Identification of Polymorphisms in Thymic Stromal Lymphopoietin (TSLP) Gene and Their Association with Allergic Rhinitis

Mei-Hua Zhang, Yong-Shin Kim, Eun-Heui Jin, Ki-Mo Kim, Jae-Hoon Lee, Chun-Shi Li, Qinggao Zhang, Ki-Jung Yun, Soo-Cheon Chae, and Hun-Taeg Chung

1 Genome Research Center for Immune Disorders, School of Medicine, Wonkwang University, Iksan, Chonbuk 570-749, South Korea
2 Department of Otolaryngology, School of Medicine, Wonkwang University, Iksan, Chonbuk 570-749, South Korea
3 Department of Pharmacology, Yanbian University Medical College, 133000 Yanji, Jilin, China
4 Department of Microbiology and Immunology, Yanbian University Medical College, 133000 Yanji, Jilin, China
5 Department of Pathology, School of Medicine, Wonkwang University, Iksan, Chonbuk 570-749, South Korea

Abstract

Dendritic cell (DC) activator, thymic stromal lymphopoietin (TSLP) induces DCs to produce Th2-attracting chemokines, causing differentiation of CD4+ and CD8+ T cells into effector cells. The differentiated cells are characterized by a typical pro-allergic phenotype. In this study, we identified four single nucleotide polymorphisms (SNPs) and one variation site (g.-305delT) from the results of scanned human TSLP gene. The SNPs are as following: g.-1914A>G and g.-847C>T in promoter, g.-82C>T in 5' UTR and g.1117C>T in intron 2. We also evaluated the association of genotype and allele frequencies of these SNPs between non-allergic rhinitis controls and allergic rhinitis patients. We further investigated possible correlations between each genotype with serum total IgE levels and peripheral blood eosinophil counts in allergic rhinitis patients. The frequencies of haplotype constructed by these SNPs between these two groups were also compared. Our results demonstrate the two SNPs (g.-1914A>G and g.-847C>T) were associated with susceptibility of allergic rhinitis (P=0.006 and P=0.015 respectively), however, serum total IgE levels were not significantly related with the SNPs.

Key words: TSLP, polymorphism, haplotype, allergic rhinitis, high resolution melting.

Introduction

Allergic diseases such as allergic rhinitis, asthma and atopic dermatitis are usually happened by an excessive immune response to certain antigens called allergens. They are characterized by the predominant secretion of interleukin (IL)-4, IL-5, and immunoglobulin (Ig) E production and recruitment of mast cell, eosinophils and basophils to the site of allergic reaction (Kay, 2001; Lichtman and Abbas, 1997; Renauld, 2001). Allergic rhinitis could be initiated by the common allergens, such as pollen, house dust and mites, in atopic individuals. A series of cellular interactions on exposure to specific allergens ultimately resulted in the inflammation of nasal mucosa which is accompanied
by elevation of serum IgE levels and recruitment of eosinophil to the site of allergic reactivity (Passali et al., 2001). Naïve T helper cells differentiate into two distinct subsets of helper T1 (Th1) cell or Th2. Th1 cells release cytokines IL-2, IFN-γ, TNF-β, and Th2 cells produce IL-4, IL-5 and IL-10. Binding of the cytokines to their target receptors results in activation of transcription factors involved in signaling pathways (Ho and Glimcher, 2002). The balance between Th1 and Th2 is very important in maintaining the healthy state of the body (Ho and Glimcher, 2002). Overproduction of Th1 cytokines has been implicated in auto-immune diseases, and aberrant regulation of the Th2 type response is associated with allergic inflammation.

Thymic stromal lymphopoietin (TSLP) gene product, a novel IL-7-like hematopoietic cytokine, was proposed to signal through a heterodimeric receptor complex composed of the TSLP receptor (TSLPR) and the IL-7R alpha chain. In murine B- and T-cell development, both IL-7 and TSLP have vital functions. Human TSLP is involved in dendritic cell maturation (Leonard, 2002; Soumelis et al., 2002; Watanabe et al., 2004). The human TSLP is produced by epithelial cells, stromal cells and mast cells. The TSLP strongly activates CD11c+ dendritic cells (DCs) and induces production of the Th2-attracting chemokines TARC (thymus and activation-regulated chemokine; also known as CCL17) and MDC (macrophage-derived chemokine; CCL22) (Soumelis et al., 2002). TSLP-activated DCs prime CD4+ T helper cells produce proallergic cytokines IL-4, IL-5, IL-13, and tumor necrosis factor-γ (TNF-γ), while down-regulating IL-10 and IFN-γ. The TSLP is highly expressed by the keratinocytes of atopic dermatitis but not in other types of skin inflammation (Soumelis et al., 2002). Recently, the expression of a keratinocyte-specific and tetracycline-inducible TSLP transgene was demonstrated in transgenic mice (Yoo et al., 2005). Interestingly, skin-specific overexpression of TSLP resulted in an atopic dermatitis (AD)-like phenotype. The increased TSLP in Th2 CD4+ T cells expressing cutaneous homing receptors, elevated serum levels of IgE (Yoo et al., 2005).

In an attempt to find out correlations between physiologic functions and single nucleotide polymorphisms (SNPs), we identified the SNPs in human TSLP gene using the genomic DNA isolated from 32 individuals by direct sequencing. The association of genotype and allele frequencies between allergic rhinitis patients and non-allergic rhinitis controls was analyzed. We further investigated the relationships between these polymorphisms and IgE levels or peripheral blood eosinophil counts in allergic rhinitis patients. Finally, we calculated the haplotype frequencies that constructed by these SNPs in both groups.

MATERIALS AND METHODS

Patients and DNA samples

Blood samples were obtained from 568 controls (354 males and 214 females) and 370 allergic rhinitis patients (242 males and 128 females). The mean ages of controls and patients were 39.8 years and 23.6 years respectively. Genomic DNA was extracted from leukocytes in peripheral blood by a standard phenol-chloroform method or by Genomic DNA Extraction kit (iNTRON Biotechnology, Seongnam, Korea) according to the manufacturer's directions. The allergic rhinitis patients were recruited from the outpatient clinic of Wonkwang University Hospital. The diagnosis was based on symptoms of sneezing, watery rhinorrhea, nasal obstruction, and the result of a positive skin test. The skin test was performed with six common aeroallergens from house dust mites, house dust, grass mix, tree pollens, animal dander, and molds (Torii Tokyo, Japan). All of the patients with allergic rhinitis had a history of the symptoms and had a positive skin test. The controls were recruited from the general population who took a comprehensive medical examination at Wonkwang University Hospital. All subjects in this study were Korean and were living in the same area.

Polymerase chain reaction (PCR) and sequencing analysis

The entire coding regions of TSLP, including 2.0 kb promoter regions, were partially amplified with using GeneAmp PCR system 9700 thermocycler (Applied Biosystem, Foster City, USA) and three primer pairs (Table 1). PCR reactions were prepared by previously described condition (Chae et al., 2004). Amplification was carried out in a GeneAmp PCR system 9700