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Down-regulation of 8-oxoguanine DNA glycosylase 1 expression in the airway epithelium ameliorates allergic lung inflammation

Attila Bacsi[a,1], Leopoldo Aguilera-Aguirre[a], Bartosz Szczesny[b], Zsolt Radak[a,2], Tapas K. Hazra[b,c], Sanjiv Sur[b,c], Xueqing Ba[a,3], Istvan Boldogh[a,b,*]

[a] Department of Microbiology and Immunology, School of Medicine, University of Texas Medical Branch, Galveston, TX 77555, USA
[b] Sealy Center for Molecular Medicine, School of Medicine, University of Texas Medical Branch, Galveston, TX 77555, USA
[c] Department of Internal Medicine, School of Medicine, University of Texas Medical Branch, Galveston, TX 77555, USA

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ABSTRACT

Allergic airway inflammation is characterized by increased expression of pro-inflammatory mediators, inflammatory cell infiltration, mucus hypersecretion, and airway hyperresponsiveness, in parallel with oxidative DNA base and strand damage, whose etiological role is not understood. Our goal was to establish the role of 8-oxoguanine (8-oxoG), a common oxidatively damaged base, and its repair by 8-oxoguanine DNA glycosylase 1 (Ogg1) in allergic airway inflammatory processes. Airway inflammation was induced by intranasally administered ragweed (*Ambrosia artemisiifolia*) pollen grain extract (RWPE) in sensitized BALB/c mice. We utilized siRNA technology to deplete Ogg1 from airway epithelium; 8-oxoG and DNA strand break levels were quantified by Comet assays. Inflammatory cell infiltration and epithelial methaplasia were determined histologically, mucus and cytokines levels biochemically and enhanced pause was used as the main index of airway hyperresponsiveness. Decreased Ogg1 expression and thereby 8-oxoG repair in the airway epithelium conveyed a lower inflammatory response after RWPE challenge of sensitized mice, as determined by expression of Th2 cytokines, eosinophilia, epithelial methaplasia, and airway hyperresponsiveness. In contrast, 8-oxoG repair in Ogg1-proficient airway epithelium was coupled to an increase in DNA single-strand break (SSB) levels and exacerbation of allergen challenge-dependent inflammation. Decreased expression of the Nei-like glycosylases Neil1 and Neil2 that preferentially excise ring-opened purines and 5-hydroxyuracil, respectively, did not alter the above parameters of allergic immune responses to RWPE. These results show that DNA SSBs formed during Ogg1-mediated repair of 8-oxoG augment antigen-driven allergic immune responses. A transient modulation of OGG1 expression/activity in airway epithelial cells could have clinical benefits.

Abbreviations: 8-oxoG, 7,8-dihydro-8-oxoguanine; AECs, airway epithelial cells; AHR, airway hyperresponsiveness; APE1, AP-endonuclease 1; AP sites, apurinic/apyrimidinic site; BER, base excision repair; BALF, bronchoalveolar lavage fluid; CA, comet assay; DSB, double-strand break; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; GSH, GSSG, reduced and oxidized glutathione, respectively; H_2DCF-DA, 2′-7′-dihydro-dichlorofluorescein diacetate; MEF, murine embryonic fibroblast; Neil1^D/Neil2^D, deficient in Neil1/Neil2 expression; Neil1^P/Neil2^P, proficient in Neil1/Neil2 expression; NOX, NAD(P)H oxidase; Ogg1, 8-oxoguanine DNA glycosylase 1; Ogg1^D, deficient in Ogg1 expression; Ogg1^P, proficient in Ogg1 expression; ROS, reactive oxygen species; RWPE, ragweed pollen grain extract; SSBs, single-strand breaks.

* Corresponding author at: Department of Microbiology and Immunology, University of Texas Medical Branch, 3.142 Medical Research Building, 301 University Blvd, Galveston, TX 77555, USA. Tel.: +1 409 772 9414; fax: +1 409 747 6869.
E-mail addresses: bacsi.attila@gmail.com (A. Bacsi), leaguile@utmb.edu (L. Aguilera-Aguirre), baszczes@utmb.edu (B. Szczesny), radak@tf.hu (Z. Radak), tkhazra@utmb.edu (T.K. Hazra), sasur@utmb.edu (S. Sur), baxq755@nenu.edu.cn (X. Ba), sboldogh@utmb.edu, bacsi.attila@gmail.com (I. Boldogh).
[1] Permanent address: Department of Immunology, Faculty of Medicine, Medical and Health Science Center, University of Debrecen, Debrecen H-4012, Hungary.
[2] Permanent address: Institute of Sport Science, Faculty of Physical Education and Sport Science, Semmelweis University, Budapest H-1025, Hungary.
[3] Permanent address: Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China.

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1. Introduction

Exposure to pollen grains is the major cause of seasonal allergic reactions in the skin, eyes, and upper and lower respiratory tracts [1,2]. Air pollutants can modify the antigenic (allergenic) properties of pollen and generate reactive oxygen species (ROS), which promote pro-inflammatory signaling in airway epithelium, thereby increase the severity and complexity of allergic symptoms in atopic subjects [3,4]. It has been reported that pollen grains, including those of short ragweed (*Ambrosia artemisiifolia*), have intrinsic NAD(P)H oxidase (NOX) activity [5,6]. Upon interaction of pollen with mucosal surfaces, pollen NOX becomes activated and generates ROS, leading to the formation of lipid peroxides (4-HNE, MDA) and oxidized glutathione (GSSG) [5], and activation of transcription factors (including NF-κB) [7]; together with antigens this provokes robust allergic inflammation in the airways and conjunctiva in sensitized subjects [5,8,9]. Additionally, we previously reported that inhibition/inactivation of the pollens' NOX activity decreases both T helper 1 (Th1) and Th2 responses [5,6,10–12].

The pollen NOX primarily generates superoxide anions ($O_2^{\bullet-}$), which are reduced to H_2O_2 and then to hydroxyl radicals ($^{\bullet}OH$), causing damage to macromolecules, including DNA [5,13]. The primary target of ROS in DNA is guanine, because it has the lowest redox potential of the four nucleobases [14,15]. Although guanine base lesions vary according to the nature of the oxidants [16], 7,8-dihydro-8-oxoguanine (8-oxoG) is the most frequent oxidation product in both DNA and RNA, and the accumulation of 8-oxoG is considered a biomarker of inflammation [17]. While its repair in RNA is poorly understood [18], 8-oxoG is repaired in DNA via the base excision/single strand break (BER/SSB) repair pathway [19,20]. In mammalian cells, repair of 8-oxoG is initiated by the 8-oxoguanine DNA glycosylase 1 (OGG1) [21]. The 3′-phospho-α,β-unsaturated aldehyde terminus (apurinic/apyrimidinic [AP] sites) produced by Ogg1 is removed by the 3′-phosphodiesterase activity of the AP endonuclease-1 (APE1) to generate 3′OH for DNA polymerases, which then incorporate the intact base followed by action of DNA ligase to complete the repair process [19,22]. When 8-oxoG is not removed by OGG1 before DNA replication, MYH (*E. coli* mutY homolog) removes adenine misincorporated opposite 8-oxoG in the DNA template [23,24]. The mammalian orthologs of *E. coli* MutM/Nei are NEIL1 and NEIL2 (Nei endonuclease VIII-like glycosylases), which remove oxidized base lesions, including 8-oxoG, during DNA replication and transcription, respectively [25,26].

In the present study, we examined the role of 8-oxoG accumulation and its repair in the DNA of airway epithelial cells in allergic immune responses. To do so, we used siRNA to ablate Ogg1 from the airway epithelium of ragweed pollen grain extract (RWPE)-sensitized animals (Ogg1-deficient: $Ogg1^{D}$ mice) before RWPE challenge. Mice treated with control siRNA were used as controls (Ogg1 proficient: $Ogg1^{P}$ mice). We depleted Ogg1 only from the epithelium because environmental oxidative pollutants primarily affect the epithelium, and its constituent cells play a decision-making role in the initiation and manifestation of innate and adaptive inflammatory processes [27–29]. We avoided using Ogg1-defficient mice, as they globally lack Ogg1 activity. For the first time, we show that supra-physiological levels of 8-oxoG have no effect, but DNA repair intermediates, including SSBs generated during the action of Ogg1, increase the expression of Th2 cytokines and allergic immune responses upon challenge of sensitized mice with RWPE. These data link DNA damage repair to allergic immune responses, and also imply that transient modulation of OGG1 activity could have clinical benefits.

2. Experimental procedures

2.1. Cell culture

MLE-12 (American Type Culture Collection), an immortalized type 2 mouse lung epithelial cell line, was grown in RPMI 1640 medium. $Ogg1^{-/-}$ and $Ogg1^{+/+}$ mouse embryo fibroblast (MEF) cells [30] were kindly provided by Dr. Deborah E. Barnes (Imperial Cancer Research Fund, Clare Hall Labs, United Kingdom) and cultured in DMEM/Ham's F-12 medium [31]. Mouse airway epithelial cells (AECs) were isolated as described previously [32]. In co-culture studies cells were incubated with neutrophils as described previously [33]. Cell viability was determined by Annexin V assay as we described previously [31].

2.2. Animals, sensitization and challenge

Six- to 8-week-old female BALB/c mice (Harlan Sprague–Dawley; San Diego, CA, USA) were sensitized via intraperitoneal (ip) injections of RWPE (certified LPS-free, Greer Laboratories, Lenoir, NC, USA) on days 0 and 4 [5]. On day 11, mice ($n = 7$–9) were challenged intra-nasally with 50 μg of RWPE (per challenge) in 60 μL of saline. All experiments were performed according to the NIH Guidelines for the Care and Use of Experimental Animals. The protocol used was approved by the University of Texas Medical Branch Animal Care and Use Committee (#0807044-05).

2.3. Evaluation of airway inflammation and hyper-responsiveness

Bronchoalveolar lavage fluid (BALF) samples were collected, processed and stained with Wright-Giemsa as we previously described [5,6,13]. Lung tissue sections were processed for staining with hematoxylin and eosin or periodic acid-Schiff, photographed, and evaluated as we previously described [5,6,13]. In parallel experiments immunofluorescence staining was carried out to identify eosinophils in lung sections. Lung tissue sections were stained with antibody to the major basic eosinophilic protein, a kind gift form Dr. G Gleich (Division of Allergic Diseases, Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, MN, USA) as we published previously [5]. Airway hyper-responsiveness (AHR) was determined three days after challenge [7,13]. AHR was expressed as enhanced pause = {(expiratory time/relaxation time) − 1} × (peak expiratory flow/peak inspiratory flow). The flow signals and the respiratory parameters were calculated using the Biosystem XA program (Buxco Electronics Inc., Troy, NY, USA).

2.4. Measurement of ROS levels

ROS levels were assessed using 2′-7′-dihydrodichlorofluorescein diacetate (H_2DCF-DA; Molecular Probes). Dichlorofluorescein (DCF)-mediated fluorescence was determined by fluorescence spectroscopy analysis (FLx800 Bio-Tek Instruments, Winooski, VT, USA) [31,34].

2.5. RNA extraction and real-time (RT)-PCR analysis

RNA was extracted using an RNeasy kit per the manufacturer's instructions (Qiagen, Valencia, CA). Total RNA (1 μg) was reverse-transcribed using a SuperScript® III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed in an ABI7000 thermal cycler [13]. Relative expression levels were calculated by the ΔΔCt method [13,35].