



Robin R. Hodges



Darlene A. Dartt

# Conjunctival **Goblet Cells** are **Direct Targets** of **Allergic Mediators**

ROBIN R. HODGES AND DARLENE A. DARTT\*

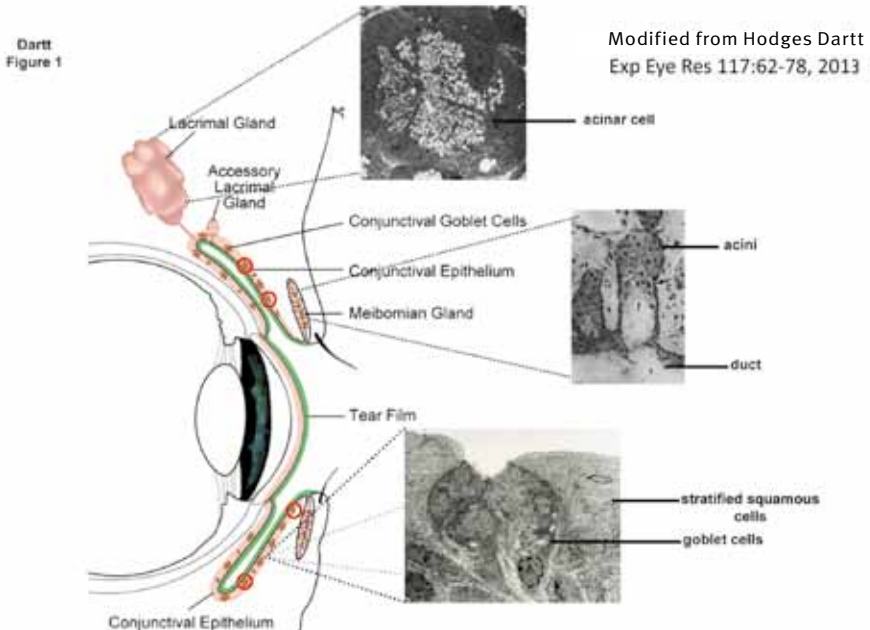
SCHEPENS EYE RESEARCH INSTITUTE/MASSACHUSETTS EYE AND EAR, DEPARTMENT OF OPHTHALMOLOGY,  
HARVARD MEDICAL SCHOOL, BOSTON, MA

**T**he tear film covers the exposed areas of the eye to protect it from various challenges including environmental (wind, light), pathogenic (bacterial, viral), and allergens<sup>1</sup>. In addition the tear film provides nourishment to and removes waste from the avascular cornea and as such is a necessarily complex fluid which is secreted by different cell types and glands (Figure 1)<sup>1</sup>. The tear film consists of a mucous layer, an aqueous layer and a lipid layer with many studies indicating that there is significant mixing of these layers<sup>2</sup>. Conjunctival goblet cells are responsible for the synthesis and secretion of the high molecular weight the mucin

MUC5AC<sup>1</sup>. This mucin, along with mucins secreted by the conjunctival stratified squamous cells, cornea and lacrimal gland comprise the mucin layer of the tear film. This layer prevents binding of pathogens and allergens to the cornea and conjunctiva. The aqueous layer is secreted by the main and accessory lacrimal glands. This layer contains proteins, many of which are anti-bacterial, water and electrolytes. The lipid layer is secreted by meibomian glands, and it is believed that this layer prevents evaporation and collapse of the thin layer of tears<sup>3</sup>.

Conjunctival goblet cells span the width of the epithelium and occur either clusters in rats or singly in humans.

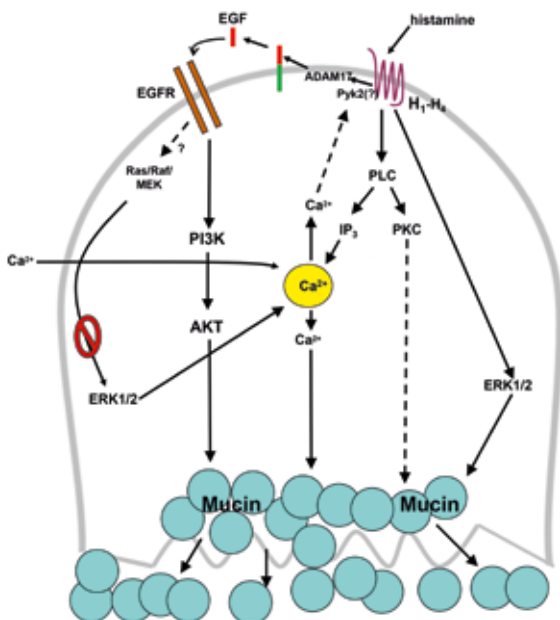
Goblet cells are packed with numerous secretory granules containing MUC5AC. The secretion of MUC5AC is tightly regulated. Too much mucin secretion, such as occurs in chronic allergy, can become excessive and contribute to disease whereas too little secretion can result in dry eye syndromes and ocular surface damage. The rate of mucin production onto the ocular surface is dependent on the number of goblet cells present in the conjunctiva i.e rate of apoptosis and proliferation, the rate of synthesis of MUC5AC, and the rate of mucin secretion. With the advent of the ability to grow pure cultures of goblet cells from excised conjunctiva, it is now possible to study human, rat, and mouse



**Figure 1. Schematic diagram of the Tear Producing Tissues of the Ocular Surface.** Acinar cells of the lacrimal gland secrete water, electrolytes, and protein. Epithelial cells of the conjunctiva, and goblet cells of the conjunctiva synthesize and secrete mucins onto the ocular surface. Meibomian glands secrete lipid into the tear film. Photographs are electron micrographs of indicated tissue. Micrograph of meibomian gland is from Jester JV, Nicolaides N, Smith RE. Meibomian gland dysfunction. I: Keratin protein expression in normal human and rabbit meibomian glands. *Invest Ophthalmol Vis Sci* 1989;30:927.

most cell stabilizers that are not always effective. In severe cases corticosteroid therapy, which has potential side effects of glaucoma, infection, and cataracts, is used. Because of the dearth of knowledge about the regulation of goblet cell mucin secretion, there are no therapies that directly regulate excessive mucin secretion that can result in blurred vision, pain and irritation, and can lead to mucus fishing syndrome<sup>10</sup>

Recently there is accumulating evidence indicating that goblet cells are direct targets of the pathogenic mediators produced during allergy and respond to these mediators with mucin secretion. This secretory activity is important in removal of allergens from the ocular surface and protection of the corneal and conjunctival epithelial cells that line the ocular surface<sup>11</sup>. In support of the hypothesis that goblet cells are direct target of allergic mediators is the finding that conjunctival goblet cells express all four of the histamine receptor subtypes (H<sub>1</sub>-H<sub>4</sub>) (Figure 2). Histamine is one the major allergic mediators and is released from mast cells and basophils. Activation of each of these receptors with either histamine or agonists specific to each receptor subtype leads to an increase in intracellular [Ca<sup>2+</sup>]<sub>i</sub> and activation of extracellular regulated kinase (ERK 1/2) which in turn leads to mucin secretion. Antagonists to each receptor inhibit these responses<sup>12</sup>. In addition, stimulation of H<sub>1</sub>-H<sub>4</sub> receptors each increased the phosphorylation of the epidermal growth factor receptor (EGFR) to stimulate an increase in [Ca<sup>2+</sup>]<sub>i</sub> and mucin secretion (Figure 2). Therefore, goblet cells can respond to



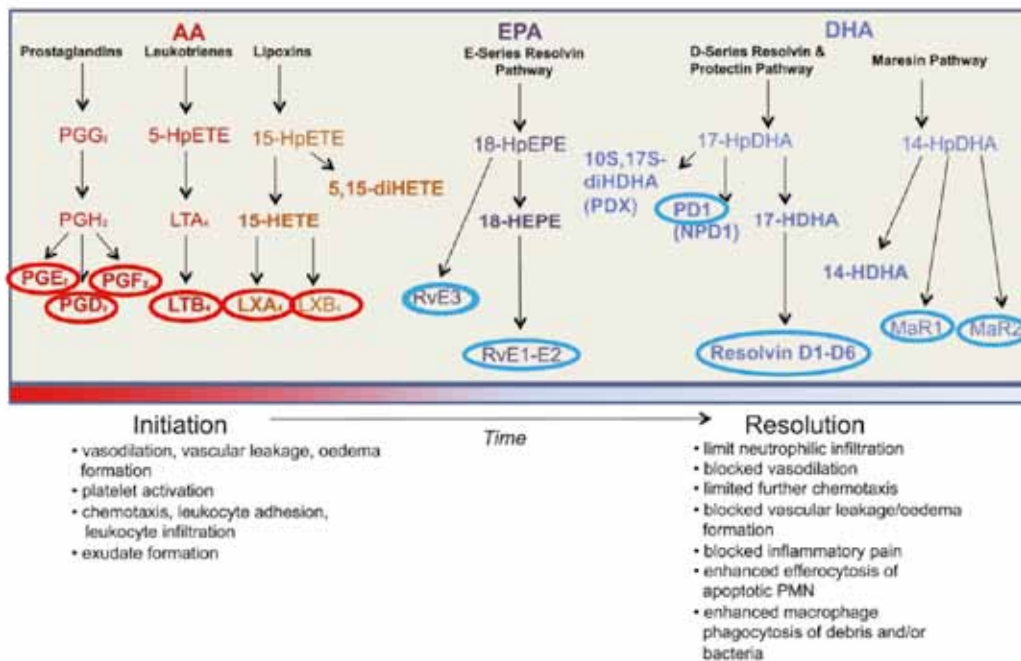
**Figure 2. Schematic diagram of the signaling pathways activated by histamine.** Solid lines indicate known pathways; dotted lines are hypothetical pathways. H<sub>1</sub>, histamine receptors; EGFR also called ErbB1; Ras, small GTPase; Raf, serine/threonine kinase; MEK, mitogen-activated protein kinase; ERK1/2 also known as MAPK; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; AKT, serine/threonine kinase, also known as protein kinase B; PKC, protein kinase C. From He M, Lippestad M, Li D, Hodges RR, Utheim T, Dartt DA. Activation of the EGF Receptor by Histamine Receptor Subtypes Stimulates Mucin Secretion in Conjunctival Goblet Cells. *Invest Ophthalmol Vis Sci* 2018;59:3543.

conjunctival goblet cells independent other cell types<sup>4,7</sup>.

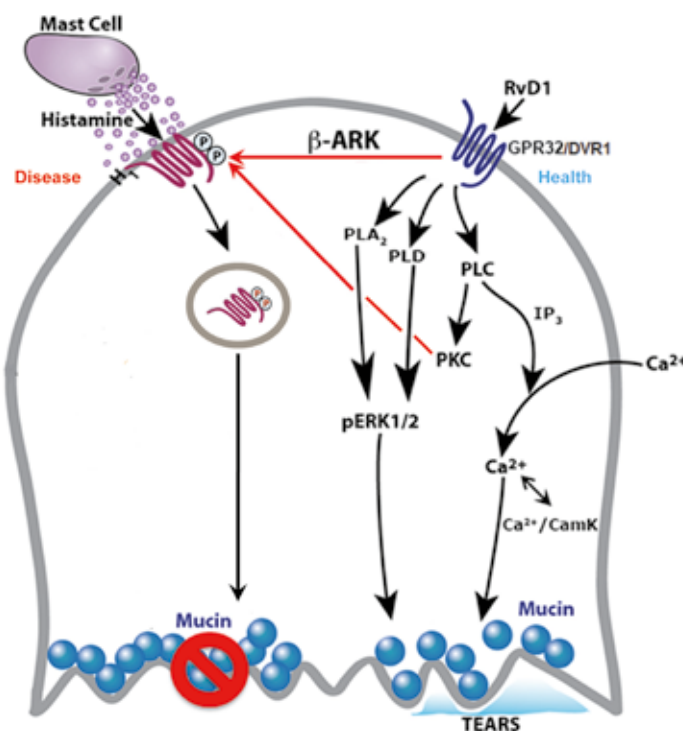
The most common types of allergic eye disease are seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis that affects 20-40% of the world's population<sup>8</sup>. Other types of allergic eye disease includes giant papillary conjunctivitis (GPC), vernal keratoconjunctivitis (VKC), and atopic keratoconjunctivitis (AKC)<sup>9</sup>. GPC is an inflammatory condition caused by mechanical irritation usually by contact lenses, while VKC is caused by seasonal allergens with more severe effects than SAC. AKC results from an increased

antibody production in response to allergens<sup>8</sup>.

Allergens, such as pollen and pet dander, interact with cells of the cornea and conjunctiva before passing into the stroma where mast cells, eosinophils, dendritic cells, and macrophages are activated<sup>4</sup>. Mast cells degranulate releasing histamine and other pro-inflammatory cytokines. Activation of these cells causes the characteristic symptoms of ocular allergies: conjunctival redness, swelling and chemosis, as well as excess tear production. Treatment regimens mostly rely on anti-histamines and



**Figure 3. Overview of eicosanoids and specialized pro-resolving mediators pathways. Each are derived from three polyunsaturated fatty acids (PUFA) namely arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Known biologic functions from the pro-inflammatory mediators involved in the initiation of inflammation and those pro-resolution families of mediators involved in the resolution of inflammation are included. Mediators detected in tear samples are highlighted in bold text and are circled. Red circles indicates mediators from omega-6 fatty acids and blue circles from omega 3 fatty acids. From English JT, Norris PC, Hodges RR, Dartt DA, Serhan CN. Prostaglandins Leukot Essent Acids 2017, 117:17-27.**

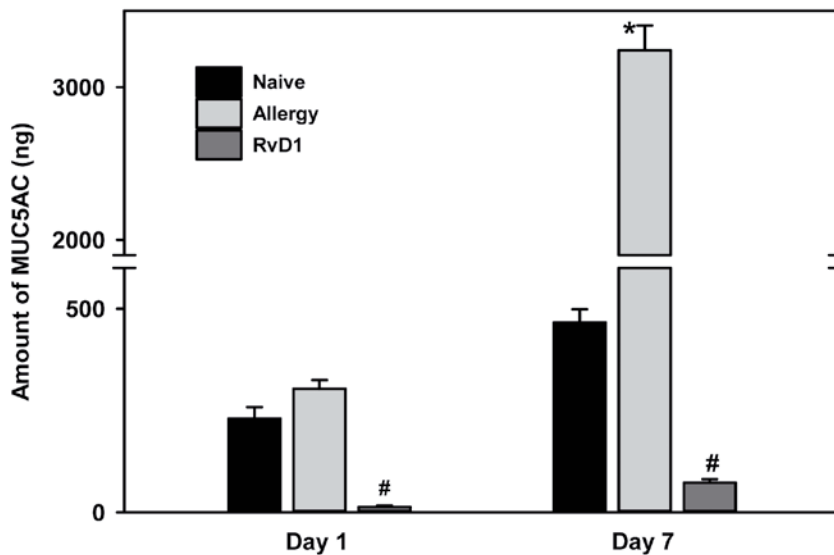


**Figure 4. Schematic diagram of signaling pathways of resolvins and its counter-regulation of the histamine receptor in conjunctival goblet cells. Histamine is released from degranulating mast cells and binds to the H1 histamine receptor subtype. This leads to activation of phospholipase C (PLC) to increase inositol trisphosphate (IP3) and intracellular [Ca<sup>2+</sup>] and to increase phosphorylation of extracellular regulated kinase (ERK) 1/2. Resolvin D1 (RvD1) binds to its receptor GPR32/DVR1 and activates phospholipase A2 (PLA2), phospholipase D (PLD) and PLC. Activation of these pathways leads to mucin secretion. In addition, RvD1 activates  $\beta$ -adrenergic receptor kinase ( $\beta$ -ARK) and protein kinase C (PKC) which phosphorylates (P) the H1 receptor to increase its internalization and inhibit mucin secretion.**

the allergic mediator histamine with mucin secretion.

Also produced and secreted during allergic responses are the pro-inflammatory autacoids such as the leukotriene (LT) LTB<sub>4</sub>, the cysteinyl leukotrienes (cysLT) C<sub>4</sub>, D<sub>4</sub> and E<sub>4</sub> and prostaglandins. The LT receptors BLT<sub>1</sub> for LTB<sub>4</sub> and cys-LT<sub>1</sub> and cys-LT<sub>2</sub> for the cysLTs are all present on goblet cells grown from rat and human conjunctiva. All four types of cysLTs stimulate mucin secretion as does another pro-inflammatory mediator, prostaglandin D<sub>2</sub><sup>13</sup>.

In addition to directly responding to allergic mediators, goblet cells are also responsible for movement of antigens from the ocular surface into the stroma. Barbosa et al have shown that antigens bind to goblet cell mucins and move into the stroma through goblet cell associated passages (GAPs)<sup>14</sup>. These GAPs can occur under homeostatic conditions as well as when cells are stimulated with cholinergic agonists that cause an opening of the GAPs within or around goblet cells. Interestingly, cholinergic agonists also increase mucin secretion from goblet cells. Antigens bind to the mucins before being transferred via GAPs to the stroma. Thus the change in the number of goblet cells and cholinergic regulation can alter



**Figure 5. Effect of RvD1 on MUC5AC secretion in mouse model of allergic eye disease.** OVA was applied topically to naïve and previously sensitized (allergy and RvD1) mice. RvD1 or vehicle were added 30 min prior to OVA challenge. After 20 min one microliter of PBS was used to wash the eye and was collected. The amount of MUC5AC was measured in eye wash by ELISA. \* indicates a significant difference from naïve values and # indicates a significant difference from allergy values. From Saban et al *Mucosal Immunol.* In Press.

antigen presentation and potentially the immune response.

As goblet cells are clearly a target for allergic mediators and responsible for allergen transport, an important goal is to treat mucin overexpression due to allergic mediators without unwanted side effects. Ocular allergies are a type of chronic inflammation. For many years, it was thought that resolution of inflammation was a passive process. However, in the past decade, it was shown to be an active process. Resolution of inflammation involves a class of compounds known as specialized pro-resolution mediators (SPMs). These mediators are biosynthesized from omega-6 and omega-3 fatty acids and are generated when there is a lipid mediator class switching from production of prostaglandins and leukotrienes (proinflammatory) to production of SPMs (proresolution) (Figure 3). SPMs include the families of resolvins (Rv), lipoxins (LX), maresins (Mar), and protectins (for a review of SPMs please see <sup>15</sup>).

Recent evidence in conjunctival goblet cells suggests that not only do many SPMs play a role in mucin secretion under inflammatory conditions, but they also play a role under normal, non-diseased conditions. Our laboratory demonstrated that family members of Rvs including RvD1 <sup>13,16</sup>, aspirin-triggered RvD1 <sup>17</sup>, RvE1 <sup>18</sup>,

and LXA4 <sup>19,20</sup> alone each stimulate an increase in intracellular  $[Ca^{2+}]_i$  and mucin secretion. In addition, we hypothesize that these SPMs are responsible in part for basal mucin secretion and may play a role in the formation of GAPS. In addition, D-series resolvins, protectin D1, and LXA4 have been identified in the tears from healthy individuals <sup>21</sup>. Under inflammatory conditions, these SPMs counter-regulate the H1 histamine receptor to inhibit histamine-stimulated mucin secretion. RvD1 counter-regulates the H1 receptor through activation of  $\beta$ -adrenergic receptor kinase (BARK) and protein kinase C (PKC) (Figure 4) <sup>17</sup>. These kinases phosphorylate the H1 receptor to increase its down regulation and internalization that decreases its signaling ability. LXA4 also counter-regulates the H1 receptor using BARK, but not PKC <sup>20</sup>. As histamine receptors are mediators of mucin secretion in conjunctival goblet cells, this downregulation could play an important role in curbing excess secretion that occurs in ocular allergy.

A recent study from our lab demonstrates the efficacy of SPMs as treatment for ocular allergies. We used a mouse model of severe ocular allergy, allergic eye disease <sup>22</sup>. Mice were sensitized with ovalbumin (OVA) via intraperitoneal injection. After two weeks, these mice received daily, topical OVA, which caused a chronic severe,

allergic eye disease complete with increases in clinical symptoms such as excess tearing and mucin secretion, chemosis, fibrosis, and conjunctival redness. Pretreatment with topical RvD1 added before OVA returned the eye to homeostasis as determined by scoring of the clinical symptoms, including MUC5AC secretion <sup>22</sup> (Figure 5). Interestingly while mucin secretion was decreased in the eye with allergic eye disease after treatment by RvD1, the number of goblet cells in the conjunctiva did not change over the course of disease or treatment<sup>22</sup>. This implies that SPMs do not alter the proliferation or death of goblet cells, but rather alter the ability of the cells to secrete mucins. Importantly, RvD1's mechanism of action was at the ocular surface only and was not a systemic effect. This study demonstrates a potential novel treatment for ocular allergies.

Goblet cells play a major role in the response of the ocular surface in the response to allergens and inflammation. Allergic mediators such as histamine and LTs act directly on goblet cells which respond by secretion of the mucin MUC5AC to protect the cornea and conjunctiva. Goblet cells also respond to SPMs that could provide unique opportunities for treatment of allergic eye disease and protection of corneal and conjunctival health.

References: [www.oftalmolog.com](http://www.oftalmolog.com) ■