CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Sept. 1994, p. 569–577 Vol. 1, No. 5
1071-412X/94/$04.00+0

Synergistic Effects of Gamma Interferon on Inflammatory Mediators That Induce Interleukin-6 Gene Expression and Secretion by Human Retinal Pigment Epithelial Cells

CHANDRASEKHARAM N. NAGINENI,[1] BARBARA DETRICK,[2] AND JOHN J. HOOKS[1]*

Immunology and Virology Section, Laboratory of Immunology, National Eye Institute, Bethesda, Maryland 20892,[1] and Department of Pathology, The George Washington University Medical Center, Washington, D.C. 20037[2]

Received 15 March 1994/Returned for modification 5 May 1994/Accepted 9 June 1994

The retinal pigment epithelial (RPE) cell is a potent regulatory cell within the retina. It helps to maintain normal retinal activity, and following gamma interferon (IFN-γ) exposure, it may express major histocompatibility complex class II molecules and function as an antigen-presenting cell. Since interleukin-1 (IL-1) and IL-6 are potent cytokines observed in ocular inflammatory processes, we initiated studies to evaluate conditions which enable RPE cells to produce these cytokines. Cultures of human RPE cells from two eye donors were established and characterized, and enzyme immunoassays were employed to screen for IL-1 and IL-6 production. Treatment of RPE cells with lipopolysaccharide (LPS) or recombinant tumor necrosis factor alpha, IL-1, or IFN-γ resulted in a significant level of secretion of IL-6. In contrast, treatment with recombinant epidermal growth factor, basic fibroblast growth factor, platelet-derived growth factor, or transforming growth factor β1 did not result in IL-6 production. IFN-γ in combination with suboptimal levels of IL-1, tumor necrosis factor alpha, or LPS can dramatically augment the secretion of IL-6 by RPE cells. Thus, these inflammatory mediators can act alone or synergistically with IFN-γ to activate RPE cells and dramatically increase the expression and secretion of IL-6. In contrast, IL-1 was not detected following stimulation with any of the above-mentioned cytokines or LPS. Characterization of IL-6 protein production by RPE cells revealed that 98% of the protein is promptly secreted by the cell, its induction is dependent upon the time and concentration of the stimulant, and the continuous presence of the stimulant is required for IL-6 production. Moreover, Western blot (immunoblot) analysis of secreted proteins revealed that IL-6 was produced in multiple molecular forms. Characterization of gene transcription by Northern (RNA) blot analysis revealed that mRNA for IL-6 was detected shortly after IL-1 treatment. However, within hours of IL-1 withdrawal, IL-6 mRNA levels returned to control levels. These data demonstrate that LPS and cytokine activation of RPE cells results in the activation of IL-6 gene transcription and IL-6 protein secretion. Moreover, these studies indicate that cytokine activation of RPE cells may be one of the critical factors in ocular inflammation and that it is an important consideration in RPE cell transplantation studies.

Retinal pigment epithelium, a single layer of tightly packed epithelial cells, acts as a barrier between the highly vascularized choroid and the delicately structured neuroretina in the posterior segment of the eye (2, 52). Because of its polarized organization and strategic location, the retinal pigment epithelial (RPE) cell plays an important role in the normal functioning of both the choroid and the retina. Diverse activities performed by RPE cells include (i) the transport of nutrients and metabolic products from the choroid to the retina and vice versa, (ii) phagocytosis of the outer segments shed by retinal rods and cones, and (iii) the absorption of light and the dissipation of heat energy derived from incident light (2, 22, 51, 52). Since many of these physiologic actions are essential for the functional and structural integrity of the retina and choroid, the RPE cell plays a vital role in degenerative, inflammatory, and infectious diseases of the eye. For example, in retinitis pigmentosa, choroideremia, proliferative vitreoretinopathy, parasitic and viral infections, and age-related maculopathy, the characteristic disorganization and destruction of retinal and/or choroidal elements are accompanied by atrophy, necrosis, and degeneration of the RPE cell (3, 9, 31, 32, 36, 50). In addition, the RPE cell has been demonstrated to actively participate in immune reactivity in the retina. It can express major histocompatibility complex (MHC) class I and II molecules and adhesion molecules, and it can process antigen and present it to helper T cells (7, 8, 13, 40).

Cytokines play an essential role in maintaining normal physiologic states and in modulating responses to aberrant conditions. In inflammatory and autoimmune diseases of the eye, infiltration of lymphocytes and macrophages into the anterior and posterior segments of the eye and secretion of cytokines, such as gamma interferon (IFN-γ), interleukin-2 (IL-2), tumor necrosis factor alpha (TNF-α), IL-1, and IL-6, are the initial events (5, 10, 26, 30, 48). The presence of IL-1-secreting macrophages in the inflamed subretinal space during neovascularization has been reported (39). In humans, IFN-γ and IL-2 were localized in infiltrating T cells in uveitis and sympathetic ophthalmia (26). Significantly higher levels of IL-6, IL-1, TNF-α, and IFN-γ were detected in vitreous aspirates of patients with proliferative vitreoretinopathy (30), uveitis, and other inflammatory eye diseases (4, 18).

Reports characterizing the cellular infiltrate and the presence of cytokines within the ocular microenvironment have been supplemented by studies on the inflammatory effects of direct inoculation of cytokines into the eye. Ocular inflammatory diseases, particularly uveitis, have been produced in rabbits after administration of endotoxin, TNF-α, or IL-1 into

* Corresponding author. Mailing address: Immunology and Virology Section, Laboratory of Immunology, National Eye Institute, Building 10, Room 6N 228, National Institutes of Health, Bethesda, MD 20892-1596. Phone: (301) 496-6578. Fax: (301) 402-0485.

the anterior or vitreous compartment of the eye (17, 42, 43). Intravitreal, but not intravenous, injection of IL-6 causes uveitis in rats, thus suggesting that local elevation of IL-6 levels may be involved in ocular inflammatory reactions (24, 25).

The presence of cytokines within the eye during pathologic states and the induction of ocular pathology following administration of cytokines suggest a critical role for these molecules in ocular disease. In vitro systems have provided one approach to exploring some of the interrelationships between cytokines and cellular activation. Recent studies show that IFN-γ can activate RPE cells to express MHC class I and II antigens (7, 8). These cells can then function in antigen processing and the presentation of retinal and nonretinal antigens to helper T lymphocytes (40). Moreover, RPE cells can respond to inflammatory cytokines by expressing MHC class I and intercellular adhesion molecule-1 molecules and/or by producing IL-6, IL-8, and monocyte chemotactic protein (7, 8, 13–16, 41, 49).

In this study, human RPE cells were found to produce and secrete IL-6 when they were incubated with lipopolysaccharide (LPS) and cytokines such as IL-1, TNF-α, and IFN-γ. Moreover, IFN-γ acts synergistically with other cytokines to induce IL-6 secretion. These results are discussed in view of the functional aspects of RPE cells in inflammatory and degenerative diseases of the retina.

MATERIALS AND METHODS

Reagents. Human recombinant IL-1α (rIL-1α) (specific activity, 5.03×10^6 U/mg), rIL-1β (specific activity, 7.47×10^6 U/mg), rIL-6 (specific activity, 1×10^9 U/mg), and basic fibroblast growth factor (bFGF) were obtained from Collaborative Research, Bedford, Mass. Human rTNF-α (specific activity, 2×10^7 U/mg), rIFN-γ (specific activity, 2×10^7 U/mg), recombinant platelet-derived growth factor (rPDGF), and nonradioactive DNA labeling and detection kits (Genius 1) were purchased from Boehringer Mannheim, Indianapolis, Ind. Human rIL-6 (CHO derived; specific activity, 4×10^7 U/mg) and rabbit anti-human IL-6 antibody were obtained from Genzyme, Cambridge, Mass. Human rIFN-α was obtained from Amgen, Thousand Oaks, Calif. Anticytokeratin monoclonal antibodies, LPS (from *Salmonella typhosa* or *Escherichia coli*), transforming growth factor β_1 (TGF-β_1), and epidermal growth factor (EGF) were obtained from Sigma Chemical Co., St. Louis, Mo. Enzyme immunoassay (EIA) kits for IL-1α, IL-1β, and IL-6 were purchased from AMAC, Westbrook, Maine. *E. coli* carrying human IL-6 cDNA plasmid (clone PT 7.7/hIL-6) was obtained from the American Type Culture Collection, Rockville, Md. A human β-actin cDNA probe was purchased from Clontech Laboratories, Palo Alto, Calif. Tissue culture media and plastic culture ware were purchased from GIBCO, Grand Island, N.Y., and Becton Dickinson, Lincoln Park, N.J., respectively.

Human RPE cell cultures. Human donor eyes were obtained within 50 h of enucleation from New England Eye Bank in Boston, Mass. Eyes were opened by cutting around the equatorial zone. The vitreous humor and retina were carefully dissected out from the posterior segment and discarded. The choroid with attached retinal pigment epithelia was gently peeled off and cut into approximately 2-mm^2 pieces with a sharp razor blade. Retinal pigment epithelium-choroid explants were seeded onto the substratum of the culture dishes, with the RPE layer facing down. Minimal essential medium (MEM) supplemented with 20% fetal calf serum (FCS), nonessential amino acids, penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (Fungizone) (50 ng/ml) was added to the cultures carefully without disturbing the explants. The cultures were incubated at 37°C in 5% CO_2 in a humidified chamber. Culture growth was monitored with an inverted microscope to identify the epithelial or fibroblastic nature of the cells. Half of the explants yielded pure epithelial cells. Explants yielding nonepithelial cells were carefully scraped off the dish. The cultures reached confluence in approximately 3 weeks. The human RPE cell cultures were subcultured at a 1:3 ratio, and MEM supplemented with 10% FCS and other components as described above was used. RPE cell cultures at passages 5 to 10 were used in all of these studies.

Immunofluorescence. RPE cells were grown to confluence on glass chamber slides (Nunc, Naperville, Ill.) and fixed with an acetone-methanol (1:1) mixture at −20°C for 5 min. After being washed with phosphate-buffered saline (PBS), the cells were incubated with a 1:50 dilution of monoclonal antibody to cytokeratin (clone 8.13), RPE-9, or PR-6 for 1 h at room temperature. RPE-9 and PR-6 monoclonal antibodies were developed in our laboratory and react specifically with antigens present in RPE cells and photoreceptors, respectively (27). Following this incubation, the cells were washed and incubated with fluorescein isothiocyanate-conjugated horse anti-mouse immunoglobulin G (1:100 dilution) for 1 h. After a final wash with PBS, the cells were mounted and evaluated.

Western blot (immunoblot) analysis. (i) Cytokeratin. Human RPE cells, human choroid fibroblast cultures, and freshly isolated bovine RPE cells were lysed by sonication in extraction buffer (50 mM Tris, pH 8.5; 4 mM EDTA; 2 mM phenylmethylsulfonyl fluoride [PMSF]; 1% [wt/vol] deoxycholate; 1% [vol/vol] Triton X-100). Cell lysates were clarified by centrifugation at 10,000 × *g* for 10 min. The supernatant fraction was subjected to sodium dodecyl sulfate–10% polyacrylamide electrophoresis (SDS–10% PAGE) and transferred to nitrocellulose membranes. After incubation of the membranes with a 1:200 dilution of anticytokeratin antibody (clone 8.13) for 6 h, they were washed and further incubated for 2 h with a 1:100 dilution of goat anti-mouse immunoglobulin G coupled to horseradish peroxidase. The color was developed with 4-chloro-1-naphthol and H_2O_2 as substrates.

(ii) IL-6. RPE cell cultures were grown to confluence in MEM containing 10% FCS. Cultures were washed twice with serum-free medium (SFM) and were incubated in SFM with or without various stimulants. After 24 h of incubation at 37°C, the media were collected. The media were concentrated 100-fold with Centriprep-10 and Centricon-10 concentrators (Amicon, Beverly, Mass.). Proteins in the concentrated media were fractionated by SDS–14% PAGE. Immunoblotting was performed as described above. Rabbit anti-human IL-6 and goat anti-rabbit immunoglobulin G-horseradish peroxidase were used as the primary and secondary antibodies.

Cytokine assays by EIA. The effects of all the stimulants were always studied in SFM, so that cytokines and growth factors in the serum would not interfere with the stimulant effects as well as in EIAs. Cells were grown to confluence in eight-well chamber slides (Nunc) in MEM containing 10% FCS. The cultures were washed twice with SFM and incubated in SFM with or without the stimulants. At the indicated time points, the media were harvested and either used for analysis immediately or frozen at −20°C. Cell counts in the representative wells were determined by removing the cells with trypsin-EDTA and counting the cells in a hemocytometer. Each experiment was conducted with cells derived from the same batch of cultures grown under similar conditions.

IL-6, IL-1α, and IL-1β were detected by the specific sandwich type immunoassay (AMAC), according to the manufacturer's instructions. The intensity of the color developed in the well strips was read on a microplate reader (Bio-Rad, Rich-

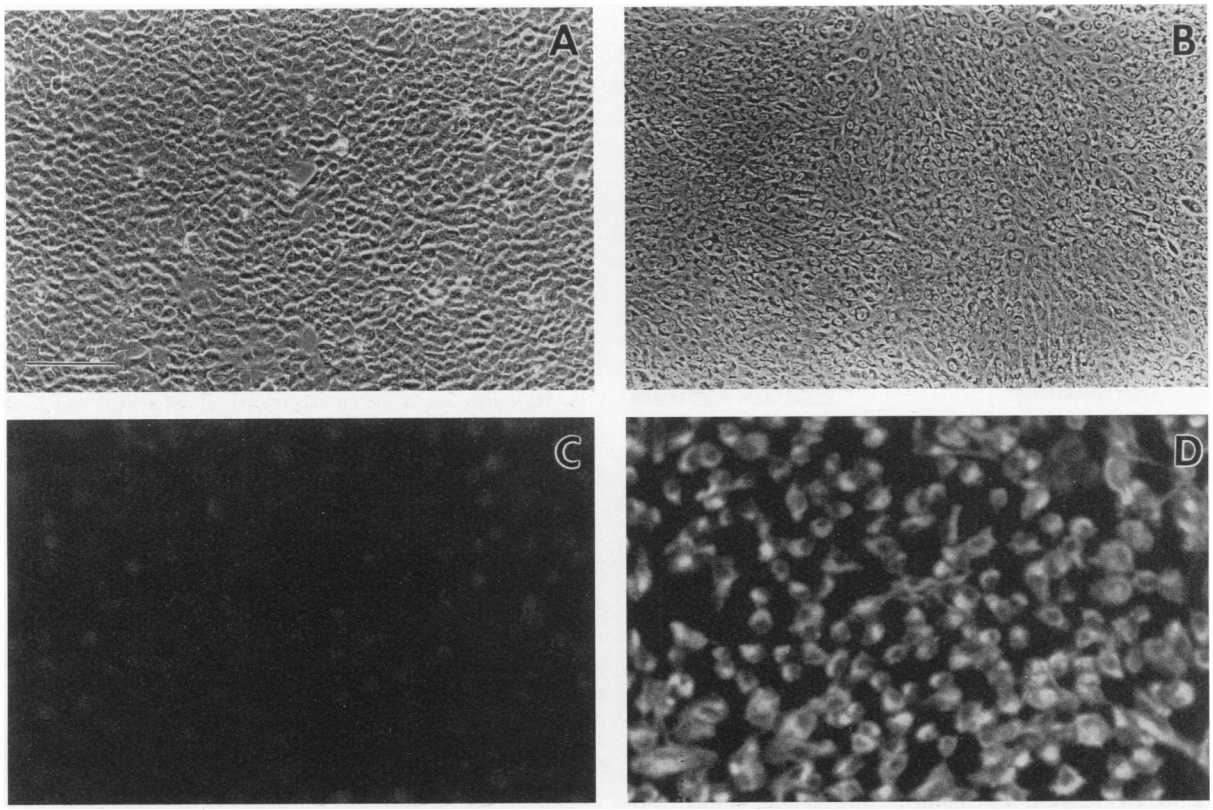

FIG. 1. Phase-contrast photomicrographs of human RPE cell cultures at passage 6 after 4 days (A) and 4 weeks (B) in culture. Shown are representative fluorescent images of RPE cell cultures stained with irrelevant monoclonal antibody as a negative control (C) or anticytokeratin (D). Bar (panel A) = 200 μm (panel B has the same magnification).

mond, Calif.) at 405 nm. Results were calculated from the standard curve prepared at the same time.

Northern (RNA) blot analysis. A human IL-6 cDNA probe of 1.3 kb was prepared by digesting plasmid DNA (clone PT 7.7/hIL-6) with *Bgl*II and *Bam*HI. The IL-6 probe was then purified by gel electrophoresis. RPE cell cultures, grown to confluence in 100-mm-diameter dishes in MEM containing 10% FCS, were washed twice with SFM and incubated in SFM in the presence of various stimulants. Total cellular RNA from the cultures was prepared with RNA extraction medium (RNA zol; Tel-Test, Inc., Friendswood, Tex.). Cellular RNA was fractionated by electrophoresis on 1.2% formaldehyde agarose gels, transferred to nylon membranes, and immobilized by UV cross-linking for 3 min. The IL-6 cDNA probe was labeled by the random primer method with digoxigenin-UTP. Membranes were hybridized to the IL-6 probe for 16 to 18 h at 42°C in high-efficiency hybridization solution with 50% formamide (Tel-Test). Membranes were washed twice with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at room temperature for 5 min; this step was followed by two washes with 0.1× SSC–0.1% SDS at 68°C for 15 min each. Immunological detection of the hybridized probe was performed by using anti-digoxigenin alkaline phosphatase conjugate followed by a color reaction with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (DNA labeling and detection kit; Boehringer Mannheim).

RESULTS

RPE cell cultures. Retinal pigment epithelium-choroid explants prepared from the eyes of Caucasian human donors aged 77, 81, and 87 were used to initiate RPE cell cultures. RPE cell cultures derived from two donor eyes, free of non-RPE-cell contamination, were selected for further propagation. Pigmentation of the cells was lost gradually. By the second passage no visible melanin granules were observed. When the cells reached confluence, they displayed the typical hexagonal shape and formed monolayers with clear intercellular boundaries (Fig. 1A). When the cultures were left in the same dish for extended periods (4 to 8 weeks), cells continued to replicate further and to form more compact sheets with the deposition of extracellular matrix-like material (Fig. 1B). Two primary RPE cell lines (passages 5 to 10) were used in this study. Cytokeratin was used to monitor the specificity of the cell lines (35). All of the cells reacted positively with anticytokeratin antibody, suggesting a homogeneous population of epithelial cells (Fig. 1C and D). In addition, immunoblotting analysis confirmed the presence of cytokeratin in the RPE cells. Cytokeratin 18 (42 kDa) was noted to be the predominant form of cytokeratin associated with these cultured RPE cells (Fig. 2). In addition, freshly isolated bovine RPE cells also contained cytokeratin 18. At the time of the first passage, RPE cells reacted positively with the anti-RPE-9, developed against RPE cells (27). However, as previously described, in vitro culture of these cells resulted in the loss of reactivity with this specific monoclonal antibody (21).

Effect of inflammatory mediators on IL-6 secretion. Confluent RPE cells were washed in SFM and incubated for 24 h in SFM alone or in SFM containing IL-1α (20 ng/ml), IL-1β (20 ng/ml), TNF-α (20 ng/ml), LPS (10 μg/ml), IFN-γ (500 U/ml), IFN-α (1,000 U/ml), EGF (50 ng/ml), bFGF (20 ng/ml), PDGF

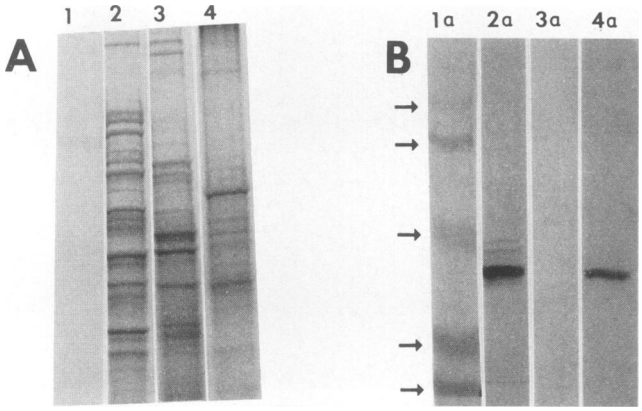

FIG. 2. Immunological detection of cytokeratin in RPE cell cultures. (A) Coomassie blue-stained gel. Cell extracts (50 μg of protein per lane) were subjected to SDS–10% PAGE and stained with Coomassie blue. Lane 1, protein molecular weight markers; lane 2, RPE cell cultures at passage 7; lane 3, human choroid fibroblast cultures at passage 4; lane 4, freshly isolated bovine retinal pigment epithelium. (B) Western blot analysis of cellular proteins for cytokeratins. The samples in lanes 1a to 4a are the same as those in lanes 1 to 4 in panel A. The experimental details are described in Materials and Methods. The blots were probed with monoclonal anticytokeratin antibody (clone 8.13). The arrows indicate the positions of prestained protein standards (top to bottom): 106, 80, 49, 32, and 27 kDa.

(20 ng/ml), or TGF-β_1 (20 ng/ml). Supernatant fluids were harvested and analyzed by EIA for the presence of IL-6. The data are summarized in Fig. 3. In untreated cultures, trace amounts of IL-6, less than 5 pg/10^4 cells, could be detected in the medium. IL-1β and IL-1α were noted to be the most potent stimulants of IL-6 production (425 and 400 pg/10^4 cells, respectively). TNF-α and LPS were the second most potent stimulants (118 and 126 pg/10^4 cells, respectively). In the presence of IFN-γ, 32 pg of IL-6 was secreted per 10^4 cells. In contrast, IFN-α and growth factors bFGF, EGF, TGF-β, and

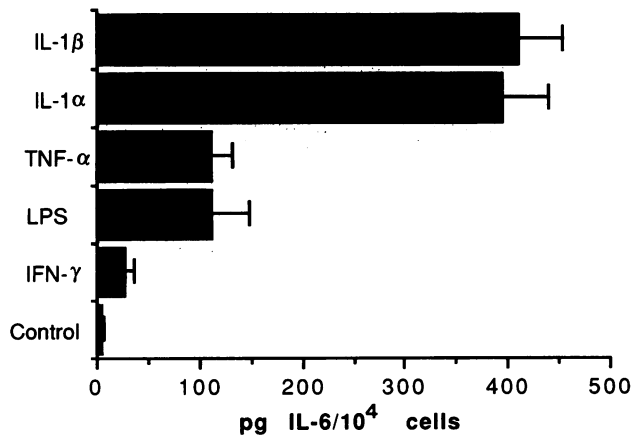

FIG. 3. Effects of various stimulants on IL-6 secretion. RPE cell cultures grown to confluence were washed with SFM and incubated for 24 h in SFM alone (Control) or in SFM containing IFN-γ (500 U/ml), LPS (*S. typhosa*) (10 μg/ml), TNF-α (20 ng/ml), IL-1α (20 ng/ml), or IL-1β (20 ng/ml). The media were harvested and analyzed for IL-6 by EIA. Results are means ± standard errors of the means for four experiments performed in duplicate.

TABLE 1. Comparison of intracellular and secreted levels of IL-6 produced by RPE cells[a]

Stimulant	Concn of IL-6 (ng/10^7 cells)		% Secreted[b]
	Cellular	Secreted	
None	0.2	14	98.4
LPS (*S. typhosa*) (10 μg/ml)	2.7	455	99.4
TNF-α (20 ng/ml)	2.2	370	99.4
IL-1α (10 ng/ml)	80.1	4,280	98.2
IL-1β (10 ng/ml)	70.7	4,050	98.3

[a] RPE cell cultures were grown to confluence in 100-mm-diameter dishes and incubated in SFM containing various stimulants at the indicated concentrations. After 24 h, the media were harvested and the cells were extracted into buffer (50 mM Tris, pH 7.4). The cell extracts were clarified by centrifugation at 12,000 × *g*. The culture media and the supernatants of cell extracts were used for IL-6 analysis. Data are the means for two experiments.

[b] Percentage of total IL-6 protein produced that was secreted into the medium.

PDGF AB and BB had no effect on IL-6 secretion (data not shown).

Two sources of LPS were used in these experiments. It was noted that the source of LPS greatly affected IL-6 secretion by RPE cells. For example, 10 μg of LPS derived from *S. typhosa* (L-7895; Sigma) per ml induced significant levels of IL-6 (94.5 pg of IL-6 per 10^4 cells), whereas 10 μg of LPS derived from *E. coli* 055-85 (L-6018; Sigma) per ml was ineffective in inducing IL-6 production above the baseline (6.5 pg of IL-6 per 10^4 cells). Data from the LPS experiments are the mean values obtained from four separate experiments. It should be noted that different LPS preparations may have differential effects on cytokine secretion. Because LPS (*S. typhosa*) induced IL-6, it was selected for use in further experiments.

Comparison of intracellular and secreted IL-6. The relationship between the amounts of cell-bound IL-6 and IL-6 secreted into the medium was studied in the presence of LPS, TNF-α, IL-1α, and IL-1β. Quantitation of IL-6 in the media and cell lysates was performed by EIA. Results indicate that more than 98% of the IL-6 produced was promptly released into the medium (Table 1).

Effect of inflammatory mediators on IL-1 production. RPE cell cultures were treated with bFGF (20 ng/ml), EGF (50 ng/ml), TGF-β_1 (20 ng/ml), PDGF (20 ng/ml), LPS (10 μg/ml), TNF-α (20 ng/ml), or IL-1α or IL-1β (20 ng/ml) for 24 h. Media and cell extracts were then analyzed for IL-1 production by EIA (11). Neither IL-1α nor IL-1β was readily detected in media or cell extracts (sensitivity of assay, 10 pg/ml).

Immunoblot analysis of secreted IL-6. The molecular heterogeneity of the secreted IL-6 was examined by immunoblot analysis. Human rIL-6 produced in mammalian cells (CHO) and in *E. coli* was used as a positive control. By using anti-IL-6 antibody, multiple molecular forms of IL-6 were detected in the IL-1α- and IL-1β-treated RPE cell cultures (Fig. 4). One major band representing approximately 26 kDa and several minor bands representing 23, 28, and 30 kDa were detected. An additional band at the 45-kDa position was also observed in the IL-1β- and IL-1α-treated cultures. In LPS- and TNF-α-treated culture samples, one faint band, located at the 45-kDa position, was seen. No bands were observed in the bFGF- or medium-treated cultures.

Kinetics and dose dependence of IL-6 secretion. The time course of IL-6 production by RPE cells in response to LPS, TNF-α, IL-1α, and IL-1β was studied. The amount of IL-6 secreted appeared linear with time, ranging from 1 to 24 h (Fig. 5). These kinetics suggest that the cells were continuously

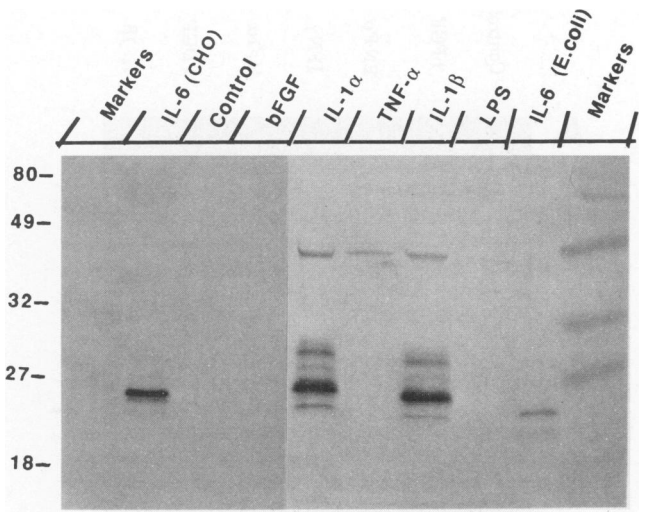

FIG. 4. Immunoblot analysis of IL-6 secreted by RPE cell cultures stimulated by inflammatory mediators. RPE cells grown to confluence were washed and incubated in SFM alone (Control) or in SFM containing bFGF (20 ng/ml), IL-1α (10 ng/ml), TNF-α (10 ng/ml), IL-1β (10 ng/ml), or LPS (*S. typhosa*) (10 μg/ml) for 24 h. Hundred-fold-concentrated media were subjected to Western blotting as described in Materials and Methods. Human IL-6 produced in CHO cells (100 ng) and in *E. coli* (50 ng) is the positive control. The positions of protein molecular weight markers (in thousands) are shown on the left.

responsive to the mediators during this 24-h period. The effects of various concentrations of these inflammatory mediators on IL-6 secretion were studied to determine a dose-response relationship (Fig. 6). Low concentrations (0.1 ng/ml) of TNF-α, IL-1α, and IL-1β produced significant amounts of IL-6. IL-6 secretion increased correspondingly with an increase in the concentration of mediators, reaching saturation at a concentration of 10 ng/ml of the mediator. In the case of LPS, IL-6 production was optimal at 10 μg/ml.

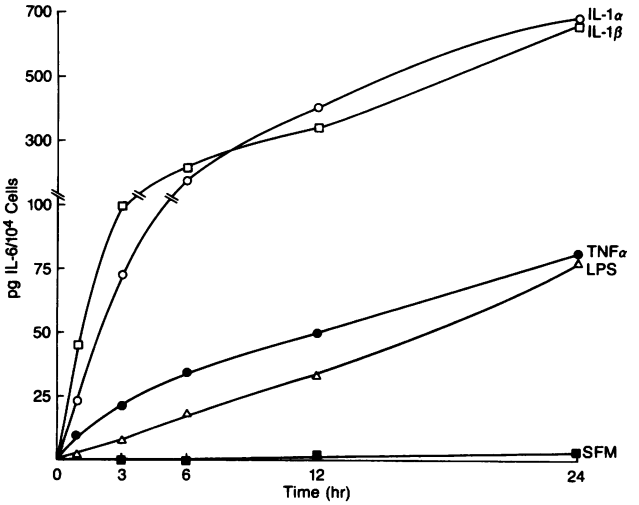

FIG. 5. Time course of IL-6 secretion by RPE cells in the presence of various inflammatory mediators. RPE cell cultures grown to confluence were washed with SFM and incubated in SFM containing LPS (*S. typhosa*) (10 μg/ml), TNF-α (20 ng/ml), IL-1α (20 ng/ml), or IL-1β (20 ng/ml). At the indicated times, the media were harvested and IL-6 levels were determined by EIA. Results are from a single experiment performed in duplicate and are representative of three such studies.

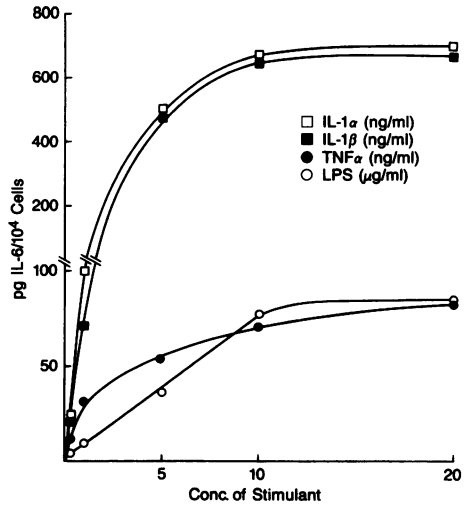

FIG. 6. Effects of various concentrations of inflammatory mediators on IL-6 secretion by RPE cells. Cultures grown to confluence in eight-well glass chamber slides were washed and incubated in SFM containing various concentrations of LPS (*S. typhosa*), TNF-α, IL-1α, or IL-1β. After 24 h, the media were harvested and IL-6 levels were determined by EIA. Results are from a single experiment and are representative of three such studies.

IL-6 secretion following withdrawal of mediators. The following experiment was designed to evaluate whether the continuous presence of the inflammatory mediator is required to induce IL-6 production. Cultures were incubated in the presence of maximal concentrations of LPS (20 μg/ml), TNF-α (20 ng/ml), and IL-1β (20 ng/ml) for 12 h. The media were collected, and the cultures were washed three times with SFM and further incubated in SFM in the absence of the mediator. At different times (4, 12, and 24 h), the media were harvested and analyzed by EIA. The data are illustrated in Fig. 7. Cultures treated with IL-1β, TNF-α, and LPS secreted 564 ± 15, 100 ± 8, and 98 ± 3 pg of IL-6 per well per h, respectively (data are means ± standard errors of the means). Four hours after removal of TNF-α and LPS, IL-6 secretion dropped to 25% of the peak value. However, withdrawal of IL-1β did not result in a significant change in IL-6 secretion. Overall, there was a progressive decrease in IL-6 secretion with time. IL-6 secretion returned to control levels within 12 to 24 h after the withdrawal of mediators. These studies indicate that the continuous presence of the stimulant is required for IL-6 secretion.

Expression of IL-6 mRNA. IL-6 mRNA levels were evaluated by Northern blot analysis of total RNA prepared from RPE cell cultures. A single band of 1.3 kb was detected in IL-1α- and IL-1β-treated cultures (Fig. 8A, lanes IL-1α and IL-1β). No reaction was observed in the bFGF-, TNF-α-, IFN-γ-, and PDGF-treated cultures. Furthermore, no reaction was observed in the LPS-treated cultures (data not shown). Although significant amounts of IL-6 were observed in the presence of LPS and TNF-α (Fig. 3, 5, and 6), IL-6 mRNA was not detectable. One possibility could be that the sensitivity of the nonradioactive method employed in this study is not high enough to detect moderate changes in IL-6 mRNA levels. However, results clearly show that the cDNA probe used is highly specific and does not cross-react with any other species of mRNA. The blots prepared with a human β-actin cDNA

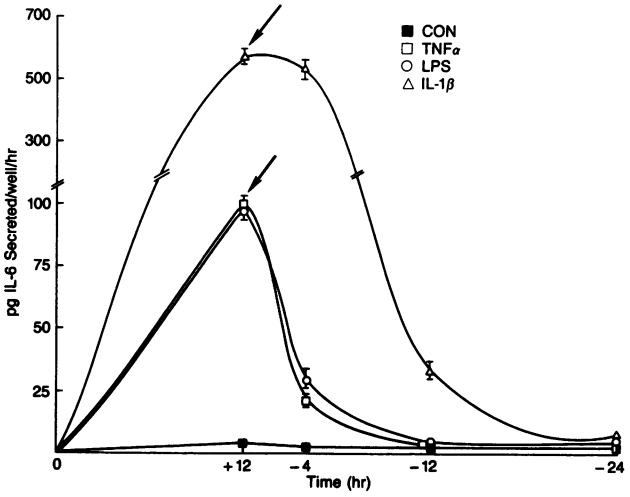

FIG. 7. IL-6 secretion by RPE cells following removal of inflammatory mediators. The cultures were grown to confluence in eight-well glass chamber slides in MEM containing 10% FCS. Then cultures were washed and incubated in SFM (350 μl of medium per well) alone (CON) and in SFM containing LPS (*S. typhosa*) (20 μg/ml), TNF-α (20 ng/ml), or IL-1β (20 ng/ml). After 12 h, the media were harvested (+12-h samples [arrows]) and the wells were washed twice with SFM to remove the mediators. Then SFM was added to the wells, and after 4 h, the media were harvested (−4-h samples), and the wells were washed and refed with SFM. The samples were prepared similarly after 12 and 24 h (−12-h and −24-h samples, respectively). The media were analyzed for IL-6 by EIA. Results are means ± standard errors of the means for four experiments.

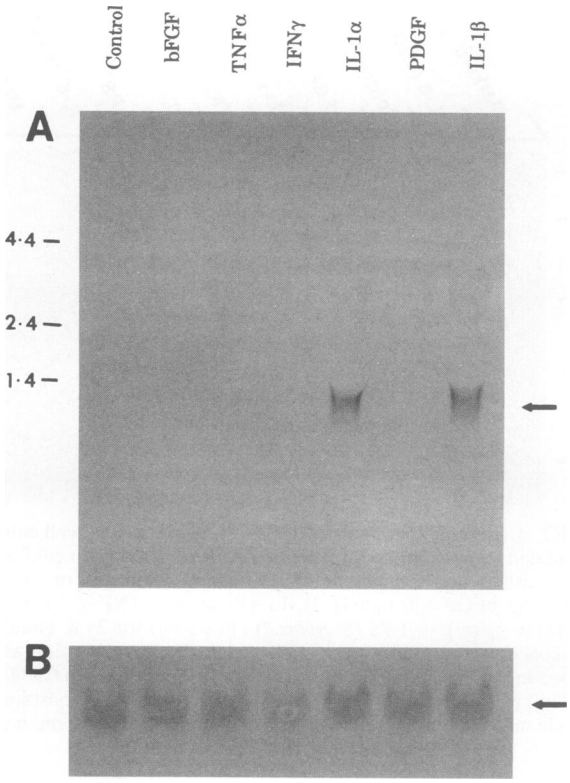

FIG. 8. Northern blot analyses of total RNA isolated from RPE cell cultures treated with various mediators. (A) Blot probed with nonradioactive digoxigenin-labeled human IL-6 cDNA as described in Materials and Methods. The positions of RNA size markers (in kilobases) are shown on the left. The arrow indicates the position of IL-6 mRNA. (B) Blot probed with human β-actin cDNA. The samples in the lanes correspond to the samples shown in panel A. The arrow indicates the position of β-actin mRNA.

probe (Fig. 8B) identified bands of similar intensity in all the lanes, suggesting that similar amounts of RNA were loaded onto the gel.

The time course of induction of IL-6 mRNA was examined by treating RPE cell cultures with IL-1β (10 ng/ml) for selected periods (Fig. 9). Within 2 h of IL-1β treatment, significant induction of IL-6 mRNA was observed. IL-6 mRNA levels increased with time, reaching a peak at 16 h which was maintained up to 24 h (densitometric data not shown). After 24 h of IL-1β treatment, the medium was removed, and the cultures were washed twice and incubated in IL-1β-free SFM for 2 or 5 h. Within 2 h of withdrawal of the stimulus (IL-1β), the IL-6 mRNA level returned to the prestimulatory level (Fig. 9A, lane −2). These results suggest that rapid IL-6 secretion by RPE cells in response to stimuli (Fig. 5) was dependent on the induction of IL-6 mRNA. Withdrawal of the stimuli resulted in a rapid reversal of IL-6 mRNA levels followed by a decrease in IL-6 production and secretion.

Synergistic effects of IFN-γ on IL-6 production. Since combinations of cytokines can result in the augmentation of the effects of individual cytokines, we evaluated the effects of human IFN-γ on IL-6 secretion by LPS, TNF-α, and IL-1β (Fig. 10). When RPE cell cultures were incubated with 100 or 1,000 U of IFN-γ alone per ml, low levels of IL-6 were produced (6.72 ± 0.64 and 10.20 ± 0.50 pg of IL-6 per 10^4 cells). Suboptimal concentrations of LPS (1 μg/ml), TNF-α (0.1 ng/ml), and IL-1β (0.1 ng/ml) also resulted in the secretion of low levels of IL-6 (<20 pg of IL-6 per 10^4 cells). In contrast, the combination of IFN-γ with LPS, TNF-α, or IL-1β resulted in a striking increase in the secretion of IL-6. For example, LPS and IFN-γ induced the secretion of 100 pg of IL-6 per 10^4 cells. Similarly, TNF-α and IFN-γ treatment resulted in the secretion of 90 pg of IL-6 per 10^4 cells. Enhanced secretion of IL-6 by RPE cells in the presence of inflammatory mediators and IFN-γ is due to the synergistic actions of IFN-γ but is not due to additive effects.

DISCUSSION

Cytokine production and cytokine-induced activation of cells are associated with a variety of cellular responses. In the eye, cytokine-induced activation of RPE cells has been demonstrated to affect a number of fundamental cellular responses such as cell adhesion and antigen processing and presentation (7, 8, 13, 40). In this report we show that inflammatory mediators, particularly the bacterial endotoxin LPS, and cytokines, such as IFN-γ, TNF-α, and IL-1, enhance gene expression and secretion of IL-6. In addition, IFN-γ in combination with suboptimal levels of IL-1, TNF-α, or LPS can dramatically augment the secretion of IL-6 by RPE cells. In fact, the effect of IFN-γ is striking, in that it can act synergistically with other mediators to induce higher levels of IL-6. This is a pattern of action which has been noted in a number of cytokine systems (12, 44).

This study also reveals that the IL-6 secreted by RPE cells appears as multiple molecular forms. Other investigators have noted that IL-6 can undergo posttranslational glycosylation and phosphorylation reactions (33, 34). Furthermore, it was observed that the removal of the stimulant, either LPS or the cytokine, results in a rapid decline in IL-6 levels. Moreover,

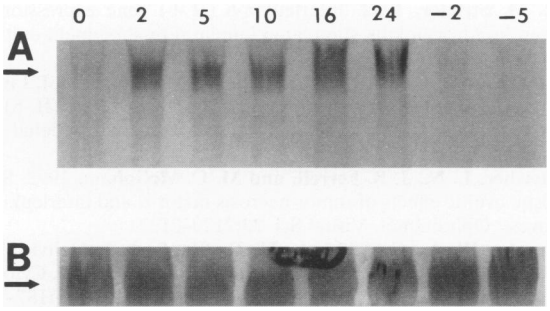

FIG. 9. Time course of IL-6 mRNA expression by RPE cell cultures treated with IL-1β. Cultures were incubated in SFM containing IL-1β (10 ng/ml), and at the times shown above the lanes (in hours), total RNA was isolated and analyzed by Northern blotting. The samples in lanes 0 to 24 were prepared after incubation of cultures for 0, 2, 5, 10, 16, and 24 h, respectively, in the presence of IL-1β. The samples in lanes −2 and −5 were prepared 2 and 5 h after the removal of IL-1β following the 24-h treatment. (A) Blot probed with human IL-6 cDNA. The arrow indicates the position of IL-6 mRNA. (B) The blot in panel A was reprobed with human β-actin cDNA. The samples in the lanes correspond to those in panel A.

mRNA analysis revealed a rapid induction of IL-6 mRNA by IL-1 and depletion of mRNA upon withdrawal. These data suggest that the transcription of the IL-6 gene in RPE cells is under stringent regulatory control. Finally, in contrast to other published reports (15, 41), we were able to document that LPS induced IL-6 production by RPE cells derived from two donor eyes. Two preparations of LPS were evaluated for their ability to induce IL-6 secretion. Only the *S. typhosa* LPS preparation was capable of stimulating significant secretion of IL-6, whereas the *E. coli* preparation failed to induce this cytokine. The differences in LPS sources may account for the opposing findings (15, 41). Alternatively, the use of primary RPE cells from different individuals may also contribute to these differences.

IL-6, previously known as B-cell stimulatory factor, IFN-β2, hepatocyte stimulatory factor, and hybridoma growth factor, is produced by circulating leukocytes and by endothelial cells, hepatocytes, smooth muscle cells, fibroblasts, and epithelial cells (23, 29, 45–47). This multipotent cytokine regulates immune responses and plays a major role in the autoimmune and inflammatory disorders. Some of the immunological actions of IL-6 are associated with its ability to induce proliferation, differentiation of B lymphocytes, and production of antibodies. Upregulated IL-6 production has been described in patients with rheumatoid arthritis, AIDS, psoriasis, sepsis, and two ocular disorders, proliferative vitreoretinopathy and uveitis (19, 20, 28, 30, 37, 38).

Several lines of evidence to support the notion that bacterial endotoxins and the cytokines TNF-α, IL-1, IL-6, IL-8, and IFN-γ play a critical role in uveitis and other inflammatory disorders of the eye now exist (10, 17, 24–26, 30, 39, 42, 43, 48). In a rabbit model system, it has been demonstrated that intravitreally injected TNF-α, IL-1, or endotoxin can induce ocular inflammation consisting of both anterior and posterior uveitis (17, 42, 43). Similarly, in rodents, the injection of endotoxin (LPS) into the footpad causes uveitis (24). Furthermore, a comparison of sera and ocular samples revealed that the intraocular preparations contained 10-fold-higher concentrations of IL-6 than the serum samples. Subsequent studies have shown that direct intravitreal injection of IL-6 also caused uveitis in rats (25). Taken together, these animal studies indicate that ocular inflammation induced by inflammatory mediators can be associated with the presence of IL-6 within the eye.

In humans, inflammatory mediators and cytokines have also been localized in the ocular microenvironment. For example, IFN-γ and IL-2 have been immunochemically identified in infiltrating T lymphocytes in uveitis and sympathetic ophthalmia (26). Moreover, elevated levels of IL-6, IL-1, and IFN-γ have been found in the aqueous humor of uveitis patients (4, 18, 37) and in vitreous aspirates of patients with proliferative vitreoretinopathy (4, 30). Since ocular IL-6 levels, rather than systemic IL-6 levels, are associated with the uveitic reaction, local production of IL-6 by ocular resident cells and inflammatory cells may be important in ocular inflammatory reactions (10). The RPE cell is a critical retinal regulatory cell of neuroectodermal origin. This ocular resident cell can be modified by cytokines and is itself a source of cytokines, such as IL-6, IL-8, and monocyte chemotactic protein (14–16, 41).

Interferons are powerful regulatory molecules. IFN-γ has been shown to exert a variety of immunoregulatory actions, including induction of MHC class I and II molecules and activation of T cells and macrophages. IFN-γ is detected within the retina during inflammatory and degenerative diseases, and its presence is closely associated with the expression of MHC class II molecules on RPE cells (7). Under normal physiologic conditions, these cells do not express MHC class II molecules. RPE cells treated with IFN-γ in vitro are activated to express these molecules. These MHC class II-positive RPE cells are now capable of processing retinal antigens and presenting them to T lymphocytes (40). In addition, IFNs may activate gene expression within the retina and contribute to retinal differentiation (1, 6). The studies presented here further substantiate the potential activity of IFN-γ within the retina. IFN-γ is one of the cytokines capable of inducing enhanced IL-6 secretion by RPE cells. Moreover, in the presence of low concentrations of IL-1 or TNF-α, IFN-γ stimulates secretion of IL-6 in amounts higher than could have been produced by simple summation effects. Thus, IFN-γ acts synergistically to augment the cytokine-induced IL-6 secretion by RPE cells.

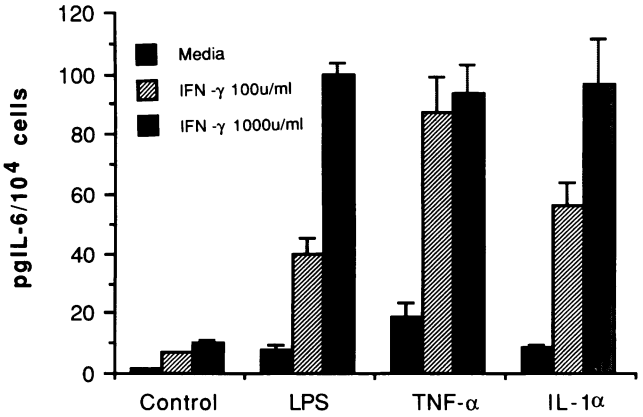

FIG. 10. Synergistic effects of IFN-γ on inflammatory-mediator-induced secretion of IL-6 by RPE cells. Cultures grown to confluence were washed with SFM and incubated in SFM alone or in SFM containing IFN-γ (100 or 1,000 U/ml). Each of these cultures was further treated with media alone (Control) or with medium containing LPS (*S. typhosa*) (1 μg/ml), TNF-α (0.1 ng/ml), or IL-1α (0.1 ng/ml). After 24 h, media were harvested and IL-6 levels were determined by EIA. Results are means ± standard errors of the means for four experiments with duplicate samples.

The mechanism of amplification may be highly effective, since several cytokines are produced simultaneously during inflammation.

In summary, these studies provide evidence that cytokines and inflammatory mediators can act alone or synergistically with IFN-γ to activate RPE cells and to dramatically increase the expression and secretion of IL-6. Since the inducer cytokines and IL-6 are potent inflammatory molecules, the data suggest that cytokine interaction with and production by RPE cells may contribute to ocular inflammatory processes. Future studies that define the specific mechanisms by which cytokines regulate IL-6 secretion by RPE cells in normal and disease states may provide important insights into biologic mechanisms for modifying inflammatory processes in retinal inflammation, degenerations, and RPE tissue transplantation. Moreover, the indication that IL-6 can also act in neuronal differentiation (29) suggests a potential role for RPE cell cytokines in retinal differentiation.

REFERENCES

1. **Barez, S., D. Boumpas, C. Percopo, D. Anastassiou, J. J. Hooks, and B. Detrick.** 1993. Modulation of major histocompatibility complex (MHC) class I genes in human retinoblastoma cells by interferons: evidence for both transcriptional and posttranscriptional regulation. Invest. Ophthalmol. Visual Sci. **34:**2613–2621.
2. **Bok, D.** 1985. Retinal photoreceptor-pigment epithelium interactions. Friedenwald lecture. Invest. Ophthalmol. Visual Sci. **26:** 1659–1694.
3. **Chan, C. C., B. Detrick, R. B. Nussenblatt, A. G. Palestine, L. Fujikawa, and J. J. Hooks.** 1986. Expression of HLA-DR antigens on RPE cells from patients with uveitis. Arch. Ophthalmol. **104:**725–729.
4. **de Boer, J. H., M. A. C. Van Hren, W. A. E. J. de Vries-Knoppert, G. S. Baarsma, P. V. T. M. de Jong, F. J. Postema, A. J. J. M. Rademakers, and A. Kijlstra.** 1992. Analysis of IL-6 levels in human vitreous fluid obtained from uveitis patients, patients with proliferative intraocular disorders and eye bank eyes. Curr. Eye Res. **11:**181–186.
5. **Deschenes, J., H. D. Char, and S. Kaleta.** 1988. Activated T-lymphocytes in uveitis. Br. J. Ophthalmol. **72:**83–87.
6. **Detrick, B., C. H. Evans, G. Chader, C. M. Percopo, and J. J. Hooks.** 1991. Cytokine-induced modulation of cellular proteins in retinoblastoma. Invest. Ophthalmol. Visual Sci. **32:**1714–1722.
7. **Detrick, B., and J. J. Hooks.** 1992. Autoimmune aspects of ocular disease, p. 345–361. *In* N. Rose and I. MacKay (ed.), The autoimmune diseases, vol. II. Academic Press, New York.
8. **Detrick, B., D. Newsome, C. Percopo, and J. J. Hooks.** 1985. Class II antigen expression and gamma interferon modulation of monocytes and retinal pigment epithelial cells from patients with retinitis pigmentosa. Clin. Immunol. Immunopathol. **36:**201–211.
9. **Detrick, B., M. Rodriguez, C. C. Chan, M. O. M. Tso, and J. J. Hooks.** 1986. Expression of HLA-DR antigens on retinal pigment epithelial cells in retinitis pigmentosa. Am. J. Ophthalmol. **101:** 584–590.
10. **De Vos, A. F., R. Hoekzema, and A. Kijlstra.** 1992. Cytokines and uveitis, a review. Curr. Eye Res. **11:**581–597.
11. **di Giovine, F. S., and G. W. Duff.** 1990. Interleukin 1: the first interleukin. Immunol. Today **11:**13–20.
12. **Elias, J. A., and V. Lentz.** 1990. IL-1 and tumor necrosis factor synergistically stimulate fibroblast IL-6 production and stabilize IL-6 messenger RNA. J. Immunol. **145:**161–166.
13. **Elner, S. G., V. M. Elner, M. A. Pavilack, R. F. Todd, L. Mayo-Bond, W. A. Franklin, R. M. Strieter, S. L. Kunkel, and A. R. Huber.** 1992. Modulation and function of intercellular adhesion molecule-1 (CD54) on human retinal pigment epithelial cells. Lab. Invest. **66:**200–211.
14. **Elner, S. G., R. M. Strieter, V. M. Elner, B. J. Rollins, M. A. Del Monte, and S. L. Kunkel.** 1991. Monocyte chemotactic protein gene expression by cytokine-treated human retinal pigment epithelial cells. Lab. Invest. **64:**819–825.
15. **Elner, V. M., W. Scales, S. G. Elner, J. Danforth, S. L. Kunkel, and R. M. Strieter.** 1992. Interleukin-6 (IL-6) gene expression and secretion by cytokine stimulated human retinal pigment epithelial cells. Exp. Eye Res. **54:**361–368.
16. **Elner, V. M., R. M. Strieter, S. G. Elner, M. Baggiolini, I. Lindley, and S. L. Kunkel.** 1990. Neutrophil chemotactic factor (IL-8) gene expression by cytokine-treated retinal pigment epithelial cells. Am. J. Pathol. **136:**745–750.
17. **Fleisher, L. N., J. B. Ferrell, and M. C. McGaham.** 1992. Synergistic uveitic effects of tumor necrosis factor-α and interleukin-1β. Invest. Ophthalmol. Visual Sci. **33:**2120–2127.
18. **Franks, W. A., G. A. Limb, M. R. Stanford, J. Ogilvie, R. A. Wolstencroft, A. H. Chignel, and D. C. Dumonde.** 1992. Cytokines in human intraocular inflammation. Curr. Eye Res. **11:**187–191.
19. **Grossman, R. M., J. Krueger, D. Yourish, A. Granellipeperno, D. P. Murphy, L. T. May, T. S. Kupper, P. B. Sehgal, and A. B. Gottlieb.** 1989. Interleukin-6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc. Natl. Acad. Sci. USA **86:**6367–6371.
20. **Guerne, P. A., B. L. Zuraw, J. H. Vaughan, D. A. Carson, and M. Lotz.** 1989. Synovium as a source of interleukin 6 in vitro: contribution to local and systemic manifestations of arthritis. J. Clin. Invest. **83:**585–592.
21. **Hamel, C. P., T. Ekaterine, E. Harris, B. Pfeffer, J. J. Hooks, B. Detrick, and T. M. Redmond.** 1993. Characterization of orpenin, a developmentally regulated 65K dalton retinal pigment epithelium specific protein. J. Neurosci. Res. **34:**414–425.
22. **Handelman, G. J., and E. A. Dratz.** 1986. The role of antioxidants in the retina and retinal pigment epithelium and the nature of preoxidant-induced damage. Adv. Free Radical Biol. Med. **2:**1–89.
23. **Hirano, T.** 1992. Interleukin-6 and its relation to inflammation and disease. Clin. Immunol. Immunopathol. **62:**s60–s65.
24. **Hoekzema, R., P. I. Murray, M. A. C. Van Haven, M. Helle, and A. Kijlstra.** 1991. Analysis of interleukin-6 in endotoxin-induced uveitis. Invest. Ophthalmol. Visual Sci. **32:**88–95.
25. **Hoekzema, R., C. Verhagen, M. V. Haren, and A. Kijlstra.** 1992. Endotoxin-induced uveitis in the rat. The significance of intraocular interleukin-6. Invest. Ophthalmol. Visual Sci. **33:**532–539.
26. **Hooks, J. J., C. C. Chan, and B. Detrick.** 1988. Identification of the lymphokines, interferon-gamma and interleukin-2, in inflammatory eye diseases. Invest. Ophthalmol. Visual Sci. **29:**1444–1451.
27. **Hooks, J. J., B. Detrick, C. Percopo, C. Hamel, and R. P. Siraganian.** 1989. Development and characterization of monoclonal antibodies directed against the retinal pigment epithelial cell. Invest. Ophthalmol. Visual Sci. **30:**2106–2113.
28. **Houssiau, F. A., J. P. Devogelaer, J. Van Damme, C. N. De Deuxchaisnes, and J. Van Snick.** 1988. Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritis. Arthritis Rheum. **31:**784–788.
29. **Kishimoto, T.** 1989. The biology of interleukin-6. J. Am. Soc. Hematol. **74:**1–10.
30. **Limb, G. A., B. C. Little, A. Meager, J. A. Ogilvie, R. A. Wolstencroft, W. A. Franks, A. H. Chignell, and D. C. Dumonde.** 1991. Cytokines in proliferative vitreoretinopathy. Eye **5:**686–693.
31. **Machemer, R., and H. Lagua.** 1975. Pigment epithelium proliferation in retinal detachment (massive periretinal proliferation). Am. J. Ophthalmol. **80:**1–23.
32. **Marmor, M. F.** 1979. Inflammations and degenerations of the retinal pigment epithelium, p. 454–477. *In* K. M. Zinn and M. F. Marmor (ed.), The retinal pigment epithelium. Harvard University Press, Cambridge, Mass.
33. **May, L. T., J. Ghrayeb, U. Santhanum, S. B. Tatter, Z. Sthoeger, D. C. Helbgott, N. Chiorazzi, G. Grieninger, and P. B. Sehgal.** 1988. Synthesis and secretion of multiple forms of B2-interferon/B-cell differentiation factor 2/hepatocyte-stimulating factor by human fibroblasts and monocytes. J. Biol. Chem. **263:**7760–7766.
34. **May, L. T., U. Santhanum, S. B. Tatter, J. Ghrayeb, and P. B. Sehgal.** 1989. Multiple forms of human interleukin-6. Phosphoglycoproteins secreted by many different tissues. Ann. N. Y. Acad. Sci. **557:**114–120.
35. **Moll, R., W. W. Franke, D. L. Schillier, B. Geiger, and R. Krepler.** 1982. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell **31:**11–24.
36. **Morris, D. A., and P. Henkind.** 1979. Pathological responses of the

human retinal pigment epithelium, p. 247–266. *In* K. M. Zinn, and M. F. Marmor (ed.), The retinal pigment epithelium. Harvard University Press, Cambridge, Mass.

37. **Murray, P. I., R. Hoekzema, M. A. C. Van Haren, F. D. De Horn, and A. Kijlstra.** 1990. Aqueous humor interleukin-6 level in uveitis. Invest. Ophthalmol. Visual Sci. **31:**917–920.
38. **Nakajima, K., O. Martinez-Maza, T. Hirano, P. Nishanian, J. P. Salazar-Gonzalez, J. L. Fahey, and T. Kishimoto.** 1989. Induction of interleukin-6 (BSF-IFN B2) production by the human immunodeficiency virus (HIV). J. Immunol. **142:**144–147.
39. **Nishimura, T., R. Goodnight, R. A. Prendergast, and S. J. Ryan.** 1990. Activated macrophages in experimental subretinal neovascularization. Ophthalmologica **200:**39–44.
40. **Percopo, C., J. J. Hooks, T. Shinohara, R. Caspi, and B. Detrick.** 1990. Cytokine-mediated activation of a neuronal retinal resident cell provokes antigen presentation. J. Immunol. **145:**4101–4107.
41. **Planck, S. R., T. T. Dang, D. Graves, D. Tara, J. C. Ansel, and J. T. Rosenbaum.** 1992. Retinal pigment epithelial cells secrete interleukin-6 in response to interleukin-1. Invest. Ophthalmol. Visual Sci. **33:**78–82.
42. **Rosenbaum, J. T., E. L. Howes, R. M. Rubin, and J. R. Samples.** 1988. Ocular inflammatory effects of intravitreally injected tumor necrosis factor. Am. J. Pathol. **133:**47–53.
43. **Rosenbaum, J. T., J. R. Samples, S. H. Hefeneider, and E. L. Howes.** 1987. Ocular inflammatory effects of intravitreal interleukin-1. Arch. Ophthalmol. **105:**1117–1120.
44. **Sanceau, J., F. Beranger, C. Gaudelet, and J. Wietzerbin.** 1989. IFN-γ is an essential co-signal for triggering IFN-B2/BSF-2/IL-6 gene expression in human monocytic cell lines. Ann. N. Y. Acad. Sci. **557:**130–142.
45. **Sehgal, P. B., G. Grieninger, and G. Tosato.** 1989. Regulation of acute phase and immune responses: interleukin-6. Ann. N. Y. Acad. Sci. **557:**1–583.
46. **Van Snick, J.** 1990. Interleukin-6: an overview. Annu. Rev. Immunol. **8:**253–278.
47. **Volvekamp, M. C. J., and R. L. Mavaret.** 1990. Interleukin-6: historical background, genetics and biological significance. Immunol. Lett. **24:**1–10.
48. **Wakefield, D., and A. Lloyd.** 1992. The role of cytokines in the pathogenesis of inflammatory eye disease. Cytokine **4:**1–5.
49. **Whitcup, S. M., C. C. Chan, Q. Li, and R. B. Nussenblatt.** 1992. Expression of cell adhesion molecules in posterior uveitis. Arch. Ophthalmol. **110:**662–666.
50. **Young, R. W.** 1987. Pathophysiology of age-related macular degeneration. Surv. Ophthalmol. **31:**291–306.
51. **Young, R. W., and D. Bok.** 1969. Participation of retinal pigment epithelium in the rod outer segment renewal process. J. Cell Biol. **42:**392–403.
52. **Zinn, K. M., and M. F. Marmor.** 1979. The retinal pigment epithelium. Harvard University Press, Cambridge, Mass.