Immunology and Pathophysiology of Dry Eye

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47th PanHellenic Ophthalmology Congress
28-31 May 2014, Thessaloniki
Dry eye disease

...one of the most frequently encountered categories of ocular morbidity
...is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface..”

The International Workshop on Dry Eye (DEWS)
http://www.tearfilm.org/dewsreport/.
Dry Eye

“... is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.”

http://www.tearfilm.org/dewsreport/.
Lacrimal functional unit (LFU)

- Main and accessory (Wolfring and Krause) lacrimal glands
- Cornea and conjunctival epithelium
- Eyelids, meibomian glands
- Interconnecting sensory and autonomic nerves

Proper functioning of the LFU is required for the production tears of normal composition.

Healthy Tear Fluid

Toxic Tear Fluid
I. Increased Osmolarity and Osmotic Stress

- One of the mechanisms responsible for inflammation
- Increased evaporation and concentration of sodium
- Occurs in all types of dry eye; cannot differentiate aqueous deficient from evaporative.
- An indicator of dry eye and now can be measured in the clinic setting in a rapid assay with the TearLab system.
- Limited and contradictory data on the usefulness of TearLab
Hyperosmolar tear film
Dehydration of cells
Osmotic stress

Mitogen-activated protein kinase (MAPK) signalling pathways

Increased inflammatory cytokines, chemokines and MMP

Activation and maturation of immature APC

Increased expression of class II MHC antigens (HLA-DR)

Increased expression of adhesion molecules ICAM-VCAM
Immunopathogenesis of Dry Eye

I. Desiccating Stress

II. Draining lymph nodes

III. Ocular surface damage

Th17, Th1, IFNγ, TNF-α, IL-6, IL-17, IL-1, IL-10, MMP-3,-9, TNFα, Epi Apo, CAM

APC, Efferent Arm, Afferent Arm
Disease involves a unique form of pathologic angiogenesis that produces lymphangiogenesis without associated hemangiogenesis.
II. Decreased Tear Stability

- Changed tear components (Lipid, mucin, protein)
- Disturbed interaction between the molecules
  - Decreased break-up time of the tear film
  - Decreased retention of the tear film on the ocular surface
- Localized dry areas
- Increased epithelial cell desquamation
III. Inflammation

* Dry eye is not simply a deficiency of any components of tear film
* An inflammatory disease of the lacrimal gland and ocular surface
  * **Primary**
  * **Secondary**
    * Osmotic stress
    * Decreased tear stability
    * Pooling due to delayed tear clearance
    * Chronic irritative stress (contact lens, mechanic)
  * Neurogenic
Primary Inflammation
a. Sjogren Syndrome

- The lacrimal and salivary glands are infiltrated by activated T-cells and B cells
- Acinar and ductal epithelial cell death
- Hyposcretion of the tears or saliva
- Expression of autoag on the ocular surface (ro, la, Fodrin) and increased serum SSA/Ro and SSB/La ribonucleoprotein antibody
- Primary or secondary (Romatoid arthritis, SLE, Scleroderma, Polymyositis, etc)
- Sjo (Nicox), diagnostic panel for Sjogren
Primary Inflammation
b. Age Related-Hormonal

* The most common cause of lacrimal gland dysfunction
* Postmenopausal women
* With aging
  * Lacrimal gland atrophy
  * Meibomian gland dysfunction
  * Mucoid tear fluid
* Thought to be due to decreased production of androgens
Androgens

* Provide **trophic support** for the LFU; increase lacrimal and meibomian gland function
* Stimulate secretion of secretory IgA, an important component of the mucosal immune system of the eye
* Antiinflammatory effect
  * Increase production of TGF-β
  * Decrease secretion of prolactin, IL-1 and TNF-β
* Increase goblet cell concentration

An immune suppressive environment

When circulating androgen levels drop, the number of reactive T lymphocyte increases resulting in lacrimal and meibomian gland dysfunction.

Cornea epithelium has approximately 7000 nerve endings per square mm.

In early phase, increased blinking and reflex lacrimation to increase tear fluid.

Continuous stimulation leads to:
- Lacrimal gland exhaustion
- Increased autoantigen expression
- Increased antigen presentation
- Inflammatory cytokine secretion

Neurogenic Inflammation
* In chronic phase, inflammation leads to
  * Decreased subbasal nerve fiber density
  * Altered morphologic structure (nerve sporouts, tortusity, thinning)
  * IL-1 and TNF-α increase opioid secretion, which inhibit neural transmission.
  * Disturbed reflex arc and decreased stimulation of lacrimal gland
  * Lacrimal gland atrophy and tear secretion
Dry eye is mainly a T-cell based inflammatory disease

- **T cell infiltration in the conjunctiva**
  - **IFN-γ secreting CD4+ Th1 (IL-12, IFN-γ)**
  - **IL-17 secreting CD4+ Th17 (IL-6, IL-23 and TGF-β)-Treg resistant Th cells**
  - **Decreased effector T cell apoptosis**

- **Decreased number of CD4- CD25- Foxp3- Treg, dysfunctional Treg**

Stern ME, et al. IOVS. 2002;43: 2609-14
**Cytokines and Clinical Correlation**

- Increased cytokine levels (IL-1, IL-2, IL-6, IL-8, TNF-α) is correlated with:
  - Ocular irritation symptoms
  - Corneal fluorescein staining
  - Decreased goblet cells
  - Decreased Schirmer scores
  - Squamous metaplasia

TNF-α expression in lacrimal gland of dry eye (a) and control (b) specimens

*Jabs DA, et al. IOVS. 2004; 45: 2293-8*
4. Growth factor deprivation

- Decreased
  - Epidermal growth factor (EGF)
  - Antimicrobial protein lysozyme
  - Antibacterial iron binding protein lactoferrin
  - Lipocalin and albumin concentrations (by lacrimal gland)
- **Tear lactoferrin concentration is correlated with**
  - Ocular symptoms
  - Schirmer test, BUT
  - Rose bengal staining
- Elevated S100AB and A9 peptides were detected in tears of patients with meibomian gland disease.
In dry eye, tear fluid plasmin, MMP-2, MMP-3, and MMP-9 levels increase.

IL-17 binds IL-17 receptors and secretion of MMP by epithelial cells.

**MMP-9**
- Destroys cornea epithelium basal membrane and tight junction proteins (ZO-1, occludine).
- Decreases the barrier function of epithelium and increases permeability.
- Epithelial cell shedding, apoptosis.
High-molecular weight glycoproteins 50-80% carbohydrate

Negatively charged mucin layer binds easily to hydrophobic cornea epithelium.

**Surfactant:** Protects ocular surface epithelial cell shedding from traumatic effect of blinking

- **Transmembrane MUC 1, 4, 16** by cornea and conjunctiva epithelium (glycocalyx)
- **Gel forming MUC 5AC** by goblet cells
- Lacrimal gland derived **soluble mucins (MUC 7)**

**In dry eye, MUC 5AC and MUC 1 decrease**

Unstable tear film, poor surface protection, mechanical damage
Epitheliopathy is one of the most easily recognizable clinical features of DED

- Ocular surface stains with fluorescein, Rose Bengal and Lissamine green
- Increased epithelial cell density and thickness, decreases epithelial cell size, and increased epithelial cell turnover.
- Increased apoptotic markers (TNF, Fas, APO 2.7,) and p53 expression
- Goblet cell loss, squamous metaplasia
Biomarkers to be used to diagnose and monitor the response to dry eye treatments

New treatment strategies that inhibit the inflammation
## Biomarkers of Dry Eye

<table>
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<th>Biomarker</th>
<th>Clinical correlation</th>
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<th>Clinical correlation</th>
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<tr>
<td>HLA DR</td>
<td>Inflammation Decrease with CS and tofacitinab</td>
<td>MUC 16</td>
<td>Tear meniscus OSOI Ocular surface staining</td>
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<tr>
<td></td>
<td></td>
<td>MUC 5AC</td>
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<tr>
<td>MMP-9 InflammaDry</td>
<td>Symptom severity, ocular surface staining</td>
<td>Tear CXCL9</td>
<td>Basal tear volume, keratoepitheliopathy, goblet cell density</td>
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<td>Tear EGF</td>
<td>Ocular surface staining with Rose Bengal, florescein, lissamine</td>
<td>Tear proteins S100A8, A9 Lactoferrin</td>
<td>In subjects with MGD Grittiness, transient blur Eye pain, tearing</td>
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<tr>
<td>Tear IL-6</td>
<td>Ocular surface staining with Rose Bengal, florescein, lissamine</td>
<td>Tear IL-8, MIP-1 alpha, IL-1beta</td>
<td>Ocular surface staining with fleorescein, lissamine</td>
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<tr>
<td>Drug name</td>
<td>Company</td>
<td>Action</td>
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<td>Dexamethasone phosphate</td>
<td>Eyegate Pharmaceut</td>
<td>Steroid</td>
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<tr>
<td>Rimexolone</td>
<td>Alcon</td>
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<td>Bromphenac</td>
<td>ISTA</td>
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<td>EBI-005</td>
<td>Eleven Biothrapeutics</td>
<td>IL-1 R1 antagonist</td>
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<td>Tocilizumab</td>
<td>F Hoffmann-La Roche</td>
<td>Anti-IL6 R</td>
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<td>IB-MECA CF101</td>
<td>Can-Fite BioPharma</td>
<td>A3 adenosine R agonist Downregulates NF-κB-TNFα</td>
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<td>Cyclosporine A (NOVA22007) cationic</td>
<td>Novagali Pharma</td>
<td>Inhibit IL-2 and Tcell activation</td>
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<td>Cyclosporine (Haporine-S)</td>
<td>Korean University</td>
<td>nanotechnology</td>
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<td>Lifitegrast (SAR 1118)</td>
<td>SARcode Bioscience</td>
<td>LFA-1 and ICAM-1 antagonist (Lymphocyte functional ag-1)</td>
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<td>Rituximab</td>
<td>IDEC Pharmaceutical</td>
<td>mAb against B lymphocyte ag (CD20) Sjogren syndrome</td>
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Dry eye disease is a multifactorial disorder involving multiple interacting mechanisms.
Regardless of the initiating event or etiology, **INFLAMMATION** has a prominent role in the development and propagation of this debilitating condition.
Thank you very much for your kind interest.