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Conjunctival Goblet Cell Function: Effect of Contact Lens Wear and Cytokines

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Abstract

This review focuses on conjunctival goblet cells and their essential function in the maintenance of eye health. The main function of goblet cells is to produce and secrete mucins that lubricate the ocular surface. An excess or a defect in those mucins leads to several alterations that makes goblet cells central players in maintaining the proper mucin balance and ensuring the correct function of ocular surface tissues. A typical pathology that occurs with mucous deficiency is dry eye disease, whereas the classical example of mucous hyperproduction is allergic conjunctivitis. In this review we analyze how goblet cell number and function can be altered in these diseases and in contact lens wearers. We found that most published studies focused exclusively on goblet cell number. However, recent advances have demonstrated that, along with mucin secretion, goblet cells are also able to secrete cytokines and respond to them. We describe the effect of different cytokines on goblet cell proliferation and secretion. We conclude that it is important to further explore the effect of contact lens wear and cytokines on conjunctival goblet cell function.

Keywords

goblet cell; contact lens; cytokines; dry eye; giant papillary conjunctivitis

Goblet cells are highly specialized epithelial cells, present in mucosal tissues along the body. The main function of these cells is to produce and secrete mucins, which hydrate and lubricate mucosal surfaces.¹ Under non-pathologic conditions in the eye, goblet cells are confined to the conjunctival epithelium.

Along with the corneal epithelium and the tear film, the conjunctival epithelium is part of the ocular surface.² Both corneal and conjunctival epithelial cells produce different mucins, but the main mucin-secreting cells are conjunctival goblet cells.

Mucins are highly glycosylated glycoproteins, consisting on a protein core and multiple side chains.³ In the human, up to 20 mucin genes have been identified. Mucins are classified into two different types: membrane-spanning mucins and secretory mucins.⁴ One of the most studied mucins is MUC5AC, a large gel-forming secretory mucin found in conjunctiva. MUC5AC or a similar mucin MUC5B are located in airway mucosa and the gastrointestinal tract.⁵ Conjunctival goblet cells are the only cells secreting MUC5AC onto the ocular surface, and for that reason, MUC5AC is one of the best markers for goblet cell identification.

In this review we analyzed what techniques have been developed to identify goblet cells and analyze their changes in different conditions. We focused on dry eye and allergic conjunctivitis due to the high prevalence of these diseases. In addition, we reviewed literature studying goblet cell variations in contact lens wearers, with special attention to patients suffering giant papillary conjunctivitis (GPC). Unfortunately, conjunctival goblet cells have not been studied as much as goblet cells in other mucosal tissues. For that reason, we summarized the effect that several cytokines have on either conjunctival or non-conjunctival goblet cells. Cytokines are immunomodulatory agents that are altered in immune diseases. Altered levels of these molecules in ocular diseases and in contact lens wearers have been broadly studied. For that reason, we aimed at drawing a global picture of the main effects exerted by cytokines in goblet cells. Bearing all this in mind, and knowing which cytokines are altered in which conditions, we hope this review will guide further research.

IDENTIFICATION OF GOBLET CELLS

Due to the impact of goblet cell alteration in ocular surface diseases, several techniques to identify these cells have been developed. Goblet cells are usually identified in biopsies or cytologies (mainly, conjunctival impression cytology⁶) using a classical chemical staining that interacts with mucopolysaccharides, namely periodic acid-Schiff (PAS) staining (Figure 1a). With this method, filled goblet cells are identified, but since the staining is based in the reaction with their mucous content, it is not possible to detect those cells that have already released their products.

Goblet cells release their products in an apocrine manner. When they receive the appropriate stimuli, they secrete all the contents of their secretory granules at once. As a consequence, those cells that have secreted their mucins are completely empty and, remain invisible to the PAS staining. The same problem remains if MUC5AC or lectins such as UEA-1 or HPA are used to identify goblet cells. In 1997, Krenzer and Freddo described that cytokeratin (CK) 7 stained the cell body of goblet cells,⁷ allowing the identification of all goblet cells, regardless whether they had already secreted their contents or not. Thus, it is possible to identify the total number of goblet cells, filled and empty, using co-immunolocalization with CK7 and HPA or MUC5AC⁸ (Figure 1b).

Another technique to identify goblet cells is *in vivo* laser scanning confocal microscopy.⁹ This technique allows evaluation of tissue structure *in vivo* identification of goblet cells¹⁰ (Figure 1c). The main advantage compared to conjunctival impression cytology, is that confocal microscopy is not invasive at all, whereas cytology is a minimally invasive method. Furthermore, cytologies or biopsies need histological processing that can introduce artifacts, what can invalidate the study or diagnosis; whereas *in vivo* confocal microscopy does not.

IN VITRO MODELS TO STUDY CONJUNCTIVAL GOBLET CELL FUNCTION

A generally accepted theory some decades ago was that the only function of goblet cells was to secrete mucins and that those mucins acted only in lubricating the ocular surface. Recent studies have demonstrated that goblet cells have more functions, and that they also produce more substances apart from mucins. Cytokine secretion has been described and an immunomodulatory function of conjunctival goblet cells has been identified based on their ability to modulate dendritic cell phenotype.¹¹ Moreover, it has recently been proven that intestinal goblet cells play a role in presenting food antigens to dendritic cells¹² which opens a new field of potential treatments against inflammatory bowel disease or celiac disease. Thus, we now know that goblet cells are much more than just mucin-secreting cells and that their mucins have more important functions than simply lubrication.

In fact, the ocular surface depends in part on the levels of mucins present in the tear film to keep its integrity, and in turn, this surface depends largely on goblet cell number and their rate of production and secretion of mucins. These mucins protect the ocular surface against desiccation, but also against pathogen access. Thus, conjunctival goblet cells are one of the first lines of defense of the ocular surface and the entire eye.¹³

Recent discoveries of unexpected functions of goblet cells makes it imperative to study further the physiology of these cells in both health and disease. However, goblet cells are slow-cycling cells, so it is difficult to culture and expand them *in vitro*. For that reason, few *in vitro* studies were performed until 10 years ago. In 2001, Shatos et al. published a method to culture primary goblet cells from rat conjunctiva.¹⁴ Later, in 2003, this technique was developed for human cells.¹⁵ Since then, the number of reported *in vitro* studies using goblet cell cultures has experienced a significant increase.

The lack of human tissue sometimes makes it difficult to advance the study of goblet cell pathophysiology. However, parallel studies using both rat and human cultured goblet cells have demonstrated that rat cells are a good model for the human ones.^{16,17} The main difference between rat and human goblet cells is distribution within the conjunctiva. Human goblet cells usually occur as single cells, mainly in the external layers of the epithelium, whereas rat cells are often associated in clusters. Regarding signaling pathways and cellular functions, results in the species are similar.

ROLE OF GOBLET CELLS IN OCULAR SURFACE DISEASE

As previously mentioned, goblet cells are altered in several diseases (Figure 2). While their specific role in pathologies affecting the gastrointestinal tract or the airway mucosa has been widely studied, the study of their function in ocular surface diseases is at an earlier stage.

Ocular surface diseases are often associated with inflammation.^{18,19} Several cell types are involved in the inflammatory reaction. An important cellular participant is the T helper (Th) cell. Depending on the pattern of signals these cells receive, different types of Th cells develop, the most studied being Th1, Th2, Th17, and regulatory cells (Treg).^{20,21} Each Th cell subtype produces a specific profile of molecules that modulate the immune response. Cytokines are one of these immunomodulatory molecules, and are also classified as Th1, Th2, or Th17 cytokines, among others. The predominant Th response pattern varies between diseases.

From the wide range of pathologies affecting the ocular surface, the role of goblet cells has been most extensively studied in two main conditions dry eye and ocular allergy. These two diseases have been classically associated with different Th responses. Dry eye is a predominantly Th1-mediated disease²² and allergic diseases are typically Th2 inflammatory responses.²³

Dry Eye Disease

Dry eye affects millions of people worldwide.^{24,25} In this inflammatory disease the lacrimal functional unit is altered.²⁶ Increased levels of several cytokines, such as IFN- γ , TNF- α , or IL-6, along with goblet cell loss have been reported in this disease (Figure 2b). In fact, the lack of goblet cells has been the topic of numerous studies on dry eye.^{27–31}

Ocular Allergic Diseases

Allergic diseases of the cornea and conjunctiva are typically associated with mucus hypersecretion. Higher goblet cell densities and/or hyperplasia are usually found in this type of disease (Figure 2c). Allergy has been classically associated with the presence of Th2 cytokines. They are not the only type of cytokines involved in these diseases, but Th2 cytokines, such as IL-4 or IL-13, are especially important in allergy. There are four types of allergic diseases affecting the eye, namely allergic conjunctivitis (seasonal or perennial), vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC), and giant papillary conjunctivitis (GPC).³² The inclusion of GPC in this group of allergic diseases is controversial because the pathophysiological features are quite different from the other diseases. For that reason, and because of its special relation with contact lenses, we will comment separately on this disease.

Giant Papillary Conjunctivitis

GPC is an adverse ocular reaction to contact lenses, and can occur with both soft and rigid contact lenses.^{33,34} Although it is predominantly associated with contact lenses, additional etiologies such as sutures, corneal foreign bodies, or prostheses have been reported.^{33,34} Common symptoms usually reported by GPC patients are decrease comfortable CL wearing time, excessive lens movement, foreign body sensation, and blurred vision.

The inclusion of GPC in the group of ocular surface allergy is controversial because this disease is a non-IgE mediated hypersensitivity.³⁵ The incorrect inclusion of GPC as an allergic disease was also pointed out by the mechanical theory that suggested an irritative and mechanical etiology for GPC, rather than an allergic etiology.^{36,37} This hypothesis is

also supported by reported cases due to sutures or foreign bodies. For these reasons, GPC is now included in the group of non-allergic hypersensitivity disorders.³⁸ However, for many years, GPC was considered an allergic disease. This mistake was probably due to the clinical symptoms that are similar to those observed in allergic diseases: itching, tearing, mucous hyperproduction, and an increase of symptoms during spring pollen season.³⁹ In addition, higher levels of IL-4 and IL-13 have also been found in this pathology. GPC is characterized by papillae on the upper tarsal conjunctiva. There is also a significantly greater number of inflammatory cells in the conjunctiva of these patients, especially in the epithelium.³⁴

In summary, GPC is an ocular surface disease, presumably induced by contact lens wear, with mucous hyperproduction as one of its main symptoms. As we previously explained, the main mucous-producers cells in the ocular surface are goblet cells, so GPC links contact lens with goblet cell function.

CONTACT LENS WEAR AND GOBLET CELL FUNCTION

The influence that contact lenses have on the ocular surface mucous system started to be explored in the 1980s. The Allansmith group published several studies regarding non-goblet epithelial cells in human conjunctiva, and concluded that there were more secretory vesicles in non-goblet epithelial cells in contact lens users.^{40–42}

An extensive review about contact lens wear and goblet cells was published in 2011 by Doughty.⁴³ In that review, the author highlighted the contradictory results found in the literature regarding the effect of contact lens wear on conjunctival goblet cells. The majority of authors found a large decrease in goblet cell numbers in contact lens users,^{44,45} several others described the opposite,^{46,47} and two studies found no differences in goblet cell density.^{48,49} As Doughty explains in his review, the reason for this variability could be inconsistency in the methodologies used.

More recently, the Tear Film and Ocular Surface society (TFOS) International Workshop on Contact Lens Discomfort also summarized the changes found in goblet cell density induced by contact lens wear.⁵⁰ As well as Doughty, they also remarked on the variations in the methodology as the potential cause for the diversity of results. Another possibility for the diverse results is that the number of goblet cells per unit area varies with location on the conjunctiva.^{51,52} If goblet cells are not sampled from the same area in each study participant, the results of a study are not valid. In addition, since the contact lens interacts differently with each area of the conjunctiva, if studies use different areas of the conjunctiva they could obtain diverse results. In fact, the TFOS study concluded that the location at which the cytology sample is obtained is likely the main reason of the variations in study results.

However, all the studies described in the previous paragraph focused on the effect of contact lens wear on goblet cell density, but not on other possible influences on goblet cell function. Surprisingly, the authors have not yet explained the reason for changes in goblet cell density. Interestingly, several recent studies have shown the effect of a variety of cytokines on goblet cells, and changes in cytokine levels (mainly increased or decreased) have been

reported in contact lens wearers.^{53–57} Research investigating the effect of contact lens wear on goblet cell function is critically needed.

CYTOKINES AND CONJUNCTIVAL GOBLET CELL FUNCTION

Cytokines are immunomodulatory agents that are secreted by different types of cells. Every cell, except the red blood cell, can produce and respond to cytokines.⁵⁸ In addition there is a wide range of cytokines, with different biochemical characteristics and biologic functions. However, all of them play an important role in cell signaling. As regulators of the immune response cytokines can exert pro-inflammatory actions, anti-inflammatory actions, or both. These compounds can be classified into different subtypes, depending on the type of T helper (Th) cell that produces them.²⁰ The main subtypes are: Th1, Th2, and Th17.⁵⁹

Th1-derived cytokines

Interferon gamma (IFN- γ)—IFN- γ is a key cytokine coordinating immune defense.⁶⁰ It is probably the most studied cytokine in relation to conjunctival goblet cell function. The main reason for this focus is the clear implication of IFN- γ in dry eye disease, as well as the extensive interest in understanding this quality of life-threatening condition. Increased levels of IFN- γ in tears of dry eye patients have been reported by several authors.^{61,62}

IFN- γ binds to its receptor IFN- γ -R to trigger a cell signaling cascade.^{60,63} The presence of IFN- γ Rs in goblet cells has been confirmed in C57/BL6 mice,^{64,65} and we have recently described it in cultured rat and human goblet cells (manuscript under review). The main signaling pathway used by IFN- γ is the JAK-STAT cascade. However, some other alternative pathways have been described.⁶⁶

IFN- γ has an apoptotic effect on goblet cells.^{30,64,65} This would explain the loss of goblet cells typically found in dry eye patients. In addition to finding a decrease in goblet cell proliferation by IFN- γ , we have also demonstrated that the secretion of the remaining cells is also altered in the presence of IFN- γ when rat cells were investigated. We showed that this cytokine by itself induced an increase in $[Ca^{2+}]_i$, which relates to high molecular weight glycoconjugate secretion. In addition to increasing $[Ca^{2+}]_i$, IFN- γ blocked the effect of a cholinergic agonist on inducing goblet cell secretion in rat goblet cells.⁶⁷ Similar results were obtained in cultured mouse goblet cells.⁶⁵

Tumor Necrosis Factor (TNF) alpha—TNF- α is another Th1 cytokine that is also involved in dry eye disease. This cytokine is upregulated in conjunctival epithelial cells of patients with Sjögren's syndrome-associated aqueous-deficient dry eye, but not in those with non-Sjögren's syndrome.⁶⁸ Due to its central role in the inflammatory state of dry eye disease, TNF- α blockers have been proposed as a treatment for dry eye disease.⁶⁹

The effect of TNF- α on conjunctival goblet cells has not been extensively explored, but it has been shown that TNF- α inhibits MUC5AC secretion induced by cholinergic agonists and increases goblet cell apoptosis.⁶⁵ These effects are similar to the ones exert by IFN- γ .

Th2-derived cytokines

Th2 cells are characterized by the production of IL-4, IL-5, and IL-13.⁷⁰ These cytokines have a critical role in allergic diseases affecting the mucosa, such as asthma or allergic conjunctivitis.

Interleukin 4—The presence of IL-4 is essential for a naïve Th cell to become a Th2 cell. IL-4 is necessary for Th2 lymphocyte differentiation and it is later secreted by these Th2 cells.⁷¹ This pleiotropic cytokine binds to IL-4 receptors to activate signal transduction, mainly through Jak1 and Jak2 kinases. We have demonstrated the presence of IL-4 receptor in cultured human goblet cells.⁷²

The effect of IL-4 on goblet cells has been studied in the airway epithelium.⁷³ These authors showed that IL-4 induced the differentiation of epithelial cells into mucous-producing goblet cells, and that after IL-4 exposure there was an increase in glycoconjugate secretion. These experiments were performed *in vitro* with a human pulmonary mucoepidermoid cell line and *in vivo* with BALB/c mice. We showed results consistent with those of Dabbagh K. et al in rat cultured conjunctival goblet cells.⁶⁷ We found that IL-4 increased conjunctival goblet cell proliferation by 1.94 fold over basal. In addition, intracellular $[Ca^{2+}]$ was also increased by IL-4 exposure. Intracellular $[Ca^{2+}]$ measurement after different stimuli is especially relevant in goblet cells, since it is directly related with mucin secretion.^{74,75}

Interleukin 13—IL-13 is, along with IFN- γ , the most extensively studied cytokine in relation to conjunctival goblet cells. IL-13 is a small glycoprotein with a broad spectrum of actions, such as promotion of eosinophil migration, upregulation of adhesion molecules, and goblet cell differentiation and mucous hypersecretion. IL-13 induces mucin synthesis and hyperplasia in airway goblet cells.⁷⁶ The role of this cytokine in asthma is well known, where it is a critical mediator in the pathology of this disease.⁷⁷ In fact, a vaccine that neutralized endogenous IL-13 has been proposed as a therapy to attenuate allergic inflammation in asthma.⁷⁸

In the eye, it has been demonstrated that IL-13 promotes conjunctival goblet cell differentiation and proliferation.^{65,79} These authors confirmed the expression of IL-13 receptor in mouse goblet cells, and we have done the same in human cultured goblet cells.⁷²

Interleukin 5—IL-5 is an important cytokine in allergic processes. Its main role is to enhance eosinophilic accumulation and activation during allergen-induced inflammation.⁸⁰ Although it has been extensively studied in allergy, where it is found elevated, the interaction of IL-5 with goblet cells is not clear. Lee et al. stated that IL-5 expression in the lung epithelium of mice leads to several pulmonary changes, including goblet cell hyperplasia.⁸¹ However, it is unclear if this hyperplasia is produced directly by IL-5, or if it is eosinophils-mediated.^{82,83}

Th17-derived cytokines

The Th17 cell subtype was described in 2005.^{59,84–86} These cells act as regulators of the immune response and produce IL-17, IL-6, IL-21, or IL-22, among other cytokines.⁸⁷

Interleukin 17—The prototypic member of the IL-17 family is IL-17A, a cytokine with high homology among mouse, rat, and human.⁸⁸ It has been associated with ocular inflammatory diseases, such as uveitis, scleritis, and dry eye syndrome.⁸⁹

IL-17 along with IL-6 is able to increase MUC5AC levels in primary human tracheobronchial epithelial cells.⁹⁰ Regarding the conjunctiva, Contreras-Ruiz et al. have recently shown an increase in proliferation of cultured goblet cells when cells were exposed to IL-17A.⁶⁵

Interleukin 6—IL-6 is another well studied Th17-type cytokine. IL-6 acts as an activator of the immune system, and is elevated in most inflammatory states.⁹¹ This cytokine is secreted mainly by monocytes/macrophages and epithelial cells, including conjunctival epithelial cells.^{92,93} Several authors have determined levels of IL-6 in tears.^{57,94,95} Boehm et al. described a 2.1 fold increase of IL-6 levels in dry eye patients compared to healthy controls. Others have studied expression levels of this cytokine on conjunctival epithelial cells.^{92,96} However, in most of these studies, goblet cells have not been analyzed.

Other cytokines—Recently, interest in the study of IL-22 and IL-33 in mucosal pathologies has increased.^{97–99} Turner et al. suggested a key role for IL-22 in intestinal goblet cells, showing IL-22-induced mucin secretion and goblet cell activation.⁹⁹ Luzina et al. reported that full-length IL-33 isoforms induced goblet cell hyperplasia in lungs⁹⁷ and Tanabe et al. showed that IL-33 acts directly on goblet cells stimulating IL-8 secretion, what may maintain mucus hypersecretion in asthma patients.⁹⁸ However, the effect of these cytokines on conjunctival goblet cells is still unexplored. The similarity previously found in goblet cells among different mucosa suggests that IL-33 would probably play an important role in the ocular surface that still needs to be discovered.

SUMMARY

Goblet cells have been studied in several diseases due to their mucin-secreting capacity. It is known that the number of these cells is lower in dry eye diseases, and that higher goblet cell densities are found in allergic diseases. The cytokine balances in these two diseases is also opposite. While a Th1 profile predominates in dry eye disease with special involvement of the Th1-cytokine IFN- γ , a Th2 profile predominates in allergic diseases that are associated with mucous hyperproduction. Recent findings on the effect of cytokines on goblet cells have shown that Th1 cytokines have pro-apoptotic effects on goblet cells, whereas Th2 cytokines promote goblet cell proliferation. A summary of the effect that cytokines have on conjunctival goblet cells is shown in Table 1.

Interestingly, several authors have studied goblet cell density in contact lens wearers. Most of studies have found a decrease in goblet cell number in these patients. However, the biological processes that lead to this decrease are not fully understood.

CONCLUSIONS

Recent studies about the influence of cytokines on goblet cells open a new field of research. It is important to observe how different types of Th cytokines seem to have opposite effects on conjunctival goblet cells, especially when comparing Th1 versus Th2-derived cytokines.

Goblet cells are becoming increasingly more important in the study of all types of pathologies affecting the ocular surface, from dry eye to allergies. It is surprising that, until now, most clinical studies regarding goblet cells were only focused on goblet cell number, but not on goblet cell function. The latest advances in this area are revealing previously unknown functions of these important cells and we know now that it is not only an issue of numbers.

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Figure 1. Goblet cell identification by several techniques. a) PAS staining in a human conjunctiva. b) Goblet cells identified by cytokeratin 7 (red) immunostaining and HPA lectin binding (green). c) In vivo laser scanning confocal microscopy image showing possible goblet cells, identified by its rounded shape and brightness.

Figure 2. Conjunctival impression cytologies (CIC) obtained from different patients and stained with PAS. a) CIC from a patient with no alterations in conjunctiva. Goblet cells can be identified by the PAS staining, and are distributed along all the cytology. b) CIC from a patient with dry eye disease. No goblet cells were found in the CIC. c) CIC from a patient with allergic conjunctivitis showing goblet cell hyperplasia.

Figure 3.
Schematic illustrating the effect of cytokines on goblet cells in dry eye disease and ocular allergy.

TABLE 1

Summary of cytokines effect on conjunctival goblet cells.

Cytokine	Effect	Specie	Authors
IFN-γ	↑ apoptosis	Mouse	Zhang et al. (2011, 2014) ^{64, 30} ; Contreras-Ruiz et al. (2013) ⁶⁵
	↓ proliferation	Rat	García-Posadas et al. (ARVO 2013) ⁶⁷
	↑ secretion	Rat	García-Posadas et al. (ARVO 2013) ⁶⁷
TNF-α	↑ apoptosis	Mouse	Contreras-Ruiz et al. (2013) ⁶⁵
IL-4	↑ proliferation	Rat; Human	García-Posadas et al. (ARVO 2013, 2014) ^{67, 72}
	↑ secretion	Rat; Human	García-Posadas et al. (ARVO 2013, 2014) ^{67, 72}
IL-13	↑ proliferation	Mouse	De Paiva et al. (2011) ⁷⁹ ; Contreras-Ruiz et al. (2013) ⁶⁵
	↑ GC differentiation	Mouse	De Paiva et al. (2011) ⁷⁹
IL-17	↑ proliferation	Mouse	Contreras-Ruiz et al. (2013) ⁶⁵
IL-6	↑ proliferation	Mouse	Contreras-Ruiz et al. (2013) ⁶⁵