Activated Retinitis Pigmentosa Peripheral Lymphocytes Adhere to and Alter Cultured Human Retinal Pigment Epithelial Cells

Lowell L. Williams,* Ho Min Lew,* Barry T. Shannon,†† Carl T. Singley,‡ Robert B. Chambers,* and Frederick H. Davidorf*

Interleukin-2 receptor (IL-2R) is an activation molecule that, when expressed on peripheral blood lymphocyte (PBL) membranes, indicates the secretion of IL-2 and initiation of an immune system activation cascade. Comparing the average IL-2R expression in 34 patients with retinitis pigmentosa (RP) syndrome (561 ± 282 cells/mm²; mean ± standard deviation) with 35 age-matched normal subjects (194 ± 39 cells/mm²), it was found that those with RP had greater numbers of IL-2R-positive cells (P < 0.001). The increased amounts of IL-2R on PBL of 29 RP and the homotypic self-aggregation of RP PBL by phase and scanning electron microscopy led to the study of the interaction of RP PBL with cultured human postmortem retinal pigment epithelial cells (RPE). A direct correlation was found between the amount of IL-2R expression and the numbers of RP lymphocytes adhering to RPE monolayers. However, the adherence effect was not unique to RP syndrome but appeared to be a nonspecific result of lymphocyte activation. Greater adherence to RPE than normal also was observed in PBL from disease control subjects with elevated IL-2R values and in PBL stimulated by the mitogen, concanavalin A (Con-A). In addition, RPE monolayers were destroyed by Con-A-stimulated PBL that showed 95-98% IL-2R expression. Similar, but less serious effects, occurring in RPE cells after 1 wk's cocultivation with RP PBL, suggested that activated RP lymphocytes can be cytotoxic to RPE during prolonged contact. Because macrophage-like cells and class II major histocompatibility complex expression have been found in RP-affected retinas, immune-mediated cytopathologic effects may contribute to retinal degeneration in RP. Invest Ophthalmol Vis Sci 33:2848-2860, 1992

Increased T-cell activation, measured by high numbers of lymphocytes positive for the Tα1 activation antigen, has been found in the peripheral blood lymphocytes (PBL) of patients with retinitis pigmentosa (RP). In addition, an unusual presence of class II major histocompatibility complex expression was associated with RP retinas. Other immune system abnormalities, including an increased expression of the interleukin-2 receptor (IL-2R), has been reported from several independent laboratories. These findings suggest that immune mechanisms may contribute partially to the pathogenesis of this heredodegenerative retinal disorder.

Increased lymphokines are secreted by peripheral immune cells when they are activated by immune stimuli. The immunoregulatory cascade, initiated by the lymphokine IL-1 from activated macrophages, leads to the production of IL-2 and other immunoregulatory secretions (such as IL-1-6, interferons, prosta-glandins, leukotrienes, and tumor necrosis factor-α). The IL-2 spreads the activation process to bone marrow and other peripheral cells, increasing the aggregation and attachment of immune cells to target tissues. An important aspect of the cascade is the increased expression of adhesion factors on lymphocyte surface membranes. The lymphocyte activation process can be detected by an increased appearance of IL-2R (also called CD25) on T- and B-cells and other circulating immune cells, using immunofluorescent flow cytometry.

The cascade of cellular responses initiated by PBL activation causes a heightened immunoregulatory state. If unabated or modulated by normal feedback control mechanisms, the increased production of lymphokines can develop into a destructive process. Autoimmune diseases represent such an event, exhibiting uncontrolled cell activation, invasion of tar-
get tissues by immune cells, and excess lymphokine secretions.\textsuperscript{13} Invading macrophage-like cells have been described in RP postmortem retinal pathologic degenerations.\textsuperscript{14-16} We reasoned that, because PBL might be activated in RP,\textsuperscript{1,3} and RP retinas exhibit class II major histocompatibility complex expression which is associated with immune activity, the immune cells found in RP retinas\textsuperscript{14-16} might contribute cytotoxic effects to the programmed degeneration of RP retinas.

To test this hypothesis, we measured the expressions of the activation epitope, IL-2R, and lymphocyte function-related antigen (LFA-1-\(\beta\) or CD18), a molecule known to cause lymphocyte adherence to activated epithelial cells, in 34 patients with RP.\textsuperscript{11,17} We compared RP lymphocyte morphology with normal not activated lymphocytes by light and scanning electron microscopy (SEM). To examine whether RP lymphocytes specifically recognize and attach to retinal pigment epithelial cells (RPE), we examined cultured human RPE for expression of intercellular adhesion molecule-1 (ICAM-1 or CD54), the counterreceptor on target cells for lymphocyte LFA-1-\(\beta\).\textsuperscript{11} We also counted the number of PBL from eight patients with RP and four normal control subjects that adhered firmly to cultured human RPE after cocultivation. The uniqueness of this PBL reaction to RP syndrome was evaluated by using PBL maximally stimulated by a mitogen, concanavalin A (Con-A). In addition, we included PBL from four disease control patients who showed increased activation expression as a result of other medical conditions. Our results suggest that, although activated PBL are not unique to RP syndrome, these cells from patients with RP could contribute to RPE pathologic findings.

Materials and Methods

Patients and Study Plan

We studied 34 patients with RP (age range, 12-78 yr; average, 42 yr), 12 male and 22 female patients, on the Retinal Service of the Ophthalmology Department at Ohio State University. All patients were evaluated by two of us (FHD and RBC); they had a history of nyctalopia, progressive loss of peripheral visual fields, pigmentary retinopathy, and reduction of electroretinogram impulses. Their diagnosis of RP was confirmed by degrees of optic nerve pallor and attenuated retinal vessels.\textsuperscript{6} Genetic analysis showed that 16 had a dominant pattern of RP inheritance, 7 had a recessive familial pattern, and 11 were classified as simplex because no other family members were affected by RP.\textsuperscript{6}

Participation in the study included donation of one blood specimen. The expression of IL-2R and LFA-1 epitopes on lymphocytes was compared with that of 35 age-matched control subjects. In the lymphocyte-adherence portion of the study, the results of eight patients with RP were compared with control subjects consisting of four normal subjects without disease and four patients with diseases other than RP (including arthritis, chronic otitis and deafness, bronchitis, and diabetes). The entire project was conducted in accordance with the guidelines of Children’s Hospital and Ohio State University Human Subjects Research Committees. After the study plan was explained and informed consent was obtained, blood samples were drawn.

Staining and Preparation of PBL

A portion of the blood sample was used for leukocyte and differential counts, using Giemsa stain and an automated Coulter cell counter (Hialeah, FL). Observation and phase photography were done with an Olympus (Valencia, PA) phase microscope. We separated PBL using density-gradient centrifugation with Ficoll-Hypaque (Pharmacia, Piscataway, NJ). After washing PBL three times in 0.15 mol/l phosphate-buffered saline (PBS), they were processed further. A portion of cells were cultured in RPMI-1640 media containing 20% fetal bovine sera (FBS), antibiotics, and 5 mmol/l glutamine (Gibco, Grand Island, NY) in humidified air with 5% CO\textsubscript{2} in 25-cm\textsuperscript{2} tissue culture flasks without or with 50 \(\mu\)g/ml of Con-A (Vector, Burlingame, CA) for observations at 48 hr. Portions of the blood or cells were processed immediately for epitope identification by flow cytometry, for SEM, or for cocultivation with cultured RPE.

Flow Cytometry of Lymphocyte Epitopes

A direct assay using whole blood was employed to label PBL with affinity-purified fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibodies (MA) to the epitopes IL-2R and LFA-1 (Coulter). Purified mouse myeloma proteins of the immunoglobulin G class (Coulter) served as negative control antigens. For staining, 10 \(\mu\)l of the fluorescent MAAb was added to 100 \(\mu\)l of whole blood and 100 \(\mu\)l of PBS without Ca++ and Mg++. After a 30-min incubation at 4°C and washing twice in RPMI-1640 containing 5% FBS for 10 min each, the erythrocytes were lysed using a lysing reagent at room temp for 5 min. After centrifugation, the cells were suspended in 1.0 ml of Isotol II (Coulter) for analysis.

Fluorescent-positive cells were counted with an automated flow cytometer (EPICS-C; Coulter). With the argon laser set at 488 nm (500 mW), the forward light scatter and right-angle green fluorescence signals were collected as a cytogram for analysis of fluorescent-
positive and -negative cells. The selected cytogram was less than 1% monocytes (as demonstrated by positive staining with OKMI; Ortho, Raritan, NJ). Using data-acquisition software to detect 10,000 cells, fluorescent-positive lymphocytes were separated from fluorescent-negative cells, and calculations were done on a logarithmic scale from two-parameter dot-plot cytograms. Absolute numbers were derived from the percent of marker-positive cells \times \text{the leukocyte count} \times \text{the differential lymphocyte count}; they were reported as the number of positive lymphocytes per cubic millimeter. Normal control specimens were included in daily assays (reproducibility differed by < 3%).

**RPE Culture for Lymphocyte Cocultivation**

Postmortem eyes, obtained from the Central Ohio Eye Bank within 48 hr of death, were used to initiate RPE cultures. After removing the anterior segment, vitreous, and sensory retina, RPE cells were detached with 0.05% trypsin and 0.53 mmol/l ethylenediaminetetraacetic acid (Gibco) during incubation at 37°C for 30 min. A stream of Ham’s F-10 medium (containing 10% FBS, antibiotics, and 5 mmol/l glutamine, Gibco) was used to dislodge and collect cells. Resuspended RPE cells were planted in 75-cm² culture flasks (Gibco) and incubated at 37°C in humidified air containing 5% CO₂ until confluent. The medium was changed at 4–6-day intervals, and the cells were subcultured weekly or biweekly with 0.5% trypsin and ethylenediaminetetraacetic acid. Early-passage (passages four to six) cells that retained their epithelial character and characteristic appearance just before confluence were used for lymphocyte coculture experiments. Expression of anti-human ICAM MAb (Vector) was detected by standard indirect immunofluorescent techniques.

**Cocultivation of Lymphocytes With Cultured RPE**

A portion of the separated lymphocytes was suspended at a concentration of 10⁶/ml in RPMI-1640 with 10% heat-inactivated FBS, 5 mmol/l glutamine, and antibiotics (Gibco). For direct cocultivation, 0.5 ml of cells were added to monolayers of RPE cells that had been plated 48 hr previously on 15-mm round plastic cover slips (Lux Thermanox; Nunc, Naperville, IL) in a flat-bottomed 24-well microtiter plate (Gibco) and incubated at 37°C in 5% CO₂. Most cocultivations were terminated at 24 hr for SEM.

To determine possible progressive effects of longer cocultivation, cover slips of RPE and lymphocytes cocultivated at the same time were removed and fixed daily to provide serial observations for up to 9 days. Supernatant fluids, removed and replaced after 4 days from combined RPE and RP PBL cultures and from RPE cultures alone, were transferred to new RPE monolayers to test the effects of RP PBL conditioned media on RPE. In some experiments, lymphocytes previously stimulated with Con-A (50 μg/ml) for 4 days were cocultivated with RPE monolayers. Washing and fixation was done as described. The RPE monolayers without lymphocytes were cultured similarly for control samples.

**Preparation of Cells and RPE Monolayers for SEM**

A portion of the lymphocytes, pelleted immediately after separation or after incubation in RPMI-1640 without or with added Con-A, were processed for SEM by fixation of the washed pellet in 2.5% glutaraldehyde in 0.1 mol/l Millonig’s PBS. In addition, other lymphocytes attached to RPE monolayers on cover slips were prepared for microscopic examination. After designated times (24 hr or longer), media and floating cells were rinsed gently three times by dipping the cover slips in PBS. Cover-slip cultures were fixed (60 min at 37°C) in 2.5% glutaraldehyde in 0.1 M Millonig’s PBS (pH 7.2). Submersion of cover slips three times in PBS at 4°C for 10 min removed the primary fixative. A second fixation followed with 1% osmium tetroxide in 0.1 Millonig’s PBS for 90 min at 4°C. After washing three times, the specimens were dehydrated using graded alcohols. Samples in absolute ethanol underwent critical point drying with a Balzer’s union dryer and were mounted on 13-mm Kent Cambridge stubs with double-sided tape. Coating with 0.02-μm palladium–gold was done under high vacuum in a Polaron coating unit (E5100; Leica, Inc., Deerfield, IL). Specimens were observed with a Hitachi (Mountainview, CA) S570 SEM.

**Assessment of Lymphocytes and RPE Morφologic Features**

To evaluate the morphologic features of lymphocytes in suspension, 10–15 photomicrographs at 10,000× of representative cells from eight patients with RP and eight control subjects were observed in a masked fashion, independently, by four observers (LLW, HML, RBC, and FHD). The lymphocytes were graded for activation on a scale of 1–4 according to the size and number of microvilli, presence of ruffled raised surface membranes, and the cell size (measurements in microns were made using the photographs). Additional photographs were observed if there was divergent opinion among observers. Autoaggregation was evaluated by the number of lymphocyte clumps (> 40 cells) seen in a suspension culture (25-cm³ polystyrene flask) at 48 hr using the low-power field (40X) of an inverted Olympus microscope.
To count adherent cells after a SEM preparation, RPE monolayers were observed at one magnification 400×. For each sample, the lymphocytes still present on five representative fields (after the washing and fixation procedures) were counted and averaged. A higher magnification was used to distinguish lymphocytes from possible cellular debris. Photographs were made of representative fields.

Data Analysis

The CRISP statistical package (Chrunch Interactive Software Corp., San Francisco, CA) was used to summarize and compare data points of patients with RP and control subjects using an analysis of variance for linkage to age and genetic pattern. Mean normal values were compared with those found in patients with RP by two-tailed student t-tests.

Results

RP Lymphocyte Counts and Morphology

The total leukocyte counts of all patients with RP (4265–9233 cells/mm³) were normal (range, 3200–9800 cells/mm³). In differential counts, numbers of RP lymphocytes and monocytes were also normal. Typical activated monocytes could be seen frequently in differential stained blood smears, but there were no abnormal forms.

Increased self- or autoaggregation, consistent with greater adhesiveness of activated lymphocytes, was observed by an unusual formation of homotypic cell clumps (average, five per field) by RP PBL visible by phase microscopy (40X) at 48 hr when in suspension culture in media without stimulants (Fig. 1A). Little clumping was seen in normal lymphocyte cultures at 48 hr (zero to one per field, Fig. 1B). However, large homotypic cell aggregates always were found in lymphocyte cultures stimulated with the mitogen Con-A by 48 hr (Fig. 2C).

Lymphocyte activation also was apparent using SEM. In each specimen of RP PBL examined by SEM, many individual cells had increased size, prominent microvilli, and ruffled ridge-like membrane projections from their surfaces (Fig. 2A). Both activated (top) and unactivated (bottom) lymphocytes with few microvilli could be found in each specimen (Fig. 2B), but the numbers of activated cells averaged 55% in RP samples and 15% in normal specimens. Lymphocytes with several attached platelets suggested the presence of surface adhesion molecules (Fig. 2C).11 Lymphocytes activated by the mitogen Con-A demonstrated maximal stimulation and lymphokine secretion by a swollen appearance with many blunted projections over their entire surfaces (Fig. 2C).20

IL-2R Activation and LFA-1 Expressions of RP Lymphocytes

Activation of RP PBL was measured in 34 patients with RP by an increased average absolute number of IL-2R-positive cells (571 ± 282 cells/mm³) compared with IL-2R of 35 laboratory control specimens (194 ± 39 cells/mm³). The enhanced IL-2R expression (> normal mean and standard deviation) was present in 29 of the 34 patients (85%). However, the five patients with normal numbers of IL-2R+ cells did not appear clinically different. In the four disease control patients who had other chronic medical conditions (Table 1), significantly increased IL-2R expression (773 ± 198 cells/mm³) was measured (P < 0.001). The disease control values were not different from those found in the average patient with RP; the four normal control subjects selected for lymphocyte attachment had IL-2R values in the normal range (236 ± 22 cells/mm³).

Statistical methods were used to evaluate possible linkage of age or genetic pattern with lymphocyte activation as expressed by the IL-2R epitope in the RP group. There were no associations with age or genetic expression. The mean absolute value of the IL-2R epitope for the 16 patients with dominant RP was 575 ± 215 cells/mm³. For the seven with recessive RP, it was 555 ± 266 cells/mm³. For the 11 with simplex RP, we found a mean absolute value of 763 ± 360 cells/mm³. Although those with simplex RP heredity patterns had the highest numbers, the differences were only significant compared with the normal population.

Expression of LFA-1 was found on 95–98% of both RP and control lymphocyte populations, indicating a potential for adherence with target cells as expected.11

Morphology and ICAM Expression of RPE Monolayer Cultures

Confluent monolayers of pavement-type epithelial cells were established easily from the separated RPE cells from postmortem human eyes (Fig. 3A).18 When used at passages three to six for cocultivation experiments, they still contained a number of pigmented cells (seen as glistening globules at this magnification; arrows, 100X; Fig. 3A) and unpigmented cells. Their ultrastructure (Fig. 3B) showed normal RPE intracellular architecture with melanin granules. The ICAM-1 expression of RPE cultured alone was absent compared with the positive control cells (tonsillar tissue) (data not shown).

Adherence of Normal and Activated PBL to Cultured RPE Cells

Cocultivation of the PBL from six patients with RP and elevated IL-2R expression on RPE monolayers...
resulted in the adherence of a significantly larger number of RP lymphocytes (average, 40 ± 25 cells; mean ± standard deviation) compared with cells from the four healthy control subjects without disease (average, six ± two cells) at the same magnification (400X, Table 1, P < 0.005). A representative culture of RPE with attached RP PBL (Fig. 4A) was compared with that of a normal healthy control with attached PBL (Fig. 4B). By contrast, the patients with RP and normal IL-2R levels (patients 5 and 7) also had few attached lymphocytes (Table 1). The PBL of the four disease control subjects who had increased activation expression with high IL-2R values as a result of other medical conditions also had PBL adherent to RPE in numbers similar to the RP group (average, 52 ± 15 cells; Table 1). We concluded that the amount of
PBL activation shown by IL-2R expression correlated with PBL adherence regardless of the underlying disease and that the process of lymphocyte activation appeared essential to the increased PBL adherence to RPE.

A marked formation of microvilli from the apical RPE membranes was associated with PBL attachment as observed by SEM (Fig. 5A). Complex interactions between microvillous projections from activated PBL and surface microvilli of RPE appeared to
be associated with the dramatic RPE cell retraction that occurred at 48 hr (Fig. 5B). This result suggested that physical contact between activated PBL and RPE may be a functional part of the cytopathologic findings after the cocultivation process (Fig. 5).

**Adherence of Mitogen Con-A-Stimulated PBL to RPE**

To test the importance of PBL activation in their adherence to RPE monolayers, we used maximal stimulation with a mitogen Con-A. Either RP or control PBL were cultured with Con-A for 4 days before their addition to RPE monolayers in cocultivation experiments. There was no difference between the PBL responses of RP and control cells after Con-A treatment. Maximal activation of IL-2 secretion was shown by the presence of IL-2R (CD25) on 95–98% of the PBL using immunofluorescence (Table 1) and by the swollen appearance of the cells as seen by SEM (Fig. 2D). In addition, prominent lymphocyte adhesion factor expression was suggested by the large homotypic cell aggregates in the suspension culture (Fig. 1C).

The Con-A-stimulated lymphocyte clumps adhered to RPE monolayers in massive numbers, producing dramatic cytopathic effects of cell retraction, elongation, and cytolysis in the RPE monolayers by 24 hr (Fig. 6, Table 2). Cell destruction and loss of the entire RPE monolayer was complete by 72 hr with either RP or control Con-A-stimulated lymphocytes.

**Results of 1-Wk Cocultivation of Activated RP PBL With RPE**

To evaluate possible cytopathologic changes that might occur during prolonged cocultivation of naturally activated RP PBL with RPE, serial cocultivations over 9 days were examined. After 1 wk, cyopathic effects were visible in the RPE cells cocultivated with normally activated RP lymphocytes (Fig. 7A, Table 2). Cell retraction and elongation suggested the presence of cytotoxic action by adherent RP PBL.

To test whether these results were a result of direct cell–cell attachment or whether secreted lymphokines were also important, supernatant fluid (including a few cells) from a RPE monolayer cocultivated with RP lymphocytes for 4 days was placed on a fresh RPE monolayer. For a control, supernatant fluid was transferred to new RPE from 4-day-old RPE cells without PBL cocultivation. Only the supernatant from the cocultivated RP PBL and RPE caused cytopathologic effects (Fig. 7B). When these cells were compared with RPE cultured for 1 wk without additions (Fig. 7C), we concluded that the secreted lymphokines transferred in the supernatant from activated RP PBL with microvillous action could affect the RPE monolayer adversely (Table 3).

**Discussion**

This study confirmed a previous report of IL-2R presence in the PBL in a proportion of patients with RP by showing elevation of this activation epitope over cells from healthy control subjects in 29 of 34 patients with well-characterized RP. Although these results were consistent with the presence of altered immunoregulation, as suggested before in RP, the precise stimulus that produced the immunologic changes in RP is not known. Moreover, as in the abnormal RP immune reactions described previously, the increased IL-2R expression in RP PBL did not appear to be related to age or a specific genetic type of RP.

Although there remain many unknown factors in RP pathogenesis, we addressed a possible relationship between the consequences of lymphocyte activation and the retinal disease process in RP by creating an in vitro model. Knowing that several pathologic studies have documented invading macrophages in deteriorating RP retina, our model tested the cytopathic potential of RP and other activated PBL cells by cocultivating them with cultured human postmortem RPE cells in vitro.

This is the first report to our knowledge in which lymphocyte activation, measured by IL-2R expression, observed by SEM, and found in RP PBL has been correlated with morphologic effects on target cells. In this instance, the relevant affected target was cultured RPE cells. Using phase microscopy and SEM, we found increased IL-2R expression in RP PBL that was measured by fluorescent flow cytome-

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**Table 1. Interleukin-2 receptor expression and RPE-adherent lymphocyte numbers of RP patients**

<table>
<thead>
<tr>
<th>RP patients</th>
<th>Age</th>
<th>Diagnosis</th>
<th>IL-2R %*</th>
<th>Absolute†</th>
<th>RPE-adherent cell numbers‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>RP</td>
<td>29%</td>
<td>883</td>
<td>76 (43-110)</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>RP</td>
<td>27%</td>
<td>746</td>
<td>45 (35-57)</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>RP</td>
<td>31%</td>
<td>713</td>
<td>53 (32-68)</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>RP</td>
<td>25%</td>
<td>484</td>
<td>34 (21-49)</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>RP</td>
<td>12%</td>
<td>211</td>
<td>8 (4-13)</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>RP</td>
<td>25%</td>
<td>574</td>
<td>39 (33-51)</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>RP</td>
<td>10%</td>
<td>240</td>
<td>4 (1-9)</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>RP</td>
<td>28%</td>
<td>695</td>
<td>62 (40-83)</td>
</tr>
</tbody>
</table>

Averages 32

* Percent of peripheral cells with positive interleukin-2 receptor (IL-2R) expression.
† Absolute number is derived from the percent X the number of lymphocytes and monocytes X the total white cell count.
‡ Average and range of adherent lymphocytes in five scanning electron microscope fields counted from photographs taken at a magnification of X400.
Fig. 3. Activated RP lymphocytes without external stimulation. (A) Lymphocytes with altered morphology consistent with activation are found in RP lymphocyte suspension cultures without stimulation. Two RP lymphocytes with prominent microvilli are flanked by an active lymphocyte with ruffled margins and knobular microvilli (bar = 1 μm). (B) An activated RP lymphocyte is seen adjacent to one that is not activated (bar = 1 μm). (C) Four or more platelets are shown adhering to an activated RP lymphocyte (bar = 1 μm). (D) Swollen, blunted surface projections form the lymphocyte surface after 4-day addition of the mitogen Con-A to suspension cultures of lymphocytes of either RP patients or normals (bar = 1 μm). This appearance is associated with maximal (95%-98%) IL-2R expression and maximal cytolysis of RPE monolayer cells after 48 to 72 h cocultivation.
PBL to RPE, similar to the disease-free normal control subjects. To confirm that this effect depended on the activation process itself, PBL were stimulated with a mitogen Con-A to complete activation expression (95–98% positive for IL-2R). The Con-A-activated PBL, not only self-aggregated in suspension culture, but they also attached in massive numbers on RPE monolayers. With their maximal lymphokine

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**Fig. 4.** Adherence of RP and control lymphocytes to cultured retinal pigment epithelial cells. (A) After 24 h cocultivation of RP or control lymphocytes with monolayers of RPE followed by thorough washing, the adherent cell number was counted at a constant magnification of X400 in five fields of SEM preparations. A representative field of RPE-adherent lymphocytes from a RP patient with 28% IL-2R expression shows approximately 85 lymphocytes attached (bar = 10 µm). (B) A representative field of a healthy control with 8% IL-2R expression shows eight adherent lymphocytes (bar = 10 µm).
Fig. 5. RPE microvilli action associated with lymphocyte adherence. (A) The attachment of lymphocytes to the apical membranes of RPE monolayers is accompanied by intense microvilli formation by 24 h. Lymphocytes (3–5 μm) and platelets (1–3 μm) are clustered among microvilli on RPE cells (bar = 10 μm). (B) Marked retraction of RPE cells with microvilli projections on all apical membranes occur after 72 hr cocultivation with activated lymphocytes (bar = 10 μm). (C) Interaction between activated lymphocytes and platelets and the microvilli of a single RPE cell is shown at 72 h (bar = 12 μm).

secretions, Con-A-activated cells destroyed the RPE monolayers effectively in 48–72 hr. Therefore, RPE cells in vitro are susceptible to destruction by the uncontrolled activation cascade. Although the adherence molecule LFA-1 was present on all RP and control lymphocytes, its counterreceptor, ICAM-1, had not been expressed on resting RPE monolayers. We presume that this molecule was induced by cytokines secreted during lymphocyte activation. Moreover, in SEM views of PBL microvilli grasping RPE cell microvilli, it appeared that both activated lymphocyte physical structures and their secreted lymphokines were participants in cell attachment to RPE and the resulting cytopathologic changes.

Major cytotoxic effects can result from an uncontrolled activation expression on target tissues found in severe autoimmune, infectious, or neoplastic conditions. In one study, adherence of activated cells to endothelium led to marked increases in cellular permeability, retraction, and destruction. These results
could be reproduced by applying the activated cell
lymphokine, leukotriene (12-hydroxytetraeicosanoic
acid).24 Adherence of activated RP PBL to RPE in our
experiments may have altered permeability of these
cells and caused retraction. The immunoregulatory
cascade released by activated PBL with elevated IL-
2R expression suggests that, when these cells are pre-
sent in RP retinas,14-16 their secreted cytokines and
morphologic changes may play a role in RP retinal
degeneration.15-16 In this nonspecific manner,7-10 ac-

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Table 2. Interleukin-2 receptor expression and RPE-adherent lymphocytes of healthy and disease controls

<table>
<thead>
<tr>
<th>Controls</th>
<th>Age</th>
<th>Diagnosis</th>
<th>IL-2R*</th>
<th>Absolute</th>
<th>RPE-adherent cell number</th>
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<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>32</td>
<td>No disease</td>
<td>8%</td>
<td>194</td>
<td>3 (2-6)</td>
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<tr>
<td>2</td>
<td>21</td>
<td>No disease</td>
<td>10%</td>
<td>245</td>
<td>6 (1-10)</td>
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<tr>
<td>3</td>
<td>25</td>
<td>No disease</td>
<td>12%</td>
<td>294</td>
<td>8 (3-13)</td>
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<tr>
<td>4</td>
<td>18</td>
<td>No disease</td>
<td>13%</td>
<td>210</td>
<td>7 (4-9)</td>
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<tr>
<td>Averages</td>
<td>24</td>
<td></td>
<td>11%</td>
<td>236</td>
<td>6</td>
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<tr>
<td>Disease</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>Deaf, arthritis</td>
<td>30%</td>
<td>845</td>
<td>54 (37-72)</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>Deaf, otitis</td>
<td>34%</td>
<td>864</td>
<td>74 (40-98)</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>Bronchitis</td>
<td>25%</td>
<td>673</td>
<td>40 (31-48)</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>Diabetes</td>
<td>27%</td>
<td>710</td>
<td>38 (28-51)</td>
</tr>
<tr>
<td>Averages</td>
<td>34</td>
<td></td>
<td>29%</td>
<td>773</td>
<td>52</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Lymphs + Con-A for 4 days</td>
<td>95-98%</td>
<td>nd</td>
<td>&gt;250</td>
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</table>

* IL-2R %, IL-2R absolute number and RPE adherent cell numbers as in Table 1.
† Results of two experiments with a 4-day lymphocyte Con-A stimulation before cocultivation.
activated RP PBL may contribute an immune mechanism to the retinal pathologic findings of RP. It would be interesting to determine whether retinas with RP express the LFA-1 counterreceptor, ICAM-1, and class II antigen. This might allow circulating activated lymphocytes to home in and adhere to RPE.

Table 3. Cytopathic effects on RPE monolayers after lymphocyte cocultivations

<table>
<thead>
<tr>
<th>Lymphocytes—character and additions*</th>
<th>Time cocult.</th>
<th>RPE cytopathic effects†</th>
<th>Retraction/elongation</th>
<th>Microvilli formation</th>
<th>Cytolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2R + RP or other diseases</td>
<td>24 hr</td>
<td></td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>IL-2R - RP or other diseases</td>
<td>24 hr</td>
<td></td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>IL-2R + RP</td>
<td>1 week</td>
<td></td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Cell-free IL-2R + 4-day cell supernatant</td>
<td>72 hr</td>
<td></td>
<td>++</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Cell-free 4-day supernatant-control</td>
<td>72 hr</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Con-A mitogen maximal stim. lymphocytes</td>
<td>24 hr</td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Lymphocytes added to RPE monolayers (10⁶/ml) without further stimulants, except Con-A as indicated.
† Cytopathic effects were observed by scanning EM. From 0 (none), mild to complete (+++). RPE cells were no longer adherent to the coverslip when +++.
Key words: activated lymphocytes, interleukin-2, interleukin-2 receptor, retinitis pigmentosa, human retinal pigment epithelium

Acknowledgment

The authors thank Christy Steffen for technical assistance and manuscript preparation and Brad Foreman and Scott Pelok for preparing scanning electron micrographs.

References

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