Tear Lactoferrin Levels in Patients With External Inflammatory Ocular Disease

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Lactoferrin, an iron complexing protein in normal tears, is an important component of the nonspecific host defense system of the external eye. We measured tear lactoferrin levels in patients with contact lens-induced giant papillary conjunctivitis (GPC) by an enzyme-linked immunosorbent assay (ELISA). Patients with active GPC (N = 26) had significantly reduced tear levels of lactoferrin (0.876 ± 0.42 mg/ml) compared with normal individuals (N = 12; 1.73 ± 0.46 mg/ml, $P < 0.0003$) and the control contact lens wearers' group (N = 11; 1.57 ± 0.92 mg/ml, $P < 0.003$). Patients with vernal conjunctivitis (N = 10), an ocular disease with similar histopathology, had slightly reduced concentrations of tear lactoferrin (1.22 ± 0.59 mg/ml). Patients with inactive GPC (N = 7) had normal tear levels of lactoferrin (1.33 ± 0.49 mg/ml). The lactoferrin to total protein ratio in the tears was significantly reduced in patients with GPC compared to normal subjects, control contact lens wearers, and patients with inactive GPC. The decreased tear levels of lactoferrin in patients with GPC may contribute to increased coating of lenses with bacteria and their products and enhanced ocular inflammation which may play a role in the pathogenesis of GPC. Invest Ophthalmol Vis Sci 28:543–545, 1987

The protein fraction of tears contains a number of antimicrobial factors which are important in protecting the external eye from infection.[1,2] These substances are produced by the main and accessory lacrimal glands.[3,4] Important components of the host defense system of the external eye include complement porteins, immunoglobulins, especially secretory IgA, lysozyme, and lactoferrin. Lactoferrin, an iron complexing protein in normal tears, has both bacteriostatic[5,6] and bactericidal[7] properties which make this tear protein an important component of the nonspecific host defense system of the external eye. Recently, Kijlstra et al[8,9] showed that lactoferrin has a strong inhibitory effect on the classical complement system by blocking the formation of the C3 convertase. This complement (C) inhibitory activity suggests that lactoferrin may play an anti-inflammatory role in addition to its antimicrobial properties.

Previous studies in our laboratory[10–12] and others[13–15] have suggested that both IgE- and IgG-mediated immune mechanisms play a role in the pathogenesis of vernal conjunctivitis (VC) and contact lens-induced giant papillary conjunctivitis (GPC). In addition, recent studies by Ballow et al[16] have demonstrated increased levels of C proteins, ie C3 and Factor B, and evidence for C activation (ie C3 anaphylatoxins) in the tears of patients with VC and GPC. Activation of the C system in the external eye could contribute significantly to the inflammatory processes and tissue damage in these ocular diseases. Activation of either the classical or alternative C pathway can lead to the generation of the anaphylatoxins, C3a and C5a. C3a causes a noncytolytic release of histamine from mast cells and basophils, contraction of smooth muscle, and an increase in capillary permeability.[17] These findings together with the studies of Kijlstra et al[9] of the anticomplementary activity of lactoferrin suggested that a study be undertaken of the tear levels of lactoferrin in patients with VC and GPC.

Materials and Methods

Ten patients with VC and 26 patients with soft contact lens-induced GPC were studied. The diagnosis of VC and GPC was based on typical symptoms and physical findings as outlined previously.[10,12] Seven patients with inactive GPC were also studied. This inactive GPC group consisted of patients who previously had active GPC, and refitted with new lenses after a 4 week rest period as described previously.[18] These patients wore their new lenses without any of the symptoms usually associated with GPC,[12] but still had a papillary reaction on the upper tarsal surface. Tears

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Table 1. Tear lactoferrin and total protein in patients with vernal conjunctivitis and giant papillary conjunctivitis

Patient group	Lactoferrin (mg/ml)	P value	Total protein (g/L)	Ratio of L/TP*
Normal (12)	1.73 ± 0.46†		7.07 ± 1.10	0.26 ± 0.08
Contact lense control (11)	1.57 ± 0.92		7.2 ± 2.2	0.25 ± 0.11
VC (10)	1.22 ± 0.59	<0.03	10.2 ± 4.5	0.13 ± 0.08
GPC, Active (26)	0.88 ± 0.42	<0.003	8.3 ± 3.9	0.13 ± 0.06
GPC, Inactive (7)	1.33 ± 0.49	NS	6.97 ± 5.90	0.26 ± 0.16

* Ratio of lactoferrin to total protein.
† Mean ± 1 SD.

were collected 3 to 6 months later after successfully wearing their new lenses. Control groups included 12 individuals without eye disease who did not wear contact lenses, and 11 subjects who wore soft contact lenses without problems. Two subjects studied had viral conjunctivitis of probable adenovirus etiology. Informed written consent was obtained prior to tear collection.

Tears (100–200 μl) were collected by glass capillary tube as previously described,[10] and stored at −70°C. Lactoferrin was measured in the tears by an indirect enzyme-linked immunosorbent assay (ELISA) as modified from the procedures of Kijlstra et al.[19] Tear total protein levels were measured by a folin method. Tear IgG and C3 were measured by ELISA, tear total IgE by PRIST, and C3a des Arg (C3a anaphylatoxin) by radioimmunoassay as previously described.[16]

Results

Normal control individuals had a tear lactoferrin level of 1.73 ± .46 mg/ml (mean ± SD). As shown in Table 1, soft contact lense control subjects had similar tear concentrations of lactoferrin (1.57 ± 0.92 mg/ml). Reduced levels of tear lactoferrin were found in patients with VC (1.22 ± 0.59 mg/ml) which was significatnly reduced ($P < 0.03$) compared to normal individuals. Patients with active GPC had markedly decreased levels of tear lactoferrin (0.876 ± 0.42 mg/ml) compared to control contact lens wearers ($P < 0.003$). In contrast, patients with inactive GPC had normal levels of tear lactoferrin (Table 1). Two patients with viral conjunctivitis were studied. One had a tear lactoferrin level of 2.3 mg/ml and the other a level of 1.1 mg/ml.

Tear lactoferrin levels were also expressed as a ratio of the tear lactoferrin level to tear total protein. As expected from our previous study, the tear total protein in patients with VC were significantly elevated ($P < 0.05$) compared to the tears of normal individuals. The tear total protein in patients with GPC were slightly, but not significantly elevated. The tear lactoferrin to total protein ratio was significantly reduced ($P < 0.001$) both in GPC and VC patients. In patients with inactive GPC, the total protein and ratio were normal (Table 1).

In both GPC and VC patients there were no significant correlations of tear lactoferrin levels with the tear concentrations of C3, C3a des Arg, or immunoglobulins, IgG and IgE.

Discussion

In this study we demonstrated reduced tears levels of lactoferrin in patients with VC and GPC. The concentrations of tear lactoferrin returned to normal in patients with inactive GPC. These reduced levels were not just due to dilutional effects of tear collection or increased tearing since tear total protein concentrations were higher in VC patients or similar to controls in GPC patients. The concentrations of total proteins in the tears of normal subjects was similar to levels reported by other investigators.[20,21] Similarly the tear transferrin concentration agreed with several reports in the literature using different methods of tear collection and assays.[6,19–22]

Reductions in tear concentrations of lactoferrin and its companion tear protein, lysozyme are found in several clinical conditions and with increasing age. According to the study of McGill et al,[22] the drop in tear lactoferrin and lysozyme is most marked after age 50. However, other laboratories have not reported a change with age.[19,23] In our study, the VC patients were between 11 and 16 years of age, and the GPC patients between 20 and 45 years of age. Other ocular diagnoses with decreased tear lactoferrin include sicca syndrome,[24,25] myotonic muscular dystrophy,[26] and during the immediate postoperative period in patients undergoing cataract surgery.[27] Since our patients did not have any of these ocular disorders, VC and GPC should be added to the list of eye diseases with decreased tear concentrations of lactoferrin.

The pathogenesis of the decreased tear lactoferrin levels in patients with VC and GPC is not known. The acinar epithelium of the main and accessory lacrimal glands are the major sources of tear lactoferrin. This suggests that decreased tear lactoferrin levels may be related to lacrimal gland dysfunction. Studies are planned to measure the tear levels of lysozyme to determine if the concentrations of this tear protein are similarly reduced in patients with VC and GPC. Lactoferrin is considered to be important as a nonspecific host defense factor. It is present in high concentrations in both tears and breast milk. The major antibacterial effect of lacterferrin is thought to be due to its iron-

binding properties, but it may also have a direct effect on certain strains of bacteria.[28] In addition, lactoferrin has anti-inflammatory properties, inhibiting the activation of the classical C pathway by preventing the formation of the C3 convertase.[9] This anti-inflammatory effect of lactoferrin may have important implications in the two ocular disorders presented in this study. Activation of the C system in the external eye of patients with VC and GPC, as we have recently shown,[16] or even activation by nonimmune mechanisms, could contribute significantly to further inflammation and tissue damage in the presence of decreased tear concentrations of lactoferrin.

Key words: lactoferrin, giant papillary conjunctivitis, vernal conjunctivitis, tears, inflammation

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