NOTE Internal Medicine

Molecular Cloning of the Feline Thymus and Activation-Regulated Chemokine cDNA and Its Expression in Lesional Skin of Cats with Eosinophilic Plaque

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Abstract. Thymus and activation-regulated chemokine (TARC) is a member of CC chemokine and plays an essential role in recruitment of CC chemokine receptor 4 positive Th2 cells to allergic lesion. To investigate the association of TARC in allergic inflammation of cats, a TARC cDNA was cloned from feline thymus by RT-PCR with 3' rapid amplification of cDNA ends (RACE) method. The feline TARC clone contained a full length open reading frame encoding 99 amino acids which shared 80.8%, 72.5%, 65.6% and 67.8% homology with dog, human, mouse and rat homologues, respectively. Expression of TARC mRNA was detected not only in thymus but also in spleen, lung, lymph node, kidney, small intestine, colon and skin of the normal cat tissues examined. Furthermore, it was found that TARC mRNA was strongly expressed in lesional skin of cats with eosinophilic plaque. The present results demonstrated that TARC might be involved in the pathogenesis of eosinophilic plaque in cats.

Key words: cDNA cloning, feline, thymus and activation-regulated chemokine.

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Thymus and activation-regulated chemokine (TARC) is a member of CC chemokine and is a functional ligand for CC chemokine receptor 4 (CCR4) which is selectively expressed on Th2 cells but not on Th1 cells [11]. It has been determined that the biological function of TARC is to initiate the selective migration of Th2 cells to sites of allergic inflammation [1]. Recent studies revealed that keratinocytes and bronchial epithelial cells produced TARC protein in allergic lesions of humans [12, 14]. In dogs with atopic dermatitis, TARC [5] and CCR4 [7] mRNA were found to be selectively expressed in lesional skin but not in non-lesional skin. Another study demonstrated that a specific antibody against TARC could inhibit the allergic inflammation in mice experimentally sensitized by ovalbumin [3]. These results suggest that TARC may be a pivotal chemokine in the pathogenesis of allergic inflammation and its regulation might offer a novel therapeutic strategy for allergic diseases.

Eosinophilic plaque is a common cutaneous disease in cats, which frequently affects the skin on the abdomen and medial thighs [11]. Although the clinical signs of cats with eosinophilic plaque have been well documented [11], the pathogenesis of this disease is not well understood. Lesional skin in eosinophilic plaque shows characteristic histological findings including hyperplastic, superficial and deep perivascular dermatitis with infiltration of eosinophils, mast cells and lymphocytes [15]. In a study of 25 cats diagnosed as having allergic skin disease based on intradermal testing, three cats had lesions compatible with eosinophilic plaque [9]. Therefore, it was suggested that allergic inflammation may be a predisposing factor of eosinophilic plaque. Recently it has been proposed that eosinophilic plaque may be associated with type I hypersensitivity against an auto-allergen such as Felis domesticus allergen I [16], which is known to induce allergic reactions in asthma or rhinitis in humans [13].

In the present study, we report the molecular cloning of feline TARC cDNA and its mRNA expression not only in various tissues but also in skin samples from cats with eosinophilic plaque to provide fundamental information on its involvement of allergic diseases in cats.

Total RNA was extracted from the thymus of a healthy cat kept for experimental purposes under a protocol approved by the Institutional Animal Care and Use Committee at the University of Tokyo with a commercially available kit (SV Total RNA Isolation System: Promega, Madison, WI). cDNA was synthesized from 0.5 µg of total RNA with a commercially available kit (RNA PCR Kit: PERKIN ELMER, Branchburg, NJ). Oligonucleotide primers to amplify the 5' region of feline TARC cDNA were designed based on the sequences conserved between human [2], dog [6] and mouse [4] TARC cDNAs: forward primer, 5'-TCCCCTCCGGGTGGCTCCTGGGACC-3' (nucleotide (nt) 31–52 in human TARC cDNA [2]) and reverse primer, 5'-TGGTGGGGTCGCCGAAACAGATGG-3' (nt 284–265 in human TARC cDNA [2]). Using the primer pair, feline TARC cDNA was amplified from the feline thymus cDNA with a commercial kit (RNA PCR Kit: PERKIN ELMER, Branchburg, NJ) according to the manufacturer's instructions. The PCR amplifications consisted of pre-denaturation (95°C, 2 min), 35 cycles of denaturation (95°C, 1 min) and annealing (60°C, 1 min) followed by extension (72°C, 7 min). The PCR generated a single DNA fragment of about 270 bp, and the product was cloned into a plasmid vector (pGEM-T easy vector; Promega, Madison, WI) followed by transformation of Escherichia coli competent cells (INVFe): Invitrogen, Carlsbad, CA). The PCR product cloned into the plasmid was sequenced by the dideoxynucleotide chain termination method.

To amplify the 3′ region of feline TARC cDNA, the rapid amplification of cDNA ends (RACE) method was employed using a commercially available kit (3′ RACE System for Rapid Amplification of cDNA Ends: Life Technologies, Rockville, MD). The sequence of the forward primer was 5′-CGGGGGAGTGTTCCAAAGAC-3′ (nt 161–180) in feline TARC cDNA obtained in this study and that of the universal amplification primer was 5′-CUACUAUCAC-UAGCCACGGTCGACTAGTAC-3′ (3′ RACE System for Rapid Amplification of cDNA Ends: Life Technologies).

By combining the sequences of the 5′ and 3′ overlapping DNA fragments obtained in this study, a linear sequence corresponding to feline TARC cDNA was obtained, which was 300 bp long and contained an open reading frame of feline TARC cDNA encoding 99 amino acid residues (GenBank/EMBL/DDBJ accession number AB084139). The length of amino acid residues encoded by feline TARC cDNA was identical to that encoded by dog homologue but was 5 or 6 residues longer than those encoded by human, mouse and rat homologues. The feline TARC cDNA cloned in this study was shown to have 80.8%, 72.5%, 65.6% and 67.8% amino acid sequence similarities with dog [6], human [2], mouse [4] and rat (GenBank/EMBL/DDBJ accession number AF312687) TARC cDNAs, respectively (Fig. 1). In hydrophilicity plot analysis, the feline TARC had a hydrophobic N-terminus corresponding to the signal peptide identified in human, dog, mouse and rat TARC cDNAs. The signal peptide cleavage site of feline TARC was assumed from its alignment with the homologues of other species, and the 24th amino acid from the initiation codon was tentatively assigned as being the first amino acid of the mature feline TARC (Fig. 1). The feline TARC cDNA was shown to have four cysteine residues including two adjacent cysteines characteristic to the CC chemokine family (Fig. 1).

Expression of TARC mRNA was examined in various kinds of healthy tissues of a cat by RT-PCR. Total RNA samples were extracted from thymus, liver, spleen, heart, colon, skin, kidney, lung, lymph node and small intestine of a healthy cat kept for experimental purposes under a protocol approved by the Institutional Animal Care and Use Committee at the University of Tokyo. cDNA samples of these tissues were prepared with the procedures described above. To amplify a part of TARC cDNA, a primer pair was prepared: forward primer, 5′-TGAAGCTGCTGCTGCTGTC-3′ (fTARCs, nt 11–30 in feline TARC cDNA obtained in this study) and reverse primer, 5′-TCTCGGGCCAGGGCTTAC-3′ (fTARCR, nt 293–274 in feline TARC cDNA). As an internal control, feline GAPDH cDNA was amplified, using a primer pair: forward primer 5′-CTCATGACCACGTGCTACG-3′ (nt 415–533 in feline GAPDH cDNA, GenBank/EMBL/DDBJ accession number AB038241) and reverse primer 5′-TGAAGCTGCCAAGGGTGTCA-3′ (nt 939–906 in feline GAPDH cDNA). Using the primer pair, PCR amplification was performed with the procedure described above. The PCR products were electrophoresed through 3% agarose gel, and stained with ethidium bromide for visualization. Distinct bands of 287 bp derived from feline TARC mRNA were detected in the thymus, spleen, colon, lung, lymph node and small intestine whereas bands were relatively faint in samples of skin and kidney (Fig. 2). No bands were detected in the liver and heart by RT-PCR.

To study the involvement of TARC in allergic diseases of cats, the expression of TARC mRNA was further examined in lesional and non-lesional skin samples of 5 cats (4 castrated male and 1 spayed female; 8.2 ± 1.9 year-old) that were diagnosed with eosinophilic plaque based on clinical signs and histological examinations. All the cats with eosinophilic plaque had compatible clinical findings of eosinophilic plaque including severe pruritus with ulcerated lesions on the skin of the abdomen or thigh. Histological examination of the lesions revealed hyperplastic superficial dermatitis with infiltration of eosinophils in all the cats. Biopsy samples of the lesional skin were taken from the

Cat 1: YNNMLKLLLLVLL1GASLQVTHQARRATNVGRSECLCLAEYFXGAIPLRALTGWYTSGEEKGKD 60
Dog 1: *T+F++W---------------G+-------------IS++R*++K++*N++E+*+ 60
Human 1: *A++*M+A------------HI+++G*+-------------K+KT*+Q*E+*N*+R+ 60
Mouse 1: *R*++*Q*++*A+++*TF+*HAR+-------------D+++I*K+VS*+K++V*+R+ 60
Rat 1: *++*G*++*A+++*T+++*HAS------------C+++D+++I*K+VT*++V*+R+++ 60

Cat 61: AIEVT1HGSIIEEDPDTRVRGTVYLRQSINFPQES 99
Dog 61: *++*VQ*++*K+++*A+++*RTWGG*+++ 99
Human 61: *++*VQ*RA++*NNK*+*NA*K+++LERS 94
Mouse 61: *++*VQ*++*L++*A+++*KH+++AI+LVQFRP 93
Rat 61: *++*E*VQ+R+FP+++*KH+++AI+H+MVQRL 93

Fig. 1. Comparison of the deduced amino acid sequence of feline TARC cDNA (GenBank/EMBL/DDBJ accession number AB084139) with those of dog, human, mouse and rat homologues. Asterisks indicate the same amino acids as those of feline TARC sequence. Numerals on the right end of each line show the numbers of amino acid residues. The amino acid sequence with underline indicates the putative signal peptide. Conserved cysteine residues are boxed.
thigh in 3 cases, from the abdomen in 1 case and from the neck in 1 case. All the samples from non-lesional skin were obtained from the lateral thoracic wall where skin lesion was not grossly found. Total RNA samples were extracted from biopsied skin samples and cDNA was prepared with the procedures described above. Using the primer pair specific to feline TARC cDNA (fTARCS and fTARC R), TARC mRNA was found to be expressed in all of the samples from both lesional and non-lesional skin in all the cats by RT-PCR, however, the intensity of the bands in lesional skin was higher than that in non-lesional skin (Fig. 3).

Although the pathogenesis of eosinophilic plaque is not understood, recent studies have proposed that allergic inflammation may be involved in pathogenesis of eosinophilic infiltration [10, 15, 16]. The objective of the present study, therefore, was to test the hypothesis that TARC would be associated with the development of skin lesion in cats with eosinophilic plaque. In the present results, the expression of TARC mRNA was higher in the lesional skin than in non-lesional and normal skin, suggesting the involvement of Th2 type immune responses in the immunopathogenesis of eosinophilic plaque in cats. Since IL-5 is known to induce infiltration of eosinophils into allergic lesions in humans [8], it is possible that Th2 cells migrated by TARC to skin lesions are sources of IL-5. To further clarify the involvement of allergic responses in eosinophilic plaque of cats in terms of cytokines, it will be necessary to perform in situ analysis of cytokine production in skin lesions of eosinophilic plaque in cats

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