Retinal Dysfunction in Basigin Deficiency

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PURPOSE. To examine the retina of basigin (Bsg) knockout mice by electrophysiological and histologic methods and thereby to determine the possible function of Bsg in phototransduction and retinal development.

METHODS. Scotopic and photopic electroretinograms (ERGs) were recorded from 11 wild-type, 12 heterozygous, and 8 homozygous Bsg gene knockout mice of different ages. The retinas were also examined by histologic and immunolabeling methods.

RESULTS. Bsg knockout mice of 5 to 41 weeks of age showed a decrease in the amplitude of all components of both the photopic and scotopic ERGs. In contrast, the fundus and the fluorescein fundus angiography and morphology of the retina at the light microscopic level appeared to be normal until 8 weeks of age in Bsg knockout mice. Thereafter, the length of outer segment and outer nuclear layers decreased with increasing age. Immunohistochemical analysis localized Bsg protein in a variety of cells in the retina, especially in the pigment epithelium, the upper outer plexiform layer and the inner segments of photoreceptor cells.

CONCLUSIONS. The results demonstrated that both rod and cone function were severely affected from an early age by the targeted disruption of the Bsg gene. In spite of abnormal ERGs, the photoreceptor cells maintained normal morphology up to 8 weeks. Thereafter, the photoreceptor cells degenerated gradually and were almost ablated by 41 weeks. (Invest Ophthalmol Vis Sci. 2000;41:3128–3135)

Basigin (Bsg) is a transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily and has two Ig domains. Its protein portion is 27 kDa, and its glycosylated form is 45 to 66 kDa.1,2 Homologous Bsgs have been found in different species of animals. Chicken Bsg (HT7/neurothelin/SA11) has been implicated in the construction of the blood–brain barrier (BBB) because of its specific localization in capillary endothelial cells in the brain,3,4 and in neuronal–glial interactions in the retina during development.5 Human Bsg (M6/EMMPRIN) is induced during leukocyte activation6 and can induce matrix metalloproteases in fibroblasts.7 Strong expression of mouse Bsg (gp42) and rat Bsg (OX-47/C99) has been detected in the brain as well as other organs, including testis, kidney, heart, liver, and small intestine.8,9,10,11,12 suggesting that Bsg has diverse biological functions in mammals.

To determine the function of Bsg in the retina, we generated Bsg knockout (Bsg−/−) mice.11,12 Many Bsg−/− mice were lost because of impaired implantation. Half of the surviving Bsg−/− mice had interstitial pneumonia and died within 4 weeks after birth. Both male and female Bsg−/− mice are infertile, and new progenies were obtained by crossing heterozygous pairs.12

Bsg−/− mice also showed poor performance in learning and memory.13 In addition, they showed decreased sensitivity to irritating odors11 and increased sensitivity to electric foot-shock.13 Unexpectedly, the BBB was not affected in the Bsg−/− mice.13 In addition to these neurologic phenotypes, we have noticed a characteristic visual behavior: Bsg−/− mice take longer to enter a dark compartment. This suggested that Bsg−/− mice may be less sensitive to light than wild-type (Bsg+/+) and heterozygous (Bsg+/−) mice.13 This desensitization to light could be caused by the absence of Bsg in the retina, where Bsg mRNA is strongly expressed.14

To determine the function of Bsg in the retina, we examined the retina of Bsg−/− mice using electroretinography (ERG) and morphologic and immunohistochemical methods.

MATERIALS AND METHODS

Animals

The Bsg−/− mice used in this study were from the same line as those used in a previous study.12 They have a hybrid background of C57BL/6J × 129/SvJ, and mice at the F5–F7 generation were used. Each Bsg−/− mouse was a littermate of a Bsg+/+, a Bsg+/−, or both. The number and ages of the mice at the time of the ERG recordings are shown in Table 1. Histologic analysis was performed on all mice within 14 days of the ERG recordings.

ERG Examination

Scotopic and photopic ERGs were recorded from the three types of mice at different ages. For scotopic ERG recordings,
the animals were dark-adapted overnight and anesthetized with an intraperitoneal injection of a saline solution (15 μl/g) containing ketamine (1 mg/ml), xylazine (0.4 mg/ml), and urethane (40 mg/ml). The animals were prepared for the ERG recordings under deep red-light illumination. The pupil was dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride. The mice were held loosely in a mouse-restraining pocket and placed on a heating pad.

ERGs were recorded from the corneal surface with a coiled platinum wire that made contact through a thin layer of 1% methylcellulose. A similar wire placed in the conjunctival sac, and a needle electrode was inserted in the tail served as the reference and ground electrodes, respectively. ERGs were amplified by 1000× with a bandpass of 1 to 300 Hz. The ERGs were stored in a computer (V 3.5; Mac Laboratory, Sladstone, Australia) for later analysis.

Strobe flash stimuli were presented in a Ganzfeld bowl (Full Field Ganzfeld Stimulator Model GS 2000; LACE Elettronica sel via Marmicciolo, Pisa, Italy), and the radius of the bowl was 20 cm. The mouse was placed in the bowl to receive uniform illumination on the cornea. This position was approximately 15 cm from the closest surface. The luminance of the stimulus was measured by placing the detector of a light meter (DR-2550; Gammma Scientific Co., San Diego, CA) at the position of the mouse’s cornea. The maximum luminance was 1.0 log cd-sec/m² (photopic unit), and neutral density filters were used to reduce the full-intensity stimulus. Nine steps of stimulus intensities ranging from −6.2 to 1.0 log cd-sec/m² were used for the scotopic ERG recordings, with an interstimulus interval of 1 minute. The photopic ERGs were recorded under a steady, white background illumination of 40 cd/m², and the stimulus intensity was attenuated with the neutral density filters.

**Immunohistochemical Analysis**

The retinas were fixed with 4% paraformaldehyde in phosphate buffer adjusted to pH 7.4 and equilibrated with 30% sucrose. According to the manufacturer’s protocol, frozen sections of 15 μm thickness were cut with a cryostat (Leica Co., Ltd., Wetzlar, Germany) and mounted on silanized glass slides. The sections were incubated first with anti-Bsg antibody diluted 1:100 with phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 0.1% Triton X-100. After washing in PBS, the sections were further incubated with FITC-labeled goat anti-rabbit IgG diluted 1:50 with PBS at room temperature for 30 minutes. The sections were examined under a fluorescence microscope after several washes with PBS. For control, sections were incubated with nonimmune rabbit serum instead of anti-Bsg antibody.

These mice were produced and maintained in the Institute for Laboratory Animal Research at Nagoya University School of Medicine and were handled in accordance with the guidelines established by the institute. These experiments adhere to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**RESULTS**

**ERG Findings**

Examples of the scotopic ERGs recorded from the three types of mice at the age of 33 weeks are shown in Figure 1. The ERGs of the Bsg<sup>+/−</sup> mouse were similar to those of Bsg<sup>+/+</sup> and were

<table>
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<th>Age (Weeks)</th>
<th>Bsg&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>Bsg&lt;sup&gt;+/−&lt;/sup&gt;</th>
<th>Bsg&lt;sup&gt;−/−&lt;/sup&gt;</th>
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<tr>
<td>5–8</td>
<td>1</td>
<td>4</td>
<td>3</td>
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<tr>
<td>11–13</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>33–41</td>
<td>5</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Total</td>
<td>11</td>
<td>12</td>
<td>8</td>
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Bsg, Basigin knock-out mouse; Bsg<sup>+/+</sup>, wild-type; Bsg<sup>+/−</sup>, heterozygous; Bsg<sup>−/−</sup>, homozygous.

![Figure 1. Scotopic ERG responses of Bsg<sup>+/+</sup>, Bsg<sup>+/−</sup>, and Bsg<sup>−/−</sup> Basigin mice at 33 weeks of age. Maximum stimulus intensity is 1.0 log cd-sec/m² and the stimulus intensity was attenuated with the neutral density filters.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932909/)

**Fundus Examination**

Fundus photographs and fluorescein fundus angiograms were taken after the ERG recordings. Sodium fluorescein of 0.05 ml was injected into the tail vein, and the fundus was photographed with a fundus camera (Kowa, Nagoya, Japan).

**Histologic Analysis**

The retinas were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at 4°C for 1.5 hour and postfixed in 1% osmium oxide in the same buffer at 4°C for 1 hour. The specimens were dehydrated with ascending series of ethanol and embedded in Polybed 812 epoxy resin (Polyscience, Warminster, PA). Semi-thin sections stained with toluidine blue were used for light microscopic observation.

**Fundus Photographs and Fluorescein Fundus Angiograms**

Fundus photographs and fluorescein fundus angiograms were taken after the ERG recordings. Sodium fluorescein of 0.05 ml was injected into the tail vein, and the fundus was photographed with a fundus camera (Kowa, Nagoya, Japan).
comparable to the ERGs recorded from other types of normal mice. The ERGs of the Bsg^−/− mouse were very different; the stimulus intensity threshold was higher, and the amplitudes were lower. In addition, the peak latency of the b-wave at all recordable intensities was slightly longer in the Bsg^−/− mice.

From ERGs such as these, the amplitude and peak latency of the b-waves were measured for the ERGs evoked by the different stimulus intensities. In addition, the stimulus intensity threshold was determined. The intensity-response function of the scotopic b-wave amplitudes (Figs. 2A, 2B) and peak latencies (Figs. 2C, 2D) were plotted. The means ± SDs are shown. The results from the relatively young group (5–13 weeks; Figs. 2A, 2C) and relatively old group (33–43 weeks; Figs. 2B, 2D) are shown separately. At both ages, only the Bsg^−/− mice showed a significant reduction in the amplitude and an elevated stimulus threshold (P < 0.001; two-way ANOVA) (Figs. 2A, 2B).

The peak latencies of the Bsg^−/− mice were slightly longer than those of the Bsg^+/− and Bsg^+/+ mice for both age groups, but these changes were not statistically significant. The photopic ERGs obtained from the three types of mice at the age of 33 weeks are shown in Figure 3, and the intensity-response functions are shown in Figure 4. Similar to the scotopic ERG, only the Bsg^−/− mice showed significantly reduced b-wave amplitudes and elevated stimulus thresholds (P < 0.001; two-way ANOVA). In addition, the b-wave peak latency did not show any significant difference among these three types of mice. The amplitudes of b-wave in the younger group did not show significant difference from those of the older group in both scotopic and photopic ERG.

**Fundus**

Fundus photographs (top) and fluorescein fundus angiography (bottom) of the three types of mice at 40 weeks of age are shown in Figure 5. The appearance of the fundus of the Bsg^−/− mice did not differ significantly from that of the Bsg^+/+ or the Bsg^+/− mice. Any kinds of significant anomaly were not observed in the appearance of the fundus of the Bsg^−/− mice. However, some dark radial lines were observed around the fundus. Vessels in fundus of Bsg^−/− mice also seemed to be slightly narrower than those in Bsg^+/+ or Bsg^+/− mice. In fluorescein fundus angiography, there was no abnormal leakage of fluorescence dye from the retinal blood vessels.
capillaries and no hyperfluorescence of the retinal pigment epithelium.

**Histology**

The retinas of Bsg1/1 mice at 8 weeks of age appeared normal anatomically as a whole (Fig. 6A). However, arrangement of outer segments was disordered partially from specimen to specimen at this age. Thereafter, the neural retina degenerated progressively with age with the characteristic changes observed in photoreceptor cell layer. At 35 weeks of age, the length of the outer segment was reduced to less than half of the normal length, and the thickness of the outer nuclear layer was reduced to four layers. These observations clearly indicate a degeneration of the photoreceptor cells. At 43 weeks of age,
most photoreceptor cells were absent from the retina (Figs. 6B, 6C), but a few photoreceptor cells still remained.

Immunohistochemistry

The anti-Bsg antibody binding sites were found predominantly in pigment epithelial cells and upper outer plexiform layer and inner segments of photoreceptor cells in Bsg+/+ and Bsg−/− mice (Fig. 7). Immunoreactivity against anti-Bsg antibody was not detected in other parts of the retina at the light microscope level. In Bsg−/− mice and in wild-type mice (Bsg+/+) incubated with nonimmune rabbit serum, labeling with anti-Bsg antibody was not observed (for experimental control).

DISCUSSION

Our observations clearly demonstrated that the Bsg gene, a member of the immunoglobulin superfamily, plays a significant role in maintaining the photoexcitation process. The absence of the Bsg gene resulted in higher stimulus intensity thresholds and depressed b-waves beginning at an early age. There were, however, some unexpected findings in the Bsg knockout mice. The fundus and fluorescein angiography showed minimum changes, and the histologic abnormalities did not appear until 8 weeks of age. As described, the amplitudes of both the photopic and scotopic components of the ERGs were reduced at an early age, when the retinas appeared morphologically normal at the light microscopic level. This suggests that the ERG abnormalities were independent of the structural degeneration of photoreceptor cells and may involve an alteration of a biochemical process.

Fluorescein fundus angiography (Fig. 5) demonstrated the absence of leakage in the Bsg−/− mice, suggesting that the blood–retinal barrier (BRB) was not severely damaged. This observation is in accord with the finding that the BBB was also not altered in Bsg−/− mice because they are both characterized by complexly arranged tight junctions between the barrier-forming cells and a paucity of endocytic vesicles within these cells. Involvement of Bsg in construction of the BBB was originally suggested because of the specific localization of chicken Bsg in the capillary endothelial cells in the brain. Our observation suggest that this does not appear to be the case in the mouse retina.

In immunohistochemical studies, Linser and Perkins and Fadool et al. reported that the anti-Bsg monoclonal antibody (5A11/H7 in the chicken) recognized a 45.5- and a 69-kDa membrane protein present on mature retinal cells, especially Müller and photoreceptor cells. Tests on the maturation of retinal Müller cells in vitro implicated this antigen in heterotypic cell–cell interactions in the developing retina. In our data (Fig. 7), however, anti-Bsg antibody binding sites were found predominantly on the retinal pigment epithelium cells and synaptic terminal of photoreceptor cells in both Bsg+/+ and Bsg−/− mice. Such differences in labeling pattern between our data and others might be due, in part, to species differences. In addition, the antibody used in this study is a rabbit polyclonal antibody against the ectodomain of mouse Bsg, although others used two monoclonal IgG antibodies (5A11 mab and 3B7). Different antibodies could recognize different epitopes. This might be another reason for the differences. Our results are in agreement with the report by Neil and Barnstable that rat Bsg (RET-PE2) is predominantly expressed on the pigment epithelium. Thus, it is difficult to estimate the role of Bsg in the photoexcitation process from the present immunohistochemical data.

Histologic study (Fig. 6) showed that the 8-week-old Bsg−/− mouse retina maintained relatively normal morphology at the light microscopic level in spite of the severely reduced ERG. As described in results, however, the arrangement of outer segments appeared variably disordered among the knockout mice of a given stage of maturation. In the 35-week-old Bsg−/− mouse retina, four layers of photoreceptor cells were observed, and the 43-week-old Bsg−/− mouse contained only one or two layers of photoreceptor cells, indicating progressive degeneration of photoreceptor cells. Such a discrepancy between morphologic and functional abnormality is striking and interesting. It is obvious that Bsg is involved in some steps of photoexcitation process but not in morphogenesis. The late morphologic degeneration of the photoreceptor might be triggered by some stress induced from the long-term malfunction of the photoreceptors. If the mechanism for the photoreceptor dysfunction can be corrected before the morphologic degeneration takes place, normal retinal function activity may be rescued. Bsg knockout mice are expected to be valuable as a model animal to treat retinal degeneration by various methods including gene transfer.

Acknowledgment

The authors thank Satoshi Yamamoto for taking care of the mice.

References