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ORIGINAL ARTICLE

Immunopathological Features of Severe Chronic Atopic Keratoconjunctivitis and Effects of Topical Cyclosporine Treatment

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ABSTRACT

Purpose: To assess differential roles of inflammatory cells in pathophysiology of severe atopic keratoconjunctivitis (AKC) and evaluate immunomodulatory effects of topical cyclosporine A (CsA).

Methods: A total of 10 patients with severe, steroid-dependent/resistant chronic active AKC were treated using frequent topical CsA 0.05% as monotherapy for 2 months. Conjunctival biopsy specimens before and after treatment were examined using immunohistochemistry. A total of 10 healthy age-matched adults served as the control group. **Results:** Baseline AKC samples revealed greater cluster of differentiation 4 (CD4), interferon gamma (IFN γ), human leukocyte antigen-D-related (HLA-DR) positive cell densities compared with healthy controls ($P < 0.05$), as well as interleukin (IL)-17 ($P = 0.08$). Topical CsA treatment induced a significant reduction in CD4 and IL-17 expressions ($P < 0.05$); post-treatment levels were same as normals ($P > 0.05$). Despite reduction after treatment ($P = 0.06$), HLA-DR expression remained higher than controls ($P < 0.05$).

Conclusions: AKC-related conjunctival inflammation appears to be mediated by delayed hypersensitivity. In this short-term trial, frequent topical CsA improved conjunctival inflammation.

KEYWORDS: Atopic keratoconjunctivitis, immunopathology, topical cyclosporine

Atopic keratoconjunctivitis (AKC) is a severe allergic disease of the ocular surface associated with atopic dermatitis/eczema.¹ Although the disease itself is uncommon, scarring of the conjunctiva and loss of vision due to corneal opacity commonly occur when chronic disease is present. Therefore, better understanding of disease pathophysiology and triggers are important to develop effective therapeutic agents.

Current literature review reveals only a few published studies examining the conjunctival biopsies from patients with AKC. An earlier study demonstrated goblet cell proliferation, pseudotubule formation, infiltration by degranulating mast cells and eosinophils in the epithelium, inflammatory cell infiltration predominantly by lymphocytes and

macrophages, and significant fibrosis in the substantia propria.² These inflammatory cells as well as conjunctival epithelial cells make up the effector arm of allergic conjunctival inflammation and are significant sources of cytokines and chemokines.³ Particularly, RANTES (Regulated-on-Activation-Normal-T-Cell-Expressed-and-Secreted, selective chemoattractant for T lymphocytes and monocytes), along with granulocyte monocyte colony stimulating factor (growth factor for white blood cells) are upregulated in the conjunctival epithelium affected by AKC. Interleukin (IL)-2, IL-3, IL-4 and IL-5 expressed by the infiltrating lymphocytes have been noted in the substantia propria.^{3,4} Tryptase, together with histamine, is released from mast-cell secretory granules.⁵ The constellation of

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these findings indicate a T helper (Th)2 predominance in AKC as in other chronic allergic diseases like asthma and atopic dermatitis.

Traditionally, the mainstay of AKC treatment included a combination of chronic antihistamines/mast cell stabilizers in topical and oral form and periodic topical corticosteroids to control the acute flare-ups. However, a previous long-term outcome study demonstrated that two-thirds of patients managed in a traditional manner eventually developed significant keratopathy and subsequent vision loss, suggesting that in chronic severe cases with conjunctiva scarring, mechanisms other than typical allergy might be involved.^{1,6} More recently, other modalities of therapy have been introduced, such as orally and topically administered calcineurin inhibitors.⁷⁻⁹

Cyclosporine A (CsA) is a hydrophobic, cyclic, polypeptide purified from the fungus *Tolypocladium inflatum*, known to selectively inhibit T lymphocytes by blocking the expression of the IL-2 receptor.^{10,11} Topical 2% CsA treatment was shown to induce a reduction in T cells, a normalization of the CD4-CD8 ratio, a decrease in T-cell activation, and a reduction in T-cell cytokine expression, especially IL-2 and interferon gamma (IFN- γ) in superior tarsal conjunctival biopsy specimens of patients with AKC.¹² Our previous study demonstrated that topical cyclosporine 0.05% is an effective therapy for AKC at high doses administered over several months.⁸ Additionally, the combination of CsA eye drops with tacrolimus ointment 0.03% applied directly to the surface of the eyelids has the potential to eliminate the need for steroids and perhaps alter the natural history of AKC by preventing flare-ups.¹³

In this study, we assessed the phenotype of the inflammatory cell infiltrate in conjunctival samples from patients with chronic, steroid-dependent/resistant AKC with conjunctival scarring, before and after intensive topical CsA 0.05% treatment and compared it to normal controls.

METHODS

This study was conducted at The Ocular Surface Diseases and Dry Eye Clinic, Wilmer Eye Institute, Baltimore, Maryland. The Johns Hopkins University Institutional Review Board approved the study protocol and related procedures. The research adhered to the tenets of the Declaration of Helsinki, and the work is Health Information Portability and Accountability Act-compliant. Signed informed consent was obtained from each subject before study enrollment.

Patients

A total of 10 adult patients with a known diagnosis of AKC as outlined by Hogan¹⁴ who were under the clinical

care of a single clinician (EKA) consented (Table 1). All patients had severe chronic active disease necessitating the use of frequent topical corticosteroids combined with topical antihistamine and mast cell stabilizers (Figure 1). Patients with other allergic diseases, such as vernal keratoconjunctivitis, contact lens-related giant papillary conjunctivitis, contact dermatitis of the lids, or patients with significant concurrent active ocular surface conditions such as bacterial blepharitis or herpetic infections were excluded.

The study group consisted of five men and five women, with a median age of 41 years (range, 24–60 years). Median age at the onset of symptoms was 32 years (range, 21–50 years). All patients had a history of eczema and atopic blepharitis. Other major atopies in the group included asthma (n = 3) and allergic rhinitis (n = 4). Additionally, five of the patients had a family history of atopic disease. Median number of years since the onset of ocular symptoms was 4 years. All patients had moderate to severe disease requiring frequent topical steroid therapy to control active flare-ups. Three of the patients had a previous history of herpetic keratitis. One patient had severe steroid-induced glaucoma on presentation with significant optic nerve cupping and vision loss. None of the patients were taking systemic immunomodulators at the time of presentation. None of the patients with a history of herpetic keratitis were on any systemic prophylactic therapy.

The control group comprised 10 age-matched adult patients with no history or clinical findings of ocular allergic disease, chronic conjunctivitis, dry eye, or blepharitis of any etiology undergoing routine cataract surgery. The control group consisted of six men and four women, with a median age of 53 years (range, 47–63 years). Median age at the onset of symptoms was 32 years (range, 21–50 years). None of the patients had an underlying inflammatory systemic disease or atopy. In addition, none of the patients were on any systemic or topical medications that could affect the

TABLE 1. Diagnostic criteria for atopic keratoconjunctivitis*.

Criteria
1. A hereditary history of allergies
2. Associated allergies, such as asthma, hay fever, rhinitis, urticaria and infantile eczema.
3. A typical persistent dermatitis affecting the face, neck, shoulders, axillae, thorax, popliteal and antecubital areas. Exacerbations and remissions over a period of years.
4. A keratoconjunctivitis, associated with activity of the skin condition and characterized by thickening and hyperemia of the conjunctiva and opacification and vascularization of the cornea.

* Adopted from Hogan MJ. Atopic keratoconjunctivitis. Trans Am Ophthalmol Soc 1952; 50:265–281.

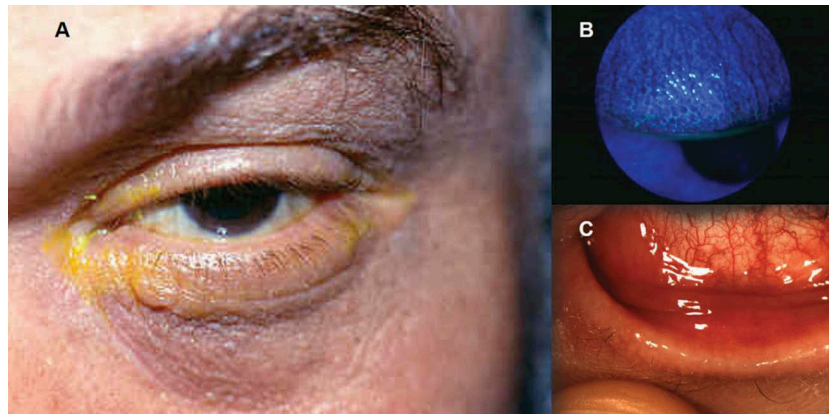


FIGURE 1. Study patient #3 with significant atopic blepharokeratoconjunctivitis. A- External appearance of the lids with typical thickening of the lid skin and lichenification mostly located in the nasal aspect. B- Upper tarsal conjunctiva, with cobalt blue light and fluorescein staining, demonstrating the papillary conjunctivitis. C- Lower forniceal and tarsal conjunctival appearance at the slit-lamp demonstrating significant inflammation.

immunological properties of the conjunctiva, including non-steroidal anti-inflammatory drugs, antihistamines and steroids.

All clinical examinations and procedures were performed by a single clinician (EKA). Prior to enrollment, patients with AKC were placed in a wash-out period for 2 weeks. All topical medications were discontinued with the exception of glaucoma drops and over-the-counter artificial tears. At the enrollment visit, patients had a complete ophthalmic examination including recording of symptoms, signs, and visual acuities. A complete history, both ocular and general medical, was also taken. All biopsies were performed in a uniform manner. First, local anesthesia was achieved using 0.1 ml of 2% lidocaine injected into the subconjunctival space on a 30-gauge needle. Then, a conjunctival specimen measuring 3 × 3 mm was harvested using a pair of fine smooth forceps and surgical scissors from the inferior forniceal conjunctiva of the worse eye according to clinical findings on the enrollment visit where sub-epithelial fibrosis was most pronounced. The patients were treated with a topical antibiotic (erythromycin ointment, four times daily) for 1 week. Individuals in the control group underwent a comprehensive ophthalmological examination including a full ocular surface examination prior to their cataract procedure. The surgical eye was biopsied in the same manner just prior to cataract removal.

Treatment

Patients with AKC were seen on day 7 after biopsy and initiated on treatment with 0.05% topical CsA eye drops (Restasis; Allergan, Inc, Irvine, CA) six times daily after ensuring that conjunctival healing was complete. One patient who had delayed epithelization of

the biopsy site was observed on topical antibiotic ointment for an additional week, and the CsA was withheld during that period. The patients were reexamined on day 28, and the dose of CsA was decreased to four times daily for an additional 4 weeks. Conjunctival biopsy was repeated on day 56 in the eye that had the biopsy previously, in the same manner, not directly adjacent to the previous site. The patients in the control group received routine post-cataract surgery care, and none had complications.

Laboratory Procedures

Specimens from patients with AKC and controls were labeled in a de-identified manner and sent to Allergan, Inc. for processing. All biopsies were cryo-sectioned at 7 μ m thickness. Immunohistochemistry staining included primary and secondary antibody incubation for CD4, human leukocyte antigen-D-related (HLA-DR), IFN γ , IL-17, IL-33 and tryptase after blocking the tissues, followed by counterstaining.¹⁵ The vendors, working concentrations of the antibodies and reagents are shown in Table 2. DAB (3,3'-diaminobenzidine) exposure times and counterstaining times of the conjunctival specimens are shown in Table 3.

Computerized image scans and area measurements were performed using Flagship image software (Flagship Biosciences, Inc. Westminster, Co) to quantify positive cell counts of incubated antibody-stained cells. Readout was performed in a masked manner by a single experienced researcher (MS). The samples were presented in a random fashion. The entire area of each biopsy specimen was reviewed to evaluate the absolute cell count from which cell densities (cell counts per square millimeter tissue area) for CD4, HLA-DR, IFN γ , IL-17, IL-33 and tryptase positivity were computed. Each specimen was reviewed

TABLE 2. Working concentrations and vendors of the antibodies and reagents used in the experiments.

Antibody	Vendor	Working Concentration
CD4	BD Biosciences CD4 Pure (346320)	1 µg/ml
IL-17	Santa Cruz Biotech Goat pAb IL-17 (E-19) (sc-6077)	10 µg/ml
IL-33	Enzo IL-33 mAb (Nessy-1) (ALX-804-840)	50 µg/ml
IFNγ	Santa Cruz Biotech Rabbit pAb IFN γ (H-145) (sc-8308)	1 µg/ml
HLA-DR	BD Biosciences Purified Mouse anti-human HLA-DR (555559)	1 µg/ml
Tryptase	Dako FLEX mouse anti-human mast cell tryptase AA1 (IR640)	Ready to use
Secondary antibody	Vector Laboratories VECTASTAIN Elite ABC Kit (Universal), R.T.U. (PK-7200)	Ready to use
CD4i	BD Biosciences Purified Mouse IgG1,k isotype control (555746)	1 µg/ml
IL-17i	SC Biotech normal goat IgG (sc-2028)	10 µg/ml
IL-33i	BD Biosciences Purified Mouse IgG1,k isotype control (555746)	50 µg/ml
IFNγi	Santa Cruz normal Rabbit IgG (SC-2027)	1 µg/ml
HLA-DRi	BD Biosciences Purified Mouse IgG2bk isotype control	1 µg/ml
Tryptasei	Dako FLEX Negative Control Mouse Cocktail (IR750)	Ready to use
Secondary antibody	Vector Laboratories	Secondary antibody

TABLE 3. DAB (3,3'-diaminobenzidine) exposure times and counterstaining times of the conjunctival specimens.

Antibody	DAB Exposure Time (min)	Counterstained Time (min)
CD4	3.5	1.5
IL-17	2	1.5
IL-33	1	-
IFNγ	2.5	1
HLA-DR	2.5	1.5
Tryptase	1	1.5

randomly in duplicate. When there was more than a 10% difference in the numbers, a third review was performed.

Statistical Analysis

Statistical analysis was performed using Excel 2016 (Microsoft Office Professional Plus 2016). The mean CD4, HLA-DR, IFN γ , IL-17, IL-33 and tryptase expressing cell densities of patients with AKC at baseline and after CsA treatment were compared to controls using a Mann-Whitney *U* test. The same parameters were also

compared between patients with AKC at baseline and after CsA treatment using a Wilcoxon test. A *P* value of < 0.05 was considered statistically significant. In the AKC group, the percentage of change in each cell density after treatment also was calculated.

RESULTS

The patients with AKC tolerated the 56-day frequent topical monotherapy CsA treatment monotherapy well. Although patients reported side effects such as discomfort, burning, or stinging, there were no discontinuations due to side effects. The clinical results have been published elsewhere.¹³ Briefly, symptoms and signs were scored at days 0, 28, and 56. A final evaluation was performed at day 63 while the patients were off therapy. There was a significant improvement in both sign and symptom composite scores over the 56-day treatment (*P* < 0.05). An increase in symptoms and signs was noted between days 56 and 63, which could be due to abrupt withdrawal of CsA treatment, causing a rebound. To date, four of the patients are still managed at the same center and doing well without complications.

Cell densities for CD4, IL-17, IL-33, IFN γ , HLA-DR and tryptase positivity per mm² in conjunctival samples from patients with AKC are displayed in Figure 2. The mean CD4, IFN γ and HLA-DR positivities were significantly greater in patients with AKC before treatment compared to the control group (*P* < 0.05) (Table 4). The mean IL-17+ cell density was also greater in patients with AKC before treatment compared to the control group, but the difference did not reach statistical significance (*P* = 0.08). The IL-33 and tryptase levels were not different (*P* > 0.05).

The mean CD4+ and IL-17+ cell densities decreased significantly in conjunctival samples from patients with AKC after treatment compared with before-treatment values (*P* < 0.05). There was no change in IL-33+, IFN γ + or tryptase+ cell densities after treatment (Table 5). Although there was a reduction in the mean HLA-DR positive cell density in patients with AKC after the CsA treatment, the difference was not statistically significant (*P* = 0.06).

The mean CD4⁺ and IL-17+ cell densities in patients with AKC after treatment were similar compared with normal controls (*P* > 0.05). The mean IFN γ and HLA-DR positivity in patients with AKC following therapy remained higher than those in control group (*P* < 0.01) (Table 6).

DISCUSSION

The results of this study demonstrate the dominance of T lymphocyte-mediated delayed type immune responses in conjunctivae affected by chronic, steroid-

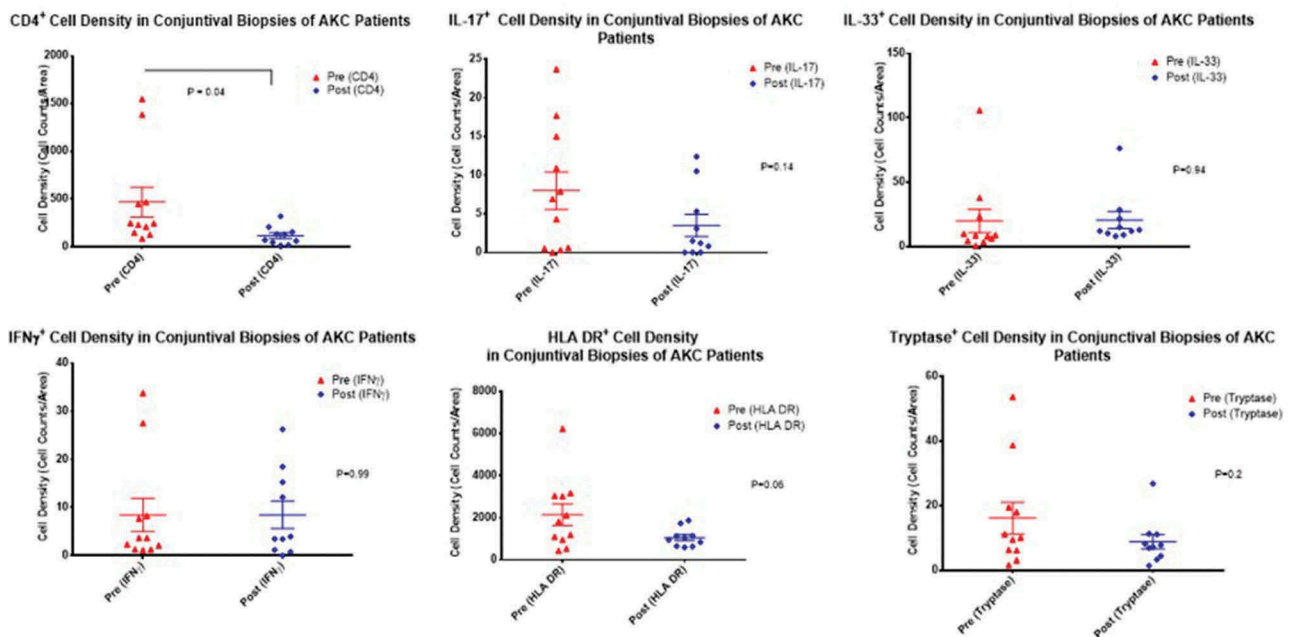


FIGURE 2. Cell densities for CD4, IL-17, IL-33, IFN γ , HLA-DR and tryptase positivity in conjunctival samples from patients with atopic keratoconjunctivitis.

dependent AKC without measurable contribution from the mediators of typical allergy, which offers an explanation for the inefficacy of typical antihistamine–mast cell stabilizer treatment. In addition, short-term topical CsA treatment was effective in reducing the expressions of CD4, HLA-DR, and IL-17, thereby alleviating the clinical findings of the disease. These findings are in agreement with previous research, which also demonstrated significant HLA-DR expression, T-cell activation with IL-2 as well as IFN- γ expression in conjunctivae affected by AKC and reduction in their expression following topical CsA treatment.¹² In addition to previous literature, we showed that the inflammation in these patients is mediated largely by Th1 and TH17 cytokines rather than Th2-associated cytokines, as reported in the literature, perhaps offering an explanation for the significant scarring of the lower forniceal and tarsal conjunctiva that invariably occurs in patients with long-standing severe AKC (Figure 3).

Other atopic conditions such as asthma and atopic dermatitis are known to be mediated by the Th2 immune responses.¹⁶ Generally, Th2-mediated immune responses are characterized by the activation of CD4⁺ Th2 cells and production of typical type 2 immunity-associated cytokines (e.g., IL-4, IL-5, and IL-13). Epithelial cell-derived type 2-initiating cytokines (e.g., IL-25 and IL-33) are also involved in initiating Th2 immune responses stimulated by various antigens (e.g., pollen, house dust mites, food allergens, parasites) that cannot directly activate Th2 cells because they are too large to be phagocytosed by antigen-presenting cells.¹⁷ Our results demonstrated greater expression of IFN γ and IL-17 positive cell densities as

well as CD4 and HLA-DR positivity in conjunctival biopsies of steroid-dependent patients with AKC compared with controls. Interestingly, IL-33 expression was not significantly different between the two groups, although atopy has been traditionally known as Th2-biased, and IL-33 has been considered crucial for the development of AKC.¹⁸ In addition, previous studies have also shown a predominantly Th2 profile in vernal keratoconjunctivitis that is less chronic, and, unlike AKC, does not typically cause scarring of the inferior forniceal conjunctivae. Indeed, the shift towards a Th1-like profile in AKC may be a potential function of disease chronicity^{4,19–21} and the reason for unresponsiveness to usual anti-allergy therapy. We also found very high levels of IFN γ expression. This has been shown in previous studies to correlate with corneal damage score, which was the case in our patients.^{22,23} The expression of tryptase that serves as a marker of mast-cell activation, on the other hand, was not significantly different in the samples from patients with AKC in our study.

One of the limitations of this study is that a limited number of cytokines were assessed in the conjunctival samples. Therefore, the results should be interpreted with caution. In addition, the subjects in the control groups were recruited on the day of their surgery after a medical records review to ensure they did not have a history/diagnosis/symptoms of any ocular surface disease or known inflammatory systemic disease, including allergy, and were not taking any medications related to those. We do not have detailed information on the control group that might be relevant to inflammation, such as

TABLE 4. CD4, IL-17, IL-33, IFN γ , HLA-DR and tryptase positive cell densities in conjunctival samples from patients with atopic keratoconjunctivitis at baseline in comparison to normal controls.

B	Cell Densities (Cell count/mm ²)											
	CD4			IL-17			IL-33			IFN γ		
	C	B	C	C	B	C	C	B	C	B	C	C
211.0	-	23.7	0.5	8.1	8.7	8.1	0	7.7	0	1097.1	130.3	10.1
129.1	245.9	6.9	5.9	17.4	3.4	17.4	0.6	3.7	0.6	533.7	998.1	53.7
245.6	2.9	10.9	0.0	4.4	4.5	4.4	0	1.2	0	1813.7	-	6.3
249.5	18.6	0.0	3.7	4.5	9.0	4.5	0.9	3.7	0.9	3024.6	382.1	3.1
86.4	48.7	0.6	1.3	15.7	7.9	15.7	0.7	1.3	0.7	3173.3	431.0	9.5
231.1	133.0	7.9	0.0	11.7	23.4	11.7	3	2.1	3	1191.1	860.8	19.5
149.2	51.3	0.5	4.1	15.9	9.9	15.9	3.1	1.3	3.1	433.1	310.8	38.7
466.6	309.8	15.0	10.3	86.6	37.9	86.6	3.9	33.8	3.9	3047.5	134.4	6.2
1547.1	36.1	17.7	0.0	2.0	105.9	2.0	3.5	27.6	3.5	6224.5	251.5	18.0
1384.6	142.4	0.3	0.0	15.3	6.4	15.3	1	2.4	1	958.9	478.1	1.6
468.26 $\pm$ 508.56	109.86 $\pm$ 107.50	7.98 $\pm$ 8.04	2.58 $\pm$ 3.44	18.16 $\pm$ 24.68	19.78 $\pm$ 30.47	18.16 $\pm$ 24.68	1.67 $\pm$ 1.52	8.46 $\pm$ 11.34	1.67 $\pm$ 1.52	2147.17 $\pm$ 1683.45	441.9 $\pm$ 303.09	16.16 $\pm$ 16.18
245.6	51.3	6.9	0.9	13.5	8.7	13.5	0.95	3.7	0.95	1813.7	382.1	10.1
0.02*			0.08	0.97			0.04*				< 0.01*	0.74

Last three rows are mean $\pm$ standard deviation, median, *P* value

B: Samples from patients with atopic keratoconjunctivitis at baseline.

C: Samples from normal controls.

**P* < 0.05 indicates statistical significance

TABLE 5. CD4, IL-17, IL-33, IFN γ , HLA-DR and tryptase positive cell densities and percentage of changes in patients with atopic keratoconjunctivitis after topical cyclosporine treatment compared to baseline.

Cell Densities (Cell count / mm ²)																													
CD4					IL-17					IL-33					IFN γ					HLA DR					Tryptase				
A	B	% Change	A	B	% Change	A	B	% Change	A	B	% Change	A	B	% Change	A	B	% Change	A	B	% Change	A	B	% Change						
130.8	211	↓38	12.4	23.7	↓47	9	8.7	↑4	3.5	7.7	↓55	597.3	1097.1	↓46	7.8	10.1	↓23												
154.4	129.1	↑20	0	6.9	↓100	28.6	3.4	↑729	12.2	3.7	↑227	1735.9	533.7	↑225	11.3	53.7	↓79												
20.3	245.6	↓92	1.2	10.9	↓89	10.3	4.5	↑126	4.0	1.2	↑244	652.6	1813.7	↓64	8.2	6.3	↑30												
16.3	249.5	↓93	1.5	0	↑100	12.1	9	↑35	15.3	3.7	↑313	1127.5	3024.6	↓63	7.2	3.1	↑137												
46.6	86.4	↓46	0	0.6	↓100	12	7.9	↑51	0.8	1.3	↓42	1059.1	3173.3	↓67	11.1	9.5	↑16												
70.6	231.1	↓69	0.8	7.9	↓89	13	23.4	↓44	1.2	2.1	↓43	941.3	1191.1	↓21	3.3	19.5	↓83												
127.8	149.2	↓14	3.1	0.5	↑535	15	9.9	↑52	0.0	1.3	↓100	845.1	433.1	↑95	26.8	38.7	↓31												
320.2	466.6	↓31	5.3	15	↓65	8	37.9	↓79	3.5	33.8	↓90	1143.6	3047.5	↓62	1.4	6.2	↓77												
62.9	1547.1	↓96	10.5	17.7	↓41	76.3	105.9	↓28	26.3	27.6	↓5	1868.6	6224.5	↓70	4.4	18	↓76												
209.4	1384.6	↓85	0	0.3	↓100	21.7	6.4	↑237	18.5	2.4	↑686	637.6	958.9	↓34	6.9	1.6	↑334												
109.12 ± 100.77	468.26 ± 508.56	↓54	3.48 ± 4.53	7.98 ± 8.04	0	20.6 ± 20.55	19.78 ± 30.47	↑108	8.53 ± 9.02	8.46 ± 11.34	↑114	1060.86 ± 439.82	2147.17 ± 1683.45	↓11	8.84 ± 7.05	16.16 ± 16.18	↑15												
95.35	245.6		1.35	6.9		12.55	8.7		3.75	3.7		1000.2	1813.7		7.5	10.1													
0.01*				0.04*		0.8	0			0.9			0.06			0.14													

TABLE 6. CD4, IL-17, IL-33, IFN γ , HLA-DR and tryptase positive cell densities in patients with atopic keratoconjunctivitis after cyclosporine A treatment as compared to normal controls.

Cell Densities (Cell count/mm ²)															
CD4			IL-17			IL-33			IFN γ			HLA DR		Tryptase	
A	C		A	C		A	C		A	C		A	C	A	C
130.8	-		12.4	0.5		9.0	8.1		3.5	0		597.3	130.3	7.8	0.6
154.4	245.9		0.0	5.9		28.6	17.4		12.2	0.6		1735.9	998.1	11.3	29.0
20.3	2.9		1.2	0.0		10.3	4.4		4.0	0		652.6	-	8.2	5.9
16.3	18.6		1.5	3.7		12.1	4.5		15.3	0.9		1127.5	382.1	7.2	11.3
46.6	48.7		0.0	1.3		12.0	15.7		0.8	0.7		1059.1	431.0	11.1	4.4
2.5	133.0		0.8	0.0		13.0	11.7		1.2	3		941.3	860.8	3.3	20.5
127.8	51.3		3.1	4.1		15.0	15.9		0	3.1		845.1	310.8	26.8	6.6
320.2	309.8		5.3	10.3		8.0	86.6		3.5	3.9		1143.6	134.4	1.4	1.0
62.9	36.1		10.5	0.0		76.3	2.0		26.3	3.5		1868.6	251.5	4.4	12.4
209.4	142.4		0.0	0.0		21.7	15.3		18.5	1		637.6	478.1	6.9	41.2
109.12 $\pm$ 100.77	109.86 $\pm$ 107.5		3.48 $\pm$ 4.53	2.58 $\pm$ 3.44		20.6 $\pm$ 20.55	18.16 $\pm$ 24.68		8.53 $\pm$ 9.02	1.67 $\pm$ 1.52		1060.86 $\pm$ 439.82	441.9 $\pm$ 303.09	8.84 $\pm$ 7.05	13.29 $\pm$ 13.22
95.35	51.3		1.35	0.9		12.55	13.5		3.75	0.95		1000.2	382.1	7.5	8.95
0.97				0.68			0.58			0.04*			< 0.01*		0.74

Last three rows are mean $\pm$ standard deviation, median, *P* value

A: Samples from patients with atopic keratoconjunctivitis after a 2-month topical cyclosporine A treatment.

C: Samples from normal controls

**P* < 0.05 indicates statistical significance



FIGURE 3. Slit-lamp picture of a patient with chronic atopic keratoconjunctivitis demonstrating significant scarring of the lower forniceal and tarsal conjunctiva.

smoking status or body mass index. In addition, we did not measure serum immunoglobulin E levels in the AKC group (to determine the severity) or the control group (to ensure they did not have underlying allergy). However, the comparisons of conjunctival inflammation were made not only between controls and AKC subjects but also within the AKC group before and after the CsA treatment. Another limitation is that the sample size is relatively small. AKC is a relatively rare disease, and it is difficult to recruit patients for clinical trials. Assessing the inflammation directly in conjunctival tissues, as opposed to tear samples or impression cytology, is one strength of the study. Also, patients with AKC were on topical treatment using benzalkonium chloride-containing medications such as their glaucoma drops. Because this may have affected the ocular surface immunological characteristics, it can be argued that the patient and control groups were not strictly similar. However, patients with AKC are likely to have co-morbidities and take topical treatment for those outside of a study setting.

Previous research has reported that topical CsA at high doses is effective in improving the clinical findings of steroid-dependent AKC.^{7,8} The combination of CsA eye drops with tacrolimus ointment (0.03%, Protopic; Astellas Pharma US, Inc., Northbrook, IL) has the potential to eliminate the need for steroids and perhaps alter the natural history of AKC by preventing flare-ups.¹³ We found a reduction in the expression of CD4, IL-17 and HLA-DR positive cells in the conjunctival samples following a short-term CsA treatment. However, levels of IFN γ and HLA-DR remained higher than normal controls. Given the possibility of disease progression leading to conjunctival and corneal scarring and visual loss, future therapeutics that target-specific cell subsets and cytokines involved might be of benefit in severe AKC.

DECLARATION OF INTEREST

Dr. Michael Stern was an employee of Allergan Inc. at the time this study was conducted.

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