Biochemical and Biophysical Research Communications 430 (2013) 895–900

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Enhancement of CGRP sensory afferent innervation in the gut during the development of food allergy in an experimental murine model

Jaemin Lee [a], Takeshi Yamamoto [a], Shusaku Hayashi [a], Hirofumi Kuramoto [b], Makoto Kadowaki [a,*]

[a] Division of Gastrointestinal Pathophysiology, Institute of Natural Medicine, University of Toyama, Toyama 930-0194, Japan
[b] Department of Applied Biology, Kyoto Institute of Technology, Kyoto 606-8585, Japan

ARTICLE INFO

Article history:
Received 29 November 2012
Available online 19 December 2012

Keywords:
Food allergy
Calcitonin gene-related peptide
CGRP
Neuro–immune interaction

ABSTRACT

Recent advances in neuroscience and immunology have revealed a bidirectional interaction between the nervous and immune systems. Therefore, the gastrointestinal tract may be modulated by neuro–immune interactions, but little information about this interaction is available. Intrinsic and extrinsic primary afferent neurons play an important role in this interaction because of their abilities to sense, process and transmit various information in the intestinal microenvironment. Calcitonin gene-related peptide (CGRP) is exclusively contained in intrinsic and extrinsic primary afferent neurons in the mouse intestine. Therefore, we investigated CGRP-immunoreactive nerve fibers in the colonic mucosa of mice induced to develop food allergy. CGRP-immunoreactive nerve fibers were specifically increased with the development of food allergy, and the fibers were juxtaposed to mucosal mast cells in the colonic mucosa of food allergy mice. Denervation of the extrinsic afferent neurons using neonatal capsaicin treatment did not affect the development of food allergy or the density and distribution of CGRP-immunoreactive nerve fibers in the colonic mucosa of food allergy mice. Furthermore, the mRNA and plasma level of CGRP was increased in food allergy mice. These results suggest that the activation of intrinsic primary afferent neurons in the intestine contributes to the development and pathology of food allergy.

1. Introduction

Food allergy is an adverse immunological reaction to food proteins, and the prevalence of food allergy has increased in developed countries over the past few decades [1]. However, no therapeutic drugs are available for food allergy because the mechanisms underlying the pathogenesis of food allergy are not well understood. Mast cells play a central role in various allergic conditions via their release of various inflammatory mediators, including proteases, eicosanoids, biogenic amines, cytokines and chemokines [2]. Mast cells are ubiquitous in the body, and these cells are often found in close proximity to nerve fibers [3,4].

Mast cells and nerves communicate bilaterally to modulate neuro-physiological effects (e.g., neural activation, neurogenic inflammation, axonal reflex and neurite growth) and mast cell functions (e.g., release of inflammatory mediators, degranulation, proliferation and differentiation) [5]. Mast cell mediators sensitize sensory neurons, which further activate the mast cells via the release of neurotransmitters or neuropeptides. Mast cells also synthesize and release nerve growth factor (NGF) [6], which induces neurite outgrowth and synaptogenesis in the brain [7] and cultured enteric neurons [8] and enhances calcitonin gene-related peptide (CGRP) expression in sensory neurons of the dorsal root ganglia [9,10].

Primary afferent neurons (i.e., sensory neurons) are key players in neuro–immune interactions in the gastrointestinal tract because primary afferent neurons recognize environmental cues and convey environmental information to other neurons and immune cells. These nerve endings also release neuropeptide mediators into the tissue microenvironment. The primary afferent neurons containing CGRP and/or substance P orchestrate mast cell–nerve interactions via direct receptor-mediated effects on surrounding mast cells [4]. These interactions contribute significantly to the neurogenic inflammation process, primarily through mast cell-derived vasodilatation, leukocyte infiltration, and tissue damage mediated by proteases [11,12].

We previously demonstrated that the number of mucosal mast cells is greatly increased in the colonic mucosa of mice with a model of food allergy [13]. We also observed alterations in cholinergic, purinergic and capsaicin-sensitive sensory neurotransmission, which affect intestinal motility in food allergy mice [14].

Abbreviations: CGRP, calcitonin gene-related peptide; NGF, nerve growth factor; OVA, ovalbumin; mMCP-1, mouse mast cell protease-1.

* Corresponding author. Address: Division of Gastrointestinal Pathophysiology, Department of Bioscience, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama-shi, Toyama 930-0194, Japan. Fax: +81 76 434 5066.
E-mail address: makotok@inm.u-toyama.ac.jp (M. Kadowaki).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.12.058

Interactions between primary afferent neurons and mucosal mast cells may therefore be involved in the pathology of food allergy. The current study investigated CGRP-containing primary afferent neurons in a murine food allergy model with intestinal mastocytosis and allergic diarrhea.

2. Materials and methods

2.1. Animals

Male BALB/c mice (5 weeks old) were purchased from Japan SLC (Shizuoka, Japan). Ten-week-old BALB/c mice were employed for mating. All mice were housed in the experimental animal facility at the University of Toyama and were provided free access to food and water. The Animal Experiment Committee at the University of Toyama approved all of the animal care procedures and experiments (authorization no. S-2009 INM-9).

2.2. Induction of food allergy in mice

The murine model of food allergy was initiated as described previously [13]. Briefly, male BALB/c mice (5 weeks old) were sensitized twice at 2-week intervals using an intraperitoneal injection of 50 μg ovalbumin (OVA, fraction V; Sigma–Aldrich, St. Louis, MO) in the presence of 1.3 mg aluminum hydroxide gel adjuvant (Sigma–Aldrich). Two weeks after systemic priming, the mice received repeated oral administrations of 50 mg/0.3 ml OVA every other day. Diarrhea resulting from food allergy was assessed by visual monitoring for up to 1 h following oral challenge. Profuse liquid stool was detected as allergic diarrhea, and the diarrhea-presenting mice were considered food allergy mice. Tissue and plasma samples were collected 1 h after oral OVA challenge.

2.3. Denervation of extrinsic primary afferent neurons using neonatal capsaicin treatment

Capsaicin (Sigma–Aldrich) is a pungent ingredient in hot chili peppers, and it is a major TRPV1 agonist that elicits a burning pain sensation via the activation of C-fiber sensory neurons. Capsaicin is a neurotoxin when given systemically to neonatal rats and mice, and it induces the cell death of C-fiber sensory neurons [15]. Experimental animals were weighed and injected subcutaneously with capsaicin (50 mg/kg) twice on postnatal days 2 and 5, and control animals received equivalent volumes of vehicle (PBS containing 10% ethanol and 10% Tween 80). The chemical denervation of C-fiber sensory neurons was verified as the absence of blinking or wiping following the application of one drop of a 0.01% capsaicin solution to the right eye in 5-week-old mice.

2.4. Immunohistochemistry

Immunohistochemistry was performed as described in our previous report [16]. The proximal colon was fixed with 4% paraformaldehyde (w/v) in 0.1 M sodium phosphate buffer (pH 7.3) and immersed for 12–18 h in the same fixative at 4 °C. The tissue was washed with 0.01 M phosphate-buffered saline (PBS; pH 7.3), cryoprotected with 30% sucrose in 0.01 M PBS and embedded in optimal cutting tissue (OCT) compound. Frozen sections (25 μm) were cut at −20 °C using a cryostat microtome (Leica Microsystems, Nussloch, Germany). The sections were soaked for 12–18 h in 0.01 M PBS containing 0.3% Triton X-100 to increase permeability, exposed to normal donkey serum (1:10; Jackson Immunoresearch Laboratories, West Grove, PA) for 30 min to reduce the nonspecific binding of antisera and washed in 0.01 M PBS. The sections were exposed to each primary antibody for 12–18 h, washed with 0.01 M PBS, and incubated with the appropriate secondary antibody for 2 h. The sections were rinsed in 0.01 M PBS and mounted in VECTASHIELD mounting medium including 4,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Peterborough, UK). All incubations were performed at room temperature. The preparations were examined using a fluorescence microscope (IX71 system; Olympus, Tokyo, Japan) with a U-MWIG3 filter set (Olympus) and photographed using an Olympus digital camera (DP70; Olympus). Furthermore, a segment of the ileum was opened along the mesenteric border and fixed with 4% paraformaldehyde to examine the immunoreactivity in nerve cell bodies in a whole-mount preparation. Longitudinal muscle with adherent myenteric plexus was dissected from the ileum, and the aforementioned procedures were performed. Whole-mount preparations were observed using confocal laser-scanning microscopy (LSM700; Carl Zeiss Japan, Tokyo, Japan). The following primary antibodies were used: sheep anti-mouse mast cell protease (mMCP)-1, a marker of mouse mucosal mast cells (1:5000; Moredun Scientific, Scotland, UK); rabbit anti-CGRP (1:10,000; Chemicon, Temecula, CA); and rabbit anti-neuronal β3-tubulin, a marker of neurons (1:10,000; Covance, Princeton, NJ). Cy3-conjugated donkey anti-sheep IgG (1:200; Jackson Immunoresearch Laboratories) and Cy3-conjugated donkey anti-rabbit IgG (1:200; Jackson Immunoresearch Laboratories) were used as secondary antibodies.

CGRP- or neuronal β3-tubulin-Immunoreactive areas per measuring square area (0.01 mm^2) in the mucosa of the colon were quantified using MetaMorph software (Molecular Devices Japan, Tokyo, Japan). The total number of mucosal mast cells was counted in 0.01 mm^2 square area of the mucosa of the colon, and the number of mucosal mast cells in close proximity to CGRP-immunoreactive nerve fibers (defined as <2 μm) was also recorded in the same area. The results are expressed as a percentage of the proximate mucosal mast cell number per the total mucosal mast cell number. Three randomly selected areas were evaluated in at least three preparations from two or three animals. The proportions of CGRP-immunoreactive neurons in the myenteric plexus were determined by the examination of β3-tubulin double-stained preparations. The cohort size of one animal was at least 100 neurons, and the data were obtained from three animals.

2.5. Measurement of CGRP level in plasma

Plasma CGRP level was measured using an enzyme immunoassay kit (SPI Bio, Massy Cedex, France). Measurement was performed according to the manufacturer's instruction.

2.6. Expression of CGRP mRNA in the colon

Total RNA was extracted from the proximal colon using the Sepasol Super kit (Nacalai Tesque, Kyoto, Japan) according to the manufacturer's instruction. Reverse transcription was performed using the Exscript RT reagent kit (Takara Bio, Ohtsu, Japan) and random primers followed by real-time PCR. Real-time PCR amplification of CGRP and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed using SYBR Premix Ex Taq (Takara Bio). The following primer pairs were used: β CGRP, forward 5′-AACACGGGCTAG CAGAGACAT-3′ and reverse 5′-GCTCTCCAAAGCGGACCTGAA-3′; and GAPDH, forward 5′-TGACCACAGTCCATGCCATC-3′ and reverse 5′-GACGGACACATTGGGGGTAG-3′. Real-time PCR was performed using the Takara TP800 (Takara Bio). The PCR reaction conditions consisted of 10 s at 95 °C followed by 40 cycles of 5 s at 95 °C and 20 s at 63 °C. Target mRNA levels were normalized to those of GAPDH as an internal control in each sample. The results are expressed as the ratio relative to the naïve group average.

2.7. Statistical analysis

The data are expressed as the mean ± SE. The statistical comparisons were performed using the Student's unpaired *t*-test and one-way repeated measures ANOVA followed by Dunnett's post hoc test or chi-square test. A significant difference was defined as $p < 0.05$.

3. Results

3.1. Upregulation of CGRP immunoreactivity in the colonic mucosa of food allergy mice

CGRP-immunoreactive nerve fibers were widely distributed in the mucosa, submucosal plexus and myenteric plexus of the proximal colon in normal mice (Fig. 1A, right picture). A dramatic increase in the fluorescence intensity of CGRP-immunoreactive nerve fibers (Fig. 1A, left picture) and mucosal mast cell hyperplasia were observed in the mucosa of the proximal colon of food allergy mice (Fig. 1B). Furthermore, CGRP-immunoreactive nerve fibers and mucosal mast cells were in close proximity in food allergy mice (Fig. 1C), the CGRP-immunoreactive areas gradually increased with the development of food allergy (i.e., after repeated oral OVA challenges) ($n = 18$, $^{**}p < 0.01$ vs. normal mice, Fig. 1D and E). The frequency of mast cells in close proximity (<2 μm) to CGRP-immunoreactive nerve fibers was not evaluable due to a lack of mucosal mast cells in the colonic mucosa of normal mice. On the other hand, a percentage of the proximate mucosal mast cell was 64.88 ± 3.78% ($n = 18$) in the mucosa of the proximal colon of food allergy mice.

3.2. Denervation of capsaicin-sensitive extrinsic primary afferent neurons

CGRP in the mouse intestine is likely the principal neurotransmitter released from both intrinsic and extrinsic primary afferent neurons [17,18]. Neonatal capsaicin treatment was used to denervate extrinsic primary afferent neurons to determine which of these two afferent neurons upregulate CGRP-immunoreactive nerve fibers in the colonic mucosa of food allergy mice. Diarrhea occurrence in neonatal capsaicin-treated food allergy mice was comparable to neonatal vehicle-treated food allergy mice ($n = 10$–17, Fig. 2A). Also, CGRP immunoreactivity in the colonic mucosa of food allergy mice was not affected by the denervation of capsaicin-sensitive extrinsic primary afferent neurons (Fig. 2B). Furthermore, the percentage of mucosal mast cells in close proximity to CGRP-immunoreactive nerve fibers was comparable between vehicle- and capsaicin-treated food allergy mice (vehicle; 64.42 ± 3.25%, capsaicin; 63.95 ± 3.23%, $n = 18$, $p = 0.92$, Fig. 2C and D). These results indicate that extrinsic primary afferents do not contribute to the development of food allergy and that the major CGRP immunoreactivity in the colonic mucosa is of intrinsic origin.

3.3. CGRP-immunoreactive afferent neurons in food allergy mice

All nerve fibers were identified using an anti-β3-tubulin antibody to investigate whether the outgrowth of nerve fibers in the colonic mucosa of food allergy mice was specific for CGRP-immunoreactive nerve fibers. No change in the fluorescent area or the distribution of β3-tubulin-immunoreactive nerve fibers in food allergy mice was observed as compared to normal mice, but the fluorescent area of CGRP-immunoreactive nerve fibers was markedly increased ($n = 9$–18, $^{**}p < 0.01$, Fig. 3A and B). In addition, CGRP-immunoreactive nerve fibers in the colonic mucosa of normal mice were observed in close proximity to the lumen and center of the villus, whereas the vast majority of nerve fibers were CGRP-immunoreactive in the mucosa of food allergy mice. We also examined the proportion of CGRP-immunoreactive neurons in the myenteric neurons. The proportion of CGRP-immunoreactive neurons in food allergy mice (22.02 ± 0.11%) was similar to that in normal mice (21.32 ± 1.87%) (Fig. 3C and D).

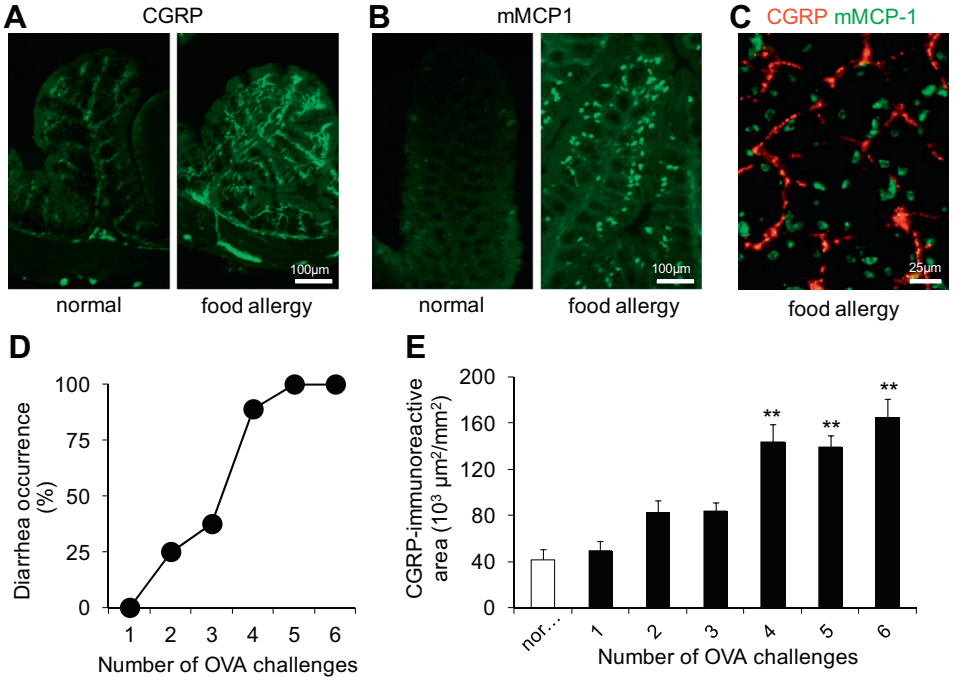

Fig. 1. Expression of CGRP-immunoreactive nerve fibers and mucosal mast cells in the proximal colon. CGRP immunoreactivity (A) and the number of mucosal mast cells stained with an anti-mMCP-1 antibody (B) were dramatically increased in the colonic mucosa of food allergy mice. (C) The majority of mucosal mast cells (64.88 ± 3.78%) was found in close proximity (<2 μm) to the CGRP-immunoreactive nerve fibers ($n = 18$). (D) Development of allergic diarrhea following repeated oral challenges of OVA. (E) CGRP immunoreactivity gradually increased in the colonic mucosa with the development of food allergy following repeated oral OVA challenges ($n = 18$, $^{**}p < 0.01$ vs. normal mice).

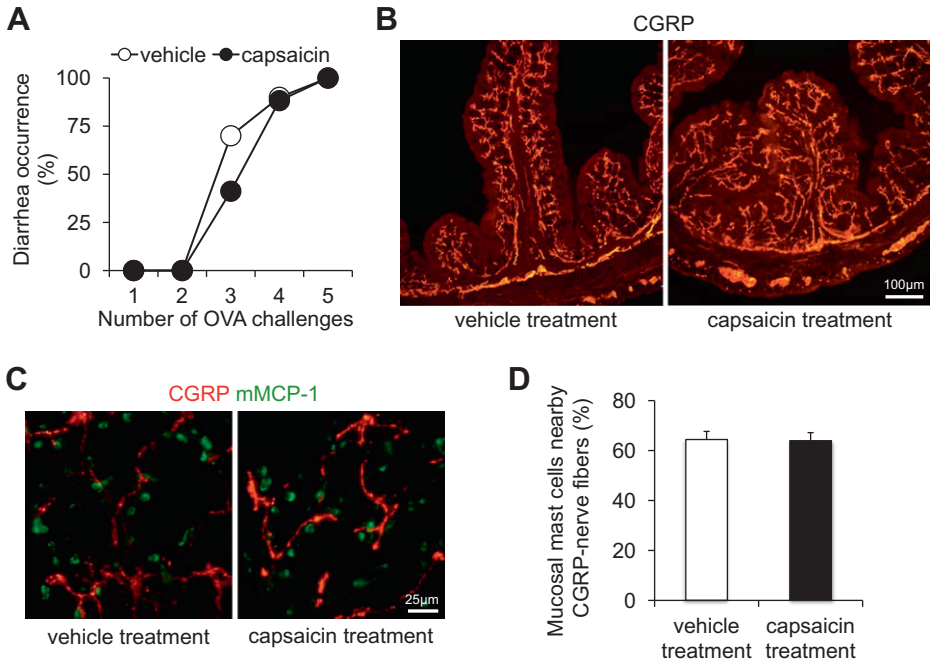

Fig. 2. Effect of extrinsic afferent denervation using neonatal capsaicin treatment in food allergy mice. The extrinsic afferent denervation did not affect the occurrence of diarrhea (A) or the expression of CGRP immunoreactivity in the mucosa of the proximal colon (B) of food allergy mice ($n = 10–17$). (C and D) Morphological interaction between CGRP-immunoreactive nerve fibers and mucosal mast cells was not affected by neonatal capsaicin treatment in the mucosa of the proximal colon of food allergy mice ($n = 18$).

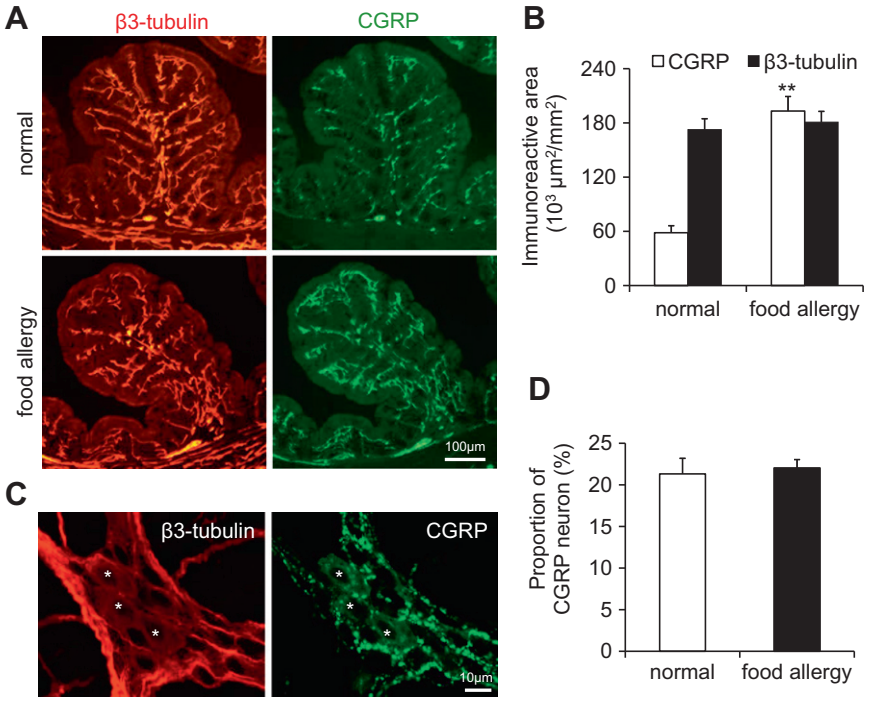

Fig. 3. No changes in the density of β3-tubulin-immunoreactive nerve fibers in the colonic mucosa or the proportion of CGRP-immunoreactive neurons in the myenteric plexus of food allergy mice. (A and B) The expression and distribution of nerve fibers stained with an anti-β3-tubulin antibody were not influenced by the development of food allergy, although CGRP immunoreactivity in nerve fibers increased in the colonic mucosa of food allergy mice ($n = 9–18$, $^{**}p < 0.01$). (C) CGRP-immunoreactive neurons (asterisks) in the myenteric ganglion. (D) The proportion of CGRP-immunoreactive neurons remained unchanged during the development of food allergy.

We next measured the mRNA expression and plasma level of CGRP, which exists in both α and β isoforms. αCGRP is prominently identified in neuronal tissues, and βCGRP is the predominant isoform in enteric neurons [19]. Therefore, we determined βCGRP mRNA expression in the enteric neurons of the colon. The expression of CGRP mRNA was significantly upregulated in the colons of food allergy mice as compared to normal mice ($n = 4–12$, $^{**}p < 0.01$, Fig. 4A). Plasma level of CGRP was also significantly elevated in food allergy mice as compared to normal mice ($n = 4–12$, $^{*}p < 0.05$, Fig. 4B).

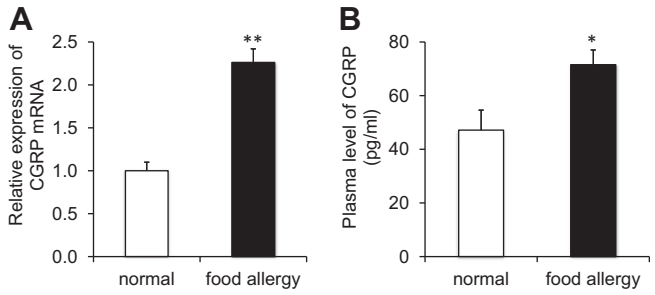

Fig. 4. Upregulation of mRNA expression and increased plasma level of CGRP in food allergy mice. (A) The expression of CGRP mRNA was significantly increased in the proximal colon of food allergy mice as compared to normal mice ($n = 4–12$, $^{**}p < 0.01$). (B) Plasma level of CGRP was significantly elevated in food allergy mice as compared to normal mice ($n = 4–12$, $^{*}p < 0.05$).

4. Discussion

The pathophysiological role of neuro–immune interactions in immune diseases has been investigated, but this interaction has not been thoroughly examined in intestinal immune diseases, especially food allergy. This study revealed that the density of CGRP-immunoreactive nerve fibers was increased in the colonic mucosa of food allergy mice, and that these nerve fibers were in close proximity to mucosal mast cells. CGRP is a marker of both intrinsic and extrinsic primary afferent neurons in the mouse intestine [17]. The percentage of mast cells that are in close proximity with PGP 9.5 (pan-neuronal marker) and/or CGRP-immunoreactive nerve fibers is significantly increased in a murine dermal contact hypersensitivity model, and mast cells significantly contribute to the increase in cutaneous PGP 9.5 and CGRP immunoreactivity in nerve fibers [20]. The food allergy mice in the present study exhibited mucosal mast cell hyperplasia in the proximal colon [13]. Mucosal-type mast cells that are derived from the murine bone marrow express CGRP receptors, and stimulation of these receptors mobilizes Ca^{2+} from intracellular stores [11]. The upregulation of CGRP nerve fibers in close proximity to mucosal mast cells may therefore affect mast cell function, which contributes to the development and pathology of food allergy.

Extrinsic sensory denervation using neonatal capsaicin treatment did not influence the development of food allergy or the expression of CGRP-immunoreactive nerve fibers in the colonic mucosa of food allergy mice. Capsaicin-sensitive sensory neurons in the gut are extrinsic primary afferent neurons, and their cell bodies are located in extrinsic ganglia, such as the dorsal root ganglia [21–23]. These results indicate that intrinsic primary afferent neurons are more deeply involved in the pathology of food allergy and that the CGRP-immunoreactive nerve fibers of intrinsic afferent neurons are more abundant than CGRP-immunoreactive extrinsic afferent neurons in the mouse colon.

We hypothesized that the enhanced intensity of CGRP immunoreactivity was due to the elongation of nerve fibers and the formation of networks in the mucosa, similar to the murine dermal contact hypersensitivity model [20]. However, the expression and distribution of β3-tubulin-positive nerve fibers remained unchanged in the colonic mucosa of food allergy mice as compared to normal mice. We previously observed alterations in cholinergic, purinergic and capsaicin-sensitive sensory neurotransmission, which affects intestinal motility in food allergy mice [14], although no change in the population of CGRP-immunoreactive neurons in the myenteric plexus was observed in food allergy mice in this study.

Our data indicated that the expression of CGRP mRNA in the colon as well as the plasma level of CGRP was significantly increased in food allergy mice as compared to normal mice. NGF also enhances CGRP expression in the sensory neurons of the dorsal root ganglia [9,10]. These results suggest that NGF and/or other mast cell mediators, which are predominantly released from hyperplastic mucosal mast cells in the colonic mucosa of food allergy mice, induced the upregulation of CGRP mRNA expression, but not neurite outgrowth, in the intrinsic primary afferent neurons. This upregulation of CGRP further activated mucosal mast cells via neurotransmitter release, which produced severe food allergy responses and neurogenic inflammation in the colon.

Here, we have provided the first morphological evidence of neuro–immune interaction in the gut and neuronal participation in the development of food allergy via the promotion of CGRP production using an experimental food allergy model. This study improves our understanding of the pathology and pathogenesis of food allergy and provides a useful strategy for the development of new therapeutic drugs for food allergy.

Acknowledgments

This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan to T. Yamamoto (No. 22790616) and M. Kadowaki (No. 24590879) and by the Knowledge Cluster Initiative Program (Second Stage) from the Ministry of Education, Culture, Sports, Science and Technology of Japan to M. Kadowaki.

References

[1] A.W. Burks, M. Tang, S. Sicherer, A. Muraro, P.A. Eigenmann, M. Ebisawa, et al., ICON: food allergy, J. Allergy Clin. Immunol. 129 (2012) 906–920.
[2] S.N. Abraham, A.L. St John, Mast cell-orchestrated immunity to pathogens, Nat. Rev. Immunol. 10 (2010) 440–452.
[3] S.A. van Diest, O.I. Stanisor, G.E. Boeckxstaens, W.J. de Jonge, R.M. van den Wijngaard, Relevance of mast cell-nerve interactions in intestinal nociception, Biochim. Biophys. Acta 2012 (1822) 74–84.
[4] J. Bienenstock, E.J. Goetzl, M.G. Blennerhassett, Autonomic Neuroimmunology, Taylor & Francis, 2003.
[5] O. Bauer, E. Razin, Mast cell-nerve interactions, News Physiol. Sci. 15 (2000) 213–218.
[6] A. Leon, A. Buriani, R. Dal Toso, M. Fabris, S. Romanello, L. Aloe, et al., Mast cells synthesize, store, and release nerve growth factor, Proc. Natl. Acad. Sci. USA 91 (1994) 3739–3743.
[7] I. Burgos, A.C. Cuello, P. Liberini, E. Pioro, E. Masliah, NGF-mediated synaptic sprouting in the cerebral cortex of lesioned primate brain, Brain Res. 692 (1995) 154–160.
[8] M.W. Mulholland, G. Romanchuk, K. Lally, D.M. Simeone, Nerve growth factor promotes neurite outgrowth in guinea pig myenteric plexus ganglia, Am. J. Physiol. 267 (1994) G716–G722.
[9] S.C. Supowit, H. Zhao, D.J. DiPette, Nerve growth factor enhances calcitonin gene-related peptide expression in the spontaneously hypertensive rat, Hypertension 37 (2001) 728–732.
[10] R.M. Lindsay, A.J. Harmar, Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons, Nature 337 (1989) 362–364.
[11] J.W. Rychter, L. van Nassauw, J.-P. Timmermans, L.M.A. Akkermans, R.H.S. Westerink, A.B.A. Kroese, CGRP1 receptor activation induces piecemeal release of protease-1 from mouse bone marrow-derived mucosal mast cells, Neurogastroenterol. Motil. 23 (2011) e57–e68.
[12] M. Kulka, C.H. Sheen, B.P. Tancowny, L.C. Grammer, R.P. Schleimer, Neuropeptides activate human mast cell degranulation and chemokine production, Immunology 123 (2008) 398–410.
[13] T. Yamamoto, K. Fujiwara, M. Yoshida, N. Kageyama-Yahara, H. Kuramoto, N. Shibahara, et al., Therapeutic effect of kakkonto in a mouse model of food allergy with gastrointestinal symptoms, Int. Arch. Allergy Immunol. 148 (2009) 175–185.
[14] Y. Leng, T. Yamamoto, M. Kadowaki, Alteration of cholinergic, purinergic and sensory neurotransmission in the mouse colon of food allergy model, Neurosci. Lett. 445 (2008) 195–198.
[15] T. Sugimoto, C. Xiao, H. Ichikawa, Neonatal primary neuronal death induced by capsaicin and axotomy involves an apoptotic mechanism, Brain Res. 807 (1998) 147–154.
[16] J. Lee, T. Yamamoto, H. Kuramoto, M. Kadowaki, TRPV1 expressing extrinsic primary sensory neurons play a protective role in mouse oxazolone-induced colitis, Auton. Neurosci. 166 (2012) 72–76.
[17] Z.-D. Qu, M. Thacker, P. Castelucci, M. Bagyánszki, M.L. Epstein, J.B. Furness, Immunohistochemical analysis of neuron types in the mouse small intestine, Cell Tissue Res. 334 (2008) 147–161.

[18] F. de Jonge, L. van Nassauw, D. Adriaensen, F. van Meir, H.R.P. Miller, E. van Marck, et al., Effect of intestinal inflammation on capsaicin-sensitive afferents in the ileum of Schistosoma mansoni-infected mice, Histochem. Cell Biol. 119 (2003) 477–484.
[19] B. Schütz, D. Mauer, A.-M. Salmon, J.-P. Changeux, A. Zimmer, Analysis of the cellular expression pattern of beta-CGRP in alpha-CGRP-deficient mice, J. Comp. Neurol. 476 (2004) 32–43.
[20] M. Kakurai, R. Monteforte, H. Suto, M. Tsai, S. Nakae, S.J. Galli, Mast cell-derived tumor necrosis factor can promote nerve fiber elongation in the skin during contact hypersensitivity in mice, Am. J. Pathol. 169 (2006) 1713–1721.
[21] L.M. Patterson, H. Zheng, S.M. Ward, H.-R. Berthoud, Vanilloid receptor (VR1) expression in vagal afferent neurons innervating the gastrointestinal tract, Cell Tissue Res. 311 (2003) 277–287.
[22] K. Funakoshi, M. Nakano, Y. Atobe, R.C. Goris, T. Kadota, F. Yazama, Differential development of TRPV1-expressing sensory nerves in peripheral organs, Cell Tissue Res. 323 (2006) 27–41.
[23] M. Kadowaki, H. Kuramoto, M. Takaki, Combined determination with functional and morphological studies of origin of nerve fibers expressing transient receptor potential vanilloid 1 in the myenteric plexus of the rat jejunum, Auton. Neurosci. 116 (2004) 11–18.