Advances in Immunopharmacology

New Mexico Pharmacists Association Annual Convention
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Objectives

1. Know the difference between adaptive and innate immunity.
2. Know some examples of the major types of immune cells and their products as drug targets.
3. Explain the concept/purpose of immunopharmacology and examples of its therapeutic niches.
4. Provide specific examples of the 'tools' of immunopharmacology, including the diseases targeted, their mechanism of action, and therapeutic objective(s).
5. Give examples of novel advances in immunopharmacology as it pertains to autoimmune disease treatment in pre-clinical models.

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What is Immunopharmacology?

- "Immunopharmacology addresses the selective regulation of immune responses and aims to uncover and exploit beneficial therapeutic options for typical and non-typical immune system-driven unmet clinical needs."
  BJP, 2015, 172:4217-4227
- In other words, immunopharmacology targets the immune system to improve disease outcomes or to slow progression of chronic conditions.

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What do you know about immunology?

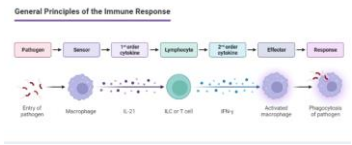

Image courtesy of biorender.com

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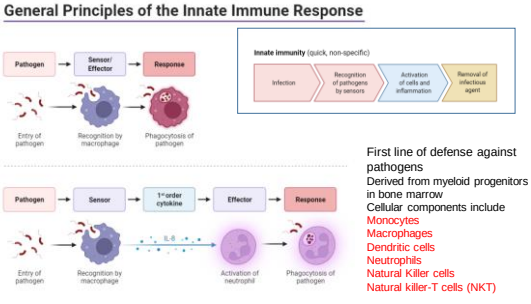

First line of defense against pathogens
Derived from myeloid progenitors in bone marrow
Cellular components include
Monocytes
Macrophages
Dendritic cells
Neutrophils
Natural Killer cells
Natural killer-T cells (NKT)

Images courtesy of biorender.com

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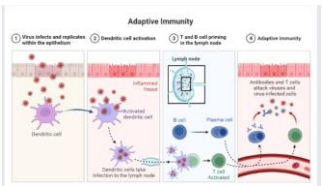
General principles of adaptive immunity

- T and B lymphocytes are the key players of the adaptive immune system.
  - They act in an "antigen-specific" manner.
- Innate immune cells (Dendritic cells or DCs) survey the tissue for invaders.
- DCs detect pathogens, migrate to draining lymph nodes and present antigen to T and B cells to become effector cells.
- T helper cells (CD4) and antibody-secreting B cells (plasma cells) are activated, generating inflammation.



Images courtesy of biorender.com

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Summary

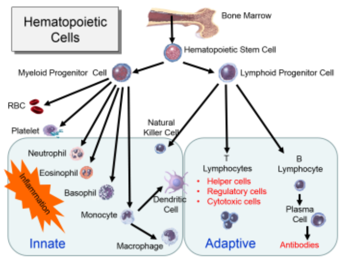

Image obtained from http://sphweb.bumc.bu.edu/otlt/MPH-Modules/PH/PH709_Defenses/PH709_Defenses3.html

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Important "tools" of immunopharmacology

- ❑ Cytokines/Growth factors
- ❑ Small molecules
- ❑ Vaccines
  - ❑ Preventive
  - ❑ Therapeutic
- ❑ Monoclonal antibodies (mAbs)

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Cellular mediators of immunity

- Cytokines
- Chemokines

Cytokines bind specific receptors on target cell membranes but may be more non-specific in downstream signaling outcomes

Cytokines are immune-modulating agents which are made up from proteins	Chemokines are a superfamily of cytokines which mediate chemotaxis
Involved in both cellular and antibody-mediated immunity in the body	Involved in directing the cells in the immune system to the site of infection
Chemokines, ILs, INFs, CSFs, TNFs and TGFs are the types of cytokines in the body	CC chemokines, C chemokines, CXC chemokines & CX3C chemokines are the structural types of chemokines

Visit www.pediaa.com

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Therapeutic biologics are generally proteins

- Complex molecules (generally proteins such as cytokines, growth factors or mAbs) manufactured using living microorganisms, plant or animal cells
- Many produced using recombinant DNA technology
- Sometimes referred to as biopharmaceuticals or biological drugs

Top 10 biologics in 2021	Global sales in $B
1) Humira®	18.4
2) Rituxan®	9.2
3) Enbrel®	7.9
4) Herceptin®	7.4
5) Avastin®	7.1
6) Remicade®	7.1
7) Lantus®	5.7
8) Neulasta®	4.7
9) Avonex®	2.1
10) Lucentis®	1.5

*Drug names will be referred to either/or generic/brand https://www.verywellhealth.com/top-biologic-drugs-2663233

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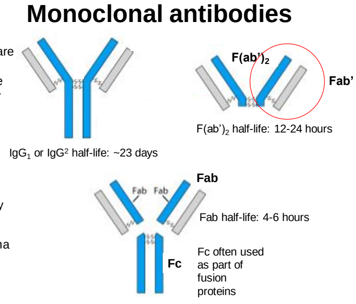
Monoclonal antibodies

Polyclonal Abs (pAbs) are produced by multiple B cells and target multiple epitopes on a particular antigen/protein.

mAbs possess many advantages over polyclonal antibodies:
- predictable dose response rates
- uniformity of antibody batches
- relative ease of production (hybridoma or rDNA technology)



Goldsby, *Immunology*, Sixth Edition, 2007 Fig 4-7 and adapted from Dr. Scott Burchiel

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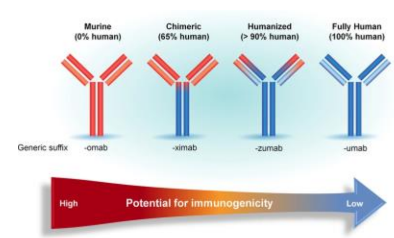
Humanization of therapeutic antibodies has reduced their immunogenicity



American Heart Association

Ian N. Foltz et al. Circulation. 2013;127:2222-2230

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Why use mAbs therapeutically?

- Use to bind soluble inflammatory cytokines ('decoy receptors')
- Use to block cell surface receptors (such as receptors for inflammatory cytokines)
- Use to target tumor associated antigens (TAMs) on surface of cancer cells
- Mediate cell killing/clearance (example cancer cells)
- PROs: SPECIFICITY
- CONs: COST

Images courtesy of biorender.com

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Niches where immunopharmacology is used

Goal: Enhance the ability of host immune cells to kill cancer cells while limiting negative side effects of chemotherapy.

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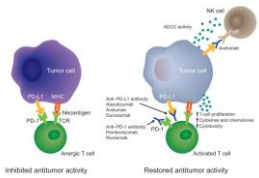
Immune checkpoint inhibitor mAbs

Opdivo®
(Nivolumab)
Breakthrough therapy approval
20-Dec-17

Monoclonal antibodies:
- ❑ Programmed cell death-1 (PD-1) receptor signaling regulates T-cell activation and proliferation.
- ❑Anti-PD-L1, anti-PD-1 antibody
- ❑ Nivolumab binds the PD-1 receptor to prevent the ligands PD-L1 and PD-L2 from binding.
- ❑ Releases PD-1 pathway-mediated inhibition of the immune response, including the antitumor immune response.
- ❑Avelumab-dual responses; PD1/PDL1 and NK cell cytotoxicity.

Pharmacodynamics/Kinetics:
- Half-life elimination: ~25 days

Combining nivolumab (anti-PD-1) with ipilimumab (anti-CTLA-4) results in enhanced T-cell function that is greater than that of either antibody alone, resulting in improved anti-tumor responses in **metastatic melanoma**.



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Cancer treatment with autologous cellular immunotherapy-cancer vaccine

PROVENGE®

Sipuleucel-T (PROVENGE)

- 1st FDA approved cancer vaccine (2010)
- For treatment of metastatic prostate cancer
- Extends median survival by 4.1 months
- Does NOT result in tumor shrinkage
- EXPENSIVE

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Sipuleucel-T (PROVENGE®)

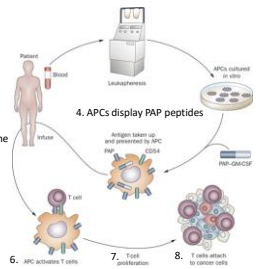
1. Three days prior to each dose of PROVENGE, Blood is collected from the patient over 2-4 hours

2. Antigen presenting cells (dendritic cells) are separated out and sent to a manufacturing facility

3. APCs ingest the PAP protein fused with GM-CSF and digest it into small fragments (peptides)

4. APCs display PAP peptides

5. PROVENGE is infused into the patient



PAP=prostatic acid phosphatase Expressed on 95% of prostate cancer Cells.

GM-CSF=immune cell activator

Di Lorenzo, G. *et al.* (2011) Immunotherapy for the treatment of prostate cancer *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2011.72

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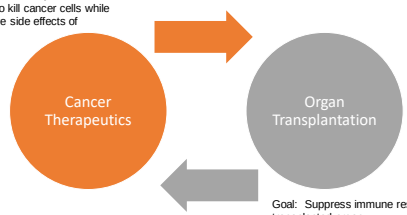
Niches where immunopharmacology is used

Goal: Enhance the ability of host immune cells to kill cancer cells while limiting negative side effects of chemotherapy.



Goal: Suppress immune response to transplanted organ

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Review of Graft Rejection

- Major Histocompatibility Antigens (HLA in humans)
  - Class I present on all nucleated cells - a person's unique ID
  - Class I (HLA-A,B,C) recognized by $CD8^+$ T cells
  - Class II (on APCs) (HLA-DP, DQ, DR) recognized by $CD4^+$ T cells that produce cytokines and inflammatory mediators necessary for T and B cell activation and antibody production
- Grafts must be matched for both Class I and II antigens
  - Degree of foreignness determines the rate of rejection
  - Previous grafts or sensitization may decrease success
- Specific immune rejection
  - **T cell mediated (T_H + T_{CTL})**
  - **B Cell mediated (antibodies) – hyperacute rejection if prexisiting antibodies**
- Continual immune suppression required to prevent rejections

Adapted from Dr. Scott Burchiel

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Three General Principals of Immunosuppressive treatment

1. Immune reactivity and likelihood of graft rejection are highest initially and decrease over time.
2. Use low doses of several drugs with non-overlapping toxicities.
3. Avoid over-immunosuppression which increases susceptibility to infection and cancer.

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Review of immunosuppressants Used in Organ Transplant

Glucocorticoids
Prednisone/ Deltasone®, PrediSONE®, Intensol®, Rayos®

T-cell depleting Abs
Anti-thymocyte globulin (Thymoglobin®- rabbit or Atgam®- equine)
Alemtuzumab/ Campath®, Lemtrada®

T-cell immunosuppression
Basiliximab/ Simulect®

Calcineurin inhibitors
Cyclosporine/ Gengraf®, Neoral®, SandIMMUNE®
Tacrolimus/ Astagraf®, Envarsus®, Hecoriaz®; Prograf®

mTOR inhibitors
Everolimus/ Afinitorz®, Afinitor®, Disperz®, Zortress®
Sirolimus/ Rapamune®

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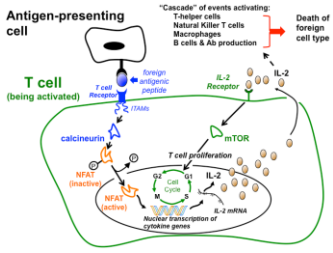
T-cells drive graft rejection



(Adapted from Wood & Goto, 2012).

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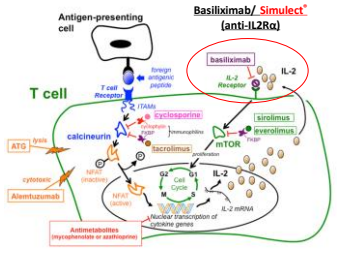
Therapeutic targets for T-cell immunosuppresion to prevent transplant rejection: blocking IL-2 signaling



MOA
IL-2 and its receptor are needed for T cell proliferation and differentiation to make cytokines

Basiliximab blocks the alpha-chain of the interleukin-2 (IL-2) receptor complex expressed on activated T lymphocytes

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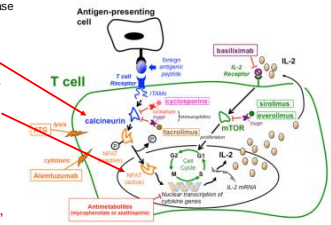
Therapeutic targets for T-cell immunosuppresion to prevent transplant rejection: blocking IL-2 production

Calcineurin Calcineurin is a protein phosphatase that activates NFAT (Nuclear Factor of Activated T-cells).

Activated NFAT is a transcription factor for **production of IL-2.**

IL-2 stimulates T-cell growth and differentiation.



Calcineurin inhibitors (CNI)
Cyclosporine/ Gengraf®, Neoral®, SandIMMUNE®

MOA: binds cyclophylin thus inhibiting calcineurin

http://tmedweb.tulane.edu/pharmwiki/doku.php/organ_transplantation

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Calcineurin inhibitors adverse effects

- Not easy to use because of adverse effects
- Increased infections
- Cyclosporin and Tacrolimus
  - Kidney damage
  - increased blood pressure
  - interactions with CPY3A4

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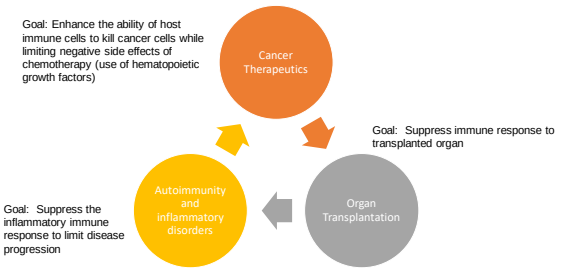
Niches where immunopharmacology is used



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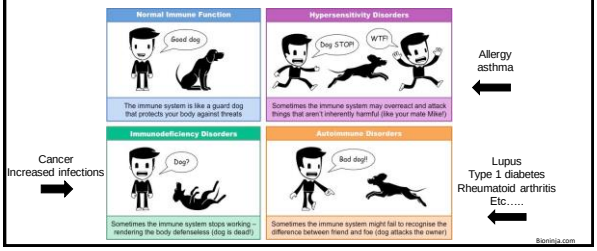
The immune system is designed to maintain normal health. Abnormal immune responses can lead to Diseases or disorders



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Disrupted immune homeostasis can lead to abnormal immune responses

Example: Autoimmune Diseases

- Impact ~5-9% of the US population
- Disproportionally affect females
- ~80-100 diseases
- Immune attack against self antigens
- Classified based on target organ pathology
- Share common underlying immunological mechanisms
  - CD4 T cell-driven
  - B cells produce autoantibodies
  - Both genetic and environmental risk factors contribute to disease

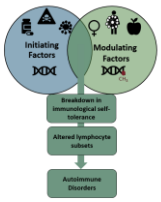

Figure from Blossom, SJ, Chapter 9. Autoimmune Disease and epigenetics in Medical Epigenetics, 2nd Edition, Vol. 29, T. Tollefsbol editor, 2021

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How do we target the immune system to treat autoimmune disease?

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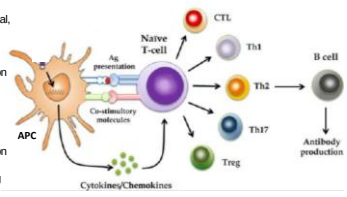
Major targets for immunosuppression

1) **T-cells**
   Activation (1st signal, 2nd signal)
   Differentiation
   Proliferation
   Cytokine production
2) **B-cells**
   Activation
   Proliferation
   Ab and Cytokine production
3) **Cytokine signaling**
4) **Immune cell trafficking**



Bruno Miguel Neves, Marta Celeste Lopes and Maria Teresa Cruz (2012). Pathogen Strategies to Evade Innate Immune Response: A Signaling Point of View, Protein Kinases, Dr. Gabriela Da Silva Xavier (Ed.), InTech, DOI: 10.5772/37771. Available from: http://www.intechopen.com/books/protein-kinases/pathogen-strategies-to-evade-innate-immune-response-a-signaling-point-of-view

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Corticosteroids have traditionally been the first line of defense

- Minimize inappropriate immune responses (autoimmune disease/asthma/allergy)
- These agents also have the potential to cause disease, increase risk for cancers and infections.

Example: Glucocorticoids are a class of corticosteroids but terms are often used interchangeably

Corticosteroid Drug	Treatment for	Molecular formula
Betamethasone	Dermatitis	$C_{22}H_{29}FO_5$
Budesonide	Asthma, noninfectious rhinitis, nasal polyposis	$C_{25}H_{34}O_6$
Cortisone	IgE-mediated allergies	$C_{21}H_{28}O_5$
Dexamethasone	Inflammation, rheumatoid arthritis	$C_{22}H_{29}FO_5$
Hydrocortisone	Dermatitis	$C_{21}H_{30}O_5$
Methylprednisolone	Arthritis, Bronchial inflammation	$C_{22}H_{32}O_5$
Prednisolone	Asthma, rheumatoid arthritis, ulcerative colitis, Crohn's disease	$C_{21}H_{28}O_5$
Prednisone	Systemic lupus erythematosus, Bell's palsy, asthma, dermatitis	$C_{21}H_{26}O_5$
Triamcinolone	Eczema, diabetic retinopathy	$C_{21}H_{27}FO_6$

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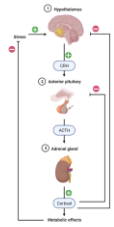
Glucocorticoids

- Mimic the action of cortisol "stress" hormone
- The powerful suppressive effect of glucocorticoids on the immune system is frequently exploited in the treatment of many immune-mediated inflammatory disorders such as asthma, and ulcerative colitis

HPA Axis



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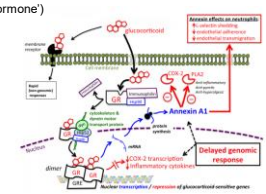
Glucocorticoid mechanism of action

- **Cortisol** is the natural glucocorticoid ('stress hormone')
- 'Fight or flight' response
- Increases access to energy substrates
- Suppresses immune system
- Binds glucocorticoid receptors (GR) in cytoplasm
- GRs move to nucleus to suppress/activate genes controlled by glucocorticoid response elements (GREs)
- Mostly suppressive (inflammatory cytokines) such as TNF-alpha



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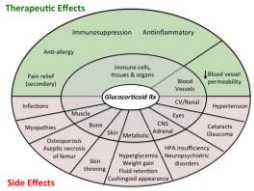
Glucocorticoids

- Completely obliterates contact hypersensitivity mediated by T cells.
- Severe allergy
- Rheumatoid arthritis



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Glucocorticoid side effects

Side Effect	Mechanism
Hyperglycemia/Diabetes	Increased hepatic glucose output & decreased peripheral glucose utilization (insulin-resistant diabetes mellitus)
Central Obesity, Moon Face, Buffalo Hump	Increases effect of lipolytic signals, leading to elevated FFA to fuel gluconeogenesis. Redistribution of fat from extremities to the trunk, back of neck & supraclavicular fossae
Muscle Weakness & Wasting	Breakdown of protein & diversion of amino acids to glucose production
Weight Gain	Increased appetite (CNS effect) and increased need for insulin over time results in weight gain
Osteoporosis	Decreased reabsorption of calcium by the kidney leading to secondary hyperparathyroidism; retardation of bone growth by direct action & decreasing GH. Inhibition of bone deposition.
Avascular Necrosis of Femur Head	Animal studies indicate GC-induced increased levels of serum lipids result in both increased formation of microemboli in the arteries supplying bone, and fat-related blockage of venous flow from bone. These mechanisms result in **increased ischemia in bone**, which **most commonly affects the femoral head**, but can occur in any skeletal site such as the knee, shoulder, ankle or hand (Wang et al, 1977; Jones, 1985; Nishimura et al, 1997; Chan & Mok, 2012; Jones & Hunt, 2014).
Thin Skin that Bruises Easily & Poor Wound Healing	Antiproliferative GC effect on fibroblasts & keratinocytes, resulting in dermal atrophy
Hirsutism & Acne	due to ACTH-mediated increase of adrenal androgens
Hyperpigmentation	direct effect of ACTH on melanocortin 1 receptors
Increased Infections	Immunosuppression related to thymic atrophy, decreased production (number) of neutrophils & monocytes, decreased production of cytokines
Hypertension	Increased cardiac contractility, increased vascular reactivity to vasoconstrictors (catecholamines, Ang II)
Hypomania, Depression, Psychosis	Normal cortisol levels (eucorticolemia) maintains emotional balance
Cataract formation & Glaucoma	Increased intraocular pressure & hypoparathyroidism

Side Effects highlighted in red are irreversible (typically requiring surgical replacement). Abbreviations: GC (glucocorticoid).

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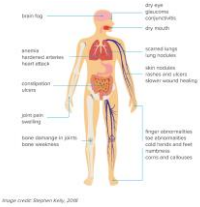
Rheumatoid Arthritis Example

- Systemic autoimmune disease
- Involves innate and adaptive immunity
  - Macrophages, T cells, B cells, autoantibodies
- Immunological attack of joint tissue resulting in inflammation
- Joint bends resulting in deformity causing nerve pain
- Without treatment-leads to fused joints
- Risk factors: family history, obesity, smoking, being female.



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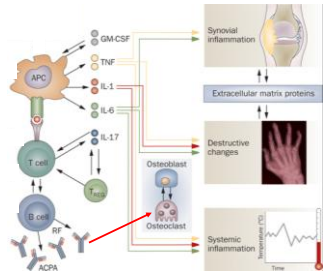
Pathophysiology of Rheumatoid arthritis



van Vollenhoven, R. F. (2009) Treatment of rheumatoid arthritis: state of the art 2009, *Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2009.182

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Treatment options?

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Disease-Modifying Anti-Rheumatic Drugs (DMARDs)

Non-biologics

Antiinflammatory agents
- Methotrexate/ Rheumatrex®, Trexall®
- Hydroxychloroquine/ Plaquenil®
- Leflunomide/ Arava®
- SulfaSALAzine

JAK inhibitor
Tofacitinib/ Xeljanz®

Biologics

Anti-TNF
- Adalimumab/ Humira®
- Certolizumab/ Cimzia®
- Etanercept/ Enbrel®
- Golimumab/ Simponi®
- InFLIXimab/ Remicade® Inflectra®

IL-1 Receptor Antagonist
Anakinra/ Kineret®

IL-6 Receptor Antagonist
Tocilizumab/ Actemra®

Anti-T cell
Abatacept/ Orencia®

Anti-B cell Ab
RiTUXimab/ Rituxan®

1. Methotrexate – 1st line
2. Methotrexate used in combination with other DMARDs
3. Etanercept & Adalimumab are the most commonly used biologics (both anti-TNFα)

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Non-biologic DMARDs are broadly anti-inflammatory

- Target immune system to slow or reverse progression of arthritis (prevent joint injury)
- Non-biologic DMARDS: non-specific immunosuppressives, anti-inflammatory and cytotoxic effects
- SLOW onset of action (weeks to months)
- Methotrexate is a 1st line DMARD

Some Examples
- Mycophenolate mofetil
  - Synthetic derivative of mycophenolic acid isolated from mold of penicillin; used in solid organ transplants for refractory rejection.
  - Also used in some immune mediated inflammatory disorders (lupus, RA, inflammatory bowel)
- Thalidomide
  - Teratogen given to pregnant women decades ago. Used now as a treatment for multiple myeloma (plasma cell cancer)
  - Angiogenesis inhibitor and has anti-inflammatory and immunomodulatory effects.
- Cytotoxic agents
  - Azathioprine
  - cylophosphamide

The older drugs can be effective and are less expensive!

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Disease-Modifying AntiRheumatic Drugs (DMARDs)

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Antiinflammatory agents
- Methotrexate/ Rheumatrex®, Trexall®
- Hydroxychloroquine/ Plaquenil®
- Leflunomide/ Arava®
- SulfaSALAzine

JAK inhibitor
Tofacitinib/ Xeljanz®

Biologics

Anti-TNF
- Adalimumab/ Humira®
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- Etanercept/ Enbrel®
- Golimumab/ Simponi®
- InFLIXimab/ Remicade® Inflectra®

IL-1 Receptor Antagonist
Anakinra/ Kineret®

IL-6 Receptor Antagonist
Tocilizumab/ Actemra®

Anti-T cell
Abatacept/ Orencia®

Anti-B cell Ab
RiTUXimab/ Rituxan®

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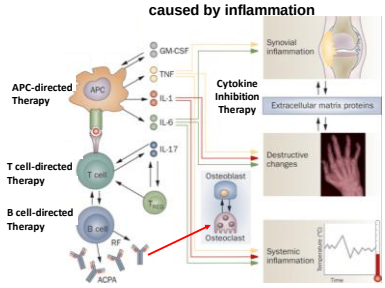
RA therapies are aimed at limiting disease progress caused by inflammation



van Vollenhoven, R. F. (2009) Treatment of rheumatoid arthritis: state of the art 2009, *Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2009.182

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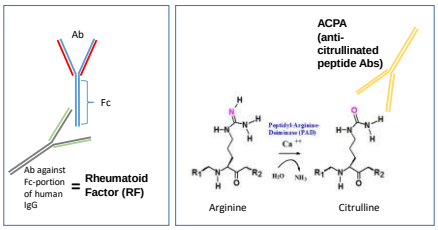
B-cells are targeted in RA therapy in part because of their production of autoantibodies (RF & ACPA)



Ag-Ab crosslinking leads to formation of immune complexes (aggregates). Binding to FcRs on immune cells induces production of inflammatory cytokines (TNFα, IL-1, IL-6).

http://www.bioxyn.com/new/citrullinated-peptide-synthesis.aspx

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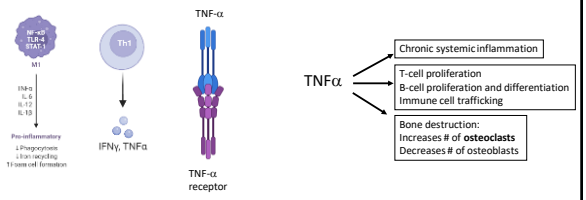
Targeting TNFα for disrupting the pathophysiology of rheumatoid arthritis



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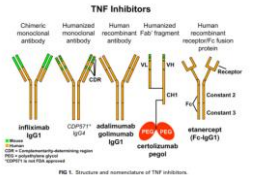
TNF inhibitors

Anti-TNFα therapeutics

Etanercept/ Enbrel®
(TNF receptor-IgG Fc fusion protein)

mAbs
InFLIXimab/ Remicade®, Inflectra®
Adalimumab/ Humira®
Golimumab/ Simponi®

Certolizumab pegol/ Cimzia®
(pegylated anti-TNF Fab')



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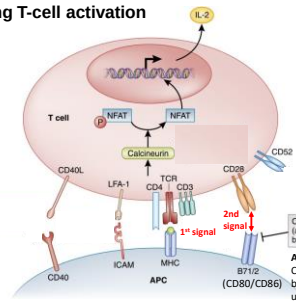
Inhibiting T-cell activation



Wiseman, *Clin J Am Soc Nephrol*. 2016 Feb 5; 11(2): 332–343.

1st signal:
TCR- Ag:MHC interaction

2nd signal
(co-stimulatory):
CD28- B7-1/2 interaction
(CD28 = '**on**' signal)

CTLA-4 (aka CD152)
expression on T-cells,
interacts with B7-1/2 to
downregulate immune
response
(CTLA-4 = '**off**' signal)

Abatacept
CTLA-4:IgG Fc fusion protein
blocks 2nd signal for T-cell activation. Sold under brand name Orencia; treats RA and other ADs

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Future directions in immunopharmacology for autoimmune disease

- Exploiting the microbiome
- Engineering T cells
  - Adapting CAR (chimeric antigen receptor) T therapy to treat autoimmunity
- Regulatory T cell therapies
- microRNAs in lupus
  - microRNA (miR-21) plays important role in regulating T cell activation.
  - Upregulated in lupus patients
  - Antagomir-21 inhibits miR-21 and reduced expansion of pathogenic T cells and lupus-like lesions in mice. (Gao et al., 2022, International Immunopharmacology 106 (2022) 108578.

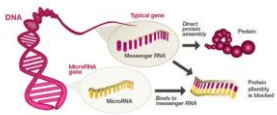

Image courtesy of MicroRNA biogenesis, functions and role in disease: microbiology tutorials

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Thank you!

Questions?

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