S 1-3

Intra-nasal Delivery of the Cytoplasmic Domain of CTLA-4 Using Novel Protein Transduction Domain Prevents Allergic Inflammation and Hyper-responsiveness

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Asthma is a chronic lung disease where inhalation and exhalation are obstructed by the production of excess mucus and the swelling of the airway membranes, giving rise to coughing and wheezing. A dysregulated Th2 immune response readily induced in the setting of the modern environment causes pulmonary inflammation, airway eosinophilia, mucus hypersecretion and airway hyperreactivity (AHR) to a variety of specific and nonspecific stimuli that result in the symptoms of asthma. Recent studies have emphasized that activation of $CD4^+$ T helper lymphocytes of the Th2 subset is important to initialize allergic asthma. While efficacy of soluble IL-4 receptor and anti-IL-5 mAb for the asthma management was demonstrated, IL-13 is thought to be considerably more important than other Th2 type cytokines in the induction of AHR, pulmonary fibrosis, and goblet cell hyper-plasia in antigen-induced murine asthma model.

CTLA-4 is an activation-induced surface molecule on T cell and essential for negative regulation of T cell activation. It binds to B7-1 or -2 on antigen presenting cells (APC) with an affinity of 10- to 20-fold higher than that of CD28 which is a positive costimulatory molecule for T cell activation. In recent study, neutralizing anti-CTLA4 mAb enhanced allergic sensitization and eosinophilic airway inflammation in genetically predisposed mice suggesting that CTLA-4 triggering represents an important regulatory mechanism for Th2 sensitization. It has been also reported that the phosphatases, SHP-1 or SHP-2 recruited to the cytoplasmic domain of CTLA-4, dephosphorylates CD3/TCR chains, ERK and JNK, thereby inhibits T cell activation. The cytoplasmic domain of CTLA-4 containing ITIM (Immunoreceptor Tyrosine-based Inhibitory Motif) has been found to be 100% conserved among different species, suggesting that this domain is important for negative regulation of CTLA-4 on T cell activation by sequestering intracellular signaling molecules. Therefore, the cytoplasmic domain of CTLA-4 can be an excellent molecular target for the development of immunotherapeutic drugs for asthma, autoimmune diseases and graft rejection.

Small domains, called protein transduction domains (PTD), have been developed for the delivery of therapeutic proteins into eukaryotic cells. Various PTDs have been identified and characterized within several cellular and viral proteins - Tat, Antp, Vp22 etc. While the fusion protein between these PTDs and intracellular signal mediators showed meaningful therapeutic effects in various in vitro and in vivo models

for many diseases, the lack of tissue specificity of PTDs limited their versatile therapeutic application.

In this study we identified the cell permeable novel PTD (amino acid sequence: YARVRRRGPRR) from mouse transcription factor Mph-1, and its in vitro and in vivo protein transduction capacity were evaluated using Mph-1-β-gal or -EGFP fusion protein especially through local administration routes such as nasal airway, skin and eye. The delivery of cytoplasmic domain of CTLA-4 using this novel Mph-1-PTD into T cells effectively blocked their activation at picomolar concentration. And in vivo delivery of Mph-1-ctCTLA-4 by intra-nasal administration markedly inhibited airway inflammation and hyper-responsiveness in antigen-induced asthma model, suggesting the promise of Mph-ctCTLA-4 as a new therapeutic agent for asthma.