Differential Staining of Ocular Goblet Cells

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Summary

Millipore filters were used to obtain sheets of cells from the ocular surface. Using Periodic Acid Schiff-haematoxylin the intracellular neutral mucus of the goblet cells stains a brilliant, bright pink and the cell nuclei dark blue making it possible to observe the epithelial cells and the goblet cell population.

In certain ocular surface diseases the size of the PAS-haematoxylin staining goblet cell population is reduced, returning towards normal as ocular health improves. These observations had previously been interpreted as showing degeneration followed by regeneration of the goblet cells.

However, Alcian blue stains an acidic mucus, which does not stain with PAS-haematoxylin. Acidic mucus containing goblet cells have been revealed using Alcian blue staining when added to the PAS stained cells in conditions that have previously been shown to have reduced goblet cell population when assessed by PAS-haematoxylin staining. This suggests that the staining properties of some of the goblet cells have changed. The extra goblet cells revealed by Alcian Blue staining makes the total of goblet cells present close to normal in numbers. Improved clearing methods and cytological preservation has revealed that there is also a population of goblet cells that do not stain with either technique. These observations must cast doubts on previous claims of reduced goblet cell populations associated with some extraocular diseases.

The use of filters for diagnostic cytology was first described in 1967.¹ The filter technique was an advantage because the filters were easy to handle and mount, and they were unaffected by most fixatives and stains. They were found to be useful in the examination of a wide range of body fluids, aspirates and washes. The use of Millipore filters on the ocular surface to perform a simple conjunctival biopsy was first described by Egbert and Maurice in 1977.²

This technique of impression cytology has proved a simple and atraumatic procedure that removes parts of the surface layer of cells from the ocular surface and includes a good sample of goblet cells in the layer (Fig. 1).

Nelson and Wright developed a classification for these surface cytology specimens, based on epithelial cell morphology and goblet cell density.³ They used PAS-Haematoxylin as their routine stain. With this technique the goblet cells were identified by the brilliant pink staining of their intracellular mucin.

Conjunctival goblet cell density is said to be fairly constant and not to drop with age, although it is known to decrease in diseases associated with a loss of vascularisation or inflammation. The reason for this is unclear but it has been suggested that a blood borne factor may be important in goblet cell differentiation and maintenance. This argument is supported by the fact that the avascular cor-

Fig. 1. PAS-haematoxylin stained cells showing normal cytology and a high density of goblet cells. Bar=100µm.

Fig. 2. Same sample as Figure 1 with PAS-haematoxylin and Alcian blue stained cells showing goblet cells containing pink and blue staining mucus. Bar=100µm.

nea does not have goblet cells, and it is possible to transform the morphology of conjunctival epithelium to that resembling corneal epithelium by reducing its blood supply and *vice versa*.⁴

Recently we took samples for examination of possible malignant change in a patient's conjunctiva. Our cytopathologist stained these specimens with a variety of dyes, including Alcian Blue. In these slides we saw blue staining mucus, as well as the characteristic pink of PAS-haematoxylin stained mucus in obvious goblet cells. This then led us to re-examine some of our previous slides. We found unstained goblet cells that appeared to be full, but were not staining with PAS-haematoxylin and Alcian Blue and we found that some of the previously non-staining goblet cells were now stained with Alcian Blue (Fig. 2).

Materials and Method

Samples were taken from the conjunctiva as previously described.⁵ Samples from eleven patients pre- and post-Botulinum toxin protective ptosis (BTPP) were obtained using impression cytology. Samples were also obtained from seven patients with suppurative keratitis over the course of the disease process, from six patients with uveitis, and from four patients with alkali injuries. Samples were normally taken from four sites in each patient: medial and lateral interpalpebral conjunctiva, inferior bulbar conjunctiva and superior tarsal plate after eversion of the upper lid. The cover slips from the slides that had previously been stained were

removed by soaking in xylene. The previous stains were removed by soaking in 70% industrial methylated spirit and hydrochloric acid until the dye was leached out of the filter papers. The filters were then rehydrated, stained in 1% Alcian Blue in 3% acetic acid for one minute, rinsed and stained with periodic acid, followed by Schiff's reagent, and haematoxylin, before being dehydrated, cleared in xylene and mounted on slides using Eukitt mounting reagent (O. Kindler, West Germany).

Results

Staining the sheets of cells obtained from the conjunctiva by impression cytology with the standard PAS-Haematoxylin technique reveals the epithelial cells and goblet cells. The intracellular mucus of the goblet cells stains a brilliant, bright pink. All goblet cells are said to stain with PAS-haematoxylin, which demonstrates the side chain sugar molecules of the mucus glycoprotein.⁶ This staining identifies the neutral mucus within the conjunctival goblet cells. As well as this neutral mucus some cells from other regions of the body have been reported to produce an acid mucus. The acid mucus can be demonstrated using the Alcian blue technique.

Using the PAS-haematoxylin-Alcian blue techniques we re-examined samples from patients with alkali burns, BTPP, uveitis and suppurative keratitis in whom we had found a loss or reduction in goblet cell density on PAS-haematoxylin staining. We found samples where PAS-Haematoxylin staining had not demonstrated all the goblet cells con-

taining mucus as we could see full goblet cells without PAS staining of the intracellular mucus.

This was seen in alkali burns (Figs. 3,4), suppurative keratitis (Figs. 5,6), corneal sur-

face disease requiring botulinum toxin protective ptosis (Figs. 7,8,9) and most obviously in samples from patients with uveitis (Figs. 10,11). We found some goblet cells that were non staining with either PAS-haematoxylin or

Fig. 3. PAS-haematoxylin stained cells in alkali burn. Many pale areas containing non-staining goblet cells can be seen. Bar=100µm.

Fig. 4. Same sample as Figure 3 with PAS-haematoxylin and Alcian blue stained cells in alkali burn showing neutral and acidic staining mucus. Bar=100µm.

Fig. 5. PAS-haematoxylin stained cells in suppurative keratitis with no obvious goblet cell staining. Bar=100µm.

Fig. 6. Same sample as Figure 5 with PAS-haematoxylin and Alcian blue stained cells in suppurative keratitis. Both pink and blue staining goblet cells are visible. Bar=100µm.

Fig. 7. PAS-haematoxylin stained cells in Botulinum toxin induced protective ptosis for indolent corneal ulceration. Polymorphs can be seen between cells. Bar=100µm.

Fig. 8. Same sample as Figure 7 with PAS-haematoxylin and Alcian blue stained cells in Botulinum toxin induced protective ptosis. Goblet cells containing pink and blue mucus can be seen. Bar=100µm.

Fig. 9. Higher resolution of Figure 8 to show pink and blue mucus with greater definition. Bar=25µm.

Fig. 10. PAS-haematoxylin stained cells in uveitis. No obvious goblet cells stained. Bar=100µm.

Fig. 11. Same sample as Figure 10 with PAS-haematoxylin and Alcian blue stained cells in uveitis. Note the goblet cells containing blue staining mucus. Bar=100µm.

Fig. 12. PAS-haematoxylin and Alcian blue stained cells with pink and blue staining mucus and a non-staining goblet cell. Non-staining goblet cell. Non-staining goblet cell arrowed. Bar=25µm.

PAS-haematoxylin-Alcian blue (Fig. 11). In some goblet cells the mucus within the cell boundaries was stained pink, whereas that spreading from the cell on the conjunctival surface stained blue

Discussion

Conjunctival mucus is important in corneal wetting, and it is a major factor in spreading the pre-corneal tear film over the ocular surface.⁷ Mucus is produced by the goblet cells which are normally present in the superficial and middle layers of the conjunctiva, and by the sub-surface vesicles that are found just below the surface of the conjunctival cells.⁸ The mucus produced by the sub-surface vesicles anchors the goblet cell mucus layer onto the ocular surface, by way of glycoprotein chains. In certain ocular surface diseases such as anaesthetic cornea, giant papillary conjunctivitis, and asymptomatic contact lens wearers, there is an increase in these sub-sur-

face vesicles, in association with increased mucus production.⁹

A trend to sulphation of mucus is said to be associated with a tendency to inflammation and conjunctival metaplasia.⁶ A change in the chemical composition of certain types of mucus influences the rheological properties of mucin and presumably different staining properties reveals some evidence for differing physico-chemical properties.¹¹ Excess mucus production can lead to tear instability and abnormal mucus can produce filamentary keratitis and mucous plaques. A specific pattern of mucus production would therefore seem to be required for ocular comfort and health.

It has been shown that goblet cells of the conjunctiva produce a neutral mucin and at least four differing types of acid mucus.⁶ PAS stains neutral mucus pink, whilst Alcian blue at pH3 will show acid muco-substances, which stain blue.¹⁰ It has been said that all goblet

cells stain with PAS. However we have demonstrated that there is evidence of goblet cells that do not stain with PAS, as well as some goblet cells that do not stain with either PAS-haematoxylin or Alcian blue, singly or in combination.

We have previously commented on the loss of goblet cells in a variety of ocular surface diseases with return of the goblet cell concentration as the eye condition improved.⁵ Examination of conjunctival cytological specimens from acute and resolving ocular conditions such as alkali burn, uveitis, suppurative keratitis and corneal disease treated by Botulinum toxin protective ptosis, have shown changes in the goblet cell population. In the acute stages there is a reduced density of goblet cells, some times they are absent, the cells returning in increasing numbers as the ocular condition resolves. Some of these samples were taken only four days apart but showed improvement in epithelial cell cytology as well as return of the goblet cells, with bright pink staining of their intra-cellular mucus. Although the epithelial repair of the cornea and conjunctiva is known to be quick, the rapid regeneration of goblet cells was surprising. We originally interpreted this as regeneration of the goblet cells simply because the early specimens had not stained with PAS-haematoxylin and we had, therefore, not noticed them.

However, from our findings that not all goblet cells stain with PAS-haematoxylin, or PAS-haematoxylin-Alcian blue, it is possible to reinterpret our results in terms of changing staining properties of the goblet cells. The cells themselves being present throughout, although it is possible that the goblet cell density may be reduced in certain conditions.

Our findings suggest that in several conditions it is the staining properties of the mucus of the goblet cells that changes in response to the disease process. When we can understand these changes and reverse them we may well have an important technique for the management of ocular disease states.

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