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Neuroendocrine regulation of innate lymphoid cells

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Summary

The activities of the immune system in repairing tissue injury and combating pathogens were long thought to be independent of the nervous system. However, a major regulatory role of immunomodulatory molecules released locally or systemically by the neuroendocrine system has recently emerged. A number of observations and discoveries support indeed the notion of the nervous system as an immunoregulatory system involved in immune responses. Innate lymphoid cells (ILCs), including natural killer (NK) cells and tissue-resident ILCs, form a family of effector cells present in organs and mucosal barriers. ILCs are involved in the maintenance of tissue integrity and homeostasis. They can also secrete effector cytokines rapidly, and this ability enables them to play early roles in the immune response. ILCs are activated by multiple pathways including epithelial and myeloid cell-derived cytokines. Their functions are also regulated by mediators produced by the nervous system. In particular, the peripheral nervous system, through neurotransmitters and neuropeptides, works in parallel with the hypothalamic-pituitary-adrenal and gonadal axis to modulate inflammatory events and maintain homeostasis. We summarize here recent findings concerning the regulation of ILC activities by neuroendocrine mediators in homeostatic and inflammatory conditions.

KEYWORDS

barrier defense, hormones, innate lymphoid cells, natural killer (NK) cells, neuroimmunology, neuropeptides

1 | INTRODUCTION

The immune and nervous systems are similar in many ways.[1] For example, both systems display a high level of plasticity in the response to external signals: the cells of each system may follow different differentiation pathways, depending on the environmental cues they receive.[2] Moreover, the cells of the immune and nervous systems have many mechanisms of cell communication in common, including morphologically similar physical connections and molecular pathways. The term "synapse" is used to define specialized membrane structures resulting from the concerted interaction of surface molecules on the communicating cells.[3] Both neural cells and cytotoxic immune cells, including NK cells, can form these extended surface communication platforms for the transfer of information and mediators between cells.[4] The most striking similarity between the two systems is their use of a common chemical language, consisting of cytokines, chemokines, neuropeptides, neurotransmitters, neurotrophins[5] and their receptors,[6] enabling the cells of the two systems to respond to the same signals and to "talk" to each other. For example, Toll-like receptors (TLRs), which were originally thought to function primarily on immune cells, have also been found on neuronal cells, in which appear to have a cell-autonomous environmental sensing function.[7] In addition, the cell-autonomous retinoic acid

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signals generated by adjacent neurons are strictly required for the development of lymphoid tissue inducer (LTi) cells, a subset of ILCs responsible for secondary lymphoid organ formation.[8,9]

The magnitude of the inflammatory response is crucial for the homeostasis of the organism. Insufficient responses result in immunodeficiency, favoring pathogen spread in the context of the infections, and leading to the uncontrolled proliferation of transformed cells in cancer development. Conversely, excessive inflammatory responses cause tissue damage, fibrosis, and immunopathology, and are associated with autoimmune diseases (such as rheumatoid arthritis, Crohn's disease, multiple sclerosis), allergy, atherosclerosis and diabetes.[10]

A balance between immunodeficiency and immunopathology is achieved through the combined action of endogenous anti-inflammatory and pro-inflammatory mechanisms, rendering protective inflammation more efficient and preventing its conversion into an excessive and potentially deleterious response. An important role for neuroendocrine pathways in this regulation is currently emerging. The neuroendocrine system can sense changes in the environment, and restore homeostasis by controlling the duration and intensity of inflammation through multiple and partially redundant mechanisms. The nervous system has two components: the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS includes the brain and the spinal cord, which are connected to the organs and limbs via the nerves and ganglia of the PNS. The PNS can be subdivided into three distinct subsystems: the autonomic, enteric, and somatic nervous systems. The autonomic and enteric nervous systems function mainly without conscious effort, whereas the somatic nerves act as intermediaries in voluntary movements. The somatic nervous system consists of afferent or sensory nerves, which transmit sensation from the body to the CNS, and efferent or motor nerves, which are responsible for sending out commands from the CNS to other parts of the body, inducing muscle contraction. The enteric nervous system controls the gastrointestinal system. The autonomic nervous system (ANS) is composed of the sympathetic and parasympathetic nervous systems, which regulate physiological functions by controlling involuntary responses. The sympathetic nervous system can be rapidly activated to mobilize energy in situations of stress or danger ("fight or flight" response). By contrast, the parasympathetic nervous system is activated when organisms are in a state of relaxation ("rest and digest" state). In the brain, hypothalamus, which is located just above the brainstem, is responsible for integrating autonomic functions. It receives regulatory ANS input from the limbic system and controls both the ANS and the release of pituitary hormones, which in turn regulates basic endocrine organ functions. The nervous and endocrine systems thus cooperate to maintain or restore homeostasis via complementary pathways. The hormones are released into the bloodstream, and, like cytokines and other humoral mediators, they act as part of a diffusible network, whereas the neural pathways act locally in the tissues.

Innate lymphoid cells (ILCs) are essential players in the acute innate immune response to infection and in tissue remodeling, and they are also involved in regulating adaptive immunity and resolving inflammation.[11] There are several subsets of ILCs: cytotoxic natural killer (NK) cells and the "helper" subsets of ILC1s, ILC2s, and ILC3s. The circulating ILCs include a major population of NK cells, that shuttle between the tissues and the blood and are rapidly recruited to target organs, in which they exert their cytotoxic and cytokine-mediated immunomodulatory functions.[12] The "helper" subsets of ILCs are found in a wide range of lymphoid and non-lymphoid tissues and are particularly abundant at mucosal sites. NK cells and tissue-resident ILCs have been shown to respond to several effector molecules of the neuroendocrine system. Many evidence support a role for circulating mediators, such as glucocorticoids and epinephrine, in the regulation of NK cell response. Consistent with their strategic localization at mucosal barriers in close contact with nerve endings, tissue-resident ILCs have been identified as targets of the neurotransmitters and neuropeptides released directly into tissues by neurons.

ILCs are known to contribute to homeostasis, but they can also initiate pathological inflammation. Under some pathological conditions, the uncontrolled action of ILC subsets can lead to tissue damage, chronic inflammation, metabolic diseases, autoimmunity, and cancer, highlighting the importance of the fine-tuning of ILC responses for the maintenance of host integrity.[13] The signals determining whether they promote homeostasis or inflammation are currently dissected. We review here recent findings showing how both circulating hormones and tissue-released neurotransmitters and neuropeptides control ILC functions in homeostatic and inflammatory conditions.

2 | ILCS AND STEROID HORMONES

Steroid hormones are derived from cholesterol and secreted by the adrenal cortex, testes, and ovaries, and by the placenta during pregnancy. They are classified into two main groups, depending on their site of secretion: adrenal cortical steroids (or corticosteroids) and sex steroids. Steroid hormones are lipophilic and are therefore generally bound to a serum binding protein when transported in the blood. Their lipophilic nature enables them to enter cells easily, by diffusing across the plasma membrane. Inside the cell, steroid hormones bind to cytoplasmic receptors, leading to their translocation into the nucleus, where they interact with specific hormone response elements present in the promoter regions of hormone-responsive genes. This binding to gene promoters directly affects gene expression. Hormone receptors can also regulate gene expression without binding directly to the DNA, through protein-protein interactions with other DNA-binding transcription factors.[14] Steroid hormones are known to regulate diverse biological responses. They have profound effects on cellular metabolism, development, and homeostasis. Moreover, hormone response elements are present in the promoters of many genes involved in immune regulation, including the TLR7, MyD88, IRF7, and TLR3 genes.[15] Hormone receptors can also interact with transcription factors involved in the production of pro-inflammatory molecules by innate immune cells, such as nuclear factor-κB (NF-κB), specific protein 1 (Sp1), CCAAT/enhancer binding protein β (C/EBPβ), and activator protein 1 (AP-1).[15]

We summarize below the most relevant data concerning the regulation of ILCs by corticosteroids and sex hormones.

2.1 | Glucocorticoid regulation of NK cells and ILC1s

Glucocorticoids (GCs, cortisol in humans and corticosterone in rodents) are corticosteroid hormones released into the bloodstream by the adrenal glands according to a circadian rhythm regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Activation of this axis leads to the secretion, by the hypothalamus, of corticotropin-releasing hormone, which acts on the anterior pituitary gland to stimulate the synthesis of adrenocorticotropic hormone (ACTH). ACTH then induces the secretion of GCs into the bloodstream by the adrenal cortex,[14] and these hormones exert multiple effects through binding to the GC receptor (GR), which is ubiquitously expressed by nearly all nucleated cells. GCs allow the optimal synchronization of physiological and behavioral processes with the external environment,[16,17] including conditions of inflammation and stress inducing systemic cytokine production. In this context, GCs are one of the principal effectors of the "stress response", which is the result of the interaction of neuroendocrine and immune systems and is responsible for maintaining physiological homeostasis. The activation of pituitary-dependent adrenal responses in response to endotoxin administration provided the first evidence of the activation of anti-inflammatory signals from the CNS by inflammatory stimuli. In particular, the IL-6, IL-1 and TNF-α produced in response to infection or bacterial lipopolysaccharide (LPS) administration can act directly on the hypothalamus to activate the HPA axis.[18] This neuroendocrine pathway is required for host protection, because excessive levels of the cytokines produced in the inflammatory cascade can have deleterious effects. Indeed, disruption of the HPA axis through adrenalectomy or the use of GR antagonists renders mice more susceptible to septic shock, due to the detrimental consequences of hyperinflammation.[19,20] In the LPS-induced septic shock model, GCs have been shown to inhibit inflammatory cytokine expression by acting directly on immune cell-subsets producing these molecules, such as monocytes and macrophages for IL-1β, TNF and IL-6[21,22] and dendritic cells (DCs) for IL-12.[23]

IFN-γ is a key cytokine of the LPS-induced inflammatory cascade, because it primes mononuclear phagocytes, causing them to adopt a pro-inflammatory phenotype.[24] NK cells and ILC1s are major sources of IFN-γ immediately after pathogen invasion,[12,25] and both these cell populations express GR.[26] NK cells and ILC1s also express the cell surface NKp46 (or NCR1) molecule encoded by the *Ncr1* gene. Using a mouse model in which the GR was conditionally deleted in NCR1⁺ ILCs (GR^Ncr1-iCre), we recently showed that endogenous GCs downregulate IFN-γ production by spleen NK cells, liver NK cells, and liver ILC1s, in response to LPS, thereby affecting the amounts of this cytokine in the serum.[26] We have shown that the GC-mediated regulation of IFN-γ production by these ILC subsets is essential and protective in situations in which endotoxins are repeatedly encountered.[26] This condition has been referred to as "endotoxin tolerance" because it corresponds to a refractory state of the immune system, which following an initial inflammatory response to LPS does not react to subsequent encounters with this bacterial endotoxin.[27] Studies in vivo in mice [28] and both in vitro and ex vivo on human peripheral blood mononuclear cells (PBMCs)[29] have shown

that multiple challenges with LPS induce myeloid cells to switch to an anti-inflammatory phenotype. Several tolerization mechanisms have been described, at the signal transduction, transcriptional, and epigenetic levels,[30-32] all of which are abolished by IFN-γ.[33-35] We have shown in our model that IFN-γ production by NK cells and ILC1s can, if not controlled by the GR pathway, reverse endotoxin tolerance, by preventing myeloid cells from releasing large amounts of IL-10 into the bloodstream.[26] Thus, upon endotoxin tolerance induction, the regulation, by GCs, of IFN-γ production by ILCs acts as a regulatory mechanism operating upstream from the GC-mediated regulation of myeloid cell cytokine production. This neuroendocrine control is required for the establishment of an immunosuppressive state, which is important for resistance to endotoxin shock.

In the LPS-induced shock model, which involves the injection of bacterial products rather than infection with living bacteria, host damage results exclusively from an excessive inflammatory response controlled by the HPA axis. The development of a GC-mediated global suppression mechanism would be paradoxical at a time at which an active immune response to an infectious agent may be critical for the survival of the organism. In this case, fine regulation of the immune response by GCs, which is essential to prevent inflammation-induced immunopathology, may increase the risk of immunodeficiency. To evaluate this possibility, we extended our investigation of the GC-intrinsic regulation of NK cell and ILC1 functions to a model of infection with murine cytomegalovirus (MCMV). NK cells are the principal mediators of early defense against both human and mouse CMV infections, through their cytokine- and killing-dependent mechanisms.[36] During the acute phase of infection, MCMV replicates in the spleen and liver,[37] in which IFN-γ, produced by NK cells and ILC1s, is essential for antiviral defense.[38,39] In this infectious model endogenous GCs have been reported to be involved in protecting against the life-threatening effects resulting from viral-elicited cytokine responses. In particular, mice rendered GC-deficient by adrenalectomy produce more IFN-γ, TNF-α, IL-12, and IL-6 and are more likely to die from MCMV infections.[40] In this study, endogenous GCs were considered to act principally as inhibitors of cytokine-mediated lethality. However, the mechanisms underlying this inhibition were unclear, as many hematopoietic and non-hematopoietic cells express the GR. We showed in GR^Ncr1-iCre mice that, as observed after LPS injection, endogenous GCs produced in response to MCMV infection prevent excessive IFN-γ production by NK cells in the spleen.[41] However, this regulation was organ-specific as the disruption of GC responsiveness in liver NCR1⁺ ILCs (ie, NK cells and ILC1) did not affect their IFN-γ production. Consistent with this differential regulation, RNA-Seq analysis revealed that the genes selectively regulated by the GR pathway were also cell type- and organ-specific (Figures 1 and 2). Importantly, expression of the *Pdcd1* gene, encoding the inhibitory receptor PD-1 (programmed cell death 1), is strictly GR-dependent and observed in the spleen, but not in the liver NK cells. PD-1 is an immune checkpoint involved, in particular, in the downregulation of T-cell activity. We showed that the GR-PD-1 pathway plays a major role in NK cells, regulating their IFN-γ production in the spleen and promoting
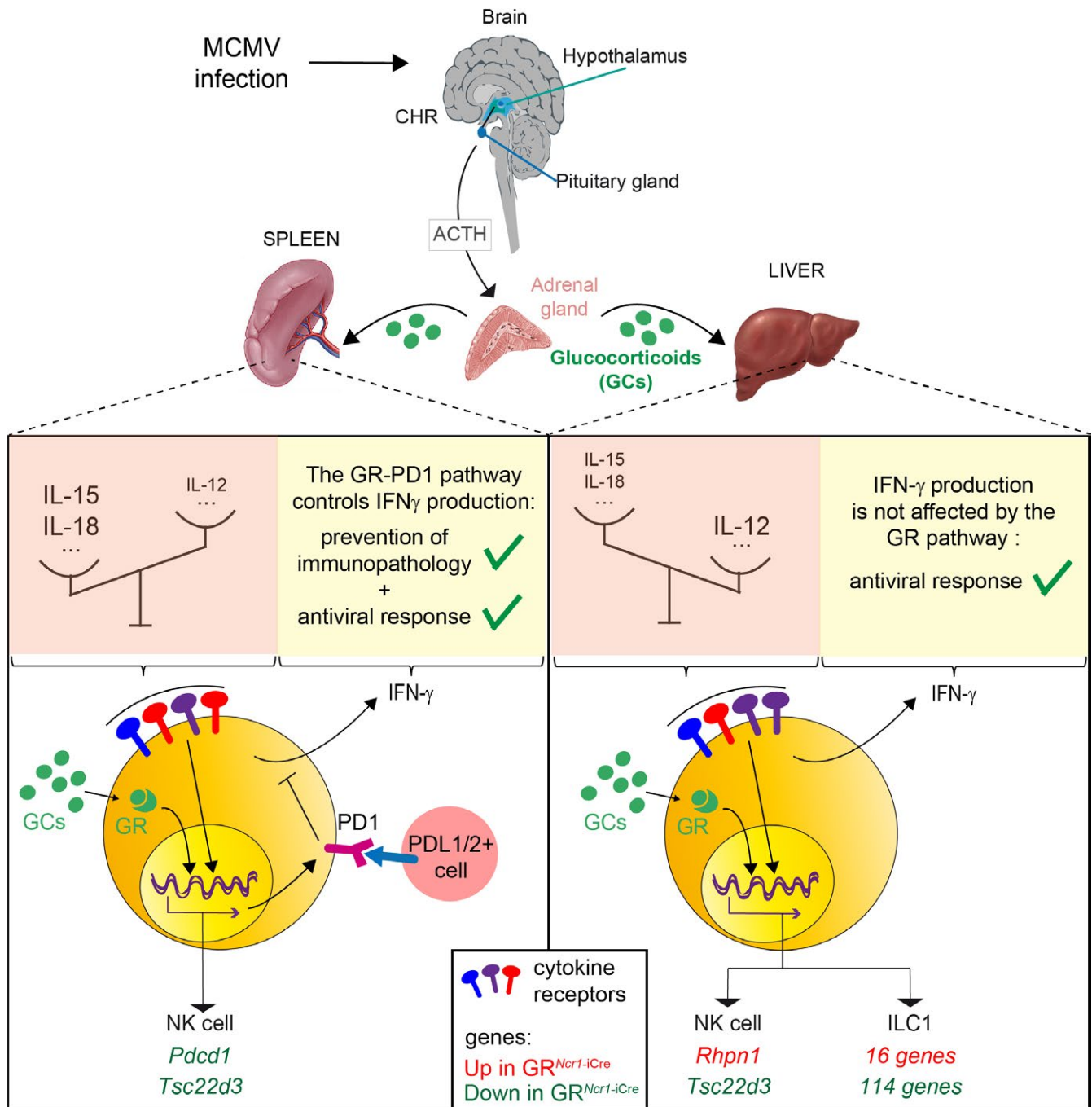
FIGURE 1 Glucocorticoids regulate NK cells and ILC1s functions upon MCMV infection. MCMV infection induces the activation of the HPA axis: the hypothalamus produces the corticotropin-releasing hormone (CHR), which activates the pituitary gland to release the adrenocorticotropin hormone (ACTH) which, finally, induces the secretion of glucocorticoids (GCs) into the bloodstream by the adrenal gland. Signaling transduced by different combinations of cytokines and other unidentified potential mediators in the spleen and liver microenvironment differentially cooperates with the glucocorticoid receptor (GR) to regulate transcription. As a result, the control of gene expression in NK cells and ILC1s is both tissue and cell type specific: the genes induced by the GR pathway in each cellular target are highlighted in green (Down in GR[Ncr1-iCre]), while the genes repressed by the GR pathway are in red (Up in GR[Ncr1-iCre]). The GR pathway inhibits IFN-γ production only in NK cells in the spleen through the induction of PD1 expression. This regulation is required to prevent immunopathology in the spleen, without affecting antiviral response

host resistance to infection.[41] This regulatory mechanism is essential to prevent IFN-γ-dependent spleen immunopathology but does not affect the local control of viral replication (Figure 1). Consistent with this finding, IFN-γ plays a dual role in MCMV infection: it has a

negligible antiviral function in the spleen, but is required to prevent viral replication in the liver, which may lead to lethal hepatitis.[42] The organ-specific mechanism by which GR regulates gene expression may depend on the different cytokine environments of the spleen
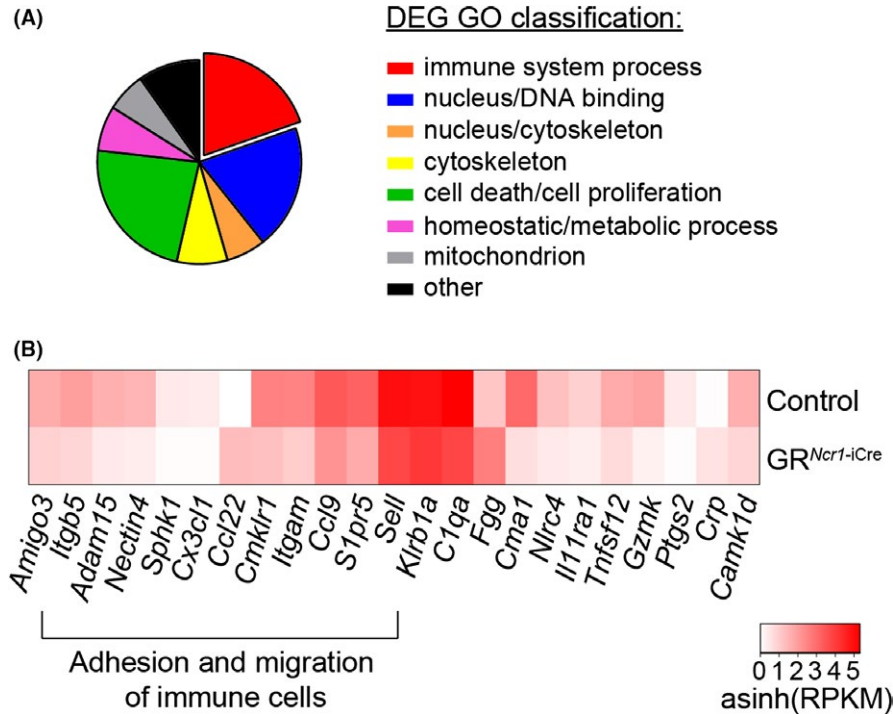
FIGURE 2 Analysis of the genes regulated by GR in liver ILC1s upon MCMV infection. Transcriptomic data (RNAseq) published in are analyzed (GSE114827).[41] The differentially expressed genes (DEGs) between liver ILC1s sorted from MCMV infected Control and GR$^{Ncr1\text{-}iCre}$ mice were classified into non-redundant functional categories by combining gene ontology (GO) analysis and literature-based manual curation (A). The DEGs involved in immune system processes were analyzed and the heat map in B represents their RNA-seq expression levels, as measured by reads per kilobase per million reads (RPKM), and asinh scaled. Among these genes, 12 transcripts encode for proteins involved in adhesion and migration of immune cells. With the exception of *Ccl22*, upregulated in GR deficient ILC1s and whose function has been related to type2 response and migration of T regs, GCs induce all of the genes listed. In particular, in liver ILC1s, GCs induce the upregulation of the genes *Amigo3*, *Nectin4*, *SelL* encoding adhesion molecules, and the genes *Itgb5* and *Itgam* encoding integrins. GCs also upregulate the expression of the genes encoding the chemokines CX3CL1 and CCL9, which attract monocytes, NK cells and neutrophils, respectively

and liver (Figure 1). Consistent with this hypothesis, we showed that PD-1 expression on NK cells in vitro is induced by simultaneous stimulation with IL-15, IL-18, and corticosterone, whereas the addition of IL-12 abolishes this effect.[41]

Remarkably, no impact on cytotoxic function was observed in either of the two models in which we investigated NK regulation by GCs, suggesting that the effects of GCs on the two main functions of these innate lymphocytes—cytokine production and cytotoxicity—are uncoupled.

Collectively, these data are consistent with the tissue microenvironment playing a determinant role in the final outcome of the GR-mediated regulation of gene expression in NK cells and ILC1s. In this model, GR signaling acts in concert with other signals from the microenvironment to generate an organ-specific effect, protecting against immunopathology without compromising viral control (Figure 1). The major role of GR-induced PD-1 expression in this regulation may have clinical implications, as PD-1 is expressed on NK cells from CMV-seropositive donors.[43] The other pathological conditions in which this GR-PD-1 pathway plays a role remain to be identified.

The control of ILC functions by GCs is not only organ-specific, but also cell-type specific. In the liver of MCMV-infected mice, the GR-dependent control of gene expression is very different in NK cells and ILC1s. Only two genes are modulated by this pathway in NK cells, whereas the transcription of 130 genes is GR-dependent in ILC1s (Figure 1).[41] Many of these genes are involved in immune cell processes, including adhesion and migration (Figure 2). Most are upregulated by the GR pathway, suggesting that GCs may increase the magnitude of the immune response in this organ, rather than damping it down. The final effect of HPA axis activation and GC production on immune responses therefore reflects a kaleidoscope of cell-specific, tissue-specific regulations.

2.2 | Sex hormones and ILC2s

Men tend to develop less vigorous adaptive immune responses than women. They are therefore more susceptible to some infectious diseases, but have a lower risk of autoimmunity. This sex bias in disease susceptibility is supported by the important role of sex hormones (estrogens, progesterone, and androgens) in immune regulation.[44] Estrogens, for example, affect many aspects of innate immunity, including the functional activity of NK cells. In vitro, exposure to 17β-estradiol (E2) enhances NK cell IFN-γ production,[45] whereas in vivo, it downregulates the expression of NK cell activating receptors (2B4,

NKp46, NKG2D) and reduces their cytotoxicity.[46] Progesterone (P4) is produced by the *corpus luteum* during the menstrual cycle and at high levels by the placenta during pregnancy. P4 has been shown to play a crucial role in NK cell recruitment to the uterus during early pregnancy, by reprogramming the chemokine receptor profile of peripheral blood NK cells exposed to the uterine microenvironment.[47] Androgens, including testosterone and dihydrotestosterone (DHT), are present at higher concentrations in post-pubertal men than in women, and generally suppress immune cell activity.[44]

Asthma is one of a number of diseases for which there is a sex bias in susceptibility. Sex hormones have been reported to regulate the inflammation associated with asthma, by acting on airway inflammation, smooth muscle contraction, mucus production, and airway mechanics.[48,49] During childhood, asthma is more prevalent in boys than in girls, but this pattern is reversed at puberty, when the levels of sex hormones increase and, as a result, adult women are about twice as likely as men to develop asthma.[48] A similar sex bias has also been observed in mouse experimental models of ovalbumin (OVA)-induced asthma,[50,51] and in house dust mite (HDM)-induced allergic airway inflammation.[52]

ILCs are involved in the regulation of pulmonary immunity, inflammation, and tissue homeostasis,[53] and ILC2s are the principal ILC subset populating the mouse lung. ILC2s were identified in the lung parenchyma of Rag$^{-/-}$ mice as a discrete population of Id2-dependent ILCs expressing CD90, c-Kit, CD127, CD125, CD44, ICOS, and IL-33R, lacking the expression of NK cell markers and ROR-γ. Functionally, these cells produce IL-5 and IL-13, but not IL-22, IL-17A, or IFN-γ in response to stimulation with IL-33 plus IL-2 and IL-7.[54,55] A population of ILC2s in human lung phenotypically and functionally analogous to that described in mice has also been identified.[54,56]

ILC2s have emerged as critical cells for the initiation of allergic inflammatory responses. Patients with allergic asthma have a higher proportion of ILC2s among their PBMCs than healthy controls,[57] and the frequency of ILC2s in the blood is higher in asthmatic women than in asthmatic men.[58] Moreover, ILC2s from women with asthma produce more IL-5 upon ex vivo restimulation than ILC2s from asthmatic men.[58] In mice, the number of ILC2 progenitors (ILC2Ps) in the bone marrow at steady state is higher in females than in males, and ILC2Ps have been shown to express high levels of androgen receptors but to have undetectable levels of estrogen receptor (ER) transcripts.[52] This difference between the sexes also extends to the lungs, in which the frequencies and total numbers of mature ILC2s are lower in intact males than in castrated males and females with and without ovarectomy.[52] Overall, these findings suggest that the sex bias in allergy is due to male hormones rather than female hormones.

The role played by sex hormones in the difference in ILC2 numbers and functions between sexes may be linked to their control of IL-2R signaling. Indeed, ILC2s in the lungs of male mice express low levels of IL-2R. In addition, ex vivo stimulation with IL-33 + IL-2 results in a greater proliferation of lung ILC2s from females than from males, together with higher levels of IL-5 and IL-13 production.[58] Through the administration of slow-release pellets containing male or female hormones to gonadectomized mice and through the ex vivo stimulation of ILC2s with hormones, DHT was shown to downregulate cytokine production.[58] In vivo models of type 2 airway inflammation (systemic administration of IL-33[52] and exposure to extracts of the fungus *Alternaria alternata*[58]) have confirmed the protective role of androgens, through their effects on ILC2s, decreasing the ability of these cells to proliferate and produce IL-5 and IL-13. However, sex hormones are also involved in other ILC-independent mechanisms regulating lung inflammation. Testosterone decreases IL-33 and thymic stromal lymphopoietin (TSLP) production,[58] whereas ovarian hormones increase mucus secretion.[59] A more specific targeting of sex hormone receptor signaling in ILC2s would be required to decipher the intrinsic contribution of these pathways in this subset of ILCs to the sex bias observed in the context of allergic airway inflammation. Moreover, the downstream targets of androgen receptors in ILC2s, the mechanism of IL-2R downregulation, and the role of androgen receptors in controlling the expression of transcription factors involved in the maintenance of ILC2Ps or their differentiation into ILC2s remain to be investigated. Studies on the T-cell differentiation pathway have shown that androgen receptors upregulate transcription of the gene encoding the phosphatase Ptpn1, thereby inhibiting Th1 polarization.[60] It remains unclear whether androgen receptors directly modulate IL-33 signaling and regulate cytokine production in ILC2s.

Lung ILC2s thus resemble the ILC2s present in gut-associated lymphoid tissue, fat-associated lymphoid clusters and the spleen.[61-63] Consistent with the sex difference displayed by ILC2Ps in the bone marrow, the numbers of ILC2s in the mesenteric lymph nodes and visceral adipose tissue have also been shown to be higher in females than in males.[52] Indeed, ILC2s have been shown to regulate thermogenesis from beige fat and to prevent metabolic syndrome and insulin resistance,[64-66] suggesting that the regulation of ILC2s by sex hormones may also be associated with differences in metabolic homeostasis between the sexes. Thus, ILC2s have pleiotropic functions beyond innate immunity that are specific to the organs in which they reside. For example, the uterus of BALB/c mice has been shown to contain a population of ILC2s phenotypically similar to lung ILC2s and capable of producing large amounts of IL-5 and IL-13 in response to stimulation with IL-33.[67] The ovariectomy leads to a decrease in ILC2 numbers in the uterus, which can be reversed by reconstitution with E2 and P4.[67] By contrast, the number of ILC2s in the lung is not affected by female sex hormones.[52] Consistent with this finding, *Esr1*, which encodes estrogen receptor alpha (ERα), is more strongly expressed by uterine than lung ILC2s, whereas *Esr2* (ERβ) expression is similar in these two cell populations.[67] As for the homeostasis of ILC2s in the lung, it would be interesting to distinguish directly between the intrinsic and extrinsic effects of estrogens and the ER in the steady-state accumulation of ILC2s in the uterus and, more importantly, in reproduction. Indeed, the effects of estrogens on uterine tissue cells, such as uterine epithelial and stromal cells, may indirectly affect the survival or proliferation of ILC2s.

These studies have demonstrated that sex hormones modulate ILC2 homeostasis, and the effects of these hormones seem to depend on the organ in which the ILC2s reside, highlighting the

importance of the tissue microenvironment in determining the regulatory effects of hormone receptors. Autoimmune and inflammatory diseases do not occur at equal frequencies in males and females.[15,68] Further studies are required to determine whether sex hormones regulate the homeostasis and function of other subsets of ILCs, and to determine the impact of this regulation in diseases for which differences between the sexes are observed.

3 | NERVOUS SYSTEM-ILC INTERACTIONS IN THE MAINTENANCE OF BARRIER DEFENSE

Barrier surfaces, like the gut, the lung, and the skin, act as interfaces at which the nervous and immune systems are in constant communication, sensing, and adapting to the challenges of the local environment. Through innate and acquired immunity, the mucosal immune system maintains immunological homeostasis over the vast surface area of the epithelium, and mediates the symbiotic relationship between the host and commensal bacteria; it also serves as the first line of physical and immunological defense against invading pathogens.[69]

The ILCs resident at barrier surfaces are an important component of the mucosal immune system. They fulfill essential roles as sentinels and local keepers of tissue functions. Recent studies have highlighted the anatomical colocalization of ILCs with nerve terminals in some tissues suggesting neuro-immune interactions.

The neurotrophic factor receptor RET constitutes the clearest example of the existence of a communication pathway between cells of the nervous and immune systems. RET is a receptor tyrosine kinase activated by glial cell-derived neurotrophic factor (GDNF) and other members of the GDNF family of ligands (GFLs). It drives hematopoietic stem cell survival, expansion, and function,[70] and is critical for the development of both Peyer's patches and the nervous system in the intestine.[71,72] In enteric neurons, RET signaling is activated in *cis* following the binding of GFLs to the GFRα1-4 coreceptors, whereas immune cells respond in *trans* to multiple GFLs.[71] ILC3s in the adult gut lamina propria have high levels of RET expression and aggregate in cryptopatches and isolated lymphoid follicles. Within cryptopatches, ILC3s are found in close proximity to stellate projections of the lamina propria glial cells. These cells are the principal producers of GFLs in response to commensal products and alarmins sensed by MYD88-dependent pathways. RET signaling in ILC3s induces the rapid phosphorylation of ERK1/2, AKT, p38/MAP kinase, and STAT3, leading to an increase in IL-22 transcription. This glial cell-ILC3 pathway is essential to shape epithelial cell reactivity, and has been shown to regulate intestinal defense in a model of infection with the attaching and effacing bacterium *Citrobacter rodentium*.[73]

In addition to this glial cell-ILC3 pathway, many other microanatomical and functional units of interaction between neural cells and ILCs have been identified at mucosal barriers. In particular, cholinergic, adrenergic, and nociceptor sensory neurons have been shown to communicate with ILCs in respiratory and intestinal tissues.

3.1 | ILC-neuron interaction in the respiratory mucosal tissue

The lungs are densely innervated by the ANS and sensory neurons. The ANS innervating the lung consists of parasympathetic neurons, the cell bodies of which reside in the brainstem and project to the lung via the vagus nerve mediating bronchoconstriction, and sympathetic neurons, the cell bodies of which reside in the paravertebral ganglia and stimulate bronchodilation (Figure 3). Sensory afferent innervation involves neurons with cell bodies residing within the dorsal root ganglia (DRG) (Figure 3). Most of the sensory fibers innervating the lung express nociceptor markers, such as the transient receptor potential (TRP) channels TRPV1 and TRPA1. Airway nociceptors initiate essential protective airway reflexes (such as coughing) in response to chemical, mechanical, or thermal stimuli. In addition to exogenous stimuli, many endogenous mediators generated during inflammation, including lipids, bradykinin and NGF, directly and indirectly activate nociceptors, resulting in the calcium-mediated release of the neuropeptides C-fiber-like calcitonin gene-related peptide (CGRP), substance P (SP), and vasoactive intestinal polypeptide (VIP). Numerous neuropeptides have been identified in lung-specific secretory structures called neuroepithelial bodies (NEBs). NEBs are clusters of pulmonary neuroendocrine cells (PNECs), highly conserved from fish to mammals and the only innervated epithelial cells in the lung.[74] NEBs form extensive synaptic contacts with afferent and efferent nerve fibers.[75] In particular, vagal sensory fibers have been shown to branch and connect NEBs, and a nerve plexus is formed by sensory neurons at the basal pole of the NEBs[76] (Figure 3). These findings suggest that the stimulation of efferent neurons may activate PNECs, which, when exposed to large quantities of airborne antigens, trigger lung immune cell activation via neuropeptide secretion.[77] Indeed, PNECs amplify allergen-induced type 2 immune responses in the mouse OVA- and HDM-induced asthma models, and, in addition to neuropeptides, they also release GABA, which stimulates goblet cell hyperplasia.[78]

IL-5 and IL-13 production by ILC2s plays a key role in promoting allergic airway inflammation in multiple models, as these cytokines are essential for eosinophilic infiltration and mucus production.[53] In addition to promoting allergen-induced type 2 cytokine responses, lung ILC2-derived IL-5, and IL-13 play a role in the context of infections with helminths, such as *Nippostrongylus brasiliensis* and *Strongyloides venezuelensis*.[79,80] ILC2s also promote beneficial tissue repair responses in the lung following epithelial damage, as they can secrete amphiregulin in response to influenza virus infection.[54]

On lung sections, ILC2s have been found in close proximity (10 µm) to SNAP-25$^+$ nerve fibers.[81] In another study, lung IL-5-producing ILC2s were found in collagen-rich regions near the confluence of medium-sized blood vessels and airways.[82] More recently, IL-5$^+$ ILC2s were found to reside close to PNECs in the airway branch junctions (nodal points), where particles entering the airways tend to concentrate.[78] ILC2s are, thus, strategically located within the airway mucosal tissue, so as to form signaling centers with the
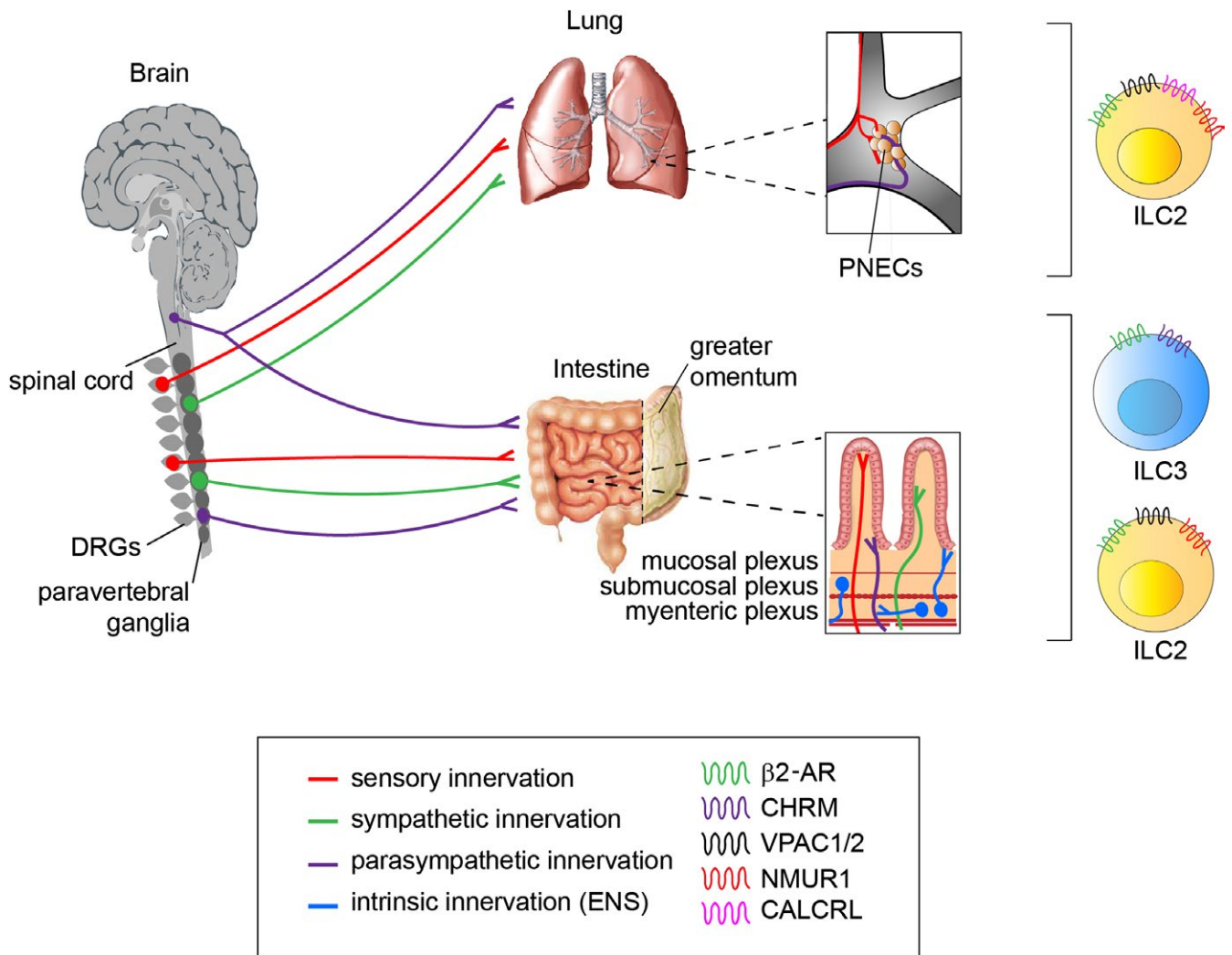
FIGURE 3 Neural pathways innervating the respiratory and intestinal mucosa. The CNS communicates with the lung and intestine through sympathetic, parasympathetic, and sensory neurons. Sympathetic neurons have their cell bodies in the paravertebral ganglia, whereas parasympathetic neurons have their cell bodies in the brainstem and sacral region of the spinal cord (S2-S4). They project to the organs via the vagus nerve or the pelvic nerve respectively. Afferent sensory innervation involves neurons with cell bodies residing within the DRG (dorsal root ganglia). The lung contains peculiar neuroendocrine cells, the PNECs (pulmonary neuroendocrine cells), which cluster together to form extensive synaptic contacts with afferent and efferent nerve fibers. The intestine is innervated by intrinsic neurons of the ENS (enteric nervous system), which have cell bodies located in the mucosal, submucosal, and myenteric plexuses and communicate with the CNS. The ILCs in the lung and intestine express receptors for the neurotransmitters and neuropeptides released in these tissues: β2-AR (β2-adrenergic receptor) for epinephrine and norepinephrine, CHRM (cholinergic receptor muscarinic) for acetylcholine, VPAC1/2 (vasoactive intestinal peptide receptor) for VIP, NMUR1 (NMU receptor) for NMU and CALCRL (calcitonin receptor-like) for CGRP

neuroendocrine system for patrolling the airways and potentially recruiting circulating immune cells to sites of damage or pathogen invasion.

3.2 | ILC-neuron interaction in the intestinal mucosal tissue

The intestine harbors the largest lymphoid cell compartment in the body, and what has been referred to as the "second brain," a neuronal network with as many neurons as the spinal cord.[83] The intrinsic innervation of the intestine (the "enteric nervous system," ENS) is often considered to be an independent branch of the ANS,

comprising neurons with cell bodies lying within the tissue. These intrinsic neural ganglia are organized into several plexuses within the intestine: the myenteric (between the circular and longitudinal muscle layers), the submucosal and the mucosal plexuses. The mucosal plexus harbors dense glial cell networks around the intestinal crypts and is in contact with mucosal immune cells, which are highly concentrated in this layer[84] (Figure 3).

In addition to the intrinsic ENS, the intestine displays extrinsic innervation from the CNS. The CNS communicates with the intestine via projections from the sympathetic and parasympathetic systems and through the HPA axis, which together form the so-called "gut-brain axis."[85] The vagus nerve is the main extrinsic parasympathetic

TABLE 1 Mediators of neuroendocrine pathways and their effects on ILC targets

Neuro-endocrine pathway	Experimental model	ILC target	Effect	Reference
Glucocorticoids	Endotoxin tolerance	ILC1, NK cells	↓IFNγ production	Quatrini (2017)
Glucocorticoids	MCMV	Spleen NK cells	*Pdcd1* induction and PD1-dependent inhibition of IFNγ production	Quatrini (2018)
Estrogens	In vitro exposure to E2	NK cells	↑ IFNγ production	Nakaya (2006)
Estrogens	Ovariectomy and E2 administration	NK cells	↓ Expression of activating receptors and cytotoxicity	Hao (2007)
Progesterone	In vitro migration assay	Human blood NK cells	Reprogramming of chemokine receptor profile	Carlino (2008)
Testosterone	IL-33, *A. alternata*	Lung ILC2	↓ IL-15 and IL-13 production and cell proliferation	Laffont (2017); Cephus (2017)
Estrogens, progesterone	Ovariectomy and E2+P4 administration	Uterus ILC2	Steady state accumulation	Bartemes (2018)
GFL	MyoD88-dependent activation of glial cells	ILC3 in the gut lamina propria	RET-dependent induction of IL-22 production	Ibiza (2016)
Cholinergic pathway	Stroke	Brain NK cells	↓ RUNX3 and inhibition of cell response	Liu (2017)
Cholinergic pathway	Vagotomy + *Escherichia coli* infection	ILC3 in the peritoneum	Ach-induced production of PCTR1	Dalli (2017)
B2adr pathway	Stroke	Spleen NK cells	↑ SOCS3 and inhibition of cell response	Liu (2017)
B2adr pathway	Noradrenergic spleen innervation	Spleen NK cells	Circadian oscillation GrzB and perforin content	Dokur (2004); Logan (2011)
B2adr pathway	In vitro stimulation	NK cells	Detachment from the endothelium	Benschop (1993)
B2adr pathway	Acute stress (restraint stress)	Lung NK cells	↓ cell number	Kanemi (2005)
B2adr pathway	Chronic stress (social disruption)	Blood NK cells	↑cell numbers, CD16, CD69, CD107, IFNγ ↓ NKG2A and Ly49A	Tarr (2012)
B2adr pathway	Enriched environment	NK cells	↑ NKG2D and CCR5 expression ↑ infiltration in pancreatic tumor model	Song (2017)
B2adr pathway	*Nippostrongylus brasiliensis*	Gut ILC2s	↓ proliferation and effector functions	Moriyama (2018)
B2adr pathway	IL33, *A. alternata*	Lung ILC2s	↓ proliferation and effector functions	Moriyama (2018)
VIP	OVA challenge	Lung ILC2s	IL13 production	Talbot (2015)
VIP	In vitro VIP + IL7	Small intestine ILC2s	↑ IL5 production	Nussbaum (2013)
VIP	Circadian/metabolic cues	Lung/small intestine ILC2s	IL-5-dependent homeostatic accumulation of eosinophils	Nussbaum (2013)
NMU	In vitro stimulation with IL25	Lung ILC2	Synergy NMU+IL25 in induction of IL13, IL5 and cell proliferation	Wallrapp (2017)
NMU	In vitro stimulation	Small intestine ILC2s	IL13 and IL5 induction comparable to PMA/iono or IL2,7,25,33 combination	Klose (2017)
NMU	*N. brasiliensis* and NMU delivery	Small intestine ILC2s	↑ IL13 production and proliferation, with ↑ eosinophilia and worm expulsion	Cardoso (2017)
NMU	*N. brasiliensis* and intranasal NMU delivery	Lung ILC2s	↑ ILC2 proliferation, maturation and cytokine expression, with ↑ lung inflammation	Klose (2017)
CGRP	In vitro stimulation	Lung ILC2s	↑ IL5 production induced by IL7+25+33	Sui (2018)
CGRP	HDM	Lung ILC2s	↓ immune cell infiltration in the lung	Sui (2018)

nerve connecting the brainstem to the gut. Multiple functions within the gastrointestinal tract are, thus, controlled by an extensive diffuse network of millions of sensory neurons, interneurons, and motor neurons[69] (Figure 3).

ILC3s are the most abundant ILC subset in mouse intestinal tract, and they use a wide range of cytokine-dependent and cell surface receptor-mediated mechanisms to exert homeostatic control over intestinal immunity.[86] ILC3s produce IL-22, GM-CSF, and IL-17 in response to IL-23 and IL-1β, which are produced principally by CX3CR1[+] monocytes and DCs.[87] IL-22 influences the composition of the intestinal microbiota in mice, is critical for the innate response to intestinal bacterial pathogens such as *Citrobacter rodentium*, and is important for epithelial cell production of antimicrobial peptides and proteins, such as defensins and mucins.[88,89] Rorγt^{+} CD3^{−} ILC3s have also been identified in the peritoneal cavity, in the greater omentum.[90] Confocal microscopy showed that these ILC3s were located close to choline acetyl transferase-positive cells,[90] suggesting that crosstalk between ILC3 and cholinergic vagus nerve fibers occurs not only in the intestine, but also in the peritoneum.

ILC2s are present at a lower frequency than ILC3s, but they nevertheless play an important role in protective innate immune responses to parasites and helminthes in the intestine, by inducing eosinophilia and goblet cell hyperplasia. As in the lung, IL-35- and IL-33-responsive ILC2s are critical for the expulsion of *N. brasiliensis* in mice lacking adaptive immune cells.[61-63] Moreover, intestinal ILC2s play a role in maintaining epithelial integrity. Indeed, the ILC2s associated with mouse gut can secrete amphiregulin in response to IL-33 and have been shown to be protective in the dextran sodium sulfate model of intestinal damage and inflammation.[91] ILC2s have also been detected in the human gut, albeit at a low frequency, where they express IL-33R (ST2), IL-25R, and the chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (CRTH2).[56]

ILC2s have been shown to form close intercellular contacts with neurons in the intestine.[92] In particular, ILC2s are found adjacent to cholinergic neurons in the small intestine lamina propria,[93] and co-localize with adrenergic neurons in the villi, submucosa, and also in the parenchyma and in mesenteric LNs, which display a high level of adrenergic innervation.[94,93] This colocalization with different kinds of nerve fibers suggests that the nervous system regulates ILC2s by multiple pathways in the intestine.

3.3 | Sympathetic and parasympathetic regulation of ILCs

The ANS is the major efferent component of the PNS. It continually regulates unconscious functions, such as heart rate, blood pressure, respiratory rate, gastrointestinal motility, and body temperature. The sympathetic (catecholaminergic) nervous system and the parasympathetic (cholinergic) nervous system can act either in synergy or in opposition, to mediate basic physiological responses in real time. For example, these two pathways have been shown to be complementary in the context of brain ischemia, as both are involved in downregulating NK cell functions, resulting in immune

system suppression and higher susceptibility to infections.[96,97] In particular, following a stroke, the cholinergic pathway is responsible for the rapid decline of NK cell response in the brain through the downregulation of runt-related transcription factor 3 (RUNX3), whereas the catecholaminergic system inhibits the NK cell response in the periphery by inducing the Jak inhibitor suppressor of cytokine signaling 3 (SOCS3).[96] These findings for peripheral NK cells are reminiscent of those for another study, in which high levels of sympathetic activity after stroke were found to induce immunosuppression through changes in the behavior of invariant NKT cells in the liver mediated by catecholaminergic signaling.[98]

Preganglionic sympathetic nerves activate postganglionic sympathetic fibers, leading to the release of norepinephrine into the innervated tissues. They also activate chromaffin cells in the adrenal medulla, leading to the release of epinephrine into the bloodstream. Epinephrine and norepinephrine (collectively known as catecholamines) are thus the neurotransmitters of the sympathetic nervous system.

Adrenergic nerves directly innervate lymphoid organs,[94,99] and catecholamines have been shown to play multiple roles in the regulation of immune cell dynamics. Lymphocytes express predominantly β2-ARs.[100] In humans, circulating CD56^{dim} NK cells have higher levels of β2AR expression than CD56^{bright} cells, suggesting that the CD56^{dim} subset can respond more vigorously to epinephrine/norepinephrine.[101]

Adrenergic nerves release norepinephrine in a circadian manner, and control the recruitment of myeloid cells to tissues by establishing circadian oscillations of adhesion molecule and chemoattractant expression by vascular endothelial cells.[102] The circadian release of norepinephrine in the spleen has been correlated with circadian oscillations in NK cell cytolytic activity, with norepinephrine acting to suppress the production of granzyme-B and perforin transcripts.[103,104] β2-AR controls the recirculation of T and B lymphocytes through LNs, contributing to the diurnal variation of lymphocyte dynamics, and translating into significant changes in the adaptive immune response.[105] However, there is currently no evidence for the sympathetic regulation of NK cell circadian recirculation between organs.

Expression of the β2-AR gene (*Adrb2*) has been compared between the ILC subsets sorted from different organs in mice: ILC2s have higher levels of *Adrb2* expression than ILC3s in the small intestine, but lower levels of the other adrenergic receptors.[95] Moreover, *Adrb2* is more strongly expressed in the ILC2s of the small intestine, colon lamina propria, and lung, compared to ILC1s of the small intestine lamina propria and spleen NK cells. By contrast, ILC2s from the mesenteric white adipose tissue have lower levels of *Adrb2* expression than other ILC2 subsets.[95] *Adrb2* gene expression has also been detected in human ILC2s sorted from the lung and blood.[95]

In inflammatory conditions, the sympathetic nervous system controls the recruitment of immune cells to sites of infection by regulating cell migration. In the lymphocytes of the adaptive immune system, signaling through β2-ARs inhibits egress from lymph nodes by enhancing retention signals from CCR7 and CXCR4 chemokine

receptors, leading to lower levels of antigen-primed T-cell recruitment to peripheral tissues in models of T cell-mediated inflammatory diseases.[106] For ILCs, the studies of the regulation of cell migration by β2-AR performed to date have been done on circulating NK cells. It has been shown in vitro that β2-AR stimulation causes NK cells to detach from endothelial cells.[107,108] In vivo, the role of β2-AR in NK cell migration has been investigated in stress-based models involving activation of the sympathetic nervous system. Beta-adrenergic stimulation reduces the number of intraparenchymal lung NK cells in a model of acute stress (restraint stress),[109] and increases the number of circulating NK cells in a model of chronic stress (social disruption).[110] In humans, direct catecholamine infusion[111] and sympathetic nervous system activation by drug administration[101] in healthy volunteers leads to an accumulation of peripheral circulating NK cells. All these lines of evidence suggest that stress responses and sympathetic nervous system activation are involved in the rapid mobilization of the innate immune system, including NK cells, to counteract incoming threats in a "fight or flight" response. The role of the adrenergic regulation of NK cell functions has also been investigated. Social stress-induced activation of the sympathetic nervous system led to an upregulation of CD16 and CD69 and a downregulation of NKG2A and Ly49A expression on NK cells.[110] In addition, splenic NK cells from chronically stressed mice had higher levels of surface CD107a expression, cytolytic activity, and IFN-γ production upon ex vivo stimulation.[110] Similarly, an enriched environment increased NK cell NKG2D expression, cytotoxicity, and CCR5 expression and tumor infiltration in a mouse model of pancreatic cancer, via a mechanism dependent on sympathetic signaling.[112]

One major limitation of these studies is that the blockade of adrenergic signaling with pharmacological antagonists or by chemical sympathectomy is not specific to NK cells, making it difficult to distinguish between the direct and indirect effects of catecholamines. Nevertheless, these studies suggest that β-adrenergic signaling "primes" NK cells, increasing the efficiency of their effector functions.

It has recently been shown that, conversely to what was shown for NK cells, β2-AR signaling impairs ILC2 responses.[95] In particular, β2-AR stimulation intrinsically suppresses the proliferation and effector function of ILC2s. Consequently, the ILC2 response and type 2 inflammation are downregulated in the gut after exposure to the parasite *N. brasiliensis*, and in the lung following intranasal IL-33 administration or exposure to extracts of the fungus *A. alternata*.[95]

The vagus nerve is the main nerve of the parasympathetic division of the ANS. It regulates metabolic homeostasis by releasing acetylcholine (Ach), thereby controlling heart rate, gastrointestinal motility and secretion, pancreatic endocrine and exocrine secretion, hepatic glucose production, and other visceral functions. The vagus nerve is also a major component of a neural reflex mechanism ("the inflammatory reflex") responsible for sensing peripheral inflammation and coordinating the innate immune response to injury and infection, to reduce collateral tissue damage.[113] Electrical stimulation of the vagus nerve attenuates the systemic inflammatory response, by reducing neutrophil adhesion and chemotaxis,[114] and by inhibiting the release of proinflammatory cytokines (TNF-α, IL-1β,

IL-6, and IL-18) by macrophages.[115] This regulatory pathway attenuates inflammation-mediated injury in sepsis and other cytokine-dependent models of inflammatory disease.[115] Vagotomy modifies the phenotype of the macrophages residing in the peritoneum, impairing their ability to clear bacteria: vagotomized mice have an exacerbated inflammatory response, with delayed resolution, and inefficient bacterial elimination during *Escherichia coli* infections.[90] The administration of human or mouse ILC3s before bacterial infection restores resolution responses in vagotomized mice.[90] Indeed, a population of peritoneum-resident ILC3s with a phenotypic profile similar to ILC3s in the small intestine lamina propria and spleen has been identified.[90] This population is significantly reduced by vagotomy, and these cells have been found to colocalize with cholinergic nerves and macrophages in the greater omentum.[90] ILC3s express the cholinergic receptors muscarinic (Chrm)1, 2, 4, and 5, and both mouse and human ILC3s respond to Ach stimulation by producing the lipid mediator PCTR1 (16R-glutathionyl, 17S-hydroxy-4Z, 7Z, 10Z, 12E, 14E, 19Z-docosahexaenoic acid).[90] PCTR1 belongs to a family of proresolution molecules with potent activities in the control of phagocyte clearance of bacterial infections and in decreasing collateral tissue injury and inflammation.[116] Tissue-resident ILC3s thus actively regulate macrophage responses during infection, by releasing cytokines such as GM-CSF,[117] and by producing proresolution mediators under the control of the vagal system.

3.4 | Neuropeptides: Role of VIP, NMU, and CGRP in the regulation of ILC2s

Neuropeptides are also crucial actors in the communication between the nervous and immune systems. Many of these molecules have very similar characteristics to cytokines, and they can also be produced directly by immune cells.[118]

The sensory fibers (mostly nociceptors) densely innervating the lung are an important source of neuropeptides. These neuropeptides are involved in the pathogenesis of allergic airway diseases, through the induction of bronchoconstriction[119] and the generation of "neurogenic inflammation," which is characterized by an increase in vascular permeability and vasodilation.[120] Lung-resident ILC2s express receptors for the sensory neuron-derived neuropeptides SP, CGRP, and VIP.[121] In particular, VIP secretion by Nav1.8[+] nociceptors has been shown to be crucial for the generation and persistence of OVA- and HDM-induced type 2 airway inflammation.[122] These neurons are activated by IL-5, leading to the production of VIP, which acts on both ILC2s and CD4^{+} cells to induce cytokine production, creating a positive loop that amplifies the immune response.[122] These data are consistent with the recognized immunomodulatory role of VIP, as a potent inducer of type 2 responses and a suppressor of type 1 responses.[123] However, VIP does not only control ILC2 functions in inflammatory conditions, it also plays an important role in regulating the homeostasis of lung and small intestine barriers. Indeed, VIP is expressed throughout the nervous system: in intestinal neurons, coordinating pancreatic secretion with smooth muscle relaxation in response to feeding,[124] and in neurons of the suprachiasmatic nucleus,

relaying the environmental cues required to synchronize central circadian oscillators.[125] Intestinal ILC2s express the genes encoding the two VIP receptors (VPAC1 and VPAC2), and produce large amounts of IL-5 in vitro when incubated with VIP in addition to IL-7.[82] In vivo, VIP stimulates the ILC2s in peripheral tissues to produce IL-5 in response to circadian and metabolic cues (ie, food intake).[82] This regulatory mechanism is essential for the homeostatic accumulation of eosinophils in the lung and small intestine, because ILC2-derived IL-5 is required to support eosinophils in peripheral tissues.

In addition to VIP, recent studies have identified cholinergic neurons as potent activators of intestinal and lung ILC2s, acting via production of the neuropeptide neuromedin U (NMU). The sequence of NMU is highly conserved between species, suggesting that it is an ancient molecule playing an important role that has been preserved throughout evolution.[126] NMU is widely distributed throughout the body; its highest concentrations are found within the gastrointestinal tract and it is usually detected in the ENS.[126] Moreover, *Nmu* expression has been detected in sensory neurons innervating the lung with cell bodies residing in the DRG, but not in the nodose/jugular ganglia.[81] *Nmu* upregulation has also been induced in vitro, by stimulating cultured DRG neurons with IL-13.[81]

The multiple functions of NMU include modulation of the immune response, and this molecule has been reported to have proinflammatory effects, by regulating cytokine secretion in various cell types. NMU was first shown to induce the synthesis and release of several different cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13) from a mouse T-helper 2 cell line.[127] It was later shown to be required for IL-6 production by macrophages.[128] NMU can activate eosinophils, is involved in allergen-induced eosinophilia,[129] and can induce smooth muscle contraction, all of which are major clinical manifestations of allergic disease.[126] Consistent with these effects, Nmu-KO mice develop attenuated airway inflammation after allergic sensitization,[130] and consistent with its role in type 2 immune responses, NMU is expressed by cholinergic neurons in the small intestine upon helminth infection.[92,93] *Nmu* expression is upregulated after infection with *N. brasiliensis* and the related nematode parasites *Trichuris muris* and *Heligmosomoides polygyrus*.[92] Its neuronal secretion is dependent on the ability of neurons to "sense" the alarmin IL-33 and parasite products through MYD88-dependent pathways.[93]

In the lung, expression of the gene encoding the NMU receptor NMUR1 is largely specific to ILCs compared to other resident cell populations. NMUR1 is strongly expressed in ILC2s, both at steady state and after the induction of airway inflammation with HDM extract.[81] In the small intestine, NMUR1 is selectively expressed in ILC2s but not in other innate or adaptive lymphocytes or myeloid cells.[92] NMUR1 gene expression has also been detected in human intestinal ILCs, but not B cells,[92] and in human blood ILC2s.[93] *Nmur2* (the other known receptor for NMU) is not detectable on lung-resident cells[81] or on the immune cells of the small intestine,[92] including ILCs, but is strongly expressed in the CNS.[131]

The stimulation of small intestine ILC2s with NMU alone is sufficient to induce the production of IL-5 and IL-13 as strongly as induction with PMA/iono or a combination of IL-2, IL-7, IL-25, and IL-33,[92]

whereas NMU alone has a minimal impact on lung ILC2 function.[81] In the lung, NMU induces the production of IL-5 and IL-13 only in synergy with IL-25, increasing inflammation. Indeed, NMU-NMUR1 signaling preferentially modulates the activation and expansion of certain IL-25-induced ILC2 subsets.[81]

In vivo, NMU-NMUR1 signaling is dispensable for ILC2 homeostasis.[93] However, upon *N. brasiliensis* infection, NMU administration by intraperitoneal injection or inhalation significantly reduces the burden of infection in the small intestine and lung, respectively, through ILC2-intrinsic pathways.[93] Upon *N. brasiliensis* infection, *Nmur1* is upregulated in the ILC2s of the small intestine, and its expression remains specific to this immune cell subset.[92] NMU administration has been associated with a robust and selective ILC2 cytokine response, an increase in eosinophilia and worm expulsion.[93] NMU stimulates ILC2 maturation in the lung, together with the cytokine production and proliferation of these cells, and is associated with increased lung inflammation.[92] Studies investigating the signaling pathway downstream from NMUR1 activation in ILC2s have shown that type 2 cytokine production is mediated by a Ca^{2+}-calcineurin-NFAT cascade and ERK1/2 phosphorylation.[93]

Pulmonary neuroendocrine cells (PNECs) represent an important source of neuropeptides in addition to the sensory nerves innervating the lung. In OVA-induced airway inflammation, they express CGRP, chromogranin A and neuropeptide Y, together with the neurotransmitter GABA.[78] Mice lacking PNECs have smaller numbers of ILC2s, eosinophils, and Th2 cells after OVA challenge. In particular, ILC2s colocalize with PNECs near airway branch points, and express the receptors for both CGRP and GABA.[78] However, whereas GABA has a negligible effect, CGRP increases IL-5 production by ILC2s cultured with IL-7, IL-33, and IL-25, and deletion of the CGRP receptor in ILCs in vivo results in lower levels of immune cell infiltration in HDM-treated mice.[78] Consistent with these findings for mice, PNECs form larger clusters in the lungs of human asthma patients than in controls.[78]

These findings demonstrate the active contribution of ILC2s to the global effects of several neuropeptides on immune responses. These neuropeptides seem to have non-redundant roles: VIP is involved mostly in maintaining homeostasis at mucosal barriers by regulating IL-5 production by ILC2s; CGRP signaling in ILC2s is required for a full Th2 immune response in allergen-induced asthma models, whereas NMU has no significant role in homeostatic conditions, but its induction upon helminth infection activates a type 2 protective immune response through the intrinsic regulation of ILC2s.

4 | CONCLUSIONS AND FUTURE PERSPECTIVES

The evidence collected to date demonstrates that hormones and neurotransmitters act in concert to regulate immune responses, controlling ILC recruitment to target organs, proliferation, cytokine production, and interplay with other cell types (Table 1). This field of investigation is currently booming, providing many opportunities to

increase our understanding of the mechanisms operating in other, as yet unexplored disease models and tissues.

For instance, it would be interesting to determine whether ILC regulatory pathways analogous to those described here and identified in studies of mouse models of infection in vivo also operate in the context of cancer. Indeed, the nervous system can play a role in tumorigenesis[132,133] and conversely, neuroendocrine tumors secrete hormones and neuropeptides and may, therefore, affect immune cell function.[134] An understanding of the role of neuroendocrine pathways in regulating the NK cell and ILC responses early in tumorigenesis and during tumor progression and metastasis is important for the design of intervention strategies.

Moreover, the studies of ILCs conducted to date have focused mostly on the lung and gut barriers. However, the skin is the outermost barrier and is continually exposed to various foreign agents. The skin contains ILCs from each subset, with ILC2s and ILC3s predominating in humans. There is considerable evidence to suggest that ILC2s play a role in atopic dermatitis: they are present in large numbers in the skin of patients, IL-33, IL-25, and TSLP are able to activate ILC2s, and ILC2-derived IL-5 and IL-13 promote disease development.[135,136] Furthermore, ILC3s have been implicated in the pathogenesis of psoriasis: in a mouse model of imiquimod-induced psoriasis-like disease, ILC3s are a source of the IL-17 and IL-22 mediating plaque formation.[137] ILCs are known to be involved in skin immunity, but the possible regulation of their function at this barrier surface by the nervous system has never been investigated. The skin is innervated primarily by somatosensory neurons, suggesting a potential role of neuropeptides in the orchestration of ILC responses in this tissue.

Studies of the neuroendocrine regulation of ILCs in the visceral adipose tissue are also required. The particular localization of ILC2 in this tissue suggests a role not only in maintaining the integrity of the barrier surface, but also in metabolic homeostasis, through the ability of these cells to induce caloric expenditure and the browning of adipose tissue.[64,66] Moreover, ILC2-derived IL-5 and IL-13 maintain eosinophil and alternative activated macrophage responses in the visceral adipose tissue, the lack of which results in increased adiposity and insulin resistance.[65,138] There is considerable evidence to suggest that the fundamental properties of adipose tissue function and biology are modulated by the ANS. Given the negative effects of adrenergic signaling on ILC2 functions in the lung and gut, it is tempting to speculate that such signaling may also affect adipose tissue deposition and metabolism, or promote weight loss and glucose tolerance. It would, thus, be interesting to study the potential contribution of sympathetic and parasympathetic innervation to the regulation of ILC function in the adipose tissue.

Based on the evidence reviewed here, we can conclude that it is difficult to predict the effect of a given neuroendocrine pathway on a cell subset, as this effect is strictly dependent on the combination of signals received simultaneously by the cell from the tissue. This is demonstrated by the comparison of the regulatory effects of GCs on NK cells in the spleen and liver during viral infection. Similarly, NMU has been shown to be a potent inducer of cytokine production by ILC2s from the small intestine, whereas it must act in synergy with IL-25 to stimulate ILC2s in the lung.[81,92] Collectively, these findings highlight the importance of the microenvironment for determining the outcome of the cell response. In addition to other signals extrinsic to ILC-nervous system communication, the ILC response depends on the integration of multiple signals conveyed by different branches of the nervous system itself. Indeed, data from groups working on different aspects of the same model of infection or inflammation have demonstrated the simultaneous activation of multiple pathways acting on the same cellular target. For example, in the parasite infection model, both the adrenergic pathway and NMU act on ILC2s, but in opposite directions: β2-AR signaling impairs ILC2 responses,[95] whereas NMU potentiates these responses.[92,93] The disruption of either of these pathways is deleterious for the host, suggesting that a balance between them is required.

All the evidence for interplay between ILCs and the neuroendocrine system presented above suggests that these two elements constitute complementary evolutionary solutions for unique challenges: they both monitor the external and internal environments of the body and they cooperate to respond to changes and restore homeostasis efficiently.

DECLARATION OF INTEREST

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