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Recombinant Derp5 allergen with α_{S1}-casein signal peptide secreted in murine milk protects against dust mite allergen–induced airway inflammation

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ABSTRACT

Recent advances in recombinant technology make transgenic animals that produce pharmaceutical proteins in their milk more feasible. The group 5 allergen isolated from *Dermatophagoides pteronyssinus* (Derp5) is one of the most important dust mite allergens in humans. The aims of this study were to develop transgenic mice that could secrete recombinant Derp5-containing milk and to demonstrate that ingesting recombinant milk protects against allergic airway inflammation. Two transgenes were constructed separately. The α-LA-Derp5f transgene consisted of the bovine α-lactalbumin (α-LA) promoter and full-length Derp5 cDNA. The α-LA-CN-Derp5t transgene included the α-LA promoter, a leader sequence of α_{S1}-casein (CN), and signal peptide-truncated Derp5 cDNA. Both species of transgenic mice were confirmed to have successful transgene integration and stable germline transmission. Western blot analysis of the milk obtained from the offspring of transgenic mice demonstrated that recombinant Derp5 was secreted successfully in the milk of αLA-CN-Derp5t transgenic mice but not in that of αLA-Derp5f transgenic mice. This study provides new evidence that transgenic mice can secrete recombinant Derp5 efficiently in milk by adding a signal peptide of α_{S1}-casein. The antigenic activity of recombinant Derp5 milk was demonstrated to have a protective effect against allergic airway inflammation in a murine model in which the ingestion of recombinant Derp5-containing milk was used as pretreatment.

Key words: *Dermatophagoides pteronyssinus*, Derp5 allergen, transgenic mice, signal peptide, allergic airway inflammation

INTRODUCTION

The house dust mite, a common indoor allergen, has been considered an important risk factor associated with asthma attack in the domestic environment (Bateman et al., 2008). *Dermatophagoides pteronyssinus* (**Derp**) is the predominant species of house dust mite in areas of Taiwan, Australia, and Western Europe (WHO, 1988). Although elevated serum IgE against group 1 and 2 allergens isolated from *D. pteronyssinus* (Derp1 and Derp2) are common among individuals with allergies (van der Zee et al., 1988), numerous studies have indicated that group 5 allergens from *D. pteronyssinus* (**Derp5**) are another important group of dust mite allergens in humans (Lynch et al., 1997; Thomas et al., 2002; Weghofer et al., 2008). In a study by Lin et al. (1994), recombinant Derp5 peptide expressed in a pGEX vector system was demonstrated to have strong reactivity with serum IgE in over 50% of asthmatic patients. The Derp1 peptide has been shown to have cysteine protease activity (Simpson et al., 1989), but the actual functions of Derp2 and Derp5 peptides in dust mites are not clearly understood. In a study by Weghofer et al. (2008), Derp5 was demonstrated to be a product secreted from gut epithelia of *D. pteronyssinus*. This finding reveals the importance of Derp5 allergen studies because house dust mites are pervasive and could excrete this allergen into the domestic environment via their feces.

Allergen-specific immunotherapy (**ASIT**) is the process of repeatedly administering a relevant allergen to induce immune tolerance in patients with allergic diseases. In past decades, many modalities of ASIT were widely investigated and developed for patients with allergic rhinitis or asthma. Recent advances in the development of immunotherapy regimens and engineering technology have improved the specificity and efficacy of ASIT (Ring and Gutermuth, 2011; Calderón et al., 2012). Recombinant allergen has the advantages

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of convenience and a high productive yield compared with traditional allergen extracts. Advances in biotechnology have also made milk-producing animals excellent bioreactors for producing recombinant proteins in their mammary glands (Houdebine, 2000). Recombinant proteins from the mammary glands of transgenic animals have the advantage of proper posttranslational modifications, and these proteins can be conveniently collected and purified. The transgenic animal systems used to produce pharmaceutical proteins in milk have improved technically, and their clinical applications have become more prevalent (Houdebine, 2009). Some researchers have attempted to engineer dust mite allergen in bioreactor platforms such as bacteria and plants (Lin et al., 1994; Hsu et al., 2004), but production of recombinant dust mite allergen from milk of transgenic animals has not been demonstrated previously.

The aim of this study was to construct an optimal transgene and produce transgenic mice that can express and secrete recombinant Derp5 into their milk. We evaluated the pretreatment efficacy of ingestion of this recombinant Derp5-containing milk in a murine model of allergic airway inflammation.

MATERIALS AND METHODS

Construction of Derp5 Transgenes

A full-length Derp5 cDNA (396 bp) encoding a 19-AA signal peptide and a 113-AA functional peptide with a molecular weight of 14 kDa was a gift from C. H. Hsu at Chinese Medicine University, Taiwan (Hsu et al., 2004). To compare the efficiency of different signal peptides for Derp5 peptide secretion from mammary glands of transgenic mice, 2 DNA constructs were engineered, each of which carried a 2.0-kb regulatory sequence of the bovine α-LA gene and either the full-length Derp5 (***Derp5f***) or the signal peptide-truncated Derp5 (***Derp5t***) cDNA sequences.

The *Derp5* cDNA containing the original leader sequence from the pGEM7 plasmid was used to generate an intact *Derp5* coding sequence by PCR amplification using the primer set of pDerp5–1(+) and pDerp5–1(−) (Table 1). After PCR amplification, the products were digested with *Xho*I (cutting sites are underlined in the primer sequences shown in Table 1) and then ligated into the α-LA/pCR3 vector (Wu et al., 2007). A linear transgene was obtained through double digestion of the α-LA-Derp5f/pCR3 vector with *Bam*HI and *Bbs*I (New England Biolabs Inc., Ipswich, MA). The 3.1-kb transgene fragment consisted of the α-LA promoter (2.0 kb), the *Derp5* cDNA encoding a 19-AA signal peptide and a mature Derp5 peptide (*Derp5f*; 0.6 kb), and the bovine growth hormone (**bGH**) polyadenylation (**poly-A**) signal sequence (0.5 kb; Figure 1A).

To construct the αLA-CN-Derp5t transgene, the 19-AA signal peptide of *Derp5* cDNA was replaced with a 15-AA signal peptide from α_{S1}-CN. The leader sequence of α_{S1}-CN was amplified by PCR using the primer set of pαS1-CN(+) and pαS1-CN(−) (Table 1). The PCR products were inserted into an α-LA promoter-containing pGEM-T plasmid by digestion with *Sac*II and *Hpa*I. Then, the α-LA-CN sequence was ligated into the pCR3 vector via digestion with *Not*I. The constructed α-LA-CN/pCR3 vector was created for mammary gland-specific expression (Tung et al., 2011). The truncated *Derp5* cDNA in the pGEM-T plasmid (Promega, Madison, WI) was used to generate a truncated *Derp5* coding sequence that did not contain the original leader sequence via PCR amplification using the primer

Table 1. Oligonucleotide primer sets used to construct, detect, and analyze the α-LA-Derp5f and α-LA-Derp5f transgenes (containing the group 5 allergen isolated from *Dermatophagoides pteronyssinus*) in this study

Primer set	Oligonucleotide sequence (5′ to 3′)[1]	Melting temperature (°C)	PCR product size (bp)
α-LA-Derp5 transgene junction			
pαLA-124(+)	CTCTCTTGTCATCCTCTTCC	47	480
pDerp5-213(−)	GAAATCTTGCGTCCACAGGC	49	
Bovine α-LA regulatory region			
pαLA-F(+)	CCTGACGCGTAGAATCGATTCATGT	53	1,956
pαLA-R(−)	GGTTACGCGTCAAGATTCTGA	48	
pαS1-CN(+)	ACCGCGGAGTCTTGGGTTCAAG	53	91
pαS1-CN(−)	TGTTAACCTGGCAAGAGCAACAG	50	
Derp5 cDNA 3′-coding region			
pDerp5–1(+)	TCTCGAGCATGAAATTCATCATTGC	49	250
pDerp5–1(−)	TTCTCGAGTGATGAAGGCAACAAG	46	
pDerp5–2(+)	GTGTTAACTGAAGATAAAAAACATGA	51	250
pDerp5–2(−)	TGGTGATATTTGGAACTGA	43	
Mouse GADPH internal control			
pGAPDH(+)	TACAGCAACAGGGTGGTGCA	62	250
pGAPDH(−)	TGGTACATGACGAGGCAGGT	62	

[1]Restriction enzyme (*Xho*I) cutting sites are underlined.