Increased Expression of Pigment Epithelium–Derived Factor in Allergic Rhinitis

Heung-Man Lee1,2, Chang Jae Choi1, Young Gi Cinn1, Hyo-Hyun Park2, Yong-Dae Kim3

1Department of Otolaryngology-Head and Neck Surgery, 2Division of Brain Korea 21 Program for Biomedical Science, 3Korea University College of Medicine, Seoul, Korea

OBJECTIVES

Allergic rhinitis is one of the most common chronic diseases in the industrialized world. It is histologically characterized by a marked infiltration of inflammatory cells, increased vascular permeability, plasma transudation, and vasodilatation in the nasal mucosa. Angiogenesis is important in the pathogenesis of many diseases such as cancer, diabetic retinopathy, and rheumatoid arthritis. Increased angiogenesis and increased expression of the angiogenic mediator vascular endothelial growth factor (VEGF) in the nasal mucosa of patients with allergic rhinitis has been documented. Although the importance of the angiostatic mediator pigment epithelium-derived factor (PEDF) in ocular and pulmonary diseases has been demonstrated in previous studies, data regarding PEDF has not been described in the nasal mucosa in the setting of allergic rhinitis. In this study, we investigated the expression and localization of PEDF in the nasal mucosa of patients with allergic rhinitis and compared them with the expression and localization of PEDF in healthy control subjects.

MATERIALS AND METHODS

1. Subjects
   Nasal mucosa specimens
   Allergic rhinitis (M : F = 5 : 5)
      Typical nasal symptoms
      Skin prick test
      Eosinophilia on nasal smear
   Normal control (M : F = 5 : 5)
      No symptoms of nasal allergy and negative results on allergy tests
      No evidence of inflammation in nasal cavity
   Exclusion criteria
      Respiratory tract infection in past 4 weeks
      Bronchial asthma
      History of smoking

2. Preparation of nasal mucosa
   Reverse transcription-polymerase chain reaction
   Flash freezing and storing at -70°C of samples
   Immunohistochemical Staining
   Fixation with 4% paraformaldehyde
   Paraffin embedding

3. Reverse Transcription-Polymerase Chain Reaction
   PEDF primers
      Sense 5'-CAGAAGAACCTCAAGAGTGCC-3'
      Anti-sense 5'-CTTCATCCAAGTAGAAATCC-3'
   GAPDH primers
      Sense 5'-GTGGAATATTGTGCCCATCAATGACC-3'
      Anti-sense 5'-GCCCCAGCTCAGTTGCTGGT-3'
   Electrophoresis: 2% agarose gel
   Densitometry: semiquantitative analysis

4. Immunolocalization of PEDF protein
   Monoclonal anti–PEDF antibody
   Monoclonal anti–VEGF antibody
   ABC method
   3,3'-diaminobenzidine tetrahydrochloride

RESULTS

1. RT-PCR
   Healthy control
   Allergic rhinitis
   PEDF: 310 bp
   GAPDH: 271 bp

2. Semi-quantitative analysis
   Healthy control
   Allergic rhinitis
   PEDF/GAPDH mRNA ratio
   P = 0.03

3. Immunolocalization of PEDF protein
   Healthy control
   Allergic rhinitis (PEDF)
   Allergic rhinitis (VEGF)
   Epithelium
   Submucosal gland

4. Western blotting analysis
   Healthy control
   Allergic rhinitis
   β-actin
   50 kDa
   43 kDa

CONCLUSIONS

Angiostatic mediator PEDF mRNA and PEDF protein are expressed in the human nasal mucosa, and their expression is increased in allergic rhinitis. We suggest a possible contribution of PEDF to the pathogenic mechanism in the chronic inflammation of the nasal mucosa in allergic rhinitis.