Acute Phase Proteins

Increase 15-10-fold

- fibrinogen – clotting
- haptoglobulin – binds iron
- C3 cleaved to make C3a – activates mast cells and C3b as an opsonin
- mannose binding protein (MBP) – opsonin

Increase 10-1000 fold

- Serum amyloid – inhibits fever & platelet activation
- C-reactive protein (CRP) – binds phosphorylcholine, opsonin

Opsonins of the Innate Immune System

opsonin	binds to	target
CRP	phosphorylcholine	bacteria, fungi, parasites, damaged cells
MBP	mannose-fucosyl residues	bacteria, fungi, damaged cells
C3b	-OH / $-NH_2$	opsonin, activated by host cells

Interferons

- IFN-α/β/γ
- inhibit viral replication in infected cell
- bind to receptors on other cells and make cell resistant to infection
- also activate macrophages and natural killer cells
- cells infected w virus produces IFN, IFN acts internally on cell / secreted
- IFN acting internally protects infected cell → inhibiting viral replication
- secreted IFN binds to receptors on nearby cells and trigger anti-viral response

Agglutination : immunoprecipitates a soluble / non-soluble antigen

neutralisation : antibodies prevent a pathogen / protein from binding to their target

opsonization : a pathogen tagged by antibodies is phagocytosed by phagocytic cells such as macrophage or neutrophil (mediated by Fc–Fc receptor binding on phagocytes)

complement activation : antibodies attached to the surface of a pathogen activate the complement system

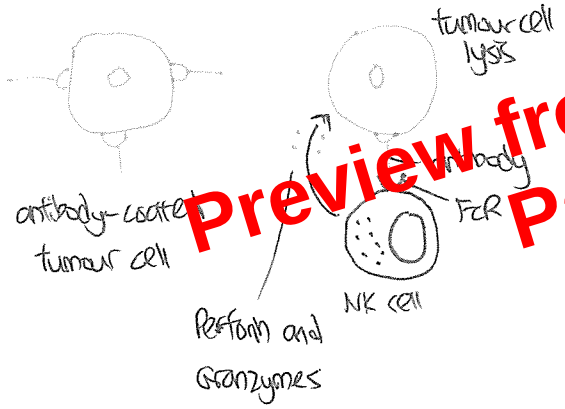
: antibody activates complement, which enhances opsonization and lyses some bacteria

Antibody-dependent cytotoxicity (ADCC)

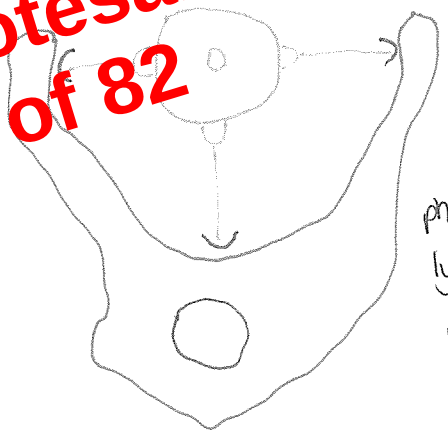
- Host cells w antigen on cell surface can bind antibodies eg: cells infected w intracellular bacteria / tumour cells)
- cells w FcR can bind the antibody eg: NK cell / macrophage
- mediates extracellular killing (NOT phagocytosis) via perforin and granzyme
- intracellular pathogens / tumours

Antibody function: Applications in Immunotherapy

a) ADCC



b) phagocytosis



Fc dependent phagocytosis and lysosomal degradation of tumour cell

Antibody classes

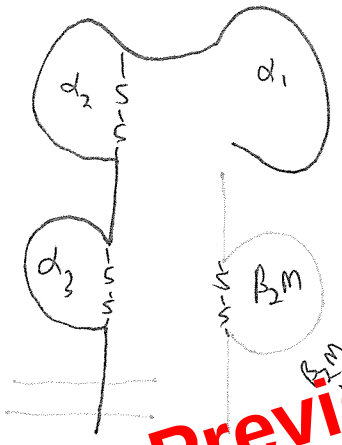
- Diff. antibody classes vary in the Fc region : class-specific structural & functional properties
- designed to do diff. things
- eg: bind to various WBC, interact w soluble immune molecules eg: complement
- an antibody can be produced which is a diff. class but have the same antigen binding specificity

MHC — class I, II, III (only I and II involved in antigen presenting to T-cells)

MHC class I
- cell surface glycoproteins expressed on all nucleated cells
- involved in T-cell recognition of antigen (T_C)
- MHC I encoded by the A, B, C loci in humans

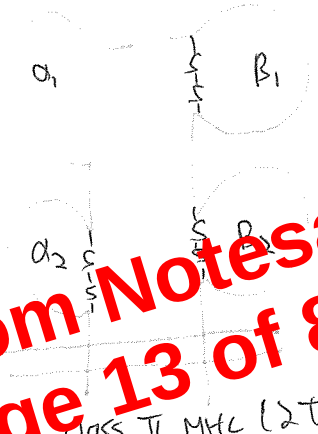
MHC class II
- cell surface glycoproteins expressed on APC eg: macrophages, dendritic cells and B-cells
- involved in T-cell recognition of antigen (T_H)
- MHC II encoded by the DP, DQ and DR regions in humans

MHC class III
- various proteins with or without immune function, including { components of complement, TNF, HSP }



β2m not MHC encoded

class I MHC (1TM)
- on most nucleated cell
- big α chain non-covalently linked to β-chain



class II MHC (2TM)
- restricted expression eg: macrophages, B-cells
- both chains have TM and cytoplasmic domains

* class I & II have similar binding clefts

Polymorphism in HLA — (1) gene from father, (1) gene from mother and both MHC genes are co-dominantly expressed (no dominant/recessive alleles)

HLA — hundreds of forms of some of the proteins

Function of class I and II MHC
- MHC class I and II are highly specialised antigen-presenting molecules that form complexes w peptide ligands
- class I and II molecules exhibit polymorphisms in the region that binds peptide
- anyone individual expresses only a small number of these proteins — upto 6 for MHC I, 12 MHC II
- class I and II MHC molecules bind peptides — I — 8-10aa, II — 12-30+ aa (class II has bigger binding clefts)

Chemokines are cytokines that control the movement of cells

- CXCL8 and CCL2 are produced at infection site
- CXCL8 released by macrophages and attracts neutrophils
- CCL2 produced by eg: epithelial cells and stromal cells and attracts monocytes
- CXCL8 (IL-8) produced by eg: macrophages & epithelial cells and mobilise naive T-cells
- cells move up chemokine gradient
- dependent on L-selectin (CD62L) expression on naive T-cells and P- and E-selectin on endothelium → slowing down of T-cell movement in HEV, allow extravasation into lymph node

What cells make antibody?

- antibodies are made by the cells of the B lymphocyte lineage
- B originally stands for "Bursa" - the bursa of Fabricius is a lymphoid organ found in chickens — the place where chickens make antibody producing cells (mammals don't have a Bursa)
- B cells originate and develop on the bone marrow and then move out to

Secondary lymphoid tissues

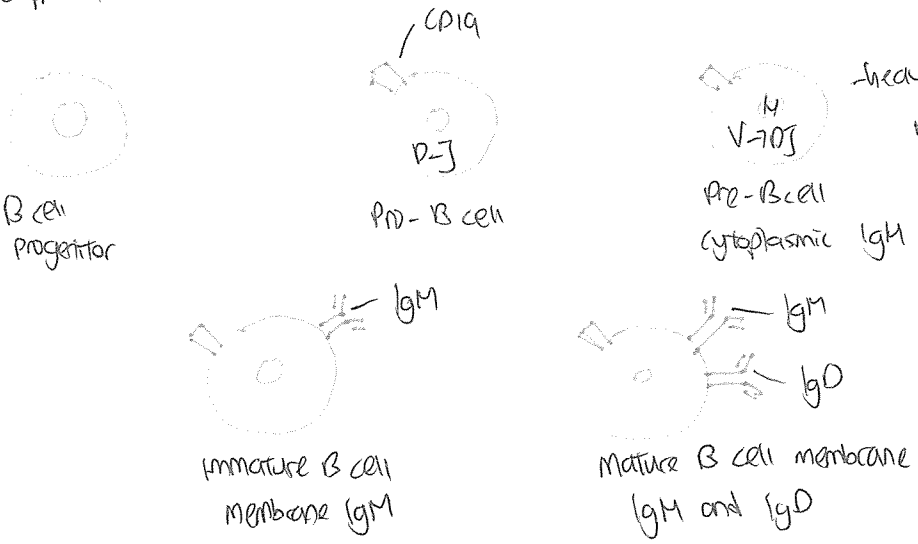
- antibodies are produced by plasma cells that differentiate from antigen-specific B-cells (NOT B-cells) ↓ heterochromatin ↑RER, ↑GA

B cell development

- B cells develop in the bone marrow
- this is where they rearrange their Ig genes
- this is independent of antigen but does depend on factors released by special cells in the bone marrow called stromal cells
- B-cells then express their re-arranged Ig molecule on the membrane surface as IgM class
- If any of these interact very strongly with self antigens in the bone marrow, they are eliminated (self-tolerance) / or its hypervariable region is modified
- these cells now mature (and additionally begin to express their re-arranged Ig molecule as an IgD class)
  (does not recognise self-antigens)

naive B cells express both IgM & IgD

B cell development



- heavy chain must be expressed, if not, B cell will die

- The B cells now leave the bone marrow and move around the body to populate 2° lymphoid organs and re-circulate
- When they encounter their specific antigen in lymph nodes, they proliferate and eventually differentiate into plasma cells and long lived memory B-cells
- B cells that leave the bone marrow cannot secrete antibody (must differentiate into plasma cells)
- B cells usually require 2 signals to become activated (like T-cells)
  - Signal ① → recognition by antigen specific membrane Ig molecule
  - ② → interacting CD4+ T-cell (T-dependent stimulation)
- In some cases, some antigens e.g. bacterial polysaccharides can deliver strong enough (via cross linking of multiple Ig molecules) antigens to stimulate B cells without T cells – (T-cell independent stimulation)

In this case, antibody is NOT usually as strong / efficient

What happens to B cells in a lymph node when antigen enters?

lymph node ↑ ↗ spleen

- antigen draining into a lymph node / spleen is collected by special macrophages (marginal sinus / zone)
- these macrophages allow conformational antigens to be recognised by any antigen specific B cell migrating through the cortex regions of the lymph node
- You need macrophage to do this because >1 BCR (Ig) on the surface of cells need to be stimulated – or cross-linked
- activated B cells move towards the border of the cortex and paracortex
- now both activated B and T cells (DC stimulated) meet up at the border of the cortex and paracortex
- form primary foci in medullary cords, some cell migrate to primary follicle

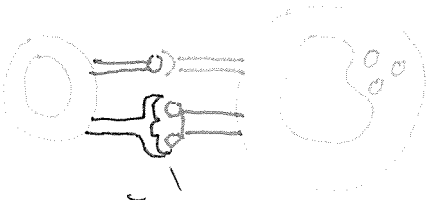
- As the activated B-cells enter the primary follicle, they down-regulate their Ig membrane receptors and proliferate extensively — they are now called centroblasts
- During this proliferation they undergo a process called affinity maturation
- This is the process where high affinity antibody is made
- As centroblasts divide they undergo hypermutation of their H and L chains of their particular Ig molecule - this will cause changes in the structure of hypervariable regions of the antibody molecules — this is a random process
- The net result of this is that some cells can now produce antibody of a slightly higher affinity for the original antigen or a slightly lower affinity

- The centroblasts now stop dividing and re-express their surface Ig — they are now called centrocytes
- They now move over the FDLs expressing antigen. If they bind antigen to high enough affinity, they will NOT die (receive a survival signal)
- only cells capable of secreting high affinity antibody survive
- As the immune response progresses, antigen levels will fall and the centrocytes compete for lesser amounts of antigen — only those of higher affinity will be selected
- affinity maturation is very effective and can result in an increase in affinity of 10,000 to 100,000

- The surviving centrocytes now meet up with activated Th cells again and differentiate into plasma cells that secrete large amounts of high affinity antibody. or turn into memory B cells
- Identification of different areas of germinal centre microscopically
  - ↳ Centroblast — dark zone
  - ↳ centrocytes, FDCs — basal light zone
  - ↳ plasma cells & memory cells - apical light zone

CTLA-4 function can be good and bad

- CTLA-4 limits T-cell activation against self-antigens
  - mice lacking CTLA-4 develop T-cell mediated autoimmunity
- CTLA-4 can limit T-cell responses during chronic infection and against tumours
  - target for therapy to enhance T-cell activation



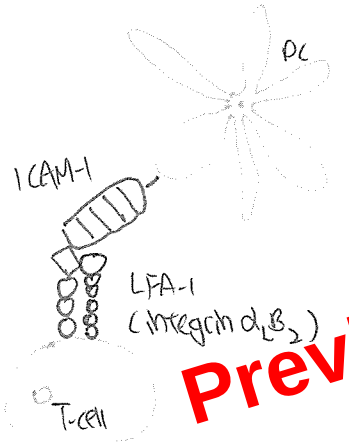
TCR interaction with MHC-presented antigen

→ In order for T-cell to scan DC looking for specific antigen, T-cells need to bind to DC

→ once bound to antigens, T-cells need to keep in contact w DC to support full activation

→ cell adhesion events are important in supporting T-cell activation

Important adhesion molecules for T-cells : DC interaction



→ LFA-1 : ICAM-1 interaction allows loose adhesion between CD4 T-cell and DC

→ allows T-cells to come into close contact w DCs (to 'sample' antigens)

If T-cell recognises* antigen on the DC:

- CD4 molecule on T-cell can interact w MHC II molecule on DC
- activation through TCR complex begins
- leads to signalling events that make the LFA1 and ICAM-1 molecules bind w higher affinity
  So more T-cell receptor / MHC II interactions can occur

If T-cell does NOT recognise antigen on DC

- loose LFA-1 : ICAM-1 interaction not strengthened
- T-cell moves on to look for DC that has antigen it recognise

Magnetic Separation of cell populations

- often used in lab to obtain purified cell populations
- may be used clinically eg: cellular immunotherapy

Immunological Memory

- specific antigen recognition is recognised / retained by the adaptive immune system
- some cells of the adaptive immune system (B cells, $CD4^+$ Th cells, $CD8^+$ Tc cells) that have recognised a foreign antigen survive in the case the same infection occurs again

Protective Immunity and Memory

- The immune system provides ↑ protection against pathogens that are encountered more than once
- Following the initial adaptive ~~immunity~~ immune response, there is a period of protective immunity, then memory responses
- once effector cells fall below the threshold for protection, protection is provided by memory responses

Features of Immunological Memory

- 1st adaptive responses to infection (primary) is often slow and weak
- 2° exposure to the same pathogen (same antigen) results in greater response
- 2° exposure to the same pathogen / antigen is more rapid
- the faster & stronger 2° response is antigen specific and better (higher affinity)

1° response : IgG ≈ IgM

2° response : IgG >>> IgM

Changes in cells of the adaptive immune system associated with memory

- adaptive immune system = acquired immunity
- expansion of "clones" of cells w/ re-arranged antigen receptor genes specific for the 1° antigen encountered
  - ↳ T cells w/ antigen specific TCR
  - B BCR / Ig
- enhanced migration & re-stimulation properties
  - ↳ adhesion molecules, rapid effector function
- survival / maintenance of these clones
  - ↳ responsive to growth / survival cytokine signals

Generation of Memory B cells

- ↑ no. of antigen specific memory B cells ie already undergone proliferation
- have already undergone antibody class switch & affinity maturation
- not yet differentiated into plasma cell
- require help from $CD4^+$ Th cells for secretion of Ab

Most Autoimmune Diseases are NOT result of single gene mutations

- They are multigenic

Why?

- Many genes are polymorphic
- structural polymorphism : different forms of protein are made (if in protein coding gene region)
- non-structural : altered protein activity / protein levels (if in protein coding region / promoter / enhancer region)
- Susceptibility to autoimmunity is due to expression of diff gene alleles (polymorphisms) and not mutation
- the degree to which an allele of a gene ↑ susceptibility is called the relative risk (RR)

Susceptibility Genes

MHC – Most important contribution especially MHC II
- estimated to account ~50% of total genetic risk
- eg: HLA-DR2 has RR of 16 for Goodpastures syndrome

Non-MHC – CTLA-4
- Sex-related genes
- eg: Hashimoto's thyroiditis 5x more likely in women, SLE 10x
- may be due to action of sex hormone on immune system

Sex-related genes are a risk factor for autoimmune diseases
- 75% autoimmune diseases found in female
- usually arises during child-bearing years
- can vary during pregnancy
- ankylosing spondylitis is one of few autoimmune diseases that is more common in male

Genetic risk includes complex multi-gene interactions
- genes identified as risk factors account for only a small proportion of genetic risk (often 10-20%)
- each gene alone carries only a small increased risk
- it is the combination of these genes that substantially enhances risk of developing a particular autoimmune disease

Histocompatibility

- Histocompatible = no immune reponse (=fully tolerant)
  - ~~syngeneic~~ syngeneic grafts
  - accepted
- Histoincompatible = immune responses (against antigenic differences)
  - allogeneic / xenogeneic grafts
  - rejected

Transplantation Antigens
- major histocompatibility antigens (strongest response) — high polymorphic class I & II MHC genes; T cell cross reactivity
- minor (weakest) — non-MHC encoded; allelic difference in any gene generating a peptide not previously tolerised against; presentation of alloppeptide on self-MHC

Major Histocompatibility Antigens

- gene loci responsible for the most vigorous graft rejection are located in MHC
  - located on human chromosome 6p21
  - closely linked genes in the HLA are usually inherited as a set - ~~haplotype~~ haplotype
    - ↳ that is why siblings are suitable for transplantation as they might inherit same haplotype

complete matching at MHC does NOT ensure graft survival

↳ gene loci responsible for less vigorous allograft rejection encode minor histocompatibility antigens

Minor Histocompatibility Antigens

- allelic difference in any non-MHC encoded gene
- leads to presentation of a peptide not previously tolerised against in the host

Allorecognition by T-cells : direct and indirect recognition

donor APC

Direct (stronger) — Donor MHC + Donor peptide — recognised by — A high frequency of T cells recognise the graft by direct cross-reaction (or donor MHC quite similar to recipient MHC) — recipient T cells

direct recognition won't happen for too long as donor APC not replenished

recipient APC

Indirect (weaker) — Recipient MHC + Processed donor peptide — recognised by — A low frequency of responsive cognate T cells — recipient T cells

processed allelic antigens (minor antigens) or processed polymorphic allo-MHC

Immune Response in Transplantation : Graft vs Host Disease (GVHD)

- a complication of bone marrow and haematopoietic stem cell transplants
- mature donor T cells recognise recipient MHC + recipient peptide
- donor cells attack cells and tissues of the host (recipient)
- very important to HLA-match
  - donors are often siblings, although GVHD can still occur in HLA-identical siblings due to allelic differences at non-MHC linked loci

Strategies to Prevent Graft Rejection

1) Avoiding graft rejection { organ retrieval; pre-screening and matching

2) preventing graft rejection - immune suppression

3) Type of organ donor - degree of damage to donated organ

Donor type	Timing of transplant	Caveats
Cadaveric (no heart-beat or breathing)	damage to organ within minutes	determination of legal time of death, rapidity of organ removal
brain dead (heart-beating, breathing to respirator)	within hours	trauma for [illegible] disconnecting the respirator
living related / non-related respiration [illegible] graft	pre-arranged	must be altruistic, potential surgical risk to donor

4) Minimise damage to donated organ during procurement

- Optimal donor management - physiological optimisation
- organ preservation - perfusion, cool
  - ↳ organ bathed in fluid for preservation

5) Screening strategies

- blood-typing
- HLA-specific antibody screening
- cross-matching (serum from recipient) → testing to avoid hyperacute rejection
- HLA typing - minimise HLA Mismatches to avoid direct recognition and vigorous acute rejection

Shock and Kill to combat latency

↳ aim to drive activation of all the infected resting cells that make up the latent reservoir so that all the hidden virus is exposed to anti-retroviral drug therapy ⇒ cleared

↳ Vorinostat : a Histone Deacetylase inhibitor (making the viral DNA non-latent, exposing it out)

↳ a possible alternative could be to use an inhibitor of the HIV protein Tat to suppress activation of transcription in latently infected cells → effectively locking down the latent reservoir

There is NO current vaccine

Prophylactic vaccine (prevention) vs therapeutic vaccine (cure)

Sterilising cure vs functional cure

Sterilising: remove all virus
functional: reduce viraemia so that virus cannot replicate

Vaccination Strategies :

- RV-144 'Prime-Boost' strategy provided some protection

Broadly neutralising Ab - neutralise against a wide spectrum of HIV variants