Investigation of the dry eye and thecorneal Langerhans cellsin inflammatory rheumatic conditions and glaucoma using in vivo confocal microscopy

Doctoralthesis

László Marsovszky, MD

Semmelweis University
School of PhD Studies, Clinical Medicine
Ophthalmology

Supervisors:	Miklós Resch, MD, PhD Attila Balog, MD, PhD
Official reviewers:	Nicolette Sohár, MD, PhD Tamás Constantin MD, PhD
Chairman of Exam Board:	Judit Fidy, MD, PhD. DSc
Members of Exam Board:	Levente Herényi, MD, PhD Ákos Skribek, MD, PhD

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INTRODUCTION

The tear film, lacrimal glands, corneal, conjunctival epithelia and Meibomian glands along with the nervous connections work together as a lacrimal functional unit (LFU) to preserve the integrity and function of the ocular surface. The integrity of this unit is essential for the health and normal function of the eye and visual system. Alteration of this unit results in the development of dry eye disease, in which the ocular surface inflammation plays a role.In these complex interactions the cellular components of both the innate and the adaptive wing of the immune system take part. The confocal cornea microscope is able to help us with in vivo exploration of the cornea in order to gain more understanding of immune processes operating on the ocular surface.

As part of the ocular surface, healthycornea plays an important rolein the cascade of immunological events to maintain its integrity and to secure its transparency. Healthy corneais devoid of lymphocytes, howeverit is endowed with a heterogeneous population of immune cells, including the antigen presenting cells (APC). Antigen presenting cells refer to specialised cells capable of capturing, processing and presenting antigens to T-cells with requisite accessory (costimulatory) signals leading to specific clonal proliferation of the T-cell lineage.

Langerhans cells (LC) belong to the APCs in the cornea, with the their extraordinary capacity to stimulate naive T-cells.Under nonpathological circumstances, the majority of LCs reside in the periphery of the cornea, with numbers of LCs tapering rapidly towards the corneal centre. Upon different stimuli and presence of cytokines like tumor necrosis factor-α and various interleukines (TNF-α, IL-1, IL-6, IL-8, IL-17), LCs are capable of transforming into an active participant of corneal immune responses characterized by the formation of dendrite-like processes. LCs have also been shown to play important roles in promoting inflammatory reactions and immunogenity, andhave a pivotal role in the regulation of ocular surface immune processes.

In several systemic autoimmune diseases,these cytokinesare overexpressed and play animportant role by shapingsubsequent adaptive immune responses and contributing to the organ specific destructive inflammatory reactions.It is also known that DED is more prevalent in systemic inflammatory autoimmune conditions, however the immunity of the cornea in the systemic inflammatory conditions and the precise mechanism leading to DED is still not fully elucidated.

Constant irritation to the ocular surface may alsoalter the homeostasis of the cornea. Glaucoma is of particular concern with regard tochronic use of topical medications which may modify the corneal immunity by exposing ocular surface epithelial cells to adverse effects of drug and preservatives employed.Benzalkonium chloride (BAK) is the principle preservative employed in topical therapy, used in ophthalmology. Although it is effective as an antimicrobial and antifungal agent, in vitrostudies have demonstrated the overexpression of cytokines like TNF-α, IL-1, IL-6on the ocular surface by the presence of BAK. Polyquaternium (PQ), like BAK is a quaternary ammonium compound which has been demonstrated to be less toxic to the corneo–conjunctival surface, however studies are controversial regarding the effect of PQ to the ocular surface.These observations drove our attention to investigate dry eye, LC and subclinical microstructural alterations of the cornea by means of confocal microscopy in inflammatory rheumatic disorders and in glaucoma.

AIMS

In order to elucidate the corneal immunity in systemic inflammatory conditions and to gain more knowledge on the possible role of systemic inflammation on the ocular surface homeostasiswe aimed to evaluatethe dry eye and investigate the LC density, distibution and morphology in three inflammatory rheumatic disorders:

1. Rheumatoid arthritis (RA),
2. Ankylosing spondylitis (AS),
3. Systemic lupus erythematosus (SLE)

Our purpose was also to study the results found in the patient groupswith regards to:

-the therapy applied,
-the disease activity,
-C-reaktive protein,
-the presence of the Human Leukocyte Antigen (HLA-B27)
-the history of uveitis

4. We also aimed to investigate the effects ofcommercially available antiglaucoma drops (travoprost) containing two different preservatives on the ocular surface.

Our purpose was to investigate dry eye, corneal epithelial andLCs and LC activity.

METHODS

The study was performed in accordance with the ethical standards set out in the 1964 Declaration of Helsinki.The investigations related to rheumatic diseases have been carried out in cooperation with theDepartment of Rheumatology, University of Szeged and the National Institute of Rheumatology and Physiotherapy, Budapest.

Inclusion criteria: Inflammatoryautoimmune rheumatic diseases (RA, AS, SLE) and primary open angle glaucoma patients using travoprost therapy have been enrolled in the studies.

Exclusion criteria: Patients with ocular symptoms requiring specific ophthalmic assistance and patients with diabetes; previous eye surgery; contact lens wear; uveitis within one year prior to examination; congenital, mechanical or toxic injury of the cornea; or illnesses causing corneal edema, haze or scars were excluded.RA, AS and SLE patients withsecondary Sjögren's syndrome diagnosed according tothe revised version of the European criteria for Sjögren's syndrome were omitted from the study. Patients with Schirmer test results of less than 5 mm/5 min were not excluded automatically from the study ifthe results of other functional tests and histology werenormal, and no anti-Ro(SSA) or anti-La(SSB) antibodies were detected.

In all cases both eyes have been examined, but only the right eye has been selected in the studies for further analysis and statistics.

1. Clinical characteristics of healthy individuals and RA patients.

Fifty-two RA patients with variable severity and duration of disease, and twenty four healthy individuals were enrolled in the study. Median age for the healthy individuals and for the RA patients were 61 years [52.5–67] and 58 years [49–66] respectively. RApatients were classified according to the latestAmerican College of Rheumatology/European LeagueAgainst Rheumatism (ACR/EULAR criteria).Disease activity score 28 (DAS28) was calculated at the time of the ophthalmic examination. Patients have been selected for group analyses according to the antiinflammatory therapy applied (anti-TNF-αand glucocorticosteroid therapy). The cut-off point for lower disease activity was DAS-28≤2,6.

All RA patients received at least one of the followingdisease-modifying anti-rheumatic drugs (DMARD): methotrexate (69%), leflunomide (17%),sulfasalazine (1%) and chloroquine (1%). Twentythreeof 52 RA patients (44%) received an anti-TNF(Tumor Necrosis Factor)

agent, and 16 of 52 RA patients were also treated withlow-dose oral glucocorticoids (GCS) and DMARDand/or anti-TNF agent together.

2. Clinical characteristics of healthy individuals and AS patients.

Twenty-four AS patients (male/female ratio: 21/3) with variable disease severity were studied along with the analysis of 24 healthy subjects (male/female ratio: 19/5). Median age was 39.5 years [34–52] for AS and 52.5 years [34–64] for healthy subjects respectively.AS patients were diagnosedaccording to the modified New York criteria.Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was calculated at the time of the ophthalmic examination.The cut-off point for lower disease activity was BASDAI≤4.Median BASDAI was 3.2 [1.5–4.4], while CRP (C-reactive protein) was 3.2 12.0[2.5–30.6] in the patient group. Twelve (50%) patients were HLA-B27-positive, and seven patients had a history of uveitis(29%).

3. Clinical characteristics of healthy individuals and SLE patients.

We have also examined twenty seven SLE patients (mean age: 42.8±11.4 years, male/female ratio: 2/25) and 27 age-, and gender-matched control subjects (mean age: 40.4±19.3 years, male/female ratio: 6/21).SLE was diagnosed according to the 1997updated American College of Rheumatology (ACR) criteria.The SLE patients had various disease duration and activity assessed by the SystemicLupus Erythematosus Disease Activity Index (SLEDAI). Patients with high disease activity had a higher score (SLEDAI >8), while patients with moderate disease activity had a lower score (SLEDAI 1-8), and patients in remission were of SLEDAI score zero (8 patients).Three patients represented the higher disease activity.We also examined the CRP level. The cut-off point for the higher systemic inflammation was CRP>5mg/l.

4. Clinical characteristics of healthy individuals and glaucoma patients.

Nineteen eyes of 19POAG (primary open angle glaucoma) patients with TravBAK (travoprost with benzalkoniumchloride preservative) (male/female ratio: 11/8,mean age: 64.8±13.6 years) and nineteen eyes of 19 POAGpatients with TravPQ (travoprost with poliquaternium preservative) (male/female ratio: 10/9, mean age:66.8±11.3 years) and nineteen age-matched controls (tenfemale and nine male, mean age: 63.8±8.2 years) were enrolledin the study.

Patients were on monotherapy (either TravBAK or TravPQ))and used one of the medication for average 1,76±0,9 and 1,69±0,8 for years respectively. No other eyedrop was used on a regular base.

A)Evaluation of dry eye

The following parameters have been examined in the studies:

-Ocular Surface Disease Index (OSDI)

Patients have been asked to fill this questionnaire, which is a 12-item scale for the assessment of symptoms related to dry eye disease. OSDI = score x25/questions answered

-Lid Parallel Conjunctival Folds (LIPCOF)

Conjunctival folds are characteristic features of dry eye disease. According to the height and morphology, gradingcan be made on a 0–3 scale reflecting the severity of dryeye.

-Tear production (Schirmer I test)

The Schirmer-strip (ST strip Ref:4701001; Haag-Streit, Harlow, UK)was used to evaluate tear production and was placed atthe temporal aspect of the lower conjunctival sac.Values were read after 5 min with the help of a ruler.Patients with Schirmer test results of less than 5 mm/5 min were not excluded automatically from the study ifthe results of other functional tests and histology werenormal, and no anti-Ro(SSA) or anti-La(SSB) anibodies were detected.

-Tear Break-up Time (TBUT)

Fluorescein was introduced into the eye by touching thelower conjunctival sac with a steril strip (Fluorescein Sodium Ophthalmic Strips U.S.P. Bio-Tech Vision Care Ltd. Khatraj, India).Tear break up time (TBUT) described the intervalbetween the last complete blink and the first appearanceof corneal black spot in seconds, and the meanvalue of three consecutive measurements was interpretedas TBUT.

B)Confocal microscopic investigations:

Image acquisition: In vivo confocal scanning laser cornea microscopy was carried out with Heidelberg Retina Tomograph with Rostock Cornea Module (HRTII/RCM) (Heidelberg Engineering, Heidelberg, Germany) on all participants. The microscope has been designed to capture two-dimensional images with a resolution of 384x384 pixels covering an area of 400x400 µm.

Forty consecutive images of the cornea were taken in each group to assess theLC density and LC morphology (LCM) along with intermedier and basal epithelial cell densities in glaucoma patients and in the corresponding control group.Epithelial cells were examined at the corneal apex only, while LC densities and morphology was assessed both at the centre and the cornealperiphery at 6 o'clock position.

Corneal image analysis: Five characteristic images wereselected, and used in thestatistics.LCM was evaluated on a 1–3 scale according to the lenght of the dendrite in relation with the individual dimension of the LC body.

Statistics:Comparisons between healthy individuals and RA patients, as well as between RA patient subgroups, were made with Cochran-Armitage trend tests in case of ordinal variables (LCM and LIPCOF). Continuous variables were compared using t-tests. Comparisons between healthy individuals and AS, SLE and glaucoma patients were made with Mann–Whitney tests. We used Spearman's rank correlation test to investigatecorrelations within groups. P-values<0.05 were considered significant. Statistical analysis was performed using STATISTICA version 8.0 (StatSoft, Tulsa, OK, USA).

RESULTS

1.The dry eye and corneal Langerhans cell investigations inRheumatoid Arthritis

Dry eye related results

The OSDI scores were higher and TBUT showed a trend to be lower than normal in RA compared to healthy individuals.There was no difference between RA and control in LIPCOF and tear production however the latterwas decreased in RApatients on Glucocorticosteroid(GCS) therapy compared to control.

Confocal microscopy data

The central and peripheral corneal LC densities were higher in RA patients compared to healthy individuals. The central LCM valuein RA was also higher than in controls. These data indicatethat mature dendritic cells accumulate in the cornea ofRA patients. The central LC density and the centralLCM values were increased, even in RA patients inremission (<2.6 DAS28).GCS therapy decreased the peripheral LC density.

2. Dry eye and corneal Langerhans cell investigations in Ankylosing Spondylitis

Dry eye related results

OSDI score was greater and tear production was significantly reduced in AS patients compared to healthy individuals. OSDI score was also significantly greater in patients positive for human leukocyte antigen HLA-B27 than in controls. Further analyses in AS subgroups showed decreased tear production in patients with a CRP level of>5 mg/l compared to patients with a CRP level of <5 mg/l.

Confocal microscopy data

Central and peripheral corneal LC density, as well as central LCM, were significantly greater in AS than in the control group. Further analyses in AS subgroups showed increased LC density (in relation to control) in patients irrespective of the disease activity, as assessed by the BASDAI index and the serum CRP levels. Correlation analysis showed a strong positive correlation between BASDAI and peripheral LC density. The activation status of the LCs, as assessed by the central corneal LCM score, was increased in patients with CRP levels >5 mg/l compared to controls. We found no further difference between AS subgroups formed according to the therapy.

3. Dry eye and corneal Langerhans cell investigations in systemiclupus erythematosus

Dry eye related results

Significant differences were detected in three out of four dry eye related parameters between SLE and control groups.: Schirmer test and TBUT values were lower in SLE patients and OSDI scores were greater in SLE patients than in control patients. Subgroup analysis showed, that in cases of complete remission (SLEDAI = 0), only TBUT was lower than in healthy subjects (Mann-Whitney test, $p<0.05$). In mild disease activity (SLEDAI 1-8), TBUT and OSDI, while in high systemic activity of SLE (SLEDAI >8), not only TBUT, but Schirmer and OSDI also showed significant difference in comparison to control. No significant difference in LIPCOF could be demonstrated in any subgroup.

Confocal microscopy data

Central corneal LC density was significantly greater in the SLE than in the control group. We have found LCs in the corneal centre in every SLE patient compared to only eight (29,6%) individuals in the control group.Further analyses in SLE subgroups showed increased central LC density even in patients in remission as assessed by the SLEDAI index.The LC number was higher in patients with CRP levels >5 mmol/l compared to controls.We found no difference between SLE subgroups formed according to the therapy.We did not find correlation between tear production and LC number.

4. Dry eye evaluation andcorneal epithelial and Langerhans cell investigations in glaucoma patients.

Dry eye related results

OSDI score was greater in the glaucoma patient group compared to control. Tear production was significantly reduced in both glaucoma patient groups irrespective of the preservative employed in comparisonto healthy individuals. Both the OSDI and LIPCOF scores were greater and TBUT was significantly reduced in patients treated with TravBAK compared to healthy subjects. No difference was detected in dry eye parameters between the two patient groups.

Confocal microscopy of the corneal epithelial cells

There was no difference in the wing cell number between patient and control subjects. Basal cell density was higher in glaucoma patients than in healthy individuals. Basal cell number was significantly greater in patients with TravBAK therapy compared to TravPQ, which may be a consequence of the proliferate stimuli secondary to BAK effect.

Confocal microscopy of the corneal LCs

Central LC density was significantly greater in all glaucoma patient groups compared to control. Central LC maturation score was significantly greater in glaucoma patient groups compared to control subjects. Central LC density was significantly greater in patients with TravBAK compared to patients on TravPQ therapy. Central LCMscoreswere significantly lower in patients on TravPQ compared to patients on TravBAK.

CONCLUSIONS

1. The role of the systemic inflammation in relation to the development of dry eye disease could be partly justified in RA as we have demonstrated the tear production to be decreased only in patients on GCS therapy.

Our team was the first to have described that LCs are in greater number both at the corneal centre and the periphery and display maturephenotype at the corneal centre representing their activity.

2. We have demonstratedfor the first time that tear production is decreased in AS even without the presence of secunder Sjögren's syndrome. We have also shown, that higher systemic inflammation and activation of the disease in AS (higher CRP, BASDAI) results in lower tear production.

We have shown for the first time that LC number is increased both at the corneal centre and the periphery and LCs at the corneal centre are active. We found, that HLA-B27 antigen does not alter LC number and activity therefore we concluded, that HLA-B27 antigen does not play an important role in corneal immunity in AS.

3. Our team was the first to demonstrate that tear production is decreased in SLE patients with higher disease activity even without secunder Sjögren's syndrome.

We have described for the first time that LC number is increased at the corneal centre and LCs at the corneal centre are of active phenotype.

4. We have demonstrated compromised tear production in glaucoma patients using travoprost eye drops regardless the preservative employed.

We have shownfor the first time there is a correlation between the preservative used in travatan eye drop and corneal LC number:patients on glaucoma drops containing benzalkonium-chloride presented with higher central LC number and LCM than those with polyquaternium.Patients on TravPQ therapy showed increased LC number and activationwhich might support the immune modulating effect of it and call for attention that poliquaternium might also interfere withthe homeostasis of ocular surface.

PUBLICATIONS

Publications related to the thesis

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