Clinical Immunology (2013) 147, 27–37

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Hypoxia potentiates allergen induction of HIF-1α, chemokines, airway inflammation, TGF-β1, and airway remodeling in a mouse model☆

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Received 4 January 2013; accepted with revision 12 February 2013
Available online 21 February 2013

KEYWORDS
Hypoxia;
Neutrophil;
Eosinophil;
KC;
Eotaxin-1

Abstract Whether hypoxia contributes to airway inflammation and remodeling in asthma is unknown. In this study we used mice exposed to a hypoxic environment during allergen challenge (simulating hypoxia during an asthma exacerbation) to investigate the contribution of hypoxia to airway inflammation and remodeling. Although neither hypoxia alone, nor OVA allergen alone, induced significant neutrophil influx into the lung, the combination of OVA and hypoxia induced a synergistic 27 fold increase in peribronchial neutrophils, enhanced expression of HIF-1α and one of its target genes, the CXC-family neutrophil chemokine KC. The combination of hypoxia and OVA allergen increased eotaxin-1, peribronchial eosinophils, lung TGB-β1 expression, and indices of airway remodeling (fibrosis and smooth muscle) compared to either stimulus alone. As hypoxia is present in >90% of severe asthma exacerbations, these findings underscore the potential of hypoxia to potentiate the airway inflammatory response, remodeling, and accelerate the decline of lung function in asthma exacerbations.

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http://dx.doi.org/10.1016/j.clim.2013.02.004

1. Introduction

Exacerbations of severe asthma are associated with hypoxemia that can persist for several days in approximately 90% of subjects as assessed by arterial blood gas analysis [1]. The cause of the hypoxemia in the majority of asthma exacerbations is due to altered ventilation perfusion ratios [1]. In addition, laboratory studies in asthmatics have demonstrated that hypoxia impairs the perception of symptoms including difficulty breathing, chest tightness, and breathlessness, all of which may contribute to treatment delay during asthma exacerbations [2]. Asthma exacerbations are also associated with neutrophilic airway inflammation in adults [3–5], eosinophilic and neutrophilic inflammation in children [6], and a greater decline in lung function [7,8]. At present there is limited information regarding whether hypoxia during exacerbations of asthma contributes to neutrophilic and/or eosinophilic airway inflammation and subsequent remodeling or decline in lung function. In this study we have used a mouse model to investigate whether mice exposed to a hypoxic environment during allergen challenge (to simulate hypoxia during an asthma exacerbation) have evidence of increased neutrophilic and/or eosinophilic airway inflammation and enhanced airway remodeling.

Hypoxia induces the transcription factor hypoxia-inducible factor (HIF) which regulates expression of over 100 genes, many of which are potentially relevant to inflammation and remodeling in asthma [9–11]. For example, hypoxia induces expression of pro-inflammatory cytokines (IL-1β, TNFα, IL-8, VEGF) [9–11], which have been detected at increased levels in the airway of asthmatics [12–14]. IL-8 in particular is a chemokine regulating neutrophil recruitment that may contribute to the neutrophilic airway inflammation noted during exacerbations of asthma [3–5]. Hypoxia in asthma exacerbations may also contribute to airway remodeling as neonatal calves exposed to chronic hypoxia develop increased airway fibrous tissue and smooth muscle [15], mice exposed to chronic hypoxia develop increase lung type III fibrillar and type IV basement membrane collagen after ten days of hypoxia [16], and hypoxia can increase the proliferation of rat airway smooth muscle cells in vitro [17].

The ubiquitously expressed and best-studied form of HIF is HIF-1, a heterodimer consisting of the oxygen-regulated alpha subunit (HIF-1α) and a constitutively expressed beta subunit HIF-1β (also known as aryl hydrocarbon receptor nuclear translocator protein or ARNT) [9–11]. Less well studied isoforms HIF-2 and HIF-3 exhibit more restricted tissue expression [9]. In previous studies we have demonstrated, using conditional myeloid HIF-1α knockout mice and pharmacologic HIF-1α inhibitors, that myeloid cell expression of HIF plays an important role in the development of airway hyperresponsiveness under normoxic conditions [18]. Interestingly, HIF may also be induced by local tissue hypoxia as opposed to systemic hypoxia in inflamed tissues that are often hypoxic as a result of decreased perfusion, edema, vascular insult and/or influx of oxygen-consuming immune cells or pathogens [19]. These localized areas of lung tissue hypoxia may be pertinent not only to severe asthma, but may also occur in mild and moderate asthmatics. Thus, activation of HIF-1α in the context of inflammation can occur in both normoxic as well as hypoxic external environments. Additional studies using mouse models of asthma have demonstrated under normoxic conditions that HIF-1 pharmacologic inhibitors [20,21], HIF siRNA knockdown [21], and conditional HIF-1β deficient mice [22] influence levels of airway inflammation and/or airway remodeling. Human studies have also demonstrated under normoxic conditions increased levels of HIF-1α in lung tissue and bronchial fluid of patients with asthma, and in the nasal fluid of patients with rhinitis after allergen challenge [22].

In this study we have used a mouse model of allergen induced asthma studied under hypoxic conditions (to simulate severe asthmatics having hypoxic asthma exacerbation) to determine the influence of hypoxia on levels of airway inflammation and remodeling. These studies under hypoxic conditions differ critically from prior studies investigating the role of HIF in allergen-induced inflammation and remodeling under normoxic conditions [18,20–22]. Overall our studies demonstrate that in a mouse model simulating hypoxia during an asthma exacerbation, the combined hypoxia and allergen stimulus significantly enhanced HIF-1α expression, airway inflammation (in particular neutrophilic but also eosinophilic), as well as lung levels of KC (the murine equivalent of IL-8), eotaxin-1, and TGF-β1, with resultant increased airway remodeling. As hypoxia is present in >90% of severe asthma exacerbations, these findings underscore the potential of hypoxia to potentiate the airway inflammatory response, increase levels of remodeling, and contribute to the decline in lung function in severe asthmatic exacerbations.

2. Material and methods

2.1. Mouse model of acute OVA challenge and/or hypoxia exposure

The following four groups of BALB/C mice aged 6–8 weeks (n = 8 mice/group) (Jackson Labs) were studied. 1) No hypoxia + no OVA; 2) No hypoxia + OVA; 3) Hypoxia + no OVA; and 4) Hypoxia + OVA. The hypoxia and allergen exposures as well as all procedures involving experimental animals were approved by the Animal Care and Use Committee of the University of California San Diego.

2.2. Hypoxia exposure

Mice in the hypoxia groups (groups 3 and 4) were placed in a plexiglass chamber maintained at 10% O_2, while normoxic groups (groups 1 and 2) were placed in a control chamber open to room air (21% O_2). The duration of the hypoxia or normoxia exposure was 7 days based on pilot time course studies. CO_2, water vapor, and ammonia were removed from the chambers by Drierite (anhydrous calcium sulfate) (Fisher Scientific, Atlanta, GA).

2.3. Acute OVA protocol

BALB/C mice were immunized s.c. on days 0, 7, 14, and 21 with 25 μg of OVA (OVA, grade V; Sigma) adsorbed to 1 mg of alum (Aldrich) in 200 μl normal saline as previously described [23]. OVA-challenged mice received intranasal OVA (20 μg OVA in 50 μl PBS) on days 27, 29 and 31 under isoflurane (Vedco, Inc. St Joseph, MO) anesthesia. The