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Stage-related central corneal epithelial transformation in congenital aniridia-associated keratopathy

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ABSTRACT

Purpose: To relate central corneal epithelial phenotype to degree of keratopathy in a limbal stem cell deficient population.
Methods: 37 patients (67 eyes) with aniridia-associated keratopathy (AAK) underwent corneal examination including slit lamp biomicroscopy to determine the Grade of AAK, Cochet-Bonnet esthesiometry, and in vivo confocal microscopy (IVCM) to assess morphology of the central corneal epithelium and subepithelial region.
Results: AAK Grade ranged from 1 (limbal involvement only) to 4 (total conjunctivalization), with progression from Grade 1 occurring after the age of 20. 30% of subjects had an asymmetric Grade between eyes. In early-stage AAK (Grades 1–2), central epithelial cells had mixed corneal-conjunctival phenotype, touch sensitivity and subbasal nerves diminished, and mature dendritic cells, inflammatory leukocytes, and blood vessels were present despite central transparency in the slit lamp. In later stages (Grades 3–4) of the LSCD, neural deficit and nerve function worsened, immune cell invasion increased, and lymphatic vessels were detected in several cases. Goblet cells and epithelial cysts were observed to varying degrees in all stages, but without clear association to AAK severity. The clinical grade and progression of AAK was strongly associated with the central corneal epithelial phenotype.
Conclusions: AAK is associated with degradation of epithelial phenotype, a neural deficit, and immune compromised status even in the clear central cornea in the earliest stages. IVCM can aid in assessing whether the conditions for limbal stem cell maintenance are likely to exist, based on morphology of the central epithelial microenvironment.
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1. Introduction

Limbal stem cell deficiency (LSCD) is defined by partial or total degradation of the limbal niche structures that support the limbal epithelial stem cells of the ocular surface, resulting in a loss of the ability to renew the cells of the transparent corneal epithelium. LSCD is characterized clinically by a breakdown of the corneo-scleral epithelial border, most often resulting in conjunctival epithelial invasion into the clear cornea. Along with invasion of the resulting pannus of conjunctival tissue, associated inflammatory cells, blood vessels, and goblet cells normally limited to the limbal and conjunctival regions also invade the cornea [1]. In cases of acute LSCD due to chemical burns and infectious keratopathy, loss of corneal transparency and conjunctivalisation can occur quickly, making investigation of the sequelae of phenotypic transformation of the epithelium difficult. In longer, protracted cases such as the LSCD characterizing congenital aniridia, transformation of the corneal epithelium to a conjunctival phenotype occurs over a time span of years to decades [2,3] in a slower degenerative process termed aniridia-associated keratopathy (AAK). This offers the possibility for closer study of the gradual transformative changes initiated by breakdown of the limbal niche function.

In earlier studies examining the cornea in congenital aniridia at the cellular level using in vivo confocal microscopy (IVCM) [3,4], we

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noted that corneal epithelial phenotypic changes manifested in early stages before conjunctival invasion of the cornea was clinically apparent by slit lamp biomicroscopy. We also noted that individual conjunctival epithelial cells or constellations of cells (termed 'conjunctival islands') began to invade the clear cornea prior to encroachment of a conjunctival pannus [3,4] and that an abnormal spiraling apical epithelial mosaic correlated precisely with the migration of the underlying subbasal nerves [4].

It is known from the 'X,Y, Z hypothesis' first proposed by Thoft and Friend [5] that corneal epithelial renewal from the basal limbus occurs in a circumferential pattern towards the central cornea, and thus as limbal stem cell function declines, we can hypothesize that abnormal or pathologic cells may be swept from the limbal region into the central cornea. The subasal nerve plexus and its associated nerves are also noted as a typical 'path of least resistance' for dendritic (Langerhans') cells normally residing in the peripheral and limbal regions to enter the central cornea in cases of subclinical and clinical inflammation [6,7]. Concomitant immune-mediated changes in LSCD may therefore also be apparent in the central cornea prior to conjunctival invasion. Examination of the detailed morphology of the central cornea at the cellular level may serve to reveal the earliest signs of LSCD in conditions such as AAK, and may improve our understanding of limbal stem cell function and the pathologic breakdown of this function. Additionally, early signs of trophic breakdown in the central cornea may serve as a diagnostic indicator of compromised limbal stem cell function or as a prognostic indicator for planned therapeutic interventions.

In this study, we report on the largest cohort of AAK eyes to be examined at the morphologic level by IVCM to date. The aim was to focus on documenting central corneal epithelial morphologic parameters in early and later stages of AAK, and to determine the relationship of these parameters with severity of LSCD as assessed by a clinical grading score.

2. Methods

2.1. Clinical examinations

All subjects were examined during a single week in Dec 2014, at the Ophthalmology Department, Second School of Medicine, Medical University of Silesia, Katowice, Poland. The examination protocol and study were approved by the Ethical Review Board of the Medical University of Silesia, Katowice (Protocol KNW/0022/KB1/35/14) and all subjects gave written informed consent prior to examination. The conduct of the examinations and the study adhered to the tenets of the Declaration of Helsinki for ethical conduct of research on human subjects. Upon receiving informed consent, subjects underwent general ophthalmic examinations, with particular focus on characterization of AAK by slit lamp biomicroscopy, contact esthesiometry and in vivo confocal microscopy.

Slit lamp biomicroscopy was performed bilaterally where possible, for all subjects. A digital camera attached to the slit lamp was used to photographically document the corneal status in examined eyes. The degree of progression or severity of AAK was determined during slit-lamp examination, consistent with a previously published grading scale [4]. Briefly, Grade 0 AAK indicates an intact limbus, Grade 1 is encroachment of conjunctiva just over the limbal border, Grade 2 is involvement of the peripheral to mid-peripheral cornea but sparing the central cornea, Grade 3 is central corneal involvement, Grade 4 is totally conjunctivalized and vascularized cornea and Grade 5 is end-stage AAK with an opaque, thick, vascularized cornea. With this scale, Grades 0–2 indicate AAK sparing the visual axis, while grades 3–5 indicate visual impairment due to AAK.

Contact esthesiometry was performed using a Cochet-Bonnet esthesiometer with nylon filament (Luneau Ophthalmologie, Chartres, France). The non-anesthetized eye was probed centrally with successively shorter nylon filament lengths to determine the threshold length to elicit a blink reflex. The test was repeated to confirm the threshold level.

Laser-scanning in vivo confocal microscopy (IVCM) was performed using the Heidelberg Retinal Tomograph 3 with Rostock Corneal Module (Heidelberg Engineering, Heidelberg Germany). The detailed procedure of confocal microscopy has been described elsewhere [3]. Here, the limbal region was scanned inferiorly and superiorly to confirm the presence or absence of limbal palisade structures (palisade ridges and/or focal stromal projections [3,8]). Based on previous observations in healthy subjects and patients with limbal stem cell deficiency it was decided to focus on the superior/inferior limbus and exclude nasal/temporal limbal regions due to the rare presence of stem cell niche structures in these latter regions [9]. The subject was asked to gaze in a forward direction, and the central and paracentral cornea were scanned at the epithelial and subepithelial depth. Due to nystagmus present in most subjects and the difficulty for subjects to keep the gaze and the head in a fixed position, the central cornea was taken to include both central and paracentral regions (away from the peripheral and limbal regions). Due to the difficulties related to nystagmus and head/eye motion and additionally the varying degree of patient compliance (particularly in younger subjects), IVCM scans were taken with the aim to confirm the presence or absence of various morphological features for semi-quantitative analysis, rather than to achieve the best possible quality of images for a fully quantitative analysis of feature density.

2.2. Assessment of morphologic parameters by IVCM

The phenotype of the epithelium as assessed by IVCM was classified into one of three categories. Corneal phenotype was present where epithelial cell layers appeared unaltered in reflectivity and morphology from a normal transparent cornea, i.e., basal and wing cell layers had low reflectivity with cell borders visible, while superficial epithelium had large squamous polygonal cells with cell nuclei visible [10]. A 'mixed' phenotype was defined as corneal epithelial phenotype with small islands of highly reflective or opaque cells indicative of conjunctival epithelium, as previously described [3]. Finally a 'conjunctiva' phenotype was characterized by total absence of corneal phenotype with highly reflective cells without distinct borders visible.

Epithelial cysts were identified based on presence in epithelial cell layers, and appeared as rounded or oval-shaped dark (hyporeflective) vacuoles of varying size (from 10 to 100 μm in diameter), sometimes containing small, rounded hyper-reflective inclusions. Goblet cells in the central and paracentral cornea were assessed in IVCM images based on their typical oval-shape, diffuse reflectance, and generally uniform size. Morphology was assessed based on published IVCM images of goblet cells in studies demonstrating a high correlation of characteristic in vivo morphology with positive staining from impression cytology samples of the epithelium [11,12].

Mature (antigen-presenting) dendritic cells (mDCs), were identified from IVCM images by their morphology, their preferential accumulation at the subbasal nerve plexus and their characteristic long dendrites [6,7]. mDCs are distinguished from immature DCs by the absence of long dendrites and only a visible cell body in the latter [6,7]. Other inflammatory cells (non-dendritic morphology) were identified based on their predominantly round, highly reflective cell bodies, indicative of leukocytes of myeloid origin [10]. These were observed in the epithelium and in the subepithelial region.

Subbasal nerves were visualized by first locating the subbasal plexus region immediately posterior to the epithelium, which

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