Experimental immune-mediated conjunctivitis (EIC): downregulation by Coversin, a dual C5 and LTB4 inhibitor

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PURPOSE
Steroid resistant atopic keratoconjunctivitis (AKC) is difficult to treat and can lead to corneal cicatrization and vision loss. Topical or systemic immunosuppression may be necessary to prevent disease progression. We used Coversin, a therapeutic peptide, in a model of experimental immune mediated conjunctivitis (EIC) to test the hypothesis that combined complement C5 and LTB4 inhibition might mitigate allergic eye surface inflammation.

METHODS
EIC was induced in 8-10 week old female C57/Bl6 mice (N=8 per group) following ip immunisation to ovalbumin (OVA) and bilateral daily challenge with topical xmg/mL OVA. Severe ocular surface inflammation was detectable from Day 14 onwards. Topical Coversin at various concentrations (0.063%-0.5%) was used twice daily in both eyes from Day 17, once inflammation peaked. Placebo- treated and unchallenged groups were used as controls. Two additional groups were treated with EV131, a histamine binding protein, alone and in conjunction with Coversin. All eyes were examined daily by a masked observer (VC) and clinical scores were assessed from Day 15 to Day 21. Animals were euthanised and eyes harvested for histology, flow cytometry and cytokine estimations on Day 21.

RESULTS

1. Timecourse of Coversin given topically from Day 3 of OVA challenge
Coversin 0.125 % and 0.25% were found to be equally effective in reducing eye inflammation compared to placebo by Day 18 [p=0.008] but no treatment was effective at earlier time points. Other concentrations and EV131 alone or in combination with Coversin were less effective. Whilst no significant differences were observed at early stages of inflammation (Days 3, 4, 5), a significant decrease in scores was detected in Coversin-treated groups on Day 6 relative to the PBS controls, as summarized in Figure 2 below.

2. Histological findings
Left hand eyes were harvested from each mouse on day 21, fixed and embedded for histological scoring. A score of 0-3 was ascribed for each fornical region, where 0=no inflammation, 1=mild (Figure 3A), 2=moderate (Figure 3B) and 3=severe. Final histology scores are shown in Figure 3C.

CONCLUSIONS
There was a significant decrease in clinical severity in the treatment groups receiving Coversin, in comparison with PBS controls, but this decrease was only detected at Day 6. This anti-inflammatory effect was also observed within the tissues histologically. A more detailed flow cytometric investigation of the CD4⁺T cells within the conjunctival tissues at the time of harvesting revealed a significant increase in CD4⁺T cells in all EIC groups, relative to the non-challenged controls. Since this did not correlate with the clinical scores, we further analysed the CD4⁺T cells for their effector subsets, and found that the PU.1⁺GATA3⁺ Th9 cells were significantly downregulated in the treatment groups. Interestingly there were no significant effects seen on Th2 cells (GATA3⁻), Th1 cells or Treg cells, suggesting that the effects of treatment were primarily targeting the Th9 cell subset.