Clin Exp Immunol 2003; **131**:248–253

Differential cytokine expression of human retinal pigment epithelial cells in response to stimulation by C5a

Y. FUKUOKA*, M. STRAINIC* & M. E. MEDOF* **Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, USA*

(Accepted for publication 3 December 2002)

SUMMARY

Human retinal pigment epithelial (RPE) cells form part of the blood–retina barrier where they potentially can regulate leucocyte function. RPE cells are known to secrete several cytokines in response to stimulation by other cytokines. Anaphylatoxin C5a, a potent inflammatory mediator produced during complement activation, binds to G-protein coupled C5a receptors (C5aR) on monocytes/macrophages and releases various cytokines from the cells. We previously reported that the human RPE cell line ARPE-19 possesses C5aR and expresses IL-8 mRNA in response to C5a stimulation. In this study, we used a primary human RPE cell line (RPE43) and found that C5a induces increased expression of IL-1β, IL-6, MCP-1 and GM-CSF mRNAs as well as IL-8 mRNA. ARPE-19 cells showed similar increases in the same cytokines. Interestingly, the kinetics of expression of the various cytokines differed. These results provide further evidence that C5a stimulation of RPE cells may play a role in regulating leucocyte function during ocular inflammation in which there is complement activation.

Keywords C5a complement cytokine receptors retinal pigment epithelium

INTRODUCTION

Retinal pigment epithelial (RPE) cells form a blood–retina barrier which limits access of blood cells and proteins to the retina. There is evidence that RPE cell dysfunction plays a role in the pathogenesis of certain chronic ocular diseases such as proliferative vitreoretinopathy [1] and age-related macular degeneration [2], disorders in which leucocyte and macrophage infiltration is often seen [3].

Physiologically, RPE cells have specific functions including transporting nutrients to photoreceptors, and phagocytosing their shed rod outer segment (ROS) fragments [4,5]. With respect to their immunomodulatory properties, they have been reported to express Fcγ receptors (FcγRIII) and iC3b receptors (CR3) as present on traditional phagocytic cells [6], express class II antigen [7,8] and express ICAM-I [9], the latter of which can be up-regulated by IFN-γ stimulation. In response to certain cytokine stimulation, they also have been found to produce a number of other cytokines which may play important roles in ocular inflammation. Among the cytokines produced, it has been shown that they elaborate (1) IL-1β in response to IL-1α stimulation [10], (2) IL-6 in response to IL-1β or TNFα stimulation [11,12], (3) IL-8 in response to IL-1β, TNFα [13] or IL-7 stimulation [14], (4) granulocyte-macrophage colony stimulating factor (GM-CSF) in response to IL-1α stimulation [10] and (5) monocyte chemotactic protein-1 (MCP-1) in response to IL-1β, TNFα [15] or IL-7 stimulation [14].

Anaphylatoxin C5a is a 9-kD soluble complement activation fragment generated by cleavage of C5 during activation of the complement cascade. C5a is a potent inflammatory factor, i.e. active at 10 pM (in chemotaxis), with several biological functions including (1) induction of smooth muscle contraction (2) augmentation of vascular permeability, and (3) release of histamine from mast cells and basophils [reviewed in [16]]. C5a has potent chemotactic activity for polymorphonuclear cells (PMN) [17], monocytes [18], eosinophils [19,20], T lymphocytes [21] and mast cells [22,23]. C5a stimulates a respiratory burst in PMN [24], up-regulates CR3 expression on PMN [25], induces P-selectin expression on endothelial cells [26,27] and increases adhesion of eosinophils to endothelial cells [28]. C5a is also a potent immune regulatory factor that activates several cell types to release cytokines. It activates macrophages/monocytes to release IL-1β and TNFα [29,30] as well as IL-6 [31] and IL-8 [32].

C5a stimulates cells via interaction with C5a receptors (C5aR). These receptors belong to a family of G-protein coupled receptors with seven transmembrane segments [33,34]. Although C5aR traditionally were believed to be expressed only on blood phagocytes and a limited number of other cell types (see Discussion), recent studies have shown that C5aR are also present on endothelial and epithelial cells in the lung [35], mesangial cells in the kidney [36], as as well as astrocytes, microglia and neurons in the brain [37–40].

Correspondence: M. Edward Medof, Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Cleveland, Ohio 44106, USA.

Present address for Y. Fukuoka: Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA, USA.

E-mail: mxm16@po.cwru.edu

Previously we showed that ARPE-19 cells (a well-established ATCC human RPE cell line) possess C5aR and exhibit increased expression of IL-8 mRNA in response to C5a stimulation [41]. In this investigation, we examined the effect of C5a stimulation on a primary cultured human RPE cell line with an emphasis on more closely approximating the *in vivo* situation and assaying for the expression of other cytokines in addition to IL-8. We found that C5a induces increases of IL-1β, IL-6, MCP-1 and GM-CSF mRNAs in these cells, as well as IL-8 as in the ARPE-19 cell line. In the cases of IL-8 and IL-6, we documented that protein production paralleled the mRNA increases. These results further support the proposition that C5a effects on RPE cells may be important in regulating inflammatory and immune responses in ocular diseases.

MATERIALS AND METHODS

Reagents and cells

Human C5a was obtained from Advanced Research Technologies (San Diego, CA, USA). It was prepared using methods excluding LPS contamination [42]. Rabbit anti-human C5aR antibody produced against C5aR's 21 N-terminal amino acids [43] was a gift from Dr T.E. Hugli (La Jolla Institute for Molecular Medicine, San Diego, CA, USA). L cell transfectants expressing human C5aR were also a gift of Dr T.E. Hugli. Human IL-6 and IL-8 proteins were quantified using Quantikine kits (R & D systems, Minneapolis, MN, USA). Mouse anti-human C5aR mAb was obtained from BD-Pharmingen, San Diego, CA. The primary human RPE cell line (RPE43) was kindly provided by Dr D.N. Henry (Michigan State University) [44,45]. Cells at 6 passages in RPMI-1640 medium containing 2 mM L-glutamine, 10% fetal calf serum (FBS) and 5 mM glucose were plated at a density of 20 000 cells/cm^2 in 100 mm dishes and grown at 37°C in humidified air with 5% CO_2. The ARPE-19 cell line was obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained in a 1 : 1 mixture of Dulbecco's modified Eagle's medium (DMEM): F12 containing 10% FBS and 2 mM L-glutamine. In some experiments, 0·4 μm pore size Transwell filters (Costar, Cambridge, MA, USA) coated with 10 μg/cm^2 of purified mouse laminin (Collaborative Biomedical Products, Bedford, MA, USA) were used and the RPE-43 cells plated on the coated filters [46].

FACS analyses

RPE43 cells or freshly isolated PMN (1×10^6) were suspended in fluorescence-activated cell sorter (FACS) buffer containing 10% FBS, 10 mg/ml goat IgG, 5 mM EDTA and 0·1% NaN_3 with 3 μg/ml rabbit anti-human C5aR antibody or control rabbit IgG, and the mixtures incubated for 1 h on ice. After washing with cold phosphate-buffered saline (PBS) containing 0·1% NaN_3, the cells were suspended in 100 μl of the same buffer containing 1 : 50 diluted fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Sigma Chemicals, St. Louis, MO, USA) in FACS buffer and the mixture incubated for 1 h on ice. The washed cells were analysed on a FACScan flow cytometer (Becton-Dickinson, San Jose, CA, USA).

Cell stimulation and RT-PCR

RPE43 cells ($1{\cdot}5 \times 10^6$) were stimulated with human C5a in serum-free medium for the indicated times. Total RNA was extracted using TRIzol reagent (Life Technologies, Rockville, MD, USA) according to the manufacturer's instructions. Following treatment for 20 min at 37°C with RQ-1 RNase-free DNAse (Promega, Madison, WI, USA), cDNA was prepared from 5 μg of RNA using Oligo(dT)12–18 primers and the SuperScript Preamplification System for first strand cDNA synthesis (Gibco BRL, Gaithersburg MD, USA). Primers for human IL-1β, IL-8, GM-CSF, and glyceroaldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Stratagene (La Jolla, CA, USA). Primers for MCP-1 [47] and C5aR [33] were designed according to the reported sequences. The primers used are listed on Table 1.

Semiquantitative RT-PCR was carried out with 1 μl of cDNA and 1 μl of Taq DNA polymerase (Promega) in a 50-μl final volume. In preliminary experiments, PCR for each product was performed at 25, 30, 35 and 40 cycles. The cycle number used for semiquantitative measurements was then selected based on the amplification results falling in the mid-linear portion of the density vs. cycle response curve, using the average for all of the time points for each cytokine, respectively. For IL-1β, 40 cycles were used; for IL-6, 35 cycles; for IL-8, 30 cycles; for GM-CSF, 30 cycles; for MCP-1, 35 cycles; and for GAPDH, 25 cycles. The PCR conditions used were: denaturation for 3 min at 94°C, 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 60°C with extension for 2 min at 72°C, and final elongation at 72°C for 10 min. PCR was performed on a model 480 DNA thermal cycler

Table 1. Oligonucleotide primers

Gene	Orientation	Primer	Product size (bp)
IL-1β	sense	5′ CTTCATCTTTGAAGAAGAACCTATCTTCTT 3′	332
	antisense	5′ AATTTTTGGGATCTACACTCTCCAGCTGTA 3′	
IL-6	sense	5′ GATGGATGCTTCCAATCTGGAT 3′	450
	antisense	5′ AGTTCTCCATAGAGAACAACATA 3′	
IL-8	sense	5′ CGATGTCAGTGCATAAAGACA 3′	200
	antisense	5′ TGAATTCTCAGCCCTCTTCAAAAA 3′	
GM-CSF	sense	5′ ACACTGCTGAGATGAATGAAACAGTAG 3′	286
	antisense	5′ TGGACTGGCTCCCAGCAGTCAAAGGGGATG 3′	
MCP-1	sense	5′ GATCTCAGTGCAGAGGCTCG 3′	152
	antisense	5′ TGCTTGTCCAGGTGGTCCAT 3′	
C5aR	sense	5′ ACCACCCCTGATTATGGGCACTATGATGAC 3′	420
	antisense	5′ GTTCTGGCACCAGATGGGTTTAAACACCAG 3′	
GAPDH	sense	5′ CCACCCATGGCAAATTCCATGGCA 3′	600
	antisense	5′ TCTAGACGGCAGGTCAGGTCCACC 3′	

(Perkin Elmer/Roche Molecular Systems, Inc., Branchburg, NJ, USA). Products were analysed on 2% agarose gels stained with ethidium bromide. All experiments were repeated at least three times.

ELISAs

Aliquots of the culture supernatants of C5a (30 nM)-stimulated RPE43 cells were removed at the indicated times. Quantifications of IL-6 and IL-8 were performed by specific ELISAs (R & D Systems) according to the manufacturer's instructions.

RESULTS

Analysis of C5aR by FACS

In initial studies to establish whether RPE43 primary culture cells, like the ARPE-19 line, express C5aR [41], the cells were stained with anti-human C5aR antibody and analysed by flow cytometry. As shown in Fig. 1, the RPE43 cells showed a significant shift relative to the nonrelevant IgG control. For comparative purposes, control studies were performed under identical conditions with PMN on which C5aR have been extensively studied [43]. Correlation of the results showed that the C5aR level on the RPE43 cells was ~33% of that of unstimulated PMN (not shown). Expression of C5aR by the RPE43 cells was independently documented by RT-PCR using primers (Table 1) for human C5aR mRNA (not shown). It additionally was confirmed on Western blots in which identical bands of the expected M_r (45–50 kD) were visualized in extracts of RPE43 and L cell transfectants expressing human C5aR, but not in untransfected L cells (not shown.)

Expression of cytokine mRNA

In the next set of studies, the effect of C5a stimulation of the cells was assessed. Based on previous dose–response curves in the APRE-19 line which showed a maximum effect at 100 nM C5a, the RPE43 cells were stimulated with 30 nM of C5a for 3, 6, 9, 24 h. Expression of IL-8, GM-CSF, IL-1β, IL-6, MCP-1 and GAPDH mRNA was then measured by semiquantitative RT-PCR. In the case of each of the cytokines, mRNA levels increased relative to constitutive levels. However, as shown in Fig. 2, the kinetics of maximum expression of each cytokine differed. The maximum increase of IL-8 mRNA was observed at 9 h, that of GM-CSF at 9 h, that of IL-1β at 3 h, that of IL-6 at 3 h, and that of MCP-1 at 24 h. Equivalent data were obtained in three independent experiments. The increase in IL-8 mRNA was inhibited by overnight pretreatment with 1 nM pertussis toxin verifying the specificity of the effect. When ARPE-19 cells were substituted for RPE43 cells and the cells identically stimulated by C5a, similar increases in mRNAs for each of the cytokines were measured and

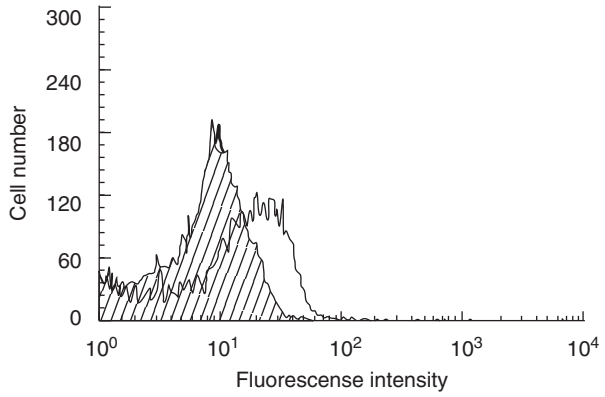

Fig. 1. FACS analysis of RPE43 cells. RPE cells were stained by rabbit anti-human C5aR IgG and FITC-conjugated goat anti-rabbit IgG. Shaded pattern indicates rabbit IgG control.

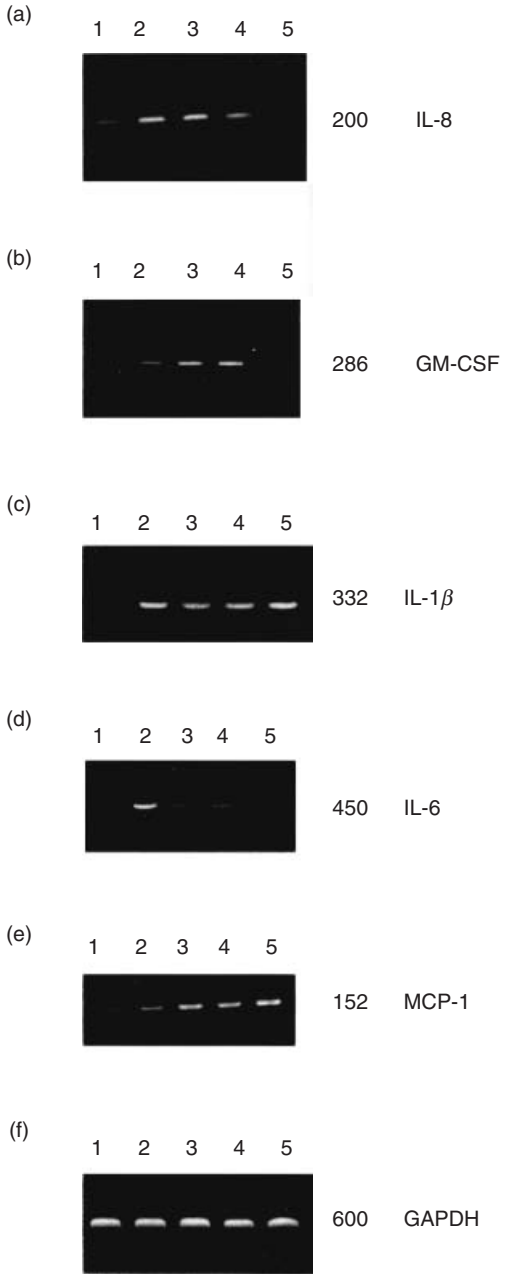

Fig. 2. RT-PCR analyses of mRNA expression of IL-8, GM-CSF IL-1β, IL-6, and MCP-1 following stimulation of RPE43 cells by C5a. RPE43 cells were stimulated with 50 nM human C5a for 3,6,9, and 24 h. Lane 1 = 0 time. Lanes 2, 3, 4, and 5 = 3, 6, 9, and 24 h (a) IL-8 mRNA expression. (b) GM-CSF mRNA expression. (c) IL-1 mRNA expression. (d) IL-6 mRNA expression. (e) MCP-1 mRNA expression. (f) GAPDH mRNA expression at the same time points.

the same kinetics were observed (not shown) as recorded for the primary RPE43 line.

Release of cytokine protein

To document that the increases in mRNAs did result in increased cytokine production, two representative cytokines, IL-6 and IL-8 were studied. For each cytokine, protein levels released in the culture supernatant after stimulation by C5a were measured by specific sandwich ELISA. As shown in the Table 1, the levels of both proteins increased in a time-dependent fashion.

Table 2. Time-dependent increases of IL-6 and IL-8 protein released from primary cultures of human RPE cells

	Time (h)			
Protein	3	6	9	24
IL-6	87·3	120·1	179·5	170·1
IL-8	16·2	33·3	53·4	109·6

Values are in pg/ml. Cells were exposed to 30 nM human C5a at 37°C. Levels of IL-6 and IL-8 at time zero were <0·1 pg/ml. Levels for IL-6 at 9 h and for IL-8 at 24 h in the absence of C5a were <30% of the values shown.

DISCUSSION

C5a is a potent mediator of inflammation which activates cells via C5aR, a G-protein coupled receptor. It binds to C5aR through its C-terminal region. As indicated, historically C5a initially was thought to stimulate only phagocytic blood elements (PMN and peripheral blood mononuclear cells (PBMC)), macrophages and mast cells. However, the cloning of C5aR cDNA and production of specific anti-C5aR antibody revealed that a number of other cells also express C5aR. Recent findings that C5aR are expressed on several brain cell types [38,39] prompted us to examine whether C5aR are present within the eye, and as a consequence, C5a ligation could play a role in ocular inflammation.

RPE cells are multifunctional cells which are uniquely positioned within the eye. They have properties similar to macrophages among which are the capacity to phagocytose and the ability to generate several cytokines including IL-1β, IL-6 and IL-8 [10–13]. By ingesting degenerating ROS, they play an essential role in maintaining photoreceptor health [4,5]. They are situated between the retina and the uveal vasculature which contains serum proteins and leucocytes. In several ocular inflammatory disorders, PMN and monocyte infiltration are usually seen.

In this study, we showed that C5a stimulates RPE43 cells derived from a primary RPE cell culture to increase mRNA levels of IL-1β, IL-6, IL-8, MCP-1 and GM-CSF. We previously reported that C5a was able to cause the APRE-19 cell line to increase mRNA levels of IL-8 [41]. RPE43 primary culture cells have properties closely simulating those of *in vivo* RPE cells, and thus avoid any alterations that could occur in the established ATCC APRE-19 line. With the use of RPE43 cells in the present study, in addition to four other cytokine mRNAs, we showed that IL-8 mRNA is similarly produced, confirming our former results.

IL-1β and IL-6 are important mediators of the acute phase response and possess biological functions that support host immune reactions. It has been reported that IL-1β is generated in low levels in response to C5a stimulation of PBMC [48,49], and its production is synergistically enhanced by endotoxin (LPS) and IFNγ [29]. Our results showed that IL-1β mRNA expression by RPE cells in response to stimulation by C5a similarly is weak, its detection needing 5 more cycles of PCR than the other cytokines identified. Based on the findings with PBMC, it is possible that synergistic effects of LPS and/or other cytokines in the vicinity of RPE cells could have effects similar to those on PBMC. It has been reported that C5a can stimulate human astrocytes to increase mRNA levels of IL-6 without protein production [50]. In view of this, we tested for IL-6 protein by ELISA and recovered it in the RPE cell supernatant (Table 2). Scholz *et al.* [31] also reported IL-6 protein production by human PBMC in response to C5a stimulation. The reason for these contradictory results is not clear. As indicated in the Results, differences in the kinetics of expression of the mRNAs for some of the cytotoxins were found. It is possible, for example, that the release of IL-1 could function in a paracrine or autocrine fashion to cause the release of MCP-1.

IL-8 and MCP-1 are known to have strong chemotactic activity for PMN and monocytes/macrophages, respectively. Since the infiltration of both cell types is commonly seen in many ocular diseases, these cytokines may play important roles in the initial stage of inflammation. Although the primary activity of GM-CSF is its haematopoietic myeloid stem cell stimulating function, it also induces the proliferation and differentiation of PMN, enhances the responsiveness of PMN to secondary stimuli, and induces the release of several cytokines from monocytes [51]. GM-CSF thus can enhance the activity of infiltrating cells both directly and secondarily by virtue of stimulating the release of cytokines.

Although we identified C5aR on primary cultured RPE cells and showed that C5a induces the generation of several cytokines in this report, there may be other roles that C5a has in the eye. In view of findings that neurons express C5aR [39,40] and a recent report [52] that C5a has a protective effect in glutamate-mediated neurotoxicity in the brain, it is possible that in addition to being an inducer/regulator of inflammation, it may have a similar role on RPE cells or other C5aR$^+$ cells in the eye. Another recent report has shown that C5a is required for hepatocyte regeneration after toxic injury [53]. If C5a can function as a growth stimulator in the eye, it could play a role in protecting against certain degenerative processes.

ACKNOWLEDGEMENTS

Supported by National Institutes of Health grant R01 EY11288. The authors thank Dr T. E. Hugli for anti-human C5aR antibody and human C5aR transfected L cells, Dr D. N. Henry for the primary human RPE line (RPE43), Sara Cechner for manuscript preparation, and Beth Ann Benetz in the Visual Science Photography lab for help with figures.

REFERENCES

1 Wiedemann P. Growth factors in retinal diseases. proliferative vitreoretinopathy, proliferative diabetic retinopathy, and retinal degeneration. Surv Ophthalmol 1992; **36**:373–84.

2 Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 2000; **14**:835–46.
3 Weller M, Heimann K, Wiedemann P. Immunochemical studies of epiretinal membranes using APAAP complexes: evidence for macrophage involvement in traumatic proliferative vitreoretinopathy. Int Ophthalmol 1988; **11**:181–6.
4 Young RW, Bok D. Participation of the retinal pigment epithelium in the rod outer segment renewal process. J Cell Biol 1969; **42**:392–403.
5 Edwards RB, Szamier RB. Defective phagocytosis of isolated rod outer segments by RCS rat retinal pigment epithelium in culture. Science 1977; **197**:1001–3.
6 Elner VM, Schaffner T, Taylor K, Glagov S. Immunophagocytic properties of retinal pigment epithelium cells. Science 1981; **211**:74–6.
7 Liversidge JM, Sewell HF, Forrester JV. Human retinal pigment epithelial cells differentially express MHC class II (HLA, DP, DR and DQ) antigens in response to in vitro stimulation with lymphokine or purified IFN-gamma. Clin Exp Immunol 1988; **73**:489–94.
8 Casella AM, Taba KE, Kimura H *et al.* Retinal pigment epithelial cells are heterogeneous in their expression of MHC-II after stimulation with interferon-gamma. Exp Eye Res 1999; **68**:423–30.
9 Elner SG, Elner VM, Pavilack MA *et al.* Modulation and function of intercellular adhesion molecule-1 (CD54) on human retinal pigment epithelial cells. Laboratory Invest 1992; **66**:200–11.
10 Planck SR, Huang XN, Robertson JE, Rosenbaum JT. Retinal pigment epithelial cells produce interleukin-1 beta and granulocyte-macrophage colony-stimulating factor in response to interleukin-1 alpha. Curr Eye Res 1993; **12**:205–12.
11 Elner VM, Scales W, Elner SG, Danforth J, Kunkel SL, Strieter RM. Interleukin-6 (IL-6) gene expression and secretion by cytokine-stimulated human retinal pigment epithelial cells. Exp Eye Res 1992; **54**:361–8.
12 Planck SR, Dang TT, Graves D, Tara D, Ansel JC, Rosenbaum JT. Retinal pigment epithelial cells secrete interleukin-6 in response to interleukin-1. Invest Ophthalmol Vis Sci 1992; **33**:78–82.
13 Elner VM, Strieter RM, Elner SG, Baggiolini M, Lindley I, Kunkel SL. Neutrophil chemotactic factor (IL-8) gene expression by cytokine-treated retinal pigment epithelial cells. Am J Pathol 1990; **136**:745–50.
14 Elner VM, Elner SG, Standiford TJ, Lukacs NW, Strieter RM, Kunkel SL. Interleukin-7 (IL-7) induces retinal pigment epithelial cell MCP-1 and IL-8. Exp Eye Res 1996; **63**:297–303.
15 Elner SG, Strieter RM, Elner VM, Rollins BJ, Del Monte MA, Kunkel SL. Monocyte chemotactic protein gene expression by cytokine-treated human retinal pigment epithelial cells. Laboratory Invest 1991; **64**:819–25.
16 Ember JA, Jagels MA, Hugli TE. Characterization of complement anaphylatoxins and their biological responses. In: Volanakis JE, Frank MM, eds. The Human Complement System in Health and Disease. New York: Marcel Dekker, 1998:241–84.
17 Fernandez HN, Henson PM, Otani A, Hugli TE. Chemotactic response to human C3a and C5a anaphylatoxins. I. Evaluation of C3a and C5a leukotaxis *in vitro* and under stimulated *in vivo* conditions. J Immunol 1978; **120**:109–15.
18 Pieters WR, Houben LA, Koenderman L, Raaijmakers JA. C5a-induced migration of human monocytes is primed by dexamethasone. Am J Respir Cell Mol Biol 1995; **12**:691–6.
19 Morita E, Schroder JM, Christophers E. Differential sensitivities of purified human eosinophils and neutrophils to defined chemotaxins. Scand J Immunol 1989; **29**:709–16.
20 Daffern PJ, Pfeifer PH, Ember JA, Hugli TE. C3a is a chemotaxin for human eosinophils but not for neutrophils. I. C3a stimulation of neutrophils is secondary to eosinophil activation. J Exp Med 1995; **181**:2119–27.
21 Nataf S, Davoust N, Ames RS, Barnum SR. Human T cells express the C5a receptor and are chemoattracted to C5a. J Immunol 1999; **162**:4018–23.
22 Nilsson G, Johnell M, Hammer CH *et al.* C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. J Immunol 1996; **157**:1693–8.
23 Hartmann K, Henz BM, Kruger-Krasagakes S *et al.* C3a and C5a stimulate chemotaxis of human mast cells. Blood 1997; **89**:2863–70.
24 Ehrengruber MU, Geiser T, Deranleau DA. Activation of human neutrophils by C3a and C5A. Comparison of the effects on shape changes, chemotaxis, secretion, and respiratory burst. FEBS Lett 1994; **346**:181–4.
25 Monk PN, Barker MD, Partridge LJ. Multiple signalling pathways in the C5a-induced expression of adhesion receptor Mac-1. Biochim Biophys Acta 1994; **1221**:323–9.
26 Foreman KE, Vaporciyan AA, Bonish BK *et al.* C5a-induced expression of P-selectin in endothelial cells. J Clin Invest 1994; **94**:1147–55.
27 Mulligan MS, Schmid E, Till GO *et al.* C5a-dependent up-regulation in vivo of lung vascular P-selectin. J Immunol 1997; **158**:1857–61.
28 DiScipio RG, Daffern PJ, Jagels MA, Broide DH, Sriramarao P. A comparison of C3a and C5a-mediated stable adhesion of rolling eosinophils in postcapillary venules and transendothelial migration in vitro and in vivo. J Immunol 1999; **162**:1127–36.
29 Okusawa S, Dinarello CA, Yancey KB *et al.* C5a induction of human interleukin 1. Synergistic effect with endotoxin or interferon-γ. J Immunol 1987; **139**:2635–40.
30 Okusawa S, Yancey KB, van der Meer JW *et al.* C5a stimulates secretion of tumor necrosis factor from human mononuclear cells *in vitro*. Comparison with secretion of interleukin 1 α and interleukin 1 α. J Exp Med 1988; **168**:443–8.
31 Scholz W, McClurg MR, Cardenas GJ *et al.* C5a-mediated release of interleukin 6 by human monocytes. Clin Immunol Immunopathol 1990; **57**:297–307.
32 Ember JA, Sanderson SD, Hugli TE, Morgan EL. Induction of interleukin-8 synthesis from monocytes by human C5a anaphylatoxin. Am J Pathol 1994; **144**:393–403.
33 Gerard NP, Gerard C. The chemotactic receptor for human C5a anaphylatoxin. Nature 1991; **349**:614–7.
34 Boulay F, Mery L, Tardif M, Brouchon L, Vignais P. Expression cloning of a receptor for C5a anaphylatoxin on differentiated HL-60 cells. Biochemistry 1991; **30**:2993–9.
35 Haviland DL, McCoy RL, Whitehead WT *et al.* Cellular expression of the C5a anaphylatoxin receptor (C5aR): demonstration of C5aR on nonmyeloid cells of the liver and lung. J Immunol 1995; **154**:1861–9.
36 Wilmer WA, Kaumaya PT, Ember JA, Cosio FG. Receptors for the anaphylatoxin C5a (CD88) on human mesangial cells. J Immunol 1998; **160**:5646–52.
37 Gasque P, Chan P, Fontaine M *et al.* Identification and characterization of the complement C5a anaphylatoxin receptor on human astrocytes. J Immunol 1995; **155**:4882–9.
38 Gasque P, Singhrao SK, Neal JW, Gotze O, Morgan BP. Expression of the receptor for complement C5a (CD88) is up-regulated on reactive astrocytes, microglia, and endothelial cells in the inflamed human central nervous system. Am J Pathol 1997; **150**:31–41.
39 Stahel PF, Frei K, Eugster HP *et al.* TNF-α-mediated expression of the receptor for anaphylatoxin C5a on neurons in experimental Listeria meningoencephalitis. J Immunol 1997; **159**:861–9.
40 O'Barr SA, Caguioa J, Gruol D *et al.* Neuronal expression of a functional receptor for the C5a complement activation fragment. J Immunol 2001; **166**:4154–62.
41 Fukuoka Y, Medof MEC. 5a receptor-mediated production of IL-8 by the human retinal pigment epithelial cell line, ARPE-19. Curr Eye Res 2001; **23**:320–25.
42 Hugli TE, Gerard C, Kawahara M *et al.* Isolation of three separate anaphylatoxins from complement-activated human serum. Mol Cell Biochem 1981; **41**:59–66.

43 Morgan EL, Ember JA, Sanderson SD *et al.* Anti-C5a receptor antibodies. I. Characterization of neutralizing antibodies specific for a peptide, C5aR-(9–29), derived from the predicted amino-terminal sequence of the human C5a receptor. J Immunol 1993; **151**:377–88.

44 Henry DN, Del Monte M, Greene DA, Killen PD. Altered aldose reductase gene regulation in cultured human retinal pigment epithelial cells. J Clin Invest 1993; **92**:617–23.

45 Henry DN, Frank RN, Hootman SR, Rood SE, Heilig CW, Busik JV. Glucose-specific regulation of aldose reductase in human retinal pigment epithelial cells in vitro. Invest Ophthalmol Vis Sci 2000; **41**:1554–60.

46 Dunn KC, Aotaki-Keen AE, Putkey FR, Hjelmeland LM. ARPE-19, a human retinal pigment epithelial cell line with differentiated properties. Exp Eye Res 1996; **62**:155–69.

47 Yoshimura T, Yuhki N, Moore SK, Appella E, Lerman MI, Leonard EJ. Human monocyte chemoattractant protein-1 (MCP-1). Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. FEBS Lett 1989; **244**:487–93.

48 Schindler R, Gelfand JA, Dinarello CA. Recombinant C5a stimulates transcription rather than translation of interleukin-1 (IL-1) and tumor necrosis factor: translational signal provided by lipopolysaccharide or IL-1 itself. Blood 1990; **76**:1631–8.

49 Kaspar RL, Gehrke L. Peripheral blood mononuclear cells stimulated with C5a or lipopolysaccharide to synthesize equivalent levels of IL-1 beta mRNA show unequal IL-1 beta protein accumulation but similar polyribosome profiles. J Immunol 1994; **153**:277–86.

50 Sayah S, Ischenko AM, Zhakhov A, Bonnard AS, Fontaine M. Expression of cytokines by human astrocytomas following stimulation by C3a and C5a anaphylatoxins: specific increase in interleukin-6 mRNA expression. J Neurochem 1999; **72**:2426–36.

51 Ruef C, Coleman DL. Granulocyte-macrophage colony-stimulating factor: pleiotropic cytokine with potential clinical usefulness. Rev Infect Dis 1990; **12**:41–62.

52 Osaka H, Mukherjee P, Aisen PS, Pasinetti GM. Complement-derived anaphylatoxin C5a protects against glutamate-mediated neurotoxicity. J Cell Biochem 1999; **73**:303–11.

53 Mastellos D, Papadimitriou JC, Franchini S, Tsonis PA, Lambris JD. A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration. J Immunol 2001; **166**:2479–86.