



Letter

Effect of nasal allergen challenge in allergic rhinitis on mitochondrial function of peripheral blood mononuclear cells

Allergic rhinitis is the most common of the atopic diseases, affecting up to 25% of the population worldwide.¹ Grass pollen sensitization has been recognized as a major cause of allergic rhinitis. On the pathophysiologic level, allergic rhinitis is an IgE-mediated inflammation of the nasal mucosa. Grass pollen allergy, commonly called hay fever, can also cause more general symptoms, such as fatigue and unwellness as seen in flulike syndromes. This might be partly related to the activation of a systemic inflammatory pathway after the local nasal inflammatory response, but data from studies concerning the systemic effects of nasal mucosal allergen exposure are limited.

Mitochondria are crucial cellular signaling organelles that are an integral part of the decision-making process when cells receive internal and external cues to trigger diverse biological outcomes ranging from metabolic adaptation, proliferation, differentiation, to cell death.² To our knowledge, it is not known whether peripheral inflammatory cells are also affected by mitochondrial dysfunction after a local nasal allergen response. We therefore examined, in patients with grass pollen allergic rhinitis, whether allergen exposure might affect mitochondrial function in peripheral blood mononuclear cells (PBMCs) (clinicaltrials.gov Identifier: NCT01992835).

Eleven patients (mean [SD] age, 26.2 [7.9] years; 5 men and 6 women), with a history of grass pollen allergic rhinitis confirmed by the presence of specific IgE to grass pollen (mean [SD], 14.02 [23.3] KU) were included. The study was approved by the local research ethics committee, and informed written consent was obtained from each participant.

A first nasal allergen challenge was performed to determine the threshold dose (Lebel score >5), obtained after increasing doses of a grass pollen extract locally delivered via a nasal pump spray device.³ Then after a washout period of 4 weeks, patients underwent 2 nasal allergen challenges with either a single threshold dose of the grass pollen allergen extract (Stallergenes) or a diluent (saline solution) in a randomized order at least 4 weeks apart. All the challenges were performed outside the grass pollen season. Peripheral blood was collected immediately before and 6 and 24 hours after the nasal challenge.

PBMCs were isolated from heparinized blood. Briefly, blood samples (20 mL) were layered on top of 3 mL of Ficoll 400 (Eurobio, Les Ulis, France) and centrifuged at 300g for 25 minutes (18°C–20°C). The white buffy coat was gently aspirated, mixed with 3 to 4 volumes of phosphate-buffered saline, and centrifuged at 300g for 10 minutes. The PBMC pellet was resuspended in

respiratory solution that contained glutamate (5 mM) and malate (2 mM). Cell count and viability were performed.^{4,5} Mitochondrial respiratory function was analyzed on 2×10^6 cells/mL at 37°C with continuous stirring in a high-resolution respirometry (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria). Briefly, cells were permeabilized with saponin (0.1 mg/mL). The maximal respiration rate and specific mitochondrial respiratory chain complex activities were determined as previously reported.⁶ Data are presented as mean (SEM) with $P < .05$ considered statistically significant.

Our results indicate that grass pollen exposure reduces maximal mitochondrial respiratory chain complex activities and mitochondrial coupling 6 hours after the nasal allergen challenge. Eight healthy individuals (mean [SD] age, 31.2 [2.2] years; 4 men and 4 women) had a combined activity of complexes I, III, and IV (5.9 [0.7] pmol/ 10^6 cells) that was not statistically different from baseline levels in allergic patients (6.1 [0.6] pmol/ 10^6 cells before placebo and 4.7 [0.9] pmol/ 10^6 cells before allergen challenge; $P > .05$). Combined activities of complexes I, III, and IV were significantly decreased 6 hours after the local allergen challenge when compared with the rate before the challenge (4.7 [0.9] pmol/ 10^6 cells at baseline vs 2.1 [0.4] pmol/ 10^6 cells at 6 hours; 63% decrease; $P < .05$) (Fig 1), although after accounting for the different baseline values, the change from baseline was not statistically different between the 2 groups. This decrease was no longer observed 24 hours after the challenge (6.2 [0.7] pmol/ 10^6 cells at 24 hours). No significant changes were observed after the placebo challenge (6.1 [0.6] pmol/ 10^6 cells at baseline vs 4.2 [0.9] pmol/ 10^6 cells at 6 hours vs 5.9 [1.3] pmol/ 10^6 cells at 24 hours). Mitochondrial respiratory chain complexes II, III, and IV activities and cytochrome c oxidase activity (complex IV) were not significantly impaired after allergen exposure, emphasizing the deleterious effect of the allergen challenge on mitochondrial complex I.

After allergen exposure, the acceptor control ratio, reflecting the degree of coupling between oxidation and phosphorylation, was significantly lower at 6 hours compared with 24 hours (1.8 [0.3] vs 3.9 [0.7], $P < .05$).

An increase in the percentage of peripheral blood eosinophils was observed 6 and 24 hours after the allergen challenge (1.8% [0.4%] at baseline vs 3.1% [0.51%] at 6 hours vs 3.7% [0.6%] at 24 hours, $P < .01$). A negative correlation was found 6 hours after the allergen challenge between the blood eosinophil level and the maximal respiration rate ($R^2 = 0.40$, $P < .05$).

This is the first report demonstrating that a nasal allergen challenge impairs circulating cell mitochondrial functions in patients presenting with allergy in general and particularly in grass pollen allergic rhinitis. The significant increase in our study of peripheral blood eosinophils observed after the allergen challenge confirms the systemic involvement in local nasal allergy

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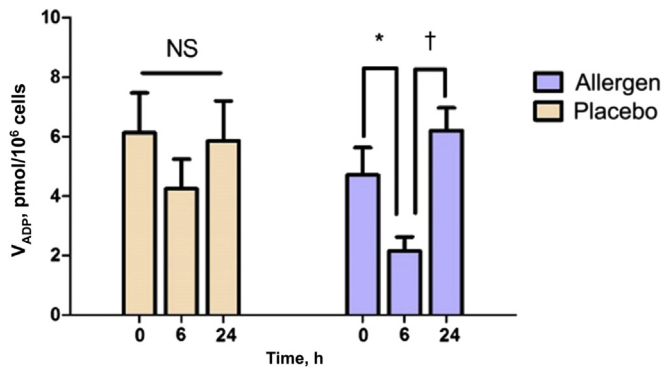


Figure 1. Mitochondrial function of peripheral blood mononuclear cells (PBMCs) is impaired 6 hours after the local nasal allergen challenge. Mitochondrial respiratory chain complex activities (maximal respiration rate [V_{ADP}]) in PBMCs at 0, 6, and 24 hours after allergen or placebo nasal challenge. * $P < .05$; $^{\dagger}P < .01$.

pathophysiology, which is further underlined by the inverse correlation between the peripheral eosinophil count and maximal respiration rate of PBMCs 6 hours after the nasal allergen challenge.

After nasal allergen provocation, the acute early local allergic nasal response in the first minutes is typically followed in a proportion of individuals by a late nasal response with an ongoing recruitment of inflammatory cells in the nasal mucosa from 3 to 24 hours.⁷ Studies reported that this late nasal response that peaks generally at 6 hours is accompanied by a systemic effect outlined by an increase in peripheral blood eosinophils.⁸ Changes in the activation status and phenotypic markers of circulating basophils, T cells, and plasmacytoid dendritic cells after nasal allergen challenge have also been reported,⁹ supporting the concept that exposure to allergen at the level of the respiratory mucosa induces systemic alterations to various components of the immune system. Interestingly, in our study, complex I respiration and coupling, main parameters of mitochondrial functions of peripheral blood mononuclear cells, were significantly impaired after the nasal allergen challenge, suggesting that such decreased mitochondrial function might participate in the allergic process. Whether such PBMC mitochondrial alteration might be used as a biomarker of allergic activity or as a therapeutic target remains unknown, but as observed in other immune diseases, the crosstalk between mitochondria and immunity gains importance, and such issue deserves further studies in the setting of allergic disease. This finding is in line with the emerging role of mitochondria in shaping the immune response from the innate to the adaptive processes.^{10,11} Extending such knowledge, our data are the first to indicate a potential role of mitochondria in allergic disease.

In conclusion, our data support the concept that allergic rhinitis is both a local and a systemic disease, affecting mitochondrial dysfunction of peripheral mononuclear cells. This work highlights the implication of mitochondria in allergic rhinitis and supports further studies aiming to elucidate the potential clinical usefulness of PBMC mitochondrial function monitoring in such setting.

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