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Cytokine Expression in Staphylococcal and Streptococcal Endophthalmitis

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Abstract
Background: Endophthalmitis is an infection of ocular tissues, often with devastating outcomes for vision. Immunomodulation is an emerging avenue for therapeutic intervention in endophthalmitis, with the expression of cytokines central to potential mechanisms. This literature review with a systematic approach characterizes the cytokine expression in both animal and human staphylococcal and streptococcal endophthalmitis. ***Method and Results:*** Four online databases were searched for studies profiling cytokine levels in animal models or human populations with staphylococcal and/or streptococcal endophthalmitis. Of the 1,060 articles identified, 14 studies were included in this review comprising eight animal models and six human populations. Mouse, rat, and rabbit models of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pneumoniae* endophthalmitis had elevated levels of IL-1β, IL-6, IFN-γ, TNF-α, and IL-8, with earlier peaks observed in *S. epidermidis* infection. Human endophthalmitis demonstrated significantly increased mediator levels compared to controls for a range of pro-inflammatory and anti-inflammatory cytokines, chemokines, and growth factors. Several associations were established between cytokine concentrations and both initial visual acuity and visual prognosis, with no consistent correlations across trials. ***Conclusions:*** It may be that virulence factors and the combinations of toll-like receptors activated influence the pathogen-specific visual outcomes observed in endophthalmitis. Furthermore, disease severity and potential therapeutic targets may be dependent on synergistic and compensatory cytokine pathways and the expression of anti-inflammatory mediators. Future research should aim to better characterize the roles of inflammatory mediators and solidify associations between pathogens, inflammation, and endophthalmitis outcomes. This has exciting implications for the prevention and treatment of endophthalmitis in clinical settings.

Introduction

Endophthalmitis is a rare but potentially blinding condition characterized by infection of the vitreous humour. Infectious agents are mostly from exogenous sources such as intraocular surgery, penetrating trauma, or intravitreal injection. Infection may also be endogenous, arising from haematogenous seeding of microbes into the eye [1]. Less commonly, endophthalmitis is associated with fungal infection. Typical presenting features include loss of vision, pain and signs of ocular inflammation such as hypopyon, and vitreous opacification [2]. Microbial iden-

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tification may aid a clinical diagnosis, but in up to 30% of patients, no pathogen is isolated [3]. Empiric intravitreal antibiotics remain the mainstay of treatment, while a vitrectomy is reserved for more severe presentations.

The microbiology of endophthalmitis varies between preceding events. Ocular surgery is a principal risk factor, accounting for over 80% of cases [4]. Cataract surgery and intravitreal injection are commonly implicated, with per-procedure complication rates of 0.265% and 0.056%, respectively [5, 6]. In post-surgical patients, major pathogens include coagulase-negative staphylococci such as *Staphylococcus epidermidis*, followed by *Staphylococcus aureus*, streptococci, enterococci, and *Bacillus* [7]. Overall, *Staphylococcus* and *Streptococcus* are the most frequently isolated genera in endophthalmitis [8].

Endophthalmitis caused by *S. epidermidis*, the most common commensal on the ocular surface [9], is associated with better visual outcomes compared to Gram-positive, non-coagulase-negative endophthalmitis such as *Staphylococcus aureus* and *Streptococcus pneumoniae* infections which cause severe visual loss (worse than 5/200 visual acuity) in up to 37% of eyes [8]. Endophthalmitis caused by Gram-negative bacteria including *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Moraxella* spp., and *Klebsiella pneumoniae* are also associated with severe visual deficits [8, 10] but remain scarcely studied in both animal and human models.

In addition to the virulence of the pathogen, the host immune response contributes to the inflammation and visual outcomes in endophthalmitis. Microbes express clusters of similar molecules known as pathogen-associated molecular patterns (PAMPs) which are recognized by pattern recognition receptors such as toll-like receptors (TLRs). TLRs have been characterized in multiple resident cell types of the eye including retinal pigment epithelial cells, retinal microglia, and Müller cells [11, 12]. TLR2 in particular is well-described in the inflammatory response in Gram-positive endophthalmitis [13, 14]. When activated by PAMPs, TLRs initiate a signalling cascade, driving the expression of inflammatory mediators such as cytokines and the recruitment of innate immune inflammatory cells such as neutrophils and monocytes [15, 16].

Cytokines are small polypeptides that act as intercellular messengers and chemoattractants to facilitate the initiation, maintenance, and convalescence of the immune response. They are secreted by leukocytes such as lymphocytes, macrophages, neutrophils, and dendritic cells, as well as resident cell types of the eye [12]. Cytokines may be involved in recognition (IL-1 and TNF-α), recruitment (chemokines), removal (IFN-γ, IL-2, and IL-6), and repair (growth factors) [12]. The expression of these major cytokines in endophthalmitis is an active area of research across rat, rabbit, and mouse models [4, 17]. Only a handful of studies have profiled cytokine levels in human endophthalmitis populations [18–23].

Despite advancements in treatments and perioperative measures, endophthalmitis remains a sight-threatening complication of eye surgery. With the development of antimicrobial resistance and inconclusive evidence surrounding the use of corticosteroids [24], there is an emerging role for immunomodulation as a potential therapeutic and/or prophylactic measure. Thus, this literature review with a systematic approach aims to examine the cytokine expression observed in animal and human endophthalmitis, with a particular focus on staphylococcal and streptococcal infection in view of its higher prevalence, severe visual outcomes, and better characterization in animal studies. Although endophthalmitis caused by other infectious agents such as Gram-negative bacteria is also associated with significant visual deficits, we considered it outside the scope of this review due to comparatively lower prevalence rates, differences in virulence factors, and sparse cytokine characterization in both animal and human models of endophthalmitis.

Methods

Databases and Search Strategy

Four online databases (Medline OVID, Embase, Web of Science Core Collection, and Cochrane Library) were searched on September 12, 2020, without date or language limits. These databases were chosen based on their applicability to endophthalmitis and cytokine expression.

The key search terms identified were "endophthalmitis" and "cytokine." "Panophthalmitis" was regarded a related term for the disease, and specific classes of cytokines including "chemokine," "interleukin," and "growth factor" were considered in the search (online suppl. Material 1; for all online suppl. material, see www.karger.com/doi/10.1159/000525330). An intentionally broad search strategy was developed because of the diversity of infectious agents and relative paucity of human studies. Selection for streptococcal and staphylococcal infection was conducted following the initial search.

Inclusion and Exclusion Criteria

Included studies investigated levels of cytokine expression in animal models or human participants with endophthalmitis. Additionally, only articles which involved staphylococcal and/or streptococcal infection were selected. One study in a human population [19] was included despite not specifying causative pathogens because the presence of staphylococcal infection was presumed given its high rates of occurrence in endophthalmitis.

Excluded articles were those which presented no data on cytokine expression, did not have live models of streptococcal and/or

staphylococcal endophthalmitis, or were in vitro experiments only. Conference abstracts, review articles, and single case reports were also not considered in this review. Five articles were excluded because they were not written in the English language.

Results

Literature Search

A total of 1,060 records after deduplication were identified through databases and reference reviews for title and abstract screening. As per the inclusion and exclusion criteria, the removal of 990 studies on screening yielded 70 records for full-text assessment of eligibility. Fifty-six full-text articles were excluded including one full-text which was not able to be retrieved. Fourteen studies therefore met the criteria for inclusion in this review (Fig. 1). All data were organized using EndNote X9.3.3.

Study Characteristics

Animal Models

Eight included studies were experimental rat [16, 25], rabbit [26], or mouse [13, 27–30] models of endophthalmitis (Table 1). Animal eyes were inoculated via intravitreal injection of *Staphylococcus aureus* in six studies [13, 16, 27–30]. *Staphylococcus epidermidis* [25] and *Streptococcus pneumoniae* [26] were introduced in one study each.

Samples were collected from vitreous humour in rats and rabbits and from retinal or eye tissue lysates in mice. The timing of samples varied across studies from 6 hours to 7 days post-infection. Cytokine levels were quantified via enzyme-linked immunosorbent assay in all but one animal study, which applied quantitative reverse transcriptase polymerase chain reaction to detect cytokine messenger RNA expression only [26].

Human Populations

Six studies quantified cytokine expression in human endophthalmitis populations (Table 2). All were prospective studies conducted in different countries, namely Brazil [18], China [19], Canada [20], France [21], India [22], and Japan [23]. Unsurprisingly, considering the low incidence of endophthalmitis, sample sizes were small, ranging from 16 to 49 samples. There was also variation among study criteria in the selection for events preceding diagnosis. Two articles did not select for specific predisposing events [22, 23], two selected post-cataract surgery patients [18, 21], one included cases post-surgery or intravitreal injection [20], and one limited their population to endophthalmitis following open globe injury [19].

Furthermore, one study did not provide analysis of microbiological aetiology [19] and two studies excluded non-bacterial infections [19, 23]. Across cited populations, staphylococcal and streptococcal endophthalmitis comprised 52–100% of culture-positive cases [18, 22]. Only three studies detected Gram-negative infections, which comprised 12–23% of culture-positive endophthalmitis [20, 22]. Samples collected from the vitreous humour [18, 20, 22, 23], aqueous humour [21], or both [19] varied in timing from 4 days [19] to 6 weeks [20] after the predisposing event, though timing was not reported in two studies [21, 23]. Additionally, control samples were a point of difference. Most control samples were collected from eyes with non-infectious conditions such as macular hole [20], idiopathic epiretinal membrane [23], retinal detachment, and diabetic retinopathy [22]. One study sampled controls from cadaveric eyes within 4–10 hours of death [19]. All studies in human populations used multiplex analysis of cytokine expression.

Findings in Animal Models of Endophthalmitis

Two studies investigated cytokine expression in the vitreous humour of rats (Table 1). In a *Staphylococcus aureus* model of endophthalmitis, Giese et al. [16] detected upregulation of the pro-inflammatory cytokines IL-1β, IFN-γ, and TNF-α, as well as the chemokine CINC, a functional rat homologue of human IL-8 [31]. Petropoulos et al. [25] described similar cytokine increases in *Staphylococcus epidermidis*-infected rats but noted earlier concentration elevations (12 vs. 24 hours). Both studies reported temporal correlations between peak cytokine levels and clinical inflammation. Interestingly, IFN-γ concentrations peaked later than the other cytokines measured. No cytokines were detected in the sera at any time point in either study.

Five studies assessed retinal or eye tissue lysate samples in mouse models of *S. aureus* endophthalmitis [13, 27–30], observing similar cytokine profiles to the previous studies. Significant upregulation was demonstrated for the pro-inflammatory cytokines IL-1β and IL-6 in all these studies, with peak levels reported by Talreja et al. [13] at 2 days and 1 day post-infection, respectively. Kumar et al. [29] similarly showed that these cytokines were most elevated at the earliest sampling time point of 36 hours. TNF-α is another pro-inflammatory cytokine which was elevated in three mouse studies [13, 27, 29]. Francis et al. [30] did not detect increased TNF-α protein levels but reported an upregulation of TNF-α messenger RNA expression. Additionally, Talreja et al. [13] reported an elevation in IL-10 levels, highest between 1 and 2 days

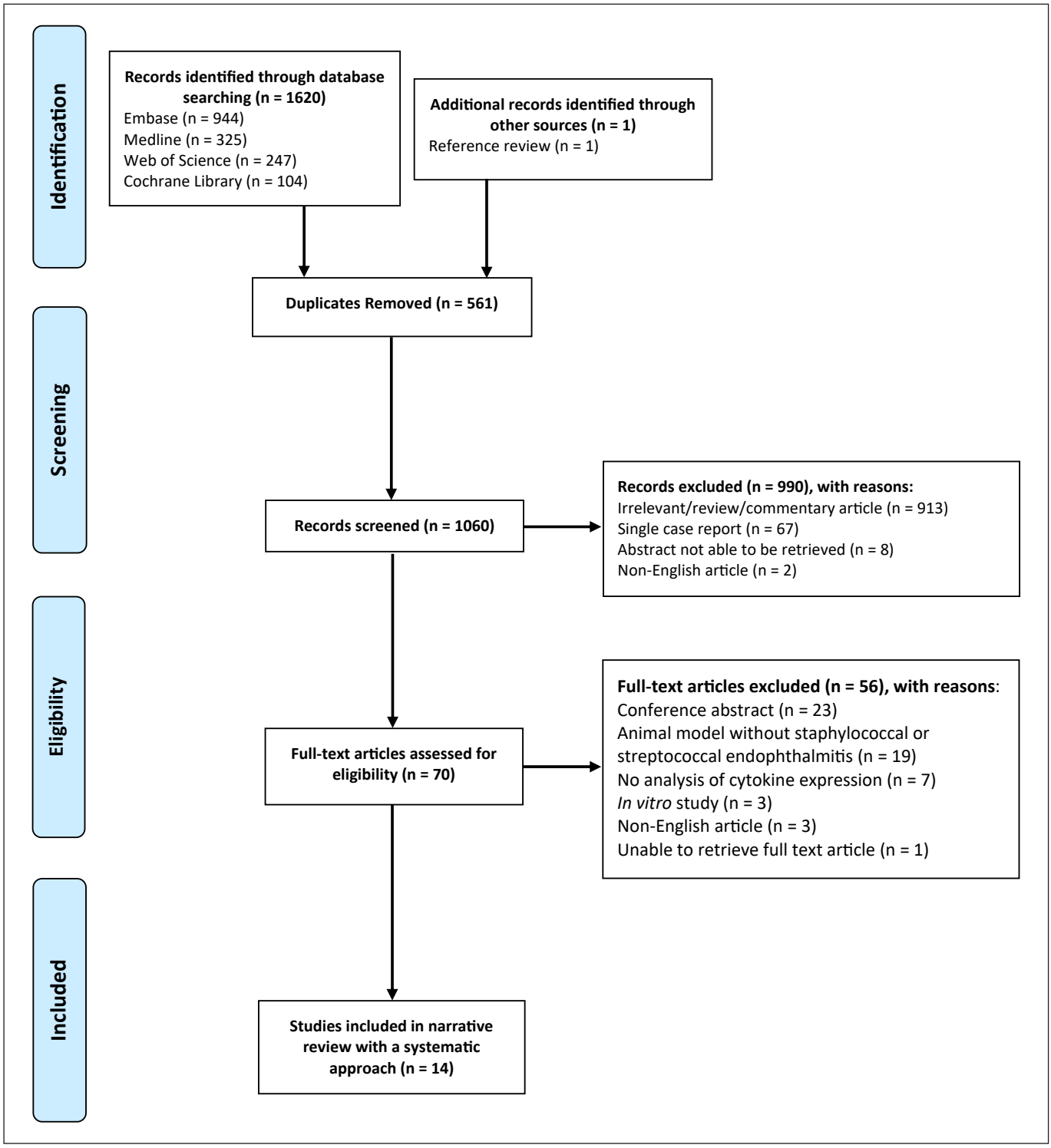

Fig. 1. Literature search flow diagram.

Table 1. Studies investigating cytokine expression in animal models of endophthalmitis

Study	Study characteristics	Cytokine expression	Chemokine expression
Giese et al. [16], 1998	Model: rat Pathogen: *Staphylococcus aureus* Sample: vitreous Time: 6, 24, 48, 72 h	↑ IL-1β (24 h)[a] ↑ IFN-γ (24–48 h)[a] ↑ TNF-α (24 h)[a]	↑ CINC (24 h)[a]
Petropoulos et al. [25], 2006	Model: rat Pathogen: *Staphylococcus epidermidis* Sample: vitreous Time: 6, 12, 24, 48, 72 h, 7 days	↑ IL-1β (12 h)[a] ↑ IFN-γ (6, 48 h)[a] ↑ TNF-α (12 h)[a]	NS
Sanders et al. [26], 2011	Model: rabbit Pathogen: *Streptococcus pneumoniae* Sample: vitreous Time: 3, 6, 9, 12, 24, 36, 48 h	↑ IL-1β[b] ↑ IL-6[b] ↑ TNF-α[b]	↑ IL-8/CXCL8[b]
Singh et al. [27], 2014	Model: mouse Pathogen: *Staphylococcus aureus* Sample: retinal tissue lysate Time: unclear	↑ IL-1β ↑ IL-6 ↑ TNF-α	↑ KC/CXCL1 ↑ MIP-2/CXCL1
Talreja et al. [13], 2015	Model: mouse Pathogen: *Staphylococcus aureus* Sample: retinal tissue lysate Time: 1, 2, 3 days	↑ IL-1β (2 days) ↑ IL-6 (1 day) ↑ IL-10 (1–2 days)	↑ KC/CXCL1 (1–2 days) ↑ MIP-2/CXCL1 (2–3 days)
Rajamani et al. [28], 2016	Model: mouse Pathogen: *Staphylococcus aureus* Sample: retinal tissue lysate Time: 24 h	↑ IL-1β ↑ IL-6	↑ MIP-2/CXCL1
Kumar et al. [29], 2016	Model: mouse Pathogen: *Staphylococcus aureus* Sample: retinal tissue lysate Time: 36, 60, 84 h	↑ IL-1β (36 h) ↑ IL-6 (36 h) ↑ TNF-α (60 h)	NS
Francis et al. [30], 2020	Model: mouse Pathogen: *Staphylococcus aureus* Sample: eye lysate Time: 24, 30 h	↑ IL-1β ↑ IL-6	↑ KC/CXCL1 ↑ MIP-2/CXCL1[c]

Time – elapsed interval between inoculation of bacteria and sampling. h – hours. (12 h) – peak cytokine level at 12 h. (24–48 h) – peak cytokine level between 24 and 48 h. (6, 48 h) – bimodal peak of cytokine levels at 6 and 48 h. ↑ – significant increase reported. ↓ – significant decrease reported. NS – no significant changes in expression or not investigated. IL, interleukin; IFN, interferon; CINC, cytokine-induced neutrophil chemoattractant; TNF, tumour necrosis factor; KC, keratinocyte-derived chemokine; MIP, macrophage inflammatory protein. [a] No cytokine levels detected in the sera. [b] No non-endophthalmitis control for reference. [c] Only measured at 24 h, not measured at 30 h.

post-infection. This study further demonstrated increased expression of KC/CXCL1 and MIP-2/CXCL2, the functional murine homologues of the chemokine IL-8 [32]. Similar patterns of expression were reported in three other studies [27, 28, 30].

Findings in Human Endophthalmitis

Included studies of human endophthalmitis profiled a large number of cytokines, chemokines, and growth factors involved in all phases of the immune response (Table 2). Generally, mediators were elevated in both aqueous and vitreous samples. The pro-inflammatory cytokines and chemokines described in animal models were observed to increase with good concordance across human studies, particularly noticeable in IL-1β, IL-6, IFN-γ, TNF-α, and IL-8.

Escarião et al. [18] reported elevated levels of twenty inflammatory mediators in the vitreous of 18 endophthal-

Table 2. Studies investigating cytokine expression in human endophthalmitis

Study	Study characteristics	Cytokine expression	Chemokine expression	Growth factor expression
Summary of findings from studies in animal models		↑ IL-1β, IL-6, IL-10, IFN-γ, TNF-α	↑ KC/CXCL1, MIP-2/CXCL2, IL-8/CXCL8, CINC	NS
Escarião et al. [18], 2014	Endophthalmitis preceded by cataract surgery *N* = 18, vitreous samples Controls: vitreous from non-infectious ocular disease	↑ IL-1Ra, IL-2, IL-4, IL-6, IL-9, IL-10, IL-15, IL-17, IFN-γ, TNF-α ↓ IL-7 (i) TNF-α	↑ IL-8/CXCL8, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, eotaxin/CCL11	↑ G-CSF, GM-CSF, FGF2/bFGF, PDGF-BB
Hao et al. [19], 2016	Bacterial endophthalmitis preceded by open globe injury *N* = 30, aqueous samples Controls: aqueous or vitreous from cadavers without systemic inflammatory or ocular disease	↑ IL-1β, IL-6, IL-17A	↑ MIP-3α/CCL20	↓ TGF-β1
	Bacterial endophthalmitis preceded by open globe injury *N* = 30, vitreous samples Controls: aqueous or vitreous from cadavers without systemic inflammatory or ocular disease	↑ IL-1β, IL-6, IL-13	↑ MIP-3α/CCL20	NS
Seamone et al. [20], 2017	Endophthalmitis preceded by intraocular surgery or intravitreal injection *N* = 18, vitreous samples Controls: vitreous from macular hole surgery	↑ IL-1β, IL-1Ra, IL-6	↑ IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCL2	↑ VEGF-A
Sauer et al. [21], 2018	Endophthalmitis preceded by cataract surgery *N* = 49, aqueous samples Controls: aqueous from uncomplicated cataract surgery	↑ IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, IFN-γ, TNF-α (i) IL-6, IL-15 (f) IL-10	↑ IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, eotaxin/CCL11 (i) IP-10/CXCL10, MCP-1/CCL2 (f) IL-8/CXCL8, MCP-1/CCL2	↑ G-CSF, FGF, PDGF-BB, VEGF (i) G-CSF, VEGF (f) VEGF
Deshmukh et al. [22], 2018	Culture-positive endophthalmitis without requirement for predisposing event *N* = 25, vitreous samples Controls: vitreous from non-infectious ocular disease	↑ IL-1α, IL-1β, IL-1Ra, IL-6, IL-10, IL-12p40, IL-13, IFN-γ, TNF-α (i) IL-1β	↑ GRO, IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, MCP-3/CCL7 (i) IL-8/CXCL8	↑ G-CSF, FGF2/bFGF, PDGF-BB, TGF-α (i) TGF-α
Fukunaga et al. [23], 2020	Bacterial endophthalmitis without requirement for predisposing event *N* = 16, vitreous samples Controls: vitreous from idiopathic epiretinal membrane	↑ IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12p70, IL-13, IL-17A, IFN-γ, TNF-α	↑ IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, eotaxin/CCL11	↑ G-CSF, GM-CSF, FGF2/bFGF, PDGF-BB, VEGF

N – number of endophthalmitis samples. ↑ – significant increase reported. ↓ – significant decrease reported. NS – no significant changes in expression or not investigated (i) – correlation reported between cytokine level and initial visual acuity at presentation. (f) – correlation reported between cytokine level and final visual acuity after 1 year. IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; KC, keratinocyte-derived chemokine; MIP, macrophage inflammatory protein; IL-1Ra, interleukin-1 receptor antagonist; MCP, monocyte chemoattractant protein; RANTES, regulated on activation, normal T cell expressed and secreted; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; FGF, fibroblast growth factor; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; IP-10, interferon-gamma-inducible protein-10; VEGF, vascular endothelial growth factor; IL-12p70, interleukin-12 subunit p70; IL-12p40, interleukin-12 subunit p40; GRO, growth-regulated oncogene.

mitis patients post-cataract surgery. Anti-inflammatory cytokines such as IL-1Ra, IL-10, and IL-4 were among those upregulated. Higher IL-8 levels were detected in culture-positive than culture-negative cases, and IL-17 was elevated in *S. aureus* endophthalmitis in contrast with other pathogens. Furthermore, in the vitreous of 25 culture-positive and 16 bacterial endophthalmitis patients, respectively, Deshmukh et al. [22] and Fukunaga et al. [23] also reported increases in pro-inflammatory and anti-inflammatory cytokines, chemokines, and growth factors. Notably, the former detected significant elevations in the growth factors FGF2, TGF-α, G-CSF, and PDGF-BB. No significant differences were established between culture-positive and culture-negative cases.

In the vitreous of 18 exogenous endophthalmitis patients, Seamone et al. [20] reported elevated VEGF-A, IL-1β, IL-1Ra, IL-6, IL-8, IP-10, and MCP-1 levels. Results were not reported for many of the other mediators investigated; however, a positive correlation was detected between VEGF-A and IL-8/CXCL8 levels. The expression patterns between culture-positive and culture-negative populations did not significantly differ.

A multi-centre study of 49 aqueous endophthalmitis samples post-cataract surgery by Sauer et al. [21] showed an increase in all inflammatory markers measured except GM-CSF. The elevation was particularly noticeable in pro-inflammatory and T-helper 1 cytokines such as IL-1β, IL-1Ra, IL-15, and IL-16 and chemokines such as IL-8, MIP-1β, MCP-1, IP-10, and G-CSF. This generalized cytokine upregulation was not reported by Hao et al. [19] who compared inflammatory mediator levels in the aqueous and vitreous humour of bacterial endophthalmitis patients post-open globe injury. IL-1β, IL-6, and MIP-3α levels were elevated in both aqueous and vitreous, with IL-17A overexpressed in aqueous only. Additionally, decreased levels of IL-2, IL-5, IL-21, and TGF-β1 were detected in the aqueous but not vitreous humour.

Correlations between Cytokine Expression and Visual Acuity

Several studies sought for correlations between cytokine concentrations and visual acuity at initial presentation and then at follow-up, with no consistent findings. Escarião et al. [18] reported a positive correlation between levels of TNF-α and initial visual acuity. Strong associations between initial visual acuity and intraocular levels of TGF-α, IL-1β, and IL-8 were also determined by Deshmukh et al. [22]. Sauer et al. [21] reported that initial visual acuity was better with increased expression of IL-15 and poorer with high levels of IL-6, MCP-1, G-CSF, IP-10, VEGF. After 1 year, final visual acuity was better with high initial levels of IL-10. A poorer prognosis at 1 year correlated with greater concentrations of IL-8, MCP-1, and VEGF.

Discussion

Staphylococcal and Streptococcal Virulence Factors in Endophthalmitis

Animal models of endophthalmitis demonstrated similar immune mediator profiles with some temporal differences. Earlier peak cytokine responses were noted in *Staphylococcus epidermidis* [25] than in *Staphylococcus aureus* studies [13, 16, 29], which may reflect its lower virulence potential [8]. Indeed, *S. epidermidis* has been shown to induce rapid inflammatory responses and clear more quickly than other pathogens in endophthalmitis [33]. With note of the eye's status of immune privilege and the associated suppression of an inflammatory milieu [34], the ability of the ocular immune system to respond quickly to bacterial challenge may therefore influence visual outcomes.

Gram-positive bacteria including *Streptococcus* and *Staphylococcus* share similar cell wall components that activate TLR2 and induce the production of inflammatory mediators [13, 35]. In addition to cell wall PAMPs such as lipoteichoic acid and peptidoglycan, Kumar and Kumar [36] demonstrated that *S. aureus*-specific virulence factors independently induce expression of IL-1β, TNF-α, and KC/CXCL1 in mouse eyes. Similar cytokine elevations were noted in studies included in this review. One such virulence factor, α-toxin, is a strong inducer of the pro-inflammatory cytokine IL-17 in humans [37] which induces the production of other pro-inflammatory cytokines and chemokines and also increases the recruitment of neutrophils [38]. Additionally, the presence of α-toxin in a rabbit model of *S. aureus* endophthalmitis has been shown to correlate with increased retinal damage when compared to infection with α-toxin-deficient strains [1]. This notion that α-toxin may be a key virulence factor in staphylococcal endophthalmitis is furthered by several human endophthalmitis studies reporting significant IL-17 upregulation [18, 19, 21, 23]. Importantly, Escarião et al. [18] also found that increased IL-17 levels distinguished *S. aureus* from other bacterial infections.

Although most streptococcal endophthalmitis is caused by other species [8], several virulence factors have been investigated in *Streptococcus pneumoniae* endo-

phthalmitis. Unencapsulated *S. pneumoniae* strains induce less severe but clinically significant disease when compared with capsulated strains [26], suggesting that bacterial factors other than the capsule are involved in mediating inflammation. Pneumolysin, an intracellular cytotoxin produced by almost all pneumococcal strains [39], has been implicated in the pathophysiology of *S. pneumoniae* endophthalmitis. Infection of rat eyes by a pneumolysin-deficient *S. pneumoniae* strain causes less severe tissue damage than pneumolysin-producing strains [40]. Furthermore, both the polysaccharide capsule and pneumolysin are recognized by TLR4 [41], which contributes to the ocular immune response in other encapsulated bacterial infections [42]. Therefore, the poor outcomes of *S. pneumoniae* endophthalmitis may reflect that pneumococcal virulence factors activate both TLR2 (cell wall components) and TLR4 (capsule and pneumolysin).

Host Immune Response in Endophthalmitis

Similar patterns of elevation in pro-inflammatory cytokines and chemokine levels were observed between animal models and human endophthalmitis populations, suggesting the validity of using animal experimentation to inform and guide human studies in endophthalmitis. This is despite intra-study and inter-study heterogeneity with respect to disease severity and time course, with the latter shown to influence cytokine expression patterns in animal models [13, 16, 25, 29]. We note that regardless of the timing between predisposing event and sampling, the invasive nature of acquiring aqueous and vitreous samples means that sampling often occurred as an adjunct to, or as part of, other routine investigations and procedures, likely coinciding with times of higher intraocular concentrations of pro-inflammatory mediators.

The pro-inflammatory cytokines IL-1β and TNF-α were upregulated across the included studies. They are expressed in the early stages of infection to initiate the immune response and recruit mediators to the eye [12], as observed by Petropoulos et al. [25] who reported that levels of both mediators peaked prior to clinical inflammation. IL-1β augments other pro-inflammatory cytokines such as IL-6, IL-17A, KC, and MCP-1 and can independently cause cellular infiltration in mouse eyes [43]. TNF-α has a further role of promoting neutrophil migration via increasing vascular permeability [44]. However, studies have suggested that the absence of TNF-α in ocular inflammatory states may be compensated by the upregulation of other cytokines and chemokines, resulting in similar disease outcomes [43, 45]. Furthermore, IL-6 was another pro-inflammatory cytokine upregulated in the studies. In a model of *Bacillus cereus* endophthalmitis, IL-6-deficient mice did not have a significantly altered clinical course compared to wild-type mice [46]. This apparent presence of both synergism and compensatory cytokine pathways presents a challenge for immunomodulatory therapies. Although targeting single inflammatory mediators such as IL-1, TNF-α, and IL-6 has evidence in certain autoimmune conditions [47], effective immunomodulation in endophthalmitis may require consideration of multiple cytokine targets and upstream regulatory pathways [28].

Several anti-inflammatory cytokines were at increased concentrations across cited studies including IL-1 receptor antagonist (IL-1Ra) and IL-10. In combination with elevated IL-1β expression, the observed upregulation of IL-1Ra supports the idea that anti-inflammatory cytokines play a role in limiting host-mediated tissue damage. IL-10, which inhibits T cell, natural killer cell, and macrophage activity, is implicated in limiting the production of pro-inflammatory cytokines and chemokines [48]. Angiogenesis, a process which is harmful to ocular tissues, is also inhibited by IL-10 [49]. While correlations between concentrations of IL-10 and other mediators in endophthalmitis have not been investigated, its safeguarding mechanism may explain the association of elevated IL-10 with the better visual outcomes reported by Sauer et al. [21]. Other anti-inflammatory cytokines such as IL-4, IL-5, and IL-13 were also upregulated; however, their significance in endophthalmitis is yet to be characterized.

Chemokines are a family of cytokines involved in the recruitment of cells in the inflammatory response. IL-8 and its functional homologues in animal models (CINC in rats [31], KC and MIP-2 in mice [32]) were consistently upregulated, functioning primarily to attract and recruit neutrophils. In an *S. aureus* endophthalmitis rabbit model, Sanders et al. [26] described temporal variations in neutrophil activity without similar changes to IL-8 expression, suggesting the presence of other neutrophil chemotaxins. Such chemokines may include growth-related oncogene (GRO) [50], IFN-γ-inducible protein-10 (IP-10), and members of the CC chemokine family more commonly associated with monocyte chemotaxis [32], many of which are upregulated in human endophthalmitis. Interestingly, included studies showed that concentrations of IL-8, monocyte chemoattractant protein-1 (MCP-1/CCL2), and IP-10 correlate with poorer initial visual acuity [21, 22]. It is possible that these specific chemotactic factors, via the recruitment of neutrophils,

monocytes, and T cells, respectively, induce the downstream expression of pro-inflammatory mediators which contributes to ocular damage and poorer visual outcomes.

The vascular endothelial growth factor family (VEGF/VEGF-A), implicated in the pathophysiology of non-infectious ocular conditions, was upregulated in several studies [20, 21, 23]. In diabetic retinopathy and age-related macular degeneration, trauma such as tissue hypoxia leads to production of VEGF and subsequent neovascularization [51]. Although hypoxia is not a recognized sequela of endophthalmitis, Seamone et al. [20] reported a positive correlation between VEGF-A and IL-8 concentrations, suggesting a hypoxia-independent mechanism for VEGF-A secretion mediated by IL-8. Moreover, the authors highlighted that VEGF-A may be upregulated upon activation of ocular immune defences regardless of endophthalmitis aetiology. This is supported by its associations with poor initial visual acuity and 1-year prognosis detected by Sauer et al. [21], who sampled from both culture-positive and culture-negative populations. It is plausible then, that by inducing monocyte and granulocyte chemotaxis and increasing the permeability of the blood-retinal barrier [52], VEGF has a key role in the ocular tissue damage caused by the host immune response, resulting in its association with poor visual outcomes.

Limitations

There are several limitations in this review. Regarding the methodology, restricting search parameters to streptococcal and staphylococcal infection resulted in the exclusion of several animal endophthalmitis studies, particularly regarding *B. cereus* infection, four of which are included in the discussion [14, 45, 46, 53]. Additionally, quantitative comparison of cytokine expression between studies is limited by the different animal models, pathogens, and cytokine detection methods, as well as varying bacterial loads inoculated to induce disease.

The paucity of available literature further restricted study selection. For human endophthalmitis populations, our inclusion criteria did not consider selection for predisposing events, severity of disease, infection time course, or any concomitant medical therapy, which may have affected the observed cytokine concentrations. Differences in sampling locations and controls may also have influenced results, particularly as one study using cadaveric control samples reported a significantly different cytokine profile compared with other cited populations [19].

More broadly, analysis of cytokine expression in endophthalmitis is limited by the rarity of the disease. The low power of small sample sizes restricts the capacity to determine significant differences between intra-study variables such as cytokine profiles induced by different organisms. This is exacerbated by traditionally low rates of culturing pathogens; however, newer PCR techniques are improving bacterial detection rates [54]. It seems likely that non-Gram-positive pathogens such as Gram-negative bacteria and fungi may exhibit distinct profiles of cytokine expression owing to their different virulence factors and subsequent immune activation pathways. Furthermore, many inflammatory mediators detected in multiplex assays have currently uncharacterized roles in endophthalmitis, reinforcing the need for future animal studies to extend knowledge and understanding in this area.

Conclusion

Gram-positive bacterial endophthalmitis remains a challenging ophthalmic emergency with high rates of vision loss. This review highlights its complex pathophysiology which involves organism-specific virulence factors, activation of TLR2, and various interactions with the host immune response. *Staphylococcus* spp. and *Streptococcus* spp., the most common endophthalmitis pathogens, also produce proteins such as α-toxin and pneumolysin that may inflict further damage either directly or via the activation of other immune system receptors such as TLR4 [36, 41].

Central to TLR activation and inflammation is the downstream expression of cytokines. Included studies demonstrated a global upregulation of inflammatory mediators across both animal models and human populations with endophthalmitis. Pro-inflammatory cytokines are the most well-studied subset of these mediators, an area of interest being the possible presence of synergistic and compensatory cytokine regulation pathways with its unique implications on directions for future research [43, 45]. Anti-inflammatory cytokines, chemokines, and growth factors similarly show promise as targets of immunomodulation and are associated in varying degrees to visual outcomes [21, 22], although most have currently indeterminate roles in the pathophysiology of endophthalmitis.

Future Directions

As recent progresses show, there remains an important place for animal models to characterize the roles of cytokines upregulated in human endophthalmitis populations and to explore the inflammatory profiles induced by other bacterial pathogens. Additionally, animal models provide a means to assess potential therapeutic treatments such as targeting metabolic pathways [29, 30] or administering phage lysins [27] or anti-VEGF [20]. Some of the less characterized mediators highlighted by this review as having potential roles in immunomodulation include IL-1Ra, IL-10, MCP-1, and IP-10.

In human populations, well-powered studies are required to identify patterns of inflammation associated with specific pathogens and to continue exploring the inconsistent associations identified between cytokine expression and visual acuity [18, 21, 22]. Furthermore, studies investigating cytokine expression in the sera of patients with endophthalmitis may reveal correlations with vision loss and ocular inflammation that pave a more clinically practical path for diagnostic and/or therapeutic intervention.

Statement of Ethics

An ethics statement is not applicable because this study is based exclusively on published literature.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Data Availability Statement

All data generated or analysed during this study are included in this article and its online supplementary material. Further enquiries can be made to the corresponding author.

References

1 Callegan MC, Engelbert M, Parke DW 2nd, Jett BD, Gilmore MS. Bacterial endophthalmitis: epidemiology, therapeutics, and bacterium-host interactions. Clin Microbiol Rev. 2002 Jan;15(1):111–24.
2 Endophthalmitis Vitrectomy Study Group. Results of the Endophthalmitis Vitrectomy Study. A randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of postoperative bacterial endophthalmitis. Arch Ophthalmol. 1995 Dec;113(12):1479–96.
3 Durand ML. Bacterial and fungal endophthalmitis. Clin Microbiol Rev. 2017 Jul;30(3):597–613.
4 Miller FC, Coburn PS, Huzzatul MM, LaGrow AL, Livingston E, Callegan MC. Targets of immunomodulation in bacterial endophthalmitis. Prog Retin Eye Res. 2019 Nov;73:100763.
5 Taban M, Behrens A, Newcomb RL, Nobe MY, Saedi G, Sweet PM, et al. Acute endophthalmitis following cataract surgery: a systematic review of the literature. Arch Ophthalmol. 2005 May;123(5):613–20.
6 Fileta JB, Scott IU, Flynn HW Jr. Meta-analysis of infectious endophthalmitis after intravitreal injection of anti-vascular endothelial growth factor agents. Ophthalmic Surg Lasers Imaging Retina. 2014 Mar–Apr;45(2):143–9.
7 Durand ML. Endophthalmitis. Clin Microbiol Infect. 2013 Mar;19(3):227–34.
8 Endophthalmitis Vitrectomy Study Group. Microbiologic factors and visual outcome in the Endophthalmitis Vitrectomy Study. Am J Ophthalmol. 1996 Dec;122(6):830–46.
9 Graham JE, Moore JE, Jiru X, Moore JE, Goodall EA, Dooley JS, et al. Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. Invest Ophthalmol Vis Sci. 2007 Dec;48(12):5616–23.
10 Stevenson LJ, Dawkins RCH, Sheorey H, McGuinness MB, Hurley AH, Allen PJ. Gram-negative endophthalmitis: a prospective study examining the microbiology, clinical associations and visual outcomes following infection. Clin Exp Ophthalmol. 2020 Aug;48(6):813–20.
11 Kumar A, Shamsuddin N. Retinal Muller glia initiate innate response to infectious stimuli via toll-like receptor signaling. PLoS One. 2012;7(1):e29830.
12 Vallejo-Garcia JL, Asencio-Duran M, Pastora-Salvador N, Vinciguerra P, Romano MR. Role of inflammation in endophthalmitis. Mediators Inflamm. 2012;2012:196094.
13 Talreja D, Singh PK, Kumar A. In vivo role of TLR2 and MyD88 signaling in eliciting innate immune responses in staphylococcal endophthalmitis. Invest Ophthalmol Vis Sci. 2015 Feb;56(3):1719–32.
14 Novosad BD, Astley RA, Callegan MC. Role of toll-like receptor (TLR) 2 in experimental Bacillus cereus endophthalmitis. PLoS One. 2011;6(12):e28619.
15 Ravindranath RM, Mondino BJ, Adamu SA, Pitchekian-Halabi H, Hasan SA, Glasgow BJ. Immunopathologic features of Staphylococcus aureus endophthalmitis in the rat. Invest Ophthalmol Vis Sci. 1995 Nov;36(12):2482–91.

16 Giese MJ, Sumner HL, Berliner JA, Mondino BJ. Cytokine expression in a rat model of Staphylococcus aureus endophthalmitis. Invest Ophthalmol Vis Sci. 1998 Dec;39(13):2785–90.
17 Astley RA, Coburn PS, Parkunan SM, Callegan MC. Modeling intraocular bacterial infections. Prog Retin Eye Res. 2016 Sep;54:30–48.
18 Escarião P, Commodaro AG, Arantes T, de Castro CMMB, Diniz MFA, Brandt CT. Analysis of cytokines in presumed acute infectious endophthalmitis following cataract extraction. J Clin Exp Ophthalmol. 2014;5(2):1000335.
19 Hao X, Yi C, Wang Y, Li J, Huang F, He L, et al. Identification of intraocular inflammatory mediators in patients with endophthalmitis. Mol Vis. 2016 Jun 2;22:563–74.
20 Seamone ME, Lewis DR, Haidl ID, Gupta RR, O' Brien DM, Dickinson J, et al. VEGF-A is increased in exogenous endophthalmitis. Can J Ophthalmol. 2017 Jun;52(3):277–82.
21 Sauer A, Candolfi E, Gaucher D, Creuzot-Garcher C, Bron A, Chiquet C, et al. Intraocular cytokine levels in post-cataract endophthalmitis and their association with visual outcome. Ocul Immunol Inflamm. 2018;26(6):964–70.
22 Deshmukh D, Chakrabarti M, Jayasudha R, Hasnat Ali M, Tyagi M, Sharma S, et al. Elevated cytokine levels in vitreous as biomarkers of disease severity in infectious endophthalmitis. PLoS One. 2018 Oct;13(10):e0205292.
23 Fukunaga H, Kaburaki T, Shirahama S, Tanaka R, Murata H, Sato T, et al. Analysis of inflammatory mediators in the vitreous humor of eyes with pan-uveitis according to aetiological classification. Sci Rep. 2020 Feb 17;10(1):2783.
24 Bui DK, Carvounis PE. Evidence for and against intravitreous corticosteroids in addition to intravitreous antibiotics for acute endophthalmitis. Int Ophthalmol Clin. 2014;54(2):215–24.
25 Petropoulos IK, Vantzou CV, Lamari FN, Karamanos NK, Anastassiou ED, Pharmakakis NM. Expression of TNF-alpha, IL-1beta, and IFN-gamma in Staphylococcus epidermidis slime-positive experimental endophthalmitis is closely related to clinical inflammatory scores. Graefes Arch Clin Exp Ophthalmol. 2006 Oct;244(10):1322–8.
26 Sanders ME, Norcross EW, Robertson ZM, Moore QC, Fratkin J, Marquart ME. The Streptococcus pneumoniae capsule is required for full virulence in pneumococcal endophthalmitis. Invest Ophthalmol Vis Sci. 2011 Feb 22;52(2):865–72.
27 Singh PK, Donovan DM, Kumar A. Intravitreal injection of the chimeric phage endolysin Ply187 protects mice from Staphylococcus aureus endophthalmitis. Antimicrob Agents Chemother. 2014 Aug;58(8):4621–9.
28 Rajamani D, Singh PK, Rottmann BG, Singh N, Bhasin MK, Kumar A. Temporal retinal transcriptome and systems biology analysis identifies key pathways and hub genes in Staphylococcus aureus endophthalmitis. Sci Rep. 2016 Feb 11;6:21502.
29 Kumar A, Giri S, Kumar A. 5-Aminoimidazole-4-carboxamide ribonucleoside-mediated adenosine monophosphate-activated protein kinase activation induces protective innate responses in bacterial endophthalmitis. Cell Microbiol. 2016 Dec;18(12):1815–30.
30 Francis R, Singh PK, Singh S, Giri S, Kumar A. Glycolytic inhibitor 2-deoxyglucose suppresses inflammatory response in innate immune cells and experimental staphylococcal endophthalmitis. Exp Eye Res. 2020 Aug;197:108079.
31 Guex-Crosier Y, Wittwer AJ, Roberge FG. Intraocular production of a cytokine (CINC) responsible for neutrophil infiltration in endotoxin induced uveitis. Br J Ophthalmol. 1996 Jul;80(7):649–53.
32 Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity. 2000 Feb;12(2):121–7.
33 Meredith TA, Trabelsi A, Miller MJ, Aguilar E, Wilson LA. Spontaneous sterilization in experimental Staphylococcus epidermidis endophthalmitis. Invest Ophthalmol Vis Sci. 1990 Jan;31(1):181–6.
34 Koevary SB. Ocular immune privilege: a review. Clin Eye Vis Care. 2000 Dec;12(3–4):97–106.
35 Schröder NW, Morath S, Alexander C, Hamann L, Hartung T, Zähringer U, et al. Lipoteichoic acid (LTA) of Streptococcus pneumoniae and Staphylococcus aureus activates immune cells via toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. J Biol Chem. 2003 May 2;278(18):15587–94.
36 Kumar A, Kumar A. Role of Staphylococcus aureus virulence factors in inducing inflammation and vascular permeability in a mouse model of bacterial endophthalmitis. PLoS One. 2015 Jun 8;10(6):e0128423.
37 Niebuhr M, Gathmann M, Scharonow H, Mamerow D, Mommert S, Balaji H, et al. Staphylococcal alpha-toxin is a strong inducer of interleukin-17 in humans. Infect Immun. 2011 Apr;79(4):1615–22.
38 Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. Nature. 2008 Jun 19;453(7198):1051–7.
39 Ng EW, Samiy N, Rubins JB, Cousins FV, Ruoff KL, Baker AS, et al. Implication of pneumolysin as a virulence factor in Streptococcus pneumoniae endophthalmitis. Retina. 1997;17(6):521–9.
40 Ng EW, Costa JR, Samiy N, Ruoff KL, Connolly E, Cousins FV, et al. Contribution of pneumolysin and autolysin to the pathogenesis of experimental pneumococcal endophthalmitis. Retina. 2002 Oct;22(5):622–32.
41 Malley R, Henneke P, Morse SC, Cieslewicz MJ, Lipsitch M, Thompson CM, et al. Recognition of pneumolysin by toll-like receptor 4 confers resistance to pneumococcal infection. Proc Natl Acad Sci U S A. 2003 Feb 18;100(4):1966–71.
42 Hunt JJ, Astley R, Wheatley N, Wang JT, Callegan MC. TLR4 contributes to the host response to Klebsiella intraocular infection. Curr Eye Res. 2014 Aug;39(8):790–802.
43 Da Cunha AP, Zhang Q, Prentiss M, Wu XQ, Kainz V, Xu YY, et al. The hierarchy of proinflammatory cytokines in ocular inflammation. Curr Eye Res. 2018 Apr;43(4):553–65.
44 Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. Physiol Rev. 2006 Jan;86(1):279–367.
45 Ramadan RT, Moyer AL, Callegan MC. A role for tumor necrosis factor-alpha in experimental Bacillus cereus endophthalmitis pathogenesis. Invest Ophthalmol Vis Sci. 2008 Oct;49(10):4482–9.
46 Parkunan SM, Randall CB, Astley RA, Furtado GC, Lira SA, Callegan MC. CXCL1, but not IL-6, significantly impacts intraocular inflammation during infection. J Leukoc Biol. 2016 Nov;100(5):1125–34.
47 Kunz M, Ibrahim SM. Cytokines and cytokine profiles in human autoimmune diseases and animal models of autoimmunity. Mediators Inflamm. 2009;2009:979258.
48 Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol. 2008 May 1;180(9):5771–7.
49 Ghasemi H, Ghazanfari T, Yaraee R, Owlia P, Hassan ZM, Faghihzadeh S. Roles of IL-10 in ocular inflammations: a review. Ocul Immunol Inflamm. 2012 Dec;20(6):406–18.
50 Geiser T, Dewald B, Ehrengruber MU, Clark-Lewis I, Baggiolini M. The interleukin-8-related chemotactic cytokines GRO alpha, GRO beta, and GRO gamma activate human neutrophil and basophil leukocytes. J Biol Chem. 1993 Jul 25;268(21):15419–24.
51 Sene A, Chin-Yee D, Apte RS. Seeing through VEGF: innate and adaptive immunity in pathological angiogenesis in the eye. Trends Mol Med. 2015 Jan;21(1):43–51.
52 Hofman P, Blaauwgeers HG, Tolentino MJ, Adamis AP, Nunes Cardozo BJ, Vrensen GF, et al. VEGF-A induced hyperpermeability of blood-retinal barrier endothelium in vivo is predominantly associated with pinocytotic vesicular transport and not with formation of fenestrations. Vascular endothelial growth factor-A. Curr Eye Res. 2000 Aug;21(2):637–45.
53 Ramadan RT, Ramirez R, Novosad BD, Callegan MC. Acute inflammation and loss of retinal architecture and function during experimental Bacillus endophthalmitis. Curr Eye Res. 2006 Nov;31(11):955–65.
54 Bispo PJ, de Melo GB, Hofling-Lima AL, Pignatari AC. Detection and gram discrimination of bacterial pathogens from aqueous and vitreous humor using real-time PCR assays. Invest Ophthalmol Vis Sci. 2011 Feb 22;52(2):873–81.