Morphological changes in the human conjunctival epithelium. II. In keratoconjunctivitis sicca

L. M. R. ABDEL-KHALEK, J. WILLIAMSON, and W. R. LEE

From the University Departments of Ophthalmology and Pathology, University of Glasgow, and the Southern General Hospital, Glasgow

SUMMARY A clinicopathological correlation was performed on 24 patients suffering from keratoconjunctivitis sicca (KCS). Examination of conjunctival biopsies by light and transmission electron microscopy revealed stratification of the conjunctival epithelium with separation of the superficial cell layers, and this was directly proportional to the clinical severity of the disease. Counts of goblet cells were found to be inversely proportional to the degree of stratification. The goblet cells were morphologically identical to, but significantly fewer in number than, those found in normal conjunctival tissue. A reduction in epithelial cell stratification and separation and an increase in goblet cell density were closely related to nasolacrimal duct occlusion—an operation performed only in the severe examples of KCS in this series. The possible causes of ‘mucous’ plaques and ‘filaments’ are discussed in relation to these phenomena. Moreover, there is a dramatic reduction and deformation in the microplicae on the surface of the conjunctival epithelium. The significance of this was considered in relation to tear film stability.

In part I of this communication the study of the normal conjunctival epithelium revealed flattening of the superficial cells and a reduction in the goblet cell density in a significant proportion of the people over the age of 79 years. These appearances were observed in very few of the younger age groups. Although it was known that the incidence of mild keratoconjunctivitis sicca (KCS) rises with age (Whaley et al., 1972), it was impracticable to examine all the individuals in this ‘normal’ control group for the presence of a dry eye. It is possible, therefore, that some of the normal group were suffering from a mild, clinically undetected form of KCS.

This background information was necessary for the purpose of the present study, in which the morphology of the conjunctival epithelium was examined in patients suffering from definite KCS in both rheumatoid arthritis (Sjögren’s syndrome) and in the sicca syndrome. An attempt has been made to reassess the observations made on the normal tissue, to establish the pathological changes which might be specific for KCS, and to assess the effect of current therapy on the epithelial morphology in KCS.

Materials and methods

 Conjunctival biopsies were obtained by the surgical technique which was described in part I except that retrobulbar anaesthesia was not required. Thirty patients volunteered for this study, and the details of age, sex, clinical severity, duration, and relevant therapy are shown in Table I. The site of biopsy was the same as that for the procedure described in part I and the methods for fixation and tissue distribution for qualitative and quantitative light microscopy and qualitative electron microscopy were identical to those previous employed. After morphological assessment 6 specimens were excluded from the group because the technical quality of the material was inadequate.

Results

From Table 1 it can be seen that the average age (± SD) of the patients, 22 of whom were female and only 2 male, was 61 ± 13. Eight patients had the sicca syndrome, there was 1 case of Still’s disease, and the remaining 15 suffered from Sjögren’s syndrome.

The clinical duration of the disease in both rheumatoid arthritis and KCS varied considerably, and this was unavoidable because the patients were selected on the basis of availability and willingness to co-operate in the study.

Light microscopy

The material was examined without prior knowledge of the patients’ clinical status and the following
morphological changes were found to be of relevance.

**Epithelial stratification**

Stratification of the epithelium (Fig. 1) was a feature common to almost every biopsy examined. In severity, however, it varied and was classified into 3 grades. Grade 0: The mildest form in which there was evidence of superficial segmental flattening and elongation in about 25% of the total length of the epithelium available for study, but this was of insufficient severity to make it readily distinguishable from the normal. Grade 1: Approximately 25 to 50% of the total length of the epithelium showed stratification. Grade 2: At least 50 to 75% or more of the total length of the epithelium showed stratification.

Eight patients were classified as grade 0, 4 as grade 1, and 12 as grade 2. This assessment was repeated on coded sections some 6 months after the first assessment and the results were essentially similar.

**Reduction in goblet cell density**

The goblet cell population was found to be reduced in nearly every case. In those specimens which showed grade 0 stratification the mean count (±SD) was 8±2, which was near to the normal level of 10±3 cells/mm. On the other hand in the specimens which showed grade 2 stratification, the goblet
cells were significantly reduced to a mean ± SD of 0.8 ± 1.0. Indeed in 4 of the 12 specimens with severe stratification there were no goblet cells in the epithelium. There appeared to be no relationship between the age of the patient, the duration of arthritis or KCS, and the conjunctival pathology and goblet cell population in this series. Moreover, with a few exceptions, those graded clinically as mild or well controlled showed little stratification, and the goblet cell counts approached the normal. The most significant observation was that those patients who were treated by nasolacrimal duct obliteration and whose treatment was considered to be satisfactory for periods varying between 2 and 5 years had few abnormal features in the morphology of the conjunctival biopsies (Table 1).

Inflammatory cells, lymphocytes, plasma cells, and polymorphonuclear leucocytes were identified in the stroma and epithelium, but the distribution and number were variable and no significant patterns emerged.

**ELECTRON MICROSCOPY**

The most marked changes in the ultrastructure of the epithelium were seen in the untreated cases of KCS in Sjögren's syndrome and in the sicca syndrome; no morphological distinctions could be made between these 2 groups of patients. The alteration in epithelial cell morphology was simply one of a change in shape, and apart from separation of the superficial cells the organelle content and the nuclear chromatin pattern remained essentially the same throughout the epithelial layers. The disordered orientation of the epithelial cells extended frequently to the intermediate cells, and in the most advanced examples of stratification the basal cell layer was involved (Fig. 2). In all cases, however, the intercellular relationships between the elongated inter-

mediate and basal cell layers were preserved, and the interdigitations and intercellular junctions were apparently normal. Nevertheless, the slight widening of the intercellular spaces, which is a feature of the normal intermediate cell layer, was less prominent in the diseased epithelium.

The principal feature of interest was a widening of the intercellular spaces between the superficial and the subjacent cells. This process of superficial cell separation was found to vary with the severity of stratification. In the mildest form no marked separation could be seen. At a later stage the intercellular space was widened and processes extended from the cells into the intercellular space, which contained fine electron-dense strands (Fig. 3). Often the underlying cells were relatively more electron-dense and contained intracytoplasmic vacuoles, which suggested involvement of this cell layer in the pathological process. The separating superficial cells contained membrane-bound structures; the rough endoplasmic reticulum showed focal dilatation and the cytoplasm was of diminished density. The microplacae were reduced in number and height. However, the anterior cell membrane was usually intact.

In the more advanced stages of separation the cytoplasm of the separating cells was electron-lucent and the organelle content was markedly diminished. Occasionally membrane-bound vesicular structures were present in the cytoplasm and the nuclear membrane was disrupted with loss of nuclear chromatin into the cytoplasm. The processes from the detaching cells were contracted, and the cytoplasm within them was electron-lucent. The anterior surface showed marked or total loss of microplacae with, in addition, disruption of the anterior cell membrane (Fig. 4). By contrast the intercellular apical junctions were preserved and the attachments
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between the cells of the superficial layer persisted, so that elongated strips of cells were detached from the subjacent layer.

In all grades of involvement the morphology of the goblet cells was identical to that described in the control group. Hyaline bodies were not observed in any of the biopsies in the KCS series.

Discussion

The most important feature in this investigation was the almost constant finding of stratification in the epithelium of patients suffering from KCS. Whether this is the result of reduced lacrimal secretion in part or whole is a matter for speculation. In this study the most severe forms of stratification were to be found in those patients classified clinically as severe forms of KCS. Furthermore, an apparent reduction in stratification and an improvement in the goblet cell count was closely related to nasolacrimal duct occlusion. Although the numbers in the series are few, this observation was consistent, and its significance is enhanced when it is noted that only severe examples of KCS were treated by this operation.

Thus, if the improvement in the tear coverage following the occlusion procedure was unrelated to the observed conjunctival changes, it would be necessary to find another causal relationship. At present it would appear that nasolacrimal duct occlusion is the only common feature to this improvement in epithelial morphology.

A reduced goblet cell population is a known feature in patients suffering from dry eye (Ralph, 1975). In the region of conjunctiva examined in this study the goblet cell count was inversely proportional to the degree of stratification. Moreover, these goblet cells were morphologically identical to those described in the normal tissue in part I. In view of this observation it is of interest to speculate on the possible cause of the accumulation of mucus in severe cases of KCS. If the samples used for this investigation are to be regarded as representative, it must be assumed that there is a reduction in the total goblet cell count throughout the conjunctiva, although it must be conceded that the assumption may not be entirely valid (Kessing, 1966).

However, if the assumption is accepted, there are 2 possible explanations for the accumulation of
mucus. Firstly, normal mucus could accumulate because there is inadequate wash-through by normal tear secretion. Secondly, there could be an alteration in the relative proportions of the 5 glycoproteins which are secreted by the goblet cells and this could change the physical and/or biochemical properties of the tear film (Wright, 1974).

Careful examination of the conjunctiva in KCS will frequently reveal the existence of tiny filaments, the cause and nature of which are controversial. This investigation suggests that the separation of sheets of superficial cells may contribute to these filaments. On the other hand reduction in the number of filaments frequently follows on treatment with mucolytic agents (Jones and Cook, 1965; Williamson et al., 1974), and it may be argued that filaments are therefore mucous threads which are attached to separating epithelial cells. Recent studies of 'mucous' plaques from the corneal surface in patients which KCS have shown these to consist of a mixture of mucus and desiccated epithelial cells (Fraunfelder et al., 1977). In the present study the epithelial cells showed a tendency to separate in sheets, and, since the intercellular junctions between these cells were preserved, it can be assumed that these changes are not entirely a degenerative phenomenon.

It is also possible that the separation of sheets of superficial cells is a mechanical process caused by the eyelids rubbing over the relatively dry surface of the bulbar conjunctival epithelium. Thus a shearing effect might account for the withdrawal of cytoplasmic processes from the intercellular space (see Fig. 4). Another feature of major importance in relation to the surface morphology is the dramatic reduction and deformation seen in the microplicae. Under these circumstances even in the presence of a normal tear secretion it would seem unlikely that a tear film could be maintained.

The increase in stratification of the conjunctival epithelium in association with the severity of the
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Fig. 4 An example of a more advanced separation of the superficial cell layer. Note the presence of the apical junction (arrow) and the severe loss of microplicae. The insert (a) shows disruption of the anterior cell membrane and (b) shows advanced stunting of the cell processes (×3350; inserts ×17 500)
KCS suggests that it is a protective mechanism evolved by the change in the tear secretion, but it may also be an intrinsic and basic response of the cells to a hostile environment. Whether or not the cell stratification is a cause or a result of the diminished goblet cell density remains a subject for further investigation.

Another well documented condition in which epithelial stratification has been observed in association with goblet cell depletion is severe vitamin A deficiency, a condition which is reversible with systemic vitamin A therapy (Sullivan et al., 1973). Vitamin A has been used in the past as a treatment for KCS, but the results were disappointing and the pathogenic mechanisms for this ultimate morphological response are probably unrelated. Nevertheless in this study we have observed a tendency to return to morphological normality in the conjunctiva of patients treated for 2 to 5 years previously by nasolacrimal duct occlusion. The stratification and superficial cell separation was less and the goblet cell count was higher than in untreated patients with KCS, and this return to near normality was associated with clinical improvement of these patients. It should be remembered at this point that the patients selected for obliteration of tear passages were all examples of severe KCS judged to be inadequately controlled by at least 1 year of tear substitute therapy (Williamson et al., 1974).

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