Ocular Immunotherapy and Immunomodulation

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Ocular Immunotherapy and Immunomodulation

- Introduction
- Antimetabolites
- Alkylating agents
- T-cell inhibitors
- Other immunomodulators (niacin, tetracycline)
- Cytokine or cytokine receptor inhibitors
- Immunosuppressive cytokines
- Oral tolerance
Immunomodulation / Immunotherapy

- Recent advancements
- Many ocular immune-mediated diseases
- Steroidal medications are the most frequently used
  - “sledge hammer” approach
  - Peripheral damage
- New medications
  - More specific
  - Fewer side effects.
To Effectively Treat Uveitis:

- **Decrease activation:**
  - Prevent systemic inflammation / activated T-cells
  - Induce tolerance to antigens by APC / dendritic
  - Minimize exposure of ocular antigens to Th17 cells
  - Inhibit T-cell function and cytokine release

- **Decrease inflammation**
  - Limit production of PG and NO
  - Decrease adhesion molecule function
  - Decrease or inhibit chemokines
  - Enhance expression of CD4+Foxp3+ Treg cells
Immunomodulation / Immunotherapy

- Most are systemically administered medications
- Local injections, implants, and eyedrops being developed.
- Ocular penetration, both topically and via the systemic route have not been studied extensively for most drugs
- The purpose of these lectures is to review existing and emerging IMT drugs to better our understanding of immunotherapy of ocular disease.
Ocular Immunotherapy and Immunomodulation

- Antimetabolites
- Alkylating agents
- T-cell inhibitors
- Other immunomodulators (niacin, tetracycline)
- Cytokine or cytokine receptor inhibitors
- Immunosuppressive cytokines
- Oral tolerance
Antimetabolites

• Most commonly used antimetabolites in ocular therapy include:
  • Azathioprine (Imuran®)
  • Methotrexate (Rheumatrex®)
  • Mycophenolate Mofetil (CellCept®)
Azathioprine (Imuran®)

- Converted in the body to the active metabolite 6-mercaptopurine
- Inhibit purine synthesis
- Necessary for the proliferation of cells
Azathioprine (Imuran®)

- Ocular treatment:
  - Nodular granulomatous episcleritis
  - Use in combination with corticosteroids
    - Uveitis
    - Steroid-responsive RD
  - Decrease dose of CS
Methotrexate (Rheumatrex®)

- Inhibits metabolism of folic acid
- Competitively and reversibly inhibits dihydrofolate reductase, that is part of the folate synthesis metabolic pathway.
- Folic acid is needed for the de novo synthesis of thymidine, required for DNA synthesis
Methotrexate (Rheumatrex®)

- Used as a chemotherapeutic agent in dogs
- No reports of use for eye disease in veterinary medicine
- Commonly used for rheumatoid arthritis in humans
- Used for autoimmune uveitis and scleritis in human
Mycophenolate Mofetil (CellCept®)

- Mycophenolate mofetil (MMF) is the mofetil ester of mycophenolic acid
- MMF has largely replaced azathioprine
  - organ transplants
  - myasthenia gravis
  - uveitis.
- Topical MMF has been shown to penetrate the cornea.
Mycophenolate Mofetil (CellCept®)

- Inhibits T and B cell proliferation
  - by blocking the production of guanosine nucleotides required for DNA synthesis.
  - inhibitor of inosine monophosphate dehydrogenase, thus blocking purine synthesis

- A clinical trial at NCSU evaluating MMF for treatment SARDS.

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Alkylating Agents

- Cyclophosphamide (Cytoxan®)
- Chlorambucil (Leukeran®)
Alkylating Agents

- General mode of action:
  - Interfere with DNA replication
  - Prevent cellular division of rapidly proliferating cells
    - Inflammatory
    - Neoplastic cells.
Cyclophosphamide (Cytoxan ®)

- Nitrogen mustard alkylating agent
- Chemotherapy
- Autoimmune disease
- Uveitis
  - With other meds
Cyclophosphamide (Cytoxan®)

- Active metabolite is 4-hydroxycyclophosphamide.
  - Forms DNA crosslinks between and within DNA strands
  - Leads to cell death.

- **Use in dogs:**
  - Steroid sparing medication in uveitis
  - Ocular lymphoma (Cave T, Billson M. *J Small Anim Pract.* 2003;44:523, 553-4)
Chlorambucil (Leukeran®)

- Nitrogen mustard alkylating agent
  - Chemotherapy (CLL)
  - Autoimmune diseases
    - Lupus erythematosus
    - Acute and chronic glomerular nephritis
    - Nephrotic syndrome,
    - Psoriasis
    - Wegener’s granulomatosis
    - Chronic active hepatitis.
Chlorambucil (Leukeran®)

- Veterinary medicine:
  - In dogs, it is used primarily as a chemotherapeutic agent but has been used in dogs as a steroid sparing medication in uveitis.

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T-cell Inhibitors

- Cyclosporine
  - (Neoral®, Atopia®, Optimmune®, Restasis®)

- Tacrolimus
  - (FK-506; Fujimycin; Prograf®)

- Sirolimus
  - (Rapamycin, Rapamune®)

- Voclosporine
  - LX214
Cyclosporine
(Neoral®, Atopia®, Optimmune®, Restasis®)

- Calcineurin inhibitor
- Isolated from the fungus, *Tolypocladium inflatum* in 1976
- First selective T-cell inhibitor
  - At therapeutic concentrations it inhibits T-cell but is non-toxic
Cyclosporine
(Neoral®, Atopia®, Optimmune®, Restasis®)

• **Mode of Action**
  - Inhibits T-cell activation
  - CsA interferes with the expression of IL2 receptors on the surface of T-lymphocytes and also with IL2 release from lymphocytes
  - Blocks transcription of mRNA specific for production of IL2, IL4, and gamma interferon.
  - CsA is bound in cytoplasm by cyclophylline or immunophylline, which binds to calcium-dependent protein phosphorase called calcineurin.
  - CsA–calcineurin–immunophylline complex blocks ability of calcineurin to translocate transcription factors into the nucleus, preventing transcription and gene expression
Cyclosporine - Mode of action

T-cell Inhibitors
Cyclosporine
(Neoral®, Atopia®, Optimmune®, Restasis®)

- Use in veterinary medicine:
  - Immune-mediated canine keratoconjunctivitis sicca (KCS).
  - Successful in the 80-90% of dogs with KCS
Cyclosporine
(Neoral®, Atopia®, Optimmune®, Restasis®)

- Use in veterinary medicine:
  - Topical CsA has also been shown to be effective in treating:
    - Chronic superficial keratitis, or pannus, in dogs.
    - Nodular granulomatous episclerokeratitis
    - Equine immune-mediated keratitis
    - Prevention of rejection of corneal grafts
  - Numerous studies demonstrating lack of corneal penetration of CsA
  - CsA is effective in immune-mediated uveitis in the horse and dogs.
    - In dogs, oral CsA is effective, but expensive (see protocol in notes)
    - Ocular implants have been evaluated in horses
      - Intravitreal
      - Deep-scleral - suprachoroidal
Ocular Implants
Equine Recurrent Uveitis

- ERU is a T-cell mediated disease
  - High IL-2, γIFN
  - CsA IL-2 inhibitor
- CsA does not penetrate intact cornea
- Development of sustained release devices
Ocular Drug Delivery

- Suprachoroidal space
- Site of potential drug delivery

H-110 lipophilic fluorescein implants
4.5 hours
Photo courtesy of Dr. M.R. Robinson, NEI
Matrix-Reservoir Implant

12 mg, 4.5 mm CsA pellet in 15% CsA PVA

Release (mg/day) vs Time (days)
Placement of Device
Clinical Results - Flare ups of Uveitis

- **Number**
  - Number of flare ups in 12 months
- **Frequency**
  - Flares/month
- * $P<0.0001$
- **$P<0.0001$**
Clinical Results

- Mean Follow-up
  - 14.3 ± 10.5 months
  - Range 1 to 34 mos

- 85% (68/80) considered visual at last recheck

From: Gilger, et. al. Invest Ophthal Vis Sci 2006;46;6:2596-2605
Current Status of CsA Implants

- implants are available through NCSU Pharmacy
- https://commerce.cashnet.com/NCSUHospitalPayments?itemcode=VH-IMPLANT
T-cell Inhibitors

- **Cyclosporine**
  - (Neoral®, Atopia®, Optimmune®, Restasis®)

- **Tacrolimus**
  - (FK-506; Fujimycin; Prograf®)

- **Sirolimus**
  - (Rapamycin, Rapamune®)
Tacrolimus  
(FK-506; Fujimycin; Prograf®)

- Macrolide lactone discovered in 1984 from a Japanese soil sample *Streptomyces tsukubaensis*.
- Calcineurin inhibitor
- 20 to 50 times more potent then CsA
- Equal in efficacy to CsA, but a better safety profile (humans)
Tacrolimus
(FK-506; Fujimycin; Prograf®)

- Mode of Action
  - Binds to the immunophilin FKB12 (FK506 binding protein)
  - Inhibits calcineurin thus inhibiting both T-lymphocyte signal transduction and IL-2 transcription
  (similar to cyclosporine)
Tacrolimus
(FK-506; Fujimycin; Prograf®)

- **Use in Veterinary Medicine**
  - Systemic tacrolimus is more toxic in dogs than CsA, so systemic use has been limited.
    
  - **Topical** 0.02% - 0.03% tacrolimus efficacy in canine KCS
    
  - No adverse effects reported
Sirolimus (Rapamycin, Rapamune®)

- Macrolide antibiotic
- Isolated from a soil sample from Easter Island containing *Streptomyces hygroscopicus*
- Inhibits T and B-cell activation
Sirolimus
(Rapamycin, Rapamune®)

- Not a calcinuerin inhibitor
- Binds to the FK506-binding protein
- Does not inhibit calcineurin-dependent signal transduction and does not affect IL2 generation.
Sirolimus
(Rapamycin, Rapamune®)

- It dampens the response of T cells to cytokines
- Inhibits the phosphorylation of protein kinases
- Interferes with T-cell activation at a later stage of the cell cycle than tacrolimus or cyclosporin
Sirolimus (Rapamycin, Rapamune®)

- Use in Veterinary Medicine:
  - No descriptions yet
  - Much promise since it does not exhibit renal toxicity and has been shown to be effective in treatment of uveitis in humans.
Rapamycin in ERU

- Efficacy and toxicity in clinical patients in ocular inflammatory disease
  - Initiated an open-label clinical efficacy trial in horses with ERU (5 to 10 mg of Rapa injected intravitreally)

- Pilot studies were effective

- Need better formulation to allow injection through small needle
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Niacin (Nicotinic acid, vitamin B₃)

- Known to lower cholesterol
- Possibly decrease heart attacks, etc
- ARMD
- Adjunctive therapy for NGE?
- Dose: 25 mg/kg/day
Tetracycline

- Immunomodulatory effects
- Anti-MMP
  - Healing of indolent ulcers
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Cytokine or Cytokine Receptor Inhibitors

**TNFα Inhibitors**
- Etanercept (Enbrel®)
- Infliximab (Remicade®)
- Adalimumab (Humira®)
- Efalizumab (Raptiva®)

**IL1 Inhibitor**
- Anakinra (Kineret®)

**IL2 Inhibitor**
- Daclizumab (Zenapax®)
Etanercept (Enbrel®)

- Human recombinant TNFα receptor inhibitor
- Used inflammatory diseases such as rheumatoid arthritis, psoriasis, ankylosing spondylitis.
- Use in treatment of human uveitis, optic neuritis, scleritis
- Safe experimentally injected intravitreally
Etanercept (Enbrel®)

- Decreases expression of adhesion molecules responsible for leukocyte migration
- Decreases serum levels of cytokines and MMPs
- Once or twice weekly SQ injection
Etanercept (Enbrel®)

- Veterinary Medicine
  - No descriptions
  - Human recombinant
  - Extremely high cost
Infliximab (Remicade®)

- Chimeric (mouse-human) monoclonal antibody that binds specifically to Hu TNFα.
- Used to treat human rheumatoid arthritis, psoriasis, psoriatic arthritis, and ankylosing spondylitis.
- Human uveitis and posterior uveitis.
- Increased respiratory infections, TB
Infliximab (Remicade®)

- Etanercept - weekly SQ, Infliximab IV every q2 mo.
- Etanercept - protein
  Infliximab - antibody
- No descriptions of use in veterinary medicine
Adalimumab (Humira®)
Efalizumab (Raptiva®)

- TNFalpha inhibitors (monoclonal antibodies).
- Adalimumab - RA
  Efalizumab - psoriasis
- One report on the effective use of adalimumab for treatment of human uveitis.

Anakinra (Kineret®)

- Recombinant antibody, nonglycosylated form of Hu IL-1 receptor antagonist
- It is approved for treatment of RA in humans
Daclizumab (Zenapax®)

- Murine-human chimaerised monoclonal antibody to the IL-2Rα receptor of T-cells.
- Used to prevent organ rejection.
- In hu non-infectious uveitis, used to decrease steroid needs. (Nussenblatt RB, et al. *Ophthalmology.* 2005;112:764-70)
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Immunosuppressive Cytokines

- Interleukin -10 (IL-10)
- Transforming growth factor beta (TGFβ)
Interleukin -10 (IL-10)

- Multifunctional cytokine
- Acts to limit and ultimately terminate inflammatory responses
- Regulates growth and/or differentiation of:
  - B cells
  - NK cells
  - Cytotoxic and helper T cells
  - Mast cells
  - Granulocytes, others
Interleukin -10 (IL-10)

- **Mode of action:**
  - (1) inhibit cytokine production by macrophages
  - (2) reduced expression of MHC class II molecules and certain co-stimulators (e.g., B7).
- **Viral IL-10 adenovirus gene transfer** has experimentally reduced uveitis in animal models\(^{43}\)
Transforming Growth Factor Beta

- Multifunctional cytokine
- Throughout body
- Plays an important role in the ocular immune response and is predominantly immunosuppressive.
- Three isoforms (β1, β2, β3) of TGF-β have been identified in mammals and they share 70-99% sequence homology across species.
- Number, maintenance and proportion of TGF-β receptors have a pivotal role in the biologic effects exerted by the TGF-β protein.
Transforming Growth Factor Beta

- The biological interactions of TGF-B are complex
- Direct therapy and anti-TGF-B therapies for specific ocular diseases are being evaluated experimentally.
- Generally suppresses responses of Th1 cells
- Stimulates the fibrotic component of the chronic inflammatory response.
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Oral Tolerance

- Induction of peripheral immune non-responsiveness as the result of the oral administration of soluble protein antigens.

- It is considered a natural, immunologic mechanism, driven by exogenous antigens, to avoid untoward immune responses in the gut.
Oral Tolerance

- In a pilot study in humans with immune-mediated uveitis fed S-antigen led to immunosuppressive medications being decreased and/or stopped

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