Interleukin-13 Stimulates the Transcription of the Human a2(I) Collagen Gene in Human Dermal Fibroblasts*

Received for publication, June 22, 2004
Published, JBC Papers in Press, July 22, 2004, DOI 10.1074/jbc.M406951200

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Interleukin-13 (IL-13) is a novel lymphokine produced by activated Type 2 helper cells. In this study, we examined the target genes of IL-13 by the cDNA microarray analysis in human dermal fibroblasts. We focused on the human a2(I) collagen gene, which was one of the IL-13-induced genes by the microarray analysis. IL-13 induced type I collagen protein as well as mRNA in a dose-dependent manner. Actinomycin D, an RNA synthesis inhibitor, significantly blocked the IL-13-mediated up-regulation of a2(I) collagen mRNA expression, whereas cycloheximide, a protein synthesis inhibitor, did not block this up-regulation. In addition, IL-13 treatment induced the promoter activity of a2(I) collagen by nuclear run-on transcription assay and chloramphenicol acetyltransferase assay. IL-13-mediated transcriptional activation of a2(I) collagen gene or type I collagen protein up-regulation was inhibited by the treatment of fibroblasts with a selective phosphoinositide 3-kinase (PI3K) inhibitor, LY294002, or STAT6 antisense oligonucleotide, but not by PD98059, a specific inhibitor of MEK/ERK, or SB202190 or SB203580, specific inhibitors of p38 MAPK; IL-13 induced the phosphorylation of PI3K p85 regulatory subunit and STAT6. These results suggest that IL-13 may play a role in the regulation of extracellular matrix and indicate the possible therapeutic value of the blockade of IL-13 signaling pathways via PI3K and STAT6 in fibrosis.

Sharing many biological properties with IL-4, IL-13 induces cell surface phenotype changes and displays immunomodulatory effects on B cells and human monocytes (4, 5). In contrast to IL-4, IL-13 has been shown not to affect T cells (5, 6). IL-13 induces proliferation and differentiation of B cells, enhances proliferative responses to anti-IgM and anti-CD40 antibodies, up-regulates major histocompatibility complex class II and CD23 expression, and induces anti-CD40-dependent IgE class switch and certain immunoglobulin isotypes such as IgG4 on B cells (1, 4, 6–8). In lipopolysaccharide-stimulated monocytes, IL-13 inhibits the production of chemokines (IL-8 and macrophage inflammatory protein 1), hematopoietic growth factors (granulocyte/macrophage colony-stimulating factor and granulocyte colony-stimulating factor), and proinflammatory cytokines (e.g. tumor necrosis factor, IL-1, and IL-6) (1, 9). Thus, IL-13 is considered to be an anti-inflammatory cytokine.

On the other hand, there have been quite a few reports that discussed its effects on fibroblasts. High affinity IL-13 receptor is reported to be expressed in normal skin fibroblasts (10). Recently, total collagen protein and tissue inhibitor of metalloproteinase-1 protein are up-regulated in normal skin fibroblast after stimulation by IL-13 (11). Moreover, IL-13 inhibits IL-1β-induced matrix metalloproteinases 1 and 3 protein production (11). Therefore, IL-13 is thought to be associated with the regulation of extracellular matrix synthesis in the skin.

In this study, we examined the target genes of IL-13 by the cDNA microarray analysis in human dermal fibroblasts. We focused on the human a2(I) collagen gene, which was induced by IL-13 by the microarray analysis. We investigated the mechanisms regulating the human a2(I) collagen gene expression by IL-13 in dermal fibroblasts.

**EXPERIMENTAL PROCEDURES**

*Reagents—Recombinant human IL-13, IL-4, transforming growth factor (TGF)-β, and pan-specific neutralizing TGF-β antibodies, which have been shown to inhibit TGF-β activity (12, 13), were obtained from R & D Systems (Minneapolis, MN). Anti-type I collagen-UNLB was purchased from SouthernBiotech (Birmingham, AL). Actinomycin D and cycloheximide were purchased from Sigma. Anti-PI3K p85 antibodies and anti-phosphotyrosine (4G10) antibodies were from Upstate Biotechnology, Inc. Interferon-γ, LY294002, PD98059, SB202190, SB203580, and wortmannin were from Calbiochem (La Jolla CA). Anti-STAT6 antibody was from Santa Cruz Biotechnology (Santa Cruz, CA).

*Cell Cultures—Human dermal fibroblasts were obtained by skin biopsy of six healthy donors. All of the biopsies were obtained with informed consent and institutional approval. Primary explant cultures were established in 25-cm² culture flasks in modified Eagle’s medium (MEM) supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamycin, as described previously (14, 15). Monolayer cultures were maintained at 37 °C in 5% CO₂ in air. The fibroblasts between the third and sixth subpassages were used for experiments.

*Microarray—The cDNA microarray hybridization experiments were performed using Intellgene Human CHIP 1K version 1.0 (codes X1071), Intelligene Human DNA CHIP for endocrine disruption study version 1.1 (codes X103) and Intelligene Human Cytokine CHIP Ver-

*This work is supported in part by a grant for scientific research from the Japanese Ministry of Education and by project research for progress in systemic sclerosis from the Japanese Ministry of Health and Welfare. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§ These abbreviations used are: IL, interleukin; PI3K, phosphoinositide 3-kinase; TGF, transforming growth factor; STAT, signal transduction and activator of transcription; MEM, modified Eagle’s medium; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
were visualized using an ECL system (Amersham Biosciences) according to the manufacturer’s recommendations. The densities of bands were measured using densitometer.

**RNA Preparation and Northern Blot Analysis**—Total RNA was extracted using an acid guanidinium thiocyanate-phenol-chloroformal method and analyzed by Northern blotting as described previously (18–20). RNA was subjected to electrophoresis on 1% agarose/formaldehyde gels and blotted onto nylon filters (Roche Applied Science). The filters were UV cross-linked, prehydrized, and sequentially hybridized with probes for α2(I) collagen and GAPDH. The membranes were then washed, exposed to x-ray film, and scanned with a densitometer.

**Plasmid Construction**—Generation of a ~3500 COL1A2/chloramphenicol acetyltransferase construct consisting of the human collagen α2(I) gene fragment (+58 to –3500 bp relative to the transcription start site) linked to the chloramphenicol acetyltransferase reporter gene was previously described (21–23). Plasmids used in the transient transfection assays were purified twice on CsCl gradients, as described previously (21–23). At least two different plasmid preparations were used for each experiment.

**Transient Transfection and Chloramphenicol Acetyltransferase Assay**—Human fibroblasts were grown to 80% confluence in 100-mm dishes in Dulbecco’s MEM with 10% fetal calf serum. Monolayers were washed, and the cells were transfected by the Lipofectin technique (FuGENE 6 Transfectin Reagent; Roche Applied Science) (12–15) with ~3500 COL1A2/chloramphenicol acetyltransferase constructs. pSV-β-galactosidase control vector (Promega) was cotransfected to normalize for transfection efficiency (12–15, 17–20). After incubation overnight, the medium was replaced with MEM containing 0.1% bovine serum albumin or with MEM containing various amounts of cytokines, and incubation was continued for 48 h. The cells were harvested in 0.25 M Tris-HCl (pH 8) and fractured by freeze thawing. Extracts, normalized for protein content as measured by the following 30 (codes X104; Takara, Tokyo, Japan) according to the manufacturer’s protocol. The protocol and the complete listing of nearly 1000, 240, and 550 genes on Intelligent Human CHIP 1K, Intelligene Human DNA CHIP for endocrine disruption study, and Intelligene Human Cytokine CHIP are available on the web, respectively. An approximately 300-bp cDNA region of each gene, which has minimal homology against other genes registered at cDNA database, is spotted on this array (BM BIO, Tokyo, Japan) (16). A gene expression ratio (Cy5/Cy3) was considered significant.

**Summary of IL-13-regulated genes identified by microarray analysis**

| Gene name | Gene accession number | Expression ratio |
|-----------|-----------------------|------------------|
| α2(I) collagen | NM_000689 | 5.96 |
| Activating transcription factor 3 | NM_004024 | 2.60 |
| Pleckstrin homology-like domain, family A, member 1 | AF220656 | 2.32 |
| G protein-coupled receptor | U67784 | 2.02 |
| Hexokinase 1 | AF013685 | 2.02 |
| Nectin helix loop helix 1 | NM6739 | 0.84 |
| Arabidopsis chlorophyll ab-binding protein | AB038714 | 1.72 |
| Carcinomembrane antigen-related cell adhesion molecule 1 | X16354 | 0.12 |
| Hepatic leukemia factor | M9585 | 0.13 |
| Neuropeptide Y receptor Y1 | NM_000909 | 0.14 |
| Protein kinase C-binding protein 2 | AP221520 | 0.15 |
| Aldehyde dehydrogenase 5 family, member A1 | AL031230 | 0.17 |
| Interleukin-16 | M90391 | 0.23 |
| Early growth response 2 | NM_000399 | 0.26 |
| Transcription factor AP-2γ | U86568 | 0.31 |
| Coagulation factor II (thrombin) receptor γ-aminobutyric acid A receptor ε | U92285 | 0.34 |
| Human proteinase activated receptor-2 | U67058 | 0.36 |
| Tumor necrosis factor superfamily, member 11 | AF053712 | 0.36 |
| G protein-coupled receptor 37 | U67460 | 0.37 |
| tumor necrosis factor superfamily, member 10b | NM_001992 | 0.34 |
| α1 integrin | X67874 | 0.41 |
| Rael/Cdc-42 guanine exchange factor 6 | D25304 | 0.41 |
| Bullous pemphigoid antigen 1 | NM_001723 | 0.44 |
| Nephroplasmin 1 | NM_00272 | 0.44 |
| Bone morphogenetic protein 4 | NM_001202 | 0.46 |
| Thyroid hormone receptor interactor 4 | NM_016213 | 0.46 |
| Mitogen-activated protein kinase kinase 6 | U39657 | 0.46 |
| Nuclear protein Sp100 | AF056322 | 0.48 |
| Corticotropin-releasing hormone receptor 1 | X72304 | 0.49 |
| Bone morphogenetic protein 7 | NM_001719 | 0.49 |
| Tumor necrosis factor receptor-III | NM_005658 | 0.50 |
| Bone morphogenetic protein 6 | NM_001718 | 0.56 |
| Interferon regulatory factor 2 | NM_002199 | 0.57 |
| Transmembrane protein 1 | AB001523 | 0.58 |
| Transforming growth factor-β-related genes | | |
| Transforming growth factor-β1 | NM_000660 | 1.01 |
| Transforming growth factor-β2 | NM_003238 | 0.34 |
| Transforming growth factor-β3 | NM_002329 | 0.42 |
| Transforming growth factor-β receptor III | NM_003243 | 0.78 |
| Transforming growth factor-β receptor II | D50683 | 0.62 |
**FIG. 1. Induction of type I collagen protein expression in human dermal fibroblasts by IL-13 stimulation.** A, cultured fibroblasts were stimulated with the indicated amounts of IL-13 for 24 h, and the conditioned medium was collected. The media were analyzed by immunoblotting. One experiment representative of four independent experiments is shown. B, Type I collagen protein levels quantitated by scanning densitometry are shown relative to the level in untreated cells (1.0). The data are expressed as the means ± S.E. of four independent experiments. *, p < 0.01 as compared with the value in untreated cells. C, the time course of the IL-13 effect (10 ng/ml) was determined. One experiment representative of four independent experiments is shown. D, Type I collagen protein levels quantitated by scanning densitometry are shown relative to the level in untreated cells (1.0). The data are expressed as the means ± S.E. of four independent experiments. *, p < 0.01 as compared with the value in untreated cells. E, cultured fibroblasts were stimulated with the indicated cytokines for 72 h. Conditioned medium or cell lysates (normalized for protein concentrations as measured by the Bio-Rad reagent) were subjected to immunoblotting with anti-type I collagen antibody or anti-β-actin antibody, respectively. One experiment representative of four independent experiments is shown. IFN, interferon. F, Type I collagen protein levels quantitated by scanning densitometry and corrected for the levels of β-actin in the same samples are shown relative to the level in untreated cells (1.0). The data are expressed as the means ± S.E. of four independent experiments. *, p < 0.01 as compared with the value in untreated cells.
Bio-Rad reagent, were incubated with butyryl-CoA and \([14]C\)chloramphenicol for 90 min at 37°C. Butyrylated chloramphenicol was extracted using an organic solvent (2:1 mixture of tetramethylpentadecane and xylene) and quantitated with scintillation counting. Each experiment was performed in duplicate. The Mann-Whitney U test was used to determine statistical significance.

**Nuclear Run-on Transcription**—Nuclear run-on transcription analysis was performed according to a modified protocol that uses digoxigenin-labeled UTP (24–26). Briefly, nuclei (2 × 10⁷) from control and IL-13-treated fibroblasts were incubated with digoxigenin-UTP (Roche Applied Science). Purified mRNA from each treatment was hybridized to nylon filters containing linearized and immobilized cDNA plasmids (5 µg) of \(\alpha_2(I)\) collagen, corresponding vector, and GAPDH. After hybridization, the digoxigenin-chemiluminescent detection procedure was performed exactly following the manufacturer’s instructions (Digoxigenin System User’s Guide for Filter Hybridization; Roche Applied Science).

**Immunoprecipitation**—To prepare the extracts of total cellular proteins, the fibroblasts were washed with phosphate-buffered saline at 4°C and solubilized in lysis buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 50 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1 µg/ml leupeptin, 1 µg/ml aprotinin, and 1 µg/ml pepstatin. 500 µg of total cellular protein were incubated with antibodies to the p85 subunit of PI3K or STAT6 at 4°C overnight, followed by 2 h incubation with protein A-agarose (Invitrogen) at 4°C. After three washes in lysis buffer, the immunocomplexes were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto a polyvinylidene difluoride membrane, and incubated with anti-phosphotyrosine antibody (1:1000). The membrane was washed and then incubated with a secondary antibody against mouse IgG (1:500) for 1 h. As a loading control, immunoblotting was also performed using antibodies against p85 or STAT6 (1:1000).

**Treatment of Dermal Fibroblasts with the STAT6 Antisense Oligonucleotide**—We used a STAT6 20-mer antisense phosphorothioate oligonucleotide (CGGTCCTCATCTCAGAGAAGGC), which overlaps the promoter of the STAT6 gene. This same sequence has been found to be
sufficient in blocking STAT6 transcription in vitro (27). A sense oligonucleotide served as the control. Transient transfection was performed using the Lipofectin technique with oligonucleotides as described above.

Statistical Analysis—Statistical analysis was done using the Mann-Whitney test for the comparisons of means. *p values less than 0.05 were considered significant.

RESULTS

Effects of IL-13 on Gene Expression Profiles as Measured by cDNA Microarray Analysis—The technique of differential hybridization of cDNA expression arrays was used to identify differences in the expression pattern of genes between control and IL-13-treated fibroblasts. Among nearly 1800 genes, over 1700 of them showed no significantly different hybridization signal between control and IL-13-treated cultures. The results for important genes are shown in Table I. Human α2(I) collagen gene showed significant induction by IL-13 (5.96-fold). However, transforming growth factor-β-related genes did not show significant induction by IL-13 (TGF-β1, 1.01-fold; TGF-β2, 0.34-fold; TGF-β3, 0.41-fold; TGF-β receptor type II, 0.62-fold; and TGF-β receptor type III, 0.78-fold).

Human α2(I) Collagen Gene Expression in Human Dermal Fibroblasts Is Up-regulated by IL-13—We investigated the effects of IL-13 on the expression of α2(I) collagen gene in human dermal fibroblasts. First, we investigated the effects of IL-13 on type I collagen protein expression in dermal fibroblasts. We investigated the dose dependence of the effect of IL-13 on the expression of type I collagen. Human dermal fibroblasts were cultured until they were confluent and then incubated for an additional 24 h under conditions of serum starvation. The cells were subsequently incubated with the indicated doses of IL-13 prior to the collection of medium. IL-13 increased the synthesis of type I collagen into conditioned medium (Fig. 1, A and B).

Next, to examine the time dependence of the effect of IL-13 on the expression of type I collagen, the fibroblasts were incubated in serum-free medium for the indicated time in the presence or absence of 10 ng/ml IL-13, which was added 12, 24, 48, 72, or 96 h prior to the collection of medium. Type I collagen protein level was elevated after 12 h and markedly increased after 24 h, and the increase was sustained up to after 72 h, compared with the level in control cells (Fig. 1, C and D). In addition, we compared the effects of IL-13, IL-4, TGF-β, or interferon-γ on type I collagen protein expression by dermal fibroblasts. IL-13 or IL-4 induced type I collagen expression modestly, whereas TGF-β had the strongest effect (Fig. 1, E and F).

To determine whether the IL-13-mediated induction of type I collagen protein expression was correlated with an increase of the mRNA level, human dermal fibroblasts were incubated in the presence or absence of 10 ng/ml IL-13 under the same conditions, and mRNA expression was analyzed by Northern blotting. The human α2(I) collagen mRNA level was elevated after stimulation with IL-13 for 6 h and increased after 12 h in comparison with the control level (Fig. 2). Therefore, the effect of IL-13 on the type I collagen protein level paralleled that on the mRNA level.

Mechanisms of IL-13-mediated α2(I) Collagen Gene Up-regulation—To establish whether the increase in α2(I) collagen mRNA levels after IL-13 treatment involves transcriptional activation, we tested the effect of actinomycin D on IL-13-induced α2(I) collagen gene expression. Fibroblasts were treated with IL-13 for 6 h in the presence or absence of a transcriptional inhibitor, actinomycin D (400 ng/ml). Cell viability was determined with trypan blue stain, which demonstrated that actinomycin D did not cause cell death. Actinomycin D completely blocked the IL-13-mediated increase in α2(I) collagen mRNA, suggesting that IL-13 stimulation of α2(I) collagen mRNA involves direct activation of transcription of the α2(I) collagen gene and/or other genes involved in α2(I) collagen gene regulation (Fig. 3). In addition, we asked whether this stimulation requires the synthesis of new proteins. Cycloheximide, an inhibitor of protein synthesis, was added to fibroblasts together with IL-13 for 6 h. Cell viability was determined with trypan blue stain, which demonstrated that cycloheximide (10 μg/ml) did not cause cell death. Cycloheximide did not change α2(I) collagen mRNA in IL-13-treated fibroblasts (Fig.,
FIG. 5. LY294002 and STAT6 antisense oligonucleotide inhibits IL-13-mediated up-regulation of α2(I) collagen promoter activity and type I collagen protein. Human dermal fibroblasts were serum-starved for 24 h and pretreated with the indicated amounts of various inhibitors for 1 h prior to the addition of IL-13 (10 ng/ml) for 24 h. LY294002, PD98059, SB202190, SB203580, and wortmannin were dissolved in Me$_2$SO. The controls were incubated with an equal concentration of Me$_2$SO. A, the plasmid carrying a 3.5-kb fragment of the human α2(I) collagen promoter was used in transient transfections of fibroblasts: The values indicate the α2(I) collagen promoter activities relative to the fibroblasts untreated, which was set at 100. The means ± S.E. of four independent experiments are shown. *, p < 0.01 as compared with the value in untreated cells. B, conditioned medium were subjected to immunoblotting with anti-type I collagen antibodies. One experiment representative of four independent experiments is shown. C, type I collagen protein levels quantitated by scanning densitometry are shown relative to the level in untreated cells (1.0). The data are expressed as the means ± S.E. of four independent experiments. *, p < 0.01 as compared with the value in untreated cells. D, human dermal fibroblasts were serum-starved for 24 h and treated with 10 ng/ml IL-13 for the indicated times. The cell lysates were immunoprecipitated with anti-p85 antibody, followed by immunoblotting with anti-phosphotyrosine (4G10) antibody. The same membrane was then stripped and reprobed with anti-p85 antibody to determine the level of total p85 protein. One experiment representative of three independent experiments is shown. The levels of phosphorylated p85 quantitated by scanning densitometry and corrected for the levels of total p85 in the same samples are shown relative to the level of untreated cells (1.0). The data are expressed as the means ± S.E. of four independent experiments. *, p < 0.01 as compared with the value in untreated cells. E, 2 μg of the plasmid carrying a 3.5-kb fragment of the human α2(I) collagen promoter was used in transient transfections of fibroblasts with 0.5 μg of STAT6 sense or antisense oligonucleotides. After 24 h of incubation, the cells were incubated for 24 h additionally in the absence or presence of 10 ng/ml IL-13. The values indicate the α2(I) collagen promoter activities relative to the IL-13-untreated fibroblasts with sense oligonucleotide, which was set at 100. The means ± S.E. of four independent experiments are shown. *, p < 0.01 as compared with the value in untreated cells. F, for immunoblot analysis using anti-type I collagen antibody, the transient transfection of 2 μg of STAT6 sense or antisense oligonucleotide in human dermal fibroblasts was performed as described under “Experimental Procedures.” After 48 h of incubation, the cells were incubated for 24 h additionally in the absence or presence of 10 ng/ml IL-13. The same membrane was then stripped and reprobed with anti-STAT6 antibody and anti-β-actin antibody to show as a loading control. One experiment representative of four independent experiments is shown. G, the same experiment as shown in D was performed using anti-STAT6 antibody. CAT, chloramphenicol acetyltransferase.
suggesting that IL-13-mediated up-regulation of \( \alpha_2(1) \) collagen mRNA is independent of new protein synthesis.

**Transcriptional Activation of the Human \( \alpha_2(1) \) Collagen Gene by IL-13**—We next examined the effect of IL-13 on the promoter activity of the human \( \alpha_2(1) \) collagen gene. The direct measurement of the specific gene transcription using nuclear run-on transcription analysis showed an elevation in the rate of \( \alpha_2(1) \) collagen gene transcription in fibroblasts treated with IL-13 (4.02-fold) (Fig. 4A). This result was confirmed by chloramphenicol acetyltransferase assay using the −3500 COL1A2/chloramphenicol acetyltransferase constructs. IL-13 induced the promoter activity of \( \alpha_2(1) \) collagen gene in a dose-dependent manner, which was maximal at 10 ng/ml (Fig. 4B). These results suggest that IL-13 mediates the transcriptional activation of the \( \alpha_2(1) \) collagen gene.

**LY294002 and STAT6 Antisense Oligonucleotide Inhibits IL-13-mediated Up-regulation of \( \alpha_2(1) \) Collagen Promoter Activity and Type I Collagen Protein**—To investigate which intracellular signaling pathway plays a crucial role in transcriptional activation of the human \( \alpha_2(1) \) collagen gene in dermal fibroblasts, we examined the effects of inhibitors of intracellular signaling, such as PI3K, MEK/ERK, and p38 mitogen-activated protein kinase (p38 MAPK), on the promoter activity of the \( \alpha_2(1) \) collagen gene. As shown in Fig. 5A, the promoter activity of the \( \alpha_2(1) \) collagen gene induced by IL-13 was inhibited by LY294002, a specific inhibitor of PI3K, in a dose-dependent manner. In contrast, the promoter activity of the gene induced by IL-13 was not changed by PD98059, a specific inhibitor of MEK/ERK, or SB203580, a specific inhibitor of p38 MAPK. Furthermore, the synthesis of type I collagen protein induced by IL-13 in dermal fibroblasts treated with IL-13 was inhibited by LY294002 in a dose-dependent manner (Fig. 5, B and C). Wortmannin, another PI3K inhibitor, decreased the synthesis of type I collagen protein induced by IL-13. In contrast, the synthesis of type I collagen protein was not changed by PD98059 or SB203580. PI3K is composed of the p110 catalytic subunit and the p85 regulatory subunit. The activity of PI3K is regulated through tyrosine phosphorylation of p85. Immunoprecipitation using anti-PI3K p85 antibodies revealed a significant phosphorylation (2.4-fold) of p85 after 5 min of treatment with IL-13, followed by a slow decrease in the cellular level of the phosphorylated p85 (Fig. 5D).

Furthermore, we examined the effect of the antisense oligonucleotide targeting STAT6 on the IL-13-mediated type I collagen up-regulation. The treatment with the STAT6 antisense oligonucleotide showed a marked reduction in the expression of \( \alpha_2(1) \) collagen gene or type I collagen protein in the presence of IL-13 (Fig. 5, E and F). The sense oligonucleotide had no such inhibitory effect. In addition, IL-13 induced STAT6 phosphorylation in a time-dependent manner (Fig. 5G). Taken together, PI3K and STAT6 signaling pathways are essential for the IL-13-mediated induction of type I collagen expression.

**TGF-\( \beta \)** is known to be a major cytokine to induce collagen expression. We investigated whether TGF-\( \beta \) is involved in the increased transcriptional activity of the \( \alpha_2(1) \) collagen gene by IL-13. To examine this possibility, we used pan-specific neutralizing TGF-\( \beta \) antibodies, which have been shown to inhibit TGF-\( \beta \) activity (12, 13). As shown in Fig. 6, neutralizing TGF-\( \beta \) antibodies did not change the promoter activity of the gene stimulated by IL-13, which suggests that TGF-\( \beta \) is not involved in the increased transcriptional activity of the \( \alpha_2(1) \) collagen gene by IL-13.

**DISCUSSION**

To our knowledge, this is the first report showing the target genes of IL-13 by the cDNA microarray analysis in human
be regulated at the transcriptional level but is independent of new protein synthesis. This is because the IL-13-mediated increase in \( \alpha 2(1) \) collagen mRNA was blocked by actinomycin D but not by cycloheximide (Fig. 3) and because IL-13 induced the promoter activity of \( \alpha 2(1) \) collagen gene by both the nuclear run-on transcription assay and the chloramphenicol acetyltransferase assay (Fig. 4).

Intracellular signaling pathways involved in the transcriptional regulation of the human \( \alpha 2(1) \) collagen gene by IL-13 were also investigated in this study. Among signaling pathways investigated in this study, PI3K and STAT6 was shown to be involved in IL-13-mediated up-regulation of \( \alpha 2(1) \) collagen promoter activity and type I collagen protein (Fig. 5). Several studies have demonstrated that IL-13 also activates both PI3K and STAT6 pathway in various kinds of cells (37–40). Furthermore, they were reported to be involved in type I collagen expression in some situations (41–43). These, including our results, suggest that they are one of the crucial signaling pathways in IL-13 signaling as well as type I collagen expression. In our study, the addition of STAT6 antisense nucleotide showed a nearly complete inhibition of IL-13 effect on \( \alpha 2(1) \) collagen gene or type I collagen protein expression. This suggests the presence of cross-talk between PI3K signaling pathway and STAT6 signaling pathway in IL-13-mediated regulation of type I collagen expression.

A recent study reported that IL-13 is a potent stimulator and activator of TGF-\( \beta 1 \) in vivo and that macrophages are the major site of TGF-\( \beta 1 \) expression (44). Moreover, TGF-\( \beta \) is known to be a major cytokine to induce collagen expression. Therefore, we investigated whether TGF-\( \beta 1 \) is involved in the transcriptional regulation of the human \( \alpha 2(1) \) collagen gene by IL-13. The cDNA microarray analysis in human dermal fibroblasts showed that IL-13 did not induce the expression of TGF-\( \beta 1 \), TGF-\( \beta 2 \), TGF-\( \beta 3 \), TGF-\( \beta \) receptor type II, or TGF-\( \beta \) receptor type III (Table I). The IL-13 stimulation of \( \alpha 2(1) \) collagen gene was shown to be independent of new protein synthesis because the IL-13-mediated increase in \( \alpha 2(1) \) collagen mRNA was not blocked by cycloheximide (Fig. 3). Furthermore, pan-specific neutralizing TGF-\( \beta \) antibodies, which have been shown to inhibit TGF-\( \beta \) activity (12, 13), did not change the promoter activity of the gene stimulated by IL-13 (Fig. 5). These results suggest that TGF-\( \beta \) is not involved in the increased transcriptional activity of the \( \alpha 2(1) \) collagen gene by IL-13.

In conclusion, we showed that IL-13 up-regulates the \( \alpha 2(1) \) collagen expression at the transcriptional level using the microarray analysis, Northern blot analysis, nuclear run-on transcription analysis, and chloramphenicol acetyltransferase assay, and we demonstrated that PI3K and the STAT6 signaling pathway are essential for the IL-13-mediated induction of the \( \alpha 2(1) \) collagen gene or type I collagen protein expression. These results suggest that IL-13 may play a role in the regulation of extracellular matrix and indicate the possible therapeutic value of the blockade of IL-13 signaling pathways via PI3K and STAT6 in fibrosis.

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*J. Biol. Chem.* 2004, 279:41783-41791.
doi: 10.1074/jbc.M406951200 originally published online July 22, 2004

Access the most updated version of this article at doi: [10.1074/jbc.M406951200](http://10.1074/jbc.M406951200)

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