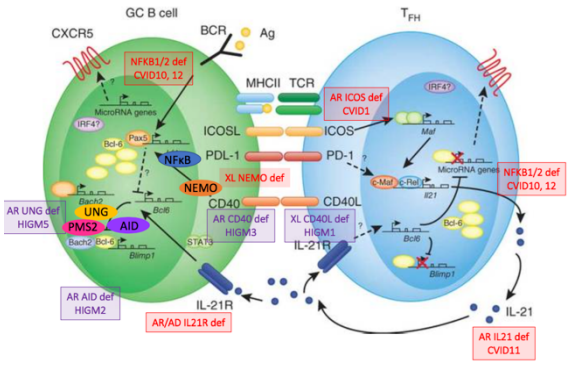
B Cell help from T Cells

There are many different ways that this process occurs
Activated B cells gather, process and present the antigen to T cells via MHCII; activated T cells upon seeing the antigen and co-stimulation provide help

- This help can include proliferation and AID-inducing signals such as CD40L and differentiation signals such as interleukins
    - CD40 stimulation activates B cells by activating the NFκB signalling pathway
- In the absence of CD4 help B cells do nothing



Defects in Help Signalling pathways lead to Primary Immunodeficiencies (PID)

- Purple defects: involve defects in B cell activation, class switch recombination and somatic hypermutation block isotype switching and thus IgG production
    - Cause hyper IgM syndromes
- Red defects: ineffective activation or differentiation of B cell commonly resulting in CVID which can have profound downstream effects, result in defects in antibody production

Hyper-IgM (HIGM) Syndromes

XL CD40L deficiency (CD40LG; HIGM1)	X-linked combined immune deficiency with cellular, humoral and innate defects Defective B cell proliferation, no germinal centres, no immune memory
AR CD40 deficiency (CD40, HIGM3)	Autosomal recessive; humoral immune deficiency, no germinal centre, no immune memory
AR/AD-AID deficiency (AICDA, HIGM2, 4)	Germinal centres form but no class switch recombination, no switched memory, no somatic hypermutation (autosomal recessive) Germinal centres form, no class switch recombination but somatic hypermutation is normal (autosomal dominant)
UNG deficiency (UNG, HIGM5)	Acts downstream of AID, GC form, no class switch recombination and no somatic hypermutation (autosomal recessive)

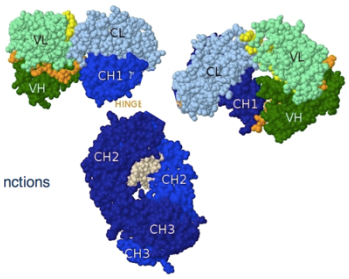
Pathogenesis:

- CSR defects: signalling/enzymatic defects (e.g. AID) that block Igμ H chain replacement by other downstream H chain isotypes; T cell defects
- SHM defects: blocks in signalling/enzymatic effectors thus preventing introduction of mutations in V region of of actively transcribed Ig genes, lack of high-affinity antibodies and T cell defects

Symptoms

- Usually present in the first or second year of life
- Recurrent pus-producing bacterial infections of upper and lower respiratory tract
- Decreased IgG, normal/abnormally high levels of IgM
- Variable symptoms and severity of disease depending on where in the gene the mutations occur

Immunoglobulin structure



Immunoglobulin structure is modular in its nature with different regions (variable/constant) playing different roles

You can break Ig into its 3 parts and each part will have the same function as they did when in the total protein

- Antigen binding site will continue to bind antigen however it will not have the effector function since F_C of heavy chain isn't present
- Constant region still has the capacity to carry out effector functions so can be used to make hybrid molecules

Modules are independent their they properties are transferrable; this means that engineering is easy because protein modules correspond to genetic modules

- You can link F_V/F_{Ab} to anything for targeting
- You can link F_C to anything for effector functions

Polyclonal vs. Monoclonal Immune Response

Polyclonal	Monoclonal
An infectious agent has many targets for an antibody response because the antigen contains many shapes that are potential targets for a specific antibody and you make antibodies for each of these shapes - This is because your system doesn't know which part of the antigen is important so instead we coat the pathogen in many antibodies (many of the antibodies we make are irrelevant in clearing or providing protection against the antigen) Hundreds of different antibodies are made against the different epitopes making a polyclonal response with many B cell clones This occurs in a normal infection Your serum is filled with lots of different antibodies that recognise the same antigen but in slightly different ways	Each B cell clone recognises and makes antibodies to a single epitope - There is one species of antibody that recognises one antigen If you could isolate a B cell clone, all of its antibodies would be identical as they derived from a single clone This allows us to determine which antibody is useful in an immune response against a particular antigen

Uses of Polyclonal Antibodies

Human cells are put into horses and then the horses recognise these as foreign thus making antibodies to it

Anti-thymocyte globulin: used in acute transplantation rejection

Acute aplastic anaemia: cancers with too many T cells, therapy prevents cancer progression by killing T cells

Anti-snake venom: covers multiple types of snake venom providing lots of coverage

Problems: batch to batch variation, lots of irrelevant antibodies are made and included, very difficult to quantify, lack of specificity

Useful because: polyclonality covers a multitude of types for one protein (cross-reactivity), easy, cheap, reliable, can recognise multiple epitopes on one antigen

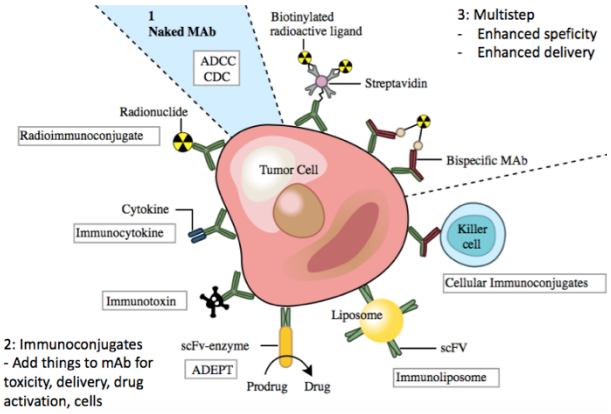
Modifying mAbs for improved therapeutic effects

Improve potency, functionality, increased half-life, tissue distribution, solubility and cost reduction

Arming mAbs for Improved Potency

Abs can be *modulated* by altering the constant region without having any effect on the variable regions so that specificity remains

- This means that things can be attached to the constant region giving the antibodies both the properties of binding to cells (e.g. tumours) whilst also conferring the function/properties of the new thing that has been added to them through variation of the constant region



Antibodies can be used as delivery systems through the process of modulation

- *Radioactive ligands:* can deliver toxic radio isotopes with high specificity to kill the tumour cell
- *Bispecific mAb:* can fuse together two different types of antibodies allowing for things to be linked together
- *Killer cell:* can be activated to induce death of the tumour cell

Mechanisms for Action of Therapeutic mAbs

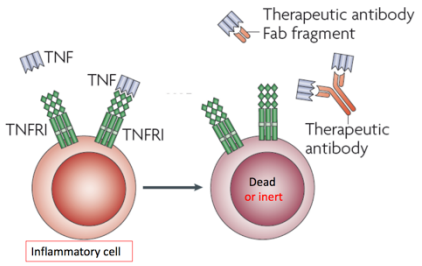
1. Ligand blockade

Bind an antibody to the ligand to block its effector function

Example: Anti-TNFα

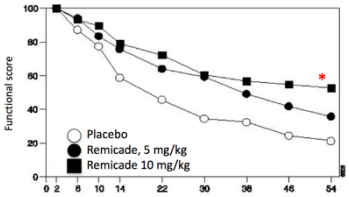
TNFα (tumour necrosis factor α) is a pro-inflammatory cytokine

- TNFα binds to its receptor on inflammatory cells stimulating the cell to produce additional cytokines that promote greater inflammation and recruit more immune cells to that site



Anti-TNFα prevents TNF from binding to the inflammatory cell thus reducing the inflammatory response

Used in Rheumatoid arthritis, Crohn's disease (inflammatory bowel disease) and psoriasis



Anti-TNFα therapy results in improved functionality of joints in rheumatoid arthritis patients

Example: Anti-VEGFA

VEGFA (vascular endothelial growth factor) is a hormone that promotes blood vessel growth

Anti-VEGFA blocks the formation of blood vessels so that large tumour masses cannot grow at new sites as they will not receive nutrients

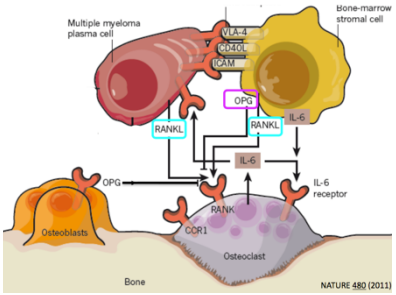
- However, cancers can adapt easily and find other ways to maintain growth

Used in metastatic colorectal, lung, breast and renal cancer resulting in a 30% increase in overall survival

Example: Anti-RANKL

RANKL is a cytokine that activates osteoclasts whose role is to destroy bone to be remodelled; during myeloma (cancer of plasma cells) and menopause there can be over-activation of osteoclasts causing brittle bones as the bone is made more thin

Anti-RANKL blocks the activation of osteoclasts allowing bones to stay at the density they were at the time of diagnosis

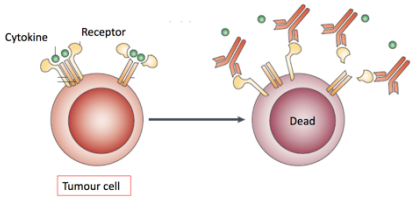


2.a. Receptor blockade

Antibodies target the receptor and bind to the receptor in such a way that they compete with the ligand for the receptor blocking the site that the ligand would usually bind

Example: anti-IL6R

IL6 is a cytokine for inflammation, anti-IL6R is used in patients with juvenile arthritis

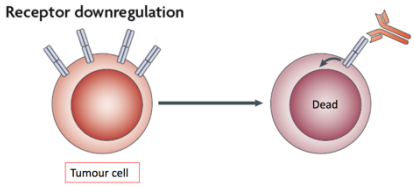


2.b. Receptor down-modulation

Antibodies bind to the receptors to cause them to be taken off the membrane to be internalised and degraded resulting in down regulation of signalling as the receptor is now no longer available to bind its ligand

Example: Herceptin and HER2 (EGFR – epidermal growth factor receptor) in breast cancer

In cancers these receptors have lost control so that they are usually on and constantly signalling; the antibody takes these receptors off the cell to be degraded meaning that tumour growth signals are not able to affect the tumour cell



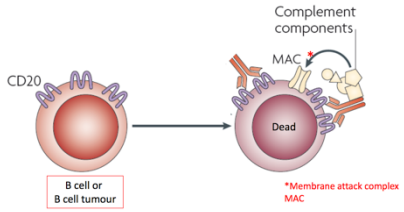
3.a. Depletion via CDC (complement dependent cytotoxicity)

B cells are labelled with antibodies for death by other immune cells

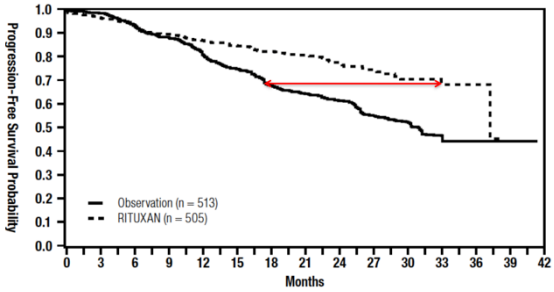
Example: Anti-CD20 (Rituximab)

CD20 is an antigen (protein) expressed on the surface of all mature B cells except for plasma cells

Anti-CD20 causes the death of all B cells by binding to them and thus labelling them for removal by other immune cells; complement components bind to the antibody and cause lysis of the B cells/B tumour cells



- Since plasma cells do not express CD20 they will not be killed, this is important as it means that individuals can still produce antibodies
- It also means that this therapy doesn't work for antibody mediated immune diseases



Often anti-CD20 is given in conjunction with traditional chemotherapy for optimum survival