Antigen processing

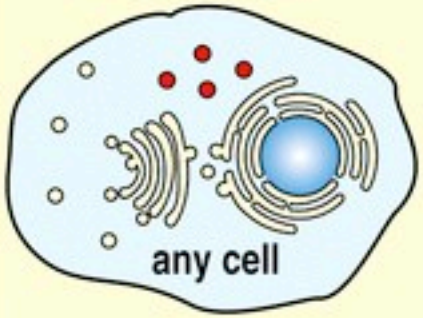
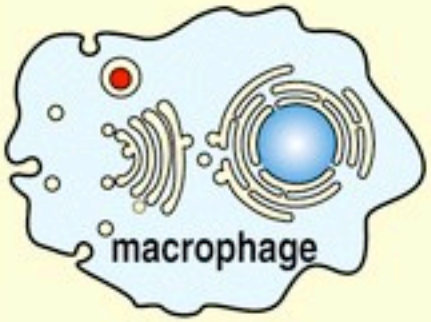
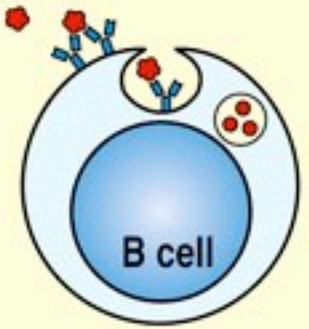
	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
			
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	CD8 T cells	CD4 T cells	CD4 T cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B cells to secrete Ig to eliminate extracellular bacteria/toxins

Figure 5-2 Immunobiology, 6/e. (© Garland Science 2005)

Class I Ag processing

- TAP= transporters associated with antigen processing
- Transport peptides into ER

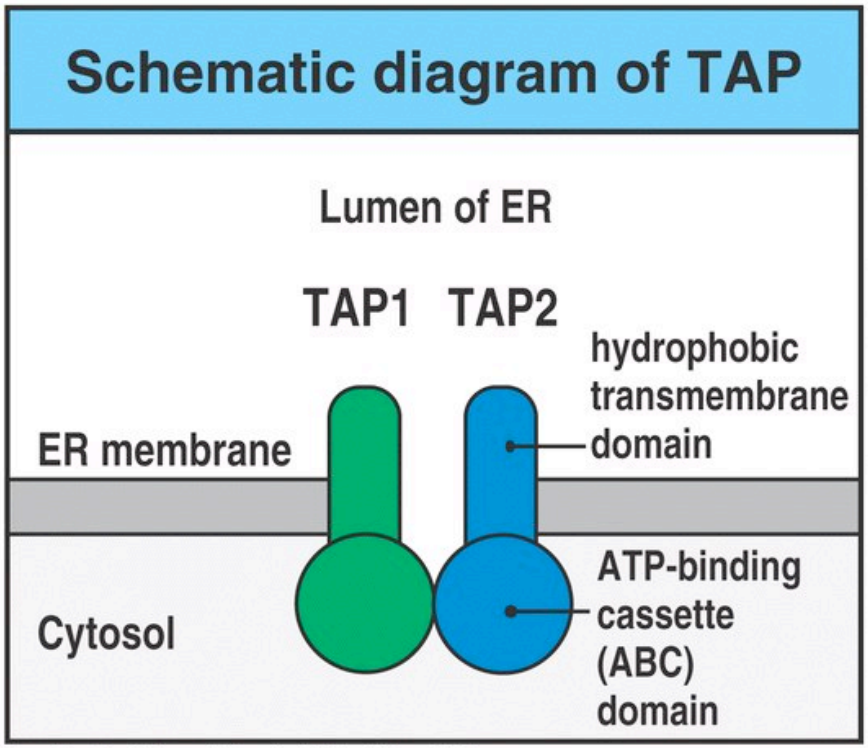


Figure 5-3 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Proteosome degrades cytosolic proteins

- Large, multi-subunit complex
- Degrades foreign and self protein
- LMP2, LMP7 and MECL-1 induced by γ-Interferon
  - Preferential proteases for class I peptides
  - Cleaves after basic, hydrophobic amino acids

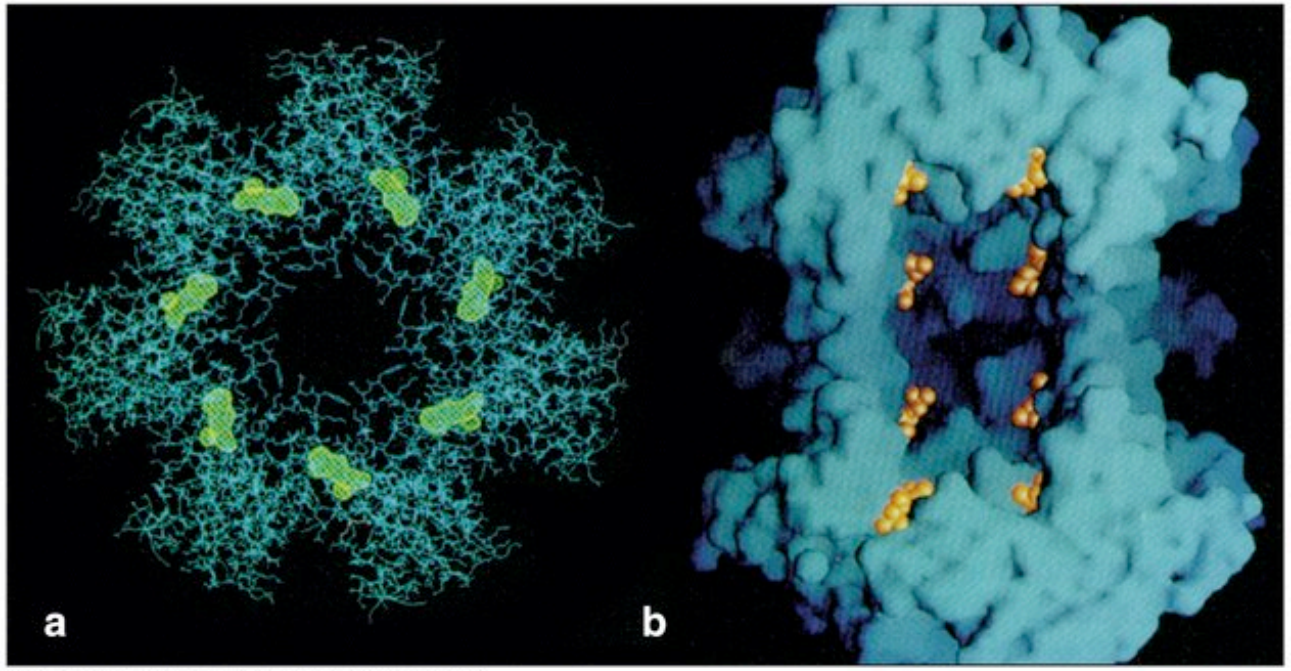


Figure 5-4 Immunobiology, 6/e. (© Garland Science 2005)

MHC class I
Ag processing pathway

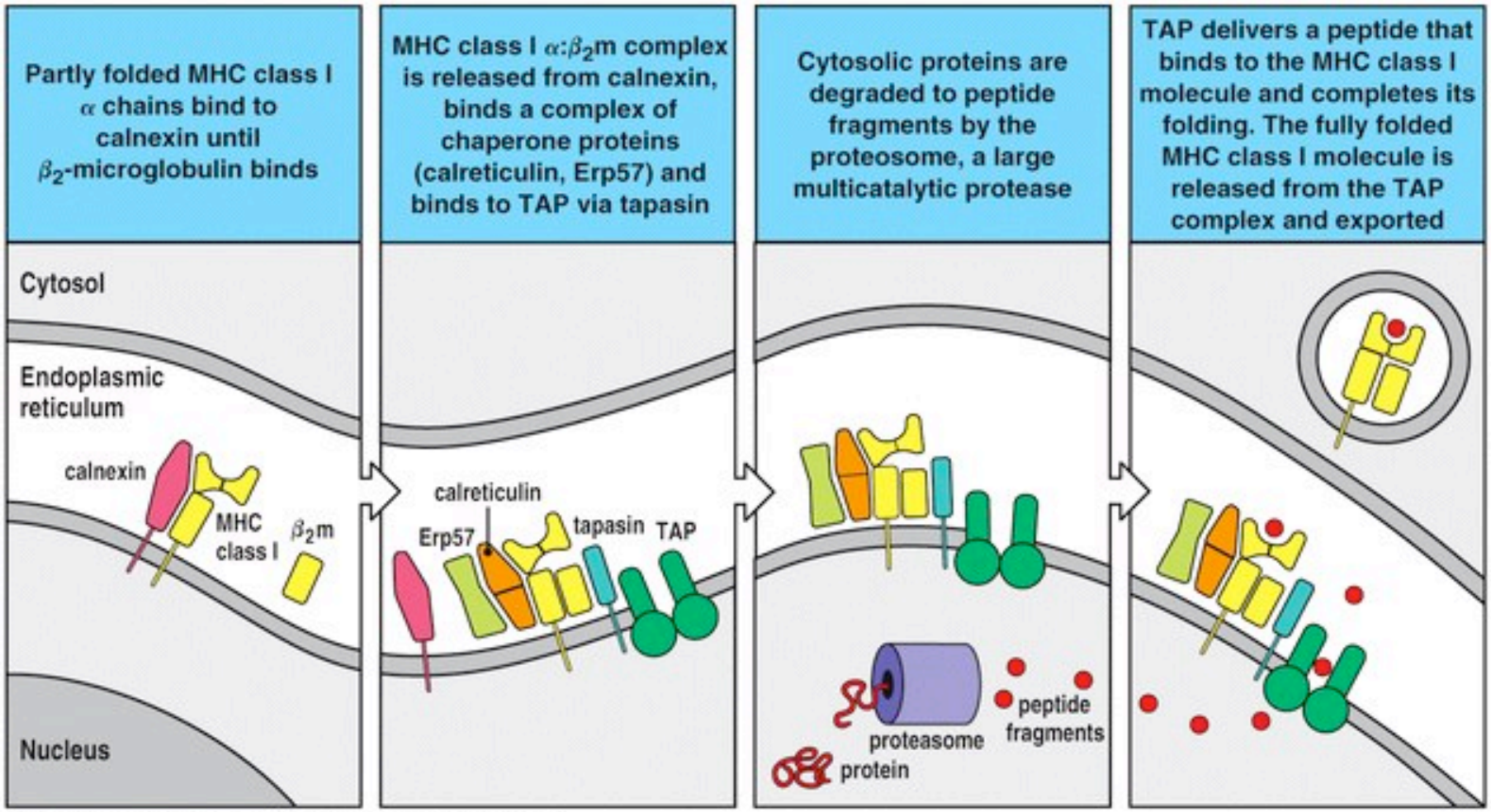


Figure 5-6 Immunobiology, 6/e. (© Garland Science 2005)

Pathogen evasion of class I Ag presentation

- Herpes simplex
  - Inhibition of antigen transport by TAP
- Adenovirus, Cytomegalovirus
  - Decreased MHC class I expression by retaining class I in ER
- Plasmodium
  - No Ag presentation since no MHC expression on RBCs

MHC class II Ag processing

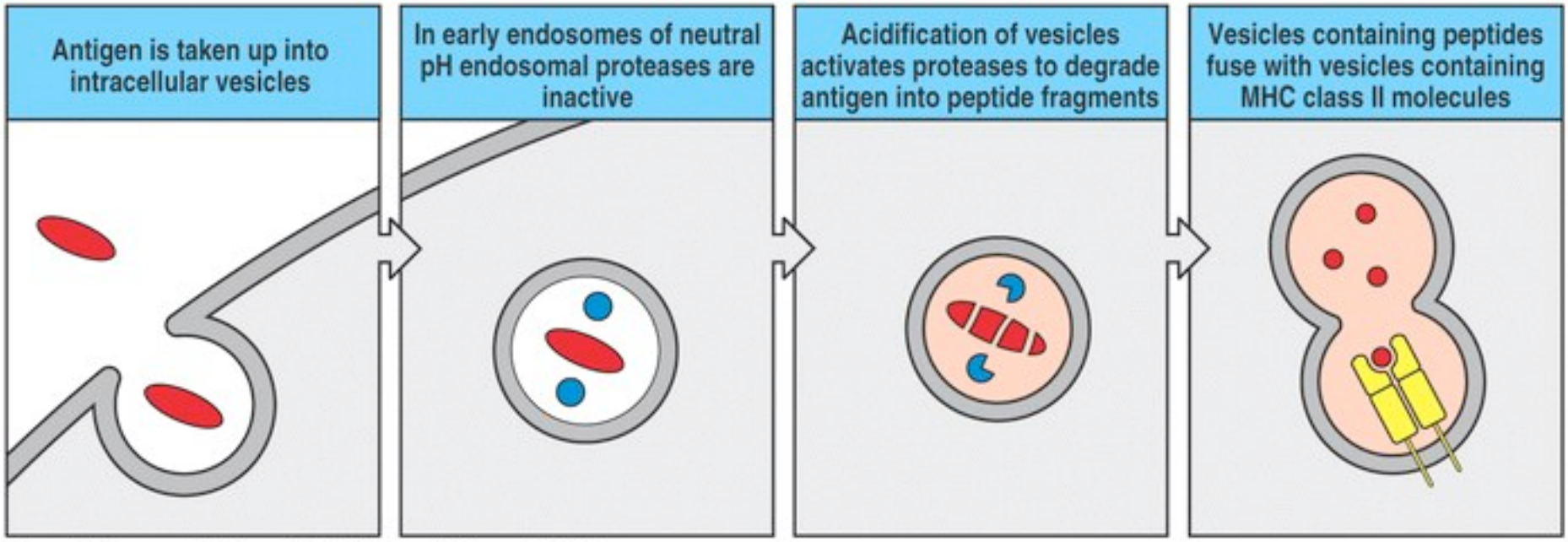


Figure 5-7 Immunobiology, 6/e. (© Garland Science 2005)

MHC class II

- Invariant chain=
  - Binds in class II groove in endoplasmic reticulum
- CLIP peptide=
  - Stabilizes class II
  - Prevents loading of peptide until fusion with phagolysozome

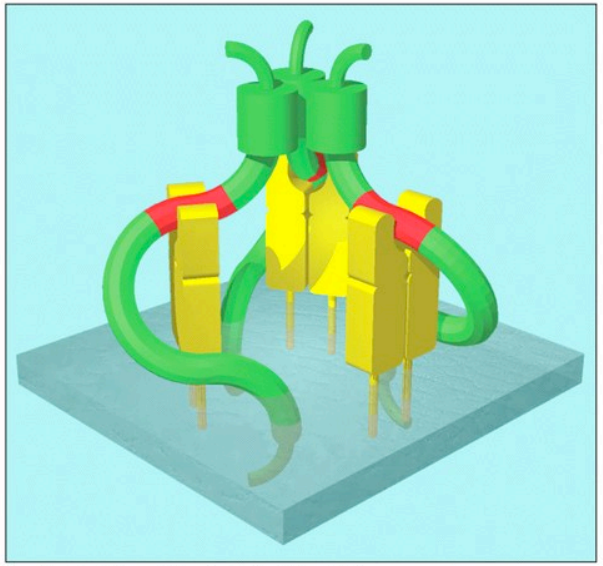


Figure 5-8 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Cleavage of Invariant chain

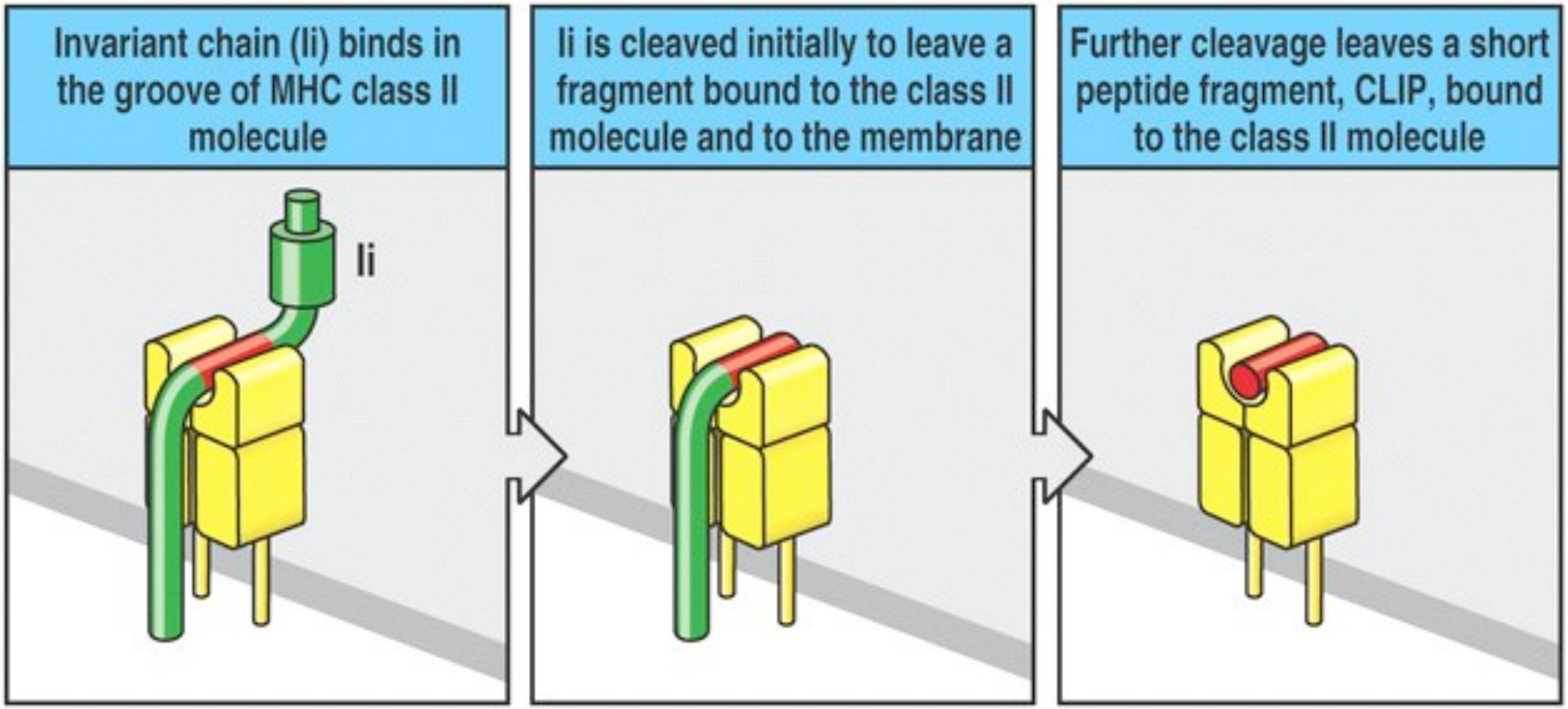


Figure 5-8 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Class II Ag processing pathway

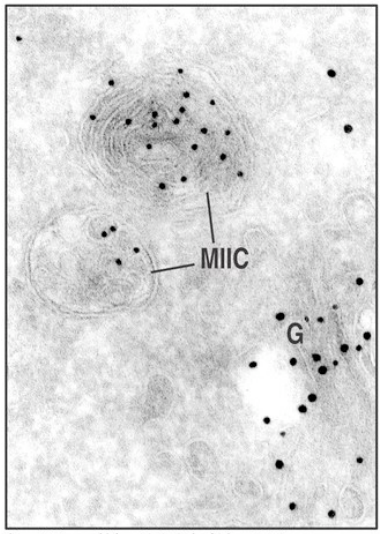
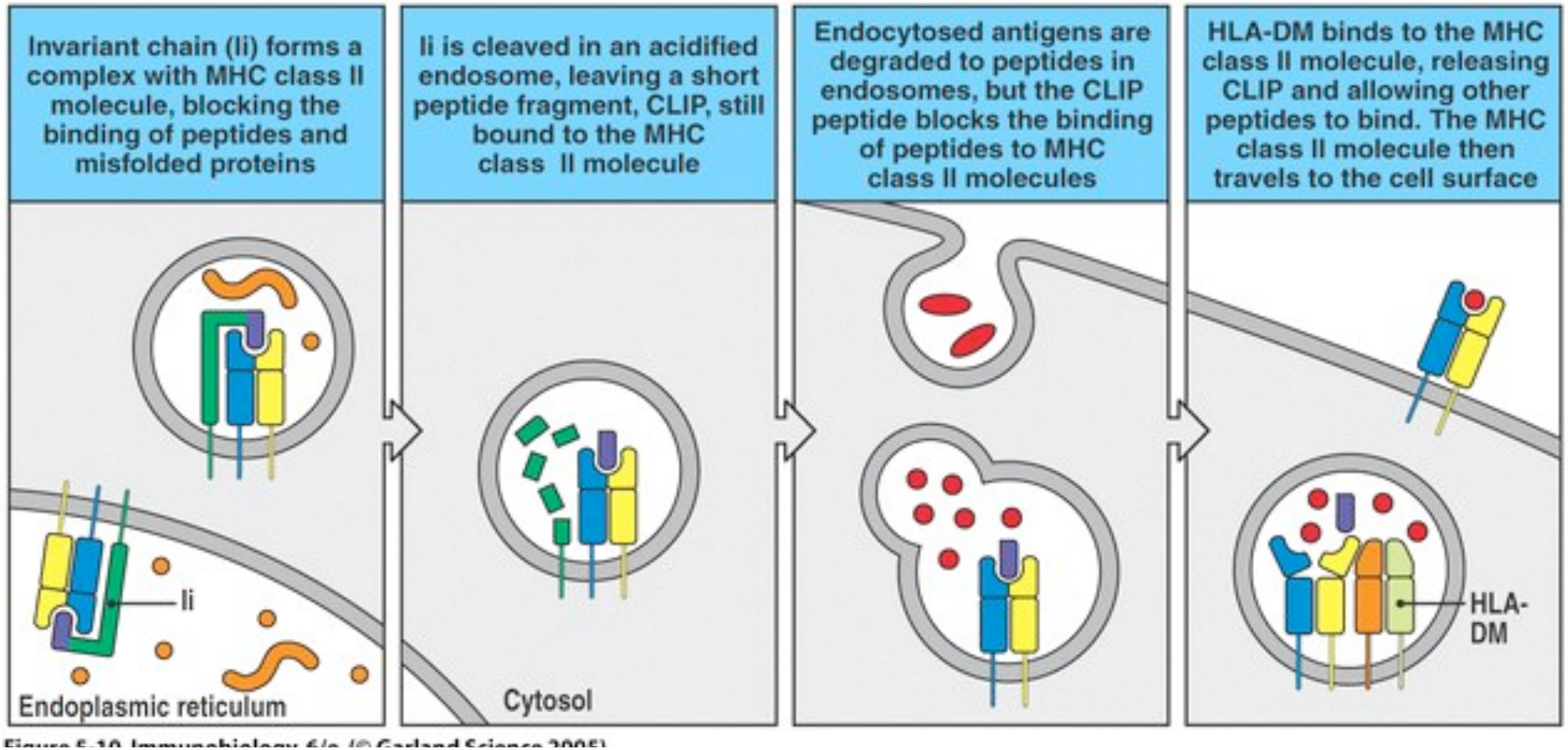


Figure 5-9 Immunobiology, 6/e. (© Garland Science 2005)



MHC and peptide stability

- Very stable binding
- Peptide not lost or gained by diffusion into groove

⇒Allows rare peptides to be presented

⇒MHC only present at surface with peptide in cleft

MHC locus

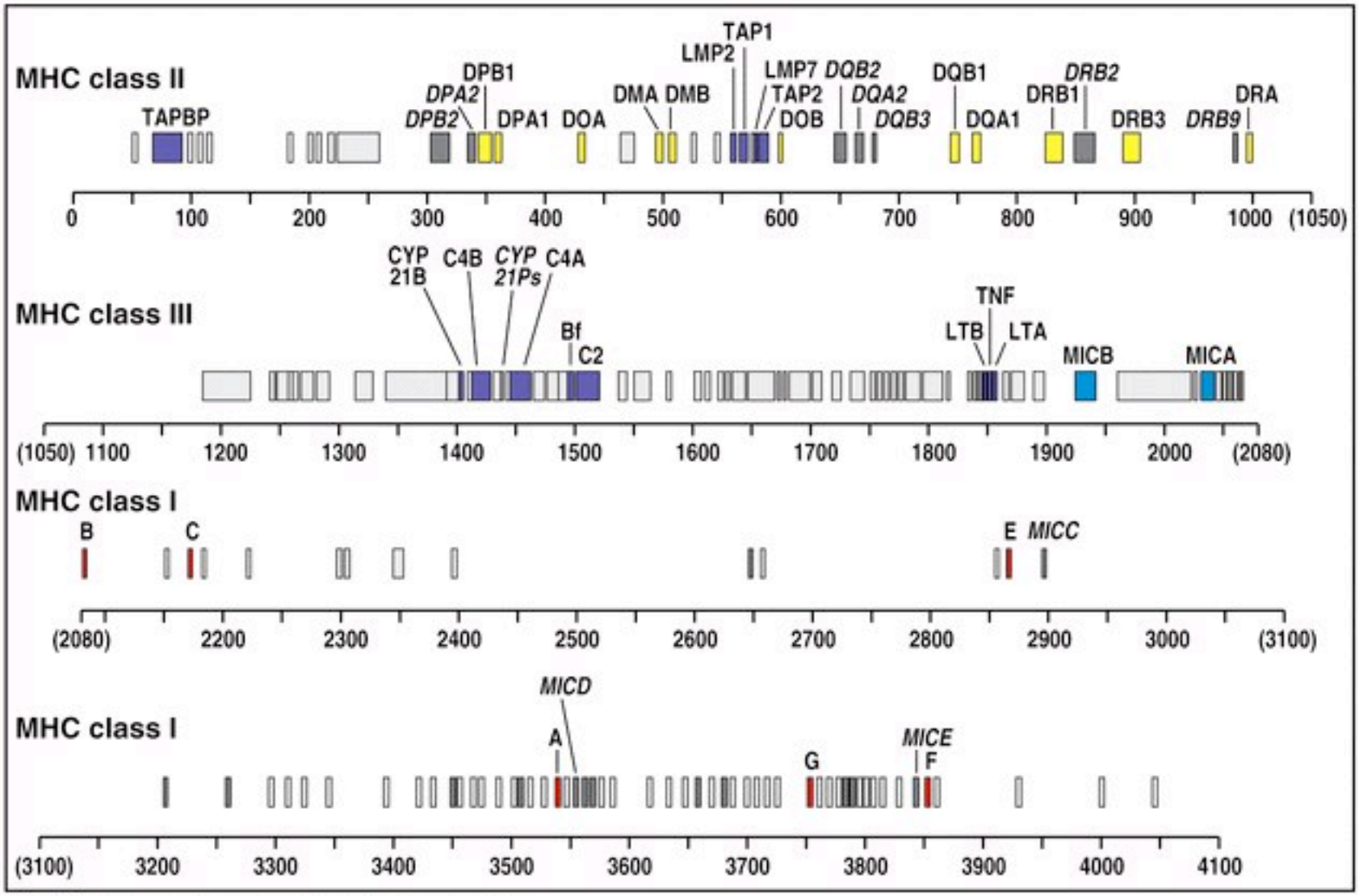


Figure 5-12 Immunobiology, 6/e. (© Garland Science 2005)

A diversity of peptides can be presented by the MHC

- Polygeny
- Codominant expression of MHC genes
- Polymorphism across population

Polygeny

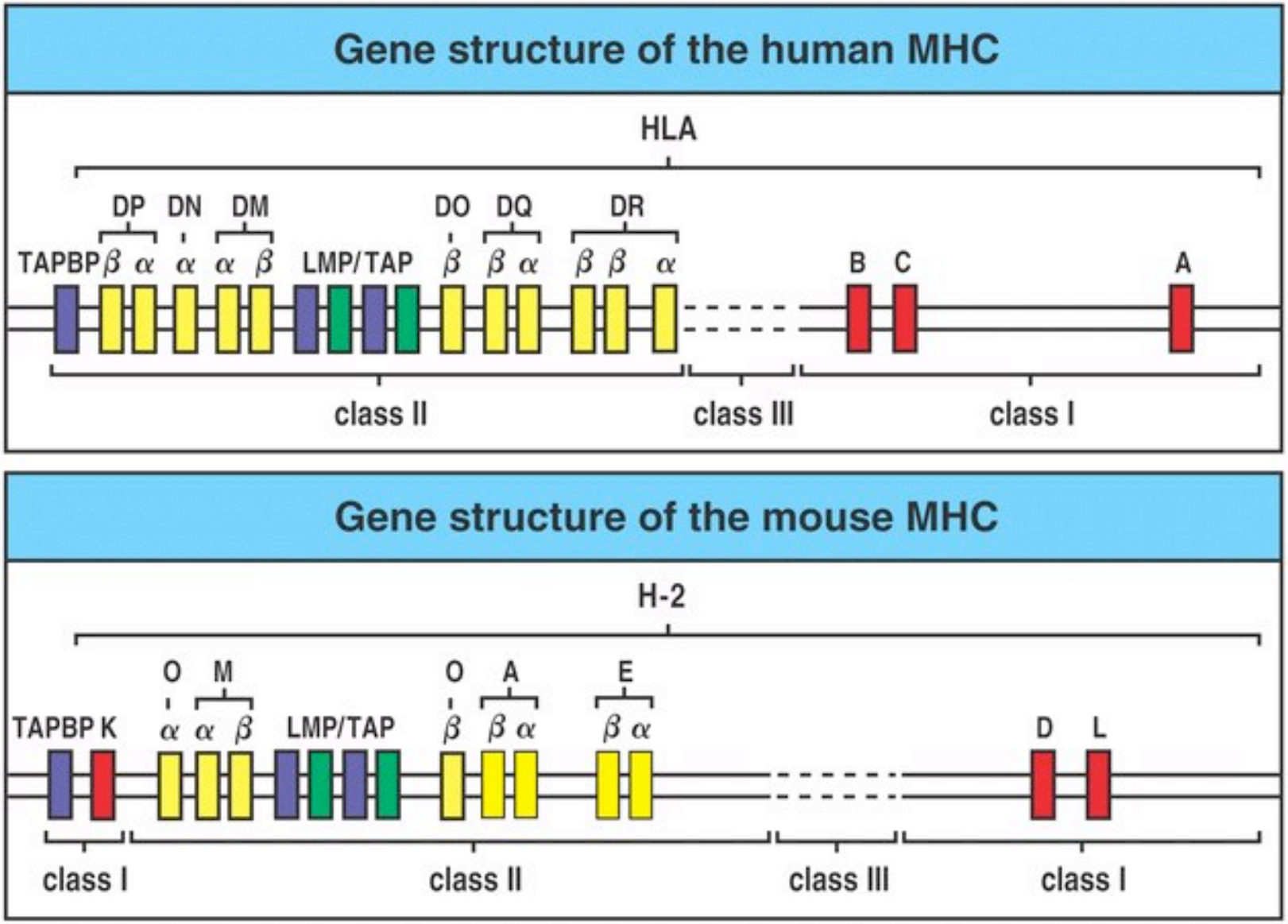


Figure 5-11 Immunobiology, 6/e. (© Garland Science 2005)

Polygeny

- Class I=
  - 3 α chains: HLA-A, HLA-B, HLA-C
  - α chain pairs with β2 microglobulin

- Class II=
  - 3 α, β pairs= HLA-DR, HLA- DP, HLA- DQ
  - Extra β chain in HLA-DR

⇨Total= 3 class I, 4 class II molecules encoded on each chromosome

Codominant expression

- 2 parents x 3 class I= 6 class I
- 2 parents x 4 class II= 8 class II
- Can mix α,β from parents

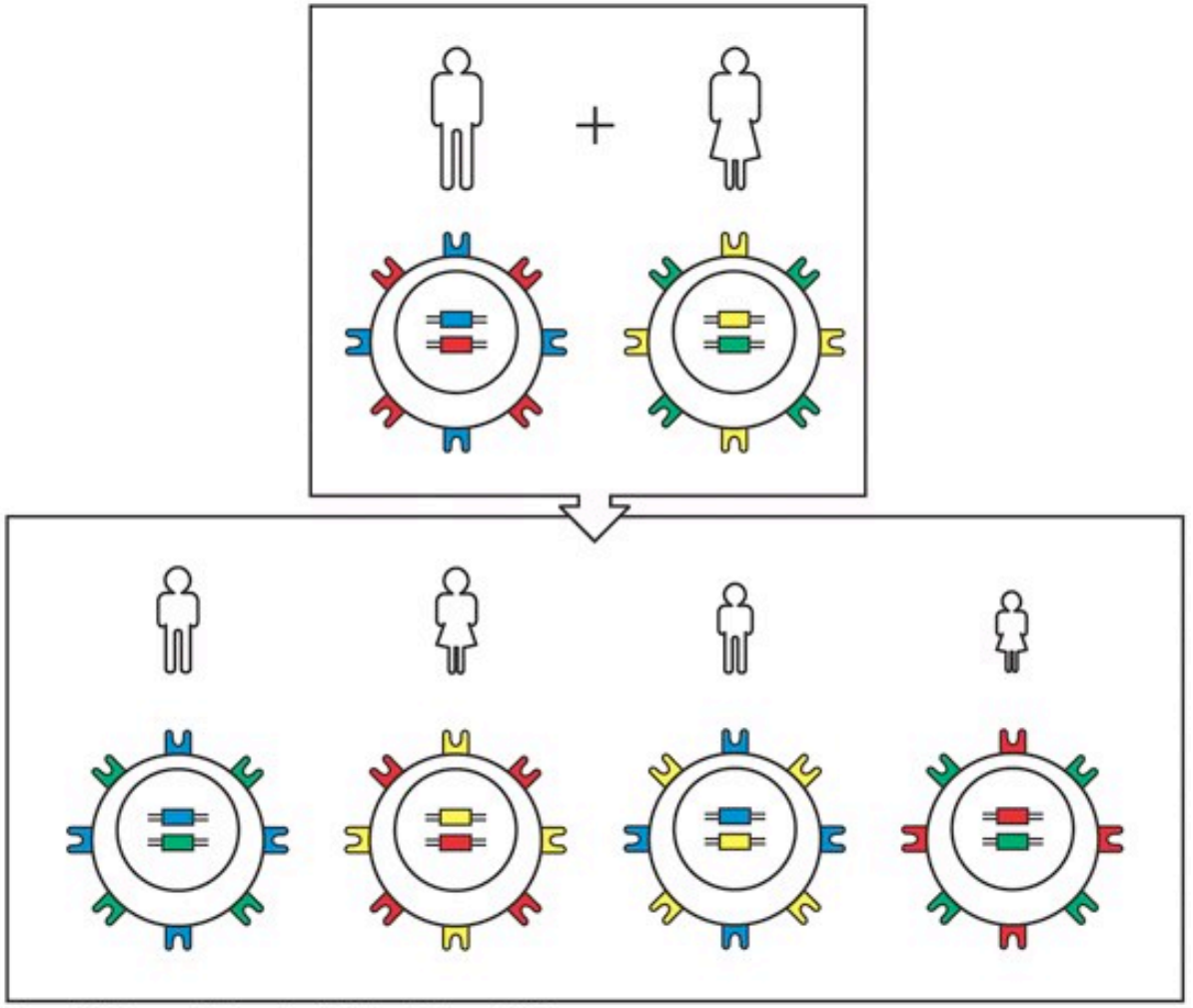


Figure 5-14 Immunobiology, 6/e. (© Garland Science 2005)

Polymorphism= different α,β alleles in population

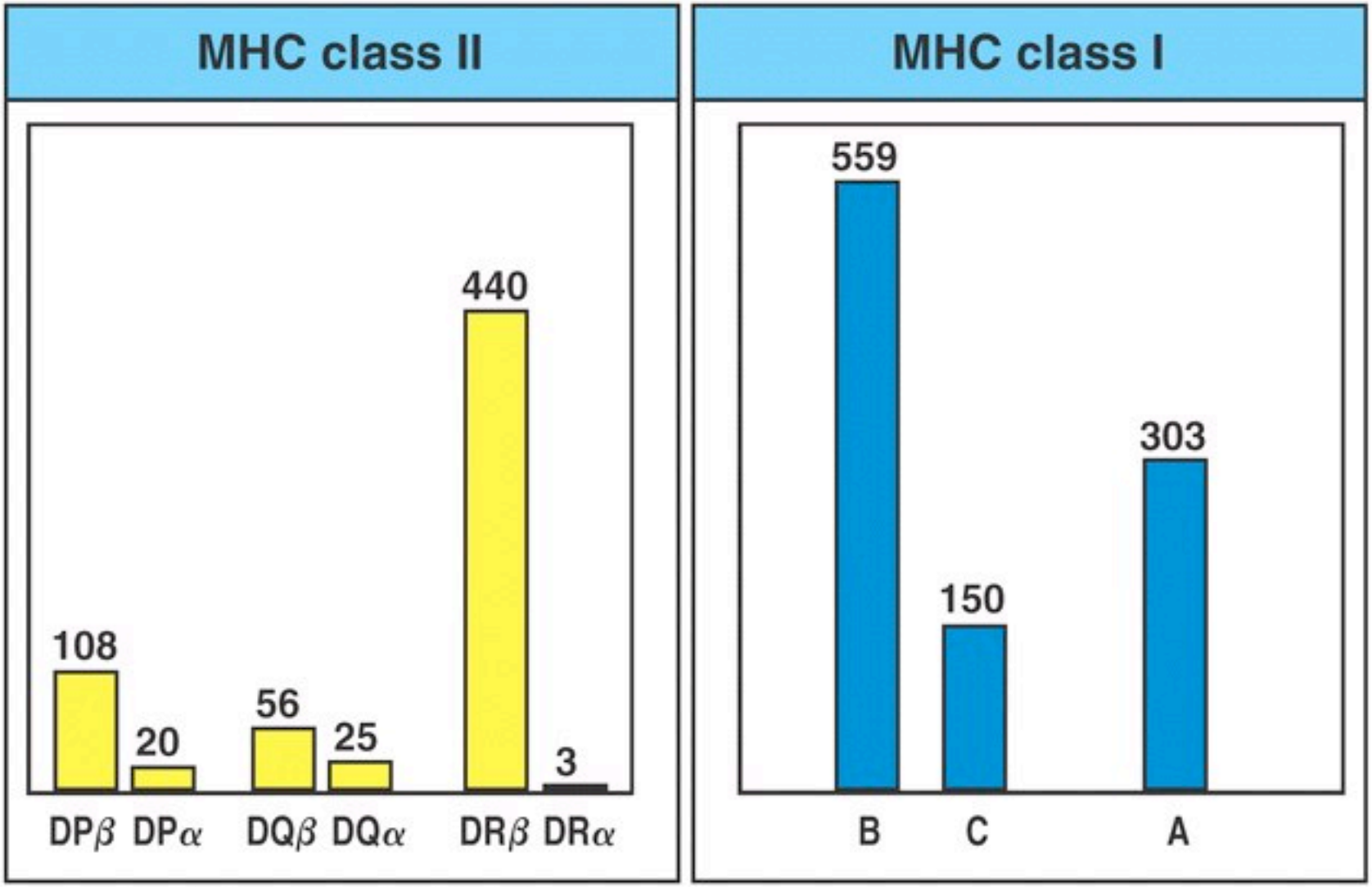


Figure 5-13 Immunobiology, 6/e. (© Garland Science 2005)

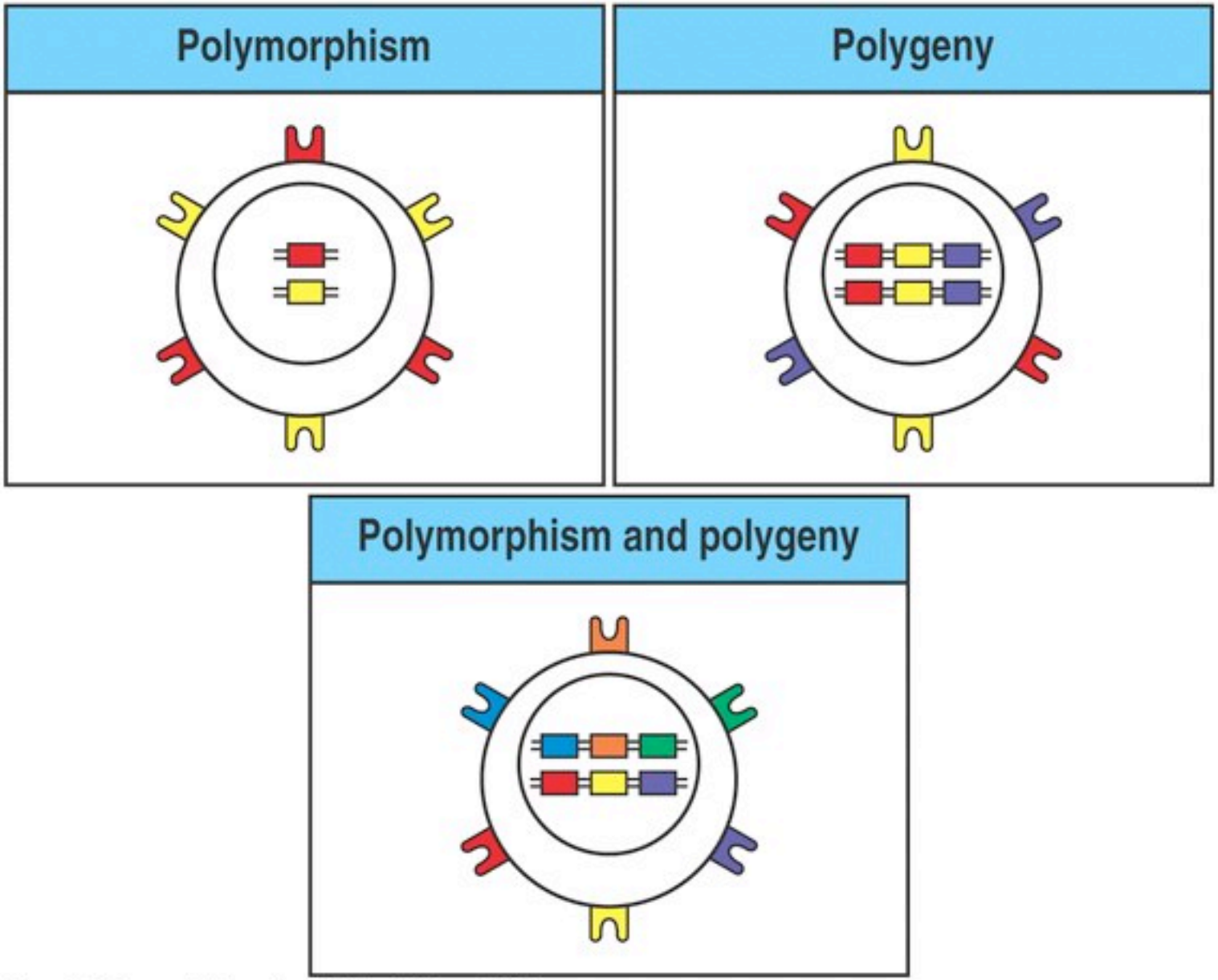


Figure 5-15 Immunobiology, 6/e. (© Garland Science 2005)

Class I variability

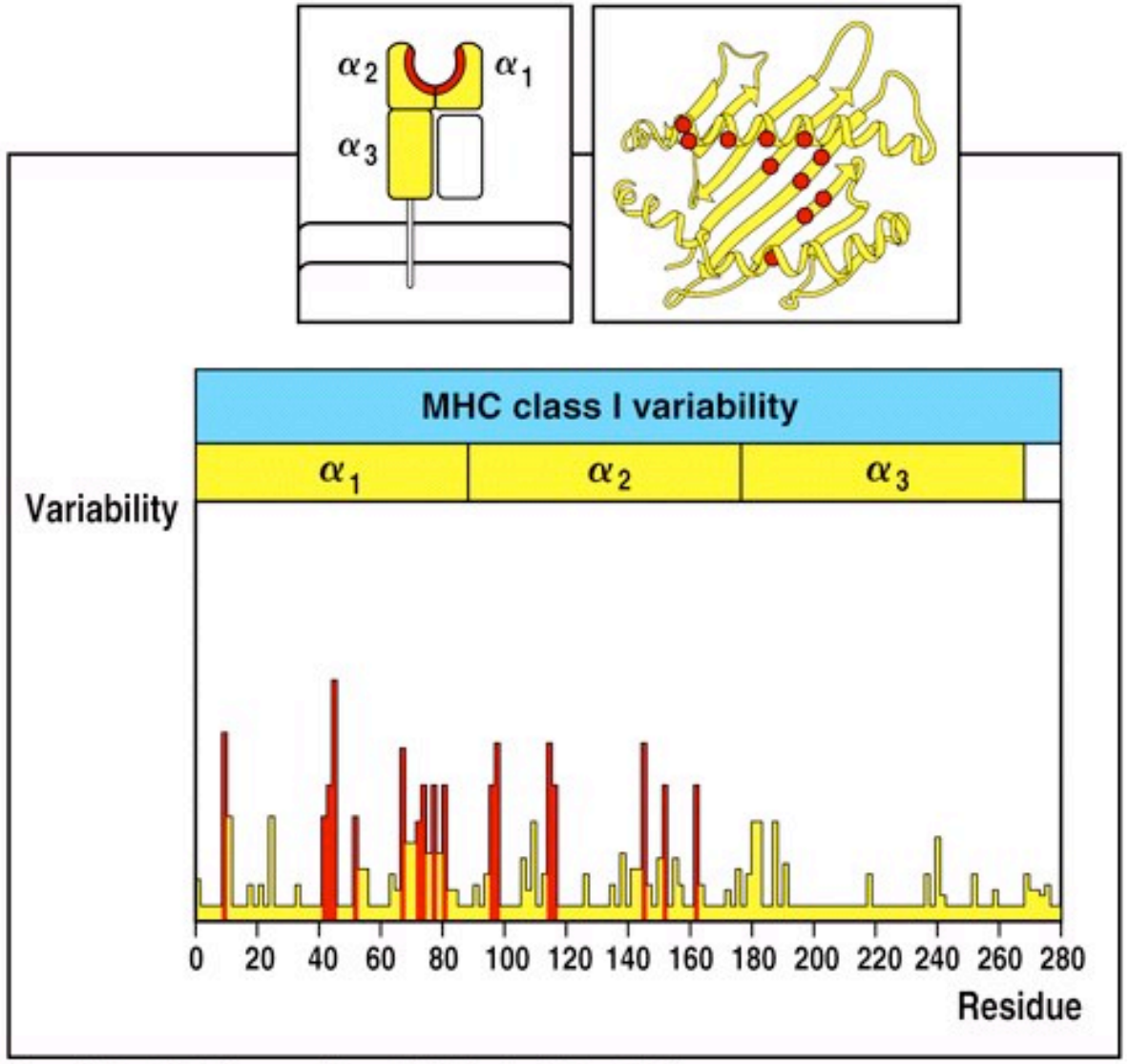


Figure 5-16 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Class II variability

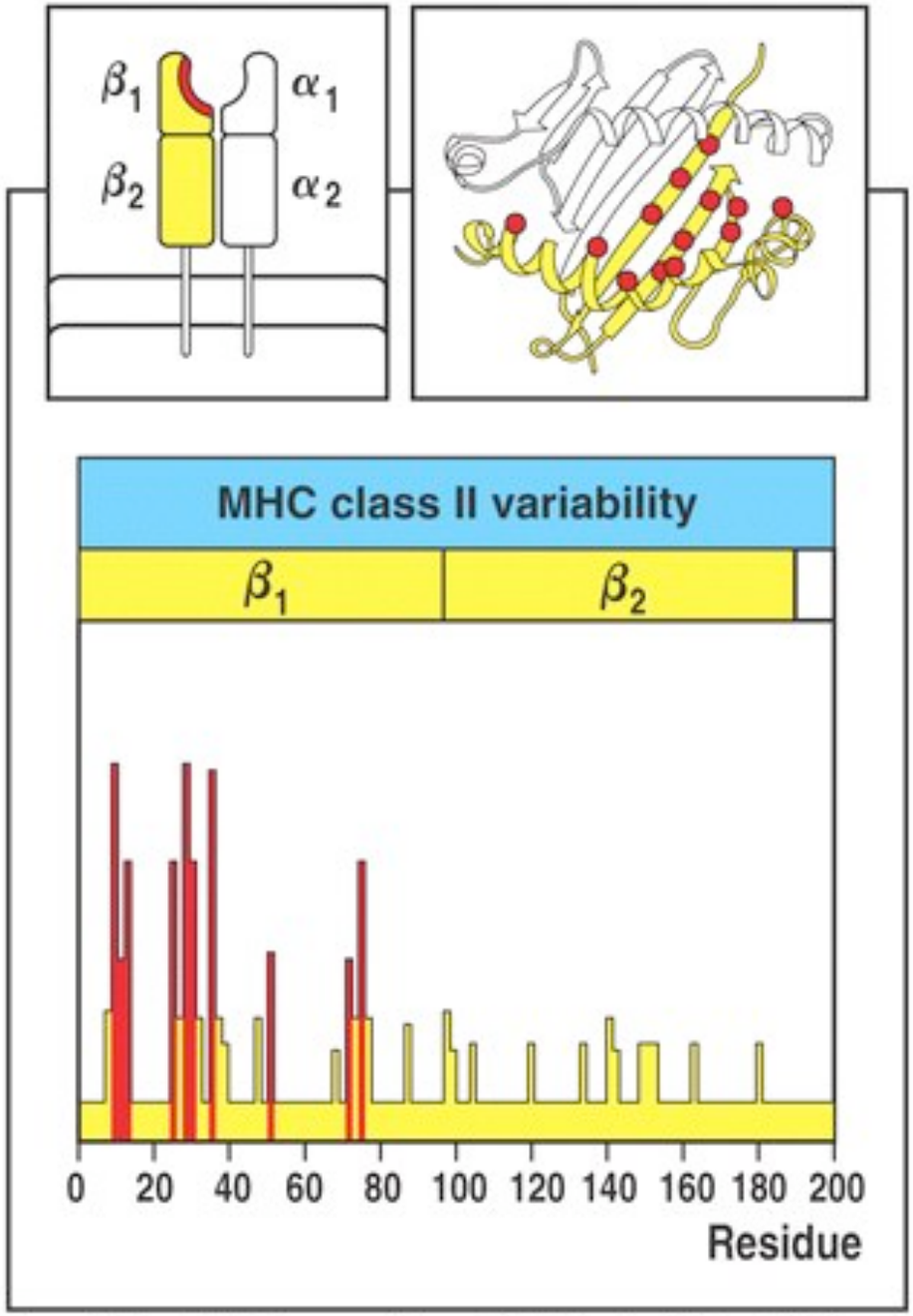


Figure 5-16 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Importance of MHC diversity

- Within population, allows presentation of some antigenic peptides from pathogen

- Problems with MHC homogeneity
  - Epstein-Barr in Papua, New Guinea
  - HIV immune evasion
  - Inbred mice

Mouse immunology vocabulary

- Isogenic= genetically identical at all loci
  - C57BL/6, BalbC

- Congenic= genetically identical at all loci except one

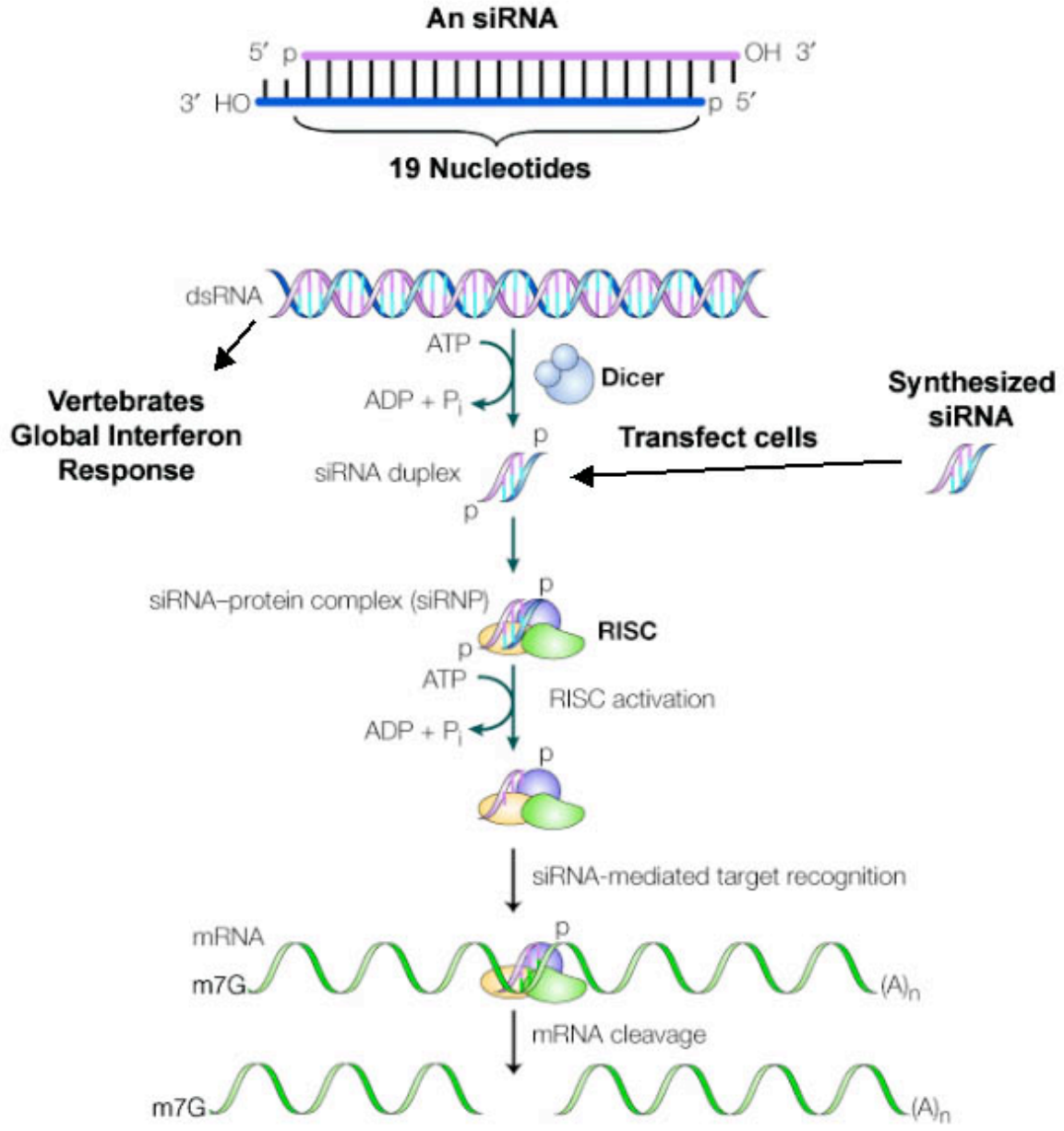
- Transgenic=
  - extra copy of gene that may be normal, altered, or different allele
  - can have tissue-specific expression

- Knock-out=
  - specific gene is deleted by homologous recombination

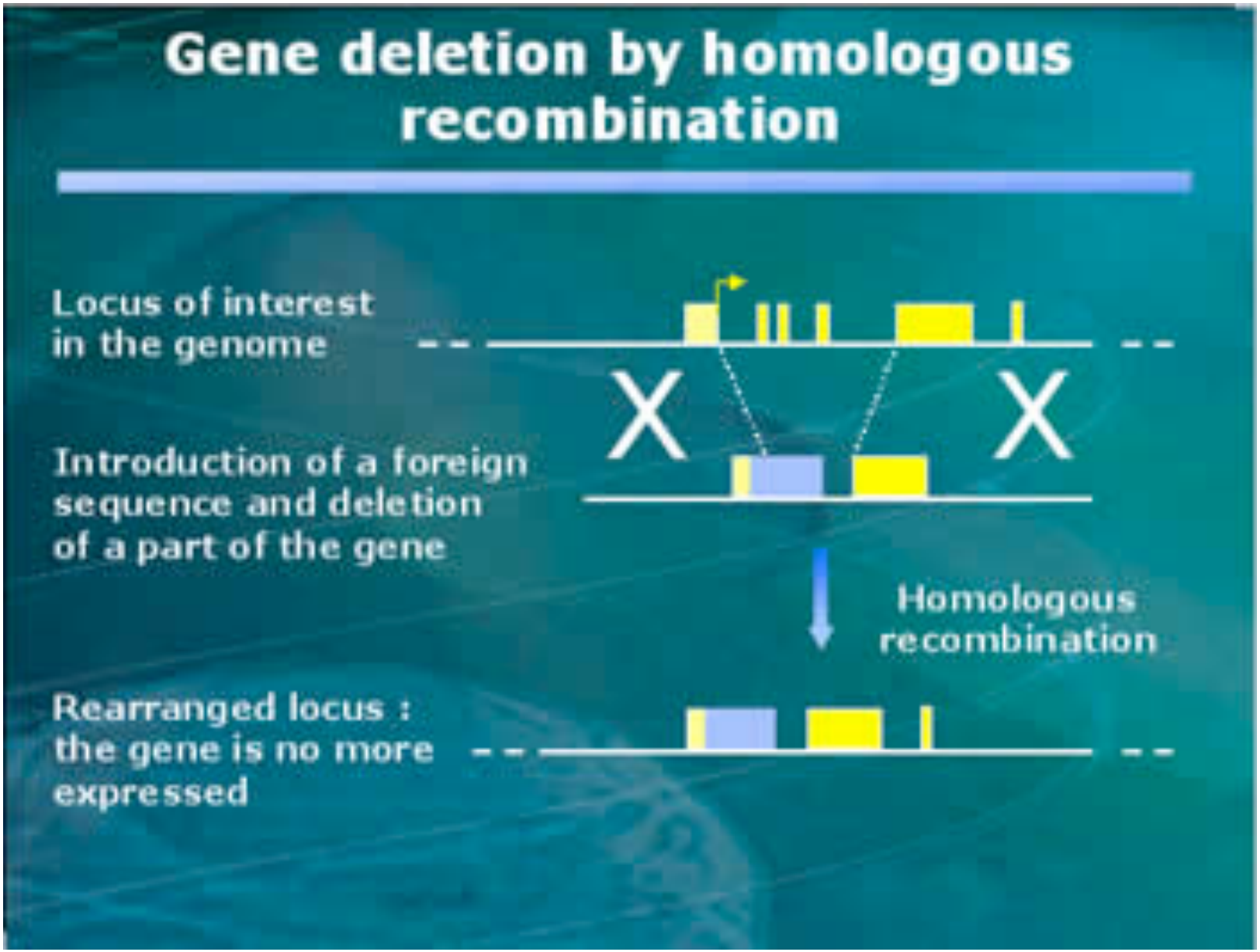
- Knock-in=
  - specific gene is replaced by altered form by homologous recombination

- Knock-down=
  - specfic mRNA is decreased by RNA interference (RNAi)
  - can have tissue-specific expression

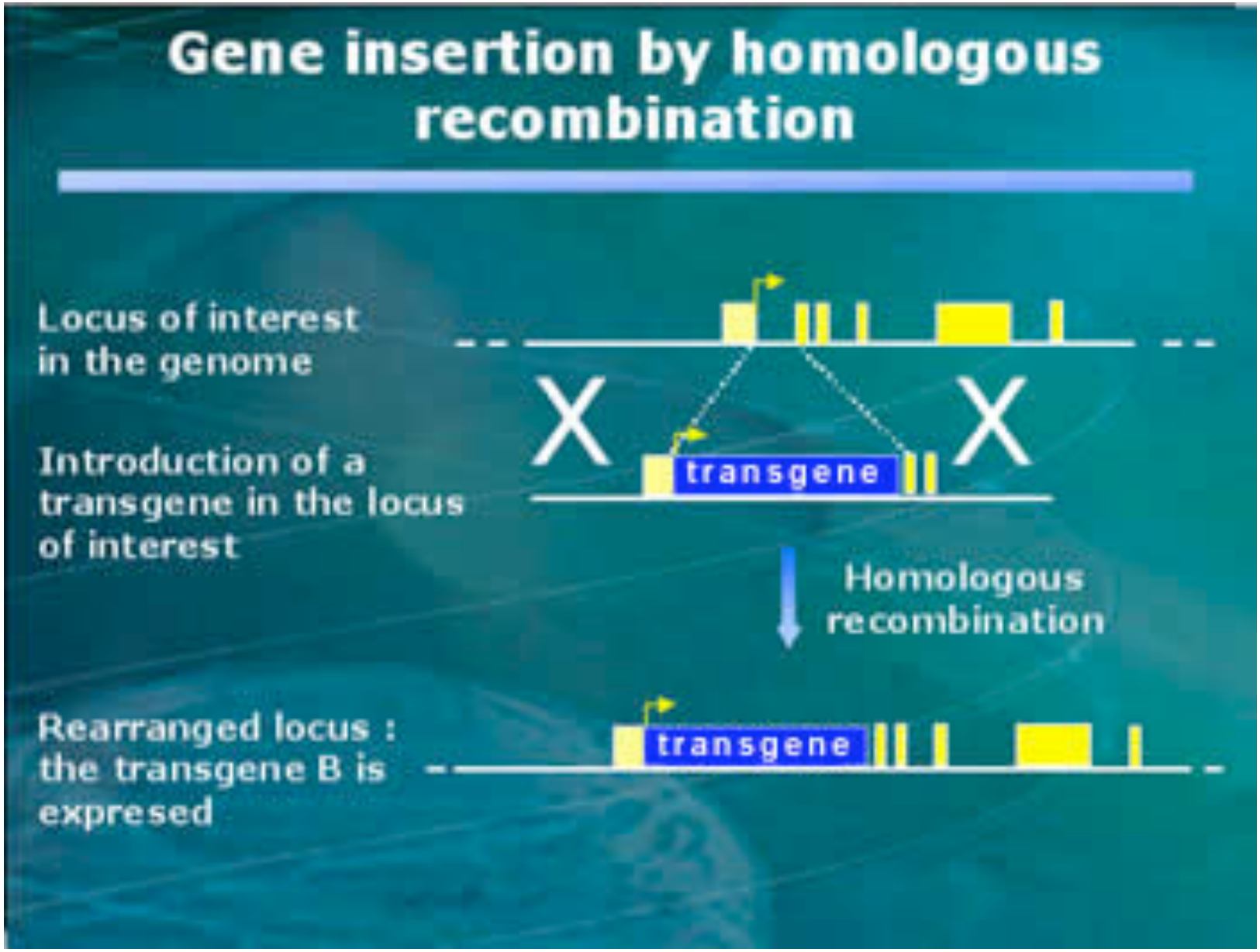
RNA interference (RNAi)



http://www.life.uiuc.edu/shapiro/RNAiApps.html



http://www.genoway.com/



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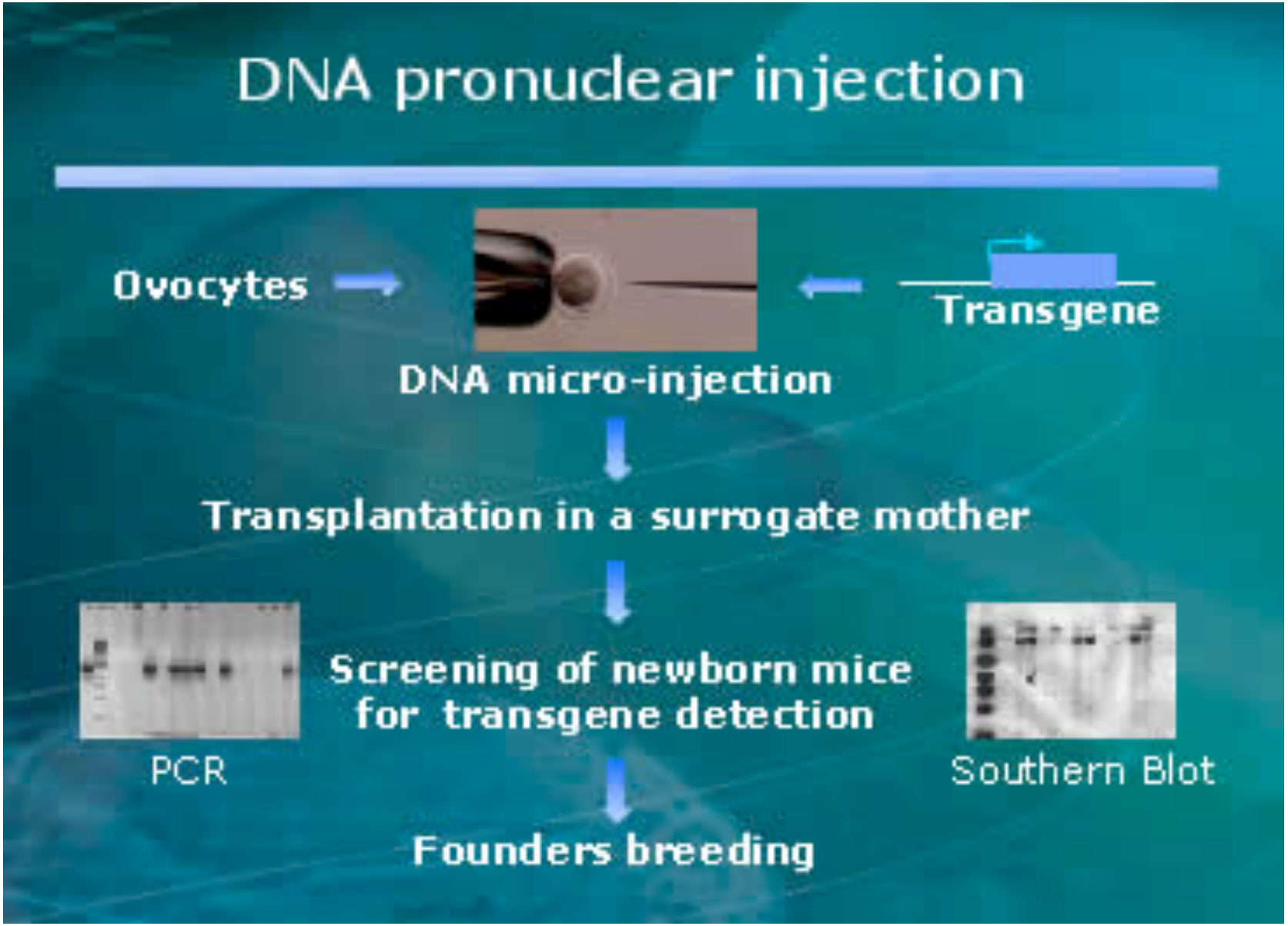

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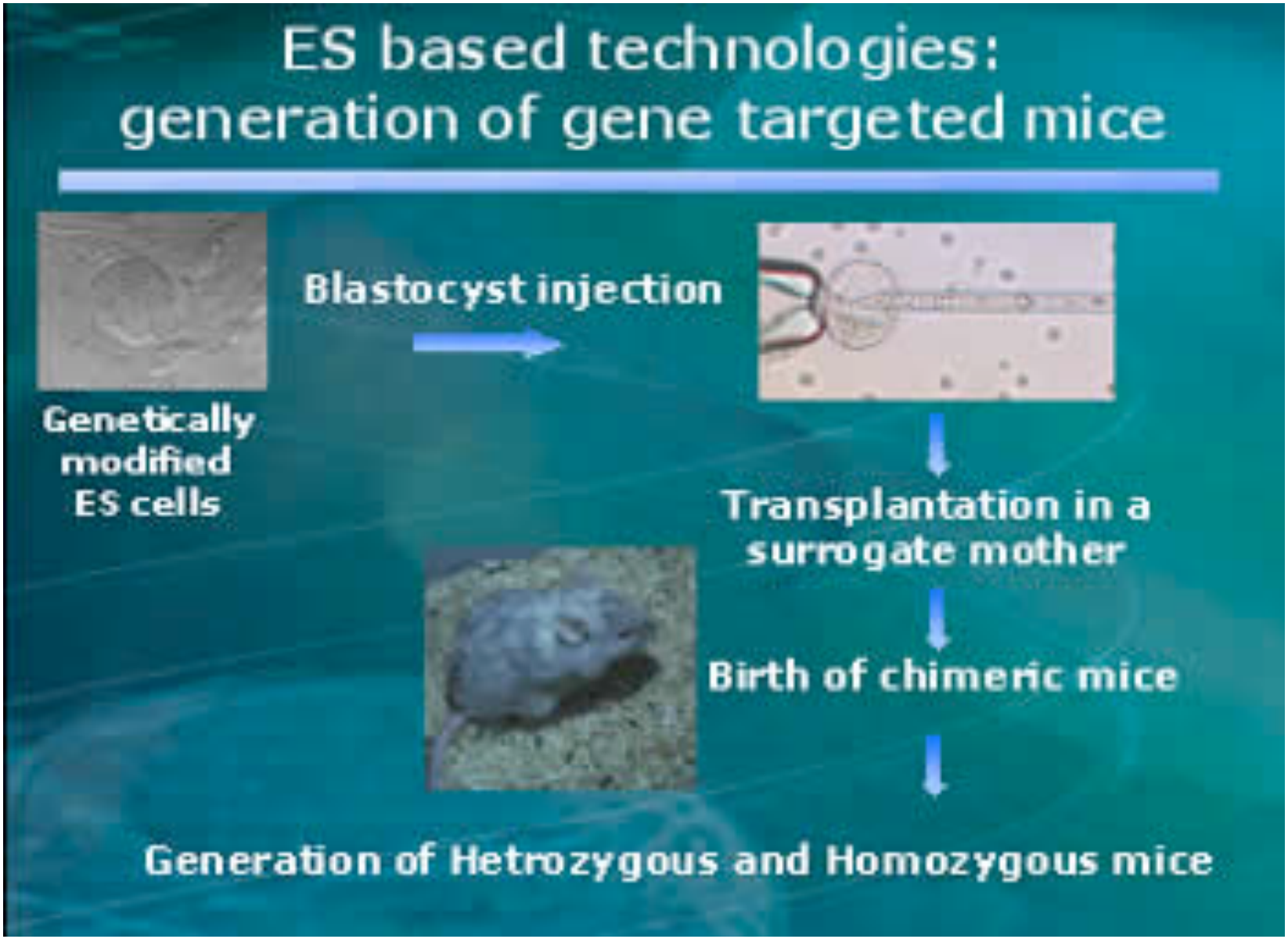


http://www.genoway.com/

Transgenic mice

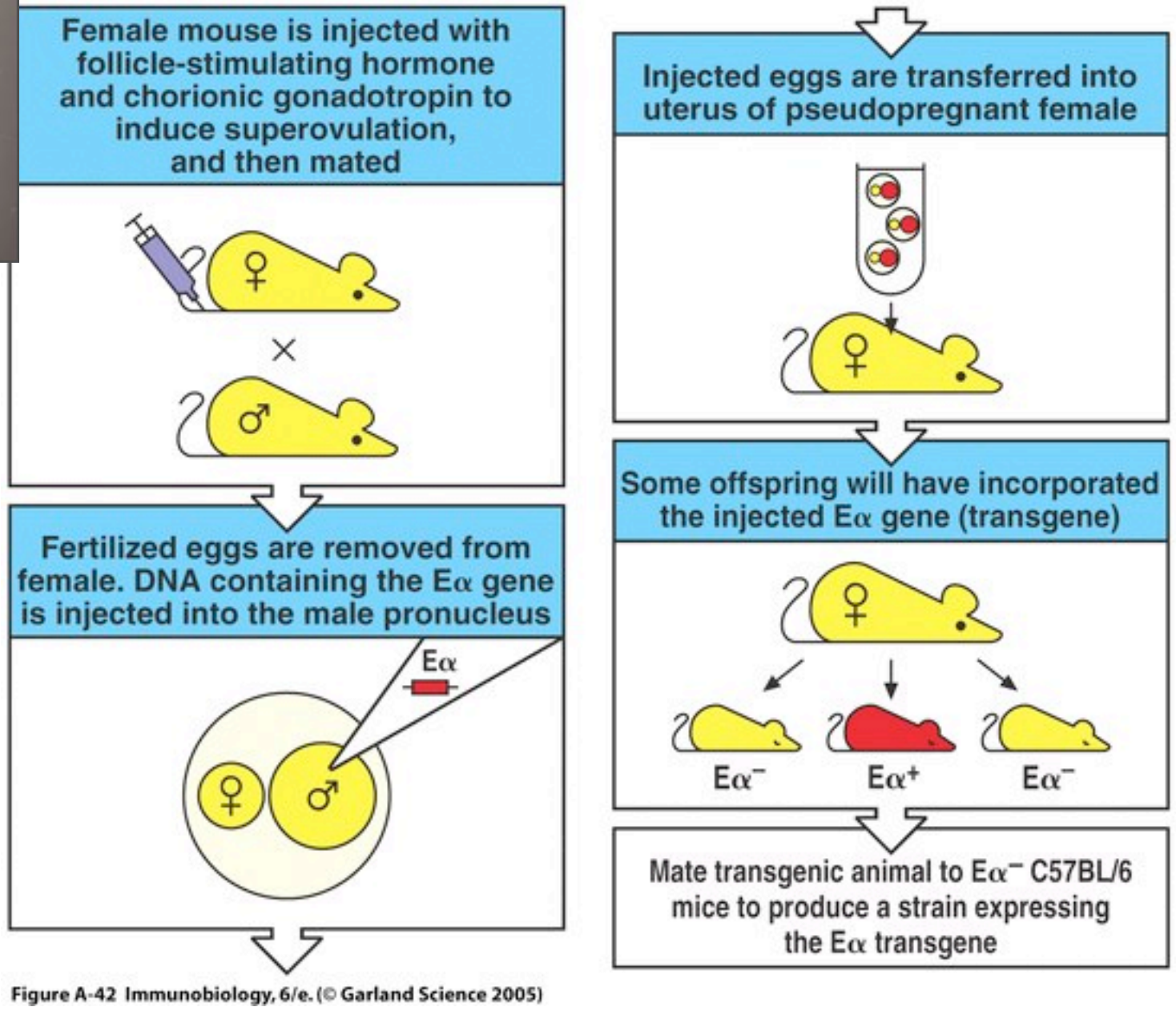


Figure A-42 Immunobiology, 6/e. (© Garland Science 2005)

Creating a gene knockout

- *neo*=
  - resistance to drug G418
- HSV-tk=
  - expression in presence of ganciclovir is lethal

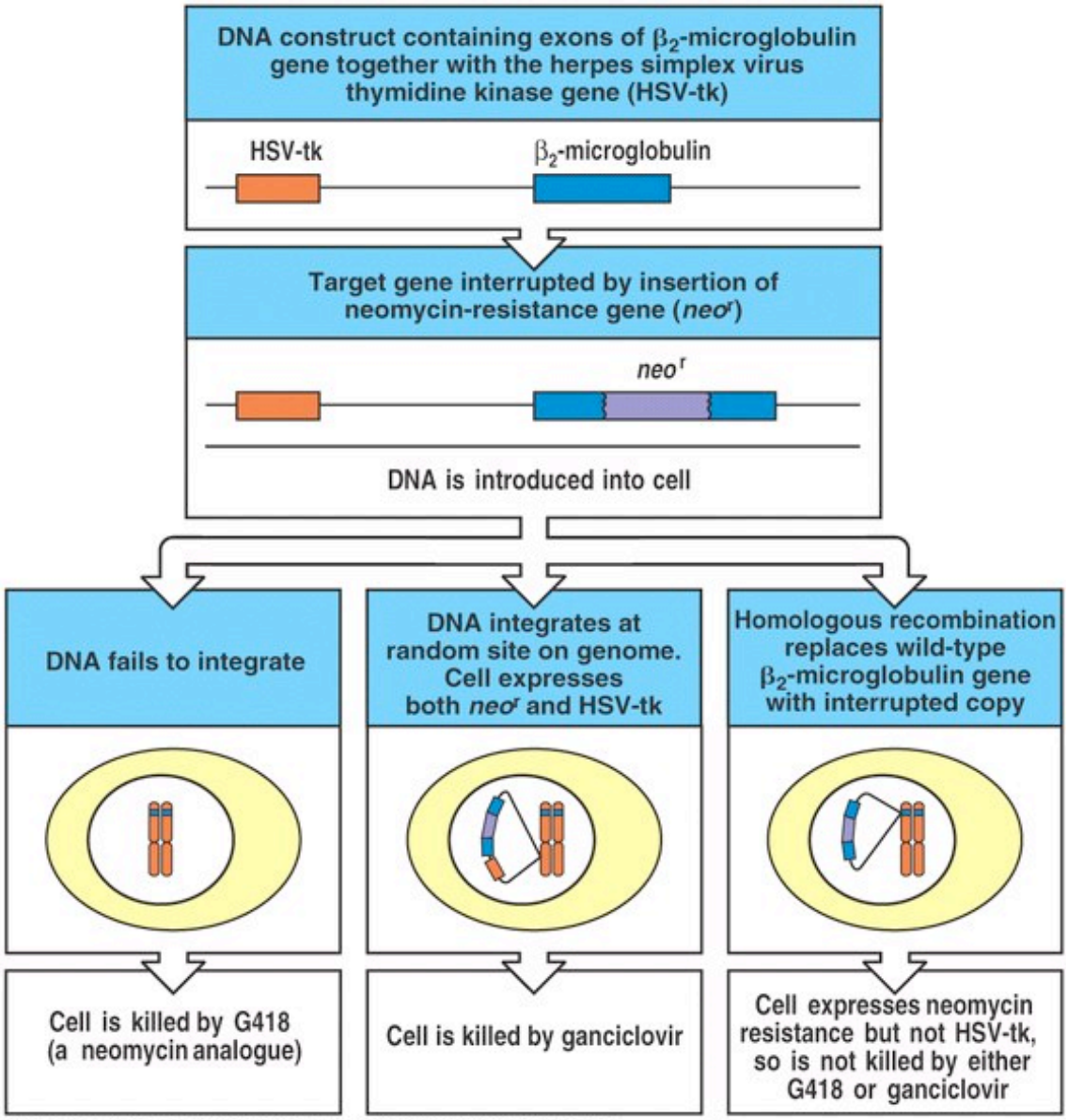


Figure A-44 Immunobiology, 6/e. (© Garland Science 2005)

Knockout mice

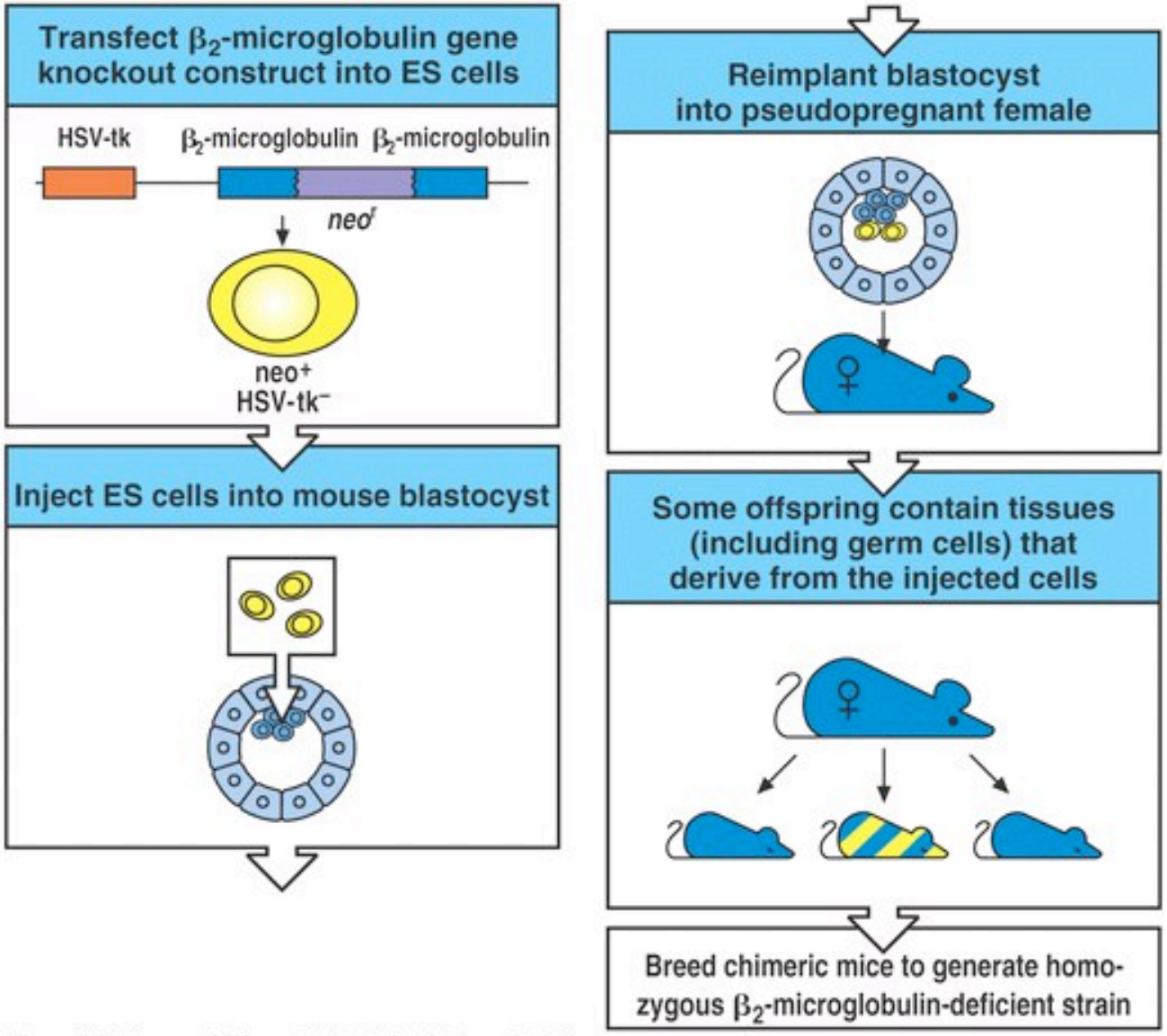


Figure A-45 Immunobiology, 6/e. (© Garland Science 2005)

L. monocytogenes infection

- Mutant mice with a defined genetic defect in the beta 2-microglobulin or the H2-I-A beta chain
- Virtually devoid of functional CD8 or CD4 alpha beta T cells
- The lethal dose of L. monocytogenes was lower, but were able to resolve low dose infection
- The course of disease was exacerbated and clearance was markedly delayed
- Granuloma infection increased

George Snell's experiment

MHC restriction

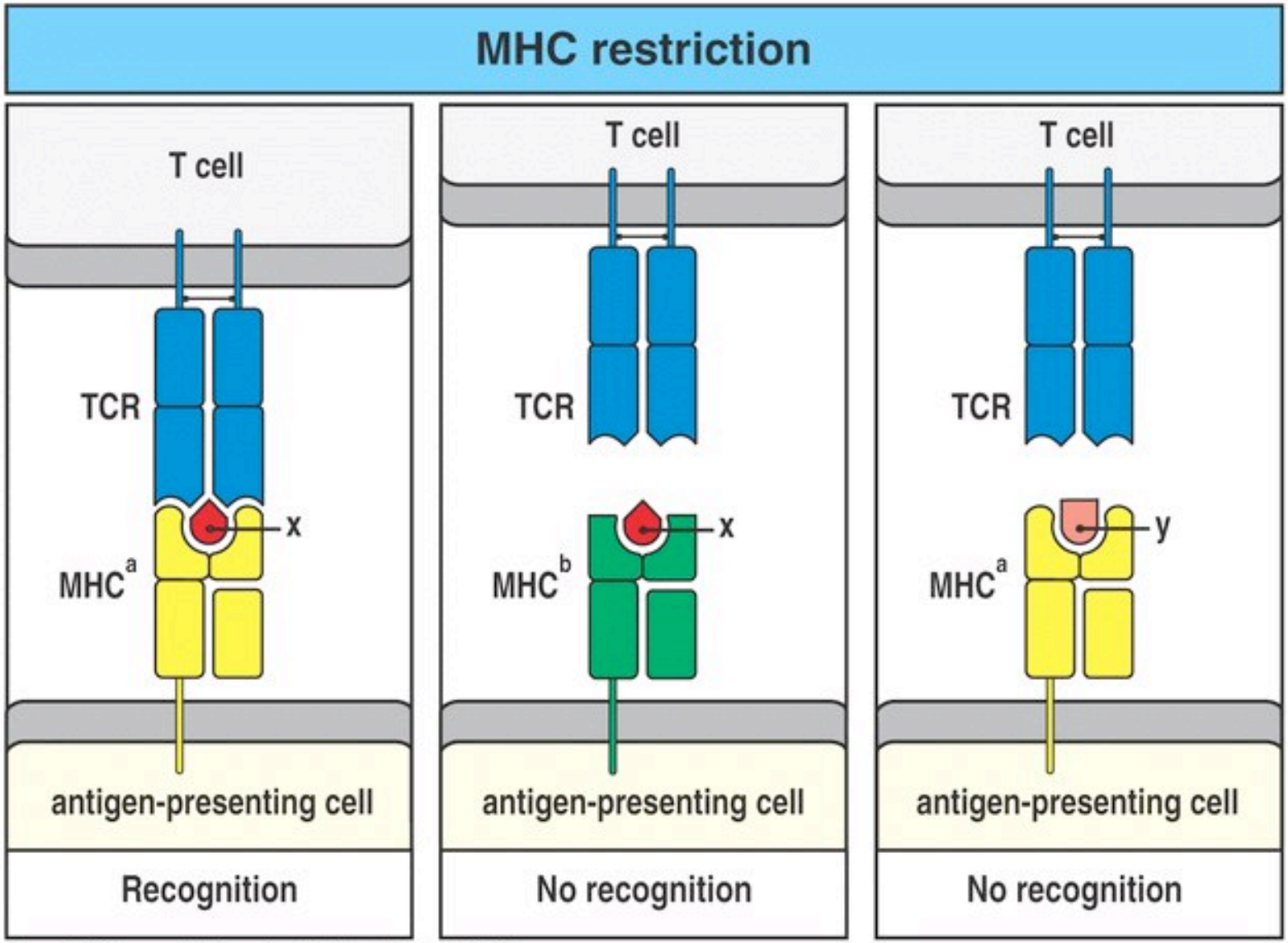


Figure 5-17 Immunobiology, 6/e. (© Garland Science 2005)

Superantigens:Disrupting MHC restriction

- Bacterial or viral in origin
  - Bacterial: *Staphylococcus, Streptococcus, Mycoplasma*
  - Viral: MMTV

- Bind to Vβ of TCR
  - Has specificity for certain Vβ genes
  - Away from CDRs

- Binds to outer portion of MHC
  - Binds to many different MHC molecules
  - Away from peptide groove

Superantigens

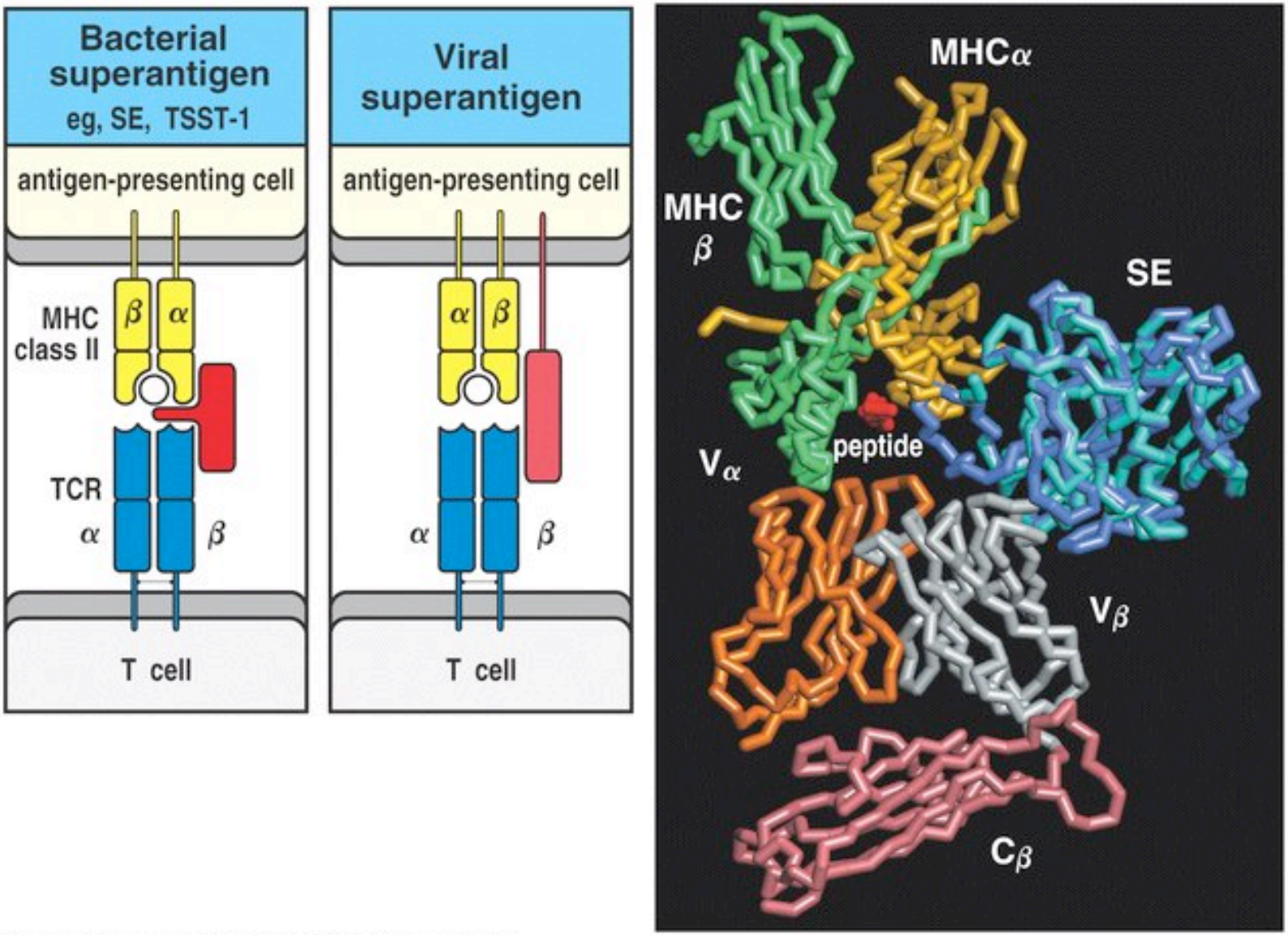


Figure 5-19 Immunobiology, 6/e. (© Garland Science 2005)

The effect of superantigens

- Stimulates 2-20% of all T cells
- Overexpression of T cell cytokines
  - IL-2: T cell proliferation
  - TNF-α: You should know!

$\Rightarrow$Can lead to shock when too many cytokines are expressed

$\Rightarrow$Can lead to immunosupression

- Deletion by apoptosis?
- T cells anergy?
- Bone marrow suppression?

$\Rightarrow$Can lead to immune system diversion

The problem with transplant rejection: T cell alloreactivity

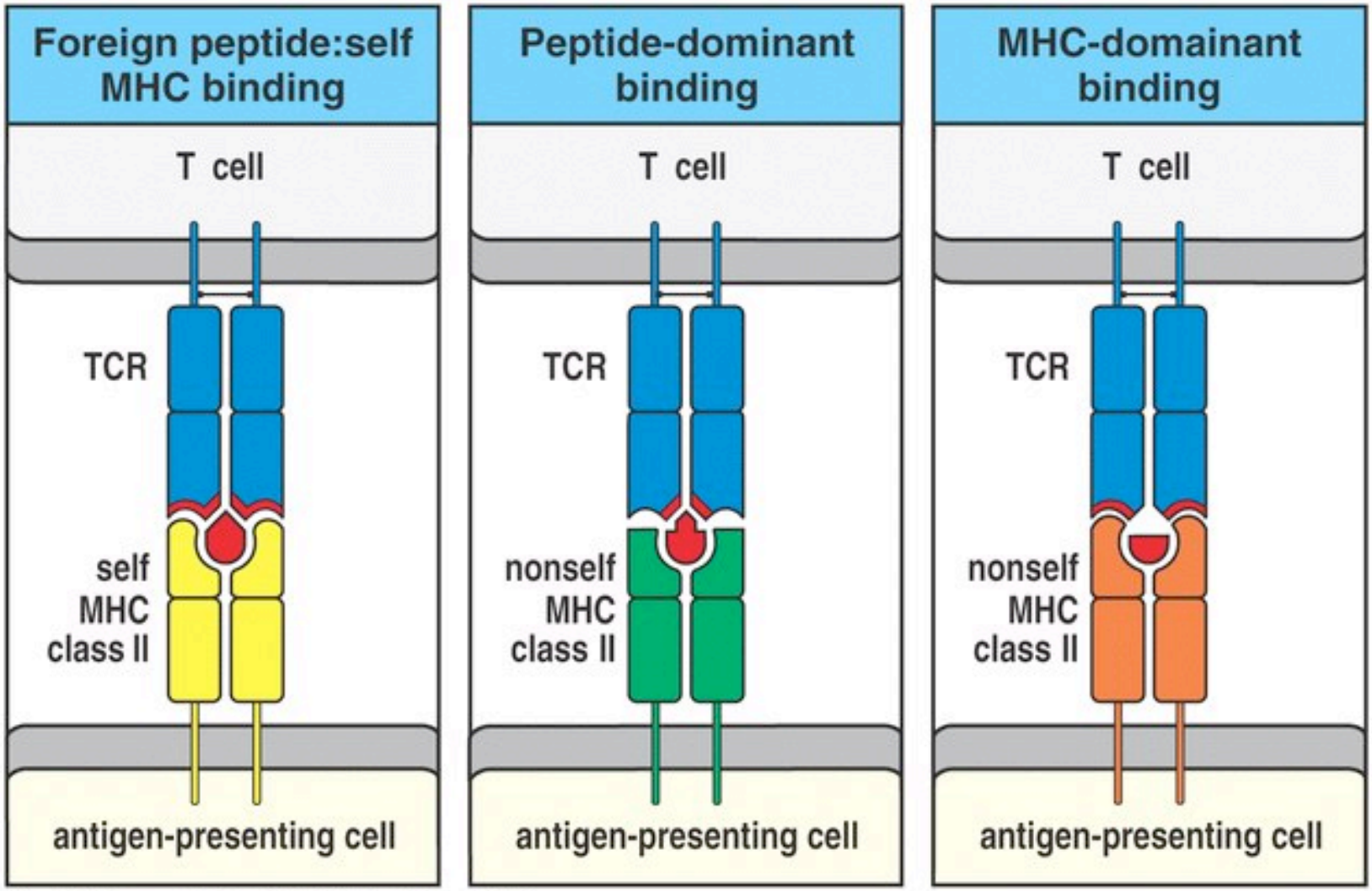


Figure 5-18 Immunobiology, 6/e. (© Garland Science 2005)

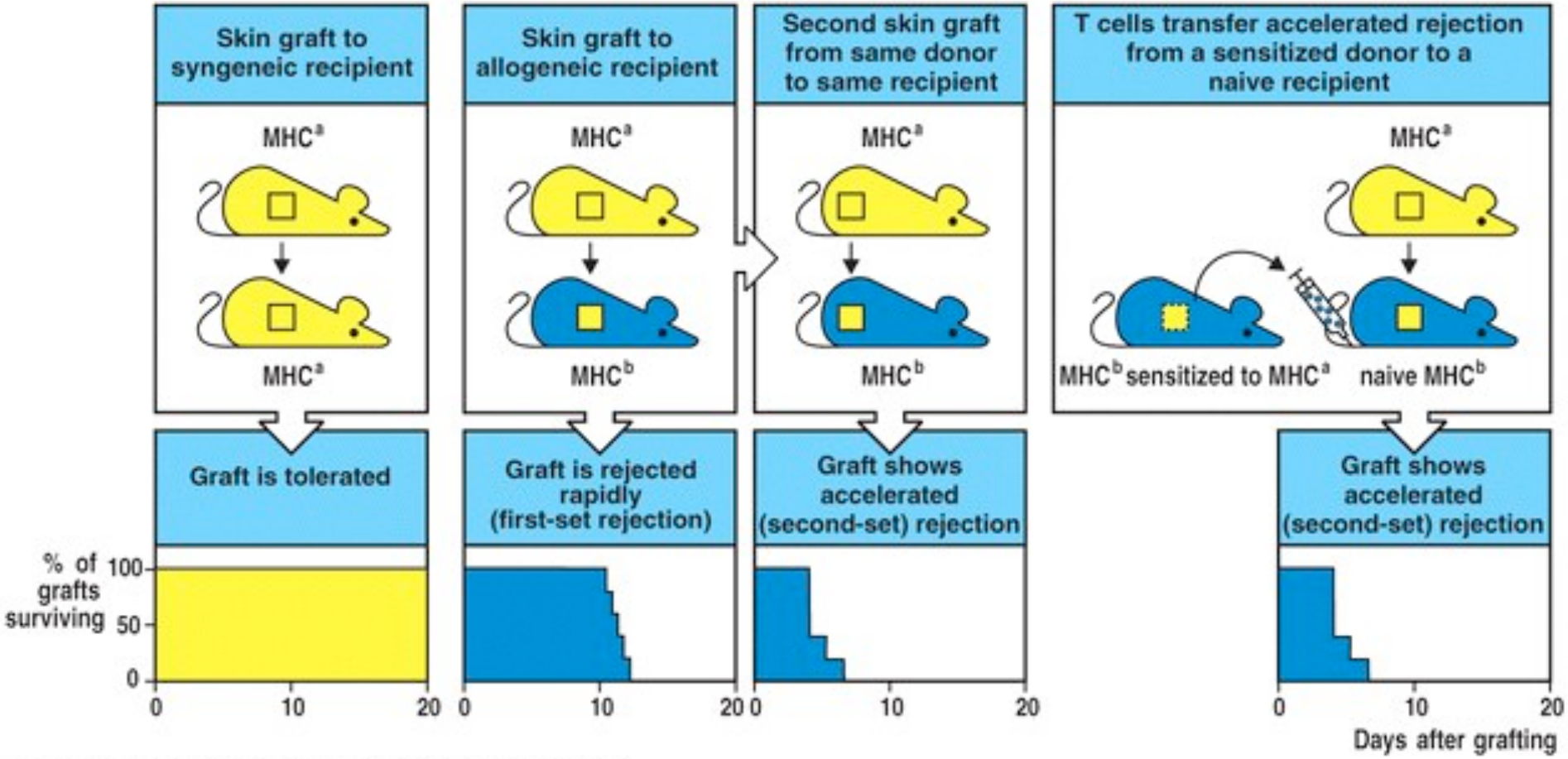


Figure 13-35 Immunobiology, 6/e. (© Garland Science 2005)

Minor histocompatability antigens

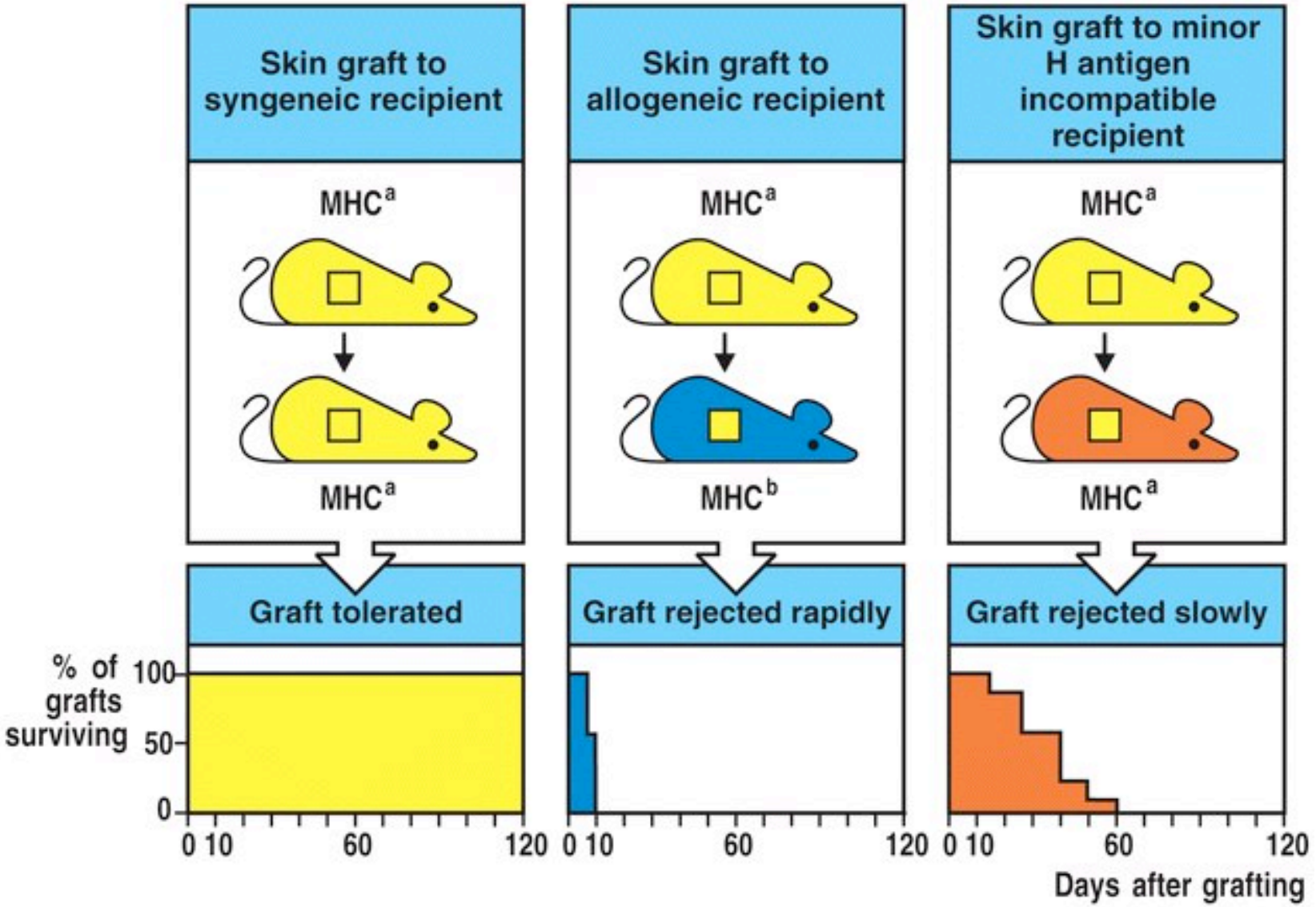
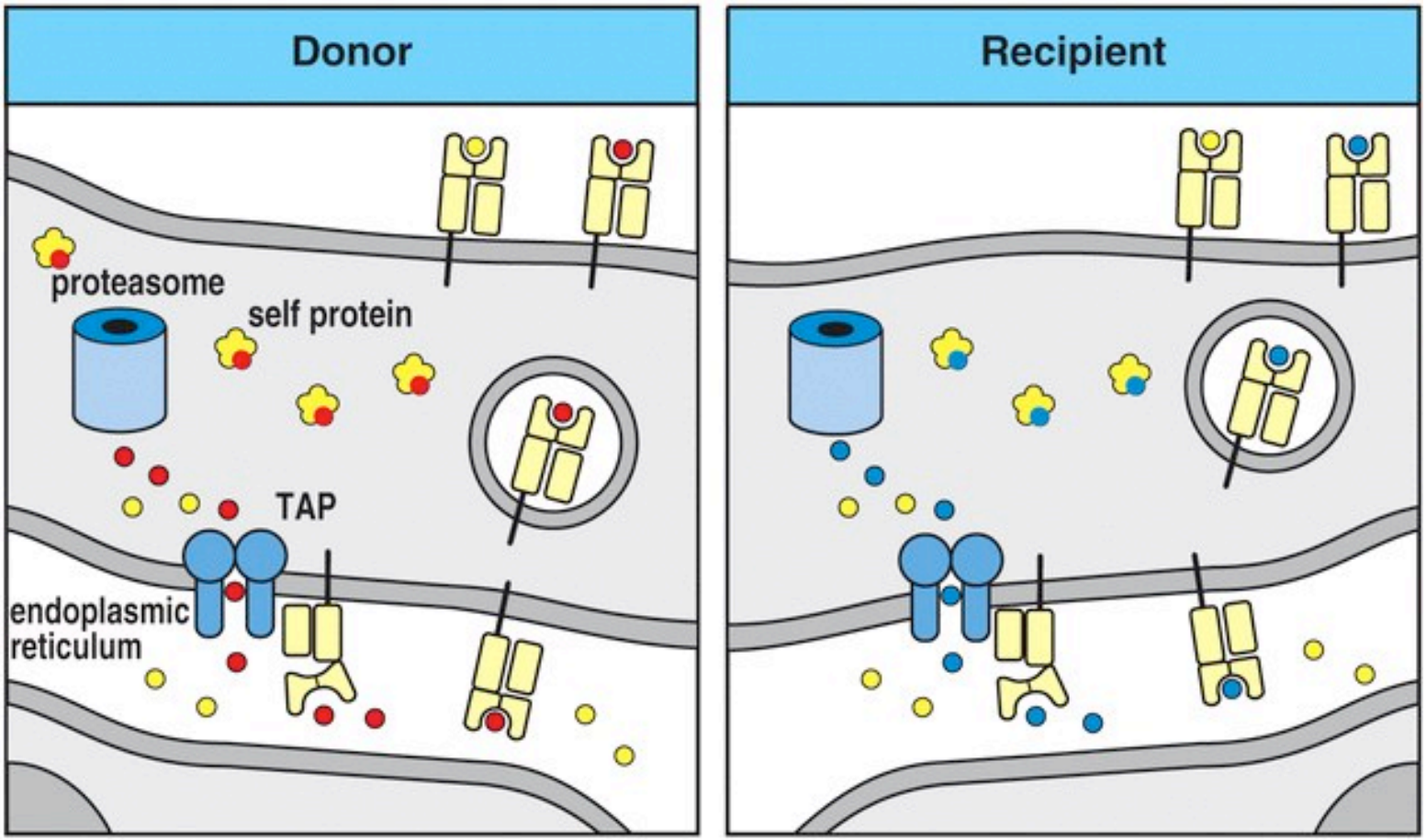


Figure 13-36 Immunobiology, 6/e. (© Garland Science 2005)

Recipient APC present minor histocompatability Ag



Polymorphic self proteins that differ in amino acid sequence between individuals give rise to minor H antigen differences between donor and recipient

Figure 13-37 Immunobiology, 6/e. (© Garland Science 2005)

APCs cause T cell proliferation

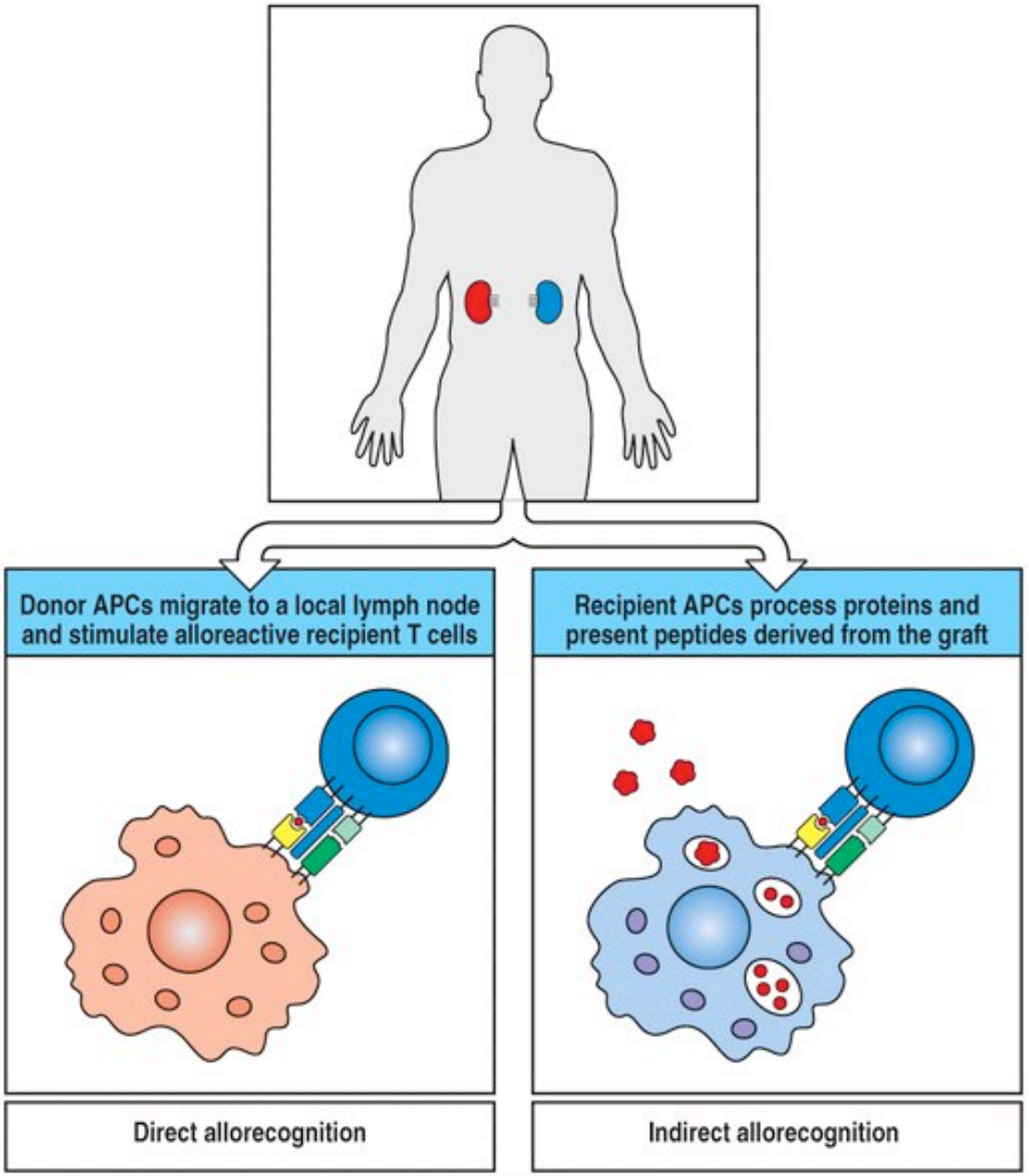


Figure 13-38 Immunobiology, 6/e. (© Garland Science 2005)

Transplant rejection

- Initial damage due to CTL response

- Helper T cell cytokines cause long-term inflammatory response

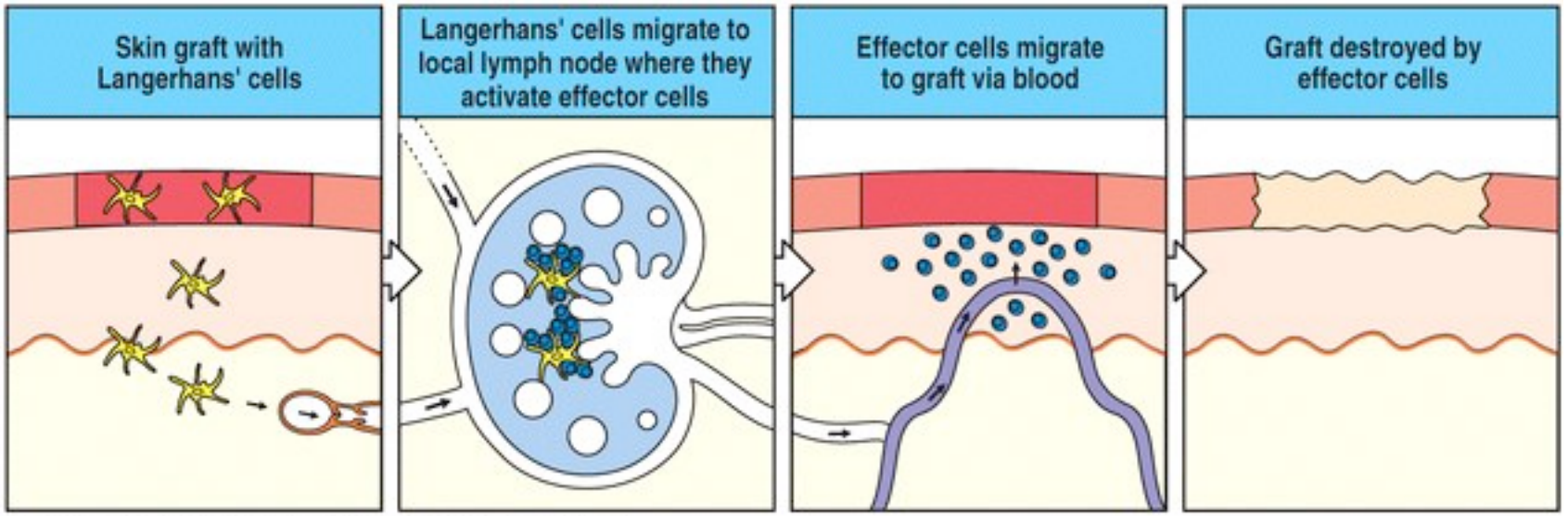


Figure 13-39 Immunobiology, 6/e. (© Garland Science 2005)

Hyperacute rejection

- Complement mediated rejection
- Preformed Ab to blood group, MHC molecules
- Problem with xenografting (α-Gal)

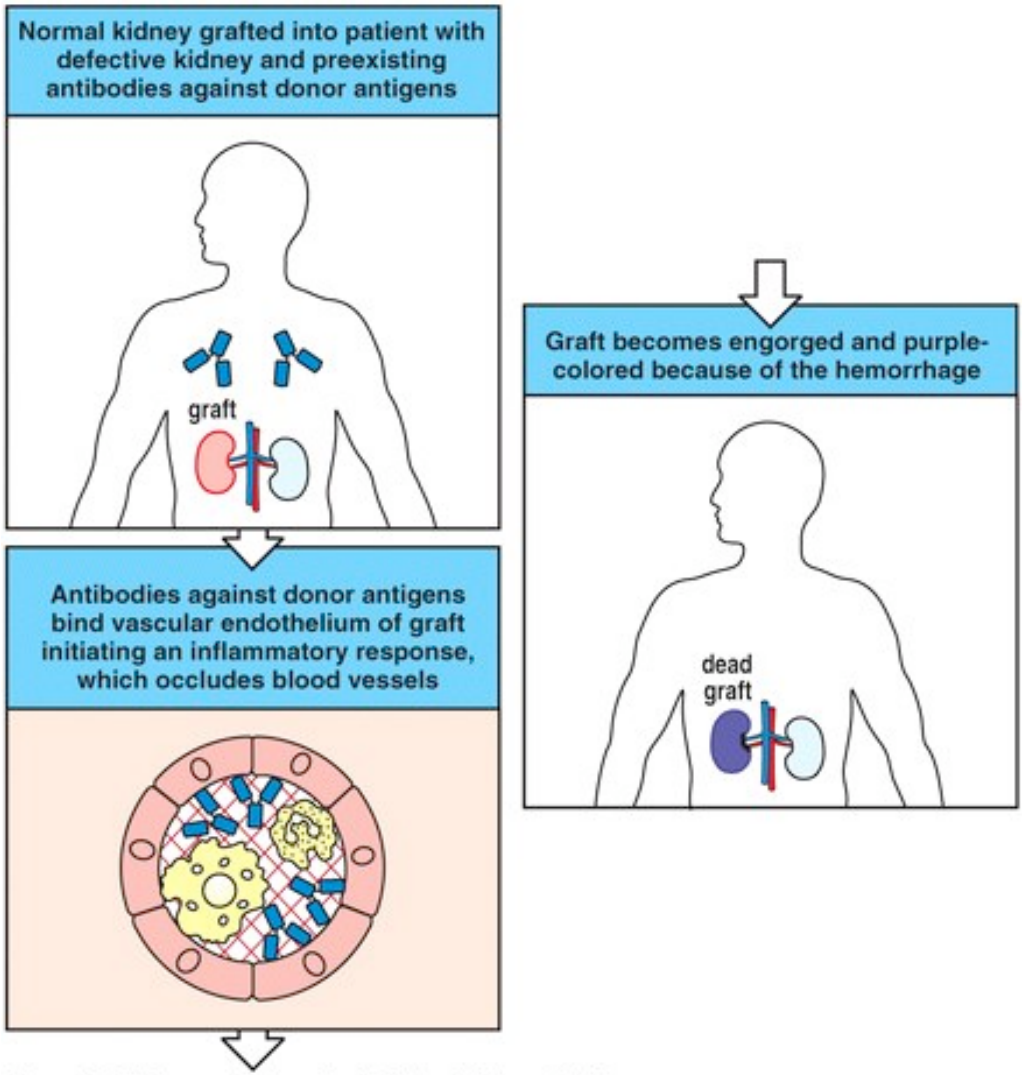


Figure 13-40 Immunobiology, 6/e. (© Garland Science 2005)

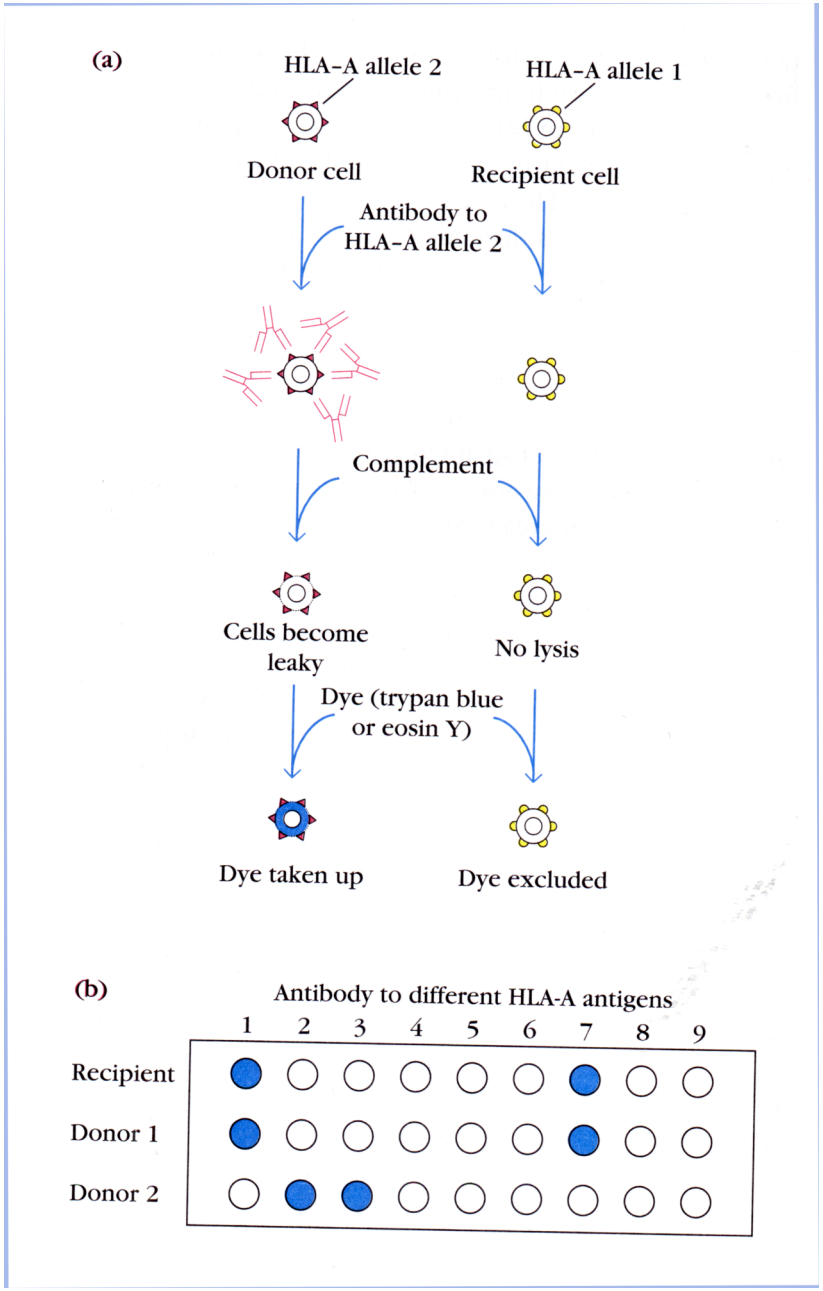
Preventing transplant rejection

- Match MHC alleles
- Immunosupression

Donor matching

- Blood type matching
- Microcytotoxicity test
- Mixed lymphocyte reaction

Microcytotoxicity testing



T cell proliferation assay

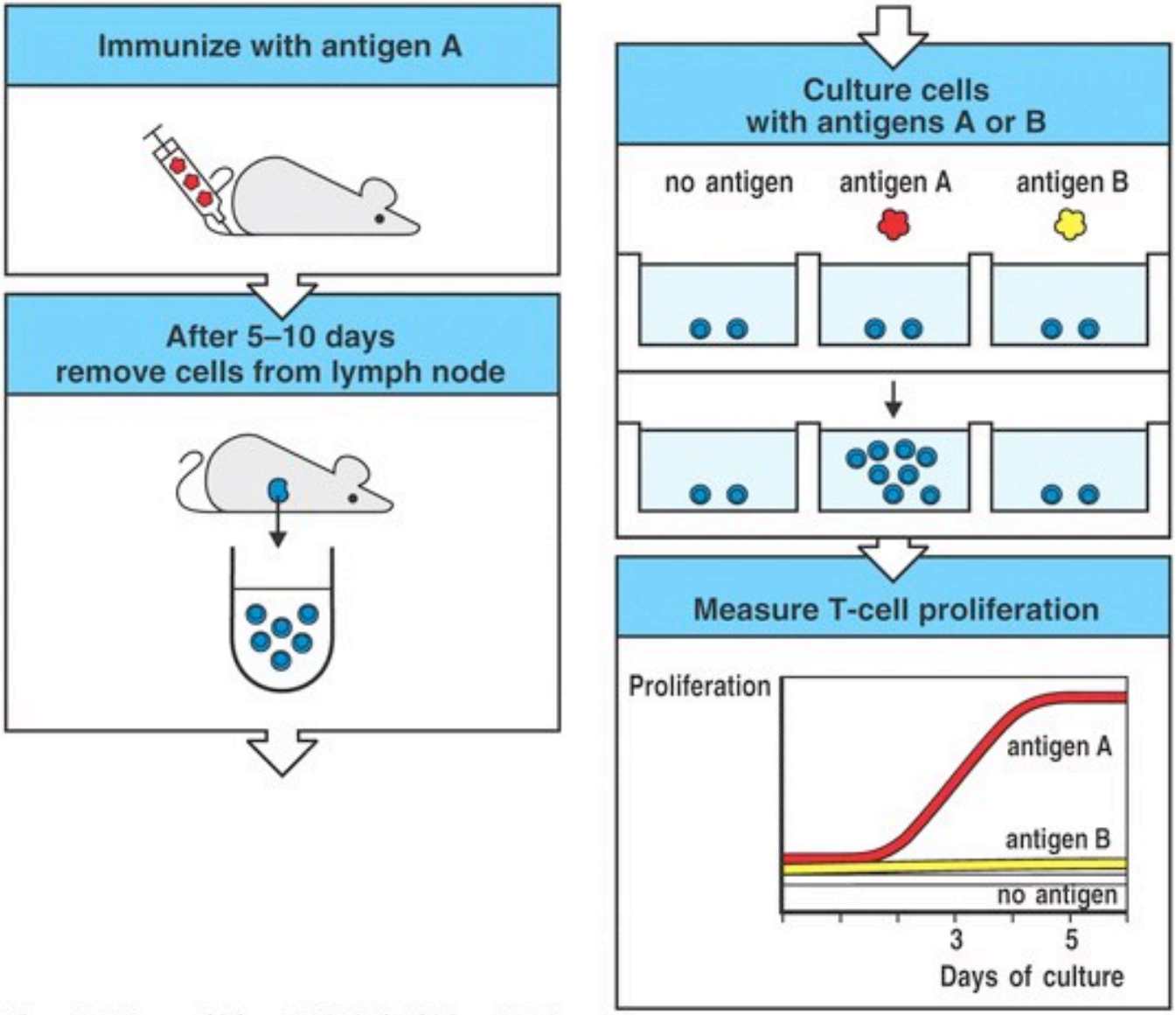


Figure A-36 Immunobiology, 6/e. (© Garland Science 2005)

CTL assay

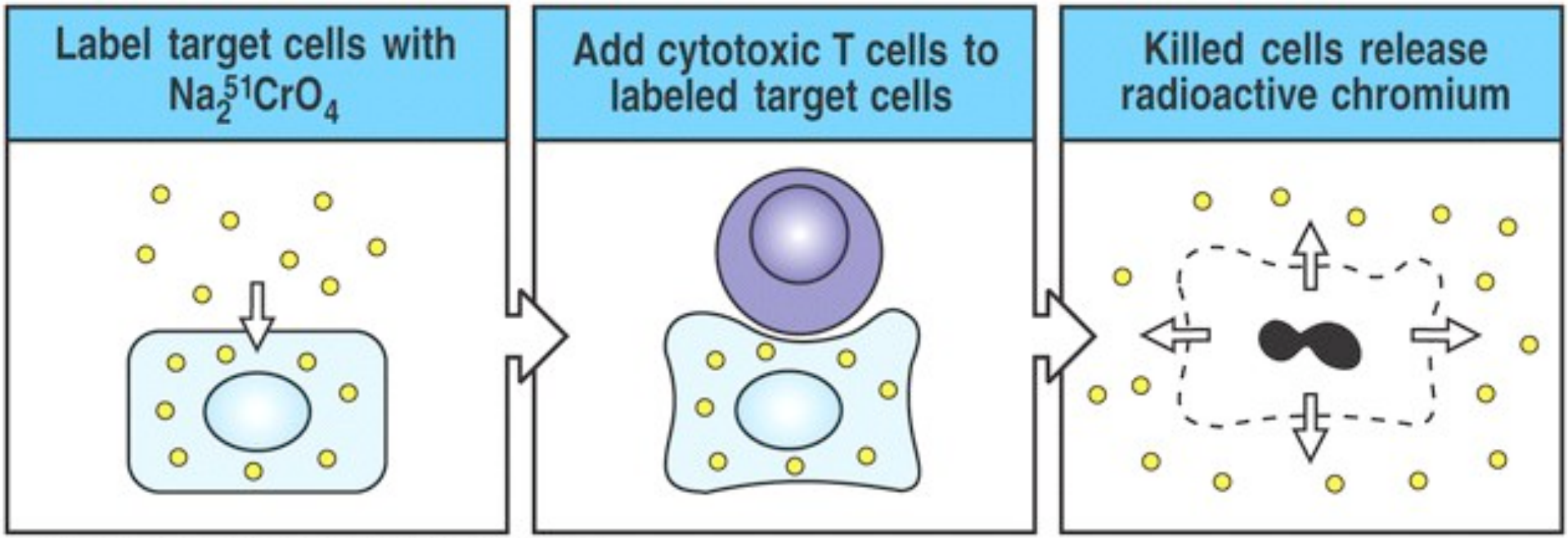


Figure A-38 Immunobiology, 6/e. (© Garland Science 2005)

Mixed lymphocyte reaction

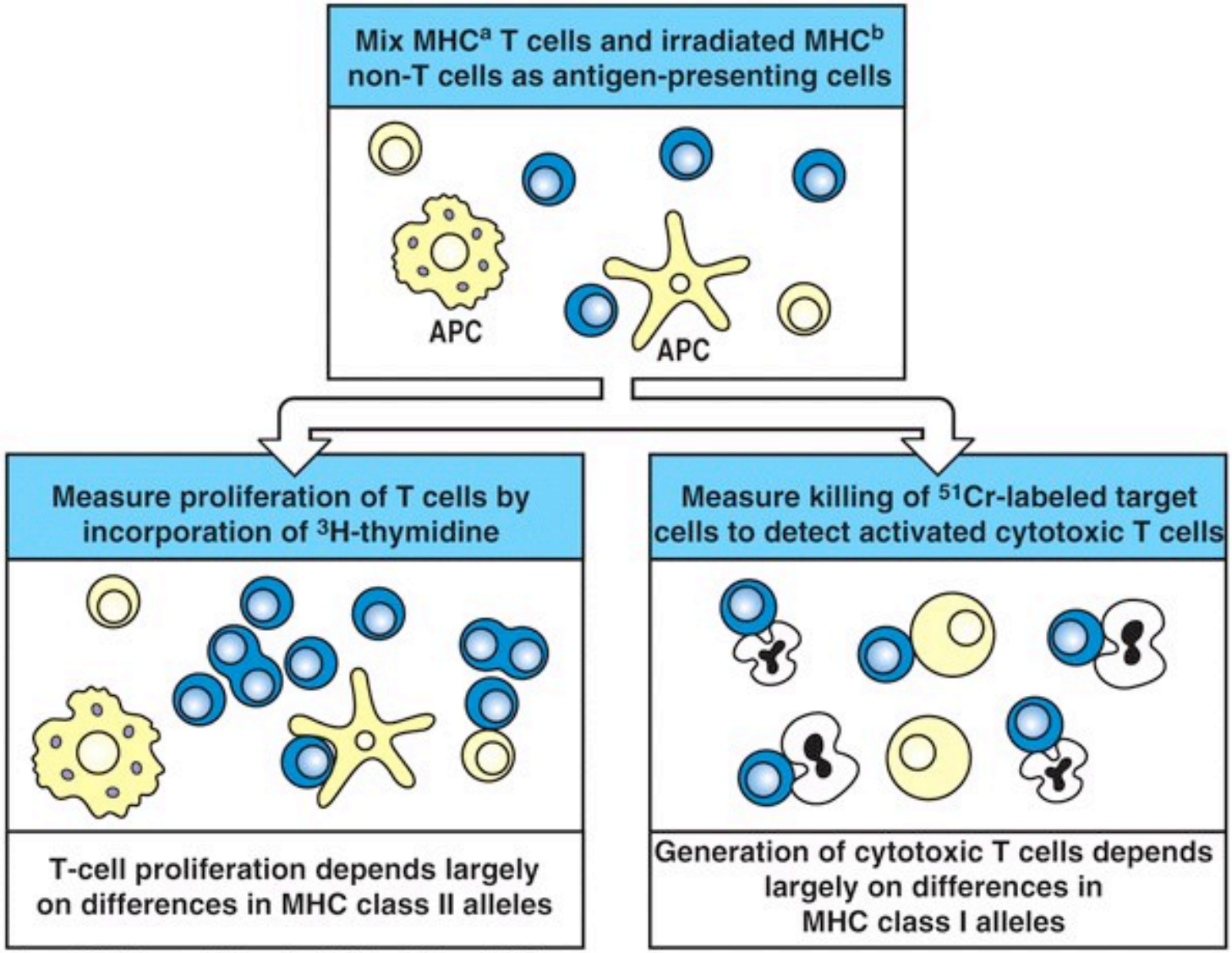


Figure 13-42 Immunobiology, 6/e. (© Garland Science 2005)

Immunosuppressive drugs

Prednisone

increases IκB production

Azathioprine/MMF

Az:purine analog that inhibits DNA synthesis

MMF:binds enzyme to prevent purine synthesis

Cyclosporin, Tacrolimus:

interferes with T cell proliferation by binding calcineurin

Rapamcyin:

interferes with serine threonine kinase, limiting costimulatory response

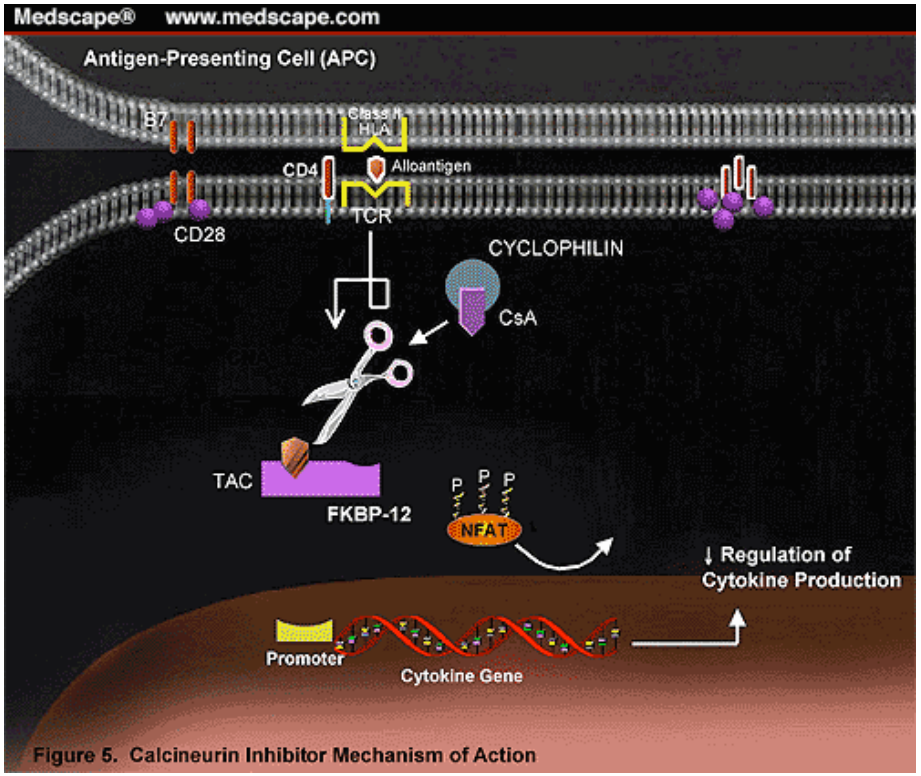


Figure 5. Calcineurin Inhibitor Mechanism of Action

Source: Duke University Medical Center

Date: 2005-10-13

Immune Therapy Could Treat Leukemias, Autoimmune Diseases, Transplant Rejection

DURHAM, N.C. -- In studies with mice, treatment with a new monoclonal antibody that targets immune system B cells has shown considerable promise for treating leukemias, autoimmune diseases and transplant rejection, according to immunologists at Duke University Medical Center.

Types of antisera

- Polyclonal=
  - Collection of antibodies from the serum
  - Multiple binding specificities to many epitopes of one or more antigens
- Monoclonal=
  - Homogeneous preparation of antibody
  - Single binding specificity to one epitope on a single antigen

Polyclonal antibody

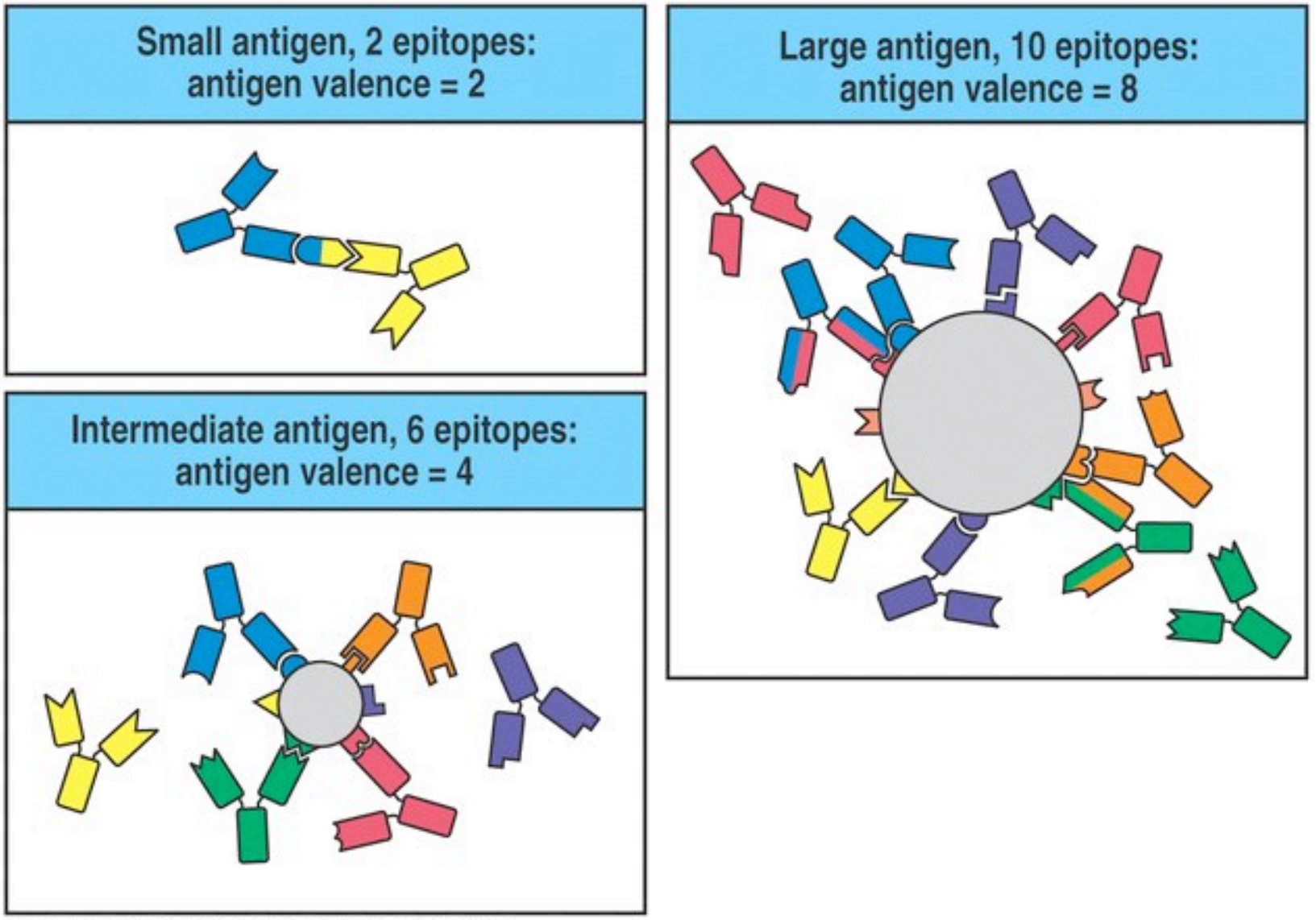


Figure A-10 Immunobiology, 6/e. (© Garland Science 2005)

Monoclonal antibody production

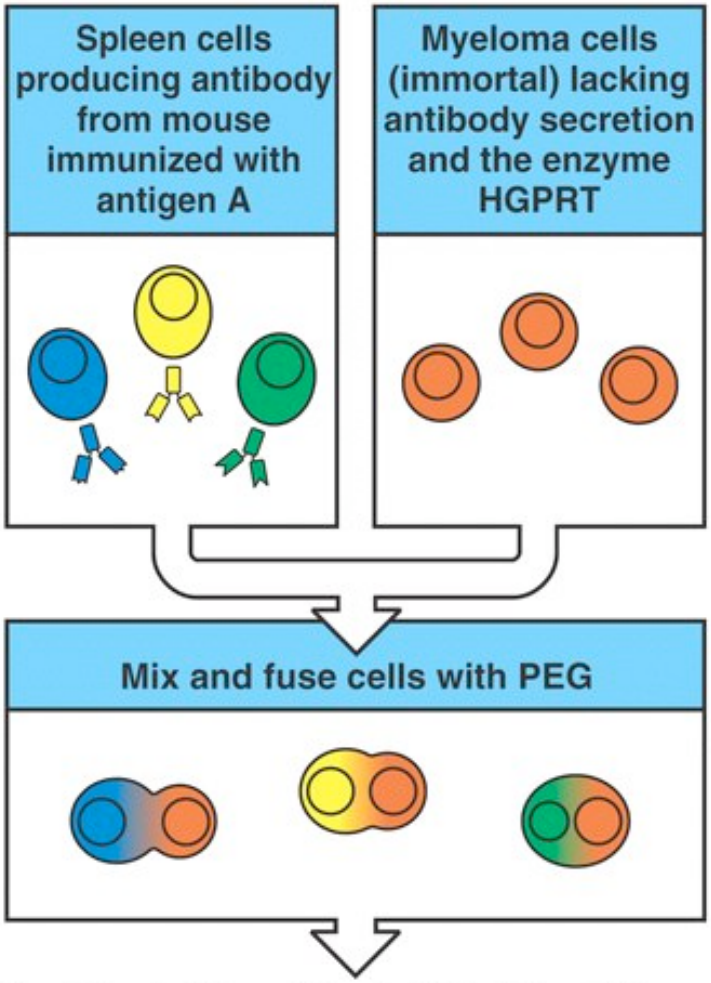


Figure A-14 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

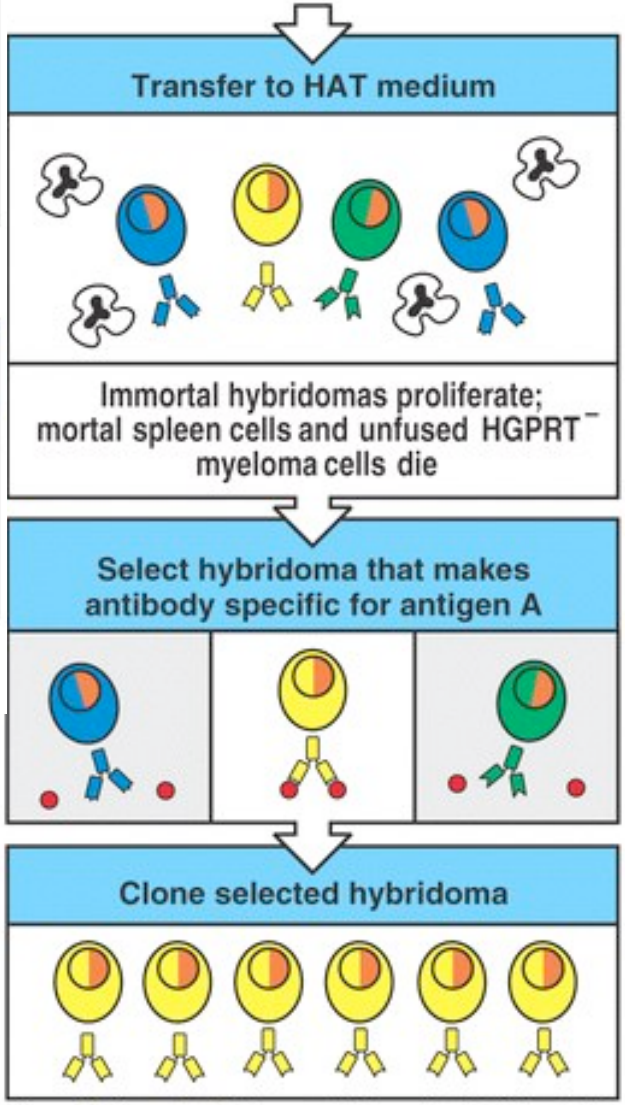


Figure A-14 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

HGPRT= hypoxanthine:guanine phosphoribosyl transferase

HAT= hypoxanthine-aminopterin-thymidine medium; lethal to cells in absence of HGPRT enzyme

Humanized monoclonal antibodies

- Variable region= murine
- Constant region= human
- CDRs from mouse cloned into human Ig
- Treatment:
  - Cancer
  - Transplantation
  - Autoimmune diseases
  - Inflammatory/allergic

Phage display

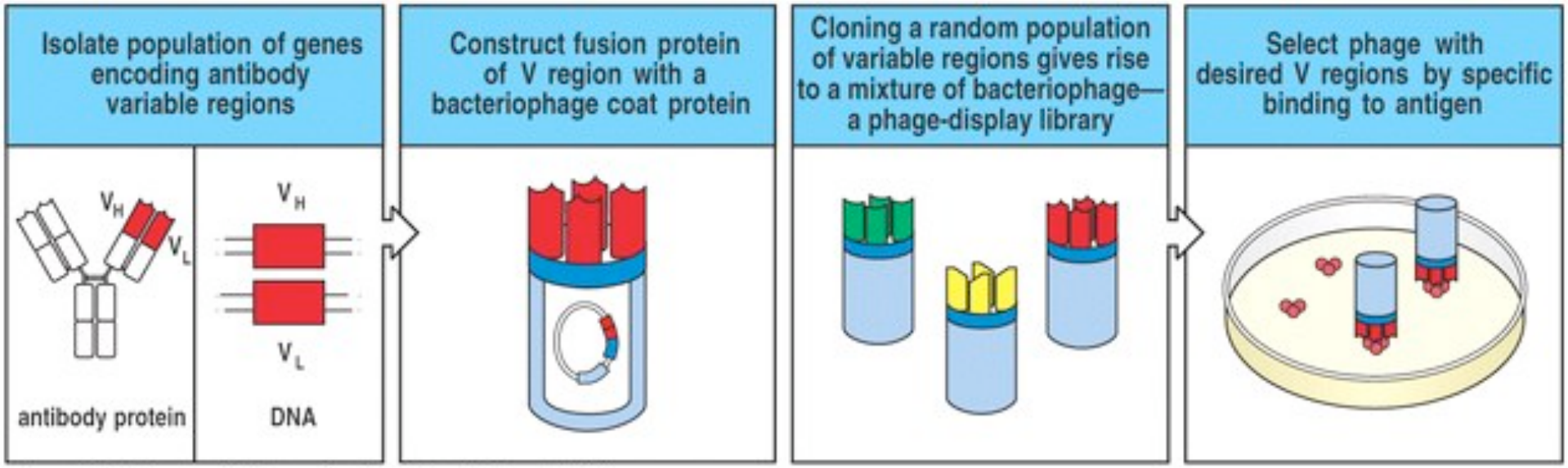


Figure A-15 Immunobiology, 6/e. (© Garland Science 2005)

Monoclonals as therapy

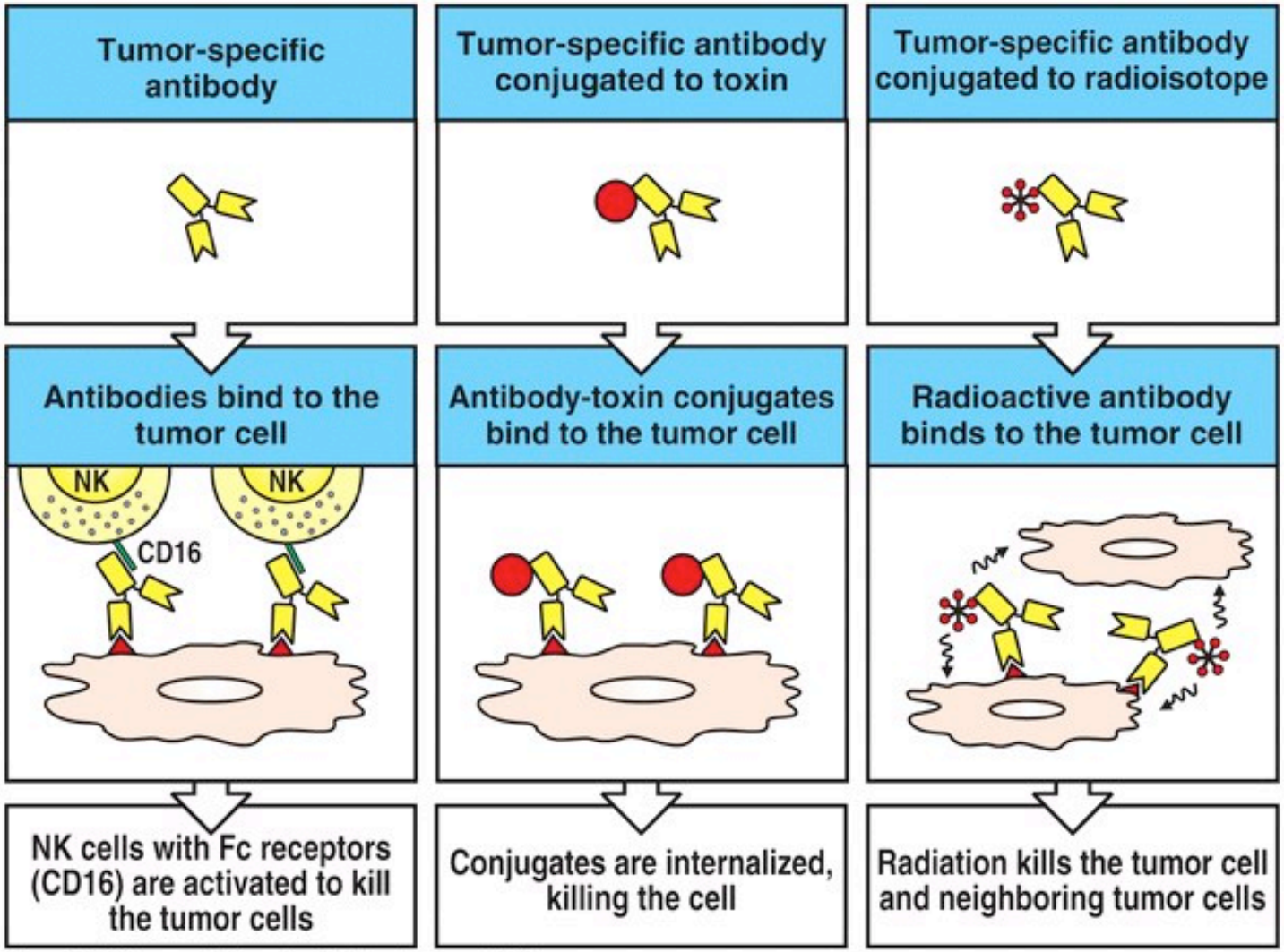
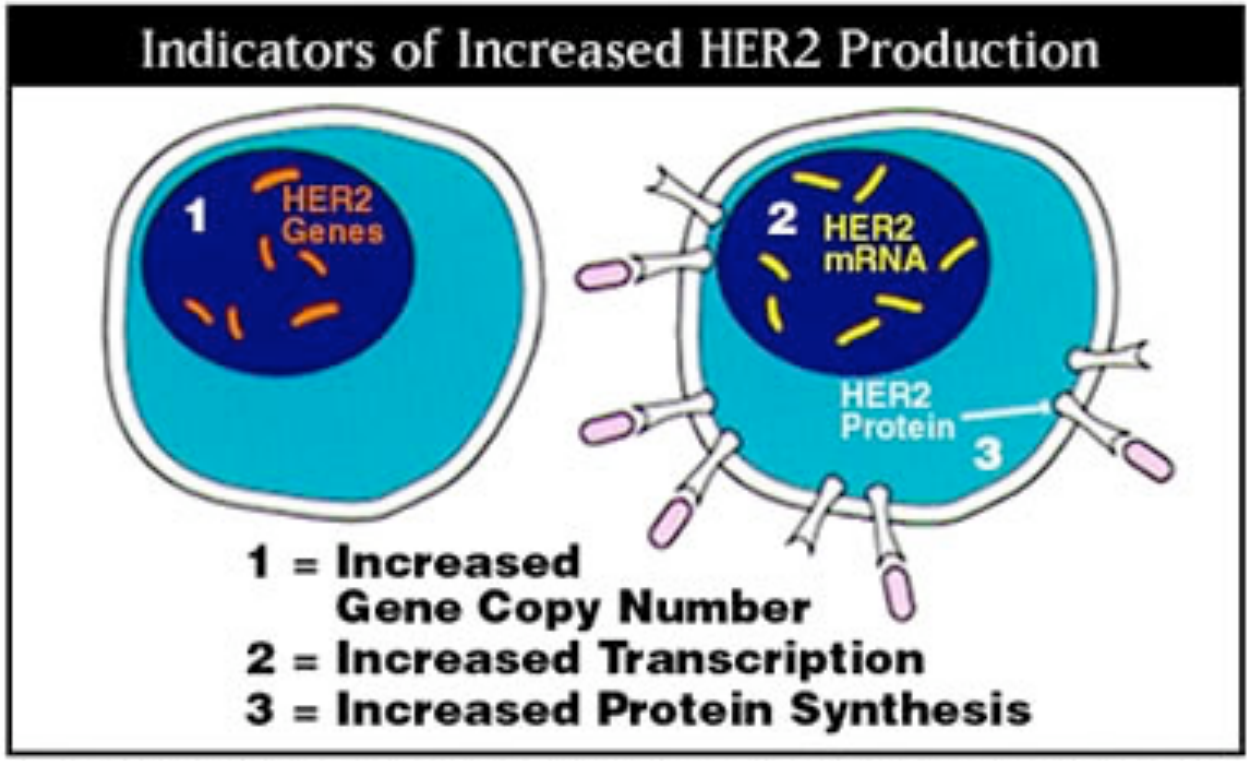


Figure 14-17 Immunobiology, 6/e. (© Garland Science 2005)

Her2 overexpression associated with some breast cancers

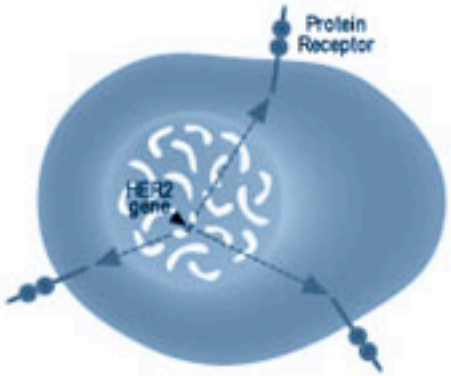
HER2 Preclinical Studies



HER2 overexpression can be achieved by 1) gene alteration resulting in additional copies of the HER2 gene; 2) increased transcription of the HER2 gene, producing increased levels of HER2 mRNA; and/or 3) increased translation of HER2 mRNA. All three alterations can result in greater HER2 synthesis and expression on the cell surface as well as increased cell proliferation.

In vitro studies demonstrated that a murine anti-HER2 monoclonal antibody, 4D5, inhibited the growth of the HER2-positive tumor cells.

- Pietras, et al.

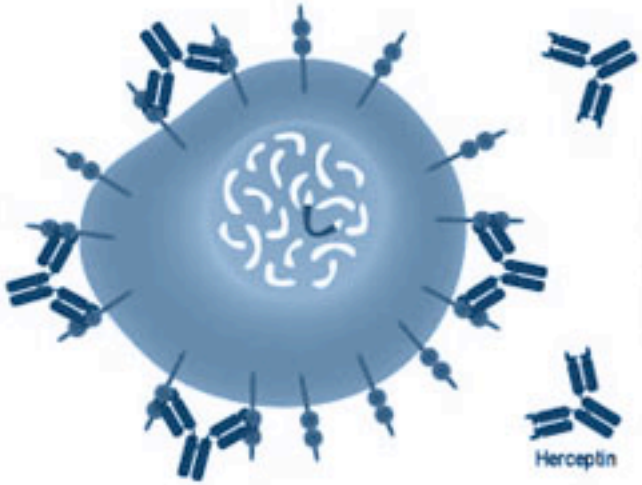


Normal Cell

In normal breast tissue cells, the HER2 gene produces a protein receptor on the cell surface. These growth factor-like receptors are thought to play a role in normal cell growth by signaling the cell to divide and multiply.

HER2 Overexpressing Cancer Cell

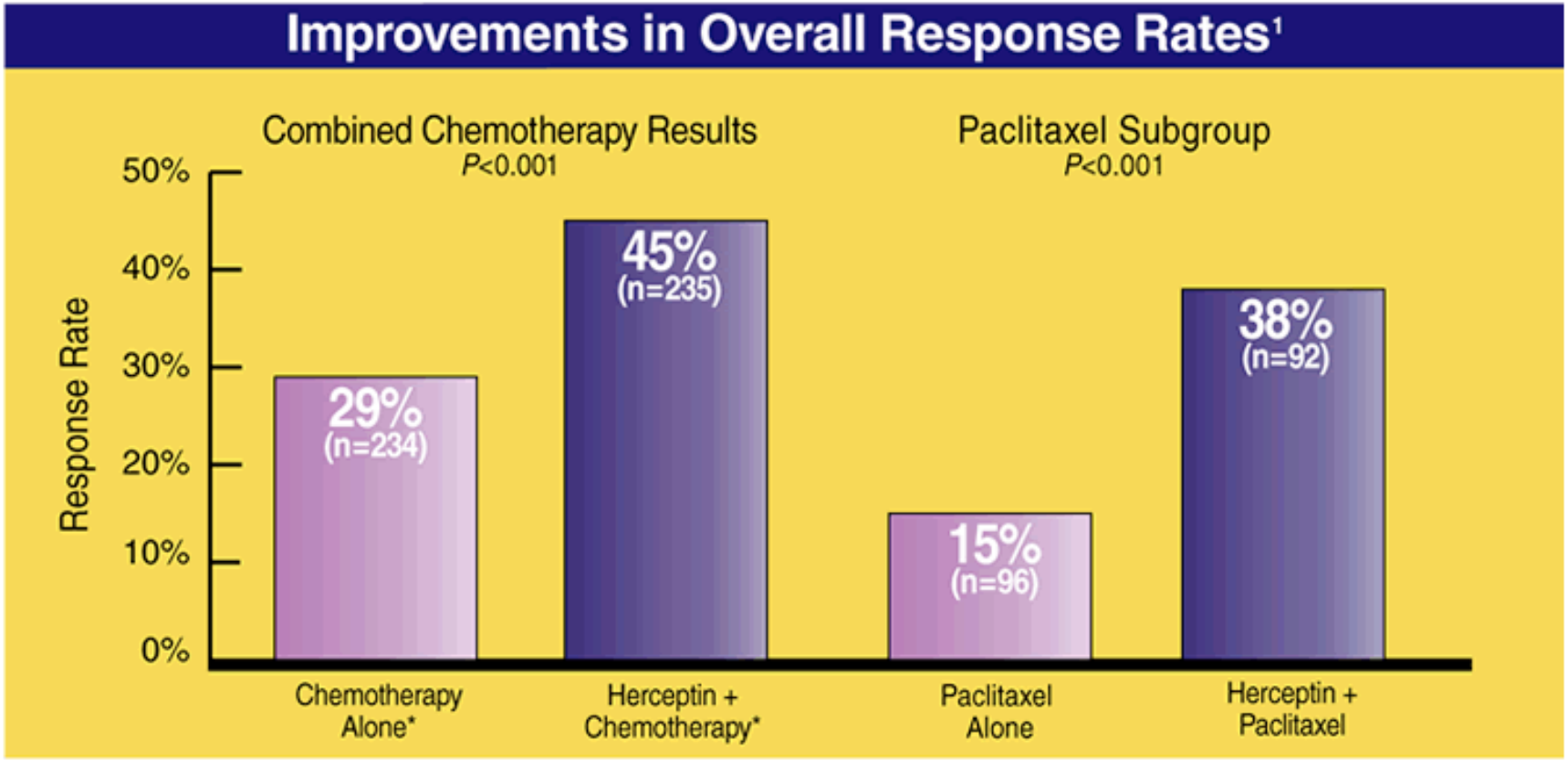
Cancerous breast tissue cells that overexpress (or overproduce) the HER2 gene produce extra protein receptors on the cell surface. The higher density of receptors triggers the cell to divide and multiply at an accelerated rate, thus contributing to tumor growth. Approximately 25-30% of all women with metastatic breast cancer overexpress the HER2 protein.



Herceptin® (Trastuzumab)

It is thought that Herceptin (a HER2 antibody) binds to numerous HER2 receptor sites found on the cell surface, blocking the receptor sites and possibly preventing further growth by interrupting the growth signal. As a result, the HER2 antibody may slow progression of the disease.

Herceptin increased efficiency of chemotherapy

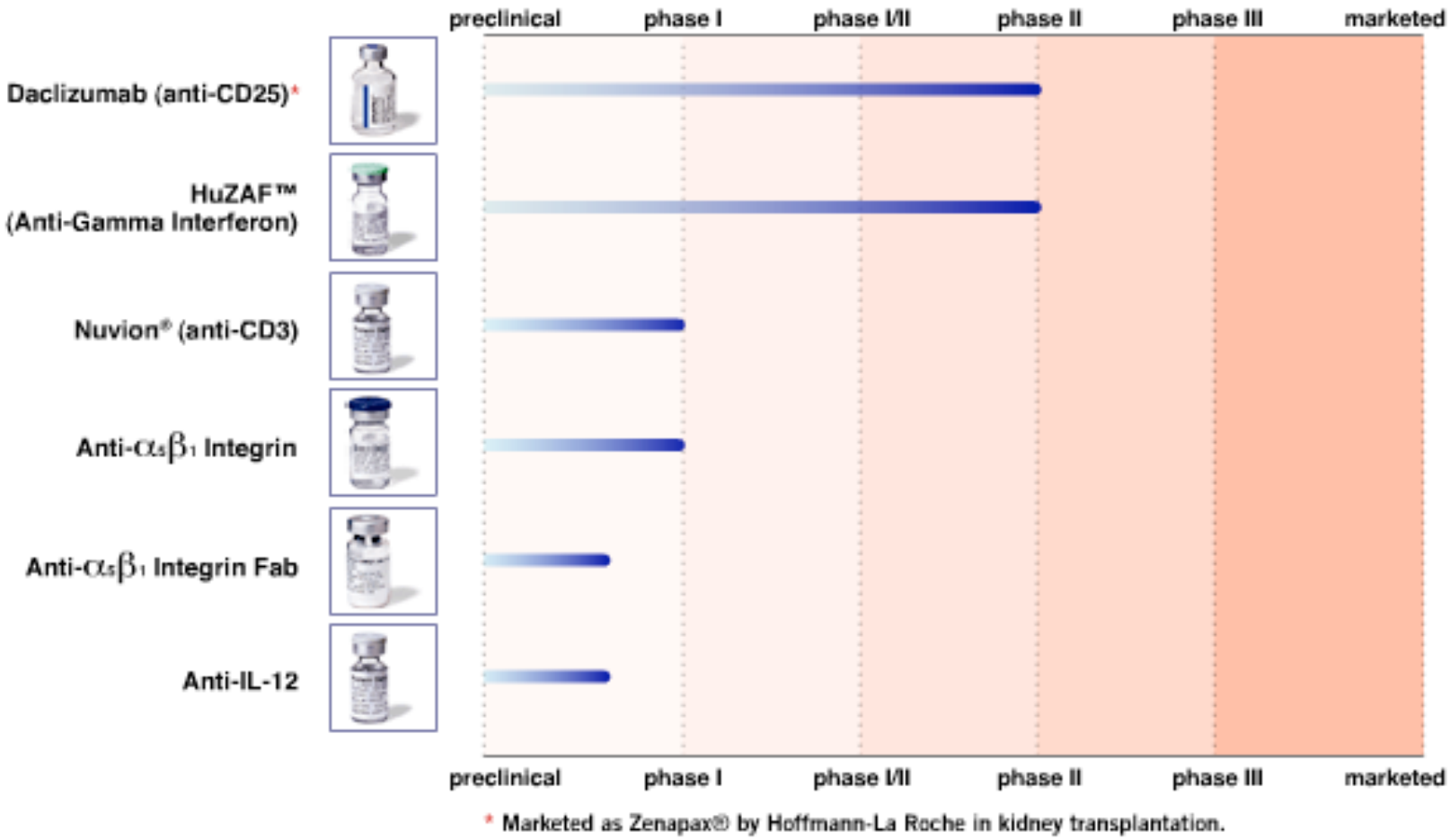


*Chemotherapy = either doxorubicin or epirubicin plus cyclophosphamide, or paclitaxel.

Targeting radiation therapy

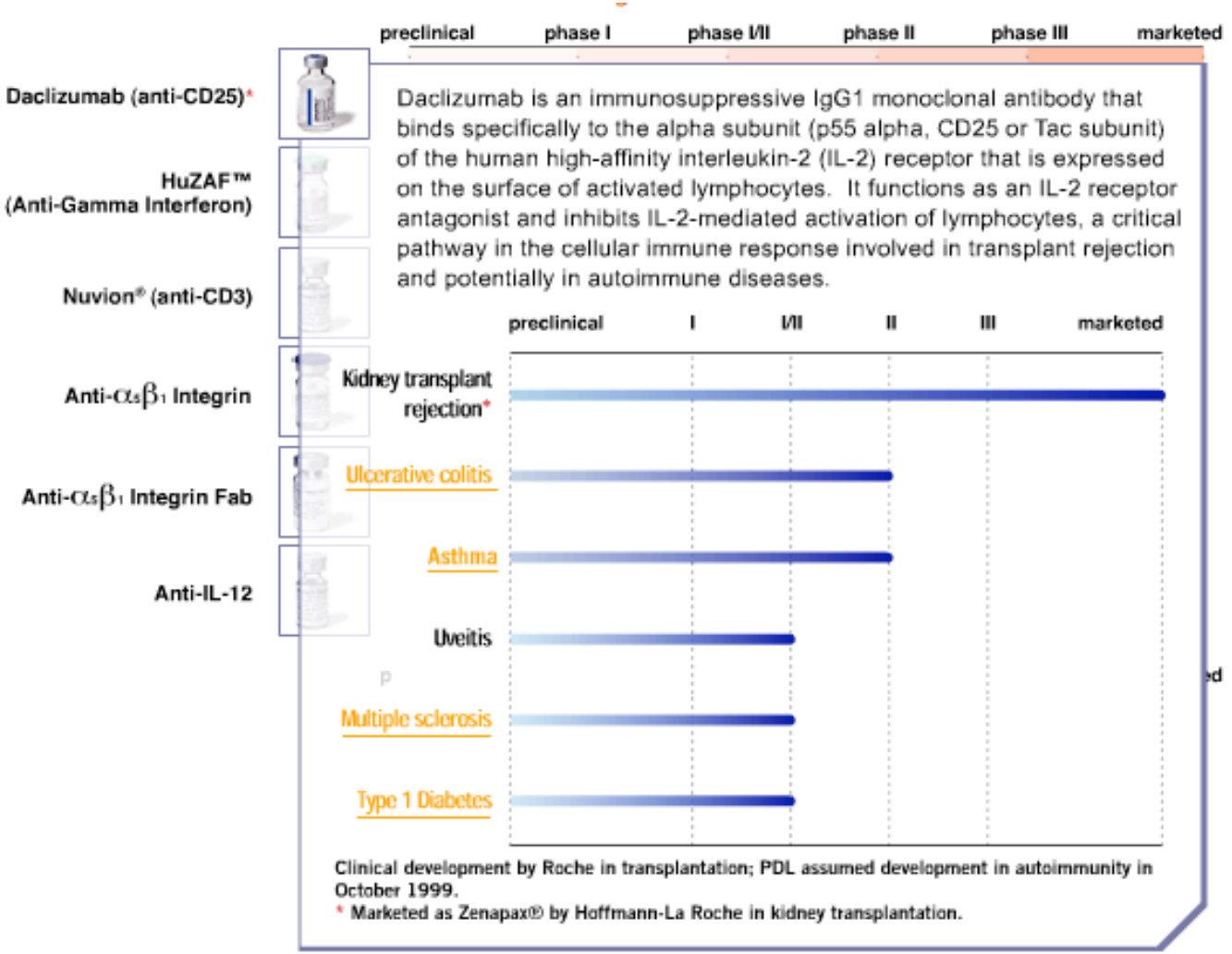
- Zevalin®, Bexxar®
  - Binds to CD20 molecule on mature B cells (and lymphomas)
  - Zevalin conjugated to either indium-111 (111In) or yttrium-90 (90Y)
  - Bexxar conjugated to iodine-131 (131I)
  - Usually used in combination with Rituxan that also binds to CD20

Humanized Ab neutralize proteins of immune response



* Marketed as Zenapax® by Hoffmann-La Roche in kidney transplantation.

Multiple uses for a single antibody



Targeting TNF-α

- Humira (adalimumab)= binds TNF-α
- Remicade (infliximab)= binds TNF-α
- Etanercept= soluble decoy receptor for TNF-α (not an antibody)

mAb to prevent transplant rejection

OKT3

- interferes with T cell proliferation by binding to CD3

Xenapax/Simulect

- interferes with T cell proliferation by binding to IL-2 receptor (α) subunit
- Daclizumab (Xenapax) is the humanized mAb and basiliximab (Simulect) is the chimeric mAb

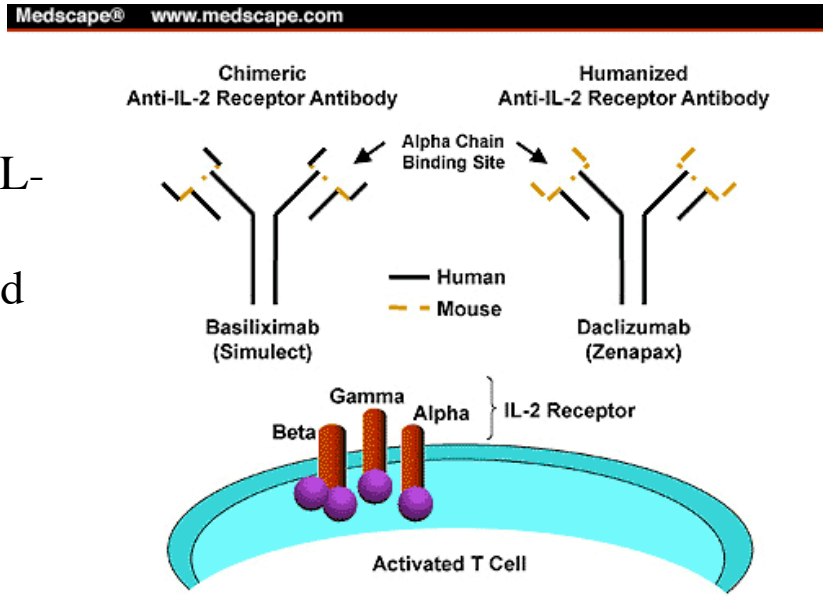
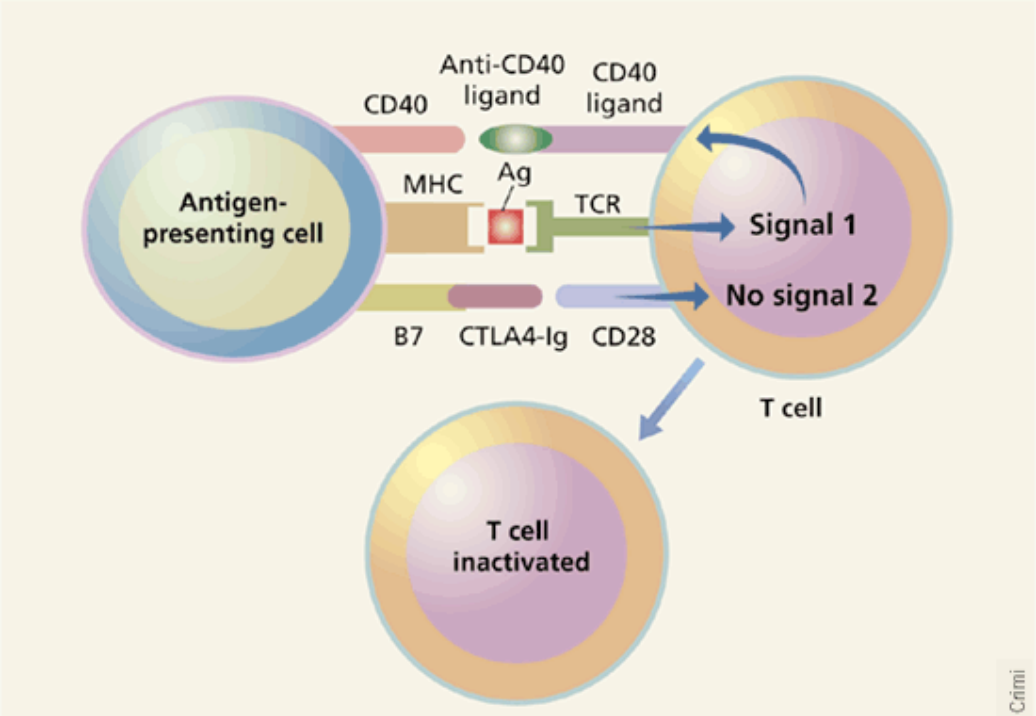


Figure 3. The IL-2 receptor antagonists.

Belatacept: Costimulation blockade



Nature Biotechnology 22, 145 - 147 (2004)

- soluble fusion protein of the B7-binding domain of CTLA4 and an Ig tail
- inhibits lymphocyte co-stimulation through CD28 for the potential treatment of solid organ transplant rejection.
- Belatacept is currently undergoing phase III clinical trials (Aug 2005)

Transplant survival rates

Tissue transplanted	5-year graft survival*	No. of grafts in USA (2002)
Kidney	65–75%	15,680
Liver	65–75%	5594
Heart	68%	2231
Pancreas	30%/80%#	1492
Lung	40%	1077
Cornea	~70%	~40,000†
Bone marrow	40%/60%	15,000‡

Figure 13-41 Immunobiology, 6/e. (© Garland Science 2005)

Fetal tolerance

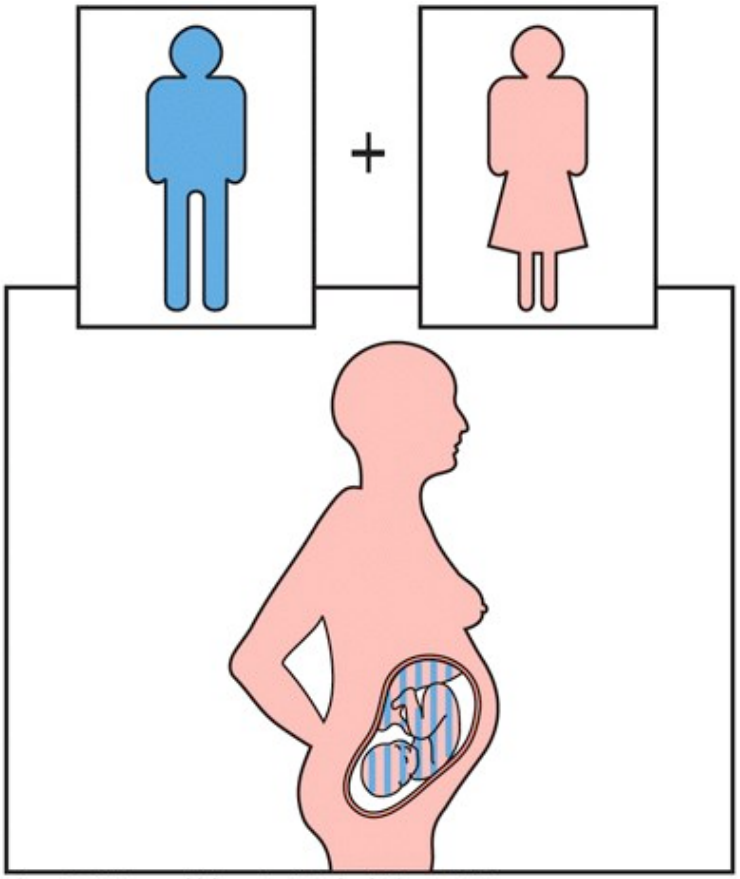


Figure 13-44 Immunobiology, 6/e. (© Garland Science 2005)

- Placenta
  - Does not exhibit classical MHC molecules
  - Expresses HLA-G that inhibits NK cells
- Maternal induced T cell tolerance to paternal alloantigens
  - Responsiveness returns after pregnancy
- Placental secretion of cytokines (IL-4, TGF-β, IL-10) that promote less harmful TH2 response
- Placental tryptophan starvation suppresses T cell activation
  - Indoleamine 3-dioxygenase catabolizes tryptophan