

Canine Epidermal Langerhans Cells Express α and γ but not β Chains of High-Affinity IgE Receptor

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ABSTRACT

Epidermal Langerhans cells (LC) express a high-affinity receptor for IgE (Fc ϵ RI), consisting of two chains (α and γ chains) in humans that allows LC to perform Fc receptor-mediated uptake of allergens. We found that canine LC express α and γ chains but not β chain of Fc ϵ RI, identical to human but not to mouse LC, which do not express functional Fc ϵ RI (only γ chain is expressed). This finding indicates that canine LC have Fc ϵ RI-mediated function similar to or identical to human LC, raising the possibility that canine species provides a better model than mouse to understand the pathogenesis of human atopic dermatitis and investigate the therapeutic effect of drugs.

Keywords: α chain, β chain, canine, γ chain, gene expression, high-affinity IgE receptor, Langerhans cells

Abbreviations: Ab, antibody; AD, atopic dermatitis; Ag, antigen; APC, antigen-presenting cells; Ig, immunoglobulin; LC, Langerhans cells; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PCR, polymerase chain reaction

INTRODUCTION

Epidermal Langerhans cells (LC) are skin-resident members of the dendritic cells (DC; professional antigen-presenting cells (APC)) that possess unsurpassed potency in initiating primary immune response. LC are also highly potent in endocytosing local environmental and skin-penetrating foreign antigens (Ag), including infectious agents, chemicals and allergens. Using these unique features, LC serve as a pivotal component in the first line of defensive mechanisms and also in the adaptive immune response.

LC are also considered to be critically involved in induction of allergic responses because of their expression of a high-affinity receptor for IgE (Fc ϵ RI). This expression is likely to allow LC to ingest allergens via Fc ϵ RI-mediated uptake, which appears more efficient than non-receptor-mediated endocytosis that LC normally use for

uptake of peptide antigens (Mudde *et al.*, 1990). Association of FcεRI expression levels with allergic diseases is supported by observations from clinical studies that LC in the lesional skin of human patients with atopic dermatitis (AD) display upregulated expression of FcεRI, compared to LC in non-lesional skin (Stingl and Maurer, 1997; Kraft *et al.*, 1998) and that LC from AD skin lesions are 1000-fold more efficient in presenting house dust allergen to T cells than are LC that lack surface IgE (Maurer *et al.*, 1995).

FcεRI is a multichain membrane glycoprotein, which is composed of α, β, and γ chains: the α chain is primarily responsible for binding to IgE (Vangelista, 2003); the β chain is responsible for transducing intracellular signalling in response to the binding (Saini and MacGlashan, 2002); and the γ chain is responsible for internalization of FcεRI α chain (Mao *et al.*, 1991). Importantly, the pattern of each chain assembly differs among cell types; for example, basophils and mast cells express a FcεRI complex of one α, one β, and two γ chains (αβγ₂) (Ravetch and Kinet, 1991), while FcεRI on monocytes, DC and LC comprises one α and two γ chains (αγ₂) (Maurer *et al.*, 1994, 1996; Jurgens *et al.*, 1995). Moreover, heterogeneity in the pattern is also found among species: human LC express a complex of αγ₂, whereas mouse LC express only γ chain (Hayashi *et al.*, 1999), indicating the inability of mouse LC to express functional FcεRI. Examination of expression of each chain will lead to better understanding of the role of LC from various animal species in initiating allergic responses and developing AD.

Canines also develop cutaneous diseases that clinically resemble human AD in many respects (Griffin and DeBoer 2001; Hill and Olivry 2001). To gain insight into the function of epidermal LC in the induction of human AD-like allergic diseases, we examined the expression pattern of genes for the three chains of FcεRI in canine LC. Here we report the effectiveness of anti-CD1a antibody (Ab)-linked magnetic beads to purify LC from canine skin and detection of mRNA expression of α and γ chain (but not β chain) in the purified canine LC. Our study suggests canine LC to be capable of performing FcεRI-mediated uptake of allergens, as shown for human LC.

MATERIALS AND METHODS

LC isolation

Canine epidermal cells were prepared from the ventral side of ear skin using the same procedures as described previously for mouse ears (Bonkobara *et al.*, 2001). Briefly, skin biopsies were prepared from three individuals (male Beagles aged 6–10 years), pooled, and then floated on 0.15% trypsin (Sigma-Aldrich, St Louis, MO, USA)/phosphate-buffered saline (PBS). After incubation at 37°C for 60 min, epidermal sheets were peeled off from the skin and treated with 0.3% trypsin/PBS at 37°C for 15 min with vigorous tapping. Epidermal cells detached from the epidermal sheets were passed through nylon mesh and collected. To purify MHC class II⁺ cells (LC) from epidermal cells, we used a positive selection method using immunomagnetic beads. Streptavidin-coated magnetic beads (Dynal Biotech, Lakesuccess, NY, USA) were incubated with

biotin-labelled rabbit anti-mouse IgG (Sigma-Aldrich) at room temperature for 30 min. After removal of unbound antibodies by washing, beads were resuspended in 50 μ l of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (cDMEM). Prepared beads (2×10^7) were then incubated at 4°C for 1 h with epidermal cells that had been treated previously with mouse anti-canine MHC class II or anti-canine CD1a monoclonal Ab (mAb) (purchased from University of California Davis, CA, USA). After washing with PBS, cells were sorted into bead-bound (MHC class II⁺ or CD1⁺ cells) and unbound (MHC class II⁻ or CD1⁻ cells) fractions using magnetic apparatus. The number of bead-bound cells was determined by counting rosette-forming cells (Figure 1).

Preparation of tumour cells

Mast cells were collected by aspiration from canine mast cell tumour (MCT) mass of patients, using a 23-gauge needle and a syringe, at the Veterinary Medical Teaching Hospital of the Nippon Veterinary and Animal Science University.

Amplification of mRNA

Total RNAs were extracted from magnetic bead-bound LC and mast cells using RNA-STAT 60 (Tel-TestB, Friendswood, TX, USA). After reverse-transcription into cDNA, PCR amplification was performed with the following primer sets for canine genes: Fc ϵ RI α chain, 5'-GCGCTGCTGCTCTCCTCTCC-3' and 5'-TAGTTGCCGCTGTTTTTGGTTGTG-3'; for Fc ϵ RI β chain, 5'-TTCTGGGGGTAACACAAAT-3' and 5'-TGCAAAACCCAGAAATAGT-3'; for Fc ϵ RI γ chain, 5'-ATTTCTGCCGTGGTCTTGCTCCTG-3' and 5'-GTCTCCTGGTTCCGGGTGCTCA-3'; for chymase, 5'-CCTGGCGTCTTGTGGTGGTTT-3' and 5'-AGGGGGCTTTGCATCATTCTG-3'; for tryptase, 5'-GCGGACATTGCCCTGCTGGAG-3' and 5'-AGTAGGCGACGCGGGTGTAGATGC-3'; and for β -actin, 5'-CTGGCCGGGACCTGACTGACTACC-3' and 5'-GCGTTCCGGAGGGGCGATGAT-3'. PCR products were size-fractionated on 1.2% agarose gel and visualized with ethidium bromide.

RESULTS

We have previously reported the effectiveness of anti-MHC class II mAb-beads for purifying epidermal LC from mouse skin (Bonkobara *et al.*, 2001), indicating that no other cell types are found in the bead-bound fraction. Epidermal cells were incubated with anti-MHC class II Ab-coated magnetic beads and fractionated into bead-bound (MHC class II⁺) (Figure 1) or unbound (MHC class II⁻) cells. However, use of anti-MHC class II Ab permitted contamination by mast cells in the MHC II⁺ cell fraction, as judged by detection of mRNA for chymase, a mast cell-specific proteinase, in the MHC class II⁺ cell fraction using RT-PCR (Figure 2). We speculate that this

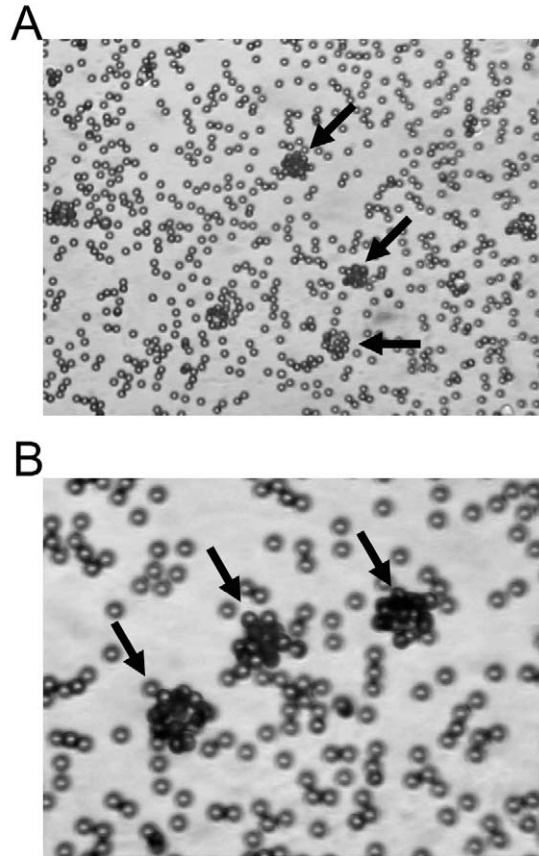


Figure 1. Rosette-forming Langerhans cells in anti-CD1a antibody-bound fraction. After incubation of epidermal cells with magnetic beads coated with anti-canine CD1a monoclonal Ab, magnetic beads were extensively washed to remove non-bead-bound epidermal cells and observed under microscope at low (A) and high (B) magnification. Cells forming rosettes with beads (shown by arrows) were observed with unbound free beads (particles in the background)

contamination might be due to the fact that, unlike in the mouse, many canine mast cells expressing MHC class II are located in the interface between epidermis and dermis. To circumvent this problem, we employed anti-canine CD1a mAb to purify LC from epidermal cells (mast cells are unlikely to express CD1a). In RT-PCR analysis, either of two mast cell-specific proteinases (tryptase or chymase) or both mRNA was clearly detected in the mast cells isolated from two different tumours (MCT#1 and #2) (Figure 2). Under the same conditions, expression of these two proteinase genes was not detectable in epidermal cells purified using anti-canine CD1a mAb-beads (Figure 2), indicating no contamination by mast cells in the CD1a⁺ cell fraction.

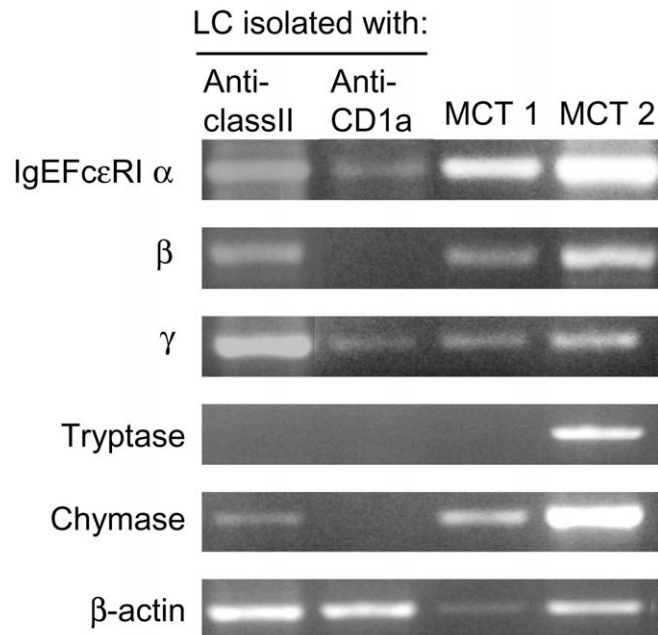


Figure 2. mRNA expression of FcεRI chains and mast cell-specific proteinase in canine LC and mast cells. Total RNA was prepared from mast cells and epidermal cells purified using Ab to MHC class II (class II) or CD1a, and subjected to RT-PCR gene expression analysis using primers specific to each chain of canine FcεRI (α , β and γ chains), mast cell-specific proteinase (tryptase and chymase) or β -actin. PCR products were size-fractionated on 1.2% agarose gel and stained with ethidium bromide. α and γ but not β chain genes of FcεRI were amplified in CD1a mAb purified LC without amplification of mast cell-specific proteinase gene, whereas MHC class II mAb purified LC expressed all three chain genes (α , β and γ) with chymase gene. The data shown here are representative of three independent experiments

Using the epidermal CD1a⁺ cells, we examined expression levels of mRNA for FcεRI α , β , and γ chain genes in canine LC (Figure 2). RT-PCR analysis showed that tumour mast cells express all three chain genes as expected, but with variable levels: highest levels with α chain gene and lower levels with the other two genes. This result verified that canine mast cells are identical to human mast cells in the expression profiles of FcεRI chains. Using the same PCR conditions, we detected expression of mRNA for α and γ chain genes in the CD1a⁺ epidermal cells (LC), although at much lower levels than detected in the tumour mast cells. By contrast, no mRNA expression for FcεRI β chain gene was noted in the canine LC. This was also true even when 10 more cycles were applied (a total of 40) (data not shown). Detection of β chain mRNA in the MHC class II⁺ cells was again due to contamination by cutaneous mast cells. These results led us conclude that canine LC express α and γ but not β chain genes.

DISCUSSION

In this study we showed that canine LC express α and γ but not β chain of Fc ϵ RI at the level of mRNA. The expression profiles are identical to those of human LC (Jurgens *et al.*, 1995) but differ from those of mouse LC, which have previously been reported to lack α chain gene expression (only γ chain mRNA was detected) (Hayashi *et al.*, 1999).

As mentioned in the introduction, Fc ϵ RI consists of three independent chains, each of which has a distinct role in the function of the receptor. Despite the necessity of their assembly for full function, expression profiles of these three genes are not always identical. Mast cells and basophils express all three genes, rendering them capable of expressing fully functional Fc ϵ RI on their cell surface in humans and mice (Ravetch and Kinet, 1991; Ra *et al.*, 1989) and also in canines (Goitsuka *et al.*, 1999). By contrast, epidermal LC and other DC subsets, even other APC, express just two genes out of the three, missing expression of β chain (Maurer *et al.*, 1994, 1996; Jurgens *et al.*, 1995). This complex missing the β chain ($\alpha\gamma_2$) is able to be transported onto the cell surface and is sufficient to mediate IgE-dependent internalization of allergens (Mao *et al.*, 1993) because α chain has a signalling motif for internalization (Turner and Kinet, 1999). However, β chain-induced intracellular signalling, which is responsible for activating immunologically functional genes, may be impaired or lacking in LC. These facts and our findings suggest that canine LC may have capacity to take up allergens via Fc ϵ RI for presentation to T cells similar to human LC. Moreover, an increased number of IgE receptor-expressing LC in epidermis was observed in canines with AD (Olivry *et al.*, 1996). Therefore, our findings may also explain why canines develop AD at as high an incidence as observed for human (Rhodes *et al.*, 1987). By contrast, mouse LC (only γ chain expression) are highly unlikely to be involved in the induction of allergic diseases such as AD. This may account for the lack of reports formally documenting AD-like skin diseases naturally occurring in mouse species. However, treatment with allergens plus adjuvant (in particular, alum) induces features of allergic responses such as high levels of serum IgE in mouse (Comoy *et al.*, 1998), in which LC-independent mechanisms may be more critically involved. The same mechanisms may be responsible for spontaneous development of AD-like diseases in a mouse model (e.g. NC/Nga) (Matsumoto *et al.*, 1999). Finally, our study raises the possibility that canine species appear better models than mouse species for understanding the role of epidermal LC in inducing human AD as well as in developing new drugs to treat AD or investigate drug effects on AD.

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