

Interferon- γ , Pulmonary Macrophages and Airway Responsiveness in Asthma

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Abstract: Asthma is increasingly being recognised as involving not only allergic inflammatory mechanisms but also a variety of other cell types and cytokines. While the role of interferon- γ (IFN- γ) in asthma remains controversial, it has been proposed to contribute to the pathogenesis of both chronic stable asthma and acute severe asthma. We have shown that in a model of chronic allergic asthma in mice, airway hyper-responsiveness (AHR) is independent of various Th2 cytokines and their signalling pathways, but is dependent on IFN- γ . In a model of an allergen-induced acute exacerbation of chronic asthma, we have demonstrated that activation of pulmonary macrophages may play a critical role in driving the inflammatory response. Furthermore, we have demonstrated that IFN- γ stimulation of macrophages can lead to steroid-resistant airway inflammation and AHR *via* the production of interleukin-27. These findings strengthen the notion that the pathogenesis of the lesions of asthma, and especially of AHR, involves both Th2 and Th1 cytokines, as well as interaction between the allergic response and the innate host defence system. Targeting the effects of IFN- γ on pulmonary macrophages may be particularly relevant to the treatment of steroid-resistant acute exacerbations of asthma.

Keywords: Acute exacerbation, airway hyperresponsiveness, asthma, interferon- γ , pulmonary macrophages, Th1 and Th2 cytokines.

INTRODUCTION

Asthma is an inflammatory disease that primarily affects the conducting airways. In approximately two-thirds of patients, it develops on a background of atopy i.e. a genetic predisposition to produce IgE antibodies in response to ordinary exposures to environmental allergens [1]. There is a background of chronic inflammation in the mucosa of the airways, with accumulation of T-lymphocytes, plasma cells and macrophages in the lamina propria, as well as of mast cells both within the lamina propria and the epithelial layer [2, 3]. This is associated with various structural changes in the airway wall, including increased smooth muscle mass, epithelial changes and airway wall fibrosis, which are collectively referred to as airway remodelling [4, 5]. Development of airflow obstruction and other clinical manifestations of asthma is believed to be associated with superimposed episodes of acute inflammation [6]. Eosinophil recruitment may be prominent during these acute episodes, including recruitment of eosinophils into the airway epithelium, which has long been recognised as a characteristic feature of asthma [7].

Asthmatics exhibit increased sensitivity and increased maximum reactivity to bronchoconstrictor agents or non-specific irritants, referred to as airway hyper-responsiveness (AHR). The airflow obstruction that develops during an acute asthmatic episode is typically reversible, although the

extent of reversibility may decline in adults with long-standing disease, possibly as a consequence of progressive airway remodelling [8].

Despite ongoing treatment, some patients with asthma acutely develop worsening clinical manifestations and airflow obstruction, referred to as an acute exacerbation [9]. Much of the serious morbidity of asthma, and most of the utilisation of healthcare resources, is related to exacerbations [10, 11]. Acute exacerbations are frequently triggered by respiratory viral infections, especially by rhinoviruses [12, 13]. Exposure to high levels of allergen is an independent risk factor, which synergises with infection [14]. In patients suffering an acute exacerbation of their asthma, there is marked recruitment of neutrophils as well as eosinophils into the airway mucosa [15].

CYTOKINE RESPONSES IN ASTHMA

A variety of chronic inflammatory cells, including T-lymphocytes, plasma cells, macrophages and mast cells, as well as airway epithelial cells and smooth muscle cells, all contribute to the pro-inflammatory cytokine environment in the airway wall in asthma. Especially in allergic asthmatics, the profile of cytokines expressed by CD4⁺ T cells is biased towards Th2 cytokines such as interleukin (IL)-4, -5 and -13. In studies in mouse models, it has often been suggested that there is a dichotomy between Th1- and Th2-biased immunological responses i.e. that suppression of the Th2 response would lead to a desirable enhancement of the Th1 response, or that promoting a Th1 response might have therapeutic value. However, patient studies indicate that allergic diseases including asthma involve more than just

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increased activity of Th2 cells. Thus intervention studies in which attempts have been made either to selectively suppress Th2 cytokines or their signalling mechanisms [16-20], or to down-regulate their activity by administration of "opposing" cytokines such as IL-12 [21], have all had limited success. The latter is perhaps not surprising, because studies in a mouse model demonstrated that whereas IL-12 could inhibit the initial induction of a Th2 response, it contributed to the subsequent development of allergic inflammation of the airways [22].

In fact, there is considerable indirect evidence for a contributory role of Th1 cells and their cytokines, notably the prototypic Th1 cytokine interferon- γ (IFN- γ), in asthma. There is a significantly increased proportion of IFN- γ -producing T cells in the peripheral blood [23, 24], and bronchoalveolar lavage fluid [25, 26] of asthmatic patients. Moreover, the presence of increased levels of IFN- γ in lavage fluid and increased numbers of IFN- γ -producing cells in sputum from asthmatics appears to be related to the severity of chronic disease and its response to treatment [27-29]. Increased production of IFN- γ has also been related to increased changes in peak expiratory flow in patients with chronic stable asthma [30] and serum levels of this cytokine appear to predict subsequent deterioration in respiratory function [31]. Furthermore, a recently-described subset of asthmatic patients who exhibit a delayed response to allergen, including airway inflammation and airflow obstruction, exhibit a parallel marked increase in production of IFN- γ by stimulated peripheral blood mononuclear cells [32].

In both severe chronic asthma and acute exacerbations of asthma, there is enhanced expression not only of Th2 cytokines [33, 34] but also relatively greater expression of IFN- γ [35], as well as expression of other pro-inflammatory cytokines such as IL-17, including by subsets of Th2 cells [36]. Our data in an animal model of an allergen-induced acute exacerbation of asthma are consistent with these observations. In this model, we have shown that pulmonary CD4⁺ T cells exhibit up-regulated expression of numerous cytokines including IL-4, IL-5, IL-13, TNF- α and IFN- γ [37].

EVIDENCE FOR A ROLE FOR IFN- γ IN AHR IN ASTHMA

There is now considerable evidence that Th1 cells and their cytokines, notably IFN- γ , contribute to the development of AHR in asthma. A large longitudinal study of asthma in childhood demonstrated a consistent association between AHR and an enhanced IFN- γ response of peripheral blood mononuclear cells to polyclonal stimulation [38]. In animal experimental studies using short-term models of allergic inflammation of the airways, transfer of allergen-specific Th1 cells elicited an enhanced inflammatory response with marked AHR [39]. In other studies, cells of mixed Th1/Th2 differentiation appeared to be the most potent inducers of AHR [40] and blockade of Th1 signalling decreased AHR [41].

The notion that IFN- γ plays a role in the development of AHR is strongly supported by our studies in an experimental model of chronic asthma. Our model involves long term

challenge of systemically sensitised mice, typically for 6 weeks, with low mass concentrations of aerosolised ovalbumin [42]. This leads to the development of lesions closely resembling mild chronic asthma, including eosinophil recruitment into the airway epithelium, chronic inflammation in the airway wall with accumulation of CD4⁺ T lymphocytes and plasma cells, and various changes of airway wall remodelling. In contrast to short-term models involving uncontrolled exposure to high levels of aerosolised ovalbumin, our model is not associated with the development of perivascular and peribronchiolar inflammation in the lung parenchyma, but instead elicits lesions confined to the conducting airways. As a consequence, AHR in response to methacholine challenge originates from the airways rather than parenchyma [43].

We have demonstrated that in this model, development of airway inflammation and remodelling are dependent on Th2 cytokines, as revealed by studies using gene-targeted animals and neutralising antibodies e.g. to IL-5 and IL-13 [44, 45]. Nevertheless, there is a mixed Th1/Th2 profile of cytokine production by local lymphocytes, as demonstrated in ovalbumin-restimulated cultures of cells from peribronchial lymph nodes. Notably, this includes a marked IFN- γ secretory response [46]. We have also shown that expression of mRNA for IFN- γ by inflammatory cells in the airway wall is significantly up-regulated [47].

Importantly, in chronically challenged mice, we have demonstrated that AHR can develop independently of IL-4 and/or IL-13, or of signalling *via* the STAT6 pathway [44, 46, 48]. Indeed, in all of the gene-targeted animals used to demonstrate that AHR was not dependent on Th2 cytokines, we found that the response to methacholine was exaggerated. Moreover, we showed that in animals with airway inflammation and AHR, established following 4 weeks of chronic challenge, administration of a neutralising monoclonal antibody to IFN- γ markedly suppressed AHR [45], despite having little effect on airway inflammation.

Recent investigations in a short-term model of allergic airway inflammation suggest that one possible mechanism by which IFN- γ may exert these effects is *via* STAT4 and IL-12 [49]. Meanwhile, our own recent studies have emphasised a potentially key role for pulmonary macrophages in the development of IFN- γ -driven AHR.

INTERACTION BETWEEN IFN- γ AND MACROPHAGES IN DEVELOPMENT OF AHR

The role of pulmonary macrophages in the biology of established asthmatic inflammation remains a neglected area. In part, this may be a consequence of the numerous studies which have emphasised the potentially important negative regulatory role of macrophages in pulmonary immune responses, including animal experimental studies showing that depletion of pulmonary macrophages leads to exaggerated AHR in models of allergic inflammation (reviewed in [50]). The perception of pulmonary macrophages as inhibitory or "tolerogenic" may have been reinforced by evidence that they function poorly as antigen-presenting cells [51, 52]. However, the suppressive activity of these macrophages is demonstrably negated in an inflammatory environment e.g. following exposure to GM-

CSF, TNF- α or a combination of these mediators [53]. This is consistent with a series of reports which have shown that (i) macrophages are activated in asthma, in terms of expression of surface receptors and co-stimulatory molecules such as CD80/CD86 [54, 55] (ii) this activation can be triggered by allergen challenge [56, 57] (iii) pulmonary macrophages from asthmatics, but not from nonatopic control subjects, are capable of functioning effectively as co-stimulatory antigen-presenting cells [55] (iv) macrophages from atopic asthmatics, but not from atopic nonasthmatics or nonatopic individuals, are able to stimulate CD4⁺ T cells to secrete type 2 cytokines such as IL-5 [58].

In our model of an experimental acute exacerbation of asthma, we have now demonstrated that pulmonary macrophages exhibit enhanced expression of a variety of pro-inflammatory cytokines. Importantly, when co-cultured with CD4⁺ T cells from chronically challenged animals, macrophages from animals in which an acute exacerbation has been induced stimulate the expression of Th2 cytokines to a significantly greater extent than control macrophages. Thus enhanced cytokine expression by pulmonary macrophages may play a critical role in triggering increased expression of cytokines by pulmonary CD4⁺ cells [37].

Furthermore, we have published three important studies characterising a novel pathway that leads to suppression of glucocorticoid responsiveness in pulmonary macrophages, in turn predisposing to steroid-resistant inflammation and AHR. Firstly, in a model in transgenic mice, we have shown that following transfer of antigen-specific IFN- γ -producing Th1 cells, challenge with both allergen and LPS is required to induce AHR. This AHR, which is resistant to systemic administration of the glucocorticoid dexamethasone, is dependent on IFN- γ , is abrogated in TLR4- or MyD88-deficient mice, and is inhibited by depletion of pulmonary macrophages [59]. Secondly, using improved methods of assessing AHR in a mouse model of allergic airway disease, we have shown that an interaction between IFN- γ and

pulmonary macrophages contributes to the prolongation of airway-specific AHR [60]. This prolonged response may be analogous to that in the subset of patients who exhibit a delayed asthmatic response [32]. Thirdly, we demonstrated that the mechanism by which IFN- γ and LPS interact is through the production of IL-27 by pulmonary macrophages, and that IL-27 and IFN- γ in turn co-operate to induce steroid-resistant AHR through a mechanism that is dependent on MyD88 expression in macrophages. Specifically, this interaction prevented steroid-induced translocation of the glucocorticoid receptor to the nucleus of pulmonary macrophages. Importantly, we also showed that expression of both IL-27 and IFN- γ is increased in the induced sputum of steroid-refractory asthmatics [61].

These findings (summarised in Fig. 1) are consistent with accumulating evidence which suggests that the innate host defence system (so-called innate immunity) is a major contributor to airway inflammation in asthma exacerbations, and that infection appears to prime an enhanced inflammatory response to allergen exposure [62, 63]. They also highlight the role of IFN- γ and pulmonary macrophages in driving AHR in patients with an acute exacerbation of asthma, potentially triggered by intercurrent infection.

POSSIBLE THERAPEUTIC IMPLICATIONS

In the light of the evidence supporting a role for IFN- γ in mild to moderate chronic asthma and/or in acute exacerbations of asthma, this cytokine may well be an appropriate target for therapeutic inhibition. Indeed, it has previously been suggested that the known efficacy of theophylline in asthma may be related to its capacity to suppress production of IFN- γ [64]. Nevertheless, given the crucial role of interferons in host defence to infection, it would be appropriate to proceed with caution.

Rational therapy needs to be based on a more complete understanding of the cellular sources of IFN- γ in acute

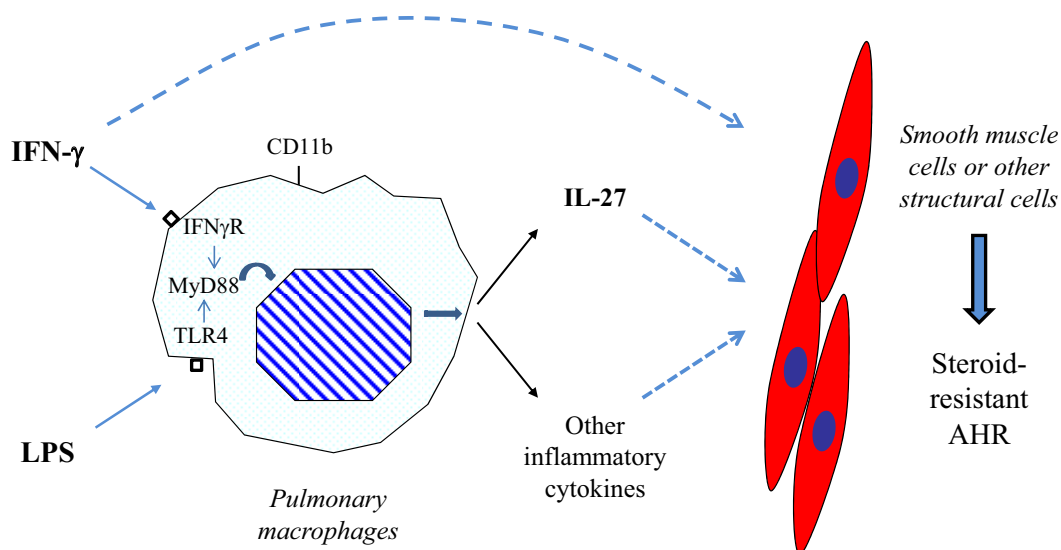


Fig. (1). Diagrammatic representation of how interferon- γ and LPS may interact with pulmonary macrophages to trigger release of IL-27 and other pro-inflammatory cytokines in an acute exacerbation of asthma, leading to steroid-resistant inflammation and airway hyper-responsiveness. For further information, see Li *et al.*, ref. [61].

asthma. While CD4⁺ T cells clearly are a major source of this cytokine, the relative importance of IFN- γ production by CD8⁺ T cells is unclear, although they appear to produce more IFN- γ [23] and may be prominent in fatal asthma [65]. Other potentially significant sources are NKT cells and eosinophils, both of which may be important in AHR [66, 67]. More information is also needed about the mechanisms by which IFN- γ induces AHR. To date, studies suggest these may include potentiation of TNF- α -induced enhanced expression of CD38, which may contribute to the pathogenesis of AHR [68], as well as up-regulation of cysteinyl leukotriene receptors (CysLTR-1 and -2) on smooth muscle cells, which may modulate contractile responsiveness to LTD4 and increase smooth muscle stiffness [69]. The role of local versus systemic production of IFN- γ also needs investigation, because at least one study has suggested that increased pulmonary expression of IFN- γ may not be related to AHR [70].

A potential approach to bypassing the problems associated with targeting IFN- γ is suggested by the evidence from our studies that it is the effect of IFN- γ on pulmonary macrophages which drives AHR. It may be realistic to selectively target pulmonary macrophages, to inhibit the inflammatory signalling pathways in these cells that contribute to the development of AHR. This might be particularly relevant in the setting of acute exacerbations of asthma, especially steroid-resistant episodes triggered by respiratory viral infection. Because inflammatory signalling in pulmonary macrophages is not known to be critical to the response to viral infections that trigger exacerbations, targeting these pathways is unlikely to have an adverse effect on host defences. A current focus of ongoing studies in our laboratories is the use of mannosylated liposomes, which are actively taken up by macrophages, as a drug delivery vehicle for selectively targeting pulmonary macrophages. These have already been demonstrated to be of potential therapeutic benefit for delivery of antimicrobial agents [71] and anti-inflammatory agents such as glucocorticoids [72]. Nuclear factor- κ B is a key transcription factor that regulates the inflammatory activation of macrophages [73]. In the context of exacerbations of asthma, it is noteworthy that inhibitors of NF- κ B can be selectively delivered to pulmonary macrophages using mannosylated liposomes [74].

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DISCLOSURE

This is an extended and updated version of the paper "Interferon- γ as a possible target in chronic asthma" by Kumar, R. K.; Webb, D. C.; Herbert C.; and Foster, P. S. previously published in *Inflammation & Allergy - Drug Targets* 2006, 5, 253-256.

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