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Neuromedin U: potential roles in immunity and inflammation

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Summary

Since the discovery of neuromedin U (NmU) from porcine spinal cord in 1985, this neuropeptide has been subsequently identified in many other species with multiple physiological and pathophysiological roles detected, ranging from smooth muscle contraction, feeding, energy balance to tumorigenesis. Intriguingly, NmU is also emerging to play pro-inflammatory roles involving immune cell activation and cytokine release in a neuron-dependent or neuron-independent manner. The NmU-mediated inflammatory responses have already been observed in worm infection, sepsis, autoimmune arthritis and allergic animal models. In this review, we focus on the roles of NmU in immunity and inflammation by highlighting the interactions between NmU and immune cells, summarizing the signalling mechanism involved in their reactions and discussing its potential contributions to inflammatory diseases.

Keywords: immunity; inflammation; neuromedin U; NmUR1; NmUR2.

Introduction

Neuromedin U (NmU) is a group of structurally conserved neuropeptides, belonging to neuromedin superfamily.[1] Four groups of neuromedins have been identified based on their structures and functions, including the bombesin-like (NmB and NmC), the kassinin-like (NmK and NmL) and the neurotensin-like (NmN) peptides and the neuromedin U group (NmU and NmS).[2] Different from other neuromedins, all NmU members, except in carp and goldfish, contain an identical C-terminal pentapeptide (-Phe-Arg-Pro-Arg-Asn-NH_2). The discovery of NmU commenced from the identification of two different versions of this peptide, NmU-8 and NmU-25, from porcine spinal cord, with the suffix U denoting their potent contractile effect on rat uterus.[3] Meanwhile, the hypertensive effect of NmU in rats was also observed.[3,4] Following the further investigations, multiple forms of NmU in various species, including fish, birds, amphibians and other mammals, have been discovered.[1] In humans, NmU peptide consists of 25 amino acid residues with the same C-terminal octapeptide (-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2) to that in porcine.[5] Two G protein-coupled receptors have been recognized as the major receptors for NmU, designated as neuromedin U receptor 1 (NmUR1) and neuromedin U receptor 2 (NmUR2).[6] NmUR1 is mainly expressed in peripheral tissues, while NmUR2 is primarily detected in the central nervous system (CNS).[6] In addition to inducing smooth muscle contraction, pleiotropic roles of NmU have been detected including control of blood pressure, stress response, food intake, metabolic homeostasis, hormone release, circadian rhythm and tumorigenesis.[2,7] Recently, NmU peptides have also been shown to contribute to inflammation with roles in both innate and adaptive immune responses.[8–11]

Neurogenic inflammation is commonly defined as an inflammatory phenomenon initiated by neuropeptides and/or neurotransmitters derived from sensory neurons.[12]

Abbreviations: CFA, complete Freund's adjuvant; CGRP, calcitonin gene-related peptide; CNS, central nervous system; CTMCs, connective tissue-type mast cells; ERK, extracellular signal-regulated kinases; GHSR, growth hormone secretagogue receptor; GIT, gastrointestinal tract; hsMCs, human skin-derived mast cells; ILC2, group 2 innate lymphoid cell; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; MRGPR, Mas-related G protein-coupled receptor; NFAT, nuclear factor of activated T cells; NmU, neuromedin U; NmUR1, neuromedin U receptor 1; NmUR2, neuromedin U receptor 2; NTSR, neurotensin receptor; PI3 K, phosphoinositide 3-kinase; PLC, phospholipase C; PMC, peritoneal mast cell; PSGL-1, P-selectin glycoprotein ligand-1; SP, substance P; TLR, Toll-like receptor; TRPV3, transient receptor potential vanilloid 3

NmU-producing neurons have been located anatomically in the vicinity of immune cells such as group 2 innate lymphoid cells (ILC2s) and T cells in some tissues such as the lung and gut, and NmU peptides released from afferent neurons have been demonstrated to activate immune cells directly, indicating a role of NmU in neurogenic inflammation.[11] However, NmU can also be produced by non-neuronal cells, including epidermal keratinocytes and immune cells, suggesting a neuron-independent inflammatory responses in an autocrine or paracrine manner.[8,9,13] So far, several papers have discussed the general structure, distribution and functions of NmU. This review will focus on the role of NmU in immunity and inflammation, elucidating the effects of NmU on immune cells and the potential signalling mechanisms mediated by NmU in inflammatory responses and diseases.

The structure of NmU

Sequence analysis confirmed that the NmU precursor is a 174-amino acid polypeptide in human being and rat, with 74% homology between the species.[5,14] Both precursors include a characteristic secretory signal sequence and several paired dibasic amino acids, which serve as putative enzymatic cleavage sites.[5,14,15] The sequence between the last two cleavage sites near the C-terminus contains the NmU peptide, 23 residues in rat and 25 in human being.[5,14]

After first discovery of NmU from porcine spinal cord in 1985,[3] the peptides have been identified in other species, including goldfish,[16] zebrafish,[17] pufferfish,[18] carp,[19] frog,[20] tree frog,[21] toad,[22] Japanese quail,[23] chicken,[24,25] rat,[26,27] guinea-pig,[28] rabbit,[29] canine,[30] and human being.[5] Based on the length of the peptides, NmU can be generally divided into two forms: the longer form (NmU-17 to 40) and the short or truncated form (NmU-8 to 9) (Fig. 1). The short form is considered as the cleaved products of their longer NmU analogues except in guinea-pig where no longer isoforms have been found.[3,25,28,30] The amino acid sequences of NmU are significantly conserved through evolution, indicating critical functional roles for the peptides. The highly homologous and asparagine amidated C-terminal region is vital for receptor binding and function, whereas the variable N-terminus controls the potency of the protein.[3] These structure–activity relationships are supported by the evidence that point mutations or modifications to the porcine NmU-8 can cause drastic reduction in smooth muscle contraction activity or receptor binding affinity.[31,32] Furthermore, porcine des-amido-NmU-8 lost its activity on smooth muscle and blood pressure,[3] while, non-amidated NmU-8 was unable to activate the receptor.[8,32] Compared with NmU-8, porcine NmU-25 induced more reinforced and repeated effect on rat uterus contractile activity.[3] Some residues such as Tyr-Gln-Gly-Pro and Ser-Gly-Gly at the N-terminus in rat have been proved to be especially important for the enhancement of the biological activity.[33,34]

The receptors of NmU

Two major NmU receptors have been established, although some other putative receptors may exist. NmUR1 (also designated as GPR66 or FM-3) was first cloned based on the high (46–47%) DNA sequence homology to human growth hormone secretagogue receptor (GHSR) and neurotensin receptor (NTSR).[35,36] Subsequently, NmUR2 (also known as TGR-1 or FM-4) was discovered on the basis of its approximately 50% amino acid similarity with NmUR1.[6,37–39] These receptors have been identified in human being, murine, chicken and goldfish,[40–42] but not in all species studied so far.

Several G protein subtypes have been reported to get involved in the signal transduction by NmURs. NmUR1 and NmUR2 from human being and mouse are coupled to $G\alpha_{q/11}$ when the receptors were overexpressed in several cell lines.[40] Binding of NmU to either receptors caused influx of Ca^{2+}.[6,8,38,39,43,44] However, $G\alpha_i$-coupling was also reported in the studies with NmUR1/NmUR2-overexpressed CHO or HEK293 cells where cAMP level was slightly decreased after the activation of the receptors.[38,45] Furthermore, a chimeric G protein study demonstrated that NmUR1 was preferentially coupled to $G\alpha_q$, but NmUR2 mainly to $G\alpha_i$.[46] Therefore, NmUR1/NmUR2 signalling could be potentially transducted through either $G\alpha_q$ or $G\alpha_i$ depending on certain physiological conditions.

Besides NmUR1/2, NmU could also interact with other potential receptors. In a complete Freund's adjuvant (CFA)-induced inflammatory model and an autoantibody-induced arthritis study, the effect of NmU was independent of NmUR1 and NmUR2.[47–49] In a non-small-cell lung cancer study, the interaction of human NmU-25 with GHSR1b/NTSR1 via a $G\alpha_s$ signalling was suggested.[36] NmU has also been reported to induce degranulation of human skin-derived mast cells (hsMCs) through Mas-related G protein-coupled receptor X2 (MRGPRX2), as well as mouse connective tissue-type mast cells (CTMCs) through Mrgprb2, a mouse analogue of MRGPRX2.[50] However, whether NmU binds to these putative receptors directly and with what binding affinity remain unclear. With further studies on NmU, additional receptors are likely to be discovered in the future.

The distribution of NmU and its major receptors

In general, NmU peptides appear to be widely produced in many tissues, organs and cells, with the highest levels detected in gastrointestinal tract (GIT) and CNS,[5,20,51–58] although the expression patterns vary between species

(Table 1). The expression pattern of NmURs shows overlap with that of NmU, but NmUR1 is mainly expressed in peripheral tissues while NmUR2 is predominant in the CNS[6,8,37,43,59–62] (Table 1). In human being, NmUR1 is broadly expressed with high levels in the pancreas, testis, small intestines,[37] adipose[43] and cardiovascular tissues.[63] NmUR2 is preferentially expressed in specific regions of the brain, particularly in the hypothalamus, medulla oblongata, pituitary and spinal cord.[6,38] However, NmUR1 has also been detected in the CNS, for example the cerebellum, dorsal root ganglion and thalamus, although these findings are not consistent between studies.[6,32,37,61] Similarly, the expression of NmUR2 in peripheral tissues such as the gastrointestinal and genitourinary tract, with high levels in the testis, has also been reported.[39,62] Currently, the precise cell types that express NmURs in these organs and tissues remain uncertain.

NmU-producing sensory neurons could also produce other neuropeptides such as vasoactive intestine peptide, substance P (SP), neuropeptide Y and calcitonin gene-related peptide (CGRP), or could be co-localized with other types of neurons producing these neuropeptides.[11,52–55,64–68] Cholinergic neurons producing NmU in the GIT and lungs were detected in close proximity to immune cells, indicating the potential role of NmU in neuroimmune crosstalk and neurogenic inflammation.[11,64,65,69–71] Moreover, indirect evidence indicated the expression of NmUR2 in microglia and astrocytes from the mouse hippocampus,[72] which suggested the potential involvement of NmU in the regulation of CNS inflammation.

NmU and NmUR1 are also expressed in non-nervous system. NmU mRNA has been detected in human bone marrow, spleen, dendritic cells, monocytes and B cells,[8,43] and significant levels of NmUR1 mRNA were also found in human bone marrow, spleen, lymphocytes,[11,64,65] platelets,[73] eosinophils[10] and mast cells.[13] NmUR1 expression has also been reported in mouse ILC2s,[11] eosinophils,[10] mast cells and macrophages,[9,13] and in murine Y-16 cell line (IL-5-dependent) and Th2 cell line (D10.G4.1).[10,74] These non-neuronal distributions provide opportunity for NmU to function as a neuron-independent mediator in inflammation.

Signalling pathways mediated by NmURs

Several signalling pathways downstream of the activation of NmURs have been documented[36,38,45,50,59,75] and reviewed.[7] Based on existing reports, the potential signalling pathways used by NmU in inflammation are mainly mediated by NmUR1 (Fig. 2) and little is known about that mediated by other NmURs.

Ca^{2+}/calcineurin/NAFT signalling pathway

It has been demonstrated that the NmU-NmUR1 pathway mediates $G\alpha_{q/11}$ signalling in cells, leading to the activation of phospholipase C (PLC), which catalyses conversion of PIP_2 (phosphatidylinositol 4,5-bisphosphate) into DAG (diacylglycerol) and IP_3 (inositol trisphosphate). Subsequently, IP_3 binding to its receptor on the endoplasmic reticulum will elicit Ca^{2+} influx into cytosol.[46,76] Liberated Ca^{2+} binds calmodulin, which activates calcineurin phosphatase. Activated calcineurin in turn dephosphorylates nuclear factor of activated T cells (NFAT), inducing its activation and translocation to the nucleus, whereupon it regulates gene transcription.[77] This calcium-dependent calcineurin-NFAT cascade downstream of NmU signalling has been observed in mouse ILC2s and murine Th2 cell line D.10.G4.1.[11,74] Preincubation of lymphocytes with selective $G\alpha_{q/11}$ inhibitor FR900359 completely abrogated ILC2-mediated cytokine production induced by NmU in mouse.[65] Preincubation of D10.G4.1 cells with PLC inhibitor U73122 or calcineurin inhibitor cyclosporin A (CsA) also resulted in a dose-dependent reduction in pro-inflammatory cytokine production.[74] Pretreatment of ILC2s with calcineurin inhibitor FK506 or CsA, and NFAT inhibitor 11-VIVIT induced a remarkable diminution of type 2 cytokine release.[11] Taken together, these results suggested that $G\alpha_{q/11}$-mediated PLC/Ca^{2+}/calcineurin/NFAT signalling pathway is critical for NmU-stimulated cytokine production in immune cells.

Mitogen-activated protein kinase signalling pathway

NmU-induced cytokine secretion also depends on the activation of the mitogen-activated protein kinase (MAPK) signalling pathway as the treatment with U0126, an inhibitor of mitogen-activated protein kinase kinase (MEK) 1/2, caused significant reduction in the cytokine production in a dose-dependent manner in D10.G4.1 cells.[74] In mouse ILC2s, administration of PD98059, an inhibitor of extracellular signal-regulated kinases (ERK) at downstream of MEK, resulted in marked impairment of cytokine production induced by NmU.[11] Crosstalk between Ca^{2+}/calcineurin/NFAT and MAPK signalling cascades in T-cell activation has been suggested.[78,79] Thus, the MAPK signalling pathway may also be used by NmU to control cytokine production in immune cells.

Phosphoinositide 3 kinase signalling pathway

The phosphoinositide 3-kinase (PI3K) signalling pathway acts as a critical modulator contributing to lymphocyte-mediated responses.[80] The use of PI3K inhibitor LY294002 caused a concentration-dependent decline in interleukin release in NmU-treated D10.G4.1 cells, which demonstrated a pivotal role for PI3K signalling in NmU-mediated cell activation.[74] NmU has been suggested to promote the secretion of IL-6 but not TNF-α from macrophages in lipopolysaccharide (LPS)-induced endotoxaemia.[9] This

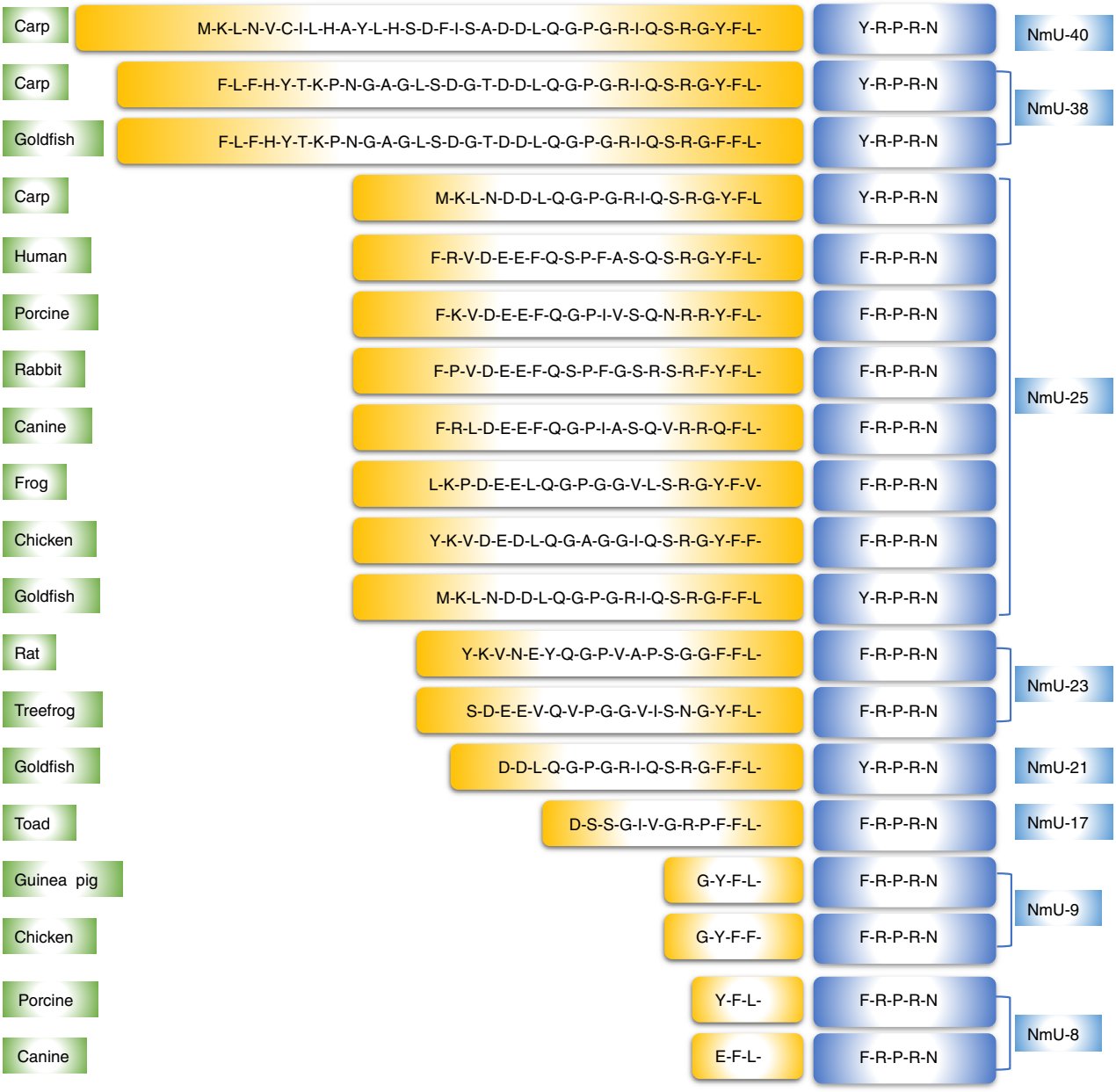

Figure 1. Amino acid sequences of NmU among different species. The highly conserved C-terminus is highlighted in blue, and other residues are labelled in yellow.

macrophage-derived IL-6 production was partially dependent on PI3K phosphorylation.[81] Interaction between Ca^{2+}/calcineurin/NFAT and PI3K signalling pathways in T-cell activation has also been reported in other studies,[82] suggesting that such crosstalk between these pathways could also exist in NmU-activated immune cells.

The pro-inflammatory roles of NmU in immune cells

The close proximity between NmU-producing sensory neurons and different immune cells, and the production of NmU by epidermal keratinocytes, endothelial cells and immune cells suggest potential involvement of this neuropeptide in immune responses. Based on previous reports, the regulatory effects of NmU on the immune systems have been summarized in Fig. 3 and Table 2.

The potential role of NmU in lymphocytes

The expression of NmUR1 has been detected in several types of lymphocytes, including T cells, NK cells and ILC2s.[8,11,64] Up to now, the functional studies of NmU in these cells were mainly conducted in mice. NmU-NmUR1

Table 1. Distribution of NmU, NmUR1 and NmUR2 in CNS and periphery of different species

Objects	Species	Samples	Methods	Key findings	References
NmU	Rat, porcine, guinea-pig, human being	CNS, gut	RIA	NmU occurred in ileum of all species, sacral spinal cord of rat, porcine and guinea-pig, cerebral cortex of human being	Domin (1986)[51]
NmU	Rat	CNS, peripheral tissues	RIA	NmU was high in pituitary anterior lobe, small intestine, nucleus accumbens, septum, hypothalamus, sacral spinal cord, vas deferens, ureter, fallopian tube, urethra NmU was confined to submucosal muscular layers of small intestine	Domin (1987)[52]
NmU	Rat, guinea-pig	Brain, intestine	RIA, IHC	In rats, NmU was highest in ileum and also stained in submucous and myenteric neurons of small intestine and in nerve fibres of these ganglionated plexuses NmU was seen in nerve terminals within ileum submucous and myenteric ganglionated plexuses of guinea-pig	Augood (1988)[53]
NmU	Rat	Brain, GIT	ICC	NmU was restricted to nerves fibres in the submucous and myenteric plexuses and mucosa of all gut areas except stomach, and NmU was also seen in ganglion cells of both ganglionated plexuses In CNS, NmU was located in fibres of all brain regions except cerebellum and stained cells were confined to rostrocaudal part of arcuate nucleus	Ballesta (1988)[54]
NmU	Guinea-pig	Small intestine	IHC	NmU was detected in myenteric and submucous nerve cells and in nerve fibres of these ganglionated plexuses NmU was co-localized in neurons containing VIP, SP, NPY	Furness (1989)[66]
NmU	Pig	Small intestine	ICC	NmU presented coexistence with neurons containing SP and CGRP in the plexus submucous internus of small intestine	Timmermans (1989)[67]
NmU	Frog	CNS, peripheral tissues	RIA	NmU showed highest concentration in small intestine	Domin (1989)[20]
NmU	Rat	CNS, digestive tract	RIA, IHC	NmU was abundant in small intestine NmU was confined to enteric nervous system	Honzawa (1990)[55]
NmU	Pig	Small intestine	ICC	NmU was found in nerve fibres of submucosal ganglionic plexuses and coexisted with SP- and VIP-containing neurons	Timmermans (1990)[68]
NmU	Rat	GIT	Northern blot	NmU was detected in all regions of GIT and highest in duodenum and jejunum	Austin (1994)[56]
NmU	Human being	GIT	Northern blot, RIA	NmU expression was similar throughout the GIT, and highest level of NmU was revealed in jejunum using RIA	Austin (1995)[5]
NmU, NmUR1	Rat	CNS, peripheral tissues	PCR	NmU was significantly expressed in pituitary and small intestine NmUR1 was obviously expressed in small intestine and lung	Fujii (2000)[59]
NmU, NmUR1	Human being	CNS, peripheral tissues	PCR	NmU showed high levels in intestine, pituitary, bone marrow and fetal liver NmUR1 was highest in adipose tissue, with moderate levels in spleen, intestine, bone marrow, lymphocytes and pancreas	Szekeres (2000)[43]
NmUR2	Rat	CNS, peripheral tissues	PCR	NmUR2 was highest in uterus, with high levels in CNS, mainly in hypothalamus, medulla oblongata and spinal cord	Hosoya (2000)[38]

Table 1. *(Continued)*

Objects	Species	Samples	Methods	Key findings	References
NmU, NmUR1, NmUR2	Human being, rat	CNS, peripheral tissues	Northern blot, ISH	NmU and NmUR1 were high in the gut, NmUR1 was widely detected in periphery but cannot be detected in brain NmUR1 showed specific expression in the goblet cells of ileum, NmUR2 was restricted to specific regions of brain	Howard (2000)[37]
NmUR1, NmUR2	Human being	CNS, peripheral tissues	PCR	NmUR1 was mainly expressed in peripheral tissues, especially in gastrointestinal and urogenital systems, with low levels in CNS such as cerebellum, DRG, hippocampus, spinal cord NmUR2 was high in CNS, particularly the medulla oblongata, pontine reticular formation, spinal cord and thalamus	Raddatz (2000)[6]
NmU, NmUR1	Human being	CNS, peripheral tissues	Dot blot, Northern blot, PCR	NmU and NmUR1 were widely expressed, with highest levels in GIT, NmUR1 was extremely low in CNS NmU was detected in DCs, monocytes and B cells, NmUR1 was expressed by NK cells and T cells	Hedrick (2000)[8]
NmUR2	Human being	CNS, peripheral tissues	Dot blot, Northern blot	NmUR2 was highly observed in testis and CNS, particularly spinal cord, medulla, corpus callosum, thalamus. Low levels of NmUR2 were also detected in GIT	Shan (2000)[39]
NmU, NmUR1, NmUR2	Mouse	CNS, peripheral tissues	PCR	In CNS, NmU and NmUR2 were highly detected in medulla and spinal cord, but no NmUR1 expression In periphery, NmUR1 was widely expressed, with high levels in lung and various types of immune cells	Funes (2002)[32]
NmU	Rat	Brain	ISH	NmU was significantly expressed in pituitary pars tuberalis	Ivanov (2002)[58]
NmUR1, NmUR2	Human being	Peripheral tissues	PCR	NmUR1 mRNA was highest in small intestine, and NmUR2 mRNA was highest in testis	Westfall (2002)[62]
NmU, NmUR2	Mouse, rat	CNS, peripheral tissues	ISH	NmU and NMUR2 showed different expression patterns between mouse and rat in hypothalamus	Graham (2003)[60]
NmUR1, NmUR2	Rat	CNS	ISH	NmUR1 was detected in DRG, and NmUR2 was expressed in laminae I and II	Yu (2003)[61]
NmUR1, NmUR2	Rat, human being	CNS, peripheral tissues	PCR	NmUR1 was mainly in peripheral tissues with highest levels in adipose tissues NmUR2 was predominantly in CNS with highest levels in hypothalamus, medulla oblongata, substantia nigra and thalamus	Garlton (2004)[57]
NmUR1, NmUR2	Human being	Cardiovascular tissues	PCR	NmU and NmUR1 were expressed in cardiovascular tissues	Mitchell (2009)[63]

DRG, dorsal root ganglia; ICC, immunocytochemistry; IHC, immunohistochemistry; ISH, in situ hybridization; RIA, radioimmunoassay.

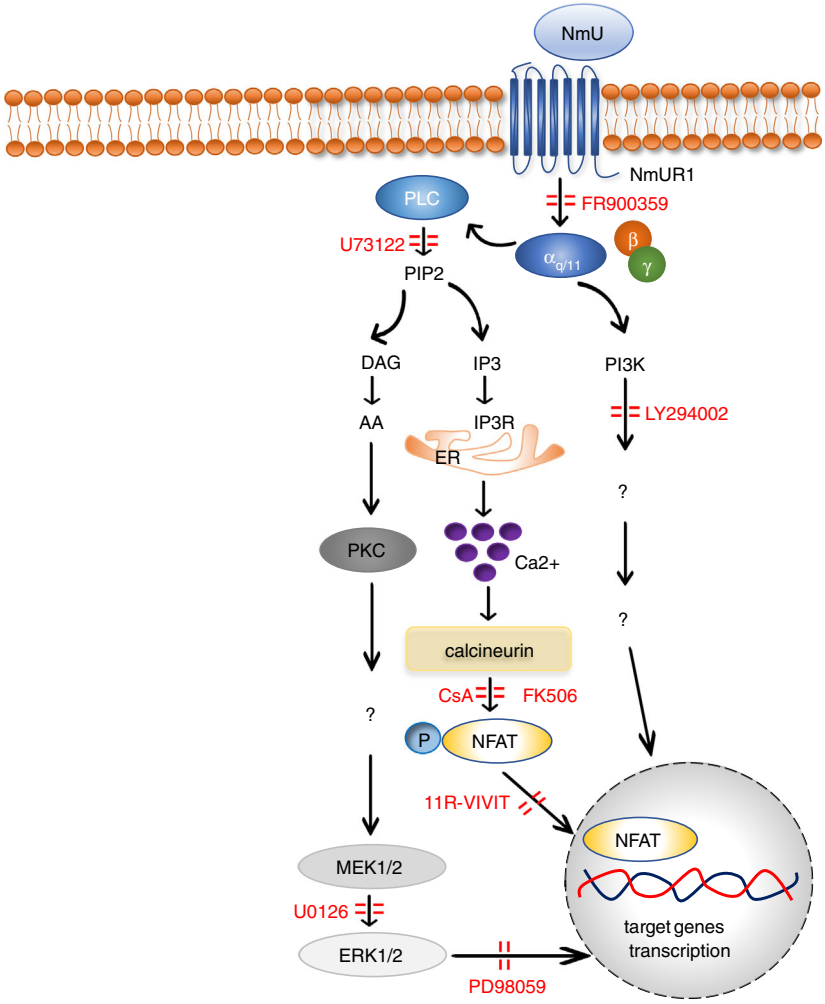

Figure 2. The signalling pathways of NmU in inflammation.

signalling promoted cytokine production, such as IL-4, IL-5, IL-6, IL-10 and IL-13, in a murine Th2 cell line (D10.G4.1) and ILC2s.[65,74,83] In mouse GIT and lungs, NmU-producing neurons were in close vicinity to NmUR1-expressing ILC2s.[11] Stimulation of ILC2s with NmU in combination of IL-25 enhanced rapid secretion of type 2 cytokines both *in vivo* and *in vitro*, whereas knockout of NmUR1 impaired the type 2 cytokine release in such stimulation, indicating the critical role of NmU-NmUR1 axis in neuron/ILC2s crosstalk.[11,64,65] NmU not only induced pro-inflammatory type 2 cytokine production, but also promoted ILC2 proliferation, as measured by the upregulation of Ki67, a biomarker of proliferation.[11,64,65]

The potential role of NmU in mast cells

In mouse model, the mRNA levels of NmUR1 in mouse bone marrow-derived mast cells and peritoneal mast cells (PMCs) were as high as that in the mouse intestine. Administration of NmU resulted in the intracellular Ca^{2+} mobilization and degranulation of PMCs in a concentration-dependent manner.[13] The pro-inflammatory role of NmU through direct activating mast cells was evidenced in the mouse treated with intraplantar injection of CFA where CFA induced intense and rapid production of NmU from keratinocytes, which in turn promoted degranulation and pro-inflammatory mediator/cytokine production of mast cells, resulting in skin oedema.[13] These immune responses were abolished by mast cell deficiency or NmU deficiency.[13]

Compared with bone marrow-derived mast cells and PMCs, mouse CTMCs expressed relatively lower level of NmUR1 but higher level of Mrgprb2. Similarly, hsMCs were found to express high level of MRGPRX2 but not NmUR1.[50] Although low or no NMUR1 was expressed in mouse CTMCs and hsMCs, NmU-induced degranulation of these cells was in a dose-dependent manner, as

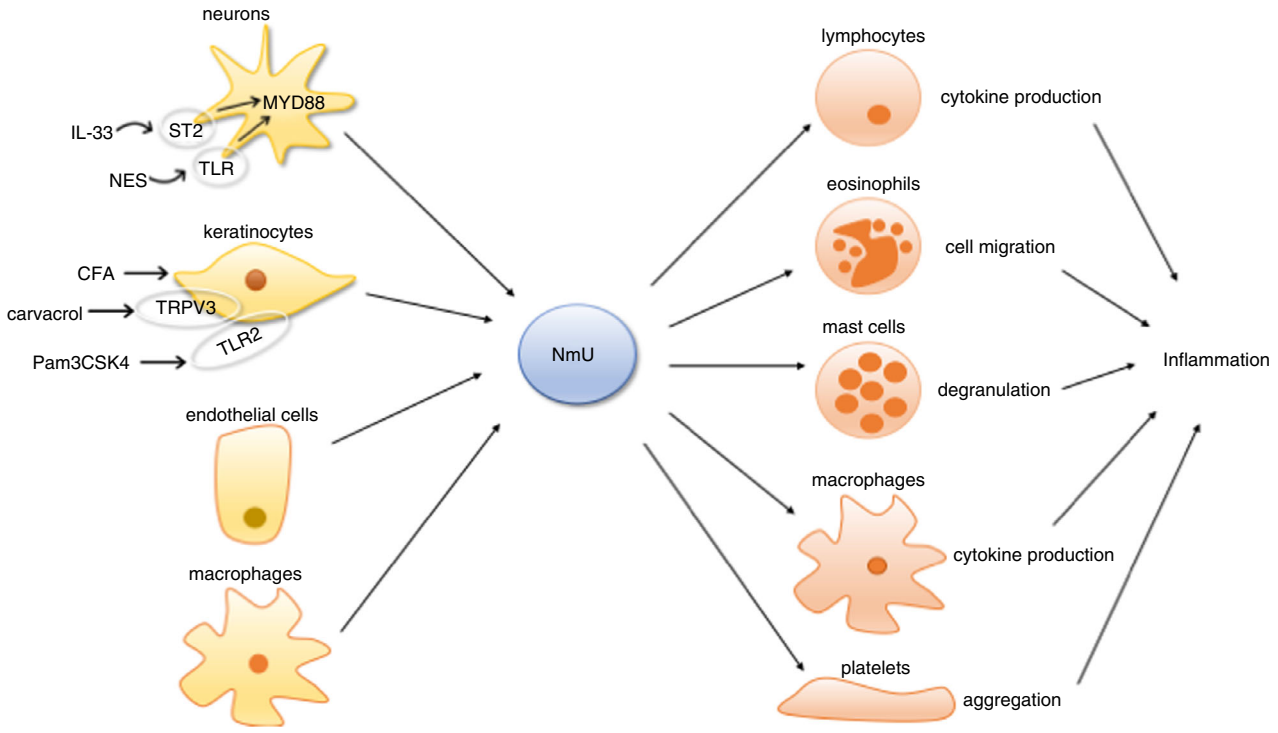

Figure 3. Role of NmU in immunity and inflammation.

detected by the release of β-hexosaminidase.[50] In cultured keratinocytes from human skin, activation of Toll-like receptor (TLR) 2 with Pam3CSK4, a synthetic triacylated lipopeptide, or activation of transient receptor potential vanilloid 3 (TRPV3) with carvacrol, a plant-derived phenol, triggered release of NmU.[50] Therefore, keratinocyte/NmU/mast cell axis could play important role in skin inflammation. As several receptors could mediate the effects of NmU in different mast cells, the function of NmU might be heterogeneous in mast cells derived from different tissues and species.[84]

The potential role of NmU in eosinophils

Neuromedin U has also been suggested to be an important player in the eosinophilic inflammation. NmUR1 mRNA was detected in a murine eosinophil cell line Y-16 and human peripheral blood eosinophils, and NmU could directly activate these cells by inducing cell adhesion to the components of extracellular matrix (fibronectin and collagen type I) or cell migration.[10] In allergic mouse model, OVA administration upregulated the level of NmU in the lungs and induced airway eosinophilia. NmU knockout reduced the enrichment of eosinophils without attenuating airway hyperresponsiveness.[10] In contrast, CGRP knockout in the same animal model significantly decreased airway hyperresponsiveness without affecting airway eosinophil infiltration.[85] These observations may ascribe that CGRP stimulates airway smooth muscles and epithelial cells while NmU targets eosinophils.[10,85]

As human eosinophils have been previously reported to be the source of vasoactive intestine peptide and SP,[86] whether eosinophils could also produce NmU is still yet to be determined.

The potential role of NmU in macrophages

Being populated in tissues, macrophages function as resident phagocytic cells involving in both tissue homeostasis and inflammation.[87] Macrophages express neuropeptide receptors, such as SP receptor NK-1 and NmU receptor NmUR1, and are believed to be involved in the regulation of neuropeptide-induced inflammatory responses.[9,88,89] Macrophages are also able to produce NmU.[9,90] Lipopolysaccharide stimulation induced upregulation of NmU but downregulation of NmUR1 in macrophages, whereas in NmU-knockout macrophages, no downregulation of NmUR1 was detected, indicating an interaction between NmU and NmUR1 via an autocrine or paracrine manner in macrophages.[9] In NmU-deficient macrophages, the release of IL-6 but not TNF-α was obviously decreased after LPS administration.[9] This may be attributed to the fact that two different signalling mechanisms are involved in the release of TNF-α and IL-6 in macrophages, and IL-6 production induced by LPS is regulated by the NmU/NmUR1-dependent signalling.[9,48,81]

Table 2. Influence of NmU on immune cells and related inflammatory disorders

Source of NmU	Target cell types	Receptors	Inflammatory mediators	Signalling transduction	Effect of NmU	Related diseases	References
–	Mouse Th2 cell line D10.G4.1	NmUR1	IL-4, IL-5, IL-6, IL-10, IL-13	Ca^{2+}, PLC, calcineurin, MEK, PI3K	Promote Th2-induced inflammation	Th2-mediated disorders such as asthma	Johnson (2004) [74]
Keratinocytes	Mast cells	NmUR1	TNF-α, IL-6, MIP-2, ICAM-1, β-hexosaminidase	Ca^{2+}	Promote mast cell-mediated inflammation	Hyperalgesia	Moriyama (2005)[13]
Macrophages	Macrophages	NmUR1	IL-6	Ca^{2+}, ERK, PI3K	Enhance IL-6 production from macrophages	Endotoxaemia	Moriyama (2006)[9]
Sensory neurons or immune cells	Y-16 cell line, eosinophils	NmUR1	–	Ca^{2+}, ERK	Promote eosinophil migration	Allergic disorders	Moriyama (2006)[10]
–	–	–	IL-6	–	No pro-inflammatory effect	–	Abbondanzo (2009)[48]
Haematopoietic cells	–	–	–	Ca^{2+}	Promote autoantibody-mediated inflammation	Arthritis	Rao (2012)[49]
Keratinocytes	–	–	IFN-γ, IL-4, IL-23	–	Negative regulator of allergic skin inflammation	Atopic dermatitis	Mizukawa (2016)[104]
Sensory neurons	ILC2s	NmUR1	IL-5, IL-13, Areg, Csf	Ca^{2+}-calcineurin/NFAT, ERK	Fight against worm infection at early stage	Worm infection, lung inflammation	Cardoso (2017)[11]
Enteric cholinergic neurons	ILC2s	NmUR1	IL-5, IL-9, IL-13	$G\alpha_q$ -dependent signalling	Promote ILC2-mediated inflammation at mucosal sites	Worm infection, allergic inflammation	Klose (2017)[65]
Neurons	ILC2s	NmUR1	IL-5, IL-13	–	Mediate alarmin or allergen-driven ILC2-mediated allergic responses	Mucosal allergic disorders	Wallrapp (2017)[64]
Vascular endothelial cells	Platelets	NmUR1	P-selectin	Ca^{2+}, P2Y12 signalling	Enhance human platelet activation and aggregation	Platelet-related diseases	Grippi (2017)[73]
Keratinocytes	Mast cells	Mrgprb2, MRGPRX2	β-Hexosaminidase	–	Induce mast cell activation	Skin allergic diseases	Matsuo (2018)[50]

Areg, amphiregulin; Csf2, colony-stimulating factor 2; ICAM-1, intracellular cell adhesion molecule 1; MIP-2, macrophage inflammatory protein 2.

The potential role of NmU in platelets

Platelets are traditionally regarded as anucleate cells that play critical roles in the regulation of immune haemostasis, and currently recognized as a regulator in both innate and adaptive immune responses.[91,92] Injured human endothelial cells could produce NmU, which enhanced platelet aggregation induced by ADP.[63,73] NmUR1 was found to be expressed in human platelets, and the activation of platelets by the combination of NmU and ADP could be proved by the upregulation of P-selectin.[73] As one of the main pro-inflammatory modulators released by platelets, P-selectin exerts important roles in the platelet–leucocyte aggregation and leucocyte–epithelium adhesion.[91] Several types of immune cells including haematopoietic progenitor cells, monocytes, neutrophils and eosinophils express the receptor for P-selectin, P-selectin glycoprotein ligand-1 (PSGL-1 or CD162),[93,94] further supporting the potential of interaction between platelet and immune cells mediated by P-selectin. Although, there is no direct evidence so far to confirm the relationship between NmU/ADP-stimulated platelets and PSGL-1-expressing immune cells, it is not difficult to conduct such interaction *in vitro*, even more, the binding of P-selectin to platelet-activated factor-activated monocytes has already been reported.[95]

NmU-related host defence and inflammatory disorders

NmU in worm infection and atopic inflammation

Type 2 immunity plays critical role in host defence against helminth infection or atopic inflammation such as asthma, dermatitis, rhinitis and other allergic disorders.[96,97] Type 2 cytokines produced by type 2 cells such as Th2, Tc2 and ILC2s are considered as important regulators for the recruitment and activation of mast cells, basophils and eosinophils and B-cell class switching towards IgE production.[96,97] NmU can be upregulated after worm infection or allergen exposure, leading to type 2 cell responses and eosinophil activation,[10,11,65] suggesting a potential bridging role for NmU between these inflammatory challenges and type 2 immunity.

In OVA-induced asthma model, NmU enhanced the recruitment of eosinophils to the inflammatory areas, and NmU knockout attenuated the OVA-induced airway eosinophilia.[10] NmU also augmented airway inflammation by promoting the maturation and proliferation of ILC2s, enhancing type 2 cytokine secretion from lung-resident ILC2s and inducing enrichment of eosinophils in bronchoalveolar lavage and lung tissues.[65] Taken together, NmU could contribute to the pathogenesis of asthma via the interactions with eosinophils and ILC2s.

Peripheral tissues such as skin, muscle, lung and GIT are highly innervated by sensory neurons.[12] NmU released from sensory neurons has been demonstrated to provide protective effects on worm infection through the interactions between cholinergic neurons and immune cells. Helminth infection can stimulate alarmin cytokine IL-33 production from epithelial cells, macrophage or dendritic cells,[98] and neurons can directly sense the excretory/secretory products from helminth parasite Nippostrongylus brasiliensis through TLR or respond to IL-33 through its receptor ST2.[99–101] Activation of TLR and ST2 in neurons efficiently induced upregulation of NmU in a myeloid differentiation primary response 88 (the adaptor protein of IL-33/ST2 and Nippostrongylus brasiliensis excretory/secretory products/TLR signalling pathway)-dependent manner.[11,102,103] Deletion of myeloid differentiation primary response 88 abolished NmU production.[11] Upregulation of NmU by parasite infection was also confirmed by using other related nematode parasites *Trichutis muris* and *Heligmosomoides polygyrus*.[65] The effect of NmU on ILC2s in response to worm infection was mediated by NmUR1 as NmUR1 knockout not only resulted in the decrease in type 2 cytokine production from ILC2s but also increased worm infection burden.[11]

The role of NmU in cutaneous inflammation has been hypothesized to be dual as a pro-inflammatory mediator at early stage and as an anti-inflammatory regulator at a later phase.[104] Initially, keratinocyte-derived NmU has been shown to promote CFA-induced mouse skin inflammation via NmUR1 in a mast cell-dependent manner.[13] Subsequently, NmU has been demonstrated to be a negative regulator in skin inflammation at later stage as depletion of NmU from epidermis resulted in dry skin and increased scratching, which would act in concert with repeated hapten treatment to induce IgE-mediated allergic inflammation.[104] Nevertheless, in another CFA-induced cutaneous inflammatory model, NmUR1 knockout did not exert any effect on the immune responses, raising the argument on the role of NmU in this skin disorder.[48] Recently, it has been reported that other receptor of NmU besides NmUR1 may contribute to skin inflammation,[50] which was mainly based on the identification of NmU-induced activation and degranulation of hsMCs through MRGPRX2.

NmU and endotoxaemia

Lipopolysaccharide, the major component of the cell wall in Gram-negative bacteria, plays an important role in the endotoxin-induced septic shock[105]. In LPS-induced endotoxaemia in mice, NmU knockout mitigated degeneration of hepatocytes and formation of thrombosis, which further resulted in the reduction in multiorgan dysfunction and mortality.[9] Additionally, serum level of IL-6 instead of TNF-α, IL-1β and IL-12p40 was significantly decreased

in NmU-deficient mice after LPS administration,[9] which was considered as an important factor associated with the reduced mortality rate.[9,106] Although NmU/NmUR1 signalling was demonstrated to promote IL-6 production from macrophages in LPS-induced endotoxaemia, the detailed molecular mechanisms were still undetermined.

NmU and arthritis

NmU released from bone marrow-derived cells has been shown to enhance the development of autoantibody-induced arthritis, as the depletion of NmU exhibited a protective influence on the arthritis mouse model.[49] The pro-inflammatory effect of NmU on arthritis was not attributed to the differentiation of immune cells as the frequency and ratio of leucocyte subsets and platelets remained unchanged after NmU knockout, but more likely to be mediated by the activation of multiple immune cells.[49] However, the exact pro-inflammatory mechanisms of NmU in arthritis have not been defined. Although NmU knockdown showed benefit, the knockout of NmU receptors such as NmUR1, NmUR2 and NTSR1 did not alleviate autoantibody-induced arthritis, indicating some unknown receptor might contribute to the role of NmU in arthritis.[49]

It has been suggested that SP-induced neurogenic inflammation was partially NmU-dependent.[13] NmU-expressing neurons were found to express SP or be co-localized with SP-containing neurons.[66,67] Increased levels of SP in synovial fluid and serum were reported to be associated with the patients with rheumatoid arthritis.[107] However, whether NmU and SP can collaborate with each other in the pathogenesis of arthritis remains unknown.

NmU and CNS inflammation

NmU has been demonstrated to promote nociception.[61,108,109] Intrathecal injection of NmU significantly increased the withdrawal reflex and nociceptive behaviours to noxious thermal stimuli in mice.[108] Meanwhile, mechanical allodynia and hyperalgesia were also observed after administration of NmU in rat spinal cord.[61] Pro-nociceptive pain was NmUR2-dependent as NmUR2 but not NmUR1 deficiency in mice led to compromised pain responses to noxious chemical and thermal stimuli.[47,110] The NmU-induced hyperalgesia in the spinal cord was suggested to be caused by the enhanced synaptic transmission by NmU/NmUR2 interaction.[110] However, the mechanism mediating NmU-associated neuropathic pain is still unclear. Given the crosstalk between neuropathic pain and chronic inflammation,[111] NmU could potentially play a promoting role in this neuropathic pain-associated inflammation.

NmU has been reported to protect mouse from LPS-induced memory damage and neuronal cell death.[72] This protective effect of NmU has been ascribed to the upregulation of brain-derived neurotrophic factor from hippocampus-derived microglia and astrocytes after NmU treatment.[72] However, the signalling mechanism involved in this process still remains unknown.

Conclusions

Since the discovery of NmU peptides in 1985, various studies have further identified the unique structure and ubiquitous distribution of NmU and their receptors. Meanwhile, the functional roles of NmU have also been investigated. It is worthy to note that, in addition to multiple physiological functions, NmU can promote inflammation through either neurogenic or neuron-independent mechanisms. At the time of writing, most reports on NmU functions in inflammation have been mainly based on mouse models. We still know little on the role of NmU in the human immune system. A better understanding of NmU-mediated inflammation may be beneficial for the development of novel therapies for NmU-involving disorders such as asthma, septic shock, atopic dermatitis and rheumatoid arthritis.

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Disclosure

The authors declare no conflicts of interest.

Data availability statement

No data were available as no data sets were generated or analysed during this article.

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