Transplantation Immunology

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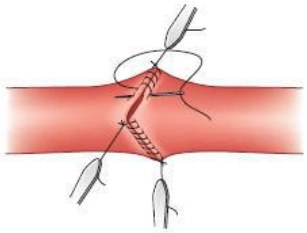
History of transplant immunology

Alexis Carrel

Sir Peter Brian Medawar



First vascular anastomosis

Breakthrough event

Surgical aspect: *Alexis Carrel* (French)

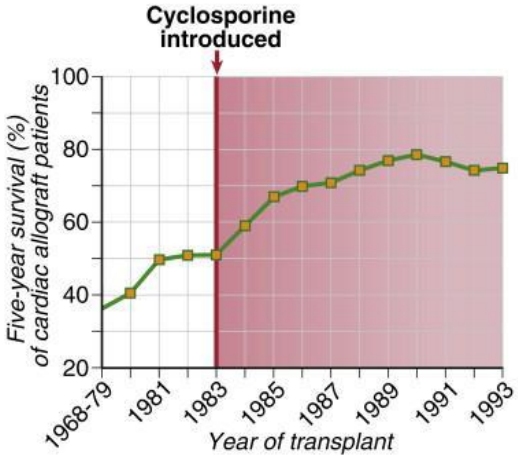
- Developed technique of vascular anastomosis

Biological aspect: *Sir Peter Brian Medawar* (1940s, British)

- Skin graft in animal models and human burn patient
- Reported allograft rejection
- → "Transplant immunology"

Timeline

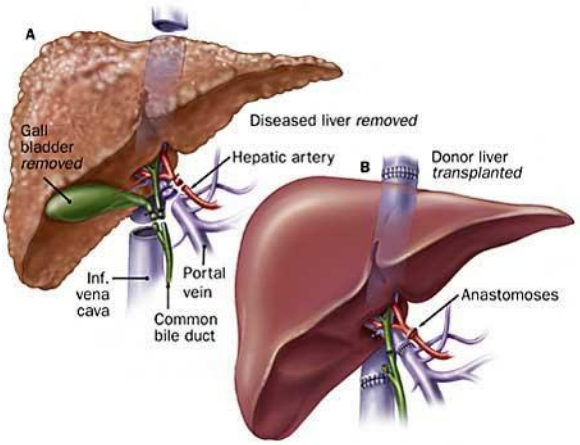
- 1954 Murray 1st Kidney Tx
- Early 1960s .... Combined immunosuppression
- 1963 Starzl 1st Liver Tx
  Hardy 1st Lung Tx
- 1966 Lillehei 1st Pancreas Tx
- 1967 Barnard 1st Heart Tx
  Lillehei 1st Small intestine Tx
- Early 1980s .... Cyclosporine



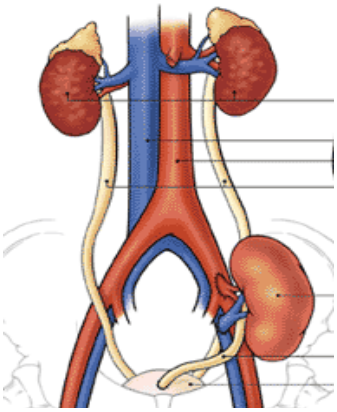
Influence of cyclosporine on graft survival

Definitions

- Transplantation: The process of transferring an organ, tissue or cell from one place to another
  Graft: The process of taking cells, tissues or organs
  Donor: The individual who <u>provides</u> the graft
  Recipient/Host: The individual who <u>receives</u> the graft
- Organ transplantation: A surgical procedure in which a failing organ is replaced by a functioning one
  Orthotopic: Implanted in <u>same anatomical</u> location e.g. heart, lung, liver, intestine



  Heterotopic: Implanted in <u>another anatomical</u> location e.g. kidney, pancreas



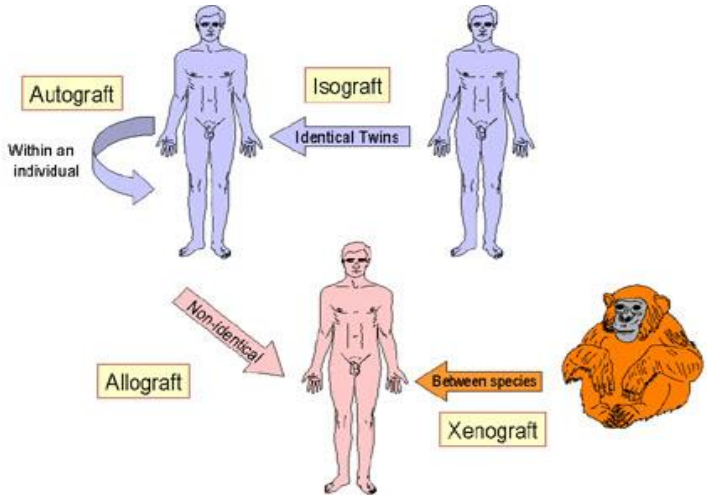
- Type of grafts

  Autograft: same person

  Isograft: genetically identical twin

  Allograft: to another same species

  Xenograft: to different species



Immune response

- Transplant Antigens
- Major histocompatibility antigens – MHC

  Main antigens of grafts rejection

  Difference of HLA types is the main cause of human graft rejection

  Several different classes of T-cells may recognize MHC molecule
- Minor histocompatibility antigens

  Also cause grafts rejection, but slow and weak

  Several minor antigens may result in rejection, even when MHC antigens are concordant between donor and recipient
- Other alloantigens

  Human ABO Bl.group antigens

  Some tissue specific antigens

  - Skin, kidney, heart, pancreas, liver
  - Vascular endothelial cell (VEC) antigen
  - Skjelbred (SK) antigen

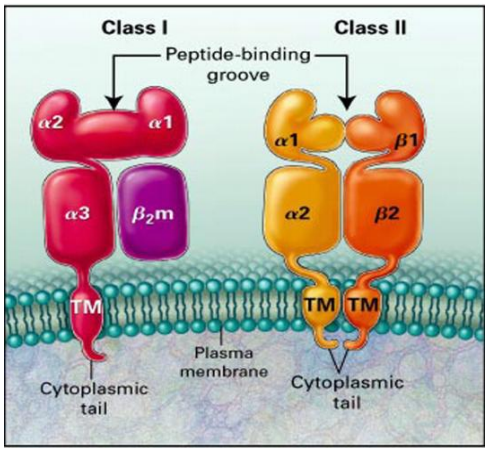
- Major histocompatibility complex (MHC)
- Highly polymorphism
- Genes are found on chromosome 6
- Essential for T-cell recognition and response

MHC class I antigens

- HLA-A, HLA-B, HLA-C
- These antigens are glycoproteins found on surfaces of all nucleated human cells and platelets
- Class I MHC antigens are involved of MHC restriction of cell mediated cytotoxicity

MHC class II antigens

- HLA-DP, HLA-DQ, HLA-DR
- These antigens are glycoprotein found on the surface of macrophages, B-lymphocytes, dendritic cells, langerhans cells, activated T cells, endothelial cells, thymic epithelium



Major histocompatibility complex

Feature	MCH class I	MCH class II
HLA	HLA-A, HLA-B, HLA-C	HLA-DP, HLA-DQ, HLA-DR
Types of APCs	All nucleated somatic cells	Macrophages, B-lymphocytes, dendritic cells, Langerhans cells, activated T cells
Responsive T cells	CD8+ Cytotoxic T-cells	CD4+ Helper T-cells
Functions	Presentation of Antigen to TC cells leading to elimination of tumor or infected host cell	Presentation of Antigen to TH cells which secrete cytokines

- Human leukocyte antigens (HLA)

- To present the fragment of foreign proteins to T-lymphocytes
- Lead to recognition → elimination of the foreign antigen
- HLA trigger rejection

  Cellular rejection by T-lymphocyte

  Humoral rejection by circulating Ab against the donor's HLA

- Cellular components

- T-lymphocyte

  Play a major role in cell-mediated immunity

  CD8-positive T-cells : *cytotoxic T-cells*

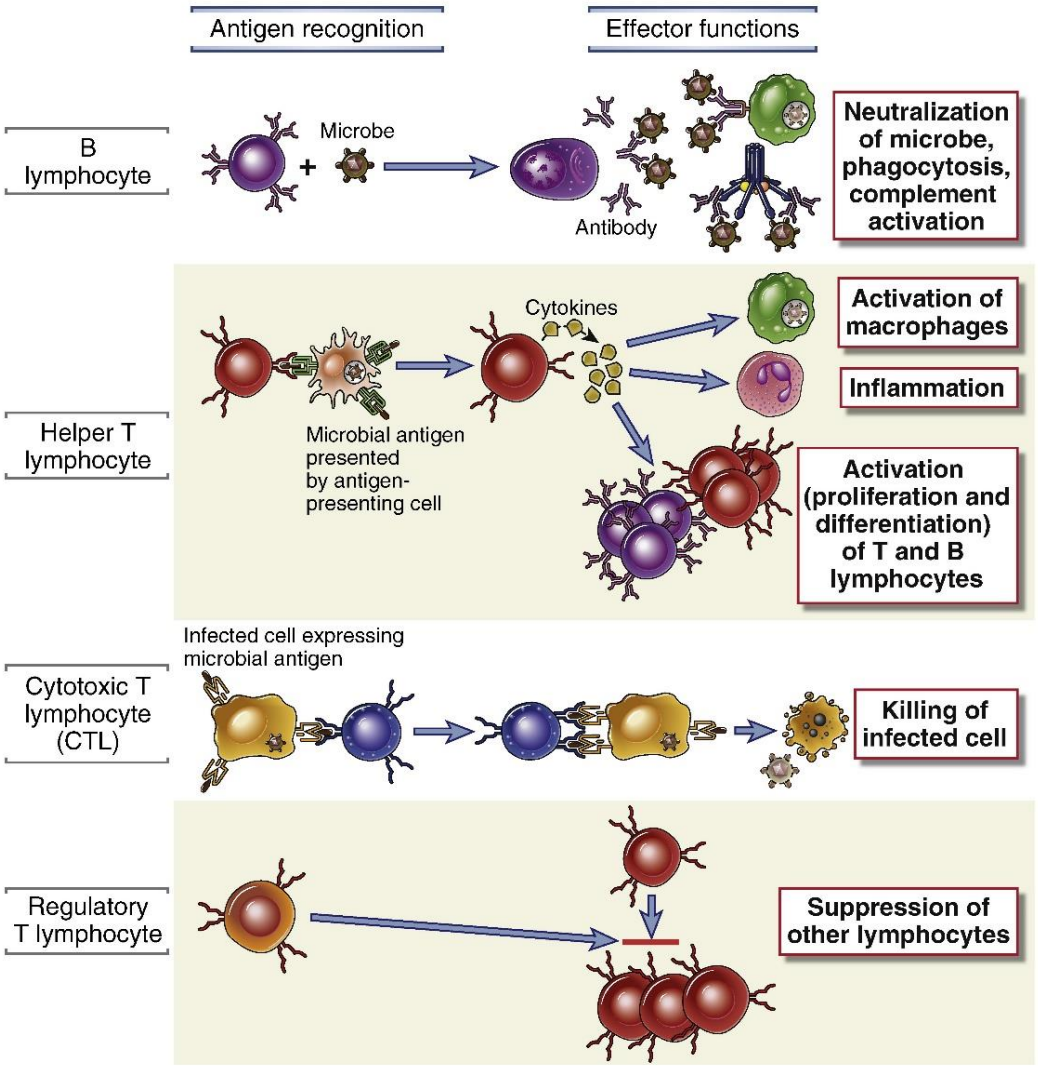
  - Response to antigenic peptide derived from intracellular protein *bound to MHC class I*
  - Function → Cell lysis

  CD4-positive T-cells : *helper T-cells*

  - Engage antigenic peptide derived from extracellular protein *bound to MHC class II*
  - Function → coordinating the efficacy of innate immune response, promoting the activation and proliferation of CD8+

- B-lymphocyte

  Play a major role in antibody-mediated immunity



Transplant rejection

- Mechanisms of Graft Rejection – Types
- T cell-mediated Reactions

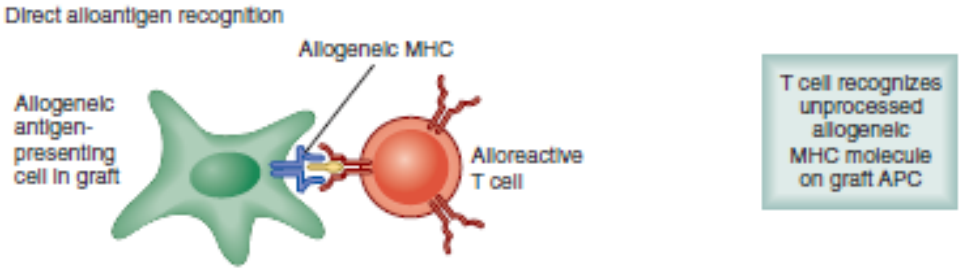
  Direct Pathway

  Indirect Pathway
- Antibody-Mediated Reactions

- Recognition of alloantigens
- Direct presentation

  Recipient's T-cells are activated by direct interaction with the donor's HLA molecules

  Involves both CD8+ and CD4+ T-cells



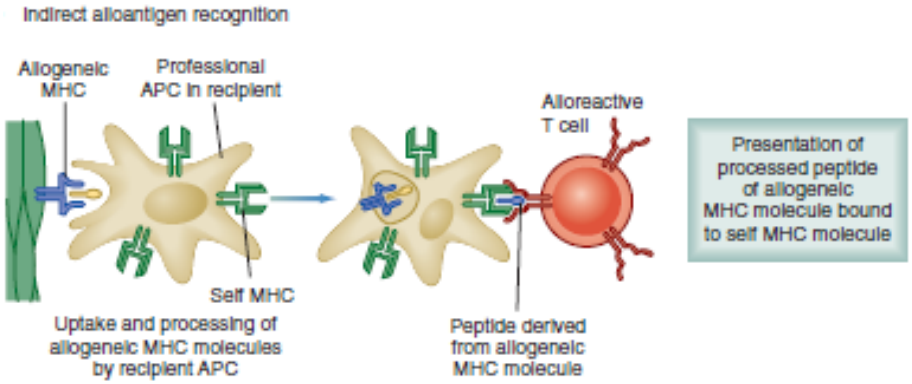
- Indirect presentation

  Recipient's T-cells are activated by interaction with APCs that have processed and presented the foreign antigen

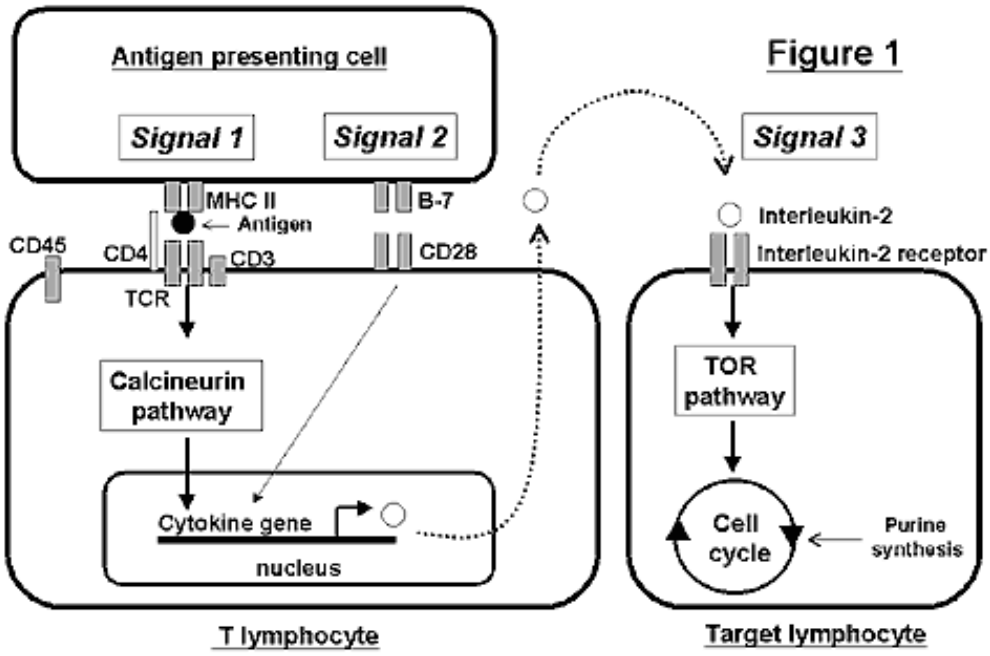
  Recipient APCs are able to process the donor Ag and present the resulting foreign peptides to T-cells using self MHC molecules

  The host APCs digest the foreign Ag through phagocytosis

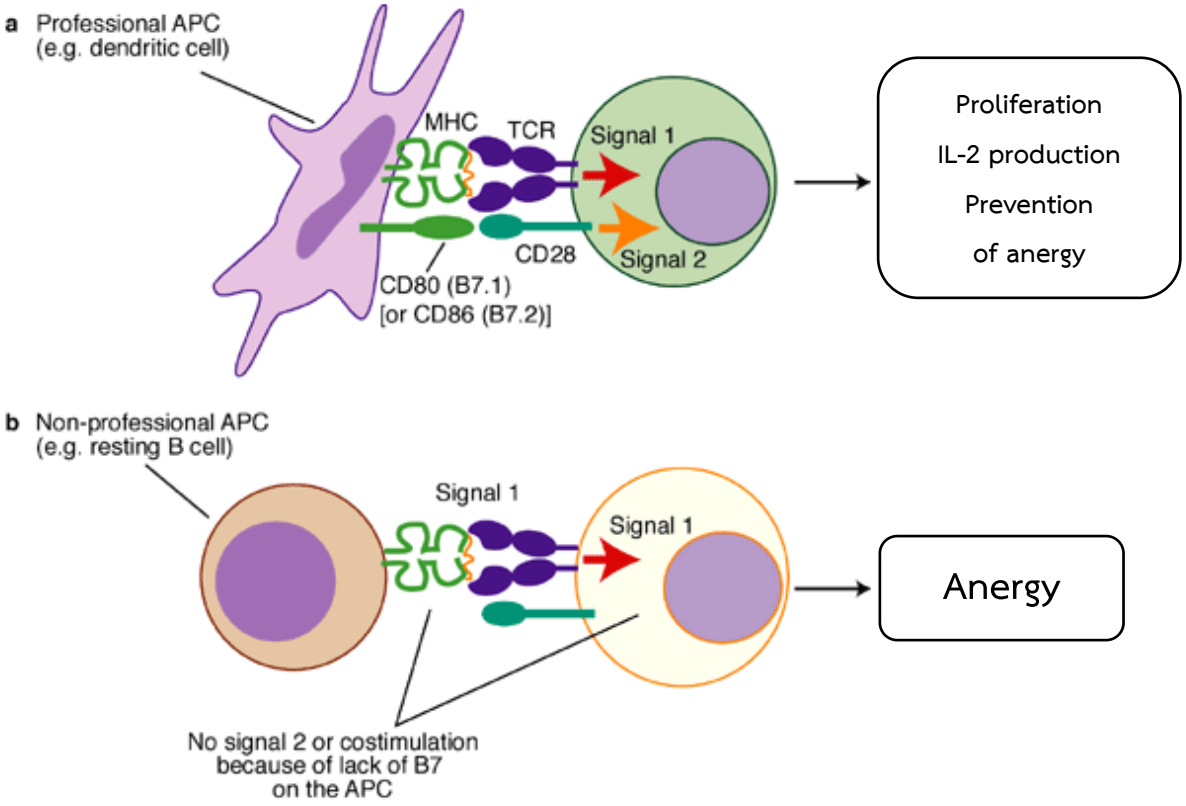
  The peptides from the digested complex ate presented by host MHC molecules to either CD4+ or CD8+ T-cells



- T-lymphocyte activation



- Signal 1: Antigen Presenting cells: T-cell receptor (TCR)
- Signal 2: Co-stimulatory molecule: CD28 (on surface T cell) → ligand CD80 (B7-1),or CD86(B7-2)
- Signal 3: Cytokine production and release



The role of signal 1 and 2 T-cell activation

- Clinical rejection

- Hyperacute rejection

  Within minute-hour following graft reperfusion

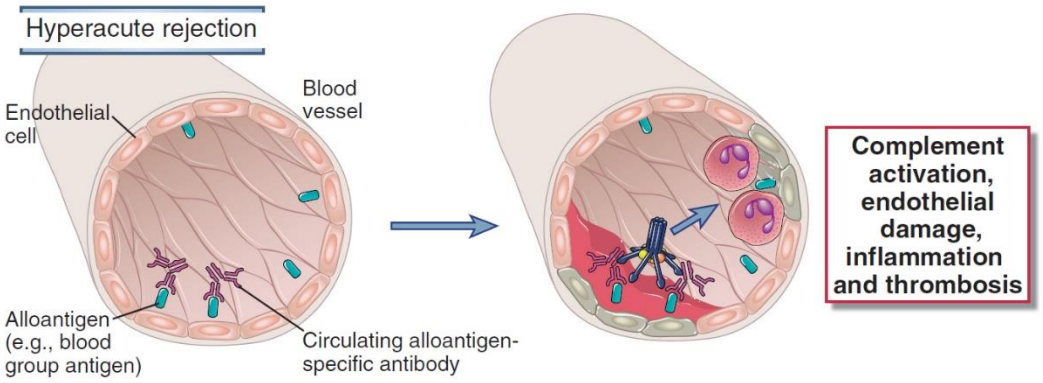
  Mediated by preformed antibody

  Irreversible damage, Untreatable

  Preventable → "pre-transplant crossmatch"

  Target is donor vascular endothelium

  Rapid diffuse intravascular thrombosis, graft necrosis

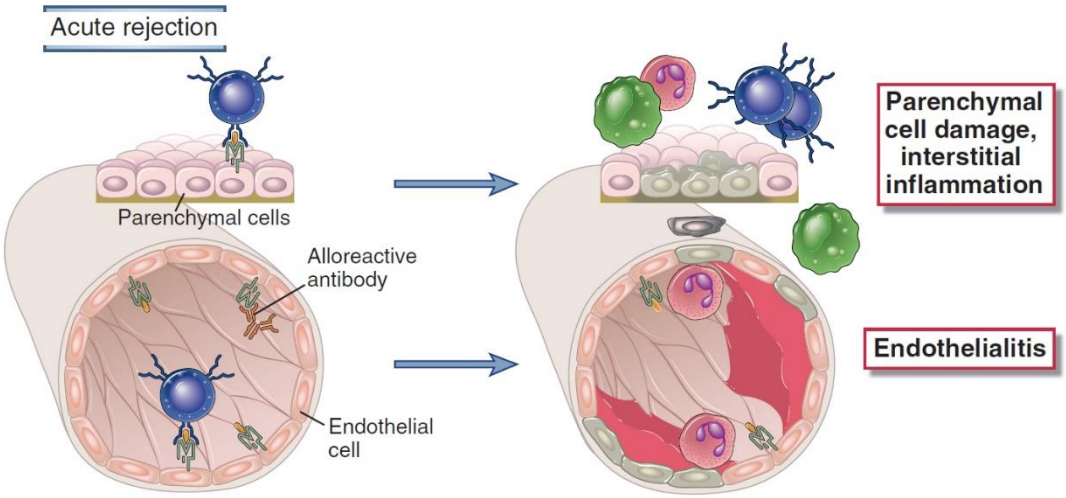


- Acute rejection

  Occur within a few days–weeks post-transplant

  Mechanism

  - T-cell mediated rejection (TCMR)
  - Antibody-mediated rejection (ABMR)- (humoral pathway)



- Chronic rejection

  Slow type of rejection

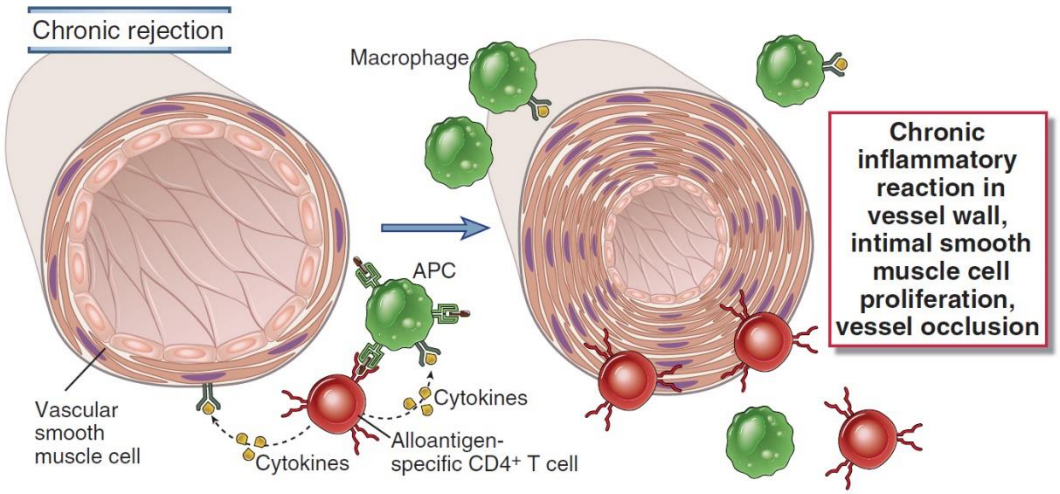
  Progress gradually over several years

  Mechanism

  - Immunologic mediated
  - Non-immunologic mediated

- Lead to fibrosis and loss of graft function

Immunologic mediated	Non-immunologic mediated
Associated with presence of anti-donor antibody Repeated/indolent TCMR or ABMR	Ischemia/reperfusion injury Oxidative stress Hypertension/dyslipidemia Side effect of medication



- Pre-transplant crossmatch

- Checking of Compatibility to prevent rejection
- Compatibility of donor and recipient must be determined before transplantation to prevent or minimize rejection
- The following tests are done:

  ABO blood group compatibility

  HLA antigens by tissue typing

  Cross-matching between recipient's serum for preformed antibodies against donor's HLA antigens

Immunosuppression

- General principle

- Immune reactivity & the likelihood of graft rejection are highest initially and decrease over time
- Use low doses of several drugs with non-overlapping toxicities
- Avoid over-immunosuppression which increases susceptibility to infection and malignancy

- Immunosuppressive drugs

- Nonbiological immunosuppression

  Immunophillin binders

  - Calcineurin inhibitors (CNI) : Cyclosporine, Tacrolimus(FK506)
  - Non Calcineurin inhibitors (mTOR): Sirolimus, Everolimus

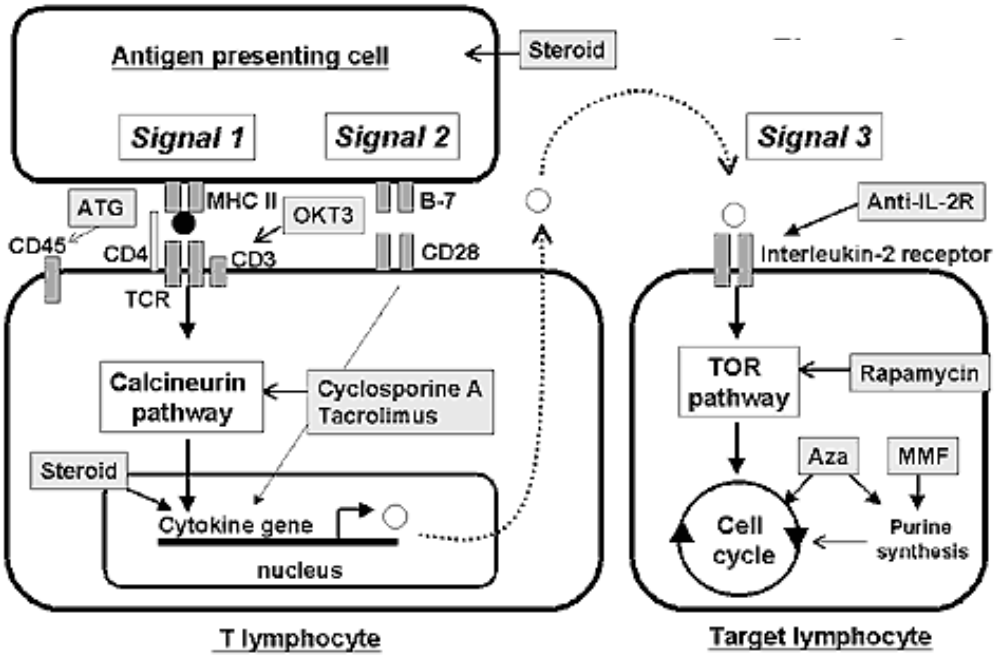
  Antimetabolites: Azathioprine, Mycophenolate mofetil (MMF)

- Biological immunosuppression

  Polyclonal antibodies

  Monoclonal antibodies: Anti-thymoglobulin AntiCD25, AntiCD3

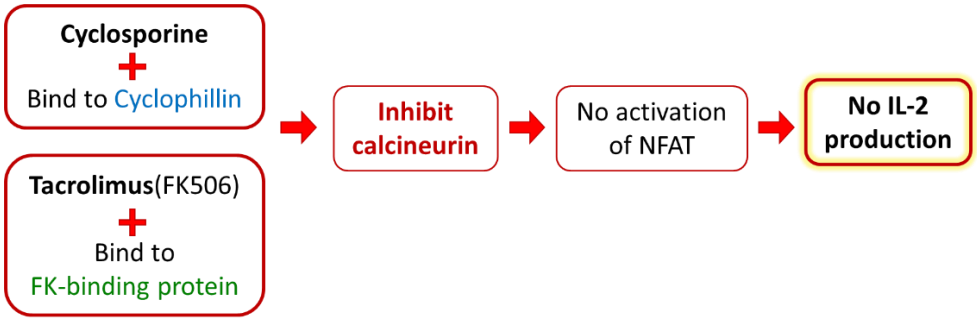
- Other: Corticosteroids



The main site of action of established immunosuppressive drugs

<u>Calcineurin inhibitors (CNI)</u>

- Mechanism of action



- Potency: Tacrolimus >>> Cyclosporine 50 -100 times

- Side Effects of CNI

  Renal toxicity

  - Vasoconstriction of the afferent and efferent glomerular arterioles and reductions in renal blood flow & GFR
  - The increase in renal vascular tone is "usually reversible" when therapy is stopped
  - Calcium channel blockers can prevent the renal vasoconstriction

  Hyperkalemia & hypertension

  - a result of their toxic effect on the kidney (High incidence of HT, dyslipidemia for Cyclosporine)

  Hyperglycemia

  - reduce insulin gene expression in pancreatic β cells → reduced insulin release (High incidence of new onset DM for Tacrolimus)

  Hypomagnesemia, alopecia, tremor, headache, seizure

  Gingival hyperplasia, hirsutism (only cyclosporine)

- CNI drug interactions

  CNI metabolized by P450 enzyme (CYP 3A4)

  Nephrotoxic Synergy

  - gentamicin, amphotericin B, cimetidine, bactrim, tobramycin, ketoconazole, ranitidine, vancomycin, diclofenac

  P-450 3A inhibitors (*Increase CNI Levels*)

  - diltiazem, ketoconazole, erythromycin, nicardipine, fluconazole, bromocriptine, methylprednisolone, verapamil, itraconazole, metoclopramide, amphotericin B

  P-450 3A inducers (*Decrease CNI Levels*)

  - rifampin, phenytoin, phenobarbital, carbamazepine

<u>mTOR inhibitors</u>

- Mechanism of action

  mTOR (mammalian Target of Rapamycin) regulates transcription, and hence protein synthesis, cell growth, cell proliferation & cell survival

  Bind FKBP but does not inhibit calcineurin

  Bind and inhibit mTOR and block the <u>response</u> to IL-2by blocking cell cycle progression in G1 → S

- Side effects

  Thrombocytopenia, Dyslipidemia, Impaired wound healing

  No renal toxicity

- Sirolimus Vs Everolimus

  Sirolimus (Rapamycin)

  - 1st mTOR inhibitor

Everolimus

- higher oral bioavailability
- shorter half-life
- more rapid time to steady state
- eliminates the need for a loading dose

Azathioprine

- Mechanism of action

  Converted to 6-mercaptopurine

  Inhibit both the de novo purine synthesis and salvagepurine synthesis

  Decrease T-cell activity and antibody production
- Side effects

  Myelosuppression, hepatotoxicity, pancreatitis, etc.
- Drug interaction

  Allopurinol (block AZA's metabolism)

Mycophenolate mofetil – MMF

- Mechanism of action

  Derivative of mycophenolic acid (MPA)-prodrug

  MPA inhibit inosine monophosphate dehydrogenase(IMPDH)

  IMPDH is rate-limiting enzyme in de novo synthesis of guanosine monophosphate

  Inhibit T & B lymphocyte proliferation
- Side effects

  Myelosuppression, GI symptoms, etc.
- Drug interaction

  Antacid

Antilyphocyte globulin (ATG)

- Polyclonal antibody

  Several immunoglobulin

  Produced by injecting immunogen into animal

  Immunogen may be lymphocyte, thymocyte or T-cell line

  Purified serum solution
- Mechanism of action

  Lymphocyte depletion

  Complement dependent cell lysis

  Cell apoptosis

- Side effects

  Cytokine release syndrome

  - Fever with chill 20%
  - Treatable with antipyretics and antihistamine

  Leucopenia, anemia

  Increase viral reactivation and primary viral infections

- Preparation

  ATGAM®

  - *horse* antithymocyte globulin

  Thymoglobulin®

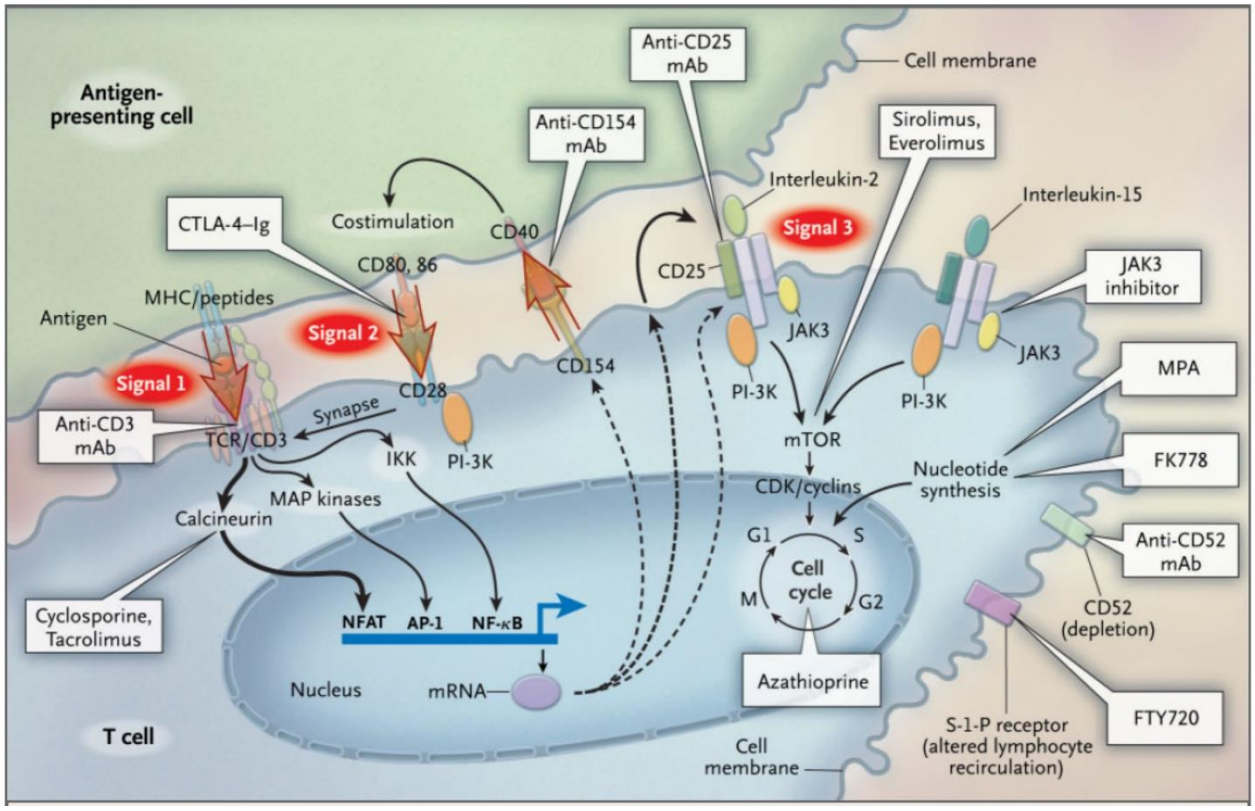
  - *rabbit* antithymocyte globulin (rATG)

- More effective than ATGAM at reducing the incidence of acute rejection episode

Monoclonal antibodies

- Anti-CD3 *Muromonab-CD3 (OKT3)*
- Anti-CD25 *Basiliximab, Dacluzimab*
- Anti-CD20 *Rituximab*
- Anti-CD52 *Alemtuzumab*

Monoclonal Ab	Mechanism of action	Side effects
Muromonab-CD3 (OKT3)	- Bind CD3 associated with the TCR - Leading to initial activation and cytokine release - Follow by blockade of function lysis - T-cell depletion	- Cytokine release syndrome - Pulmonary edema - ARF - CNS change
Basiliximab # chimeric	- Bind to high affinity chain of IL-2R (CD25) on activated T-cells causing depletion - Preventing IL-2 mediated activation	- Hypersensitivity (uncommon)
Dacluzimab # humanized		
Rituximab	- Binds to CD20 on B cells and causes depletion	- Hypersensitivity (uncommon)
Alemtuzumab	- Binds to CD52 - Expressed on most T and B-cells, macrophages, NK cells, monocytes; causing lysis and prolong depletion	- Mild cytokine release syndrome - Neutropenia, anemia - Autoimmune thrombocytopenia



Molecular mechanism of immunosuppressive drugs

Corticosteroids

- Mechanism of action

  Inhibit macrophage function

  Reduce cytokine production and adhesion molecule expression

  Induced lymphocyte apoptosis

- Side effects

  Diabetes, osteoporosis, weight gain, psychological disturbances, ulcers, wound healing, adrenal suppression

- Difference phases of therapy

- Induction phase: *T cells are the primary mediators of rejection*

  Administered immediately post-transplant to induce immunosuppression

  Potent immunosuppressive drugs

  For T cell depletion, interrupt T cell activation & proliferation

  - Depleting (polyclonal) antibodies / Non-depleting antibodies

- Maintenance therapy

  Administered to maintain immunosuppression after recovered from operative procedure

  Combination of low dose of drugs

  Non-overlapping toxicities

Induction regimens

- High dose of conventional immunosuppressive agents

  Corticosteroids: Prednisone

  Antimetabolites: MMF or Azathioprine

  Calcineurin inhibitors: Cyclosporine or Tacrolimus

- PLUS

  One or more of the following antibody reagents:

  - IL-2 receptor antagonist: Basiliximab
  - Polyclonal anti-thymocyte Ab: ATG
  - Monoclonal anti-thymocyte Ab: Alemtuzumab

Maintenance regimens

- Multiple drug therapy using combinations of two or three of the following types of immunosuppressant

  Calcineurin inhibitor: Cyclosporine or tacrolimus

  Anti-metabolite: Azathioprine or MMF

  Corticosteroid: Prednisone (tapered over the 1st year)

  mTor inhibitors: Sirolimus or Everolimus (in place of a CNI or antimetabolite)

- Complication of immunosuppression

- Risk of infection

  Highest 6 -12 weeks after transplantation

  Prophylactic regimen is needed

- Risk of malignancy

  10-fold increase in malignancy rate

  Skin cancers (SCC)

  Virally mediated tumors – HPV (CA cervix), HBV, HCV (HCC), HSV (Kaposi's sarcoma), EBV (PTLD)

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