Myeloid body associations in the frog pigment epithelium. MICHAEL T. MATTHE5 AND SCOTT F. BASINGER.

Myeloid bodies are found in the retinal pigment epithelium of certain vertebrate species. They are organized structural forms of the smooth endoplasmic reticulum which are usually seen as stacks of flattened, smooth sacculles having a circular or lens-shaped configuration. Our findings in the frog Rana pipiens suggest that changes occur in the structure of the myeloid bodies which are related to the phase of the diurnal lighting cycle. At certain times, the myeloid bodies are found closely associated with other cytoplasmic organelles, notably the nucleus and oil droplet. In addition these associations can be induced by incubation of the isolated eyecup in the presence of guanosine 3',5'-monophosphate.

During a study of rod outer segment shedding and phagocytosis in the frog retina,1 we observed diurnal changes in the structure and location of various cytoplasmic inclusions in the pigment epithelium (PE). Among the most significant were changes in the structure of myeloid bodies and in their association with other cytoplasmic organelles. This report presents our preliminary observations on the association of myeloid bodies with the nuclei and oil droplets of the PE.

Myeloid bodies were first noted by Kühne in 1879 and are found in the retinas of birds, reptiles, and amphibians. They are specific structural forms of the smooth endoplasmic reticulum which lack a limiting membrane and are most commonly observed as stacks of flattened, smooth sacculles, often having a circular or lens-shaped appearance. For a time, these structures were confused with phagosomes and thought to play a role in light reception by the PE, but even though they have been studied in detail by a number of investigators, virtually nothing is known about their function.2 However, during certain times of the diurnal lighting cycle, we have noted their close association with nuclei and oil droplets and have been able to induce some of these associations by in vitro incubation of frog eye cups in the presence of guanosine 3',5'-monophosphate (cGMP).

Methods. Adult Rana pipiens (northern variety) were obtained from West Jersey Biological Supply, Wenonah, N. J. They were maintained at room temperature in large plexiglass chambers with constantly running water and were fed crickets weekly. Automatic timers provided a 14:10 lighting cycle of 645 lux/m² with fluorescent ceiling illumination (Vita-lite; Duro-Test Corp., Grand Prairie, Texas).

Frogs entrained for at least 1 month to the 14:10 lighting cycle were killed at specified times during the diurnal cycle. After frogs were decapitated and pithed, the eyes were fixed in freshly prepared 3% glutaraldehyde–0.087M phosphate buffer, pH 7.2, at room temperature. After 30 min in fixative, the anterior segment and the lens were removed, and the eyecup was fixed for an additional 4 to 8 hr. During the dark phase of the lighting cycle, frogs were killed under dim red light, and the eyes were maintained dark for the first 4 hr of fixation. After fixation, small pieces were cut from the posterior pole on each side of the optic nerve head, postfixed in phosphate-buffered 1% OsO₄ for 1 hr, dehydrated in graded ethanols, and embedded in an Epon-Araldite mixture. After longitudinal orientation, 0.5 to 1.0 μm sections were cut and stained with toluidine blue for observation with the light microscope. Morphological evaluations were made on at least three frogs at each time point. Observations were reported only if we found them in greater than 80% of at least 100 pigment epithelial cells from each of the three frogs examined. After morphological evaluation, appropriate areas were thin-sectioned, stained with uranyl acetate and lead citrate, and photographed with an AEI 801A electron microscope.

For in vitro incubations, light-adapted, isolated eyecups were incubated in room light at 22° in bicarbonate-buffered Ringer's solution containing 10 mM glucose and presaturated with 95% O₂/5% CO₂, according to the procedure of Basinger and Hall.3 During incubation, the flasks were gassed with humidified O₂/CO₂ at 1.2 R³/hr. Where noted, dibutyryl guanosine 3',5'-monophosphate (cGMP) was added to the incubation medium to determine its effect upon the morphology of the PE. After 2 hr of incubation, the eyecups were removed and fixed as above.

Results. Examination of the structure of the frog PE at regular intervals during the 14 light:10 dark diurnal cycle revealed a constantly changing PE morphology. Some of the most dramatic changes were seen in the structure of the myeloid bodies and in the association of the myeloid bodies with the cell nucleus and oil droplet. Examples of the extremes of myeloid body and nucleus association...
are seen in Figs. 1 and 2. Fig. 1 shows a portion of the PE from a frog examined at 6 P.M. which is typical of myeloid body and nucleus morphology during the middle portion (1 P.M. to 8 P.M.) of the light phase of the diurnal cycle. During this time, the nucleus was usually circular in shape, with a large area of heterochromatin located centrally. The myeloid bodies were principally seen as small lens-shaped structures and were only infrequently associated with the cell nucleus (arrow). In general, during the middle of the light phase, myeloid bodies occupied a relatively small portion of the total PE cell cytoplasm.

Fig. 2 shows a pigment epithelial cell from a frog killed at 4 A.M. and demonstrates PE morphology typical of frogs examined during the last 5 hr (3 A.M. to 8 A.M.) of the dark phase of the diurnal cycle. The nucleus at this time was usually irregularly shaped and contained multiple areas of heterochromatin, located both centrally and around the periphery. Numerous myeloid bodies were present during this period and were most commonly seen as large, lens-shaped structures. Many of them were in direct contact with the nucleus, particularly in areas where heterochromatin lay adjacent to the nuclear membrane (arrow). It is now well accepted that the outer membrane of the nuclear envelope is often continuous with the smooth endoplasmic reticulum, so that it was not surprising to see this type of structural association between the nucleus and the myeloid body. During the late dark phase, the myeloid bodies occupied a much larger proportion of the PE cytoplasm. Thus myeloid body associations with the nucleus were more prevalent during the latter portion of the dark phase and occurred almost exclusively on the nucleus adjacent to areas of heterochromatin.

When isolated eyecups from light-adapted frogs were incubated in vitro for 2 hr under room light and then fixed for microscopy, the myeloid bodies in the PE were few in number and were almost always seen as small, lens-shaped structures. The PE morphology was similar to that seen in Fig. 1. Virtually no associations of myeloids with the cell nucleus were found. However, eyecups incu-
Fig. 3. Electron micrograph of a pigment epithelial cell from a light-adapted isolated eye cup incubated in vitro in the presence of 500 μM cGMP for 2 hr under room light. The nucleus contains multiple areas of heterochromatin located throughout the nuclear matrix and scattered around the periphery. A large, elongated, lens-shaped myeloid body partially surrounds the nucleus, making multiple contacts at the sites of peripherally located heterochromatin. (×11,000.)

Fig. 4. Higher magnification of the top portion of Fig. 3. The myeloid body lies in direct contact with the nuclear membrane only at the sites of peripherally located heterochromatin. (×24,000.)

Fig. 5. Electron micrograph of a pigment epithelial cell from a frog killed at 6 P.M. Three small lens-shaped myeloid bodies are in direct apposition (arrows) with the large oil droplet (OD.). (×11,800.)

Fig. 6. Electron micrograph showing the detail of the association between a myeloid body and oil droplet (OD). There are numerous areas of contact between the inner membranes of the myeloid body and the limiting membrane of the oil droplet. (×50,000.)
bated under identical conditions with the addition of cGMP demonstrated a dose-dependent increase in myeloid body-nucleus associations. Fig. 3 shows a portion of a PE cell after 2 hr incubation in the presence of 500 μM cGMP. No inhibitors of cyclic nucleotide phosphodiesterase were present. Under these conditions, the PE nucleus contained numerous areas of heterochromatin, which when located peripherally, were often found associated with myeloid bodies, now seen as larger, elongated lenses similar to those seen in Fig. 2. In some cases, the myeloid bodies were almost entirely wrapped around the PE nucleus. The contacts between myeloid body and nucleus are shown at higher magnification in Fig. 4, where the direct apposition of the myeloid membranes and the nuclear envelope can be seen. As a general rule, although there were peripheral areas of heterochromatin where no myeloid body contact was observed, there were virtually no points of contact between myeloid membranes and nuclear envelope in areas where heterochromatin was absent peripherally. Incubations in the presence of less than 50 μM cGMP had no effect on PE morphology. Incubation in the presence of 100 or 200 μM cGMP showed similar, though not as pronounced effects.

The other common type of association which myeloid bodies make is with the large oil droplet found in the frog PE cell. An example can be seen in Fig. 5, where three lens-shaped myeloid bodies lie adjacent to the oil droplet. One of these is shown in higher magnification in Fig. 6, where it appears that the myeloid membranes were continuous with the limiting membrane of the oil droplet. However, close examination at higher magnification revealed that although the innermost membranes of the myeloid body were in direct contact with the oil droplet, there was no fusion between the myeloid membrane and the limiting membrane of the oil droplet. As in the case of their association with the nucleus, the myeloid body–oil droplet associations were more numerous during the late dark phase. However, the myeloid body–oil droplet associations seemed less influenced by both lighting conditions and incubation in the presence of cGMP.

Discussion. Our work confirms earlier observations on the association of myeloid bodies with nuclei and oil droplets and demonstrates that these interactions are more prevalent during certain phases of the diurnal lighting cycle and are also favored by incubation in the presence of cGMP. Clearly, myeloid bodies are not merely static structural components of the frog PE but, instead, demonstrate cyclic variations in both morphology and association with other PE cell organelles.

Since the myeloid bodies of the PE represent a structurally organized form of the smooth endoplasmic reticulum, they may be involved in some type of cell metabolism which is usually associated with the smooth endoplasmic reticulum, e.g., lipid metabolism. Additionally, specific association with the nuclear envelope at the site of heterochromatin aggregation during certain times of the diurnal cycle might indicate that they are involved in transferring and/or processing RNA. However, at the present time, the functional significance of these structural associations can be only speculative. The retina has been found to be resistant to lipid and vitamin A depletion, and with the constant turnover of rod outer segment membranes, the PE must play a significant role in the conservation of lipid and vitamin A metabolites. Thus the association of myeloid bodies with both the nucleus and oil droplet might suggest that they are involved in lipid and/or vitamin A metabolism.

To date, very little is known about the role of cGMP in cell metabolism. The outer retina is particularly rich in cGMP, and within the photoreceptors, cGMP levels fall rapidly after the onset of light. In frogs maintained under cyclic lighting, nucleus–myeloid body associations occur during the latter portion of the dark phase of the diurnal cycle, a time of high cGMP concentration in the outer retina. In addition, nucleus–myeloid body associations can be induced during in vitro incubations in light by the addition of cGMP. Therefore cGMP may play a role in controlling these association and thus in part regulate PE cell metabolism. Whatever its role, cGMP can exert a pronounced effect upon the morphology of the frog PE.

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REFERENCES


Characterization of retinoblastoma immune complexes. Paul C. Stein, Mary Christensen, and Devron C. Char.

Immune complexes from retinoblastoma sera were characterized with molecular sieves chromatography, affinity chromatography, and polyacrylamide gel electrophoresis (PAGE). Retinoblastoma patients' sera had two well-defined peaks of immune complex activity after molecular sieves chromatography. These protein fractions had a molecular weight of approximately 1.6 x 10^6 and 2.0 x 10^6 daltons. Affinity chromatography with Sepharose 4B-protein A and analytical PAGE demonstrated that IgG was the predominant immunoglobulin in these immune complexes. Immune complexes also had affinity for Sepharose-concanavalin A, indicating the glycoprotein nature of the antigen component.

Elevated levels of immune complexes have been demonstrated in retinoblastoma. In some tumors the levels of immune complexes have correlated with disease status and prognosis, and the complexes consist of tumor-associated antigens. In most human neoplasms, including retinoblastoma, the nature of the antigenic component and the correlation between immune complex level and prognosis are unclear. We have studied retinoblastoma sera with high levels of immune complexes using molecular sieves chromatography, affinity chromatography, polyacrylamide gel electrophoresis (PAGE), and immunoelectrophoresis (IEP) to partially characterize these immune complexes and their antigen and antibody components.

Material and methods. Sera from retinoblastoma and control subjects examined under anesthesia were obtained following informed consent. The sera were aliquoted and stored at -70°C. Immune complex levels were determined before and after chromatography, with the Raji cell radioimmunoassay as previously described. All retinoblastoma sera samples were found to be negative for autoantibodies, carcinoembryonic antigen (CEA), and Australia antigen. Immunoglobulin levels were determined by radial immunodiffusion or rocket IEP.

Purification and characterization procedures were performed on retinoblastoma sera with high levels of immune complexes (>200 μg/ml aggregated human IgG equivalents) and on normal control sera. The procedures included molecular sieves chromatography with Ultragel AcA-22 (LKB Products, Pleasant Hill, Calif.) or Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) and affinity chromatography with Sepharose 4B-concanavalin A (Con A) or Sepharose 4B-Protein A (Pharmacia).

Immune complexes from some sera aliquots were initially precipitated with 5% polyethylene glycol (PEG) prior to Sepharose 4B-protein A gel chromatography in order to concentrate the immune complexes and reduce the concentration of monomeric IgG; other sera samples were applied directly to the chromatography columns. After molecular sieves chromatography, protein peaks with significant immune complex activity (Raji assay) were pooled, concentrated, and then applied to affinity columns. Unbound proteins were washed off with phosphate buffered saline (PBS), pH 7.2. Bound proteins on the Sepharose-Con A columns were eluted with 0.1M and 0.5M alpha-methyl-D-mannoside in 0.01M Tris buffer, pH 7.4, containing 0.14M NaCl or PBS. Bound proteins on the Sepharose-protein A columns were eluted with 0.1M acetic acid and then im-