Received: 8 March 2023 | Revised: 19 April 2023 | Accepted: 28 April 2023

DOI: 10.1111/all.15760

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

NEWS AND VIEWS

Mapping the way to strengthening epithelial barriers: Neuronal circuits in mucus regulation

The skin, airways, and gastrointestinal epithelium are physical barriers that protect individuals against environmental threats including toxins, pollutants, pathogens, and allergens. Certain aspects of the industrialized lifestyle are thought to contribute to the increasing prevalence of inflammatory diseases, such as allergy. In this regard, it has been proposed that epithelial barrier impairment caused by environmental insults, among others, favors the passage of allergens and bacterial translocation to subepithelial areas. This event, combined with the underlying inflammation and the alarmins involved, is thought to mislead dendritic cells, which perceive allergens as a threat and acquire an activation phenotype that is inductive of Th2 differentiation upon cognate CD4 T-cell encounter. Thus, barrier impairment can lead to the development of type 2 inflammatory diseases.[1,2]

The gut epithelium is the largest barrier of the organism. By and large, it consists of epithelial cells and their junctional complexes, overlaid by a mucus layer and microbes.[1,3] The mucus is composed by mucins (MUC) and other proteins secreted by specialized goblet cells.[4] Altogether works as a built-in system that facilitates nutrient absorption while defending itself from outer insults. On this point, sensory neurons communicate with gut epithelial cells to preserve gastrointestinal functionality.[3] The relevance of this neuroepithelial axis is highlighted by the association between a *leaky gut* and neurological disorders,[1] or by the direct cross-talk between enteroendocrine cells and sensory neurons that results in metabolic adaptation.[4] Therefore, neuroimmune communication is crucial for maintaining epithelial barriers integrity and, thus, may play a role in the development of allergy.

The recent work by Yang et al. describes that mucin secretion by goblet cells is directly regulated by nociceptor sensory neurons. They show that the gut mucus layer was reduced in nociceptor sensory neuron-deficient mice (Nav1.8^{DTA}). Moreover, in a hMD3q-designer-receptor reporter mice (Nav1.8^{hM3Dq}), whose nociceptor sensory neurons are activated by clozapine N-oxide, they demonstrate that the activation of these neurons was sufficient to elicit mucus production.[4] The role of the mucus layer is not limited to forming a non-specific physical barrier, but also has an immunoregulatory function. The hyperglycosylated-MUC2, secreted by goblet cells, is a main mucus component of the gastrointestinal epithelium. It has been demonstrated that MUC2-associated glycans reduce inflammation by impairing NFκβ-driven responses in dendritic cells.[5] By contrast, alterations in composition and the amount of the airway MUC5AC and MUC5B are associated with airway obstruction and mucus plugging in both murine asthma models and disease-affected individuals.[6] This excessive mucus production is accompanied by both an airway remodeling and an increase of epithelial permeability, which may favor luminal allergen access and bacterial translocation, exacerbating an inflammatory response and further damaging the epithelium.

The nociceptor sensory neuron-mucus axis discovered by Yang et al. is triggered by the α-calcitonin gene-related peptide (CGRPα), which activates the receptor activity modifying protein 1 (Ramp1) signaling pathway in intestinal goblet cells inducing mucin-2 production (Figure 1). CGRPα is a 37-amino-acid neuropeptide product of the alternative splice of the *calcitonin* gene.[7] They observe that mice lacking Ramp1 expression in gut epithelial cells (Ramp1^{Villin}) had a significantly thinner mucus layer thickness with no reduction in goblet cell number. These data are strengthened by the release of CGRPα by nociceptor sensory neurons in Nav1.8^{hM3Dq} mice upon clozapine N-oxide challenge.[4] In addition, Yang et al. report that either the absence of these neurons or Ramp1 expression in goblet cells resulted in dysbiosis in mice and an increased susceptibility to inflammatory colitis, which was reversed by CGRPα administration.[4]

Meanwhile, the CGRPα-Ramp1 axis has also been described in allergic diseases such as asthma, where it can regulate airway tone and type 2 immune response. However, this axis may have an opposite effect depending on the tissue. For example, it has been observed that CGRPα could have a protective function in the airway epithelium by increasing intracellular levels of cyclic AMP, causing bronchodilation.[8] Precisely, lower levels of Ramp1 have been found in biopsies of asthma patients as compared to controls.[9] In addition, CGRPα promotes regulatory T lymphocyte differentiation, thus dampening the Th2 response and the release of inflammatory mediators by type 2 innate lymphoid cells. In contrast, the CGRPα-Ramp1 axis could also favor asthma pathogenesis as it promotes airway inflammation by inducing a Th9 profile, IL-6 secretion by bronchial epithelium and IL-5 synthesis by type 2 innate lymphoid cells.[7,10]

In conclusion, Yang et al. add insight on a novel neuroepithelial axis controlling mucus integrity in the gastrointestinal epithelium. Understanding the complex regulatory role of the CGRPα-Ramp1 pathway in mucus production may be a promising therapeutic

Abbreviations: CGRPα, α-calcitonin gene-related peptide; MUC, mucin; Ramp1, receptor activity modifying protein 1.

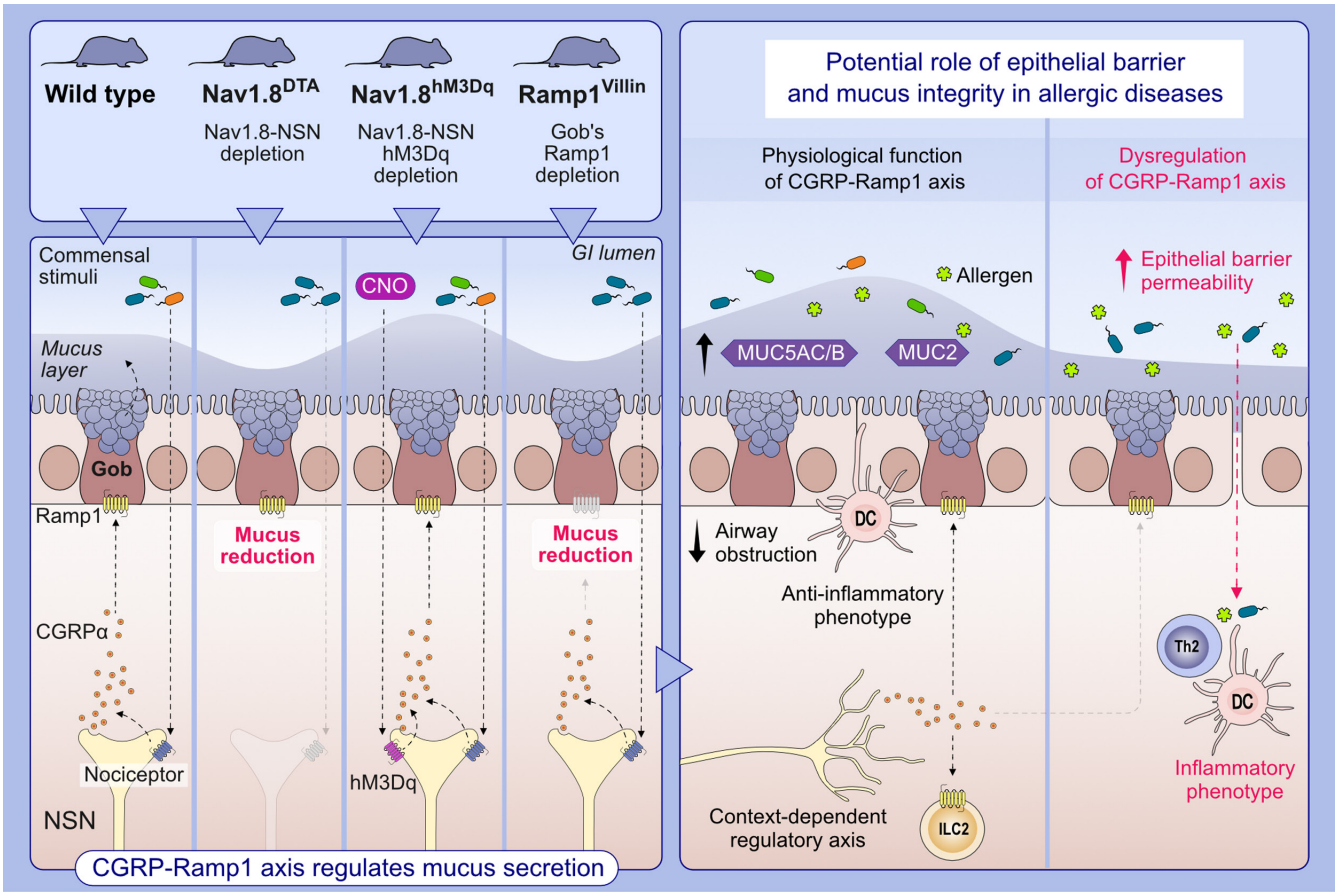

FIGURE 1 Nociceptor sensory neuron-goblet cells axis CGRPα-Ramp1 regulates mucus production in the gastrointestinal tract. The absence of both nociceptor sensory neurons in Nav1.8^{DTA} or Ramp1 in Ramp1^{Villin} murine models resulted in a reduction of mucus thickness. However, nociceptor sensory neurons induced signaling upon bacterial insults or clozapine N-oxide challenge in the hMD3q-designer-receptor reporter mice Nav1.8^{hM3Dq} retrieve mucus production (left panel). Mucus in epithelial barriers is key in maintaining homeostasis. In the gastrointestinal tract, an altered mucus layer converges in dysbiosis and colitis, while in the airways affects the disease course of asthma patients. Thus, the lack of an adequate mucus layer may contribute to enhance epithelial permeability to noxious threats such as allergens, increasing allergic inflammation (right panel). CGRPα: α-calcitonin gene-related peptide; CNO: clozapine N-oxide; DC: dendritic cell; GI: gastrointestinal; Gob: goblet cell; ILC: innate lymphoid cell; MUC: mucin; NSN: nociceptor sensory neurons; Ramp1: receptor activity modifying protein 1; Th: T helper lymphocyte.

avenue for enhancing barrier integrity and, therefore, preventing dysregulated inflammatory responses in allergic diseases. Yet, it is important to further characterize the protective function of the CGRPα-Ramp1 axis in allergic diseases, as its impact appears to be context-dependent. From a broader perspective, given its importance for gastrointestinal health, mucus may be targeted as a biomarker, particularly considering the fast-advancing technological developments for ingestible microdevices.[11] Along this line, it is not farfetched to consider the application of synthetic biology to engineer CGRPα-secreting bacteria that enhances mucus production.[12]

AUTHOR CONTRIBUTIONS

The authors approved the final version of the manuscript as submitted and agreed to be accountable for all aspects of the work. CLS and ENB drafted the manuscript and the graphical abstract. RJS provided critical feedback and revisions.

KEYWORDS

epithelial barriers, goblet cells, mucus layer, neuroimmunology

ACKNOWLEDGMENTS

The authors thank Dr. Anna Globinska for graphical abstract design.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare in relation to this manuscript.

FUNDING INFORMATION

RJS's laboratory is funded by the FSE/FEDER through the Instituto de Salud Carlos III (CP20/00043; PI22/00236; Spain), The Nutricia Research Foundation (NRF-2021-13; The Netherlands), New Frontiers in Research Fund (NFRFE-2019-00083; Canada), and SEAIC (BECA20A9; Spain).

Celia López-Sanz[1]
Rodrigo Jiménez-Saiz[1,2,3,4]
Emilio Nuñez-Borque[5]

[1]*Department of Immunology, Instituto de Investigación Sanitaria Hospital Universitario de La Princesa (IIS-Princesa), Universidad Autónoma de Madrid (UAM), Madrid, Spain*
[2]*Department of Medicine, McMaster Immunology Research Centre (MIRC), Schroeder Allergy and Immunology Research Institute (SAIRI), McMaster University, Hamilton, Ontario, Canada*
[3]*Department of Immunology and Oncology, Centro Nacional de Biotecnología (CNB)-CSIC, Madrid, Spain*
[4]*Faculty of Experimental Sciences, Universidad Francisco de Vitoria (UFV), Madrid, Spain*
[5]*Department of Allergy and Immunology, Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD), Universidad Autónoma de Madrid (UAM), Madrid, Spain*

Correspondence
Emilio Nuñez-Borque, Department of Allergy and Immunology, Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD), Av. de los Reyes Católicos 2, 28040 Madrid, Spain.
Email: enbli7@hotmail.com

ORCID

Celia López-Sanz https://orcid.org/0000-0001-7203-0290
Rodrigo Jiménez-Saiz https://orcid.org/0000-0002-0606-3251
Emilio Nuñez-Borque https://orcid.org/0000-0003-2928-410X

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