ORIGINAL ARTICLE

Cellular Localization of Interleukin 13 Receptor α2 in Human Primary Bronchial Epithelial Cells and Fibroblasts

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Abstract

Background: Interleukin (IL) 13 is a key cytokine in asthma, regulating fibrosis, airway remodeling, induction of immunoglobulin E synthesis by B cells, bronchial hyperresponsiveness, and mucus production. IL-13 signals through the type II IL-4 receptor (IL-4R), which is composed of the IL-4Rα and the IL-13Rα1 chains. Another IL-13 binding chain, IL-13Rα2, binds IL-13 with high affinity but has no known signaling capability and is thought to serve as a decoy receptor providing tight regulation of IL-13 responses.
Methods: In this study, we investigated the cellular localization of IL-13Rα2 in human primary bronchial epithelial cells and fibroblasts using flow cytometry and confocal microscopy, as well as the in vivo expression of IL-13Rα2 in the human bronchial mucosa by means of immunohistochemistry.
Results: IL-13Rα2 is predominantly an intracellular rather than a membrane-bound molecule in both human primary bronchial epithelial cells and fibroblasts and displays a diffuse granular cytoplasmic distribution in both cell types. IL-13Rα2 protein is expressed in vivo in the human bronchial mucosa with its expression being higher in bronchial epithelial cells than bronchial fibroblasts both in vivo and in vitro.
Conclusions: IL-13Rα2 is expressed by both human primary bronchial epithelial cells and fibroblasts as an intracellular protein with a diffuse cytoplasmic distribution. In vivo, IL-13Rα2 is expressed in the human airway mucosa mainly by bronchial epithelial cells.

Key words: IL-13 receptor α2. Intracellular protein. Cellular localization. Cytoplasmic compartment. Bronchial epithelial cells. Bronchial fibroblasts.

Resumen

Antecedentes: La interleucina (IL) 13 es una citocina que tiene un papel clave en el asma, ya que regula la fibrosis, la modificación de las vías respiratorias, la inducción de la síntesis de inmunoglobulina E mediante los linfocitos B, la hiperreactividad bronquial y la producción de mucosidad. La IL-13 transmite señales a través del receptor IL-4 de tipo II (IL-4R) que consta de las cadenas IL-4Rα e IL-13Rα1. Otra cadena de unión de IL-13, la IL-13Rα2, une la IL-13 con una gran afinidad pero no se conoce que tenga ninguna capacidad de transmitir señales y se cree que actúa como un receptor señuelo, proporcionando una rigurosa regulación de las respuestas de la IL-13.
Métodos: En este estudio, se investigó la localización celular de la IL-13Rα2 en células epiteliales bronquiales primarias humanas y en los fibroblastos, a través de la citometría de flujo y la microscopia confocal, así como también la expresión de la IL-13Rα2 in vivo en la mucosa bronquial humana, por medios inmunohistoquímicos.
Resultados: La IL-13Rα2 es una molécula predominantemente intracelular y no de superficie, ya sea en células epiteliales bronquiales primarias humanas, como en fibroblastos, y presenta una distribución citoplasmática granular difusa en ambos tipos de célula. La proteína IL-13R α2 se expresa in vivo en la mucosa bronquial humana y su expresión es más elevada en las células epiteliales bronquiales que en los fibroblastos bronquiales, ya sea in vivo o in vitro.
Conclusiones: La IL-13Rα2 se expresa mediante células epiteliales bronquiales primarias humanas y fibroblastos como una proteína intracelular con una distribución citoplasmática difusa. La IL-13Rα2 in vivo se expresa en la mucosa de las vías respiratorias principalmente mediante las células epiteliales bronquiales.

Palabras clave: Receptor α2 de IL-13. Proteína intracelular. Localización celular. Compartimiento citoplasmático. Células epiteliales bronquiales. Fibroblastos bronquiales.

Introduction

Interleukin (IL) 13 plays a key role as an effector molecule in asthma through many mechanisms including induction of immunoglobulin (Ig) E synthesis by B cells [1], airway hyperresponsiveness [2], airway eosinophilia [3], mucus production [4], and airway remodeling [5,6]. IL-13 exerts its activity via its receptor complex, which consists of the heterodimeric proteins IL-13Rα1 and IL-4Rα [7]. The IL-13Rα1/IL-4Rα heterodimer also serves as an alternative receptor for IL-4, especially in nonhematopoietic cells that do not express the common gamma chain (IL-2Rγ) [8]. Another receptor chain, IL-13Rα2, binds IL-13 with high affinity (Kd of approximately 250 pM) [9].

IL-13Rα2 has no known signaling capability and its short cytoplasmic domain of 17 amino acids does not contain the conserved box-1 region that is critical for downstream signal transduction [10]. IL-13Rα2 is thought to be a decoy receptor or a dominant negative inhibitor that provides tight regulation of IL-13 responses without affecting IL-4 signaling [11]. This hypothesis has been supported by Kawakami and co-workers [12], who found that transfection of Chinese hamster ovary cells (CHO-K1) with IL-13Rα1 and IL-4Rα induced STAT6 phosphorylation, whereas co-transfection with the IL-13Rα2 chain abolished STAT6 activation. Similarly, overexpression of IL-13Rα2 in renal carcinoma cell lines reduced their ability to respond to IL-13 [13], while the pro-B cell line Ba/F3 expressing IL-13Rα2 was unable to proliferate in response to IL-13 [14]. IL-13Rα2 can also inhibit IL-13 activities through other mechanisms. For instance, in the glioblastoma cell line 293T, transient expression of IL-13Rα2 inhibited IL-13 and IL-4-mediated STAT6 activation, possibly through the physical interaction of the IL-13Rα2 protein and the IL-4Rα chain [15]. In vivo studies have also highlighted the role of IL-13Rα2 in the inhibition of IL-13-mediated responses. IL-13Rα2-deficient mice displayed enhanced macrophage development and IgE production [16], and *Schistosoma mansoni*-infected IL-13Rα2-deficient mice showed a marked exacerbation in hepatic fibrosis compared to wild-type mice [17]. However, recently, an alternative explanation for the actions of IL-13Rα2 has been put forward in which it is suggested that in fact signaling through IL-13Rα2 in macrophages results in the activation of an AP-1 variant containing c-jun and Fra-2 and consequent production of transforming growth factor ß in a model of murine lung fibrosis [18]. These apparently conflicting roles for IL-13Rα2 remain to be reconciled. IL-13Rα2 has a more restricted pattern of expression when compared to that of IL-13Rα1 and was found to be expressed in fibroblasts, airway smooth muscle cells, and airway epithelial cells [19].

Although IL-13Rα2 is predicted to be a membrane-bound protein, in fact, it has been shown by flow cytometry to be predominantly an intracellular molecule in various human cell types including monocytes and nasal epithelial cells [20], airway fibroblasts [21], and bronchial epithelial cells [22]. It has previously been reported that A549 cells and human primary nasal epithelial cells exhibited diffuse cytoplasmic IL-13Rα2 expression when cells were visualized under confocal microscopy [20]. However, the cellular localization of IL-13Rα2 in human primary bronchial epithelial cells and fibroblasts has not been described, and its in vivo expression in the human airways has not been reported. The purpose of this study was to investigate the cellular localization of IL-13Rα2 in human primary bronchial epithelial cells and fibroblasts, and to assess the pattern of IL-13Rα2 expression in the human bronchial mucosa.

Materials and Methods

Cells and Reagents

Primary bronchial epithelial cells and primary bronchial fibroblasts were established from bronchial brushings and bronchial biopsies, respectively, as previously described [6,23]. Cells were obtained by fiberoptic bronchoscopy from 3 mild asthmatic subjects with a mean age of 32 years and mean forced expiratory volume in 1 second (FEV_1) of 86% (range, 72% – 98%), all receiving inhaled $ß_2$-agonists only (salbutamol). Primary airway epithelial cells were cultured at 37ºC, 5% CO_2, in bronchial epithelium growth medium (BEGM; Clonetics, San Diego, California, USA) supplemented with 50 IU/mL penicillin (Sigma-Aldrich, Poole, UK), and 50 µg/mL streptomycin (Sigma-Aldrich) in collagen-coated tissue culture flasks. When confluent, the cells were passaged using trypsin (Sigma-Aldrich) and were allowed to further expand until used for assays at passage 3. Primary bronchial fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM; GIBCO Invitrogen, Paisley, UK) containing 10% heat-inactivated fetal calf serum (FCS) (Sigma-Aldrich), 2 mM L-glutamine (Biowhittaker, Wokingham, UK), 50 IU/mL penicillin, and 50 µg/mL streptomycin. Cells were used for experimentation between passages 5 to 8. A549 lung adenocarcinoma (American Type Culture Collection, ATCC) and 16HBE human bronchial epithelial cell lines were grown in DMEM containing 10% heat-inactivated FCS, 50 IU/mL penicillin, 50 µg/mL streptomycin, and 2 mM L-glutamine. NCI-H292 lung mucoepidermoid adenocarcinoma (ATCC) and U937 promyelomonocytic (ATCC) cell lines were maintained in RPMI 1640 (GIBCO Invitrogen) supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 50 IU/mL penicillin, and 50 µg/mL streptomycin. The Jurkat T cell leukemia cell line (European Cell and Animal Culture Collection, Salisbury, UK) was cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, and 50 µM ß-mercaptoethanol (Sigma-Aldrich).

Reverse-Transcriptase Polymerase Chain Reaction

Total RNA was extracted from established primary cell cultures and cell lines using TRIzol (Life Technologies, Paisley, UK) and from whole blood (1.5 mL) using the RNeasy Blood Kit (Qiagen, Crawley, UK) according to the manufacturers' instructions. Reverse transcription was performed for 1 hour at 37ºC using 1 µg total RNA with 1 µM oligo(dT)15 as a primer and 4 U Omniscript reverse transcriptase (Qiagen) in the presence of 0.5 mM dNTPs, 10 U RNase inhibitor (Ambion, Austin, Texas, USA), in a total volume of 20 µL. Polymerase chain reaction (PCR) involved 2 µL cDNA template (from the 20 µL cDNA reaction), standard PCR buffer, 0.6 µM of each primer, 0.2 mM dNTPs, and

0.025 U/μL Jumpstart Taq DNA polymerase (Sigma-Aldrich) in a total volume of 25 μL using a TETRAD thermocycler (MJ Research, Boston, Massachusetts, USA). The following $MgCl_2$ concentrations and primers were used: IL-13Rα2 (amplicon size: 426 base pairs) amplification was performed with 2 mM $MgCl_2$, forward 5'-GGA GCA TAC CTT TGG GAC CT-3', and reverse 5'-TTG GCC ATG ACT GGA AAC TG-3'; APRT (amplicon size: 245 base pairs) amplification was done with 1 mM $MgCl_2$, forward 5'-GCT GCG TGC TCA TCC GAA AG-3', and reverse 5'-CCT TAA GCG AGG TCA GCT CC-3'. Thermal cycling included a single cycle at 95ºC for 5 minutes followed by 35 cycles at 94ºC for 30 seconds, annealing at 64ºC for IL-13Rα2 and 56ºC for APRT for 30 seconds, extension at 72ºC for 30 seconds, and finally a 72ºC soak for 10 minutes. Aliquots of PCR reactions were run on 2% agarose gels and visualized by ethidium bromide staining. All amplicons were designed to span introns and were tested to ensure they would not amplify genomic DNA. The specificity of amplicons was confirmed by direct sequencing using a BigDye Terminator version 3.0 (Applied Biosystems, Warrington, UK) with the products run on an ABI PRISM 377 DNA Sequencer (Applied Biosystems).

Flow Cytometry

Primary bronchial epithelial cells, primary bronchial fibroblasts, and airway epithelial cell lines were detached from flasks using trypsin (Sigma-Aldrich). Cells were rinsed with phosphate-buffered saline (PBS) containing 0.1% sodium azide (Sigma-Aldrich) and resuspended in PBS containing 2% FCS at a concentration of 1×10^6 cells/mL. Then, 1×10^5 cells were incubated for 1 hour at 4ºC with a monoclonal anti-IL-13Rα2 antibody (B-D13, Diaclone, Boldon, UK) in a final antibody concentration of 2.5 μg/mL, then washed 3 times with PBS and resuspended in PBS containing a 1:50 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse IgG secondary antibody (Becton Dickinson, Oxford, UK) and 2% FCS. After incubation for 1 hour in the dark at 4ºC, cells were washed in cold PBS and then processed for analysis using a FACScan flow cytometer (Becton Dickinson). Data were analyzed using WinMDI 2.8 software. For intracellular staining, cells were fixed in 1% paraformaldehyde and 0.2% saponin (both Sigma-Aldrich), which was added to the antibody and wash solutions as permeabilization with saponin is reversible. The correct antibody dilutions for flow cytometry were established by titration. Isotype controls were used to confirm specificity and to enable subtraction of the background staining. Nonviable cells were excluded by the addition of 2.5 μg/mL propidium iodide (Sigma-Aldrich) in PBS.

Immunohistochemistry

Glycol methacrylate-embedded bronchial biopsies, obtained from 3 mild asthmatic subjects, were incubated with a monoclonal anti-IL-13Rα2 antibody (BD-13) for 1 hour at room temperature. Immunohistochemistry was performed using the avidin-biotin complex immunoperoxidase kit (Dako, Ely, Cambridgeshire, UK) according to the manufacturer's instructions. Slides were developed using the liquid diamino benzidine substrate-chromogen system (Dako) according to the manufacturer's instructions and then lightly counterstained with hematoxylin.

Confocal Microscopy

Cells derived from 3 mild asthmatic subjects were grown on tissue culture collagen-coated slides, washed 3 times in cold PBS with 1% FCS and subsequently fixed in 2% paraformaldehyde pH 7.4 for 15 minutes. Cells were incubated with a monoclonal anti-IL-13Rα2 antibody (B-D13) at a final concentration of 2.5 μg/mL for 1 hour in a solution containing PBS, 1% FCS, and 2% goat serum. Following PBS washes, an Alexa Fluor 633-conjugated goat anti-mouse secondary antibody (Molecular Probes European BV, Leiden, The Netherlands) was added for 1 hour. Coverslips were washed in PBS and counterstained with the nuclear dye SYTOX Orange (Molecular Probes European BV) (1μL in 1mL PBS) for 2 minutes. For permeabilized cells, 0.2% saponin was added to the antibody solution and to all subsequent washes. After the final wash, slides were covered with a coverslip and the edges sealed with nail polish. Cells were observed on a Leica SP2 confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany).

Ethical Approval

Ethical approval for this study was granted by the Southampton and South West Hampshire Joint Research Ethics Committee and written informed consent was obtained from all subjects undergoing bronchoscopy.

Results

Expression of IL-13Rα2 in Airway Cells and Bronchial Mucosa

We initially established that IL-13Rα2 specific transcript is expressed in primary bronchial epithelial cells and primary bronchial fibroblasts (Figure 1). In addition, various airway epithelial cell lines including NCI-H292, A549, and 16HBE, as well as the promonocytic U937 cells also expressed IL-13Rα2 mRNA. IL-13Rα2 transcript was not detected in Jurkat cells, whereas total blood leukocytes displayed only weak expression (Figure 1). We subsequently investigated the in vivo expression of IL-13Rα2 in bronchial biopsies derived from 3 mild asthmatic subjects. The columnar epithelial cells of the airway mucosa were the main sites of IL-13Rα2 immunoreactivity (Figure 2). Basal epithelial cells and fibroblasts displayed a weak staining pattern, while infiltrating inflammatory cells showed no positive staining.

IL-13Rα2 is an Intracellular Protein in Primary Bronchial Epithelial Cells and Primary Bronchial Fibroblasts

We evaluated the cell-surface expression of IL-13Rα2 on primary bronchial epithelial cells, primary bronchial fibroblasts, various airway epithelial cell lines, and U937 cells by flow cytometry. We used a commercially available specific monoclonal mouse anti-human IL-13Rα2 antibody (B-D13) that recognizes epitopes of the extracellular domain of the protein. No cell-surface IL-13Rα2 expression was detected in any of the cell types examined (data not shown). In contrast, permeabilized cells exhibited strong intracellular expression

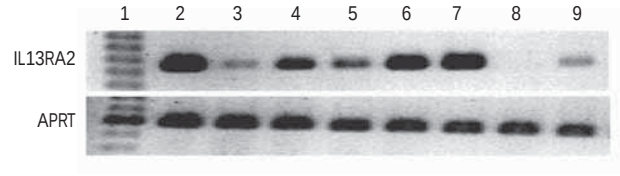

Figure 1. mRNA expression of the interleukin 13 receptor α2 (IL-13Rα2). mRNA expression of IL-13Rα2 and adenosine phosphoribosyltransferase (APRT) was investigated by reverse-transcriptase polymerase chain reaction in primary bronchial epithelial cells (lane 2), U937 cells (lane 3), 16HBE cells (lane 4), A549 cells (lane 5), NCI-H292 cells (lane 6), primary bronchial fibroblasts (lane 7), Jurkat cells (lane 8), and peripheral blood leukocytes (lane 9); lane 1 shows DNA size ladder. IL-13Rα2 is expressed by bronchial epithelial cells and fibroblasts, as well as airway epithelial cell lines. There is no IL-13Rα2 expression by Jurkat cells (lane 8), and only weak expression in U937 cells (lane 3) and white blood cells (lane 9). APRT was used as an internal positive control. Results are representative of 4 separate experiments. The number of cycles selected resulted in product being multiplied in the linear range of the reaction.

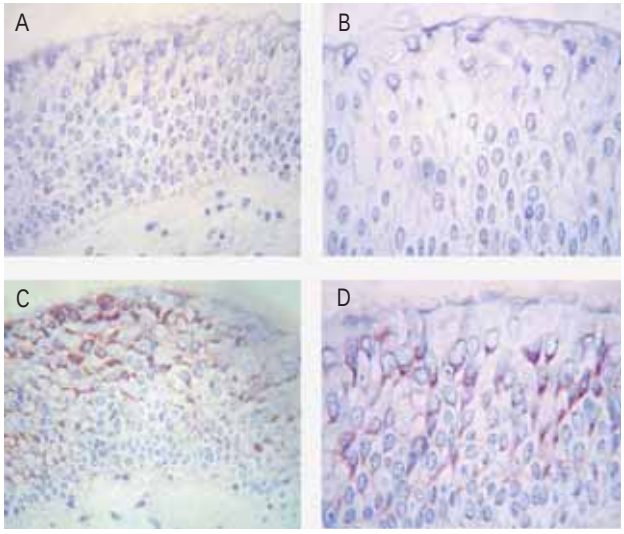

Figure 2. Expression of IL-13Rα2 in the human bronchial mucosa. Bronchial biopsies were sectioned and stained for the presence of IL-13Rα2. The columnar epithelial cells show intense immunoreactivity, whereas basal epithelial cells exhibit a weaker staining pattern (C, D). No staining was seen in the control sections using an isotype-matched control antibody (A, B). Original magnification, ×40 (A, C) or ×63 (B, D). Representative sections from biopsies of 3 mild asthmatic subjects are shown.

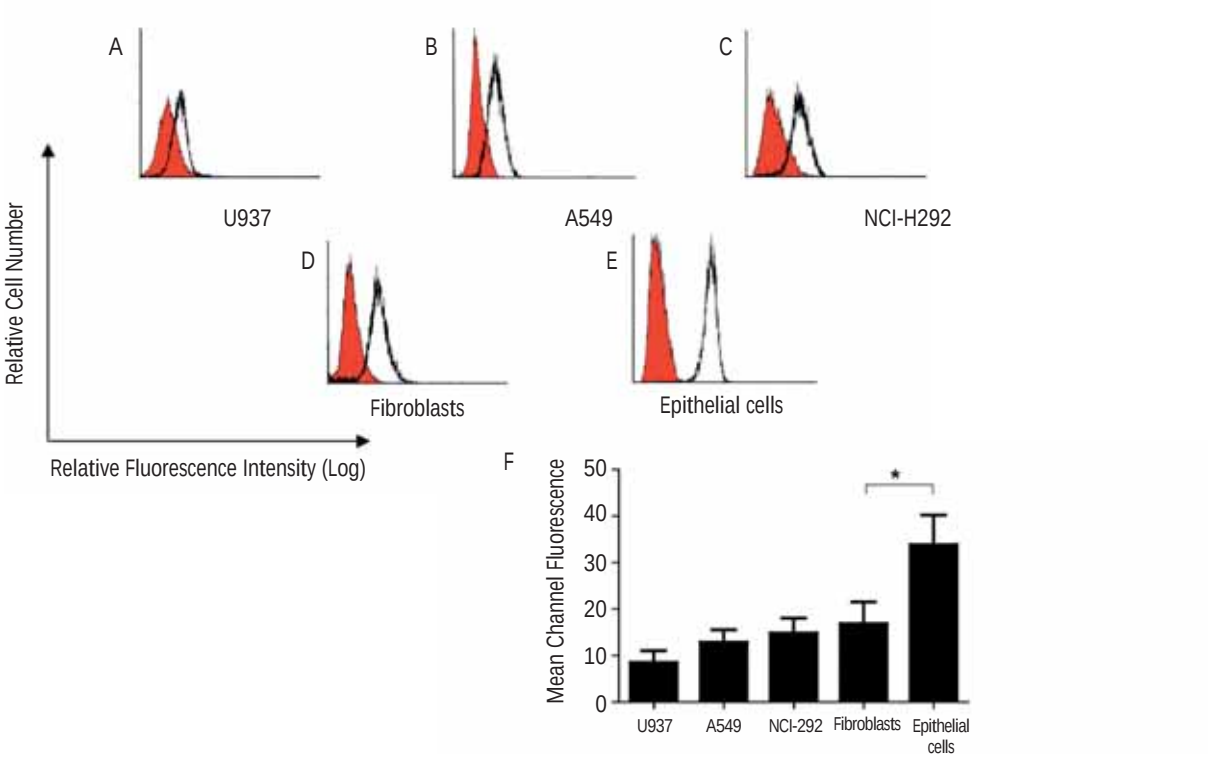

Figure 3. Intracellular stores of interleukin 13 receptor α2 (IL-13Rα2) in various cell types. U937 cells (A), A549 cells (B), NCI-H292 cells (C), primary bronchial fibroblasts (D), and primary bronchial epithelial cells (E) were permeabilized with saponin and stained with a specific monoclonal anti-IL-13Rα2 antibody. Fluorescence intensity by flow cytometry is represented by open histograms with a black line; red filled histograms correspond to background staining of isotype-matched controls. Representative histograms are shown in panels A-E. F, Mean channel fluorescence (arbitrary units) in permeabilized cells stained for IL-13Rα2. Bars show the mean of 3 experiments; whiskers indicate SD. Statistical significance was determined by *t* test. Asterisk (*) indicates $P = .008$.

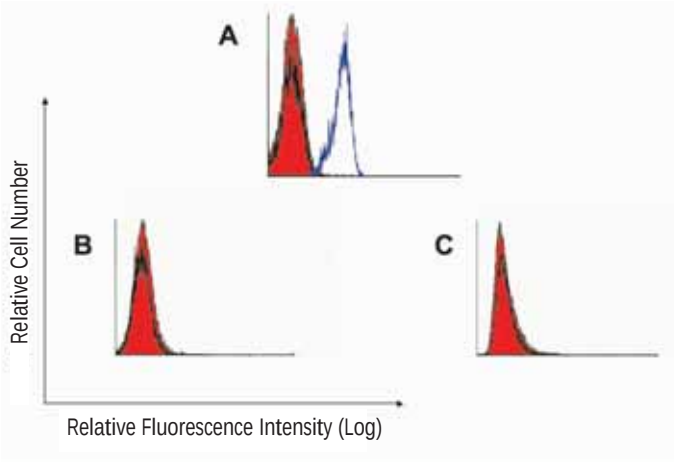

Figure 4. Specificity of the monoclonal anti-IL-13Rα2 antibody analyzed by flow cytometry. A, Primary bronchial epithelial cells were either left intact (black line) or permeabilized with saponin (blue line) and stained with anti-IL-13Rα2 antibody. The red filled histogram shows isotype control staining. There is no staining on intact cells, whereas permeabilized cells have positive staining. Jurkat cells, either intact (B) or permeabilized (C), were stained with a specific monoclonal IL-13Rα2 antibody and analyzed by flow cytometry. Fluorescence intensity is represented by open histograms with a black line; red filled histograms refer to the background staining of isotype-matched controls. There is no staining either on intact or in permeabilized Jurkat cells.

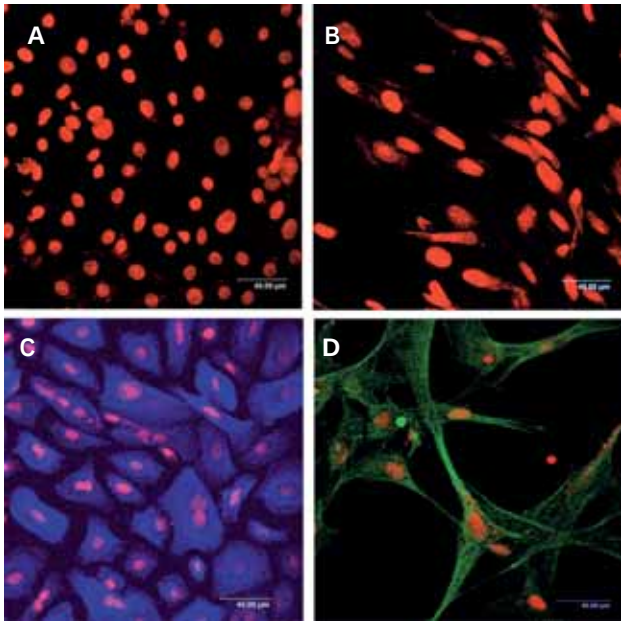

Figure 5. Localization of interleukin 13 receptor α2 (IL-13Rα2) in permeabilized human primary bronchial epithelial cells and fibroblasts. Primary bronchial epithelial cells (A, C) and primary bronchial fibroblasts (B, D) were cultured on glass coverslips, fixed, permeabilized, and stained with an anti-IL-13Rα2 antibody (C, D), or an isotype-matched control antibody (A, B). Maximal projections of optical sections taken at 1 µm intervals by confocal laser scanning microscopy are shown. Specific cytoplasmic IL-13Rα2 staining is shown in blue (C) or green (D), whereas nuclei, counterstained with SYTOX Orange, are shown in red. IL-13Rα2 has a uniform granular cytoplasmic fluorescent staining pattern (C, D), whereas the specimens incubated with the control antibody show no staining (A, B). Representative images from 3 separate experiments are shown. Scale bar = 40 µm.

of IL-13Rα2, suggesting that IL-13Rα2 is predominantly an intracellular protein (Figure 3). Expression of IL-13Rα2 in primary bronchial epithelial cells was approximately twice as high as in primary bronchial fibroblasts (Figure 3). We next examined whether the intracellular expression of IL-13Rα2, shown by flow cytometry, might be due to nonspecific binding of the antibody to intracellular proteins. We had previously shown by reverse-transcriptase PCR analysis that Jurkat cells do not express IL-13Rα2 mRNA, and would therefore not be expected to express IL-13Rα2 protein. Analysis of intact and permeabilized Jurkat cells by flow cytometry, using the monoclonal anti-IL-13Rα2 antibody (BD-13), revealed that Jurkat cells do not express either cell-surface or intracellular IL-13Rα2 protein (Figure 4), indicating that antibody binding in the intracellular compartment was specific.

Cellular Localization of IL-13Rα2

To identify the cellular localization of IL-13Rα2, we performed confocal laser scanning microscopy on both nonpermeabilized and saponin-permeabilized primary bronchial epithelial cells and primary bronchial fibroblasts. Intact primary bronchial epithelial cells and intact primary bronchial fibroblasts had negligible surface staining (data not shown). In contrast, both permeabilized primary bronchial epithelial cells and permeabilized primary bronchial fibroblasts had a bright uniform granular pattern of cytoplasmic staining in both cell types (Figure 5). Analysis of individual confocal planes revealed that IL-13Rα2 expression is confined to the cytoplasm with no nuclear staining in both bronchial epithelial cells and bronchial fibroblasts (data not shown). In all experiments, use of appropriate isotype-matched control antibodies revealed no staining.

Discussion

In this report, we describe the cellular localization of IL-13Rα2 in human primary bronchial epithelial cells and fibroblasts, as well as the pattern of IL-13Rα2 expression in the human bronchial mucosa.

Initial experiments showed that specific IL-13Rα2 mRNA transcripts are present in primary bronchial epithelial cells, primary bronchial fibroblasts, U937 cells, and various airway epithelial cell lines, in accordance with previous studies [20-22]. In addition, weak IL-13Rα2 mRNA expression was found in peripheral leukocytes. Flow cytometry showed no

cell-surface IL-13Rα2 expression in either primary bronchial epithelial cells or primary bronchial fibroblasts. This was also confirmed by confocal microscopy, which showed negligible surface IL-13Rα2 expression on both cell types. Bernard and co-workers [13] found high cell-surface IL-13Rα2 expression in unstimulated human glioma cell lines, but not in renal carcinoma cell lines, by flow cytometry, suggesting that IL-13Rα2 surface expression varies among different human cell types. We found IL-13Rα2 to be an intracellular molecule in primary bronchial epithelial cells and fibroblasts, as well as various airway epithelial cell lines and U937 cells, in agreement with previous studies [20-22]. In vitro expression of IL-13Rα2 protein in primary bronchial epithelial cells was approximately twice that of expression in bronchial fibroblasts when analyzed by flow cytometry. Similarly, in vivo data showed that bronchial epithelial cells are the main site of IL-13Rα2 immunoreactivity in the human airways. We demonstrated that intracellular binding of the monoclonal anti-IL-13Rα2 antibody used in our studies is specific, since no IL-13Rα2 protein expression was detected in the intracellular compartment of permeabilized Jurkat cells, previously shown to lack IL-13Rα2 mRNA expression.

Confocal microscopy showed that the intracellular pool of IL-13Rα2 has a diffuse granular pattern in the cytoplasmic compartment of both primary bronchial epithelial cells and primary bronchial fibroblasts. To our knowledge, this is the first study demonstrating the cellular localization of IL-13Rα2 in primary bronchial epithelial cells and fibroblasts. Our findings are in accordance with those of Daines and Khurana Hersey [20], who showed by confocal microscopy that IL-13Rα2 has a diffuse cytoplasmic distribution in primary human epithelial nasal cells and A549 cells. In agreement with their study, we also observed some bright localized cytoplasmic staining in both bronchial epithelial cells and fibroblasts that may represent distinct vesicles containing IL-13Rα2. Although the functional role of intracellular IL-13Rα2 has not yet been fully elucidated, in a recent in vitro study using U937 cells and murine splenocytes stably transfected with Flag-human IL-13Rα2, intracellular IL-13Rα2 was shown to serve as a reservoir of biologically active soluble IL-13Rα2 [24]. In addition, soluble IL-13Rα2 was found in bronchoalveolar lavage fluid from asthmatics and normal controls [25], while the intracellular pool of IL-13Rα2 was shown to mobilize to the cell surface in response to interferon γ and phorbol myristate acetate in U937 cells and primary human monocytes [20].

In conclusion, we have demonstrated that IL-13Rα2 is predominantly an intracellular rather than a membrane-bound protein in human primary bronchial epithelial cells and fibroblasts and displays a diffuse granular cytoplasmic distribution in both cell types. Furthermore, it is expressed in vivo in the human airway mucosa mainly by bronchial epithelial cells. Further studies are warranted to determine the functional role of intracellular IL-13Rα2 in the human airways.

Acknowledgments

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