OCULAR IMMUNOLOGY

Christine Watté, DVM, Dipl.ECVO
BSC 20 June 2018; NCSU
Plan of the lecture

1. GENERAL PRINCIPLES OF IMMUNOLOGY
2. DEFENSE MECHANISMS OF TEARS AND OCULAR SURFACE
3. IMMUNE PRIVILEGE OF THE CORNEA
4. OCULAR IMMUNE PRIVILEGE, ACAID, THERAPEUTIC APPLICATIONS
5. IMMUNE MECHANISMS OF SELECTED DISEASES
Why study ocular immunology?
PART 1:
GENERAL PRINCIPLES OF IMMUNOLOGY
What is Immunology?

• Not just the host response against infectious agents
• Study of all mechanisms leading to recognition and response against what is recognized as foreign (non-self)
• Purpose of the immune system:
  • Fight off pathogens (bacteria, viruses, fungi, parasites)
  • Promote tolerance to commensal microbes
  • Regulate auto-reactivity
Innate and adaptive immunity

1st Line defense
- Innate immunity
  - Microbe
  - Phagocytes
  - Dendritic cells
  - Complement
  - NK cells

2nd Line defense
- Adaptive immunity
  - B lymphocytes
  - T lymphocytes
  - Antibodies
  - Effector T cells

Time after infection
- Hours: 0, 6, 12
- Days: 1, 4, 7
### Specificity of innate and adaptive immunity

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>For pathogen-associated molecular patterns (PAMPS)</td>
<td>Different microbes</td>
<td>Different microbes</td>
</tr>
<tr>
<td>Identical mannose receptors</td>
<td>Identical mannose receptors</td>
<td>Distinct antibody molecules</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encoded in germline (pattern recognition receptors)</td>
<td>Toll-like receptor</td>
<td>Encoded by lymphocyte genes produced by somatic recombination</td>
</tr>
<tr>
<td>N-formyl methionyl receptor</td>
<td>Mannose receptor</td>
<td>Ig</td>
</tr>
<tr>
<td>TCR</td>
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</tr>
</tbody>
</table>
Innate Immunity
Pattern Recognition Receptors

Examples:
- Toll-like Receptors (TLR)
- Nod-like receptors (NLR)
- RIG-I-like Receptors (RLR)
- C type lectin Receptors (CLR)

Present in all cellular compartments

PRR can differentiate between broad classes of pathogens
PRRs signal the earliest events in inflammation

- Acute inflammation
- Stimulation of adaptive immunity

TLR signaling

Expression of inflammatory genes:
- Cytokines (TNF, IL-1, IL-6)
- Chemokines (MCP-1, IL-8, others)
- Endothelial adhesion molecules (E-selectin)
- Costimulatory molecules (CD80, CD86)

Expression of type 1 interferon (IFN α/β) genes

Secretion of type 1 IFNs

Antiviral state

INFKb: Nuclear factor-Kb; IRFs: Interferon regulatory factor
**PRR recognize microbes & danger**

Molecular structures that are characteristic of microbes

**Pathogen-Associated Molecular Patterns (PAMPs)**

Host molecules released from damaged or dying cells

**Damage-Associated Molecular Patterns (DAMPs)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Example</th>
<th>Source(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acids</td>
<td>ssRNA, dsRNA, Cpg</td>
<td>Virus, Virus, bacteria</td>
</tr>
<tr>
<td>Proteins</td>
<td>Pilin, Flagellin</td>
<td>Bacteria, Bacteria</td>
</tr>
<tr>
<td>Cell wall lipids</td>
<td>LPS, Lipoteichoic acid</td>
<td>Gram-negative bacteria, Gram-positive bacteria</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Mannan, Dectin glucans</td>
<td>Fungi, bacteria, Fungi</td>
</tr>
<tr>
<td>Stress-induced proteins</td>
<td>HSPs</td>
<td></td>
</tr>
<tr>
<td>Crystals</td>
<td>Monosodium urate</td>
<td></td>
</tr>
<tr>
<td>Nuclear proteins</td>
<td>HMGB1</td>
<td></td>
</tr>
</tbody>
</table>

*Cpg, cytidine-guanine dinucleotide; dsRNA, double-stranded RNA; HMGB1, high-mobility group box 1; HSPs, heat shock proteins; LPS, lipopolysaccharide; ssRNA, single-stranded RNA.*
Innate Immunity
Complement Proteins

Initiation of complement activation

Early steps

Late steps

Microbe
Alternative pathway
Classical pathway
Antibody
Mannose binding lectin
Lectin pathway

C3a: Inflammation
C3b: opsonization and phagocytosis
C5a: Inflammation
C5b-9 Lysis of microbe

Effector functions

C3b: is deposited on microbe
Membrane attack complex (MAC)
C5
C5b
C5a

6/18/2018
Adaptive Immunity

- **B lymphocyte**
  - Microbe + Antibody
  - Neutralization of microbe, phagocytosis, complement activation

- **Helper T lymphocyte**
  - Microbial antigen presented by APC
  - Cytokines
  - Activation of macrophages
  - Inflammation
  - Activation of T and B lymphocytes

- **Cytotoxic T lymphocyte (CTL)**
  - Infected cell expressing microbial antigen
  - Killing of infected cell
CD4 Th cells can differentiate in subsets with distinct functions

- **TH1 cell**
  - Signature cytokines: IFN-γ
  - Immune reactions: Macrophage activation; IgG production
  - Host defense: Intracellular microbes

- **TH2 cell**
  - Signature cytokines: IL-4, IL-5, IL-13
  - Immune reactions: Mast cell, eosinophil activation; IgE production; "alternative" macrophage activation
  - Host defense: Helminthic parasites

- **TH17 cell**
  - Signature cytokines: IL-17A, IL-17F, IL-22
  - Immune reactions: Neutrophilic, monocytic inflammation
  - Host defense: Extracellular bacteria; fungi
Phases of adaptive immune responses

- **Antigen recognition**
- **Lymphocyte activation**
- **Antigen elimination**
- **Contraction (homeostasis)**
- **Memory**

- **Clonal expansion**
- **Differentiation**
- **Elimination of antigens**
- **Humoral immunity**
- **Cell-mediated immunity**
- **Apoptosis**
- **Surviving memory cells**

Days after antigen exposure: 0, 7, 14, 21
Specificity Memory and Contraction

- Anti-X B cell
- Anti-Y B cell
- Plasma cells
- Plasma cell
- Memory B cells
- Memory B cells
- Memory B cells
- Memory B cells
- Naive B cells
- Primary anti-X response
- Primary anti-Y response
- Secondary anti-X response
- Secondary anti-Y response

Weeks

Serum antibody titer
Regional Immunity

Specialized Immune Responses in Epithelial and Immune Privileged Tissues
Diverse ocular tissues with different challenges

- Microbes at the ocular surface
- Preserve optical clarity
- Little regenerative capacity
Immune responses in the eye are adapted to suit the functional needs of the diverse ocular tissues
PART 2: DEFENSE MECHANISMS OF TEARS AND OCULAR SURFACE
Healthy ocular surface

Lubrication Commensal flora

Resist pathogenic microbes
Multiple layers of protection

- Anatomic and physical barriers
- Tears
- Ocular epithelia
- Eye associated lymphoid tissue
Eyelids & Eyelashes

Eyelids

- Protect the eyeball
- Wipe the eye clean
- Distribute the tear film
- Shunt tears to the lacimal puncta
- Secrete the lipid layer

Eyelashes

- Prevent fine particles from entering the eye

McCurnin; Small Animal Physical Diagnosis and Clinical Procedures (1991)
The tear film

L lipid

Aqueous

Mucus
Tears: more than just water

Aqueous tears

Antimicrobial proteins and peptides

Mucins

Immunoglobulins
Mucins:
High molecular weight glycoproteins

Glycan structures of ocular surface mucins in man, rabbit and dog display species differences
Louis Rock, Elizabeth Matthews, Anthony Corfield, Monica Horsley, Pauline M. Ruddle, Raymond A. Dox, Stephen R. Corns.
Mucin producing tissues of the eye

Lacrimal gland

Cornea

Conjunctiva & Goblet cells

Gipson IK, Exp Eye Res 2004
## Type and origin of mucins

<table>
<thead>
<tr>
<th>Source</th>
<th>Mucin gene</th>
<th>Mucin type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial cells</td>
<td>MUC1, MUC4, MUC16</td>
<td>Membrane Associated</td>
</tr>
<tr>
<td>Goblet cells</td>
<td>MUC5AC, MUC5B, MUC2</td>
<td>Gel Forming</td>
</tr>
<tr>
<td>Lacrimal gland</td>
<td>MUC1, MUC4, MUC5B, MUC7</td>
<td>Small Soluble</td>
</tr>
</tbody>
</table>

Paulsen; Int Rev Cyto (2006)
Mucins: The swiss army knife of the ocular surface

- **Immunological properties**
  - Repulse or trap microorganisms
  - Reservoir of antimicrobial proteins and peptides
  - Binding sites for surveilling neutrophils
  - Maintain a healthy CALT
  - Wound healing

- **During infection:**
  - Cleavage of transmembrane mucins of the glycocalix
  - Increased mucus secretion
  - Intracellular signaling to increase cellular resistance to infection
  - Altered glycosilation

CALT: Conjunctiva Associated Lymphoid Tissue
Tears: more than just water

Aqueous tears

Antimicrobial proteins and peptides

Mucins

Immunoglobulins
Antimicrobial proteins and peptides (AMP): A chemical barrier against microbes

Source:
- Lacrimal glands and epithelium
- Serum exudates
- Infiltrating cells

Target:
- Bacteria, fungi and viruses

MS Gregory, Innate immune system and the eye, 2011
AMP: the shortlist

Lactoferrin
Lipocalin
Complement
α and β Defensins
Cathelicidin
Secretory phospholipase A2
Surfactant proteins
Secretory IgA

..................
Dog tear film protein analysis

125 different proteins in the tear film of healthy dogs

Dog Tear Film Proteome In-Depth Analysis.
Winiarczyk M et al. (2015) PLoS ONE
Redundancy If the immune system falls short of one factor, most of the time it can compensate for this deficit with other factors
## Dynamic regulation of AMP

<table>
<thead>
<tr>
<th></th>
<th>Open eyes</th>
<th>Lysozyme, lactoferrin, lipocalin, sIgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed eyes</td>
<td>Increased sIgA, complement, serum derived proteins and PMN</td>
<td></td>
</tr>
<tr>
<td>Ocular infection</td>
<td>More potent defensins</td>
<td></td>
</tr>
</tbody>
</table>
Neutrophil Chasing Bacteria

bacteria

David Rogers (1950s)
### Effect of AMP on Microorganisms

<table>
<thead>
<tr>
<th>Action</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disrupt microbial membrane</td>
<td>Lysozyme, secretory phospholipase A2, cathelicidin, defensin, SLP1, Complement factors</td>
</tr>
<tr>
<td>Interfere with microbial growth</td>
<td>Lactoferrin, Lipocalin A, SP-D</td>
</tr>
<tr>
<td>Interfere with microbial adherence</td>
<td>Lactoferrin</td>
</tr>
<tr>
<td>Neutralize and/or aggregate toxins and microorganisms</td>
<td>slgA, Defensin</td>
</tr>
</tbody>
</table>

SLP1: secreted leukocyte protease inhibitor-1
## Effect of AMP on the Epithelium

<table>
<thead>
<tr>
<th>Action</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promote proliferation</td>
<td>defensins, cathelicidins</td>
</tr>
<tr>
<td>Inhibit bacterial adhesion and invasion</td>
<td>SP-D, lactoferrin</td>
</tr>
<tr>
<td>Amplify apoptosis of infected cells</td>
<td>lactoferrin</td>
</tr>
</tbody>
</table>

SP-D: surfactant protein D
## Effect of AMP on Immune Cells

<table>
<thead>
<tr>
<th>Action</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemoattract Immune cells</td>
<td>SP A-D</td>
</tr>
<tr>
<td>Opsonization</td>
<td>IgA, complement</td>
</tr>
<tr>
<td>Promote phagocytosis</td>
<td>Defensins, complement</td>
</tr>
<tr>
<td>Potentiate bactericidal activity of Neutrophils</td>
<td>Lactoferrin</td>
</tr>
<tr>
<td>Reduce inflammation (through neutralization of bacterial toxins)</td>
<td>Cathelicidin, defensins</td>
</tr>
</tbody>
</table>

SP A-D: surfactant protein A&D
Lysozyme

• Function:
  • Enzyme: disrupts bacterial cell wall (Gram +)

• Quantity:
  • Major tear protein in people
  • Present in sheep, goats, llamas, cattle, horses, dogs, and rabbits
Tears: more than just water

Aqueous tears

Antimicrobial proteins and peptides

Mucins

Immunoglobulins
Immunoglobulins

- Secretory IgA, IgG, IgM
- Variable concentrations within healthy canine tears
  - Age
  - Time of day
  - Day to day variations

Measurement of IgG, IgM and IgA concentrations in canine serum, saliva, tears and bile

A.J. German\textsuperscript{a,b,*}, E.J. Hall\textsuperscript{a}, M.J. Day\textsuperscript{b}

\textsuperscript{a}Department of Clinical Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU, UK
\textsuperscript{b}Department of Pathology and Microbiology, University of Bristol, Langford, Bristol BS40 5DU, UK

Accepted 25 February 1998
Secretory IgA

- Dimeric antibody
  - Junction chain
  - Secretory component
- Plasma cells in the conjunctiva and lacrimal glands
- Transported through the epithelium
Secretory IgA: How does it work?

- Form Immune Complexes (IC)
  Agglutinate and neutralize microbes

Antigen-specificity

SEM IgA-mediated agglutination of *Salmonella typhimurium*
**Secretory IgA**

- Immune complexes reinforce epithelial barrier function
- Favors commensals close to the epithelium
- Inhibit pathogenic invasion of the epithelium
- IC uptake by conjunctival M cells

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Mantis N.J. Mucosal Immunology 2011
Tear IgG

- Low concentrations
- Produced during re-exposure
- Diffuses passively or actively transcytosed
- Mode of action:
  - Activate the complement cascade
  - Promote phagocytosis by macrophages

**Kill Microbes**
Tear IgM

- Low concentrations (pentamer, large molecular weight)
- Produced during primary infection
- Mode of action:
  - Activate the complement cascade

Kill Microbes

M. R. Ehrenstein & C. A. Notley; Nature Reviews Immunology 2010
Epithelial defense mechanisms

• Tight junctions
• High cell turn-over
• Pattern Recognition Receptors (e.g. TLRs)

Abelson, Rev Ophthalmol; 2009
PRR: Toll-Like Receptors

- Total of 10 TLR in humans
- All 10 TLRs are present in the eye
  - Ocular surface
  - Iris, ciliary body, choroid and RPE
- Key receptors that signal the earliest events in inflammation
  - Release of inflammatory mediators
  - Chemotaxis of immune cells
  - Expression of adhesion molecules
Regulation of TLR-signaling

• Spatial regulation of TLR expression
  • Basal and wing cells (e.g. TLR5 for flagellin)
  • Intracellular compartments (e.g. TLR2&4 for lipopeptide and LPS)
• Modulation of inflammatory signals after TLR5 stimulation by flagellin from pathogenic bacteria versus commensal bacteria

LPS: Lipopolysaccharide
TLRs on the equine ocular surface

TLR mRNA expression in the corneal, limbal and conjunctival epithelium

Expression of Toll-like receptors 2, 3, 4, 6, 9, and MD-2 in the normal equine cornea, limbus, and conjunctiva

Kara Gornik, Phillip Moore, Monica Figuiredo† and Michel Vandenplas‡

<table>
<thead>
<tr>
<th>TLR 2</th>
<th>TLR 3</th>
<th>TLR 4</th>
<th>TLR 6</th>
<th>TLR 9</th>
<th>MD-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Limbus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dorsal Conjunctiva</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ventral Conjunctiva</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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K. Gornik et al, Vet Ophthalmol 2011
Eye Associated Lymphoid Tissue

- Functionally linked to the mucosal immune system
- Eye Associated Lymphoid Tissue
  - Lacrimal Gland Associated Lymphoid Tissue (LGALT)
  - Conjunctiva Associated Lymphoid Tissue (CALT)
  - Lacrimal Drainage Associated Lymphoid Tissue (LDALT)

= Atomically and immunologically connected
Eye associated lymphoid tissue

LGALT

CALT

LDALT

Knop & Knop; Conjunctiva immune surveillance; 2011
Lymphoid follicles of the third eyelid
Follicle Associated Epithelium

- Thinner epithelium
- No goblet cells
- M-cells

Conjunctival follicles

- M-cells sample surface antigens
- APCs present Antigens to CD4+Th cells
- CD4+Th cells help B cells transform into Ab producing plasma cells
Eye Associated Lymphoid Tissue

Knop & Knop, 2011, Conjunctiva immune surveillance

Diffuse lymphoid tissue (Immune effector sites)

Blood circulation

Lymphoid follicles (Immune inductive sites)
When pathogens evade ocular defense mechanisms

- Stimulation of TLR
- Signal inflammation:
  - vasodilation
  - inflammatory cytokines
  - chemoattractants
  - adhesion molecules
- Recruitment of Innate Immune cells
- Inflammatory APCs initiate the adaptive immune response
- Generation of antigen-specific T and B cells
- Conventional adaptive immune response
Anti- vs Pro-inflammatory responses

Knop & Knop, 2011, Conjunctiva immune surveillance
Anti- vs Pro-inflammatory responses
DRY EYE AND ASSOCIATED OCULAR SURFACE INFLAMMATION
Tear production:
Integrated Lacrimal Functional Unit

Dysfunction of any component:
- Destabilize the tear film
- Alter volume, composition, or distribution of tears

Dry eye in canine patients

Quantitative disorder

= Aqueous tear deficiency
= Keratoconjunctivitis sicca
Immune-mediated KCS: Lacrimal gland inflammation

- Genetic predisposition
- Environmental stress
- Hormonal imbalances
- Lacrimal and/or Immune dysregulation
Immune-mediated KCS: Lacrimal gland inflammation

Regardless of the inciting cause dry eye is always accompanied by
Redness - Irritation – Inflammation
Self-perpetuating cycle of surface inflammation

- Activates autoreactive B and T cells
- Phagocytosis of necrotic cells
- Invasion of immune cells

- Decreased goblet cells
- Decreased mucin production
- Disrupts tight junctions
- Squamous metaplasia
- Cell death

- Adhesion molecules
- Vascular permeability
- Inflammatory cytokines

- Altered TF quantity, quality, stability

- Altered tear osmolarity, dessication
Self-perpetuating cycle of surface inflammation

- Damage to the lacrimal glands & reduced tear production
- Cellular debris are shed into tears
- Inflammatory mediators impede neural transmission at the ocular surface
- Sensory isolation of the lacrimal gland
- Neuronal reflex signaling to the lacrimal gland is interrupted
- Ocular surface inflammation

Damage to the lacrimal glands & reduced tear production

Cellular debris are shed into tears

Inflammatory mediators impede neural transmission at the ocular surface

Sensory isolation of the lacrimal gland

Neuronal reflex signaling to the lacrimal gland is interrupted

Ocular surface inflammation
Integrated Lacrimal Functional Unit

Dysfunction of any component destabilizes the tear film
PART 3:
IMMUNE PRIVILEGE OF THE CORNEA
Corneal transparency

Relies on several factors:

- Anatomic
- Physiologic
- Immunologic
Immune Privilege of the cornea

Cornea possess tissue specific immune regulatory mechanisms that aim to prevent destructive immune responses

- Corneal Angiogenic Privilege
- Corneal Immune Privilege
1. Angiogenic Privilege of the cornea

- Absence of blood and lymph vessels from the cornea
- Corneal avascularity is an active process:
  - Balanced production of angiogenic factors
  - Excess of inhibitors of angiogenesis
Angiogenic and anti-angiogenic factors in the normal cornea

Angiogenic: VEGFs, bFGFs, MMPs

Anti-Angiogenic: TSP, Endostatin, Angiostatin, sVEGFR

VEGF: vascular endothelial growth factor; bFGF: basic fibroblastic growth factor; MMP: matrix metalloproteinase; sVEGFR: soluble VEGF receptor; TSP: Thrombospondin; PEDF: pigment epithelium derived factor
Angiogenic and anti-angiogenic factors in the normal cornea

Angiogenic

- MMPs
- bFGFs
- VEGFs

Anti-Angiogenic

- TSP
- Endostatin
- Angiostatin
- sVEGFR

VEGF: vascular endothelial growth factor; bFGF: basic fibroblastic growth factor; MMP: matrix metalloproteinase; sVEGFR: soluble VEGF receptor; TSP: Thrombospondin; PEDF: pigment epithelium derived factor
Inhibition of VEGFs by soluble VEGFRs

R.J-C. Albuquerque; Blood 2013

R.J-C. Albuquerque; Blood 2013
Pathologic conditions modify the balance

Epithelial loss
Hypoxia
Inflammation

Angiogenic

Anti-Angiogenic
Blood and lymphatic vessels co-migrate into the cornea:

Confocal Immunohistochemistry of the cornea

C. Cursiefen, et al., J Clin Invest; 2004
Macrophages stimulate corneal vascularization

Importance of corneal vascularization in the immune response

Corneal vascularization in animal species

Masson’s trichrome stain of the Manatee cornea. Stroma: blue, epithelium: red-purple, arrow: blood vessels

J.Y Harper, Vet Ophthalmol; 2005
Corneal avascularity is due to sVEGFR1

- Manatees have vascularized corneas because they are genetically deficient in sVGFR1
VEGFR 1-2 expression in the dog

- VEGFR1 in corneal epithelium and endothelium
- VEGFR1-2 expression increases in vascularized corneas (soluble and membrane bound forms)
VEGFA expression in the horse

Equine deep stromal abscesses:
- Fungal hyphae
- Delayed vascularization
- Low VEGF-A expression (IHC)

Equine deep stromal abscesses (51 cases – 2004–2009) – PART 2: the histopathology and immunohistochemical aspect with attention to the histopathologic diagnosis, vascular response, and infectious agents

Michala de Linde Henriksen,*1,‡; Pia Hasbro Andersen‡; Kristy Mietelka‡; Lisa Furina,** Preben D. Thoennes,† Caryn E. Plummer,† Berndan G. Mangan,† Steffan Hergaard,**‡‡; James K. Coleman,** Nils Tofte‡ and Dennis E. Brooks‡
2. Immune privilege of the cornea

- Low antigenicity of the corneal tissue
- Few and immature APCs
- Local immune suppression
- Direct contact with aqueous humor
Low antigenicity of the corneal tissue

- Low Cellularity
- Little antigenicity
  - Low MHC I, MHC II virtually absent
  - Epithelium > endothelium
- Endothelium
  - Increased protective mechanisms

### Few and immature APCs in the cornea

Resident population of APC

<table>
<thead>
<tr>
<th>Central cornea</th>
<th>Peripheral cornea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Few APCs</td>
<td>More APCs</td>
</tr>
<tr>
<td>Immature (no MHC II)</td>
<td>Some MHC II, connect via dendrites</td>
</tr>
</tbody>
</table>

MHC II: Major Histocompatibility Complex class II molecules; serve for antigen presentation

Hamrah, Antigen presenting cells in the eye and ocular surface; 2011
# Corneal suppressive factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>FasL</td>
<td>Apoptosis T cells, PMNs</td>
</tr>
<tr>
<td>PDL-1</td>
<td>Inhibit of T cell proliferation</td>
</tr>
<tr>
<td></td>
<td>Inhibit cytokine secretion</td>
</tr>
<tr>
<td></td>
<td>T cell apoptosis</td>
</tr>
<tr>
<td>Non-classical MHC class Ib</td>
<td>Inhibit NK cell activity</td>
</tr>
<tr>
<td>Complement regulatory proteins</td>
<td>Protect from complement-mediated cellular lysis</td>
</tr>
<tr>
<td>Contact with Aqueous humor</td>
<td>Multiple Factors that modulate adaptive and innate immunity</td>
</tr>
</tbody>
</table>

Survival of corneal grafts relies in part on Anterior Chamber Associated Immune Deviation (ACAID)
CORNEAL TRANSPLANTATION
Corneal transplantation

1905 - The man who received the first cornea transplant was given no antibiotics, no drugs to stop him rejecting the tissue

Dr E. Zirm, 1905, Czech Republic
Transplantation trends

Lamellar keratoplasties
GOAL: Replace only the diseased corneal layers
Improve graft acceptance

- Penetrating Keratoplasty
- Endothelial Keratoplasty
Corneal transplantation

- 200,000 corneal grafts/year worldwide
- No HLA matching (human equivalent of MHC)
- Limited immunosuppression
- High success
  - tissue characteristics and ocular immune privilege
  - Low risk corneas
  - Replace only diseased tissue
  - Refined surgical techniques
  - Low antigenicity of the endothelium
  - Immunosuppressive factors in the anterior chamber

- 2 year success rate DMEK 99%
  PK 82%
What modifies graft survival?

<table>
<thead>
<tr>
<th>Decrease survival</th>
<th>Increase survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal inflammation</td>
<td>Deplete macrophages</td>
</tr>
<tr>
<td>Corneal vascularization</td>
<td>Inhibit vascularization (Lymphatic &gt; blood vessels)</td>
</tr>
<tr>
<td>Interfere with ACAID</td>
<td>Induce ACAID prior to transplantation</td>
</tr>
</tbody>
</table>

ACAID: Anterior Chamber Associated Immune Deviation
Effect of inflammation and vascularization on graft survival

Acceptance

Normal risk
(no blood/lymph vessels)

Avascular High risk
(no vessels but inflamed)

Alymphatic High risk
(only blood vessels)

High risk
(blood/lymph vessels)

Rejection

Modified from: T. Dietrich, J. of Immunol, 2010
## New therapies that target vascularization

<table>
<thead>
<tr>
<th>Block VEGF production</th>
<th>Block VEGF-VEGFR binding</th>
<th>Block intracellular signaling</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Insuline receptor substrate inhibitor-1 (Aganirsen)</td>
<td>• Blocking Ab (Avastin) or Ab fragments (Lucentis)</td>
<td>• Tyrosine kinase inhibitors</td>
</tr>
<tr>
<td></td>
<td>• VEGF-trap fusion protein (Aflibercept)</td>
<td>• mTor inhibitor</td>
</tr>
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</table>

![Diagram showing VEGF and VEGFR binding and signaling](image-url)

- **VEGF** (vascular endothelial growth factor)
- **VEGFR** (vascular endothelial growth factor receptor)
- **Anti-VEGF therapy**
- **Tyrosine kinase inhibitors**
- **mTor inhibitor**
- **Hemangiogenesis**
- **Lymphangiogenesis**

**Critical binding site**

- **sVEGFR-1**
- **sVEGFR-2**
- **sVEGFR-3**

- **VEGF-A**
- **VEGF-C**
- **VEGF-D**
Corneal transplantation in veterinary patients

- Great species variations
  - Better tolerated in feline patients
  - Rejection in canine and equine patients

- Individual / breed variations:
  - Make up of the immune system
  - Tendency to develop corneal vascularization: Boxer

- Indications:
  - Optical
  - Cosmetic
  - Therapeutic
  - Tectonic/reconstructive

= High risk corneas
Corneal transplantation in veterinary patients

Pictures gift from R. Stoppini, Italy
NON-ULCERATIVE IMMUNE-MEDIATED KERATOPATHIES
Immune mediated keratopathy

- Corneal pathology that results from abnormal activity of the immune system (exaggerated activity or autoimmunity)
  - Equine IMMK
  - Canine CSK
  - Feline stromal keratitis
Canine chronic superficial keratitis (CSK)

- **Clinical appearance:**
  - Bilateral fibrovascular infiltration and inflammation of the cornea

- **Infiltrating cells:**
  - Predominantly CD4+ T cells
  - Macrophages
  - Plasma cells
  - Neutrophils

- **Increased expression of:**
  - IFNγ
  - MHC II

- **Systemic:**
  - Increased serum levels of VEGF
CSK – Risk factors

• **Immunogenetic predisposition**
  - GSD /Greyhounds
  - Leukocytes of affected dogs react to corneal antigens
  - MHC II genes
    - DLA-DRB1*01501/DQA1*00601/DQB1*00301
      - Risk for disease development:
        - 2.7x heterozygotes
        - 8x homozygotes
    - SNP in the regulatory region that controls MHC transcription
      - Affects level/pattern of MHC expression

• **Environmental factors**
  - UV exposure /Altitude

• **Hormonal imbalance**
  - female
CSK - Treatment

Reduce stimulation
• Sun protection

Immune response
• Topical steroids
• CsA / Tacrolimus
• Adjunct therapy
  Radiation (Sr90/soft-X rays)

Target both avenues for maximum therapeutic effect
Feline stromal keratitis

Progressive inflammation, edema and vascularization of the cornea

**Trigger**
- FHV-1

**Effector**
- Immune response

**Virus-induced immunopathology**
Develops with repeated viral reactivation
Spectrum of FHV-1 related ocular surface disease
FHV-1 life cycle

- Severe infections
  - Viremia, pneumonia, encephalitis
  - Viral DNA recovered in multiple sites: trigeminal, ciliary, vestibular ganglia; cornea

Immuno-pathogenesis experimental cats

Experimental cat model (Nasisse 1989 - 1995)
- Subconjunctival CCS + FHV-1 = Stromal keratitis
  - Suppression of the local immune response
  - Increased viral load, increased cellular damage, delayed clearance
  - Viral particles in the stroma
  - Cellular infiltrates: neutrophils /leukocytes
  - Continued disease process after viral clearance
Immuno-pathogenesis experimental rodents

Rodent model
- Phase 1: Innate immunity
  - Cytokines/VEGF
- Phase 2: CD4Th cells
  - Cytokines
  - Orchestrate stromal keratitis
  - Virus-specific CD4^+T
  - Bystander activated CD4^+T
    - (Auto-reactive T cells)
- Phase 3:
  - Exacerbation of disease
Treatment

- Trigger
  - FHV-1

- Immune response
  - Topical steroids
  - CsA / Tacrolimus

Target both avenues for maximum therapeutic effect
Targeting the immune response

- Topical steroids
  - + Reduced cellular infiltration, scarring, vascularization
  - - Exacerbate active viral infection, opportunistic infection

- Cyclosporine
  - + T cell specific suppression, reduces vascularization
  - - Suppressing T cells may interfere with viral control. No direct effect on bystander activated T cells

Weigh the benefits against the risk
Comparative comments:
Herpes Simplex Keratitis in people

- Topical or oral antivirals
- Topical corticosteroids (fluo -)
- Topical cyclosporine
- Long-term oral antiviral therapy
PART 4: OCULAR IMMUNE PRIVILEGE & ACAID THERAPEUTIC APPLICATIONS
Ocular Immune Privilege - landmarks

- 1873 Van Dooremaal studied cataractogenesis: prolonged survival of mouse skin grafts in canine anterior chamber
- 1905 First successful human corneal transplant
- Sir Peter Medawar 1950’s recognized prolonged survival of skin grafts in the eye and brain = Immune Privilege
- Streilein et al in the 1970s show immune deviation when antigen placed in AC
- Today: 4th generation of ocular immunologists
Meaning of immune privilege

Medawar:
Foreign tissue grafts placed in the immune privileged site are tolerated and survive, whereas placement of such grafts at conventional body sites leads immune rejection.

Passive immunological ignorance

Streilein:
Local and systemic immunoregulatory mechanisms allow for graft survival in the eye

Multiple active immune-mechanisms
Immune privilege in the year 2018

Multiple mechanisms that provide the eye with immune protection, while avoiding the damaging effects of excessive inflammation induced by conventional immune responses.
Multiple aspects of Immune Privilege

- Physical barriers: blood-ocular barriers
- Local immune regulation
- Systemic immune deviation
Blood ocular barrier:
Blood-Aqueous & Blood-Retina barriers

CB & Iris epithelium
Iris vessels

RPE
Retinal vessels

Tight Junctions
Vascular endothelium

- Iris and retinal vessels
- Non-fenestrated endothelium
- Tight junctions

**True barrier system**
Ciliary Body and RP Epithelia

- Tight junctions
- Immune modulating properties

R. Shechter et al.; Nat Rev Immunol; 2013
Blood-ocular barriers

- Effect of immunomodulatory factors:
  - Suppress cells with an inappropriate phenotype (deletion, anergy or active suppression)
  - Convert of immune cells to a regulatory phenotype (Treg)

**Barrier and immunomodulatory gate**

= Preferential survival of immune cells with a desired phenotype
Multiple aspects of Immune Privilege

- Physical barriers: blood-ocular barriers
- Local immune regulation
- Systemic immune deviation

Membrane bound molecules
Reprogramming of T cells into regulators
Soluble immunomodulatory factors

Streilein-foundation.org
Local immune regulation:
Membrane bound molecules

<table>
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<tr>
<th>Immune response</th>
<th>Effect</th>
<th>Factors</th>
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<td><strong>Innate immunity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK cells</td>
<td>suppression</td>
<td>MHC class Ib</td>
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<tr>
<td>PMN</td>
<td>Activation</td>
<td>FasL</td>
</tr>
<tr>
<td>Complement</td>
<td>Suppression</td>
<td>CD46, CD55, CD59, Crry</td>
</tr>
<tr>
<td><strong>Adaptive immunity</strong></td>
<td></td>
<td></td>
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<tr>
<td>T cells</td>
<td>Apoptosis/suppression</td>
<td>PDL-1, FasL, CD86, MHC class Ib</td>
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PDL-1: Programmed death ligand-1
### Local immune regulation: Soluble factors

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<td>CGRP, αMSH, MIF, sFasL</td>
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<tr>
<td>Complement</td>
<td>Suppression</td>
<td>CRP</td>
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<td><strong>Adaptive immunity</strong></td>
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<td></td>
</tr>
<tr>
<td>APC</td>
<td>Suppression/tolerance</td>
<td>αMSH, CGRP, VIP, TGFβ2, TSP-1</td>
</tr>
<tr>
<td>T cell</td>
<td>Suppression/Apoptosis</td>
<td>TGFβ2, αMSH, VIP, SOM, FasL</td>
</tr>
<tr>
<td></td>
<td>Treg</td>
<td>Soluble: TGFβ2, αMSH, SOM Membrane bound: CTLA-2α, CD86, mTGFβ, mTSP</td>
</tr>
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</table>

CGRP: calcitonine gene related peptide; αMSH: αmelanocyte stimulating hormone; MIF: macrophage inhibitory factor; CRP: complement regulatory proteins; VIP: vasointestinal peptide; TSP: trombospondin; SOM: somatostatin; TGFβ: transforming growth factorβ; CTLA-2α: cytotoxic T lymphocyte associated antigen; mTSP: membrane bound TSP.

Immunomodulatory factors in the eye
Multiple aspects of Immune Privilege

- Physical barriers: blood-ocular barriers
- Local immune regulation
- Systemic immune deviation

Streilein-foundation.org
Systemic Immune Deviation

- ACAID: Anterior chamber-associated immune deviation
- VCAID: Vitreous cavity-associated immune deviation
- Immune deviation induced through the sub-retinal space
AACAID

- Antigen-specific systemic immune deviation to an antigen that has been introduced (injected) in the anterior chamber
- Can be induced to various types of antigens:
  - Soluble protein antigens
  - Viral antigens
  - Allo-transplantation antigen
  - Tumor antigens
AIDA

- **Cellular responses** are suppressed: Th1, Th2 and Th17 induced inflammation

- **Antibody responses** modified
  - Suppresses complement fixing Ab
  - Generation of non-complement fixing Ab

- **Cytokine profile:**
  - Th1 cytokine (IFNγ): suppressed
  - Th2 cytokine (IL-4): not required
  - Regulatory cytokine (IL-10): produced
Anterior Chamber Associated Immune Deviation is sustained through the cooperation of various immune cells in organs other than the eye itself
Organs required for ACAID induction

- ACAID induction requires an intact
  - Eye (not inflamed)
  - Thymus
  - Spleen
  - Sympathetic nervous system
- Impaired ACAID induction:
  - Certain types of antigens/excessive ocular inflammation
  - Removal of the eye within 3 days of anterior chamber injection of antigen
  - Splenectomy, thymectomy or sympathectomy prior to anterior chamber injection of antigen

= Development of a normal immune response
ACAID Thymic phase

ACAID Splenic phase

Marginal Zone Metallophilic Macrophages
ACAID APC
ACAID Tregs

Lymph Node

Th1

Th0

Infiltrating Th1/Th2 effector T cells

Eye

CD8 Treg suppress effector T cells

Spleen

CD4 Treg

CD8 Treg

CD4 Treg inhibit Th1 differentiation
In vitro ACAID

IV Cell therapy

Lymphocytes

ACAID-APC
TGFβ + Ag

Antigen

Anterior chamber

Trabecular meshwork

Tregs
Cell therapy to restore tolerance

- In vitro generated ACAID-APC have been used to treat:
  - Rodent models of
    - Experimental autoimmune uveitis (EAU)
    - Experimental autoimmune encephalitis (EAE)
    - Pulmonary interstitial fibrosis
    - Spinal cord injury
    - Corneal transplantation
  
  - Humanized mouse model of allergic asthma
Tolerogenic cell therapy

Tolerogenic dendritic cells and negative vaccination in transplantation: from rodents to clinical trials

Aurélie Monne, Emilie Vervy, Guillaume Bérian, Matthieu Haw, Laurence Bouchez-Delbœuf, Mercedes Segovia and Madeleine Cotturi

[Image of the journal page]
Is ocular immune privilege unique?
Are there other sites capable of deviating the immune response?

- Eye
- Brain
- Testis
- Pregnant uterus
- Gut
- Cheek pouch in rodents
- Hair follicle
- Certain tumors
Is there a benefit to having Ocular Immune Privilege?

• All animals tested possess ocular immune privilege
• Protection against minor day-to-day insults
• Evolutionary benefit
Is there a downside to ocular immune privilege?

- Failure to reject certain tumors
- Delayed clearance of certain pathogens
- Incomplete immune-tolerance to ocular antigens
- May leave the eye more vulnerable to autoimmune attack when immune privilege breaks down
PART 5:
IMMUNE MECHANISMS OF UVEITIS
Immune mediated diseases of the uvea

• Failure of ocular immune privilege
• Inflammation of the uvea intended to protect the eye by eliminating a potential harmful stimulus
• High vascularity of the uvea makes it very responsive to inflammatory mediators
A common pathway to inflammation

Pathogen signals
PAMP

Endogenous danger signals
DAMP

PRR

Pro-inflammatory factors

Innate Immunity

Context determines the nature of the immune response

Adaptive Immunity
Autoimmunity and the eye

Genetic susceptibility
- ERU in Appaloosas
- UDS and Akitas

Tolerance to ocular antigens

Environmental triggers
- Infections
Tolerance to ocular antigens?

- Central and peripheral tolerance to self antigens

- Less than perfect peripheral tolerance to ocular antigens
Infection and autoimmunity

Primary infection somewhere

Migration to the eye

Autoreactive T cells

C. Münz et al; Nature Reviews Immunology (2009)
Infection and autoimmunity

Molecular mimicry

Tissue damage & Epitope spreading

C. Münz et al; Nature Reviews Immunology (2009)
Autoimmunity is a failure of self-tolerance

<table>
<thead>
<tr>
<th>Systemic IMT</th>
<th>Several organs UDS</th>
<th>Organ specific ERU</th>
</tr>
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Imbalance between lymphocyte activation and control mechanisms
## Canine Uveodermatologic Syndrome

- Vogt-Koyanagi-Harada syndrome (eyes-skin-brain)
- Uveitis and depigmentation (uvea/RPE)
- Skin and fur depigmentation (vitiligo/poliosis)
- Young adult dogs – otherwise healthy
- Akita breed
Immunopathology

- Autoimmune reaction to melanocyte antigens (tyrosinase proteins)
- Genetic predisposition: Akitas
  - MHC II (DLA DQA1*00201)
- Infectious trigger?
  - Suspected in people
  - Molecular mimicry between human cytomegalovirus (HHV-5) and tyrosinase proteins
- Anti-retinal antibodies aggravate uveitis
- Histopathology:
  - Mixed inflammatory reaction with pigment laden macrophages
Feline lymphoplasmacytic uveitis

- Persistent uveitis
- No infectious agents
Feline lymphoplasmacytic uveitis

- LPU: Histological diagnosis
- Immunological mechanisms?
- Target antigen(s)?
Lens Associated Uveitis

**Phacoclastic uveitis**
capsular rupture

**Phacolytic uveitis**
cataractous lens intact capsule
Immune tolerance to lens proteins

- Crystallins: structural proteins of the lens
- Crystallins are present in other tissues
- Serum and aqueous contain soluble crystallins
- Central / peripheral immune tolerance to crystallins
  - T cells: tolerant
  - B cells: Naturally occurring anti-crystallin antibodies are present in a majority of healthy people and dogs (60%)
Phacoclastic uveitis

- E.g. Cat scratch-injuries
- Tissue injury
  - = Danger Associated Molecular Patterns
- Inoculation of microorganisms
  - = Pathogen Associated Molecular Patterns
- PRR stimulation
- Innate and adaptive immune reactions
Phacolytic uveitis

- Uveitis associated with leaking of proteins through an intact capsule of a cataractous lens
Immunological basis of phacolytic uveitis

- Mild lymphoplasmacytic uveitis
- Cataract in dogs is not associated with an increase circulating anti-lens antibodies or anti-lens antibodies in the aqueous humor
- Protein modifications with cataract development could constitute new antigenic epitopes