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Review

Role of lactoferrin in the tear film

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Abstract

The surface of the eye provides an inert barrier against infection. Through its unique combination of antimicrobial action and anti-inflammatory activities lactoferrin (Lf) in the tear film plays an important role in the maintenance of ocular health. In order to maintain clarity the eye must provide immunological defense without immunopathology. Along with physical barriers, soluble plasma factors and other proteins such as lysozyme, Lf produced by the acinar cells of the lacrimal gland serves a number of roles in defense for this purpose. Lf in tears provides antimicrobial efficacy by binding free iron thus reducing the availability of iron necessary for microbial growth and survival as well as pathogenesis. Lf has been shown to inhibit biofilm formation and thus may play a role in protecting contact lens surfaces from colonization. Virus particles' entry into epithelial cells is inhibited by Lf while an excess of Lf in tear film is thought to limit the opportunistic Lf-mediated bridging of adenovirus and host cell that occurs in other tissues. Lf dampens the classical complement activation pathway by binding to markers of inflammation and immune activation while pathogen-associated molecular patterns such as lipopolysaccharide (LPS) are targeted by Lf for removal through tears and hydrodynamic flushing.

This review focuses on the role of Lf in human tear film and its contribution to ocular health during contact lens wear.

Keywords: Lactoferrin; Tears; Microbial keratitis; Contact lens

1. Introduction

The epithelial surface of the eye is continuously exposed to potential pathogens but rarely becomes infected. This is due in large part to the tear film which contains a number of antimicrobial components that are renewed during tearing. The tear film provides the human cornea and conjunctiva with an elaborate system of defenses including an assortment of proteins that alter antimicrobial activity by a variety of mechanisms. The tear film historically has been described as comprising three layers; an extensive aqueous layer situated between a mucin layer and lipid layer [1,2]. The aqueous layer which is mainly secreted from the lacrimal gland [3] contains locally synthesized and serum-derived proteins. Quantitatively the major locally synthesized proteins are lysozyme, tear lipocalin, secretory immunoglobulin A (sIgA) and Lf [4].

To help prevent corneal or conjunctival infection, the anterior eye harbors a variety of antimicrobial defenses which do not manifest damaging inflammatory immunopathological mechanisms [5]. Tear proteins such as Lf, lysozyme and complement comprise the non-adaptive antimicrobial factors along with anatomical barriers and mucous secretions [5].

Tear Lf, first reported by Masson in 1966 [6], is an 82 kDa protein [7] produced in the acinar cells of the lacrimal gland [8]. Lf is present in normal tears of mammals including humans [8], cattle and bison [9] rabbits, dogs, cats, mice, koalas [10] and guinea pigs [11]. Kijlstra et al. found Lf in normal human tears to constitute approximately 25% by weight of total tear protein at a concentration of around 2.2 mg/ml [12]. This was found to be invariant through age and independent of sex. However others have reported

Abbreviations: Lf, lactoferrin; sIgA, secretory immunoglobulin; RTF, reflex tear fluid; CTF, closed eye tear fluid; LPS, lipopolysaccharide.

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variation in normal tear Lf content between subjects ranging from 0.63 to 2.9 g/l with an average 1.42 g/l [13–17] and reduced levels with increased age [18] and in certain diseases such as Sjögren syndrome (an autoimmune disease of the lacrimal gland) [19,20], idiopathic dry eye [18,19], myotonic muscular dystrophy [21], vernal conjunctivitis, contact lens-induced giant papillary conjunctivitis [22], trachoma, herpes simplex keratitis, chronic irritative conjunctivitis keratoconjunctivitis sicca [15,18,19], ocular pemphigoid when it occurs concomitantly with dry eye [18], patients suffering cutaneous pemphigus and clinical dry eye with a marked keratopathy who exhibited reduced Lf levels [18,23] and post-operative cataract surgery [7]. Lf is also reduced in the tears of asymptomatic HIV-positive patients [17], in patients with chronic hepatitis C [24] and patients suffering Type 2 reactions in leprosy [25].

In tear film Lf is thought to be antimicrobial and anti-inflammatory. Although the lacrimal gland is the major source of tear Lf, Santagati et al. have shown that ocular surface epithelial cells also produce detectable amounts of Lf with higher expression in conjunctival than corneal epithelial tissue [26] and more recently there have been reports suggesting the presence of Lf mRNA transcripts in the mouse meibomian gland while proteome analysis of human meibomian gland secretions also identified Lf [27] suggesting that the meibomian gland might synthesize and secrete Lf and serve as a source of Lf in tear film.

2. Changes in lactoferrin concentration in tears

2.1. Waking and sleeping

There is some suggestion that Lf levels differ between waking and sleeping. In the open eye state the protein profile of lacrimal secretions consists of three major proteins: lysozyme, tear-specific lipocalins and Lf [28,29]. For antimicrobial efficacy open-eye tear fluid is enriched with lysozyme and Lf [20]. Main or accessory lacrimal glands are induced neurologically to secrete open-eye or ‘‘reflex’’ tears (reflex tear fluid or ‘‘RTF’’). Open-eye tears may contain slightly higher levels of Lf compared with reflex tears [30].When eyes are closed, reflex secretion ceases or decreases greatly; the pump action due to blinking ceases and tear flow is restricted. In addition oxygen and carbon dioxide exchange is reduced and a subclinical inflammation manifests at the ocular surface [29].

Our studies have demonstrated that tear film composition changes during sleep [31]. sIgA changes in abundance from ~2% of total protein in RTF to about 58% in closed eye tear fluid (CTF) with a concomitant decrease in abundance of lysozyme, while Lf and tear-specific lipocalin (measured collectively) are reduced from 85–88% to ~30% [32]. Sack et al. [32] reported no change in Lf concentration between reflex tears and (basal) open-eye tears (~30% relative to total protein) but noted a decrease to 10% of total protein in the closed eye. However another study has demonstrated that open-eye tears contain slightly higher levels of Lf compared with reflex tears but this could be due, as the authors suggest, to differences in tear flow rate [30].

2.2. Lactoferrin and dry eye

Dry eye syndrome is caused by abnormalities in the quality or quantity of the tear film. Sjögren syndrome is an autoimmune disorder that predominantly affects middle-aged women, with a female:male ratio of 9:1 [33]. In the eye it is characterized by decreased lacrimal gland function [3]. Lymphocytes infiltrate the lacrimal epithelium causing cytolysis of lacrimal gland cells, resulting in severe dry eye [4]. Conversely in the dry eye disorder non-Sjögren syndrome there is no lymphocytic infiltration or lacrimal gland destruction in the dry eye. Tears are still produced but only maximally in response to strong stimuli. Dry eye in this instance is in part due to aqueous tear deficiency through an inability to produce water and tear proteins [34].

There is increasing evidence of a degree of mutual pathophysiology of Sjögren syndrome and dry eye not associated with Sjögren syndrome since markers of immune involvement occur in both types, but there are also important differences. In Sjögren syndrome Vitali et al. [35] found variable Lf results, which were not concordant with other more common diagnostic tests such as Rose Bengal staining, Schirmer test, and ocular symptoms [36]. However, Ohashi and colleagues identified significant correlations between Lf and epidermal growth factor (EGF) and clinical indices such as tear function index, Rose Bengal scores and Schirmer tests [37]. Decrease in Sjögren syndrome Lf levels was attributed to dysfunction of the lacrimal gland while a decrease observed in non-Sjögren syndrome patients was attributed lack of neural stimulation of the lacrimal gland [37].

Lf and lysozyme in the normal tear fluid provide oxygen free radical and hydroxyl scavenging activities [38] (discussed further in Section 5) but in dry eye these activities are reduced [39]. The reduced Lf and other tear factors increase susceptibility to infection exposing the eye to additional oxidative metabolites [39].

Generally it has been reported that decreases in Lf concentration are associated with decreases in tear production from the lacrimal gland in dry eye [36,40]. Lf concentration has been shown by some researchers to be a good predictor of tear film stability or volume but by others to be an unreliable indicator [36,40,41]. Contact lens-induced dry eye is a major cause of contact lens intolerance and discontinuation. Lens-associated reduced tear volume has been implicated [42] suggesting concomitant decreases in Lf amount, if not concentration. However Glasson et al. [43] found that there was no significant change in Lf concentration in either tolerant or intolerant contact lens wearers.

2.3. Lactoferrin and contact lens wear

As mentioned in Section 1 the normal range of Lf concentration in the non-contact lens wearing population varies from around 0.63 to 2.9 g/l [13–17]. In relation to

contact lens wear, Carney et al. [44] reported no change in Lf concentration in tears during extended wear (defined as six or more consecutive nights [45]) in a study comprising a small number of subjects trialing extended wear anionic hydrogel lenses. Further, it was reported in this same study that concentration of tear Lf in contact lens wearers is not significantly different from that reported for non-wearers [29]. To address issues relating to tolerant versus intolerant contact lens wearers (tolerance to lens wear is defined as the ability to wear lenses regularly during one working day (9 h) or longer [36]) Glasson et al. [43] assessed tear film and ocular surface characteristics of each cohort before and after 6 h contact lens wear of high water content hydrogel lenses. Within this period no statistically significant change in concentration for either tolerant wearers or the intolerant group was observed (2.68 ± 0.6 to 2.58 ± 0.95 g/l).

Contact lens papillary conjunctivitis (CLPC) is a milder form of giant papillary conjunctivitis (GPC) (but often these terms are used interchangeably [46]). CLPC is one of the most common reasons for contact lens wear discontinuation [47]. Velasco Cabrera and colleagues [16] showed that there was no change in Lf concentration after 270 days of wear of hydrogel lenses in subjects who did not develop CLPC while in subjects who did develop CLPC there was a significant reduction in Lf under the same conditions. Rapacz et al. [15] measured tear Lf levels in soft contact lens wearing patients with GPC and found that those with active GPC had significantly reduced levels of Lf in tears ((0.876 ± 0.42 mg/ml) compared with normal individuals ($N = 12$; 1.73 ± 0.46 mg/ml, $p < 0.0003$) and the contact lens wearing control group, ($N = 11$; 1.57 ± 0.92 mg/ml, $p < 0.0003$) while patients presenting with inactive GPC had normal levels of Lf.

Overnight use of reverse geometry lens and rigid gas-permeable (RGP) lens materials during orthokeratology, a contact lens technique used to produce short-term reduction in myopia via contact lens-induced flattening of the corneal curvature, has raised concerns about associated ocular surface health [48]. Although there were significant changes detected in some tear constituents following overnight wear of orthokeratology lenses there were no significant changes in Lf [48].

Finally, though most studies of contact lens–tear film interactions have focused on the effect of the lens on the tear film, contact lenses accumulate proteins and other tear components on their surfaces and within their matrix with a very short period of wear [49]. Protein deposition occurs across lens materials despite attempts to improve cleaning regimens or modify polymer materials and wear schedules. Deposits are implicated in discomfort, dryness, mechanical irritation and reduced visual acuity. They have also been associated with development of hypersensitivity reactions [50] and microbial contamination that can lead to infection [51]. Ionic and higher water content materials tend to attract more protein than non-ionic materials [52]. It has been speculated that drying and thinning of tear film influences protein deposition [49].

Lf has been shown to be a common deposit on contact lenses [44]. In one study using a mass-spectrometric-based approach Lf was shown to be one of six proteins identified in all lens-solution combinations tested. Some contact lenses have a more negative charge and tend to attract more Lf through electrostatic interactions [53]. The specific implications of Lf deposition on contact lenses have yet to be fully elucidated.

In summary normal use of contact lenses in healthy eyes does not show any significant changes in either basal or reflex tear Lf concentration. However some contact lens-related adverse conditions such as CLPC/GPC do result in a decrease in Lf concentration. The reduced amount of Lf might contribute to the inflammation associated with CLPC/GPC. Changes in Lf concentration could be an important factor in ocular infection that often arises following a mechanical adverse event and are discussed below.

3. Role of Lf during infection

3.1. Antimicrobial action of lactoferrin in tears

Lf can occur as holo-Lf which consists of a single polypeptide chain folded into two globular lobes, each with one binding site for iron [54,55] and apo-Lf (less than 5% iron saturation [56]) which is more susceptible to proteolysis due to its molecular conformation that is characterized by lobes that are more 'open'[57,58]. Lf found in most secretions is almost entirely as apo-Lf [59] and thus has the ability to tightly bind any free iron and effectively compete with bacteria for this essential cofactor. Lf has been shown to bind free iron in tears, reducing the availability of iron required for bacterial growth [20]. The ability to acquire iron *in vivo* is thought to be an essential requirement for colonization and invasion by pathogenic bacteria [60]. Indeed, Lf has been shown to inhibit the growth of a number of bacterial species implicated in adverse events in tear film including *Escherichia coli* [61], *Haemophilus influenzae* [62], *Bacillus subtilis* [63], *Streptococcus* spp., *Staphylococcus* spp. [64] and *Pseudomonas* spp. [65]. Lf possesses a high isoelectric point with positively charged amino acids clustered at the N-terminus. This overall positive charge at physiological pH allows Lf to interact with the negatively charged surface components of bacteria. Lf can directly bind both Gram-positive and Gram-negative bacteria perturbing bacterial membranes. Due to the cationic nature of the N-terminus, Lf binding to bacteria can be a non-specific interaction due to the negative charge on bacterial membranes [66]. Direct interactions between the lipopolysaccharide (LPS) from Gram-negative cell membranes and Lf have been investigated. Human Lf binds to the lipid A region of LPS with high affinity [67] resulting in a concomitant increase in membrane permeability. This action is due to lactoferricin (Lfc), a peptide obtained from Lf by enzymatic cleavage, which is active not only against bacteria, but even against fungi, protozoa and viruses [68–76]. Lf can also bind to porins present on the outer membrane of Gram-negative bacteria [77] disrupting and releasing LPS from the bacterial surface thus potentiating susceptibility of bacteria to osmotic shock and the action of other antibacterial molecules [78].

Staphylococcus epidermidis, the most common bacterial isolate from tears appears to be susceptible to Lf only in the presence of lysozyme [78], a situation that would occur naturally in tears. The mechanism of the synergy between Lf and lysozyme has been studied. Leitch and Willcox found initial Lf binding to cell-bound lipoteichoic acid (LTA) was required prior to bacteria becoming susceptible to lysozyme. It was proposed that, on binding to the anionic LTA of *S. epidermidis*, the cationic protein Lf decreases the overall negative charge on the bacterial surface, allowing greater accessibility of lysozyme to the underlying peptidoglycan [79].

Interestingly, holo-Lf can be utilized by bacteria for growth, turning a host defense to bacterial advantage. Bacteria such as *Neisseria* spp, *Haemophilus* spp. and *Vibrio cholerae* possess surface receptors that specifically bind holo-Lf [80]. Ocular isolates of *P. aeruginosa*, *Serratia marcescens*, *E. coli* and *Stenotrophomonas maltophilia* were all able to grow in an artificial tear fluid containing natural human Lf [81] in which the degree of iron saturation varies from about 10 to 30% [82]. The mechanism of growth was not studied, although it is known that several of these bacteria have developed mechanisms to compete with Lf for available Fe^{3+}. Kim et al. [82] examined the effects of apo-, holo- and natural Lf on the growth of *Lactbacillus acidophilus* and found that growth was stimulated by bovine holo-Lf but not by apo-Lf. Lf, independent of iron saturation, affected Bifidobacteria more modestly in a strain-dependent manner. Lf-associated growth stimulus appeared related to the presence of membrane-bound Lf-binding proteins.

Gram-positive *Staphylococcus aureus* [83,84] and Gram-negative families Neisseriaceae (which can infect intact non-keratinized ocular epithelium), Moraxellaceae (including *M. lucanata*, one of the causes of blepharoconjunctivitis in humans [60]) and Pasteurellaceae (including causative agents of microbial keratitis) possess energy-dependent, iron-repressible, saturable Lf receptors. The role of these receptors in iron acquisition from Lf has been unequivocally demonstrated [85–88].

Adding a further level of complexity, a patient with Lf deficiency was reported to not exhibit increased susceptibility to infection suggesting that though Lf plays an important role, if absent its role might be compensated by other mechanisms [33].

3.2. Lactoferrin and biofilm formation

During contact lens wear, tear film proteins are deposited onto the lens surface. Williams et al. [89,90] have shown that Lf deposited on the contact lens surface can kill *P. aeruginosa* cells that attempt to colonize the surface. Singh and colleagues [91] have shown that *P. aeruginosa*, the most common bacteria implicated in microbial keratitis, are 100-times more resistant to Lf once they are in a biofilm. However before the biofilm is formed, in the presence Lf concentrations lower than those required for killing or preventing growth, *P. aeruginosa* are unable to form biofilms. Apo-Lf, by chelating iron, stimulates twitching, a specialized form of surface motility, with the result that bacteria wander across biomaterial surfaces [91]. This may be important in reducing the ability of bacteria to form biofilms on contact lens surfaces and contact lens cases that might otherwise increase the risk of infection. Unlike *P. aeruginosa*, biofilms formed by *S. epidermidis* strains isolated from the eye are only susceptible to the action of Lf in the presence of lysozyme [64]. As well, Lf increases the susceptibility of *S. epidermidis* biofilms to the antimicrobial action of vancomycin [64]. This synergistic response is thought to be due to Lf binding of lipoteichoic acid, neutralizing the negative charge of this structure and hence allowing great access of lysozyme to the peptidoglycan [78].

Thus although bacteria in biofilms show increased resistance to Lf as to other antimicrobial agents, in tears Lf may be effective against biofilms through inhibition of initial attachment and the formation of microcolonies. Working in concert with lysozyme and/or topical applications of antibiotics, Lf may increase susceptibility to these bactericidal agents by increasing penetration into the biofilm.

3.3. Viral keratitis

Herpes simplex keratitis caused by herpes simplex virus (HSV) can result in blindness. Susceptibility to infection is due to a combination of viral factors and immune responses to the virus and host antigens [92]. A 2005 study of 50 patients found 92% of adults actively shed HSV in tear fluid. Yet of these only two had a history of ocular HSV infection [93]. Lf antiviral activity has been well documented (see ref. [94] and references therein). Keijser et al. [95] detected a polymorphism in Lf that predisposes subjects to primary HSV keratitis. In tear fluid, Lf aids in control of HSV infections by preventing HSV particles from binding and entering epithelial cells [96–98]. Keijser et al. [95] found no significant differences in Lf concentration among the Lf genotypes and Lf concentration was not correlated with severity of disease. Two N-terminal polymorphisms were not correlated with the disease suggesting that the cationic peptide function (lactoferricin) of Lf is not involved in protection against HSV.

3.4. Adenovirus

It is well known that Lf is a potent inhibitor of several enveloped and naked viruses, such as rotavirus, enterovirus and adenovirus [99]. However Lf has also been reported to be supportive of adenovirus infection under certain circumstances [100]. The anti-adenovirus action of Lf takes place during virus attachment to cell membranes through competition with viral particles for common glycosaminoglycan receptors inserted in target cell membranes [99,101]. It can also neutralize infection by binding to adenovirus particles and targeting viral III and IIIa structural polypeptides [101]. In promoting infection adenovirus co-opts Lf attached to its cognate receptor on host cell surfaces and uses this ‘‘bridge’’ to enter the cell leading to infection. Adenovirus serotypes 19 and 37 have been shown to be important causes of epidemic

keratoconjunctivitis outbreaks [102]. Johansson and colleagues [100] reported that tear fluid did not affect the infectivity of Ad37, but enhanced infectivity of Ad5 in human corneal epithelial cells (HCE). HLf alone was found to promote binding of adenovirus to epithelial cells in a dose-dependent manner and also to promote infection of epithelial cells by adenovirus. Further, HLf was shown to promote gene delivery from an adenovirus-based vector [100]. Finally, holo-HLf was reported to be a more effective promoter of Ad5 binding to HCE cells than apo-HLf.

However when Lf concentration was high the excess Lf saturated binding to both virus and cell separately inhibiting the opportunistic conduit. Thus the role of Lf in viral infection is complex and specific to viral subtype and site of infection. In tears, due to the high concentration of free lactoferrin, it is most likely that lactoferrin provides a protective role against viral adhesion and pathogenesis.

3.5. *Acanthamoeba*

One infecting ocular pathogen that Lf has been shown to be ineffective against is *Acanthamoeba*. Lf was not able to inhibit adherence of *Acanthomoeba* to human corneal epithelial cells (HCEC) *in vivo* [103]. In support of these findings, the tear film also did not offer protection against *Acanthamoeba* infection.

4. Anti-inflammatory actions of lactoferrin in tear film

Mechanisms of action of the well-established immunomodulatory role of Lf in excessive inflammation have not been fully elucidated. Lf has been reported to play a role in myelopoiesis, primary antibody response and lymphocyte proliferation [20] and to enhance monocyte and natural killer cell cytotoxicity [104]. Results from a number of studies (see [105] for a comprehensive review of this subject) have suggested that although Lf acts variously to stimulate and/or repress production of certain cytokines, the overall effect is attenuation of excessive inflammation in host response to pathogens [105]. Lf and Lf-derived peptides are involved in suppression of classical complement activation [106–108]. Unregulated complement activation in tear film may lead to undesirable host tissue destruction. To reduce risk of trauma the open-eye tear fluid lacks the capacity to support either the classical or the alternative pathways of complement activation [31]. Lf can inhibit the classical pathway of complement activation [20]. Samuelsen et al. found antimicrobial peptides derived from the N-terminal region from both human and bovine Lf (lactoferricin H and lactoferricin B, respectively), inhibit the classical complement pathway suggesting the N-terminal region of Lf is the important part in the inhibition of complement activation [108]. Willcox et al. [31] found Lf was in similar concentrations in both closed-eye and reflex tears. However Lf inhibition of complement was much reduced in closed eyes [31], suggesting that during sleep a low level complement activation may occur in order to help remove any entrapped bacteria.

Evidence exists to support the hypothesis that inflammation is a feature of all forms of dry eye [109]. Markers of inflammation and immune activation, such as ICAM-1 are highly expressed in diseases such as Sjögren syndrome-associated keratoconjunctivitis sicca (KCS) [110]. Epithelial cells resident on the ocular surface and inflammatory cells are potential sources of ICAM-1 [111]. In KCS ICAM-1 is upregulated on lymphocytes and/or vascular endothelial cells resulting in lymphocytic diaspedesis to the lacrimal and conjunctival tissue [111]. Levels of ICAM-1 are positively correlated with disease progression and severity [111]. Lf has been shown to inhibit ICAM-1 [110] and may play a role in modulating this inflammation. Lf has been shown to bind and inhibit inflammatory mediators elevated in conjunctivitis such as ICAM.

CD14 proteins are detected in reflex human tears [112]. Human lacrimal glands and corneal epithelia express CD14 mRNAs and proteins. In the corneal epithelium, CD14 expression is limited to the wing and basal epithelial cells [112]. ICAM is induced by LPS–CD14 complex. CD14 binds with high affinity to human Lf (hLf). In a dose-dependent manner, tear CD14 and LPS-binding protein (LBP) mediate the secretion of interleukin (IL)-6 and IL-8 by corneal epithelia cells when challenged with LPS [112]. Thus the anti-inflammatory effects of hLf are due not only to its ability to chelate LPS but also to its ability to interact with both ICAM and secreted CD14 (sCD14), and with the sCD14 complexed to LPS [110].

Tear CD14 and LBP complement the LPS receptor complex expressed by the corneal epithelia to trigger an immune response in the presence of LPS and thus work together to modulate ocular innate immunity [112].

Furthermore, Blais and colleagues [112] have suggested that CD14 could prevent shed LPS monomers from interacting with the intact corneal epithelium by transferring them to Lf thus removing LPS from the ocular surface through tears and hydrodynamic flushing, preventing the LPS from attaching to corneal epithelial cells. Therefore, the reduction in Lf seen in some studies during dry eye might result in increased activation of complement (especially during sleep), increased expression of ICAM and increased CD14–LBP-mediated cytokine expression, and so increased overall inflammation in the cornea and conjunctiva.

5. Protection from oxidative stress

Iron-catalyzed generation of hydroxyl radicals plays a role in the severity of ocular inflammation and tissue damage [113]. Hydroxyl radicals can damage tissue through peroxidation of cell membrane lipids, by oxidative damage to proteins, and through the production of other free radicals. Normal Lf levels can deplete free iron and inhibit the pro-inflammatory effects of the hydroxyl radicals [114].

Hypoxic injury to corneal epithelium is well known. Lf has been shown to protect the cornea tissue from reoxygenation injury after extensive hypoxia [115]. Reoxygenation injury after extended hypoxia causes increased cellular damage to corneal epithelial cells. Because the most potent agents that

protect cells from reoxygenation injury are iron-chelating agents such as Lf that sequester cytoplasmic iron [116,117], the underlying mechanisms are distinct from hypoxic injury. Lf is taken into corneal epithelial cells as an iron transporter or antioxidant against excessive free iron. Lf in tears may also protect the corneal epithelium from solar UV irradiation [118].

Activated macrophages produce metabolites such as superoxide and hydrogen peroxide which are important mediators of initial tissue injury [119]. The highly reactive hydroxyl radical is formed through an iron-catalyzed reaction involving superoxide and hydrogen peroxide [113,120]. Bacterially mediated elastase cleavage of transferrin generates iron-chelates which are able to catalyze formation of the highly cytotoxic hydroxyl radical from neutrophil-derived superoxide and hydrogen peroxide via the Haber–Weiss reactions [121]. However bacterially mediated cleavage of di-ferric or apo-Lf conversely has minimal alteration to Lf function [122]. Sequestration of iron by Lf can thus decrease the potential for oxidant-mediated tissue injury via its ability to bind iron in such a way as to prevent iron-dependent formation of hydroxyl radicals [20].

6. Concluding remarks

In order to maintain corneal transparency antimicrobial defenses manifest specific and non-specific mechanisms to preserve a delicate balance between effective antimicrobial challenge and harmful inflammatory response. The presence of Lf in tears provides a major contribution to this balance through complex modulation of the activities of bacteria, viruses and the host immune response. The involute interaction of Lf with bacteria can effect host defense through competition for free iron, binding of bacterial LPS and inhibition of bacterial biofilm formation or adversely can result in inadvertent promotion of pathogenesis through bacterial competition for Lf iron reserves. Adenovirus too can take advantage of Lf to enter target cells using Lf as a bridge while higher Lf levels inhibit this action. Thus the concentration and levels of iron saturation must be finely tuned to effect positive actions of Lf and reduce opportunistic microbial exploitation. Beyond these antimicrobial actions Lf provides protection from immune-mediated and hypoxic oxidative stress as well as protection against UV damage.

Tear Lf works to protect corneal clarity through diverse actions as part of a robust ocular defense system with the result that sight threatening infection or sequela even amongst the contact lens wearing population remains a rare event.

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