CXCL14-like Immunoreactivity Exists in Somatostatin-containing Endocrine Cells, and in the Lamina Propria and Submucosal Somatostatinergic Nervous System of Mouse Alimentary Tract

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In the present study, we investigated the distribution of CXCL14 immunoreactive endocrine cells and neurons in mouse alimentary tract by immunohistochemistry. CXCL14 immunoreactive endocrine cells were found as closed-type cells in the stomach and open-type cells in the small intestine. The immunostaining of these endocrine cells corresponded with that of the somatostatin-containing endocrine cells. Only a few CXCL14 immunoreactive endocrine cells were seen in the large intestine. CXCL14 immunoreactive fibers were observed in the muscular layer from the stomach to the rectum with most abundance in the rectum. Many CXCL14 immunoreactive fibers were observed in the lamina propria and submucosal layer from the duodenum to the rectum with most abundance in the rectum; these fibers corresponded to the somatostatin-containing nerve fibers. Some CXCL14 immunoreactive neuronal somata that were also immuno-positive for somatostatin, were noted in the submucosal layer of the rectum. However, the remaining parts of the alimentary tract presented with almost negligible immunoreactive somata. The co-localization of CXCL14 and somatostatin suggests that CXCL14 contributes to the function of somatostatin, which include the inhibition of other endocrine and exocrine cells and the enteric nervous systems.

Key words: CXCL14, chemokine, somatostatin, gut, mouse

I. Introduction

Chemokines are basic proteins (molecular size, 8–12 kDa) that fundamentally possess chemotactic activity for leukocytes and lymphocytes. Based on the position of the cysteines near the amino terminal region, chemokines are divided into four subfamilies, CC, CXC, C, and CX3C [9]. So far, 17 CXC type chemokines have been identified [56]; the CXC subfamily is further divided into ELR+ and ELR− subtypes depending on the presence of “Glu-Leu-Arg” residues at the N-terminals [29].

CXCL14 is a member of the CXC subfamily and belongs to the ELR− subtype. CXCL14 was first isolated from human breast and kidney cells and originally termed as BRAK [19]. The full-length cDNA of CXCL14 encodes 111 amino acids with a putative signal peptide of 34 amino acids [7]. CXCL14 is expressed in epithelial tissues and mesenchyme-derived cells throughout the body [32]. However, it is not expressed in majority of the head and neck squamous cell carcinomas, and other established human cancers cell lines [11, 19]. Several physiological functions...
of this chemokine such as recruitment and maturation of macrophages and renewal of Langerhans cells in the skin have been proposed [24, 45]. The other functions of CXCL14 include stimulation of trafficking of activated natural killer cells to the sites of inflammation or malignancy [50], infiltration of macrophages into white adipose tissue under a high-fat diet [34], and inhibition of angiogenesis [47]. Suppression of tumor formation by this chemokine has also been reported [36]. In addition to these functions in the peripheral organs, CXCL14 is thought to possess neuron-associated functions as neuromodulators [3, 46, 55] and regulators of neuronal migration [37].

Although CXCL14 mRNA is present in normal and cancerous tissues of the alimentary tract [28], its protein expression in various cell types within the alimentary tract is not fully elucidated. The gastro-enteric endocrine cells regulate exocrine and endocrine gastrointestinal secretion, motility, blood perfusion and cell renewal under physiological and pathological conditions in tandem with the gastroenteric nervous system. These endocrine cells, in general, are divided into two groups depending on the extent of the apical cytoplasmic processes in the intestinal lumen. The apical processes in open-type endocrine cells contact the intestinal lumen and receive chemical information from the intestinal contents. On the other hand, the processes in the closed-type endocrine cells do not contact the intestinal lumen; therefore, the cells regulate themselves and/or other endocrine and exocrine cells through autocrine, paracrine, and/or endocrine fashions. To date abundant peptidergic and monoamnergic gastro-enteric endocrine cells have been discovered and are classified into six categories based on their structural and functional similarities [8]. The enteric nervous system controls and modulates various functions of the gut [12]. Neurons located in the submucosal (Meissner’s) and intermuscular (Auerbach’s) nerve plexus control gut functions by releasing a mixture of different inhibitory and/or excitatory neurotransmitters [13]. These neurotransmitters are expressed in distinct classes of enteric neurons with different functions [5]. In the present study, we investigated the presence of CXCL14-like immunoreactivity in mouse alimentary tract. This study demonstrates the co-existence of CXCL14-like immunoreactivity with somatostatin in gastro-enteric endocrine cells and in somatostatinergic neurons of the lamina propria and submucosal layer.

II. Materials and Methods

Animals and fixation

Male mice (C57BL/6NCrSlc; n = 7) were deeply anesthetized with Halothane Fluthane (Takeda Pharmaceutical Co. Ltd., Osaka, Japan). The animals were then perfused with 0.85% NaCl, and subsequently, with 4% formaldehyde and 0.2% picric acid in 0.1 M sodium phosphate buffer (PB, pH 6.9). The alimentary tract from the stomach to the rectum was rapidly dissected and separated into the follow-
CXCL14 immunoreactive endocrine cells in mucosal epithelial cell layer (A, B) and fibers in muscular layer (C) in the columnar epithelial regions of the stomach. Sagittal (A) and transverse (B) sections obtained from the villi. Note that these cells appear to be the closed-type of endocrine cells. Arrows in A and B indicate CXCL14 immunoreactive cells, and arrows in C indicate CXCL14 immunoreactive nerve fibers.

Fig. 1. CXCL14 immunoreactive endocrine cells in mucosal epithelial cell layer (A, B) and fibers in muscular layer (C) in the columnar epithelial regions of the stomach. Sagittal (A) and transverse (B) sections obtained from the villi. Note that these cells appear to be the closed-type of endocrine cells. Arrows in A and B indicate CXCL14 immunoreactive cells, and arrows in C indicate CXCL14 immunoreactive nerve fibers.

York, N.Y., USA) diluted to 1:500 in PBS-BSAT. Immunoreactivities were visualized by Alexa Fluor 555-labeled donkey anti-rabbit IgG (Abcam, Cambridge, UK) for CXCL14 and Alexa Fluor 488-labeled donkey anti-rat IgG (Abcam) for somatostatin.

**Immunoelectron microscopy**

Immunoelectron microscopy was performed as described in our previous studies [23, 38, 54]. For the pre-embedding method, portions of the stomach and rectum dissected from similarly perfused mice were used. Small blocks of the fixed samples were cut into 20 μm-thick sections and processed; CXCL14 immunoreactivity was detected by DAB reaction. The sections were then fixed in 1% OsO₄ for 1 hr at room temperature, dehydrated and embedded in Quetol 812 (Nissin EM, Tokyo, Japan). Subsequently, ultrathin sections were obtained and mounted on nickel grids. After staining with uranyl acetate and lead citrate for 1 min each, the sections were examined under an electron microscope (JEM 1220: JEOL, Tokyo, Japan).

**III. Results**

**Stomach**

In the esophageal region of the mouse stomach no CXCL14 immunoreactive cells was observed in the stratified squamous cell layers; however, a few CXCL14 immunoreactive fibers were seen in the myenteric nervous plexus. On the other hand, in the columnar epithelial regions, CXCL14 immunoreactive cells were scattered in the mouse stomach. They were especially abundant in the fundus, and whereas some cells were found in the neck, none was noted in the surface mucous cell layer. These immunoreactive cells often surrounded other types of cells in the epithelial cell layer of the stomach and appeared to be the closed-type of gastro-enteric endocrine cells (Fig. 1A, B). The shapes of the immunoreactive cells varied from triangular to bipolar (width, 5.8–8.2 μm; length, 8.2–

Fig. 2. Electron micrographs showing a CXCL14 immunoreactive cell in the columnar epithelial cell layer (A) and an immunoreactive cell bearing a cilium (B) in the stomach. Arrow in B indicates a cilium protruding from the CXCL14 immunoreactive endocrine cell.
23.5 μm). Many immunoreactive fibers were seen running along with long axes of the smooth muscle in the muscular layers (Fig. 1C). Nevertheless, no immunoreactive fibers were noted in the lamina propria and submucosa.

Electron microscopic studies indicated that the CXCL14 immunoreactive cells contained spindle- to horseshoe-shaped nuclei and secretory granules (approximately 160–330 nm in diameter; Fig. 2A). These cells sometimes harbored the cilium that penetrated between the CXCL14 immunoreactive cells and other epithelial cells (Fig. 2B).

**Small intestine**

In the duodenum, characterized by the presence of Brunner’s glands beneath the lamina muscularis propria, a few immunoreactive cells, considered to be the open-typed gastro-enteric endocrine cells (data not shown), were found scattered between the duodenal epithelial cells. Unlike in the stomach, some immunoreactive fibers were observed in the lamina propria and submucosa (data not shown), but only a few in the muscular layer.

A number of immunoreactive open-typed endocrine cells (the apical processes seemed to reach the lumen) were seen in the epithelial cell layer of the jejunum (Fig. 3A, B). Immunoreactive fibers were abundant in the lamina propria and submucosa of the jejunum (Fig. 3A, C) when compared to those of the duodenum. These fibers were found to innervate the tip of villi and often surrounded the neuronal somata in the Meissner’s nerve plexus (Fig. 3D). A few fibers were also localized in the muscular layer, including the myenteric nervous plexus. In the ileum, only a few immunoreactive cells were observed with some immunoreactive fibers in the lamina propria and submucosa, and only a few in the muscular layer (data not shown).

**Large intestine**

Only a few immunoreactive cells were observed in the epithelial cell layer of the cecum. Some immunoreactive
fibers were seen in the lamina propria, submucosa and muscular layer (data not shown). In the colon, no immunoreactive cells were seen in the epithelial layer. However, immunoreactive fibers were abundant in the lamina propria, submucosa and muscular layer (data not shown). In the rectum, we found a few immunoreactive cells that seemed to be of the closed-type of endocrine cells in the epithelial cell layer (data not shown). On the other hand, abundant immunoreactive fibers were seen in the lamina propria, submucosa and muscular layer (Fig. 4A–D). In the rectum, some immunoreactive somata were noted in the submucosa (Fig. 5A, B). The short and long axes of these somata were...
approximately 4.0 μm and 12.0 μm, respectively. Electron microscopic studies indicated that CXCL14 immunoreactive fibers in the submucosa contained clear synaptic vesicle-like structures (approximately 45–60 nm in diameter; Fig. 6), which is one of the characteristic features of the nerve fibers.

**Double labeling**

A double labeling study indicated that all CXCL14 immunoreactive cells were immuno-positive for somatostatin in the stomach (Fig. 7A–D) and jejunum (Fig. 7E, F). CXCL14 immunoreactive fibers in the lamina propria of the small and large intestines were immuno-positive for somatostatin (Fig. 8A, B). CXCL14 immunoreactive neuronal somata in the submucosa of the rectum were also immuno-positive for somatostatin (Fig. 8C, D). However, the CXCL14 immunoreactive fibers in the muscular layer from the stomach to the rectum were immuno-negative for somatostatin (Fig. 8E, F).

Table 1 shows the relative densities and co-localization states with somatostatin of CXCL14 immunoreactive endocrine cells and nerve fibers.

**Pre-absorption tests**

Pre-absorption of the antibody with CXCL14 resulted in the disappearance of immunoreactive cells in the colum-
nar epithelial cells of the stomach (Fig. 9A, B); however, pre-absorption of the antibody with somatostatin had no effects on CXCL14 staining profiles (Fig. 9B, C).

**IV. Discussion**

Gastro-entero-pancreatic (GEP) endocrine cells contribute to the regulation of gastrointestinal systems [8]. We have previously demonstrated that CXCL14-like immunoreactivity is localized in somatostatin immunoreactive cells in mouse pancreatic islets [51]. In the present study, we further confirmed the co-localization of these two peptides. In explanation, CXCL14 immunoreactive cells in the stomach were closed-type of somatostatin-containing cells and those in the small intestine were open-type of somatostatin-containing cells. In general, the open-type of GEP endocrine cells have apical cytoplasmic processes that make contact with and receive chemical information from the gastrointestinal contents. In contrast, the closed-type of GEP endocrine cells seem to sense local chemical and mechanical information [8]. The presence of open-type and closed-type of CXCL14/somatostatin cells is accord with the previous studies demonstrating that somatostatin immunoreactive cells contained both open- and closed-type GEP endocrine cells in other animals, including rat, dog, cat, and sheep [1, 6, 22, 25, 27]. Somatostatin suppresses gastrin release from G cells located in the stomach [17]. Although the functions of CXCL14 located on GEP endocrine cells are not known so far, co-localization suggests that CXCL14 contributes to the endocrine functions of somatostatin, similar to the pancreatic somatostatin cells [51].

A principal function of somatostatin is the suppression of all activities in the gastrointestinal tract [26], such as exocrine activities [30] and the inhibition of gastrointestinal hormone secretion including gastrin release as noted above [26, 30]. Furthermore, somatostatin inhibits gastrointestinal motility and decreases visceral blood flow, cell growth and cell renewal in concert with somatostatin-containing enteric neuronal system. On the other hand, somatostatin release is induced by various factors, which include not only fat and proteins [39], but also various hormones such as CCK, VIP and gastrin [10, 18]. Interestingly, in accordance with previous studies [16, 43], CXCL14/somatostatin immunoreactive endocrine cells in the stomach were found to contain cilia that protruded into the space between the immunoreactive and mucous epithelial cells. Cilia are fundamentally sensory in nature and mainly detect their environment [44, 48]. Somatostatin cells in the stomach are regarded as chemical sensory cells not only for meal but also hormones. Evidently, the cilia themselves contain hormone receptors...
such as somatostatin receptors [2, 15, 20]. Therefore, the cilia in the CXCL14/somatostatin cells may be responsible for sensation of local hormonal and chemical conditions. In addition, stomach CXCL14/somatostatin immunoreactive cells belong to the closed-type of endocrine cells, which suggests its role as a mechanoreceptor that detects the extension of the mucous layer and contributes to bowel reflex. Supportingly, it is reported that somatostatin cells react to the extension of stomach [43].

The enteric nervous system consists of two main plexuses, submucosal plexus and myenteric plexus, and regulates gastrointestinal absorption, secretion and motility [12]. It contains various types of neurons, which based on morphological, physiological and neurochemical characteristics, are classified into 18 types in the guinea pig small intestine [5]. CXCL14 immunoreactive fibers in the lamina propria and submucosal layer corresponded to somatostatin-containing fibers. CXCL14 immunoreactive neuronal somata were mainly located in the submucosal nerve plexus. Submucosal somatostatin-containing neurons belong to secretomotor neurons that project into the mucosa and mucosal glands [5, 49]. On the other hand, myenteric somatostatinergetic neurons are inhibitory interneurons with descending (anally) axons [5, 40]. Based on the absence of co-localization of CXCL14 with somatostatin in the muscular layer, CXCL14 may associate more likely with the secretomotor functions of somatostatin, rather than function as an inhibitory interneuron. The nature of CXCL14 as a neuromodulator and/or neurotransmitter is not fully elucidated at present. Nevertheless, the existence of CXCL14 in neurons has been demonstrated in hypothalamic neurons [55] and GABAergic neurons [3, 46]. Furthermore, CXCL14 modulates the expression and maintenance of tyrosin hydroxylase (dopamine synthesis enzyme) levels in mesencephalic dopaminergic neurons [52] and inhibits tonic and phasic effects of synaptically released GABA [3]. Other chemokines also perform not only as chemotactants but also as neuromodulators. For example, CXCL12 stimulates the release of glutamate from astrocytes and affects the synaptic activity independently of its receptors on the neurons [4]. CXCL8 reduces the calcium current in cholinergic neurons [41], and CXCL1 and 8 modulate calcium transients, enhance synaptic activity and suppress the induction of long-term depression in cerebellar Purkinje cells through CXCR2 (a chemokine receptor) [14]. Furthermore, several CC and CXC chemokines are able to reduce calcium oscillation in hippocampal neurons [31]. All these lines suggest that CXCL14 may also serve as a neuromodulator in addition to its chemotactic functions.

To date, the purpose of the coexistence of CXCL14 with somatostatin in GEP endocrine cells and part of the gastrointestinal nervous plexuses remains unclear; moreover, the CXCL14 receptor has not yet been identified. In contrast, somatostatin receptors are well characterized and classified into five subtypes. These somatostatin receptors are often targeted for the therapeutic treatment of various...
neuroendocrine tumors [42]. Although underlying mechanisms of these two peptides-associating functions are not elucidated yet, it is worthy to note that clinically, both CXCL14 and somatostatin have antitumor activities [21, 33, 35, 53]. Further studies on the genetic and protein expression profiles of CXCL14 in various neuroendocrine tumors may clarify the association of these two peptides and their antitumor mechanisms.

V. Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

VI. Acknowledgments

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