Inflammation: Can we silence the secret killer?

Prakash Nagarkatti, Ph.D.
Vice President for Research
University of South Carolina
Columbia, SC 29208
Inflammation

Inflammation is a natural response from the immune system against infections or tissue injury.
Cells of Innate Immunity

No antigen-specificity
No Memory
Cells of Adaptive Immunity: Lymphocytes-B and T

Innate Response
- Macrophage
- Dendritic cell
- IF-Liposome
- IL-6
- IL-12
- NK Cell
- Neutrophils

Adaptive Response
- Liposome phagocytosis
- CD4 T cells
- CD4 T cell
- B cell
- Plasma B cells
- Antibodies
- Memory B cells
- Th1
- Th2
- IL-12
- IFN-g
- IL-4
- IL-10
- Liposome fusion
- MHCI
- CD8 T cell
- CTL
- Memory CD8 T cells
- Lysis

Antigen-specificity
Memory
Nature of inflammation in Type-I Hypersensitivity (allergies)

- Histamine, tryptase, kininegenase, ECFA
- Leukotriene-B4, C4, D4, prostaglandin D, PAF
- Newly synthesized mediators

Macrophage/Dendritic cells

IL-4, IL-5, IL-13

TH2

B cell

Plasma cell

IgE

Mast cells

Leukotriene-B4, C4, D4, prostaglandin D, PAF

Newly synthesized mediators
Anaphylaxis

Anaphylaxis:

Egg Albumin

Repeat Inj. 2 Weeks later

Dies from asphyxia

Guinea Pig dies from anaphylaxis.
Egg albumin → IgE Abs → Mast cells

Lungs
- Bronchoconstriction
- Vasodilation

Histamine

Loss of intravascular fluid → Shock
Epinephrine → Life saving drug.
List of Allergens

- Grasses/Pollens
- Weeds
- Foods --> nuts, soy, fish, shell fish, eggs, wheat, dairy, etc.
- Epidermal --> dog, cat, mouse
- Insect bites
- House dust
- Molds
- Drugs
- Chemicals
Skin test for allergy
Inflammation mediated by IgE Abs and mast cells
Type II hypersensitivity

Role of complement and phagocytes in inflammation

Ab-dependent Cell-mediated cytotoxicity
Type II hypersensitivity induced by exogenous agents

Drug-induced reaction to red blood cells

RBC antigen

Absorbed drug or drug metabolite

Ab to drug

Complement

Lysis

Examples of drug-induced type II hypersensitivity

- **Red cells:**
  - Penicillin, chloropromazine, phenacetin

- **Granulocytes:**
  - Quinidine, amidopyridine

- **Platelets:**
  - Sulphonamides, thiazides
Abs against blood group Ags are naturally present and are IgM type.
Transfusion Reaction

Result:
Anaphylactic Shock due to Complement activation.
Hemolytic Disease of the New Born

RhD-ve mother

Anti-RhD Abs

RhD +ve fetus

RhD +ve fetus

Prophylaxis (RhoGAM)

Anti-RhD Abs

RhD-ve mother

RhD +ve fetus

RhD +ve fetus

Mid-term injection of RhoGAM and a second injection within a few days of delivery

Type III Hypersensitivity
Inflammation driven by immune complexes

Immune complexes not cleared

Activation of Complement

C3a & C5a

Mast cell degranulation

Inflammation

Systemic lupus erythematosus (Lupus)

Butterfly rash

Rheumatoid Arthritis

- Auto Abs against Fc portion of IgG often called rheumatoid factor.
- Abs against collagen.
- Immune complexes deposited in joints.
- Complement activation leads to inflammation---Type III hypersensitivity.

Type IV Hypersensitivity

- Inflammation driven by T cells: Th1 and Th17 cells that activate macrophages

- Delayed, takes 24-48 hours

Examples:
- Contact Hypersensitivity:
  - Nickel
  - Chromate
  - Poison Ivy
Mechanism of Type IV Hypersens.

Skin

poison Ivy

urushiol

serum protein

Inflammation

MØ

APC

Th1

Th1
Contact dermatitis to Nickel

Contact Dermatitis

Reaction to Poison Ivy
Tuberculin-type Hypersensitivity

- Tuberculosis Patient
- PPD (Ag derived from M. tuberculosis)
- Erythema & Induration
- Used as a diagnostic Test
- Not accurate.
Experimental Autoimmune Encephalomyelitis (EAE) (Multiple Sclerosis)

Inject Myelin Basic Protein (MBP) in an adjuvant

MBP is processed by dendritic cells and presented to Th cells

Th1/Th17 cells get activated

Th1/Th17 cells

Macrophage

Myelin Sheath

Axon

hind leg paralysis

Th cells cross blood-brain barrier
MS: Inflammation in Brain causing demyelination
Detecting immunomodulatory properties of chemicals

- **Tier I:** Broad effect on naïve immune system: Effect on BM, thymus, LNs: Histopathology and studying markers on immune cells

- **Tier II:** Effect following immunization: Study the immune response to antigens: Ab response by B cells, cytokine response by T cells, cytotoxicity by T cells, NK cells, hypersensitivity reactions, inflammation, etc
Summary
Inflammation is a double-edged sword

- Acute inflammation is critical for clearing infections but sometimes it can have serious consequences as seen in many hypersensitivity reactions.

- If the inflammation persists and become chronic, that can also have deleterious effects on the host as seen in autoimmune diseases.
Chronic Inflammation

- Chronic Inflammation is the underlying cause of all major clinical disorders.

- Clinical disorders with inflammatory component:
  1. Autoimmune diseases: > 80 disorders
  2. Cancer
  3. Cardiovascular disease
  4. Neurodegenerative diseases
  5. Obesity
  6. Diabetes

Obesity: Role of inflammation
M1 and M2 macrophages
What are the mechanisms that control inflammation?
Activation-induced cell death

Activation of T cells → Fas (CD95) → Fas ligand → Apoptosis (cell-death)
Fas-FasL interactions leading to apoptosis
Mutations in Fas or FasL leads to ALPS

- Mutations in Fas or Fas ligand genes in humans leads to development of **Autoimmune lymphoproliferative syndrome** (ALPS).
- Patients with ALPS have chronic, enlargement of lymph nodes and spleen.
- Increased risk of B-cell lymphomas, and autoimmune complications.
Regulatory T cells (Tregs) 
FOXP3+
Role of Foxp3 on Tregs in controlling inflammation

- Foxp3 is a transcription factor that is critical for the differentiation of Tregs.
- Genetic mutations in Foxp3 in humans leads to fatal autoimmune disorder known as Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome.
- Foxp3 mutations lead to massive lymphoproliferation, diabetes, dermatitis, thyroiditis and enteropathy.
Treatments for Inflammation in allergies: Blocking endproducts

- **Symptomatic**
  - Receptor blockers
    - antihistamine, antileukotriene
  - Bronchodilators
    - β-agonists (inhahants)
- **Prevent mast cell degranulation**
  - Ca influx inhibitor (chromolyn sodium)
  - Phosphodiesterase inhibitor (theophylline)
- **Immunotherapy:** Desensitization: Triggers IgG and Tregs
  - Anti-IgE(Fc) Ab
Treatment of Inflammatory and Autoimmune diseases

Immunosuppressive drugs:
- Corticosteroids
- Imuran (Azathioprine)
- Cytoxan (cyclophosphamide)
- Methotrexate
- Cyclosporin A

Biologics:
- Rituximab: Antibody against CD20 expressed on B cells. Used against B cell cancers and autoimmune diseases
Treatment of Inflammatory and Autoimmune diseases

- Adalimumab: (Humira): Antibody against TNF—reduces pain and joint destruction
- Tocilizumab (Actemra): Antibody against IL-6 receptor
- The above increase the risk of severe infections.
Impact of Imbalance

Effector

Th1/Th17
Pro-Inflammation

Botanicals

Regulatory

Tregs
Fas-FasL
Anti-Inflammation

Botanicals
Almost half of all pharmaceuticals are derived from natural products:

- morphine (opium)
- digitalis (foxgloves)
- quinine (Cinchona tree)
- vincristine (periwinkle)
- taxol (Pacific yew tree)
- aspirin (willow tree)

Image Source: Wikimedia Commons
Aryl hydrocarbon (AhR) signaling pathway: Do some dietary supplements act through AhR?

Role for dietary ligands of AhR in immune regulation?

Image Source: Wikimedia Commons
Screening natural compounds on FoxP3 induction

**Figure 7.** Effect of natural compounds on *TCDD*-induced CYP1A1 activity.

**Figure 9.** Effects of natural compounds on *FoxP3-Luc* expression (percentage change of response unit compared to the control.)
AhR dietary ligands

- Resveratrol: Plant polyphenol in red grapes
- Extends the lifespan in yeast, worms, flies, mice
- Plant-derived indoles: I3C (Indole-3-carbinol) and DIM (3,3'-diindolylmethane).
Experimental Autoimmune Encephalomyelitis (EAE): A model for MS

MOG35-55 peptide (s.c.): Day 0

PTX (i.p.): Day 0 and 2

Treatment: Day 2
Vehicle or Resveratrol (100-250 mg/kg) by Oral gavage

5 = complete paralysis
4
3
2
1
0 = no symptoms

Image Source: Wikimedia Commons
Resveratrol treatment significantly suppresses development and progression of EAE.
Inflammatory Bowel Disease

- The two most common forms: Ulcerative colitis and Crohn’s disease.
- It is estimated that ~1.4 million people in the US suffer from IBD.
- There are various animal models of colitis.

Image Source: Wikimedia Commons
Resveratrol attenuates dextran sodium sulfate (DSS)-induced colitis

J Pharmacol Exp Ther. 2010 Mar;332(3):829-39
Treatment with I3C and DIM attenuates inflammation in the CNS of EAE mice.
RES and indoles trigger Tregs

Tregs in SJL/J mice with EAE: D15

VEH

RES100/D1

RES200/D1

RES100/D8

CD4

FoxP3
Epigenetic Regulation of gene expression

Resveratrol-mediated regulation of miRs
miRNA Profiles induced by various AhR ligands
AhR ligands sharing miRs

# 1

# 2

# 3

# 4

# 5

FoxP3  miR-190
miR-217
miR-490

IL-17  miR-203
miR-320
miR-494

miR-190
miR-217
miR-490
Some Botanicals act as AhR ligands and reciprocally regulate Tregs & Th17 cells through epigenetic mechanisms.
THC and Cannabidiol (CBD)

Psychoactive

Non-psychoactive
Cannabinoid Receptors

- CB1 receptor
- CB2 receptor
- Endocannabinoids
- Psychotropic Effects

Δ⁹ THC

Immune System

- Tonsils and adenoids
- Lymph nodes
- Lymph nodes
- Thymus
- Spleen
- Peyer’s patches
- Appendix
- Bone marrow

???
Cannabidiol (CBD) treatment attenuates hepatitis

- a) Vehicle;
- b) ConA+Veh;
- c) ConA+CBD (50mg/kg);
- d) CBD alone

* $p<0.05$, ** $p<0.01$
THC-induced hypermethylation of IFN-γ and hypomethylation of IL-4 and IL-10 promoters

Genome wide DNA Methylation

DNA methylation in the promoter region of Arg-1 by MeDIP-Seq
Histone methylation and gene expression in THC treated activated T lymphocytes

J. Biol. Chem. 289:18707, 2014
J. Biol. Chem. 201:15460, 2016
Histone methylation pattern affecting gene expression

- H3K27me3
- H3K4me3
- H3K36me3
- H3K9me3

Vehicle

THC

Ifn-γ → 10 kb

Tbx21 → 10 kb
Gut microbiota play an important role in regulating inflammation.
Effect of botanicals on microbiota

- **TNBS**
- **TNBS+I3C**
- **Control**

![Graph showing the effect of botanicals on microbiota](image_url)
Summary

Acute inflammation

Dysregulation

Toxicity to Tissues/Organs

Dysregulation

Chronic Inflammation

Infection

Clinical disorders:

- Autoimmune
- Cardiovascular
- Neurological
- Obesity
- Cancer

Botanicals:

AhR, CB receptors

Epigenetic Regulation/Microbiota

FoxP3+ Tregs Fas-FasL
