Induction of DNA Synthesis by Co-Culture of Retinal Glia and Pigment Epithelium

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Retinal glia (RG) and retinal pigment epithelial cells (RPE) have been previously identified in intravitreal cellular membranes of patients with proliferative vitreoretinopathy (PVR). This study was undertaken to determine if the co-presence of both cell types might lead to increased membrane growth due to some heterotypic cell interactions that enhance cell proliferation. Cell proliferation in co-cultures of RPE plus RG, RPE plus dermal fibroblasts (DF), or RG plus DF was evaluated by quantitation of labeled nuclei in radioautographs prepared from monolayer co-cultures exposed to 3H-thymidine. In each co-culture, one cell type was premarked by phagocytosis of latex particles so that the cells could be identified and the labeling rates in each cell type could be assessed. In co-cultures of RPE and RG, both cell types exhibited a higher labeling rate. In RG-DF co-cultures, DF-labeling was increased, but RG-labeling was unaffected or suppressed. Co-culture of RPE and DF did not affect labeling in either cell type. Studies of conditioned media suggested that RG stimulate RPE and DF by means of a product secreted into the culture medium by the RG. Further, cell-cell contact might modulate the growth response at least for RG. The interaction of RPE and RG in co-culture differed from the interaction of either cell type with DF in that the co-culture of RPE and RG resulted in a higher apparent proliferation rate for both cell types. If similar interactions occur in vivo, the presence of both types of retinal cells in intravitreal membranes of PVR might result in greater growth than in lesions derived from a single retinal cell population. Invest Ophthalmol Vis Sci 26:636–642, 1985

Retinal glial cells were previously shown to exhibit a contact-mediated stimulation of proliferation. In that study, increasing numbers of cell–cell associations in subconfluent monolayer culture promoted glial cell DNA synthesis. The study reported here was undertaken to determine if this growth stimulation required glial-specific interactions, or if co-culture with other cell types also affected glial cell growth. We were particularly interested to determine if interactions between retinal pigment epithelial cells and retinal glia affected the growth of either cell type because, not only are these cell types closely related developmentally, but they also have a potential for association in some ocular disorders such as proliferative vitreoretinopathy (PVR).

PVR is a condition in which cells invade the vitreous and proliferate therein to form cellular membranes that can lead to traction detachment of the retina. Examination of human surgical specimens revealed that glial cells and pigment epithelial cells are major cellular constituents of vitreal membranes. In an experimental animal model of PVR it was demonstrated that cells of many types could establish intravitreal membranes and, further, that cell proliferation was significant for achieving the critical mass of cells necessary for the development of traction retinal detachment. The study reported here, therefore, was also undertaken to examine interactions between glial and pigment epithelial cells to determine if cellular association might provide part of the stimulus for proliferation in PVR.

Materials and Methods

Cell Cultures

Retinal glial cell cultures (RG) were derived from pigmented rabbit retinas using methods that were previously described. The cultures consist predominantly of Muller cells. Cultures of retinal pigment epithelial cells (RPE) were prepared from pigmented rabbits or humans by the method of Flood et al.
Dermal fibroblast cultures (DF) were derived from explants of rabbit skin by standard procedures. Cultures of all cell types were maintained in Eagle's Minimum Essential Medium (MEM-Gibco; Madison, WI) supplemented with 10% fetal bovine serum (FBS), antibiotics, and antymycotics. Cultures were passaged by trypsin treatment and used in the first or second passage (for retina-derived cells) or second to fourth passages (for DF).

**Proliferation Assay**

For the basic proliferation assay, cells were plated in Lab-Tek (Naperville, IL) 8-chamber tissue culture chamber slides at a density of $1.5 \times 10^4$ cells/cm$^2$ in MEM containing 10% FBS. After cell adherence (approximately 1 hr), medium was replaced with MEM containing low serum levels (0.5–1% FBS) to slow cell proliferation. After 24 hr, cultures received fresh medium and were then labeled for 24 hr with $^3$H-thymidine (1 μCi/well) to quantitate DNA synthesis as a measure of the induction of cell proliferation. For serum-free tests, cells were incubated in serum-free MEM containing 0.2% bovine serum albumin (BSA) for 24 hr prior to the labeling period. After labeling, the slides were air-dried and processed for radioautography using Kodak (Rochester, NY) NTB2 liquid emulsion and an exposure period of 6 days. Labeling rates were assessed by counting 300–500 nuclei per well.

**Co-Culture Proliferation Experiments**

For co-culture experiments, one cell type was pre-marked by phagocytosis of 1 μm latex particles (Polysciences; Warrington, PA) prior to plating. The presence of latex particles in one type of cell could then be used to distinguish the cell types on the radioautographs. To particle mark cells, visually confluent 75-cm$^2$ flasks were briefly treated with trypsin, then refed with MEM containing 10% FBS and maintained for approximately 1 hr. The enzyme treatment appeared to enhance subsequent particle uptake. Particles were then added to the serum-containing medium using 100 μl of the commercial suspension in 10 ml culture medium with a 1-hr incubation period. The medium was then replaced, and cells were allowed to internalize adherent particles for 1 hr. Using this protocol, examination of 2,000 cells indicated that more than 99% of the cells contained at least five particles.

For use in the proliferation assay, particle-marked cells were detached by trypsinization, pelleted by centrifugation, and the supernate discarded to avoid contamination of the co-cultures with any released particles. To seed the co-cultures, resuspended particle-marked cells were mixed in varying proportions with the other unmarked cell type and plated at a final common subconfluent density of $1.5 \times 10^4$ cells/cm$^2$. The intention here was to vary the cell ratio and, thus, the number of homotypic versus heterotypic cell–cell associations. Although the mixing ratio of the two cell populations was known prior to seeding the co-cultures, there are variations in plating efficiency when using secondary cultures. The actual ratio of cells plated on the culture substrate was, therefore, determined by assessing the percentage of particle-marked cells in the resulting radioautographs.

Co-cultures were incubated with $^3$H-thymidine as described above in the proliferation assay, and labeling rates for each cell type in the co-cultures were evaluated by counting the number of labeled nuclei in both particle-marked and particle-free cell types. In co-culture experiments, 700–1,000 total nuclei were counted per well using three or four replicates for each test group. In reporting some co-culture results, the labeling percentage for one cell type was related to the number of cells of the other cell type (the heterotypic cell) present in the co-culture.

To determine if the presence of the marker particles was responsible for any changes in labeling in co-cultures, an experiment was conducted in which growth was compared between RPE-RG co-cultures using RPE with, and RPE without, latex particle markers. For this experiment, paired flasks of RPE were used in which cells in one flask only phagocytosed the latex particles. Each population of RPE was mixed with RG, using the same mixing ratios, and plated in test wells as described above. Solitary cultures of RG, RPE with particles, and RPE without particles were also prepared.

**Conditioned Media Proliferation Experiments**

To prepare conditioned media, 75-cm$^2$ subconfluent flasks of each cell type were incubated for 24 hr with 10 ml serum-free MEM containing 0.2% BSA. Conditioned media were centrifuged to remove any cell debris and maintained at 4°C prior to use. To evaluate conditioned media effects, test cultures were exposed to the conditioned media during the 24-hr labeling period of the basic proliferation assay.

**Proliferation Experiments in Mixed Cultures Containing both Homotypic and Heterotypic Regions**

In these experiments, cultures were generated that contained a region with only RG, a region with only RPE, and a region with RPE and RG plated together.
Fig. 1. In these radioautographs of co-cultures there is a high density of grains overlying the $^3$H-thymidine-labeled nuclei. A, RG and particle-marked human RPE. Double arrow indicates labeled RG; single arrows indicate labeled RPE. B, RG and particle-marked DF. Arrows indicate labeled DF. C, DF and particle-marked human RPE. Double arrow indicates labeled RPE; single arrows indicate labeled DF. D, Higher magnification view of adjacent labeled DF and human RPE seen in 1C to show the marker particles in the RPE.

All three regions were in the same culture well and exposed to the same culture medium. The aim was to keep medium conditioning equivalent to determine the effects on proliferation of different types of cell-cell associations. To prepare the mixed cultures, RG were first plated in Lab-Tek one-chamber slides that were tilted so that the cell-containing medium covered approximately two thirds of the substrate. After cell

Fig. 2. Percent labeling in medium containing 1% fetal bovine serum of RG and human RPE when mixed in varying ratios and plated in co-culture. Data are mean ± sd of quadruplicate counts. sd: standard deviation.

Fig. 3. Percent labeling in serum-free medium of RG and human RPE when mixed in varying ratios and plated in co-culture. Data are mean ± sd of quadruplicate counts.
adherence (about 30 min), the plated cells were washed with fresh medium, then the culture chamber was tilted in the opposite direction and particle-marked RPE were similarly seeded. After this second cell population was adherent, the cultures were washed again and medium was replaced with MEM containing low serum (0.5% FBS) for 24 hr. Cultures were then fed with fresh low-serum medium and labeled for 24 hr with $^3$H-thymidine as described above. Using this protocol, approximately one third of the mixed culture substrate was occupied by RG alone and about one third by RPE alone. In these regions, cells have only homotypic cell-cell contacts. In the middle one third of the slide, RG and RPE were mixed and, therefore, also had heterotypic associations. All cells, notwithstanding their intercellular contacts, were exposed to common culture medium.

The plating dynamics were such that part of each of the three regions had a cell density equivalent to that in cultures used for other experiments and it was these regions that were used to assess $^3$H-thymidine labeling rates. Solitary cultures of each cell type were also established in quadruplicate test wells as controls.

The provisions of the ARVO Resolution on the Use of Animals in Research were observed in this study.

Results

Using latex particles as a marker, two populations of cells in co-culture could be distinguished and the labeling rates in each separately assessed. Figure 1 shows co-cultures of RPE and RG (1A), RG and DF (1B) and RPE and DF (1C). A higher magnification view of adjacent RPE and DF cells is provided to demonstrate the marker particles (1D). The plating protocol yielded subconfluent cultures with most cells having one to five apparent cell–cell contacts.

Co-cultures of RPE and RG had a higher labeling rate than expected at all cell ratios (Table 1). Seven

Table 1. Total $^3$H-thymidine labeling in co-cultures of RG and RPE*

<table>
<thead>
<tr>
<th>Cell ratio (%)</th>
<th>Expected labeling (%)†</th>
<th>Observed labeling (%)</th>
<th>Percent increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE/RG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32/68</td>
<td>16.1</td>
<td>19.2</td>
<td>19</td>
</tr>
<tr>
<td>51/49</td>
<td>22.4</td>
<td>32.3</td>
<td>44</td>
</tr>
<tr>
<td>58/42</td>
<td>24.8</td>
<td>39.9</td>
<td>61</td>
</tr>
<tr>
<td>70/30</td>
<td>28.8</td>
<td>52.5</td>
<td>82</td>
</tr>
<tr>
<td>77/23</td>
<td>31.1</td>
<td>47.6</td>
<td>53</td>
</tr>
<tr>
<td>90/10</td>
<td>35.5</td>
<td>58.7</td>
<td>65</td>
</tr>
</tbody>
</table>

* Human RPE and rabbit RG were plated in varying ratios and assayed in medium containing 1% fetal bovine serum. Labeling data are means of triplicate counts.

† The expected labeling percentages are computed from the labeling rate of each cell type in solitary culture and its proportion in the co-culture. The labeling rates in solitary culture were 38.8% for RPE and 5.4% for RG.
growth in those co-cultures was also observed (not was due to the presence of the phagocytosed latex than RPE were marked with particles and increased labeling. An expected labeling rate of 8.4% for all co-cultures was used to compute the same (RG, 8.3%; RPE with particles, 8.3%; RPE without particles, 8.7%).

Experiments of this type were completed using different cultures of RPE and RG. Although a variable basal labeling rate was observed in the proliferation assay for both retinal cell populations, in every experiment the mixed RPE-RG co-cultures showed increased labeling over the basal rates of the cells used in that experiment (data not shown). In the experiment shown in Table 1, the greatest observed increase in labeling was in cultures containing 70% RPE/30% RG. The ratio at which maximal labeling occurred in the seven RPE-RG pairings ranged from 27% to 82% RPE.

Increased DNA synthesis in RPE-RG co-cultures was not due to the presence of the phagocytosed latex particles in the RPE. In some experiments, RG rather than RPE were marked with particles and increased growth in those co-cultures was also observed (not shown). In the experiment shown in Table 2, proliferation in RPE-RG co-cultures in which RPE contained latex particles was compared with proliferation in co-cultures in which RPE was not premarked by the particles. The lack of particle markers makes cell identification difficult so the actual cell ratio in the resulting radioautographs could not be determined and only total culture labeling could be assessed. In this experiment, however, the basal labeling rate in solitary cultures of all test cell populations was the same (see legend to Table 2) so increased labeling in the co-cultures could be detected whatever the actual ratio of the cell types. As shown in Table 2, all co-cultures showed elevated labeling and, further, the increase over the basal labeling rate was similar notwithstanding the presence of latex markers in the RPE.

Elevated labeling in RPE-RG co-cultures was due to increased DNA synthesis in both cell populations and was demonstrable in the presence (Fig. 2) and absence (Fig. 3) of serum. Labeling rates in both cell types increased with increasing numbers of the heterotypic cell population, but the relationship between apparent proliferation and cell ratios in co-culture was not simple. In RPE (Figs. 2, 3), and also in RG (in other experiments not shown), labeling percents fell at high proportions of the heterotypic cell type. Similar results were obtained for RPE from rabbits and humans.

Co-culture of RPE and DF promoted proliferation in DF, whereas proliferation in RG was either unaffected (in serum-free medium) or suppressed (in the presence of serum) by the presence of DF (Figs. 4, 5). Co-culture of RPE and DF did not affect the proliferation of either cell type (Fig. 6). RPE-DF co-cultures were similar in the presence and absence of serum; the data shown are for serum-free cultures (Fig. 6).

To determine if any changes in DNA synthesis in co-culture were due to factor(s) secreted into the medium, each cell population in solitary culture was incubated with a variety of conditioned media (Table 3). Both RPE and DF showed relatively higher labeling in medium conditioned by exposure to RG than to conditioned media from other cell types. DF were more sensitive to medium conditioning than RPE, and RG were unaffected by conditioned medium from any cell source.

When co-cultures of RPE and RG were seeded by tilting the chamber as described in Materials and Methods, a region of the mixed culture substrate was occupied by RG alone and a region by RPE alone (homo- and heterotypic regions), and a central region was occupied by both RPE and RG (heterotypic region). The labeling rates for each cell type in the homotypic and

**Table 2. Comparison of total 3H-thymidine labeling in RPE-RG co-cultures using paired cultures of RPE with and without latex particle markers**

<table>
<thead>
<tr>
<th>Cell ratio</th>
<th>Observed labeling (%)</th>
<th>Percent increase†</th>
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<tbody>
<tr>
<td></td>
<td>RG plus RPE with particles</td>
<td>RG plus RPE without particles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increasing RPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.1/68</td>
<td>140.0/67</td>
<td></td>
</tr>
<tr>
<td>11.9/42</td>
<td>118.8/40</td>
<td></td>
</tr>
<tr>
<td>12.1/44</td>
<td>11.7/39</td>
<td></td>
</tr>
<tr>
<td>12.6/50</td>
<td>13.6/62</td>
<td></td>
</tr>
<tr>
<td>9.6/14</td>
<td>10.0/19</td>
<td></td>
</tr>
</tbody>
</table>

* Rabbit RPE and RG were plated in varying ratios and assayed in serum-free medium. Labeling data are means of quadruplicate counts.
† The labeling rate in solitary culture of all cell populations was approximately the same (RG, 8.3%; RPE with particles, 8.3%; RPE without particles, 8.7%). An expected labeling rate of 8.4% for all co-cultures was used to compute the percent increase.

**Table 3. Differential effects of conditioned medium on 3H-thymidine labeling**

<table>
<thead>
<tr>
<th>Target cell</th>
<th>Medium conditioning*</th>
<th>Percent labeling†</th>
</tr>
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<tbody>
<tr>
<td>RPE</td>
<td>None</td>
<td>14.6 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>25.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>RPE</td>
<td>16.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>DF</td>
<td>13.6 ± 3.1</td>
</tr>
<tr>
<td>RG</td>
<td>None</td>
<td>6.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>6.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>RPE</td>
<td>7.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>DF</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>DF</td>
<td>None</td>
<td>18.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>46.4 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>RPE</td>
<td>26.9 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>DF</td>
<td>29.9 ± 3.6</td>
</tr>
</tbody>
</table>

* Serum-free conditioned medium was prepared by incubation for 24 hr with a subconfluent flask of cells as described in Materials and Methods. This medium was then added to the target cells in the proliferation assay and cells were maintained in the conditioned medium during labeling with 3H-thymidine.† Labeling data are mean ± standard deviation of quadruplicate counts.
heterotypic regions, as well as in separate solitary culture, are shown in Table 4. RG had the same labeling rate in solitary culture and in the homotypic region of the mixed culture, where they had contact only with other RG. In the heterotypic region of the mixed culture, where they also had RPE contacts, the RG labeling rate was nearly two-fold higher. In contrast, RPE had a higher labeling rate in mixed culture, notwithstanding the type of cell contacts, than they did in solitary culture. All cells in mixed culture were exposed to the same medium.

**Discussion**

The data reported here indicate that co-culture of retinal glial cells and retinal pigment epithelial cells promoted a reciprocal induction of DNA synthesis. The phenomenon was not species-specific since similar results were obtained with human and rabbit RPE. Further, the in vitro interaction of RG and RPE differed from the interaction of either retinal cell type with dermal fibroblasts. RG stimulated DNA synthesis in DF, but the growth of RG was unaffected or suppressed in RG-DF co-cultures. Co-culture of DF and RPE did not affect the proliferation rate of either cell type.

The basal proliferation rates, both in the presence and absence of serum, varied among different populations of RPE and RG. The variation was partly due to differences in the number of population doublings the cells had undergone prior to assay, and in vitro age is known to affect cell proliferation.9 Further, RG senesce rapidly in culture and soon exhibit a low proliferation rate even in the presence of serum.10 Despite differences in basal growth rates, the stimulation of proliferation in RPE-RG co-cultures was observed both in the presence and absence of serum.

The magnitude of the proliferation stimulation was greater in the presence of serum, but serum was not required for stimulation to occur. This observation suggests that elevated proliferation in co-cultures was not due to an increased sensitivity to serum-derived growth promoters but rather to some interactions among the cells themselves.

The experiments using conditioned media were conducted to determine if part of the stimulation of proliferation observed in some co-cultures was due to factors secreted into the medium. The data suggest that RG release a stimulant(s) for both RPE and DF since RG conditioned medium was more effective in elevating the DNA synthetic rates in both target cells than were conditioned media from other sources. The data from the mixed culture experiments in which RG and RPE were plated so as to yield regions occupied by RPE alone, by RG alone, and by RG and RPE together, supported the observation that RPE are sensitive to growth stimulants in the culture medium. RPE showed equivalent growth in mixed cultures in the region where they were plated alone and in the region where they were in contact with RG, and the growth was elevated over that in solitary RPE cultures containing no products of RG.

Although RG showed an elevated DNA synthetic rate in co-culture with RPE, RPE conditioned medium did not promote RG proliferation. Further, RG proliferation in the mixed culture experiment was the same in the region occupied by RG alone as in RG solitary cultures. RG proliferation was, however, elevated in the mixed culture in the region in which they also had heterotypic contacts with RPE. The available data suggest, therefore, that in RPE-RG co-culture the stimulation in DNA synthesis observed for RPE was predominantly due to humoral factor(s) secreted by RG, whereas the stimulation for RG was mediated by intercellular association between RG and RPE. It remains possible, however, that cell–cell contact between RG and RPE increased the sensitivity of RG to some humoral components. It is also possible that intercellular association among RPE modulated RPE growth. The observation of a decline in RPE proliferation at high proportions of RG in co-culture (Figs. 2, 3) and the observation that maximal cell growth occurred at a variety of cell ratios, might be an indication of complex interactions between cell contact-mediated and humoral signals.

In a previous study we had reported that increasing numbers of homotypic contacts among RG increased the DNA synthetic rate.1 The RPE-RG co-culture data from this study suggest that, not only can RPE substitute for RG to support contact-mediated growth, but contact with RPE (or some combination of homotypic and heterotypic contacts) is a more effec-

### Table 4. Tritiated thymidine labeling in solitary cultures and in mixed cultures containing regions of RG only, of RPE only, and of mixed RPE and RG

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Solitary culture†</th>
<th>Mixed culture homotypic regions‡</th>
<th>Mixed culture heterotypic region§</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG</td>
<td>12.1 ± 2.3</td>
<td>11.3</td>
<td>23.9</td>
</tr>
<tr>
<td>RPE</td>
<td>4.0 ± 0.7</td>
<td>9.3</td>
<td>9.4</td>
</tr>
</tbody>
</table>

* The mixed culture was established by separate seeding of RG and rabbit RPE by tilting the culture substrate as described in Materials and Methods. Labeling was in medium containing 0.5% fetal bovine serum.
† Data are mean ± standard deviation of quadruplicate counts.
‡ Data are counts of 800 nuclei of each cell type.
§ Data are counts of 1600 nuclei where the cell ratio was 29% RPE/71% RG.
tive stimulator of RG. In contrast, contact of RG
with DF in co-culture was less effective in supporting
RG growth and, as the relative number of DF in-
creased, the RG growth rate often declined. The
inhibition of RG growth in co-culture with DF may
be a reflection of the density-dependent suppression
of proliferation known to occur in confluent cultures
of many cell types. RG, therefore, may be sensitive
to both contact-mediated growth promotional and
growth inhibitory signals where the former type of
stimulus might be peculiar to retina-derived cells.

The growth properties of retinal cells are of partic-
ular interest to students of proliferative vitreoretin-
opathy because, in this clinical condition, intravitreal
cell proliferation is a significant component of the
pathology. Examination of human vitreal membranes
has led to the suggestion that mixed cell membranes,
membranes containing cells of more than one type,
are more common in severe PVR. Our observation
of elevated growth in co-cultures of RPE and RG
might provide a partial explanation for the increased
severity of mixed PVR membranes involving these
cell types. If similar phenomena occur within the
vitreous as we have observed in vitro, growth-pro-
moting interactions between RPE and RG might
help to generate the critical mass of cells required to
effect traction retinal detachments.

We conclude that, although RG may be the source
of a growth promoter(s) for other cell types (eg, DF),
RG and RPE interact in co-culture so as to elicit an
increased proliferation in both cell types. This reci-
procral growth promotion may be due to both cell–cell
association and to product(s) released by cells into
the medium. We also suggest that similar cellular
interactions in intravitreal membranes containing RPE
and RG might contribute to cell proliferation in PVR.

Key words: retinal glia, Muller glia, retinal pigment epithe-
lium, proliferative vitreoretinopathy, cell–cell contact

References
1. Burke JM: Cell–cell contact promotes DNA synthesis in retinal
2. Kampik A, Kenyon KR, Michels RG, Green WR, and de la
Cruz ZC: Epiretinal and vitreous membranes: comparative
massive periretinal proliferation: in vitro characteristics of
comparison of different cellular inocula in an experimental
model of massive periretinal proliferation. Am J Ophthalmol
of cellular proliferation in an experimental model of massive
6. Burke JM and Kower HS: Collagen synthesis by rabbit neural
7. Burke JM: Cultured retinal glial cells are insensitive to platelet-
8. Burke JM and Foster SJ: Culture of adult rabbit retinal glial
cells: methods and cellular origin of explant outgrowth. Curr
and ultrastructure of human retinal pigment epithelium in
10. Smith JR and Lincoln II DW: Aging of cells in culture. Int
tenberger B, and Woolsey T: Growth control by cell to cell
12. Kampik A, Green WR, Michels RG, and Nase PK: Ultrastruc-
tural features of progressive idiopathic epiretinal membrane