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Involvement of retinoic acid-inducible gene-I in the IFN-γ/STAT1 signalling pathway in BEAS-2B cells

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ABSTRACT: Bronchial epithelial cells play an important role in airway host defence, and interferon (IFN)-γ controls immune reactions by regulating the expression of various genes in bronchial epithelial cells. Signal transducer and activator of transcription 1 (STAT1) is the key transcriptional factor in IFN-γ signalling. Retinoic acid-inducible gene-I (RIG-I) is a member of the DExH box family of proteins and designated a putative RNA helicase. RNA helicases play diverse roles in regulation of gene expression and cellular functions, and RIG-I is implicated in antiviral responses.

The aim of the present study was to investigate the effect of IFN-γ on RIG-I expression in a cell line derived from human bronchial epithelial cells, BEAS-2B.

Induction of RIG-I in response to IFN-γ was found in BEAS-2B cells. Induction of RIG-I by IFN-γ was also demonstrated in another pulmonary epithelial cell line, NCI-H292. Transfection of BEAS-2B cells with RIG-I complementary DNA resulted in the upregulation of STAT1. Induction of IFN-γ-inducible protein 10 by IFN-γ was enhanced in the cells overexpressing RIG-I.

It is concluded that retinoic acid-inducible gene-I may play an important role in the regulation of immunological reactions in bronchial epithelial cells elicited by interferon-γ.

KEYWORDS: BEAS-2B, interferon-γ, interferon-γ-inducible protein 10, retinoic acid-inducible gene-I, signal transducer and activator of transcription 1

Bronchial epithelial cells play an important role in host defence against microbial infections by producing a wide variety of factors involved in inflammatory reactions [1]. Cytokines, such as tumour necrosis factor-α, interferon (IFN)-γ and interleukin (IL)-4, serve as agonists for bronchial epithelial cells, and these factors are involved in the pathogenesis of inflammatory lung diseases. IFN-γ exerts pleiotropic effects by regulating the expression of various genes. In bronchial epithelial cells, IFN-γ induces expression of IFN-γ-inducible protein 10 (IP-10) [2], monocyte chemoattractant protein (MCP)-1 [3], intercellular adhesion molecule-1 [4], c-Met/hepatocyte growth factor receptor [5], fractalkine [6], p11 [7] and eotaxin [8]. Expression of these IFN-γ-stimulated genes is known to be mediated through the signalling pathway of Janus kinase (JAK) 1/2/signal transducer and activator of transcription 1 (STAT1) [9]. Expression of STAT1 is also stimulated by IFN-γ [10].

Retinoic acid-inducible gene-I (RIG-I) is a member of the DExH box family of proteins, and has two caspase recruitment domains (CARDs) at its N-terminus and a C-terminal helicase domain. RIG-I is regarded as a putative helicase and suggested to play an important role in innate antiviral responses [11]. IFN-γ is a key cytokine for antiviral responses, and it has previously been shown that IFN-γ induces expression of RIG-I in vascular smooth muscle cells [12], bladder epithelial cells [13] and MCF-7 breast cancer cells [14]. However, details of the biological function of RIG-I are unknown, and no information is available on the expression of RIG-I in bronchial epithelial cells.

The present study was undertaken to address the effect of IFN-γ on RIG-I expression in human bronchial epithelial cells, BEAS-2B.

MATERIALS AND METHODS

Cells

BEAS-2B cells, a cell line derived from human bronchial epithelial cells [15], were cultured using Dulbecco's modified Eagle medium (InVitrogen, Carlsbad, CA, USA) supplemented with 10% foetal bovine serum (FBS) (InVitrogen) as previously described [16]. NCI-H292 pulmonary epithelial cells [17] were cultured using RPMI

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1640 (InVitrogen) supplemented with 10% FBS. Cells were incubated with recombinant human (rh) IFN-γ (Roche Diagnostics, Mannheim, Germany) or rhIL-4 (R&D Systems, Minneapolis, MN, USA) for the indicated time. The effect of pre-treatment with a selective inhibitor of JAK2, JAK3 and epidermal growth factor receptor autophosphorylation, α-cyano-(3,4-dihydroxy)-*N*-benzylcinnamide (AG490; Calbiochem, La Jolla, CA, USA), was examined. The pre-treatment was performed according to a report on another type of lung epithelial cell [18]: AG490 was dissolved in dimethyl sulphoxide, and the cells pre-incubated for 30 min with 50 μM AG490 prior to stimulation with IFN-γ. AG490 and its vehicle did not affect cell viability.

RT-PCR

Total RNA was isolated from the cells using an RNAeasy total RNA isolation kit (Qiagen, Hilden, Germany). First-strand complementary DNA (cDNA) was synthesised from 1 μg total RNA using Moloney murine leukaemia virus reverse transcriptase and primer Oligo$(dT)_{12–18}$ (both InVitrogen). The cDNAs for RIG-I, STAT1, IP-10, MCP-1, eotaxin and glyceraldehyde-3-phosphate dehydrogenase were amplified by PCR using *Taq* DNA polymerase (Qiagen). The sequences of primers (custom synthesised by Greiner Japan, Atsugi, Japan), annealing temperature and product size are shown in table 1. Eotaxin cDNA was amplified by nested PCR: the first-round PCR was performed using eotaxin forward (F) and reverse (R) primers F1 and R1, and, subsequently, 2 μL of a 1:20 dilution of the first-round product was subjected to second-round amplification using eotaxin primers F2 and R2. The products were analysed by electrophoresis on 1.0–2.0% agarose gels, which were stained with ethidium bromide.

Western blot analysis

Cells were washed twice with 20 mM cold PBS (pH 7.4) and lysed using Laemmli's lysis buffer. Cell lysates were subjected to electrophoresis on 6–9% (RIG-I) or 4–20% (STAT1) gradient polyacrylamide gels. The proteins were transferred to a polyvinylidene difluoride membrane, and RIG-I was detected using rabbit anti-RIG-I antiserum (1:20,000 dilution), a horseradish peroxidase-labelled anti-rabbit immunoglobulin G (Kirkegaard Perry, Gaithersburg, MD, USA) and a Supersignal West Pico chemiluminescent substrate (Pierce, Rockford, IL, USA) [19]. Western blotting for STAT1 and phosphorylated STAT1 (pSTAT1) was performed using rabbit antibodies directed against STAT1 and pSTAT1 (both Cell Signaling Technology, Beverly, MA, USA) as previously described [20].

Transfection with RIG-I complementary DNA

In order to examine the biological effect of RIG-I expression in BEAS-2B cells, the cells were transfected with cDNAs encoding full-length RIG-I (RIG-I (full); amino acids 1–925) or deletion mutants of RIG-I: RIG-I (N) (amino acids 1–284), which lacks the C-terminus and encodes only the CARDs (''ΔRIG-I'' in [11]), and RIG-I (C) (amino acids 218–925), which lacks the N-terminus and encodes only the helicase domain. The cells, at 50–80% confluence in a six-well plate, were transfected with cDNA (400 ng·well^{-1}) using an Effectene transfection reagent (Qiagen) for 24 h. In order to assess the efficiency of the transfection, cells were transfected with cDNA encoding green fluorescent protein (GFP) in a similar manner. GFP expression was observed in ~70% of the cells. The transfected cells were treated with 5 ng·mL^{-1} IFN-γ, and cellular RNA and protein were collected after additional incubation for 6 and 24 h, respectively. RT-PCR and Western blot analyses were performed as described above. More than 90% of cells were viable throughout the experiments, and the transfection reagent alone had no effect on cell viability.

The transfection experiment was also attempted with NCI-H292 cells; however, the efficiency was <10% for transient transfection using GFP cDNA.

TABLE 1 Oligonucleotide primers for RT-PCR

	Primers	Annealing temperature ℃	Cycles	Product size bp
RIG-I	F: 5′-GCATATTGACTGGACGTGGCA-3′	58	29	644
	R: 5′-CAGTCATGGCTGCAGTTCTGTC-3′			
STAT1	F: 5′-CAGTTCTCCCAAGGGAGTTAG-3′	55	27	649
	R: 5′-GTATGCAGTGCCACGGAAAGC-3′			
IP-10	F: 5′-ACCTCCAGTCTCAGCACCATG-3′	60	31	761
	R: 5′-TGGGAGGATGGCAGTGGAAG-3′			
MCP-1	F: 5′-AAACTGAAGCTCGCACTCTCGC-3′	60	25	353
	R: 5′-ATTCTTGGGTTGTGGAGTGAGT-3′			
Eotaxin	F1: 5′-TCACGCCAAAGCTCACACCT-3′	55	30	
	R1: 5′-TTATGGCTTTGGAGTTGGAGAT-3′			
	F2: 5′-CCCAACCACCTGCTGCTTTAACCTG-3′	59	15	208
	R2: 5′-TGGCTTTGGAGTTGGAGATTTTTGG-3′			
GAPDH	F: 5′-CCACCCATGGCAAATTCCATGGCA-3′	60	30	598
	R: 5′-TCTAGACGGCAGGTCAGGTCCACC-3′			

bp: base pair; RIG-I: retinoic acid-inducible gene-I; STAT1: signal transducer and activator of transcription 1; IP-10: interferon-γ-inducible protein 10; MCP: monocyte chemoattractant protein; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; F: forward; R: reverse; G: guanine; C: cytosine; A: adenine; T: thymidine.

IP-10 ELISA

The cells transfected with RIG-I (full) cDNA were treated with 5 ng·mL^{-1} IFN-γ for 24 h, and the conditioned medium was collected. The level of IP-10 in the conditioned medium was measured using an ELISA kit (R&D Systems).

RESULTS

RIG-I is induced in BEAS-2B cells stimulated with IFN-γ

IFN-γ stimulated BEAS-2B cells to express RIG-I (fig. 1b) and the corresponding mRNA (fig. 1a) in a concentration-dependent manner. The time course of RIG-I mRNA expression is shown in figure 2a. RIG-I mRNA expression was induced shortly after stimulation with 5 ng·mL^{-1} IFN-γ and reached a maximal level in 2–4 h. IFN-γ also induced expression of STAT1, IP-10 and MCP-1 mRNA. Figure 2b shows the time-dependent induction of RIG-I by IFN-γ; protein expression lagged several hours behind mRNA expression. STAT1 was also upregulated by IFN-γ. Elimination of FBS from the culture medium had no effect on RIG-I expression (data not shown).

The effect of IL-4 on expression of RIG-I mRNA is shown in figure 3a. IL-4 induced expression of eotaxin mRNA, but did not affect expression of RIG-I in response to IFN-γ. Pre-treatment of cells with the JAK2/3 inhibitor AG490 partially inhibited expression of MCP-1 mRNA induced by IFN-γ, but had no effect on RIG-I expression (fig. 3b).

Overexpression of RIG-I enhances STAT1 expression

The effect of RIG-I overexpression on the expression of other IFN-γ-inducible genes is shown in figure 4a. Transfection of the cells with RIG-I (full) cDNA resulted in enhanced expression of STAT1 mRNA, but IFN-γ-induced upregulation of STAT1 was not affected. Conversely, transfection with RIG-I (full) cDNA did not induce IP-10 mRNA, but IFN-γ-induced expression of IP-10 mRNA was enhanced. Expression of MCP-1 mRNA was not affected by overexpression of RIG-I. Upregulation of STAT1 in the cells transfected with RIG-I (full) cDNA was confirmed (fig. 4b). Transfection of the cells with cDNA encoding deletion mutants had no effect on expression of STAT1, IP-10 or MCP-1 mRNAs. Expression of RIG-I was confirmed in the cells transfected with RIG-I cDNA (fig. 4c).

Next, the effect of RIG-I on the level of pSTAT1 was examined (fig. 4c). Transfection with RIG-I (full) cDNA alone did not induce phosphorylation of STAT1, but the level of pSTAT1 after IFN-γ treatment was increased in the cells overexpressing RIG-I.

Effect of RIG-I transfection on IP-10 production

RIG-I cDNA transfection alone did not induce expression of IP-10, but the IFN-γ-induced secretion of IP-10 was significantly increased in cells transfected with RIG-I cDNA (fig. 5).

IFN-γ induces expression of RIG-I in NCI-H292 cells

The results of RT-PCR analyses of RIG-I mRNA expression in NCI-H292 cells are shown in figure 6a–c. IFN-γ induced expression of RIG-I mRNA in a concentration- and time-dependent manner. Neither IL-4 nor AG490 affected

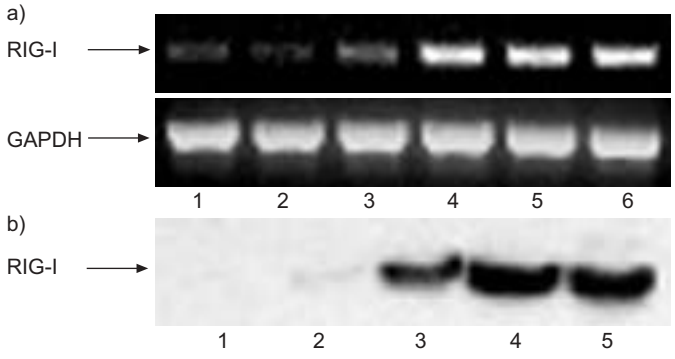

FIGURE 1. Interferon (IFN)-γ-induced expression of retinoic acid-inducible gene-I (RIG-I) and the corresponding mRNA in BEAS-2B cells. a) BEAS-2B cells were incubated with 5 pg·mL^{-1}–50 ng·mL^{-1} IFN-γ (lane 1: 0 pg·mL^{-1}; lane 2: 5 pg·mL^{-1}; lane 3: 50 pg·mL^{-1}; lane 4: 500 pg·mL^{-1}; lane 5: 5 ng·mL^{-1}; lane 6: 50 ng·mL^{-1}) for 4 h and the cells subjected to total RNA extraction. Single-strand complementary DNA was synthesised from 1 μg total RNA, and RT-PCR analysis of RIG-I and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was performed. b) Cells were stimulated with 5 pg·mL^{-1}–50 ng·mL^{-1} IFN-γ (lane 1: 0 pg·mL^{-1}; lane 2: 50 pg·mL^{-1}; lane 3: 500 pg·mL^{-1}; lane 4: 5 ng·mL^{-1}; lane 5: 50 ng·mL^{-1}) for 24 h. The cells were then lysed and subjected to sodium dodecylsulphate-polyacrylamide gel electrophoresis, and proteins were transferred to a polyvinylidene difluoride membrane. RIG-I, with a molecular mass of 101 kDa, was detected using rabbit anti-RIG-I antiserum and horseradish peroxidase-labelled anti-rabbit immunoglobulin G.

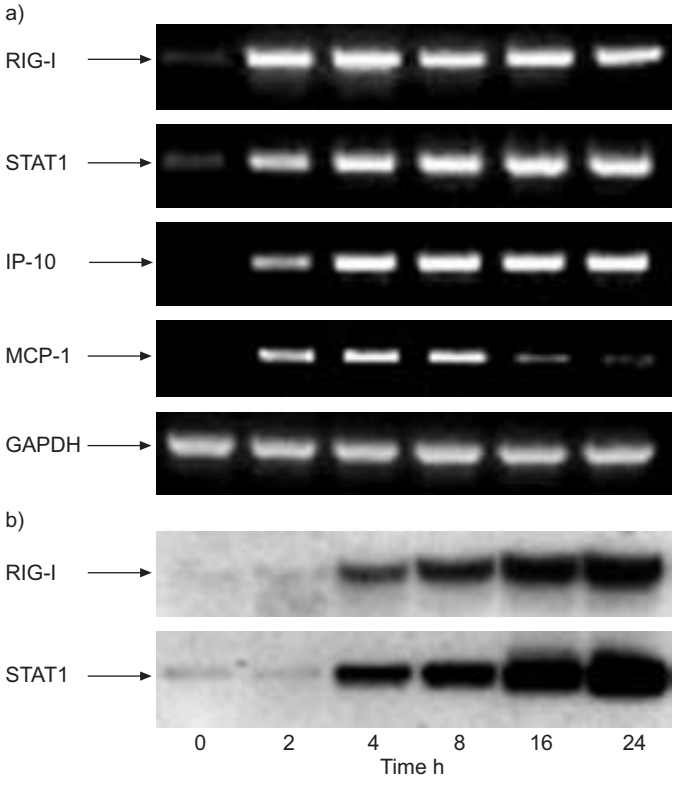

FIGURE 2. Interferon (IFN)-γ upregulates expression of retinoic acid-inducible gene-I (RIG-I) and the corresponding mRNA in a time-dependent fashion in BEAS-2B cells. Cells were stimulated with 5 ng·mL^{-1} IFN-γ for up to 24 h. a) RNA was extracted and RT-PCR analysis performed for RIG-I, signal transducer and activator of transcription 1 (STAT1), IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein (MCP)-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). b) Cell lysate was prepared and subjected to Western blot analysis for RIG-I and STAT1.

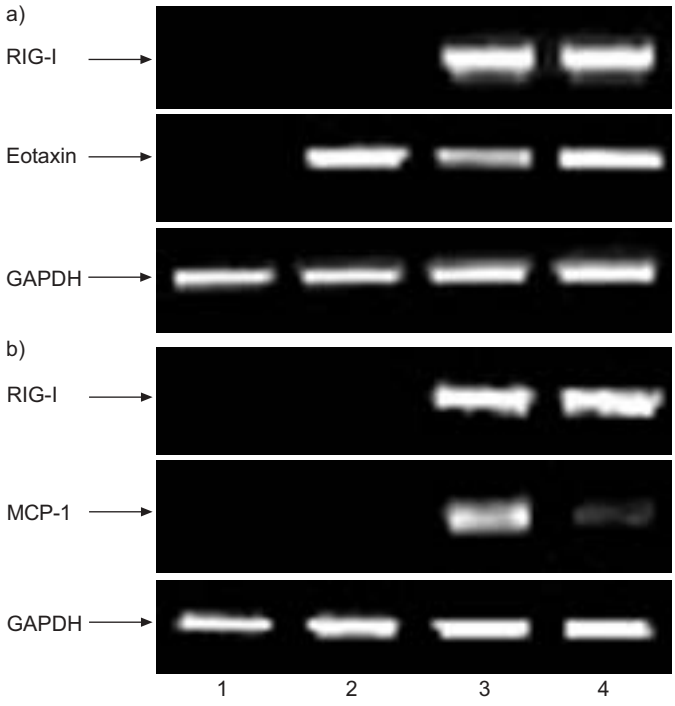

FIGURE 3. Effects of interleukin (IL)-4 and α-cyano-(3,4-dihydroxy)-*N*-benzylcinnamide (AG490) on expression of retinoic acid-inducible gene-I (RIG-I) mRNA induced by interferon (IFN)-γ. a) The cells were co-treated with 5 ng·mL^{-1} IFN-γ (lanes 3 and 4) and 5 ng·mL^{-1} IL-4 (lanes 2 and 4) for 4 h and RNA was extracted. RT-PCR was performed for RIG-I, eotaxin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). b) The cells were pre-treated with 50 μM AG490 (lanes 2 and 4) for 30 min, and then treated with 5 ng·mL^{-1} IFN-γ (lanes 3 and 4) for 4 h. RNA was extracted from cells and RT-PCR was performed for RIG-I, monocyte chemoattractant protein (MCP)-1 and GAPDH.

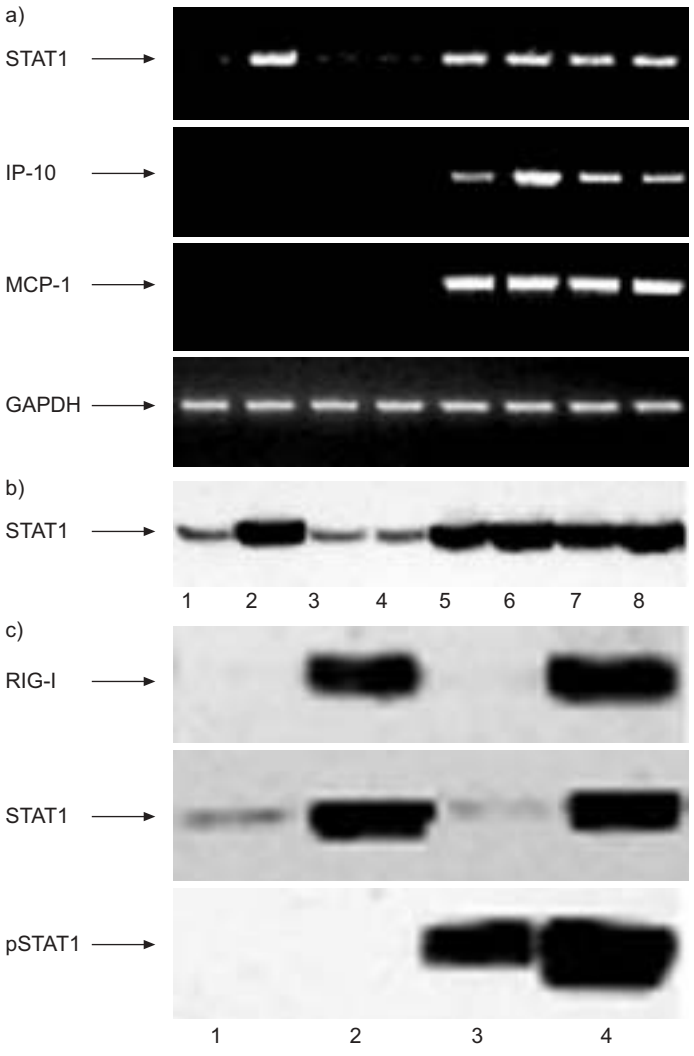

FIGURE 4. Effect of retinoic acid-inducible gene-I (RIG-I) overexpression on the expression of other interferon (IFN)-γ-inducible genes and signal transducer and activator of transcription 1 (STAT1) in BEAS-2B cells. cDNA encoding full-length RIG-I (RIG-I (full); lanes 2 and 6) or a deletion mutant (RIG-I(N) (lanes 3 and 7) or RIG-I(C) (lanes 4 and 8)) was transfected into BEAS-2B cells, and, after 24 h, the cells were treated with 5 ng·mL^{-1} IFN-γ (lanes 5–8) (lanes 1 and 5: transfection control). a) After incubation for a further 6 h, RNA was extracted and RT-PCR analyses were performed for STAT1, IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein (MCP)-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). b) Cell lysate was prepared after a further 24-h incubation and subjected to Western blot analysis for STAT1. c) The cells transfected with RIG-I (full) cDNA (lanes 2 and 4) were stimulated with IFN-γ (lanes 3 and 4) for 15 min and lysed. The lysates were subjected to Western blot analyses for RIG-I, STAT1 and phosphorylated STAT1 (pSTAT1).

expression of RIG-I mRNA induced by IFN-γ. IFN-γ-induced RIG-I expression was also demonstrated in NCI-H292 cells by Western blot analysis (fig. 6d and e).

DISCUSSION

T-lymphocytes play a central role in the regulation of immune responses through the secretion of different sets of cytokines. T-helper cells (Th) type 1 are considered to be involved in cell-mediated immunity, and Th2 in allergic reactions; the Th1/Th2 balance may be important in various pathological conditions. IFN-γ is a potent cytokine with a wide range of functions, including antiviral and immunomodulatory activities, and its major source is Th1. IL-4 is known as a major cytokine expressed by Th2. In bronchial epithelial cells, the Th1 cytokine IFN-γ is considered important in immunological reactions against pathogens, and the Th2 cytokines to play a role in allergic diseases such as asthma. In addition, clinical bronchial inflammation is often associated with a combination or imbalance of Th1 and Th2 reactions. Therefore, it is important to examine and compare the effects of IFN-γ and IL-4 on the function of bronchial epithelial cells. In the present study, it was found that IFN-γ upregulates the expression of RIG-I in BEAS-2B and NCI-H292 bronchial epithelial cells. Conversely, IL-4 did not induce expression of RIG-I or affect the RIG-I expression induced by IFN-γ. The induction of eotaxin mRNA [8] in response to IFN-γ and/or IL-4 was confirmed in a parallel experiment. These results suggest that RIG-I mediates the immunological or inflammatory reactions induced by IFN-γ in bronchial epithelial cells. IFN-γ activates JAK1 and/or JAK2, and this is followed by the phosphorylation of transcriptional factor STAT1. Pre-treatment of cells with AG490, a specific JAK2/3 inhibitor, partially inhibited the IFN-γ-induced MCP-1 mRNA expression, although it did not

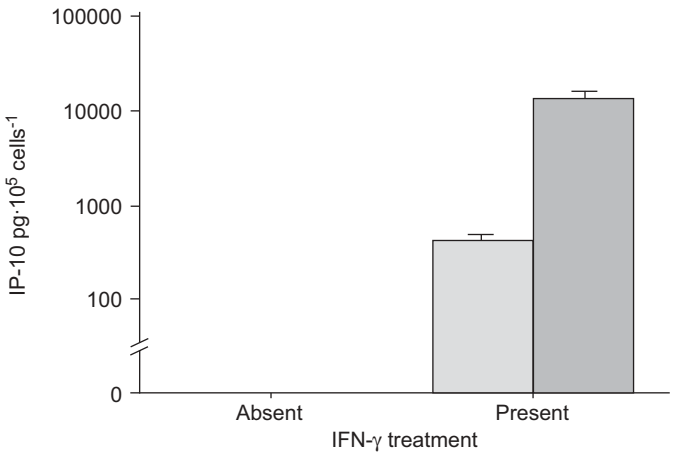

FIGURE 5. Effect of retinoic acid-inducible gene-I (RIG-I) on production of interferon-(IFN)-γ-inducible protein 10 (IP-10) by BEAS-2B cells treated with IFN-γ. The cells were transfected with full-length RIG-I complementary DNA (■) (■: transfection control). After 24 h, the cells were washed twice and incubated for a further 24 h in fresh medium containing 5 ng·mL^{-1} IFN-γ. The conditioned medium was collected and an IP-10 ELISA was performed. Data are presented as mean±SD (n=3). Note that the concentration of IP-10 is shown on a logarithmic scale.

affect the expression of RIG-I mRNA; JAK2 may not be involved in the expression of RIG-I in response to IFN-γ.

RIG-I is induced by retinotic acid in a promyelocytic leukaemia cell line [21] and in vascular endothelial cells stimulated with lipopolysaccharide [19]. Expression, in response to RIG-I overexpression, of cyclooxygenase-2 was found in T24 bladder epithelial cells [19] and IFN-γ-stimulated gene 15 in MCF-7 breast cancer cells [14]. Transfection of BEAS-2B cells with RIG-I (full) cDNA resulted in the induction of STAT1 and the corresponding mRNA. Since expression of MCP-1 mRNA was not affected by RIG-I cDNA transfection, each of the IFN-γ-inducible genes is differentially regulated by RIG-I.

STAT1 is a key mediator in IFN-γ signalling. In cells stimulated with IFN-γ, JAK1 and/or JAK2 activate STAT1 by tyrosine phosphorylation. Activated STAT1, in turn, serves as a transcription factor and directly regulates the expression of key proteins that control cellular responses [22]. In addition to STAT1 phosphorylation status, the mechanisms that regulate STAT1 expression may play an important role in controlling cellular responses. It has been reported that transcription factor zinc finger binding protein 89 is required for constitutive expression of STAT1 [23], and that interferon regulatory factor 1 is important in the induction of STAT1 mRNA [10]. The present findings suggest that RIG-I may be a new member of the factors regulating STAT1 expression and implicated in the IFN-γ signalling pathway.

IP-10 is a member of the CXC chemokine family and plays an important role in the recruitment of activated T-cells. Expression of IP-10 in BEAS-2B cells was induced by IFN-γ, as previously reported in other types of cell [24]. RIG-I cDNA transfection enhanced expression of IP-10 in response to IFN-γ, and RIG-I may be involved in the IFN-γ-induced expression of IP-10. However, RIG-I cDNA transfection alone did not induce expression of IP-10. Therefore, the expression of RIG-I and

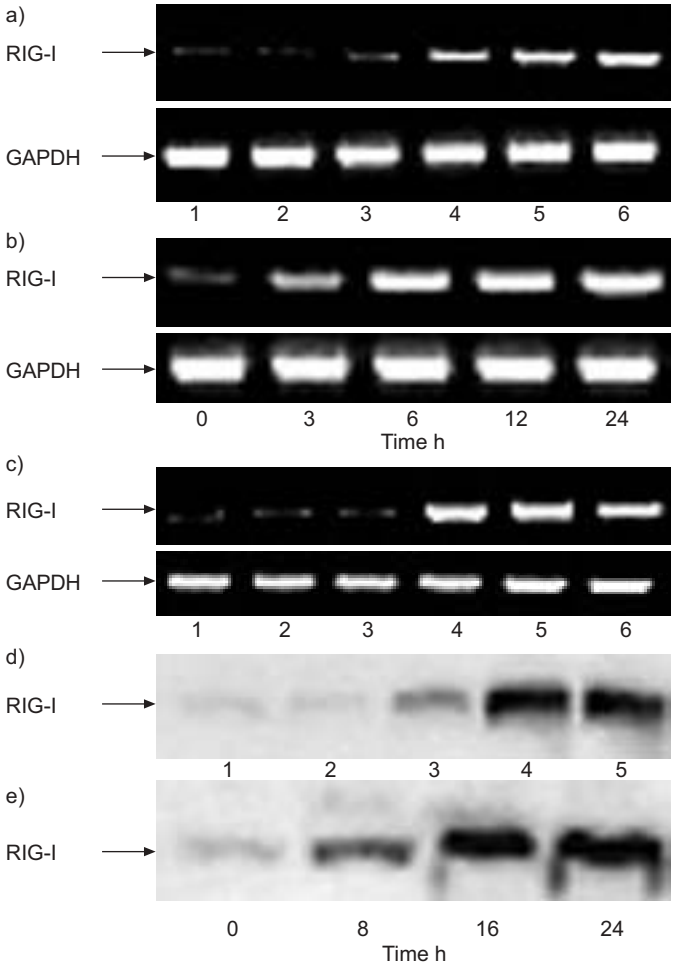

FIGURE 6. Expression of retinoic acid-inducible gene-I (RIG-I) in NCI-H292 cells treated with interferon (IFN)-γ. NCI-H292 cells were treated with IFN-γ in a similar manner to that described in figures 1–3. a–c) RNA was extracted, and RT-PCR analyses were performed for RIG-I and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): a) IFN-γ concentration-dependent (lane 1: 0 pg·mL^{-1}; lane 2: 5 pg·mL^{-1}; lane 3: 50 pg·mL^{-1}; lane 4: 500 pg·mL^{-1}; lane 5: 5 ng·mL^{-1}; lane 6: 50 ng·mL^{-1}); and b) time-dependent expression of RIG-I mRNA; and c) effects of interleukin (IL)-4 (lanes 2 and 5) or α-cyano-(3,4-dihydroxy)-*N*-benzylcinnamide (AG490) (lanes 3 and 6) (lanes 1–3: no IFN-γ; lanes 4–6: IFN-γ). d, e) The cells lysates were subjected to Western blotting for RIG-I. RIG-I was induced in a concentration-dependent (lane 1: 0 pg·mL^{-1}; lane 2: 50 pg·mL^{-1}; lane 3: 500 pg·mL^{-1}; lane 4: 5 ng·mL^{-1}; lane 5: 50 ng·mL^{-1}) (d) and time-dependent (e) manner by IFN-γ.

IP-10 are not regulated by a simple cause–effect link, and RIG-I may serve in a mechanism for amplifying induction of IP-10. Enhanced expression of STAT1 caused by RIG-I resulted in increased pSTAT1 levels after IFN-γ stimulation. This may explain, at least in part, the enhanced expression of IP-10 induced by IFN-γ in the cells transfected with RIG-I cDNA. However, MCP-1 mRNA expression was not altered by RIG-I overexpression despite an increased pSTAT1 level. This suggests the existence of different regulatory mechanisms for IP-10 and MCP-1 expression in response to IFN-γ.

RIG-I is a member of the DExH box family of proteins [25] and has two CARDs at the N-terminus and a C-terminal helicase

domain. DExH box proteins are involved in various biological processes related to nucleic acid metabolism, such as transcription, pre-mRNA splicing, ribosomal biogenesis, RNA transport, RNA interference and RNA degradation [26]. The effects of deletion mutants lacking the N- or C-terminus were examined. Transfection of the cells with cDNA encoding these mutants had no effect on the expression of STAT1, which was induced by overexpression of RIG-I (full). This suggests that both the CARDs and the helicase domain are essential in the induction of STAT1. Overexpression of RIG-I alone did not increase pSTAT1 levels, and the induction of STAT1 by RIG-I is independent of STAT1 phosphorylation. Details of the mechanisms by which RIG-I induces the expression of STAT1 are still unknown, and should be clarified in future studies.

BEAS-2B cells were derived from human bronchial epithelial cells by introducing viral H-*ras* [15], and have been shown to possess characteristics quite similar to those of primary bronchial epithelial cells. BEAS-2B and primary bronchial epithelial cells show a similar spectrum of responsiveness to various pathogens and stimuli [27–30]. BEAS-2B cells exhibit an intracellular signalling system almost identical to that found in primary bronchial epithelial cells [2, 29]. IFN-γ-induced expression of RIG-I was also observed in NCI-H292, another cell line derived from respiratory epithelial cells. RIG-I may be involved in Th1-type responses in airway epithelial cells, and the role of RIG-I in airway inflammation and related diseases should be examined in animal models and clinical samples.

It can be concluded that interferon-γ induces expression of retinoic acid-inducible gene-I in BEAS-2B human bronchial epithelial cells, and that retinoic acid-inducible gene-I may be involved in the expression of signal transducer and activator of transcription 1 and interferon-γ-inducible protein 10. Retinoic acid-inducible gene-I may contribute, at least in part, to the cellular responses induced by interferon-γ during immunological and inflammatory reactions in bronchial epithelial cells.

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REFERENCES

1 Alder KB, Fischer BM, Wright DT, Cohn LA, Becker S. Interactions between respiratory epithelial cells and cytokines: relationships to lung inflammation. *Ann N Y Acad Sci* 1994; 725: 128–145.
2 Sauty A, Dziejman M, Taha RA, *et al.* The T cell-specific CXC chemokines IP-10, Mig, and I-TAC are expressed by activated human bronchial epithelial cells. *J Immunol* 1999; 162: 3549–3558.
3 van der Velden VH, Verheggen MM, Bernasconi S, *et al.* Interleukin-1β and interferon-γ differentially regulate release of monocyte chemotactic protein-1 and interleukin-8 by human bronchial epithelial cells. *Eur Cytokine Netw* 1998; 9: 269–277.
4 Subauste MC, Choi DC, Proud D. Transient exposure of human bronchial epithelial cells to cytokines leads to persistent increased expression of ICAM-1. *Inflammation* 2001; 25: 373–380.
5 Nagahori T, Dohi M, Matsumoto K, *et al.* Interferon-γ upregulates the c-Met/hepatocyte growth factor receptor expression in alveolar epithelial cells. *Am J Respir Cell Mol Biol* 1999; 21: 490–497.
6 Fujimoto K, Imaizumi T, Yoshida H, Takanashi S, Okumura K, Satoh K. Interferon-γ stimulates fractalkine expression in human bronchial epithelial cells and regulates mononuclear cell adherence. *Am J Respir Cell Mol Biol* 2001; 25: 233–238.
7 Huang XL, Pawliczak R, Yao XL, *et al.* Interferon-γ induces p11 gene and protein expression in human epithelial cells through interferon-γ-activated sequences in the p11 promoter. *J Biol Chem* 2003; 278: 9298–9308.
8 Meyer-Hoffert U, Lezcano-Meza D, Bartels J, Montes-Vizuet AR, Schroder JM, Teran LM. Th2- and to a lesser extent Th1-type cytokines upregulate the production of both CXC (IL-8 and gro-α) and CC (RANTES, eotaxin, eotaxin-2, MCP-3 and MCP-4) chemokines in human airway epithelial cells. *Int Arch Allergy Immunol* 2003; 131: 264–271.
9 Decker T, Kovarik P, Meinke A. GAS elements: a few nucleotides with a major impact on cytokine-induced gene expression. *J Interferon Cytokine Res* 1997; 17: 121–134.
10 Wong LH, Sim H, Vhatterjee-Kishore M, *et al.* Isolation and characterization of a human STAT1 gene regulatory element: inducibility by interferon (IFN) type I and II and role of IFN regulatory factor-1. *J Biol Chem* 2002; 277: 19408–19417.
11 Yoneyama M, Kikuchi M, Natsukawa T, *et al.* The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004; 5: 730–737.
12 Imaizumi T, Yagihashi N, Hatakeyama M, *et al.* Expression of retinoic acid-inducible gene-I in vascular smooth muscle cells stimulated with interferon-γ. *Life Sci* 2004; 75: 1171–1180.
13 Imaizumi T, Yagihashi N, Hatakeyama M, *et al.* Upregulation of retinoic-acid inducible gene-I in T24 urinary bladder carcinoma cells stimulated with interferon-γ. *Tohoku J Exp Med* 2004; 203: 313–316.
14 Cui XF, Imaizumi T, Yoshida H, Borden EC, Satoh K. Retinoic acid-inducible gene-I is induced by interferon-γ and regulates the expression of interferon-γ stimulated gene 15 in MCF-7 cells. *Biochem Cell Biol* 2004; 82: 401–405.
15 Amstad P, Reddel RR, Pfeifer A, Malan-Shibley L, Mark GE 3rd, Harris CC. Neoplastic transformation of a human bronchial epithelial cell line by a recombinant retrovirus encoding viral Harvey ras. *Mol Carcinog* 1998; 1: 151–160.
16 Kumagai M, Imaizumi T, Suzuki K, *et al.* 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits the IL-1β-induced expression of granulocyte-macrophage colony-stimulating factor in BEAS-2B bronchial epithelial cells. *Tohoku J Exp Med* 2004; 202: 69–76.
17 Shibuya Y, Hirasawa N, Sakai T, *et al.* Negative regulation of the protein kinase C activator-induced ICAM-1 expression in the human bronchial epithelial cell line NCI-H292

by p44/42 mitogen-activated protein kinase. *Life Sci* 2004; 75: 435–446.
18 Liu J, Kern JA. Neuregulin-1 activates the JAK-STAT pathway and regulates lung epithelial cell proliferation. *Am J Respir Cell Mol Biol* 2002; 27: 306–313.
19 Imaizumi T, Aratani S, Nakajima T, *et al.* Retinoic acid-inducible gene-I is induced in endothelial cells by LPS and regulates expression of COX-2. *Biochem Biophys Res Commun* 2002; 292: 274–279.
20 Imaizumi T, Kumagai M, Nishi N, *et al.* 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits IFN-γ-induced galectin-9 expression in cultured human umbilical vein endothelial cells. *Int Arch Allergy Immunol* 2003; 131: 57–61.
21 Sun YWL. RIG-I, a human homologue gene of RNA helicase, is induced by retinoic acid during the differentiation of acute promyelocytic leukemia cells. PhD thesis. Shanghai, Shanghai Institute of Haematology, 1997.
22 Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. *Annu Rev Biochem* 1998; 67: 227–264.
23 Bai L, Merchant JL. Transcriptional factor ZBP-89 is required for STAT1 constitutive expression. *Nucleic Acids Res* 2003; 31: 7264–7270.
24 Wang X, Yue TL, Ohlstein EH, Sung CP, Feuerstein GZ. Interferon-inducible protein-10 involves vascular smooth muscle cell migration, proliferation, and inflammatory response. *J Biol Chem* 1996; 271: 24286–24293.
25 Walker JE, Saraste M, Runswick MJ, Gay NJ. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J* 1982; 1: 945–951.
26 Schwer B. A new twist on RNA helicase: DExH/D box proteins as RNPases. *Nat Struct Biol* 2001; 8: 113–116.
27 Thomas LH, Friedland JS, Sharland M, Becker S. Respiratory syncytial virus-induced RANTES production from human bronchial epithelial cells is dependent on nuclear factor-κB nuclear binding and is inhibited by adenovirus-mediated expression of inhibitor of κB α. *J Immunol* 1998; 161: 1007–1016.
28 Pichavant M, Delneste Y, Jeannin P, *et al.* Outer membrane protein A from *Klebsiella pneumoniae* activates bronchial epithelial cells: implication in neutrophil recruitment. *J Immunol* 2003; 171: 6697–6705.
29 Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP. Activation of airway epithelial cells by Toll-like receptor agonists. *Am J Respir Cell Mol Biol* 2004; 31: 358–364.
30 Ieki K, Matsukura S, Kokubu F, *et al.* Double-stranded RNA activates RANTES gene transcription through co-operation of nuclear factor-κB and interferon regulatory factors in human airway epithelial cells. *Clin Exp Allergy* 2004; 34: 745–752.