IL-1R2 gene expression is downregulated in obesity-associated asthma

Bantulà M\textsuperscript{1,2,*}, Arismendi E\textsuperscript{1,2,3,*}, Roca-Ferrer J\textsuperscript{1,2}, Picado C\textsuperscript{1,2,3}

\textsuperscript{1}Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona, Spain.
\textsuperscript{2}Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain.
\textsuperscript{3}Department of Pneumology, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain.

* The two authors contributed equally to this work

**Corresponding author**
Marina Bantulà, PhD
ORCID: 0000-0002-3586-2351
Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)
C/ Casanova 143, 08036 Barcelona, Spain
mbantulafonts@gmail.com

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0928
IL-1R2 is the first decoy receptor identified of the IL-1 family and, in contrast to other members of the family, is unable to trigger signal transduction following interaction with its ligands. Regulating IL-1R2 expression helps counterbalance the exacerbated inflammation triggered by endogenous and exogenous stimuli [1].

The NLRP3 inflammasome is an intracellular sensor that detects harmful signals, representing a key component of innate immune responses [2]. Activated NLRP3 interacts with caspase-1, which further cleaves IL-1β into its active forms [2].

NLRP3 inflammasome is involved in the pathogenesis of airway inflammation in asthma [3,4]. IL-1β and caspase-1 are detected at high levels in sputum and peripheral blood of asthmatic patients with neutrophilic airway inflammation [3]. Moreover, IL-1β in sputum predicts poor lung function in neutrophilic asthma [5].

Obesity is a risk factor for asthma, and obese asthmatics experience more severe symptoms and decreased responsiveness to existing therapies [6,7]. IL-1β is elevated in obese individuals’ blood resulting, at least in part, from the activation of caspase-1 and its assembly with the NLRP3 inflammasome by fatty acids [8].

Obese asthmatic patients have increased concentrations of IL-1β and NLRP3 in blood and sputum [9,10]. Activation of NLRP3 inflammasome due to a high-fat diet increased IL-1β production and enhanced airway hyperreactivity [9]. The role of IL-1R2 in asthma and how obesity affects its expression remains unclear.

Obesity is associated with increased inflammation and oxidative stress, which are closely related and refuel each other [11]. The IL-1R2 action as a decoy receptor antagonising IL-1β proinflammatory...
effects supports the hypothesis that obesity, via IL-1R2 downregulation, may contribute to enhancing inflammatory responses and oxidative stress in patients with comorbid asthma-obesity.

We recruited 22 obese asthmatics (OA) [Body mass index (BMI) ≥ 30 kg/m²], 12 non-obese asthmatics (NOA) (BMI < 25 kg/m²), 11 obese non-asthmatics (O), and 13 non-obese non-asthmatic controls (C). The clinical characteristics of the participants are depicted in Supplementary Table 1. The study was approved by the Ethics Committee of Hospital Clínic of Barcelona (2018/4015) and all subjects gave informed consent to participate.

Ten millilitres of whole blood were collected from each patient and peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep™ (Stem Cell TM, Germany). 1 µg of mRNA, isolated from PBMCs using TRIzol (Life Technologies, UK) was converted into cDNA with High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher, Lithuania). Final cDNA was diluted 10-fold prior to use in qPCR. The IL-1R2 expression was analysed via real-time qPCR in the Viia7 Real-Time PCR system (Applied Biosystems, USA). Specific mRNA expression was normalised against 18S rRNA and GAPDH genes.

Moreover, 8-isoprostane in serum was analysed as a marker of oxidative stress using an enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, USA).

Results are expressed as median and interquartile range. Non-parametric comparisons were performed using Mann-Whitney U test. Statistical analyses were performed using GraphPad Prism (version 8.4) with an alpha level set at p<0.05.

We found that IL-1R2 gene expression was elevated (almost statistically significant) in NOA with respect to control subjects. In contrast, IL-1R2 was significantly reduced in both OA and O individuals compared with NOA and controls (Figure 1A). IL-1R2 gene expression levels were inversely correlated (r=−0.439, p<0.0006) with the BMI of all participants (Figure 1B). IL-1R2 levels were higher in severe asthmatics, both with and without obesity, with respect to mild/moderate asthmatics, however, differences were not statistically significant (data not shown).
Serum 8-isoprostane levels were significantly higher in O subjects compared with OA, NOA, and C. No differences were found between OA and NOA patients with respect to C, nor between OA and NOA participants (Figure 1C).

IL-1R2 expression and serum 8-isoprostane levels were positively correlated (r=0.4081, p<0.002) (Figure 1D).

Patients with comorbid asthma-obesity experience greater disease severity and are less responsive to therapy [6]. Weight loss results in clinical and lung function improvement and better response to corticosteroid therapy [6,12]. Given the potentially relevant role that the NLRP3 inflammasome seems to play in asthma and obesity [3,5,6,9,10], we investigated IL-1R2 expression, a decoy receptor with an important role in counterbalancing IL-1β-related exacerbated inflammatory responses [1,2].

Our data show that obesity markedly downregulates IL-1R2 expression in asthmatic and non-asthmatic individuals. To our knowledge, this work is likely to be the first study implicating IL-1R2 in the mechanisms potentially linking asthma and obesity-related inflammation. However, it is unclear whether the combination of obesity and asthma necessarily causes a synergistic effect, leading to even greater oxidative stress [13]. The results of the present study show that systemic oxidative stress significantly differs between obesity and asthma.

Although both diseases are often considered inflammatory diseases, in asthma, the inflammatory process occurs predominantly in the airways with limited systemic extension, whereas the impact of obesity-dependent inflammation spreads widely from adipose tissue affecting other organs and systems, causing a serious alteration of whole body homeostasis. Elevated 8-isoprostane levels have been found in samples of exhaled breath condensate or induced sputum from asthma patients revealing that, in contrast to obesity, the inflammatory process in asthma is predominantly local rather than systemic [14,15].

Interestingly, the lower serum levels of 8-isoprostane detected in OA compared with O subjects suggests that the presence of asthma contributes to downregulating the intensity of obesity-dependent systemic oxidative stress. We are tempted to speculate that the mechanisms that
contribute to regulating excessive ROS release in the airways of asthma patients might counteract the systemic oxidative stress associated with obesity.

Our study has several limitations, including the relatively small number of patients evaluated. Results need to be examined in a larger cohort. Neither did we use induced sputum to analyse the possible differences that distinct asthma phenotypes may have for the studied variables.

With all these limitations in mind, the results of this study reveal the complex interrelation that exists between asthma and obesity. On the one hand, obesity downregulates the expression of the anti-inflammatory IL-1R2 receptor in asthmatic patients, while on the other hand, asthma contributes to reducing 8-isoprostane levels in obese asthmatic subjects.

Conflicts of Interest
None.

Funding
The study was supported by grants from the Fundación Respira (SEPAR) 167/2016 and 736/2018, Fundació Catalana de Pneumologia (FUCAP) and by an unrestricted grant from Menarini.

REFERENCES
1. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev. 2018;281:8–27.*


Figure 1. IL-1R2 gene expression and 8-isoprostane serum levels.

A. Differences in IL-1R2 expression expressed as Fold Change (FC) between non-asthmatic non-obese (C) subjects, non-obese asthmatics (NOA), obese asthmatics (OA), and non-asthmatic obese (O) subjects. B. Negative correlation (r=-0.439, p<0.0006) between IL-1R2 expression and body mass index (BMI). C. 8-isoprostane serum levels comparison between non-asthmatic non-obese (C) subjects, non-obese asthmatics (NOA), obese asthmatics (OA), and non-asthmatic obese (O) subjects. D. Positive correlation (r=0.4081, p<0.002) between 8-isoprostane serum levels and IL-1R2 gene expression. Data presented as individual values and medians (25th–75th percentile). Mann-Whitney U test for differences between groups and Spearman’s rank method for correlation data.