論 文 の 内 容 の 要 旨

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論文題目　**Studies on endothelin-1 and pathophysiology of allergic dermatitis in dogs**
（犬におけるエンドセリン - 1 とアレルギー性皮膚炎の病態に関する研究）

Endothelin (ET) is a peptide hormone composed of 21 amino acids which was originally found in the culture supernatant of porcine endothelial cells. There are three subtypes of human ET, ET-1, ET-2, and ET-3, encoded by the gene family in distinct chromosomal locations. Among these subtypes, ET-1 is produced by endothelial cells and well known as a potent vasoconstrictor. Besides this function, ET-1 shows various functions such as cell proliferation and chemotaxis. Additionally, ET-1 is known to have many pathophysiological roles in various diseases including cardiovascular disorders and tumors. Based on these findings, many kinds of antagonists for blocking ET receptor-mediated cell signaling have been developed and applied to human medicine.

Many reports indicate that ET-1 is up-regulated and mediates inflammatory reactions in the lesional tissues caused by various types of allergic diseases. For example, in human patients with asthma, ET-1 immunoreactivity was detected in bronchial epithelium and elevated ET-1 levels were found in bronchoalveolar lavage fluid and peripheral blood. Moreover, in a mouse model of allergic lung inflammation, ET receptor antagonists were shown to be effective to prevent granulocyte infiltration into the lung. However, it has not been clearly understood whether ET-1 has a pathophysiological role in allergic dermatitis such as atopic dermatitis and food hypersensitivity. Therefore, a series of the present studies were carried out to understand the pathophysiological roles of ET-1, endothelin receptor A (ET_A), and endothelin receptor B (ET_B) in canine allergic

dermatitis.

In Chapter I, as a first step to elucidate the function of the ET system in dogs, full-length cDNAs of canine ET receptors were cloned and sequenced. As s result, high sequence similarities of the canine ET receptors to the counterparts of mouse, rat, and human were shown. Additionally, the expression analysis revealed that ET-1, ET_A, and ET_B genes were ubiquitously expressed in various organs in the dog as in the case of other mammals (Fig.1). These results suggest that ET system has a wide range of functions in the dog as shown in the mouse, rat, and human.

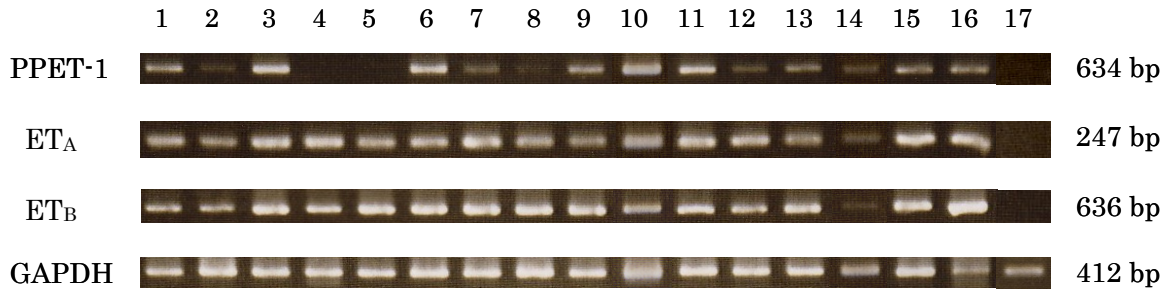

Fig.1. Reverse transcription (RT)- polymerase chain reaction (PCR) for detection of the expression of preproendothelin-1 (PPET-1), ET_A, and ET_B mRNAs in normal tissues and blood of dogs (lane 1, skin; lane 2, thymus; lane 3, lung; lane 4, heart; lane 5, liver; lane 6, pancreas; lane 7, spleen; lane 8, adrenal grand; lane 9, kidney; lane 10, testis; lane 11, uterus; lane 12, bladder; lane 13, stomach; lane 14, duodenum; lane 15, colon; lane 16, mesenteric lymph node; and lane 17, blood).

Next, in the Chapter II, plasma ET-1 concentrations and the cutaneous mRNA expression levels of ET-1, ET_A, and ET_B were measured in dogs with allergic dermatitis. As the results, plasma ET-1 level was significantly lower in dogs with allergic dermatitis than healthy dogs (Fig.2), however, PPET-1 expression level was not statistically different among the non-lesional and lesional skin samples in dogs with allergic dermatitis and the skin sample from healthy dogs (Fig.3). Meanwhile, mRNA expression levels of ET receptors were significantly lower in the lesional skins than normal skins (Fig.3). These results indicated that ET system was down-regulated in the skin of dogs with allergic dermatitis. Its difference from the phenomenon of the up-regulation in the allergic bronchitis in human and mouse might be due to the phase of the disease because most of the dogs with allergic dermatitis were in the chronic phase.

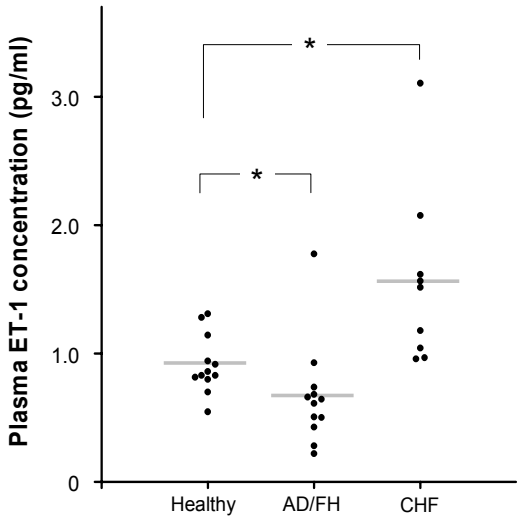

Fig.2. Plasma ET-1 concentration in dogs with allergic dermatitis in comparison to healthy dogs and dogs with congestive heart failure. The mean values of each group are indicated by crossbars. AD: atopic dermatitis, FH: food hypersensitivity, CHF: congestive heart failure. *P< 0.05 vs. healthy dogs.

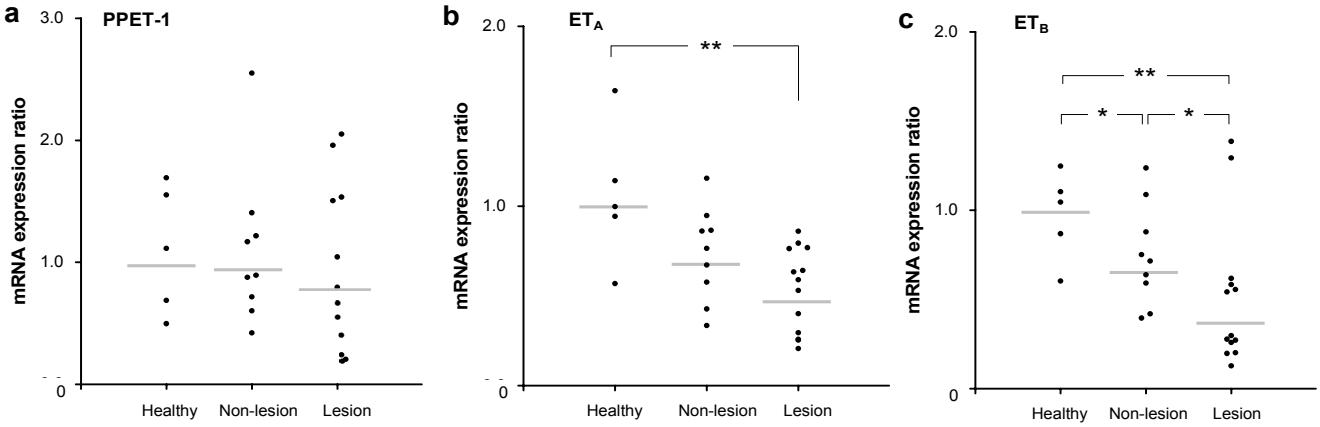

Fig.3. Relative expression of PPET-1 (a), ET_A (b), and ET_B (c) mRNAs in the lesional and non-lesional skin samples from dogs with allergic dermatitis and normal skin samples from healthy dogs. The mean values of each group are indicated by crossbars. *P< 0.05 vs. normal skin. **P=0.001 vs. normal skin.

Lastly, in Chapter III, to interpret the physiological role of ET system in allergic dermatitis, a study was focused on the chemotactic activity of ET-1 in the dog. Several studies have revealed the roles of ET-1 in leukocyte accumulation into the inflamed tissues. For the chemotaxis assay, a novel assay system allowing real-time observation of the movement of the cells (KK-chamber system) was employed in this study. I found that blood-derived neutrophils showed chemotaxis to ET-1, however, other leukocytes such as monocytes and lymphocytes did not, suggesting that ET-1-mediated chemotaxis was almost specific to neutrophils among canine leukocytes (Fig.4). The chemotaxis of canine neutrophils was shown to be mediated by receptors other than ET_A and ET_B, because ET receptor antagonists for selectively blocking ET_A or ET_B did not eliminate the cell migration (Fig.5). Previous studies indicated that neutrophil adhesion to vascular endothelial cells was mediated by enhancing the expression of adhesion molecules

via ET receptor signaling. These findings indicate that ET system provides a comprehensive support to the neutrophil infiltration into inflammatory site.

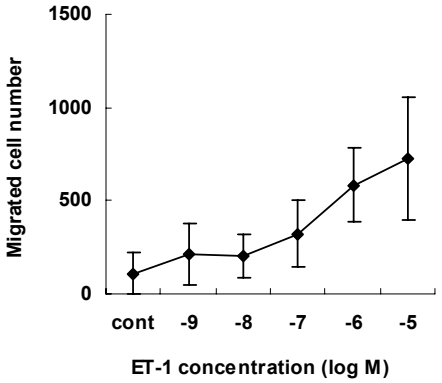

Fig.4. Chemotaxis of canine neutrophils to ET-1. The number of migrated cells were counted by KK-chamber system in the presence of a serially diluted ET-1. The average values from the results in 3 dogs are plotted.

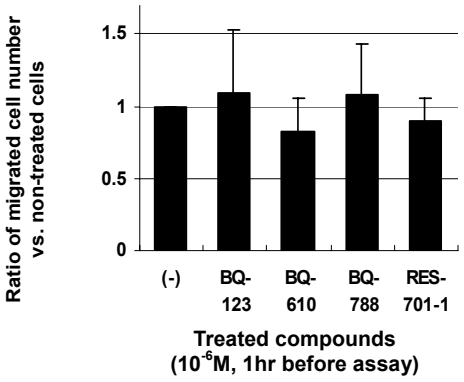

Fig.5. Effects of ET receptor antagonists on the chemotaxis of canine neutrophils to ET-1. Ratios of the migrated cell number in a presence of 4 kinds of ET receptor antagonists (BQ-123, BQ-610, BQ-788, BQ-701-1) to that without treatment of the antagonists are shown. The average values of the results in 3 dogs are indicated.

In Chapter II, I found that decreased ET-1 concentration in the plasma and decreased ET receptor expression in the lesional skin in dogs with allergic dermatitis. Considering the chemotactic activity of ET-1 to canine neutrophils as shown in Chapter III, the down-regulation of ET system may affect the neutrophil recruitment into the lesional skin of dogs with allergic dermatitis. Interestingly, it is known that accumulation of neutrophils does not occur in the lesional skin of dogs with allergic dermatitis, meanwhile, increase of neutrophils in the bronchial or bronchoalveolar tissues has been described in human asthmatic patients. These findings suggest the presence of different mechanism on controlling the neutrophil infiltration between allergic dermatitis and allergic bronchitis. This difference could be resulted from variation in the immune reaction and cytokine production from the cells between the two diseases. The present studies revealed the association of ET system with the pathophysiology in canine allergic dermatitis and will provide a novel therapeutic approach to allergic diseases which are now one of the most problematic diseases in dogs as well as humans.