MEETING SUMMARY

2023 Workshop: Neuroimmune Crosstalk in the Gut – Impact on Local, Autonomic and Gut–Brain Function

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Keywords: Gastrointestinal; Neuroimmune Interactions; Enteric Nervous System; Immune System.

Meeting Summary: Overview

The gastrointestinal (GI) tract is a sensory organ that mediates the detection of mechanical stretch, dietary, and microbial stimuli and, in this capacity, also serves as a protector from potentially noxious luminal contents, such as toxins and pathogens. The reciprocal influence between cells in the enteric nervous system (ENS; eg, neurons, glia, and interstitial cells of Cajal) and cells in the immune system, including epithelial cells, is central to human health maintenance and disease, as the interaction between these two systems underlies the ability to sense, interpret, and respond to internal and external stimuli. It has become increasingly apparent that neuroimmune communication is critical for GI homeostasis and prevention of disease. The highly diverse and numerous entrants and inhabitants of the GI tract (eg, nutrients, gut bacteria, and toxins) require the GI immune system and ENS to not only sense current conditions, but also to recall previous challenges and events in order to mount memory responses that both anticipate and efficiently adapt to ever-changing conditions to maintain balance. Thus, when neuroimmune communication in the gut is dysfunctional, symptoms of debilitating and common GI disorders, such as inflammatory bowel diseases (IBDs) and disorders of gut–brain interaction (DGBI), may increase in frequency and severity. The current therapies available for these conditions are limited, and prior research has, in large part, targeted the immune system or nervous system in a siloed manner. Given the increased understanding that these two systems necessarily interact in ways that impact critical GI functions, such as inflammation, nociception, and motility, as well as gut–brain communication, it is imperative that researchers in both areas combine expertise and resources to unravel the key neuroimmune mechanisms that provide for GI homeostasis yet also contribute to common and debilitating GI conditions that have few effective therapies, such as IBD and DGBI.

From June 29–30, 2023, the National Institute of Diabetes and Digestive and Kidney Diseases hosted a public workshop with the following objectives: to facilitate in-person networking among mucosal immunologists, enteric neuroscientists, and microbiologists to expand the scope of current National Institute of Diabetes and Digestive and Kidney Diseases funding in this area; to explore recent advances in state-of-the-art molecular, imaging, and genetic technologies that can be leveraged across these disciplines; and to better characterize the nature and specificity of neuroimmune interactions in the gut to allow for more precise targeting for development of novel therapeutics for GI disorders. The workshop was composed of six sessions beginning with Neuroimmune Perspectives, which provided an overview of the field from the immune and neural viewpoints and laid the groundwork for how gut–neuroimmune interactions are relevant to GI physiology, health, and disease. The remaining five sessions focused on specific aspects of neuroimmune interactions to include "ENS Development and Immune Interactions," "Visualizing Neuroimmune Interactions," "Mechanisms of Neuroimmune Communication," "Neuroimmune Interactions and Gut Sensation," and "Neuroimmune Interactions along the Gut–Brain Axis." This workshop summary provides a synopsis of the meeting presentations, panel discussions, and breakout sessions on the current state of knowledge in the field of gut–neuroimmune crosstalk and how it relates to GI homeostasis, symptoms, and diseases (highlights included in Figure 1), inclusive of the gaps in knowledge and future directions. Table 1 summarizes the identified gaps and opportunities, as well as resources needed to move the field forward.

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Abbreviations used in this paper: DGBI, disorder of gut–brain interaction; ENS, enteric nervous system; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases.

0016-5085
https://doi.org/10.1053/j.gastro.2024.03.023

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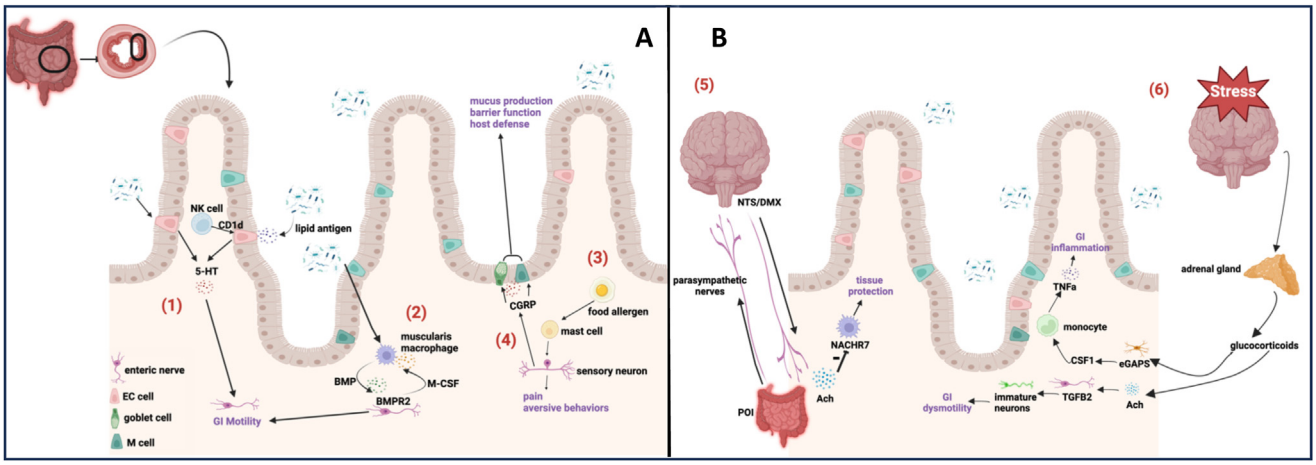

Figure 1. Selected examples of neuroimmune interactions that modulate diverse GI functions. Examples noted are those discussed at the workshop. (*A*) (*1*) Gut microbiota releases products that can directly stimulate enterochromaffin (EC) cells, which release 5-HT to stimulate gut motility and pain. Natural killer (NK) T cells also interact with EC cells via microbial lipid antigens to modulate motility; (*2*) Muscularis macrophages (MMs) influence GI motility by secreting bone morphogenetic protein 2 (BMP2), that activates BMP receptors (BMPR) on enteric neurons. Enteric neurons, in turn, secrete colony stimulatory factor 1 (CSF1), a growth factor required for macrophage development; (*3*) food allergens elicit mast (M) cell activation of sensory neurons, resulting in pain-related and aversive behaviors; (*4*) sensory neuron stimulation elicits calcitonin gene–related peptide (CGRP) secretion, which signals to epithelial cells (eg, M cells and goblet cells) to induce mucus production, to increase barrier function and to provide host defense against bacterial pathogens. (*B*) (*5*) Intestinal inflammation induced by postoperative ileus (POI) signals to the nucleus tractus solitarius (NTS) by way of parasympathetic/vagal afferent signaling. In turn, activation of vagal efferent nerves in the dorsal motor nucleus (DMX) stimulate cholinergic enteric neurons to secrete acetylcholine (ACh). ACh activates $\alpha 7$ nicotinic receptors on MMs to down-regulate inflammation; (*6*) stress causes adrenal release of glucocorticoids (GCs) that stimulate intestinal inflammation and dysmotility by stimulating an inflammatory subset of glia (enteric glia associated with psychological stress [eGAPS]) to secrete CSF1, inducing monocyte secretion of tumor necrosis factor α (TNFa), resulting and causing transcriptional immaturity in enteric neurons, acetylcholine deficiency, and dysmotility via transforming growth factor $\beta 2$ (TGFB2).

Current Knowledge

The processes by which the ENS and immune system engage in a bidirectional crosstalk involve ligand–receptor interactions initiated by the secretion of neurotransmitters and/or cytokines by immune cells or neurons onto the receptors of the other. In this way, immune cells have been found to influence ENS development, plasticity, and activity and, conversely, enteric neurons signal to immune cells to modulate immune cell transcription and function. These interconnections, which often also involve other parts of the gut ecosystem, including gut mucosal cells and/or the gut microbiota, work to maintain GI homeostasis. Yet, when individual components of this ecosystem go awry, dysfunctional interactions can result in GI dysmotility, inflammation, and pain to cause and/or perpetuate medical conditions, such IBD and DGBI. Conversely, disease states can change the frequency and types of neuroimmune interactions. For example, in intestinal inflammation, immune cells are a source of inflammatory mediators that can affect neural activity, neuronal survival, and glial function and phenotype.

Neuroimmune interactions are important from fetal development through adulthood. Throughout development, enteric neural cell types are specified and connections between cells are made and refined. The ENS has a multifaceted origin with contributions from the vagal neural crest, sacral neural crest, nerve-associated progenitor cells/Schwann cell precursors, and possibly putative non-neural crest/mesodermal cells. Vagal neural crest cells populate the gut in a rostral-to-caudal gradient, while the hind gut receives progenitors from the sacral crest. Defects that affect ENS development and migration during development are recognized to cause conditions like Hirschsprung disease. In individuals with Hirschsprung disease, ganglionic sections of bowel exhibit a lower number of cholinergic fibers and abnormalities in mucosal immunity.[1] Yet little is known about how these differences in neuronal specification and immune system function precisely affect neonatal ENS development and the neuroimmune actions that ensue post development.

In contrast to fetal development, the maintenance of mature enteric neurons and neurogenesis under specific conditions has been found to involve crosstalk with immune cells. To what extent constitutive enteric neurogenesis occurs is controversial, but it has been shown that this process can be induced by means of injury or inflammation and involves serotonergic- and/or Toll-like receptor–related signaling. These new neurons appear to arise from glia or at least glial-like progenitor cells in the myenteric plexus. The involvement of Toll-like receptor–mediated mechanisms implies a role for neuroimmune crosstalk with the microbiota in neurogenesis and enteric neuronal and glial maintenance. The microbiota has also been found to regulate gene expression in enteric neurons, which allows the ENS to adjust function according to microbiota composition. Although inflammation and/or microbiota differences have

Table 1. Summary of Breakout Group Discussions Identifying Gaps, Opportunities, and Resources Needed to Advance the Field[a]

Group 1	Group 2	Group 3
Kara Margolis (leader)	Brian Gulbransen (leader)	Isaac Chiu (leader)
Terez Shea-Donohue (NIDDK)	Patricia Greenwel (NIDDK)	Diana Cummings (NIDDK)
Polina Anikeeva	Laren Becker	Dwayne Lunsford (NIDDK)
Diego Bohorquez	Jaime Belkind-Gerson	Esther Borges-Florsheim
Kirsteen Browning	Gianluca Cipriani	Milena Bogunovic
Alejandra Mendoza	Julia Ganz	Eric Chang
Timothy Sampson	Yuki Obata	Hongzhen Hu
Christoph Thaiss	Colin Reardon	Ruaidhri Jackson
Michael Wheeler	Kristen Smith-Edwards	Francisco Quintana
Helen Vuong	Michelle Southard-Smith	Chuan Wu
Li Ye	Jakob von Moltke	

Gaps	Opportunities	Needed resources
Determine the key players in the bidirectional neuroimmune communication in the ENS and other parts of the autonomic nervous system, and gut–brain axis	Mechanisms to increase the visibility of this area as it relates to IBD, DGBI, motility disorders, metabolic disease, neurodevelopmental disorders, neurodegeneration, and other relevant GI pathologies	Development of new tools and adaptation of current state-of-the-art technologies (eg, adapted from central nervous system–based tools) to study neuroimmune interactions in the gut
Define functional correlates of neuroimmune interactions in GI disease	Team science to support multidisciplinary research relevant to neuroimmune communication in the gut	Development of mice for intersectional genetics targeting specific neuronal cell types
Recognition of complexity of the neuroimmune communications, including regional differences, immune context, and individual variation	Increase and enhance basic and clinical collaborations	Application of novel genetic tools (RABID-seq, FIND-seq, SPEAC-seq)
High-throughput analyses of luminal molecules and their function in neuroimmune interactions	Leverage existing human studies to incorporate research on neuroimmune interactions	Centralized resources for translational studies
Integration of sensory biology into neuroimmune circuits	Identification of neuroimmune interactions as therapeutic targets	Cross-disciplinary training opportunities and formal education on GI neuroimmunology
Build a neuroimmune connectome		

FIND-seq, focused interrogation of cells by nucleic acid detection and sequencing; RABID-seq, rabies barcoding in droplets followed by sequencing; SPEAC-seq, systematic perturbation of encapsulated associated cells followed by sequencing.
[a]Key discussion points covered by breakout groups 1–3: gaps in our current knowledge; opportunities to address these gaps in knowledge; and resources needed by the field to advance knowledge in this area.

been noted in a variety of GI diseases, including DGBI and IBD, how these differences are related to changes in ENS morphology and function in humans with these conditions is not yet known. Important scientific advances, however, have provided fundamental insights into the neuroimmune cells and circuits by which gut homeostasis is maintained. For example, one mapped circuit that modulates intestinal inflammation is the cholinergic anti-inflammatory pathway,[2] in which the vagus nerve detects inflammatory mediators via afferent fibers, and vagal efferents then signal to choline acetyltransferase^{+} CD4^{+} T cells, which inhibit cytokine production to protect against immunopathology and mediate host defense.[3,4] These findings have paved the way for multiple clinical trials that have sought to evaluate the role of vagal nerve stimulation in patients with IBD, with mixed results.[5] Other studies have demonstrated the important influence of neuroimmune interactions on maintenance of GI motility; enteric neurons and gut-resident muscularis macrophages have been found to communicate through growth factors and cytokines, which together coordinate smooth muscle contractility and gut motility.[6] Interestingly, this communication pathway is at least partially gut microbiota–dependent and elucidates an important mechanism by which gut microbiota disruptors (eg, antibiotics and infections) alter gut motility. Conversely, the immune system has also been found to coordinate the detection of microbes and signaling to the ENS to impact gut motility. For example, natural killer T cells, which recognize lipid antigens from gut microbes, crosstalk directly with enterochromaffin cells, which signal to the ENS via serotonin.[7]

Given that the immune system has developed a vast capability to detect foreign antigens, how neural and immune cells interact with diverse gut entrants, such as dietary components, needs to be studied further. Recent work has shown that adaptive immune cells participate in neural

sensing of food antigens. Mice sensitized to an allergen (ie, ovalbumin) develop aversion behaviors to drinking water containing that allergen, mediated by IgE-dependent mast cell activation, and release leukotrienes that signal to the brain to induce avoidance, decreasing consumption.[8,9] This could be one key mechanism of food quality control and also a way that a dysfunctional immune system recognizes nonthreatening dietary components as pathogenic in conditions like food allergies and/or eosinophilic esophagitis, although more study is needed.

Studying neuroimmune interactions in diseases in a holistic manner can be quite complex. A more targeted yet relevant alternative approach is to study neuroimmune interactions in the underlying symptoms that are diagnostic or heavily associated with a disease. For example, a major problem in IBD and DGBI is pain, which is often unassociated with apparent inflammation and is termed *visceral hypersensitivity*. Pain is common, associated with significantly decreased quality of life scores, and can be exceptionally challenging to treat.[10,11] The etiology of visceral hypersensitivity, or biomarkers for its diagnosis, are largely unknown, and both of these obstacles have limited therapeutic development. Visceral hypersensitivity, and gut sensation in general, have been found to be modulated, in part, by interactions between immune cells and the ENS; enteric neurons express ion channels and/or G-protein–coupled receptors tuned to detect mechanical, thermal, and chemical stimuli,[12,13] and the immune system possesses adaptive and innate receptor mechanisms to detect pathogens, along with the ability to discriminate between self and non-self. Given their complementary but distinct sensory capabilities, coordination among neurons, immunocytes, epithelial cells, and the gut microbiome is critical for gut function.[14] Visceral pain in patients with irritable bowel syndrome (IBS), one of the most common DGBI, has been linked to abnormalities in the gut microbiome, dysregulation of inflammatory activation, and/or abnormal sensory responses in enteric neurons, although direct clinical correlations are limited.[15] Furthermore, it is important to recognize that nociceptive sensory neurons are not only involved in nociception, but also play roles in coordinating gut immune function by signaling via neuropeptides, including calcitonin gene–related peptide and substance P, to intestinal immune and epithelial cells to protect against specific pathogens (eg, *Salmonella* and *Citrobacter rodentium*)[16,17] and maintain gut barrier integrity to protect against colitis.[18,19]

Although GI homeostasis and disease can be maintained and perpetuated, respectively, by intrinsic neuroimmune mechanisms within the GI tract, as discussed above, there is a highly complex matrix of interactions also involving the extrinsic (eg, vagal, spinal, and sympathetic) nervous systems. This communication is facilitated by a vast array of neurotransmitters, hormones, metabolites, and short-chain fatty acids, among others. An important body of literature has highlighted some of the underlying cells and mechanisms involved in afferent and efferent gut–brain signaling, as well as their functional outputs. For example, enterochromaffin cells within the intestinal mucosa are sensory cells that, when stimulated by means of specific bacterial metabolites, GI irritants or hormones, secrete serotonin that binds to 5-HT_3 receptors on spinal afferent neurons, eliciting sex-specific responses to visceral pain caused by colorectal distention.[20,21] Conversely, a recent study focusing on the efferent effects of stress on gut pain and motility demonstrated how neuroimmune crosstalk facilitated the relationship between stress and the enhancement of IBD-like inflammation. The investigators found that chronically elevated glucocorticoid levels do this by means of propelling a subset of enteric glia to induce monocyte- and tumor necrosis factor–mediated inflammation and generating transcriptional immaturity of enteric neurons and acetylcholine deficiency that were linked with dysmotility.[22]

Investigating the nature and function of enteric–neuroimmune interactions requires methods suitable for interrogating and manipulating defined cell types in organs and whole animals. Many prior neuroimmune studies emphasized anatomic associations and did not demonstrate functional interactions or give information regarding whether cellular exchanges were actually occurring and, if so, their directionality. This emphasizes a critical need for approaches to study cellular activity in their native environment to better understand the nature of neuroimmune interactions.

Contemporary technologies, including optogenetic actuators and reporters, viral tracing strategies, novel chemical biology, and biologically compatible devices for recording and stimulation in vivo, are enabling scientists to target interactions or functions more precisely. As an example, genetically encoded sensors have revolutionized this area by allowing investigators to study the activity of cells in their natural environment. The most popular of these sensors are for calcium, and there are multiple examples of successfully using genetically encoded calcium sensors to study calcium channel–based intercellular communication between cells of the ENS, including neurons, glia, and interstitial cells of Cajal. Genetically encoded calcium sensors can be targeted to defined cell populations using cre-lox technology and combined with optogenetic actuators to control and record cellular activity with light. Together, these technologies are allowing investigators to identify which specific cells in the ENS control factors like motility and then determine drug targets for those specific cell types. Despite these advances, there have been limitations regarding the targeting of some specific cell types or organs secondary to a lack of available animal models. Some of these challenges are being overcome by the development of novel viral approaches that exhibit optimal tropism in peripheral neurons, such as retrograde viruses optimized for organ tracing, that can be used to transfect specific neurons innervating defined peripheral locations. Approaches combining retrograde viruses optimized for organ tracing with genetically encoded calcium sensors and optogenetic actuators, such as channelrhodopsin, now allow investigators an unprecedented opportunity to target,

manipulate, and record the activity of neurons or immune cells in peripheral organs, including the intestine. It is not yet fully known, however, whether ligand-gated ion channels, such as channelrhodopsin, are as relevant a mode of stimulation for immunocytes or whether calcium has the overlapping well-defined roles in immunocyte activity that it has in neurons.

Gaps in Knowledge and How These Gaps Can Be Addressed

The current data are highly supportive of the notion that neuroimmune crosstalk is important for GI tract functions, including motility, inflammation, and nociception. There remains, however, a critical need to understand precisely when, where, how, and under what conditions these interactions occur.

When?

Perturbations during development, disease progression, and chronicity, such as abnormal neuroimmune crosstalk, can lead to long-term changes in gut function. The extent to which neuroimmune interactions shape normal ENS and immune system development, maintenance, and turnover, and also how these processes are altered in disease, remain incompletely understood. For example, fundamental questions remain regarding the nature of the neuroimmune interactions and signaling molecules that regulate ENS development and/or plasticity by controlling both access and migration of the vagal and sacral neural crest cells. It is also unclear how neurons and immunocytes interact to impact one another's developmental lineages.

Where?

Although much of the data thus far have focused on intrinsic gut neuroimmunity, virtually all cells involved in gut–brain crosstalk are also likely to engage in interactions with the immune system. There has been a concerted effort to characterize the complexity of the multiple participants in gut–brain communication, including the gut epithelium, as well as vagal, spinal, and enteric neurons. Future directions, however, will require technologies that help investigators understand how these different cell types function in neuroimmune crosstalk to stimulate afferent gut signaling and, ultimately, brain functions, such as mood and cognition. Deciphering where and how gut immune sensing throughout the intestine differs, and how diverse types of mechano- and chemosensation in the different parts of the intestine signal at the brainstem and central nervous system levels may involve technologies that incorporate in vivo tracing and/or 2 photon calcium imaging systems. There have also been advances in the elicitation of sensory cell excitation in vivo, using bioengineered optogenetic-reliant fiberoptic rods placed within the gut lumen. These devices have been leveraged to evaluate appetitive behaviors, yet more optimized versions are now available that could be used to look at other potential effects of gut afferent signaling, such as mood and pain. In addition, these technologies will help enable scientists to determine how sensory cells in the gut epithelium detect dietary components or gut microbiota differences, and how, once detected, these signals proceed to the central nervous system to affect behavior in both health and disease states. The neurotransmitters involved in afferent signaling are also understudied, but are likely to be important. For example, as noted above, enterochromaffin cell–derived serotonin plays roles in pain and motility, as well as brain and intestinal development and ongoing function. How serotonin interacts with the immune system to impact brain and gut development and brain–gut communication, however, is not yet rigorously defined.

How?

Overarching goals of research in this field are currently directed toward understanding the enormous cellular and functional diversity of the ENS and the immune system, as well as how these cells interact with one another in health and disease. These investigations will require novel approaches through single-cell analyses (eg, single-cell RNA sequencing); the systematic mapping of gut–neuroimmune interactions through viral tracing, in vivo calcium imaging, and spatial transcriptomics, as well as functional interrogation using chemo- and optogenetics.

During development, technological advances that permit visualizing neural crest migration in vivo are important to advance the understanding of ENS formation. Such methods would permit investigating signaling molecules and processes that regulate access by neural crest streams. Likewise, new insights from single-cell transcriptomics have the potential to uncover key transcription factors controlling neuron diversity in development, but more functional studies in this area are needed. For example, these approaches would be valuable in identifying genomic programs that dictate the developmental patterns of early born enteric neuron subsets, such as serotonergic neurons and other neuronal subsets that have been shown to play important roles in intestinal motility, secretion, and/or sensation.

Single-cell analysis has revealed a myriad of networks of gut-intrinsic and extrinsic neurons and immune and epithelial cells. Higher-throughput techniques that also permit studying the function of multiple genes or regulatory gene networks simultaneously are needed to identify mechanisms regulating immunocyte, neuron, and glial diversity. RABID (rabies barcoding in droplets) followed by sequencing allows for an unbiased approach in mapping neuroimmune connections by the use of barcoded viruses that jump from targeted cell types to adjacent cells and can be used to map all interacting partners and their transcriptional profiles, leading to identification of novel ligand–receptor interactions between cells.[23] In addition to defining these interactions at the molecular level, spatially mapping them using transcriptomic or proteomic approaches will also be required. Mapping of receptor–ligand pairs and signaling pathways can be complemented with technologies such as systematic

perturbation of encapsulated associated cells, followed by sequencing that will enable investigators to understand how manipulation of the involved cells affect functional outcomes.[24] Future work in this area will benefit from incorporating new tools that permit accurate readouts of relevant modes of activity and targeting probes to subsets of immune cells, neurons, glia, interstitial cell of Cajal, and epithelial cells. Coupling such techniques with new methods of manipulating cell activity through opto-, chemo-, and magnetogenetics will provide powerful approaches to study cell–cell communication in the gut.

On a whole-organ level, intestinal studies have been impeded by issues with accessibility and light scattering. These challenges are now being overcome by new strategies for whole-body clearing and wireless devices suitable for use in organs. Tissue clearing was originally developed for the brain and has now been adapted for whole-body or organ applications using a balanced approach that clears tissue and lessens fluorophore quenching. Combining this method with new chemical approaches for imaging small molecules now allows investigators to study the tissue and cellular distribution of drugs that were classically characterized in a much more nonspecific way by in vitro biochemical assays. These approaches also allow for large-scale, unbiased analysis of cellular anatomy and circuits in intact systems. Taking advantage of this information to stimulate specific cells or circuits has been limited by the availability of devices and techniques suitable for internal organs. New flexible light-emitting devices, however, are being designed that are biocompatible, wireless, and able to incorporate additional components to record cellular responses or deliver drugs.[25] These, and other approaches, such as chemo- and magnetogenetics, can overcome prior barriers to manipulating peripheral neuroimmune circuits and studying their functions. Adopting these tools will open doors to new research avenues and allow for pursuit of questions that were previously inaccessible.

Under What Conditions?

It is recognized that neuroimmune circuits may be altered under diverse conditions, yet it is relatively unknown how neuroimmune circuits are changed or altered in most GI-related diseases and disorders, including IBD and DGBI like IBS. Although visceral pain can be a prominent component of all of these conditions, the peripheral and central pathways, neural circuits, and behaviors originating from GI immune sensing remain to be mapped fully. Furthermore, nociceptive neurons have been found to modulate not only nociceptive pathways but also inflammation. Deciphering the specific stimulatory factors for selective pathway control could thus be useful in order to target actions that are focused on alleviating GI pain versus those that are centered on controlling inflammation. How the immune system plays a role specifically in defining the signals that regulate traffic of immune cells from gut to brain and how they impact pain is also an important research objective.

Future Directions

Increasing data confirm that neuroimmune interactions are important for gut homeostasis and disease. Despite these integral relationships, research has been largely siloed until recently. Given the diversity and complexity of sensory neurons (ie, enteric, dorsal root ganglion, vagal, and pelvic), and the multitude of innate and adaptive immune cells, future collaborative research is necessary to determine how visceral sensation, gut motility, inflammation, and immunity are coordinated.

Targeting key neuroimmune pathways involved in intestinal inflammation could also lead to novel therapeutic approaches for gut inflammatory conditions. Defining molecular mechanisms by which neurons regulate immune cells, including neuromodulators (eg, acetylcholine and calcitonin gene–related peptide) and receptors (eg, nicotinic a7 receptor and RAMP1/CALCRL) can identify pharmacologic targets for immune diseases. Conversely, defining immune mediators (eg, cytokines and growth factors) that affect ENS function can produce novel approaches to treat visceral pain, dysmotility, and neurologic diseases. Engineered microorganisms, such as bacterial or yeast probiotics, can be used to deliver modulators of neuroinflammation.[26,27] Bioelectronic or ultrasound approaches can be used to stimulate specific neurons and their downstream neuroimmune circuits to modulate inflammation.[28]

A multidisciplinary approach involving experts in neurobiology, immunology, gastroenterology, microbiology, and dietary sciences would thus be useful to fully understand the mechanisms of this crosstalk. Defining these neuroimmune circuits can yield fundamental insights into homeostasis and host defense that are necessary to develop novel treatments for conditions such as DGBI and IBD. Synergistic investigative teams, combined with the emerging technologies that allow for coordination of comprehensive single-cell analyses, spatial interactions, and functional outcomes will enable scientists to answer questions, such as those noted below, that are critical to developing novel therapeutics for patients with GI conditions. On a more basic or translational level, key overarching questions that remain include:

1. How do intestinal epithelial cells sense microbial products and dietary components and how do they communicate with neurons in gut homeostasis and inflammation?
2. What luminal cues are involved in regulating the function and genesis of neurons and glia in homeostasis and inflammation?
3. How do intrinsic and extrinsic peripheral neurons signal to epithelial and immune cells?
4. How does the gut microbiome impact the neuro–immune signaling axis?
5. How do specific dietary components and other microbiome-dependent antigens regulate

immune–neuron sensory circuits to initiate pain- or inflammation-related stimuli?

6. How are gut–neuroimmune circuits altered in conditions of human disease, such as IBD, DGBI such as IBS, or food allergies?

Insights into these fundamental questions will enable scientists and clinicians to reveal the contributions of neuroimmune crosstalk in diseases that affect the GI tract (eg, food allergies, IBD, and IBS) and ultimately translate these findings into novel therapeutic targets.

References

1. Schill EM, Floyd AN, Newberry RD. Neonatal development of intestinal neuroimmune interactions. Trends Neurosci 2022;45:928–941.
2. Reardon C. Neuro-immune interactions in the cholinergic anti-inflammatory reflex. Immunol Lett 2016; 178:92–96.
3. Rosas-Ballina M, Olofsson PS, Ochani M, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science 2011;334:98–101.
4. Ramirez VT, Godinez DR, Brust-Mascher I, et al. T-cell derived acetylcholine aids host defenses during enteric bacterial infection with *Citrobacter rodentium*. PLoS Pathog 2019;15:e1007719.
5. Serafini MA, Paz AH, Nunes NS. Cholinergic immunomodulation in inflammatory bowel diseases. Brain Behav Immun Health 2022;19:100401.
6. Muller PA, Koscso B, Rajani GM, et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. Cell 2014;158:300–313.
7. Luo J, Chen Z, Castellano D, et al. Lipids regulate peripheral serotonin release via gut CD1d. Immunity 2023; 56:1533–1547.e7.
8. Plum T, Binzberger R, Thiele R, et al. Mast cells link immune sensing to antigen-avoidance behaviour. Nature 2023;620:634–642.
9. Florsheim EB, Bachtel ND, Cullen JL, et al. Immune sensing of food allergens promotes avoidance behaviour. Nature 2023;620:643–650.
10. Sinopoulou V, Gordon M, Akobeng AK, et al. Interventions for the management of abdominal pain in Crohn's disease and inflammatory bowel disease. Cochrane Database Syst Rev 2021;11(11):CD013531.
11. Knowles SR, Skvarc D, Ford AC, et al. Negative impact of disorders of gut-brain interaction on health-related quality of life: results from the rome Foundation Global Epidemiology Survey. Gastroenterology 2023; 164:655–668.e10.
12. Furness JB. The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 2012; 9:286–294.
13. Basbaum AI, Bautista DM, Scherrer G, et al. Cellular and molecular mechanisms of pain. Cell 2009;139:267–284.
14. Yang D, Almanzar N, Chiu IM. The role of cellular and molecular neuroimmune crosstalk in gut immunity. Cell Mol Immunol 2023;20:1259–1269.
15. Vanuytsel T, Bercik P, Boeckxstaens G. Understanding neuroimmune interactions in disorders of gut-brain interaction: from functional to immune-mediated disorders. Gut 2023;72:787–798.
16. Lai NY, Musser MA, Pinho-Ribeiro FA, et al. Gut-innervating nociceptor neurons regulate Peyer's patch microfold cells and SFB levels to mediate salmonella host defense. Cell 2020;180:33–49.e22.
17. Ramirez VT, Sladek J, Godinez DR, et al. Sensory nociceptive neurons contribute to host protection during enteric infection with *Citrobacter rodentium*. J Infect Dis 2020;221:1978–1988.
18. Zhang W, Lyu M, Bessman NJ, et al. Gut-innervating nociceptors regulate the intestinal microbiota to promote tissue protection. Cell 2022;185:4170–4189.e20.
19. Yang D, Jacobson A, Meerschaert KA, et al. Nociceptor neurons direct goblet cells via a CGRP-RAMP1 axis to drive mucus production and gut barrier protection. Cell 2022;185:4190–4205.e25.
20. Bayrer JR, Castro J, Venkataraman A, et al. Gut enterochromaffin cells drive visceral pain and anxiety. Nature 2023;616:137–142.
21. Bellono NW, Bayrer JR, Leitch DB, et al. Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. Cell 2017;170:185–198.e16.
22. Schneider KM, Blank N, Alvarez Y, et al. The enteric nervous system relays psychological stress to intestinal inflammation. Cell 2023;186:2823–2838.e20.
23. Clark IC, Gutierrez-Vazquez C, Wheeler MA, et al. Barcoded viral tracing of single-cell interactions in central nervous system inflammation. Science 2021; 372(6540):eabf1230.
24. Wheeler MA, Clark IC, Lee HG, et al. Droplet-based forward genetic screening of astrocyte-microglia crosstalk. Science 2023;379:1023–1030.
25. Sahasrabudhe A, Rupprecht LE, Orguc S, et al. Multifunctional microelectronic fibers enable wireless modulation of gut and brain neural circuits. Published online June 22, 2023. Nat Biotechnol. https://doi.org/10.1038/s41587-023-01833-5.
26. Sanmarco LM, Rone JM, Polonio CM, et al. Engineered probiotics limit CNS autoimmunity by stabilizing HIF-1alpha in dendritic cells. Preprint posted online March 21, 2023. bioRxiv. https://doi.org/10.1101/2023.03.17.532101.
27. Scott BM, Gutierrez-Vazquez C, Sanmarco LM, et al. Self-tunable engineered yeast probiotics for the treatment of inflammatory bowel disease. Nat Med 2021; 27:1212–1222.
28. Pavlov VA, Tracey KJ. Bioelectronic medicine: preclinical insights and clinical advances. Neuron 2022; 110:3627–3644.

Received September 7, 2023. Accepted March 15, 2024.

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MEETING SUMMARY

Author Contributions
All authors contributed to the organization of the workshop and drafting of the text. All authors approved the final version of the manuscript before submission.

Conflicts of interest
The authors disclose no conflicts.

Funding
Funding for this meeting was provided by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Preparation of this article was supported by NIDDK and the following grants: R01DK127257, Food Allergy Science Initiative, Kenneth Rainin Foundation to Isaac M. Chiu; R01DK103723, NIDDK R01DK120862, NHLBI P01HL152951-A1 to Brian D. Gulbransen; and RO1DK130518-01, RO1DK126644, NS015547; DoDW81XWH-22-PRMRP-IIRA, DoD W911NF-21-S-0008, and a North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Takeda Award to Kara G. Margolis. This report does not represent the official view of the NIDDK, NIH, the Department of Health and Human Services, or any part of the US Federal Government. No official support or endorsement of this article by the NIDDK or NIH is intended or should be inferred.