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Production of a monoclonal antibody to canine thymus and activation-regulated chemokine (TARC) and detection of TARC in lesional skin from dogs with atopic dermatitis

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Abstract

A monoclonal antibody to canine thymus and activation-regulated chemokine (TARC/CCL17) was developed to examine the association of TARC with the immunopathogenesis of canine atopic dermatitis (AD). Recombinant canine TARC was prepared using an *E. coli* expression system. Results of transwell chemotaxis assay demonstrated that the recombinant canine TARC showed chemotactic activity for canine lymphoid cells expressing CC chemokine receptor 4 (CCR4). Mice were then immunized with the recombinant canine TARC to obtain monoclonal antibodies. Among the monoclonal antibodies thereby obtained, one monoclonal antibody (CTA-1) was found to react with both recombinant and authentic canine TARC in ELISA and flowcytometric assays, respectively. Immunohistochemical analysis using the monoclonal antibody CTA-1 demonstrated that keratinocytes were major TARC producing cells in lesional skin of dogs with AD.

Keywords: Dogs; Atopic dermatitis; TARC/CCL-17; Monoclonal antibody; Chemokine

1. Introduction

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases in dogs. Dogs with AD have severe pruritus and develop skin lesions showing erythema, hair loss, hyperpigmentation and lichenification at the ventral abdomen, thigh or axilla. An immunohistochemical study revealed that CD4+ cells

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are predominantly infiltrated in lesional skin of dogs with AD (Sinke et al., 1997), suggesting that CD4+ cells may play an important role in the immunopathogenesis of canine AD. In acute skin lesions of human AD, it has been shown that infiltrated CD4+ cells produce Th2-type cytokines such as IL-4, IL-5 and IL-13, which may conceivably initiate and maintain allergic inflammation (Leung and Soter, 2001). Recent studies in dogs demonstrated that expression of IL-4 mRNA was up-regulated in lesional skin of dogs with AD (Nuttall et al., 2002; Olivry et al., 1999). These results suggest that infiltrated CD4+ cells in atopic lesions in dogs may be composed of Th2 cells which contribute to the development of skin lesions as in human AD.

Chemokines are known to be important molecules regulating the trafficking of leukocytes (Zlotnik and Yoshie, 2000). Previous studies indicated that CC chemokine receptor 4 (CCR4) is selectively expressed on Th2 cells in humans (Imai et al., 1999) and mice (Andrew et al., 2001), implying that CCR4 may play important roles in selective infiltration of Th2 cells in atopic skin lesions. It was reported that CCR4+ cells are predominantly infiltrated in atopic lesions of humans (Nakatani et al., 2001), which are most likely induced by a biological ligand for CCR4, thymus and activation-regulated chemokine (TARC/CCL17). In humans with AD (Kakinuma et al., 2001; Zheng et al., 2003) and in NC/Nga mice (Vestergaard et al., 1999), keratinocytes distributed in lesional skin are a major source of TARC production. These results suggest that the association between TARC and CCR4 may be involved in the recruitment of Th2 cells into atopic lesions.

Our previous studies indicated that the mRNAs of both TARC (Maeda et al., 2002a) and CCR4 (Maeda et al., 2002b) are selectively expressed in lesional skin of dogs with AD but not in non-lesional or normal skin. Furthermore, it was also found that the number of CCR4+ cells is increased in peripheral CD4+ cells from dogs with AD (Maeda et al., 2004). These results imply that chemokines may also play important roles in the immunopathogenesis of canine AD, as in that of human AD.

In this study a monoclonal antibody to canine TARC was developed in order to further clarify the association of chemokines with the pathogenesis of canine AD.

2. Materials and methods

2.1. Preparation of recombinant canine TARC

A cDNA fragment encoding the mature form of canine TARC was amplified from canine thymus cDNA by PCR using a forward primer (5′-ACC-*GAATTC*GCTCGAGGCACCAACGTGGGCCGG-3′, nucleotides (nt) 70–93 in canine TARC cDNA (Maeda et al., 2001); the italic sequence is an *Eco* RI adaptor) and a reverse primer (5′-AGG*GTCGAC*TCAG-GACTCTTGGGGCCCTCCCTT-3′, nt 300–277 in canine TARC cDNA; the italic sequence is a *Sal* I adaptor) with a commercially available kit (RNA PCR Kit, Applied Biosystems, Foster City, CA). After digestion with *Eco* RI and *Sal* I, the TARC cDNA was ligated into the *Eco* RI–*Sal* I sites of pGEX-4T-1 (Amersham Biosciences, Piscataway, NJ) to enable the expression of TARC as a fusion protein with glutathione *S*-transferase (GST), using a commercially available kit (DNA Ligation Kit Ver. 2, Takara Bio Inc., Shiga, Japan). The expression vector, pGEX-TARC, was prepared in *Escherichia coli* (*E. coli*) competent cells (Top10, Invitrogen Corp, Carlsbad, CA) and purified using a commercially available kit (Qiagen Endotoxin Maxi Kit, Qiagen, Chatsworth, CA). The plasmid was then introduced into *E. coli* strain BL21 (Novagen Inc., Madison, WI) and incubated in Luria-Bertani medium (LB media) at 37 °C overnight. One milliliter of this culture was further expanded to 1 l at 37 °C for 90 min followed by induction of expression with isopropyl-β-D-thiogalactopyranoside (1 mM) for 4 h. The cells were then pelleted and suspended in PBS for sonication. The solubilized bacterial suspension was centrifuged at 10,000 × *g* at 4 °C for 10 min and the supernatant was rotated with Glutathione Sepharose 4B (Amersham Biosciences, Piscataway, NJ) at 4 °C overnight. The fusion protein combined with Glutathione Sepharose was then separated from the supernatant using a Glutathione Sepharose 4B column (Amersham Biosciences, Piscataway, NJ). The fusion protein was incubated with thrombin (100 U/ml, Amersham Biosciences, Piscataway, NJ) at room temperature for 16 h in order to separate the complex between the fusion protein and GST-Glutathione Sepharose from the fusion protein. Recombinant TARC protein was then purified by a gel infiltration chromatography