Deterioration of Visual Function as Examined by Electroretinograms in *Toxoplasma gondii*–Infected IFN-γ-Knockout Mice

Kazumi Norose,¹ Fumie Aosai,¹ Atsushi Mizota,²,⁵ Shuichi Yamamoto,⁴ Hye-Seong Mun,¹ and Akibiko Yano¹

**PURPOSE.** To investigate by ERG the effects of *Toxoplasma gondii* infection on the visual function of interferon gamma knockout (GKO) mice, as a model of immunocompromised hosts.

**METHODS.** Susceptible wild-type (WT) C57BL/6 and GKO C57BL/6 mice were infected with five cysts of the avirulent *T. gondii* perorally. ERGs were recorded before and after the infection. The eyes of WT and GKO mice were enucleated and prepared for histologic studies 4 weeks and 12 days after infection, respectively.

**RESULTS.** The a- and b-waves of ERGs did not change significantly up to 1 month after infection in WT mice, but those of GKO mice were significantly reduced 11 days after infection. Histopathology revealed focal retinitis and vasculitis in WT mice 4 weeks after infection. Mild inflammation and sludging of blood in the retina and choroid were found in GKO mice 12 days after infection, just before death. Cysts were found in the inner nuclear layer, with little disturbance of the surrounding retinal architecture in both WT and GKO mice.

**CONCLUSIONS.** ERG clearly showed deterioration of visual function in GKO but not in WT mice after *T. gondii* infection. ERG is a sensitive and reliable method for observing activity in mice severely affected with experimental toxoplasmic retinochoroiditis. (Invest Ophthalmol Vis Sci. 2005;46:317–321) DOI:10.1167/iovs.04-0580

The protozoan parasite *Toxoplasma gondii* can cause severe, life-threatening disease, especially in immunocompromised patients. Toxoplasmosis is a major cause of ocular disease in immunocompetent subjects but is more destructive in immunocompromised individuals, such as patients with human immunodeficiency virus (HIV),¹ cancer patients, and organ transplant recipients.² Because a mouse model of ocular toxoplasmosis would enhance the ability to evaluate various aspects of its pathogenicity, we reasoned that the development of a mouse model of ocular toxoplasmosis³,⁴ is essential.

Interferon (IFN)-γ is the pivotal mediator that induces anti-*T. gondii* effector mechanisms.⁵–¹¹ The use of IFN-γ-knockout (GKO) mice as a model of immunocompromised hosts, such as patients with acquired immunodeficiency syndrome (AIDS), seemed reasonable, because HIV-infected individuals have decreased ability to produce IFN-γ, and the deficiency allows the development of opportunistic infections,¹² such as toxoplasmic retinochoroiditis. Our precise measurements, in which we used quantitative competitive polymerase chain reaction (QC-PCR) assays and reverse transcription (RT)-PCR for stage conversion markers, allowed us to determine the location, number and interconversion of *T. gondii* in the retina.¹³ In addition, conventional fluorescein angiography and histopathology were used to assess alterations of the retina after infection by *T. gondii*.¹⁴,¹⁵ However, evaluation of the functional retinal status in murine models of toxoplasmosis has yet to be established.

Electroretinography (ERG) is one of the most reliable methods for evaluating retinal function objectively in humans.¹⁴ Recently, ERGs were used to assess panretinal functions in experimental eye diseases in rabbits,¹⁵ rats,¹⁶,¹⁷ and even mice,¹⁸–²⁰ and several articles have reported ERG changes in gene-altered mice.²¹–²⁵

The purpose of this study was to determine whether ERG can be used to assess the functional status of the retina in our recently established mouse model of toxoplasmic retinochoroiditis in GKO mice.¹⁵

**MATERIALS AND METHODS
Toxoplasma gondii**

Cysts of the avirulent Fukaya strain of *T. gondii* were obtained from B10.A(4R) mouse brain 8 weeks after peroral infection with five cysts.⁸

**Experimental Animals**

Eight- to 10-week-old inbred wild-type (WT) C57BL/6 mice were purchased from SLC Co. (Hamamatsu, Japan). The creation of mice lacking the *IFN-G* gene has been described in detail elsewhere,²⁴ as have been the methods used for genotyping. Age- and sex-matched GKO mice, which were backcrossed to WT C57BL/6 mice for more than eight generations, were used in the experiments. The mice were kept in animal quarters with ambient temperature of 25°C to 30°C and a 12-hour light–dark cycle. All mice had normal findings in physical and ophthalmic examinations and had no detectable serum antibodies to *T. gondii* before the infection.

**Induction of Toxoplasmosis in Mice**

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mice were infected perorally with five cysts of the Fukaya strain of *T. gondii* administered with a syringe fitted with a 19-gauge round-ended needle on day 0. All the GKO mice died 11 or 12 days after infection, whereas
all the WT mice survived for at least 30 days. This difference in susceptibility between GKO and WT mice was statistically significant (\( P < 0.01 \)).

**Electroretinography**

Retinal function was assessed by analyzing dark-adapted ERGs recorded before and at 4 weeks after infection in WT mice, and at 0, 6, and 11 days after infection in GKO mice. The mice were dark adapted for 12 hours before the recordings, and all procedures were performed under dim red light. The treatments and procedures were conducted with animals anesthetized by intramuscular injection of ketamine (11 mg/kg), xylazine (14 mg/kg), and urethane (500 mg/kg). Pupils were dilated with a single drop containing 0.5% tropicamide and 0.5% phenylephrine hydrochloride.

For the ERG recordings, a cotton-wick electrode was placed on the cornea and was referred to an electrode placed subcutaneously on the nasal bone. The animal was grounded by another electrode placed subcutaneously in the neck region. Stimulus light was obtained from a 100-W quartz halogen light bulb. The light was collected and focused onto a 3-mm-diameter fiber-optic bundle, and the tip of the other end of the bundle was placed 5 mm in front of the cornea. Illuminance of unattenuated stimulus on the cornea surface was 140,000 lux, and neutral density filters (NDFs) were used to reduce the stimulus intensity. Stimulus duration of 5 ms was controlled by electromagnetic shutter. The responses were amplified with a preamplifier (VC-11; Nihon Kohden, Tokyo, Japan), and single-flash ERGs were recorded (PowerLab/200; ADI, Castle Hill, Australia).

The a-wave amplitude was measured from the baseline to the trough of the a-wave, and b-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave. Each experimental group consisted of three to four mice.

**Pathologic Examinations**

The eyes freshly collected for pathologic examinations were fixed in phosphate-buffered formaldehyde (15%), dehydrated, and embedded in paraffin. Sections were stained with hematoxylin and eosin.

**Statistical Analyses**

Two-tailed Student’s \( t \)-tests were used for the a- and b-wave amplitudes recorded at saturation points before and after infection. \( P < 0.05 \) was considered significant.

**RESULTS**

**Electroretinograms**

ERGs elicited by different stimulus intensities were recorded before and after the infection. Representative ERGs by \(-2.5\) log stimulus from a WT mouse before (Fig. 1A) and 4 weeks after (Fig. 1B) demonstrated that there was no significant change in ERGs, even 4 weeks after the \( T. gondii \) infection. The intensity response curves for the mean amplitudes of the a- and b-waves of WT mice elicited by increasing stimulus intensities are shown in Figures 1C (a-wave) and 1D (b-wave). The amplitudes of the a- and b-waves increased with increasing stimulus intensities until they became saturated at approximately 0.5 and 1.0 log units below maximum intensity. The differences in the amplitudes of the a- and b-waves before and after infection were not significant.

Representative ERGs elicited with \(-2.5\) log stimulus from GKO mice are shown in Figures 2A, 2B, and 2C. Although the amplitudes of a- and b-waves on day 6 after infection (Fig. 2B) were not different from those before infection (Fig. 2A), they were smaller on day 11 after infection (Fig. 2C). The intensity–response curves for the mean a- and b-waves in GKO mice before infection were similar to those in WT mice. The mean a- (Fig. 2D) and b- (Fig. 2E) wave amplitudes increased until becoming saturated at approximately 0.5 and 1.0 log units below maximum intensity, as in the WT mice. Although the a- and b-wave amplitudes on day 6 after infection were smaller than those before infection, the differences were not significant. The a- and b-wave amplitudes on day 11 after infection, however, were significantly smaller than those before infection (\( P < 0.05 \)).
Although the mean a- and b-wave amplitudes in WT mice were larger than in GKO mice before infection, the differences were not significant.

**Histopathology**

In WT mice, there were no obvious histopathological changes at 12 days after infection. However, at 4 weeks after infection, they showed mild inflammation in the eyes, such as focal retinitis accompanying inflammatory cell and pigmented cell infiltrations and disorganization of the retinal architecture (Fig. 3A), focal vasculitis of the inner retinal vessels associated with mononuclear cell infiltrate (Fig. 3B), and vitreitis. Cysts were present in the inner nuclear layer, with little disturbance of the surrounding retinal architecture (Fig. 3C).

GKO mice showed mild eye inflammation 12 days after infection, including sludging or congestion of blood in the retina and choroid (Fig. 3D), as we have already reported, and vasculitis (Fig. 3E) in the inner retinal vessels. Cysts accompanying retinal hemorrhage were detected in the inner nuclear layer of GKO mice (Fig. 3F) at 12 days after infection. They were smaller than those in the retina of WT mice (Fig. 3C) 4 weeks after infection.

**DISCUSSION**

We previously analyzed the distribution of *T. gondii* in the retina, which was cut along a line halfway between the optic nerve head and ora serrata, and they were used in QC-PCR as central and peripheral retinas, respectively, of WT and GKO mice. Our published results showed that the number of *T. gondii* in the retina was much larger in GKO mice, even 12 days after infection, than in WT mice 4 weeks after infection in both the central and peripheral areas. In addition, the number of protozoans in the central retina exceeded that in the peripheral retina (*P* < 0.05) in WT mice, but not in GKO mice.

Our present results showed that retinal function, as assessed by ERGs, remained normal for up to 4 weeks in WT mice with murine toxoplasmic retinochoroiditis. However, in GKO mice, both the a- and b-wave amplitudes on day 11 after infection were significantly smaller than those before infection.

**FIGURE 2.** Representative ERGs of GKO mice elicited with the −2.5 log stimulus before infection (A) and at days 6 (B) and 11 (C) after infection. The mean a- (D) and b- (E) wave amplitudes versus log stimulus intensity of GKO mice before and at 6 and 11 days after infection. Solid, dotted, and dashed curves: before infection and 6 and 11 days after infection, respectively.

**FIGURE 3.** Photomicrographs of retinas in WT (A–C) and GKO (D–F) mice 4 weeks and 12 days after infection, respectively. An area of retinitis (♀) with the accumulation of inflammatory cells, proliferation of pigmented cells, and disorganization of the retinal architecture (A), retinal vasculitis (arrowhead) of the inner retinal vessels (B) accompanying vitreitis, and a cyst (arrow) in the inner nuclear layer with little disturbance of the surrounding retinal architecture (C), in WT mice 4 weeks after infection. Sludging or congestion of blood in the retinal and choroidal vessels (♂♂) and mild inflammation (D), retinal vasculitis (arrowhead) of the inner retinal vessels (E), and a smaller cyst (arrow) accompanied by retinal hemorrhaging in the inner retinal layer in GKO mice (F) 12 days after infection, just before death. Original magnification: (A, B, D, E) ×400; (C, F) ×1000.
These findings indicate that *T. gondii* affected both the photoreceptor cells and cells in the inner retina of GKO mice, whereas the degree of inflammation in WT mice was mild and probably focal, although that in GKO mice was greater and more widespread. Thus, our present ERG data confirmed our previous findings using QC-PCR.

Histopathologic findings revealed mild retinitis and vasculitis in WT mice 4 weeks after infection, and sludging or congestion of blood in the inner retinal and choroidal vessels only in GKO, not in WT mice. In our previous article, 1,3 fluorescein angiography clearly demonstrated retinal vasculitis in GKO mice. As the b-wave is the ERG-component most susceptible to ischemia,25 its suppression has been taken as an electrophysiological indicator of reduced retinal blood flow in humans26 and experimental animals.27 Although the a-wave is usually less affected by changes in blood flow, suppression of this component is seen when complete retinal ischemia is induced.28 Our results suggest that the alterations of ERG in GKO mice 11 days after infection were probably due to the direct cytopathic effect of the parasite, together with these vascular and/or hemodynamic changes, not only in the retina but also in the choroid.

Based on the close agreement among the distribution and number of *T. gondii*, ERG results, and morphologic changes, our findings demonstrate that the ERG method, a noninvasive technique, is a valid, reliable, and simple way to assess the disease status in our mouse model of ocular toxoplasmosis without having to kill the animal.

There are reports on the relationship between uveitis and ERGs. Riemslag et al.1 reported that ERGs can be of use to identify the location of the scar in patients with toxoplasmic retinochoroiditis who have dense vitreous clouding. Horio et al.2 examined patients with endophthalmitis after intraocular lens implantation and examined whether single-flash ERGs can predict the virulence of the causative organism and the visual outcome of the endophthalmitis. They reported that the combined findings of a b- to a-wave ratio <1.0 and early onset of endophthalmitis (within 1 week) may indicate high virulence of causative organisms and poor prognosis for endophthalmitis after intraocular lens implantation. In rabbits, the b-wave amplitudes of the scotopic ERGs have been reported to decrease within 2 or 3 days after the induction of bacterial endophthalmitis.50,51 These investigators also concluded that changes in ERGs were dependent on the virulence of the causative organisms. Mizota et al.20 reported that in BALB/c mice, the depression of the b-wave began on days 3 to 4 after inoculation of murine cytomegalovirus, and that in B- and T-cell–deficient severe combined immune-deficient mice, the ERGs were extinguished. Hamasaki et al.17 followed and correlated the physiologic and morphologic changes occurring in experimental autoimmune uveitis induced by peptide G of the S-antigen. They concluded that light and electron microscopy of the retina shows better correlation of the pathologic changes with b-wave depression than with the interval after inoculation. All these observations indicate that there is a close relationship between the degree of inflammation and the depression of ERGs.

It is well known that ocular toxoplasmosis in immunocompromised hosts differs distinctly, both clinically and histopathologically from that in immunocompetent patients.32 The features differentiating these two groups are that there are multiple active lesions, massive necrosis in all areas of the retina, a greater number of organisms in the retinal lesions, and a larger size of retinochoroiditis in immunocompromised patients.1,53-55 The present ERG data are consistent with these features. Therefore, ophthalmologists should be very suspicious of the possibility of toxoplasmic retinochoroiditis in immunocompromised patients who report visual disturbance and treat the patients as early as possible to prevent diffuse retinal damage.

In summary, ERG clearly showed deterioration of visual function in GKO but not in WT mice after *T. gondii* infection. ERG is a sensitive and reliable method for observing disease activity in mice severely affected with experimental toxoplasmic retinochoroiditis.

**Acknowledgments**

The authors thank Emiko Adachi-Usami (Department of Ophthalmology, Sannoh Medical Center, Chiba, Japan) for useful suggestions regarding the experiments, Duco I. Hamasaki (Bascom Palmer Eye Institute, Miami, FL) for useful comments during preparation of the manuscript, and Akihiko Uemura (Department of Ophthalmology, Graduate School of Medicine, Chiba University, Chiba, Japan) for help with the ERG recording.

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