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sPSGL-1 Inhibits Eosinophil Recruitment in an Ocular Allergic Inflammatory Model

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Atopic diseases which include bronchial asthma, allergic rhinitis, atopic dermatitis, and ocular allergic disorders are among the most frequent conditions experienced by patients. Allergic conjunctivitis, an atopic ocular disease, is one of the most common problems encountered in general ophthalmic practice and the only ocular inflammatory disease that represents a pure type-I hypersensitivity response. Other ocular diseases, some of which are associated with blinding corneal complications, have more complex immunological and inflammatory components. However, a unifying component of all forms of ocular allergy is the presence of eosinophils, a major inflammatory cell in the IgE-mediated late-phase reaction.

Current medical therapy for ocular allergic disorders is primarily symptomatic. To assess the efficacy of new treatment modalities and study the pathophysiology of ocular allergy, we have used a murine model that closely resembles human allergic conjunctivitis. SWR/J mice develop clinical (conjunctival congestion and erythema) and histological (eosinophil infiltration) signs of allergic conjunctivitis after exposure to short ragweed pollen. Moreover, the SWR/J mouse model represents a pure type-I hypersensitivity response to a natural allergen. As eosinophils are a hallmark of the late-phase response, the mechanisms that mediate their recruitment are integral to an understanding of allergic inflammation.

In response to a localized inflammatory stimulus, leukocyte recruitment involves the distinct phases of rolling, firm adhesion, and diapedesis. The selectin family consists of three transmembrane adhesion receptors that mediate the initial rolling interaction between leukocytes and the vascular endothelium prior to extravasation. P- and E-selectin are expressed differentially on activated endothelial cells and interact with ligands on leukocytes while L-selectin is constitutively expressed on leukocytes and binds to inducible ligands on the endothelium. The selectin family members have a similar structural organization including an amino-terminal lectin domain that binds a variety of sialylated and fucosylated oligosaccharides. However, physiologically relevant ligands for the selectins appear to be restricted to a discrete group of cell surface glycoproteins. P-selectin glycoprotein ligand-1 (PSGL-1) is the high affinity counter-receptor for P-selectin on leukocytes and is also a ligand for E-selectin. PSGL-1 is a 220 kd mucin-like transmembrane glycoprotein expressed on the cell surface of most leukocytes. A soluble, extracellular form of human PSGL-1 (sPSGL-1), constructed and expressed in COS cells, binds P- and E-selectin. As rolling is an early event in leukocyte recruitment, the inhibition of selectin function disrupts the

subsequent steps of firm adhesion and diapedesis of inflammatory cells. For example, gene-targeted mice lacking selectin expression display deficiencies in leukocyte rolling and leukocyte-dependent inflammatory responses. In addition, recent evidence suggests that oligosaccharide competitors of specific selectin ligands can provide a protective effect against inflammation in animal models. These findings offer persuasive support for the development of antiselectin therapeutic strategies to inhibit acute inflammatory responses. In this study, we assess the efficacy of human sPSGL-1 to antagonize eosinophil recruitment and ocular allergic inflammation in the SWR/J mouse model. This investigation provides strong evidence that sPSGL-1 is a potent inhibitor of eosinophil recruitment and late-phase allergic inflammation.

To determine the effects of sPSGL-1 on eosinophil recruitment, we have modified the previously described experimental protocol for the SWR/J mouse model of allergic conjunctivitis. Modifications were introduced to specifically permit an evaluation of the role of sPSGL-1 on eosinophil recruitment and allergic inflammation. Animals were sensitized by topical treatment of the nasal mucosa with 2.5 mg of short ragweed pollen for 6 consecutive days. The conjunctival inferior fornix of the right eye was subsequently challenged with the same allergen three days later. Mice received an intraperitoneal injection of either 300 μ l of sterile phosphate buffered saline (PBS) or were treated with PBS containing 200 μ g of sPSGL-1 one hour prior to challenge. During the 12 hour post-challenge period sPSGL-1 or PBS was administered every 4 hours. Four experimental groups designated A-D were studied. Group A consisted of normal control mice that were neither sensitized nor challenged with allergen. In group B, mice were sensitized but not challenged with allergen. Group C consisted of mice sensitized and challenged with allergen. Finally, group D mice were sensitized, challenged with allergen, and treated with sPSGL-1. Each group was evaluated on the basis of gross appearance, histology, and immunofluorescence. Figure 2 illustrates the appearance of the right eye of representative mice from each group. Animals that were neither sensitized nor challenged (panel A) and mice that were sensitized

but not challenged display a normal appearance with the eye and periorcular regions failing to show any signs of irritation or inflammation. This indicates that the modified sensitization protocol alone did not affect the clinical appearance of the ocular or periorcular structures. In striking contrast, group C mice that were sensitized and challenged display prominent, gross changes to the conjunctiva and periorcular area. These include conjunctival hyperemia, lid redness and edema, and tearing, all classical signs of allergic conjunctivitis. However, as shown in panel D, when mice were sensitized, challenged, and treated with sPSGL-1, the physical appearance of the right eye and its surrounding external structures were indistinguishable from the control groups. In the sPSGL-1 treated group, there was essentially no visible irritation or inflammation. These gross observations suggest that sPSGL-1 treatment prevented the signs of allergic conjunctivitis and imply that sPSGL-1 could represent a novel treatment for selectin-mediated allergic inflammation.

To correlate the clinical findings with potential histologic changes, tissue sections of the right eye from groups A-D were prepared for light and immunofluorescent microscopy. Serial sections were examined by hematoxylin-eosin staining and immunofluorescence with a rabbit polyclonal serum generated against the murine major basic protein (mMBP) and prebleed negative control serum. mMBP is a granule protein found within the eosinophil secondary granule. The anti-mMBP polyclonal antibody identified tissue eosinophils allowing us to assess eosinophil infiltration. Group A (unsensitized, unchallenged) mice illustrate the normal conjunctiva and adjacent ciliary body and iris. In the hematoxylin-eosin section, the conjunctival epithelium is a discrete 1-2 cell layer and the conjunctival stroma appears as a compact structure. A few endogenous cells lacking characteristic features of eosinophils are evident in the subepithelial region of the stroma. Anti-mMBP staining was indistinguishable from a serial section stained with the negative prebleed control, demonstrating that resident eosinophil populations are essentially absent from the normal conjunctiva. Group B (sensitized, unchallenged) animals showed that the normal structural characteristics of the conjunctival epithelium and stroma have been maintained, and there is no evidence of eosinophil infiltrate. We conclude that allergen sensitization by topical administration to the nasal mucosa did not alter the architecture of the conjunctiva or result in eosinophil influx or inflammation. However, group C mice that were sensitized and challenged exhibited dramatic, reproducible changes in histology. One of the most striking features was the overall expansion of the tissue dimensions as a consequence of edema associated with late-phase inflammation. The conjunctiva from group C occupies the entire frame and essentially excludes the ciliary body and iris from visual field. In addition, a substantial eosinophil infiltrate was evident; the specific presence of eosinophils was confirmed by anti-mMBP immunofluorescent staining. Two distinct anti-mMBP staining patterns were routinely observed; however, both correspond to infiltrating eosinophils. Although there was massive eosinophil infiltration into the conjunctiva by immunofluorescence, extensive degranulation was not apparent. This observation is similar to findings in a mouse model of pulmonary allergic inflammation. In group D animals, sPSGL-1 administration abrogated the pronounced inflammatory changes. The conjunctival epithelium and stroma

were normal in appearance although a few eosinophils were apparent by hematoxylin-eosin and immunofluorescent staining. The number of eosinophils in the sPSGL-1 treated group was similar to SWR/J mice that were never sensitized but merely challenged with allergen.

To acquire a more definitive appreciation of sPSGL-1 effects on eosinophil recruitment, eosinophil infiltrate in hematoxylin-eosin tissue sections was quantified for each experimental group. A comparison of groups A and B revealed no statistical difference in the number of eosinophils in the conjunctiva. Thus, sensitization did not contribute to eosinophil influx into the conjunctiva. In group C, there was almost a 500-fold increase in eosinophil numbers. However, treatment with sPSGL-1 resulted in a 35-fold reduction in eosinophil recruitment which corresponds to a 97% inhibition of eosinophil influx. Taken together, our data provide strong evidence that sPSGL-1 is a potent antagonist of eosinophil recruitment and late-phase allergic inflammation, suggesting a potential therapeutic role for this agent in ocular allergic inflammation.

To assess the relative involvement of the endothelial selecting in eosinophil recruitment in our model of allergic conjunctivitis, we have also performed blocking studies with monoclonal antibodies against P- and E-selectin. Mice that were sensitized, treated with either anti-P-selectin mAb or anti-E-selectin mAb, and subsequently challenged displayed characteristic eosinophil infiltration and epithelial and stromal edema of the conjunctiva. These results indicated that blocking by passive immunization had no effect and was essentially identical to PBS or rat IgG controls at 12 hours post-challenge. In contrast, coadministration of P- and E-selectin blocking antibodies prior to challenge prevented the late-phase inflammatory response. These findings suggest that either P- or E-selectin can mediate the initial adhesion requirements for eosinophil infiltration in ocular allergic inflammation and that the inhibitory effects of sPSGL-1 on eosinophil recruitment were mediated primarily through blockade of both the endothelial selectins. These results do not rule out the possibility of functional heterogeneity between P- and E-selectin during the initial phases of eosinophil recruitment. However, our data indicate eosinophil recruitment accompanying the late-phase response of allergic inflammation can occur in the presence of a single endothelial selectin, suggesting functional redundancy between these adhesion molecules.

The striking inhibitory effect of sPSGL-1 was achieved without any apparent adverse side effects and appears to be mediated by specific blockade of P- and E-selectin. However, recently L-selectin has also been shown to bind sPSGL-1, although at reduced affinity relative to P- and E-selectin. This observation suggests that appropriate sPSGL-1 dosage regimens may provide effective blockade of all three selectins. Although no apparent adverse side effects were associated with short-term sPSGL-1 treatment in our allergic conjunctivitis model, it is conceivable that chronic systemic administration would interfere with leukocyte trafficking. Moreover, our study has demonstrated the efficacy of intraperitoneal administration of sPSGL-1; however, alternative routes of delivery to target tissues is of considerable

interest. In a mouse model of thioglycolate induced peritonitis, intravenous infusion of sPSGL-1 has reduced neutrophil influx; however, preliminary experiments with subcutaneous injection have achieved only minimal success. In ocular inflammation, competitive inhibition of selectins with sPSGL-1 could be restricted to topical administration of the eye. Investigations are in progress to address these issues. In addition, alternative constructs and chimeric forms of sPSGL-1 are being developed to enhance the duration of anti-inflammatory activity associated with this antagonist.

The use of soluble cell-surface glycoproteins as therapeutic agents against viral infections and to modulate immune function has been investigated. Previous studies also suggest that competitors targeted against specific selectins may inhibit the inflammatory response. In a rat model of P-selectin-dependent lung injury, the intravenous infusion of sialyl-Lewis X containing oligosaccharides provided a protective effect against neutrophil-dependent inflammation. Furthermore, in mouse models of thioglycolate induced peritonitis, administration of an L-selectin immunoglobulin chimera and heparin oligosaccharides that bind L- and P-selectin reduced neutrophil recruitment. Our data provides persuasive evidence that sPSGL-1 is an efficacious antagonist of ocular allergic inflammation in a mouse model system. With the rational design of alternative sPSGL-1 structures that permit specific delivery to appropriate target tissues, this strong competitive inhibitor may have extensive applications beyond ocular allergic inflammation, for example, acute asthma therapy to inhibit eosinophil recruitment and the onset of pulmonary inflammation.