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Water-soluble chitosan-based antisense oligodeoxynucleotide of interleukin-5 for treatment of allergic rhinitis

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Abstract

Interleukin (IL)-5 produced by allergen specific T cells is a major cytokine in the allergic inflammation such as allergic rhinitis (AR). To inhibit the production of IL-5, water-soluble chitosan (WSC)-based IL-5 antisense oligodeoxynucleotide (AS-ODN) complex was generated. WSC, a biocompatible cationic polymer, was used as a non-viral vector for the improvement of stability and transfection efficiency. After condensation IL-5 AS-ODN with WSC, the size, morphology and zeta potential analysis of IL-5 AS-ODN/WSC complexes were performed. The protective effect of complex was also observed against the enzymatic degradation. *In vitro* transfection efficiency into H1299 epithelial cells was investigated by flow cytometer and inhibition effect of IL-5 levels was also evaluated in D10.G4.1 cells. In the murine model with AR, the IL-5 and IgE levels closely related to the allergic inflammation were significantly reduced after the intranasal administration of IL-5 AS-ODN/WSC complexes. Based on these results, the condensation with WSC improved the physicochemical stability and transfection efficiency of IL-5 AS-ODN/WSC complex. Our results suggest that AS therapy using IL-5 AS-ODN/WSC complex can be an effective strategy in regulating IL-5 and may be applied to the treatment of allergic disorder related to IL-5.

Keywords: Interleukin-5; Water-soluble chitosan; Antisense oligodeoxynucleotide; Allergic rhinitis

1. Introduction

Allergic rhinitis (AR) is induced by the development of nasal inflammation in response to natural allergens such as pollen, ragweed, dust mite and pet dander. Statistically 20–40 million people, 10–30% of adults and up to 40% of children, suffer from AR in USA [1]. AR, either seasonal or perennial, has nasal symptoms which include sneezing, itching, runny nose and nasal congestion and can have a significant impact on quality of life, psychological well-being and capacity to function in activities of daily life [2,3]. The inflammatory allergic response described above is characterized by the production of numerous cytokines and chemokines by activated cells including T cells, mast cells, macrophages and eosinophils. In particular, an imbalance in the cytokines in Th1 and Th2 cells that leads to Th2 predominance occurs in patients with allergic disorders [4].

Among the cytokines or chemokines associated with Th2 cells, IL-5 has been described as the major haematopoietin responsible for the terminal differentiation of eosinophils and it has been shown to be involved in eosinophilic inflammation when there is a raised level of IL-5 in body fluids [5]. IL-5 has also been implicated in local eosinophil recruitment into tissue during allergic reactions and it promotes eosinophilc maturation, adhesion, survival, and activation [6]. Many studies regarding the function of IL-5 and eosinophils in allergic disease have recently been reported. IL-5 levels have been found to be significantly increased in symptomatic subjects with allergic diseases [7]. Positive correlations have also been shown between the levels of eosinophil mediators and IL-5 and the symptoms of seasonal AR [8]. Other reports have indicated that IL-5

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acts directly as a chemoattractant and as an activator for eosinophil recruitment in allergic inflammatory disease [9,10].

In keeping with this concept, an IL-5 antisense oligodeoxynucleotide (AS-ODN) was designed for the treatment of AR by reduction in IL-5 levels due to the blocking of translation. Although AS therapy is theoretically effective, several obstacles have been reported. First, the transfection efficiency of AS-ODN itself is low because oligomers have a negative charge and their molecular shape is linear. Secondly, AS-ODN is instable *in vivo* because oligomers are easily cleaved by enzymatic degradation under physiological conditions. The modification of phosphodiester bonds in the AS-ODNs and the incorporation of AS-ODNs into viral or non-viral vectors have been attempted in order to avoid these problems [11]. Among these approaches, the viral vector system has a high transfection efficiency, but is liable to endogenous virus recombinations, oncogenic effects and immunological reactions [12,13]. The non-viral vector system is relatively safe and less immunogenic than the viral vector system. It is also easier to control the size of the DNA complex in the non-viral vector and to scale it up [14].

In the previous reports, several vehicles for the gene delivery system have been described. These include cationic liposomes, which are nucleic acid–cationic lipid complexes, and polyplexes, which are complexes of nucleic acids and cationic polymers, mainly polyethylenimine (PEI) and chitosan [(1,4)-2-amino-2-deoxy-β-D-glucan]. Chitosan is a suitable natural cationic polymer vehicle as it has low toxicity and high positive charges. Due to these advantages, chitosan has been used in gene delivery systems to enhance the transfection efficiency and stability, and to reduce cytotoxicity [15,16]. Chitosan–DNA nanoparticles have also been studied for oral gene delivery and a chitosan–plasmid delivery system as a condensing carrier has been also attempted [17,18]. Therefore, water-soluble chitosan (WSC, 40 kDa) was used in this study since common chitosan has a low solubility due to its crystalline structure [14,15]. The aims of this study were to produce IL-5 AS-ODN/WSC complexes (chitoplexes) and to evaluate its physicochemical properties and therapeutic effects in the treatment of AR.

2. Materials and methods

2.1. Reagents

IL-1 alpha was purchased from R&D Systems (Minneapolis, MN, USA). Conalbumin (ConA) and ovalbumin (OVA) were purchased from Sigma (St. Louis, MO, USA), and alum was purchased from Pierce (Rockford, IL, USA). Rat T-STIM factor, recombinant mouse IL-5, purified rat anti-mouse IL-5 monoclonal antibodies (mAbs), and biotinylated rat anti-mouse IL-5 mAbs were purchased from Becton Dickinson (Bedford, MA, USA). Water-soluble HFP-Chitosan® (40 kDa), as a cationic natural polymer, was purchased from Jakwang Co. Ltd. (South Korea).

2.2. Synthesis and design of ODN

All ODNs containing IL-5 AS and nonsense were synthesized at Bioneer Corporation (Daejeon, South Korea). These ODNs were phosphorothioated to increase their stability while their sequences were being designed. The sequences of AS-ODN corresponded to 5′-AAGCCTCATCGTCTCATTGCTT-3′ and NS-ODN corresponded to 5′-GGTCTCACCTCC CAACTGCTTC-3′.

2.3. Cell culture and animals

IL-5 secreted from the D10.G4.1 murine Th2 cell line was obtained from Dr. E.J. Park (Institute of Molecular Biology and Genetics, Seoul, South Korea). Cells were maintained in RPMI 1640 medium supplemented with 0.05 mM 2-mercaptoethanol, 10 pg/mL IL-1α, 10% fetal bovine serum (FBS), and 10% rat T-STIM™ supplement. In order to induce the D10.G4.1 cell line to produce IL-5, the cells were treated with ConA (250 ng/mL) for 2 days. The amount of IL-5 in the medium of these stimulated cells was analyzed via an enzyme-linked immunosorbent assay (ELISA). Another lung cell line, H1299, was seeded and cultured in 24-well plates for the analysis of transfection efficiency.

Female BALB/c mice (6–8 weeks old) were housed in the specific-pathogen free (SPF) animal facility of Seoul National University (Seoul, South Korea). All animal experiments were performed in accordance with the regulations of the Korean National Board for Laboratory Animals.

2.4. Preparation of the WSC-based murine IL-5 AS-ODN complex

WSC (40 kDa) was dissolved in a 100 mM sodium acetate buffer (pH 5.5) to give a 2% stock solution. Chitoplexes for the *in vitro* transfection study were prepared by adding 0, 1, 3, 5 and 10 equivalents of chitosan nitrogen per DNA phosphate (N/P ratio) in 50 μL of saline to 2 μg (0.6 pmol) of ODN in 50 μL of saline. The amount of ODN was increased to100 μg for the intranasal treatment of AR. AS-ODN chitoplexes were produced by mixing the WSC solution and the ODN for 30 s. After letting the mixture stand for 30 min, IL-5 AS-ODN chitoplexes were ready for use in the experiments [19].

2.5. DNA retardation assay and fluorescence decay assay

The condensation of the IL-5 AS-ODNs with the WSC was determined using the agarose gel retardation assay. Five μg IL-5 AS-ODNs and increasing amounts of WSC were mixed in 25 μL of saline and incubated for 20 min. Aliquots of the samples (20 μL) were resolved onto a 0.6% agarose gel and visualized with a UV transilluminator.

A fluorescence decay assay was performed to confirm complex formation and condensation of the chitoplex. In this case, FITC-labeled IL-5 AS-ODNs (2 μg) at different charge ratios were prepared in saline. The IL-5 AS-ODNs were labeled with FITC prior to their complexing with WSC. The samples were loaded onto a 96-well microplate and green fluorescence was measured (Ex = 485, Em = 535 nm) using a microplate fluorimeter.

2.6. DNase I protection assay

Three μg IL-5 AS-ODNs and increasing amounts of WSC were added to 50 μL Tris–HCl buffer (10 mM) and mixed for 20 min. Ten units DNase I were added and the mixtures were incubated at 37 °C for 20 min. Ten μL EDTA (0.5 M) were added and the samples were placed on ice to block DNase I activity. Fifty μL (1250 units) of heparin were added and the samples were incubated at 4 °C for 1 h to complete the dissociation of the chitoplexes. Subsequently, ODNs were extracted using phenol/chloroform and subjected to agarose gel electrophoresis (0.6%) as described above.