

Transplantation

Course	 Immunology
Date	@April 13, 2024
Type	Lecture
Status	Completed
Reading	<input type="checkbox"/>

Historical perspective

Tissue transplantation is the preferred, and often life-saving, treatment pathway for a range of clinical conditions including end-stage renal, liver, heart, and lung failure, and certain causes of blindness.

Until the mid-20th century, transplantation was no more than an interesting surgical experiment since attempts at tissue transplantation inevitably ended in failure.

The idea that certain terminal illnesses could be treated by transplantation was vindicated when the first successful kidney transplant was performed in 1954 between identical twins, by Joseph Murray and colleagues in Boston.

What can we transplant

- | | | | |
|---------------|----------------|-----------------|--------------------------|
| 1. Heart | 7. Small bowel | 12. Penis | 18. Hands |
| 2. Lung | 8. Cornea | 13. Uterus | 19. Leg |
| 3. Heart-lung | 9. Skin | 14. Thymus | 20. Ovary |
| 4. Kidney | 10. Blood | 15. Heart valve | 21. Islets of Langerhans |
| 5. Liver | 11. HSC | 16. Bone | 22. Ligament and tendons |
| 6. Pancreas | | 17. Face | |

Grey means experimental

Terminology

Normal immune homeostasis is characterised by tolerance to antigens expressed on the individual's own cells, a process known as self-tolerance.

- Transplants or grafts from one site of the body to another in the same individual (**autograft**) or between genetically identical individuals (**isograft**) are therefore accepted.

However, skin, cells (e.g. blood) or solid organs (e.g. kidney) transplanted from one person to another (**allograft**) or from an animal to a human (**xenograft**) express non-self antigens, to which the recipient is not tolerant, and which are promptly recognised as foreign, thereby setting in motion a chain of immunological events which can lead to rejection of the graft.

Transplantation antigens:

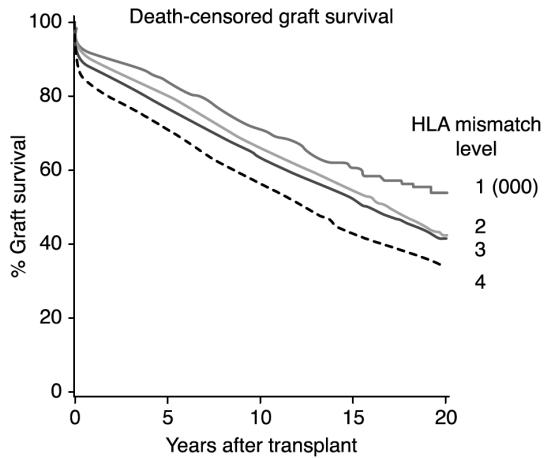
Antigens on transplanted cells recognised as foreign by the immune system.

Several types of antigens relevant to transplantation have been characterised:

- Major histocompatibility complex (MHC) molecules
- Minor histocompatibility antigens
- ABO blood group antigens

From a transplant perspective, the most important of these are the MHC antigens, also known in humans as human leucocyte antigens (HLA)

- Highly polymorphic gene complex, i.e., many different types in the population, encoding human leukocyte antigen (HLA)
- Contained on the short arm of chromosome 6
- Divided into 3 main classes (regions)
 - Class I: HLA-A, B, C
 - Class II: HLA-DR, DQ, DP
 - Class III: Densely packed with genes including complement factors and tumour necrosis factor
- The more the HLA mismatch, the lower percentage of the graft survival



Tissue typing

- HLA antigens can be typed by serological techniques
- PCR using allele-specific oligonucleotide primers (more usual nowadays)



Transplant rejection may be mediated by either T cells or B cells

Phases and Mechanisms of rejection

Rejection of the allograft can occur at any time after transplantation and may be driven either by cellular or antibody-mediated mechanisms.

- Usually don't know the exact mechanism in rejections
- **Hyperacute rejection**
 - Immediate, minutes or hours post transplantation
 - Occurs only if the recipient has pre-existing antibodies against the transplantation antigens.
 - Due to screening, it is very rare
- **Acute rejection**
 - a few days and 3 months post-transplant (early acute rejection)
 - 3 months to one year (late acute rejection)
 - Involves activation of lymphocytes and adaptive immunity.
- **Chronic rejection**
 - many months or even years post-transplant

- Mechanism poorly understood, and can be due to multiple immune mechanisms.
- Kidney transplants last about 10-15 years, probably due to chronic rejection
- Chronic rejection is the major cause of graft loss

Rejection (acute and chronic) is mediated by cellular and/or humoral mechanisms.

- Cellular infiltrate consists of CD4+ and CD8+ T lymphocytes destroying parenchymal structures
- Alloantibodies and complement (C4d) damage vascular endothelium

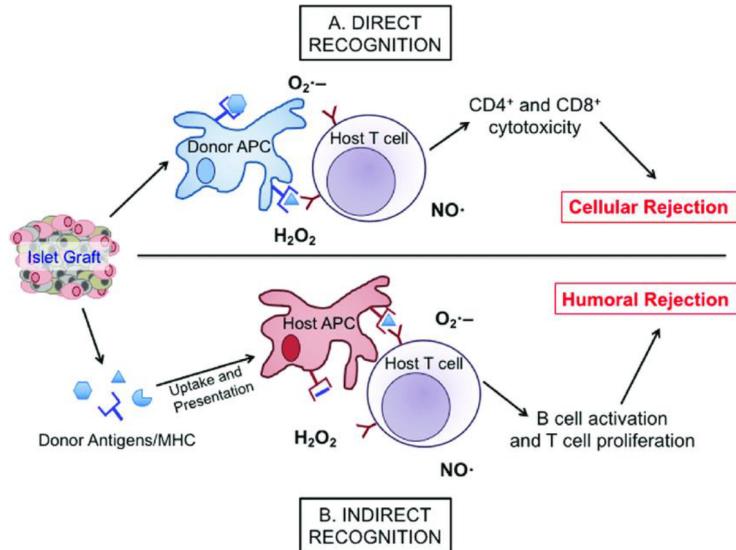
The transplantation antigens, just like microbial antigens, induce an **adaptive immune response** (with **specificity** and **memory**).

Previously grafted strain A mouse	Transfer T-cells	Naive strain A mouse	Rejection
1 			2nd set
2 			1st set
3 			1st set

When graft recipients have rejected tissues/organs once ("1st set" rejection), they mount a faster immune response to subsequent grafts from the same donor, resulting in an accelerated "2nd set" rejection.

Allorecognition

A defining characteristic of transplantation is that T-cell recognition of alloantigen can occur through pathways that do not operate in conventional responses to conventional protein antigens



- MHC alloantigen can be recognised “**directly**” by T cells as conformationally intact protein on the surface of **donor APCs**
 - Unique to transplantation
 - Processing of allogeneic MHC molecules is not required for T cell recognition
 - A high percentage (~2%) of a graft recipient's T cells are capable of directly recognizing the MHC molecules encoded by a single non-self MHC allele
 - All the different MHC molecules expressed on a graft cell can potentially be directly recognized by recipient T cells, even if they carry different peptides in the peptide binding clefts
 - Memory T cells can be involved in direct recognition of allogeneic MHC molecules, even if the recipient has never been exposed to the donor MHC molecules before
 - In direct presentation, peptide bound to the foreign MHC molecule is **not** involved in TCR recognition
- T cells are able to recognise MHC alloantigen after it has been internalised and processed by **recipient APCs** as self-restricted allopeptide (the **indirect pathway**)

Sources of organs

- Solid organ transplantation is limited by a shortage of organs

- Procurement of organs need to be ethical and coordinated on an international scale
 - Exclusion criteria of donor: HIV, HBV, Cancer

Prevention of graft rejection

- **HLA matching**
- **Immunosuppression**
- **Gene editing**
 - E.g. to make animal organs more similar to human's
- **Cell therapy**
 - Modified Treg that target HLA to tolerate the body to the new graft

The severity of graft rejection is partly determined by the number of genetic differences in the MHC molecules between the donor and recipient.

- Therefore, one way to prevent graft rejection is to try to reduce these differences and select donors and recipients as closely **matched** for the different MHC alleles as possible.
- The most important for matching are the class II MHC **HLA-DR**, followed by the class I MHC **HLA-B** and then **HLA-A**.

Transplantation within families

- Grafts between genetically identical twins are completely accepted, as there are no MHC differences
- However, although most individuals don't have an identical twin, a reasonably good 'match' is more likely within a family

Immunosuppression



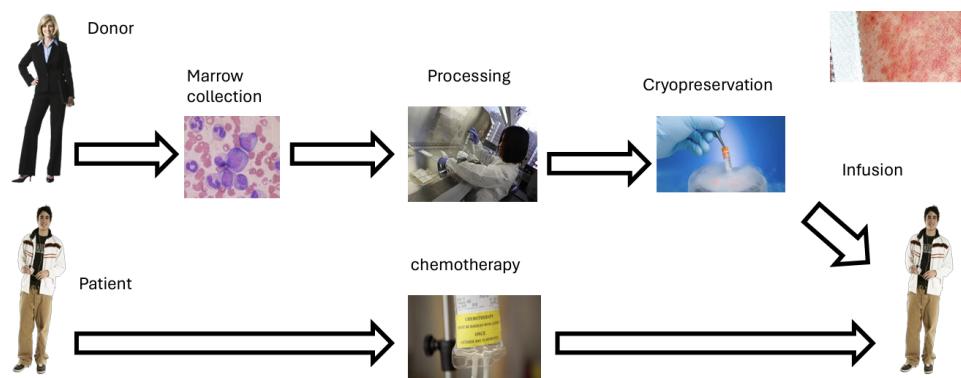
Immunosuppressant medications are required lifelong for all patients and non-selectively inhibit the immune response

- Unless a transplant occurs between genetically identical individuals (e.g. identical twins), some degree of immune response will occur
 - If unchecked, can cause rejection

A combination of immunosuppressant drugs is used to try and prevent this

- Calcineurin inhibitors, such as **Cyclosporin** and **Tacrolimus** act to **inhibit cytokine**, particularly **interleukin-2**, production,
- Anti-proliferatives such as **mycophenolic acid** and **azathioprine** prevent T and B cell activity
- Note that such drugs are non-antigen specific, and result in general immunodeficiency, leading to increased risks of infection and cancer
- Biologics such as belatacept (a soluble form of CTLA-4) which block co-stimulatory interactions are also entering routine clinical use antigen-specific approaches (i.e. **tolerance induction**), are being explored in animal models

Allogeneic haematopoietic stem cell (HSC) transplantation



Transplanting a whole immune system to the body, which can react to any organ in the body.

- Solid transplantation: host immune system attacking the graft
- HSCT: Graft attacking the recipient

Compared to other transplantation

- HSCT need to remove the the whole immune system of the recipient before the transplant
- Kidneys: put in a third kidneys
- Heart and lung: remove during the operation

Indications of Allogeneic HSCT

MALIGNANT DISEASES

- Acute myeloid leukaemia
- Acute lymphoblastic leukaemia
- Chronic myeloid leukaemia (CML)
- Chronic lymphocytic leukaemia
- Myelodysplastic syndromes
- Myeloproliferative Disorders
- Non-Hodgkin lymphoma
- Hodgkin lymphoma
- Multiple myeloma
- Juvenile CML

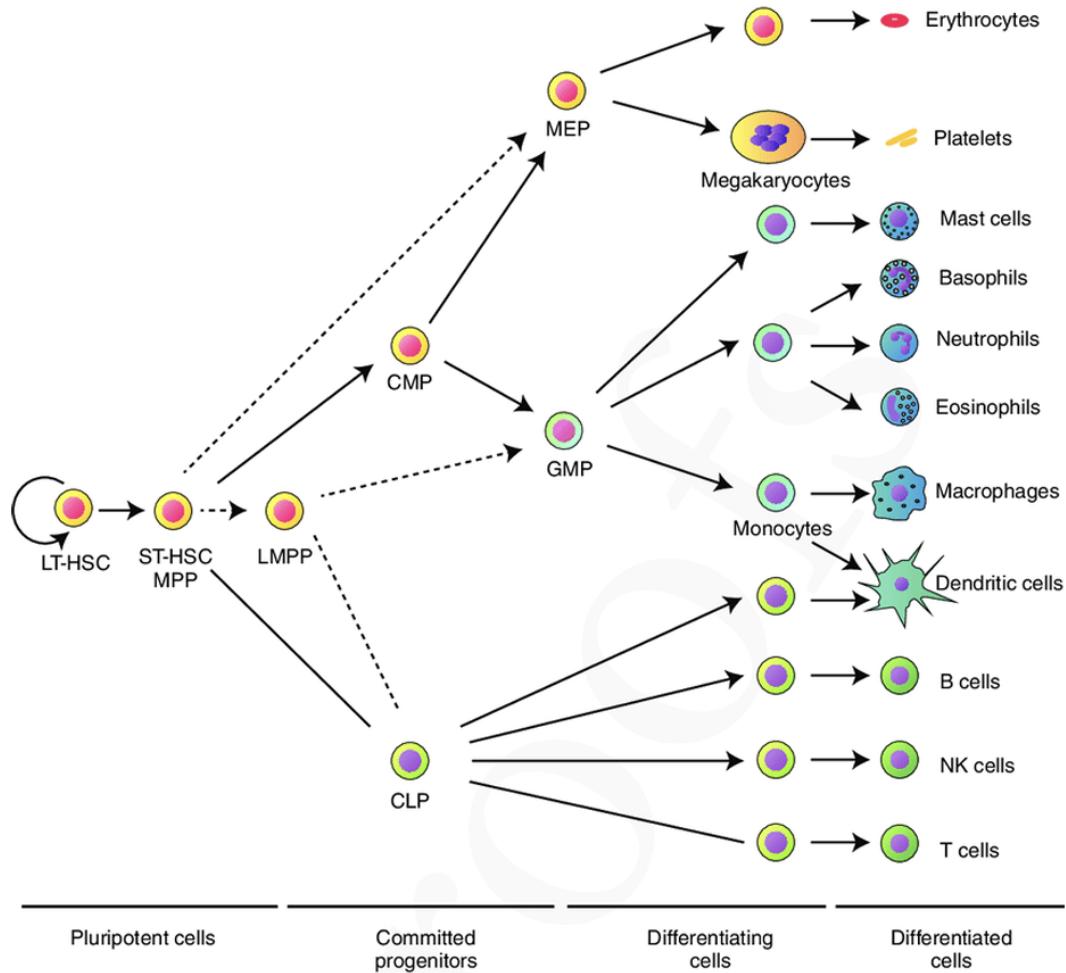
NON-MALIGNANT DISEASES

- Aplastic anaemia
- Paroxysmal nocturnal hemoglobinuria
- Fanconi anaemia
- Blackfan-Diamond anaemia
- Thalassemia major
- Sickle cell anaemia
- **Severe combined immunodeficiency (SCID)**
- **Inborn errors of immunity**
- **Inborn errors of metabolism**

- Can also do HSCT to inborn errors with immunity

Importance of HSCT

- Replacing the HSC can replace all the cells in the immune system



Aims of HSCT

- Eliminate underlying disease by chemoradiotherapy and/or allow engraftment of donor cells to generate immunotherapeutic effect
- Restore function of haematopoietic and immune systems

Types of HSCT

- Choice of transplant is based largely on
 - disease type
 - patient condition (age, co-morbidities)
 - availability of compatible donor
- There are two basic types of transplants:
 - Autologous: cells come from the patient themselves
 - E.g. rescue the patient's immune system after high dose of chemotherapy
 - Allogeneic: cells come from a source other than the patient
 - A matched related or unrelated donor, including identical twin (syngenic) donors
 - A mismatched related (e.g. haplo-identical) or unrelated donor
 - Cord blood

Matched Related Donors (siblings)	Matched Unrelated Donors (MUD)	Haploidentical Donors	Umbilical Cord Blood Donors
<ul style="list-style-type: none">• 25% chance a sibling will be a match• Syngenic donors are a very specific subtype (100% will be a match)	<ul style="list-style-type: none">• Sourced through registry• Risk of severe graft versus host disease higher• Higher transplant related mortality	<ul style="list-style-type: none">• Parent or child will be haplo-identical• 50% chance a sibling will be haplo-identical• Increased risk of graft versus host disease	<ul style="list-style-type: none">• Specific sub-type of MUD donors• Stored in cord banks• Small HSC dose• Can target specific ethnic groups

Allogeneic HSCT considerations

- Advantages
 - Graft versus malignancy effect
 - Recognise the cancer as foreign

- Given a new immunity may restore the immune surveillance
 - Potential to harvest additional stem cells or WBC if required later
 - Stem cells from healthy donors are free of cancer cells
- Disadvantages
 - The graft may be rejected
 - **Graft versus host disease (GVHD)**
 - Increased risk of infection through use of immunosuppressive drugs
 - Higher transplant related mortality (10-40%)

Sources of HSCs

- Primary sources
 - Bone marrow
 - Peripheral blood
 - Cord blood
 - Problems: may not be enough cell for adults, but enough for pediatrics
 - Growing in culture dish may cause the SC to change their properties
- Cultured sources

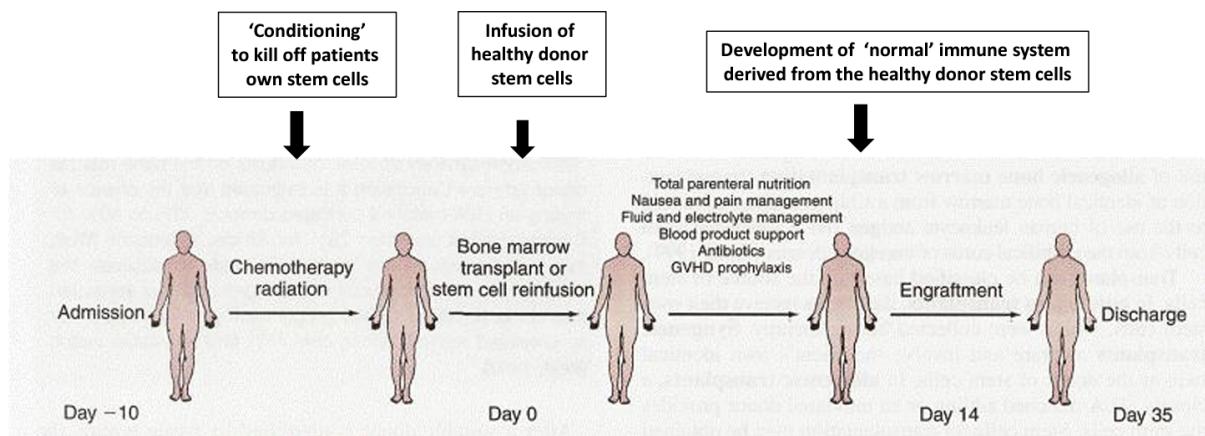
Harvesting HSCs

- Peripheral blood derived (PBSCs)
 - Donor given haematopoietic growth factors to increase the number of stem cells released into the bloodstream
 - Stem cells are identified by CD34+ express during flow cytometry
 - Stem cells are collected through an apheresis catheter, typically takes 4-6 hours
 - The stem cells are then frozen until they are given to the recipient in the case of autologous transplants or returned fresh in the case of allogeneic transplants
- Bone marrow derived

Basic concepts

- Donor BM contains B and T lymphocytes, macrophages and other APCs - hence necessity for recipient to be HLA-matched to prevent graft rejection or acute GVHD
 - Usually deplete the T cells of the donor BM and just keep the stem cells
- Only 30-40% of patients have an HLA-matched sibling - hence need for alternative donors
- Compared to autografting, engraftment of allogeneic stem cell requires **additional recipient immunosuppression to prevent graft rejection** and **prolonged post-transplant immunosuppression to prevent GVHD.**

Allogeneic HSCT today: the first 5-6 weeks



Human Leukocyte Antigen (HLA) matching

- HLA matching required
 - Molecular typing of major antigens - class I and class II
 - Class II MHC **HLA-DR** > Class I MHC **HLA-B** > **HLA-A** (mentioned above)
- Identical twin: full match
- Parents: 50% match, high risk of GVHD
 - Chemotherapy after the transplantation to prevent reactive T cells

Minor histocompatibility antigens

- Minor antigens (mHAGs) play an important role in pathogenesis of GVHD

Immunology of Allogeneic HSCT

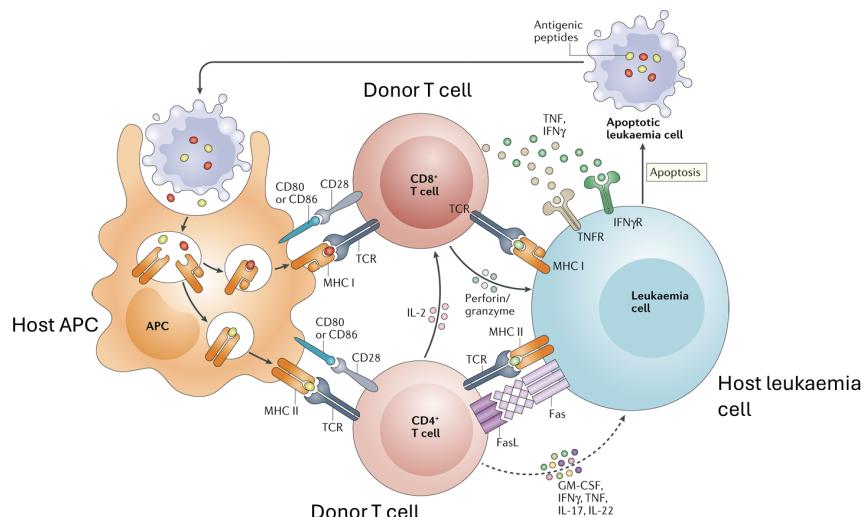
Non-self-HSCs give rise to non-self innate and adaptive immune cells

- Graft versus Malignancy effect
- Graft versus Host disease
 - But for leukemia patients, taking the risk is more beneficial than death

Graft versus Malignancy (GvM) effect

- Immunotherapeutic effect of allogeneic HSCT.
- Occurs when **donor T cells** recognise the tumour as 'foreign'.
- Confers a lower risk of relapse (of leukemia)
- This lower rate of relapse accounts for the increased success rate of allogeneic transplants if transplant-related mortality is not too high.
- Provides rationale for transplants that do not employ the most intensive myelo-suppressive regimens (so-called reduced intensity transplants).
- In some patients who relapse after transplant, the GvM capacity of the donor's lymphocytes can be harnessed again, by infusing the patient with more of the donor's lymphocytes (DLI).

Mechanism



Graft versus host (GVH) disease

- The graft may contain HLA-incompatible T cells and attack host tissues
 - e.g. a bone marrow or peripheral blood haematopoietic stem cell graft

- Especially skin, lung and gut, leading to life-threatening **GVH disease**.
- **a systemic disorder that occurs when the graft's immune cells recognize the host as foreign and attack the recipient's body cells**
- The risk of this occurring can be reduced by removing the T lymphocytes prior to transplantation and using immunosuppressive drugs.

Symptoms of chronic GVHD

- The earliest sign is often an itchy, dry rash. A fever may also develop

Decreased appetite	Diarrhoea	Abdominal cramps	Weight loss
Jaundice	Enlarged liver	Bloated abdomen	Pain in the upper right part of the abdomen
Increased levels of liver enzymes in the blood	Tightening of the skin	Dry, burning eyes	Dryness or ulceration in the mouth
Burning sensations when eating acidic foods	Bacterial infections	Shortness of breath or cough	



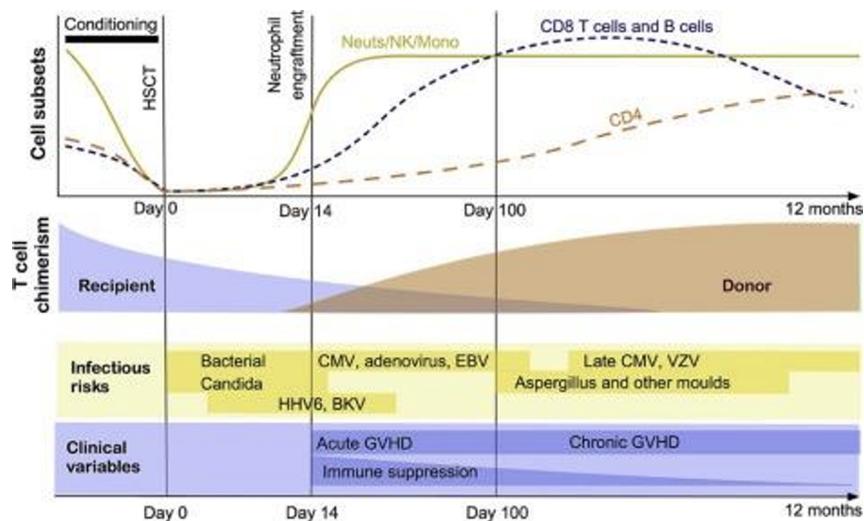
GVHD involves activation of donor T cells.

Graft rejection

- *Graft rejection* refers to a primary lack of donor engraftment or a secondary loss of donor engraftment
 - For HSCT the most serious form is that the counts of the immune cells never come up after Day 0 - host immune system rejected the new bone marrow
- *Graft failure* is the term generally applied to persistent cytopenias 血球減少 in the presence of proven donor engraftment
- Both are rare, because the pre-transplant treatment (chemo and/or radiation) mostly destroys the host immune system.

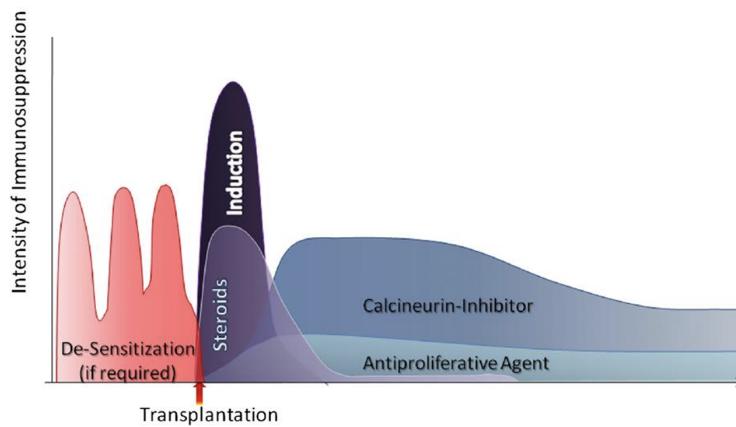
- More common in patients whose donor is not well matched and in patients who get stem cells that have had the T-cells removed
 - Older patients: More gentle chemotherapy to remove the existing bone marrow and higher risk of rejection
- It can also happen in people who get a low number of stem cells, such as a single umbilical cord unit.
- Graft rejection can be treated by reconditioning the patient and administration of a second dose of stem cells.

Immune recovery post allogeneic HSCT

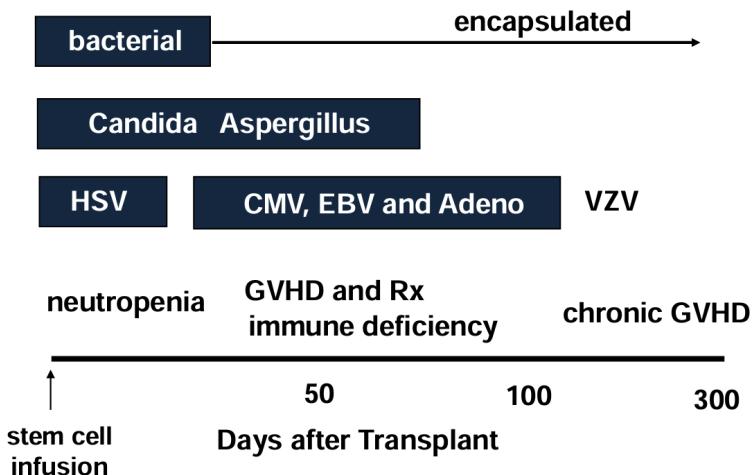


- No immune system for 11 days and immune cells are produced again start from innate cells followed by CD8 T cells and B cells, lastly CD4
 - Risk of infections
- Gradual shift of T cells from recipient to donor

Complications of immune suppression



Infections (Timing)



Immune reconstitution

- Median time to total lymphocytes $>10E9/l$ post T cell depleted Allo and RI
Allo approx 4 and 9 months respectively
- Delayed by chronic GVHD (prolonged immunosuppression with CSA and steroids, and often hypogammaglobulinaemia)
- Permanent loss of T cell repertoire
- Protective Ab generation occurs > 1 year post transplant**
- Re-vaccination protocols vary, typically start at around 12 months post HSCT

Late complications

- Relapse of underlying disease
- Infertility

- Harvest sperm or eggs before
- Endocrine failure
- Secondary malignancies
- Cataracts
- Cardiorespiratory failure & accelerated atherosclerosis

Summary

- Solid organ transplant is performed to treat end-organ failure
- Many organs can now be allografted. Some established, some experimental.
- The immune response is critical to understanding the basis of transplantation and its complications.
- AlloHSCT is performed for lots of indications (not just bone marrow failure)
- AlloHSCT differs from solid organ transplant in that the graft becomes tolerised to the recipient.

Transplantation is clearly an effective treatment for end-stage organ failure, and improvements in immunosuppression have ensured that few grafts are lost through acute rejection.

However, there has been little improvement in long-term kidney graft survival over the past 15 years, and chronic allograft rejection continues to be the main cause of graft failure more than 1 year after transplantation.

Certain immunosuppressive agents that effectively prevent acute rejection may themselves contribute to chronic graft damage, and the requirement for long-term immunosuppression increases the risk of infections, malignancy, and cardiovascular damage.

Moreover, transplant waiting lists continue to expand more quickly than organ donation rates, leading to ever-increasing numbers of patients who are unable to receive a transplant.

These two problems are driving significant research efforts into strategies for inducing immunological tolerance and alternative sources of tissue for transplantation.

Immunological tolerance to the graft would obviate the need for immunosuppression and was first demonstrated in experimental studies more than five decades ago.

However, it remains an elusive goal in clinical transplantation.

Alternative sources of organs include xenotransplantation and regenerative medicine.

Xenografts

- (especially pig organs) are potentially an answer to the paucity of donor organs
- This poses particular problems in the form of natural human antibodies to sugars expressed on the surface of pig cells.
- Pigs have therefore been genetically modified to remove the target of these antibodies, but concerns remain regarding the feasibility of xenotransplantation.

Organ cloning using differentiated stem cells

- another approach to overcoming donor shortage and organ rejection
- Considerable progress has been made in generating organs, especially by using acellular organ scaffolds as a substrate for differentiating stem cells