Conflict of interest
The authors declare no conflict of interest.

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A Hedayatfar[1] and SP Chee[1,2,3]

[1]Ocular Inflammation and Immunology Department, Singapore National Eye Centre, Singapore, Singapore
[2]National University of Singapore, Singapore, Singapore
[3]Singapore Eye Research Institute, Singapore, Singapore
E-mail: Chee.soon.phaik@snec.com.sg

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Sir,
Response to Hedayatfar *et al*

We thank Hedayatfar and Chee[1] for their interest in our article.[2] In the below we hope we have answered the questions they raised.

1. We speculated the pink hypopyon was caused by severe necrosis because *Klebsiella pneumonia* causes classic cases of pneumonia, characterized by brick-red or 'currant jelly' sputum. The biological effects of *Klebsiella pneumonia* in animal study produced fever, capillary haemorrhage, hypotension, and circulatory collapse in animals, symptoms that are similar to those seen in humans with Gram-negative sepsis.[3] We agree that red blood cells were not revealed on the aqueous smear. It might be caused by haemolysis before fixation.
2. This reported case is a healthy individual who does not have diabetes mellitus or other systemic diseases. The pink hypopyon in our case was most likely caused by *Klebsiella pneumonia*, based on the clinical course and the culture reports of vitreal aspirates and liver abscess. This patient had rapid visual loss in 2 days with topical and oral steroids, and her right eye orbital cellulitis and liver abscess resolved after she received intravenous ceftriazone. The aim of our reporting this case is to raise the issue that *Klebsiella pneumonia* is one of the causes of a pink hypopyon.

Conflict of interest
The author declares no conflict of interest.

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A-N Chao

Department of Ophthalmology, Chang Gung Hospital and Chang Gung University, College of Medicine, Taoyuan, Taiwan
E-mail: anning@adm.cgmh.org.tw

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Sir,
Expression of tumor necrosis factor-α and interleukin-6 in corneal cells after excimer laser ablation in Wistar rats

The release of tumor necrosis factor-α (TNF-α) has been reported to be increased in human tear fluid during the first 2 postoperative days following excimer laser phototherapeutic keratectomy (PTK).[1] Interleukin-6 (IL-6) has also been found to be increased in the human tear fluid from 12 PTK patients, as measured 24 h after PTK.[2] In this study we have analyzed the gene expression of TNF-α and IL-6 in rat corneas after PTK.

In all, nine eyes of nine Wistar rats received excimer laser PTKs (B&L Kerakor 217 laser (Bausch and Lomb, Chiron Technolas GmbH, Dornach, Germany), optical zone 4 mm, 1600 pulses, nominal ablated depth 50 μm). Three groups of three rats each were killed at 1, 12, and 24 h after treatment, respectively. An additional group of three rats without previous PTK served as control group. From all the collected eyes, 4 to 5 μm paraffin sections were obtained on RNAase-free silan-coated slides and further analyzed by nonradioactive mRNA *in situ* hybridizations, using the DIG-labeling and detection kit from Roche Diagnostics (Mannheim, Germany), as described.[3] Statistical analysis was performed using the two-tailed Mann–Whitney *U*-test, and differences were considered significant at $P<0.05$.

At 1 h after PTK, the gene expression of the cytokines TNF-α and IL-6 was higher than in untreated controls, but lower than 12 h after treatment (Table 1). The increases observed between 1 and 12 h after PTK were statistically significant for both cytokines ($P=0.0005$ and $P=0.0078$, respectively; Table 1). The expression of the inflammatory cytokines TNF-α and IL-6 was detected not only in epithelial, endothelial, and infiltrating cells,[4] but also in the keratocytes from the corneal stroma (Figure 1). Whereas at 24 h after PTK, the expression of both cytokines remained higher than in the controls, slight decreases could be observed when compared with the results at 12 h after PTK treatment ($P=0.0244$ and $P=0.0142$, respectively; Table 1).

Table 1 TNF-α and IL-6 gene expression in corneal sections from Wistar rats, as detected by specific nonradioactive *in situ* hybridizations, at 1, 12, and 24 h after PTK[a]

Time after PTK (h)[b]	*TNF-α gene expression*		*IL-6 gene expression*	
	Score	*Means ± SEM* (P (t, t-*1*))	*Score*	*Means ± SEM* (P (t, t-*1*))
0 (controls)	−	0.3 ± 0.2 −	+/−	1.0 ± 0.3 −
1	+/−	1.0 ± 0.3 $P = 0.1359$	+	2.0 ± 0.4 $P = 0.0939$
12	+ +	3.3 ± 0.3** $P = 0.0005$	+ + +	3.7 ± 0.3** $P = 0.0078$
24	+	2.0 ± 0.3* $P = 0.0244$	+ +	2.7 ± 0.2* $P = 0.0142$

[a]Scores used for evaluation of the *in situ* hybridization results: (− = 0) negative reaction/not detected; (+/− = 1) positive reaction, <5% positive cells; (+ = 2) clearly positive reaction, ~5–10% positive cells; (+ + = 3) extended positive reaction, 10–20% positive cells; (+ + + = 4) very extended positive reaction, 20–50% or more positive cells. The evaluation procedure was repeated at least three times, by three independent evaluators, and was always performed without any sample reference. The numerical data are presented as score means ± SEM.
[b]Time indicates the time point in which animals were killed, in hours after the PTK treatment was completed.
*$P < 0.05$; **$P < 0.01$, statistically significant values when compared with the corresponding values observed for the time point immediately before (P (t, t–1)). The P-values were calculated using the nonparametric two-tailed Mann–Whitney U-test.

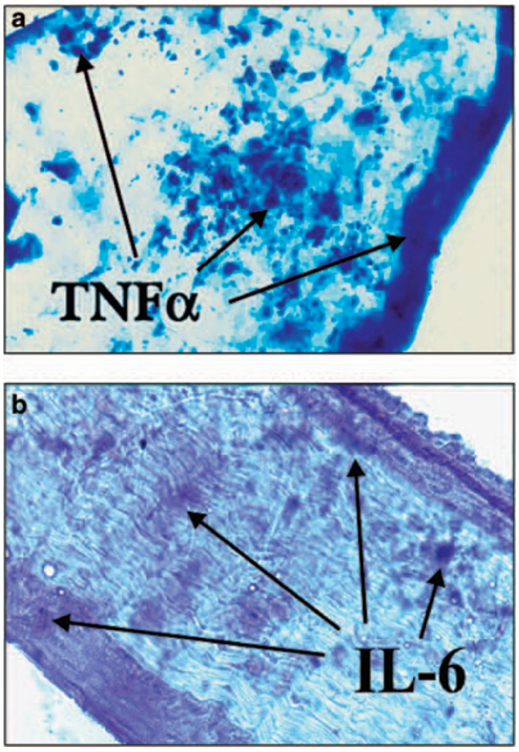

Figure 1 Detection of TNF-α (a) and IL-6 (b) gene expression using nonradioactive *in situ* hybridizations in paraffin corneal sections from Wistar rats at 1, 12, and 24 h after PTK. (a) Representative TNF-α mRNA hybridization pattern in a paraffin section from rat 2 of group 4 (24 h after PTK, hybridization score 3 = + +). (b) Representative IL-6 mRNA hybridization pattern in a paraffin section from rat 1 of group 4 (24 h after PTK, hybridization score 3 = + +). A positive reaction was evaluated as a dark blue–violet color, which was the result of the corresponding reaction using the DIG detection kit from Roche Diagnostics. TNF-α, TNF-α mRNA; IL-6, IL-6 mRNA. Original magnification: × 1000.

TNF-α derived from epithelial cells after laser treatment has been found to modulate apoptosis and expression of chemokines, which would further attract monocytes and granulocytes into the corneal stroma.[5] Moreover, IL-6 released from epithelial cells and keratocytes after PTK has been reported to modulate the synthesis of collagen and the production of metalloproteinases.[2] All these effects may have a relevant role in wound healing after laser treatment.

Conflict of interest

The authors declare no conflict of interest.

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We hereby certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during this research.

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J Prada[1,2], S Schruender[2], T Ngo-Tu[2,3], H Baatz[2], C Hartmann[2] and U Pleyer[2]

[1]Biomedizinisches Forschungszentrum, Charité-Universitätsmedizin Berlin, Campus Virchow-Klinikum, Humboldt University of Berlin, Berlin, Germany
[2]Department of Ophthalmology, Charité-Universitätsmedizin Berlin, Campus Virchow-Klinikum, Humboldt University of Berlin, Berlin, Germany
[3]Department of Microbiology, University of Hanoi, Hanoi, Vietnam
E-mail: javier_prada@yahoo.de

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