Immuno\ology

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I: Transplantation

Introduction

**Autograft:** within the same individual e.g. skin from buttock to face

**Isograft:** between genetically identical individuals of the same species

**Allograft:** between different individuals of the same species

**Xenograft:** between individuals of different species e.g. pig or cow heart valves

Deceased donors can provide anything from solid organs to corneas, heart valves, skin and bone. Living donors can provide bone marrow, a kidney, and also some partial liver transplants.

There are two ‘types’ of deceased donor, after **brain death** (DBD) or **cardiac death** (DCD).

**DBD**s often become available following **road traffic accidents**, where there has been catastrophic cerebral haemorrhage. Brain death must be confirmed before the organs are harvested and cooled. To confirm someone as braindead while their heart is still beating, there must be:

- irremediable structural brain damage from a **known cause**
- apnoeic coma **not** due to:
  - depressant drugs
  - metabolic / endocrine disturbance
  - hypothermia
  - neuromuscular blockers
- demonstrable lack of **brainstem** function, via:
  - eyes unresponsive (pupillary light reflex, corneal reflex, and cold caloric reflex test)
  - cranial nerve motor reflexes absent
  - gag reflex absent
  - no respiratory movements on disconnection from ventilator

Reasons for **exclusion** are:

- viral infection, especially HIV, HBV, HCV
- malignancy
- drug abuse, overdose, or poisoning
- disease of the organ itself
A braindead person’s **family** refusing to consent for his/her organs to be transplanted is the #1 obstacle to donation. The UK has an obscene 43% family refusal rate (compared the Czech Republic’s 4.7% for example).

Potential strategies for increasing transplantation include:
- including more marginal donors (i.e. slightly relaxing some criteria for the deceased’s organs)
- exchange programmes to acquire better tissue matches
- xenotransplantation and stem cell research for the future

**Immunology of transplantation**

**ABO blood group** matching between donor and recipient is crucial. If an organ from an A group donor is transplanted into a group B recipient, **hyperacute rejection** occurs as the foreign cells are lysed by complement and/or phagocytosed all with massive inflammation, platelet activation etc.. Recently, it has become possible to **remove the recipient’s A/B antibodies** with good outcomes.

**HLA** (human leukocyte antigen) is the other most important protein variation to match. **Class I** are expressed on all cells, and **Class II** on immune cells (but can also be upregulated on other cells). HLA is **highly polymorphic** i.e. there are lots of potential alleles for each locus. Exposure to foreign HLA molecules stimulates an **immune response** mediated by both **B cells** and **T cells**. HLA does not have to be perfectly matched (as that’s impractical) but there must be <6 mismatches.

**Rejection** is diagnosed by histological examination of a graft biopsy, and is treated (prophylactically, ideally) by immunosuppressive drugs. Some organs’ rejection can be tested clinically e.g. via creatinine (kidney), serum amylase/lipase/glucose (pancreas). Hearts cannot.

A standard **immunosuppressive regime** entails:
- an **induction agent** e.g. cytokine blockade, T cell depletion
- **baseline immunosuppression**
  - signal transduction blockade e.g. calcineurin inhibitor (cyclosporin), mTOR inhibitor
  - antiproliferative agent e.g. azathioprine
  - corticosteroids
- **treatment** of episodes of **acute rejection**
  - cellular: steroids, anti-T cell agents
  - antibody-mediated: IVIg (intravenous immunoglobulin), anti-C5, plasma exchange

Immunosuppression is all about a **balance**: rejection versus infection, malignancy, or drug toxicity.
2: **Hypersensitivity and allergy**

Inappropriate immune responses are known as **hypersensitivity** reactions. They are in response to:
- harmless foreign antigens, as in **allergy** (e.g. foods or pollen)
- **autoantigens**, as in **autoimmune disease**
- **alloantigens**, as in **graft rejection**

There are four types of hypersensitivity reaction:
- **type I**: immediate
- **type II**: antibody-dependent
- **type III**: immune complex-mediated
- **type IV**: delayed cell-mediated

Many diseases involve a **mix** of these.

**Type I – immediate hypersensitivity**
e.g. **anaphylaxis, asthma, hayfever, food allergy**.

Upon first exposure to an antigen, specific **IgE** is produced by B cells. The IgE becomes incorporated onto **basophil** and **mast cell** membranes. Upon subsequent exposures, much more IgE is produced much more rapidly, and the antigens form cross-links between membrane-bound IgE, stimulating **degranulation** and release of **mediators**.

**Type II – antibody-dependent hypersensitivity**

Here, the clinical presentation depends on the **autoantibodies’ target**:
- **myasthenia gravis**: postsynaptic nicotinic acetylcholine receptor at neuromuscular junctions
- **pemphigus vulgaris**: desmoglein (links keratinocyte desmosomes to the basement membrane)
- **glomerulonephritis**: glomerular basement membrane
- **pernicious anaemia**: intrinsic factor

Autoantibodies can also target **blood cells**, so can cause things like:
- anaemia
- neutropenia
- thrombocytopenia

Tests can be done to find specific autoantibodies via immunofluorescence or ELISA e.g. **anti-CCP** (cyclic citrullinated peptide) antibodies for **rheumatoid arthritis**.

**Type III – immune complex-mediated hypersensitivity**

**Antigen-antibody complexes** form in the blood and become deposited in tissues. This causes the activation of the clotting cascade, complement cascade, and the recruitment of immune cells.

All of this leads to **vasculitis** and tissue damage. e.g. **systemic lupus erythematosus** (SLE or lupus) and **vasculitides** (which also cause systemic inflammation, such as **polyarteritis nodosum**).
Type IV – delayed cell-mediated hypersensitivity

- **Type IV** are the most common hypersensitivity reactions.
  
  The cell types which mediate type IV reactions are:
  
  - **Th1**
    
    Th1s have antigen presented to them, then produce **interferon-γ (IFN-γ)** which activates **macrophages** to produce lots of **TNF-α**. Th1s also produce **IL-2**, which stimulates **CD8+ CTLs**. If the resulting **inflammation** is chronic, **fibroblast growth factor (FGF)** is also made by the Th1s, leading to fibrosis and angiogenesis.
  
  - **Th2**
    
    Involved in allergic responses, in chronic asthma and hayfever.
  
  - **CTL**
    
    Kill virally infected cells.

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**Summary**

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<td><strong>Immune reactant</strong></td>
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<td>IgE</td>
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<td>Complement, FcR+ cells</td>
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<td>Chronic asthma, chronic allergic rhinitis</td>
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A common feature of all hypersensitivity reactions is inflammation, and everything it entails.
Types of inflammation/hypersensitivity in allergy

Type I: anaphylaxis, angioedema, urticaria
Type II: urticaria (chronic or idiopathic)
Type I + IV: asthma, hayfever, eczema

Sensitisation

The thing to note above is that normally, when an allergen is presented to a naïve T cell, regulatory T cells stop memory generation via IL-10. This is blocked in atopy, hence sensitisation proceeds fully.
**Eosinophils**
- 2-5% of circulating WBCs
- more are in tissues than in the bloodstream
- recruited during allergic inflammation
- generated in bone marrow
- two-lobed nucleus
- contain large granules of toxic proteins which can lead to tissue damage

**Mast cells**
- only reside in tissues
- have IgE as receptors on their cell membrane
- crosslinking of IgE as antigen binds causes release of mediators:
  - pre-formed: histamine, cytokines, toxic proteins
  - newly synthesised: leukotrienes, prostaglandins

**Neutrophils**
- 55-70% of circulating WBCs
- important in asthma and atopic eczema
- many-lobed nucleus
- granules contain digestive enzymes to follow phagocytosis
- also synthesise reactive oxygen species (radicals), cytokines, and leukotrienes

**Asthma**
The immunopathogenesis involves both *acute and chronic inflammation*.

**Acute** inflammation (an 'asthma attack') is caused by *mast cell* activation and degranulation, and leads to acute airway narrowing via:
- **vascular leakage** (oedema)
- **mucus secretion**
- **smooth muscle contraction**

There is then **chronic** inflammation, involving:
- **cellular infiltrate** of Th2 cells and eosinophils (as in diagram on previous page)
- **mucus plugging**
- **smooth muscle hypertrophy**
- **epithelial shedding**
- **sub-epithelial fibrosis**

Asthma is **reversible generalised airway obstruction**, leading to a chronic **episodic** wheeze. The bronchi are hyperresponsive and irritable. It leads to cough, mucus production, and breathlessness.

**Treatment** can involve inhaled β2-adrenoceptor agonists (salbutamol), either short acting and/or longer-acting inhaled steroids, oral steroids, leukotriene antagonists, or anti-IgE monoclonal antibodies.
**Hayfever**

Hayfever is **seasonal allergic rhinitis**. If year-round, it is called **perennial allergic rhinitis** instead. Perennial allergic rhinitis is not due to pollen, but other allergens such as animal fur or dust mites. **Symptoms** of any allergic rhinitis include:

- sneezing
- runny nose (**rhinorrhea**)
- itchy nose and/or eyes
- nasal blockage, sinusitis, and subsequent loss of smell/taste

**Treatment** involves **antihistamines** to combat sneezing, itching, and rhinorrhea, and nasal steroid spray for the blockage. **Cromoglycate** can help children's eyes.

**Eczema**

This is a **chronic itchy rash** on the skin. It is most common at the **knee** and **elbow flexures**. It results in dry, cracked skin and can be complicated by infections (majority bacterial, sometimes HSV). It is generally a disease of childhood, as 50% of cases clear by age 7 and 90% by adulthood.

**Treatment** involves topical **steroid cream** and **emollients** (moisturisers).

**Food allergy**

**Mild** allergy causes an itchy mouth and lips, angioedema, and urticaria. **Severe** allergy causes nausea, abdominal pain, diarrhoea, and in the worst cases **anaphylaxis**.

**Treatment** is ideally by **avoidance**, or with adrenaline, antihistamines, and steroids for anaphylaxis.

**Diagnosing allergy**

A **careful history** is essential, but tests can be performed to confirm:

- skin prick testing
- radioallergosorbent test (**RAST**), which checks for specific IgE antibodies in the blood
- total IgE levels
- lung function tests (for asthma)

**Immunotherapy**

This can be very useful for single antigen hypersensitivities (bee/wasp venom, pollen, dust mites). There is **subcutaneous** immunotherapy which requires 3 years of regular clinic attendance for injections, and **sublingual** immunotherapy which can be done at home with pills, but we don’t know how long sublingual immunotherapy needs to continue to be successful.
3: Tolerance and autoimmunity

Autoimmunity

~5% of people in developed countries are affected by autoimmune disease. ~75% of these are female. The incidence of autoimmunity, like hypersensitivity/allergy, is increasing (via the hygiene hypothesis). The major autoimmune diseases are:

- rheumatoid arthritis (RA)
- type I diabetes mellitus (T1DM)
- multiple sclerosis (MS)
- systemic lupus erythematosus (SLE / lupus)
- autoimmune thyroid diseases e.g. Hashimoto’s and Graves’ disease

Others include pernicious anaemia, myasthenia gravis, primary biliary cirrhosis, ulcerative colitis…

Autoimmunity is the presence of adaptive immune responses with specificity for ‘self’ antigens.

Autoimmune reactions use the same mechanisms as immune responses to pathogens. Their coming about involves breaking T cell tolerance. Since ‘self’ tissue is always present, autoimmune diseases are always chronic conditions. The effector mechanisms resemble type II, III, and IV hypersensitivity.

Autoantibodies (aAbs) and levels thereof have a direct link with autoimmune disease. For example, autoantibodies to RBCs are responsible for haemolytic anaemia via clearance and also complement activation, which causes systemic effects beyond a lack of RBCs. Type II and type III hypersensitivity pathways are both involved in that example.

Sometimes the autoantibody has a very different effect, such as in Graves’ disease where it binds to and activates the TSH receptor on thyroid follicular cells, causing hyperthyroidism.

Type IV, cell-mediated responses are also important, for example in T1DM where T cells react to the pancreatic β-cell antigen and kill off all those cells.

Human MHC (major histocompatibility complex) / HLA (human leukocyte antigen) genes are the dominant genetic factor affecting susceptibility to autoimmune disease. The two terms mean the same thing. MHC is the general name across vertebrates; HLA is the human version of MHC.
Immunological tolerance

“The acquired inability to respond to an antigenic stimulus.”

It is the ‘3 As’:
- **acquired**: tolerance involves cells of the acquired/adaptive immune system and is ‘learned’
- **antigen-specific**
- an active process in neonates, the learnings of which are maintained through life

Central tolerance

Immature T cells in the thymus recognise peptides presented on MHCs. Normally, those that can’t recognise any peptides die by apoptosis, those that recognise ‘self’ strongly are actively signalled to die by apoptosis, and those that see MHC weakly (~5% of immature T cells) are signalled to survive.

A disease resulting from failure of central tolerance is **APECED** (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy). It’s a rare disease resulting from the thymus failing to delete self-reactive T cells, caused by genetic mutations in a **transcription factor** called AIRE (autoimmune regulator) which stop it working, so other-tissue-specific genes for tolerance-building aren’t expressed.

During B cell selection in the bone marrow, it is cross-linking of B cell surface immunoglobulins by polyvalent antigens on bone marrow stromal cells that facilitates deletion versus survival.

Peripheral tolerance

Some antigens are not expressed in the thymus or bone marrow, and so are only seen after the immune system has matured. Hence, mechanisms to stop mature lymphocytes responding are needed.

**Anergy** is an absence of costimulation. Most cells on the body lack costimulatory molecules like CD80/86/40 (found on APCs). Without costimulation, T cells do not proliferate or produce factors.

**Immunological ignorance** occurs:
- when antigen concentration is too low in the periphery
- when the antigen presenting molecule is absent (most cells have no MHC class II)
- at immunologically privileged sites where immune cells can’t normally penetrate, so they lack tolerance to the autoantigens there e.g. the eyes, testes, nervous system

Failure of this occurs in sympathetic ophthalmia, when eye trauma allows autoantigens into the blood and into contact with intolerant T cells. The activated T cells then attack those antigens in both eyes.

**Regulation** by T-reg cells can control autoreactive T cells. **IPEX** (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance) is a fatal genetic disease (recessive) presenting early in childhood. It’s caused by a mutation in FOXP3, the gene encoding a transcription factor essential for T-reg development. Due to the lack of T-reg, autoreactive T-cells accumulate and lead to T1DM, eczema, severe enteropathy and infections, and crazier autoimmune phenomena.

**Infections** can ‘break’ peripheral tolerance by changing expression of ‘self’ proteins (e.g. MHC, costimulatory molecules), affecting T-reg cells, and damaging tissue at privileged sites.
4: **Tumour immunology**

**A case study**

A previously healthy 53-year-old woman awoke dizzy. She had severe vertigo, unintelligible speech, truncal and appendicular ataxia. She was unable to sit, stand, or use her hands.

It turned out she had breast cancer! The tumour was ectopically producing a protein called *cerebellar degeneration-related antigen 2* (CDR2), which stimulated an autoimmune response. Unfortunately, this sensitised the immune system to the CDR2 present normally in the cerebellum’s Purkinje cells (the nervous system is an immunologically privileged site, hence no tolerance) and the autoimmune response destroyed them. This *paraneoplastic cerebellar degeneration* caused the patient’s symptoms.

This example shows that:
- tumours can express antigens that are absent from the corresponding ‘normal’ tissue
- the immune system can detect this abnormality and, as a result, attack the tumour
- in some cases, this might result in autoimmune destruction of other ‘normal’ tissues too

**Tumour immunology**

**Tumour growth** will inevitably, eventually, result in *inflammation* (via the innate immune system) due to the tumour’s size causing necrosis and/or other complications. Inflammation brings macrophages, natural killer (NK) cells, and dendritic cells to the site.

Dendritic cells “see inside” cells via the molecules expressed on their surface, for example some new protein expressed on a tumour cell’s membrane. They then present those antigens to naïve T helper cells at lymph nodes, activating CTLs to kill the tumour cells and B cells to produce specific antibodies (via the adaptive immune system).

There are two main problems with *immune surveillance of cancer*:
- it takes a while for a tumour to grow enough to cause inflammation (without which there will be no immune cell recruitment and no immune response).
- antigenic differences between normal and tumour cells can be very subtle (often just 1 or 2 amino acid changes) so they can be difficult to recognise at all, let alone produce specific antibodies against.

**Viruses and cancer**

Some viruses can cause *opportunistic malignancies* (only possible during immunosuppression):
- EBV leading to B cell lymphoma
- HIV leading to Kaposi’s sarcoma

However, some viruses can lead to cancer in *healthy individuals*:
- HTLV1 (human T lymphocyte virus 1) leading to T cell lymphoma
- HBV and HCV leading to hepatocellular carcinoma
- HPV leading to cervical cancer
Vaccines and cancer

The HPV vaccine uses recombinant HPV coat proteins (called L1 and L2 because they’re produced in the late phase of the virus’ replication). An HPV vaccine can also be administered therapeutically, to help combat neoplasia in the event of immune failure.

>99% of unvaccinated HPV infectees don’t develop any neoplasia; the infection is cleared normally with no lasting issues.

**MAGE-3** is an example of the melanoma-associated antigen family, whose proteins are usually only expressed in male germ cells and the placenta but can be ectopically expressed in melanoma and other cancers. MAGE-3 specific immunisation, in 10% of cases, reduces a tumour to almost nothing.

Unfortunately, vaccinations for all types of tumour are not possible. Many tumours secrete different levels of pre-existing proteins but not new, unique molecules, and lymphocytes cannot be made to react to certain levels of a molecule, only to its presence.

Immunotherapy against melanoma can be accompanied by vitiligo (depigmentation) due to autoimmune destruction of melanocytes. Sometimes this vitiligo can happen without immunotherapy though: