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Myeloid Bodies in the Mammalian Retinal Pigment Epithelium

Gareth A. Tabor and Steven K. Fisher

In the retinal pigment epithelium (RPE) of a mammal, the Eastern gray squirrel, a type of cytoplasmic organelle, the so-called "myeloid body" that was previously thought to be restricted to the RPE of lower vertebrates was observed. In the squirrel, these organelles are continuous with the smooth endoplasmic reticulum (SER), lack an enclosing membrane, and in general exhibit all the morphologic criteria used to identify myeloid bodies. The presumptive myeloid bodies in the squirrel RPE are most prevalent in animals killed during the early hours of the dark period of a 12L:12D lighting cycle. They are rarely observed in animals killed just prior to or during the light period. Thus, these findings document for the first time the occurrence of myeloid bodies in the mammalian RPE, and indicate that their presence is influenced by a diurnal lighting cycle. Invest Ophthalmol Vis Sci 24:388–391, 1983

During a study monitoring the pattern of photoreceptor disc shedding and phagocytosis in light-entrained gray squirrels,[1] we observed structures in the retinal pigment epithelium (RPE) that closely resemble the myeloid bodies found in the RPE cells of a wide range of nonmammalian species. Myeloid bodies are actually specialized regions of the smooth endoplasmic reticulum (SER), comprising a stack of flattened cisternae.[2] They are generally believed to occur in the RPE of amphibians, reptiles, and birds, but neither in the RPE of fish nor mammals.[2,3] Myeloid bodies in the amphibian RPE have been shown to undergo striking alterations in structure in response to a cyclic lighting regime.[4]

In this report, we describe our observations on a type of cytoplasmic organelle present in the RPE of Eastern gray squirrels that resembles the myeloid bodies described in other species. In the squirrel, the presumptive myeloid bodies are prominent structures of the RPE cells of animals killed during the first 1–3 hrs of the dark period, but are rarely observed in light-killed animals. The latter finding may provide an explanation for the common belief that myeloid bodies do not exist in the mammalian RPE.

Materials and methods. Adult Eastern gray squirrels (*Sciurus carolinensis*) were entrained for several weeks to a 12L:12D lighting cycle. For this study, observations were obtained from animals killed at 30 mins, 1, 2, 3, 4, 5, 7, 8, and 11 hours after the onset of darkness. Details of the lighting regime and fixation

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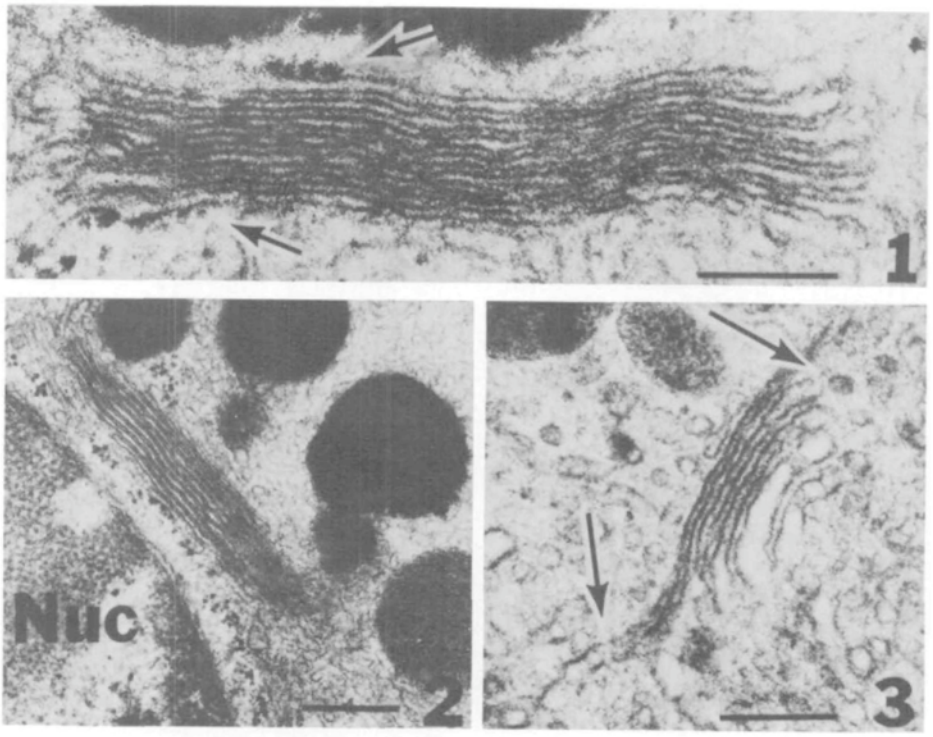

Fig. 1. Electron micrograph of a presumptive myeloid body in the RPE of a gray squirrel killed 1 hr after dark onset. The inclusion comprises seven to eight cisternae of uniform thickness. Ribosomes (arrows) are closely associated with the outermost cisternae. A continuity between the cisternae and the endoplasmic reticulum can be seen in the lower right-hand corner of the micrograph. Bar = 0.2 μM. **Fig. 2.** A myeloid body consisting of 9–12 cisternae is shown in close proximity to the cell nucleus on one side, and to pigment granules on the other. Elements of rough endoplasmic reticulum are sandwiched between the myeloid body and the nuclear envelope. Bar = 0.5 μM. **Fig. 3.** A representative profile of a gray squirrel RPE cell Golgi body. Cis and trans faces can be discerned based on the differing morphology of the respective cisternae. Numerous cytoplasmic vesicles (presumably Golgi-derived) are evident (arrows). Several in the lower left-hand corner of the micrograph appear to be either fusing with, or detaching from the Golgi body. Bar = 0.25 μM.

procedure have been published elsewhere.[1] Briefly, the animals were killed by intracardiac perfusion of phosphate-buffered aldehydes under deep sodium pentobarbital anesthesia. The eyes were removed, the anterior structures dissected away, and the eyecups fixed overnight in fresh fixative at 4 C. The tissue was then washed in buffer, osmicated, dehydrated, and embedded in Araldite 6005. Toluidine blue-stained thick sections were obtained for orientation of the tissue. Silver-gold thin sections were stained with uranium and lead salts and examined by electron microscopy.

Between one and four tissue blocks per animal were sectioned for this study. For each block, at least 12 grids containing 5–8 sections each were examined. In some cases, formvar-coated slot grids were used allowing uninterrupted examination of entire sections, but in others, mesh grids (75 × 300) were used. The tissue sections were approximately 0.75 mm in length; since RPE cells in this species measure approximately 15 μM across their lateral extent,[5] each of the sections contained the profiles of about 50 RPE cells. Thus, at the minimum, 600 RPE cell profiles were examined for each animal assuming that the equivalent of one entire tissue section was observed for each of the mesh grids. The number of cell profiles available for examination would, of course, be higher in the case of the slot grids.

Results. A presumptive myeloid body is shown in profile in Figure 1, taken from an animal killed one hour after the onset of darkness. The inclusion appears as a stack of seven to eight flattened cisternae and is devoid of an enclosing membrane. The peripheral edges of the cisternae are slightly distended, an observation consistent with numerous descriptions of myeloid bodies in other species.[2] In general though, all cisternae are of a uniform intracisternal thickness of 80–100 Å, and are separated by a uniform intercisternal space. By electron microscopy, the myeloid body-like inclusions in squirrel RPE measure approximately 1–2 μm long by 0.2–0.5 μm wide, and contain up to 10–12 cisternae (Figs. 1, 2, and 4b). We have been unable to discern these structures using light microscopy, presumably because their staining characteristics match those of the rest of the smooth endoplasmic reticulum (SER).

Continuities occur between the presumptive myeloid bodies and the SER, and ribosomes are frequently associated with the outermost cisternae (Figs. 1, 2, and 4b). The inclusions exhibit no structural polarization of the lamellar stack such as that seen in the Golgi (compare Figs. 1 and 2 with Fig. 3).

The RPE cells of the animal killed 1 hr into the dark period exhibited numerous presumptive myeloid bodies, located primarily in the perinuclear cytoplasm. Each of the RPE cells in scores of thin sec-

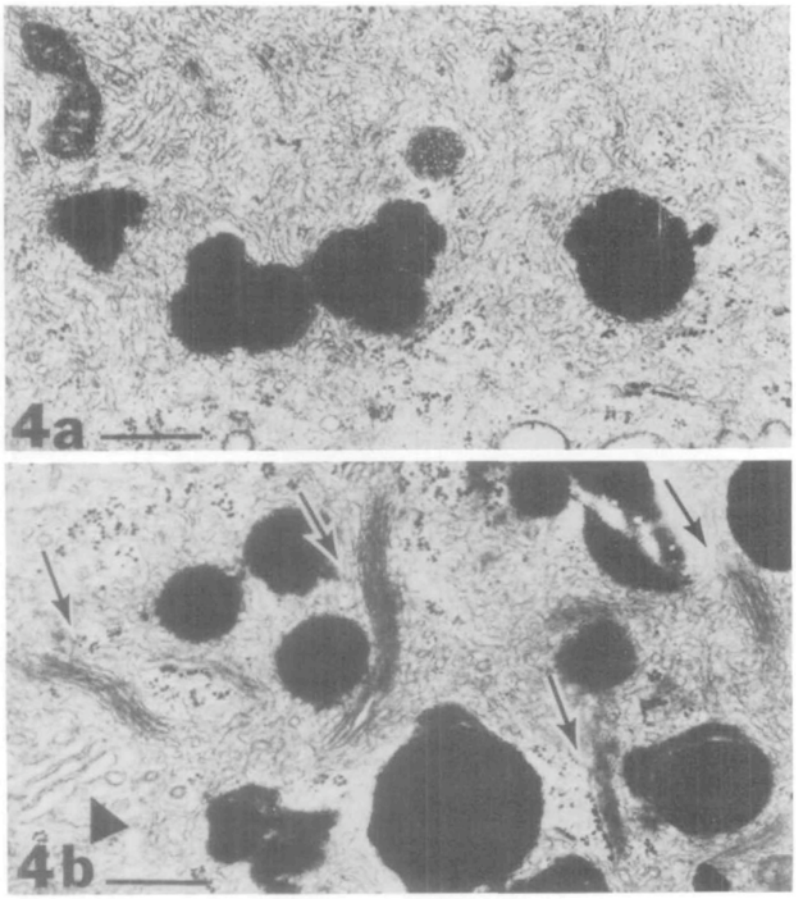

Fig. 4. Apical RPE cytoplasm from squirrels killed in either the light (4a; 4 hrs after light onset) or the dark (4b; 1 hr after dark onset) of a 12L:12D lighting cycle. a. SER is abundant throughout the field shown here, except in the cytoplasm bordering the apical processes originating at the bottom margin of the micrograph. Electron-dense, lipofuscin-containing pigment granules are present, as are scattered elements of rough endoplasmic reticulum. Bar = 0.5 μM. b. In addition to the RPE structures present in light-killed animals, numerous myeloid bodies (arrows) appear in animals killed during the early dark hours. The large arrowhead denotes a Golgi body and its associated cytoplasmic vesicles. Bar = 0.5 μM.

tions contained several of the myeloid body-like profiles. This is in striking contrast to the RPE cells of late-night (approximately 1–2 hrs prior to light onset) or light-killed animals in which the occurrence of these inclusions was exceedingly rare; indeed, the great majority of the RPE cells examined in these animals were devoid of the presumptive myeloid bodies (Fig. 4).

Although a strict quantitative analysis of the occurrence of these inclusions was not performed in this study, their concentration declined apparently in animals killed between 3–7 hrs following dark onset. This was noted by the presence of fewer of these structures in fewer of the RPE cells examined in these animals. When present, the presumptive myeloid bodies were often associated with other RPE organelles, especially the cell nucleus and lipofuscin granules (Figs. 2 and 4b).

Discussion. Rodieck[3] credits Angelucci[6] and Kuhne[7] with the discovery of myeloid bodies based on their descriptions of the amphibian retina. Porter and Yamada[8] studied the frog RPE using electron microscopy and found that myeloid bodies are specializations of the SER. By light microscopy, myeloid bodies appear as small, lens-shaped or circular structures. Ultrastructurally, they appear in cross section as a stack of parallel, flattened cisternae of uniform thickness; adjacent cisternae are separated by a distance similar to the intracisternal space giving these inclusions an overall lamellar appearance. Because of this, myeloid bodies have been mistaken for phagosomes and/or Golgi bodies, resulting in early confusion regarding their presence in several species.[9–13] However, myeloid bodies lack an enclosing membrane and therefore can be distinguished from phagosomes. Unlike Golgi bodies, myeloid bodies exhibit no structural polarization of the lamellar stack such as that embodied by the cis/trans configuration of the Golgi. Ribosomes may be associated with the outermost cisternae of myeloid bodies, and continuities with the SER are often discernible.[14] Furthermore, myeloid bodies are devoid of cytoplasmic vesicles such as those commonly associated with the Golgi cisternae.

We conclude from our observations that the presumptive myeloid bodies present in gray squirrel RPE meet the morphologic criteria used to identify this type of inclusion in a wide variety of other species. The myeloid bodies in squirrel RPE are continuous with the SER, lack an enclosing membrane, and usually have ribosomes on their outermost cisternae. Therefore, our findings indicate that the heretofore

held belief that myeloid bodies are absent from the mammalian RPE is no longer tenable. The presumed absence of these inclusions from the mammalian RPE may be explained on the basis of our finding that in the squirrel RPE, the occurrence of myeloid bodies is largely restricted to the first few hours of darkness. Assuming that most of the previous ultrastructural studies of the mammalian RPE were carried out on animals that had been killed during the normal daytime working hours, the chances of finding one of these inclusions would have been exceedingly remote as shown by our semi-quantitative evidence regarding the relative frequency of these structures in light-vs dark-killed gray squirrels. Perhaps further observations will reveal that myeloid bodies are a common feature of the RPE of other mammalian species.

Our findings indicate that squirrel myeloid bodies are similar to those of the amphibian RPE, both in morphology and in their response to cyclic light, and with regard to their associations with other RPE organelles. However, several important distinctions deserve comment. In *Rana*, myeloid bodies reach a peak in size and number during the last five hours of a 14L:10D lighting cycle. During this time, they appear as large, biconvex lens-shaped structures, and occupy a significant proportion of the total RPE cell volume.[4] In contrast, myeloid bodies in the squirrel RPE are most numerous during the early hours of the dark period. Furthermore, myeloid bodies in the squirrel resemble the "small" myeloid bodies seen in light-killed frogs (see Matthes and Basinger[4]), but they undergo few, if any structural alterations in shape or size during the light cycle. The findings in frog and squirrel may reflect species differences with respect to the influence of cyclic light on the presence of myeloid bodies. On the other hand, our semi-quantitative data precludes making any firm conclusions regarding the exact temporal pattern of the occurrence of myeloid bodies in the RPE of light-entrained gray squirrels.

Myeloid bodies in the frog are frequently observed to be in close association with the nucleus and/or oil droplet of the RPE cell. Direct contacts occur between the myeloid bodies and the nuclear envelope in *Rana*.[4] In the squirrel, myeloid bodies are usually found lying close to the cell nucleus, or to lipofuscin-containing pigment granules. However, we have found no direct contacts between either of these organelles and myeloid bodies in the squirrel. We have frequently observed continuities between the myeloid bodies and elements of the endoplasmic reticulum (ER) that are lying near the nucleus (Fig. 2). Since it is known that the ER may be continuous with the nuclear envelope, the continuity between myeloid bodies and the ER may provide an indirect contact between myeloid bodies and nuclei in the squirrel RPE.

Key words: Myeloid bodies, retinal pigment epithelium, cyclic light, retina

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