GOBLET CELLS: THE UNSUNG HERO

the story of how ocular surface inflammation effects goblet cell density and function.

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GOBLET CELLS HAVE MANY FUNCTIONS

- Guard and Protector
- First Responder
- Diplomat and Mitigator
- Sensitive Side
GOBLET CELLS AS GUARDS/PROTECTORS

- Secrete a variety of mucins
  - improve the wetability and spreadability of tears (think of RainX for your windshield)
  - provide protective glycocalyx to epithelial cell microvilli
  - reduce the surface tension sheer forces on the corneal and conjunctival epithelium
  - lubricate the lid wiper epithelium and support lid wiper function

GOBLET CELLS AS FIRST RESPONDERS

- First line of ocular surface defense and rapid protection from environmental challenges
- Mucus layer traps allergens, pathogens, debris and is eliminated in the NLD
- Increase mucin production in the allergic response (Th 2 cytokines IL6 and IL13)
- Respond rapidly to K and Conj sensory nerve activation with mucin secretion

GOBLET CELLS AS DIPLOMATS AND MEDIATORS

- Dendritic Cell modulation:
  - Immunoreactive to Immunotolerant phenotype
  - Important for limiting bystander damage,
GOBLET CELLS ARE HURT BY:

- Inflammatory Cytokines generated from:
  - infection (bacterial, viral, fungal)
  - Dry Eye Disease: TNF and IFN cause apoptosis
- Allergic reactions: acute phase and chronic phase (reference)
- Medications:
  - oral anti-histamines have anti-M3 R promiscuity which directly decreases goblet cell output
  - Beta-Blockers
  - Anti-cholinergics
  - BAK
THE CYTOKINE DANCE

❖ DEWS Report 2007:
  ❖ summarized the best science to date for clinicians and researchers
  ❖ organized our thinking around DED
  ❖ magic and wonder for discovery in the red box
  ❖ DED results from aberrant activation of innate and adapted immune responses
LOSS OF IMMUNOREGULATION IS ASSOCIATED WITH:

- Decreased IL-10 and TGF
- Increased Th1 activated T cells, leading to Goblet Cell apoptosis as Th1 cytokines increase (Contreras-Ruiz et al.)
- TNF — acute phase cytokine and cytokine of perpetual inflammation as Th1 population increases.
- IFN — directly limits goblet cell output and directly induces goblet cell apoptosis. Also acts as a hyperkeratinizing stimulus.
- As goblet cell population declines, there is less immunoregulation (decreased TGF) signaling to the Dendritic Cells (part of the self-perpetuating inflammatory cycle)

Contreras-Ruiz A et al. Modulation of Conjunctival Goblet Cell Function by Inflammatory Cytokines. Mediators Inflamm. 2013: 636812
CORE MECHANISMS: HYPEROSMOLARITY, DESICCATING STRESS AND LOSS OF IMMUNOREGULATORY CONTROL

- Acute phase cytokines IL-1 and TNF activate resident Dendritic Cells
- Lymph node takes MHC II presented Ag and drives Th-1 vs Th-17 differentiation
- Activated Th 1 or Th 17 cells arrive via ICAM/LFA, CCL5/CCR5 and CXCL9/CXCL10:CXCR3
- Tregulatory cells are overpowered
  - immunoregulatory cytokines IL-10 and TGF-B decrease
  - loss of IFN regulation resulting in goblet cell damage, increasing tear instability and further desiccating stress

Pflugfelder S and Stern M. Immunoregulation on the Ocular Surface. OcSurf 2009 7(2) 67-77
As the chronic inflammation increases, Th1 cell infiltration increases.

Th1 cells secrete IFN.

IFN induces Goblet Cell apoptosis resulting in further tear film instability and further Desiccating Stress.

Cycle repeats in a chronic downward spiral (goblet cell apoptosis, decreased GC-TGF-dendritic cell immunoregulatory signals).

IFN induces Goblet density (apoptosis) mucin squamous metaplasia epith apoptosis

IL-1, TNF, IL-6 (CsA), RANTES
TH17 CELLS...TH1 CELLS’ MISCHIEF MAKER SIBLING

- Th17 activity results in MMP-9 release
  - 1. MMP-9 disrupts corneal epithelial tight junctions (punches holes in the wall)
  - 2. IL-17 and MMP-9 induce the release of more IL-1 and TNF (more Thing 1 and Thing 2)
  - 3. MMP-9 correlates with disease activity
  - 4. IL-17 does not induce Goblet Cell apoptosis

- anti-MMP-9 strategies:
  - steroids, doxycycline, azithromycin, cyclosporine

- Th17
  - IL-17

- MMP-3
- MMP-9
  - gap junction damage

- epith injury
- Toll-Like R activ
  - MAP-K
  - more IL-1, TNF, IL-6, RANTES
IN SUMMARY, GOBLET CELLS ARE LIKE SUPER HEROES THAT ACT AS GUARDS, FIRST RESPONDERS AND DIPLOMATS WITH A SENSITIVE SIDE:

- **Guard/Protect**: Critical contributors to a healthy ocular surface and tear film

- **First Responders**: rapidly increase mucin in response to allergens, nerve stimulation, infectious agents, foreign bodies

- **Diplomats**: Contributors to the immunoregulation of Dendritic Cells

- **Sensitive Side**: Apoptosis and reduced Goblet Cell Density are induced by TNF and IFN induced by Dry Eye Disease
THANK YOU KINDLY FOR YOUR ATTENTION AND INTEREST
THE OSD CYTOKINE DANCE, WHY NEW DRUGS FAIL FDA APPROVAL

And Why Your Mama Don’t Dance and Your Daddy Don’t Rock and Roll

Advanced Refractive Congress, August 2-3, 2015 Deer Valley, Utah

Laura M Periman, MD
Seattle, WA
THE CYTOKINE DANCE

- DEWS Report 2007:
  - summarized the best science to date for clinicians and researchers
  - organized our thinking around DED
  - magic and wonder for discovery in *The Red Box*
  - DED is a disease of the LFU with loss of homeostatic control. Tear instability and hyperosmolarity are core mechanisms

DED RESULTS FROM ABERRANT ACTIVATION OF INNATE AND ADAPTIVE IMMUNE RESPONSES

- TLRs are the epithelial cells’ early warning system for bacteria, fungus and virus.
- NF-κB and MAP-K signal transduction
- Hyperosmolarity and Desiccating Stress trip-wire this native defense mechanism
- DED results from aberrant activation of innate and adaptive immune responses

Figure 1. The lipopolysaccharide (LPS) receptor, Toll-like receptor 4 (TLR4) is expressed by the ocular surface epithelial cells. It shares signaling components with the interleukin-1 (IL-1) receptor (IL-1R), including TIRAP, MYD88 and IRAK. Binding of LPS to its receptor activates NFκB.
CORE MECHANISMS: HYPEROSMOLARITY, DESICCATING STRESS AND LOSS OF IMMUNOREGULATORY CONTROL

- Acute phase cytokines IL-I (IL-1R) and TNF-a activate resident Dendritic Cells (clodronate)
- Lymph node takes MHC II presented Ag and drives Th-1 vs Th-17 differentiation
- Activated Th 1 or Th 17 cells have three doors to reach the ocular surface:
  - Tregulatory cells get drowned out
  - immunoregulatory cytokines IL-10 and TGF-B decrease
  - loss of IFN regulation (and increasing IFN levels) result in goblet cell damage, increasing tear instability and further desiccating stress

Figure 4. Immunoregulation can be disrupted on the ocular surface by a number of mechanisms. Corneal and conjunctival epithelial barrier can be disrupted by proteolysis of tight junction proteins. Squamous metaplasia of the conjunctival epithelium with goblet cell loss can be induced by IFN-y. Inflammatory cytokines, such as IL-1 and TNF-a activate dendritic antigen-presenting cells and increase production of activation markers by these cells, including MHC class II antigen, VEGF3 and CCR7. Increased production of chemokines, chemokine receptors and adhesion molecules by the ocular surface vascular endothelium and epithelium facilitates lymphocyte recruitment. Targeted CD4+ T cells migrate into the conjunctival epithelium, while regulatory intraepithelial CD8+ T cells decrease in number. (Artist: Eilaine Kurfe, Sparta, NJ)
TISSUE-SPECIFIC ACTIVATED TH1 AND/OR TH17 CELLS HAVE THREE DOORS TO ENTER THE OCULAR SURFACE HOUSE PARTY:

- The auto-reactive, tissue-specific T-cells enter the ocular surface through any one of three doors:
  - ICAM-1:LFA-1 *
  - CCL5:CCR5
  - CXCL9/10:CXCR3
- *=ICAM downregulators: steroids, CsA, doxy, azithro

ACTIVATED TH1 OR TH17 CELLS TISSUE-SPECIFICALLY DELIVERED THEN SECRETE TOXIC CYTOKINES THAT BOX GOBLET CELLS AND CORNEAL EPITHELIUM

- Th1 cells secrete IFN which:
  - decreases goblet cell density
  - induces squamous cell metaplasia
  - induces epithelial cell apoptosis
- Th17 cells secrete IL-17 (IL-17 R) which:
  - induces MMP3/MMP9 which:
    - damages epithelial cell tight junctions and basement membrane (RCE)
- IL-2 is the shameless self-promoter autocrine and paracrine cytokine of Th1 and Th17 cell amplification and recruitment (CsA)

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Th 17 activity results in MMP-9 release (THE KITES)

1. MMP-9 disrupts corneal epithelial tight junctions (punches holes in the wall)
2. IL-17 and MMP-9 induce the release of more IL-1 and TNF (more Thing 1 and Thing 2)
3. MMP-9 correlates with disease activity (chaos levels increase in a Thing Frenzy)

anti-MMP-9 strategies:
- steroids, doxycycline, azithromycin, cyclosporine
Immunopathophysiology is a highly diversified, redundant and complex system.

- Full of gas pedals and brakes, amplification and modulation
- A single intervention or approach is unlikely to modulate the system.
- Mother nature builds in work around and back up systems
COMPLEX DISEASE STATE, MULTIPLE FEEDBACK LOOPS, SELF-AMPLIFICATION, MODULATIONS

Explains the need for multiple medications with increasing disease severity
FDA APPROVAL DENIALS: WHY??

- 14 FDA denials over 10 years. No new dry eye disease specific prescription ophthalmics approved by US FDA since 2002.

- Need Statistical Significance for 1 sign AND 1 symptom, EXCEPT total corneal clearing is accepted without a symptom improvement. (diquafasol failure reason)

- Meeting SS for 1 sign AND 1 symptom is problematic:
  - high complexity or “noise” in the disease state, limited sensitivity and specificity metrics/endpoints. *It’s like trying to pick out one conversation at the dance party.*

FDA TASKS AND TACKS

1. Ensure safety and efficacy

2. Uphold the law that drug trials must be “adequate and well-controlled”
   - Typically, this means 2 trials (usually Phase III). Can also mean 1 good trial with “substantial other evidence” (e.g. European data/trials)
   - Some trials include 2 symptom trials and 2 signs trials to help demonstrate drug effect
   - The signs and symptoms metrics used are chosen by the clinical trial designers
MAMA DON’T DANCE

- Study Design Determined by Clinical Trials Designers, NOT FDA:
  - placebo choice: do you compare drug effect against a top-shelf artificial tear or basic saline? Drug needs to demonstrate SS superiority to tear/vehicle.
  - controls: no treatment group, positive control against established therapy (e.g. PGE vs Timolol). Risky.

- Sample Size, Statistics, Time of year, Study Center location

- S/Sx Endpoints: Schirmer’s notoriously imprecise (51% specificity)\(^1\) and Subjective measures are variable.

- Duration: time points chosen by clinical trial designers. Based on when they think their drug will take effect and have a measurable improvement. Staining takes time to improve. Maybe this is the wrong surrogate to measure improvements in signs (IL-17R ?)

- Exclusion Criteria, Patient Selection: pure ADDE, pure EDE or hybrid?

1. AAO Preferred Practice Patterns. Dry Eye Disease. 2013. [www.aao.org](http://www.aao.org)
AND DADDY DON’T ROCK N ROLL: SCIENTIFIC RIGOR OR STACK THE DECK?

- Rigor: best controls, best vehicles, repeatability
- Stack the deck?:
  - lousy vehicle
  - deny rescue AFT use
  - seasonality/location of clinical trial centers
  - end-point design
Improved metrics (Dr Wiley Chambers’ “Ideal DED Trial”):

1. Symptoms: The patient names and grades symptoms to measure Sx effect. (e.g. what is itch to you might be FBS to me)

2. Signs: Switch to biomarkers to improve statistical reliability and ability to measure impacts on the disease state activity. E.g. Goblet cell density, micro-array testing of cytokines (IL-1, IL-6, IL-8, TNF-a, MMP-9, etc). All are acceptable endpoint choices to demonstrate evidence of Sign improvement.

Newer trials incorporating specific biomarkers
PATHWAYS TO FDA APPROVAL

- Set up the beat: Improved Symptoms measurements
- Clear the dance floor: Improved Signs metrics (biomarkers)
- Then, we can have a good beat and dance to it.
THANK YOU

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