Airway Smooth Muscle Cell as an Inflammatory Cell

Lessons Learned from Interferon Signaling Pathways

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The present article will describe the potential role of airway smooth muscle (ASM) in mediating both deleterious/beneficial effects of interferons (IFNs) in asthma. First described as beneficial in treating the main features of asthma, the interplay between IFNs and ASM could explain their deleterious actions recently described in a number of different studies. Through multiple mechanisms, including the suppression of steroid action, the synergistic pro-inflammatory actions when combined with other cytokines, and the modulation of calcium metabolism, IFNs are now seen as critical mediators in the pathogenesis of asthma.

Keywords: signaling cross talk; interferons; asthma; cytokines; TNF- α

In recent years, airway smooth muscle (ASM) has been seen as a significant player in the pathogenesis of asthma. Studies using bronchial thermoplasty that ablates ASM have demonstrated the importance of ASM in the abnormal airway narrowing seen in symptomatic individuals with asthma. Although the long-term side effects of this therapy are yet unknown, alternative studies need to concentrate on the pro-inflammatory function of ASM. A myriad of articles showed that ASM is a target for and a source of different pro-asthmatic factors that could play a role, via autocrine and paracrine actions, in the orchestration and/or perpetuation of the chronic inflammatory process that occurs in the airways (1–5). This review will describe the clinical importance of interferon (IFN) signaling pathways in the regulation of the immunomodulatory functions of ASM. Apart from being important for immune defense mechanisms, evidence described in this review shows that IFN action on structural airway cells such as ASM seems to represent an interesting hypothesis to explain their dual protective/deleterious effects in asthma.

AN OVERVIEW OF IFNS COMPLEX SIGNALING PATHWAYS

Receptors, Transcription Factors, and Regulation

The classical components of the IFN signaling cascade include the Janus tyrosine kinases (JAKs) and signal transducers and activators of transcription (STATs) factors. Activation of each IFN receptor complex stimulates different receptor-associated tyrosine kinases—namely, JAK1 and Tyk2 by IFN- α/β (type I), or JAK1 and JAK2 by IFN- γ (type II) (6). JAKs-mediated phosphorylation of STAT proteins results in STAT assembly in dimeric or oligomeric forms, which translocate to the nucleus, where they can regulate gene expression via DNA binding motifs called either γ -activated sequence (GAS) elements (recognized by STAT1 homodimers) or IFN-stimulated response element

(ISRE, recognized by STAT1-STAT2 heterodimers) (7, 8). A number of signaling molecules, including (1) tyrosine phosphatases SHP1 or SHP2 (both present in the nucleus and cytoplasm), (2) suppressors of cytokine signaling (SOCs previously termed under various names), and (3) nuclear inhibitors called PIAS (proteins that inhibit activated STATs), serve as negative regulators of the JAK/STAT pathways (9). Viral infection is the emblematic inducer of type I IFNs, which are critical to mediate the establishment of antiviral response (10). The type II IFN- γ , mainly produced by CD4⁺, CD8⁺ T lymphocytes and natural killer (NK) cells as well as antigen-presenting cells (APC), plays an important role in T helper type 1 (Th1)-mediated innate immunity and regulates Th2-mediated responses (11). Immunohistochemistry studies showing an up-regulation of STAT-1 and STAT-1-dependent genes such as intercellular adhesion molecule (ICAM)-1 and IFN regulatory Factor-1 (IRF-1) in asthmatic airways (12) suggest the potential contribution of IFN-associated JAK/STATs in the regulation of immunomodulatory genes associated with allergic asthma. As discussed below, there is still controversy on whether IFNs are beneficial or deleterious in asthma.

Signaling Cross Talk between IFN- γ and Other Pro-Asthmatic Cytokines

Evidence suggests that IFNs may promote the airway allergic responses by their ability to interact with other inflammatory factors. Both *in vitro* and *in vivo* studies confirmed that IFN- γ effectively potentiates the expression of immunoregulatory genes induced by either viruses (13) or pro-asthmatic cytokines such as IL-13 (14) or TNF- α (15, 16). When given together, the IL-13 and IFN- γ combination leads to greater lung inflammation in mice, supporting the inflammatory potential of Th1 and Th2 cytokine interaction (14). These amplifying properties of IFN- γ may explain, at least in part, why viral infection, which increases production of IFNs, is an important trigger for asthma and chronic obstructive pulmonary disease exacerbation (17). Most studies that used a combination of IFN- γ and TNF- α showed that the synergistic action involves several molecular mechanisms (see Figure 1). In some instances, their cooperativity may be explained by the IFN- γ -induced up-regulation of TNF- α receptors (18–20) or vice-versa (21, 22). IFN- γ also enhances TNF- α receptor-associated signaling by increasing nuclear factor (NF)- κ B-dependent pathways in murine macrophages and rat cell lines such as PC12 (23, 24). Furthermore, both cytokines collaborate at the gene level by increasing promoter activation through a synergistic interaction between transcription factors activated by IFN- γ (STATs) and TNF- α (NF- κ B). Thus, a functional cooperation has been demonstrated between NF- κ B and STAT1 in the regulation of genes activated by IFN- γ and TNF- α , including ICAM-1 (25), RANTES (regulated on activation, normal T cells expressed and secreted) (26), and Caspase 11 (27). Recent evidence also showed that STATs cooperatively interact with IRF-1 to regulate gene transcription by involving multiple mechanisms. For example, IRF-1 regulates many immunomodulatory genes by physically binding and activating ISRE DNA binding elements that are normally recognized by

(Received in original form May 14, 2007; accepted in final form June 4, 2007)

This study was supported by NIH grant R01 HL064063 (Y.A.).

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Proc Am Thorac Soc Vol 5, pp 106–112, 2008

DOI: 10.1513/pats.200705-060VS

Internet address: www.atsjournals.org

STAT1/STAT2 heterodimer (28–30). This strongly suggests that the joint activation of IRF-1 and STATs by different types of cytokines may represent a key mechanism to regulate an overlapping set of genes. Collectively, besides their individual action, the synergistic inflammatory action resulting from IFN- γ /TNF- α combination may involve (1) enhanced receptor-associated signal pathways, (2) transcriptional synergy, or (3) functional co-operation between STAT members and other transcription factors (31).

Interestingly, some reports, but not all, showed the ability of IFN- α or IFN- γ to inhibit TNF- α -induced NF- κ B pathways in different cell types, including 2fTGH fibroblasts (32), Ewin's sarcoma EW-7 cells (33), and Jurkat T cells (34). We also demonstrated a similar finding in TNF- α -treated human ASM cells (discussed below in ANTAGONISTIC MODULATION OF INFLAMMATORY GENES BY TNF- α /IFN- γ) (35). Multiple mechanisms underlying IFNs inhibitory effect on NF- κ B pathways have been proposed including (1) inhibition of NF- κ B-DNA interaction (33), (2) prevention of I κ B α degradation (34), or (3) tight regulation of TNF- α receptor 1 activity induced by direct interaction with STAT1 (36–38). These studies strongly suggest that the antagonistic/synergistic actions exerted by IFNs and other cytokines are highly dependent on the cell types.

AMBIGUOUS ROLE OF IFNS IN THE PATHOGENESIS OF ASTHMA

The observation that viral infection, the common inducer of IFNs, is a well-known trigger for asthma exacerbations supports the concept that IFNs could play a negative role in lung diseases (39). Typically, viral syndromes are characterized by intense inflammatory responses in the airways, with marked leukocyte trafficking and production of Th1-type cytokines such as IFNs (40). The subsequent interaction of IFNs with airway resident cells could initiate asthma exacerbations via multiple mechanisms, including production of chemokines/cytokines or recruitment of different inflammatory cells (41). Increased levels of IFN- γ have been detected in asthmatic airways (42, 43); the immunopathogenic role of the IFN-associated pathways in asthma is still controversial. While little information is known about the role of type I IFNs (IFN- β) in asthma, previous *in vivo* evidence shows that type II IFN- γ could have suppressive activities against key features of allergic responses including immunoglobulin E production, airway hyperresponsiveness and eosinophilic influx (44). It is important to mention that most of these studies were performed in experimental asthma models that used either exogenously administered IFN- γ , IFN- γ knockout mice, or transgenic mice overexpressing IFN- γ (reviewed in Reference 45) or inhibitors such as the double-stranded decoy oligonucleotide sequences (46). Whether IFNs solely exert a protective role in asthma, however, needs to be re-evaluated in light of recent evidence showing that, on the contrary, IFNs could be detrimental to the pathogenesis of asthma. One study performed in allergen-exposed patients with asthma failed to demonstrate any therapeutic effects of increasing levels of IFNs in the airways using immunostimulatory sequences (47). In a chronic model of allergic asthma, blocking antibodies revealed that IFN- γ is a major player in mediating ovalbumin-induced airway hyperresponsiveness to methacholine (48). A similar observation was reported in toluene diisocyanate-exposed subjects where anti-IFN- γ blocking antibodies also abrogated the development of airway hyperresponsiveness to methacholine (49). In addition to their role in airway hyperresponsiveness, IFNs could also regulate airway inflammation. Targeted expression of IFN- γ in the airways of IFN-KO mice significantly increased allergen-induced responses including IL-5 and IL-13 expression, BAL eosino-

philia, and airway inflammation (50). Another report also shows that IFN- γ enhanced allergen-induced cytokine production (eotaxin, RANTES) as well as accumulation of eosinophils and lymphocytes in the BAL (51). Finally, an elegant study convincingly showed a critical role of IFN- γ and dendritic cells in enhancing Th2-dependent allergic responses after viral infection (52).

The reasons underlying the controversial role of IFNs in asthma are not clear but several reasons could be implicated. First, it appears that the actions of IFNs on allergic responses differ according to the route of administration; it was reported that systemic but not nebulized IFN- γ is able to suppress markers of lung inflammation (53). Second, IFN- γ protective action in the airways is closely dependent on the duration of treatment as one study demonstrated that a prolonged 4-week treatment with subcutaneous IFN- γ was no longer able to reverse allergen-induced airway hyperresponsiveness to methacholine in contrast to the protection observed after 1 week of treatment (54). Finally, a number of studies (discussed in SIGNALING CROSS TALK BETWEEN IFN- γ AND OTHER PRO-ASTHMATIC CYTOKINES ABOVE) show the biological activities of IFNs can be influenced by the inflammatory milieu which can synergize or antagonize their inflammatory actions. Because a variety of inflammatory mediators are present in asthmatic airways, there is a strong likelihood that interaction between IFNs with other mediators is a critical factor in determining the final biological responses of IFNs in the airways. Additional studies are needed to determine whether the dual inflammatory actions of IFNs (i.e., antagonism versus synergism) in the airway resident cells may explain the controversial effects of IFNs in the pathogenesis of asthma.

CLINICAL SIGNIFICANCE OF IFNS ACTION ON AIRWAY SMOOTH MUSCLE

Synergistic Modulation of Inflammatory Genes by TNF- α /IFN- γ

Several lines of evidence demonstrate that TNF- α could play a role in the pathogenesis of asthma through a direct immunomodulatory action on ASM (3). In cultured ASM cells, TNF- α , alone or in combination with other cytokines such as IL-1 β or IL-13, has been shown to stimulate the expression of various "pro-asthmatic" mediators, including cytokines (IL-6), chemokines (eotaxin, RANTES, IL-8) as well as adhesion molecules (ICAM-1, VCAM-1) (3). Interestingly, TNF- α also cooperates with IFN- γ to synergistically induce different pro-inflammatory proteins including RANTES (55), fractalkine (56), CXCL10 (57), COX-2 (58), or TLR2 and TLR3 receptors (59). These inflammatory molecules could participate in the regulation of airway inflammation via multiple mechanisms, including recruitment of mast cells and T cells. We also found that induction by TNF- α of CD38, an ecto-enzyme involved in calcium signaling (60, 61), was synergistically increased by both type I and type II IFNs (62). IFN- γ also acts as an "amplification" factor with other cytokines such as IL-1 β to enhance the expression of nerve growth factor in bronchial ASM cells (63). Both NGF and CD38 have been associated with the development of airway hyperresponsiveness, another defining feature of asthma (61, 64, 65). Table 1 summarizes the details about the TNF- α /IFNs targeted genes in ASM cells and their clinical relevance in asthma. The underlying mechanisms of such cooperation have not been elucidated, but we made the interesting observation that induction of defined genes including RANTES as well as CD38 by TNF- α occurred, at least in part, via activation of the autocrine action of IFN- β (62, 66).

Figure 1. Mechanisms underlying interferon (IFN) and tumor necrosis factor (TNF)-α synergistic/antagonistic actions in airway resident cells. Based on studies performed in other cell types as well as in airway smooth muscle (ASM) cells, it is possible to hypothesize that the dual inflammatory actions induced by IFN-γ/TNF-α combination may involve different molecular mechanisms including (1) changes in the expression and/or function of TNF-α or IFN receptor; (2) functional or physical interactions between IFN and TNF-α-dependent transcriptional factors, including IRF-1, NF-κB, and STAT1/2; or (3) direct activation of JAK/STATs pathway by TNF-α or via the autocrine secretion of IFN-β. All these mechanisms could play an essential role in determining the final biological activities of IFNs in asthma.

We found that TNF-α, via the autocrine action of IFN-β, activates JAK1 and Tyk2, and STAT1- and STAT2-dependent gene expression in human ASM cells (67). In this study, we showed that autocrine IFN-β differentially regulates TNF-α-induced inflammatory gene expression, by suppressing IL-6 expression and promoting RANTES secretion while ICAM-1 expression was not affected. Although functional cross talk between TNF-α and IFN signaling molecules has also been reported in other cell types (mostly hemopoietic cells), our

recent study is the first evidence to demonstrate secretion of IFN-β by TNF-α in airway structural cells. In previous studies performed in 3T3-L1 adipocytes, TNF-α induces the phosphorylation of STAT-1 by directly interacting with both JAK1 and JAK2 (68), whereas in Hela cells, STAT-1 was shown to physically interact with TNFR1 and the adaptor proteins TNF receptor-associated death domain (TRADD), but not TRAF-2 (36). TNF-α also induces STAT1 phosphorylation at serine 727 in macrophages (69). These data clearly show that autocrine

Figure 2. Hypothetical clinical model showing the combined inflammatory actions of IFNs and TNF-α in the pathogenesis of asthma. Synergistic action can occur between IFNs produced by virally infected airway cells (epithelium) and TNF-α possibly originating from immunoglobulin E-activated mast cells within the ASM bundles. The concomitant presence of both TNF-α and IFNs in the airways of individuals with asthma exposed to respiratory viruses can act on different resident cells including ASM to elicit pathological changes associated with asthma exacerbations. IFNs/TNF-α interaction with ASM has been shown to increase inflammation through the production of a number of inflammatory mediators (chemokines and adhesion molecules), to impair steroid function via the dominant-negative activity of GRβ isoform, and to regulate airway hyperresponsiveness via changes in ASM contractility through calcium regulatory proteins including CD38.

TABLE 1. EXAMPLES OF PRO-ASTHMATIC GENES SYNERGISTICALLY INDUCED BY INTERFERONS AND OTHER CYTOKINES IN AIRWAY SMOOTH MUSCLE CELLS AND THEIR POTENTIAL ROLE IN ASTHMA

Molecule	Relevance in Asthma	Mechanisms
Fractalkine	Airway inflammation	Chemoattractant for Mast cells, T cells, NK cells, and eosinophils
RANTES		
CXCL10		
COX-2	Airway hyper-responsiveness	Secretion of inflammatory prostanoids and cytokines
TLR 2,3		
CD38		Modulation of GPCR-induced calcium signaling and contractility
NGF	Steroid resistance	Impaired GR α activity
GR β		

Definition of abbreviations: COX = cyclooxygenase; GPCR = G-protein coupled receptor; GR β = glucocorticoid receptor beta isoform; NK = natural killer; RANTES = regulated on activation, normal T cells expressed and secreted; TLR = Toll-like receptor.

IFN- β is a novel signaling component by which TNF- α regulates ASM function in human ASM cells. This raises the hypothesis that the synergistic action of TNF- α /IFN- γ combination in ASM cells could result from an enhanced activation of IFN receptor-associated JAK/STAT signaling pathways.

Antagonistic Modulation of Inflammatory Genes by TNF- α /IFN- γ

The antagonistic action of TNF- α /IFN- γ combination has been supported by others who showed that exogenous IFN- γ suppressed TNF- α -inducible inflammatory genes including vascular endothelial growth factor (70), IL-17 receptor expression (71), and TLR3 mRNA expression (59). We also found that, in the presence of blocking anti-IFN- β antibodies, TNF- α -induced IL-6 production was significantly increased (72). This hypothesis was recently confirmed in another study where we found that exogenous IFN- γ dose dependently suppressed the expression of not only IL-6 but also IL-8 and eotaxin expression after TNF- α exposure (35). These data suggest that, in addition to having synergistic actions, IFNs also act as negative regulators of TNF- α -inducible genes in ASM cells. Although the mechanisms underlying these suppressive effects are not yet known, the use of soluble extracellular signal-regulated kinases (ERK) inhibitor showed the involvement of mitogen-activated protein (MAP) kinases pathways in IFN- γ -mediated IL-17 receptor down-regulation. Ito and colleagues recently confirmed the role of ERK pathway in IFN- γ -induced ADAM33 down-regulation (73). Our group showed that IFN- γ is a potent inhibitor of TNF- α -induced NF- κ B transcriptional activities (35). The use of trichostatin A, a specific histone deacetylase (HDAC) inhibitor, partially reverses IFN- γ inhibitory effects on TNF- α -inducible genes and NF- κ B-dependent gene expression (35). Immunoblot analyses confirmed that increased acetylation of NF- κ B p65 subunit after TNF- α exposure was considerably reduced in the presence of IFN- γ . These findings suggest that IFN- γ suppresses the expression of some, but not all, pro-inflammatory genes induced by TNF- α by interfering with NF- κ B transcriptional activity, possibly through the changes of acetylation levels of key regulatory proteins. These observations suggest that HDAC could represent a promising therapeutic target for the suppression of NF- κ B-dependent inflammatory genes. Interestingly, overexpression of a specific isoform of HDACs, HDAC2, in glucocorticoid-insensitive alveolar macrophages from patients with COPD was able to restore glucocorticoid sensitivity (74). How-

ever, further studies are needed to examine whether HDAC up-regulation in ASM cells modulates glucocorticoid signaling.

Modulation of Steroid Responsiveness by TNF- α /IFN- γ

Most anti-inflammatory effects of steroid are mediated via glucocorticoid receptor alpha isoform (GR α), which suppresses expression of inflammatory genes through mechanisms known as transactivation or transrepression (75). Transactivation occurs via direct binding of activated GR α to DNA sequences termed glucocorticoid-responsive elements (GRE) present on the promoter of steroid-inducible genes. Transrepression defines a direct interaction of activated GR α with different transcription factors, such as NF- κ B and Activated Protein 1 (AP-1), thus repressing expression of pro-inflammatory genes (76). As a result of alternative splicing mechanisms, another glucocorticoid receptor isoform, namely, GR β , has been described (77).

Although steroids have been largely investigated in ASM cells (78), their modulatory actions on inflammatory genes remain complex and poorly characterized. (1) The anti-inflammatory effects of glucocorticoids are gene-specific. For example, reports showed that dexamethasone effectively inhibits cytokine-induced IL-6, RANTES, or eotaxin expression, while the same steroid has little effect on cytokine-induced ICAM-1 expression (79–81). (2) Glucocorticoid-suppressive effects are time dependent, since dexamethasone partially abrogates cytokine-mediated ICAM-1 expression at early time points, but has no effect at later time points (79). (3) Glucocorticoid inhibitory action is also stimuli-specific. While dexamethasone significantly inhibits IL-1 β -induced granulocyte macrophage-colony stimulating factor (GM-CSF) secretion, it has only partial effect on GM-CSF secretion induced by thrombin (82). (4) We and others recently showed that the specific combination of TNF- α with IFNs, but not with IL-1 β or IL-13, impairs the ability of fluticasone, dexamethasone, and budesonide to inhibit the expression of different pro-asthmatic genes such as CD38, RANTES, ICAM-1, fractalkine, and TLR2 (56, 59, 72). Interestingly, we demonstrated that GR β overexpression in cells expressing endogenous GR α prevents the abilities of steroid to (1) induce transactivation activity, and (2) inhibit cytokine-induced pro-inflammatory gene expression (72). Although the pathological role of GR β isoform is not well understood, previous reports demonstrate a strong correlation between steroid resistance in individuals with asthma and the expression levels of GR β (83). More importantly, increased GR β in the airways has been detected in patients who died of fatal asthma (84). Indeed, by its ability to act as a dominant-negative inhibitor of steroid action in other cell types (85), GR β has been associated with steroid resistance in different inflammatory diseases (76). Our study demonstrating increased functional GR β in ASM is unique since most investigators studied GR β function in immune or transformed cells; its role in stromal, nontransformed effector cells remains unexplored. Accordingly, the transfection studies investigating the dominant-negative activities of GR β were performed using exogenously expressed GR α and GR β in cells that do not express endogenous GR α such as COS-1 and COS-7 cells (85, 86). Collectively, upon pro-inflammatory cytokine stimulation, ASM cells become insensitive to steroid action by a mechanism involving GR β up-regulation, thus providing a novel *in vitro* cellular model to dissect steroid resistance in primary cells.

CONCLUSIONS AND FUTURE DIRECTIONS

The controversial roles of IFNs in asthma could be due to different reasons including their different actions on the airways when administered systemically or locally, their treatment duration (short-term versus long-term) and the animal models

of asthma used (chronic versus acute models). In addition, the interplay between IFNs and airway resident cells represents an important parameter in determining the biological activities of IFNs in asthma. Thus, ASM could play an essential role in the deleterious actions of IFNs in asthma by involving several mechanisms including (summarized in Figure 2): (1) their ability to stimulate different pro-inflammatory mediators, (2) their synergistic actions with other "pro-asthmatic" stimuli such as Th1- or Th2-type inflammatory cytokines (TNF- α or IL-13), and (3) their suppressive effects on steroid signaling, possibly via the up-regulation of negative regulators such as GR β . Another surprising lesson learned from IFNs-ASM interaction is their antagonistic effects on some but not all genes possibly through the suppression of NF- κ B transcriptional activity. These dual IFN effects on airway resident cells could explain, at least in part, their ambiguous actions seen in both preclinical studies and in patients with asthma. There are still clinical lessons to be learned from studying ASM-IFN interactions.

Conflict of Interest Statement: O.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. Y.A. received \$30,000 in 2006 from Centocor as a research grant.

Acknowledgment: The authors thank Mary McNichol for her assistance in the preparation of the manuscript.

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