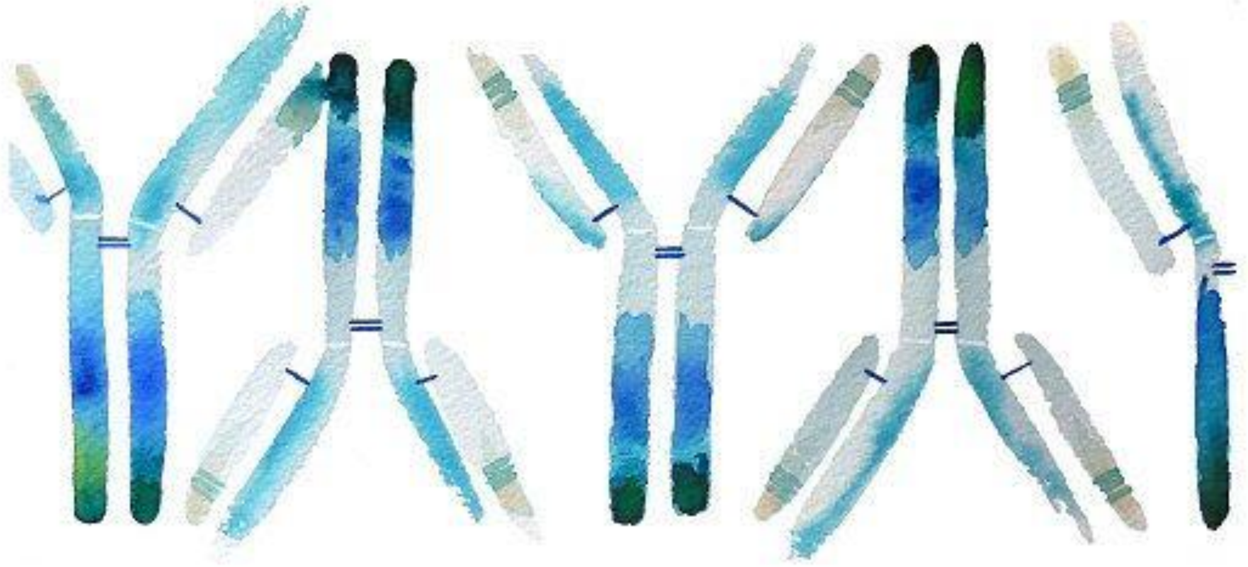
Medical Immunology



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M.D. Ph.D.

In this lecture we will discuss:

- MHC restriction
- Transplantation immunology

Major histocompatibility complex (MHC)/ discovery

- First it was found that individuals who had received multiple blood transfusions and patients who had received kidney transplants contained **antibodies** that **recognized cells from the donors**. and **multiparous women** had circulating antibodies that **recognized paternal cells**.

- Those antibodies bound to antigens called **human leukocyte antigens** (HLA) (leukocyte because the antibodies were tested by binding to the leukocytes of other individuals).

- Then, mice injected with a pathogen were found to have a variable response, better responder strains, which can mount immune responses to a particular polypeptide antigen, inherit MHC genes whose products can bind peptides derived from these antigens, forming peptide- MHC complexes that can be recognized by helper T cells.

Major histocompatibility complex (MHC)

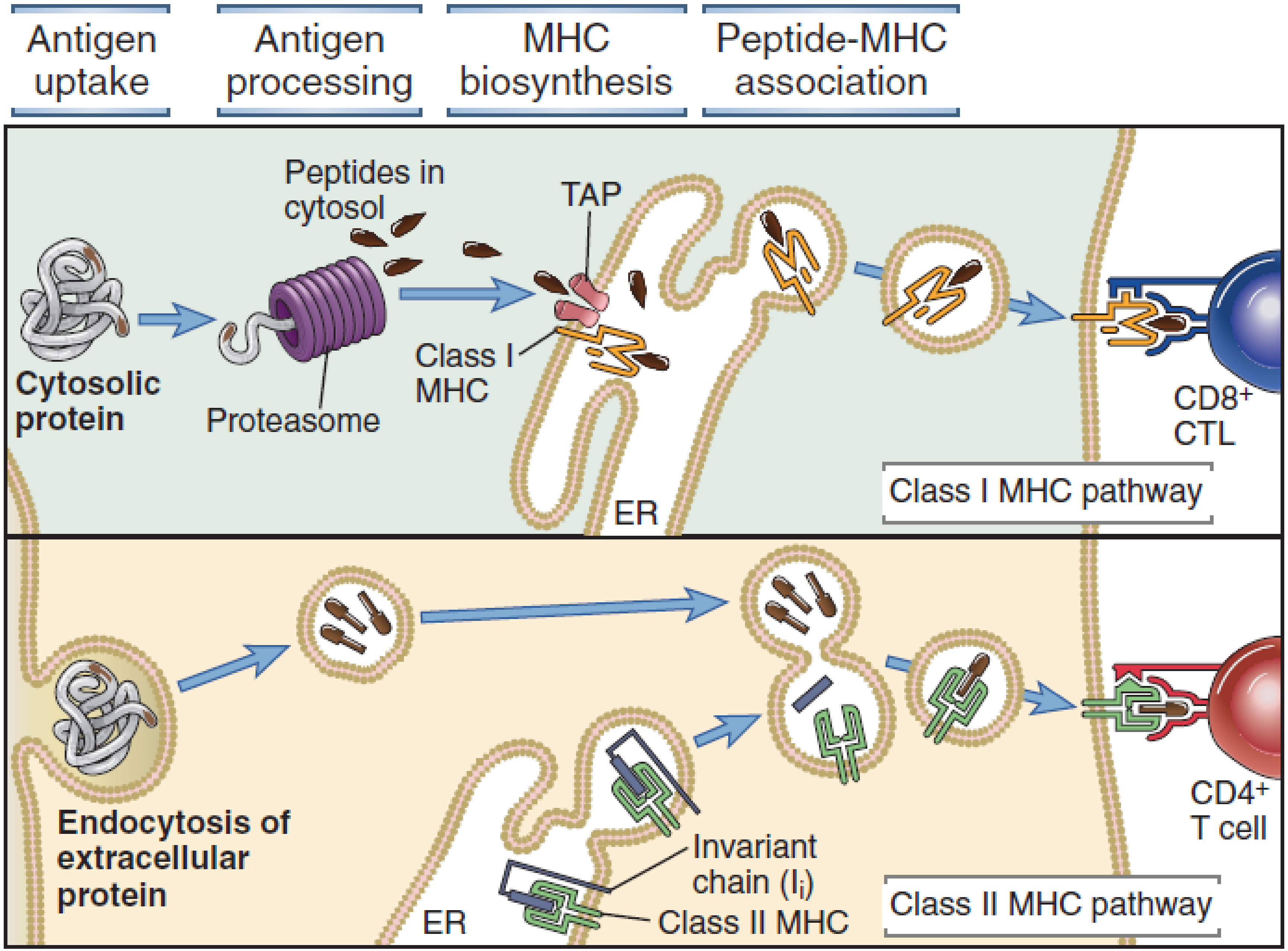


FIGURE 6–14 Pathways of antigen processing and presentation. In the class I MHC pathway *(top panel)*, protein antigens in the cytosol are processed by proteasomes, and peptides are transported into the endoplasmic reticulum (ER), where they bind to class I MHC molecules. In the class II MHC pathway *(bottom panel)*, extracellular protein antigens are endocytosed into vesicles, where the antigens are processed and the peptides bind to class II MHC molecules. Details of these processing pathways are in Figures 6-16 and 6-17.

Major histocompatibility complex (MHC)

- T-cell receptors **recognize features both of the peptide antigen and of the MHC molecule** to which it is bound.

- Any given T-cell receptor is specific not simply for a foreign peptide antigen, but for **a unique combination of a peptide and a particular MHC molecule**, this is known as **MHC restriction.**

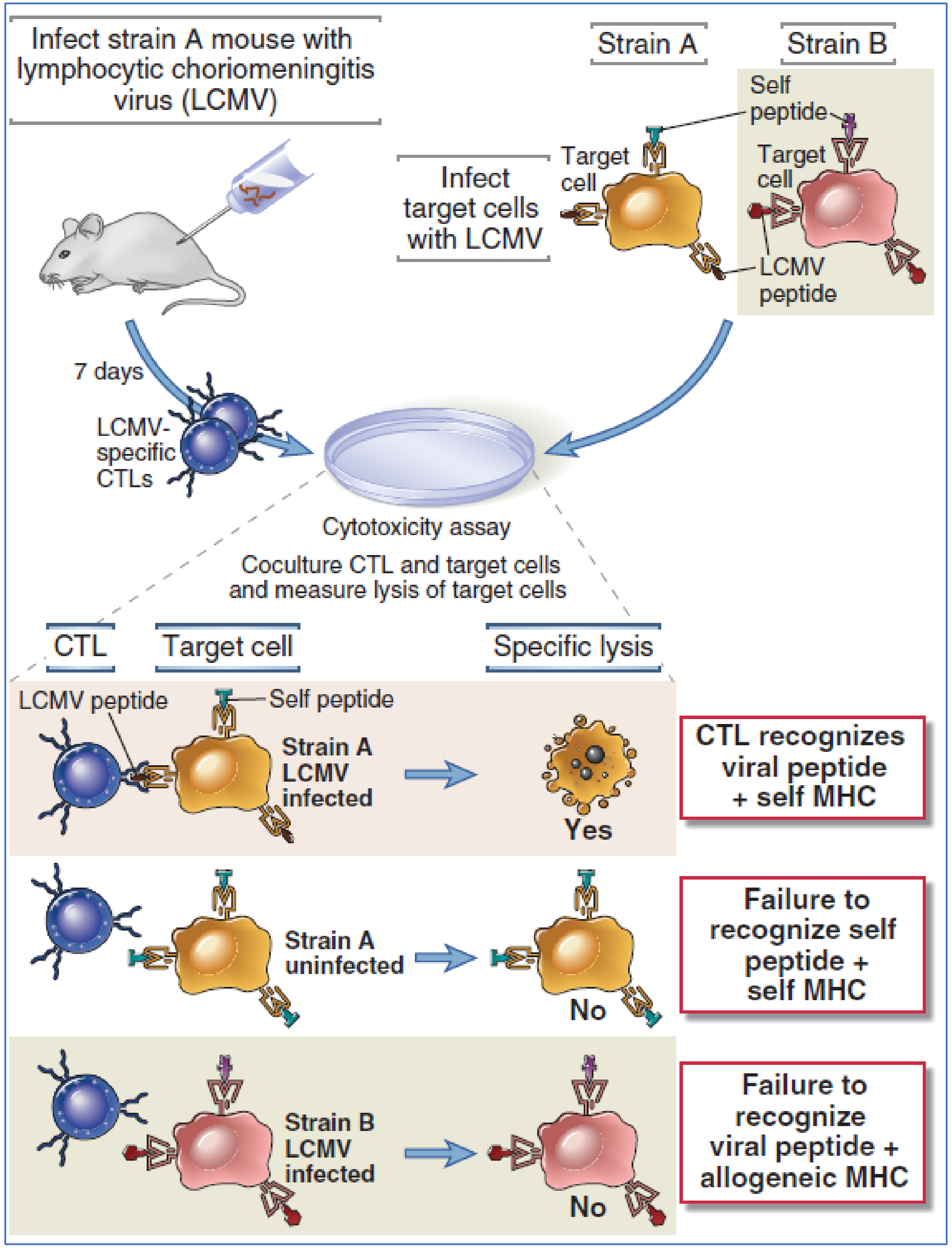


FIGURE 6–6 Experimental demonstration of the phenomenon of MHC restriction of T lymphocytes. Virus-specific cytotoxic T lymphocytes (CTLs) generated from virus-infected strain A mice kill only syngeneic (strain A) target cells infected with that virus. The CTLs do not kill uninfected strain A targets (which express self peptides but not viral peptides) or infected strain B targets (which express different MHC alleles than does strain A). By use of congenic mouse strains that differ only at class I MHC loci, it has been proved that recognition of antigen by $CD8^+$ CTLs is self class I MHC restricted.

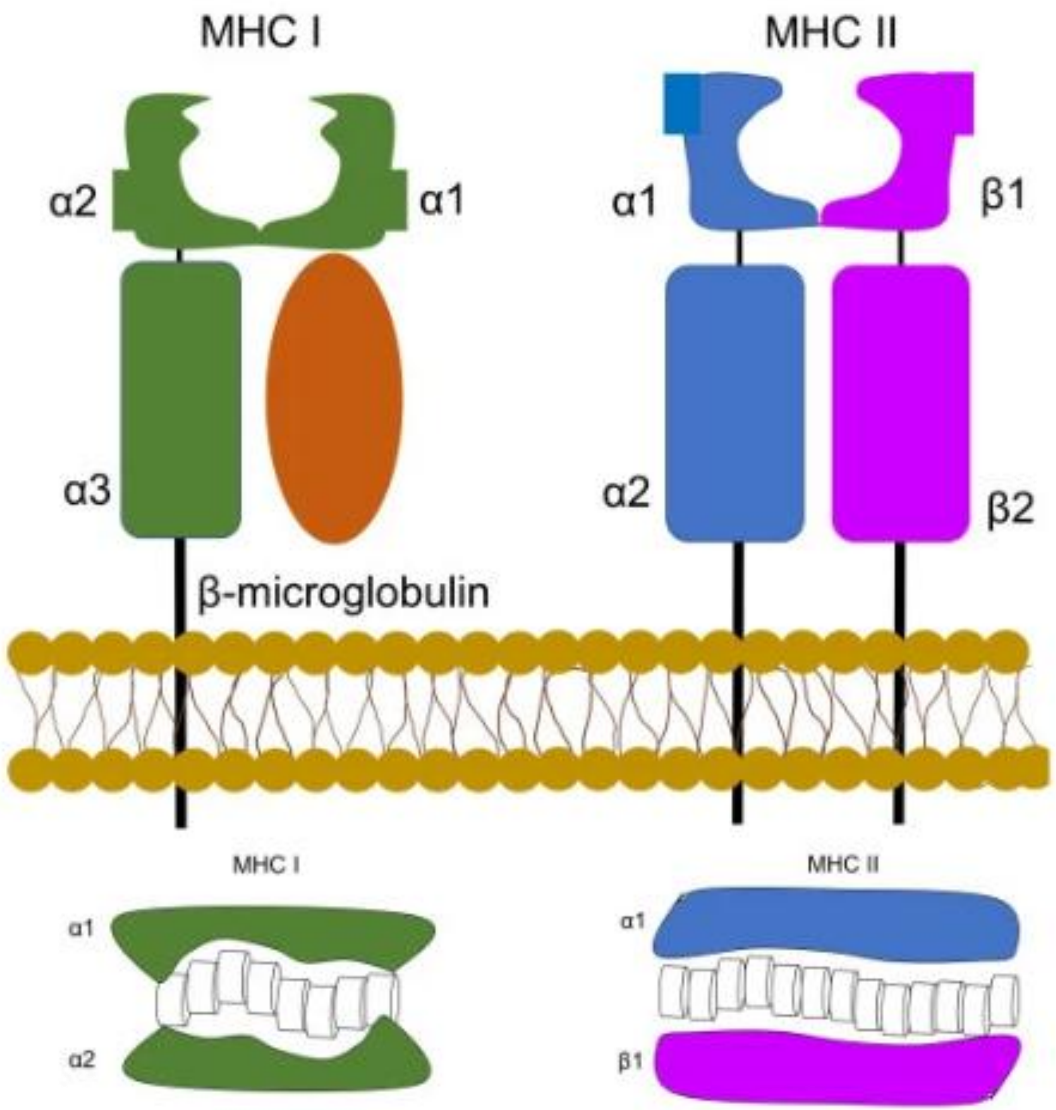
Major histocompatibility complex (MHC)

- The MHC molecules are glycoproteins encoded in the large cluster of genes known as the major histocompatibility complex (MHC). Their most striking structural feature is **a cleft running across their outermost surface, in which a variety of peptides can be bound.**

- Each class I or class II MHC molecule has a **single peptide-binding cleft** that binds one peptide at a time, but each MHC molecule **can bind many different peptides.**

- MHC molecules show great genetic variation in the population, and each individual carries up to 12 of the possible variants which increases the range of pathogen-derived peptides that can be bound. (Molecular sequencing has shown that a single serologically defined HLA allele may actually consist of multiple variants that differ slightly).

Major histocompatibility complex (MHC)



The Classical MHC molecules

MHC Class I and Class II present peptides to immune cells as part of routine immune surveillance.

LEFT: MHC Class I is comprised of three alpha subunits and beta macroglobulin. The binding groove (lower left) of Class 1 is deep, with closed ends and binds peptides of 8-10 amino acids in length.

RIGHT: MHC Class II is a heterodimer of a 2-unit alpha chain and a 2-unit beta chain. The binding groove of class II is shallow and open at each end, allowing binding of peptides 13-17 amino acids in length.

Major histocompatibility complex (MHC)

- Human MHC class I and II are also called human leukocyte antigen (HLA).

- The set of alleles that is present in each chromosome is called the MHC haplotype. In humans, each HLA allele is named with a number. For instance, for a given individual, his haplotype might be HLA-A2, HLA-B5, HLA-DR3, etc... Each heterozygous individual will have two MHC haplotypes, one each from the paternal and maternal chromosomes. MHC alleles are expressed in a codominant fashion. This means the alleles (variants) inherited from both parents are expressed equally.

- The MHC genes are highly polymorphic; many different alleles exist in the different individuals inside a population.

- Each person carries 2 alleles of each of the 3 class-I genes, (HLA-A, HLA-B and HLA-C), and so can express six different types of MHC-I. one heterozygous individual can inherit six or eight functioning class-II alleles, three or more from each parent

HLA polymorphism

- Genes of the MHC are the most polymorphic genes of the human genome. Polymorphisms in this locus were first defined phenotypically by acceptance or rejection of tissue or by reaction with defined antibodies (serological typing).
- The HLA types are detected by **phenotypic** or **genotypic** typing methods. Molecular typing methods reveal HLA polymorphisms as base changes in the DNA sequence.
- Each HLA gene can differ in sequence from one individual to another, except for identical twins. A set of alleles on the same chromosome is a haplotype.

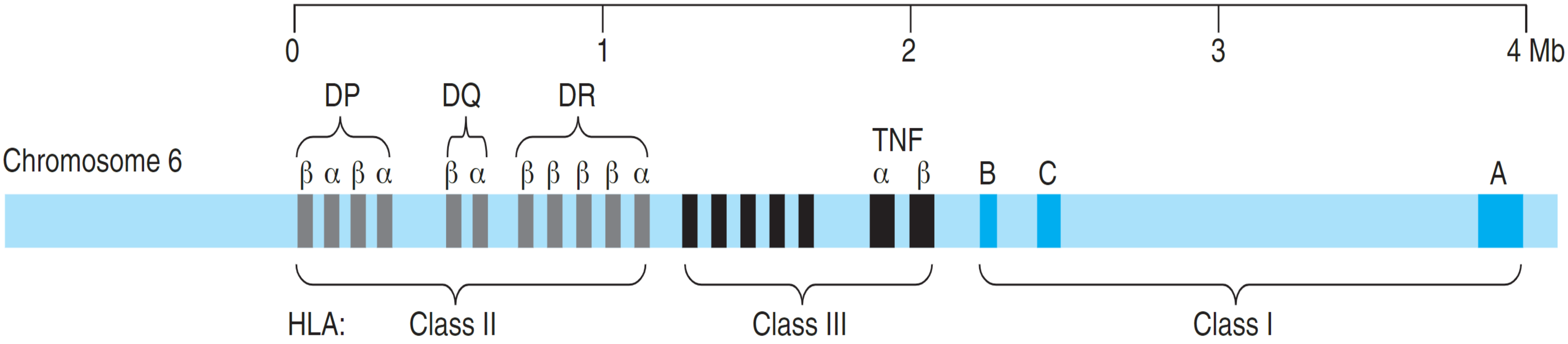


Figure 15-1 The MHC locus on chromosome 6 covers about 4 Mb of DNA, depending on the individual. Class I genes are 3–6 kb long, and class II genes are 4–11 kb in length. TNF-α and TNF-β are not part of the polymorphic HLA system.

The MHC locus includes genes other than those that code for the HLA. Cytokine genes and genes encoding tumor necrosis factor β (TNF-β) and tumor necrosis factor α (TNF-α) are located inside of the main HLA complex.

- The alleles of the haplotype are inherited together as a block of chromosomal sequence, unless a rare recombination within the region separates the alleles.
- An HLA haplotype is, therefore, the combination of polymorphic sequences or alleles in the HLA gene regions.
- The maternal and paternal HLA antigens are expressed codominantly on cells.

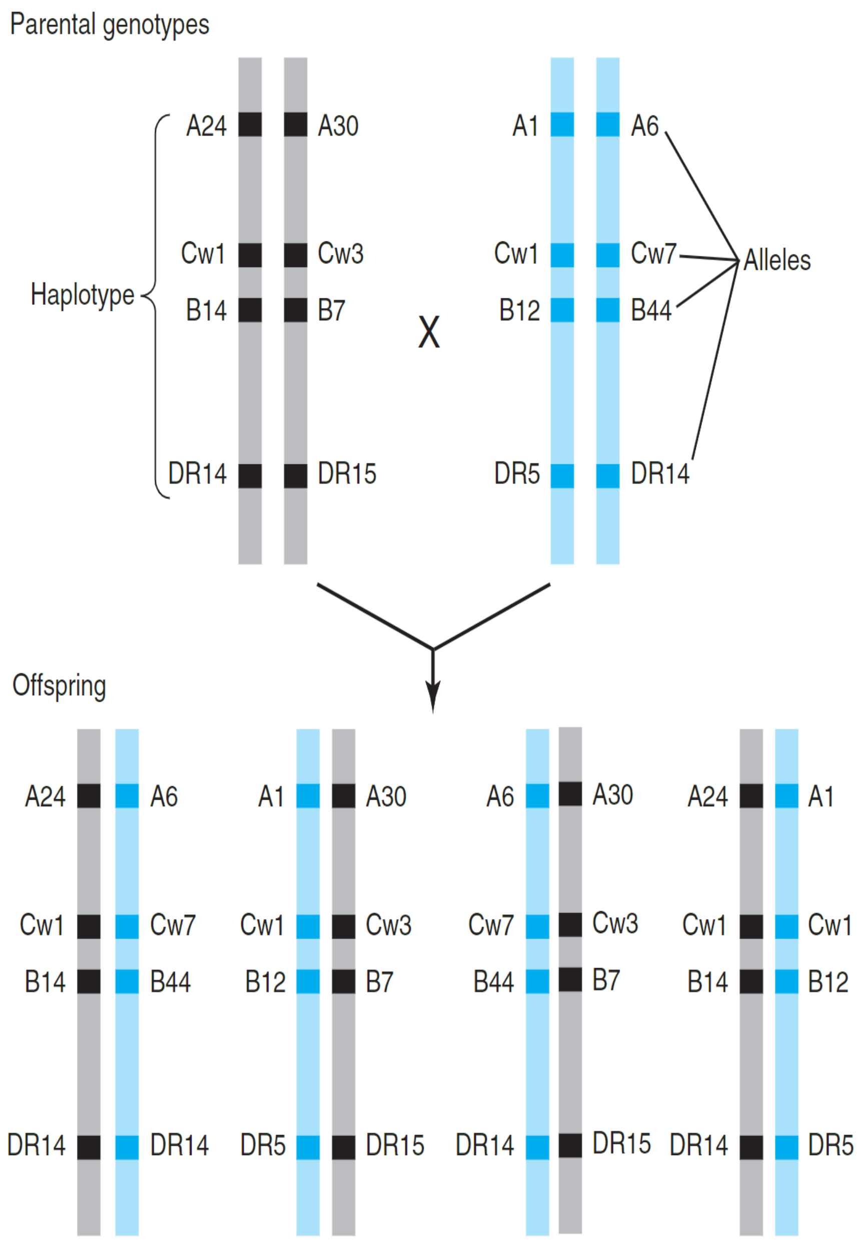


Figure 15-4 A haplotype is the combination of alleles that are inherited together. In this example, parental genotypes (top) can produce four possible genotypes in the offspring (bottom).

Table 15.1 **Genes of the Major Histocompatibility Locus**

MHC Region	Gene Products	Tissue Location	Function
Class I	HLA-A, HLA-B, HLA-C	All nucleated cells	Identification and destruction of abnormal or infected cells by cytotoxic T cells
Class II	HLA-D	B lymphocytes, monocytes, macrophages, dendritic cells, activated T cells, activated endothelial cells, skin (Langerhans' cells)	Identification of foreign antigen by helper T cells
Class III	Complement C2, C4, B	Plasma proteins	Defense against extracellular pathogens
Cytokine genes	TNF-α, TNF-β	Plasma proteins	Cell growth and differentiation

- Class I and II are the strongest antigens expressed on cells.

Table 2

The three most common HLA-allele group frequencies for the total population, specific ethnic groups, and donor groups.

	Total (N = 1,151)	**African descent (N = 641)**	**European descent (N = 380)**	**Mixed Ancestry (N = 107)**	**All Donors (N = 406)**	**African descent donors (N = 189)**	**European descent donors (N = 165)**
HLA-A	HLA-A∗02 (15.3%), HLA-A∗03 (9.1%), HLA-A∗68 (9.1%)	HLA-A∗30 (13.1%), HLA-A∗68 (12.8%), HLA-A∗02 (12.3%)	HLA-A∗02 (22.4%), HLA-A∗03 (15.7%), HLA-A∗24 (9.4%)	HLA-A∗02 (11.7%), HLA-A∗30 (11.2%), HLA-A∗68 (9.6%)	HLA-A∗02 (17.6%), HLA-A∗68 (11.8%), HLA-A∗03 (9.3%)	HLA-A∗68 (15.2%), HLA-A∗02 (12.8%), HLA-A∗30 (11.8%)	HLA-A∗02 (22.5%), HLA-A∗03 (14.6%), HLA-A∗24 (11.4%)
HLA-B	HLA-B∗15 (11.1%), HLA-B∗44 (10.1%), HLA-B∗07 (10%)	HLA-B∗15 (12.6%), HLA-B∗58 (11.7%), HLA-B∗44 (9.9%)	HLA-B∗07 (14.6%), HLA-B∗44 (10.6%), HLA-B∗51 (7.3%)	HLA-B∗15 (17%), HLA-B∗07 (10.3%), HLA-B∗44 (9.3%), HLA-B∗58 (9.3%)	HLA-B∗07 (11.3%), HLA-B∗15 (10.3%), HLA-B∗44 (9.7%)	HLA-B∗15 (11.9%), HLA-B∗58 (11.4%), HLA-B∗44 (9.5%)	HLA-B∗07 (15.5%), HLA-B∗44 (9.5%), HLA-B∗51 (7.9%)
HLA-C	HLA-C∗07 (19.3%), HLA-C∗06 (14.6%), HLA-C∗04 (11.5%)	HLA-C∗07 (16.8%), HLA-C∗06 (16.4%), HLA-C∗04 (12.8%)	HLA-C∗07 (24.6%), HLA-C∗03 (12.6%), HLA-C∗06 (11.5%)	HLA-C∗02 (14.5%), HLA-C∗06 (14.5%), HLA-C∗07 (13.4%)	HLA-C∗07 (20%), HLA-C∗06 (14.7%), HLA-C∗04 (11%)	HLA-C∗06 (18.8%), HLA-C∗07 (18%), HLA-C∗04 (10.5%)	HLA-C∗07 (25.3%), HLA-C∗03 (12.7%), HLA-C∗04 (11%)
HLA-DRB1	HLA-DRB1∗03 (15.2%), HLA-DRB1∗13 (14.2%), HLA-DRB1∗15 (13.9%)	HLA-DRB1∗03 (17.2%), HLA-DRB1∗13 (16.1%), HLA-DRB1∗11 (16%)	HLA-DRB1∗15 (15.2%), HLA-DRB1∗04 (14.3%), HLA-DRB1∗03 (11.5%)	HLA-DRB1∗13 (16.1%), HLA-DRB1∗03 (15.5%), HLA-DRB1∗15 (14.3%)	HLA-DRB1∗15 (17.9%), HLA-DRB1∗03 (14.7%), HLA-DRB1∗13 (12%)	HLA-DRB1∗03 (19.8%), HLA-DRB1∗15 (16.7%), HLA-DRB1∗11 (14.2%)	HLA-DRB1∗15 (19.4%), HLA-DRB1∗04 (12.4%), HLA-DRB1∗13 (12.4%)

Transplantation Immunology

- **Transplantation** is the process of moving cells, tissues or organs from one site to another for the purpose of replacing or repairing damaged or diseased organs and tissues.
- **The immune system** poses a significant barrier to successful organ transplantation when tissues/organs are transferred from one individual to another.
- **Rejection** is caused by the immune system identifying the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue.
- Donor and recipient are carefully **matched** prior to transplantation to minimise the risk of rejection.
- **Immunosuppressive drugs** are used to prevent and to treat transplant rejection by dampening the overall immune response.
- Research on the immunological mechanisms of rejection will help improve cross matching, diagnosis and treatment, as well as facilitating the discovery of novel strategies for preventing rejection.

Transplantation Immunology

Clinical transplantation to treat human diseases
has increased steadily during the past 45 years, and
transplantation of kidneys, hearts, lungs, livers, pancreata,
and bone marrow is widely used today

Since 1990, 1-year survival of kidney allografts has been better than 90% but the
10-year survival has remained about 60% despite
advances in immunosuppressive therapy.

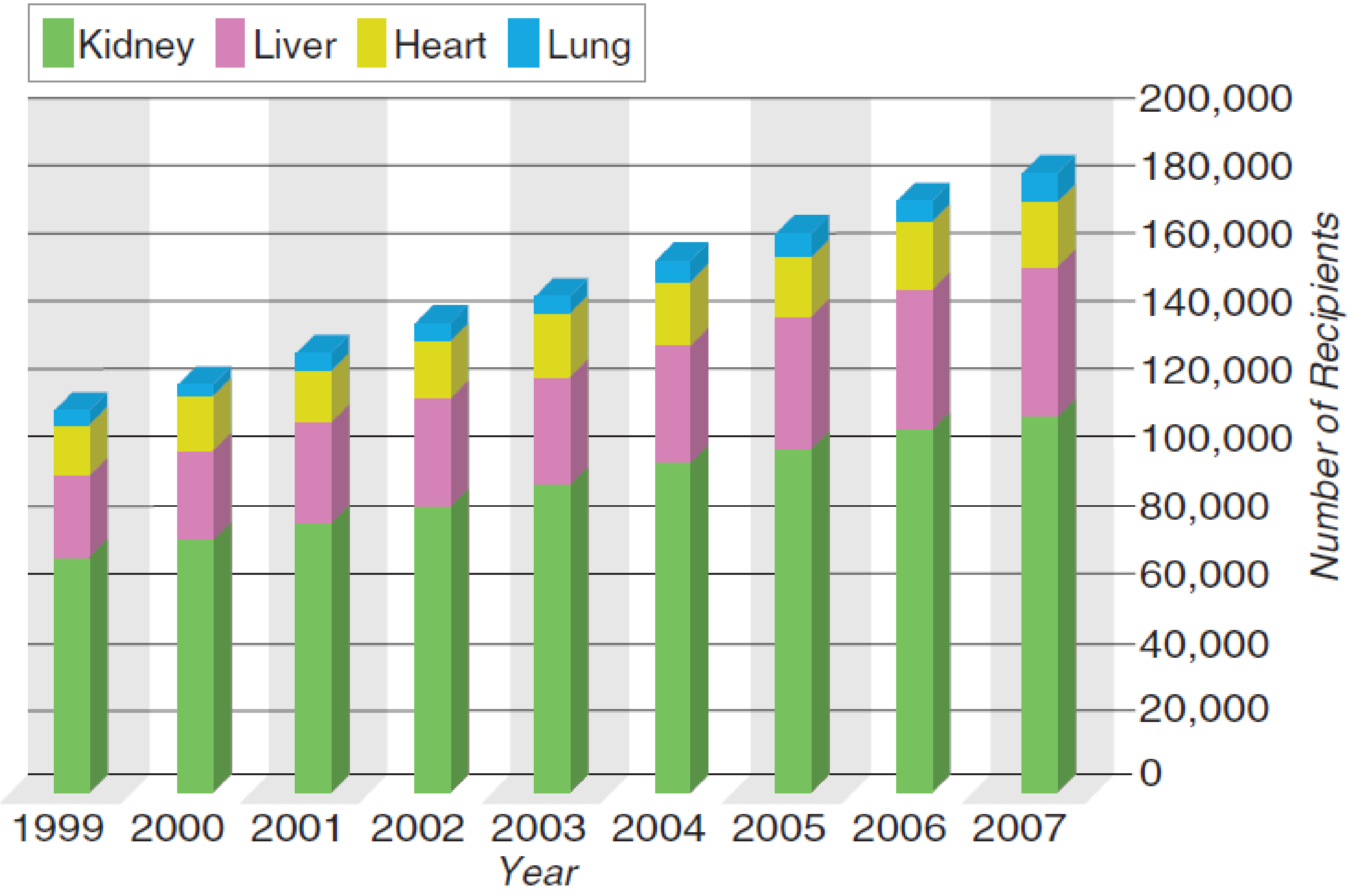
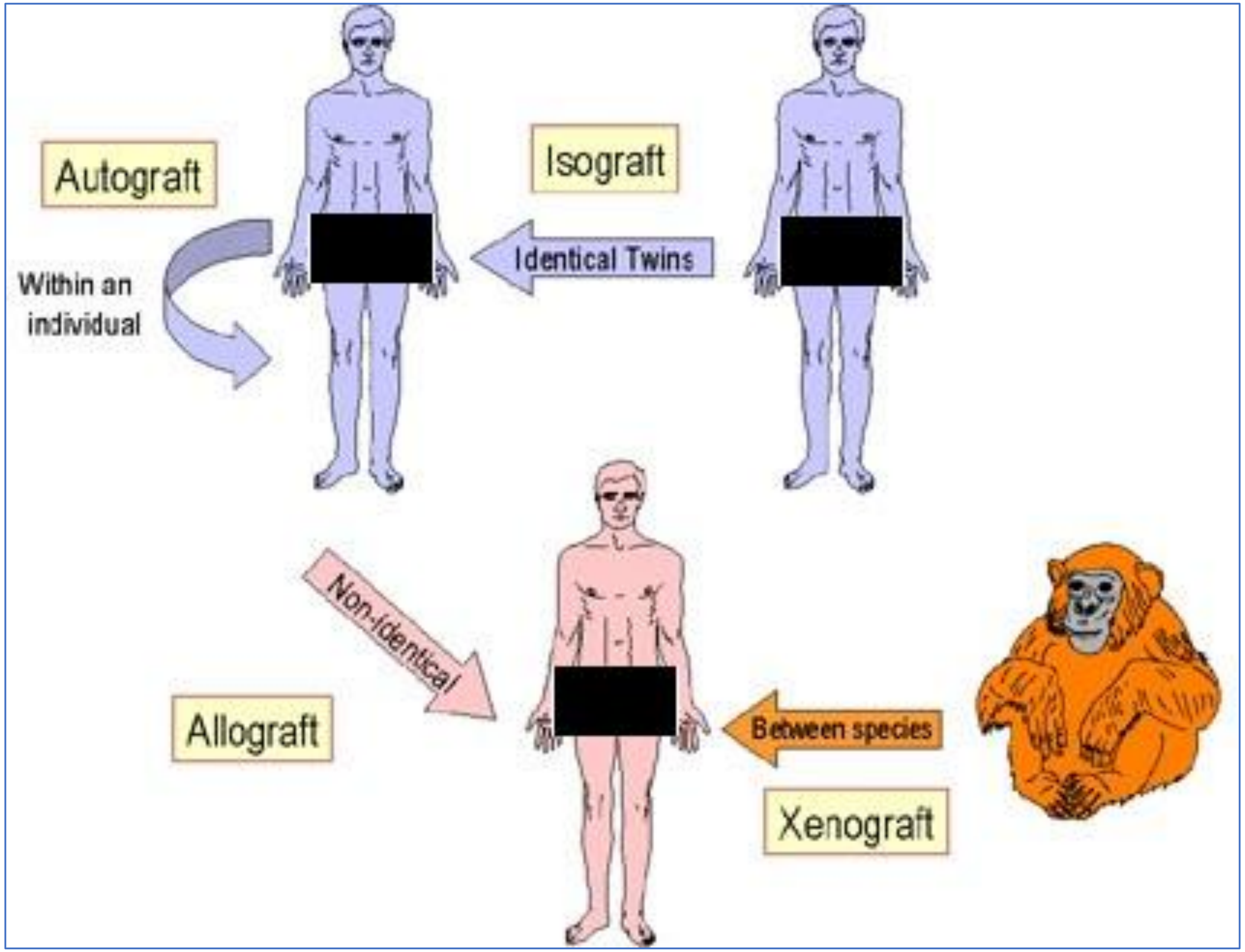


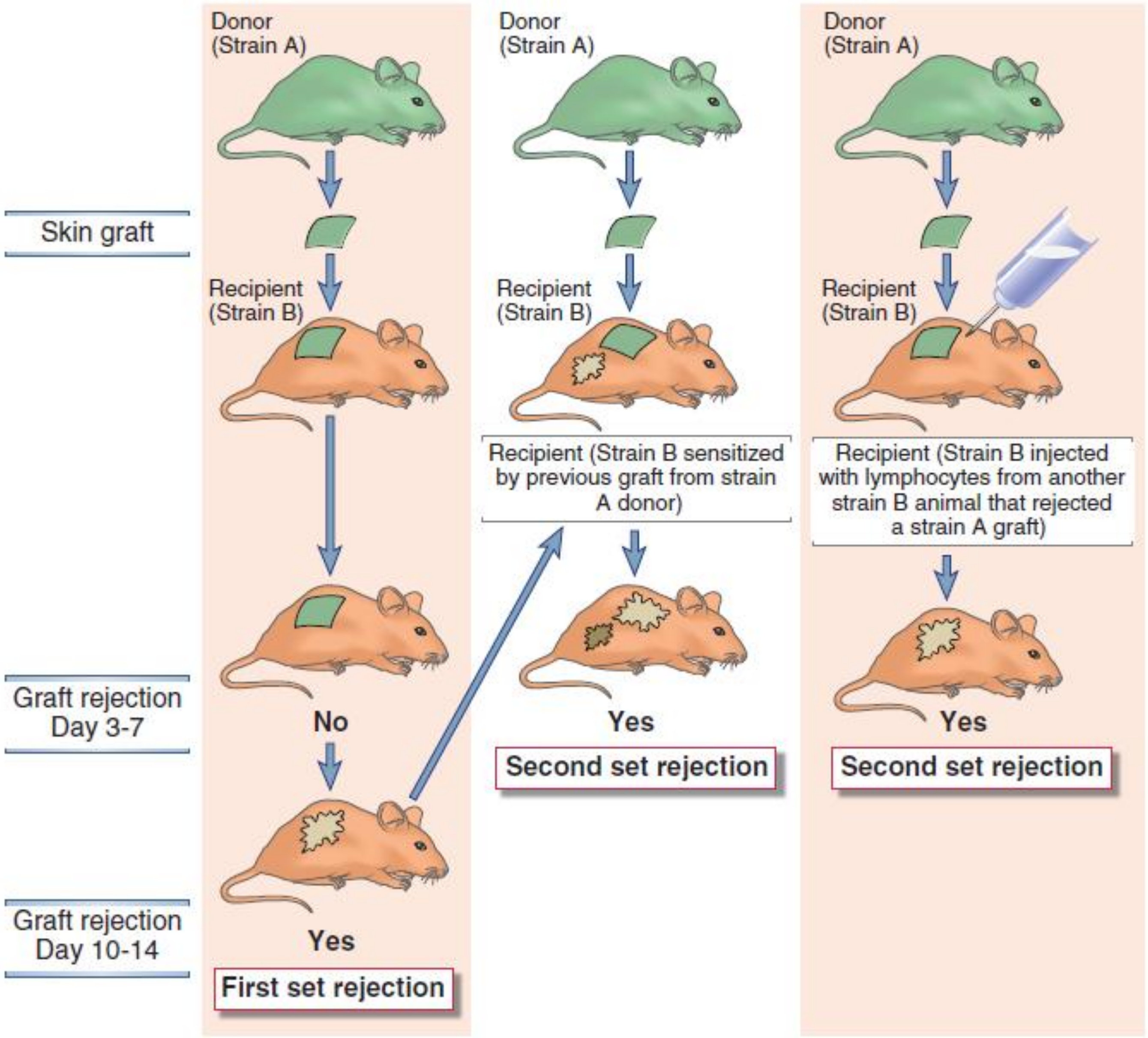
FIGURE 16–1 People in the United States living with functioning organ grafts, 1999-2007. *(Data from OPTN/SRTR Annual Report 2009. Available at: http://www.ustransplant.org/csr/current/fastfacts.aspx. Accessed April 2010.)*

Types of transplantation

- **Autograft** –Transplantation of cells, tissues or organs between sites within the same individual e.g. skin grafts in burn patients.
- **Allograft** – Transplantation of organs or tissues from a donor to a non-genetically identical individual of the same species. Allografts are the most common type of transplant.
- **Isograft -** Transplantation of organs or tissues from a donor to a genetically identical individual (i.e. identical twin).
- **Xenograft** – Transplantation of an organ or tissue between two different species. 'Pig valves', for example, are commonly used to repair or replace a defective heart valve in humans.



Transplantation Immunology

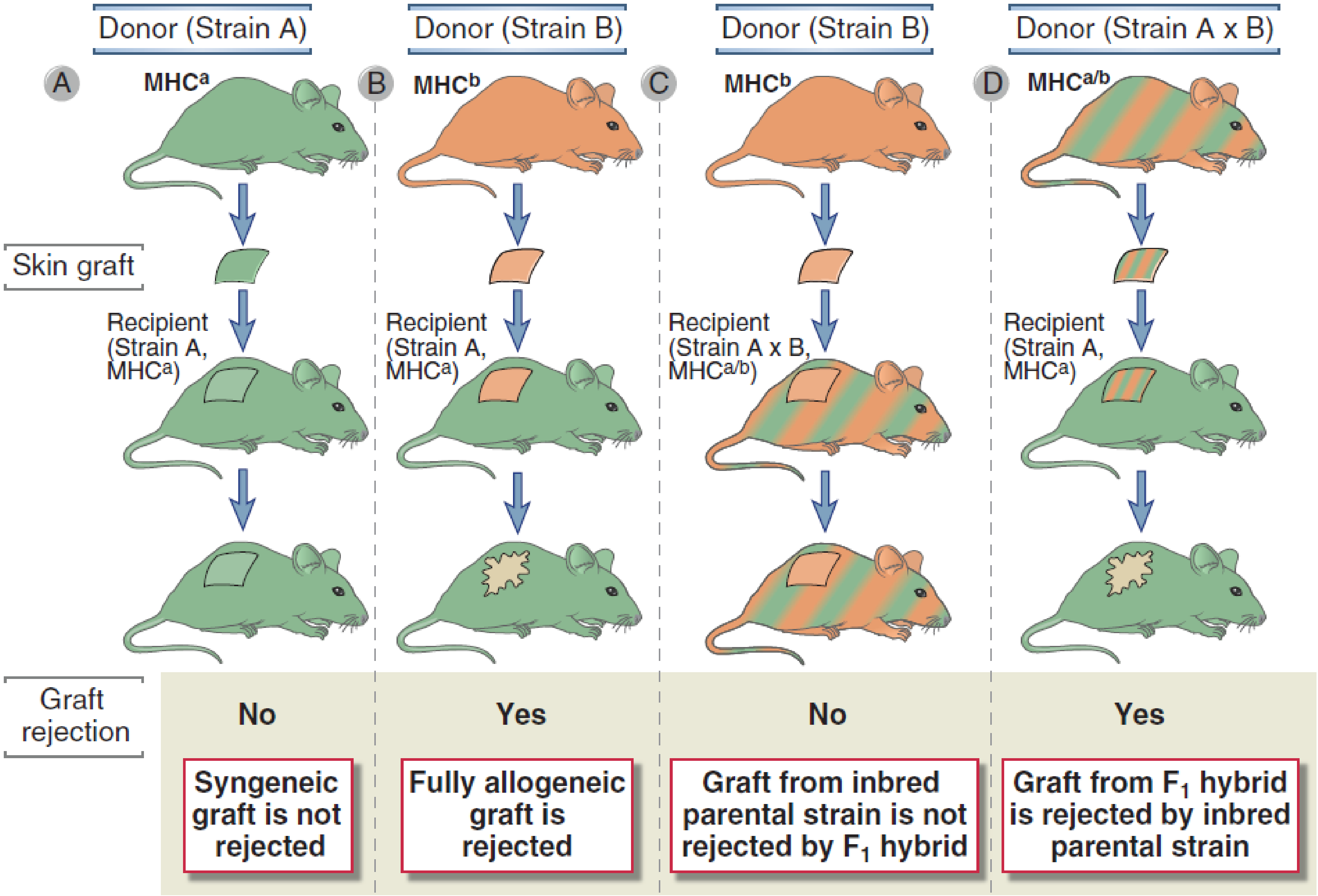


Transplantation Immunology

The molecules responsible for almost all strong (rapid) rejection reactions are called major histocompatibility complex (MHC) molecules.

Allogeneic MHC molecules of a graft may be presented for recognition by the T cells of the recipient in two fundamentally different ways, called direct and indirect

Transplantation Immunology



Transplant Immunology

- Allogeneic MHC molecules of a graft may be presented for recognition by the T cells of the recipient in two fundamentally different ways, called direct and indirect

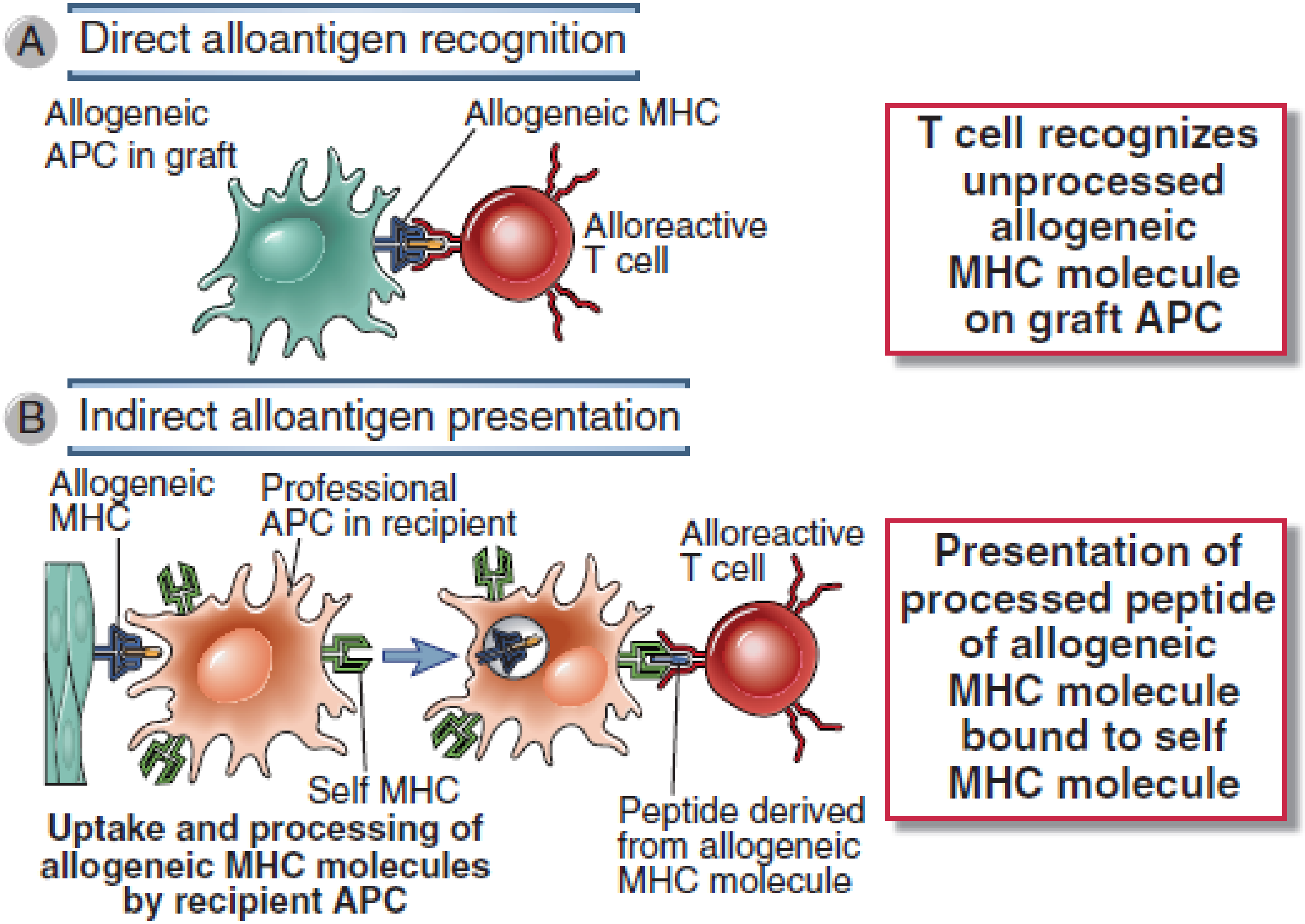
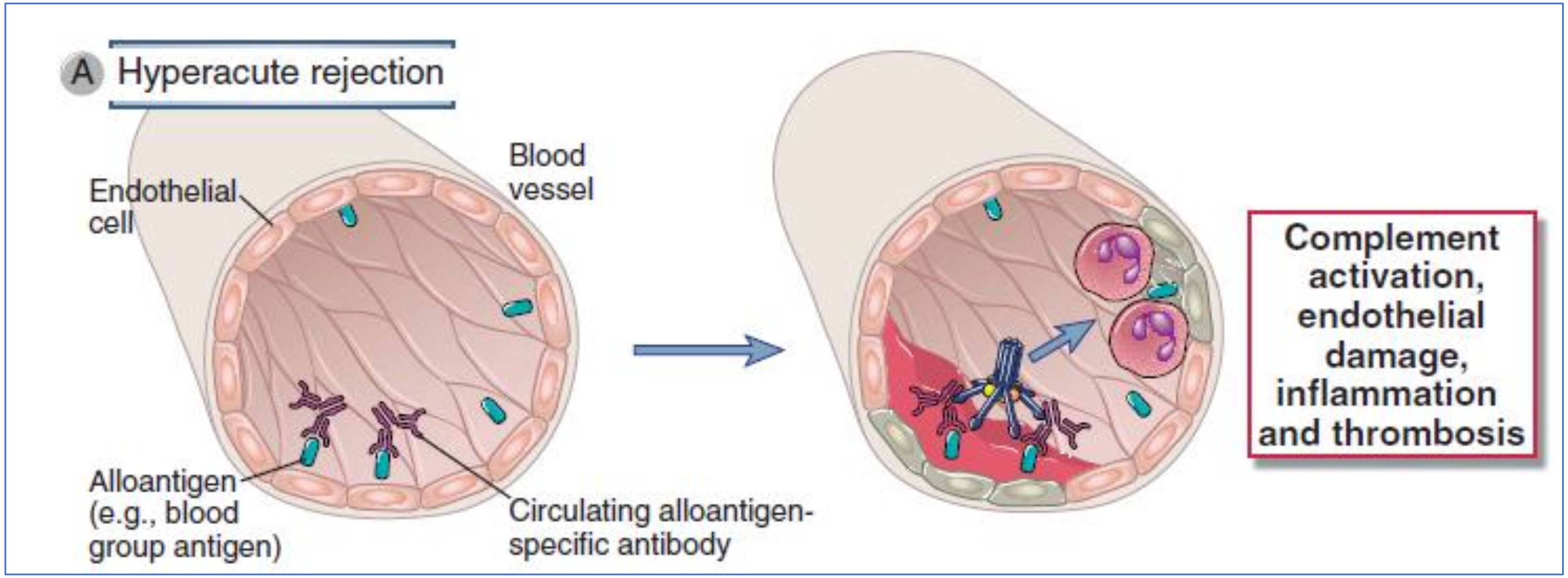


FIGURE 16–4 Direct and indirect alloantigen recognition. A, Direct alloantigen recognition occurs when T cells bind directly to an intact allogeneic MHC molecule on a graft (donor) antigen-presenting cell (APC). **B,** Indirect alloantigen recognition occurs when allogeneic MHC molecules from graft cells are taken up and processed by recipient APCs and peptide fragments of the allogeneic MHC molecules containing polymorphic amino acid residues are bound and presented by recipient (self) MHC molecules.

Rejection of graft (types/stages)

- **Hyperacute rejection** occurs within minutes or hours after a transplantation and is caused by the presence of preexisting antibodies of the recipient, that match the foreign antigens of the donor, triggering an immune response against the transplant.

- The antibodies react with cells in the blood vessels of the graft, causing blood clots to form, which will prevent blood supply from reaching the graft resulting in immediate rejection of the transplant



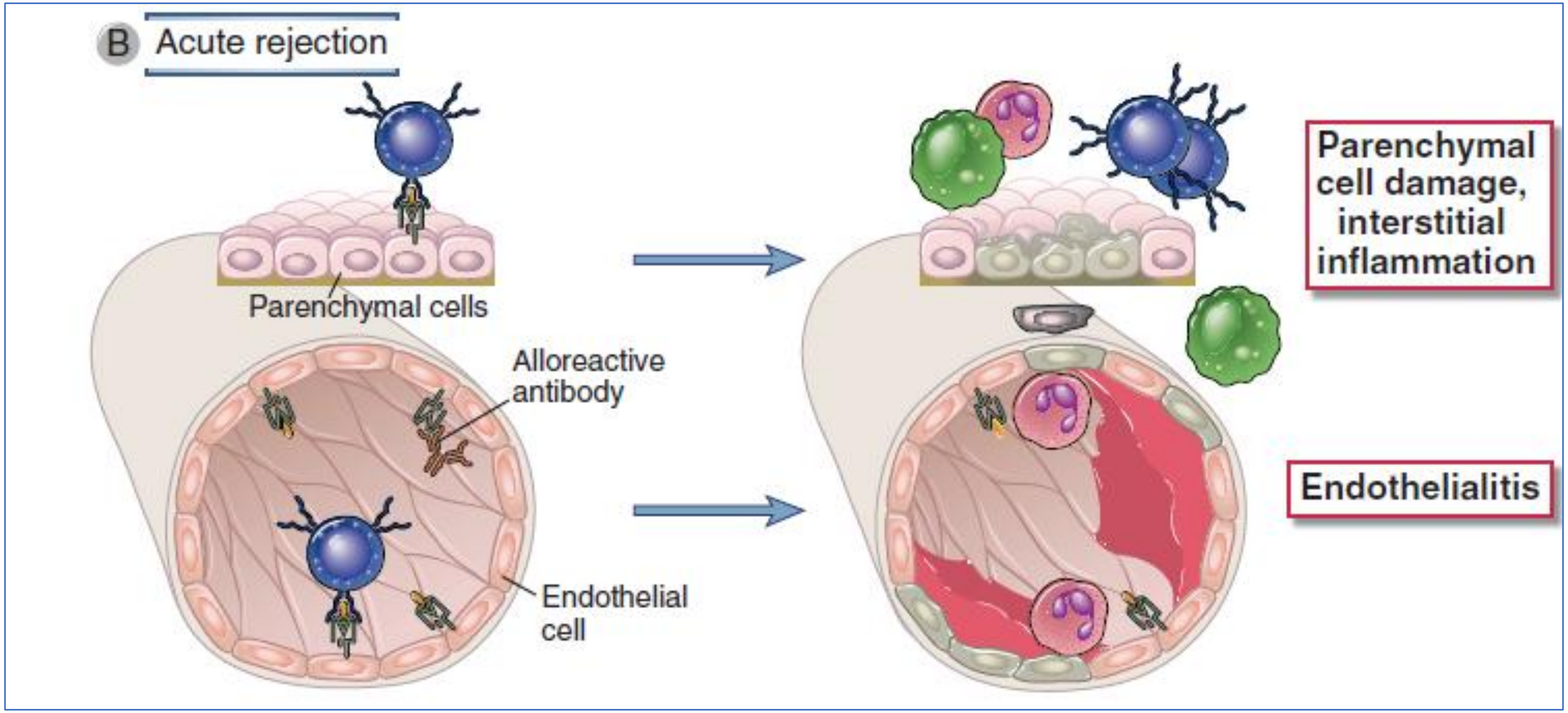
Rejection of graft (types/stages)

- **Acute rejection** usually takes several days-weeks, and occurs within the first 6 months after transplantation. Some degree of acute rejection will occur in all transplantations, except between identical twins.

- In addition to direct killing of the graft cells by **CTLs**, activated **CD4+ helper T** cells and CTLs produce cytokines that recruit and activate inflammatory cells, which also injure the graft.

- **Alloantibodies** cause acute rejection by binding to alloantigens, mainly HLA molecules, on vascular endothelial cells, causing endothelial injury and intravascular thrombosis that results in graft destruction.

Rejection of graft (types/stages)

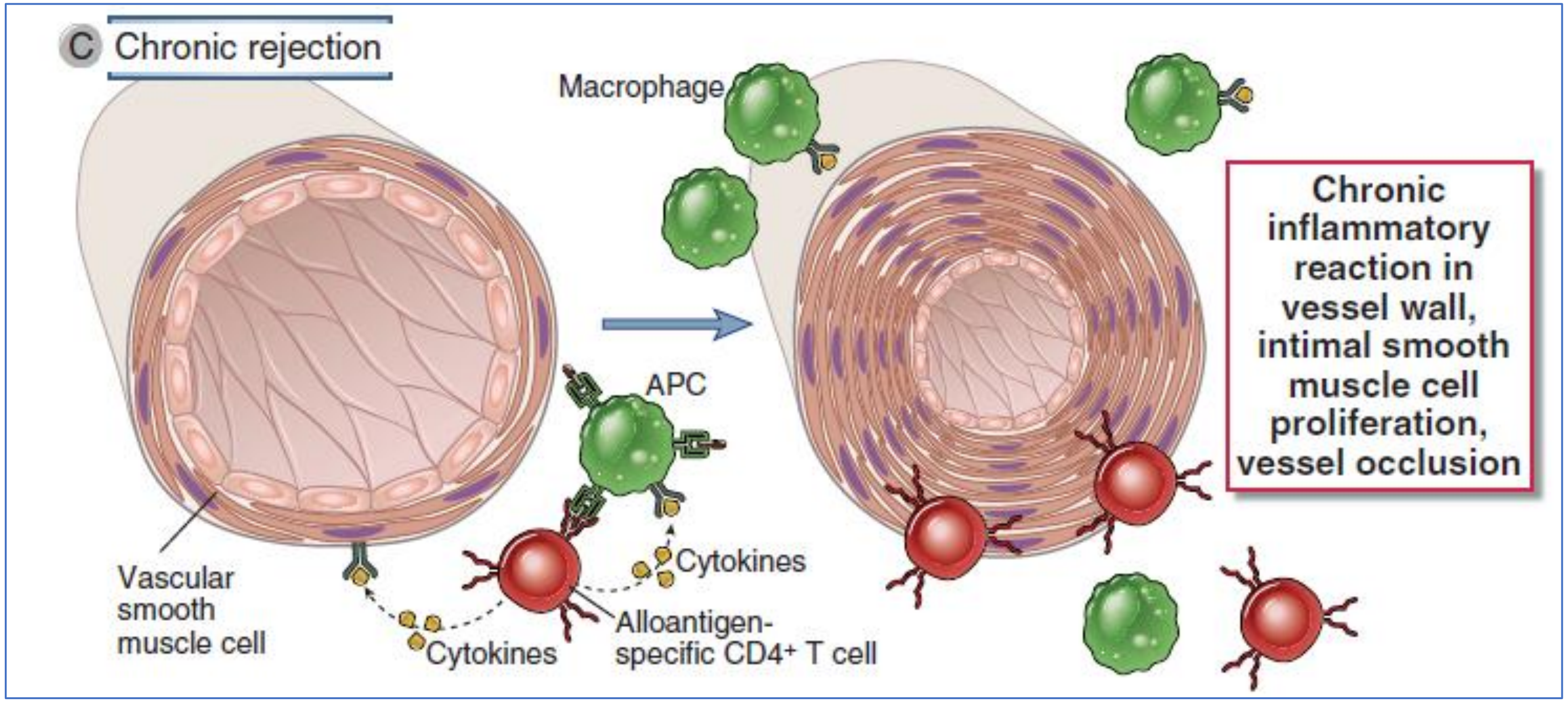


Acute rejection is a process of injury to the graft parenchyma and blood vessels mediated by alloreactive T cells and antibodies

Rejection of graft (types/stages)

- **Chronic rejection**. Repeated episodes of acute rejection can ultimately lead to chronic rejection of the graft and failure of the transplant. Chronic rejection commonly manifests as scarring of the tissue or organ which can occur months to years after acute rejection has subsided.

- A dominant lesion of chronic rejection in vascularized grafts is arterial occlusion as a result of the proliferation of intimal smooth muscle cells, and the grafts eventually fail mainly because of the resulting ischemic damage



Compatibility testing (matching)

- Rejection can be **minimised** by carefully matching the donor and recipient for compatibility prior to transplantation. The better matched the donor and recipient are the more successful the transplantation is likely to be. Several tests are commonly done including:

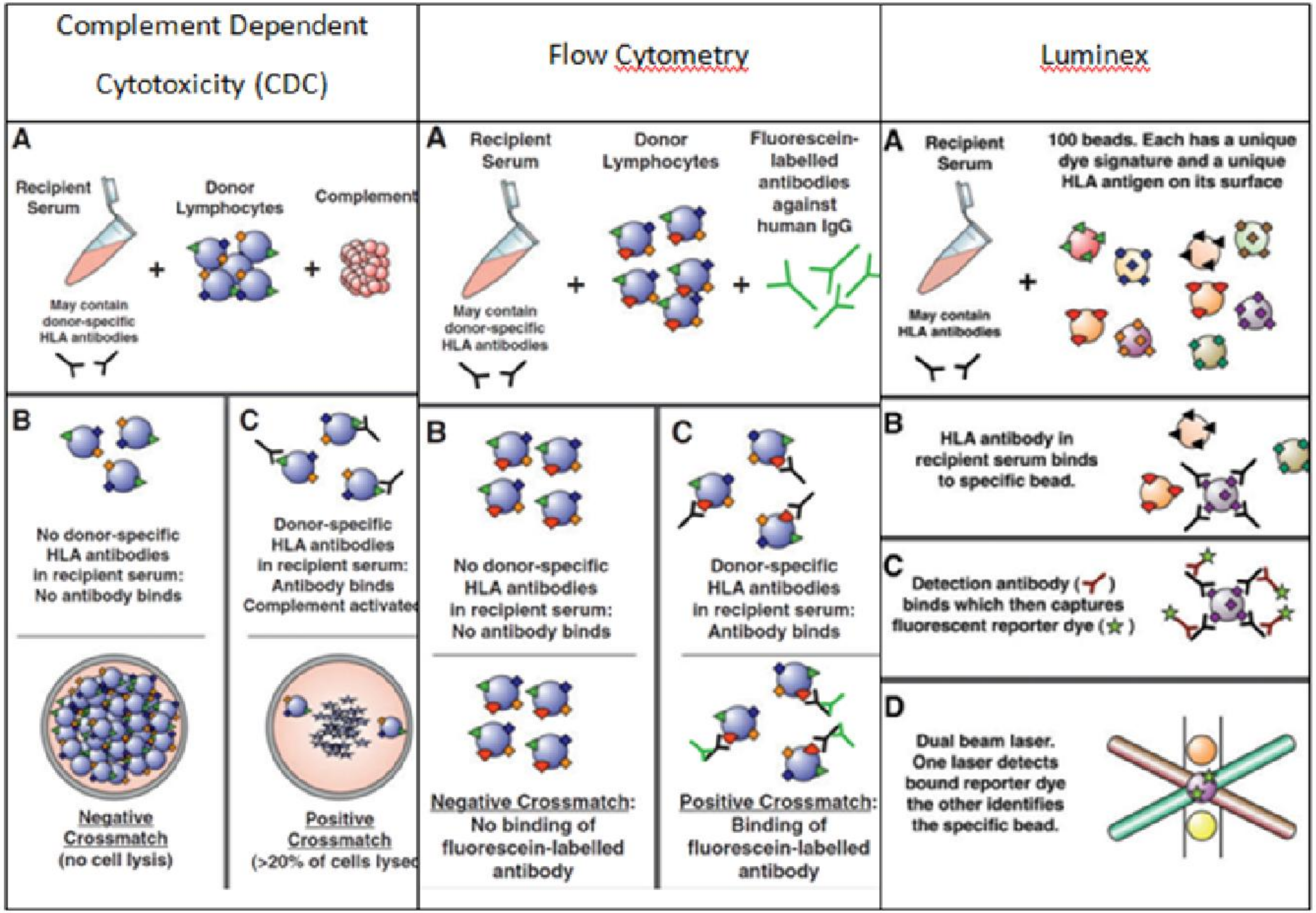
- **ABO blood group** compatibility – The donor and recipient are tested for compatible blood groups.

- **Tissue typing** – A blood sample is taken from the recipient to identify the HLA antigens present on the surface of the their cells to help find a compatible donor. **Siblings** offer the best donors usually.

- **Cross matching** – Blood samples are taken from both the recipient and donor, and the cells of the donor are mixed with the blood serum of the recipient. If the recipient's antibodies attack the donor cells, they are considered a positive match and transplantation will not be suitable due to increased risk of hyper-acute rejection.

- **Panel reactive antibody test** – The blood serum of patients awaiting transplantation are tested for reactive antibodies against a random panel of cells. The more HLA antibodies present, the higher he panel reactive antibody (PRA) level denoted to the patient, and the greater the chance of graft rejection.

Tissue typing and crossmatching



Compatibility testing (matching)

- In kidney transplantation, the larger the number of MHC alleles that are matched between the donor and recipient, the better the graft survival

- Past clinical experience with older typing methods had shown that of all the class I and class II loci, matching at **HLA-A, HLA-B, and HLA-DR** is most important for predicting survival of kidney allografts

- Zero-antigen mismatches predict the best survival of living related donor grafts, and grafts with one-antigen mismatches do slightly worse.

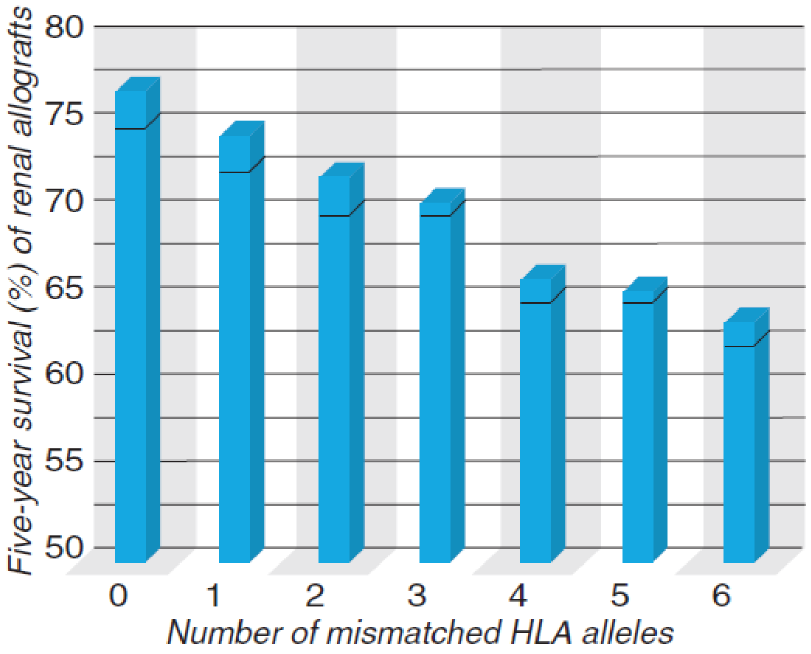


FIGURE 16–12 Influence of MHC matching on graft survival. Matching of MHC alleles between the donor and recipient significantly improves renal allograft survival. The data shown are for deceased donor (cadaver) grafts. HLA matching has less of an impact on survival of renal allografts from live donors, and some MHC alleles are more important than others in determining outcome. *(Data from Organ Procurement and Transplantation Network/Scientific Registry annual report, 2010.)*

Immunosuppressive drugs

- To reduce the risk of transplant rejection, patients are treated with immunosuppressive drugs that will dampen their immune response.
- Immunosuppressive drugs are given in two phases; an initial induction phase involving a high dose, and a later maintenance phase which involves using the drug in the long term at a lower dose.
- The combination of drugs, and dosage given, will vary depending on the type of transplant and the chosen treatment regime.
- Examples include: The calcineurin inhibitors cyclosporine and tacrolimus, steroids, Target of Rapamycin Inhibitors, Azathioprine.

Graft vs host disease (GVHD)

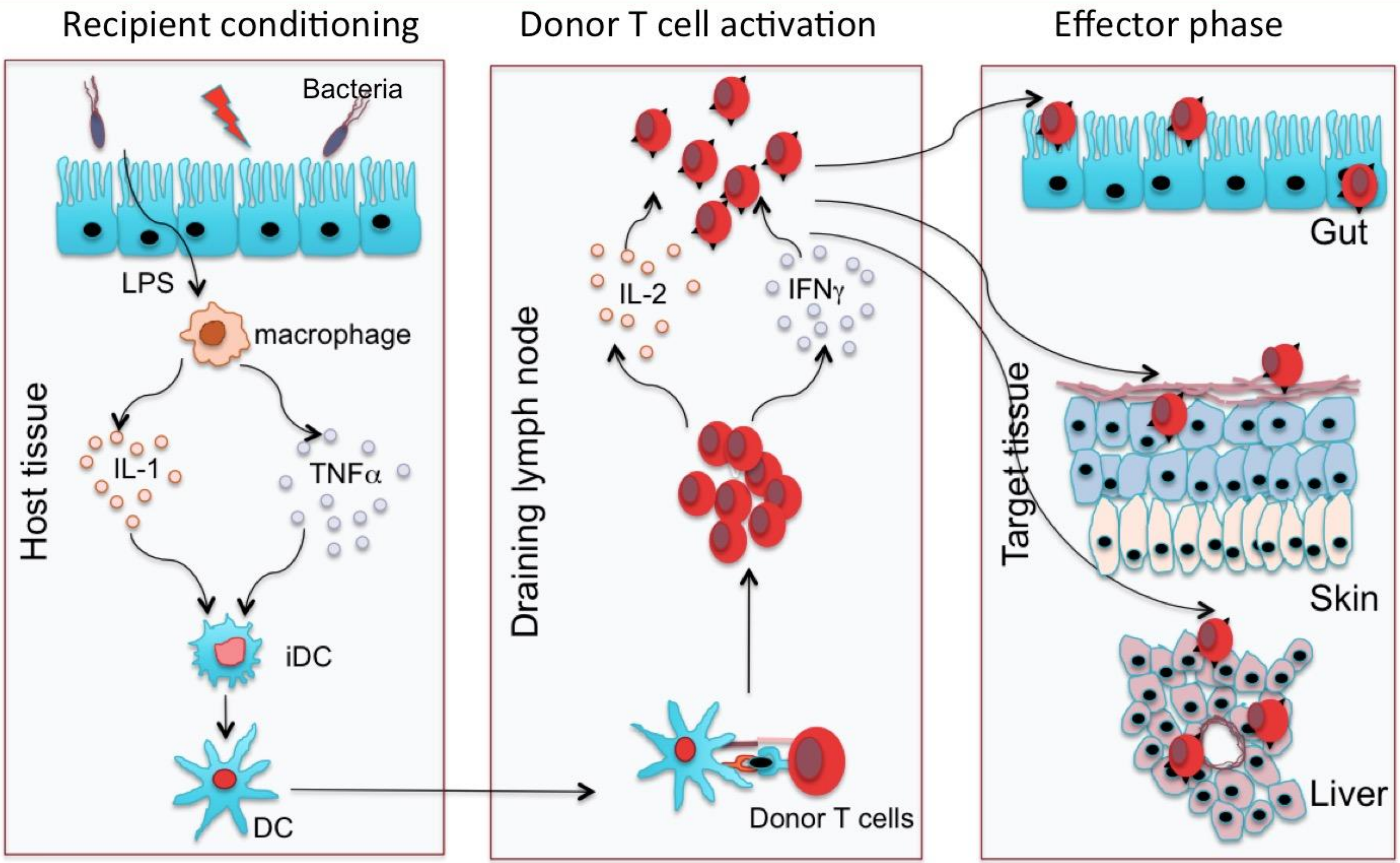
- Allogeneic hematopoietic stem cell transplantation (HSCT) is used for treatment of several hematological malignancies as well as immune disorders.

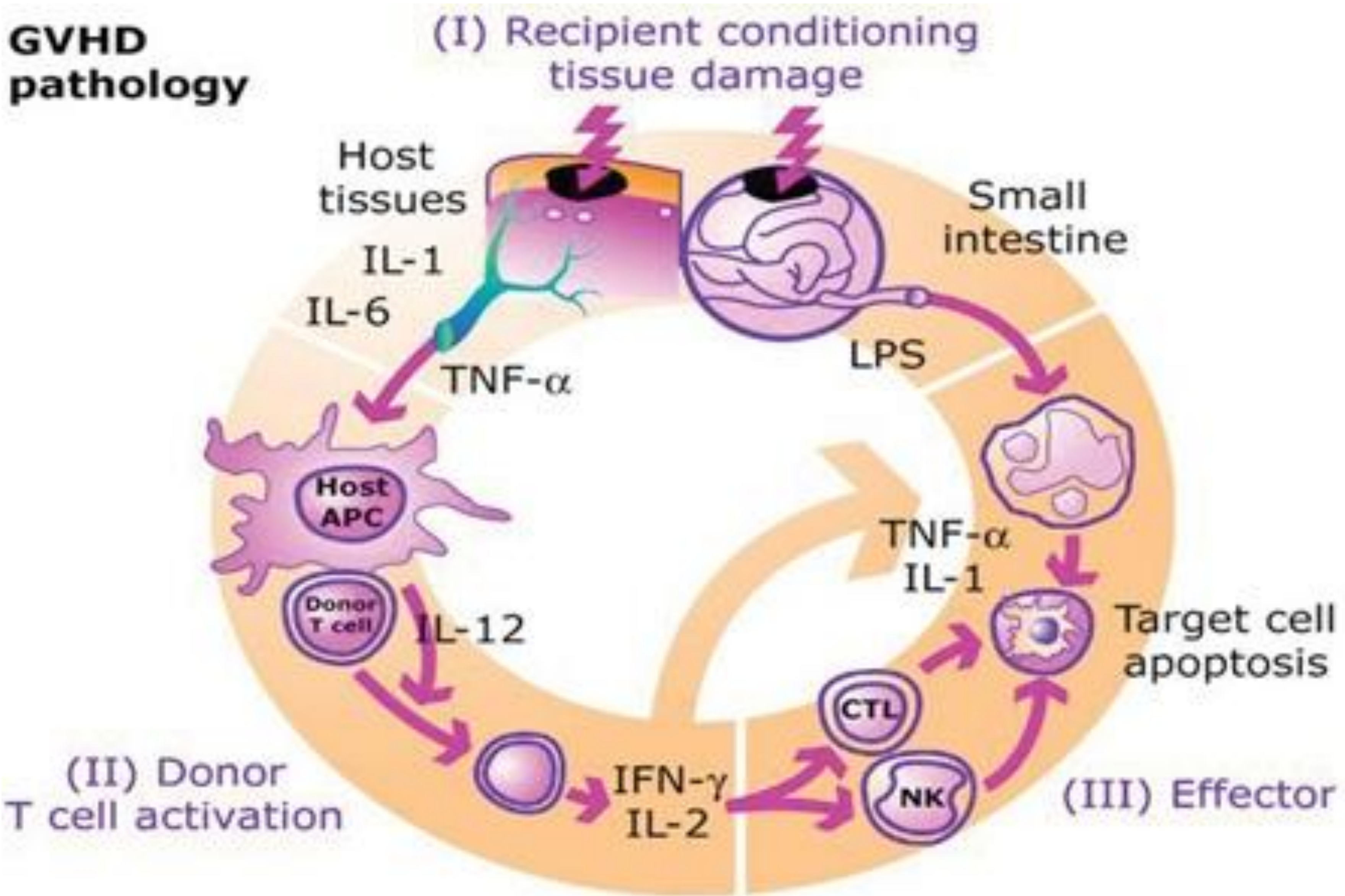
- **GVHD** is initiated by mature $CD4^+$and/or $CD8^+$ **T cells** that accompany **allogeneic HSCT**.

- GVHD can occur in **HLA identical** individuals, due to differences in **minor** histocompatibility antigens (miHA). Many miHA are encoded on the Y chromosome.

- Diagnosis of GVHD is based on signs and symptoms the affected tissue.

Patho-physiology of GVHD




GVHD
pathology
(I) Recipient conditioning
tissue damage
Host
tissues
Small
intestine
IL-1
IL-6
TNF-α
LPS
Host
APC
Donor
T cell
IL-12
TNF-α
IL-1
Target cell
apoptosis
CTL
NK
IFN-γ
IL-2
(II) Donor
T cell activation
(III) Effector

What is a graft between different members of the same species termed?

A Autograft

B Isograft

C Xenograft

D Allograft

E None of the above

Mitosis occurs when mixing lymphocytes of two individuals:

A In presence of mitomycin C

B In presence of anti-CD4

C Who are identical twins

D Of differing MHC class II haplotype

E Of differing MHC class I, but identical MHC class II, haplotype

Which of the following statements is NOT TRUE? Rejection of a second (set) skin graft from the same allogeneic donor:

A Can be blocked by azathioprine (an antimitotic agent)

B Proceeds at the same speed as the first graft rejection

C Shows specificity for the graft donor

D Can be transferred to a naive recipient with lymphocytes

E Involves recognition of MHC differences

Which of the following statements is TRUE of the human major histocompatibility complex (MHC)?

A MHC provokes the most intense allograft reactions

B MHC is termed H-2

C MHC contains only class I and class II genes

D MHC is not expressed as codominant antigens on the cell surface

E MHC encodes the human leukocyte antigens (HLA) expressed only on leukocytes

Further reading:

- Cellular and Molecular Immunology. 7th Edition..

Chapter 6. Major histocompatibility complex molecules ….

Chapter 16. Transplantation immunology