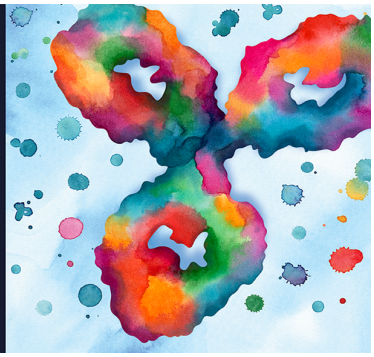

The Journal of Immunology
RESEARCH ARTICLE | APRIL 01 1996

IL-15 promotes cytokine production of human T helper cells. FREE

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J Immunol (1996) 156 (7): 2400–2405.
https://doi.org/10.4049/jimmunol.156.7.2400

IL-15 Promotes Cytokine Production of Human T Helper Cells

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IL-15 is a newly identified cytokine that has T cell and B cell growth factor activity similar to that of IL-2. In this study, a novel biologic function of IL-15 to promote cytokine production by human Th cells has been elucidated. *Dermatophagoides farinae* II (a major allergen of house dust mite)-specific human T cell clones produced IL-5 in response to recombinant human IL-15 as well as to either anti-CD3 or IL-2 stimulation. IL-5 mRNA became detectable 3 h after IL-15 stimulation and reached a maximum at 9 h. Human IL-5 promoter/enhancer-luciferase gene construct transfected to T cell clones was clearly transcribed in response to IL-15, indicating that the approximately 500-bp human IL-5 promoter/enhancer segment 5′ upstream of the coding region sufficiently responded to IL-15. IL-15-induced IL-5 synthesis was completely inhibited by the tyrosine kinase inhibitor, herbimycin A, suggesting the involvement of tyrosine kinases in the signal transduction leading to IL-5 synthesis as well as to proliferation of T cells induced by IL-15. Whereas IL-5 production by human peripheral T cells was abolished by the addition of anti-IL-2-neutralizing Abs into the culture, IL-15 restored the IL-5 synthesis despite effective IL-2 neutralization. IL-15 produced at the site of allergic inflammation may play a role in the recruitment and activation of eosinophils by inducing IL-5 (a Th2 cytokine) production by T cells. *The Journal of Immunology,* **1996, 156: 2400–2405.**

IL-15 is a newly identified cytokine having T cell and B cell stimulatory activity similar to IL-2 (1, 2). Whereas IL-15 has no sequence homology with IL-2, IL-15 interacts with components of the IL-2R (3). The β- and γ-chain of the IL-2R complex are shared by the IL-15R, suggesting that common signal transduction mechanisms may be utilized by these two cytokines.

The importance of persistent eosinophilic inflammation in the bronchial mucosa is well recognized in the pathogenesis of chronic asthma (4–11). Activated Th cells and T cell cytokines, especially IL-5, have been strongly implicated in the local infiltration and activation of eosinophils (12–16), since human IL-5 is a selective growth factor (17), a chemoattractant, and a potent activator for eosinophils (18). Indeed, administration of anti-IL-5-neutralizing Ab suppressed eosinophil recruitment into bronchial mucosa (19–21) and abrogated late phase bronchoconstriction induced by the inhalation of specific Ags (22) in several experimental asthma models.

Our previous report indicated that IL-5 production by $CD4^+$ T cells is enhanced in both atopic and nonatopic asthmatics (23, 24). We have recently demonstrated that IL-5 synthesis by human T cells is totally dependent on the presence of IL-2 and that human recombinant IL-2 induced human Th cell clones to express IL-5 mRNA and synthesize IL-5 protein (25). As IL-2-induced IL-5 synthesis was partially inhibited by anti-IL-2Rβ-chain Ab, the signal transduced via IL-2Rβ- and γ-chain made by IL-15 may induce IL-5 gene transcription. In this study we also tested the possibility that IL-15 can replace the requirement of IL-2 in IL-5 production by T cells. The results elucidated a novel biologic function of human IL-15, which is to stimulate gene transcription and protein synthesis of human IL-5. Locally produced IL-15 may participate in eosinophilic inflammation by inducing IL-5 synthesis by T cells.

Materials and Methods

Reagents

Recombinant human IL-15 was purchased from Pepro Tech (Rocky Hill, NJ). Recombinant human IL-2 was provided by Shionogi Pharmaceutical Co. (Osaka, Japan). The sp. act. of the recombinant materials for IL-2 and IL-15 were both approximately 1 U/ng protein. Anti-CD3 mAb was from Ortho (Raritan, NJ). FITC-labeled anti-CD3 (Leu4), anti-CD4 (Leu2), and anti-CD8 (Leu3) mAbs were from Becton Dickinson (San Jose, CA). Anti-IL-2- and anti-IL-4-neutralizing mAbs were obtained from Genzyme (Cambridge, MA) and R&D Systems (Minneapolis, MN). Polyclonal goat anti-IL-2 Ab was from R&D Systems. Anti-IL-2R α-chain Ab was from Genzyme. Anti-IL2R β-chain Ab (Mik 2β) was from PharMingen (La Jolla, CA). Herbimycin A was from Wako Chemicals (Tokyo, Japan). Recombinant *Dermatophagoides farinae* II (r*Der f* II)[2] protein was prepared as described previously (26). Con A and PHA were purchased from Sigma Chemical Co. (St. Louis, MO). AIM-V medium (Life Technologies, Inc., Gaithersburg, MD) was used for T cell cultures. RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin was used for the electroporation.

Establishment of Ag-specific T cell clones

Der f II-specific human T cell clones were derived from PBMC of atopic asthmatic patients by Ag stimulation followed by the limiting dilution method, as described previously (25).

Briefly, PBMC (2×10^6/ml) were cultured with r*Der f* II protein (1 μg/ml) for 10 days in 24-well culture plates, and nonadherent cells were recovered. Then 10^2 to 10^4 live cells were cultured in 96-well round-bottom culture plates (Nunc, Roskilde, Denmark) with Ag and 2,500 rad-irradiated autologous PBMC (5×10^4 cells). Fresh medium containing 10 U/ml rIL-2 was added once a week. When less than 1 of 10 wells contained proliferating cells, the resulting cell lines were considered to have originated from a single clone. To ensure their clonality, these T cell

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Received for publication August 11, 1995. Accepted for publication January 31, 1996.

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[2] Abbreviations used in this paper: *Der f, Dermatophagoides farinae*; rh, recombinant human.

0022-1767/96/$02.00

clones were further subcloned by limiting dilution using irradiated autologous PBMC and Ag. After 10 to 14 days, expanding cultures were transferred to 24-well culture plates (Becton Dickinson). T cell clones were maintained by antigenic stimulation with irradiated autologous PBMC (2×10^6/well) and r*Der f* II protein every 2 to 3 wk.

Stimulation of T cell clones

T cells were harvested at least 10 days after the last antigenic stimulation, layered onto Ficoll-Paque, and centrifuged. The interface was recovered, washed twice, and resuspended in fresh medium. The resulting preparation usually consisted of more than 98% $CD3^+$ cells, as determined by flow cytometry. Cells (10^5/well) were cultured in triplicate with various stimuli in 96-well round-bottom culture plates for designated time periods. For proliferation analysis, cells were cultured for 72 h. [^{3}H]Thymidine (0.5 μCi/well) was pulsed for the last 16 h. To obtain cytoplasmic RNA, T cells (4×10^6/well) were cultured in 24-well culture plates with various stimulants for the designated time periods.

Stimulation of PBMC

PBMC (2×10^6/well) was incubated with PHA (1 μg/ml) in 24-well culture plates for 3 days. They were washed and cultured in a fresh medium containing rIL-2 (5 ng/ml) for 4 days. The T cell fraction was obtained using a glass bead column (Accurate Chemical, Westbury, NY) according to the manufacturer's directions. The resulting cell population consisted of more than 80% $CD3^+$ cells, as determined by FACS analysis. Then the T cell-enriched fractions (2×10^6/well) were cultured in triplicate with rhIL-15 (0, 10, and 100 ng/ml) in 24-well culture plates for 24 h. For the proliferation analysis, cells (10^5/well) were cultured in 96-well round-bottom plates for 72 h.

Quantitation of cytokines

IL-5 was measured by a sandwich ELISA using monoclonal anti-human IL-5 (D138) as the capture Ab and biotinylated purified rabbit anti-human IL-5 as the second Ab, as described previously (27). The linear portion of the standard curve was between 3.9 and 500 pg/ml. IL-2 and IL-4 were measured by specific ELISA (Quantikine ELISA kits; R&D Systems), according to the manufacturer's directions.

Reverse transcription-PCR assay

IL-5 gene expression was analyzed by the reverse transcription-PCR method, as reported previously (23). Briefly, RNA was extracted from the pelleted cells essentially following the one-step acid guanidinium isothiocyanate/phenol-chloroform extraction method of Chomczynski and Sacchi (28) using Isogene (Nippongene, Tokyo, Japan). cDNA was synthesized from 1 μg of cytoplasmic RNA using random primers and murine Moloney leukemia virus reverse transcriptase (Life Technologies, Inc.). PCRs were performed using the following primers (Clontech, Palo Alto, CA):

IL-5: 5′-GCTTCTGCATTTGAGTTTGCTAGCT-3′
5′-TGGCCGTCAATGTATTTCTTTATTAAG-3′
β-actin: 5′-ATGGATGATGATATCGCCGCG-3′
5′-CTAGAAGCATTTGCGGTGGACGATGGGGGCC-3′

To 50 μl (final volume) of amplification solution (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.2 mM of each deoxynucleotide triphosphate; Toyobo, Osaka, Japan), 2 μl of cDNA (corresponding to about 250 ng of starting RNA material), 0.4 μM of each primer, and 2 U of Taq DNA polymerase (AmpliTaq; Perkin-Elmer Cetus, Norwalk, CT) were added. The mixture was heated at 95°C for 5 min, followed by 25 cycles, each consisting of incubations for 1 min at 95°C, 2 min at 60°C, and 3 min at 72°C. The PCR products were analyzed by 1% agarose gel electrophoresis in the presence of ethidium bromide.

Plasmid constructs

Human genomic DNA isolated from HeLa cells was used as a template. IL-5 promoter/enhancer gene (−511 to +4 relative to the transcription initiation site) was PCR amplified using a "sense" strand primer (5′-ATACTCGAGGGATCCTAATCAAGACCC-3′), and an "anti-sense" strand primer (5′-TGCAAGCTTTGCATAGTACAAGACTGC-3′) according to the DNA sequence reported by Tanabe et al. (29). This 515-bp PCR product was inserted into the *Xho*I-*Hin*dIII site of the pGL2 basic vector (Promega, Madison, WI) to construct pIL-5(-511)Luc (Fig. 1). Identity of the promoter/enhancer sequence with the originally reported DNA sequence was confirmed by chain termination sequencing using Sequenase 2.0 (Strategene, La Jolla, CA). pCMV-β-gal control vector (Riken DNA Bank, Tokyo, Japan) was used as a transfection control.

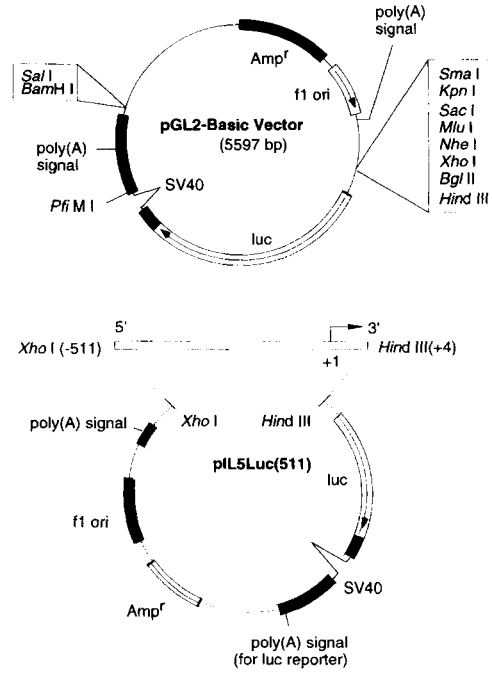

FIGURE 1. Plasmid maps.

Transient transfections, luciferase assay, and β-galactosidase assay

T cell clones were transfected by electroporation using a Gene Pulser (Bio-Rad, Richmond, CA). Briefly, 2.5×10^7 T cell clones in 500 μl RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 ng/ml rIL-2 in a 0.4-cm electroporation cuvette were incubated with 50 μg of pIL-5(-511)Luc and 10 μg of pCMV-βgal and electroporated at 270 V, 960 μF with a mean time constant of 19. After transfected cells were cultured with or without stimulation for 24 h, protein extracts were prepared and luciferase activity was assayed using the Luciferase Assay system (Promega). The relative luciferase unit was calculated by the following formula:

relative luciferase unit =

luciferase activity in cell lysate/protein content (mg/ml).

β-Galactosidase activity was assayed using chlorophenol red-β-D-galactopyranoside as the substrate. First, 150 μl of cell lysates diluted with Z buffer (0.1 M Na_2HPO_4-NaH_2PO_4, pH 7.5, 10 mM KCl, 1 mM $MgSO_4$, 50 mM 2-ME) and 30 μl of 15 mM chlorophenol red-β-D-galactopyranoside was incubated at 37°C for 30 min. Then the reaction was stopped by the addition of 75 μl of 1 M Na_2CO_3, and OD_{574} was measured.

Protein assay

Protein concentrations were determined using a bicinchoninic acid protein assay reagent (Pierce, Rockford, IL), according to the manufacturer's directions.

Statistical analysis

Statistical analysis was performed using Student's t test. A value of $p < 0.05$ was considered statistically significant. Responses are presented as mean ± SEM.

Results

Recombinant IL-15 induced IL-5 production by T cell clones

The first experiment was conducted to examine the production of IL-5 by T cell clones stimulated with recombinant human IL-15 (rhIL-15). T cell clones were used for experiments at least 10 days after the last antigenic stimulation. As described in *Materials and Methods*, T cells obtained from the interface of Ficoll-Paque density gradient consisted of more than 98% pure $CD3^+CD4^+$ cells.

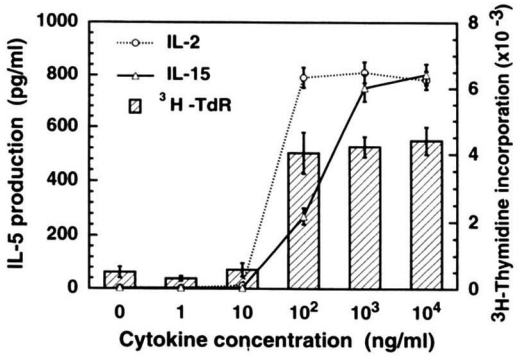

FIGURE 2. IL-5 production induced by IL-15. T cell clones (HK5, 10^5/well) were cultured in 96-well round-bottom culture plates with various concentrations of rhIL-2 (○) or rhIL-15 (△). Culture supernatants were harvested after 24 h and assayed for IL-5 by a specific ELISA. For proliferation assays (hatched bars), cells were cultured with various concentrations of rhIL-15 for 72 h. [^{3}H]Thymidine (0.5 μCi/well) was pulsed for the last 16 h. Data are expressed as the mean of triplicate cultures ± SEM.

Table I. *The profiles of cytokine production by human T cell clones*[a]

	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)		
Clone	Anti-CD3[b]	Anti-CD3[b]	Anti-CD3[b]	rIL-2[c]	rIL-15[d]
YA5	1740 ± 102	840 ± 40	424 ± 29	300 ± 17	220 ± 21
YA7	2620 ± 98	410 ± 29	829 ± 72	1034 ± 39	782 ± 39
YA8	420 ± 32	720 ± 52	182 ± 12	224 ± 31	142 ± 28
YA9	3410 ± 201	142 ± 31	729 ± 49	410 ± 29	220 ± 27
YA10	1642 ± 92	1820 ± 102	170 ± 42	242 ± 19	124 ± 8
YA11	884 ± 48	1042 ± 91	725 ± 36	802 ± 49	620 ± 51
HK2	229 ± 29	847 ± 52	429 ± 21	410 ± 29	372 ± 31
HK3	1024 ± 32	420 ± 31	990 ± 45	724 ± 30	490 ± 19
HK4	2024 ± 89	792 ± 82	180 ± 17	260 ± 13	202 ± 16
HK5	1064 ± 82	1320 ± 41	620 ± 82	708 ± 70	202 ± 12

[a] 10^5 cells per well were incubated in a 96-well round-bottom culture plate for 24 h with the designated stimuli. The resulting supernatants were assayed for IL-2, IL-4, and IL-5 by specific ELISAs. Values are expressed as the mean of triplicate cultures ± SEM. IL-2, IL-4, and IL-5 productions in the unstimulated cultures were always below the detection limit; IL-2 <6 pg/ml, IL-4 <3 pg/ml, IL-5 <3 pg/ml.

[b] Wells were pretreated with OKT3 mAb (10 μg/ml) in 0.05 M carbonate-bicarbonate buffer, pH 9.6, at 4°C overnight and then washed three times with fresh medium before use.

[c] Cells were incubated with rIL-2 (100 ng/ml) for 24 h.

[d] Cells were incubated with rIL-15 (100 ng/ml) for 24 h.

They were washed three times, resuspended in fresh medium, and cultured with various concentrations of rhIL-2 or rhIL-15 for 24 h. A representative result is shown in Figure 2. IL-5 production was clearly induced by rhIL-15 in a dose-dependent manner. rIL-2 also induced IL-5 production as previously reported by Mori et al. (25). IL-4 was not detected in the culture supernatants of T cells stimulated with rIL-2 or rhIL-15 at any concentrations tested (data not shown). T cell proliferation was also induced by rhIL-15 at concentrations of 10 to 10^3 ng/ml. Essentially the same dose response was observed in six other T cell clones (YA5, YA7, YA8, YA9, HK3, and HK4). All the 10 T cell clones that produced IL-5 upon either immobilized anti-CD3 Ab or rIL-2 stimulation responded to rhIL-15 by producing IL-5 (Table I).

Analysis of IL-5 gene expression induced by IL-15

To examine IL-5 gene expression in T cell clones, a T cell clone (YA5) was stimulated with rhIL-15 (100 ng/ml) for designated time periods, and total cytoplasmic RNA was extracted. RNA was then reverse transcribed and amplified by PCR using primer sets

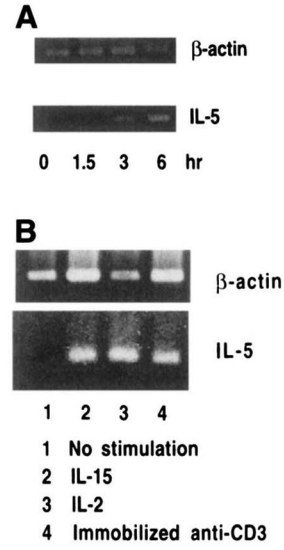

FIGURE 3. IL-5 gene expression of human T cell clones induced by IL-15. *A*, T cell clones (YA5, 2 × 10^6/well) were cultured in 24-well culture plates with rhIL-15 (100 ng/ml). Cells were harvested after 0, 1.5, 3, or 6 h. Total RNA was extracted, reverse transcribed, and amplified by PCR. 279- and 1128-bp products correspond with the expected size of IL-5 and β-actin amplification products, respectively. *B*, YA5 cells were stimulated with rIL-2 (100 ng/ml), rhIL-15 (100 ng/ml), or immobilized OKT3 for 6 h. Total RNA was extracted, reverse transcribed, and amplified by PCR.

specific for human IL-5 and β-actin. The number of cycles was titrated before the experiment and reactions were repeated for 25 cycles, at which time PCR products were amplified exponentially. As shown in Figure 3*A*, IL-5 mRNA was clearly detected 3 h following IL-15 stimulation and still increased at 6 h.

The level of IL-5 mRNA was compared among T cell clones stimulated for 6 h with rhIL-15, rIL-2, or immobilized anti-CD3 mAb. A representative result is shown in Figure 3*B*. Comparable amounts of IL-5 mRNA were detected following either rhIL-15, rIL-2, or immobilized anti-CD3 stimulation. Essentially the same results were obtained when PCR was performed for 23 and 27 cycles (data not shown), indicating that the amounts of PCR products at 25 cycles well reflect the relative quantity of mRNA in the original RNA preparations. IL-5 mRNA was also detected in three other clones (YA7, HK2, and HK3) stimulated with rhIL-15 (data not shown).

Human IL-5 promoter/enhancer activity induced by IL-15

To further delineate the molecular mechanisms of IL-5 gene transcription, we constructed a human IL-5 promoter/enhancer-luciferase reporter gene plasmid, pIL-5(-511)Luc, and analyzed the inducible transcriptional activity of the activated T cells using a transient expression system.

Transfection of T cell clones (YA5) with pIL-5(-511)Luc followed by stimulation with rhIL-15 (100 ng/ml) resulted in a 30-fold increase in luciferase activity compared with that in unstimulated cells (Fig. 4), whereas β-galactosidase activity derived from pCMV-β-gal, a transfection control, did not differ significantly between stimulated and unstimulated T cell clones (A574/μg protein; 0.421 in stimulated vs 0.450 in unstimulated cells). To maintain the viability of unstimulated cells, rIL-2 at a concentration of 5 U/ml was included in the culture medium of both stimulated and unstimulated cells. This concentration of rIL-2 was enough to maintain viability determined by a trypan blue dye exclusion test, but not enough to induce IL-5 synthesis or proliferation of T cell clones (data not shown). These findings clearly indicate that the

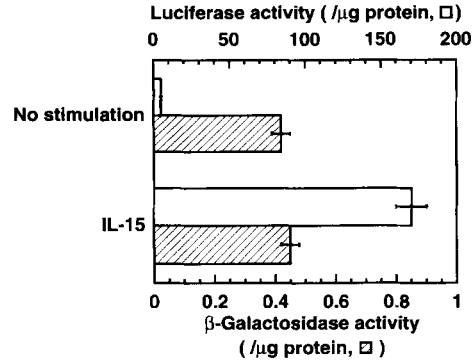

FIGURE 4. Human IL-5 promoter/enhancer activity induced by rhIL-15. T cell clones (YA5; 2.5×10^7) were transiently transfected with pIL-5Luc(-511) and pCMV-β-gal by electroporation. Cells were incubated with or without rhIL-15 (100 ng/ml) for 24 h. Cell lysates were assayed for luciferase (white bars) and β-galactosidase activities (hatched bars). Values are expressed as the mean of triplicate cultures ± SEM.

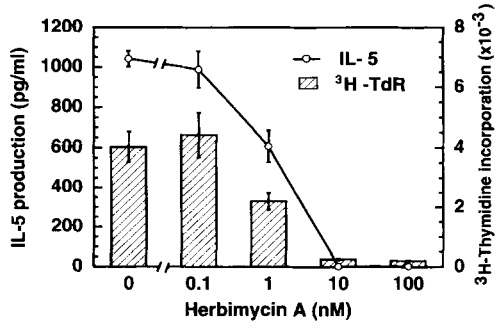

FIGURE 5. IL-15-induced IL-5 production and proliferation are inhibited by herbimycin A. T cell clones (YA5) were stimulated with rhIL-15 (100 ng/ml) for 24 h. Various concentrations of herbimycin A were added to some cultures throughout the culture period. Resulting supernatants were assayed for IL-5 (O) by a specific ELISA. For proliferation assays (hatched bars), cells were cultured for 72 h. [^{3}H]Thymidine (0.5 μCi/well) was pulsed for the last 16 h. Data are expressed as the mean of triplicate cultures ± SEM.

515-bp promoter/enhancer segment used in this experiment is functionally sufficient to respond to IL-15 stimulation. Effective transcription of pIL-5Luc in response to rhIL-15 was also confirmed in four other clones (YA7, YA8, HK3, and HK5) tested so far (data not shown).

Herbimycin A inhibited IL-5 production induced by IL-2

Tyrosine phosphorylation is one of the earliest biochemical events in the signal transduction cascade of the IL-2R system (30–34). It has been well established that the cellular proliferation induced by IL-2 is dependent on protein tyrosine kinases (35, 36). As the IL-15 signal is transduced by components of IL-2R, the β- and γ-chains (1–3), it is of interest to know whether tyrosine kinases are also involved in the IL-15 signal cascade. It has been shown that herbimycin A has strong inhibitory activity against most tyrosine kinases, but does not inhibit cyclic nucleotide-dependent protein kinases, protein kinase C, phosphorylase kinase, or phospholipase C (37). As shown in Figure 5, both IL-5 production and proliferation were inhibited by herbimycin A in a dose-dependent manner. Herbimycin A (100 nM) did not affect the cell viability or β-actin mRNA expression of T cell clones, thereby excluding nonspecific toxicity of the agent (data not shown). The effect of herbimycin A was also confirmed in five other clones (YA8, YA10, YA11, HK2, and HK5; data not shown).

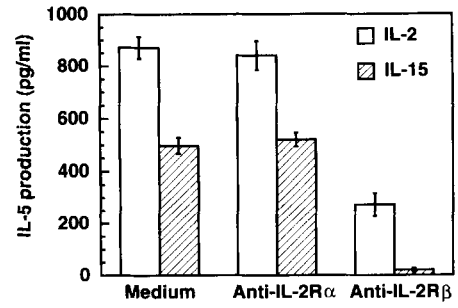

FIGURE 6. IL-15-induced IL-5 production is inhibited by IL-2Rβ mAb. T cell clones (HK5; 10^5 cells/well) were cultured with IL-2 (100 ng/ml, white bars) or IL-15 (100 ng/ml, hatched bars) for 24 h and the resulting supernatants were assayed for IL-5 by a specific ELISA. Either anti-IL-2Rα- or β-chain mAb (10 μg/ml) was included in some cultures. Data are expressed as the mean of triplicate cultures ± SEM.

Anti-IL-2Rβ Ab inhibited induction of IL-15-induced IL-5 production

The β-chain of the IL-2R complex is reported to be involved in IL-15 signaling (2, 3). Therefore, we tested the effect of anti-IL-2R Abs on IL-15-induced IL-5 production by T cell clones. As shown in Figure 6, anti-IL-2Rβ Ab (10 μg/ml) completely inhibited IL-5 production induced by rhIL-15, whereas it only partially inhibited that induced by rhIL-2. Anti-IL-2Rα Ab did not affect IL-5 production. Essentially the same results were obtained in three other clones (YA2, YA5, and HK2; data not shown).

IL-15 can replace IL-2 in IL-5 production by human PBMC

The role of IL-15 in IL-5 production by human peripheral T cells was examined. PBMCs of atopic asthmatics were stimulated with Con A (10 μg/ml) for 24 h. Significant amounts of IL-4 and IL-5 were detected in the supernatants. We have reported that the principal source of IL-5 produced by activated PBMC was $CD4^+$ T cells in this system (23). Representative results were shown in Figure 7. IL-5 production, but not IL-4 production, was totally inhibited by the presence of anti-IL-2-neutralizing Ab (2 μg/ml), as previously reported by Mori et al. (25). Neutralization of IL-2 activity was confirmed by the demonstration that this concentration of the Ab shifted the proliferation curve of an IL-2-dependent cytotoxic T cell clone (CTLL-2 cells) approximately 100-fold (data not shown). Addition of rhIL-15 (100 ng/ml) into the culture restored IL-5 production by activated PBMC despite the presence of anti-IL-2-neutralizing Ab. Neither rhIL-2 nor rhIL-15 by itself was sufficient to induce IL-5 production by PBMC. Anti-IL-4-neutralizing mAb (10 μg/ml) did not affect IL-5 production. Essentially the same results were obtained in two other donors using three different preparations of anti-IL-2 Abs and two anti-IL-4 Abs (data not shown). The results indicated that the activating signal mediated by IL-2R is required for IL-5 synthesis by human peripheral $CD4^+$ T cells and the signal through IL-15R can substitute it.

IL-15 responsiveness of human peripheral blood T cells

IL-15 responsiveness of human PHA blasts was next determined. PBMC was stimulated with PHA (1 μg/ml) for 3 days, washed, and cultured in a fresh medium containing rIL-2 (5 ng/ml) for 4 days. Then T cells were enriched, using a glass bead column, to more than 80% purity and incubated with rhIL-15. As shown in Table II, rhIL-15 clearly induced IL-5 synthesis and proliferation of these T cell preparations obtained from three different asthmatic

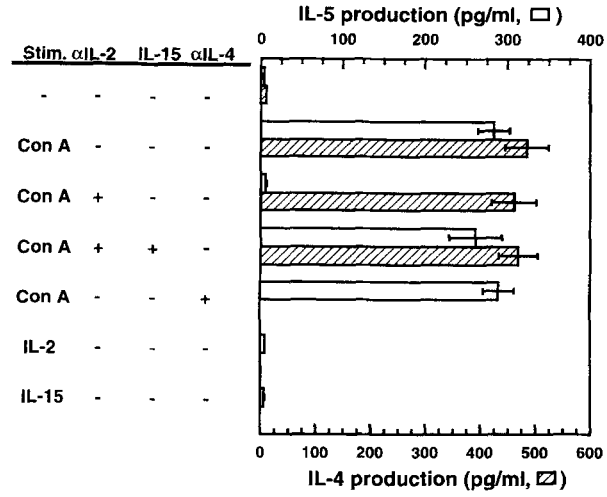

FIGURE 7. IL-15 can replace IL-2 in the production of IL-5 by Con A-stimulated PBMC. PBMC (2×10^6/well) obtained from atopic asthmatics were stimulated with either Con A (10 μg/ml), rIL-2 (100 ng/ml), or rhIL-15 (100 ng/ml) for 24 h. Anti-IL-2-neutralizing Ab (2 μg/ml), anti-IL-4-neutralizing Ab (10 μg/ml), and rhIL-15 (100 ng/ml) were added into some cultures as indicated in the figure from the start. Resulting supernatants were assayed for IL-5 by a specific ELISA. Data are expressed as the mean of triplicate cultures ± SEM.

donors, indicating that not only T cell clones but also human bulk T cells in general can respond to IL-15.

Discussion

Our results clearly demonstrate that human IL-15 induced mRNA expression and protein synthesis of IL-5 in human $CD4^+$ T cell clones. The approximately 500-bp human IL-5 gene segment 5′ upstream of the transcription initiation site was functionally sufficient for the gene transcription induced by IL-15. It has been reported that IL-15 promotes growth and differentiation of T cells, B cells, and NK cells (1–3, 38). The finding that IL-15 can promote cytokine production by $CD4^+$ T cells was demonstrated here for the first time. As T cell preparations used in the present study consisted of more than 98% pure $CD3^+CD4^+$ T cells as determined by FACS analysis, it is evident that IL-15 can act directly on T cells for the IL-5 production. Moreover, the present findings may be applicable to human peripheral Th cells in general, as human bulk PHA blasts also responded to IL-15 as well.

IL-5 production by $CD4^+$ T cell clones is dependent on IL-2 (25). IL-5 production by the activated PBMC was totally inhibited by anti-IL-2-neutralizing Abs (Fig. 7). Addition of IL-15 into the culture restored IL-5 production despite the presence of anti-IL-2 Ab. The IL-2R is composed of three subunits; α-, β-, and γ-chains (39). It has been postulated that the IL-15R is composed of three subunits, of which the β- and γ-chains are common with IL-2R (1–3), and thus the signal transduction pathway may be shared by IL-2R and IL-15R. The finding that human IL-15 also induced IL-5 production by human T cell clones suggests that either the β- or γ-chain is involved in the signaling pathway leading to IL-5 production. Indeed, IL-5 production was completely abolished by anti-IL-2Rβ-chain Ab (Fig. 6). IL-2-induced IL-5 production was only partially inhibited by the same Ab, consistent with the report by Armitage et al. in which it was demonstrated that the proliferation of B cells induced by IL-15 was more susceptible to anti-IL-2Rβ-chain Ab than that induced by IL-2 (2). It is possible that the IL-2-induced response was more intense than the IL-15-induced one and could not be suppressed by the same concentration

Table II. *IL-15 responsiveness of human PHA-activated T cells*

Cells[a]	IL-15 (ng/ml)	IL-5 Production[b] (pg/ml)	S.I.[c]
Donor 1	0	<3	1
	10	4.8 ± 2.0	1.0
	100	78.2 ± 4.3	5.2
Donor 2	0	<3	1
	100	60.4 ± 6.5	4.4
Donor 3	0	<3	1
	100	39.2 ± 3.0	7.2

[a] PBMC obtained from atopic asthmatics were stimulated with PHA for 3 days, washed, and kept in IL-2 containing medium for 4 days. T cell-enriched fraction was then incubated with various concentrations of rIL-15.

[b] Culture supernatants were recovered after 24 h and IL-5 production was measured with a specific ELISA. Data are presented as the mean of triplicate cultures ± SEM.

[c] Proliferation response was measured in 72 h cultures by pulsing [^{3}H]thymidine for the last 16 h. S.I. is calculated as TdR in the stimulated cultures/TdR in the unstimulated cultures. Data are presented as the mean of triplicate cultures.

of the Ab. The reason why anti-IL-2Rα Ab did not inhibit IL-5 production induced by IL-2 is not fully clear at this moment. The signal through IL-2Rβ and -γ may be sufficient for IL-5 synthesis, as the Ab preparation used in these experiments effectively inhibited IL-2-induced proliferation of the IL-2-dependent cytotoxic T cell clone (CTLL-2 cell line; data not shown).

It has been reported that the proliferative response induced by IL-2 is mediated by several tyrosine kinases (30–36). The γ-chain is associated with Jak3 and the β-chain is associated with Jak1 and *lck* (40–42). Mutations introduced in several cytoplasmic regions of the β- or γ-chains abrogated the association of these kinases with IL-2R and the transmission of the IL-2 signal at the same time (43). Our present work suggests that not only the proliferation of T cells but also IL-5 synthesis induced by IL-15 is dependent on tyrosine kinases (Fig. 5).

IL-15 might possibly activate the IL-5 gene directly or induce production of another factor from T cells that consequently activates the IL-5 gene. The rapid induction of IL-5 mRNA, which appeared only 3 h after IL-15 stimulation (Fig. 3*A*), strongly suggested that IL-15 directly induces IL-5 gene transcription. Indeed, it was shown for the first time that the approximately 500-bp-long promoter/enhancer segment of the human IL-5 gene located 5′ upstream of the coding region was functionally sufficient for mediating the transcriptional activity induced by IL-15 (Fig. 4). In this 500-bp segment we have found several possible transcriptional elements having more than 80% homology to AP-1, NF-AT, NF-κB, and Oct-1, previously identified in the IL-2 gene (44, 45). It has been reported that transcription factors such as AP-1 (c-*jun*, c-*fos*) (46, 47), c-*myc* (48), and NF-κB (49) are induced by IL-2 stimulation in cytotoxic T cells and B cells. Which specific transcription factor(s) or element(s) is functional in $CD4^+$ T cells following IL-15 stimulation now is under investigation using EMSA (electrophoretic mobility shift analysis) and footprint analysis.

In conclusion, we have demonstrated a novel function of IL-15 to potentiate IL-5 synthesis by human Th cells. Unlike IL-2, which is produced mainly by T cells, IL-15 is produced by a wide variety of tissues and cells, including monocytes/macrophages (1). Locally produced IL-15 may contribute to eosinophilic inflammation by inducing IL-5 production by T cells.

Acknowledgments

The authors thank Dr. Shigeru Morimura for helpful discussions, Dr. Wendy A. Gray for reviewing this manuscript, and Ibuki Ota for secretarial assistance.

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