Chapter 16

Manipulation of Immune Response
免疫反應的操控

陳炳宏副教授
KMU生物科技學系
第一教學大樓N1020/1023 (分機: 2676)
bhchen@kmu.edu.tw
http://allergy.kmu.edu.tw

Learning objectives

- Understand how unwanted immune responses can be extrinsically regulated.
- How tumors can be regulated by immune responses.
- How can we manipulate the immune responses to fight infections.

Extrinsic regulation of unwanted immune responses

1. Anti-inflammatory drugs
2. Cytotoxic drugs
3. T-lymphocyte signaling inhibitors
4. Anti-lymphocyte Ab

Conventional immunosuppressive drugs in clinical use

Fig. 16-1

Conventional immunosuppressive drugs in clinical use	
Immunosuppressive drug	**Mechanism of action**
1 Corticosteroids	Inhibit inflammation; inhibit many targets including cytokine production by macrophages
2 Azathioprine, cyclophosphamide, mycophenolate	Inhibit proliferation of lymphocytes by interfering with DNA synthesis
3 Cyclosporin A, tacrolimus (FK506)	Inhibit the calcineurin-dependent activation of NFAT; block IL-2 production and proliferation by T cells
3 Rapamycin (sirolimus)	Inhibits proliferation of effector T cells by blocking Rictor-dependent mTOR activation
3 Fingolimod (FTY270)	Blocks lymphocyte trafficking out of lymphoid tissues by interfering with signaling by the sphingosine-1-phosphate receptor

Figure 16.1 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

1. Corticosteroids (皮質類固醇)

- Refers to both glucocorticoids and mineralocorticoids ; steroid hormones
- Example:
  - cortisol (natural), prednisone (synthetic)
- Cortisol receptors are widely expressed on almost all cell types in the body!!
- Cortisol-cortisol receptor interaction will result (mostly) the increased expression of responsive genes
  - Could have both beneficial and harmful effects

Structures of anti-inflammatory corticosteriod drugs

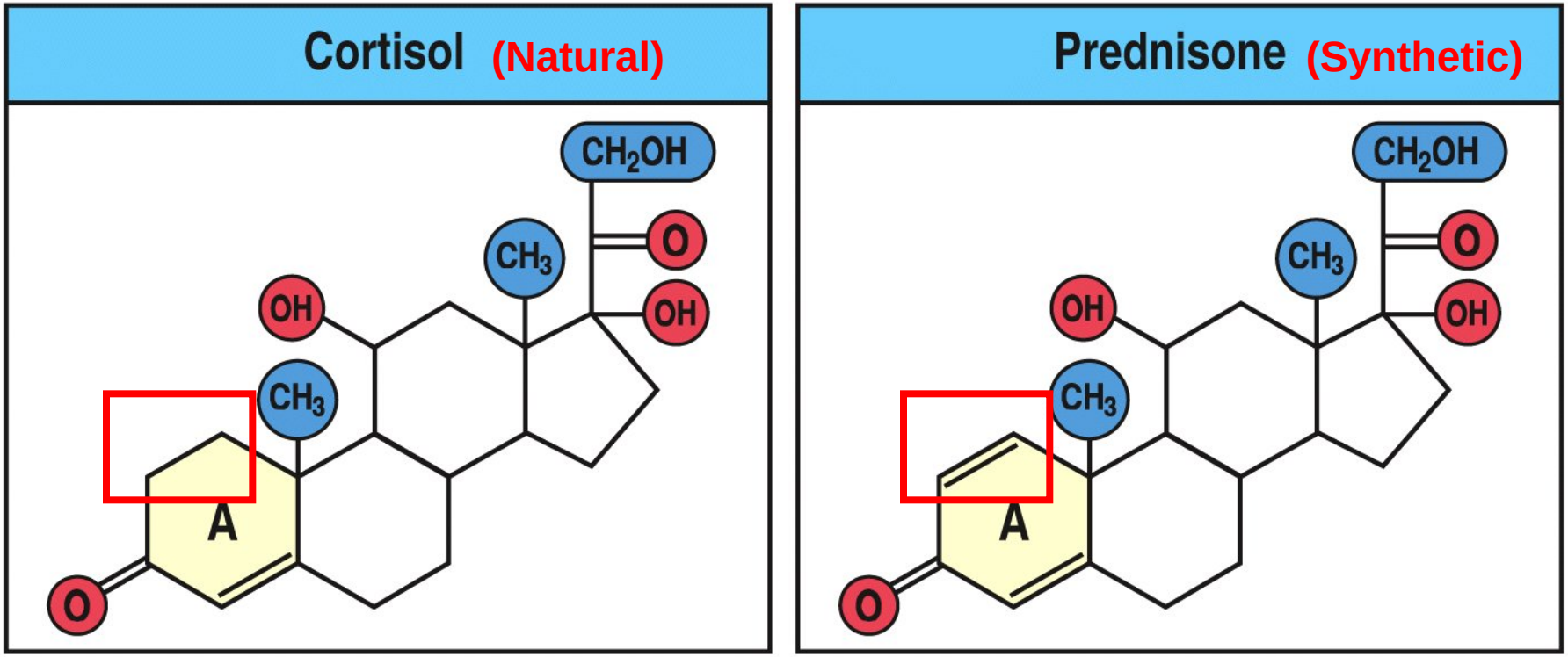


Figure 14-1 Immunobiology, 6/e. (© Garland Science 2005)

Introduction of a double bond enhances the anti-inflammatory potency

Mechanism of steroid hormone

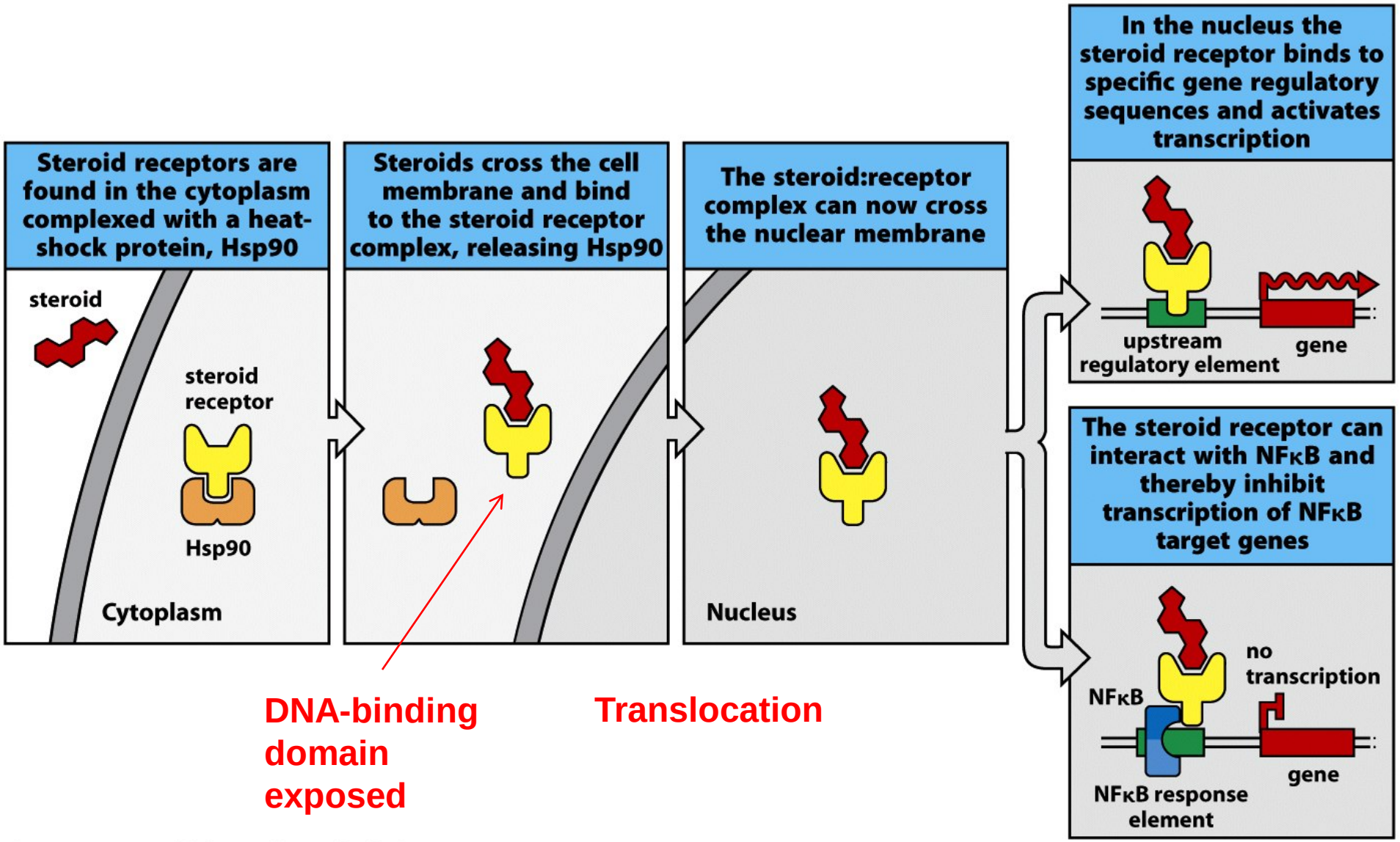


Figure 15-1 Immunobiology, 7ed. (© Garland Science 2008)

Fig. 16-2

Anti-inflammatory effects of corticosteroids

Corticosteroid therapy	
Effect on	**Physiological effects**
↓ IL-1, TNF-α, GM-CSF ↓ IL-3, IL-4, IL-5, CXCL8	↓ Inflammation caused by cytokines
↓ NOS (Nitric oxide synthase)	↓ NO
↓ Phospholipase A_2 ↓ Cyclooxygenase type 2 ↑ Lipocortin-1 (Annexin 1)	↓ Prostaglandins and Leukotrienes
↓ Adhesion molecules	Reduced emigration of leukocytes from vessels
↑ Endonucleases	Induction of apoptosis in lymphocytes and eosinophils

Figure 14-3 Immunobiology, 6/e. (© Garland Science 2005)

2. Cytotoxic drugs

- Interfere with DNA synthesis
  - Originally developed for the treatment of cancers
  - Affect dividing tissues and lymphocytes
- Wide range of toxic effects
  - Decreased immune function
    - Anemia, leukopenia, and thrombocytopenia
  - Tissue damages
    - Hair loss
    - Intestinal epithelial necrosis
- Commonly used to destroy all dividing lymphocytes
  - Usually requires subsequent B.M transplantation
- e.g. Azathioprine, mycophenolate, cyclophosphamide

3. Inhibitors on T-cell signaling

- Cyclosporin A
- A fungal peptide derived from *Tolypocladium inflatum* (initially discovered in 1970s)
- Blocks T-cell proliferation
  - Inhibits action of phosphatase **calcineurin**
    - Calcineurin is found in T cells (lower conc.) and other cell types (higher conc.)
    - Signal transmitted from TCR to nucleus is disrupted
  - Only requires nanomolar (10^{-9}) level to be effective !!
  - Results: reduced expression of several cytokine genes (e.g. IL-2) activated upon T-cell activation
- Widely used in organ transplant recipients

Cyclosporin A and Tacrolimus inhibit T-cell activation be interfering with calcineurin

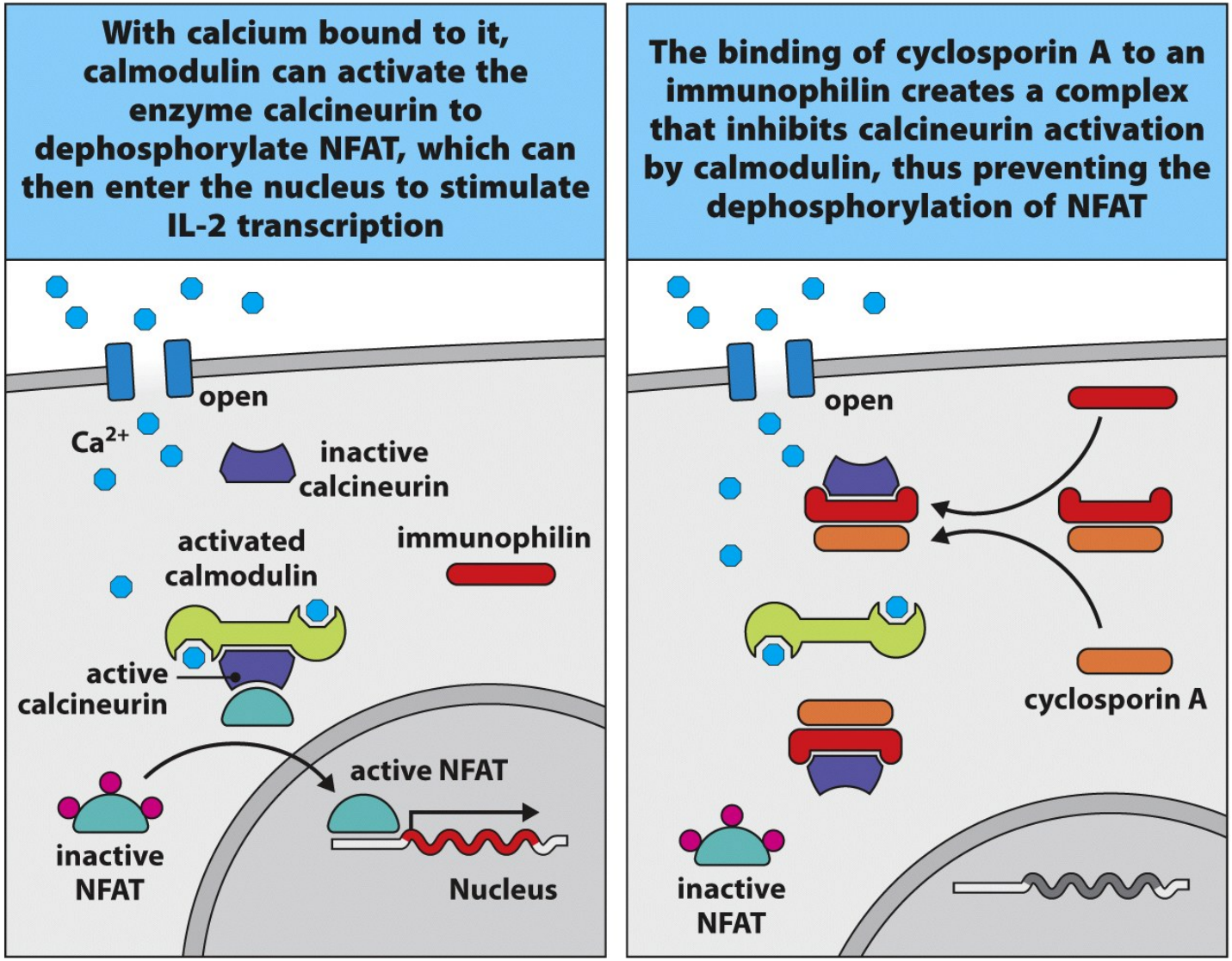


Figure 16.4 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Fig. 16-4

Effects of cyclosporin A and tacrolimus

Fig. 16-3

Immunological effects of cyclosporin A and tacrolimus	
Cell type	**Effects**
T lymphocyte	**Reduced expression of IL-2, IL-3, IL-4, GM-CSF, TNF-α** **Reduced proliferation following decreased IL-2 production** **Reduced Ca^{2+}-dependent exocytosis of granule-associated serine esterases** **Inhibition of antigen-driven apoptosis**
B lymphocyte	**Inhibition of proliferation secondary to reduced cytokine production by T lymphocytes** **Inhibition of proliferation following ligation of surface immunoglobulin** **Induction of apoptosis following B-cell activation**
Granulocyte	**Reduced Ca^{2+}-dependent exocytosis of granule-associated serine esterases**

Figure 15-3 Immunobiology, 7ed. (© Garland Science 2008)

Rapamycin inhibits cell growth & proliferation by blocking mTOR activation

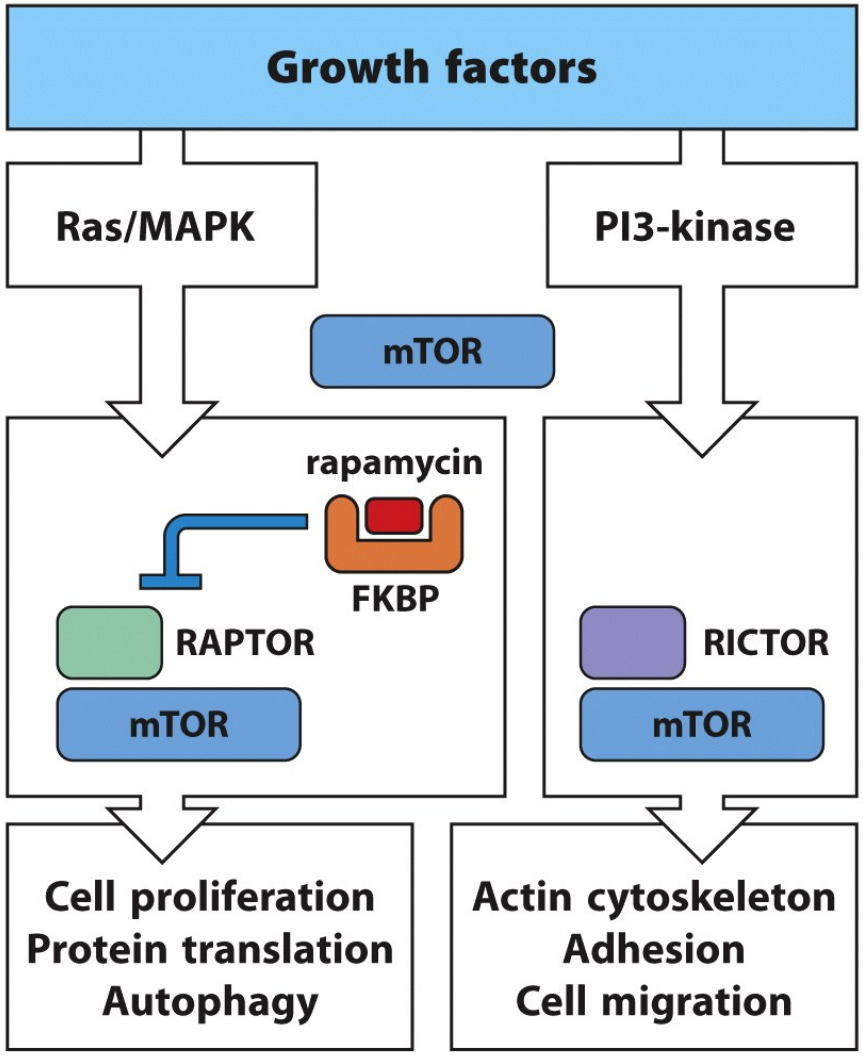


Figure 16.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Fig. 16-5

Downsides of immunosuppressive drugs

- Affect all immune responses **indiscriminately**
  - Cause generalized immunosuppression
  - Balance the doses being administered
- Affect other organs/tissues
  - e.g. high toxicity to kidneys (kidney failure)
- Expensive
  - Most are natural products
  - Required prolonged treatment (toxicity)
  - The need for semisynthetic products with comparable efficacy

4. Anti-lymphocyte antibodies

- Advantage of Ab
  - can interfere with immune responses in a non-toxic and more selective/specific manner!!!
- Antibodies can be *engineered* for the therapeutic use in humans
  - Because most therapeutic Abs come from animal sources (e.g. mouse, rat, rabbit, donkey,…etc.)
  - Animal Abs will cause a 'human-against-animal' Ab responses

Depleting vs non-depleting Abs

- Depleting Abs
  - Trigger the destruction of lymphocytes *in vivo*
  - Examples:
    - Anti-lymphocyte globulin (polyclonal)
      - Immunize horse with human lymphocytes
      - Can be used to treat acute graft rejection
      - Disadvantage: can cause serum sickness with high doses
    - Campath-1H (also called "alemtuzumab") (monoclonal)
      - Similar to anti-lymphocyte globulin
      - Against CD52 common lymphocyte marker
- Non-depleting Abs
  - Blocking functions of lymphocytes

How to reduce immunogenecity of engineered antibodies?

- Clone human Ig V gene segments into phage display library
- Mice deficient of Ig genes can be made transgenic of human Ig genes (e.g. KM mice)
- "Humanization" of mouse Ig into human backbone
  - Graft CDRs from mice source into human Ig cassette (usually human IgG backbone)

Monoclonal Abs for treating human diseases

Fig. 16-6

mab = monoclonal Ab

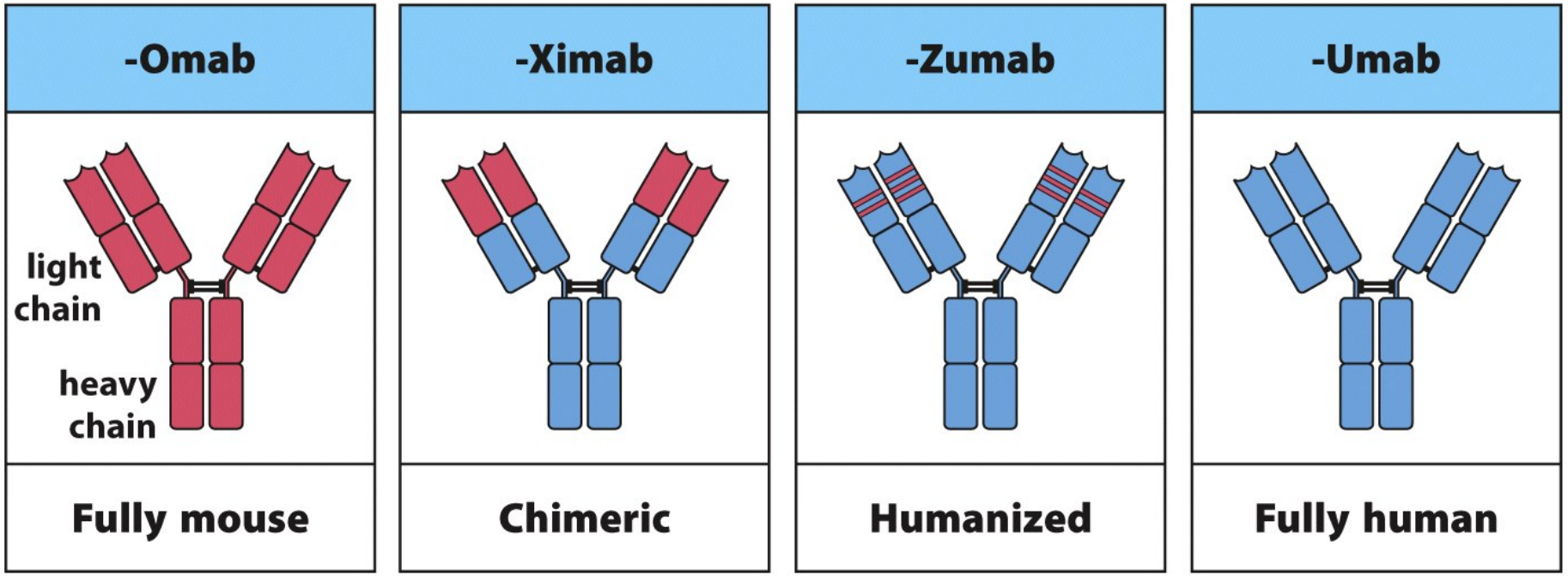


Figure 16.6 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Mouse Mouse x Human Humanized Fully

Also, refer to Fig. 16-7 (參考用, 不會考)

Management of autoimmune diseases

How to manage autoimmune diseases?

- Treatment with anti-inflammatory agents
- Reduction of autoimmune responses
- Treatment aimed at compensating pathological damages caused by autoimmune responses
  - ‘biological agents’
    - Treatments comprising
      - Natural proteins (e.g. Abs, cytokines), or fragment of proteins
      - Synthetic peptides
  - Examples
    - insulin to compensate the damaged pancreatic β cells
    - anti-TNFα Ab or TNFα receptor:Ig Fc recombinant proteins for the removal of TNFα in various rheumatoid arthritis

Potential targets of immune intervention strategies

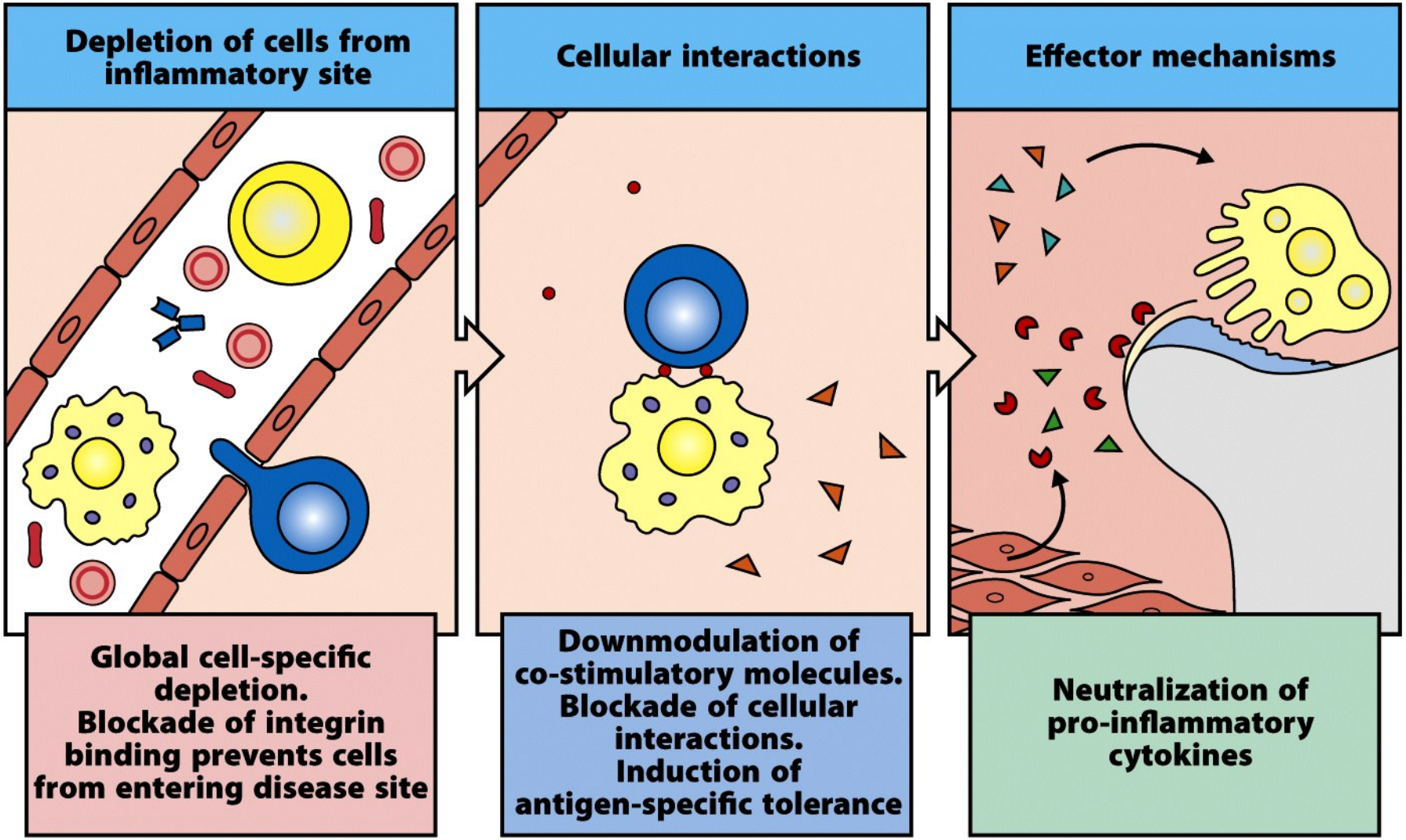


Figure 15-6 Immunobiology, 7ed. (© Garland Science 2008)

Anti-TNFα therapies currently in use

- Infliximab
  - Humanized anti-TNFα mAb
- Etanercept
  - Recombinant fusion protein
    - TNF receptor:Ig Fc complex
  - Binds TNFα, thereby neutralizing it
- Both are currently used in clinical settings

Anti-inflammatory effects of anti-TNFα therapy in rheumatoid arthritis

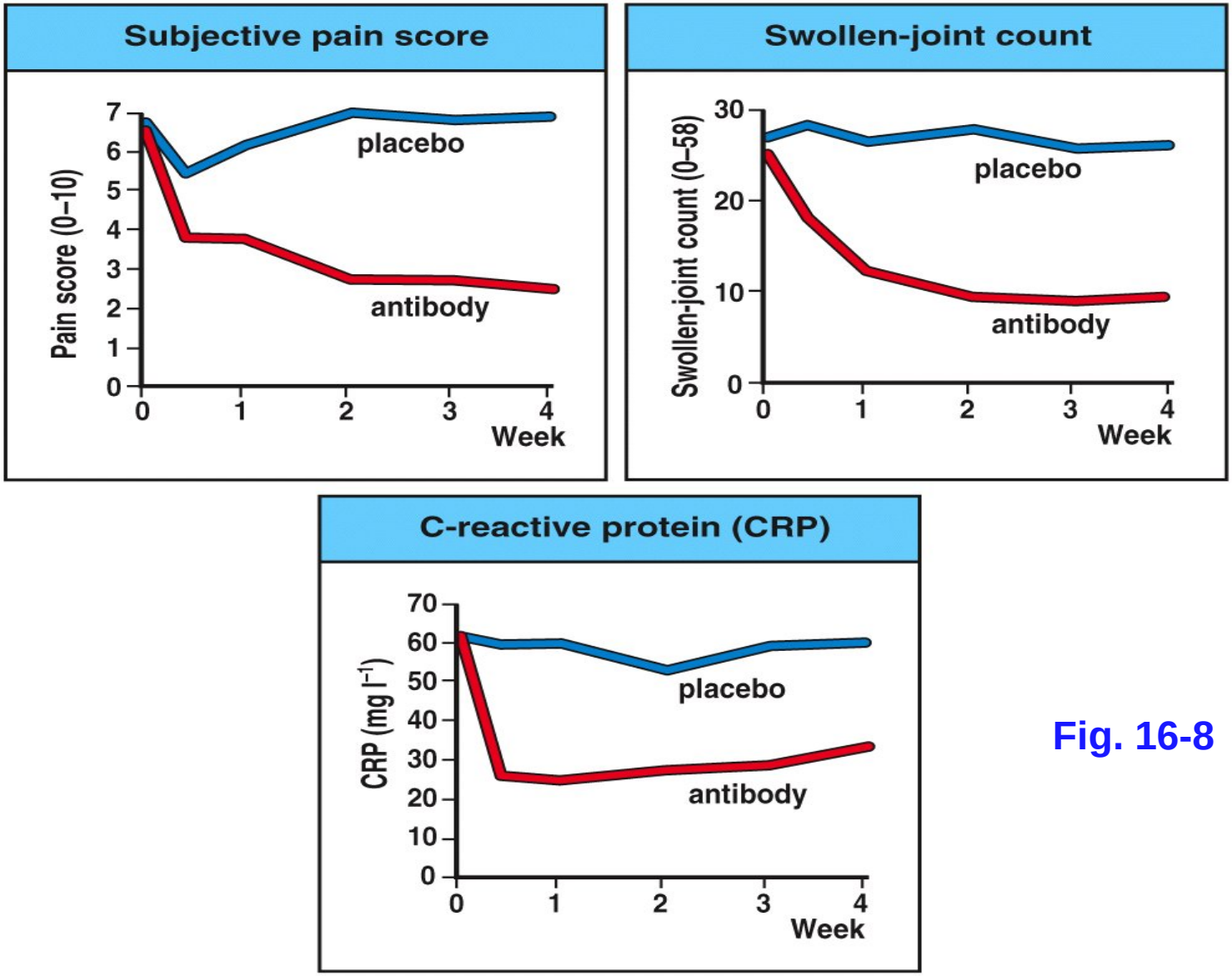


Fig. 16-8

Figure 14-7 Immunobiology, 6/e. (© Garland Science 2005)

Antibodies for the blocking of cell migration to inflammatory sites of brain in multiple sclerosis (natalizumab)

Fig. 16-9a

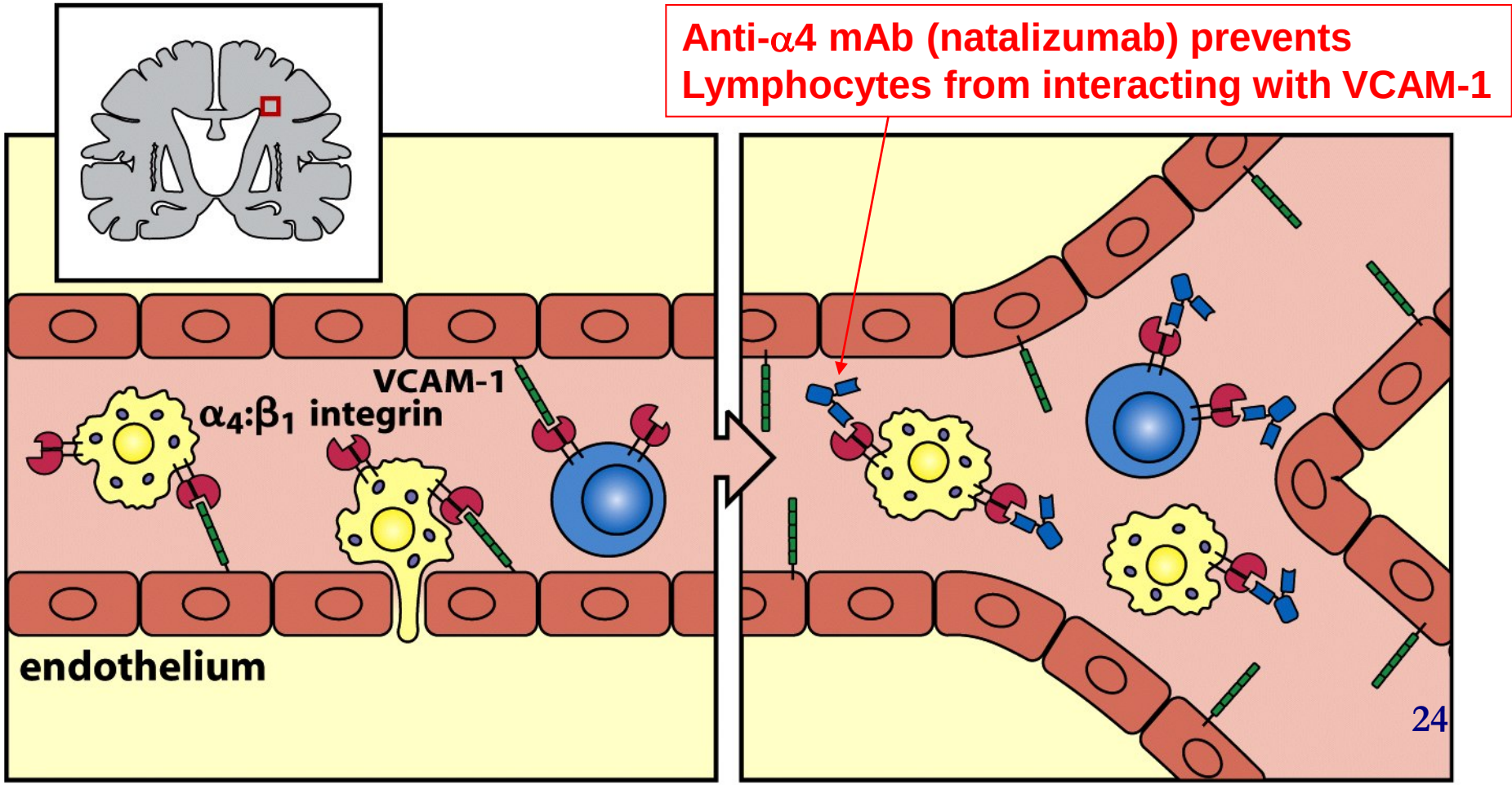


Figure 15-8 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Natalizumab effectively reduces inflammatory lesions caused by lymphocytes and monocytes

Fig. 16-9b

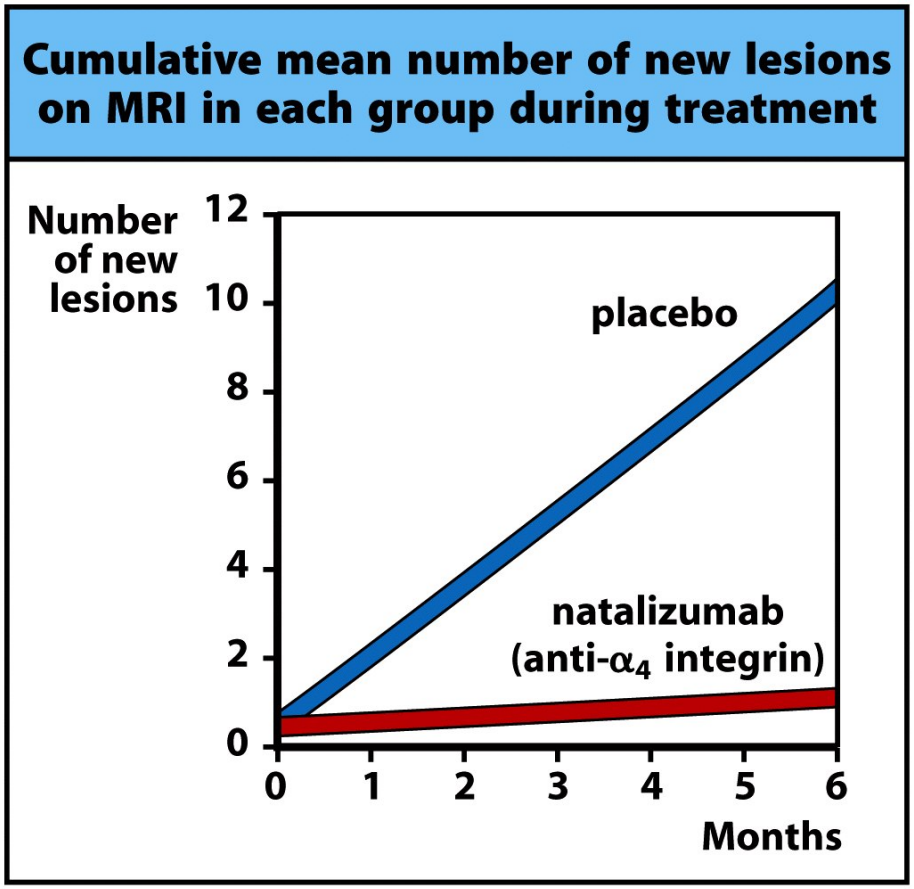


Figure 15-8 part 2 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Immune surveillance and tumor immunology

Tumor

- Tumor vs. cancer
- One of the leading causes of death among most countries
- Resulted from uncontrolled proliferation of a single transformed cell ('self' cell)
- How to attack and eliminate tumors?
  - Induction of immune responses ***solely*** against tumor cells
  - Therefore, the identification of specific 'tumor antigen' becomes crucial!!

T cells are the most critical mediator of tumor immunity

- Tumors can be induced via chemical carcinogens in experimental animals (esp. mice)
- Induced tumors are 'transplantable' among inbred mice harbors matching MHC types

T cells are the most critical mediator of tumor immunity

- When transplanted into an inbred mouse of matching MHC, an irradiated tumor, can induce protective immunity
  - against a subsequent injection of viable tumor cells of the same tumor type
  - not seen in T-deficient mice (nude mice)
  - protection can also be demonstrated via 'adoptive transfer' (BM transfer)

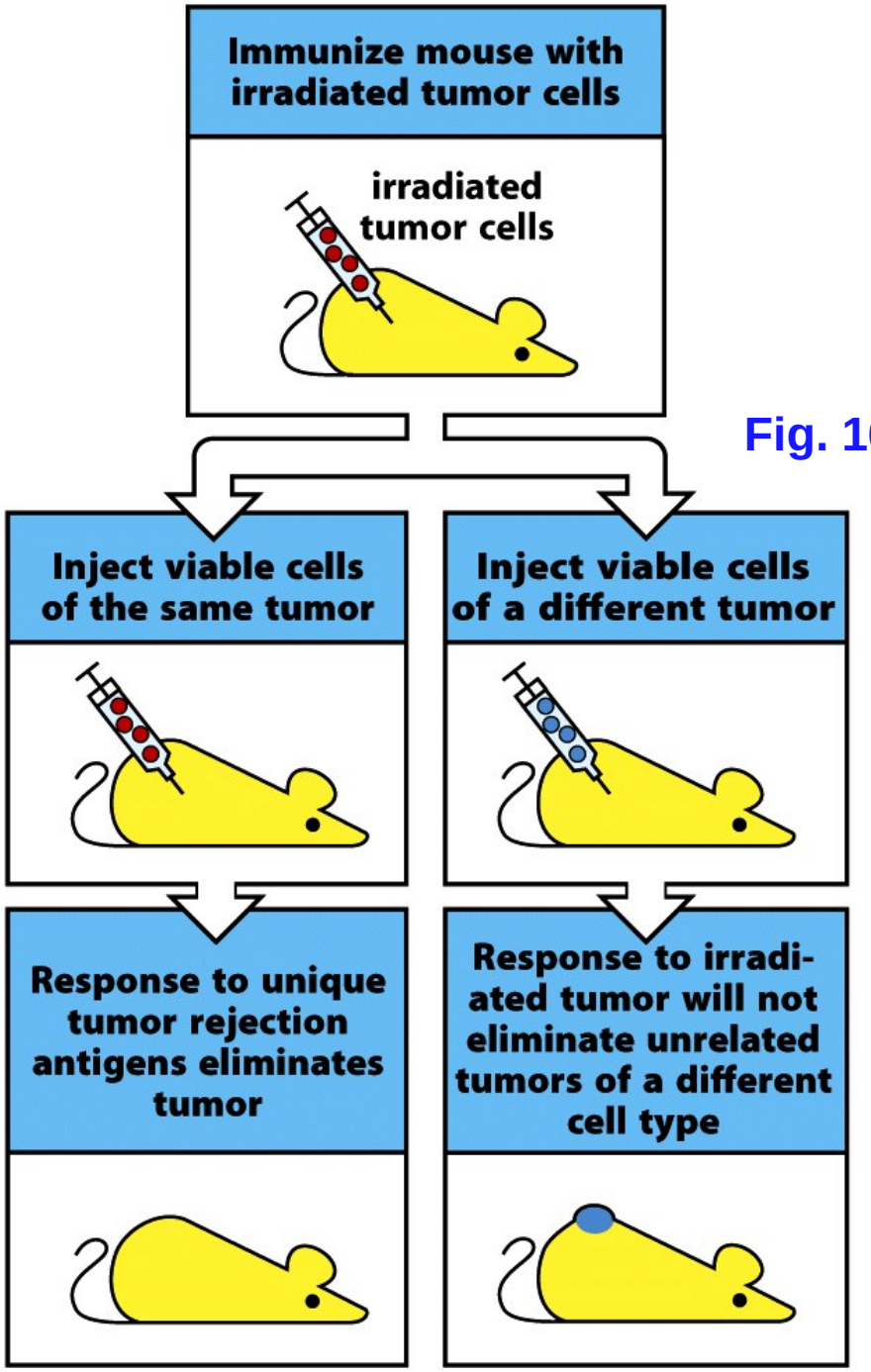


Figure 15-12 Immunobiology, 7ed. (© Garland Science 2008)

Tumor rejection is a specific process

Fig. 16-11

- Tumor-specific transplantation antigens (TSTAs)
- Also called “tumor rejection antigens” (TRAs)
- Not expressed on normal cells

Tumors can escape rejection in many ways

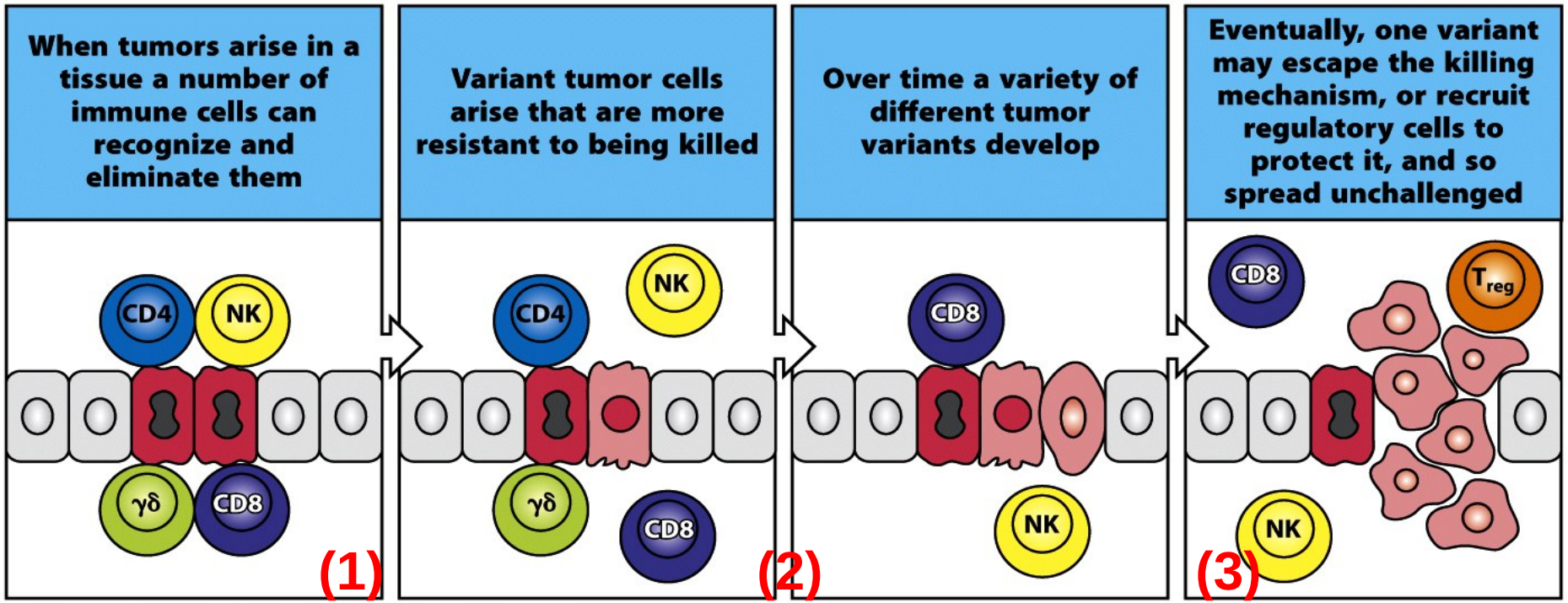


Figure 15-13 Immunobiology, 7ed. (© Garland Science 2008)

Fig. 16-12

Immune surveillance

(1) Elimination phase

(2) Equilibrium phase

(3) Escape phase

How do tumors escape immune surveillance?

(often occurs at the equilibrium phase)

1. Low immunogenicity
2. Tumor Ags treated as self-Ags
3. Antigenic modulation

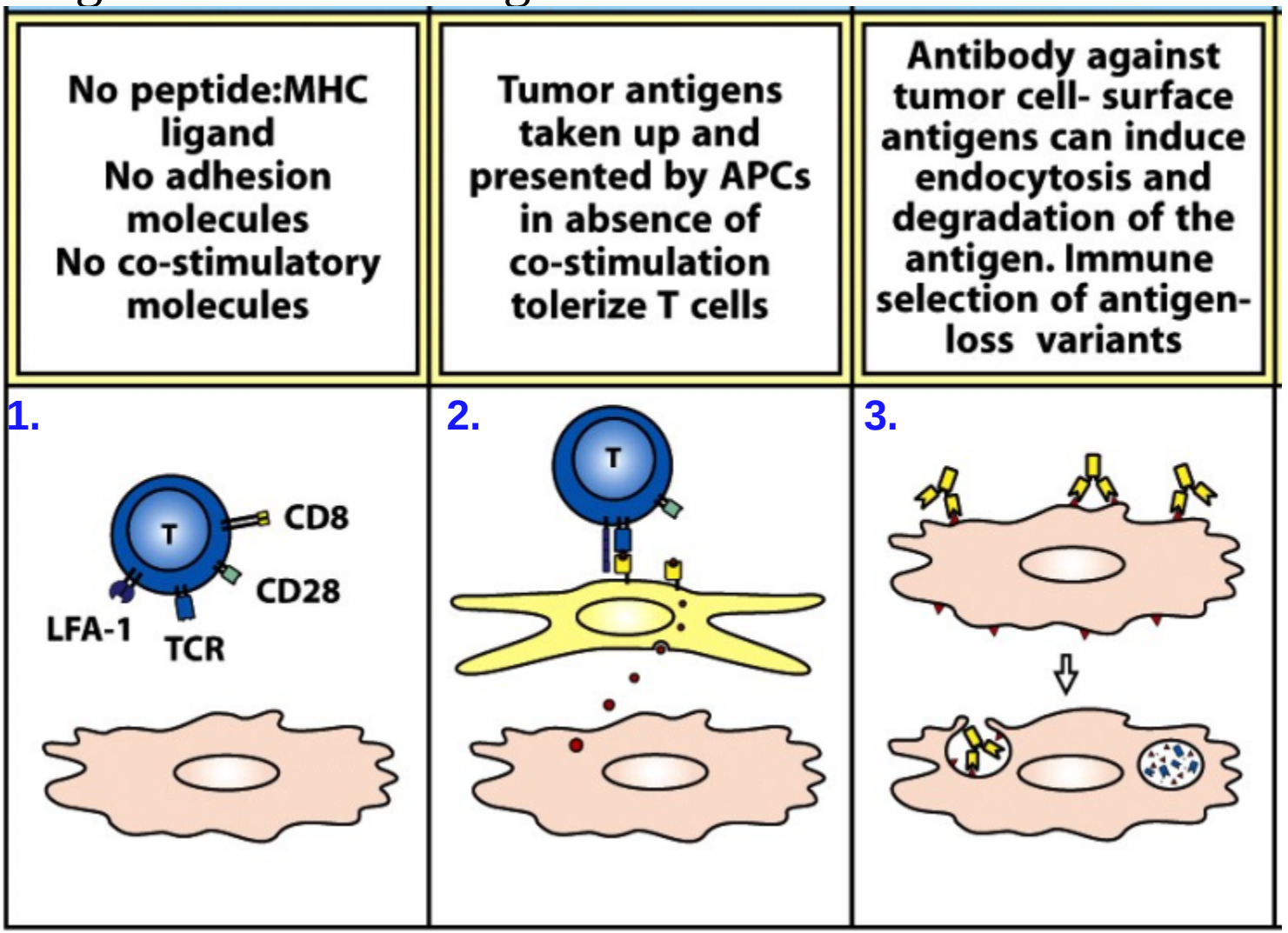


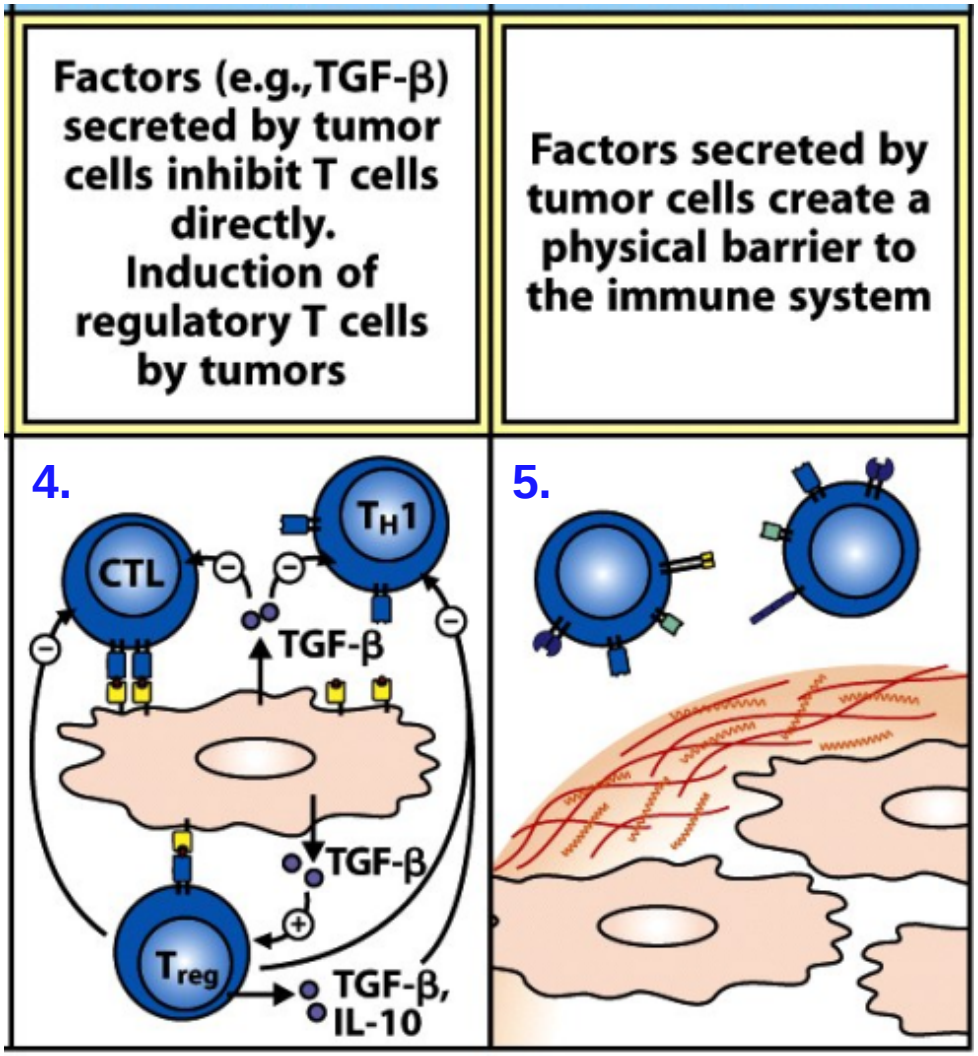
Fig. 16-13

How do tumors escape immune surveillance?

(often occurs at the equilibrium phase)

4. Direct immunosuppression by tumor-secreted factors (e.g. TGF-β)
5. Induction of tumor privileged site

- Could be either singular or multifactorial events



Categories of known human tumor-specific antigens

1. Point mutation (or gene rearrangement) of self-protein during the process of oncogenesis
2. Germ cell-encoded proteins
   - When normally expressed in male germ cells (lack of MHC molecules), not processed nor presented to T lymphocytes
   - When abnormally expressed in tumor, presented by MHC-I to T lymphocytes
3. Differentiation antigens
   - Genes expressed only in particular tissue types

Categories of known human tumor-specific antigens

4. Abnormal gene expression - overexpression of HER-2/neu in ovarian cancer
5. Abnormal post-translational modification
   - underglycosylated MUC-1 in breast or pancreatic cancer
6. Abnormal post-transcriptional modification
   - retention of introns in mRNA
7. Oncoviral proteins
   - viral transforming gene products

Point mutations in self proteins may give rise to new tumor rejection antigens

Fig. 16-17

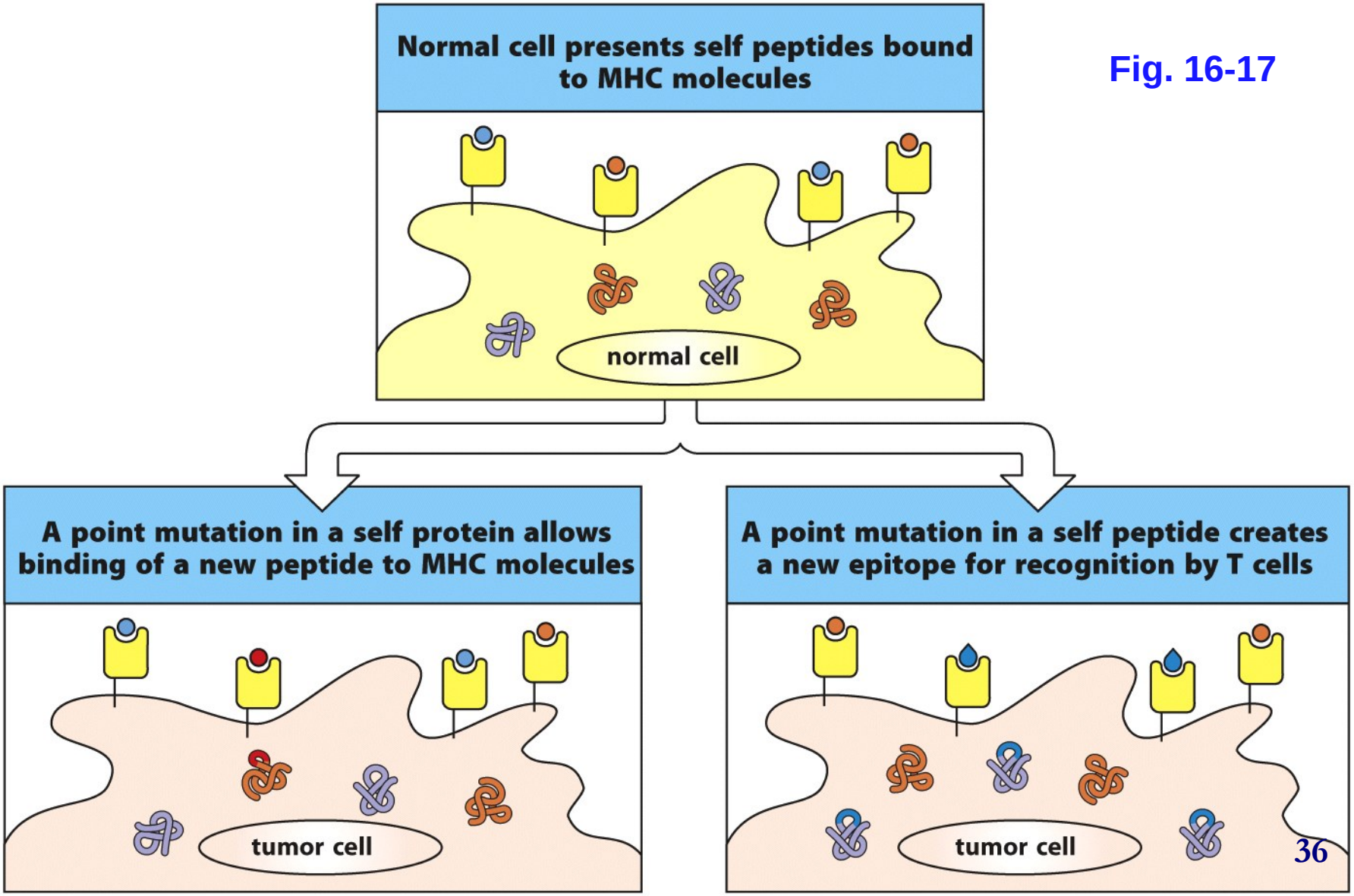


Figure 16.17 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Transplanted tumor without MHC-I expression is more susceptible to NK-killing

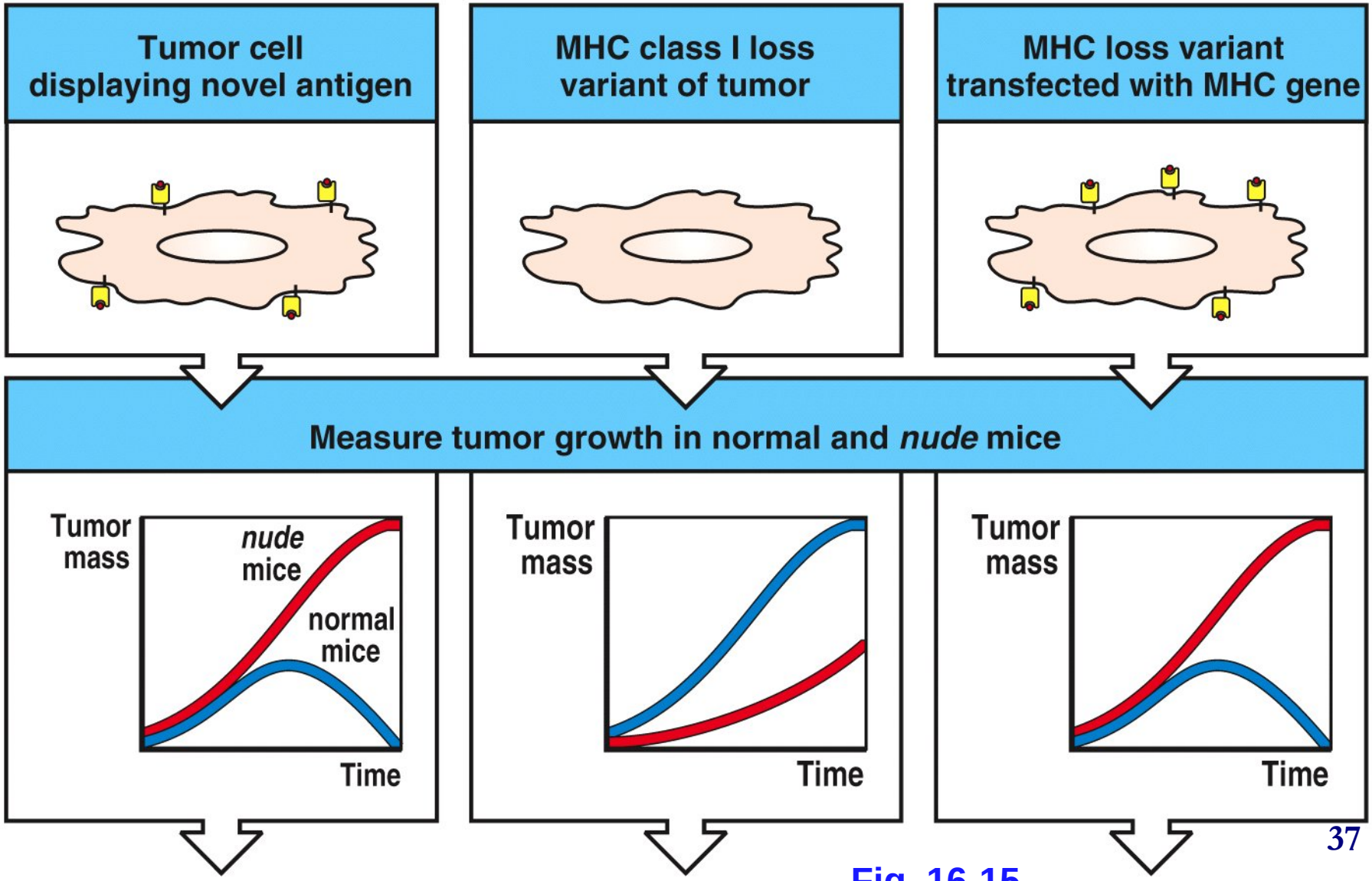


Figure 14-16 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Fig. 16-15

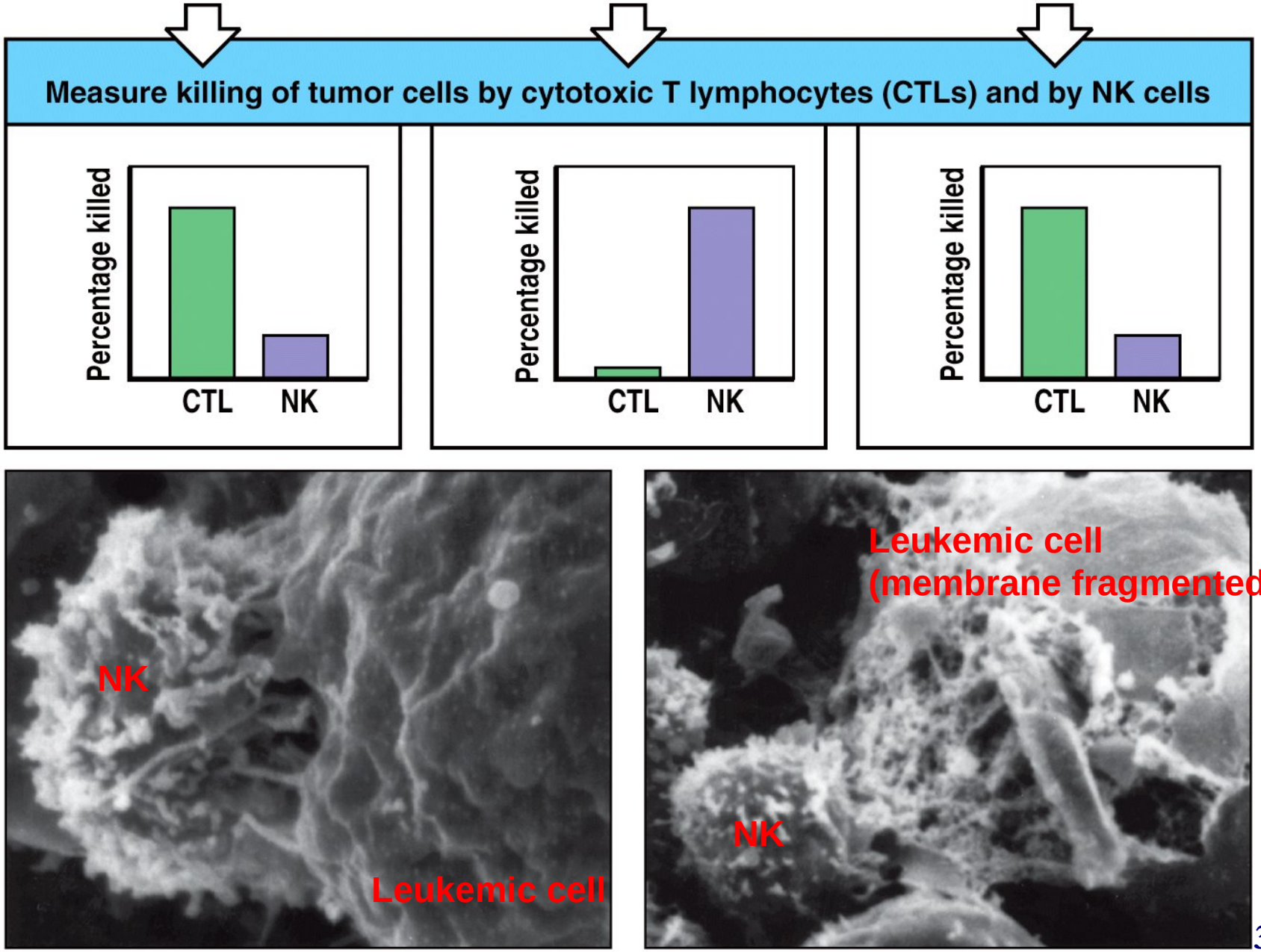


Figure 14-16 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

mAbs as therapeutic agents against tumors

Fig. 16-18

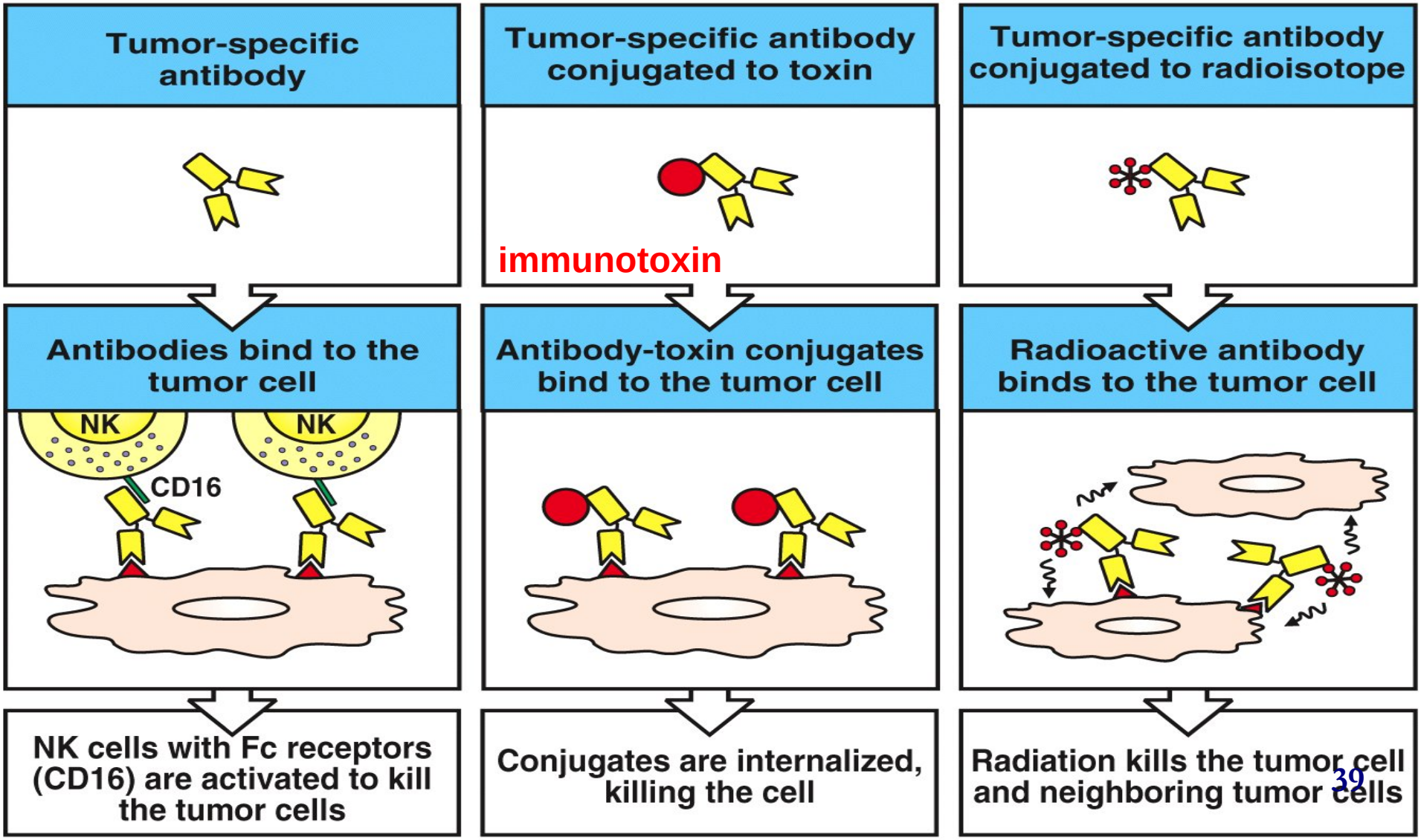


Figure 14-17 Immunobiology, 6/e. (© Garland Science 2005)

Tumor tissue origin	Type of antigen	Antigen	Tumor type
Lymphoma/ leukemia	Differentiation antigen	CD5 Idiotype CAMPATH-1 (CDw52)	T-cell lymphoma B-cell lymphoma T- and B-cell lymphoma
	B-cell signaling receptor	CD20 **Rituximab**	Non-Hodgkin's B-cell lymphoma
Solid tumors	Cell-surface antigens Glycoprotein Carbohydrate	CEA, mucin-1 Lewis^y CA-125	Epithelial tumors (breast, colon, lung) Epithelial tumors Ovarian carcinoma
	Growth factor receptor	Epidermal growth factor receptor **Herceptin** p185^{HER2} IL-2 receptor	Lung, breast, head, and neck tumors Breast, ovarian tumors T- and B-cell tumors
	Stromal extracellular antigen	FAP-α Tenascin Metalloproteinases	Epithelial tumors Glioblastoma multiforme Epithelial tumors

Fig. 16-19

Figure 14-18 Immunobiology, 6/e. (© Garland Science 2005)

Vaccination

- Manipulating the immune response to fight infection

Common vaccines

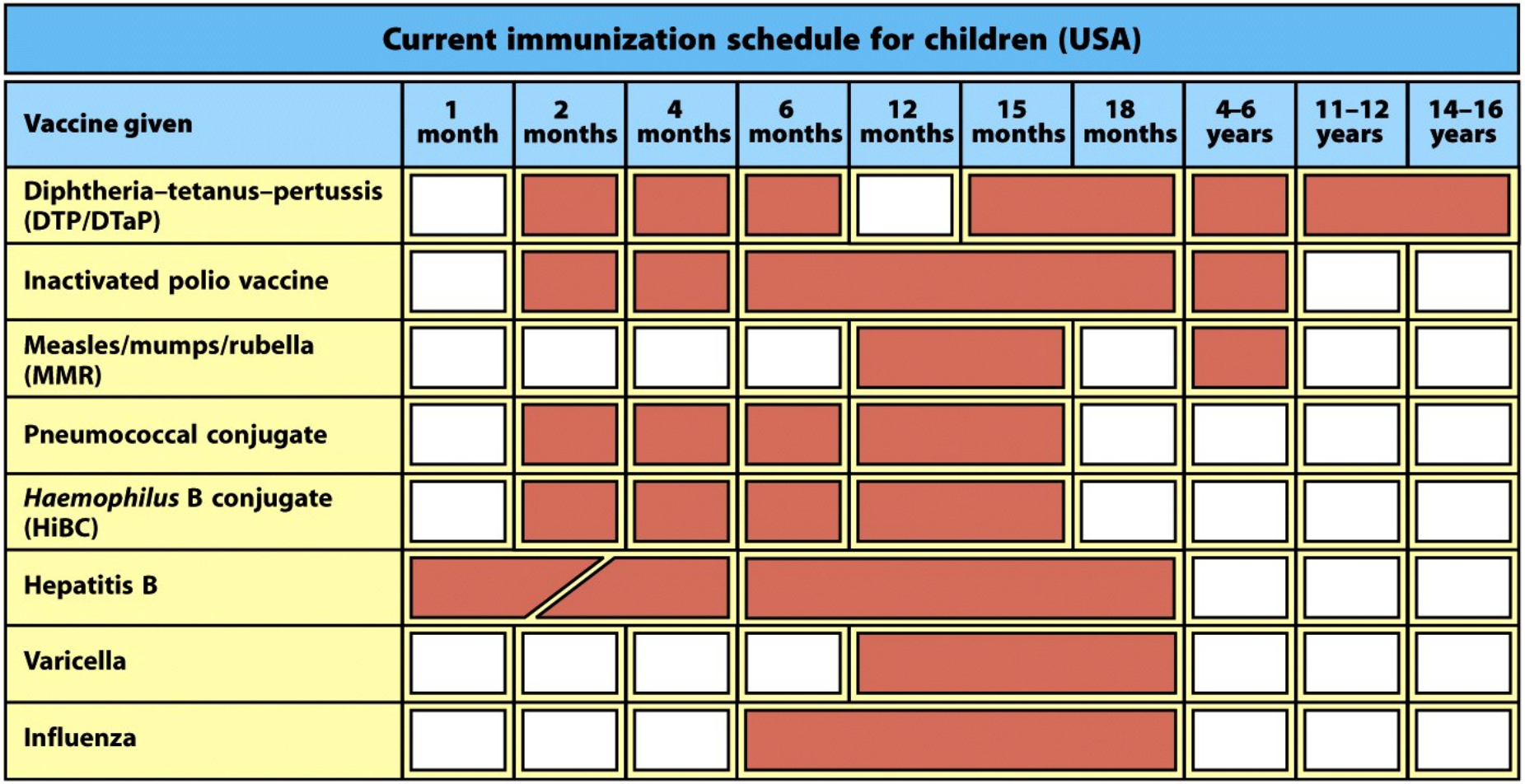
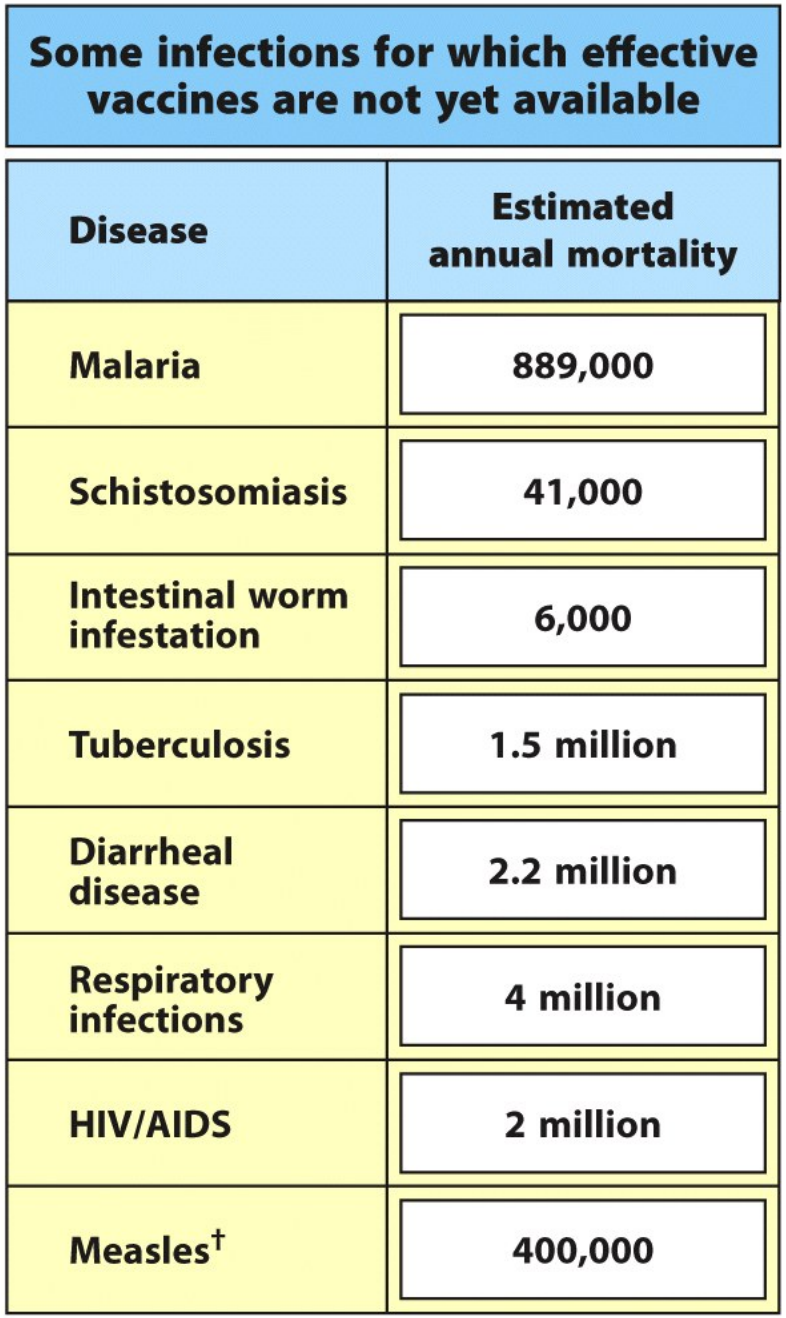


Figure 15-25 Immunobiology, 7ed. (© Garland Science 2008)

Herd immunity: when a large proportion of the population is immune (vaccinated), the pathogen reservoir is reduced and transmission of the pathogen is difficult. Thus, even those that are not immune are at reduced risk for infection



Some infections for which effective vaccines are not yet available

Disease	Estimated annual mortality
Malaria	889,000
Schistosomiasis	41,000
Intestinal worm infestation	6,000
Tuberculosis	1.5 million
Diarrheal disease	2.2 million
Respiratory infections	4 million
HIV/AIDS	2 million
Measles†	400,000

Figure 16.22 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Effective vaccines

Fig. 16-23

Features of effective vaccines	
Safe	Vaccine must not itself cause illness or death
Protective	Vaccine must protect against illness resulting from exposure to live pathogen
Gives sustained protection	Protection against illness must last for several years
Induces neutralizing antibody	Some pathogens (such as polio virus) infect cells that cannot be replaced (e.g., neurons). Neutralizing antibody is essential to prevent infection of such cells
Induces protective T cells	Some pathogens, particularly intracellular, are more effectively dealt with by cell-mediated responses
Practical considerations	Low cost per dose Biological stability Ease of administration Few side-effects

Figure 15-27 Immunobiology, 7ed. (© Garland Science 2008)

Types of vaccines

Dead organism

Live attenuated organism (減毒疫苗)

Toxoid (類毒素)

Purified protein

Recombinant protein

Require adjuvants

DNA vaccine

Attenuation of virus for the use of vaccine

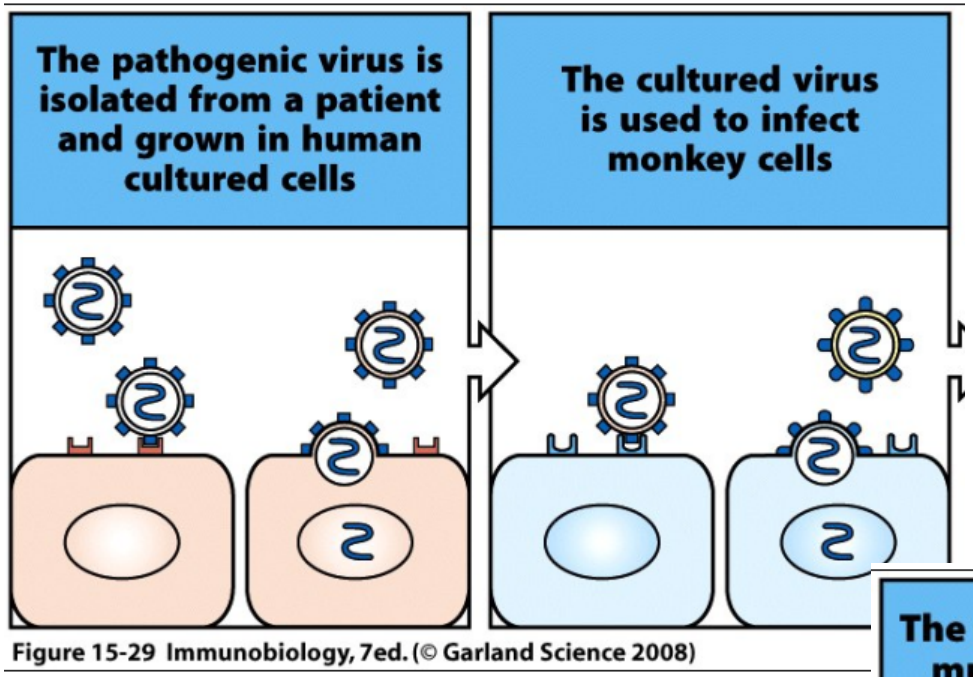


Figure 15-29 Immunobiology, 7ed. (© Garland Science 2008)

Fig. 16-24

Could be a time-consuming process!!!

Attenuation by recombinant DNA technology

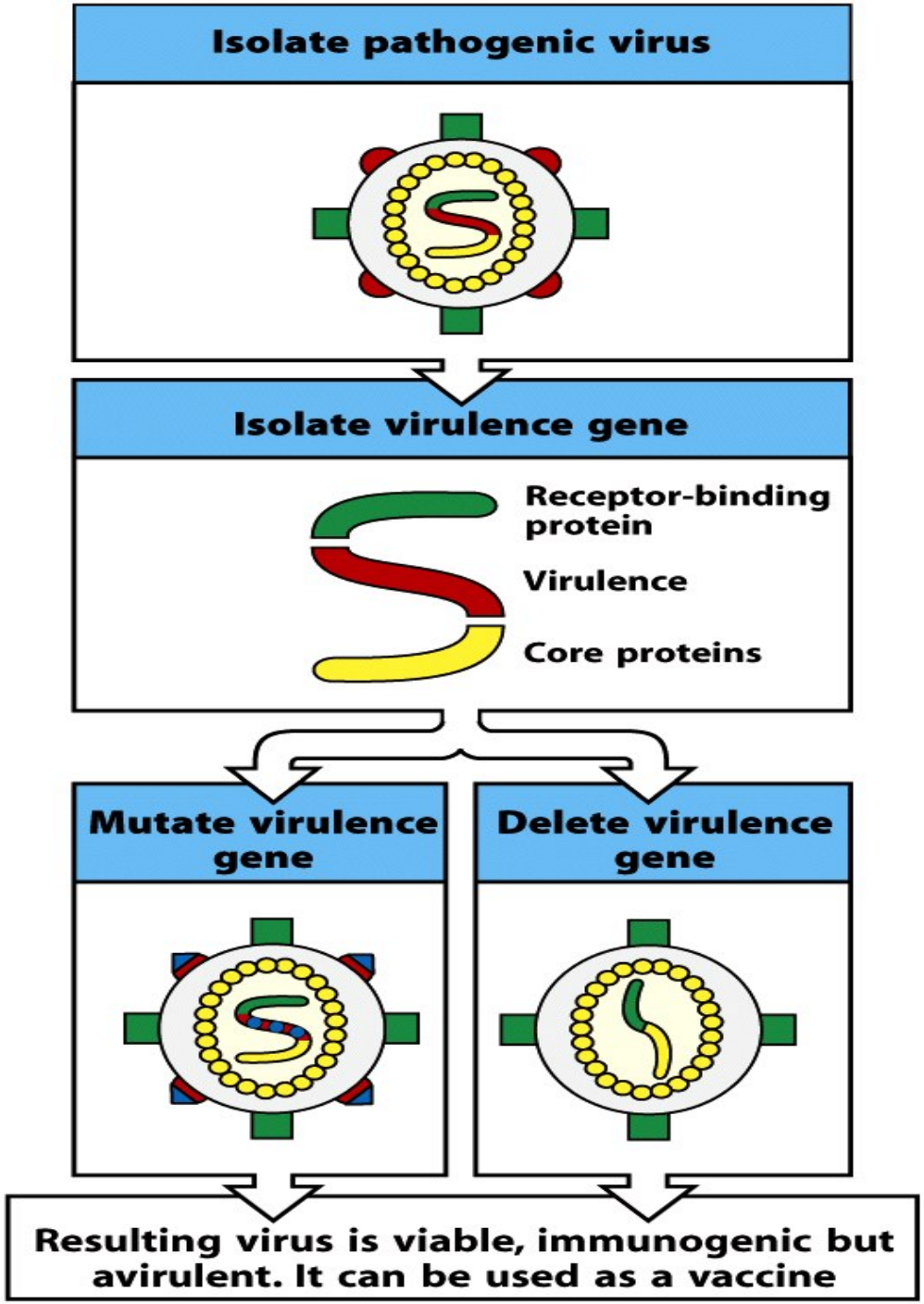


Figure 15-30 Immunobiology, 7ed. (© Garland Science 2008)

Fig. 16-25

Summary

- Unwanted immune responses can be regulated by the use of various drugs and biological agents.
- Controlling activity of T cells can best impose regulation on unwanted immune responses.
- Vaccination can help manipulate the immune responses to fight both bacterial and viral infections.

End of Chapter