Purpose. To investigate the findings of infrared fundus autofluorescence in eyes with central serous chorioretinopathy (CSC).

Methods. This study was an observational follow-up of 83 eyes of 80 consecutive patients with CSC recruited from a hospital referral practice. Infrared autofluorescence (IR-AF) findings and those of other clinical studies, including short-wave autofluorescence (SW-AF), fundus color photography, and optical coherence tomography were assessed. The IR-AF changes that appeared during the follow-up period were recorded. The relationship between IR- and SW-AF was analyzed by comparing the categories of focal autofluorescence (granular hyper-AF, granular hypo-AF, and mixed AF). The influence of final clinical findings on final best corrected visual acuity (BCVA) was analyzed.

Results. Twenty-three of 83 (27%) eyes showed granular hyper-IR-AF, whereas 53 (64%) eyes showed granular hyper-SW-AF. Most of the eyes with granular hyper-IR-AF (92%) showed granular hyper-SW-AF. On the contrary, the eyes with granular hyper-SW-AF showed various patterns of IR-AF. The deposits with hyper-IR-AF corresponding to hyper-SW-AF turned into hypo-IR-AF with hyper-SW-AF in four eyes. Final BCVA was significantly worse in eyes with granular hypo-IR-AF compared with the eyes without the findings ($P = 0.055$).

Conclusions. Granular hyper-IR-AF from the deposits in CSC appeared concurrently with hyper-SW-AF. Granular hyper-IR-AF changed from hyperautofluorescence to hypoautofluorescence during the follow-up period. This change of IR-AF characteristics was different from that of SW-AF. The changes are attributable to the modification of melanin in the RPE. The authors speculate that the lipofuscin-like materials contribute to the characteristic changes of IR-AF through the modification of melanin in the RPE. The present study especially focused on focal IR-AF of subretinal deposits.

Central serous chorioretinopathy (CSC) is characterized by serous retinal detachment (SRD) at the macula with a focal area or multifocal areas of leakage at the level of the retinal pigment epithelium (RPE) noted on fluorescein angiography (FA). Most patients with acute CSC have spontaneous resolution of their macular detachments and a good visual prognosis. The eyes with long-standing SRD show widespread alteration of the RPE with a decline in visual acuity. Imaging technology has provided important information about the disease. Indocyanine green angiography (IA) has indicated dysfunction of the hemodynamics and fluid dynamics in the choroid. Optical coherence tomography (OCT) has demonstrated morphologic changes in eyes with CSC, such as swelling of the sensory retina, disruption of the RPE, thickening of the outer retinal surface, and loss of the boundary of the photoreceptor inner/outer segments.

Fundus autofluorescence (AF) imaging is a novel imaging technology that can show characteristics of eyes with CSC. A recent report has suggested that fundus AF can predict visual acuity in eyes with CSC. Fundus AF mainly originates from lipofuscin in the RPE, which is a residue of phagocytized photoreceptor outer segments. A major fluorophore of lipofuscin is pyridinium bisretinoid (A2E), which is derived from retinol. A recent study suggested that the precursors of A2E in the sensory retina also showed AF and had cytotoxic effects. Dot-like deposits located in the sensory retina or subretinal space show hyper-AF and may be involved in the pathologic changes in CSC. However, the process causing the degenerative changes in the retina and the RPE remain unclear.

Melanin in the RPE plays an important role in the protection of eyes against phototoxicity that may be involved in age-related dysfunction of the fovea. So far, we do not have a method of evaluating melanin in living eyes. High-definition imaging systems, such as the confocal scanning laser ophthalmoscope, make it possible to visualize faint infrared autofluorescence (IR-AF). Fundus IR-AF can originate from melanin in the RPE or choroidal tissue. Imaging IR-AF may provide new information on the pathologic process in eyes with CSC. We assessed the characteristics of IR-AF in comparison with short-wave autofluorescence (SW-AF), color fundus images, and OCT findings. The present study especially focused on focal IR-AF of subretinal deposits.

Methods. Eighty consecutive patients were examined and CSC with SRD involving the fovea was diagnosed and then confirmed by OCT and FA at Fukushima Medical University Hospital, Fukushima, Japan, between July 2006 and October 2008. Sixty-one (73.4%) men and 19 (26.6%) women were observed longitudinally for 3 to 32 months (mean, 11.8). Three patients were affected with CSC in both eyes. As a result, we studied 83 eyes of 80 patients. After informed consent, each patient was initially examined by visual acuity testing, ophthalmoscopy, color and red-free monochromatic photography, FA, and OCT imaging, and fundus AF. The tenets of the Declaration of Helsinki were observed. The Institutional Review Board of the University of Fukushima Medical School granted approval for this study design. Eyes were excluded if they had undergone laser photocoagulation and had melanotic lesions in the fundus and if additional disease was present that could compromise visual acuity.

Fundus Imaging. Visual acuity testing, fundus photography, fundus AF, and OCT images were taken at all visits. Fundus photography was performed with a...
fundus camera (TRC-501X; Topcon, Tokyo, Japan). SW- and IR-AF were recorded with a confocal scanning laser ophthalmoscope (HRA2; Heidelberg Engineering, Heidelberg, Germany). The pairs of excitation laser and detection filters were 488 and >500 nm in SW-AF and 787 and >800 nm in IR-AF, respectively. The field was 30° × 30° (768 × 768 pixels). AF images were taken after maximum pupillary dilation with 0.5% tropicamide and 0.5% phenylephrine. Focusing was achieved at 815 nm, and reflectance images were taken. After a switch to the 787-nm excitation (indocyanine green mode), the sensitivity was increased until the vessels and the disc were recognized. Sixteen serial images were processed instantly by the averaging method of the system software (HRA2; Heidelberg Engineering) to gain contrast of the images. SW-AF images (excitation, 488 nm) were acquired in the same manner as the IR-AF images. SW images were taken after sufficient light exposure, because the intensity of SW-AF is changed by light exposure. Images taken within 1 month after IA were eliminated from the analysis to avoid misreading due to residual fluorescence.

OCT scans (OCT-3000-TM, Carl Zeiss Meditec, Jena, Germany; 3D-OCT-TM system Topcon, Tokyo, Japan; Spectralis-TM, Heidelberg Engineering, Heidelberg, Germany) with single scans in the horizontal and vertical orientations were made through the center of the fovea, routinely. OCT-3000-TM was used until July 2007, 3D-OCT-TM was used from August 2007 to March 2008, and Spectralis-TM was used after February 2008. Multihorizontal scans were performed through extrafoveal areas of subretinal fluid in the posterior pole as well. All sets of images obtained during the visits of all patients were analyzed.

Image Analysis

The AF classification and the clinical findings are shown in Table 1. We evaluated three types of AF: background AF, focal AF, and atrophic lesion AF. Background AF was classified into two categories: reduced AF (Figs. 1A, 1B) and diffuse hyper-AF in the area affected by the SRD (Figs. 1D, 1G, 1H). Focal AF was classified into three categories: granular hyper-AF (Figs. 1G, 1H, 1J), granular hypo-AF (Fig. 1N), and mixed AF (Fig. 2). Herein, focal AF was spotty AF corresponding to deposits or precipitates; granular hyper-AF was composed of dot-like (<65 μm) or fleck-like (65 or >65 μm) hyper-AF exclusively; and granular hypo-AF described the lesions composed of dot- or fleck-like hypo-AF exclusively. Autofluorescence associated with atrophic lesions showed extensive diffuse hypo-AF with unclear boundaries (Fig. 3).

In color fundus photographs, the deposits and fibrin were evaluated. Deposits included both yellow precipitates (<65 μm) and flecks (65 or >65 μm). The OCT findings were classified into four categories: SRD, deposit on the outer retinal surface, deposit on the RPE, and pigment epithelial detachment (PED).

The findings from all image sets of 83 eyes were entered on a computer spreadsheet. Each image was evaluated by two independent graders, with discrepancies resolved by open adjudication with the third grader. The incidence of each finding was calculated for the 83 eyes. The relationships between IR- and SW-AF were analyzed by comparing the characteristics of focal AF.

Final Visual Acuity

To evaluate the relationships between the findings listed (Table 1) and the final best corrected visual acuity (BCVA), final BCVA in the eyes with the clinical findings was compared with that in the eyes without findings by univariate analysis.

Statistical Methods

Logarithm of the minimum angle of resolution (logMAR) was used for statistical analysis and was converted to decimal acuity equivalents. The data obtained were analyzed with frequency and descriptive statistics. The final BCVA was compared between the group with the clinical findings and without findings by using the Mann-Whitney U test. *P < 0.05 was considered significant in the statistical analyses (JMP 7.0; SAS, Cary, NC).

RESULTS

The mean age of the patients was 48.9 years (SD, 8.9; range 25–65). No patient had choroidal neovascularization. The mean of the final BCVA was 0.92 (range, 0.15–2.0). One hundred four leakage points were identified by FA in the 83 eyes. The eyes with two or more leaking points were included. The type of the leakage points in FA included inkblot 84.3% (70/83 eyes), smokestack 15.6% (13/83 eyes), and diffuse leakage 9.6% (10/83 eyes). In IR-AF images, leakage points showed hypo-AF 83.7% (87/104 leakage points), hyper-AF 3.8% (4/104 leakage points), and iso-AF 12.4% (13/104 leakage points), respectively. Fourteen eyes showed a recurrence after complete resolution of SRD, which was confirmed by OCT. Forty-seven of the 83 eyes were observed for more than 6 months.

AF Findings

A summary of the clinical findings that appeared throughout the follow-up period is shown in Figure 4. For background AF, reduced IR-AF appeared in 31 (37%) eyes in the acute stages of CSC, and diffuse hyper-IR-AF appeared in 69 (83%) eyes. Reduced IR-AF turned into diffuse hyper-IR-AF in 20 (64%) of 31 eyes during the follow-up period. The incidence of the focal IR-AF findings in 83 eyes was as follows: granular hyper-IR-AF, 23 (28%); granular hypo-IR-AF, 21 (25%), and mixed IR-AF, 19 (23%) (Fig. 4). To assess the interobserver variability in background AF and focal AF, *P* statistics were calculated. The interobserver variability was 0.78 (background IR-AF), 0.75 (background SW-AF), 0.83 (focal IR-AF), and 0.84 (focal SW-AF).

Twenty-one of 25 eyes with granular hyper-IR-AF appeared concurrently with diffuse hyper-IR-AF, whereas the remainder of two eyes appeared with reduced IR-AF. Granular hyper-AF...
appeared more frequently in SW-AF imaging compared with IR-AF imaging, whereas granular hypo-AF was observed less frequently in SW-AF imaging. Two eyes with mass deposits that showed homogenous hyper-SW-AF were classified as mixed SW-AF (see Fig. 7). In color fundus photographs, 83% (72/83) of the eyes showed deposits. In OCT images, the deposit appeared on the outer retinal surface in 56 (67%) eyes and on the RPE in 54 (65%). PED away from the leakage point was noted in 19 (23%) eyes.

Focal AF findings included four categories: no focal AF, granular hyper-AF, granular hypo-AF, and mixed AF. Therefore, the combinations of focal IR- and SW-AF findings include 16 pairs of AF findings. To analyze the relationships between IR- and SW-AF, we evaluated the appearance of 16 pairs of AF categories during the follow-up period (see Fig. 8). A total of 117 paired AF findings were observed in 83 eyes throughout the follow-up period. Twenty-two (92%) of 24 eyes with granular hyper-IR-AF showed granular hyper SW-AF. The eyes with granular hyper-IR-AF showed neither granular hypo- nor mixed SW-AF. On the contrary, 64 eyes with granular hyper-SW-AF showed hypo (8 eyes/13%) or mixed IR-AF (12 eyes/19%). Dot-like hyper-SW-AF showed iso- or hypo- IR-AF in the acute stages of CSC (Figs. 1, 5). Fleck-like deposits with hyper-SW-AF and hypo-IR-AF appeared after resolution of SRD (Fig. 1). Granular hyper-IR-AF turned into granular hypo-IR-AF in 4 of 24 eyes during the follow-up period. In OCT images, some deposits with hyper-SW-AF showed hypo-IR-AF (M, N).

**Final BCVA**

The difference in final BCVA between the group with and the one without clinical findings was examined in 47 eyes that were observed for more than 6 months. Analyzed categories are follows: reduced-IR-AF, diffuse hyper-IR-AF, granular hyper-IR-AF, granular hypo-IR-AF, and mixed IR-AF.
IR-AF, granular hypo-IR-AF, mixed IR-AF, diffuse hypo-IR-AF, reduced-SW-AF, diffuse hyper-SW-AF, granular hyper-SW-AF, granular hypo-SW-AF, mixed SW-AF, diffuse hypo-SW-AF, deposits, fibrin, SRD, PED, deposits on the outer retina, and deposits on the RPE. Final BCVA decreased significantly in the eyes showing granular hypo-IR-AF ($P_{/H11005} 0.035$) by the Mann-Whitney test. BCVAs of the eyes with deposits on fundus photograph and PED in OCT were worse than in eyes without the findings; however, the difference was not statistically significant (deposit; $P_{/H11005} 0.09$, PED; $P = 0.09$).

**DISCUSSION**

We compared the findings of IR-AF with SW-AF and other clinical findings to study the characteristics of IR-AF in the eyes with CSC. Characteristics of IR-AF changed during the course of CSC. Background AF within SRD showed hypo-IR-AF and hypo-SW-AF in the acute stage, which then turned into diffuse hyper-AF during the follow-up period in both SW-AF and IR-AF images. In focal AF, although the dot-like deposits in SRD showed hyper-AF in SW-AF imaging, they showed iso- or hypo-AF in IR-AF imaging. Fleck-like deposits with hyper-SW-AF concurrently showed hyper-IR-AF, just after resolution of SRD. IR-AF of these deposits turned into hypo-IR-AF after more than a year.

Lipofuscin in the RPE is an assortment of major chromophores derived from photoreceptor breakdown and accumulates in the RPE throughout life. A2E and its precursors, which are the main components of lipofuscin, show SW-AF and provide functional information on the RPE. Melanin is an insoluble high-molecular-weight polymer derived from the enzymatic oxidation of tyrosine and dihydroxyphenylalanine. Melanin in the RPE plays an important role in the protection of eyes against phototoxicity. The protective effects of melanin are ascribed to its antioxidant property and photo-screening effects. It is presumed that the reduction of its protective effects, due to diminished melanin with age, is the pathogenesis of age-related macular degeneration. So far, we do not have a method of evaluating melanin in living eyes. Recent reports suggested that IR-AF was generated from the melanin in the RPE and the choroid. The combination of IR- and SW-AF imaging is potentially a useful tool for assessing the functional aspects of the RPE. To explain the interpretation of IR-AF in CSC, we studied the IR-AF findings and their relation to the other clinical findings.

**FIGURE 2.** AF images classified as mixed-AF. SW-AF image (A) shows granular hypo-AF among granular hyper-AF. Granular hypo-AF was more prominent in the IR-AF images (B). They were classified as mixed-AF.

**FIGURE 3.** AF images show diffuse hypo-AF. (A) SW-AF image. (B) IR-AF image.

**FIGURE 4.** Summary of findings. The numbers represent the number of eyes affected of the 83 examined. Depo, deposit.

**FIGURE 5.** AF and OCT images of the right eye of patient 2, a 38-year-old man. (A) Red-free photograph. Precipitate appeared within the area of SRD. (B) Vertical OCT image shows dot-like deposits on the outer retinal surface. (C) An SW-AF image showing hyper-SW-AF within the area of SRD. (D) An IR-AF image showing dot-like hyper-SW-AF corresponding to dot-like hypo-IR-AF (arrowheads).
AF Characteristics of Deposits

Subretinal deposits are considered to be accumulations of photoreceptor outer segments or phagocytized outer segments by macrophages. They may contribute to retinal degeneration in eyes with CSC. In attempting to evaluate the characteristics of the deposits by using IR-AF, one obstacle is the diversity of IR-AF. For example, one of the fleck-like hyper-SW-AF images shows hyper-IR-AF and another fleck-like hyper-SW-AF image shows hypo-IR-AF. The diversity of IR-AF can be attributed to the masking effect of fibrin or lipid in the subretinal space. In addition, the difference in sensitivity between IR- and SW-AF using the HRA2 system may modify the IR-AF findings.

To reduce modification factors, the focal AF was classified into three categories from the appearance of focal AF within the affected area. Dot-like hyper-SW-AF in SRD without fibrin or lipids, showed iso- or hypo-IR-AF. In addition, aggregation of the RPE can show hyper-IR-AF. The diversity of IR-AF can be attributed to the masking effect of fibrin or lipid in the subretinal space. In addition, the difference in sensitivity between IR- and SW-AF using the HRA2 system may modify the IR-AF findings.

Causes of Hyper-IR-AF

The eyes with granular hyper-IR-AF showed granular hyper-SW-AF concurrently in the course of CSC, but not vice versa. In most deposits, hyper-IR-AF colocalized with hyper-SW-AF (Fig. 8). Aggregation of the RPE can show hyper-IR-AF. Since the RPE contains melanin and lipofuscin, the masses of RPE cells can generate both SW- and IR- hyper-AF. High-resolution OCT revealed that the hyper-IR-AF appearance corresponded to the focal mound of the RPE (Fig. 6). However, aggregation of the RPE could not explain all the kinds of hyper-IR-AF, especially the diffuse hyper-IR-AF in the area without protrusion of the RPE on the OCT images (Fig. 1H).

FIGURE 6. AF and OCT images of the left eye of patient 3, a 56-year-old man. The retina was detached 6 months after onset of SRD. SW-AF (A) and IR-AF (B) at high magnification and pairs of IR-AF images and spectral domain OCT (C, D). The affected area showed diffuse hyper-SW-AF and hyper-IR-AF. Dot-like hyper-IR-AF was not observed at the fovea (B). Some fleck-like hyper-IR-AF corresponded to hyper-SW-AF. Some flecks of hyper-IR-AF corresponded to the mound of the RPE (arrows) (C, D).

FIGURE 7. AF and OCT images of patient 4 shows a mass of hyper-SW-AF in a color fundus photograph. (A) Yellow deposit accumulated in the inferior half of SRD. (B) Vertical OCT image demonstrated that the deposit occupied more than half of the SRD. (C) Three quarters of the affected area showed hyper-SW-AF. (D) An IR-AF image did not show hyper-AF corresponding to hyper-SW-AF. Hyper-AF spots appeared at the edge of the yellow deposit.

FIGURE 8. The relationships of focal AF. One hundred seventy-nine paired AF findings appeared during the follow-up period in 116 eyes. No focal, eye with no focal AF; Hyper, eye with granular hyper-AF; Hypo, eye with granular hypo-AF; Mixed, eye with mixed AF.
fuscin-like materials, during CSC (Fig. 7). Melanogenesis in the RPE may be possible, and experimental reports suggest that melanogenesis is induced by feeding of the outer segments of photoreceptors.\textsuperscript{28–31} This melanogenesis was presumed to occur by using the disc membrane of outer segments as a substrate. OCT revealed the elongation of outer segments of photoreceptor in the eyes with CSC.\textsuperscript{52} Reattachment of the retina may give similar circumstances to the outer segment feeding model. However, postnatal melanogenesis in the RPE cells is controversial.\textsuperscript{53–55} A final possibility is that the changes in the IR-AF property by phototoxication may be involved in hyper-IR-AF.\textsuperscript{36} At this point, increases in IR-AF induced by melanogenesis and photooxidation have not been shown in the living human eye. A further experimental study is needed to address this point.

Causes of Hypo-IR-AF

SRD and PED showed hypo-IR-AF, caused by the attenuation of IR-AF from the RPE or the choroid by the accumulated fluid. The other types of hypo-IR-AF were shown in focal IR-AF. Hypo-IR-AF with hypo-SW-AF is thought to be due to the loss of RPE or a strong masking effect such as could be caused by debris or components of blood. However, hypo-IR-AF appeared on the deposits with hyper-SW-AF. In this study, it was found that some of the deposits with hyper-IR-AF showed hypo-IR-AF after reattachment of the retina. This phenomenon occurred at the site of subretinal deposits showing hyper-SW-AF (Fig. 1). We hypothesized that lipofuscin-like materials may reduce IR-AF. A2E and its derivatives are known to elicit photooxidation in the RPE cells\textsuperscript{157–160} and thereby reduce the amount of melanin in the RPE cells,\textsuperscript{159,41–45} which, could cause a reduction in IR-AF. Furthermore, upregulation of oxidative stress in the RPE cells due to decreased melanin may facilitate apoptosis of the RPE. Of note, the final BCVA in the eyes observed for more than 6 months decreased in the eyes with granular hypo-IR-AF suggesting that the dysfunction of the RPE cells can affect the visual function.

The limitation of our study is that increasing IR-AF by melanogenesis and photo-oxidation have not been shown in a living human eye. Also, the magnitude of IR-AF induced by melanogenesis or photooxidation in vivo may not be enough to be detected by HRA2. Since the IR-AF contains AF generated from the choroidal tissue and is attenuated by the lesions of the retina, we may take the condition of the choroid and overlaying retina into consideration when we interpret IR-AF images. In conclusion, the IR-AF findings in CSC can be explained hypothetically by the modification of melanin in the RPE (Fig. 9). Regarding this hypothesis, we must be aware that the source of IR-AF remains unknown and future mechanistic studies are needed to validate this notion. Since IR-AF indicates the presence of the RPE, IR-AF can be used as a biomarker for the retinal pigment epithelial cells. IR-AF in combination with SW-AF may be useful to identify the cells participating in retinal lesions. In addition, as the intensity of IR-AF can be reduced by oxidative stress to the RPE, application of IR-AF for age-related macular degeneration may provide useful information. IR-AF can be an effective tool for investigating the RPE and the retina noninvasively.

References
