Similarly $(\partial'^{X}_{*})_1 : H^{\alpha}_1(X) \to H^{\beta}_0(X)$ is an isomorphism. Since $(\partial^{Y}_{*})_1 \circ (f^{\beta}_{*})_1 = (f^{\alpha}_{*})_0 \circ (\partial^{X}_{*})_1$ and $(\partial'^{Y}_{*})_1 \circ (f^{\alpha}_{*})_1 = (f^{\beta}_{*})_0 \circ (\partial'^{X}_{*})_1$, $(f^{\alpha}_{*})_1 : H^{\alpha}_1(X) \to H^{\alpha}_1(Y)$ and $(f^{\beta}_{*})_1 : H^{\alpha}_1(X) \to H^{\beta}_1(Y)$ are non-zero homomorphisms. By induction, we can prove that $(f^{\alpha}_{*})_q : H^{\alpha}_q(X) \to H^{\alpha}_q(Y)$ and $(f^{\beta}_{*})_q : H^{\alpha}_1(X) \to H^{\beta}_q(Y)$ are non-zero homomorphism for each $0 \le q \le n$. By Proposition 2.4, $H^{\alpha}_{n+1}(Y) \cong H_{n+1}(Y/C_p)$. Hence $H^{\alpha}_{n+1}(Y/C_p) = 0$ and $(j^{Y}_{*})_n : H^{\beta}_n(Y) \to H_n(Y)$ is injective. Therefore, $(j^{Y}_{*})_n \circ (f^{\beta}_{*})_n$ is a non-zero homomorphism.

On the other hand, $(j^{Y}_{*})_n \circ (f^{\beta}_{*})_n = (f_{*})_n \circ (j^{X}_{*})_n = 0$ because $H_n(X) = 0$. This is a contradiction. Therefore, the proof is complete. □

Proof of Theorem 1.3. Suppose that there is no point $x \in X$ such that $f(x) = f(gx) = \cdots = f(g^{k-1}x)$. Then the map $F : X \to Y^{*}$ defined by $F(x) = (f(x), f(gx), \ldots, f(g^{k-1}x))$ is a continuous C_k-map. This contradicts Theorem 1.1. □

3. Proof of Theorem 1.4 and Theorem 1.6

To prove Theorem 1.4, we need a definable version of Proposition 2.4.

Definable fiber bundles are introduced in [9].

Proposition 3.1 (Corollary 1.5 [8]). *Let X be a definable set with a free definable C_k-action. Then $(X, \pi, X/C_k, C_k)$ is a principal definable C_k-fiber bundle, where $\pi : X \to X/C_k$ denotes the orbit map. In particular, $\pi : X \to X/C_k$ is a definable covering map.*

Let $p : E \to X$ be a definable map. We say that p has the *definable homotopy lifting property* if for any definable set Y, each definable homotopy $h : Y \times [0, 1]_R \to X$ and a definable map $F : Y \to E$ such that $p \circ F(y) = h(y, 0)$ for all $y \in Y$, there exists a definable homotopy $H : Y \times [0, 1]_R \to E$ such that $p \circ H = h$ and $H(y, 0) = F(y)$ for all $y \in Y$.

Theorem 3.2 (Proposition 4.10 [1]). *Every definable covering map has the definable homotopy lifting property.*

The following corollary is a definable version of Proposition 2.4.

Corollary 3.3. *Let X be a definable set with a free definable C_k-action. Then the orbit map $\pi : X \to X/C_k$ has the definable homotopy lifting property.*

Proof of Theorem 1.4 and Theorem 1.6. Using Corollary 3.3 instead of Proposition 2.4, we can prove Theorem 1.4 by a way similar to the proof of Theorem 1.1. A similar proof of Theorem 1.3 proves Theorem 1.6. □

References

[1] E. Baro and M. Otero, On o-minimal homotopy groups, Quart. J. Math. (2009) (to appear).

[2] Carlos Biasi and Denise de Mattos, A Borsuk-Ulam theorem for compact Lie group actions, Bull. Braz. Math. Soc. (N.S.) 37(1) (2006), 127-137.

[3] L. van den Dries, Tame topology and o-minimal structures, London Mathematical Society Lecture Note Series 248, Cambridge University Press, Cambridge, 1998.

[4] L. van den Dries, A. Macintyre and D. Marker, The elementary theory of restricted analytic field with exponentiation, Ann. of Math. (2) 140(1) (1994), 183-205.

[5] L. van den Dries and C. Miller, Geometric categories and o-minimal structures, Duke Math. J. 84(2) (1996), 497-540.

[6] L. van den Dries and P. Speissegger, The real field with convergent generalized power series, Trans. Amer. Math. Soc. 350(11) (1998), 4377-4421.

[7] Y. Hemmi, T. Kobayashi and T. Yoshida, The Borsuk-Ulam theorem for a $\mathbb{Z}_q$-map from S^{2m+1} to a $\mathbb{Z}_p$-complex, Mem. Fac. Sci. Kochi Univ. Ser. A Math. 8 (1987), 27-30.

[8] T. Kawakami, Definable C^r groups and proper definable actions, Bull. Fac. Ed. Wakayama Univ. Natur. Sci. 58 (2008), 9-18.

[9] T. Kawakami, Homotopy property of definable fiber bundles, Bull. Fac. Ed. Wakayama Univ. Natur. Sci. 53 (2003), 1-6.

[10] K. Kawakubo, The Theory of Transformation Groups, The Clarendon Press, Oxford University Press, New York, 1991.

[11] T. Kobayashi, The Borsuk-Ulam theorem for a $\mathbb{Z}_q$-map from a $\mathbb{Z}_q$-space to S^{2n+1}, Proc. Amer. Math. Soc. 97 (1986), 714-716.

[12] C. Miller, Expansions of the real field with power functions, Ann. Pure Appl. Logic 68(1) (1994), 79-94.

[13] I. Nagasaki, Isovariant Borsuk-Ulam results for pseudofree circle actions and their converse, Trans. Amer. Math. Soc. 358 (2006), 743-757.

[14] I. Nagasaki, The converse of isovariant Borsuk-Ulam results for some abelian groups, Osaka J. Math. 43(3) (2006), 689-710.

[15] I. Nagasaki, The weak isovariant Borsuk-Ulam theorem for compact Lie groups, Arch. Math. (Basel) 81(3) (2003), 348-359.

[16] I. Nagasaki, T. Kawakami, Y. Hara and F. Ushitaki, The Borsuk-Ulam theorem in a real closed field, Far East J. Math. Sci. (FJMS) 33(1) (2009), 113-124.

[17] I. Nagasaki and F. Ushitaki, Isovariant maps from free C_n -manifolds to representation spheres, Topology Appl. 155(10) (2008), 1066-1076.

[18] Pedro L. Q. Pergher, Denise de Mattos and Edivaldo L. dos Santos, The Borsuk-Ulam theorem for general spaces, Arch. Math. (Basel) 81(1) (2003), 96-102.

[19] J. P. Rolin, P. Speissegger and A. J. Wilkie, Quasianalytic Denjoy-Carleman classes and o-minimality, J. Amer. Math. Soc. 16(4) (2003), 751-777.

[20] M. Shiota, Geometry of subanalytic and semialgebraic sets, Progress in Mathematics 150, Birkhäuser Boston, Inc., Boston, MA, 1997.

[21] J. W. Walker, A homology version of the Borsuk-Ulam theorem, Amer. Math. Monthly 90(7) (1983), 466-468.

[22] A. Worheide, O-minimal homology, Ph.D. Thesis, University of Illinois at Urbana-Champaign, 1996.

Downloaded from bjo.bmj.com on February 25, 2011 - Published by group.bmj.com

Prostaglandin E receptor subtype EP3 downregulates TSLP expression in human conjunctival epithelium

Mayumi Ueta, Toshiyuki Matsuoka, Norihiko Yokoi, et al.

Br J Ophthalmol published online September 29, 2010
doi: 10.1136/bjo.2010.188748

Updated information and services can be found at:
http://bjo.bmj.com/content/early/2010/09/29/bjo.2010.188748.full.html

These include:

References This article cites 5 articles
http://bjo.bmj.com/content/early/2010/09/29/bjo.2010.188748.full.html#ref-list-1

P<P Published online September 29, 2010 in advance of the print journal.

Email alerting service Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/

Downloaded from bjo.bmj.com on February 25, 2011 - Published by group.bmj.com
BJO Online First, published on September 29, 2010 as 10.1136/bjo.2010.188748

LETTER

Prostaglandin E receptor subtype EP3 downregulates TSLP expression in human conjunctival epithelium

Prostanoids are a group of lipid mediators that form in response to various stimuli. They include prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane (TX) A_2. There are eight types of prostanoid receptors that are conserved in mammals, ranging from mice to humans: the PGD receptor (DP), four subtypes of the PGE receptor (EP1, EP2, EP3 and EP4), the PGF receptor (FP), the PGI receptor (IP) and the TXA receptor (TP). In regard to PGE receptor subtype EP3, it is reported that the PGE_2-EP3 pathway negatively regulates allergic reactions in a murine allergic asthma model[1] and that it inhibits keratinocyte activation and exerts anti-inflammatory actions in mouse contact hypersensitivity.[2] We also previously reported that PGE_2 acts as a ligand for EP3 in murine conjunctival epithelium and downregulates the progression of murine experimental allergic conjunctivitis.[3] On the other hand, thymic stromal lymphopoietin (TSLP) plays a key role in allergic inflammation[4] and is induced by polyI:C stimulation in epithelial cells, including human conjunctival epithelial cells (HCjECs)[5] or keratinocytes. In this study, we examined whether an EP3 agonist could suppress the production of TSLP in HCjECs.

This study was approved by the institutional review board of Kyoto Prefectural University of Medicine, Kyoto, Japan. All experimental procedures were conducted in accordance with the principles set forth in the Helsinki Declaration. Reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry, ELISA and quantitative RT-PCR were performed using previously described methods (supplemental methods available online at http://bjo.bmj.com).[3 5 6]

The presence of *PTGER3* (gene encoding EP3) mRNAs in HCjECs was examined by RT-PCR. The *PTGER3* mRNA was detected in normal HCjECs (figure 1A). The sequences obtained from these PCR products were identical to the human *PTGER3* cDNA sequence. The EP3 protein was also detected in human conjunctival epithelium (figure 1A).

Next, we examined the function of EP3 in TSLP production by polyI:C-stimulated primary HCjECs, using an EP3 agonist, ONO-AE248. Primary HCjECs that were untreated or pretreated with 10 μg/ml of ONO-AE248 (which was the optimal dose among 10, 1 and 0.1 μg/ml) or vehicle were incubated for 24 h with 10 μg/ml of polyI:C. While we found high levels of TSLP in the supernatants from polyI:C-stimulated ONO-AE248-untreated primary HCjECs cultures, cultures pretreated with ONO-AE248 demonstrated significantly lower levels of TSLP compared with vehicle-treated groups (figure 1B). The TSLP mRNA levels at 6 h after polyI:C stimulation were also significantly lower in primary HCjECs cultures pretreated with ONO-AE248, compared with the vehicle-treated cultures (figure 1B).

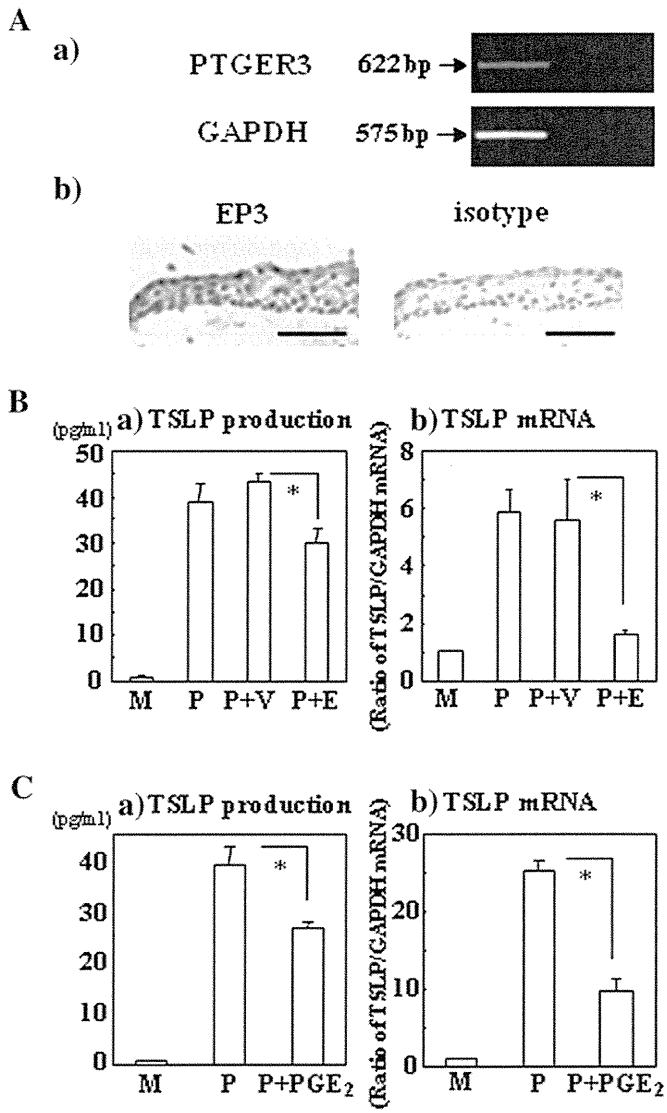

Figure 1 (A) Expression of EP3 in human conjunctival epithelium. (a) Reverse transcription-polymerase chain reaction (RT-PCR) analyses of the expression of *PTGER3* (gene encoding EP3)-specific mRNA in human conjunctival epithelium derived from a healthy volunteer. RT+, left lane; RT−, right lana. (b) Immunohistochemical analysis for EP3 in human conjunctival epithelium derived from human conjunctiva obtained during conjunctivochalasis surgery. Each bar represents a length of 50 μm. (B) Suppression of thymic stromal lymphopoietin (TSLP) production by an EP3 agonist. Primary human conjunctival epithelial cells (HCjECs), either untreated or pretreated with 10 μg/ml ONO-AE248, an EP3 agonist, were incubated for 24 h (a) and 6 h (b) with 10 μg/ml polyI:C (M, medium; P, polyI:C; P+E, polyI:C plus EP3 agonist; P+V, polyI:C plus vehicle). (C) Suppression of thymic stromal lymphopoietin (TSLP) production by PGE_2. Primary HCjECs, either untreated or pretreated with 100 μg/ml PGE_2, were incubated for 24 h (a) and 6 h (b) with 10 μg/ml polyI:C. Protein concentrations of TSLP in supernatants of cultured primary HCjECs were detected by ELISA (a), and the levels of TSLP mRNA were detected by quantitative RT-PCR (b) in (B) and (C). (B, C) Data are representative of three separate experiments and show the mean±SEM from an experiment carried out in four wells per group. *$p<0.05$ for the comparison between polyI:C plus vehicle and polyI:C plus EP3 agonist group (B) or between polyI:C and polyI:C plus PGE2 group (C) (Student t test). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Finally, we examined whether the PGE_2, an endogenous ligand for various EP receptors including EP3, could also suppress the TSLP production in primary HCjECs. Cultures pretreated with PGE_2 (100 μg/ml) (which was the optimal dose among 100, 10, 1 μg/ml (supplemental figure 1 available online at http://bjo.bmj.com)) produced significantly

Copyright Article author (or their employer) 2010. Produced by BMJ Publishing Group Ltd under licence.

Downloaded from bjo.bmj.com on February 25, 2011 - Published by group.bmj.com

lower levels of TSLP (figure 1C) and the TSLP mRNA levels were also significantly lower in primary HCjECs cultures pretreated with PGE_2, compared with the untreated cultures (figure 1C).

It is reported that TSLP is highly expressed by airway epithelial cells of asthma patients and keratinocytes in skin lesions of patients with atopic dermatitis. Conjunctival epithelium of the giant papillae obtained from vernal keratoconjunctivitis and atopic keratoconjunctivitis patients also was reported to be highly expressed.

The PGE_2-EP3 pathway might function to suppress development of human allergic conjunctivitis through the suppression of TSLP production in human conjunctival epithelium. It is evident that ocular surface epithelial cells regulate the inflammation of allergic conjunctivitis.

In summary, the results of this study showed that EP3 agonist and PGE_2, endogenous EP3 ligands, could suppress the TSLP production in human conjunctival epithelial cells and suggested that stimulating the PGE_2-EP3 pathway with a selective agonist may be useful for treating allergic conjunctivitis in humans.

Acknowledgements We thank C Endo for technical assistance.

Mayumi Ueta,[1,2] Toshiyuki Matsuoka,[3] Norihiko Yokoi,[1] Shigeru Kinoshita[1]

[1]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [2]Research Center for Inflammation and Regenerative Medicine, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan; [3]Hyogo Prefectural Amagasaki Hospital, Hyogo, Japan

Correspondence to Dr Mayumi Ueta, Department of Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajiicho, Hirokoji, Kawaramachi, Kamigyoku, Kyoto 602-0841, Japan; mueta@koto.kpu-m.ac.jp

► Supplementary methods and figure 1 are published online only. To view these files visit the journal online (http//bjo.bmj.com).

Funding This work was supported in part by grants-in-aid for scientific research from the Japanese Ministry of Health, Labour and Welfare, the Japanese Ministry of Education, Culture, Sports, Science and Technology, CREST from JST, a research grant from the Kyoto Foundation for the Promotion of Medical Science, the Intramural Research Fund of Kyoto Prefectural University of Medicine and a research grant from the Japan Allergy Foundation.

Competing interests None.

Ethics approval Ethics approval was provided by the institutional review board of Kyoto Prefectural University of Medicine, Kyoto, Japan.

Provenance and peer review Not commissioned; externally peer reviewed.

Accepted 9 August 2010

Br J Ophthalmol 2010;■:1–2.
doi:10.1136/bjo.2010.188748

REFERENCES

1. **Kunikata T,** Yamane H, Segi E, *et al*. Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. *Nat Immunol* 2005;**6**:524–31.
2. **Honda T,** Matsuoka T, Ueta M, *et al*. Prostaglandin E(2)-EP(3) signaling suppresses skin inflammation in murine contact hypersensitivity. *J Allergy Clin Immunol* 2009;**124**:809–18 e802.
3. **Ueta M,** Matsuoka T, Narumiya S, *et al*. Prostaglandin E receptor subtype EP3 in conjunctival epithelium regulates late-phase reaction of experimental allergic conjunctivitis. *J Allergy Clin Immunol* 2009;**123**:466–71.
4. **Soumelis V,** Reche PA, Kanzler H, *et al*. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;**3**:673–80.
5. **Ueta M,** Mizushima K, Yokoi N, *et al*. Gene-expression analysis of polyI: C-stimulated primary human conjunctival epithelial cells. *Br J Ophthalmol* 2010 Jul 23. [Epub ahead of print]
6. **Ueta M,** Uematsu S, Akira S, *et al*. Toll-like receptor 3 enhances late-phase reaction of experimental allergic conjunctivitis. *J Allergy Clin Immunol* 2009;**123**:1187–9.

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

Blue light and near-infrared fundus autofluorescence in acute Vogt-Koyanagi-Harada disease

Hideki Koizumi, Kazuichi Maruyama and Shigeru Kinoshita

Br J Ophthalmol published online December 3, 2009
doi: 10.1136/bjo.2009.164665

Updated information and services can be found at:
http://bjo.bmj.com/content/early/2009/12/02/bjo.2009.164665

These include:

P<P Published online December 3, 2009 in advance of the print journal.

Email alerting service Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To order reprints of this article go to:
http://bjo.bmj.com/cgi/reprintform

To subscribe to *British Journal of Ophthalmology* go to:
http://bjo.bmj.com/subscriptions

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com
BJO Online First, published on December 3, 2009 as 10.1136/bjo.2009.164665

Blue light and near-infrared fundus autofluorescence in acute Vogt-Koyanagi-Harada disease

Hideki Koizumi, MD, PhD, Kazuichi Maruyama, MD, PhD, and Shigeru Kinoshita, MD, PhD

From the Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Correspondence to: Hideki Koizumi, MD, PhD, 465 Kajii-cho, Kamigyo-ku, Kyoto 602–0841, Japan; Tel: (81) 75-251-5578; Fax: (81) 75-251-5663;
E-mail: hidekoiz@koto.kpu-m.ac.jp

Running Title: Autofluorescence in acute VKH disease

Key Words: Vogt-Koyanagi-Harada disease, Blue light fundus autofluorescence, Near-infrared fundus autofluorescence, Choroid, Retinal pigment epithelium

Word Count: 1926 words

Presented in part at the Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, Ft. Lauderdale, Florida, May 7, 2009.

The authors have no commercial or proprietary interest in products mentioned in the article.

Copyright Article author (or their employer) 2009. Produced by BMJ Publishing Group Ltd under licence.

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

ABSTRACT

Background/aims: To investigate the characteristics of fundus autofluorescence (FAF) in acute Vogt-Koyanagi-Harada (VKH) disease.

Methods: FAF photography with blue light (BL-FAF) and near-infrared light (NIR-FAF) was performed on 10 eyes of 5 patients using a confocal scanning laser ophthalmoscope before and after treatment. The FAF images were followed for 6 months and retrospectively reviewed with comparisons of the other imaging modalities.

Results: At presentation, 4 eyes of 2 patients who presented soon after the initial ocular symptoms showed mild and uniform hyperautofluorescence in the macula mixed with hypoautofluorescence in the areas of serous retinal detachment. After immediate treatment with an intravenous high-dose steroid, the abnormal FAF returned to normal at 6 months. The other 6 eyes of 3 patients, who presented weeks after the symptoms, initially demonstrated diffuse and mottled hyperautofluorescence over the posterior pole, mixed with hypoautofluorescence induced by serous retinal detachment in 4 eyes. After treatment with an intravenous high-dose steroid, all 6 eyes showed scattered and widespread hyperautofluorescence, which gradually became evident and concentrated in the macula, partially resulting in some hypoautofluorescent dots at 6 months. The BL-FAF and the NIR-FAF demonstrated similar FAF patterns, but more evidently in NIR-FAF.

Conclusion: FAF photography noninvasively visualized sequential metabolic and functional changes in the retinal pigment epithelium (RPE) in acute VKH disease. The results suggest that early and sufficient treatment with a high-dose steroid might prevent persistent RPE damage. In addition, NIR-FAF can be an alternative method for the early detection of RPE abnormality.

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

Introduction

Vogt-Koyanagi-Harada (VKH) disease is a bilateral panuveitis accompanied by systemic disorders of the nervous, auditory, and integumentary systems.[1] In spite of diffuse choroidal inflammation, inflammatory cell infiltration is thought not to encroach into the choriocapillaris and the overlying retina.[2] However, the exudative retinal detachment and the pinpoint leakage on fluoresecein angiogaphy typically seen during the acute stage of VKH disease indicate alterations in the retinal pigment epithelium (RPE). In addition, chorioretinal depigmented lesions or RPE clumping are seen as the late manifestations of VKH disease.[2-4] Therefore, subclinical stress on the RPE should be present in the course of VKH disease.

Fundus autofluorescence (FAF) photography with short wavelength light enables the visualization of lipofuscin in RPE and provides information about functional and metabolic changes in RPE. This method has been applied on various disorders involving RPE, such as age-related macular degeneration[5-9] and central serous chorioretinopathy,[10] as well as inflammatory disorders.[11,12] Recent reports utilized near-infrared light for FAF photography and provided additional information for the characterization of several disease entities.[13-16] Melanin, or melanin compounds, are thought to be the main source of FAF induced by near-infrared light, although some contributions from other possible fluorophores cannot be excluded.[14] In this study, we investigated sequential changes in FAF characteristics in acute VKH disease before and after treatment by means of the two different wavelength lights.

Materials and Methods

In this study, the FAF images of 10 eyes of 5 patients with acute VKH disease seen at Kyoto Prefectural University of Medicine between April 2008 and September 2008 were retrospectively reviewed. The 5 patients consisted of 2 men and 3 women who were observed for at least 6 months before and after treatment at our hospital.

The diagnosis of VKH disease was made based on the revised diagnostic criteria.[3] Serous retinal detachment documented as gradual dye pooling of subretinal fluid and optic nerve staining were revealed by fluorescein angiography.[3] Indocyanine green (ICG) angiography was also performed before treatment and showed irregular and multifocal hypofluorescence, patchy or delayed filling, and indistinct choroidal vessels in the earlier phases with some hyperfluorescence over the posterior pole in the late phase.[17-20]

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

All of the angiography and FAF photography were performed with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2, HRA2; Heidelberg Engineering, Heidelberg, Germany). Blue-light FAF (BL-FAF) photography was performed using a 30-degree field of view and 512 x 512 pixel resolution centered on the macula. The modality uses blue light at 488 nm for excitation and a barrier filter at 500 nm. In addition, near-infrared FAF (NIR-FAF) photography was performed with an excitation filter of 789 nm and a barrier filter of 800 nm normally used for ICG angiography. The best 9 and 15 images were taken and averaged to obtain a single image for BL-FAF and NIR-FAF, respectively; representative images of BL-FAF and NIR-FAF in a normal eye are shown in Figure 1. Optical coherence tomography (OCT) images were obtained with a time-domain OCT (Stratus OCT, version 4.0.1; Carl Zeiss Meditech, Inc., Dublin, CA) or a spectral-domain OCT (3D-OCT 1000 Mark II; Topcon Corp, Tokyo, Japan).

Results

All 10 eyes of the 5 patients presented with serous retinal detachment in the macula at the initial visit (figs 2, 4). The mean age of the 5 patients was 40 years. The clinical characteristics of the 5 patients are summarized in Table 1.

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

Table 1. Characteristics of the Patients with Acute Vogt-Koyanagi-Harada Disease

Case No.	Gender /Age	Eye	Duration from Onset to Steroid Pulse	Abnormal FAF at Initial Visit	Abnormal FAF at Month 1	Abnormal FAF at Month 6	BCVA at Initial Visit (Decimal)	BCVA at Month 6 (Decimal)
1	F/16	R	4 Days	+	+	-	0.6	1.5
		L		+	+	-	1.0	1.5
2	M/19	R	4 Days	+	+	-	0.04	1.5
		L		+	+	-	0.04	1.5
3	F/53	R	4 Weeks	+	+	+	0.7	0.6
		L		+	+	+	0.4	0.6
4	F/40	R	7 Weeks	+	+	+	1.0	0.9
		L		+	+	+	1.2	0.9
5	M/74	R	3 Weeks	+	+	+	0.2	0.8
		L		+	+	+	0.3	0.8

BCVA, best corrected visual acuity; F, female; FAF, fundus autofluorescence; L, left eye; M, male; R, right eye

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

In accordance with the revised criteria for VKH disease established by the International Nomenclature Committee,[3] all 5 patients were classified as incomplete VKH.

Of these 5 patients, 2 patients (Cases 1 and 2) presented to our hospital immediately after the ocular symptoms. Two patients (Cases 3 and 4) were insufficiently treated with low-dose oral prednisolone for 4 and 7 weeks, respectively, before the initial visit to our hospital. The other patient (Case 5) received no treatment for 3 weeks after the initial ocular symptoms.

All 5 patients were hospitalized at our hospital soon after the initial visit, and were treated with steroid pulse (intravenous drip infusion of 1000 mg methylprednisolone/day for 3 consecutive days) followed by a 50- to 60-mg daily dose of oral prednisolone, resulting in complete resolution of the retinal detachment within 2 weeks. The dose of oral prednisolone was gradually tapered over more than 6 months.

Four eyes of 2 patients (Cases 1 and 2), who had undergone steroid pulse immediately after the onset of ocular symptoms, demonstrated mild and uniform hyperautofluorescence in the macula mixed with hypoautofluorescence inside, but not in all areas of the serous retinal detachment at the initial visit, particularly in NIR-FAF (fig 3A,B). That hyperautofluorescence was seen in the areas of irregular hypofluorescence in the macula observed on ICG angiography, but the multifocal hypofluorescence on ICG angiography was more numerously and more extensively observed (fig 2D). After resolution of the serous retinal detachment, the hyperautofluorescence remained for at least 1 month after steroid pulse, wider and more clearly in NIR-FAF than in BL-FAF (fig 3C,D). With time, the hyperautofluorescence in the macula decreased in both size and intensity, and there appeared to be no abnormal FAF at 6 months after steroid pulse (fig 3E,F). OCT did not show any abnormalities at 6 months (fig 3H).

Six eyes of the other 3 patients (Cases 3, 4, and 5), who had not undergone steroid pulse until 3 to 7 weeks after the onset of the ocular symptoms, initially showed diffuse and mottled hyperautofluorescence over the posterior pole, more clearly demonstrated in NIR-FAF (fig 5A,B). In addition, 4 eyes of 2 patients (Cases 4 and 5) demonstrated hypoautofluorescence in the areas of serous retinal detachment, but 2 eyes of 1 patient (Case 3) showed no hypoautofluorescence in spite of the retinal detachment. After resolution of the retinal detachment, all 6 eyes gradually showed placoid hyperautofluorescence in the macula, and surrounding scattered hyperautofluorescence accompanied by radial patterns of hyperautofluorescence around the optic disc (fig 5C,D).

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

That hyperautofluorescence corresponded to irregular and multifocal hypofluorescence on ICG angiography, but the hypofluorescent spots on ICG angiography were more numerous and extensive. The radial hyperautofluorescence appeared to be consistent with choroidal folds seen in the angiograms (figs 4D and 5C,D).[21] The pattern of hyperautofluorescence in NIR-FAF was similar to that in BL-FAF, but more evident in NIR-FAF. Such hyperautofluorescence in BL-FAF and NIR-FAF gradually decreased in size, yet contrary to Case 1 and Case 2, increased in intensity in the fovea, showing granular hyperautoflurescence with some hypoautofluorescent dots surrounded by larger placoid hyperautofluorescence at 6 months (fig 5E,F). Some areas of that granular hyperautofluorescence in the fovea appeared to correspond to yellowish or punctate pigmentary dots at the level of the RPE seen in the color photographs (fig 5G), but the distribution of the granular hyperautofluorescence was more widespread. In 1 patient (Case 5), the choroidal inflammation relapsed two times in conjunction with the choroidal folds (at 3 months and 5 months after steroid pulse), but the BL-FAF and the NIR-FAF did not change before and after the recurrent episodes. OCT showed multifocal thickening of the RPE monolayer, some of which was consistent with granular hyperautofluorescence in the fovea (fig 5I). In the areas of placoid hyperautofluorescence, the OCT showed defects in the boundary between the photoreceptor inner and outer segments (IS/OS) with no RPE abnormality (fig 5H).

Discussion

This study investigated 10 eyes of 5 patients with acute VKH disease, and demonstrated their sequential changes in FAF. We found that the abnormal FAF occasionally persisted at 6 months after steroid therapy even after complete resolution of exudative retinal detachment in patients who received late effective steroid treatment.

The FAF patterns after the resolution of retinal detachment seen in this study can be divided into 2 patterns. The first pattern was seen in the patients who had undergone immediate intensive treatment with steroid pulse and showed mild hyperautofluorescence during the early phase of the disease. The hyperautofluorescence then decreased in size and intensity with time, resulting in normal FAF at 6 months. The second pattern was seen in the patients who had not undergone immediate treatment with steroid pulse and showed scattered and widespread hyperautofluorescence during the early phase. The hyperautofluorescence concentrated centripetally with time, resulting in some

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

hyperautofluorescent dots mixed with hypoautofluorescent dots more evident in NIR-FAF than in BL-FAF at 6 months. Previous studies have found that the interval between the development of ocular symptoms and treatment seemed to be an important predictor for visual outcome.[22,23] Early and sufficient treatment with a high-dose steroid may be important to protect the RPE from metabolic and functional stress and preserve the visual function. FAF photography longitudinally enabled the visualization of such stress on the RPE.

In this study, we used NIR-FAF photography in addition to the conventional BL-FAF photography. This method was recently introduced,[13,14] and is thought to visualize oxidized melanin or compounds closely associated with melanin.[14] Although the BL-FAF has been applied to many posterior diseases, the absorption of the short wavelength light by the macular pigment often obscures the precise condition of the RPE in the macula. In this study, the NIR-FAF showed similar patterns to the BL-FAF, but more evidently in the NIR-FAF. The NIR-FAF is known to originate in part from the RPE,[14] and it is not blocked by the macular pigment. Therefore, the NIR-FAF may have potential to be an alternative method for detection of early RPE disturbance seen in acute VKH disease, as well as other posterior diseases.[15,16]

Although our study included a small number of patients, comparisons between FAF photography and the other imaging modalities indicates some implications. The hyperautofluorescence seen in the early course of the disease corresponded to irregular and multifocal hypofluorescence on ICG angiography. Such hypofluorescence on ICG angiography is thought to represent the choroidal inflammatory foci in acute VKH disease,[17] suggesting that choroidal inflammation affected the RPE metabolism in all 5 cases. However, early treatment with a high-dose steroid might have prevented the persistent damage in Case 1 and Case 2. On the other hand, the 3 cases treated late after the onset of symptoms showed a gradual concentration of abnormal FAF in the macula, exhibiting granular hyperautofluorescence and hypoautofluorescence in the central part surrounded by placoid hyperautofluorescence at 6 months. As shown in Figure 4, the areas of placoid hyperautofluorescence showed a normal RPE monolayer with the defect of IS/OS in OCT, thus suggesting that there was RPE dysfunction affecting the photoreceptor layer. The central granular hyperautofluorescence corresponded in part to multifocal thickening of the RPE monolayer visualized in OCT and to yellowish granules or pigmentary dots seen in the color photographs. Future studies using higher-resolution OCT

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

may clarify the precise relationship between the morphologic alterations and the FAF findings.

It should be noted that this study has limitations due to the small number of patients and the limited follow-up period. In addition, retrospective design did not allow us to show more detailed correlation between the FAF photography and the other imaging modalities. We also acknowledge that our results might have been influenced by other factors such as age, since a younger age at onset was found to be a prognostic factor for better visual outcome.[23-25] However, and to the best of our knowledge, this is the first study to demonstrate the sequential changes in FAF and provide information about the condition of the RPE in acute VKH disease. It remains unclear at this point whether adjusting the dosage of the oral steroid treatment or other immunosuppressive agents following the initial immediate and sufficient treatment with a high-dose steroid can alter the course of the abnormal FAF and visual outcome. Future studies are required to elucidate this matter.

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

Competing interests: None

Ethics approval: Approval from the institutional review board was not required for this retrospective study.

Patient consent: Informed consent was obtained from all of the patients prior to initiation of this retrospective study.

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non-exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in the British Journal of Ophthalmology and any other BMJPGL products to exploit all subsidiary rights, as set out in our licence (http://bjo.bmj.com/ifora/ licence.pdf).

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

Figure Legends

Figure 1

The healthy right eye of a 32-year-old male. The blue-light fundus autofluorescence (BL-FAF) photography (A) and the near-infrared FAF (NIR-FAF) photography (B) demonstrated hypoautofluorescence caused by the major retinal vessels and the optic disc. In the BL-FAF photography, the FAF from the central fovea is obscured by the macular pigment which absorbs short-wave-length light, whereas the NIR-FAF showed subtle hyperautofluorescence in the central macula.

Figure 2

Case 1. A 16-year-old woman with acute Vogt-Koyanagi-Harada (VKH) disease. This patient presented to our hospital the day after her initial ocular symptoms. (A) The left eye showed multilobular serous retinal detachment in the macula. (B) Optical coherence tomography (OCT) (the location is indicated with arrow in (A)) demonstrated elevation of the neurosensory retina with accumulation of subretinal fluid. The fluorescein angiogram (C) showed multiple pinpoint leakages in the macula. The middle phase of indocyanine green (ICG) angiography (D) demonstrated numerous and extensive hypoautofluorescent spots (arrows) observed in more confluent and irregular form in the macula (arrowheads). Some parts of the highly elevated retinal detachment were also visualized as hypofluorescence (asterisks).

Figure 3

Case 1 (Cont.). At the initial visit, the BL-FAF (A) and the NIR-FAF (B) demonstrated mild and uniform hyperautofluorescence in the macula, but some parts of the serous retinal detachment (See fig 2D, asterisks) caused hypoautofluorescnce. At 1 month after the steroid pulse, the BL-FAF (C) showed subtle hyperautofluorescence (arrows) around the central fovea, whereas the NIR-FAF (D) showed wider and more evident hyperautofluorescence in the macula (arrows). At 6 months, the BL-FAF (E) and the NIR-FAF (F) returned to normal. The color photograph (G) and the OCT (H, the location is indicated with an arrow in (G)) at 6 months showed no abnormal findings. Arrowheads in (G) indicate the intact boundary between the photoreceptor inner and outer segments (IS/OS).

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

Figure 4

Case 3. A 53-year-old woman who had not undergone immediate steroid pulse for 4 weeks. The left eye (A) initially showed serous retinal detachment in the macula evidenced by OCT (B)(the location is indicated by arrow in (A)). Fluorescein angiography (C) demonstrated diffuse dye leakage over the posterior pole and optic nerve staining. The middle phase of ICG angiography (D) revealed numerous hypofluorescent spots (arrows), which were observed in confluent and irregular form in the central macula. Note that there are radial patterns of hypofluorescence representing choroidal folds (yellow arrowheads).

Figure 5

Case 3 (Cont.). At the initial visit, the BL-FAF (A) and the NIR-FAF (B) demonstrated diffuse and mottled hyperautofluorescence over the posterior pole, but the intensity was weak. At 1 month after the steroid pulse, the BL-FAF (C) and the NIR-FAF (D) showed placoid and scattered hyperautofluorescence in the macula in association with radial hyperautofluorescence around the optic disc (yellow arrowheads). At 6 months, the BL-FAF (E) and the NIR-FAF (F) demonstrated granular hyperautofluorescence in the fovea and surrounding placoid FAF, more evidently demonstrated in NIR-FAF. Note that there appeared to be some hypoautofluorescent dots (E, F, arrows) in the fovea. The color photograph at 6 months (G) demonstrates some yellowish granules and pigmentary dots in the fovea. OCT (the locations are indicated with arrows in (G)) showed normal hyperreflective RPE monolayer and defect in the IS/OS in the area of placoid hyperautofluorescence (H), whereas it demonstrated multifocal thickening of the RPE line (I, arrows) with intact IS/OS boundary (I, arrowheads).

Downloaded from bjo.bmj.com on March 8, 2010 - Published by group.bmj.com

References

1. Moorthy RS, Inomata H, Rao NA. Vogt-Koyanagi-Harada syndrome. *Surv Ophthalmol* 1995;39:265-292.

2. Rao NA. Pathology of Vogt-Koyanagi-Harada disease. *Int Ophthalmol.* 2007;27:81-85.

3. Read RW, Holland GN, Rao NA, *et al.* Revised diagnostic criteria for Vogt-Koyanagi-Harada disease: report of an international committee on nomenclature. *Am J Ophthalmol* 2001;131:647-652.

4. Inomata H, Rao NA. Depigmented atrophic lesions in sunset glow fundi of Vogt-Koyanagi-Harada disease. *Am J Ophthalmol* 2001;131:607-614.

5. Holz FG, Bindewald-Wittich A, Fleckenstein M, Dreyhaupt J, Scholl HP, Schmitz-Valckenberg S. Progression of geographic atrophy and impact of fundus autofluorescence patterns in age-related macular degeneration. *Am J Ophthalmol* 2007;143: 463-472.

6. Spaide RF. Fundus autofluorescence and age-related macular degeneration. *Ophthalmology* 2003;110:392-399.

7. Vaclavik V, Vujosevic S, Dandekar SS, Bunce C, Peto T, Bird AC. Autofluorescence imaging in age-related macular degeneration complicated by choroidal neovascularization: a prospective study. *Ophthalmology* 2008;115:342-346.

8. McBain VA, Townend J, Lois N. Fundus autofluorescence in exudative age-related macular degeneration. *Br J Ophthalmol* 2007;91:491-496.

9. Hwang JC, Chan JW, Chang S, Smith RT. Predictive value of fundus autofluorescence for development of geographic atrophy in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2006;47:2655-2661.

10. Spaide RF, Klancnik JM, Jr. Fundus autofluorescence and central serous chorioretinopathy. *Ophthalmology* 2005;112:825-833.

11. Koizumi H, Pozzoni MC, Spaide RF. Fundus autofluorescence in birdshot chorioretinopathy. *Ophthalmology* 2008;115:e15-20.

12. Haen SP, Spaide RF. Fundus autofluorescence in multifocal choroiditis and panuveitis. *Am J Ophthalmol* 2008;145:847-853.

13. Weinberger AW, Lappas A, Kirschkamp T, *et al.* Fundus near infrared fluorescence correlates with fundus near infrared reflectance. *Invest Ophthalmol Vis Sci* 2006;47:3098-3108.