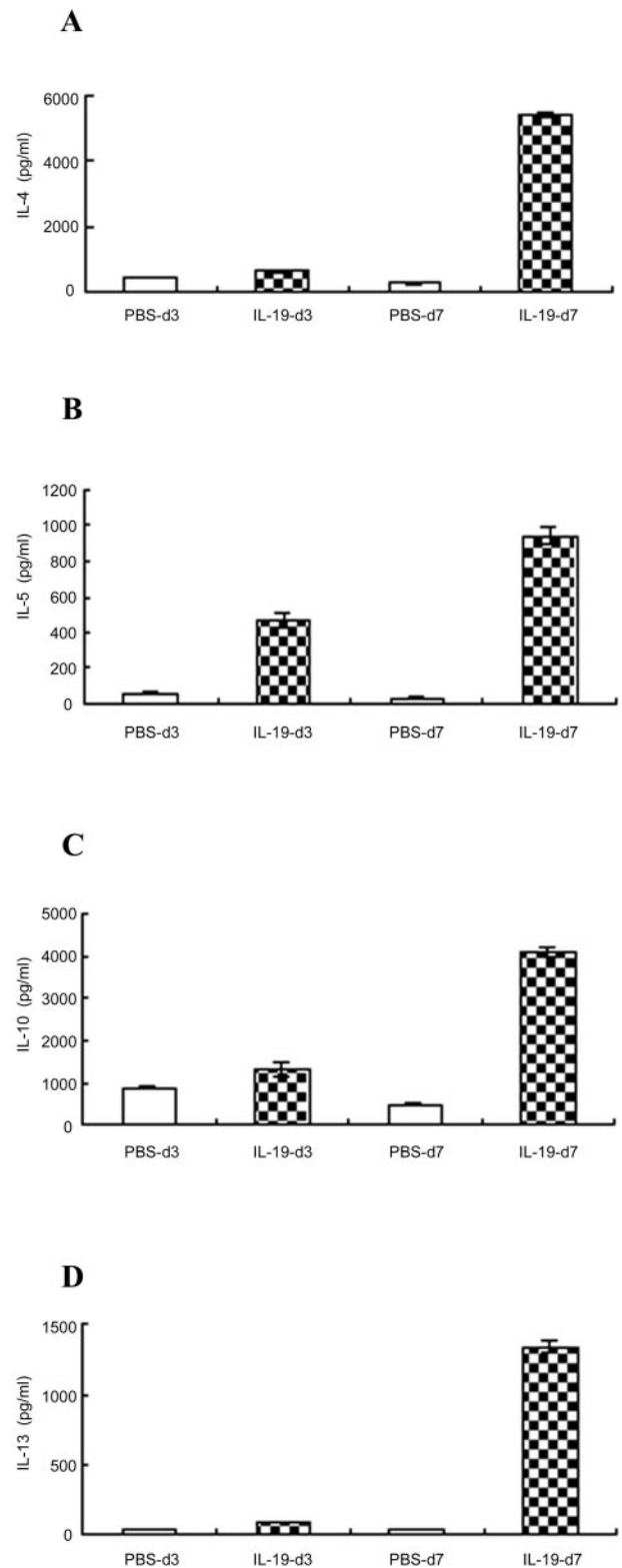


FIGURE 7. IL-19 up-regulated production of Th cytokines on activated CD4⁺ T cells. CD4⁺ T cells were isolated from mouse spleens and incubated on a plate coated with anti-CD3 and anti-CD28 mAbs for 2 days, and then PBS or IL-19 was added. CD4⁺ T cells were incubated with IL-19 (200 ng/ml) for another 3 or 7 days. Condition medium was collected on days 3 (d3) and 7 (d7). Cytokine levels in the condition medium in IL-19-treated T cells from days 3 and 7 were analyzed using ELISA. A, IL-4 level; B, IL-5 level; C, IL-10 level; D, IL-13 level. Data are the average of duplicate samples in ELISA. The experiment was repeated three times with similar results.

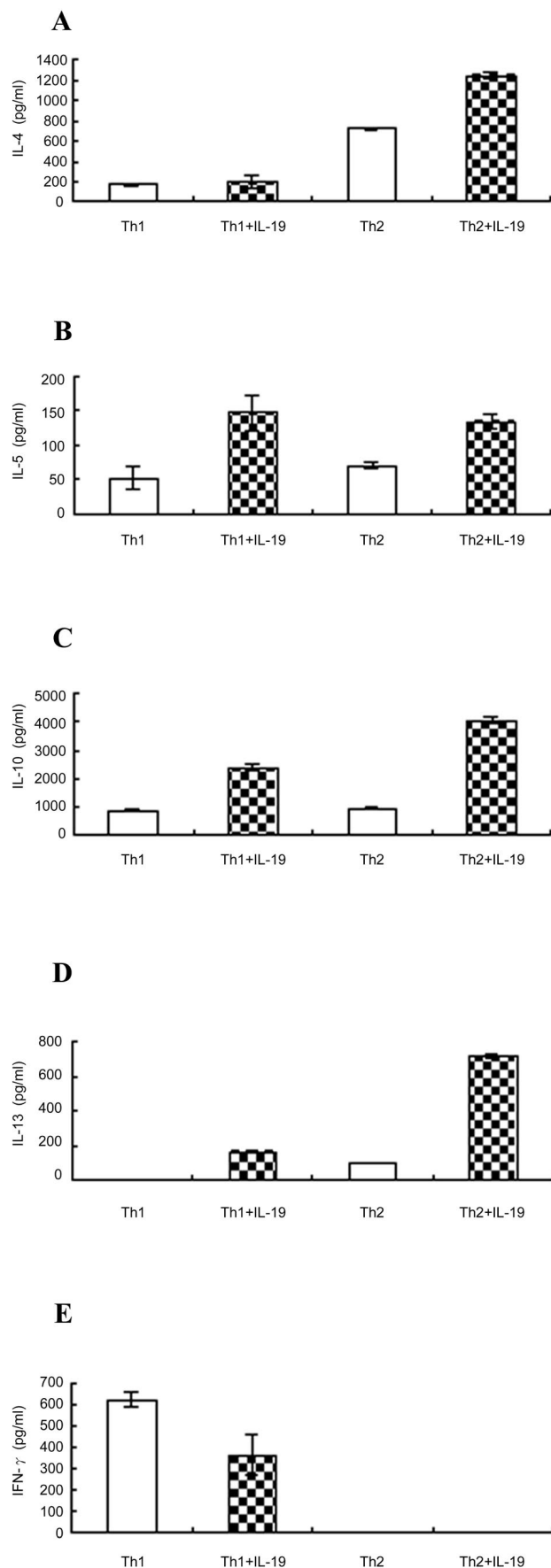


FIGURE 8. IL-19 up-regulated production of cytokines on activated Th2 cells. $CD4^+$ T (1×10^6) cells isolated from mouse spleens were incubated on a plate coated with anti-CD 3/CD 28 mAbs for 2 days. Then

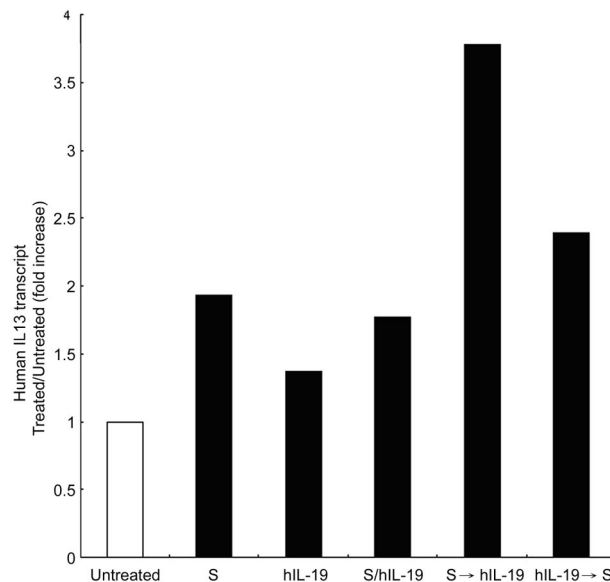


FIGURE 9. IL-19 enhanced transcription of IL-13 in activated Jurkat T cells. Jurkat T cells (1×10^6) were treated with Con A ($6.5 \mu\text{g/ml}$)-PMA (10 ng/ml ; S), or IL-19 (200 ng/ml), or both reagents added at different times (8 h apart). S/hIL-19, Jurkat T cells stimulated with both Con A-PMA and IL-19 together at the same time for 16 h; S→hIL-19, Jurkat T cells stimulated with Con A-PMA for 8 h first followed by IL-19 for another 8 h; hIL-19→S, Jurkat T cells treated with IL-19 for 8 h followed by Con A-PMA for another 8 h. Sixteen hours after incubation with one single reagent or both reagents, RNA was isolated and reverse transcription-quantitative real time PCR was performed to analyze IL-13 transcript. The experiment was repeated for six times with similar results.

demonstrated that i.m. electroporation of IL-19 could induce proliferation of B cells in vivo.

IL-5 is a key factor for eosinophil maturation and egress from bone marrow. Induction of IL-5 in vivo by IL-19 suggests that IL-19 may also play some role in the maturation of eosinophil in asthmatic patients. Whether IL-19 induces eosinophil maturation and egress from bone marrow in vivo awaits further study.

The immunostimulatory CpG oligonucleotide sequences present in most plasmid DNAs of vector are potent inducers of cytokines (24). Therefore, we used empty vector without IL-19 insert as negative controls in all of our studies of in vivo delivery of IL-19 cDNA into mice by i.m. electroporation. Induction of cytokine protein or up-regulation of cytokine transcript by IL-19 were compared with this negative control to rule out the effect of CpG oligonucleotides. The slight induction of IL-4, IL-5, and IL-10, shown in Fig. 4, by the empty vector may have been due to the CpG sequences in the vector.

In addition to the induction of Th cytokine in vivo, we demonstrated that IL-19 could induce Th cytokine production in vitro on activated $CD4^+$ T cells. To further differentiate which Th cells subset was affected by IL-19, we tested the effect of IL-19 on

cells were incubated with IL-2 plus IL-12 and anti-IL-4 mAb for Th1 cells or IL-2 plus IL-4, anti-IL-12, and anti-IFN-γ mAbs for Th2 cells. Either IL-19 (200 ng/ml) or PBS was added to these two groups of Th cells at the same time. Condition medium was collected on day 7. Levels of IL-4 (A), IL-5 (B), IL-10 (C), IL-13 (D), and IFN-γ (E) in the conditioned medium from Th1, and Th2 cells were analyzed using ELISA. Data are the average of duplicate samples in ELISA. The experiment was repeated three times with similar results.

cytokine production of Th1- and Th2-polarized T cells. IL-19 induced IL-4, -5, -10, and -13 production by Th2 cells but inhibited IFN- γ production by Th1 cells. Our result is similar to the observation by Gallagher et al. (25), who also demonstrated that IL-19 up-regulated IL-4 and down-regulated IFN- γ in whole PBMC culture. Furthermore, at the end of 7 days of incubation with IL-19, if Th2 cells were collected and washed twice with fresh medium and recultured on the plate without anti-CD3 and anti-CD28 mAbs and incubated with IL-19 alone, Th2 cytokine were found to be up-regulated by IL-19, except for IL-13, which was slightly down-regulated by IL-19 in Th2 cells (data not shown). These data further supported the hypothesis that IL-19 induces IL-13 production only on activated T cells. Studies with the Jurkat T cell line confirmed that the activation of T cells is required for IL-19 to induce IL-13 production from T cells. This result is also consistent with the observation that IL-19 up-regulated IL-13 only in the asthmatic mouse model in which the T cells have been activated. IL-13 is a crucial molecule in the pathogenesis of asthma. The data presented here demonstrate that IL-19 was up-regulated in asthma patients and induced IL-13 production. Therefore, it may play an important role in the pathogenesis of asthma.

In summary, we demonstrated that IL-19, another member of the IL-10 family, was associated with asthma by its modulatory effect on Th2 cytokines, including IL-4, IL-5, and IL-13. Regulation of IL-13, a critical molecule in the pathogenesis of asthma, was dependent on the prior activation of Th cells. The discovery of a new contributor to asthma provides further understanding of the complex mechanism of this disease.

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