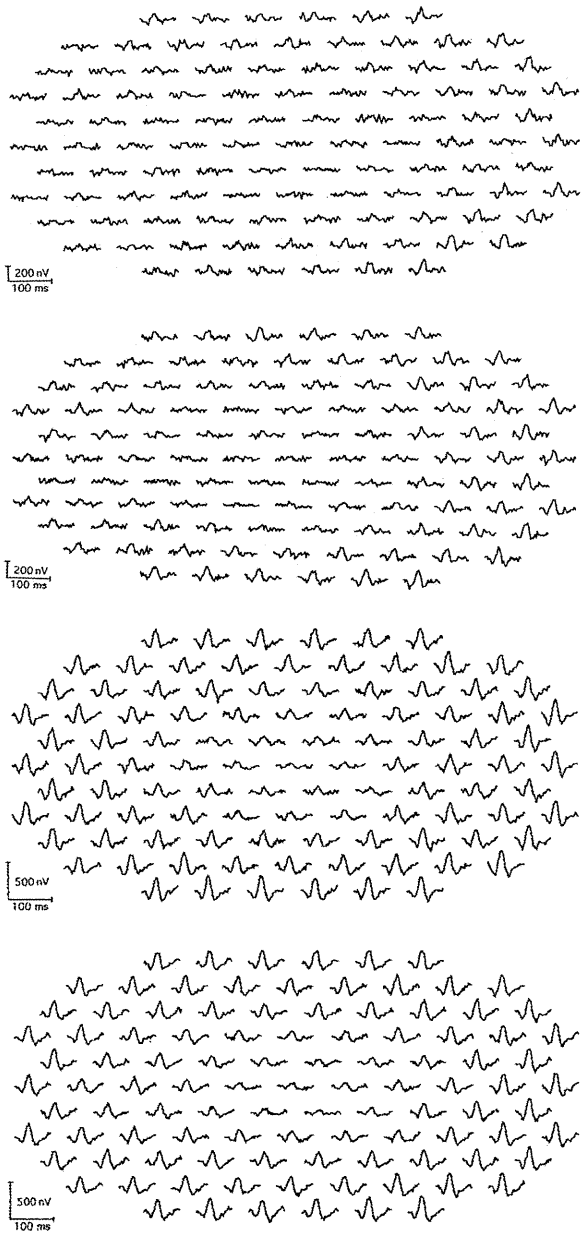
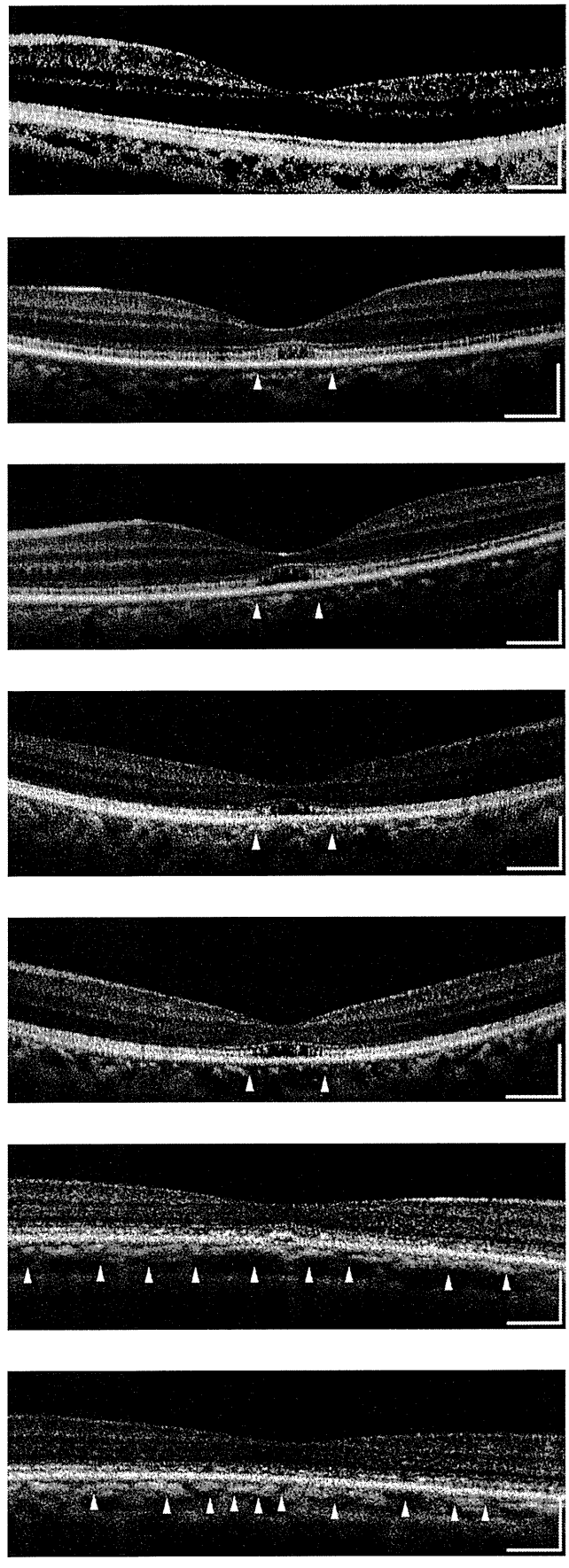


Figure 1 Multifocal electroretinogram (ERGs) of right and left eye of patient 1 (**A, B**), patient 2 (**C, D**), patient 3 (**E, F**), and patient 4 (**G, H**). Foveal amplitudes are decreased in all eyes. Especially in patient 3, amplitudes are attenuated widely including ring 5 and 6, although full-field ERG showed normal amplitude.

external limiting membrane (ELM) and the retinal pigment epithelium (RPE) (Figures 2B–2E). In patient 3, the IS/OS line and the RPE line were disrupted, and the retina was thinner (Figures 2F and 2G). The outer segment layer between the IS/OS line and RPE line of the FD-OCT images was visible only in the center of the fovea in the left eye of patient 4 (Figure 2I).

AO fundus images

The AO images showed patchy dark areas in all eyes, which disrupted the mosaic of bright spots in the fovea



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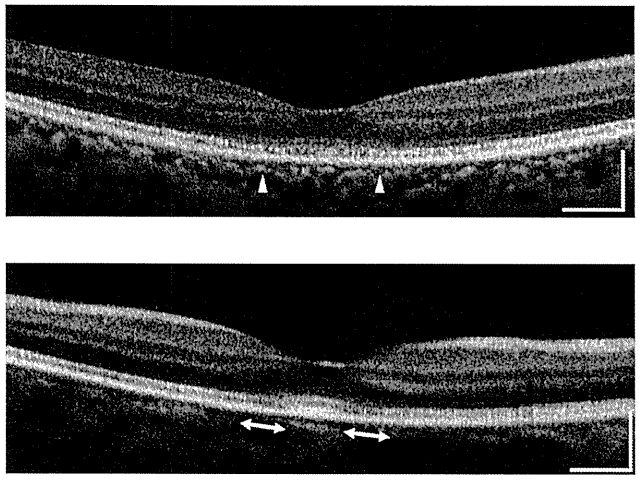

Figure 2 Fourier-domain optical coherence tomography (FD-OCT) images (horizontal scan) of normal eye (**A**), and right/left eyes of patient 1 (**B, C**), patient 2 (**D, E**), patient 3 (**F, G**), and patient 4 (**H, I**). Horizontal bars represent 500 μm, and vertical bars represent 200 μm. The eyes in patients 1–4 had a bilateral symmetric decline in visual acuity, whereas those in patient 5 had an assymmetric decline (20/200 right eye, 20/20 left eye). FD-OCT in normal eye provided clear images of the retinal layers. The external limiting membrane (ELM), photoreceptors inner and outer segment (IS/OS) junction, third line, and retinal pigment epithelium (RPE) are distinguishable. Meanwhile, the retinal photoreceptor layer is not clear in the eyes of the patients. Although ELM was visualized in all eyes, IS/OS is elevated and disrupted in fovea (**B, C, D, E, H**), widely disrupted and not clear (**F, G**), and clearly visualized in one eye (**I**). The third line was visualized only in one eye (**I**), just in the fovea.

(Figures 3B–3H), compared with normal control (Figure 3A). This suggested a degeneration of some of the photoreceptors in this area. Nonuniform bright spots with irregular shapes and higher brightness appeared around the dark areas. In patient 3, the normal cone mosaic was replaced by dark areas, and nonuniform bright spots appeared to be all that remained (Figures 3F and 3G). In the left eye of patient 4, the mosaic of blight spots were in relatively good order with fewer dark areas in the center of the image, although the mosaic was disrupted in the peripheral area (Figure 3I).

Discussion

We had hypothesized that the main structures affected in eyes with OMD were the photoreceptors as in other types of retinal dystrophy, and the morphological changes of the photoreceptor could be detected by high-resolution retinal imaging techniques. This is important in eyes with OMD because histopathological sections of eyes with OMD have not been published. In cone-rod dystrophy, a loss of cones in the perifoveal area has been reported, and the number of cones is reduced in the extrafoveal and peripheral areas.[25,26] In addition, the length of photoreceptor outer segments has been reported to be shortened,[25,26] and an accumulation of lipofuscin granules in the RPE has also been reported in these eyes.[26]

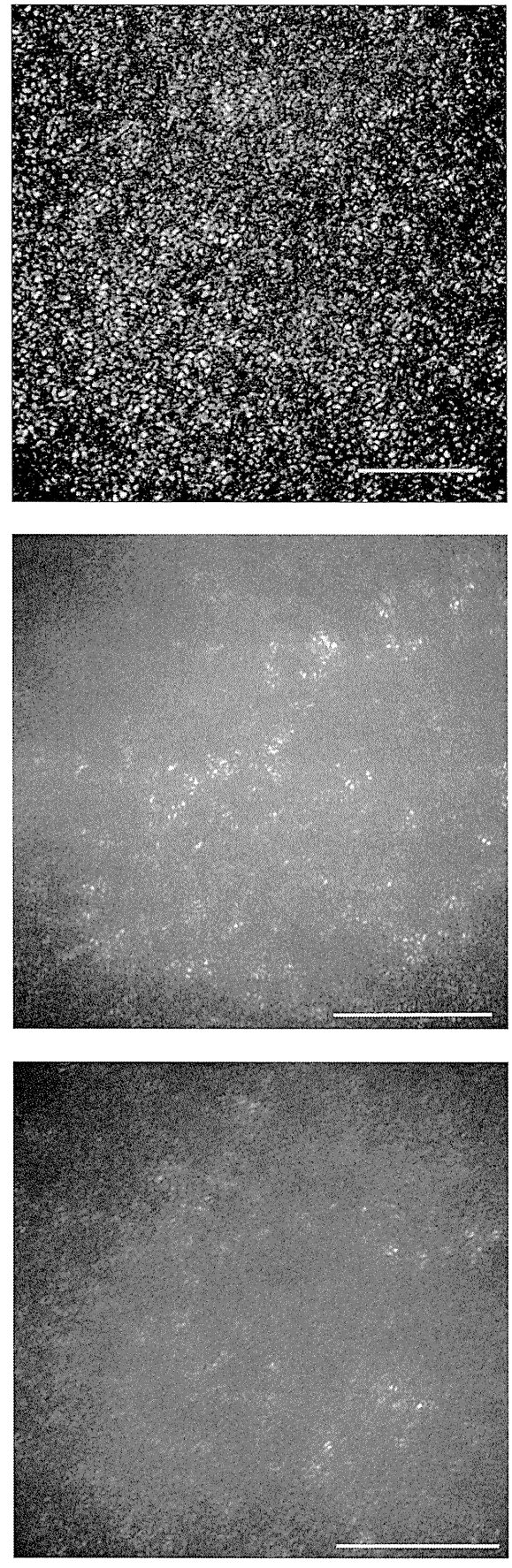

Figure 3 *(Continued)*

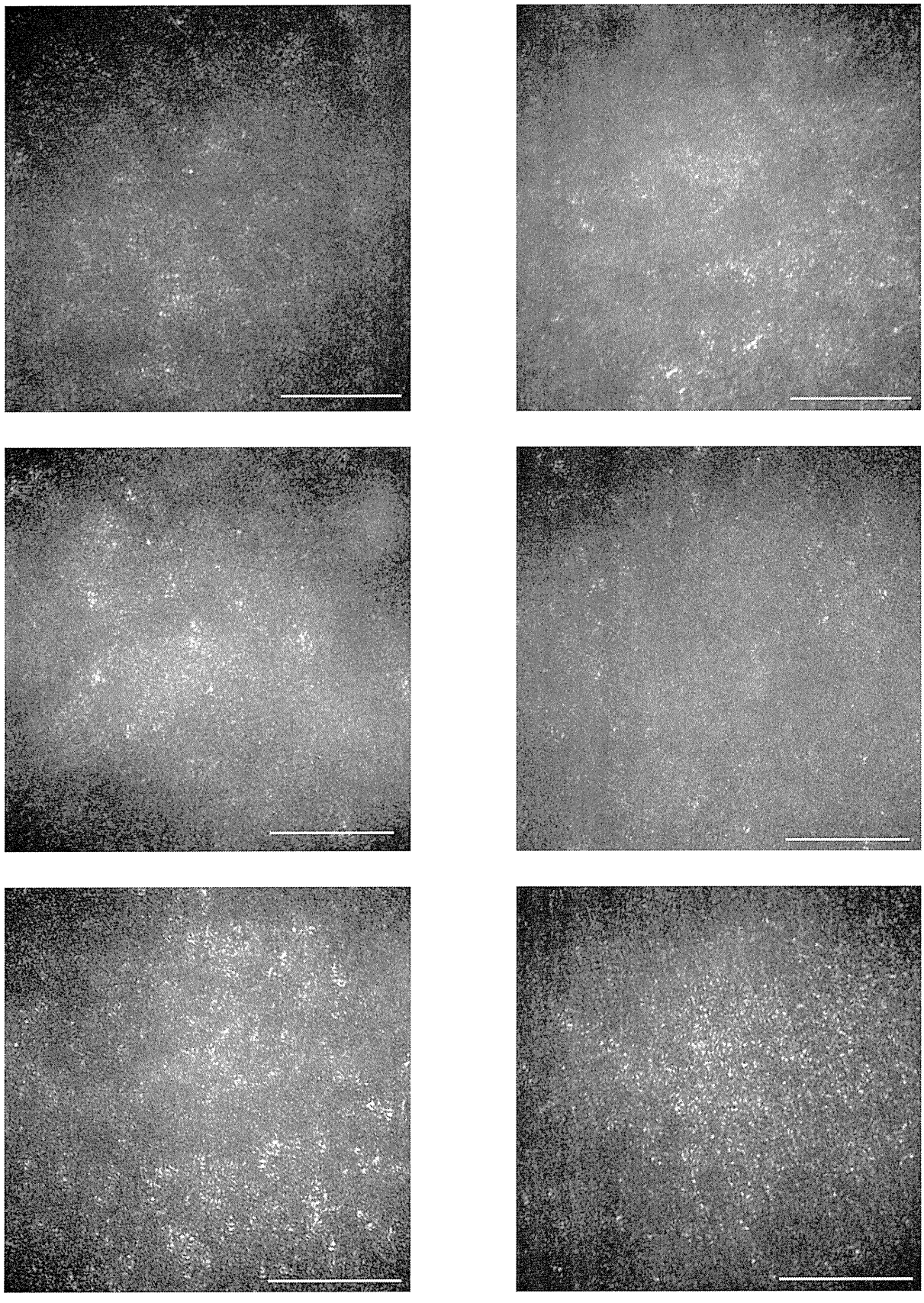

Figure 3 Adaptive optics (AO) images of the fovea of a normal eye (**A**), and right/left eyes of patient 1 (**B, C**), patient 2 (**D, E**), patient 3 (**F, G**), and patient 4 (**H, I**). Bars represent 100 μm. In the eyes of the patients, signals from the cone mosaic were attenuated, and the bright spots were distorted (**B–H**). One eye which had normal visual acuity had an almost normal appearance in the foveal center, with some dark areas around the fovea.

The FD-OCT images showed a disruption of the IS/OS line and a loss of the third highly reflective line in the center of the fovea in all eyes except for the left eye of patient 4 whose BCVA was good. These findings are consistent with recent reports that there was a significant correlation between the disturbance of the IS/OS junction and the BCVA.[7,10,11]

The origin of the third bright line in the FD-OCT images has not been determined. It cannot be detected in highly myopic eyes even if the patient has good visual acuity. This suggests that the third line cannot be resolved if the length of photoreceptor outer segments is not long enough. In our patient, the third line was not detected even though they were not highly myopic. We suggest that the shortening of the photoreceptor outer segments is the reason why the third bright line cannot be seen in the FD-OCT images. However, the third line was seen in the center of the foveal area of the left eye of patient 4. In this case, we suggest that the photoreceptor outer segments were long enough in this area, and the visual acuity had not yet decreased. The AO images showed the lateral extent of the photoreceptor changes with patchy dark areas and irregular bright spots around the foveal center. There are reports of the AO findings in patients with cone-rod dystrophy (CRD) with increased cone spacing.

Until now, ophthalmologists could detect photoreceptor degeneration only by conventional ophthalmoscopy and electroretinography. In OMD patients, the photoreceptor damage is mild, and it cannot be detected by conventional ophthalmoscopy. The mfERGs are useful for detecting reduced cone function, although the result of mfERGs may be unreliable in subjects with fixation problems, such as young children and patients with eccentric fixation.[27] FD-OCT and AO are noninvasive and effective methods to observe photoreceptor damage and confirm a diagnosis.

The future applications of AO fundus examinations include monitoring disease progression and measuring the effect of treatment. Further investigations are needed to interpret and quantify the features of these images.

In conclusion, the morphological changes of OMD patients can be seen tangentially by FD-OCT and en-face by AO.

Disclosure

The authors report no conflicts of interest in this work.

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Ocular surface molecule after transconjunctival vitrectomy

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ABSTRACT

Aim To evaluate ocular surface molecules after transconjunctival pars plana vitrectomy (PPV).

Methods A total of 28 eyes of 28 patients who received PPV were examined. Tears (10 μl) were collected before and after 20-gauge PPV or 23-gauge PPV. The concentrations of interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70 and tumour necrosis factor (TNF)-α were measured. The concentrations of each cytokine before and after surgery were compared. The change ratios of each cytokine (post-/presurgery) between 20-gauge and 23-gauge PPV were compared.

Results IL-1β, IL-6, IL-8 increased after PPV (Wilcoxon rank sum test; IL-1 β, $p=0.009$; IL-6, $p<0.001$; IL-8, $p<0.001$), while the other cytokines did not. Among them, only IL-8 increased significantly in 20-gauge PPV than in 23-gauge PPV ($p=0.01$, Mann–Whitney U test). Multivariate analysis showed that 20-gauge PPV was a significant factor behind the increase in postoperative IL-8 (OR 5.21, 95% CI (1.46 to 18.55), $p=0.018$), but surgical time and simultaneous cataract surgery were not significant factors.

Conclusions The increase in inflammatory cytokine IL-8 in tear was significantly less after 23-gauge PPV than 20-gauge PPV, indicating that transconjunctival PPV is less invasive from the viewpoint of the ocular surface molecular biology. This information may be important when deciding the method of PPV.

INTRODUCTION

The use of small-gauge transconjunctival vitrectomy has increased since its first introduction, and several benefits of this new system have been emphasised.[1–9] One benefit is a reduction in the time required for surgery because sclera and conjunctiva do not have to be opened and closed separately, although there is some discussion regarding overall surgical time.[5–8] This subsequently minimises surgically induced trauma and discomfort otherwise caused by manipulation and possible reactions. Indeed, a more comfortable experience with the ocular surface after small-incision transconjunctival sutureless vitrectomy is achieved during the first week after surgery in comparison with 20-gauge standard vitrectomy.[7–9] Because the surgical results and complications of small-incision vitrectomy are comparable with those of standard 20-gauge vitrectomy, the lack of discomfort after surgery may be of merit for patients.

So far, the evaluation of comfort after transconjunctival vitrectomy has been done by questionnaire or interview.[5–9] Although the questionnaire is superior for coding, quantifying and interpreting the results, it is not suitable for gathering information with depth and detail. At the same time, the sensory evaluation is dependent upon the respondent and is strongly influenced by the interviewer. This is an inevitable problem of a questionnaire or interview methodology. Therefore, a more objective methodology is necessary to reduce discomfort after surgery. Recently, we reported that the multicytokine assay system enables us to measure cytokine from a minimal amount of tear, which reflects the ocular surface condition.[10 11] More importantly, it is not influenced by subjective feelings. The aim of this study was to clarify the relationship between postoperative conjunctival inflammation and vitrectomy-induced chemokines in both vitrectomy techniques.

In this study, we measured the amounts of several different inflammatory molecules of tear before and after surgery, and found that pro-inflammatory cytokine IL-8 exclusively increased more significantly in 20-gauge vitrectomy than in 23-gauge vitrectomy. To our knowledge, there are no reports on the ocular surface molecule after vitrectomy, and this is the first study to show the superiority of transconjunctival vitrectomy from the viewpoint of the molecular biological mechanism and provides important information to assist surgeons when deciding on the vitrectomy method to be used for patients.

METHODS

The study was carried out with the approval of the institutional review board, and was performed in accordance with the ethical standards laid down by the 1989 Declaration of Helsinki. After checking that each eye was free from ocular surface diseases using slit-lamp microscopy, the experimental nature of this study was explained to the participants. Those who understood it were enrolled in the study. All of the participants gave written informed consent to take part in this study. One eye of each patient was used in the following study.

COLLECTION OF TEARS

Tear samples were obtained by capillary flow, with no nasal stimulation or previous instillation of drugs or vital dyes.[10 11] To avoid diurnal variations in tear cytokine, tear samples were collected around noon.[10] No anaesthetic drops were instilled. The samples were collected non-traumatically from the inferior meniscus. The collected tears were frozen at −80°C immediately and stocked until measurement. Samples collected the day before surgery

were used as presurgical samples, and those collected 4–6 days after surgery were used as postsurgical samples.

Measuring cytokines

The amounts of six inflammatory molecules, interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70 and tumour necrosis factor (TNF)-α were measured using a cytometric bead array (BD Biosciences, San Diego, California), as in our previously described method.[10 11] Data were acquired and analysed using BD cytometric bead array software.

Surgical procedures

All patients underwent a standardised surgical procedure as follows. Transconjunctial pars plana vitrectomy (PPV) system (23-gauge PPV group, n=15). We used a 23-gauge vitrectomy system (Alcon Acuras, Fort Worth, Tx) as described previously.[2 3 8] Three 23-gauge cannulas were inserted obliquely and trans-conjunctively into the eye by means of a trocar. A central core vitrectomy was performed, and detachment of the posterior hyaloid was induced. Removal of preretinal membrane, endo-photocoagulation and/or a fluid–SF_6 gas tamponade were performed when needed. Standard phecoemulsification and intraocular lens implant were performed when needed. Surgery was completed by removing the entry site alignment cannulas without suturing the conjunctiva and sclera. When apparent leakage was observed from the wound, it was closed by a suturing with 8/0 braded silk (Mani, Tochigi, Japan). Standard PPV (20-gauge PPV group, n=12): a standard PPV was performed as described previously.[12] Conjunctival incision was performed, and then 20-gauge sclerotomies were applied. The following procedures were almost the same as the 23-gauge PPV mentioned above. After surgery, the sclerotomies sites were closed with 8/0 vicryl absorbable sutures (Mani). The conjunctiva was closed with 8/0 braded silk as in 23-gauge PPV. In both groups, post-operative treatment consisted of a fixed-dose combination of 0.1% betamethasone and 0.5% levofloxacin eye-drops (Santen Pharmaceutical Co, Osaka, Japan) applied topically four times a day for 1 week.

Main outcome measures and statistics

The primary goal of the study was to evaluate the effects of 23-gauge PPV and 20-gauge PPV on molecular profile after surgery. First, the concentrations of each molecule before and after surgery were compared using the Wilcoxon rank sum test. As in our previous reports, the baseline concentrations of cytokine and chemokine vary in each eye regardless of serum cytokine.[10 11] Therefore, the change in each cytokine was also expressed as a change ratio (concentration at postsurgery/concentration at presurgery). The molecule, which was found to be significantly different between 20-gauge PPV and 23-gauge PPV, was further analysed to detect the affecting factors as follows. The following potential factors were hypothesised to affect the results: size of the number of sutures, conjunctival incision, surgical time, presence of diabetes, simultaneous cataract surgery and 23-gauge PPV. Each factor was treated as a categorical variable. An eye with the number of conjunctival sutures that was equal to or more than the median was categorised into the sutured group. The others were categorised into the suture-less group. An eye with a conjunctival incision length that was equal to or longer than the median was categorised into the large incision group, and the others were categorised into the small incision group. In terms of surgical time, an eye that received surgery requiring an equal amount of time or a longer time to complete than the median was categorised into the longer surgical time group, and the others were categorised into the shorter time group. The presence of diabetes was determined by medical history or HbA1c. Each eye was coded either 1 (for the presence) or 0 (for the absence) for the variable. Age was used as a continuous variable, and the other factors were treated as categorical variables.

Before the multivariate analysis, a subgroup analysis for each variable was carried out using the unpaired Student t test. When a variable was found to be statistically significant, it was further subjected to a multivariate analysis. We estimated the multiple-adjusted ORs and the 95% CIs for the change in selected cytokine concentration and the other variables using the multivariate analysis. The multivariate analysis used was a multiple regression analysis. Each value was derived from a logarithmic transformed IL-8 increase because of its scattered distribution. ORs and 95% CIs were evaluated by reindex translation. Statistical analyses were performed using SAS (Proprietary Software Release 8.2, SAS Institute, Cary, North Carolina) and JMP (Version 7.01, SAS Institute). p Values <0.05 were considered statistically significant.

RESULTS

Backgrounds of patients

Age, gender, disease, side of the eye or simultaneous cataract surgery was not significantly different between 20-gauge and 23-gauge groups (table 1). The time for surgery ranged from 34 to 177 min with a median of 75 min in total group of patients, ranging from 35 to 177 min with median of 90 min in the 20-gauge PPV group, ranging from 34 to 120 min with a median of 52 min in the 23-gauge PPV group. There was a significant difference between the two groups (Mann–Whitney U test, p=0.018). The length of incision was from 0 to 33 mm with a median of 16.5 mm in total, 16.5 to 33 mm with a median of 25 mm in the 20-gauge PPV group and from 0 to 33 mm with a median of 8 mm in the 23-gauge PPV group. There was a significant difference between the two groups (Mann–Whitney U test, p<0.001). The number of sutures was 0 to 5 with a median of 4 in total, 4 to 5 with a median of 4 in 20 gauge PPV group and 0 to 3 with a median of 0 in the 23-gauge PPV group. There was a significant difference between the two groups (Mann–Whitney U test, p<0.001).

cytokine levels

The concentration of IL-1β, IL-6 and IL-8 increased more significantly after surgery than baseline (IL-1β, p=0.009; IL-6, p<0.001; IL-8, p<0.001; IL-10, p=0.570; IL-12p70, p=0.253; TNF-α, p=0.375, Wilcoxon rank sum test; figure 1A). Each case was expressed also by change ratio (figure 1B). Of note is that IL-10, IL-12p70 and TNF-α did not show any significant change after surgery. Upon comparing the results of the 20-gauge group and 23-gauge group, only IL-8 showed significance regarding the change ratio after surgery (p=0.01, Mann–Whitney U test; figure 2). There were no significant differences in other cytokines or chomokines (IL-1β, p=0.305; IL-6, p=0.13; IL-10, p=0.433; IL-12p70, p=1.0; TNF-α, p=0.86).

Factors affected IL-8 increase after PPV

Next, to detect the factors that affected IL-8 change, a subgroup analysis was performed. As a result, eyes with a large conjunctival incision, with many sutures, with a longer surgical time or with 20-gauge PPV had a significant relation to change in IL-8 after surgery. Age, gender and diabetes did not have any significant influence (table 2). Although, cataract surgery was

Fujita A, Uchino E, Otsuka H, *et al*. *Br J Ophthalmol* (2010). doi:10.1136/bjo.2010.185058

Table 1 Background of eyes

		Total	20-gauge pars plana vitrectomy	23-gauge pars plana vitrectomy	p Value
No		27	15	12	
Age		31–82	31–82	34–80	0.86
Mean±SD		63.6±12.1	63.9±12.5	63.0±12.1	
Gender	Male: female	12:17	8:9	4:8	0.48
Disease	Diabetic retinopathy (+): diabetic retinopathy (−)	8:19	5:10	3:9	0.39
Side	Right: left	19:8	9:6	10:2	0.1
Simultaneous cataract surgery					
	Yes:no	20:7	10:5	10:2	0.45
Surgical time (min)		34–177	35–177	34–120	0.018*
	Median	75	90	52	
Length of incision (mm)		0–33.0	6.5–33.0	0–33.0	<0.001*
	Median	16.5	25	8	
No of suture		0–5	4–5	0–3	<0.001*
	Median	4	4	1	

*Mann–Whitney U test.
†χ^2 test.

not a significant factor, it can be assumed that simultaneous surgery might have affected wound healing after surgery. Hence, cataract surgery was involved in the following multivariate analysis. Besides, an analysis of the estimated values of assumed parameters showed that the 20-gauge PPV was exactly the same as the length of conjunctival incision or the number of sutures (table 2), and they were excluded from the multivariate analysis.

Multivariate analysis was performed with variables of 20-gauge PPV (large conjunctival incision or more sutures), surgical time and cataract surgery. As a result, 20-gauge PPV was found to be a significant factor increasing IL-8 (p=0.018; table 3). Neither simultaneous cataract surgery nor surgical time was a significant factor increasing IL-8.

DISCUSSION

The recovery process of the ocular surface after PPV is the wound-healing process of the ocular surface, which is similar to cutaneous wound-healing associating with complex, multiple orchestrated-processes. Since neutrophils are highly abundant blood-cell populations in the circulation, a significant number of neutrophils are collected passively at the site of a wound with a blood clot as a result of blood-vessel disruption or surgical incision.[13] Neutrophil recruitment is an initial event followed by macrophage invasion, which is a strong source of cytokines and chemokines. In particular, in the human wound-healing process, inflammatory cells expressing chemokines such as IL-8 were restricted to the wound edge.[14] Although this report related to the cutaneous wound-healing process, it is likely that similar processes occur in the conjunctival wound-healing process after PPV. Therefore, it is reasonable for the amounts of tear cytokines and chemokines to increase in the early phase after PPV.

Of note is that the 20-gauge PPV group showed the significant increase only for IL-8, but not IL-1β, IL-6 or TNF-α in comparison with the 23-gauge PPV group. If the increase in cytokines or chemokines reflected just the result of the wound-healing process, it cannot explain why only IL-8 showed a significant difference with the two surgical methods, because the eyes belonging to the 20-gauge PPV group were exactly the same as those of the large conjunctival incision group in this study. The larger the size of wound is, the greater should be the amounts of cytokines and chemokines accumulating at the edge of the wound. We do not have any specific answer to account for this phenomenon, but it can be speculated from the previous studies. First, IL-8 has a unique expression pattern responsive to mechanical stress. Recently, mechanical stress has become known to induce expression of bioactive molecules in various organs and cells.[15–18] These cells were reported to produce IL-1β, IL-6, IL-8 and TNF-α through several different pathways. Ricard *et al* showed that IL-1β, TNF-α and macrophage inflammatory protein (MIP)-2, equivalent to IL-8 in human, were produced under strong mechanical stress in rats; however, MIP-2 was exclusively produced under a mild or moderate mechanical stress, but not IL-1β or TNF-α.[18] During the procedures of 20-gauge PPV, the conjunctival flap produced by the large

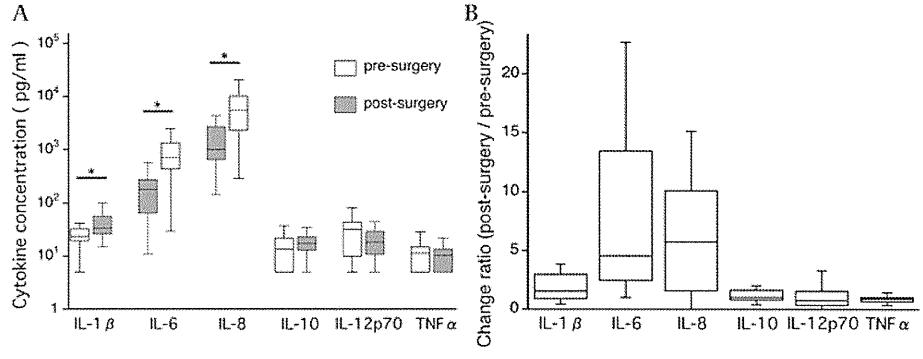

Figure 1 Tear cytokine change before and after pars plana vitrectomy. The concentration of interleukin (IL)-1β, IL-6 and IL-8 increased more significantly (*) after surgery than baseline (Wilcoxon rank sum test: IL-1β, p=0.009; IL-6, p<0.001; IL-8, p<0.001; IL-10, p=0.570; IL-12p70, p=0.253; tumour necrosis factor (TNF)-α; p=0.375). (A) The baseline concentration (presurgery) differed greatly depending upon each case. Therefore, each case was expressed also by the change ratio (B). Of note is IL-10, IL-12p70 and TNF-α did not show any significant change after surgery.

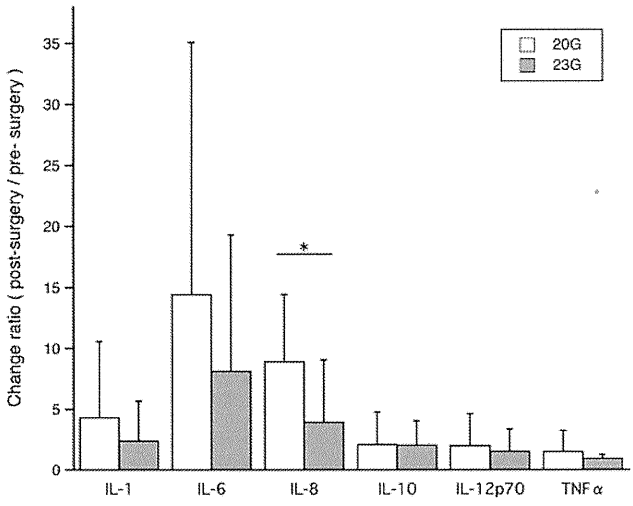

Figure 2 Tear cytokine change before and after 20-gauge (20G) and 23-gauge (23G) pars plana vitrectomy. When comparing the results of the 20G group and 23G group, only interleukin (IL)-8 showed significance regarding the change ratio after surgery (p=0.01, Mann–Whitney U test). There was no significant difference in other cytokines or chemokines; IL-1β, p=0.305; IL-6, p=0.13; IL-10, p=0.433; IL-12p70, p=1.0; tumour necrosis factor (TNF)-α, p=0.86.

conjunctival incision is apparently exposed to mechanical stress intentionally or unintentionally—for example, folding/unfolding, being stretched and being compressed in scleral indentation. These manoeuvres are not common in 23-gauge PPV. Therefore, IL-8 might have been produced exclusively by mechanical stress during the manoeuvre of 20-gauge PPV. However, the mechanical stress itself would not be strong enough, and it would be likely to cause significant effects together with cellular reactions of wound-healing for a large incision and the sutures. So far, there have been no reports on the effects of mechanical stress on the expression of chemical mediator by conjunctiva. Further study is necessary to prove this hypothesis.

There are clear limitations in this study. First is the non-randomised nature of sampling. Second, the number of samples might be small to obtain a general conclusion. When sampling tears, moderate patience is required for the patients. Because this study was not therapeutic but rather experimental, the sampling numbers were decided to be as small as possible for ethical reasons advised by the review board. Additionally, the relation between the severities of vitreous diseases and tear cytokine levels were not studied. Since higher numbers of eyes are necessary to answer this question, another study is being planned. It should be stressed that the care must be taken to interpret the present results. It is easily speculated that the conjunctival suture might be the major cause of discomfort after vitrectomy. At the same time, a large conjunctival would be a cause of discomfort as well. Indeed, in this study, the eyes with many conjunctival sutures were exactly the same as those with a large incision, and both of them are clearly associated with 20-gauge PPV. One may misunderstand we argue that the suture is not the cause of discomfort after 20-gauge PPV, but IL-8 is. On the contrary, one should discern that conjunctival sutures, a large incision, mechanical stretch and other factors are the characteristics of 20-gauge PPV, and all of these caused the increase in IL-8 in tears.

Table 2 Subgroup analysis of factors to increase interleukin-8

	Yes	No	p Value
Diabetes mellitus	6.06	6.98	0.706
Duration of surgery (longer than median)	9.09	4.13	0.029*
The number of sutures (more than median)	8.91	3.94	0.029*
Length of incision (longer than median)	8.91	3.94	0.029*
20 gauge-pars plana vitrectomy	8.91	3.94	0.029*
Cataract surgery	7.16	5.39	0.456
Age (older than median)	6.26	7.06	0.735
Female	5.21	8.89	0.158

†Unpaired Student t test.
Values are expressed as change ratio of concentration (post-/presurgery).

Table 3 Multivariate analysis of factors affected changes of interleukin-8

Variables	References	OR (95% CI)	p Value
20-gauge PPV	23-gauge PPV	5.21 (1.46 to 18.55)	0.018
Surgical time	Shorter group	0.82 (0.24 to 2.86)	0.76
Cataract surgery	PPV alone	1.45 (0.47 to 4.47)	0.52

Each value was derived from common logarithmic transformed interleukin-8 increase.
PPV, pars plana vitrectomy.

The relation between sense of discomfort/irritation and cytokine has been studied for skin, respiratory organ and ocular surface.[19–21] In these reports, increases of proinflammatory cytokines are often associated with these symptoms. For example, IL-8 is induced in irritated skin.[22] Cytokines themselves may not solely induce the sense of discomfort or irritation, but associated events, such as oedema and cell infiltration, are likely to induce these symptoms. Although the patients who received 20-gauge PPV had a tendency to complain of strong discomfort in comparison with those who received 23-gauge PPV (data not shown), the objective evaluation of each patient was not performed in this study. This is another limitation to interpret the present data, which may suggest the relation between IL-8 and ocular surface discomfort.

To our knowledge, this is the first report to show the merits of transconjunctival PPV from molecular biological evidence. Therefore, transconjunctival PPV is strongly recommended for eyes with dry eye syndrome, vernal conjunctivitis or Sjögren syndrome, because IL-8 is a deteriorating factor in these diseases. The present information is important not only to better understand the molecular mechanism but also to decide on a suitable method of PPV for each patient.

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Competing interests None.

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Review

HYALOCYTES: ESSENTIAL CELLS OF VITREOUS CAVITY IN VITREORETINAL PATHOPHYSIOLOGY?

TAIJI SAKAMOTO, MD, PHD,* TATSURO ISHIBASHI, MD, PHD†

Purpose: To review the present understanding of hyalocytes.

Methods: A review of recent studies that investigated the roles of hyalocytes in the pathophysiology of vitreous cavity.

Results: Studies on immunocytochemistry and chimeric mice with green fluorescent protein transgenic mice show that hyalocytes belong to the monocyte/macrophage lineage and derive from bone marrow. The effects of hyalocytes on vitreous cavity environment can be divided into three categories: synthesis of extracellular matrix, regulation of the vitreous cavity immunology, and modulation of inflammation. In noninflamed eyes, vitreous cavity is an immune-privileged site that is maintained by a system called vitreous cavity–associated immune deviation, in which hyalocytes play the role of antigen-presenting cells. However, cultured hyalocytes proliferate in response to inflammatory molecules and secrete vascular endothelial growth factor and urokinase-type plasminogen activator. A collagen gel embedded with hyalocytes contracts over time, which is enhanced by transforming growth factor-β but is inhibited by Rho kinase inhibitor. These results suggest that hyalocytes can be an exacerbating factor in inflamed eyes. Clinically, hyalocytes are frequently found in the surgically removed specimens of epiretinal membrane or proliferative vitreoretinopathy.

Conclusion: Elucidating the properties of hyalocytes is important to understand the biology of vitreous cavity and to develop novel treatments for vitreoretinal diseases.

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Recently, a number of novel therapies have been emerging in clinical ophthalmology, especially for vitreoretinal diseases. They include a new type of pars plana vitrectomy, novel drugs such as ranibizumab and pegaptanib, and gene-mediated therapy. Of note is that these therapies often use the vitreous cavity as a therapeutic place or platform; thus, a more detailed knowledge of the environment of the vitreous cavity is required.

The vitreous cavity is composed mainly of collagen fibers, hyaluronan, and some cells.[1] It has been reported that there are groups of cells in the cortical or peripheral vitreous.[1–5] These cells, currently called hyalocytes, have a lobulated nucleus, cytoplasmic projections, and moderate numbers of mitochondria. Hyalocytes are located at an average distance of 50 mm from the inner surface of the retina and are concentrated anteriorly at the vitreous base and posteriorly in the vicinity of optic disk. According to previous publications,[6–8] hyalocytes were regarded as resting cells, and hyalocytes have been studied less

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The authors report no conflicts of interest.

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extensively in comparison with other intraocular cells, such as retinal pigment epithelial cells. However, recent studies[9–12] have shown that hyalocytes play a significant role in maintaining the vitreous body as a transparent and avascular system actively rather than passively. Hyalocytes have been found to be present in various aspects of pathophysiology, which involve epiretinal membrane (ERM) formation, diabetic macular edema, proliferative vitreoretinopathy, and others.[10,12–14] Therefore, we would like to review our present knowledge of hyalocytes to better understand vitreoretinal pathophysiology and to develop new treatments.

Origin, Morphology, and Turnover of Hyalocytes

Hyalocytes are variously described by light microscopy as being spindle-shaped, rounded, or even star-shaped cells. Their nuclei are lobulated, and the cytoplasm is characterized by the presence of many secretory granules and a well-developed Golgi apparatus[4,15] (Figure 1, A–D). They are mainly distributed close to the retina at the vitreous base and in the posterior hyaloid.[15] Morphologic studies demonstrate that hyalocytes belong to the monocyte/macrophage lineage.[4,7,17–19] However, Hogan et al[15] reported that hyalocytes differ from macrophages because of a paucity of lysosomes. Immunocytochemical analysis shows that hyalocytes express a monocyte/macrophage cell marker but not CD68, glial fibrillary acidic protein, cellular retinaldehyde-binding protein, and cytokeratin.[9,20–23] These results indicate that hyalocytes are derived from a monocyte/macrophage lineage but not from glial cells or retinal pigment epithelial cells. Furthermore, a study on rat hyalocytes revealed a positive reaction for ED2 but not for ED1, confirming that hyalocytes have characteristics of tissue macrophages.[16]

Recently, enhanced green fluorescent protein transgenic mice have been generated; the tissues of enhanced green fluorescent protein transgenic mice are green under excitation light. Using enhanced green fluorescent protein transgenic mice, cell movements can be tracked in an in vivo model. We created chimeric mice by transplanting bone marrow from enhanced green fluorescent protein transgenic mice into irradiated wild mice.[10,16] The results show that hyalocytes were green fluorescent protein negative directly after bone marrow transplantation in chimeric mice; however, the number of green fluorescent protein–positive hyalocytes increased over time. More than 60% of hyalocytes were replaced by green fluorescent protein–positive cells within 4 months, and 90% of hyalocytes were green fluorescent protein positive within 7 months after bone marrow transplantation (Figure 2). The levels of residual macrophages might not have been maintained by their proliferation but by being produced in bone marrow under a physiologic condition with a turnover time of several months.[16] However, van Meurs et al[24] showed the half-life of vitreous macrophage was 4.8 days by allowing vitreous macrophages to phagocytose ^{141}Cerium (γ-emitter)-labeled microspheres. It is difficult to conclude that these groups studied the same type of vitreous cells; however, there might be several different cell lineages within the so-called hyalocytes.

Conversely, Gloor[25] described that hyalocytes would be in an independent tissue layer, in which the cells are replaced by reproduction because the hyalocytes showed increased mitotic activity after photocoagulation. Haddad and André observed that ^{3}H-thymidine was detected in the hyalocytes of the cortical vitreous after ^{3}H-thymidine injection and concluded that hyalocytes renew themselves inside the eye.[26] It is not clear whether hyalocytes are composed of cells of different origins or those of the same origin at different developmental stages. Although more detailed studies are necessary to answer these questions, it is safe to say that most hyalocytes originate from bone marrow, at least under physiologic conditions.

Functions of Hyalocytes

The functional properties of hyalocytes can be divided into the following three categories: synthesis of extracellular matrix (ECM), modulator of immune reaction, and modulator of inflammation.

Synthesis of Extracellular Matrix

Because hyalocytes appear in the vitreous at an early embryonic stage, it is reasonable to assume that hyalocytes produce vitreous collagen. It has been reported that chick vitreous collagen is synthesized by the neural retina at early embryonic stages, whereas the major contribution derives from cells within the vitreous body later in the development.[27] Also, hyalocytes are reported to be responsible for the production of hyaluronic acid in calf and primate.[28,29] Recently, it has been further confirmed that production of hyaluronan is modulated by cytokines, such as transforming growth factor (TGF)-β or platelet-derived growth factor-BB using cultured hyalocytes.[30] It was also shown that cultured porcine hyalocytes produce glycosaminoglycans and ECM, which is modulated by basic fibroblast growth factor and

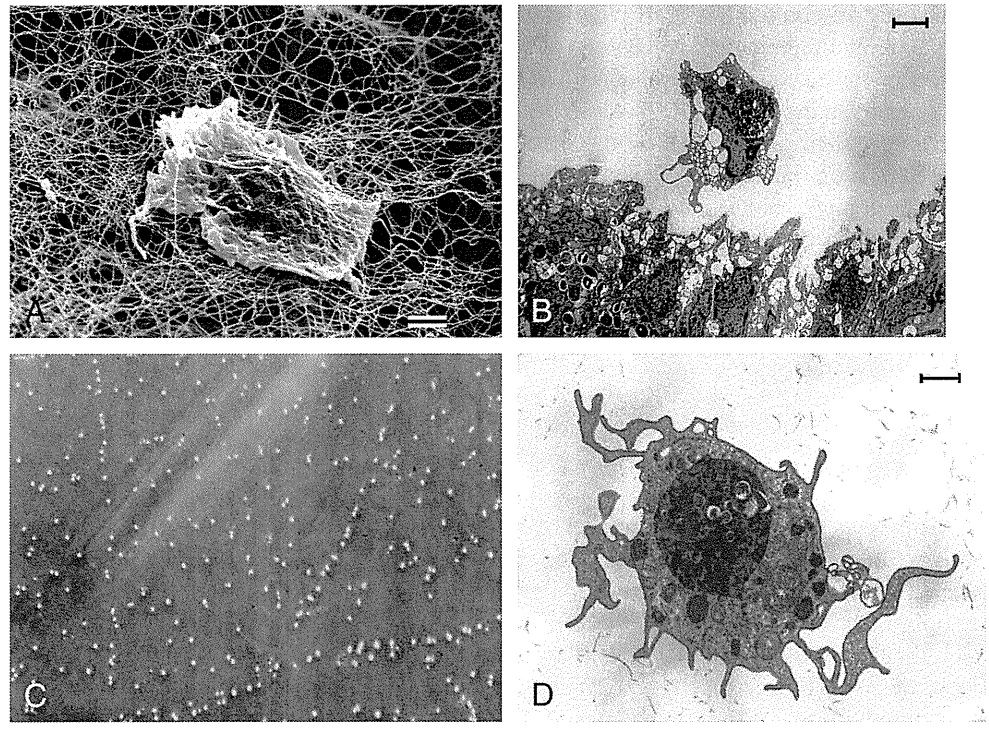

Fig. 1. Hyalocytes in animals. **A.** Scanning electron microscopic photograph of rat hyalocytes. Hyalocytes are present in collagen fibers. Bar 1 mm. **B.** Transmission electron microscopic photograph of rat hyalocytes. Hyalocytes are located close to the ciliary epithelium (arrow). Bar 1 mm. **C.** Phase-contrast microscopic photograph of bovine hyalocytes. Numerous hyalocytes are scattered in the vitreous (original magnification, ×10). **D.** Transmission electron microscopic photograph of bovine hyalocytes. Bar 1 mm. Reproduced with permission from Qiao et al,[16] Noda et al,[11] and Sakamoto.[10]

TGF-β1.[31] Because glycosaminoglycans are the stimulators of contraction of ECM with cells, the formation of a membrane with cells and ECM might be an important first step in the progression of ERM or proliferative vitreoretinopathy.[10,32] It is likely that hyalocytes play a certain role in this pathology by producing ECM in addition to other cells.[10,33–38]

Modulator of Intraocular Immune System: Vitreous Cavity–Associated Immune Deviation

The eye is an immune-privileged site that is styled to keep the visual pathway clear while at the same time to provide defenses against invading organisms.[39] Above all, the anterior chamber–associated immune deviation is a unique system to keep the eye immune privileged. Anterior chamber–associated immune deviation can be induced by antigen injection for peripheral tolerance to that antigen.[40] It is demonstrated that anterior chamber–associated immune deviation is induced by bone marrow–derived antigen-presenting cells, which are positive for F4/80, a marker of a wide range of mature tissue macrophages, localized in the iris and ciliary body in the eye and carrying an antigen-specific signal to the spleen.[39,41]

We investigated the mechanisms by which ocular inflammation associated with the vitreous cavity is reduced by injecting either ovalbumin or allogeneic splenocytes into the vitreous cavities of mice and assessed the effects of this on delayed-type hypersensitivity responses. After antigen inoculation into the vitreous cavity, antigen-specific delayed-type hypersensitivity responses were significantly impaired, and we named this phenomenon the vitreous cavity–associated immune deviation.[42] Vitreous cavity–associated immune deviation could also be induced by inoculating antigen-pulsed macrophages into the

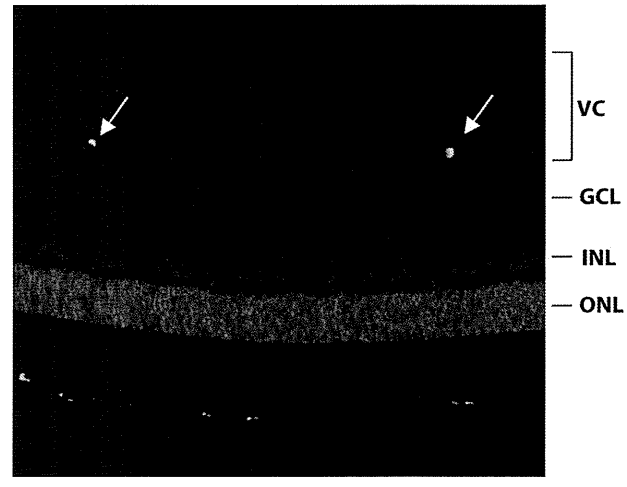

Fig. 2. Fluorescent microscopic photograph of GFP chimeric mouse. The hyalocytes (arrows) are GFP positive, indicating their bone marrow origin. VC, vitreous cortex; GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; GFP, green fluorescent protein (original magnification, ×40).

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vitreous cavity. However, vitreous cavity–associated immune deviation did not develop either in mice with inflamed eyes, whether as a result of experimental autoimmune uveitis or coadministration of interleukin-6 in the vitreous cavity, or in knockout mice deficient in natural killer T cells.[42] In this system, we found that hyalocytes are the only cells present in the vitreous cavities. Interestingly, hyalocytes express F4/80, suggesting that hyalocytes are candidate antigen-presenting cells responsible for mediating vitreous cavity–associated immune deviation (Figure 3).[42] These findings suggest that hyalocytes would play a pivotal role in inhibiting intraocular inflammation in noninflamed eyes. F3

Modulator of Intraocular Inflammation

Almost three decades ago, human hyalocytes were reported to have characteristics of macrophages, such as phagocytic activity with surface receptors for IgG and complement components.[20] Macrophages are major cells in the inflammation of most tissues, so it is natural to assume that hyalocytes play a major role in intravitreous inflammation.

Cultured bovine hyalocytes proliferate in response to platelet-derived growth factor and secrete urokinase-type plasminogen activator (uPA).[11] Because uPA has a strong fibrinolytic activity by converting proenzyme plasminogen into serine protease plasmin, it might be beneficial to keep the vitreous cavity clear by removing fibrin and fibrin-related materials. At the same time, uPA is a multifunctional protein that also affects growth factor bioavailability; for example, uPA is a potent inducer of angiogenesis and tissue remodeling, so the role of uPA in ocular pathology would be complicated.[43] Similarly, cultured hyalocytes secrete VEGF, which is upregulated by hypoxia-inducible factor-1 or tumor necrosis factor-α.[44] Hyalocytes might be one of the cellular sources of intravitreous VEGF, which is a well-proven angiogenic and vascular permeability factor in diabetic retinopathy and exudative age-related macular degeneration.

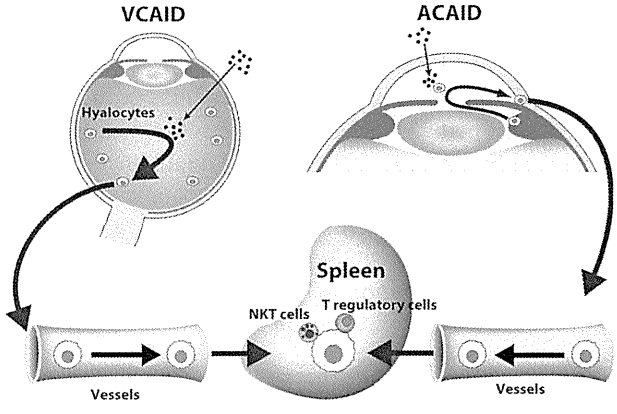

Fig. 3. Schema of vitreous cavity–associated immune deviation (VCAID) and anterior chamber–associated immune deviation (ACAID). Antigens inoculated into the vitreous cavity are captured by resident macrophage hyalocytes and carried via the bloodstream to the spleen. Both VCAID and ACAID require eye-derived antigen-presenting cells and CD1d-restricted natural killer T cells to induce antigen-specific regulatory T cells.

Recently, the role of hyalocytes in ocular pathology has been investigated from a different viewpoint. Contraction of the preretinal cortical vitreous is one of the most critical steps in various intraocular diseases. Three-dimensional collagen gel preparations have been used to assess the mechanism of membrane contraction in vitro, and this system is suitable for evaluating the cortical hyaloid contraction.[45] Using this system, the contractile property of hyalocytes was studied (Figure 4). As a result, a collagen gel embedded with hyalocytes contracts significantly in response to various stimulants, such as TGF-β2, and this effect is mediated through Rho and Rho kinase (ROCK)–dependent pathways.[46] This in vitro cell-mediated collagen gel contraction is more potent with hyalocytes than with retinal glial cells or retinal pigment epithelial cells.[10] Therefore, the presence of hyalocytes might be a potent exacerbating factor of preretinal membrane contraction in proliferative vitreoretinopathy after retinal detachment. F4

Clinical Implication

In histologic studies, several types of cell were found in preretinal membrane or posterior hyaloids, and macrophage-like cells were found frequently.[9,12,47–52] It is certain that some of them are hyalocytes (Figure 5). Kohno et al[33] found that cells located at the contractile epicenter of ERMs are mostly hyalocytes, not glial cells. Because hyalocytes have a strong contractile property, it may be assumed that hyalocytes play a critical role not only in the pathology of ERM but also in tractional retinal detachment.[32] Gandorfer et al[14] studied specimens of flat mount internal limiting membrane and found that macular hole formation is caused by the insertion of the cortical vitreous into the foveal internal limiting membrane and that cellular proliferation including hyalocytes is involved in vitreofoveal traction, resulting in a foveal tear. F5

To treat these pathologic conditions, removing ERM and posterior hyaloid is the preferred and logical approach at present because these membranes contain a number of cells including hyalocytes. The

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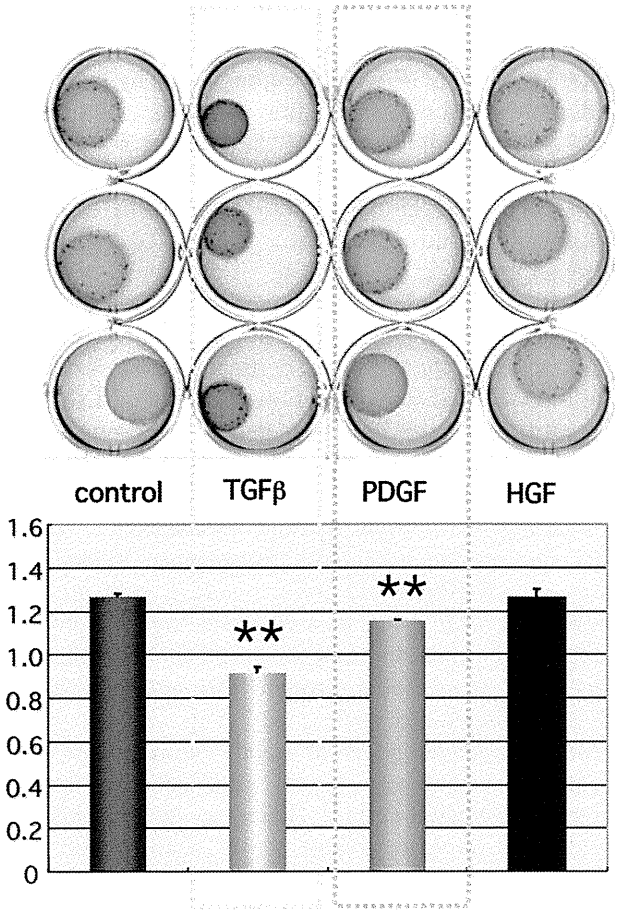

Fig. 4. Contraction of collagen gel embedded with hyalocytes. A collagen gel embedded with hyalocytes was stimulated with cytokines, such as TGF-β, platelet-derived growth factor (PDGF), or hepatocyte growth factor (HGF), and the size of the collagen gel was measured after 24 hours. The contraction of gel was significantly enhanced by TGF-β or PDGF (**$P < 0.01$). Reproduced with permission from Sakamoto.[10]

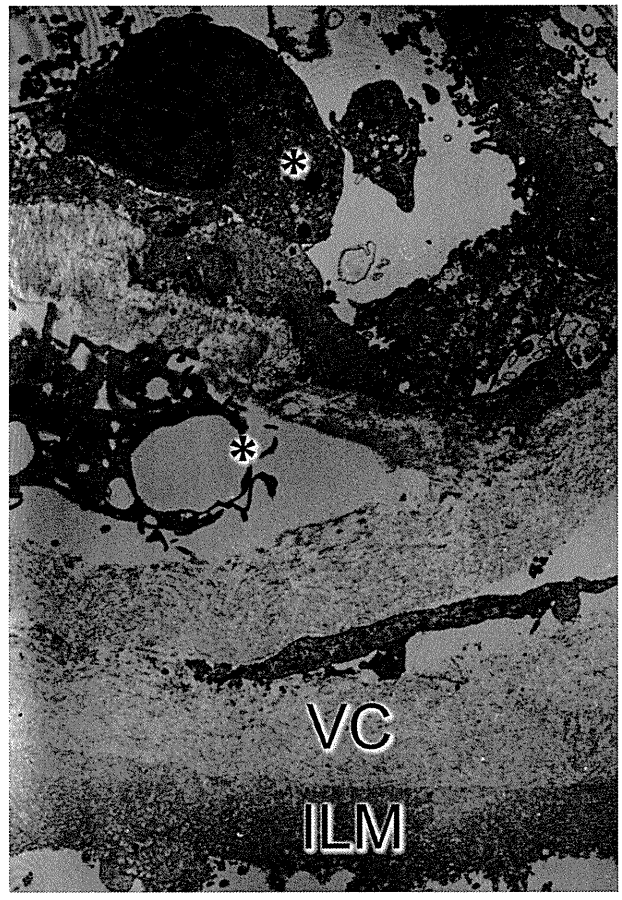

Fig. 5. Transmission electron microscopic photograph of surgically removed internal limiting membrane (ILM) from the eye of ERM. Macrophage-like cells (*) are present on the vitreous cortex (VC) and ILM. They are presumably hyalocytes. Reproduced with permission from Sakamoto[10] (original magnification, ×1800).

maneuver itself is not necessarily easy and recurrence is not rare. Adjunctive use of triamcinolone acetonide in vitrectomy is beneficial to remove these membranes securely and effectively.[53,54] Although this procedure is not always necessary in most of the cases, it might be beneficial for selected cases to reduce the incidence of postoperative preretinal fibrotic complications.[54] Complete removal of ERM together with internal limiting membrane resulted in a lower recurrence rate than incomplete removal, probably because the residual hyaloid or membrane becomes a scaffold of cell proliferation and ECM production by these cells.[55] If the residual hyaloid or internal limiting membrane is left alone without any cells, recurrence, namely reproduction of ECM by cellular elements after surgery, will not occur.

There are novel pharmacologic approaches to the disease. In an in vitro study, Rho and ROCK were found to play an important role in phosphorylation of the myosin light chain and the subsequent contraction; thus, a specific Rho kinase (ROCK) inhibitor fasudil could block contraction of collagen gel embedded with hyalocytes.[36] In rabbits, fasudil significantly inhibited the progression of experimental proliferative vitreoretinopathy without affecting the viability of retinal cells. ROCK, a key downstream mediator of TGF-β and other factors, might become a unique therapeutic target.[36] Of course, there is a distance between an animal study and the bedside; however, a pharmacologic approach to modulate hyalocytes might be a novel treatment of intraocular diseases.

Summary

As described above, there is no strict definition of "hyalocytes," but cells located at the periphery of vitreous cavity are called hyalocytes. The accumulating evidence shows that these hyalocytes can act as

COLOR

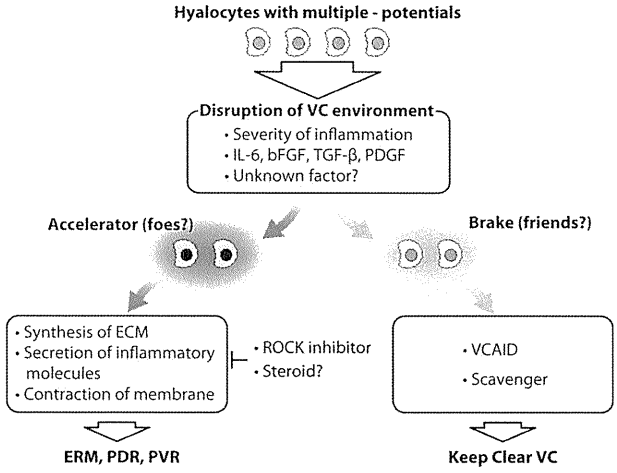

Fig. 6. Schema of possible roles of hyalocytes in ocular pathology. Hyalocytes are residual cells in vitreous cavity (VC) with multiple potentials. In the disruption of the VC environment, hyalocytes may act as an accelerator or a brake to destroy the clear vitreous dependent on unknown mechanisms. IL-6, interleukin-6; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; PDR, proliferative diabetic retinopathy; PVR, proliferative vitreoretinopathy.

"friends" to keep the vitreous cavity clear by inhibiting immune reaction through vitreous cavity–associated immune deviation. At the same time, hyalocytes can act as "foes" by producing inflammatory cytokines and ECM followed by contraction of the membrane. Unfortunately, at present, it is difficult to tell what makes hyalocytes "friends" or "foes" (Figure 6). Further studies to answer this question might provide a key to a better understanding of microenvironment of the vitreous cavity and to developing an effective treatment for intraocular diseases.

F6

Key words: antigen-presenting cells, fibronectin, macular edema, VCAID, vitrectomy.

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Results of One-Year Follow-Up Examinations after Intravitreal Bevacizumab Administration for Chronic Central Serous Chorioretinopathy

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Key Words
Central serous chorioretinopathy · Bevacizumab · Serous retinal detachment · Pigment epithelium detachment

Abstract
Background: Our purpose was to report the results of 1-year follow-up examinations after intravitreal bevacizumab injection for the treatment of chronic central serous chorioretinopathy (CSC). ***Methods:*** Five eyes in 5 patients with chronic CSC were intravitreally injected with 1.25 mg/0.05 ml of bevacizumab. The need for retreatment was evaluated if spectral-domain optical coherence tomography showed the presence of subretinal fluid at the time of a 1-month follow-up examination. Best-corrected visual acuity and central foveal thickness were compared between baseline and 1 year after the first injection. ***Results:*** The mean logarithm of the minimum angle of resolution (logMAR) best-corrected visual acuity improved from 0.23 ± 0.46 to 0.17 ± 0.47 and the mean central foveal thickness significantly decreased from 323 ± 98 μm to 171 ± 63 μm ($p < 0.05$). ***Conclusion:*** The intravitreal injection of bevacizumab is well tolerated in maintaining vision and reducing serous retinal detachment in patients with chronic CSC, as evaluated at a 1-year follow-up examination.

Introduction

Central serous chorioretinopathy (CSC) is characterized by a serous neurosensory detachment of the central macula associated with idiopathic leakage at the level of the retinal pigment epithelium (RPE) [1]. It is reported that CSC is a benign and self-limited disease which shows spontaneous resolution of a neurosensory elevation [2]. However, there exist some cases continuing with persistent pigment epithelial detachment (PED) and subretinal fluid, which means chronic CSC. Chronic CSC can cause RPE atrophy and photoreceptor degeneration, resulting in irreversible functional and anatomical damage [3–5].

The cause of CSC has been reported to be associated with choroidal vascular hyperpermeability [6]. Therefore, preventing the hyperpermeability of the choroidal vessels may play an important role for the treatment of CSC. Bevacizumab, a recombinant humanized monoclonal antibody to inhibit vascular endothelial growth factor (VEGF), is expected to reduce a serous neurosensory detachment caused by CSC. However, studies examining the use of intravitreal bevacizumab for CSC are rare, and none of the patients in these reports were followed for >1 year.

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