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The role of complement activation in the pathogenesis of Fuchs' dystrophy

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ABSTRACT

Purpose: Inflammation can be an etiologic factor of Fuchs' dystrophy according to previous studies. Our aim was to analyse the activation of the complement system in the aqueous humor in this pathological condition.
Methods: 100 μl aqueous humor sample was taken during keratoplasty of 11 Fuchs' dystrophic patients and during phacoemulsification surgery of 18 control patients. The samples were mixed with EDTA and stored at −80 °C. Concentrations of C1rC1sC1Inh and C3bBbP complexes as markers of the activation of the classical and alternative complement pathways, respectively, were measured with ELISA method. The results of the patient group and the control group were compared with statistical analysis (non-parametric Mann Whitney test).
Results: Both the concentrations of C1rC1sC1Inh [4.3 (3.2–20.2) AU/ml] and of C3bBbP [15.3 (7.8–22.6) AU/ml] were significantly higher in the Fuchs' dystrophic group than in the control group [C1rC1sC1Inh: 0.0 (0.0–5.6) AU/ml, C3bBbP: 1.4 (0.0–7.8) AU/ml]. The median value is shown along with the (25% and 75% percentiles).
Conclusions: Based on our results, the complement system may be activated both through the classical and alternative pathways in the aqueous humor of the patients with Fuchs' dystrophy.

1. Introduction

The endothelium is the most inner layer of the five layers (epithelium, Bowman layer, stroma, Descemet layer, and endothelium) of the cornea. It is most important roles are feeding the cornea and keeping it dehydrated. Keeping the cornea dehydrated is very important for maintaining good vision, as in case the cornea is not dehydrated – oedematous –, it losts its transparency. Normally the endothelium is composed of regular hexagonal cells with unique size and with density of at least 1500–2000 cells even in elderly people.

Fuchs' dystrophy is a bilateral disease of the cornea, which is characterized by progressive loss of endothelial cells, where the cells' shape and size are different from each other. Furthermore the Descemet membrane – the fourth layer of the cornea, the basement membrane of the endothelium – is irregularly thickened and central guttae appear. The prevalence of cornea guttata, which is the preliminary phase of the Fuchs' dystrophy, is about 10% in the population over 60. If the endothelial cell count falls under about 500/mm^2, cornea endothelial decompensation occurs, which means that stromal and epithelial oedema develops, which leads to rising visual loss and pain (Adamis et al., 1993).

There are numerous theories on the etiology of the endothelial destruction in Fuchs' dystrophy, which are partly of genetic origin and partly environmental. The abnormality of the endothelium may originate from the failure of the final endothelial cell differentiation in the perinatal period (Bahn et al., 1984). It can be related to hormonal discrepancy (Johnston et al., 1982). Increased apoptosis may take place among the endothelial cells (Szentmary et al., 2005; Borderie et al., 2000) as well. The numerous fibroblastic metaplasia among the endothelial cells and the fact that similar clinical picture may develop as a consequence of trauma or infection raises the possibility of the inflammation's role (Iwamoto and DeVoe, 1971).

The complement system is a complex system of more than 30 proteins that act in concert to help eliminate infectious microorganisms. Specifically, the complement system causes the lysis (bursting) of foreign and infected cells, the phagocytosis (ingestion) of foreign particles and cell debris, and the inflammation of surrounding tissue. The interacting proteins of the complement

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system, which are produced mainly by the liver, circulate in the blood and extracellular fluid, primarily in an inactivated state. The system is activated by appropriate signals, which set off a chain reaction in which one activated complement protein triggers the activation of the next complement protein in the sequence. There are three pathways of activation: the classical pathway, the alternative and the lectin pathways. In all three pathways, C3-convertase cleaves and activates component C3, creating C3a and C3b, and causing cascade of further cleavage and activation events. When the complement system is activated, biologically active anaphylatoxins and activation products appear. The detection of these products demonstrates the activation of the complement system. The marker of the activation through the classical and the alternative pathway is – among others – the C1rC1sC1inh and the C3bBbP complex, respectively.

The possible role of the complement activation in the pathomechanism of Fuchs' dystrophy was raised by one group in a paper published more than 30 year ago (Bramsen and Ehlers, 1977). Tranexamic acid was given systemically for patients suffering in Fuchs' endothelial dystrophy. In all the 20 cases the central corneal thickness decreased, symptoms and signs improved. In another study from 1990 the C3a complement activation product in the aqueous humor of cataract patients was measured, and the concentration was significantly increased in patients with Fuchs' dystrophy, compared to patients with healthy cornea (Tuberville and Wood, 1990). The mechanism of the activation of the complement system in the pathological processes in the endothelium leading to Fuchs' dystrophy is, however, still unknown.

Therefore our present study aimed to gather information on the role of the complement system by measuring the levels of the activation products both of the classical and alternative pathways in the aqueous humor of patients with Fuchs' dystrophy and control patients.

2. Patients and methods

2.1. Study subjects

Eleven patients with Fuchs' dystrophy were involved into the study. The patients were in good health state, only one (patient 10) of them had seasonal allergy, treated with systemic antihistamine and nasal steroid spray. Two patients (patients 7 and 10) used steroid eye drops for the inflammatory state of the ocular surface caused by the Fuchs's dystrophy. The mean age was 71.3 ± 10.5 years and there were 8 women and 3 men in the group. In case the problem was bilaterally symmetrical, only one eye was included, which was chosen randomly. The eyes in the Fuchs' dystrophic group were phakic with no history of previous surgery, and except for patient No. 8 all of them had cataract. Data on the duration of the symptoms were collected from all patients with Fuchs' dystrophy. Stage of the disease was assessed according to the system described by Elhalis et al. (2010). Briefly, four stages were assessed. In stage 1, only guttae are present. In stage 2, the guttae start to coalesce and the patients begin to experience decrease in vision and glare symptoms due to increasing edema of stromal layers. In stage 3, stromal edema further progresses towards the epithelial layer and causes formation of epithelial and subepithelial bullae. The rupture of these bullae causes episodes of pain. In stage 4, the cornea becomes densely opaque and vascularized, there is subepithelial fibrous tissue deposition (Table 1).

The control group consisted of 18 eyes of 18 patients who were listed for cataract surgery and with healthy cornea without history of previous eye surgery. The patients were in good health state, but three of them had seasonal allergy, one person treated with systemic antihistamine and nasal steroid spray, the other two took only local antihistamine. Another patient had rheumatoid arthritis in quiet stage, beside using low dose systemic steroid drug. The mean age was 73.1 ± 10.7 years, there were 12 women and 6 men. (This group of patients has already served as control in our previous study (Füst et al., 2013).) The study protocol adhered to the tenets of Declaration of Helsinki and the Institutional Ethics Committee of Semmelweis University provided ethical approval for the study. The written informed consent was obtained from all the subjects.

Table 1
Demographic data in the Fuchs' dystrophy group (F: female, M: male).

Patients	Gender	Age (year)	Duration of symptoms (month)	Stage
1	F	78	24	2
2	F	61	20	3
3	F	87	12	3
4	F	79	n.a.	2
5	M	73	12	2
6	F	83	48	3
7	M	76	7	3
8	F	54	20	2
9	F	66	12	2
10	M	68	12	2
11	F	59	60	2

2.2. Measurement of complement activation products in the aqueous humor

Approximately 100 μl aqueous humor sample was collected through the first corneal incision of the keratoplasty in Fuchs' dystrophy patients. The same procedure took place at beginning of the cataract operation in the control group. The samples were divided into two tubes containing EDTA, and they were stored in −80 °C. There was a <1 h long gap between the collection time and the start of the storage in either group.

The method of measurement of C1rC1sC1Inh and C3bBbP was described in detail in our previous papers (Füst et al., 2013; Kerenyi et al., 2002).

2.2.1. Measurement of C1rC1sC1Inh

The aqueous humor concentration of C1rC1sC1Inh was determined by enzyme-linked immunosorbent assay (ELISA). In brief: plates (Nunc, Maxisorp F96) were coated with 1:500 diluted rabbit anti-human C1-inhibitor (Dako, Denmark) at 4 °C for overnight. After blocking the plates (PBS, 1% BSA), 1:3 diluted aqueous humor samples and standards (normal human serum activated with heat-aggregated IgG, 1:4000–1:128,000 diluted) were incubated. In the next step, 1:500 diluted goat anti-human C1s (DiaSorin, USA) was added as second antibody. Thereafter, 1:1000 diluted peroxidase-conjugated rabbit anti-goat IgG (Jackson Immunoresearch, United Kingdom) was added. The reaction was visualized by ABTS substrate (Sigma–Aldrich, Germany) and the reaction was stopped with 0.2 M oxalic acid. Optical density was measured at 405/492 nm. Concentrations are expressed in units per ml of sample substrate; 1000 U correspond to the C1rC1sC1Inh-content of 1 ml undiluted, heat-aggregated, IgG-treated normal serum. Intra- and interassay variation did not exceed 15%.

2.2.2. Measurement of C3bBbP

The aqueous humor concentration of C3bBbP was determined with sandwich enzyme-linked immunosorbent assay (ELISA). In brief: plates (Nunc, Maxisorp F96) were coated with 1:1000 diluted goat anti-human properdin factor B (Incstar Corporation, USA) at 4 °C for overnight and washed two times in washing buffer (PBS containing 0.1% Tween 20, pH 7.4). After blocking (PBS, 1% BSA), 1:3 diluted aqueous humor samples and standards (normal human serum activated with zymosan, 1:100–1:12,800 diluted) were applied to the plates. In the next step, 1:2000 diluted