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REVIEW ARTICLE

Roles of IL-8 in Ocular Inflammations: A Review

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ABSTRACT

Introduction: This review presents the current in vitro and in vivo animal and human research on the roles of IL-8 in ocular inflammatory diseases.

Materials and Methods: Data sources were a literature review using Pub Med, Medline, and ISI databases (from 1990 to 2011). Search items included interleukine-8 (IL-8), CXCL8, chemokines, cytokines, alone or in combination with the, serum, aqueous, vitreous, eye, ocular, ocular tissues, ophthalmic, and review.

Results: IL-8 may be involved in primary or secondary ocular inflammations. Ocular effects of IL-8 differ based on the source of the secretion and site of the action. The most important effects of IL-8 in the eyes are angiogenic activities and induction of ocular inflammation.

Conclusion: IL-8 plays important roles in ocular inflammation and angiogenesis in conjunctiva, cornea, iris, retina, and orbit. Anti-IL-8 targeted immunotherapy has been introduced as an important treatment modality, provided that IL-8 signal blocking takes place in desired areas and tissues.

Keywords: angiogenesis, chemokine, cytokine, interleukine-8, ocular inflammation

Interleukin 8, the first chemokine to be characterized, was discovered in 1987.[1] IL-8, known as a pro-inflammatory chemokine, induces accumulation of neutrophils along the vascular wall. The release of this cytokine is triggered by especial inflammatory signals from different types of cells. This diversity indicates multiple functionality of this cytokine. IL-8 has a key role in the defense mechanism through the effects on neutrophil activity, but continued and prolonged presence of IL-8 in circulation in response to inflammatory conditions may cause variable degrees of tissue injuries. Like most of the peptide hormones or mediators, IL-8 transmits its signals through proper cell surface heptahelica receptors. In addition, IL-8 stimulates the mitogen-activating protein kinase (MAPK) and tyrosine phosphorylation of cellular proteins. Blocking of IL-8 actions could be considered for therapeutic purposes.[2] The primary receptor-binding domain of all chemokines are near terminal NH2, and its antagonists can be obtained by truncation or substitutions in this region.[1] Chemokines or chemotactic cytokines are structurally small (7–14 kDa) protein molecules regulating various types of cells trafficking through interactions with 7-transmembrane, G protein coupled receptors. To date, about 50 chemokines have been identified in humans. Based on the arrangement of N-terminal cysteine residues, chemokines can be divided into two major subfamilies, CC and CXC, depending on whether the first two cysteine residues are next to each other (CC) or have an amino acid linking them (CXC). Two small groups, CX3C chemokines and C chemokines, also have been identified.[3] The CX3C formula (fractalkine) has 4–6 cysteines and is the only membrane-bound chemokine with a mucin-like stalk, and the C chemokine (lymphotactin), unlike the typical chemokine structure, lacks the first and third cysteines in its formula.[4]

Ocular effects of IL-8 differ based on the source of production and the site of action. As expected, IL-8 plays many inflammatory roles in the ocular tissues that have been partially discovered and many others will be discovered in the future. One of the exciting effects of IL-8 is the angiogenic activity in any parts of the eye. Chemokines regulate angiogenesis in a

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receptor-dependent manner, for instance, CXCL8/IL8 as a pro-angiogenic chemokines, interacting with the CXCR2 receptor and interferon-inducible protein related to IL-8 (CXCL10/IP10) as an anti-angiogenic (i.e., angiostatic) chemokine, interacting with the CXCR3 receptor. Some chemokines also regulate angiogenesis in a receptor-independent manner such as bFGF and VEGF.[5] To the best of our knowledge there are no ophthalmic-related IL-8 review articles in the medical literature. Therefore, the purpose of this review is to clarify the complexity and the functional diversity of IL-8 in ocular inflammatory disorders.

MATERIALS AND METHODS

The data sources were a literature review using Pub Med, Medline, and ISI databases (from 1990 to 2011). Search items included interleukin-8 (IL-8), CXCL8, chemokines, cytokines, alone or in combination with the, serum, aqueous, vitreous, eye, ocular, ocular tissues, ophthalmic, and review. More than 19,000 articles related to IL-8 are present in the pub Med, Medline, and ISI databases, in which more than 850 articles relating to the eye, ocular, and ophthalmic items were recognized and were considered as the general basic data framework. About 385 papers were more relevant to the eyes and were used as the ocular data bank; finally, 131 full text articles were selected and used in this review. Selected language was in English. Human beings and animals as in vitro and in vivo studies were included to review the mechanisms of action and clinical effects of IL-8 focused on ocular manifestations of this cytokine in human ocular diseases.

INTERLEUKIN-8 STRUCTURE, RECEPTORS, AND SIGNALING

IL-8 is the most recognized CXC chemokine. The molecular structure of IL-8 has been determined by X-ray crystallography[6] and nuclear magnetic resonance spectroscopy.[7] The three-dimensional structure of IL-8 forms a homo-dimer in vitro at high concentrations. Each monomer helix comprises an N-terminal loop, three β-strand loops, and a C-terminal. The monomer strand has full action on neutrophils and its three-dimensional form acts like the subunits of the IL-8 dimers.[8] Beta-1 and β2 regions of the loop are potentially involved in receptor recognition and residue mutations[9] (Figure 1). The N-terminal ELR motif preceding the first cysteine has a major role in binding and activation of the cytokine.[10] Transmitted signals across the cellular membranes induce structural changes and expose the epitopes on the intracellular loops and C-terminal at the end of the receptor. These epitopes facilitate the coupling with the functional heterotrimeric G proteins.[11,12] At least 18 human chemokine

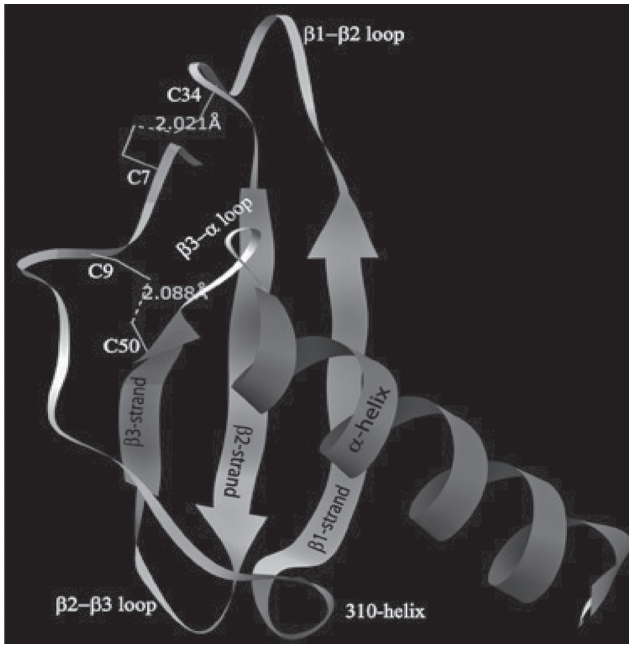

FIGURE 1 Human three-dimensional structure of interleukin-8. The ribbons include two helices (red), one before and the second after the three β-strands (green) forming a β-sheet. The hydrogen bonding between the strands that are anti-parallel forms a β-sheet. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081). Reprinted from IOS Press BV, 8(3-4), Kanagarajadurai K, Sowdhamini R, Sequence and structural analyses of interleukin-8-like chemokine superfamily, 307-30, Copyright (2008), with permission from IOS Press.[9]

receptors have been identified to date.[13] There are some limited overlaps in binding of CXC chemokines to their receptors (i.e., CXCRs): some receptors bind to more than one chemokine and some chemokines bind to more than one receptor. These receptors have been found to be located on leukocytes, endothelial cells, epithelium, neurons, astrocytes, and microglia in the brain, as well as on a variety of tumor cells.[14–16] Chemokine receptors are classified similarly according to which group of chemokines they bind and are classified as CXCR1–CXCR6, CCR1–CCR11, CX3CR1, and XCR1.[17] CXCL8 bind to CXCR1 with more affinity than CXCR2[9] (Figure 2). Another chemokine receptor, that is located on red blood cells (and to a lesser extent on endothelial and epithelial cells) is a 40- to 45-kDa glycosylated protein expressed as Duffy antigen receptor for cytokines (DARC).[18] It is possible that this receptor participates in chemokine transport[19] (Figure 2).

INTERLEUKIN-8 SUPER FAMILY

Because of the potent pro-inflammatory properties of IL-8, this chemokine is tightly regulated, and its expression is low or undetectable in normal tissues.[19] IL-8 is a small, soluble peptide with an 8- to 10-kDa molecular weight. The chemokine family has been divided into

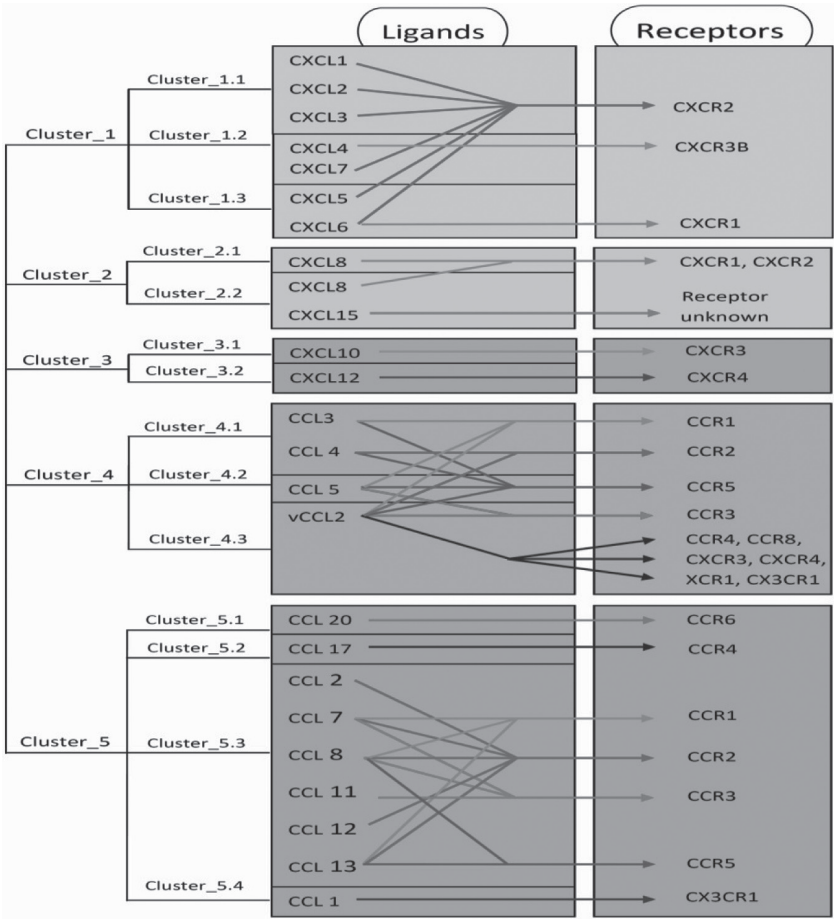

FIGURE 2 Cluster and subclusters of CXC and CC chemokines (left sided) and their ligands and corresponding receptors (right sided). Clusters 1, 2, and 3 (Megacluster A) include members of CXCL1 to CXCL8, CXCL10, CXCL12, and CXCL15. Clusters 4 and 5 (Megacluster B) include mainly CC subfamily members and few CX3C subfamily members. Reprinted from IOS Press BV, 8(3-4), Kanagarajadurai K, Sowdhamini R, Sequence and structural analyses of interleukin-8-like chemokine superfamily, 307-30, Copyright (2008), with permission from IOS Press.[9]

four major groups, CXC, CC, C, and CX3C, with individual classification based on the number and location of conserved cysteines within the N-terminal amino acid sequence.[1]

Interleukin-8 is the prototype of the CXC group. The other members include growth-regulated oncogene (GRO) α, β, and γ (CXCL1–3), platelet factor 4 (PF4; CXCL4), epithelial-cell-derived neutrophil-activating protein (ENA78; CXCL5), granulocyte chemotactic protein-2 (GCP-2; CXCL6), neutrophil-activating protein-2 (NAP-2; CXCL7), monokine induced by interferon (Mig; CXCL9), interferon-inducible protein 10 (IP-10; CXCL10), interferon-inducible T-cell α chemoattractant (I-TAC; CXCL11), stromal-cell-derived factor-1 (SDF-1; CXCL12), BCA-1 (CXCL13), and BRAK (CXCL14). Human genes for CXC group are clustered on chromosome 4 between 4q12 and 4q21.[19] About 252 interleukin-8 proteins and homologues have been recognized, which are shared among humans, birds, and fishes. The sequences can be organized into five major known clusters. IL-8 belongs to the second cluster in which two subclusters for mammalian and avian have been considered[9] (Figure 2). IL-8 and related chemokines bind to receptors belonging to the large family of G-protein-coupled receptors. Some of them cause cell migration, such as neutrophils and eosinophils, and some are implicated in angiogenic activities. More than 40 subfamilies of these ligands are known that share poor sequence similarity and display receptor specificity. Small fold of the ligand could display remarkable receptor specificity. Beta-1 and β2 loops of the fold are potentially involved in receptor recognition and could be potential sites for mutations.[9]

SOURCES AND INTERACTION

Among the first chemokines discovered, IL-8 was identified as a chemotactic factor secreted by activated monocytes and macrophages that promotes the directional migration of neutrophils, basophils, and T lymphocytes.[20,21] Human mast cells, macrophages, and neutrophils in response to proper stimuli secrete several cytokines, including IL-8.[22,23] IL-8 is expressed

by vascular endothelial cells, smooth muscle cells, and macrophages and is a potent chemoattractant for circulating monocytes and T cells. It has been also shown to contribute in smooth muscle cell proliferation and migration.[24] Despite the fact that IL-8 has been thought to act predominantly on neutrophils, it induced firm adhesion of rolling monocytes to endothelial monolayers expressing E-selectin.[25]

Treatment of monocytes with IL-2 increased IL-8 mRNA expression, but IFN-gamma, which is also a potent monocyte activator, not only failed to induce IL-8 expression but inhibited the stimulation of IL-8 by IL-2.[26] Lipopolysaccharides, IL-1β, and TNF-α are able to increase IL-8 production, in contrast to, IL-10 is a potent inhibitor of IL-8 synthesis and appears to play an antagonism role for IL-8.[27] IL-17, a cytokine represents an early event in the development of the inflammatory reaction. IL-17 selectively increased the secretion of an array of angiogenic CXC chemokines, including CXCL1, CXCL5, CXCL6, and CXCL8, and its ability to recruit neutrophils may also explain its pro-angiogenic activity.[28]

IL-8 BIOLOGY

The biological activity of chemokines is made possible by binding to specific cell surface receptors. IL-8, like most chemokine receptors, has high affinity for multiple ligands.[24] Interleukin-8 is synthesized primarily as a 99 amino acid peptide. IL-8 mature formula contains 72 amino acids that are actively secreted as a result of types of cellular stimuli. Transcription of the IL-8 gene encodes for a protein of 99 amino acids that is either cleaved to 77 amino acids in nonimmune cells or 72 amino acids in immune cells of monocytes and macrophages.[29] Most of the chemokines are first biologically inactive signal propeptides with 90 amino acids. They gradually cleave to the bioactive formulas during the secretion process from the cells. Biological activities of chemokines initiate after binding to specific cell surface receptors. Most chemokine receptors have high affinity for multiple ligands.[24] Signal sequence cleavage and N-terminal proteolysis entails biologically relevant residues with 72 and 77 amino acids that are active IL-8 isoforms.[19] The 72 amino acid molecule secreted by immune cells of monocytes and macrophages, whereas the 77 amino acid variant secretes by nonimmune cells. The monomer molecule of IL-8 contains an NH2-terminal loop, three antiparallel beta strands connected by loops, and a C-terminal alpha helix (Figure 1). While IL-8 readily forms dimers in solution, the monomer is believed to be the biologically relevant and active form of this chemokine.[8,30] Expression of IL-8 can be enhanced by IL-1, TNF-α, IL-6, interferon-γ, lipopolysaccharide, phytohemagglutinin, phorbol myristate acetate, reactive oxygen species, and other cellular stressors.[31] Potent inhibitors of IL-8 production include dexamethasone, IL-4, and IL-10.[32] The biological activities of CXC chemokines, such as IL-8, are in part dependent on their sequence of ELR amino acid motif (Glu–Leu–Arg). These three specific amino acids play crucial role in binding of IL-8 to these receptors. IL-8 is relatively resistant to temperature, proteolysis, and acidic environments. These biochemical natures make IL-8 an ideal molecule for acute inflammation sites, where it must stand firm in these destructive conditions. IL-8 molecule is produced in early inflammatory phase and remains active for a long period of time, even up to weeks at the site of inflammation. This is in contrast to most of the other inflammatory cytokines, which disappear from the site of inflammation after a few hours in vivo.[33,34] IL-8 gene expression is highly sensitive to oxidants, and anti-oxidants.[31] Two cell surface G-protein-coupled receptors of CXCR1 and CXCR2 have been discovered to mediate biological activities of IL-8. These receptors have considerable structural similarity with nearly identical biological activities.[35,36] It has been demonstrated that CXCR2 has high affinity for all CXC chemokines that attract neutrophils, including IL-8, while CXCR1 has high affinity only for IL-8.[37] This biological preference in receptors may refer N-terminal four residues that are absent in the truncated form.[38]

IL-8 EXPRESSION AND RECEPTORS IN VIVO

Once the IL-8 molecule is internalized by the endothelial cells, it is transported through the cellular plasma vesicles and released onto the luminal surfaces from the tips of membrane protrusions either directly from the Golgi apparatus or following storage in Weibel-Palade bodies.[39] In vivo, IL-8 provokes a massive neutrophil accumulation at the injection site. Five molecules structurally similar to IL-8 of neutrophil-activating cytokines have been identified. IL-8 and the related cytokines are thought to be the main cause of local neutrophil accumulation following infection, inflammation, ischemia, or trauma.[40] IL-8 and its homologues are potent signals for leukocyte migration and cell adhesion, but deletion of the IL-8 receptor homologue had minimal effect on cell rolling or arrest of in vivo video imaging.[41] Predominantly, the proangiogenic activity of IL-8 seems to occur following binding to CXCR2, but CXCR1 appears to contribute as well through independent, small-GTPase activity.[19] Immunohistochemical methods, Western blotting, and real-time PCR examinations of normal human and rabbit retinas revealed a positive immuno-reactivity for CXCL8, CXCR1, and CXCR2 in virtually all cultured glial cells and in the human Müller cell line and distinct expression of CXCR1 and CXCR2 in several neuronal cell types.[42]

ROLE OF IL-8 AND IL-8RS IN OCULAR ANGIOGENESIS

In the CXC chemokine family, angiogenic activity refers to the presence or absence of a sequence of amino acids (Glu–Leu–Arg) in their molecular structure known as the ELR motif, which is located immediately before the first N-terminal cysteine residue. These three sequences of amino acids are important in ligand/receptor interactions and biological activation of neutrophils. The ELR-positive α-chemokines, CXCL8 (IL-8), CXCL7 (NAP-2), CXCL1 (MGSA/GRO-α), and CXCL5 (ENA-78) promote angiogenesis, whereas interferon-inducible α-chemokines that lack the ELR motif, such as CXCL4 (PF4), CXCL10 (IP-10), CXCL9 (MIG), and platelet factor 4 (PF4), are potent inhibitors of both CXC (ELR) chemokine, and basic fibroblast growth factor (bFGF)-induced angiogenesis CXCR2 binds to all of the ELF^{+} CXCs involved in angiogenesis, including IL-8, but CXCR1 binds with high affinity only to IL-8 and GCP-2; therefore, CXCR2 is a more suitable candidate for mediating the proangiogenic effects of IL-8.[43–45] Clinically important angiogenic factors other than IL-8, include vascular endothelial growth factor, angiogenin, IL-6, MCP-1, and TGF-β1.[46] Some of the proinflammatory cytokines, such as IL-1β, induce the synthesis of IL-8 in human mast cells via the leukotriene B4 receptor, thus contributing to angiogenesis.[47] Neutrophils make up an essential part of the innate immune system, and are involved both in the initial responses to pathogens, and in orchestrating later immune responses. Neutrophils recognize pathogens through recently discovered pattern-recognition receptors. The Nod-like receptors (nucleotide-binding domain leucine-rich) activation resulted in interleukin-1β secretion and facilitated neutophil migration and interleukin-8 secretion.[48] Generally, IL-8 has at least four distinct proangiogenic properties, each of which is manifested through an endothelial cell response: (1) enhancement of endothelial cell proliferation by stimulation of both endothelial proliferation and capillary tube formation, both of which can be blocked by monoclonal antibodies to IL-8[49,50]; (2) chemotaxis via directional migration of neutrophils, basophils, and T lymphocytes[21]; (3) survival by ability to inhibit the apoptosis of endothelial cells[49]; and (4) protease activation by stimulation of endothelial cell mRNA to increase expression of matrix metalloproteinases (MMPs) MMP-2, 9 and gelatinase activity.[49]

In Vitro Eye Studies

In ocular surface, corneal epithelial cells play an important role in neutrophil chemotaxis, through the secretion of IL-8 in the early stages of corneal herpetic keratitis.[51] IL-8 may play a role in the development of subepithelial infiltrates in adenovirus keratitis.[52] The expression of IL-8 and monocyte chemotactic protein-1 (MCP-1) were unregulated after mitomycin C (MMC) exposure of corneal fibroblasts in a time- and dose-dependent manner.[53] Human corneal endothelial and stromal cells produce IL-8, known as the neutrophil and lymphocyte chemotaxine, especially in response to stimulation by IL-1β or TNF-α.[54] *Staphylococcus aureus* colonization in atopic keratoconjunctivitis upregulates TNF-α and IL-8 release in a dose-dependent manner.[55] In intraocular tissues, thrombin extravasation at the sites of blood-retina barrier breakdown induced retinal pigment epithelial cells (RPE) secretion of IL-8 and MCP-1. This is enhanced in RPE cell/monocyte co-cultures, and inhibited by anti-TNF-α antibody.[56]

Animal Eye Studies

Cornea and Ocular Surface Effects

IL-8 plays a bifunctional role in corneal inflammations by both neovascularization and wound healing.[45,57] Overexpression of IL-8 in the corneal tissues induced by adenoviral stimulation causes the formation of some types of corneal ulcers.[58] In the cornea IL-8 increase collagen degradation by stimulation of polymorphonuclear neutrophils (PMNs) but TNF-α promoted collagen degradation by keratocytes.[59] IL-8, produced by keratocytes and neutrophils, may be involved in the development of diffuse lamellar keratitis (DLK) after laser in situ keratomileusis in the rabbit cornea.[60] Some of the cytokines and mediators have angiogenic activity such as IL-8, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and their receptors. This activation mediated by TNF-α and inhibited by the administration of anti-IL-8, anti-VEGF, and anti-bFGF antibodies and nuclear factor kappa B (NF-kappaB) in the rabbit cornea.[61]

Uveal Effects

Intraocular injections of IL-1, IL-6, IL-8, TNF, and GM-CSF have been shown to induce inflammation in experimental animals. IL-1α initiates the inflammatory cascade containing IL-8, which acts more specifically on a smaller population of leukocytes in induced rat uveitis.[62] Intravitreal injection of an anti-IL-8 antibody (WS-4) in endotoxin-induced uveitis in rabbit eye caused a decrease in the clinical and histologic grade of inflammation.[63]

Vitreo-retinal Effects

Angiogenesis by the same mechanism has been proved in the mouse retina.[64] Animal studies have showed IL-8 mediated angiogenesis inhibited by the mechanisms of decreased intravascular volume and dynamic forces and the direct effects of diabetes on cell growth or

angiogenesis but these mechanisms have little effects on VEGF.[65] Anterior-chamber-associated immune deviation (ACAID) may be due to the intraocular effects of some cytokines, including IL-8. Common complications of ocular inflammation, such as glaucoma, keratic precipitates, retinal (macular) edema, and neovascularization, may be mediated by theses cytokines.[66]

Human Eye Studies

Normal Human Tear IL-8 Values

Undiluted basal and reflex tear fluid IL -8 levels are 731.4 ± 116.2 and 276.1 ± 47.5 pg/mL. IL-8 levels in reflex tears were significantly lower than those in basal tears.[67] The amount of some tear cytokines changed during daytime based on a diurnal rhythm with a specific pattern and this may influence on the symptoms of ocular surface diseases during the course of a day. IL-8 tear levels remain low throughout the day.[68]

Ocular Surface and Corneal Effects

Among levels of 15 cytokines and chemokines measured by multiplex bead analysis, IL-6, IL-8/CXCL8, and epidermal growth factor (EGF) levels correlated with pain and with clinical parameters measuring tear stability, tear production, or ocular surface integrity.[69] Tear fluid levels of IL- 6 and IL- 8 increased following penetrating keratoplasty, acute bacterial conjunctivitis, or corneal foreign body. This may be used as an indicator of various inflammatory reactions in the early postoperative period.[70] Mechanical stimulation of the corneal surface by rubbing or rigid contact lenses leads to increased tear levels of epidermal growth factor and IL-8.[71] In a patient with Stevens-Johnson syndrome (SJS), tear IL-8 levels and ocular inflammation decreased more rapidly in the eye with cultivated limbal stem cell transplantation (LSCT) in contrast with conventional LSCT in the next eye. More severe corneal scarring and opacification were noted in the eyes with conventional LSCT 4 years later.[72] Tear IL-6 and IL-8 levels increased in parallel with the severity and stages of the diseases in patients with conjunctivochalasis, especially when punctal occlusion and delayed fluorescein clearance test are also present.[73]

Conjunctival epithelial cells in response to stimulation by gram-positive *Staphylococcus aureus* activate the innate immune response by IL-8 gene expression and secretion.[74] IL-8 and, to some extent, IL-6 and VEGF are expressed by pterygium epithelium. This expression is enhanced by exposure to ultraviolet (UV) radiation and causes abnormal blood vessel formation, cellular proliferation, tissue invasion, and inflammation.[75] In severe allergic eye diseases the levels of IL-8 in the extracellular spaces of conjunctival epithelium increased significantly in correlation with the percentages of neutrophils and eosinophils.[76]

Uveal Effects

Serum levels of IL-8 and IL-6 were significantly elevated in patients with active uveitis and were decreased during remission. IL-8 levels were higher in patients with anterior and acute uveitis, while IL-6 is higher in chronic uveitis.[77] There is association between the disease activity and increased reflex tears levels of IL-8 induced by soft contact lens wearing in Behçet syndrome.[78] IL-8 plays a role in the progression of intraocular inflammation, and lens-induced endophthalmitis in Behçet disease and granulocytes are thought to be a possible source of IL-8 in these patients.[79] In the aqueous humor from patients with active uveitis, IL-8, levels are significantly increased. HLA-B27-associated uveitis had the highest IL-8 levels. In Behçet disease with hypopyon, the mean levels of IL-8 were significantly higher than in patients without hypopyon.[80] IL-8 participates in the inflammatory processes in the eye by attracting and degranulating neutrophils. It is suggested that these processes contribute to the pathogenesis of tissue destruction in uveitis.[81] Lipopolysaccharide-induced uveitis causes IL-8-mediated neutrophil infiltration and MCP-1-mediated mononuclear cell infiltration and protein leakage.[82]

Vitreous and Retinal Effects

IL-8 levels in the vitreous may play a role in the pathogenesis of proliferative vitreoretinopathy (PVR).[83] Vitreous gel levels of IL-8 were significantly increased in patients with active proliferative diabetic retinopathy (PDR).[84] Vitreous gel levels of IL-6, IL-8, and nitric oxide (NO) increased significantly following retinal laser photocoagulation.[85] Diabetic individuals showed significant elevation of IL-8, VEGF, and angiogenin in the vitreous but not in serum samples compared to control subjects.[86] NF-kappaB protein overlapped with angiogenic factor of IL-8 in formation of epiretinal membranes (ERMs) after PDR and PVR.[87] Mechanical stress to RPE cells, such as exposure to magnetic force, induces the RPE cells to express MCP-1 and IL-8, the two inflammatory cytokines that may be involved in the early stage of primary retinal detachment.[88] Retinal pericytes, glial cells, ganglion cells, and ciliary epithelium release VEGF and/or IL-8 in response to hypoxia and cause endothelial cell proliferation and neovascularization.[89] Serum levels of IL-8 increased during the acute phase of nonarteritic anterior ischemic optic neuropathy (NAION), but decreased during the follow-up period; these changes are in accordance with other acute vascular thromboses.[90] Serum levels of IL-8 and IL-6 and aqueous humor levels of IL-8 are higher immediately following acute retinal artery occlusion.[91] Aqueous humor levels of IL-6, IL-8, VEGF, and MCP-1 are significantly higher in clinically significant macular edema (CSME).[92] The pro-inflammatory homozygous gene for IL-8 is an important risk factor for age-related macular degeneration (ARMD).[93] Higher vitreous IL-8

levels at vitrectomy surgery, significantly increased risk of recurrent vitreous hemorrhage, this association may be explained by the role of IL-8 in angiogenesis.[94]

Orbital Effects

In thyroid-associated ophthalmopathy, progression of inflammation refers to the types of fibroblasts and amount of IL-8 production in these cells.[95] In orbital inflammations, orbital fibroblasts in response to cytokines, such as IL-1β, TNF-α, lipopolysaccharides (LPS), and IFN-γ stimulation, produced dose-dependent IL-8 and MCP-1.[96]

IL-8-TARGETTED IMMUNOTHERAPY

Pretreatment of human monocyte-derived macrophages with methylprednisolone, dexamethasones, and hydrocortisone potentiate the phagocytosis of apoptotic neutrophils, and this effect is blocked by the glucocorticoid receptor antagonist. Methyl prednisolone enhancement effects did not trigger the release of the chemokines IL-8 and monocyte chemoattractant protein-1. This property of glucocorticoids aims to the resolution of inflammatory diseases.[97] Only a limited amount of data is available concerning the therapeutic potential of IL-8. Anti-IL-8 monoclonal antibody medications may effectively improve multiple human diseases.[98] IL-8 cytokine expression in numerous xenograft and orthotopic in vivo models correlates with the angiogenesis, tumorigenicity, and metastasis of tumors. Therefore, suppressing the effects of IL-8 signaling may be a beneficial therapeutic intervention in targeting the tumor microenvironment.[29] The ability of some members of CC chemokines, such as CCL19 (ELC) and CCL21 (SLC), in inhibition of angiogenesis and attraction of immune effector cells is under investigation for antitumor therapy.[99] Antibodies directed to CXCR2 effectively inhibit chemotaxis and neovascularization induced by ELF^+ chemokines such as IL-8 and ENA-78.[100] Admixture of pentoxifylline to corneal tissue culture media leads to a suppression of IL-6 and IL-8 secretion by corneal tissue and improvement in endothelial cell survival.[101] Calcitriol (1, 25-dihydroxyvitamin D3) inhibits the expression of both IL-6 and IL-8 and may have a potential anti-inflammatory effect in ocular surface inflammatory disease.[102] It is reported that TGF-β1 and IL-8 are involved in ocular allergic inflammations. Amniotic membrane matrix is capable of decreasing fibrotic responses in ocular allergic inflammation by significantly suppressing TGF-β1 and IL-8 production.[103] In addition, antihistaminic eyedrops reduced IL-8 production dose-dependently in allergic eye diseases.[104] Hypoxia-induced NF-κB activation and increased IL-8 and VEGF. Vitreous fluid levels of IL-8 in patients with retinal neovascularization were significantly higher than that of patients without neovascular disease. Neutralizing antibodies to IL-8 or to VEGF suppresses neovascular activity.[105] Recent data showed that not only are antiangiogenic molecules inhibitors of angiogenesis, they are also efficient at reversing some levels of immunosuppression. This link may lead to the emergence of a new class of antiangiogenic and immunotherapy medications.[98] Most of the antiangiogenic molecules affect not only VEGF signaling pathways but also alternative pathways such as IL-12.[106]

IL-10 seems to be the most suppressive cytokine on secretion of IL-8 and MCP-1 by RPE cells, suggesting potential therapeutic effects of IL-10 on the ocular inflammatory and proliferative diseases.[105] Thalidomide as an immunomodulatory, anti-inflammatory, and antiangiogenic medication acts by decreasing circulating $CD4^+$ T cells and stimulating $CD8^+$ T cells. Thalidomide also inhibits proliferation of stimulated T cells and leukocyte chemotaxis. Thalidomide inhibits TNF-α, IL-5, IL-6, IL-8, and IL-12 and increases IL-2, IL-10, and IFN-γ production. Moreover, thalidomide plays an important role in inhibition of VEGF and FGF-2 mediated angiogenesis.[107]

DISCUSSION AND CONCLUSION

IL-8 is one of the most important inflammatory cytokines and has been noticed in many scattered research articles. Despite more than 30 years from discovery of IL-8 and the presence of many wide-ranging published articles about this cytokine, there are very few specific review articles concerning the body organs and especially no review article focused on the ocular effect of the IL-8. This review introduces the ocular effects of this cytokine as an accessible collection.

Several functions for IL-8 have been suggested in different cell types and tissues, including neutrophil recruitment, cell adhesion, homing of neutrophils and lymphocytes, tumor growth, angiogenesis, neuronal protection, and even brain development.[21] PMN are the primary effectors cells in acute inflammatory responses. Appropriate interactions of these cells with macrophages in injured, stressed, or infected tissues are required for the successful performance of the routine tissue response. Any deviation from these fundamental programs as a major problem may lead to many ocular diseases. Counter-regulatory signals are critical for controlled activation of immune responses in the cornea, uvea, and/or retina. The role of these circuits may provide insight to maintain a pre-inflammatory state, namely homeostasis, rather than limiting therapeutic options to palliative inhibition of pro-inflammatory circuits.[108]

Monocyte infiltration is a prominent feature of inflammatory ocular disorders such as uveitis, ARMD and epiretinal membranes.[109–111] Interleukin (IL) 8 and monocyte chemoattractant protein (MCP) 1 play major roles in pro-inflammatory human retinal pigment epithelial (HRPE) -derived leukocyte chemotactic activity.[112] IL-8 is

primarily chemotactic for neutrophils and eosinophils,[21] whereas MCP-1 attracts and stimulates monocytes and lymphocytes.[113] These chemokines are increased in PVR, uveitis and diabetic retinopathy disorders.[81,105] Direct interactions between monocytes and vascular endothelial cells resulted in the increased expression of IL-8 and MCP- 1. Hypoxia or oxidative stress caused rapid upregulation of IL-6 and IL-8 mRNA expression.[123] Better understanding of such basic mechanisms may help to improve methods for treatment of the ocular inflammatory disorders. Th2 cytokines such as IL-4, -10, and -13 are able to downregulate the pro-inflammatory cytokine release by stimulated monocytes.[114,115] These cytokines, thus, may have an immunoregulatory role in clinical and experimental ocular inflammation.

In vitro and in vivo eye studies all support the angiogenic and inflammatory effects of IL-8.[45,51,57,62] Angiogenesis in most of the cases is harmful for the ocular tissues. In humans, the most frequent type of angiogenic activity on ocular surface is pterygium.[74,116] During active process of ocular cicatricial pemphigoid, tear angiogenic molecules of IL-8 and matrix metalloproteinase-9 (MMP-9), in particular, were elevated, but decreased after systemic immunomodulatory therapy.[117] IL-8, which is smaller than the other chemokines and shows powerful attractive chemotactic properties on T cells and neutrophil polynuclear cells, dramatically increased in the tears of dry eye patients.[118]

Corneal angiogenesis are encountered in many frequent pathologic disorders, such as penetrating keratoplasty or corneal foreign body[70,71] and in rare conditions such as SJS[72] and sulfur mustard (SM) intoxicated patients.[119–121] Intraocular angiogenesis may be encountered in vitrous and retina (diabetic retinopathy)[122] and orbital tissues.[95,96] Pro-angiogenic cytokines such as IL-8 are more highly represented than anti-angiogenic cytokines such as IL-4 and IL-12 in the tears of diabetic patients with retinopathy.[123]

In different diseases of anterior segment of the eye IL-8 plays special roles. A significantly increased IL-6 and IL-8 mRNA expression was found in the ciliary process and iris in eyes with early PEX syndrome. IL-6 and IL-8 mRNA expression levels were correlated in early stages of PEX syndrome, indicating a concerted expression of both cytokines in this condition.[124] Significantly increased levels of IL-8 could be measured in aqueous samples of eyes with early stages of pseudo-exfoliation (PEX) syndrome (but not in late stages or in PEX glaucoma) as compared with control eyes with cataract. Patients with POAG displayed an increased IL-8 levels, however, the levels did not reach statistical significance. Patients with a history of inflammatory ocular disease displayed a significant increase of aqueous IL-8 compared with cataract controls. Aqueous levels of IL-8 were positively correlated both in patients with early stages of PEX syndrome and in patients with previous ocular inflammation. The latter showed further positive correlations between IL-8 and IL-1.[124] Ciliary processes are the most potential sources for aqueous IL-6 and IL-8 (about 100 times higher than that of all other normal ocular tissues). Aqueous IL-8 levels highly increased following cataract surgery, whether done by local or general anesthesia.[125] Aqueous humor levels of IL-8 concentration in primary open-angle glaucoma are significantly elevated. In patients with asymmetric glaucoma, the more severely affected eye had a significantly higher IL-8 concentration. This elevation is also correlated with the severity of visual field test abnormalities. Normal retina and optic nerve are of potential sources for aqueous humor IL-8 secretion.[126] Significantly increased concentrations of VEGF, IL-6, and IL-8 were observed in aqueous humor samples of patients with CRVO. During therapy with bevacizumab, a decrease in aqueous VEGF levels was accompanied by a decrease in central retinal thickness (CRT) and improvement in visual acuity.[127]

Interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) are significantly elevated in vitreoretinal diseases, including diabetic macular edema (DME), proliferative diabetic retinopathy (PDR), branch retinal vein occlusion (BRVO), central retinal vein occlusion (CRVO), and rhegmatogenous retinal detachment. In PDR patients, the elevation of VEGF was significantly correlated with the three factors—IL-6, IL-8, and MCP-1—while no significant correlation was observed with CRVO patients. These three major factors were strongly correlated with each other except for IL-8/MCP-1 in CRVO, indicating a common pathway involved in inflammation process in vitreoretinal diseases. VEGF may independently play a role in the pathologic process in PDR and CRVO. Presumably IL-6, IL-8, and MCP-1 in the vitreous cavity promote vascular permeability that causes DME. Retinal ischemia leads to an excessive production of VEGF that in turn causes further progression of DME to PDR. On the other hand, a substantial amount of VEGF can be initially produced by the sudden profound retinal ischemia, which in turn induces the major three factors afterward.[128] The duration of DM was found to correlate with serum VEGF, IL-8, MCP-1, and EGF levels, but the progression of diabetic retinopathy (DR) was found to be significantly correlated with the median values of serum monocyte chemoattractant protein (MCP-1). In patients with DR, no correlation was found between macular thickness and serum cytokines of IL-6, IL-8, IL-10, and VEGF levels but only increased serum levels of VEGF and MCP-1 may act as a key regulator of DR and provide a potential tool for risk assessment in diabetic patients.[129]

In this review we noticed that vitreoretinal effects of IL-8 have been recently taken into specific consideration by many investigators. This is due to the attractive and important subjects of ARMD, PVR, PDR, retinopathy of prematurity (ROP), retinal vascular accidents, and other causes of vitroretinal angiogenesis or inflammatory disorders in this field. These broad-spectrum effects of IL-8 in ocular disease had encouraged the investigators to use

IL-8-targetted immunotherapy with anti-IL-8 cytokines such as IL-10,[130] or medications.[107] As in any immune-modulating therapeutic approach, there are several disadvantages regarding IL-8 blocking that need to be considered. IL-8 is an important mediator in numerous aspects of immune response, and complete blockade of its signaling pathways may not be desirable. Therefore, specific techniques are needed to ensure that IL-8 signal blocking takes place in desired areas and tissues.[131]

In conclusion, IL-8 plays important roles in the ocular inflammation and inducing ocular angiogenesis in any parts of the eyes, such as the conjunctiva, cornea, iris, retina, and orbit. Anti-IL-8 targeted immunotherapy with specific immune products or medications and with specific techniques to act just at the inflammation sites has been introduced as new treatment modalities in IL-8 related ocular angiogenesis or inflammations. As with other vital organs, many of the inflammatory phenomena in the eyes are essential for health and vision survival. Thus, inhibition of inflammatory cytokines and chemokines such as IL-8 should be considered only in critical circumstances to avoid the dangers of such treatments.

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REFERENCES

[1] Baggiolini M, Dewald B, Moser B. Human chemokines: an update. *Annu Rev Immunol*. 1997;15:675–705.
[2] Atta UR, Harvey K, Siddiqui RA. Interleukin-8: an autocrine inflammatory mediator. *Curr Pharm Res*. 1999 Apr;5(4):241–253.
[3] Jin H, Hayes GL, Darbha NS,et al. Investigation of CC and CXC chemokine quaternary state mutants. *Biochem Biophys Res Commun*. 2005 Dec 16;338(2):987–999.
[4] Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity*. 2000 Feb;12(2):121–127.
[5] Rosenkilde MM, Schwartz TW. The chemokine system—a major regulator of angiogenesis in health and disease. *APMIS*. 2004 Jul;112(7–8):481–495.
[6] Baldwin ET, Weber IT, St CR,et al. Crystal structure of interleukin 8: symbiosis of NMR and crystallography. *Proc Natl Acad Sci U S A*. 1991 Jan 15;88(2):502–506.
[7] Clore GM, Appella E, Yamada M,et al. Three-dimensional structure of interleukin 8 in solution. *Biochemistry*. 1990 Feb;29(7):1689–1696.
[8] Rajarathnam K, Sykes BD, Kay CM,et al. Neutrophil activation by monomeric interleukin-8. *Science*. 1994 Apr 1;264(5155):90–92.
[9] Kanagarajadurai K, Sowdhamini R. Sequence and structural analyses of interleukin-8-like chemokine superfamily. *In Silico Biol*. 2008;8(3-4):307–330.
[10] Lowman HB, Fairbrother WJ, Slagle PH,et al. Monomeric variants of IL-8: effects of side chain substitutions and solution conditions upon dimer formation. *Protein Sci*. 1997 Mar;6(3):598–608.
[11] Moser B, Loetscher P. Lymphocyte traffic control by chemokines. Nat Immunol. 2001 Feb;2(2):123–128.
[12] Schraufstatter IU, Chung J, Burger M. IL-8 activates endothelial cell CXCR1 and CXCR2 through Rho and Rac signaling pathways. *Am J Physiol Lung Cell Mol Physiol*. 2001 Jun;280(6):L1094–L1103.
[13] Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer*. 2004 Jul;4(7):540–550.
[14] Flynn G, Maru S, Loughlin J,et al. Regulation of chemokine receptor expression in human microglia and astrocytes. *J Neuroimmunol*. 2003 Mar;136(1-2):84–93.
[15] Murdoch C, Monk PN, Finn A. Cxc chemokine receptor expression on human endothelial cells. *Cytokine*. 1999 Sep;11(9):704–712.
[16] Murphy PM, Baggiolini M, Charo IF,et al. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev*. 2000 Mar;52(1):145–176.
[17] Horuk R. Chemokine receptors. *Cytokine Growth Factor Rev*. 2001 Dec;12(4):313–335.
[18] Tournamille C, Blancher A, Le Van KC,et al. Sequence, evolution and ligand binding properties of mammalian Duffy antigen/receptor for chemokines. *Immunogenetics*. 2004 Jan;55(10):682–694.
[19] Brat DJ, Bellail AC, Van Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro Oncol*. 2005 Apr;7(2):122–133.
[20] Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest*. 1989 Oct;84(4):1045–1049.
[21] Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol*. 2000;18:217–42.
[22] Bradding P, Okayama Y, Howarth PH,et al. Heterogeneity of human mast cells based on cytokine content. *J Immunol*. 1995 Jul 1;155(1):297–307.
[23] Scapini P, Lapinet-Vera JA, Gasperini S,et al. The neutrophil as a cellular source of chemokines. *Immunol Rev*. 2000 Oct;177:195–203.
[24] Apostolakis S, Papadakis EG, Krambovitis E,et al. Chemokines in vascular pathology (review). *Int J Mol Med*. 2006 May;17(5):691–701.
[25] Gerszten RE, Garcia-Zepeda EA, Lim YC,et al. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature*. 1999 Apr 22;398(6729):718–723.
[26] Gusella GL, Musso T, Bosco MC,et al. IL-2 up-regulates but IFN-gamma suppresses IL-8 expression in human monocytes. *J Immunol*. 1993 Sep 1;151(5):2725–2732.
[27] Pease JE, Sabroe I. The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. *Am J Respir Med*. 2002;1(1):19–25.
[28] Ciree A, Michel L, Camilleri-Broet S, Jean LF,et al. Expression and activity of IL-17 in cutaneous T-cell lymphomas (mycosis fungoides and Sezary syndrome). *Int J Cancer*. 2004 Oct 5;20;112(1):113–120.
[29] Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. *Clin Cancer Res*. 2008 Nov 1;14(21):6735–6741.
[30] Horcher M, Rot A, Aschauer H,et al. IL-8 derivatives with a reduced potential to form homodimers are fully active in vitro and in vivo. *Cytokine*. 1998 Jan;10(1):1–12.

[31] DeForge LE, Preston AM, Takeuchi E,et al. Regulation of interleukin 8 gene expression by oxidant stress. *J Biol Chem*. 1993 Dec 5;268(34):25568–25576.
[32] Xie K. Interleukin-8 and human cancer biology. *Cytokine Growth Factor Rev*. 2001 Dec;12(4):375–391.
[33] DeForge LE, Fantone JC, Kenney JS,et al. Oxygen radical scavengers selectively inhibit interleukin 8 production in human whole blood. *J Clin Invest*. 1992 Nov;90(5): 2123–2129.
[34] Apostolakis S, Vogiatzi K, Amanatidou V,et al. Interleukin 8 and cardiovascular disease. *Cardiovasc Res*. 2009 Dec 1;84(3):353–360.
[35] Holmes WE, Lee J, Kuang WJ,et al. Structure and functional expression of a human interleukin-8 receptor. *Science*. 1991 Sep 13;253(5025):1278–1280.
[36] Murphy PM, Tiffany HL. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science. 1991. 253: 1280–1283.
[37] Ahuja SK, Murphy PM. The CXC chemokines growth-regulated oncogene (GRO) alpha, GRObeta, GROgamma, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. *J Biol Chem*. 1996 Aug 23;271(34):20545–20550.
[38] Koradi R, Billeter M, Wuthrich K. MOLMOL: a program for display and analysis of macromolecular structures. *J Mol Graph*. 1996 Feb;14(1):51–32.
[39] Rot A, Hub E, Middleton J,et al. Some aspects of IL-8 pathophysiology, III: chemokine interaction with endothelial cells. *J Leukoc Biol*. 1996 Jan;59(1):39–44.
[40] Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett*. 1992 Jul 27;307(1):97–101.
[41] Becker MD, O'Rourke LM, Blackman WS,et al. Reduced leukocyte migration, but normal rolling and arrest, in interleukin-8 receptor homologue knockout mice. *Invest Ophthalmol Vis Sci*. 2000 Jun;41(7):1812–1817.
[42] Goczalik I, Ulbricht E, Hollborn M,et al. Expression of CXCL8, CXCR1, and CXCR2 in neurons and glial cells of the human and rabbit retina. *Invest Ophthalmol Vis Sci*. 2008 Oct;49(10):4578–4589.
[43] Belperio JA, Keane MP, Arenberg DA,et al. CXC chemokines in angiogenesis. *J Leukoc Biol*. 2000 Jul;68(1):1–8.
[44] Strieter RM, Kunkel SL, Arenberg DA,et al. Interferon gamma-inducible protein 10 (IP-10), a member of the C-X-C chemokine family, is an inhibitor of angiogenesis. *Biochem Biophys Res Commun*. 1995 May 5;210(1):51–57.
[45] Strieter RM, Polverini PJ, Kunkel SL,et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem*. 1995 Nov 10;270(45):27348–27357.
[46] Forooghian F, Das B. Anti-angiogenic effects of ribonucleic acid interference targeting vascular endothelial growth factor and hypoxia-inducible factor-1alpha. *Am J Ophthalmol*. 2007 Nov;144(5):761–768.
[47] Kim GY, Lee JW, Ryu HC,et al. Proinflammatory cytokine IL-1beta stimulates IL-8 synthesis in mast cells via a leukotriene B4 receptor 2-linked pathway, contributing to angiogenesis. *J Immunol*. 2010 Apr 1;184(7):3946–3954.
[48] Ekman AK, Cardell LO. The expression and function of Nod-like receptors in neutrophils. *Immunology*. 2010 May;130(1):55–63.
[49] Li A, Dubey S, Varney ML,et al. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *J Immunol*. 2003 Mar 15;170(6):3369–3376.
[50] Shono T, Ono M, Izumi H,et al. Involvement of the transcription factor NF-kappaB in tubular morphogenesis of human microvascular endothelial cells by oxidative stress. *Mol Cell Biol*. 1996 Aug;16(8):4231–4239.
[51] Miyazaki D, Inoue Y, Raki-Sasaki K,et al. Neutrophil chemotaxis induced by corneal epithelial cells after herpes simplex virus type 1 infection. *Curr Eye Res*. 1998 Jul;17(7):687–693.
[52] Chodosh J, Astley RA, Butler MG,et al. Adenovirus keratitis: a role for interleukin-8. *Invest Ophthalmol Vis Sci*. 2000 Mar;41(3):783–789.
[53] Chou SF, Chang SW, Chuang JL. Mitomycin C upregulates IL-8 and MCP-1 chemokine expression via mitogen-activated protein kinases in corneal fibroblasts. *Invest Ophthalmol Vis Sci*. 2007 May;48(5):2009–2016.
[54] Elner VM, Strieter RM, Pavilack MA,et al. Human corneal interleukin-8. IL-1 and TNF-induced gene expression and secretion. *Am J Pathol*. 1991 Nov;139(5):977–988.
[55] Cook EB, Stahl JL, Esnault S,et al. Toll-like receptor 2 expression on human conjunctival epithelial cells: a pathway for Staphylococcus aureus involvement in chronic ocular proinflammatory responses. *Ann Allergy Asthma Immunol*. 2005 Apr;94(4):486–497.
[56] Yoshida A, Elner SG, Bian ZM,et al. Thrombin regulates chemokine induction during human retinal pigment epithelial cell/monocyte interaction. *Am J Pathol*. 2001 Sep;159(3):1171–1180.
[57] Strieter RM, Kunkel SL, Elner VM,et al. Interleukin-8: a corneal factor that induces neovascularization. *Am J Pathol*. 1992 Dec;141(6):1279–1284.
[58] Oka M, Norose K, Matsushima K,et al. Overexpression of IL-8 in the cornea induces ulcer formation in the SCID mouse. *Br J Ophthalmol*. 2006 May;90(5):612–615.
[59] Nagano T, Nakamura M, Nishida T. Differential regulation of collagen degradation by rabbit keratocytes and polymorphonuclear leukocytes. *Curr Eye Res*. 2002 Mar;24(3):240–243.
[60] Sano-Kato N, Toda I, Shimmura S,et al. Detection of neutrophils and possible involvement of interleukin-8 in diffuse lamellar keratitis after laser in situ keratomileusis. *J Cataract Refract Surg*. 2003 Oct;29(10):1996–2000.
[61] Yoshida S, Ono M, Shono T,et al. Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. *Mol Cell Biol*. 1997 Jul;17(7):4015–4023.
[62] Ferrick MR, Thurau SR, Oppenheim MH,et al. Ocular inflammation stimulated by intravitreal interleukin-8 and interleukin-1. *Invest Ophthalmol Vis Sci*. 1991 Apr;32(5): 1534–1539.
[63] Verma MJ, Mukaida N, Vollmer-Conna U,et al. Endotoxin-induced uveitis is partially inhibited by anti-IL-8 antibody treatment. *Invest Ophthalmol Vis Sci*. 1999 Oct;40(11):2465–2470.
[64] Yoshida S, Yoshida A, Ishibashi T. Induction of IL-8, MCP-1, and bFGF by TNF-alpha in retinal glial cells: implications for retinal neovascularization during post-ischemic inflammation. *Graefes Arch Clin Exp Ophthalmol*. 2004 May;242(5):409–413.
[65] Bek EL, McMillen MA, Scott P,et al. The effect of diabetes on endothelin, interleukin-8 and vascular endothelial growth factor-mediated angiogenesis in rats. *Clin Sci (Lond)*. 2002 Aug;103 Suppl 48:424S–429S.
[66] Wakefield D, Lloyd A. The role of cytokines in the pathogenesis of inflammatory eye disease. *Cytokine*. 1992 Jan;4(1):1–5.
[67] Nakamura Y, Sotozono C, Kinoshita S. Inflammatory cytokines in normal human tears. *Curr Eye Res*. 1998 Jun;17(6):673–676.
[68] Uchino E, Sonoda S, Kinukawa N,et al. Alteration pattern of tear cytokines during the course of a day: diurnal rhythm analyzed by multicytokine assay. *Cytokine*. 2006 Jan 7;33(1):36–40.
[69] Enriquez-de-Salamanca A, Castellanos E,et al. Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease. *Mol Vis*. 2010 May; 19;16:862–873.

[70] Fodor M, Facsko A, Rajnavolgyi E,et al. Enhanced release of IL-6 and IL-8 into tears in various anterior segment eye diseases. *Ophthalmic Res*. 2006;38(4):182–188.
[71] Kallinikos P, Efron N. On the etiology of keratocyte loss during contact lens wear. *Invest Ophthalmol Vis Sci*. 2004 Sep;45(9):3011–3020.
[72] Ang LP, Sotozono C, Koizumi N,et al. A comparison between cultivated and conventional limbal stem cell transplantation for Stevens-Johnson syndrome. *Am J Ophthalmol*. 2007 Jan;143(1):178–180.
[73] Erdogan-Poyraz C, Mocan MC, Bozkurt B,et al. Elevated tear interleukin-6 and interleukin-8 levels in patients with conjunctivochalasis. *Cornea*. 2009 Feb;28(2):189–193.
[74] Venza I, Cucinotta M, Caristi S,et al. Transcriptional regulation of IL-8 by *Staphylococcus aureus* in human conjunctival cells involves activation of AP-1. *Invest Ophthalmol Vis Sci*. 2007 Jan;48(1):270–276.
[75] Di GN, Wakefield D, Coroneo MT. UVB-mediated induction of cytokines and growth factors in pterygium epithelial cells involves cell surface receptors and intracellular signaling. *Invest Ophthalmol Vis Sci*. 2006 Jun;47(6):2430–2437.
[76] Miyoshi T, Fukagawa K, Shimmura S,et al. Interleukin-8 concentrations in conjunctival epithelium brush cytology samples correlate with neutrophil, eosinophil infiltration, and corneal damage. *Cornea*. 2001 Oct;20(7):743–747.
[77] Kramer M, Monselise Y, Bahar I,et al. Serum cytokine levels in active uveitis and remission. *Curr Eye Res*. 2007 Jul; 32(7-8):669–675.
[78] Hashida N, Ohguro N, Yamamoto S,et al. Unusual neutrophil infiltration under the soft contact lens in a patient with Behcet's disease. *Jpn J Ophthalmol*. 2003 Sep;47(5):469–472.
[79] Sakamoto H, Sakamoto T, Kubota T,et al. Interleukin-8 expressed in the granulocytes of the eye in a patient with Behcet's disease complicated by lens-induced endophthalmitis. *Jpn J Ophthalmol*. 2003 Nov;47(6):548–550.
[80] Bu El-Asrar AM, Al-Obeidan SS, Kangave D,et al. CXC chemokine expression profiles in aqueous humor of patients with different clinical entities of endogenous uveitis. *Immunobiology*. 2011 Sep;216(9):1004–1009.
[81] De Boer JH, Hack CE, Verhoeven AJ,et al. Chemoattractant and neutrophil degranulation activities related to interleukin-8 in vitreous fluid in uveitis and vitreoretinal disorders. *Invest Ophthalmol Vis Sci*. 1993 Nov;34(12):3376–3385.
[82] Mo JS, Matsukawa A, Ohkawara S,et al. Role and regulation of IL-8 and MCP-1 in LPS-induced uveitis in rabbits. *Exp Eye Res*. 1999 Mar;68(3):333–340.
[83] Aksunger A, Or M, Okur H,et al. Role of interleukin 8 in the pathogenesis of proliferative vitreoretinopathy. *Ophthalmologica*. 1997;211(4):223–225.
[84] Elner SG, Strieter R, Bian ZM,et al. Interferon-induced protein 10 and interleukin 8. C-X-C chemokines present in proliferative diabetic retinopathy. *Arch Ophthalmol*. 1998 Dec;116(12):1597–1601.
[85] Er H, Doganay S, Turkoz Y,et al. The levels of cytokines and nitric oxide in rabbit vitreous humor after retinal laser photocoagulation. *Ophthalmic Surg Lasers*. 2000 Nov;31(6):479–483.
[86] Maier R, Weger M, Haller-Schober EM,et al. Application of multiplex cytometric bead array technology for the measurement of angiogenic factors in the vitreous. *Mol Vis*. 2006;12:1143–1147.
[87] Harada C, Harada T, Mitamura Y,et al. Diverse NF-kappaB expression in epiretinal membranes after human diabetic retinopathy and proliferative vitreoretinopathy. *Mol Vis*. 2004 Jan 15;10:31–36.
[88] Zhang XG, Hui YN, Han QH,et al. Effects of mechanical stress on expressions of monocyte chemoattractant protein-1 and interleukin-8 of cultured human retinal pigment epithelial cells. *Zhonghua Yi Xue Za Zhi*. 2005 Aug 24;85(32):2264–2268.
[89] Inomata H, Ishibashi T, Murata T,et al. Intraocular neovascularization. *Nippon Ganka Gakkai Zasshi*. 1997 Dec;101(12):906–926.
[90] Goldenberg-Cohen N, Kramer M, Bahar I,et al. Elevated plasma levels of interleukin 8 in patients with acute anterior ischaemic optic neuropathy. *Br J Ophthalmol*. 2004 Dec;88(12):1538–1540.
[91] Kramer M, Goldenberg-Cohen N, Xer-Siegel R,et al. Inflammatory reaction in acute retinal artery occlusion: cytokine levels in aqueous humor and serum. *Ocul Immunol Inflamm*. 2005 Jul;13(4):305–310.
[92] Roh MI, Kim HS, Song JH,et al. Effect of intravitreal bevacizumab injection on aqueous humor cytokine levels in clinically significant macular edema. *Ophthalmology*. 2009 Jan;116(1):80–86.
[93] Goverdhan SV, Ennis S, Hannan SR,et al. Interleukin-8 promoter polymorphism -251A/T is a risk factor for age-related macular degeneration. *Br J Ophthalmol*. 2008 Apr;92(4):537–540.
[94] Forooghian F, Kertes PJ, Eng KT,et al. Alterations in the intraocular cytokine milieu after intravitreal bevacizumab. *Invest Ophthalmol Vis Sci*. 2010 May;51(5):2388–2392.
[95] Koumas L, Smith TJ, Phipps RP. Fibroblast subsets in the human orbit: Thy-1$^+$ and Thy-1$^-$ subpopulations exhibit distinct phenotypes. *Eur J Immunol*. 2002 Feb;32(2):477–485.
[96] Elner VM, Burnstine MA, Kunkel SL,et al. Interleukin-8 and monocyte chemotactic protein-1 gene expression and protein production by human orbital fibroblasts. *Ophthal Plast Reconstr Surg*. 1998 Mar;14(2):119–125.
[97] Liu Y, Cousin JM, Hughes J,et al. Glucocorticoids promote nonphlogistic phagocytosis of apoptotic leukocytes. *J Immunol*. 1999 Mar 15;162(6):3639–3646.
[98] Tartour, E., Pere, H., Maillere, B., et al."Angiogenesis and immunity: a bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy", *Cancer Metastasis Rev*. 2011 Mar;30(1): 83–95.
[99] Fernandez EJ, Lolis E. Structure, function, and inhibition of chemokines. *Annu Rev Pharmacol Toxicol*. 2002;42:469–99.:469–499.
[100] Addison CL, Daniel TO, Burdick MD,et al. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. *J Immunol*. 2000 Nov 1;165(9):5269–5377.
[101] Ventura AC, Bohnke M. Pentoxifylline influences the autocrine function of organ cultured donor corneas and enhances endothelial cell survival. *Br J Ophthalmol*. 2001 Sep;85(9):1110–1114.
[102] Xue ML, Zhu H, Thakur A,et al. 1 alpha,25-Dihydroxyvitamin D3 inhibits pro-inflammatory cytokine and chemokine expression in human corneal epithelial cells colonized with *Pseudomonas aeruginosa*. *Immunol Cell Biol*. 2002 Aug;80(4):340–345.
[103] Solomon A, Wajngarten M, Alviano F,et al. Suppression of inflammatory and fibrotic responses in allergic inflammation by the amniotic membrane stromal matrix. *Clin Exp Allergy*. 2005 Jul;35(7):941–948.
[104] Pauly A, Brignole-Baudouin F, Guenoun JM,et al. Comparative study of topical anti-allergic eye drops on human conjunctiva-derived cells: responses to histamine and IFN gamma and toxicological profiles. *Graefes Arch Clin Exp Ophthalmol*. 2007 Apr;245(4):534–546.
[105] Yoshida A, Yoshida S, Khalil AK,et al. Role of NF-kappaB-mediated interleukin-8 expression in intraocular neovascularization. *Invest Ophthalmol Vis Sci*. 1998 Jun;39(7): 1097–1106.
[106] Albini A, Brigati C, Ventura A, et al. Angiostatin antiangiogenesis requires IL-12: the innate immune system as a key target. *J Transl Med.* 2009;7:5. [PMID: 19144161]

[107] Maruotti N, Cantatore FP, Ribatti D. Thalidomide in treatment of connective diseases and vasculities. *Reumatismo*. 2006 Jul;58(3):187–190.
[108] Gronert K. Resolution, the grail for healthy ocular inflammation. *Exp Eye Res*. 2010 Oct;91(4):478–485.
[109] Kampik A, Kenyon KR, Michels RG,et al. Epiretinal and vitreous membranes: comparative study of 56 cases. *Arch Ophthalmol*. 1981 Aug;99(8):1445–1454.
[110] Vinores SA, Campochiaro PA, McGehee R, et al. Ultrastructural and immunocytochemical changes in retinal pigment epithelium, retinal glia, and fibroblasts in vitreous culture. *Invest Ophthalmol Vis Sci*. 1990 Dec;31(12):2529–2545.
[111] Chan CC, BenEzra D, Hsu SM,et al. Granulomas in sympathetic ophthalmia and sarcoidosis: immunohistochemical study. *Arch Ophthalmol*. 1985 Feb;103(2):198–202.
[112] Elner VM, Strieter RM, Elner SG,et al. Neutrophil chemotactic factor (IL-8) gene expression by cytokine-treated retinal pigment epithelial cells. *Am J Pathol*. 1990 Apr;136(4):745–750.
[113] Taub DD, Proost P, Murphy WJ,et al. Monocyte chemotactic protein-1 (MCP-1), -2, and -3 are chemotactic for human T lymphocytes. *J Clin Invest*. 1995 Mar;95(3):1370–1376.
[114] Kucharzik T, Lugering N, Weigelt H,et al. Immunoregulatory properties of IL-13 in patients with inflammatory bowel disease; comparison with IL-4 and IL-10. *Clin Exp Immunol*. 1996 Jun;104(3):483–490.
[115] Hart PH, Ahern MJ, Smith MD,et al. Regulatory effects of IL-13 on synovial fluid macrophages and blood monocytes from patients with inflammatory arthritis. *Clin Exp Immunol*. 1995 Mar;99(3):331–337.
[116] Di GN, Kumar RK, Coroneo MT,et al. UVB-mediated induction of interleukin-6 and -8 in pterygia and cultured human pterygium epithelial cells. *Invest Ophthalmol Vis Sci*. 2002 Nov;43(11):3430–3437.
[117] Chan MF, Sack R, Quigley DA,et al. Membrane array analysis of tear proteins in ocular cicatricial pemphigoid. *Optom Vis Sci*. 2011 Aug;88(8):1005–1009.
[118] Massingale ML, Li X, Vallabhajosyula M,et al. Analysis of inflammatory cytokines in the tears of dry eye patients. *Cornea*. 2009 Oct;28(9):1023–1027.
[119] Ghasemi H, Ghazanfari T, Yaraee R,et al. Evaluation of relationship between the serum levels of inflammatory mediators and ocular injuries induced by sulfur mustard: Sardasht-Iran Cohort Study. *Int Immunopharmacol*. 2009 Dec;9(13–14):1494–1498.
[120] Ghasemi H, Ghazanfari T, Ghassemi-Broumand M,et al. Long-term ocular consequences of sulfur mustard in seriously eye-injured war veterans. *Cutan Ocul Toxicol*. 2009;28(2):71–77.
[121] Ghasemi H, Ghazanfari T, Yaraee R, et al. Long-term ocular consequences of sulfur mustard in lung-injured war veterans. *Cutan Ocul Toxicol*. 2011 Jul 21: [PMID: 21774734].
[122] Canataroglu H, Varinli I, Ozcan AA,et al. Interleukin (IL)-6, interleukin (IL)-8 levels and cellular composition of the vitreous humor in proliferative diabetic retinopathy, proliferative vitreoretinopathy, and traumatic proliferative vitreoretinopathy. *Ocul Immunol Inflamm*. 2005 Sep;13(5):375–381.
[123] Liu J, Shi B, He S,et al. Changes to tear cytokines of type 2 diabetic patients with or without retinopathy. *Mol Vis*. 2010 Dec 31;16: 2931–2938.
[124] Zenkel M, Lewczuk P, Junemann A,et al. Proinflammatory cytokines are involved in the initiation of the abnormal matrix process in pseudoexfoliation syndrome/glaucoma. *Am J Pathol*. 2010 Jun;176(6):2868–2879.
[125] Tu KL, Kaye SB, Sidaras G,et al. Effect of intraocular surgery and ketamine on aqueous and serum cytokines. *Mol Vis*. 2007 Jul 12;13:1130–7.:1130–1137.
[126] Kuchtey J, Rezaei KA, Jaru-Ampornpan P,et al. Multiplex cytokine analysis reveals elevated concentration of interleukin-8 in glaucomatous aqueous humor. *Invest Ophthalmol Vis Sci*. 2010 Dec;51(12):6441–6447.
[127] Funk M, Kriechbaum K, Prager F,et al. Intraocular concentrations of growth factors and cytokines in retinal vein occlusion and the effect of therapy with bevacizumab. *Invest Ophthalmol Vis Sci*. 2009 Mar;50(3):1025–1032.
[128] Yoshimura T, Sonoda KH, Sugahara M,et al. Comprehensive analysis of inflammatory immune mediators in vitreoretinal diseases. *PLoS One*. 2009 Dec 4;4(12):e8158.
[129] Ozturk BT, Bozkurt B, Kerimoglu H,et al. Effect of serum cytokines and VEGF levels on diabetic retinopathy and macular thickness. *Mol Vis*. 2009 Sep; 19;15: 1906–14.:1906–1914.
[130] Yoshida A, Elner SG, Bian ZM,et al. Differential chemokine regulation by Th2 cytokines during human RPE-monocyte coculture. *Invest Ophthalmol Vis Sci*. 2001 Jun;42(7):1631–1638.
[131] Simonini A, Moscucci M, Muller DW,et al. IL-8 is an angiogenic factor in human coronary atherectomy tissue. *Circulation*. 2000 Apr 4;101(13):1519–1526.