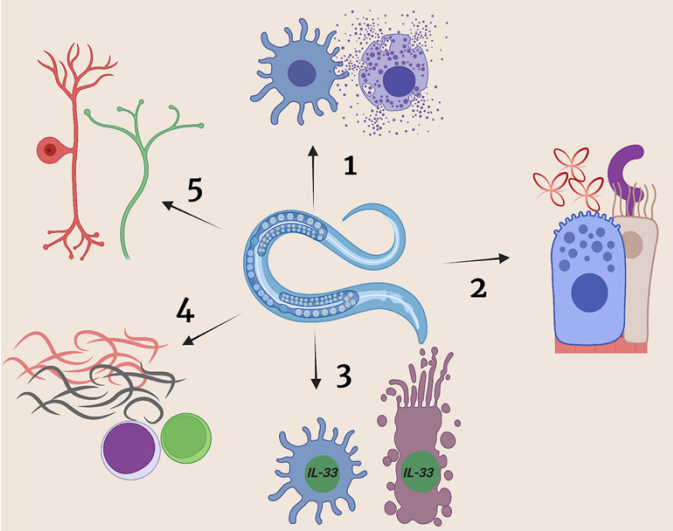
Herbert, D.R.

Research Statement



Research topics: **1**) M2 macrophages and myeloid APC in host defense and wound healing **2**) Trefoil factor family proteins in tissue repair and mucosal barrier function **3**) Cell-specific role for interleukin 33 in homeostasis and disease **4**) Gene targeting in parasitic nematodes and **5**) Role of sensory neurons in allergy and host defense.

The over-arching goal of my research program is to use parasitic organisms as a guide to investigate basic mechanisms of host immunity, inflammation and wound healing. I have been continuously NIH-R01 funded by the National Institutes of Health (NIH) since 2007 and have authored 55 manuscripts published in journals that include: *Science*, *Science Immunology*, *Nature Medicine, Nature Communications, Immunity, JEM, PNAS, PLoS Pathogens*, and the *Journal of Immunology*. Most of this work has focused on the immune response to parasitic helminths, organisms that are the likely evolutionary driving force for Type 2 immunity. Helminths are a major cause of disease in impoverished populations (~2-3 billion people) and biomedical research focused on parasitic helminths has been a fertile ground for scientific discovery.

Helminths such as hookworms pose a formidable challenge to the host immune system regarding their large size, morphological complexity and the host tissue niches they occupy. While infectious larval stages can cause tremendous damage to host tissues as they invade and migrate, the hematophagous nature of adult stages can cause persistent injury during feeding. Surprisingly, most helminth species can survive for years, even decades in their hosts due to a variety of mechanisms including those that suppress and evade host immunity. Given this complex biology, research projects based in my lab (and most collaborative work) focus on the mechanisms that initiate Type 2 responses, those that drive tissue repair, and requirements for host protective immunity at the sites where worms invade and reside (e.g., skin, respiratory tract, and intestine). Given the widely held notion that allergic inflammation is due to maladaptive Type 2 responses that initially evolved to deal with helminths, our work has broad relevance to both infectious and non-infectious disease. Below is an overview of the research topics covered by my laboratory, including key collaborative projects depicted in Figure "**Research topics**"

1. M2 macrophages

Early in my scientific career, it dawned on me that evolutionary pressure on the host to deal with damage to skin and mucus membranes caused by helminth parasites would drive the resultant tissue repair processes to adopt immunoregulatory function(s). Work during my postdoctoral days revealed that Type 2 cytokines like interleukin 4 were essential for an "alternative" form of macrophage activation (now termed M2 macrophage) that was essential for survival during infection with the trematode blood fluke *Schistosoma mansoni*. *Schistosoma spp.* infect over 250 million people across the globe. During infection, the parasite eggs cause tissue injury resulting in chronic fibrosis, particularly in the liver and lung. However, *S. mansoni* adult worms residing in the portal vein have evolved to pass their eggs in the stool, which results in moderate intestinal inflammation that is rarely lethal for the host. Our work demonstrated that M2 macrophages were critical for limiting egg-induced inflammation, inasmuch as mice lacking M2 macrophages developed hemorrhagic foci around intestinal eggs and a sepsis-like syndrome that was prevented by administration of broad-spectrum antibiotics[1]. One implication of this work was that M2 macrophages kept microbial-driven inflammation in check thereby ensuring host survival. That study led to a number of other studies designed to understand the cytokine signals that instructed M2 activation[2-4] and the molecules used by tissue macrophages to contain otherwise lethal inflammation in the liver and bowel during worm infection[5,6]. I became intensely interested in immunoregulatory cytokines like interleukin 10 and Transforming growth factor beta (TGF-β), and macrophage-derived products like arginase[7]. This interest broadened to mechanisms responsible for controlling inflammatory bowel diseases[7]. Currently, our studies that focus on mechanisms controlling inflammatory bowel disease are being led by Nicole Maloney Belle, MD/PhD, a gastroenterologist and Instructor in the Department of Medicine working in my laboratory.

2. Trefoil factor family proteins

Upon establishing my own laboratory, I decided to focus on understanding the mechanisms that governed immunity during the earliest steps in the host-parasite interaction. This led to a keen interest in mucosal epithelial cells and the processes regulating wound healing at the mucosal interface. It was at this point that I came across Trefoil factor family (TFF) proteins, which are small reparative proteins highly expressed at mucosal surfaces, but whose role in immunity was then unknown[8-10]. The decision to turn my focus to TFF proteins was risky yet exhilarating, and ultimately extremely rewarding. Through collaborative studies with Dr. Marsha Wills Karp and others, we found that TFF2 was upregulated in pediatric asthma patients and in mice experiencing allergic airway disease or hookworm infection[11]. Unexpectedly, we found that TFF2 functioned to promote expression of IL 33, a cytokine that had gained considerable attention from GWAS studies implicating it in human asthma[12-14]. Additionally, we found that TFF2 served an antagonistic role for Type 1 inflammatory responses against protozoan parasite infections[15]. Although originally thought to be solely an epithelial cell-derived product, we now know that myeloid antigen-presenting cells (APCs) can serve as a critical source of TFF2, driving tissue repair by inducing proliferative expansion of nascent epithelial cells following airway damage caused by parasites or chemical injury[16].

However, by far the greatest barrier to scientific progress in the field of TFF biology was the lack of a bona fide TFF receptor(s) [8]. It was clear that TFFs could induce conventional signaling cascades involving MAPK and EGFR signaling to mediate their reparative functions, which implied receptor-ligand interations, but this highly debated. Using a variety of biochemical and molecular approaches, we identified leucine rich repeat nogo interacting protein 2 (LINGO2) as a type I transmembrane receptor for TFF3[17]. Our data demonstrated that LINGO2 functioned as a negative regulator of epidermal growth factor receptor (EGFR) signaling and that TFF3 facilitated activation of EGFR by sequestering LINGO2 away from EGFR, thus acting as a rheostat to govern the extent of EGFR activity[17]. This provided a conceptual framework for potentially explaining how TFF3 could drive mucosal wound healing through EGFR activation. While we are still working on identifying a TFF2 receptor[18], these studies have opened the door for investigating the role of LINGO receptors in mucosal wound healing, which was completely unexplored prior to our investigations. Upon moving my lab to UPenn, we started collaborating extensively with Noam Cohen MD/PhD (UPenn) and Andrew Vaughan (PennVet) to further investigate how epithelial cell populations in the upper and lower airway respond to the injurious effects of allergen exposure and viral infection[19-21].

3. Interleukin 33

Ever since we demonstrated that TFF2 was an early damage-induced molecule that facilitated IL-33 expression, we have become increasingly interested in this peculiar IL-1 family cytokine[22]. Indeed, there has been an explosion of interest in IL-33 recently given its role in allergic disease and host protection against helminths, but over the past 5-7 years it has become evident that IL-33 is involved in a much wider array of immune responses and diverse biological processes including tissue repair, stem cell function, neural development, and thermoregulation[23-25]. Perhaps one of the most interesting aspects of its biology is that it is a cytokine constitutively tethered in the nucleus, thought to be released only upon necrotic cell death to function as an alarmin that initiates inflammation[26]. However, it is now clear that IL-33 also has profound immunoregulatory effects through enhancing the immunosuppressive functions of T regulatory cells (Treg). Whether IL-33 could be delivered from the cell in a manner independent of cell death and whether myeloid lineage cells served as biologically relevant sources of IL-33 have been outstanding questions that many investigators have sought to answer.

It was known that IL-33 could drive expansion of both pro-inflammatory group 2 innate lymphoid cells (ILC2) and immunoregulatory/suppressive Foxp3$^+$ Tregs. Given that our TFF2 studies and studies from others have indicated that both myeloid and epithelial cell lineages could be important sources of IL-33[11,27], we formed a hypothesis that cell source could define the biological role for this cytokine. Our recent work demonstrates that indeed, the cellular source of IL-33 can have a radical impact on its biological function: whereas mice lacking intestinal epithelial cell (IEC)-derived IL-33 have impaired Type 2 cytokine production, reduced ILC2 responses, and defects in host resistance to GI nematode infection, mice lacking myeloid APC-derived IL-33 show defects in GATA3$^+$ST2$^+$Foxp3$^+$ Treg numbers and augmented resistance to GI nematodes[28].

Moreover, due to the lack of signal peptide, it has remained a puzzle whether IL-33 could be released through a specific mechanism. While cytokines alone can influence lymphocyte lineage decisions and activation status, it is clear that naive T cell priming requires simultaneous delivery of signals 1 (peptide:MHC) , 2 (co-

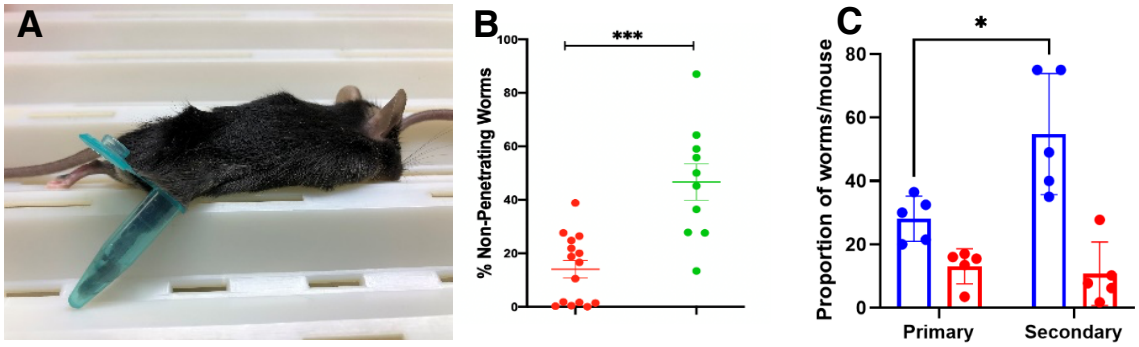
stimulation) and 3 (cytokine). Thus, was reasonable to speculate that cytokine release during cell necrosis would influence T cell function differently from cytokine release from a myeloid APC during synapse formation with a T lymphocyte. Our recently published work demonstrated that the pore-forming protein Perforin-2 is expressed by conventional dendritic cells (cDC) expressing cytoplasmic IL-33 protein, and that the release of IL-33 from cDC was Perforin-2-dependent[28]. While we do not yet possess direct evidence that Perforin-2 localizes to the immunological synapse, our data revealed that Perforin-2 localizes to the APC-T cell interface during Foxp3$^+$ Treg engagement and that blocking Perforin-2 activity inhibits proliferative expansion of GATA3$^+$ ST2$^+$Foxp3$^+$ Tregs[28]. We interpret this data that a specific releas mechanism exists and this topic is a very active area of research in the lab.

4. *Strongyloides* transgenesis

In collaboration with James "Sparky" Lok, who is arguably the father of transgenesis in parasitic nematodes[29], we have engaged in a very challenging project that explores the localization, expansion and phenotype/function of helminth antigen-specific responses. This issue of T cell recognition of helminth antigen is critical to understand if there is ever to be a successful vaccine against these pathogens. Many laboratories across the globe have attempted to make transgenic worms for studying immunology, but few, if any, have been successful. Together with Sparky, we recently demonstrated stable transgenesis in the GI nematode *Strongyloides ratti* where expression of the immunodominant CD4$^+$ T cell epitope 2W1S fused to green fluorescent protein (GFP) was achieved to facilitate tracking of CD4$^+$ T cell responses *in vivo*[30]. In this system, the parasite expresses a single 2W1S epitope under an actin promoter, which is most active in nematode muscle cells along the body wall. C57BL/6 mice infected with this stable transgenic line (termed *Hulk*) undergo a dose-dependent expansion of activated CD44^{hi}CD11a^{hi} 2W1S-specific CD4$^+$ T cells. Our results indicate that pathogen context, as opposed to TCR specificity, exerted a dominant influence over CD4$^+$ T cell phenotype. As new tissue-specific promoters are employed in this model system, it will become possible to engineer stable transgenic lines that express fluorescent reporters or immunogenic molecules in different nematode anatomical compartments to test how antigen accessibility impacts helminth antigen recognition by T cells. Altogether, this new model system allows us to test questions that were previously not possible.

6. Neurons

Keeping with my long-standing interest in understanding the earliest mechanisms responsible for Type 2 immunity and host responses to parasitic helminths, we have recently turned our attention to the nervous system. Nociceptors are a heterogeneous population of skin sensory neurons (responsible for pain and itch) that upon activation release neurotransmitters and neuropeptides, which are molecules that possess immunomodulatory function(s). This raises the intriguing possibility that nociceptors may participate in host protection against skin-penetrating larval stages of various helminth species. Most of the published work describing experimental infection with skin-penetrating helminths uses a hypodermic needle to administer the infectious inoculum, which bypasses the epidermal and dermal skin layers. Because the afferent endings of nociceptors are in the epidermis and dermis, the role of nociceptors in host protective immunity remains unexplored. To address this gap, we have developed a percutaneous infection procedure in mice that allows us to study a variety of helminth species with a skin penetrating larval stage (i.e., *Strongyloides ratti*, *S. mansoni* and *N. brasiliensis*). We can quantify numbers of infectious larvae that enter or fail to penetrate skin, thereby providing a readout by which to interrogate the level of host protection.



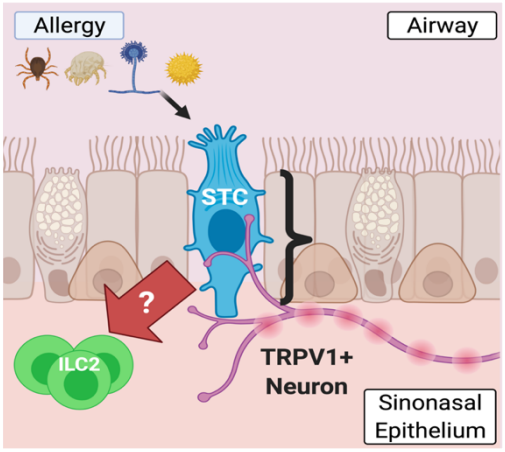
***S. ratti* percutaneous infection.** (A) Hind paw is bathed in a 100μL solution containing 2000 iL3 for 30-min under anesthesia with distal half of plantar portion of foot submerged. (**B**) iL3 remaining in solution are determined. Note how more worms are unable to penetrate skin upon second exposure. (**C**) WT mice (blue) exhibit significantly increased non-penetrating worms upon secondary challenge, while TRPV1 KO mice (red) do not. * p<0.05, ** p<0.01 ***p<0.001

Our preliminary data in the *S. ratti* model shows that during primary infection of WT mice, up to 80% of

S. ratti infectious stage larvae (iL3) penetrate the skin, whereas only 20-30% penetrate during secondary infection (**see above figure**). Moreover, mice genetically deficient in the major cation channel in nociceptors called transient receptor potential vanilloid 1 (TRPV1) show significant defects in resistance to worm entry during both primary and secondary challenge infection (**see above figure**). It is entirely possible that skin nociceptors directly sense helminth-derived products, and/or that helminth products interfere with nociceptor function as an evasion strategy. To address these possibilities, we have also established primary neuronal culture systems to study neuron activation as measured by calcium influx (in collaboration with Bruce Freedman, PhD). Preliminary studies reveal that pre-exposure of dorsal root ganglia (structures containing nociceptor cell bodies) to excretory secretory (ES) products from 2 helminth species (*S. ratti* and *S. mansoni*) suppresses calcium influx in TRPV1+ neurons, suggesting that worm secretions impair neuron activation. We are currently in the process of establishing whether and if so, which worm ES products modulate distinct subpopulations of nociceptors responsible for pain and/or itch. This work is being done in close collaboration with Ishmail Abdus-Saboor, Ph.D.

Tuft cell-neuron interactions in sinonasal allergic disease

Our interests in dissecting the critical neuro-immune interactions that control inflammation and homeostasis also extends to understanding allergic disease within the upper airway. This project has grown out of our highly productive collaboration with Noam Cohen, MD/PhD. Noam has a keen interest in chronic rhinosinusitis (CRS), and together we have established a mouse model of sinonasal Type 2 allergic inflammation evoked by a 3-week intranasal administration of *Alternaria alternata* and *Aspergillus fumigatus*-fungal allergen mix (FAM) in C57BL/6J mice. This model replicates key features of allergic fungal rhinosinusitis (AFRS), which is a subtype of CRS observed in patients, including the expansion of sinonasal Tuft cells (STC), tissue ILC2, eosinophils, goblet cells, and increased levels of IL-25 and IL-33 in the sinonasal fluid. While we have primarily focused on STC to date, we are continuing to use this model to investigate whether sensory neurons of the sinonasal mucosa that emanate from the trigeminal ganglion (TG) are instrumental in driving Type 2 allergic inflammation. Given that STC are innervated by TG neurons, the latter of which express the transient receptor potential vanilloid 1 (TRPV1+) ion channel, the same mouse models that we are building for investigating the role of skin sensory neurons in host protection against helminths are being used to investigate the role of neuronal inputs in allergic respiratory disease. Specifically, we are testing whether local interactions between STC and neurons in the sinonasal tract are required for development of allergen-induced Type 2 inflammation (**model figure on right**).



Sinonasal Type 2 inflammation
Ongoing studies are designed to test whether selective deletion of STC or TRPV1 neurons can disrupt chronic Type 2 inflammation elicited by fungal allergen administration in a mouse model that mimics certain features of human chronic rhinosinusitis (CRS).

Commitment to Diversity in Teaching and Mentoring

My experiences gained from 5 institutions across 2 continents has brought a unique perspective and outlook in working with a wide variety of people and personalities. During the completion of my PhD thesis between the years of 1995-2000, I was the only African-American male among all the different graduate programs and only one of two students of color. The array of challenges that I faced during this period (both personal and professional) left indelible marks on my identity and a fierce resolve to overcome the odds. As tenured faculty at UPenn/PennVet, it is my greatest aspiration to create a rich diversity of outstanding individuals to work with, and to serve as a key resource for students from historically underrepresented groups looking for mentoring and support during their formative years of scientific training. Currently, I serve informal advisory roles for 15 students who are either Latinx or Black, Indigenous, and people of color (BIPOC) enrolled in veterinary, graduate, and medical school programs here at UPenn and across the country. It is striking just how many trainees have approached me for insight, support, and guidance over the years, particularly following the murder of George Floyd. In a formal capacity, I have served as the Immunology Graduate Group diversity chair (2017-2020) and the Diversity Initiative Leader for Keystone Symposia, and I am currently the lead PI for our NIH funded Parasitology T32. I believe that diversity of thought, background, and perspective is vital for innovation in STEM. Recently, I have become Director of Education and International Affairs in the newly formed UPenn Institute for Innovation in Infectious Disease, where my role is to build

fellowship and mentoring programs for URM students at multiple levels of training (i.e., high school, graduate and post-graduate level), with the goal of increasing representation of Latinx and BIPOC students in biomedical research and veterinary medicine. I am building partnerships between UPenn and Historically Black Colleges and Universities (e.g., Xavier University of Louisiana) through a joint venture with the Institute for Translational Medicine and Therapeutics (ITMAT). Also, I am building an international network of graduate student mentees located in low-and-middle income countries (LMIC) paired with U.S.-based scientists, with the intent of increasing the pool of graduate and post-doctoral candidates from South American and African countries. Current partnerships include students from Ghana, Nigeria, and Cameroon.

References

1 Herbert, D. R. *et al.* Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* **20**, 623-635, doi:10.1016/s1074-7613(04)00107-4 (2004).

2 Rani, R., Jordan, M. B., Divanovic, S. & Herbert, D. R. IFN-gamma-driven IDO production from macrophages protects IL-4Ralpha-deficient mice against lethality during Schistosoma mansoni infection. *Am J Pathol* **180**, 2001-2008, doi:10.1016/j.ajpath.2012.01.013 (2012).

3 Fontana, M. F. *et al.* Myeloid expression of the AP-1 transcription factor JUNB modulates outcomes of type 1 and type 2 parasitic infections. *Parasite Immunol* **37**, 470-478, doi:10.1111/pim.12215 (2015).

4 Fontana, M. F. *et al.* JUNB is a key transcriptional modulator of macrophage activation. *J Immunol* **194**, 177-186, doi:10.4049/jimmunol.1401595 (2015).

5 Herbert, D. R. *et al.* Arginase I suppresses IL-12/IL-23p40-driven intestinal inflammation during acute schistosomiasis. *J Immunol* **184**, 6438-6446, doi:10.4049/jimmunol.0902009 (2010).

6 Herbert, D. R., Orekov, T., Perkins, C. & Finkelman, F. D. IL-10 and TGF-beta redundantly protect against severe liver injury and mortality during acute schistosomiasis. *J Immunol* **181**, 7214-7220, doi:10.4049/jimmunol.181.10.7214 (2008).

7 Rani, R., Smulian, A. G., Greaves, D. R., Hogan, S. P. & Herbert, D. R. TGF-beta limits IL-33 production and promotes the resolution of colitis through regulation of macrophage function. *Eur J Immunol* **41**, 2000-2009, doi:10.1002/eji.201041135 (2011).

8 Taupin, D. & Podolsky, D. K. Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol* **4**, 721-732, doi:10.1038/nrm1203 (2003).

9 Kinoshita, K., Taupin, D. R., Itoh, H. & Podolsky, D. K. Distinct pathways of cell migration and antiapoptotic response to epithelial injury: structure-function analysis of human intestinal trefoil factor. *Mol Cell Biol* **20**, 4680-4690, doi:10.1128/MCB.20.13.4680-4690.2000 (2000).

10 Taupin, D. R., Kinoshita, K. & Podolsky, D. K. Intestinal trefoil factor confers colonic epithelial resistance to apoptosis. *Proc Natl Acad Sci U S A* **97**, 799-804, doi:10.1073/pnas.97.2.799 (2000).

11 Wills-Karp, M. *et al.* Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. *J Exp Med* **209**, 607-622, doi:10.1084/jem.20110079 (2012).

12 Savenije, O. E. *et al.* Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. *J Allergy Clin Immunol* **134**, 170-177, doi:10.1016/j.jaci.2013.12.1080 (2014).

13 Bonnelykke, K. *et al.* A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. *Nat Genet* **46**, 51-55, doi:10.1038/ng.2830 (2014).

14 Ho, J. E. *et al.* Common genetic variation at the IL1RL1 locus regulates IL-33/ST2 signaling. *J Clin Invest* **123**, 4208-4218, doi:10.1172/JCI67119 (2013).

15 McBerry, C. *et al.* Trefoil factor 2 negatively regulates type 1 immunity against Toxoplasma gondii. *J Immunol* **189**, 3078-3084, doi:10.4049/jimmunol.1103374 (2012).

16 Hung, L. Y. *et al.* Trefoil Factor 2 Promotes Type 2 Immunity and Lung Repair through Intrinsic Roles in Hematopoietic and Nonhematopoietic Cells. *Am J Pathol* **188**, 1161-1170, doi:10.1016/j.ajpath.2018.01.020 (2018).

17 Belle, N. M. *et al.* TFF3 interacts with LINGO2 to regulate EGFR activation for protection against colitis and gastrointestinal helminths. *Nat Commun* **10**, 4408, doi:10.1038/s41467-019-12315-1 (2019).

18 Zullo, K. M. *et al.* LINGO3 regulates mucosal tissue regeneration and promotes TFF2 dependent recovery from colitis. *Scand J Gastroenterol* **56**, 791-805, doi:10.1080/00365521.2021.1917650 (2021).

19 Patel, N. N. *et al.* Sentinels at the wall: epithelial-derived cytokines serve as triggers of upper airway type 2 inflammation. *Int Forum Allergy Rhinol* **9**, 93-99, doi:10.1002/alr.22206 (2019).

20 Patel, N. N. *et al.* Fungal extracts stimulate solitary chemosensory cell expansion in noninvasive fungal rhinosinusitis. *Int Forum Allergy Rhinol* **9**, 730-737, doi:10.1002/alr.22334 (2019).

21 Rane, C. K. *et al.* Development of solitary chemosensory cells in the distal lung after severe influenza injury. *Am J Physiol Lung Cell Mol Physiol* **316**, L1141-L1149, doi:10.1152/ajplung.00032.2019 (2019).

22 Hung, L. Y., Pastore, C. F., Douglas, B. & Herbert, D. R. Myeloid-Derived IL-33 Limits the Severity of Dextran Sulfate Sodium-Induced Colitis. *Am J Pathol* **191**, 266-273, doi:10.1016/j.ajpath.2020.11.004 (2021).

23 Vainchtein, I. D. *et al.* Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development. *Science* **359**, 1269-1273, doi:10.1126/science.aal3589 (2018).

24 Odegaard, J. I. *et al.* Perinatal Licensing of Thermogenesis by IL-33 and ST2. *Cell* **166**, 841-854, doi:10.1016/j.cell.2016.06.040 (2016).

25 Mahapatro, M. *et al.* Programming of Intestinal Epithelial Differentiation by IL-33 Derived from Pericryptal Fibroblasts in Response to Systemic Infection. *Cell Rep* **15**, 1743-1756, doi:10.1016/j.celrep.2016.04.049 (2016).

26 Cayrol, C. & Girard, J. P. Interleukin-33 (IL-33): A nuclear cytokine from the IL-1 family. *Immunol Rev* **281**, 154-168, doi:10.1111/imr.12619 (2018).

27 Hsu, C. L., Neilsen, C. V. & Bryce, P. J. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PLoS One* **5**, e11944, doi:10.1371/journal.pone.0011944 (2010).

28 Hung, L. Y. *et al.* Cellular context of IL-33 expression dictates impact on anti-helminth immunity. *Sci Immunol* **5**, doi:10.1126/sciimmunol.abc6259 (2020).

29 Lok, J. B. CRISPR/Cas9 Mutagenesis and Expression of Dominant Mutant Transgenes as Functional Genomic Approaches in Parasitic Nematodes. *Front Genet* **10**, 656, doi:10.3389/fgene.2019.00656 (2019).

30 Douglas, B. *et al.* Transgenic expression of a T cell epitope in Strongyloides ratti reveals that helminth-specific CD4+ T cells constitute both Th2 and Treg populations. *PLoS Pathog* **17**, e1009709, doi:10.1371/journal.ppat.1009709 (2021).