IL-22 suppresses IFN-γ–mediated lung inflammation in asthmatic patients

Davide Pennino, MSc,[a,d] Pankaj K. Bhavsar, MD,[b] Renate Effner, BSc,[a] Simona Avitabile, PhD,[c] Pascal Venn, BSc,[d] Maria Quaranta, PhD,[a] Viviana Marzaioli, PhD,[a] Liliana Cifuentes, MD,[a,e] Stephen R. Durham, MD, FRCP,[d] Andrea Cavani, MD,[c] Kilian Eyerich, MD,[e] Kian Fan Chung, MD,[b] Carsten B. Schmidt-Weber, PhD,[a] and Stefanie Eyerich, PhD[a] *Munich, Germany, London, United Kingdom, and Rome, Italy*

Background: IL-22 controls tissue homeostasis by both proinflammatory and anti-inflammatory effects. However, the anti-inflammatory mechanisms of IL-22 remain poorly investigated.
Objective: We sought to investigate the anti-inflammatory role for IL-22 in human asthma.
Methods: T-cell lines derived from lung biopsy specimens of asthmatic patients were characterized by means of flow cytometry. Human bronchial epithelial cells from healthy and asthmatic subjects were stimulated with IL-22, IFN-γ, or the combination of both cytokines. Effects of cytokine stimulation were investigated by using whole-genome analysis, ELISA, and flow cytometry. The functional consequence of cytokine stimulation was evaluated in an *in vitro* wound repair model and T cell–mediated cytotoxicity experiments. *In vivo* cytokine expression was measured by using immunohistochemistry and Luminex assays in bronchoalveolar lavage fluid of healthy and asthmatic patients.
Results: The current study identifies a tissue-restricted antagonistic interplay of IL-22 and the proinflammatory cytokine IFN-γ. On the one hand, IFN-γ antagonized IL-22–mediated induction of the antimicrobial peptide S100A7 and epithelial cell migration in bronchial epithelial cells. On the other hand, IL-22 decreased epithelial susceptibility to T cell–mediated cytotoxicity by inhibiting the IFN-γ–induced expression of MHC-I, MHC-II, and CD54/intercellular adhesion molecule 1 molecules. Likewise, IL-22 inhibited IFN-γ–induced secretion of the proinflammatory chemokines CCL5/RANTES and CXCL10/interferon-inducible protein 10 *in vitro*. Consistently, the IL-22 expression in bronchoalveolar lavage fluid of asthmatic patients inversely correlated with the expression of CCL5/RANTES and CXCL10/interferon-inducible protein 10 *in vivo*.
Conclusions: IL-22 might control the extent of IFN-γ–mediated lung inflammation and therefore play a tissue-restricted regulatory role. (J Allergy Clin Immunol 2013;131:562-70.)

Key words: *T_H22 cells, IL-22, IFN-γ, asthma, human bronchial epithelial cells, epithelial regulation*

From [a]ZAUM–Center of Allergy and Environment, Technische Universität and Helmholtz Center Munich; [b]the Airways Disease Section, National Heart and Lung Institute, Imperial College London; [c]the Laboratory of Experimental Immunology, IDI-IRCCS, Rome; [d]Molecular Immunology, Department of Allergy and Clinical Immunology, National Heart and Lung Institute, Imperial College, London; and [e]the Department of Dermatology and Allergy, Technische Universität München, Munich.

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Corresponding author: Davide Pennino, MSc, ZAUM–Center for Allergy and Environment (ZAUM), Technische Universität and Helmholtz Center Munich, Germany Biedersteiner Straße 29, 80802 Munich, Germany. E-mail: Davide.Pennino@lrz.tu-muenchen.de and davide.pennino@gmail.com.

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Asthma is a chronic inflammatory disorder of the airways characterized by airway obstruction with characteristics of remodeling and evidence of ongoing epithelial injury and repair.[1] T cells contribute to chronic asthma by inducing direct tissue damage in epithelial airways, secreting proinflammatory cytokines, and releasing factors that contribute to epithelial remodeling. T_H2 cells are primary effector cells in asthmatic patients because they secrete IL-4, IL-5, and IL-13, leading to IgE production of B cells and eosinophil-, mast cell–, and basophil-mediated inflammation. T_H1, T_H17, and cytotoxic T cells (T_c) contribute to lung inflammation through the release of a large number of cytokines, such as IFN-γ, IL-17, and IL-22, and induction of apoptosis in lung epithelial cells.[2,3]

IFN-γ is a key proinflammatory cytokine in lung inflammation. It stimulates epithelial cells to release chemokines[4] relevant for the recruitment of immune cells and therefore amplifies the ongoing immune reaction.[5-7] IFN-γ also promotes the induction of MHC class I (MHC-I), MHC class II (MHC-II), and intercellular adhesion molecule 1 (ICAM-1) expression on epithelial cells, thereby enhancing adhesion of T cells and induction of apoptosis by $CD8^+$ and $CD4^+$ T cells.[8-11]

Although the role of IFN-γ in the inflammatory process has been extensively investigated, the contribution of IL-22 remains unclear. IL-22 is a member of the IL-10 cytokine family, which is produced by many immune cells, such as natural killer cells, $CD11c^+$ myeloid cells, lymphoid tissue inducer–like cells, T_H1 cells, T_H17 cells, and the recently described T_H22 T-cell

Abbreviations used
BALF: Bronchoalveolar lavage fluid
DHBE: Asthmatic human bronchial epithelial cell
ICAM-1: Intercellular adhesion molecule 1
IL-22R: IL-22 receptor
IP-10: Interferon-inducible protein 10
MHC-I: MHC class I
MHC-II: MHC class II
NHBE: Normal human bronchial epithelial cell

subset.[12-18] The IL-22 receptor (IL-22R) is a heterodimer that consists of IL-22R and the IL-10 receptor β subunit and is expressed by epithelial cells of nonhematopoietic origin mainly in the skin, kidney, liver, gut, and lung.[19] The expression pattern of IL-22R implies that IL-22 exerts its effects exclusively on tissue cells.[20] The engagement of IL-22R was demonstrated to be essential for innate immune defenses in the gut,[15] skin,[21,22] and airways.[23] IL-22 protects the mucosal surface from extracellular pathogens by inducing the secretion of antimicrobial peptides in epithelial cells.[12,21,23] Furthermore, IL-22 maintains epithelial integrity by preventing injury and accelerating epithelial repair after a variety of lung insults.[19]

The inflammatory properties of IL-22 in the lung are conflicting. IL-22 has been shown to induce the recruitment of granulocytes synergistically with IL-17 and thus increases inflammation in mouse models of lung fibrosis and allergic asthma. However, mice lacking IL-17 production in these disease models show less inflammation, decreased numbers of infiltrating cells, and reduced airway tissue damage after injection of IL-22.[24,25] Although IL-22 has shown to be involved in lung inflammatory disorders, its anti-inflammatory role in human lung diseases has been poorly investigated.

Here we demonstrate that IL-22 and IFN-γ have reciprocal antagonistic effects on human bronchial epithelial cells. IFN-γ impairs the main IL-22 effects, such as the induction of S100A7 and migration of epithelial cells.

On the other hand, IL-22 inhibits IFN-γ–mediated upregulation of MHC-I and MHC-II, protecting the epithelium from T cell–mediated damage. Moreover, it impairs IFN-γ–mediated regulation of proinflammatory chemokines, such as CCL5/RANTES and CXCL10/interferon-inducible protein 10 (IP-10), both *in vitro* and *in vivo*. Thus IL-22 might protect the lung epithelium from IFN-γ–mediated inflammation.

This new immune axis is of special interest because it provides the first indication, to our knowledge, of a T-cell cytokine inhibiting the proinflammatory effects of IFN-γ on tissue cells.

METHODS

Patients

Healthy subjects (n = 11) and asthmatic patients (n = 28) were included according to the American Thoracic Society Workshop on Refractory Asthma.[26] Patients affected by mild (n = 14) and severe (n = 14) asthma were included in the study. Pulmonary function tests were performed and biopsy specimens and bronchoalveolar lavage fluid (BALF) were taken during baseline symptoms. The baseline symptoms correlated with pulmonary function test results. Bronchial biopsies and bronchial alveolar lavage were performed according to the local ethics committee. Each participant provided informed consent.

Cytokines and antibodies

The following antibodies were used for flow cytometric analysis: CD4–peridinin-chlorophyll-protein complex (SK3), IFN-γ–V450 (B27; both from BD Biosciences, San Jose, Calif), CD8-phycoerythrin (RPA-T8), CD8–APC-Cy7 (SK1), IL-17A–Alexa Fluor 488 (N49-653; all from BD PharMingen, San Jose, Calif), IL-4–phycoerythrin (3010.211, BD FastImmune), IL-22–allophycocyanin (142928), CD54–fluorescein isothiocyanate (BBIG-I1; both from R&D Systems, Minneapolis, Minn), HLA-DR–allophycocyanin (LN3), and HLA A-B-C–fluorescein isothiocyanate (W6/32; both from eBioscience, San Diego, Calif). For cell culture, stimulation, and blocking experiments, the following recombinant cytokines and antibodies were used: IL-2 (Novartis, Basel, Switzerland), IL-22 and IFN-γ (R&D Systems), purified anti-CD3 (UCHT1) and anti-CD28 (CD28.2; both from BD Bioscience), anti–IFN-γR1 (MAB6732), anti–IL-22Rα1 (AF2770), mouse IgG_1 (MAB002), and polyclonal goat IgG (AB-108-C; all from R&D Systems).

Isolation and expansion of lung-derived T cells

Lung biopsy specimens of asthmatic patients were cultured in complete RPMI 1640 supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin (all from Invitrogen, Carlsbad, Calif), 5% human serum (Sigma, St Louis, Mo), and 20 U of IL-2/mL (Novartis). Emigrating cells were expanded by means of anti-CD3/anti-CD28 stimulation. After 10 to 13 days, T-cell lines were collected and characterized by using flow cytometry.

Flow cytometric analysis

Surface and intracellular cytokine staining were performed with the Cytofix/Cytoperm kit (BD Biosciences), according to the manufacturer's instructions. Acquisition and analysis were performed with the FACSCanto II (BD Biosciences).

Human bronchial epithelial cell culture and stimulation

Primary human bronchial epithelial cells derived from 3 healthy subjects (NHBE cells) and 3 asthmatic patients (DHBE cells) were purchased from Lonza (Basel, Switzerland). Bronchial epithelial cells were cultured in complete human bronchial epithelial cells (BEGM, Lonza). Cells of the first, second, and third passages were used in experiments. Confluent and subconfluent epithelial cells were stimulated with recombinant 50 ng/mL IL-22 and 10 ng/mL IFN-γ in growth factor–free BEBM (Lonza). After 36 hours of stimulation, flow cytometric staining of MHC-I (HLA-A, B, C), MHC-II (HLA-DR), and CD54 (ICAM-1) were performed. Supernatants were collected after 48 hours of stimulation, and the content of CCL5/RANTES was analyzed by using ELISA (R&D Systems).

Whole-genome microarray analysis and real-time PCR

The total RNA sample was amplified and Cy3 labeled by using the 1-color Low Input Quick Amp Labeling Kit, according to the manufacturer's protocol. Hybridization to SurePrint G3 Human Gene Expression 8x60K Microarrays was performed by using the Gene Expression Hybridization Kit. Differential gene expression was analyzed with the Genespring Software GX 11.0 (Agilent Technologies, Santa Clara, Calif). Genes regulated more than 2-fold change were further analyzed by using the paired Student *t* test and filtered for *P* value ($P \leq .05$). The primers listed in Table E1 in this article's Online Repository at www.jacionline.org were used in real-time PCR to validate the microarray data.

Wound repair assay

Confluent monolayers of NHBE cells and DHBE cells were scratched with the tip of a pipette to create a uniform cell-free zone in each well. Wounded monolayers were then incubated with IL-22 (50 ng/mL) alone or in combination with IFN-γ (10 ng/mL). BEBM basal medium represented the negative

control. In some experiments blocking anti–IFN-γ receptor 1 and the relative isotype control were added to the cultures. Closure of the wounded area was monitored microscopically at 0 hours and 16 hours after stimulation and recorded with a digital camera. The residual gap between migrating cells was measured with a computer-assisted image analysis system (AxioVision 4.5; Carl Zeiss, Oberkochen, Germany) and expressed as a percentage of the initial scratched area.

T cell–mediated cytotoxicity experiments

The cytotoxicity assay was performed with the cytotoxicity detection kit LDH, according to the manufacturer's instructions (Roche, Mannheim, Germany). Briefly, NHBE cells were seeded in flat-bottom 96-well plates in complete BEGM. Confluent NHBE cells were washed with PBS and stimulated with recombinant cytokines (10 ng/mL IFN-γ and 50 ng/mL IL-22) in basal BEBM. After 36 hours, NHBE cells were extensively washed and cocultured for 6 hours with heterologous $CD8^+$ and $CD4^+$ cells and isolated with the $CD8^+$ and $CD4^+$ T-cell isolation kit (Miltenyi Biotech, Bergisch Gladbach, Germany), respectively.

Immunohistochemistry

Lung biopsy specimens of asthmatic (n = 6) and healthy (n = 3) donors were paraffin embedded. Five-micrometer sections were dewaxed in xylene and ethanol and boiled in Tris-EDTA buffer (pH 9.0) at 96°C. After quenching endogenous peroxidase, the slides were incubated in PBS containing 1% BSA and subsequently incubated with anti-human IL-22 rabbit polyclonal or polyclonal rabbit IgG (both from Novus Biologicals, Littleton, Colo). The staining was developed with the avidin-biotin-immunoperoxidase system (Vector Laboratories, Burlingame, Calif), followed by counterstaining with hematoxylin.

BALF

BALF collected from asthmatic (n = 22) and healthy (n = 8) donors was concentrated 50- to 70-fold by using the Amicon Centripep Ulatracel-3K Centrifugal Filter Devise (Millipore, Temecula, Calif). ELISA (R&D Systems) and 27 multiplex analysis (Bio-Rad Laboratories, Hercules, Calif) were performed to measure cytokine and chemokine content. The results were corrected for concentration factor and thus represent the unconcentrated content of proteins in BALF (Table I).

Statistical analysis

Mann-Whitney *U* analysis and 1-way ANOVA with the Bonferroni multiple comparison test were used to determine significant differences between groups. The Spearman test was used to evaluate correlations (Prism 5; GraphPad Software, La Jolla, Calif). Data are expressed as means ± SEMs. Statistically significant differences were defined as *P* values of less than .05, less than .01, and less than .001.

TABLE I. Study subjects' characteristics

	Healthy control subjects	Asthmatic patients
Sex (F/M)	5/6	14/14
Age (y)	28.6 ± 11.7	44.6 ± 12.5
Duration of asthma (y)	NA	32.9 ± 19
FEV_1 (% predicted)	94.8 ± 8.1	74.6 ± 13.7†
FEV_1/FVC ratio (%)	79.3 ± 5.4	68.9 ± 10.4
Bronchodilator response§	NA	14.4 ± 12.7
BDP equivalent (μg/d)	NA	1373 ± 640
Atopy	NA	24/28
Total IgE (IU)	NA	423 ± 139
Smokers	0/11	0/28
BALF: total cell count (× 10^6)	9.33 ± 6	6.39 ± 2.65
Macrophages (%)	98.8 ± 1.1	90.82 ± 11.7
Lymphocytes (%)	0.46 ± 0.64	5.41 ± 11.04
Eosinophils (%)	0.04 ± 0.1	1.25 ± 1.94
IFN-γ (pg/mL)	0.92 ± 0.18	1.82 ± 1.48
IL-4 (pg/mL)	0.058 ± 0.019	0.076 ± 0.036
IL-5 (pg/mL)	0.0059 ± 0.011	0.080 ± 0.09*
IL-13 (pg/mL)	0.1162 ± 0.040	0.1594 ± 0.0086
IL-17 (pg/mL)	0.279 ± 0.3285	0.7564 ± 0.4612‡
IL-22 (pg/mL)	0.159 ± 0.17	0.709 ± 1.104*

Values represent means ± SDs.
BAL, Bronchoalveolar lavage; *BDP*, beclomethasone dipropionate; *F*, female; *FVC*, forced vital capacity; *M*, male; *NA*, not applicable.
*$P < .05$, †$P < .01$, and ‡$P < .001$ compared with healthy subjects.
§Measured as percentage increase in FEV_1 after 400 mg of aerosolized albuterol.

RESULTS

T cells from lung biopsy specimens of asthmatic patients display distinct IL-22–producing T-cell subsets

T-cell lines were generated from lung biopsy specimens of asthmatic patients and analyzed by using multicolor flow cytometric staining to characterize IL-22–producing T cells in asthmatic patients. On ionomycin/phorbol 12-myristate 13-acetate stimulation, the majority of lung-derived T cells produced IFN-γ (Fig 1, *A* and *B*). The frequency of $IL\text{-}22^+$ cells was comparable with that of $IL\text{-}17^+$ and $IL\text{-}4^+$ infiltrating T cells (Fig 1, *A* and *B*, and see Fig E1 in this article's Online Repository at www.jacionline.org). Only a minority of total lung $IL\text{-}22^+$ T cells coexpressed IL-17, confirming that IL-22 and IL-17 are weakly associated in human tissues (Fig 1, *A*). The majority of the IL-22–producing T cells belonged to the $CD4^+$ subset (Fig 1, *C*), whereas $CD8^+IL\text{-}22^+$ cells represented a minor population (Fig 1, *F*). Among the $IL\text{-}22^+$ lung T cells, 4 $CD4^+$ T-cell subsets were identified on the basis of their cytokine profile: $T_H1/IL\text{-}22^+$, T_H17, $T_H0/IL\text{-}22^+$, and T_H22 cells (Fig 1, *D*). $T_H1/IL\text{-}22^+$ cells were the major IL-22–producing T-cell subset, indicating that during asthma, IL-22 can be cosecreted commonly with IFN-γ (Fig 1, *D* and *E*). The frequency of T_H22 cells ranged between 10% to 15% of the total $IL\text{-}22^+$ population, suggesting that T_H22 is not a skin-restricted T-cell subset (Fig 1, *D* and *E*). Among $CD8^+IL\text{-}22^+$ cells, $T_C1/IL\text{-}22$ cells were most frequent, whereas T_C22 and T_C17 cells were rarely detected (Fig 1, *G*). $CD8^+IL\text{-}22^+$ cells were almost exclusively producing IFN-γ alone or together with IL-17 (Fig 1, *G* and *H*). Together, these data suggest that T cells produce IL-22 alone or in combination with IFN-γ in patients with chronic lung inflammation.

IL-22 and IFN-γ mediate reciprocal antagonistic effects on primary bronchial human epithelial cells

To understand the potential pathologic mechanism of IL-22 and IFN-γ cosecretion in the context of airway inflammation, we investigated the gene expression profile of primary human bronchial epithelial cells (NHBE cells) exposed to either IL-22 or IFN-γ alone or in combination.

Genes regulated more than 2-fold compared with untreated cells were hierarchically clustered (Fig 2, *A-C*) and filtered for *P* value ($P \leq .05$). Hierarchic gene expression analysis highlighted clusters of antagonism between IL-22 and IFN-γ (Fig 2, *A-C*). IL-22 was antagonizing genes regulated by IFN-γ (Fig 2, B, and see Tables E10 and E11 in this article's Online

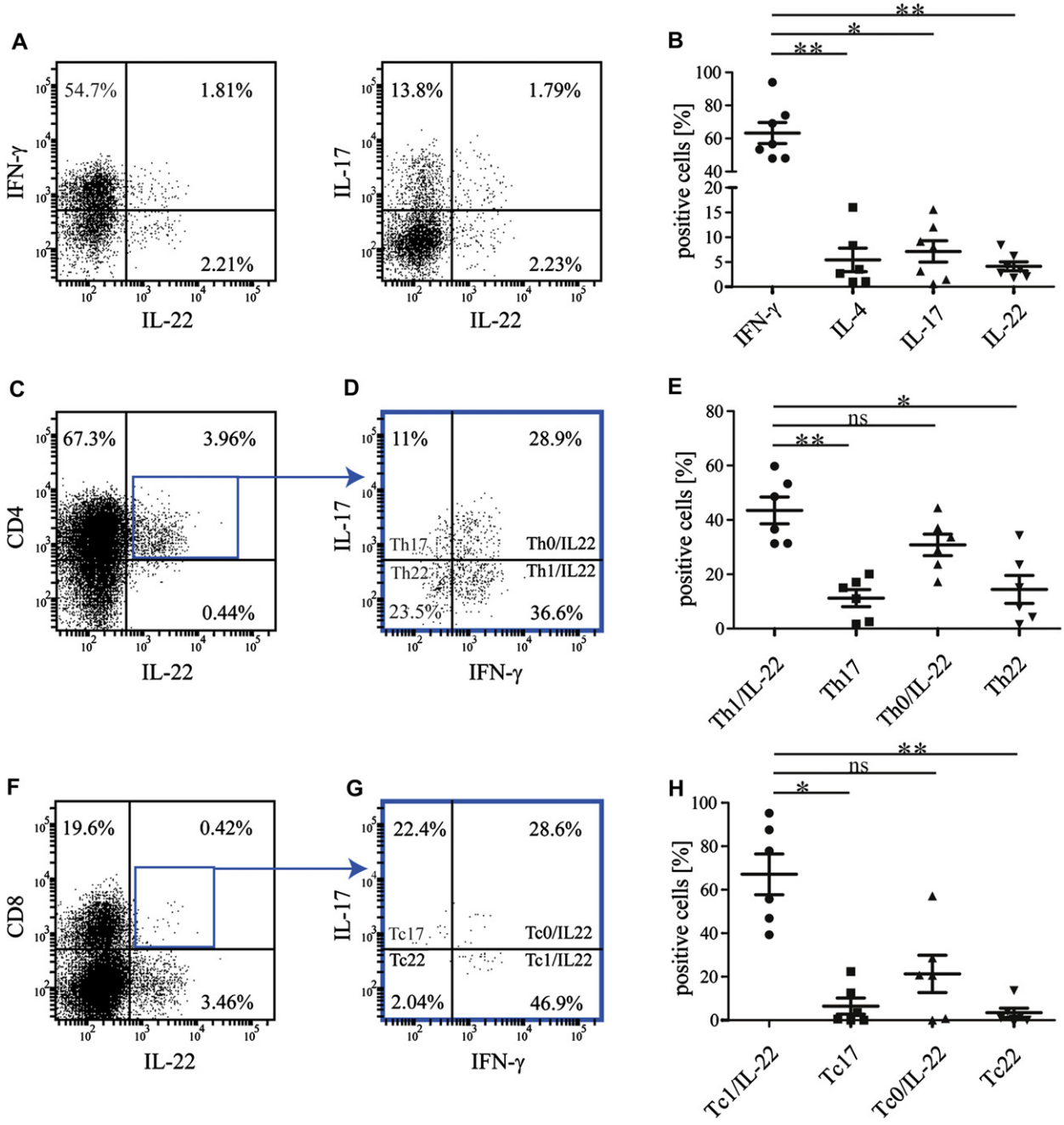

FIG 1. Distinct IL-22–producing T-cell subsets infiltrate the lungs of asthmatic patients. **A, C,** and **F,** Lung-derived T cells showing IL-22 expression in IFN-γ^+ and IL-17^+ (Fig 1, *A*), $CD4^+$ (Fig 1, *C*), and $CD8^+$ (Fig 1, *F*) infiltrating T cells. **B,** Total IFN-γ–, IL-17–, IL-4–, and IL-22–expressing T cells. **D,** Relative distribution of the T_H22, T_H17, T_H1/IL-22, and T_H0/IL-22 subsets. **G,** Relative distribution of the T_C22, T_C17, T_C1/IL-22, and T_C0/IL-22 subsets. **E** and **H,** Summary of values of the IL-22^+ subsets in 6 asthmatic patients. $^*P < .05$ and $^{**}P < .01$. *ns,* Not significant. *Error bars* represent means ± SEMs.

Repository at www.jacionline.org), and conversely, IFN-γ inhibited IL-22–regulated genes (Fig 2, *C*, and see Tables E8 and E9 in this article's Online Repository at www.jacionline.org). IFN-γ treatment induced 2920 genes in NHBE cells compared with untreated cells (see Tables E4 and E5 in this article's Online Repository at www.jacionline.org). Among those genes, many proinflammatory mediators were upregulated, such as the T_H1 chemoattractants CXCL10, CXCL9, and CXCL11 and the eosinophil-recruiting chemokines, CCL5, CCL2, CCL7, and CCL8, thus supporting the propagation of inflammation (see Table E4). In contrast, IL-22 significantly regulated only 118 genes (see Tables E2 and E3 in this article's Online Repository at www.jacionline.org). Consistent with its role in host defense, IL-22 specifically induced the antimicrobial peptide psoriasin (S100A7) in NHBE cells. Furthermore, IL-22 modulated several genes involved in cell survival and remodeling, such as *SERPINB3* and *SERPINB4* involved in mucin secretion and cell survival,[27,28] *VWA1* involved in matrix deposition,[29,30]

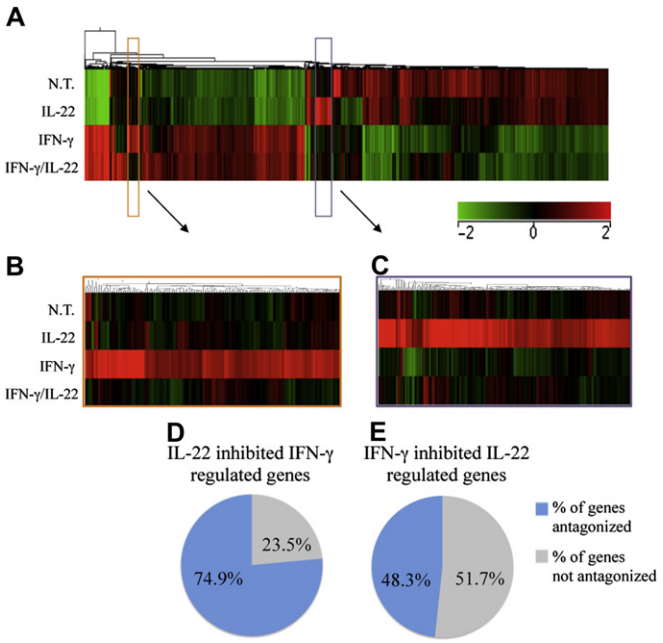

FIG 2. Antagonistic gene regulation in primary human bronchial epithelial cells by IL-22 and IFN-γ. **A,** Hierarchic clustering analysis of genes regulated at least 2-fold induction compared with untreated *(N.T.)*. **B** and **C,** Representative cluster of IFN-γ–induced genes suppressed by IL-22 (Fig 2, *B*) and IL-22–induced genes suppressed by IFN-γ (Fig 2, *C*). **D** and **E,** Percentage of the number of IFN-γ–regulated genes significantly antagonized by IL-22 (Fig 2, *D*) and IL-22–regulated genes significantly antagonized by IFN-γ (Fig 2, *E*). $*P \leq .05$.

Asprv1 involved in skin regeneration,[31] and the transcription factor *NFE2* involved in cell maturation[32] (see Fig E2 in this article's Online Repository at www.jacionline.org). The IFN-γ/IL-22 combination regulated 2623 genes compared with untreated cells (see Tables E6 and E7 in this article's Online Repository at www.jacionline.org). Consistent with antagonistic features, IFN-γ inhibited the expression of both host defense and remodeling genes regulated by IL-22 (see Fig E2 and Tables E8 and E9), whereas IL-22 inhibited most of the proinflammatory IFN-γ–regulated genes, such as chemokines and HLA molecules (see Fig E3 and Tables E10 and E11 in this article's Online Repository at www.jacionline.org).

Among the genes significantly regulated, IFN-γ inhibited 57 (48.3%) of 118 genes regulated by IL-22 (Fig 2, *E*), whereas IL-22 inhibited 2203 (74.9%) (Fig 2, *D*) of 2920 genes modulated by IFN-γ (Fig 2, *B-D*, and see Figs E2 and E3).

Taken together, these data suggest that IL-22 and IFN-γ can act antagonistically on bronchial epithelium.

IFN-γ inhibits IL-22–mediated *in vitro* wound healing

To evaluate the functional consequence of the IFN-γ/IL-22 antagonism, we investigated the effect of IFN-γ on IL-22–mediated wound closure in an *in vitro* wound-healing model performed on both NHBE and DHBE primary epithelial cells. IL-22 but not IFN-γ significantly enhanced epithelial migration. In line with the antagonism of both cytokines, IL-22–induced wound healing was impaired by IFN-γ in bronchial epithelial cells from both healthy and asthmatic subjects (Fig 3 and see Fig E4 in this article's Online Repository at www.jacionline.org). The IFN-γ effect was reversed by blocking the IFN-γ receptor (see Fig E5 in this article's Online Repository at www.jacionline.org).

These results confirm that IL-22 induces epithelial migration and suggest that when coproduced with IFN-γ, IL-22 functions might be inhibited.

IL-22 impairs proinflammatory activities of IFN-γ

On IFN-γ exposure, epithelial cells upregulate a plethora of proinflammatory cytokines, chemokines, and adhesion molecules that promote recruitment of immune cells and T cell–mediated damage.[9] IFN-γ induced many proinflammatory mediators in primary bronchial epithelial cells (see Table E4). Consistent with the antagonistic activities of IL-22 on IFN-γ, IL-22 diminished the induction of HLA haplotypes, CD54/ICAM-1, and the chemokines CXCL10, CXCL9, CXCL2, CXCL17, CXCL16, CXCL11, CCL8, CCL5, CCL7, and CCL2 by IFN-γ in the microarray assay. Confirming observations at the level of gene expression, IL-22 inhibited the IFN-γ–mediated expression of CCL5/RANTES at the protein level (Fig 4, *A*). To confirm the MHC-I and MHC-II regulation observed in the microarray, we performed flow cytometric analysis of ICAM-1 (Fig 4, *C*), the MHC-II haplotype HLA-DR (Fig 4, *B*), and the MHC-I haplotypes HLA-A, HLA-B, and HLA-C (Fig 4, *D*). Consistently, IL-22 suppressed the IFN-γ–mediated induction of MHC-I (Fig 4, *D*), MHC-II (Fig 4, *B*), and ICAM-1 (Fig 4, *C*) on the surface of both NHBE and DHBE cells (see Fig E4). The IL-22 inhibitory effects were reverted by blocking IL-22R (see Fig E6 in this article's Online Repository at www.jacionline.org).

Taken together, these data suggest that IL-22 suppresses IFN-γ–mediated inflammation.

Inhibition of IFN-γ–mediated MHC-I expression leads to a reduced CD8-dependent cytotoxicity

MHC-I, MHC-II, and CD54/ICAM-1 upregulation on epithelial cells enables CD8- and CD4-mediated cytotoxicity.[8,9] To investigate the functional consequences of the IL-22–mediated inhibition of MHC-I and MHC-II upregulation, we measured the T cell–mediated cytotoxicity. Untreated epithelial cells were slightly prone to CD4- and CD8-mediated cytotoxicity, whereas IFN-γ–treated epithelial cells upregulated MHC-I and MHC-II and became highly susceptible to T cell–mediated cytotoxicity. Treatment of primary epithelial cells with the combination of IFN-γ and IL-22 greatly reduced CD8-mediated cytotoxicity compared with IFN-γ–treated NHBE cells (Fig 4, *F*). A similar but not significant tendency was observed for $CD4^+$-mediated cytotoxicity (Fig 4, *E*).

Together, these results suggest that by impairing IFN-γ–mediated expression of MHC-I, MHC-II, and ICAM-1 molecules, IL-22 can protect the lung epithelium from T cell–mediated damage.

IL-22 expression inversely correlates with IFN-γ–dependent proinflammatory mediators *in vivo*

To investigate the expression and *in vivo* relevance of IL-22–mediated suppression of IFN-γ, we performed immunohistochemical staining of sections from lung biopsy specimens from healthy and asthmatic subjects and measurement of BALF content (Table I). Immunohistochemistry showed IL-22–producing cells infiltrating

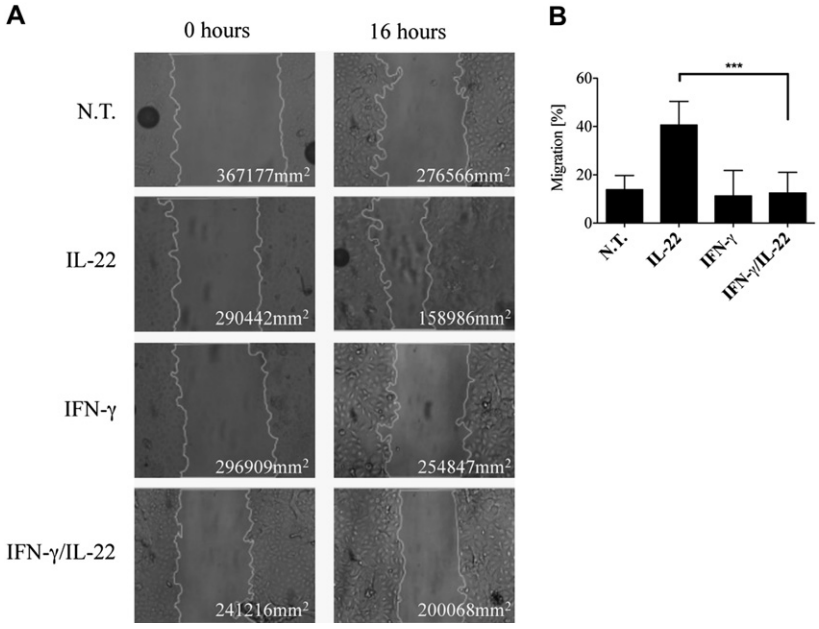

FIG 3. IFN-γ suppresses IL-22–induced wound healing in a functional *in vitro* injury model. **A,** Representative wound-healing experiment. **B,** Data from 6 independent experiments are shown. ***$P < .001$. *Error bars* represent means ± SEMs. *N.T.*, Untreated.

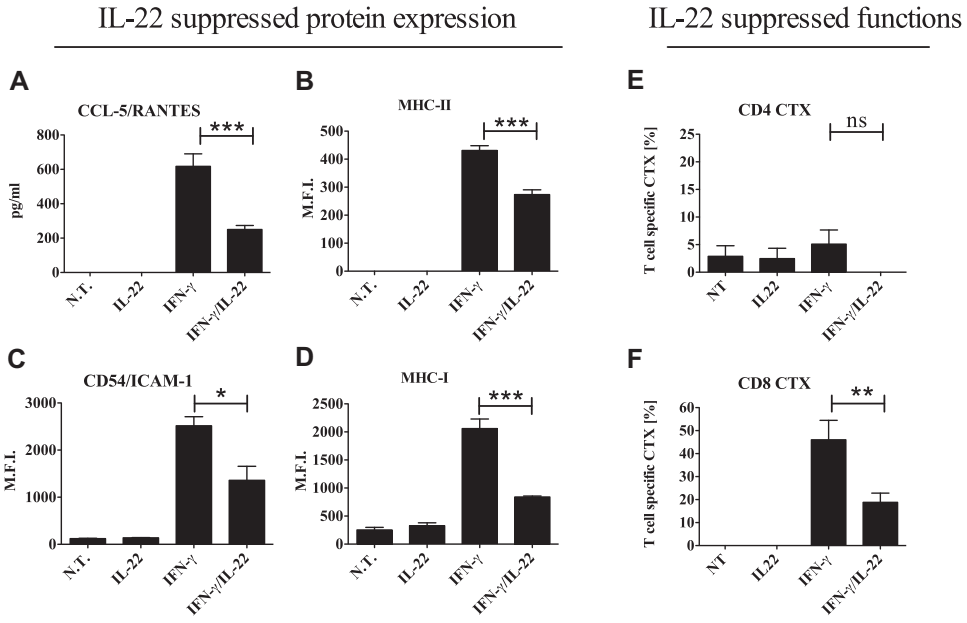

FIG 4. IL-22 suppresses IFN-γ–induced molecules and CD8-meditated cytotoxicity. Primary epithelial cells were treated with recombinant cytokines. **A,** CCL5/RANTES secretion. **B-D,** MHC-II (Fig 4, *B*), CD54/ICAM-1 (Fig 4, *C*), and MHC-I (Fig 4, *D*) expression shown as mean florescence intensity *(MFI)*. **E** and **F,** After 36 hours of stimulation, NHBE cells were cocultured with heterologous $CD8^+$ and $CD4^+$ cells, and specific cytotoxicity *(CTX)* was evaluated. *Error bars* indicate SEMs. *$P \leq .05$, **$P \leq .01$, and ***$P \leq .001$. *ns*, Not significant.

the epithelium of the bronchial mucosa in asthmatic patients, whereas few IL-22^+ cells were detected in healthy subjects (Fig 5, *A*). Because cytokines are normally undetectable in neat BALF of asthmatic patients, we measured cytokine levels in concentrated BALF. We corrected the values for concentration factor and expressed them as representative of the unconcentrated BALF. The expression of IL-22 was increased in BALF of asthmatic patients compared with that of healthy control subjects (Fig 5, *B*). To evaluate whether IL-22 might have a suppressive effect on IFN-γ–mediated inflammation *in vivo*, we examined whether there was a correlation between the IL-22/IFN-γ ratio and the content of the IFN-γ–inducible chemokines CXCL10/IP-10 and

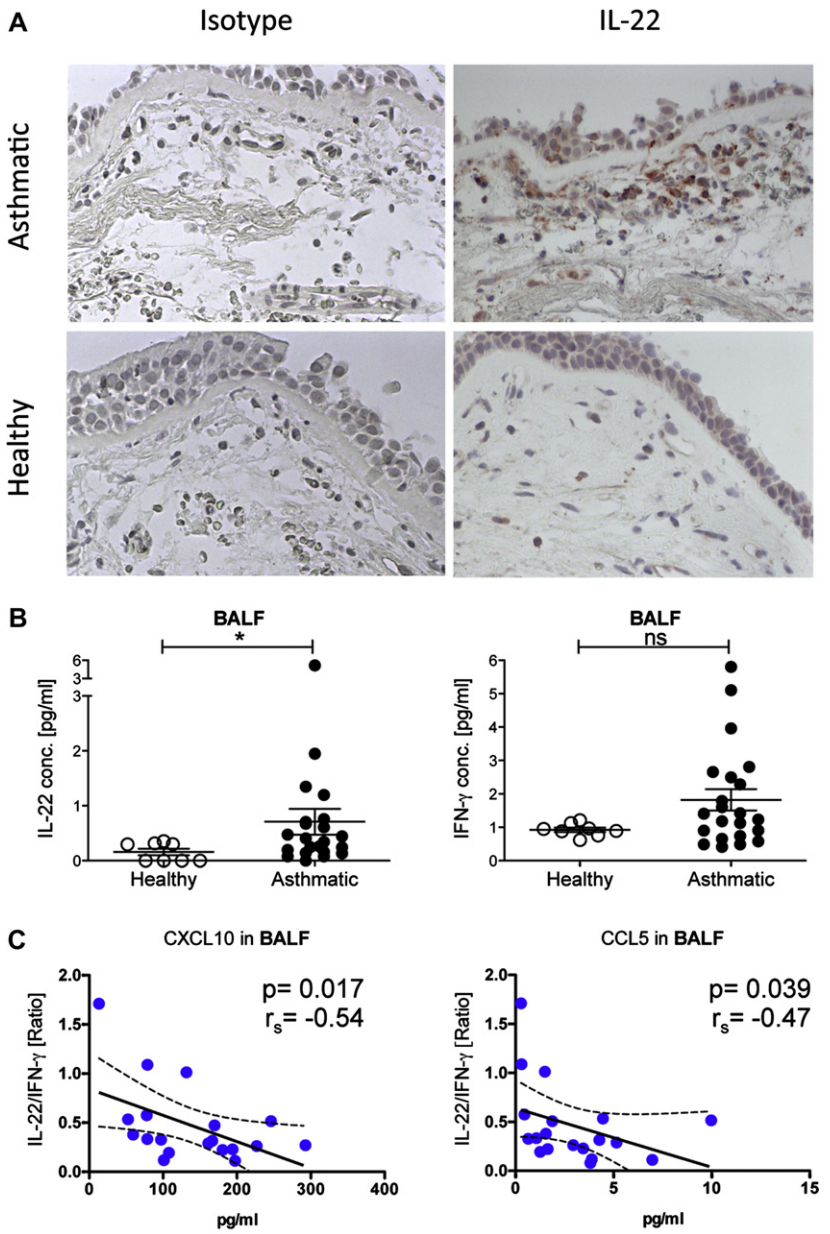

FIG 5. IL-22/IFN-γ ratio inversely correlates with CXCL10/IP-10 and CCL5/RANTES levels in BALF. **A,** Representative IL-22 immunohistochemistry staining with relative controls of an asthmatic and healthy lung biopsy specimen. **B,** IL-22 and IFN-γ expression in BALF. **C,** Correlation between the IL-22/IFN-γ ratio and CXCL10 (IP-10) and CCL5 (RANTES) content in BALF. Correlation was measured with the Spearman rank correlation test *(r_s)*. The Mann-Whitney test was performed to compare the groups of asthmatic and healthy donors. *Error bars* indicate SEMs. $*P \leq .05$, $**P \leq .01$, and $***P \leq .001$. *ns,* Not significant.

CCL5/RANTES (Fig 5, *C*). In line with the *in vitro* data, the IL-22/IFN-γ ratio but not other ratios investigated inversely correlated with the expression of CXCL10/IP-10 and CCL5/RANTES. Taken together, these results suggest that an IL-22–dominated environment might suppress the IFN-γ–mediated inflammation *in vivo*.

DISCUSSION

The current study identifies a regulatory role for IL-22 in the context of IFN-γ–mediated inflammation. We demonstrated that IL-22 and IFN-γ mediate antagonistic effects in lung epithelial cells. These antagonisms become manifest in impaired protective and remodeling activities of IL-22, as well as in dampened IFN-γ–mediated upregulation of proinflammatory molecules. To our knowledge, IL-22 is the first T-cell cytokine described to inhibit IFN-γ–mediated proinflammatory effects on human primary epithelial cells.

In murine models IL-22 shows either proinflammatory or anti-inflammatory properties. Consistently, 2 reports in animal models of lung inflammation conclude that IL-22 can act as an anti-inflammatory cytokine in the absence of IL-17, whereas in the presence of IL-17, IL-22 contributes to the recruitment of

inflammatory cells.[24,25] However, both reports lack a mechanistic explanation for this finding.

We find that T cells infiltrating the lungs of asthmatic patients are a source of IL-22. Both $CD4^+$ and $CD8^+$ cells produce IL-22. Similar to reports studying the skin,[13] different subsets of IL-22–producing T cells were detected. As expected, IL-22 was produced by some T_H17 cells but primarily by T_H1 cells. An additional source of IL-22 in the lung is the recently described T_H22 subset. T_H22 cells were originally described in the skin on the basis of their expression of CCR10, CCR6, and CCR4.[14] However, this chemokine receptor repertoire is also adequate for migration of T cells to the lung.[33] Thus T_H22 seems to be a tissue-restricted rather than a skin-restricted T-cell subset.

In line with previous reports, IL-22 induces antimicrobial peptides in airway epithelial cells, epithelial migration, and genes involved in mucin secretion and matrix deposition.[23] IFN-γ antagonized the majority of IL-22–mediated effects. In fact, antifibrotic properties were previously assigned to IFN-γ, such as inhibition of collagen synthesis, cell-cycle arrest, induction of apoptosis, and activation of natural killer cell cytotoxicity.[34] The role of IL-22 in this context remains to be investigated.

The current study demonstrates that IL-22 impairs IFN-γ–induced chemokine release in airway epithelial cells, which confirms previous studies showing that exogenous IL-22 administration in a mouse model of lung fibrosis reduces inflammation through inhibition of CXCL9, CXCL10, and CXCL11 expression.[35] Consistently, it was shown that administration of IL-22 in an OVA mouse model of asthma impaired the secretion of CCL11 and CCL5 and consecutive eosinophil infiltration.[25] Thus IL-22 antagonism of IFN-γ might explain the anti-inflammatory effects of IL-22 in mice and human subjects.

Interestingly, the IL-22/IFN-γ antagonism only partially affects the innate related molecules. In fact, molecules such as Toll-like receptors 2 and 3 induced by IFN-γ on epithelial cells (see Table E4) were not inhibited by IL-22. This observation suggests that the interplay between IFN-γ and IL-22 results in a different rather than diminished response to microbial invasion compared with the effects of the single cytokines.

Notably, IL-22 is the first T-cell cytokine that diminishes IFN-γ–induced expression of MHC-I and MHC-II on primary epithelial cells and reduces CD8-mediated cytotoxicity. This finding is consistent with reports showing that IL-22 is an effective cytokine for the clearance of extracellular, but not intracellular, pathogens in which MHC-I recognition by $CD8^+$ cells is critical.[23,36,37] In fact, it was previously demonstrated that lower lung viral titers were observed after treatment with anti–IL-22 in a mouse model of influenza virus, and this suggests that IL-22 might promote influenza virus replication.[38]

Viruses play an important role in asthma exacerbation and are implicated in the development of chronic asthma.[39,40] In this context IL-22 might have a Janus-faced role: on the one hand, IL-22 can protect the lung from an excessive IFN-γ–mediated inflammation, and on the other hand, it might be involved in the propagation, perpetuation, or both of virus-mediated asthma or unwanted remodeling of the airway structure.

IFN-γ–mediated inflammation and MHC-I recognition are of critical relevance in several medical fields, such as tumor immunology, transplantation immunology, autoimmunity, and intracellular infectious diseases. Further studies are needed to clarify the role of IL-22 in MHC-I–dependent diseases and to further investigate its role in pathogen-dependent aggravation of inflammation.

The present report links the murine evidence to human data and investigates in detail how and in what context IL-22 might act as an anti-inflammatory cytokine.

In summary, IL-22 might control the extent of IFN-γ–mediated lung inflammation and therefore plays a regulatory role in tissue inflammation. These results raise possibilities for new therapies using recombinant IL-22 to limit tissue inflammation and anti–IL-22 to enhance IFN-γ–mediated tissue immunity.

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Key messages

- **T_H22 cells infiltrate the lung during asthma.**
- **IL-22 and IFN-γ exert tissue-restricted antagonistic functions in the lung.**
- **IL-22 protects the epithelium from T cell–mediated cytotoxicity by reducing the IFN-γ–induced MHC-I and MHC-II expression on bronchial epithelial cells.**
- **IL-22 might limit tissue inflammation, whereas anti–IL-22 might enhance IFN-γ–mediated tissue immunity.**

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