



Löser, S. and Maizels, R. M. (2018) Immunology: the neuronal pathway to mucosal immunity. *Current Biology*, 28(1), R33-R36, (doi:10.1016/j.cub.2017.11.025).

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

<http://eprints.gla.ac.uk/155737/>

Deposited on: 1 March 2018

Enlighten – Research publications by members of the University of Glasgow_
<http://eprints.gla.ac.uk>

Immunology : The nervous pathway to mucosal immunity

Stephan Löser and Rick M Maizels*

Wellcome Centre for Molecular Parasitology
Institute of Infection, Immunity & Inflammation
College of Medical, Veterinary & Life Sciences
University of Glasgow
120 University Place
Glasgow G12 8TA, UK

* Corresponding Author : e-mail : rick.maizels@glasgow.ac.uk

SUMMARY

Type 2 immunity at mucosal surfaces is thought to be initiated by type 2 innate lymphoid cells (ILC2s). New studies report that ILC2s are themselves activated by the neuropeptide neuromedin U (NMU), produced by cholinergic neurons in the gut and airways.

Type 2 immunity is the arm of the immune system typically associated with both allergy, and effective defense against helminth worm infections [1, 2]. Type 2 cells release a range of soluble multifaceted effector cytokines, accelerating intestinal nematode expulsion and wound repair, but also exacerbating disease in allergic patients when dysregulated. At the heart of type-2 immunity are two different lymphoid-derived cells; T helper type-2 (Th2) cells of the adaptive immune axis, that release cytokines when activated through their antigen-specific receptors, and their mirror image in the innate immune system, type-2 innate lymphoid (ILC2) cells, representing potent first-responders to generic immune stimuli released at barrier surfaces [2, 3].

ILC2s are local amplifiers of the type-2 immune response, releasing the key cytokine drivers of eosinophil infiltration (IL-5), goblet and tuft cell hyperplasia (IL-13) and fibrosis, in response to signals such as alarmins secreted by epithelial cells [4]. Attention has focused on a troika of epithelial alarmins, namely IL-33, a DNA-associated cytokine released upon cell damage; IL-25, produced by tuft cells in a taste-receptor signaling-dependent manner, and Thymic Stromal Lymphopoietin (TSLP), a predominant ILC2 activator in the skin [4, 5]. These mediators have been depicted in a simple bi-directional stromal cell/immune cell dialogue, that allows epithelial cells to flag and report an immune insult to ILCs, which in turn instruct the epithelial barrier to condition itself against the immediate challenge, while kick-starting adaptive immunity to counteract chronic infections. A more profound network is now revealed by three novel reports [6-8] introducing a multidirectional communication circuit between stromal, immune system and nerve cells in which ILC2s are the central cell type coordinating the crosstalk between the mucosa and the innervation (**Figure 1a**). In this system, it is the release of the peptide neuromedin U (NMU) by cholinergic neurons [7, 8] which is the key event in ILC activation, showing that type 2 immunity is not dictated exclusively by epithelial cells.

NMU is a neuropeptide, 25 amino acids in length in the human. It is a potent activator of two NMU receptors (NMUR), of which *Nmur1* is specifically expressed by ILC2 cells in the lung and small intestine [6-8], but only marginally [8] in other lymphoid and myeloid populations, suggesting ILC2s are selectively responsive to the neuropeptide. ILC2-specific *Nmur1* expression rises following either infection with the gastrointestinal helminth *Nippostrongylus brasiliensis* or intranasal allergen challenge [6-8], concomitant with increased NMU expression in helminth-infected intestinal and lung tissue [7, 8]. In particular, increased *Nmur1* expression correlated with the expression of the IL-33 receptor (ST2), but not the IL-25 receptor [6], suggesting that *Nmur1* may discriminate between subsets of alarmin-responsive ILCs. Additionally, IL-33 stimulation results in *Nmur1* down-regulation, thereby limiting NMU action on ILCs following alarmin release. These data highlight a conserved mechanism of nerve-ILC2 crosstalk in diverse settings of type-2 pathophysiology, with its own intricate selectivity and regulation.

A fascinating insight from these new studies is the intimate anatomical connection between the neuronal and innate lymphoid cells (**Figure 1a**). The closer proximity between the innervation of the intestinal lamina propria and lung parenchyma and resident ILC2 cells in comparison to T cells, leads to several interesting, but yet unanswered, questions regarding the function of this co-localization. Does NMU enjoy a very limited range of spatial activity within tissues, and are ILCs instructed by nerve-derived NMU to migrate from the fixed sites of enteric innervation, and/or do they do so in response to the epithelial alarmins? In fact, in an *ex vivo* assay of ILC2 cell stimulation, NMU proved considerably more potent in promoting ILC2 cytokine production than either IL-25 or IL-33 [8] or even a combination of both [7]. However, the physiological NMU concentration inside the lamina propria or lung tissue has yet

to be elucidated, and the degree of redundancy among the various ILC2 stimuli *in vivo* may be very considerable. Certainly, bone marrow chimera experiments testing both *Nmur1*-sufficient and -deficient cells showed a reduced but not abrogated type-2 cytokine expression in lung *Nmur1*-deficient ILCs, demonstrating that no one ligand fulfills a unique and indispensable role [8].

The significance of ILC2s was initially established in the setting of helminth infection, and hence it is not surprising that these parasites elicit increased NMU production in murine gut tissue [7]. Cardoso et al. and Klose et al. focused their *in vivo* characterization on ILC2-neuronal crosstalk (**Figure 1b**) during infection with *N. brasiliensis*, which migrates through, and induces strong type-2 immunity within, both the lung and the small intestine. *Nmur1*-deficient mice show increased worm burdens at early time points (days 2-7) in both tissues, alongside compromised type-2 cytokine levels, airway eosinophilia and mast cell recruitment [7, 8]. However, *N. brasiliensis* was still effectively expelled by day 9 p.i. [8], emphasising again that the immune system can compensate, albeit tardily, through redundant mechanisms. As both lung and gut immunity is attenuated in *Nmur1*-deficient mice, it was not clear if delayed expulsion is a result of impeded type-2 immune mechanisms in the lung or a consequence of malfunctioning intestinal immunity. To circumvent this issue, Klose et al. i.p. injected NMU days 2, 4 and 6 p.i., leading to a mildly, but significantly, reduced worm count at day 7 p.i., suggesting that intestinal NMU may indeed affect nematode survival. As NMU can also provoke intestinal smooth muscle contraction [9] which is instrumental in helminth expulsion [10], it was also important that to pinpoint the role of haematopoietic cells. Thus, both investigators injected bone marrow from *Nmur1*^{+/+} and *Nmur1*^{-/-} mice into alymphoid *Rag2*^{-/-} *Il2rg*^{-/-} mice. Worm burdens were augmented in *Nmur1*^{-/-} bone marrow chimeras compared to mice reconstituted with *Nmur1*-sufficient cells [7, 8], emphasizing the importance of NMU-activated ILC2s in anti-helminth immunity.

To test the function of neuronal NMU release into the respiratory system without infection, two approaches were taken, with direct intranasal administration of NMU [7] and intranasal instillation of the highly allergenic house dust mite (HDM) extract [6]. In the lung, NMU proved to strongly induce ILC2 cytokine production and eosinophil recruitment, including in ILC2-reconstituted *Rag2*^{-/-} *Il2rg*^{-/-} mice [7]. In an elegant single cell RNA sequencing (scRNAseq) approach Wallrapp et al. addressed the heterogeneity of ILC2 cells in resting, epithelial cytokine stimulated and HDM-treated immune states. Strikingly, IL-25 and IL-33 induced different transcriptional profiles in ILC2s, with high *Nmur1* expression at steady state and after IL-25 treatment, but downregulated by IL-33. In particular, NMU amplifies the pro-inflammatory capacity of IL-25, not only *in vitro*, but also *in vivo* when pulmonary pathophysiology is evaluated. In the allergic setting, however, *Nmur1* deficiency resulted only in minor differences in terms of immunological readouts, other than the phenotype of ILC2s themselves. Despite reduced ILC2 cells and cytokines in *Nmu* KO mice after HDM challenge, this had no effect on the increased level of total inflammation and augmented eosinophilic infiltration, which may be explained by compensatory CD4⁺ T-cell recruitment to the site of inflammation. Similarly when exposed to HDM, *Nmur1* deficient mice only showed a mildly but significantly improved inflammatory score compared to WT mice [6].

By opening a new window into the generation of type 2 immunity, the authors also provoke many further questions. For example, the nature of the molecular patterns from helminths or allergens which select the type 2 pathway remain unresolved [11, 12]. Interestingly, the secretions of *N. brasiliensis* (termed NES, previously reported to drive type 2 responses *in vivo* [13]) elicit NMU production from neurons indicating a pathogen recognition system in these cells. Direct neuronal sensing of microbial

products has also been reported, most recently in an intestinal organ culture model [14]. Certainly, MyD88 (which also transduces the IL-33 signal) is required within neuronal cells for optimal immunity, implying that there may be a positive feedback loop in which NMU production is amplified by IL-33 or other epithelial/ILC2-derived signals [8] (**Figure 1b**). Equally, although mast cell- and eosinophil-deficient mice show intact NMU-driven ILC2 activation [7], it is known that these and other innate populations do participate in a fully orchestrated type 2 response to attack (appropriately) helminths and (inappropriately) allergens, and their interaction with neuronal cells seems highly likely.

These exciting papers open up a new dimension in the immunological network, and add to growing appreciation of how pivotal the nervous system may be in driving the immune system [15, 16]. NMU is one of many small molecule mediators that act on immune cells, including vasoactive intestinal peptide (VIP) which regulates a circadian ILC-eosinophil interaction [17] and acetylcholine (ACh) produced by the NMU-producing neurons in the intestine [7]. Interestingly, ACh signaling is known to be required for immunity to *N. brasiliensis* [18]. Returning the compliment, many immune cells produce neuroactive molecules including ACh [19], histamine, serotonin and catecholamines [20], which in the latter case enable alternatively-activated macrophages to regulate thermogenesis. While the full circuitry has yet to be mapped, it is clear that the integration of neuro-immune signaling will reveal many more critical interactions that will aid understanding of neuroimmunology and our ability to regulate immune dysfunction through neurological pathways.

REFERENCES

1. Allen, J.E., and Maizels, R.M. (2011). Diversity and dialogue in immunity to helminths. *Nat Rev Immunol* **11**, 375-388.
2. Licona-Limón, P., Kim, L.K., Palm, N.W., and Flavell, R.A. (2013). TH2, allergy and group 2 innate lymphoid cells. *Nat Immunol* **14**, 536-542.
3. McKenzie, A.N.J., Spits, H., and Eberl, G. (2014). Innate lymphoid cells in inflammation and immunity. *Immunity* **41**, 366-374.
4. Klose, C.S., and Artis, D. (2016). Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. *Nat Immunol* **17**, 765-774.
5. Ziegler, S.F., and Artis, D. (2010). Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* **11**, 289-293.
6. Wallrapp, A., Riesenfeld, S.J., Burkett, P.R., Abdulnour, R.E., Nyman, J., Dionne, D., Hofree, M., Cuoco, M.S., Rodman, C., Farouq, D., et al. (2017). The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. *Nature* **549**, 351-356.
7. Klose, C.S.N., Mahlakoiv, T., Moeller, J.B., Rankin, L.C., Flamar, A.L., Kabata, H., Monticelli, L.A., Moriyama, S., Putzel, G.G., Rakhilin, N., et al. (2017). The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature* **549**, 282-286.
8. Cardoso, V., Chesne, J., Ribeiro, H., Garcia-Cassani, B., Carvalho, T., Bouchery, T., Shah, K., Barbosa-Morais, N.L., Harris, N., and Veiga-Fernandes, H. (2017). Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature* **549**, 277-281.
9. Prendergast, C.E., Morton, M.F., Figueroa, K.W., Wu, X., and Shankley, N.P. (2006). Species-dependent smooth muscle contraction to Neuromedin U and determination of the receptor subtypes mediating contraction using NMU1 receptor knockout mice. *Br J Pharmacol* **147**, 886-896.
10. Vallance, B.A., Blennerhassett, P.A., and Collins, S.M. (1997). Increased intestinal muscle contractility and worm expulsion in nematode-infected mice. *Am J Physiol* **272**, G321-327.
11. Camberis, M., Prout, M., Tang, S.C., Forbes-Blom, E., Robinson, M., Kyle, R., Belkaid, Y., Paul, W., and Le Gros, G. (2013). Evaluating the in vivo Th2 priming potential among common allergens. *J Immunol Methods* **394**, 62-72.
12. Tyagi, N., Farnell, E.J., Fitzsimmons, C.M., Ryan, S., Tukahebwa, E., Maizels, R.M., Dunne, D.W., Thornton, J.M., and Furnham, N. (2015). Comparisons of allergenic and metazoan parasite proteins: allergy the price of immunity. *PLoS Comput Biol* **11**, e1004546.
13. Holland, M.J., Harcus, Y.M., Riches, P.L., and Maizels, R.M. (2000). Proteins secreted by the parasitic nematode *Nippostrongylus brasiliensis* act as adjuvants for Th2 responses. *Eur. J. Immunol.* **30**, 1977-1987.
14. Yissachar, N., Zhou, Y., Ung, L., Lai, N.Y., Mohan, J.F., Ehrlicher, A., Weitz, D.A., Kasper, D.L., Chiu, I.M., Mathis, D., et al. (2017). An intestinal organ culture system uncovers a role for the nervous system in microbe-immune crosstalk. *Cell* **168**, 1135-1148 e1112.
15. Veiga-Fernandes, H., and Mucida, D. (2016). Neuro-immune interactions at barrier surfaces. *Cell* **165**, 801-811.
16. Yoo, B.B., and Mazmanian, S.K. (2017). The enteric network: interactions between the immune and nervous systems of the gut. *Immunity* **46**, 910-926.
17. Nussbaum, J.C., Van Dyken, S.J., von Moltke, J., Cheng, L.E., Mohapatra, A., Molofsky, A.B., Thornton, E.E., Krummel, M.F., Chawla, A., Liang, H.E., et al. (2013). Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* **502**, 245-248.
18. Darby, M., Schnoeller, C., Vira, A., Culley, F.J., Bobat, S., Logan, E., Kirstein, F., Wess, J., Cunningham, A.F., Brombacher, F., et al. (2015). The M3

- muscarinic receptor is required for optimal adaptive immunity to helminth and bacterial infection. *PLoS Pathog* 11, e1004636.
19. Rosas-Ballina, M., Olofsson, P.S., Ochani, M., Valdes-Ferrer, S.I., Levine, Y.A., Reardon, C., Tusche, M.W., Pavlov, V.A., Andersson, U., Chavan, S., et al. (2011). Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* 334, 98-101.
 20. Nguyen, K.D., Qiu, Y., Cui, X., Goh, Y.P., Mwangi, J., David, T., Mukundan, L., Brombacher, F., Locksley, R.M., and Chawla, A. (2011). Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature* 480, 104-108.

Figure Legend

Figure 1

Type-2 innate lymphoid cells (ILC2s) and nerve cells form a neuro-immunological unit within mucosal tissues.

(a) The intestine is innervated by both sympathetic and parasympathetic nerves and is thereby connected to the central nervous system, but also encompasses an independent neurological network, the enteric nervous system (ENS). Nerve cells of the ENS, which extend into the villi, are organized in interconnected enteric ganglia forming submucosal and myenteric plexuses inbetween the intestinal muscle layers [15, 16]. In very close proximity to enteric neurons are ILC2 cells, innate immune cells capable of releasing type-2 cytokines in response to immune challenges and poised to be instructed by both neurological and epithelial mediators. Similarly, but not shown in the figure, ILC2s have been shown to be located close to neurons of the pulmonary innervation.

(b) In the mucosa, ILC2s, epithelial cells and neurons form a neuro-immunological triangle, in which ILC2s resemble central effector cells instructed by both epithelial cytokines, e.g. IL-25 and the neuron-derived peptide NMU to release type-2 cytokines in response to an immune challenge such as helminth infection, resulting in epithelial conditioning and amplification of local type-2 immunity. New evidence suggests the NMU release might be regulated directly by helminth-derived products, as well as epithelial cytokines and - possibly - type 2 cytokines from ILCs.

