IMMUNOMODULATORS

IMMUNODODULATION. By immunomodulation we mean the use of drugs, alone or in combination with other maneuvers, to change the function of all, or part, of the immune system. In general, the drugs and other agents we have available are not the ones we'd like to have; they are insufficiently specific, and thus side-effects are always a significant problem. We have to affect most good responses to suppress a few rogue clones, for example. In the last two decades, and especially the last 5 years, newer ideas, better drugs, and interesting biological response modifiers have become available, and it's exciting to consider how we manipulate immunity.

CATEGORIES OF DRUGS. Many of the drugs that are used to alter immune responses are also used in other conditions; this is most true of the older drugs. Here we'll consider drugs that are true immunomodulators, and touch briefly on other drugs that don't really affect the immune system but are commonly used in the treatment of immune diseases. These are some of the main categories:

1. **Non-steroidal anti-inflammatory drugs (NSAIDs).**
2. **Disease-modifying antirheumatic drugs (DMARDs).**
3. **Glucocorticoids**.
4. **Biological response modifiers.**
5. **Tumor-specific monoclonal antibodies.**
6. **Other antibodies.**
7. **Miscellaneous drugs**.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS are a big topic, and better covered in a pharmacology course. They are mostly cyclooxygenase inhibitors, and the older ones inhibit both COX-1 and COX-2. Cyclooxygenases are involved in making prostaglandins. The effect on COX-1, which is a constitutively expressed enzyme, is not desirable, and leads to the most severe side effect of drugs like aspirin: GI bleeding and ulceration. So rational drug design methods were used for the first time to produce the breakthrough selective COX-2 inhibitors, which are 40-4000 fold more selective for COX-2, a homolog of COX-1 that is induced in inflammation. These drugs were wonderful for people who could not tolerate the older NSAIDs, but were eventually shown to increase cardiovascular accidents, and most have been withdrawn. Note that in chronic inflammatory conditions like rheumatoid arthritis, NSAIDs are often used, to relieve pain or inflammation, but they do not modify the course of the disease, and side effects are such that only brief, low-dose treatment is recommended.

DISEASE-MODIFYING ANTI-RHEUMATIC DRUGS. Up to quite recently, most patients with rheumatoid arthritis (RA) got NSAIDs, but relatively few also got DMARDs. This was because of a general belief that DMARDs are toxic and should be reserved for severe or late-stage disease. But this reasoning was flawed: if they really can prevent the *progression* of symptoms and joint erosions, they should be used early. Studies have shown that they are less toxic than previously thought, and in combinations often have even less toxicity, hardly more so than NSAIDs. Now almost all RA patients get DMARDs. Treatment of other autoimmune diseases is similar to RA, with emphasis on the most distressing symptoms, and then addition of specialized drugs for specific indications.

DMARDs	
Antimetabolites	**methotrexate**, **leflunomide,** azathioprine, 6-mercaptopurine
Salicylate	**sulphasalazine**
Gold -- parenteral	myocrisin
Gold -- oral	auranofin
Antimalarials	**hydroxychloroquine**, chloroquine formulations
Antibiotics	**minocycline**
Alkylating agent	cyclophosphamide
Cyclic polypeptides	cyclosporine, tacrolimus
JAK kinase inhibitor	**tofacitinib** (Xeljanz™)

DMARDS OF PARTICULAR INTEREST TO IMMUNOLOGY:

Cyclosporine and **tacrolimus** are sometimes used in severe autoimmune disease, in conjunction with glucocorticoids or methotrexate. Their use is decreasing in autoimmunity as newer BRMs become available; they are frequently used for immunosuppression in organ transplantation.

Mechanism of action: In a Th cell, TCR engagement causes Ca^{2+} mobilization which activates calcineurin, a protein phosphatase. Calcineurin in turn activates NFAT, a transcription factor, which activates the genes for certain cytokines and their receptors, and the cell moves into cycle. Within the cell, cyclosporine binds to cyclophilin (and tacrolimus binds to FKBP-12). Thus bound, they now can bind to calcineurin, blocking it, so that the Th cannot become activated. Helper T cells are the main target. IL-2, IL-4, and IFNγ production are all suppressed. Cyclosporine and tacrolimus have no functional effects on phagocytic cells, and do not cause bone marrow suppression. Most toxicity is seen in the kidney. This is awkward when you're using these drugs in kidney transplantation.

Tofacitinib, the first new DMARD in 10 years, blocks the JAK/STAT pathways involved in the activation many immune cells. Among many others, the secretion of IL-2, IL-4, IL-6, IL-12, and IFNγ is blocked. Approved and very effective ± methotrexate in RA, but on hold for psoriasis and other conditions due to side effects (mostly, infections.) Effective in 3 recent trials for ulcerative colitis. Baricitinib**,** approved in Europe, is breathing down Xeljanz's neck in late 2017.

GLUCOCORTICOIDS (GCs). GCs are the anti-inflammatory drugs par excellence, and are used in most (auto)immune diseases, including asthma, rheumatoid arthritis, SLE, inflammatory bowel disease, atopic dermatitis, and psoriasis. They are effective in both Type II and Type IV conditions. They are more anti-inflammatory than immunosuppressive.

EFFECTS OF GLUCOCORTICOIDS ON DIFFERENT CELLS	
Monocytes and macrophages	GCs induce a protein, lipocortin, that inhibits phospholipase A2, and hence the inflammatory mediators derived from arachidonic acid (prostaglandins and leukotrienes). Production and release of IL-1, IL-6, and TNFα are also inhibited. Macrophage motility and response to IFNγ are decreased so fewer cells arrive at inflammatory sites.
Endothelial cells	Adhesion molecules are downregulated, reducing inflammatory cell adherence and extravasation.
Mast cells	IgE-dependent degranulation is inhibited by GCs.
Eosinophils	Eosinophils are induced to undergo apoptosis by GCs. This partially explains their great usefulness in asthma.
Neutrophils	Conversely, PMN survival is *enhanced* by GCs, so they are not very effective in diseases dominated by PMNs, for example ARDS (acute respiratory distress syndrome).

BIOLOGICAL RESPONSE MODIFIERS. These are a loose class of substances targeted mostly at cytokines or their receptors. They can be antagonists or agonists. They can be genetically-engineered **receptor antagonists**. And they can be cloned, mass-produced **normal gene products**. Included in this category are **antibodies** to various components of the immune or inflammatory system (which stimulate, inhibit, or opsonize, depending on the designer's intentions). Some of the leading-edge products are extraordinarily clever. Most are too expensive in the long run for any imaginable medical care system.[1]

►**ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY**, or ADCC. Here's an example of the phenomenon, then we'll explain it: If antibody against a surface protein on tumor cells is added to them in culture, the antibody binds but has no observable effect. If normal blood leukocytes are now added, the tumor cells are killed by induced apoptosis (without the addition of the antibody, nothing happens.) Anyone's leukocytes can be used; the phenomenon is not MHC-restricted the way CTL-mediated killing is.

The effectors are the ►large granular lymphocytes (LGL) which make up 5-10% of blood lymphocytic cells. They are dual-function: they have NK receptors which recognize molecules on the surface of 'stressed' cells, which they then kill; therefore, they are part of the innate immune system. But they also have receptors for the Fc end of IgG (FcγR), and so they have a second, antibody-dependent, way to interact with target cells. (*See* the diagram in the Tumor Immunology notes.) The mechanism of ADCC is this: IgG antibody binds to the target cell, then the NK cell binds to the Fc end of the antibody. Just like a killer T cell, the NK cell now delivers lethal signals to the target, which dies by apoptosis. We know that many of the new therapeutic monoclonal antibodies (used to modulate the immune response, or treat cancer) work by triggering ADCC. The normal role of ADCC has been hard to define; some HIV elite controllers have particularly strong ADCC that destroys their HIV-infected cells soon after infection.

> *Slightly confusing point:* When an NK cell is doing its ADCC thing, it is sometimes called a K cell, probably because K cells were named before it was realized they and NK cells are the same LGLs. Note, NK cells have several weird relatives, like iIELs and NKT cells.

ANTIBODIES. Going to monoclonal antibodies was a good idea; they can be manufactured under ideal conditions, and any quantity desired can be made, with complete uniformity of the product. The main problem, of course, is cost. Genentech has a 100,000 L GMP mAb production facility which cost $400 million. Production costs are about $1,000/g (most small molecules cost drug companies about $5/g to produce). There are more than 400 mAbs in tests around the world; there is no way they would all be able to be commercialized at this cost. A very exciting possibility is mAb production in transgenic animals, secreted into milk; and transgenic plants that make mAbs (a big effort by Monsanto). **ZMapp**, the monoclonal antibody cocktail used in the Ebola epidemic, is made in transgenic tobacco plants.

The lists of BRMs and mAbs below are primarily for your interest and reference. Not all licensed drugs are listed (some are just copies of pioneering drugs) but the most important ones are mentioned. While you are reading, just get a feel for the wide range of pathways that have been targeted by researchers and pharmaceutical manufacturers. We'll discuss a few of the most interesting and surprising examples.

[1] For a sobering view on the unsustainability of ultra-priced cancer therapy, see: Sullivan, Pramesh and Booth (2017) Cancer patients need better care, not just more technology, Nature 549:325.

SOME IMMUNOMODULATOR MONOCLONALS IN CLINICAL USE		
infliximab $[2]	[Remicade® J&J]	Chimeric mAb against **TNFα.** Approvals: Crohn's, ulcerative colitis, RA (with methotrexate), ankylosing spondylitis, psoriasis and psoriatic arthritis.
adalimumab $	[Humira® Abbott]	Fully human mAb to **TNFα**. It has been shown to slow the progress of RA, and thus is a BRM and a DMARD, technically. Approvals: many inflammatory conditions.
certolizumab pegol	[Cimzia®USB]	Humanized PEGylated Fab fragment of mAb against **TNFα**. Polyethylene glycol does not cross placenta so cautiously approved in pregnancy. Approved for use in refractory Crohn disease and in RA.
abciximab	[ReoPro® Lilly]	Chimeric mAb to the **glycoprotein IIb/IIIa receptor**, which is key for platelet aggregation. Used with stenting, in coronary artery disease & stroke.
basiliximab	[Simulect® Novartis]	Chimeric mAb to the **IL-2 receptor α chain**. For prophylaxis of acute renal transplants rejection. Immunosuppressive regimen includes cyclosporine (or tacrolimus), and glucocorticoids.
efalizumab	[Raptiva®Genentech]	Humanized mAb against CD11a (**LFA-1**), an accessory binding molecule on T cells involved in activation, adherence, and migration. It was phase-III tested in psoriasis (about 30% effective) and was released in 2004.
ustekinumab	[Stelara®Jansssen]	Human mAb against **IL-12 and IL-23**, which share a p40 subunit. For psoriasis.
tildrakizumab	In Phase 3 trials	Humanized **anti-IL-23p19** mAb, so it binds only IL-23, not IL-12. Highly effective against psoriasis. IL-23 is necessary for the development of Th17 cells.
secukinumab	[Cosentyx® Novartis]	mAb against **IL-17A**. Effective in psoriasis and psoriatic arthritis, after TNF blockers have failed. Approved 2015.
omalizumab	[Xolair® Genentech]	Humanized mAb against human **IgE**, blocks its binding to the FcεR. Approved for severe refractory asthma, and in trials for other indications, including chronic urticaria.
mepolizumab	[Nucala®, GlaxoSmithKline]	Humanized mAb against **IL-5**, for use in severe asthma to reduce blood eosinophil levels.
natalizumab	[Tysabri®Biogen/Elan]	Humanized mAb to **alpha-4 integrin**, it blocks lymphocyte migration; approved for MS and Crohn disease with black box restrictions due to risk of progressive multifocal leukoencephalopathy (JC virus).
tocilizumab	[Actemra®Genentech]	Anti-**IL-6 receptor**. For use in RA when response to DMARDs is inadequate. Also approved for JRA. Used off-label for cytokine release syndrome.
belimumab	[Benlysta® GlaxoSmithKline]	Human mAb against the cytokine **BLyS** (B-lymphocyte stimulator) indicated for the treatment of adult patients with active, autoantibody-positive, systemic lupus erythematosus who are receiving standard therapy. First new SLE drug in 56 years.
rituximab	[Rituxan® Genentech]	Chimeric mAb to **CD20**. For RA when anti-TNF therapy fails, and for Wegener granulomatosis. Off-label for MS, SLE, some rarer conditions.
alirocumab **evolocumab**	[Praluent® Sanofi-Aventis] [Repatha™Amgen]	Human mAb to **PCSK9**, blocks it from binding to and facilitating degradation of the LDL receptor. For uncontrolled LDL hypercholesterolemia. LDL lowered 77% more than statin alone. Top seller.

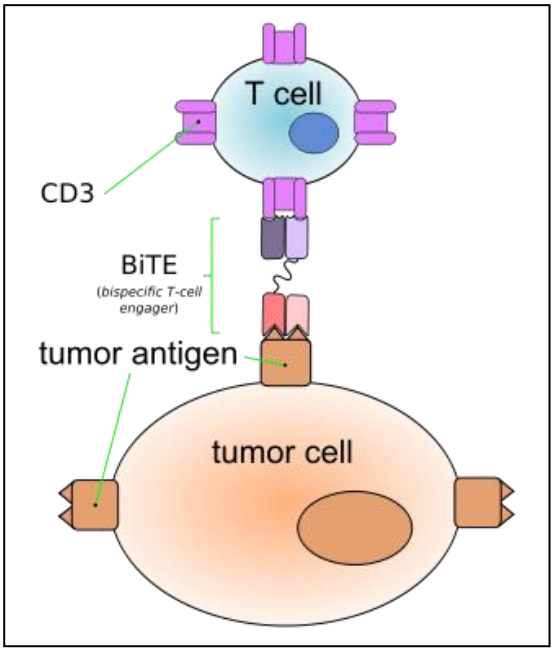
[2] **$** means the drug was a top-20 dollar earner among all drugs, USA, 2016. Humira, for example, made $16 billion. In 2016, eight of the 10 best-selling medicines globally were biologics, with about $67 billion in combined sales.

PASSIVE ANTIBODY THERAPY IN CANCER. Antibody to tumor-associated antigens (a term we'll define more specifically in tumor immunology) should be useful, and quite a few monoclonal antibodies (mAb) are already available. A few activate complement, and the tumor is lysed or phagocytosed; ►more often they invoke ADCC. Antibodies can also be tagged with a poison or a radioisotope (such modified antibodies are called **immunotoxins**). They provide highly-targeted delivery of the toxic moiety. At least one mAb is available for use as both an imaging and a therapeutic drug, depending on which radioisotope is attached.

SOME MONOCLONALS USED IN ONCOLOGY		
rituximab $	[Rituxan® Genentech]	Chimeric mAb to **CD20**. Used to treat non-Hodgkin's lymphoma and chronic lymphocytic leukemia.
ibritumomab tiuxetan	[Zevalin® IDEC]	A fully murine mAb against **CD20** (same specificity as rituximab) but to which is coupled covalently a molecule that can chelate ^{111}Indium and ^{90}Yttrium. It is the first FDA-approved radioimmunotherapy. You first use the mAb with ^{111}I to image the tumor, then with ^{90}Y to treat it (5 mm mean free path means "crossfire" radiation).
alemtuzumab	[Campath® Schering]	Humanized mAb to **CD52**, on malignant B cells in B-CLL.
trastuzumab $	[Herceptin® Genentech]	Humanized mAb to the extracellular domain of the **EGF receptor HER2**. It downregulates the receptor, and it sensitizes the cell for killing by ADCC. For HER2-positive breast and gastric adenocarcinoma.
ado-trastuzumab emtansine	[Kadcyla® Genentech]	**Trastuzumab** linked via a novel bridge to DM1, a **cytotoxic** maytansinoid. Taken up by the target, the bridge is cleaved in the phagolysosome, releasing the toxin. Works when trastuzumab has failed. Approved late 2015.
margetuximab	no name yet MacroGenics	In phase 3 trials for breast cancer. The binding of **trastuzumab**, but with a **better Fc** engineered to bind exclusively to *activating* FcR on LGL.
cetuximab	[Erbitux® ImClone]	The infamous Martha Stewart-jailing chimeric mAb to the **EGF receptor**. Approved for use with irinotecan in EGF-positive colorectal cancer; and for advanced head and neck cancer.
bevacizumab $	[Avastin® Genentech]	Humanized mAb to vascular endothelial growth factor, **VEGF**, which is made by many kinds of tumor to increase their blood supply. Approved for certain lung, renal, colon, glioblastoma tumors.
ipilimumab	[Yervoy® Bristol-Myers Squibb]	Human blocking mAb against **CTLA-4**; binds CTLA-4 and prevents its inhibitory signal, so CTLs are activated. Used in melanoma. Side effects due to immune activation.
pembrolizumab	[Keytruda® Merck]	mAb against **PD-1**, another effector T cell inhibitory molecule. Approved late 2014. Approved for **any** PD-L1 positive tumor, May 2017 (first tissue-agnostic approval by FDA)
nivolumab $	[Opdivo® Bristol-Myers Squibb]	Another mAb against **PD-1.** Approved for use against ipilimumab-resistant melanoma, in trials for several solid tumors. Also available is a combined Yervoy/Opdivo combination.
durvalumab atezolizumab avelumab	[Imfinzi™AstraZeneca] [Tecentriq™Genentech] [Bavencio™Pfizer]	All 3 are mAbs against **PD-L1**, and are looking as good or better than the PD-1 antibodies in a variety of cancers.

MONOCLONALS APPROVED SO FAR IN 2017 BY FDA			
Tremfya	guselkumab (compare to ustekinumab, p.4)	**IL-23 subunit alpha** (p19 subunit) (which is not part of IL-12); blocks interleukin-23, not IL-12.	certain adults with moderate to severe plaque psoriasis
Kevzara	sarilumab	IL-6 receptor ($39,000/yr)	adult rheumatoid arthritis
Imfinzi	durvalumab	PD-L1	urothelial carcinoma
Ocrevus	ocrelizumab	CD-20 (humanized)	relapsing and primary progressive multiple sclerosis
Dupixent	dupilumab	IL-4α receptor subunit (both IL-4 and IL-13 receptors)	adults with moderate-to-severe eczema (atopic dermatitis)
Bavencio	avelumab	PD-L1	for Merkel-cell carcinoma of skin
Siliq	brodalumab	IL-17 receptor	adults with moderate-to-severe plaque psoriasis
Besponsa	inotuzumab ozogamicin	CD-22	adults with relapsed or refractory B-cell precursor acute lymphoblastic leukemia.
Mylotarg	gemtuzumab ozogamicin	CD-33	Approved 2000, withdrawn 2010, reapproved 2017 for AML

BISPECIFIC ANTIBODIES. You might think the following approach[3] seems unlikely to work, but it has: A group coupled together two single-chain engineered antibodies, one against CD19 and one against CD3 (remember, CD3 is the signaling component of the T cell receptor). This construct can bind T cells via their CD3 to CD19+ B cell lymphoma cells.



Formula:
[V_L1-linker-V_H1]-big linker-[V_H2-linker-V_L2]

The concept was named **BiTE** for **Bi**specific **T**-cell **E**ngager. Small doses given to lymphoma patients resulted in some cases in complete clearance of the tumor cells! Approved by the FDA after "breakthrough therapy" designation on 3 December 2014, 6 months ahead of schedule. It's for use in Philadelphia-chromosome negative acute lymphoblastic leukemia (ALL). The drug is called **blinatumomab** [Blincyto®, Amgen.]

[3] Bargou et al. (2008) Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science 321: 974-977.

OTHER BRMs		
etanercept $	[Enbrel® Immunex]	A chimeric fusion protein (made in CHO cells) consisting of the human p75 **TNF receptor** and CH2 and CH3 of human IgG1. Approved for severe RA, JRA, ankylosing spondylitis, and psoriasis.
abatacept	[Orencia® BMSquibb]	Fusion protein between the extracellular part of **CTLA-4** and Fc of IgG1. For RA and psoriasis.
anakinra	[Kineret® Amgen]	**IL-1 receptor antagonist** (an inert form of IL-1, it binds to and blocks the receptor). Used in RA. Developed here at AMC.
interleukin-2	[Proleukin®Chiron]	Used in renal cancer and melanoma treatment with other agents.
interferon-γ	[Actimmune® InterMune]	Approved to treat chronic granulomatous disease and osteopetrosis. In neither case is the mechanism understood.
G-CSF, filgrastim	[Neupogen® Amgen]	Used to treat lymphomas, leukemias, and in bone marrow transplantation. Also as an adjunct in cancer chemotherapy
G-CSF, filgrastim-sndz	[Zarxio™ Sandoz]	The first FDA-approved **biosimilar**. 2015.
G-CSF, pegfilgrastim $	[Neulasta® Amgen]	As above, but it's **pegylated** and needs only to be given once per chemotherapy cycle.
GM-CSF	[Sargramostim® Immunex]	Used to treat leukemias, and in bone marrow transplantation (both to mobilize stem cells, and after reconstitution).
Imiquimod	Generic; many brands.	Synthetic drug developed by 3M Co. It stimulates **TLR-7**, thus producing several cytokines; and activating dendritic cells. Used in cutaneous warts, and several kinds of skin tumors (which it may make more immunogenic.)
denosumab	[Prolia®Amgen] [Xgeva®Amgen]	Human mAb against RANK ligand (**RANKL**). For use in severe (Prolia), or cancer-related (Xgeva), osteoporosis. Stimulation by RANK causes osteoclast maturation, which denosumab blocks.
eculizumab	[Soliris®Alexion]	Humanized mAb against **complement C5**, which blocks C-mediated hemolysis. For treatment of paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome. *The most expensive drug at $500,000/year.*
palivizumab	[Synagis®MedImmune]	Humanized mAb against **respiratory syncytial virus**, used to protect at-risk infants (preemies, congenital heart disease) from the disease.
IVIg	[many suppliers]	Originally used only in immunodeficiency disease, IVIG is now widely used as an anti-inflammatory in a variety of conditions including many autoimmune diseases. It stimulates inhibitory receptors on phagocytes. It is surprisingly effective.

When you compare a traditional drug, like a corticosteroid, to a modern BRM, for example a monoclonal antibody, it's easy to be impressed with the greater specificity and mechanism-based design of the latter. We're not surprised that corticosteroids (which are themselves related to hydrocortisone, a natural hormone) have many side effects. What was perhaps unanticipated was how many, and how serious, the side effects of BRMs can be; and that most of them are only partially effective. So, they're the present of medicine; but are they also the future? Can you think of approaches to correcting diseased metabolic pathways, confused immunity, or uncontrolled growth that might be safer, more specific, and—dare we say it—affordable?

Learning objectives for Immunomodulators

1. Distinguish between NSAIDs and DMARDs as drug classes in terms of: Mechanisms of action; use in autoimmune and rheumatic diseases; effects on those diseases.

2. Define monoclonal antibodies, and describe in principle how they are made.

3. Discuss the use of monoclonal antibodies as anti-inflammatory agents.

4. Define NK cells and ADCC. Discuss the effect of Class I MHC expression levels on susceptibility of target cells to CTL and NK cells, respectively. Describe the mechanism for ADCC.

5. Discuss the use of modified (drugs, isotopes) monoclonal antibodies in tumor diagnosis or therapy.

6. Discuss strategies for use of monoclonal antibodies to modify immune responses.

7. Discuss how the use of BRMs, which target known pathways of the immune system, can lead to serious and often predictable side effects.

8. Describe a generic BiTE (bi-specific T cell engager) and speculate on possible use of BiTEs with CD3 and any other binding ability.