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Sir,

Reply to Limbal stem cell deficiency: a clinical chamaelon

We read this case report with great interest. The authors described a case of a persistent corneal epithelial defect, stromal changes, and wound leak following a penetrating keratoplasty in an eye which had undergone a pterygium excision and conjunctival autograft.[1] The authors allude the epithelial defect noticed on the first postoperative day to be due to limbal stem cell deficiency. It may be plausible to provide an alternative explanation for the reported finding. The epithelial defect and corneal stromal changes were noticed to a site adjacent to the previous pterygia. Recent studies have shown evidence to suggest that the development of pterygia is linked to matrix metalloproteinases (MMPs) overexpressed by altered limbal epithelial basal cells.[2] MMPs are a family of more than 21 genetically distinct proteases, which are produced in small amounts under normal physiological conditions by fibroblasts and epithelial cells.[3] These MMP's being proteases dissolve and remodel extracellular matrix that includes fibronectins, collagen, and basement membrane.[3] During the development of pterygia, there is overexpression of MMPs that go on to dissolve Bowman's layer, which in turn triggers the fibrovascular pannus formation.[2]

The epithelial and stromal changes observed might have resulted from abnormal activity of MMPs from the previous site of the pterygium. Further, the figure shows the epithelial defect to be involving the donor corneal button as well on day 1. This was a very rapid change and manifestations of limbal stem cell deficiency are generally slow in onset.[4] The epithelial defect in this patient may be due to altered MMP expression resulting in dissolution of Bowman's layer leading to a corneal epithelial defect. The rapid healing of the epithelial defect following limbal stem cell graft may be contributed to the removal of source of the MMPs.

We would be most grateful for the view of the authors

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Sir,

Limbal stem cell deficiency: a clinical chameleon

Zaidi *et al*[1] infer that the corneal donor epithelial defect noted day 1 postkeratoplasty is secondary to limbal stem cell deficiency. How can this be so? A donor epithelial defect day 1 is surely due solely to loss of donor epithelium and has nothing to do with host limbal stem cell function. They have treated the donor epithelial defect with cyclosporin drops and intensive topical preservative-free steroids. This is inappropriate management for both stem cell dysfunction and persistent donor epithelial defect. They have then performed a limbal stem cell graft along with repeat keratoplasty, used the same inappropriate line of clinical management and observed a similar but less severe course of events. Therefore, they have neither demonstrated that limbal stem cell deficiency was the cause of the problem nor shown any convincing benefit from the stem cell graft.

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Sir,

Matrix metalloproteinase expression in transplanted corneas

We are grateful to the comments from both centres which enable us to expand on the case of limbal stem cell deficiency (LSCD) that we originally described.[1] Firstly, donor epithelial defects can be intimately related to limbal stem cell function as early as the first day postoperatively—Ahmed and Ahmed have shown that corneal epithelial cell migration rate is 61 μm/h during the active healing phase of the epithelium, which means that the defect may close in 18–48 h, or even faster.[2] This rapid rate of recovery is a response to wounding, which has been conclusively shown by Lehrer *et al*[3] using double-labelling techniques. The latter experiments demonstrate three mechanisms to account for the persistent epithelial defect on day 1 following corneal grafting. First, replication of limbal stem cells. Second, additional cycles of cell proliferation that normally remain in reserve. Third, enhancement of transient amplifying cell (TAC) proliferation via a shortening of the cycling time. These processes may also be under circadian control.[4,5] The observed clinical epithelial defect day 1 postop in our patient is a clinical correlate of this upregulation of cell turnover in the limbus. This cellular upregulation has a further possible significance, as we shall discuss later specifically in response to Dr Zaher and Dr Ramesh's correspondence.

Regarding concerns about the rationale for our treatment of the first and second grafts, immunosuppression (steroid and cyclosporin) was not started to treat **either** the stem cell deficiency **or** the persistent epithelial defects. Rather, we started immunosuppression to treat **transplant rejection** which was causing stromal inflammation and, secondary to this, exacerbating poor healing at the site of the epithelial defect due to limbal stem cell deficiency. Immunosuppresion also served to reduce or prevent any further rejection occurring as a result of a persistent epithelial defect.

We appreciate Mr Morgan drawing attention to our use of cyclosporin drops and intensive topical preservative-free steroids. There is at present no hard verdict on the appropriateness of this choice over systemic administration of immunosuppressants. Opinions have varied since Kenyon and Tseng's[6] pioneering operations in the field were reported in 1989. For example, oral immunosuppression was used by Tsai and Tseng[7] in their work with limbal stem cell allografts, while other workers such as Tsubota *et al*[8] used a combination of both intensive systemic immunosuppression with cyclosporin and steroids, together with topical cyclosporin and two different types of topical steroid applied intensively (a total of 10 steroid drops per day).[7,8] Indeed Tan *et al*[9] noted in 1996 that the issue of immunosuppression was then under evaluation. More recently Xu *et al*[10] have convincingly shown *in vivo* in mammals that cyclosporin-A administered topically or systemically is equally effective in maintaining limbal stem cell grafts and the ocular surface. However, Tsubota *et al*[11] noted that limbal stem cell grafts may be more prone to tissue rejection than conventional corneal transplants, and this led them in at least one fairly recent clinical series to use systemic over topical immunosuppression. On the other hand, Shimazaki *et al*[12] have also in a fairly recent clinical series used intensive topical steroids for the treatment of LSCD with transplantation of limbal grafts and amniotic membrane grafts, as we did.

We disagree with Mr Morgan over the outcome of the course of events following the two corneal transplants, the first without and the second with limbal stem cell grafting, which, we feel, were markedly dissimilar. This is simply as the second corneal transplant survived.

It is in fact the similarities in both grafts that point to LSCD as the cause of the epithelial defects that occurred in both grafts—these being the identical shape *and* locations of the epithelial defects in both corneal grafts. These additionally matched those of the pterygium in the original host cornea, and, further, pterygium is also associated with LSCD.[12–14] Indeed, in addition to these identical anatomic and topographic defects across three