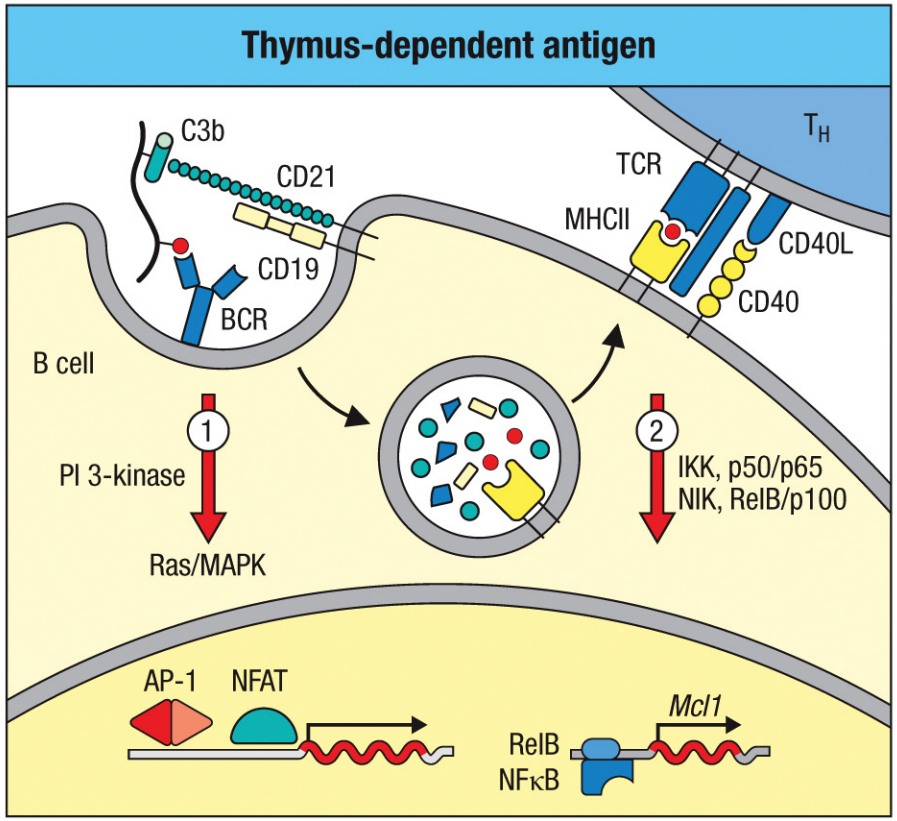
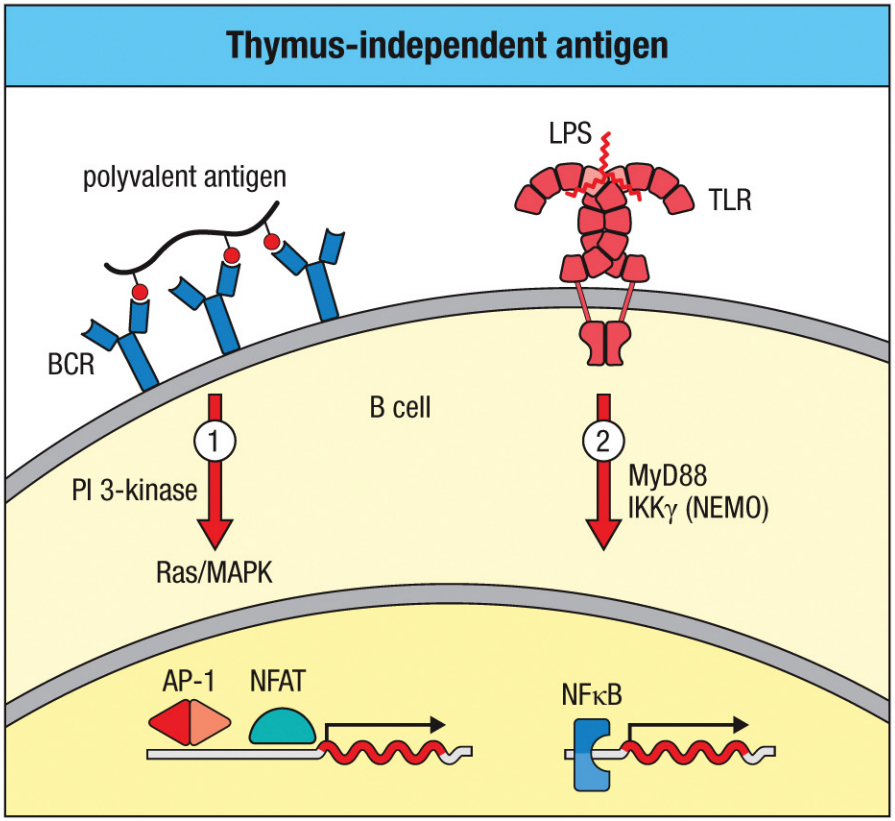
Two Signals Are Required for B Cell Activation





1. Crosslinking of the BCR
2. Signal from T helper Cells

1. Extensive cross linking of BCR (IgM)
2. Activation of TLR

Immunoglobulin Diversification

Diversification mechanism	*Effect*
Somatic hypermutation	Antigen specificity
Class switching	Effector activity

Irreversible changes at the DNA level

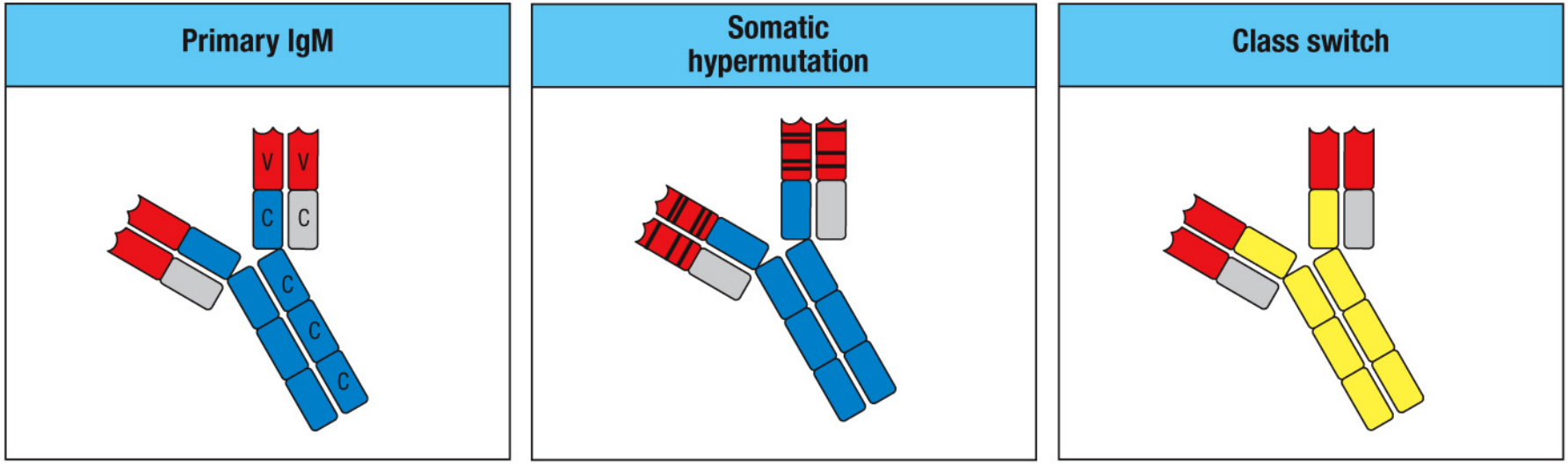
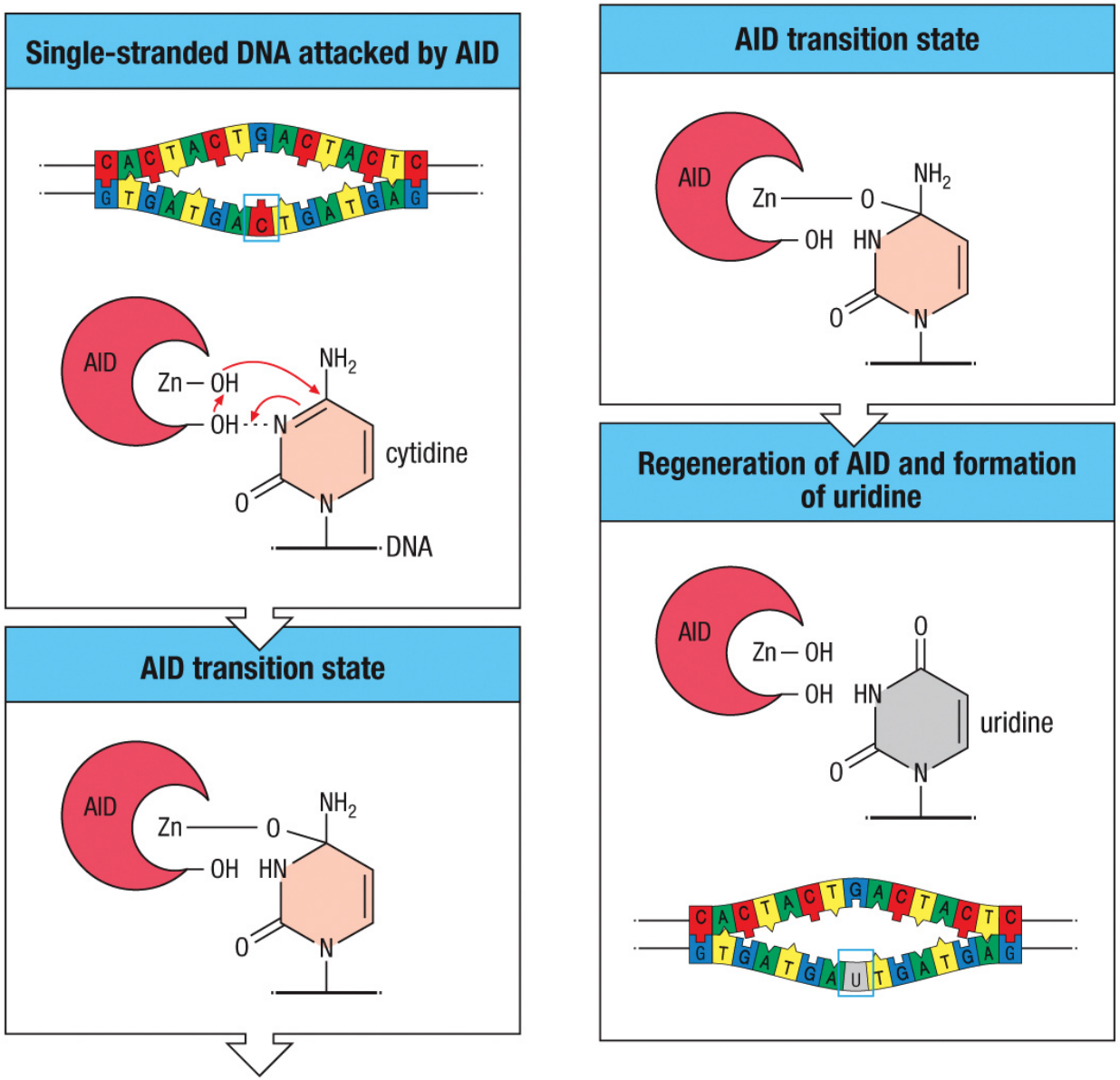


Figure 10.13 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

C-U Switch at Single-Stranded DNA



Outline

- Biological Functions of antibodies
  - Neutralization
  - Opsonization
  - Complement activation
  - Mast cell activation
  - Antibody dependent toxicity
- Application of antibodies
  - Biomedical
  - Technical

Functions of Ig Classes

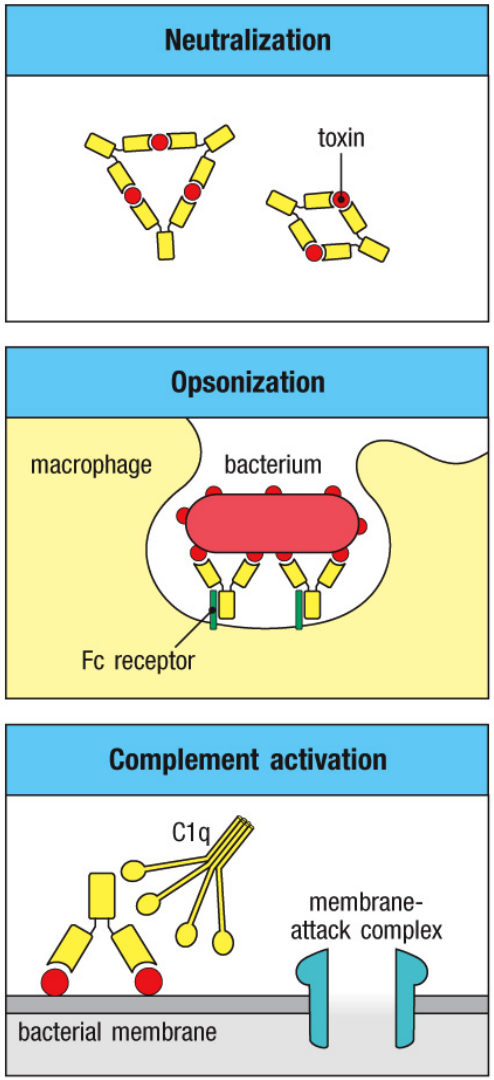
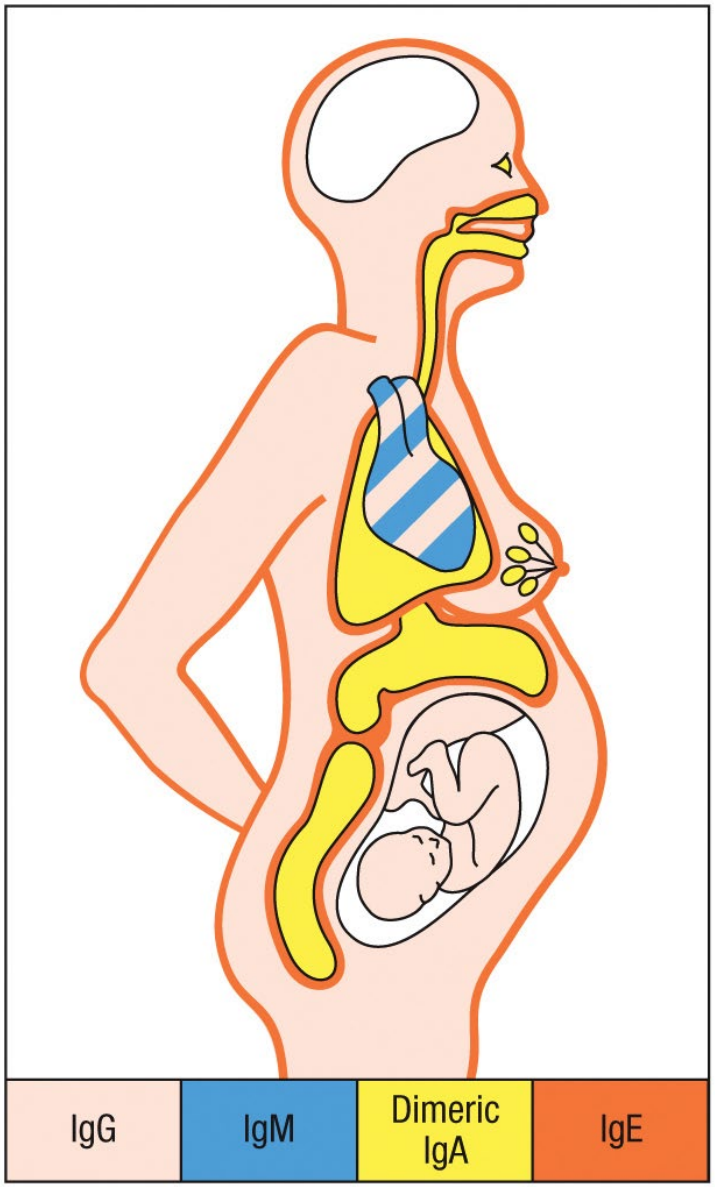


Figure 10.1 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Distribution of Ig Classes



IgM:
Pentamor, circulation

IgG:
Monomer, Circulation, extravascular Spaces
Penetrates the placenta

IgA:
Dimer, gut lumen, exocrine organ
Monomer, extravascular Spaces

IgE:
Fixed on cell surface, underneath epithelium

Distribution of Ig Classes

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

IgM and IgA Form Multimers

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ε
Molecular mass (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2

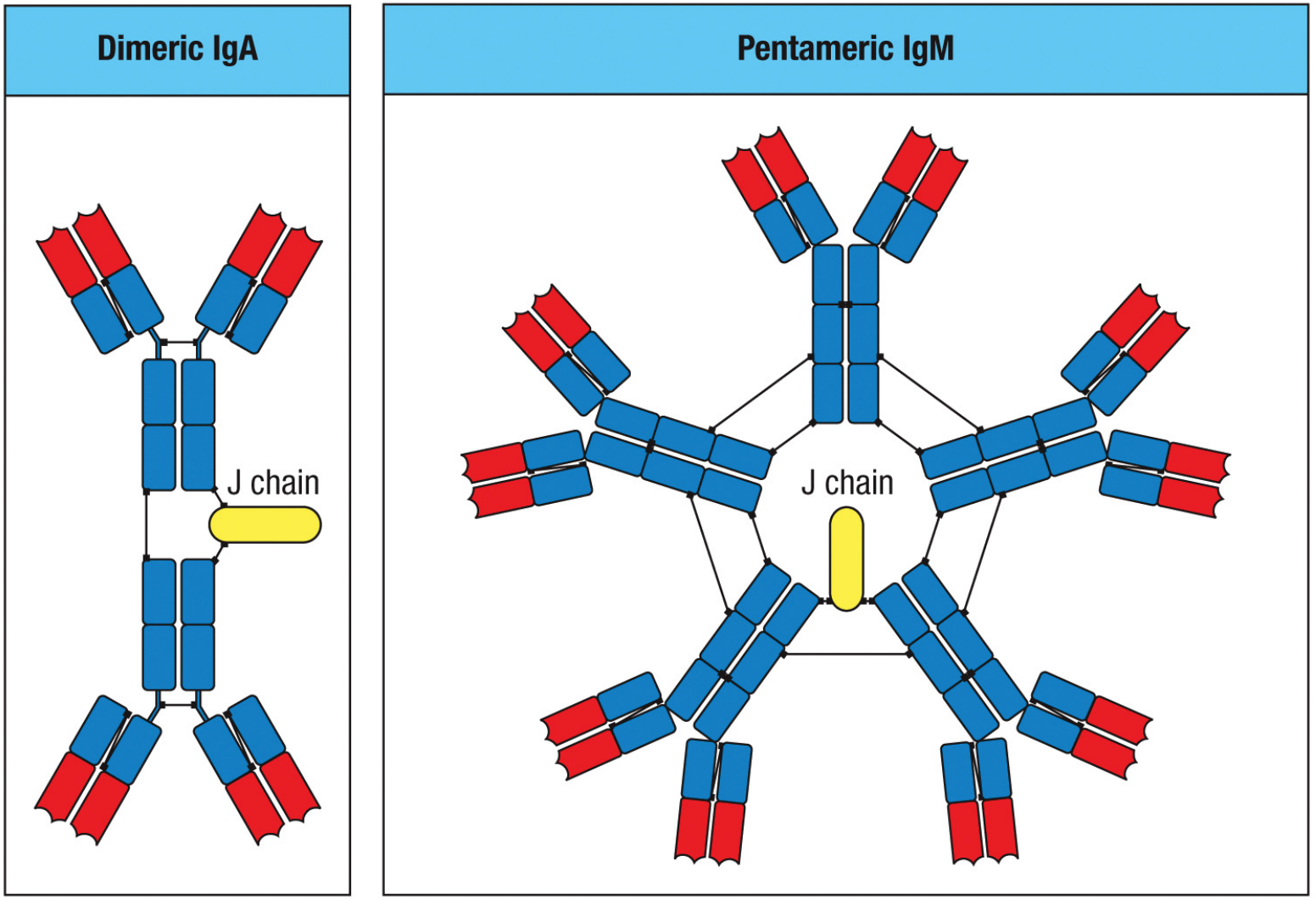
Pentamer in plasma

Dimers

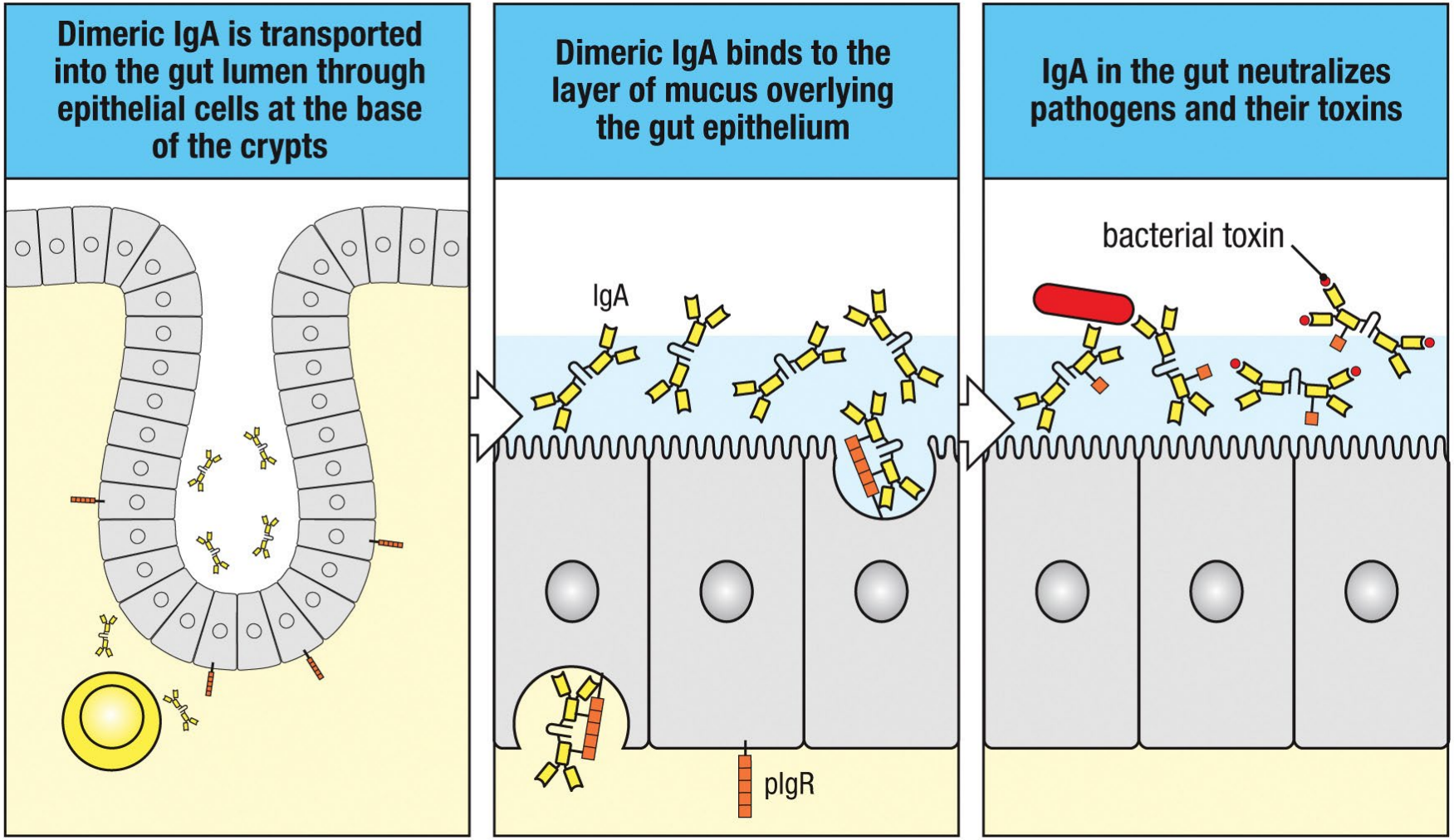
Higher levels in mucous membranes (gut, etc.)

Longer $t_{1/2}$ when fixed to mast cells

IgM and IgA Form Multimers



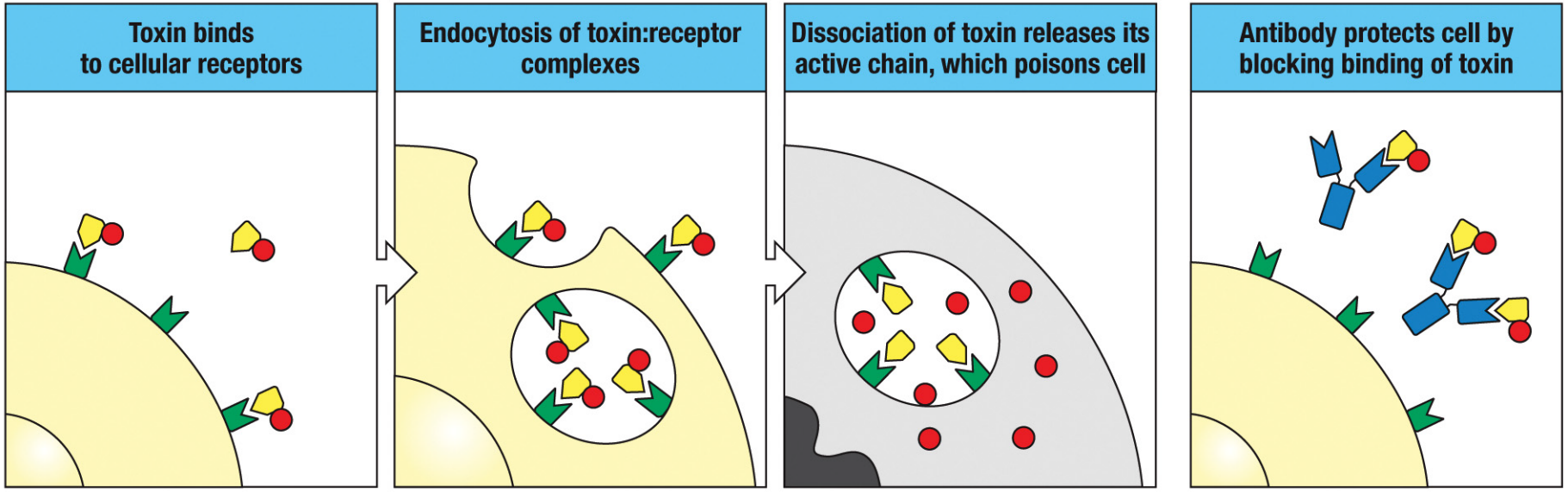
Dimeric IgA is transported into the gut Lumen



Polymeric immunoglobulin receptor and J chain

Neutralization - Prevent Binding

IgA=IgG>IgM



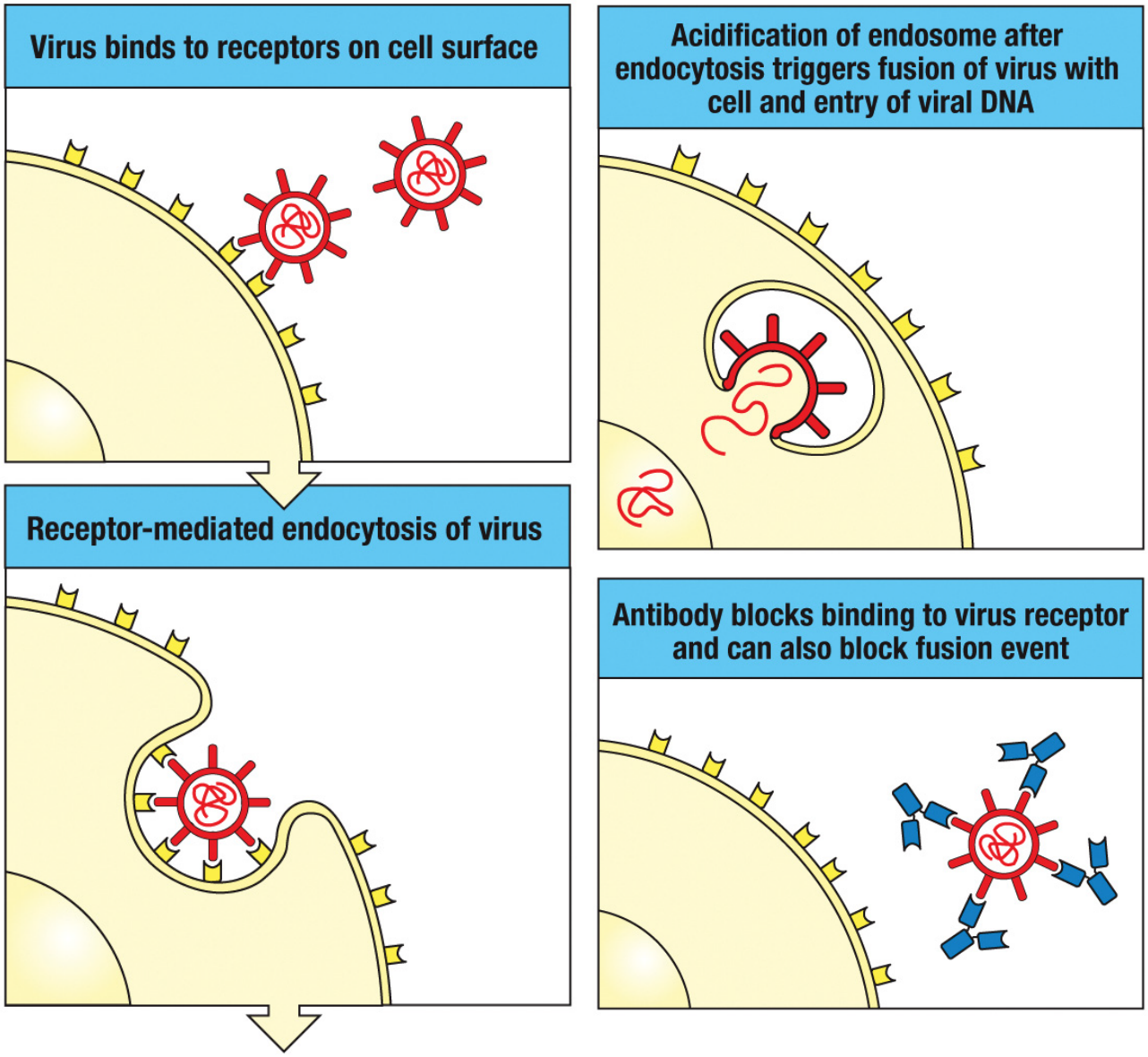
Neutralization - Prevent Binding

Disease	Organism	Toxin	Effects *in vivo*
Tetanus	*Clostridium tetani*	Tetanus toxin	Blocks inhibitory neuron action, leading to chronic muscle contraction
Diphtheria	*Corynebacterium diphtheriae*	Diphtheria toxin	Inhibits protein synthesis, leading to epithelial cell damage and myocarditis
Gas gangrene	*Clostridium perfringens*	Clostridial toxin	Phospholipase activation, leading to cell death
Cholera	*Vibrio cholerae*	Cholera toxin	Activates adenylate cyclase, elevates cAMP in cells, leading to changes in intestinal epithelial cells that result in loss of water and electrolytes
Anthrax	*Bacillus anthracis*	Anthrax toxic complex	Increases vascular permeability, leading to edema, hemorrhage, and circulatory collapse
Botulism	*Clostridium botulinum*	Botulinum toxin	Blocks release of acetylcholine, leading to paralysis

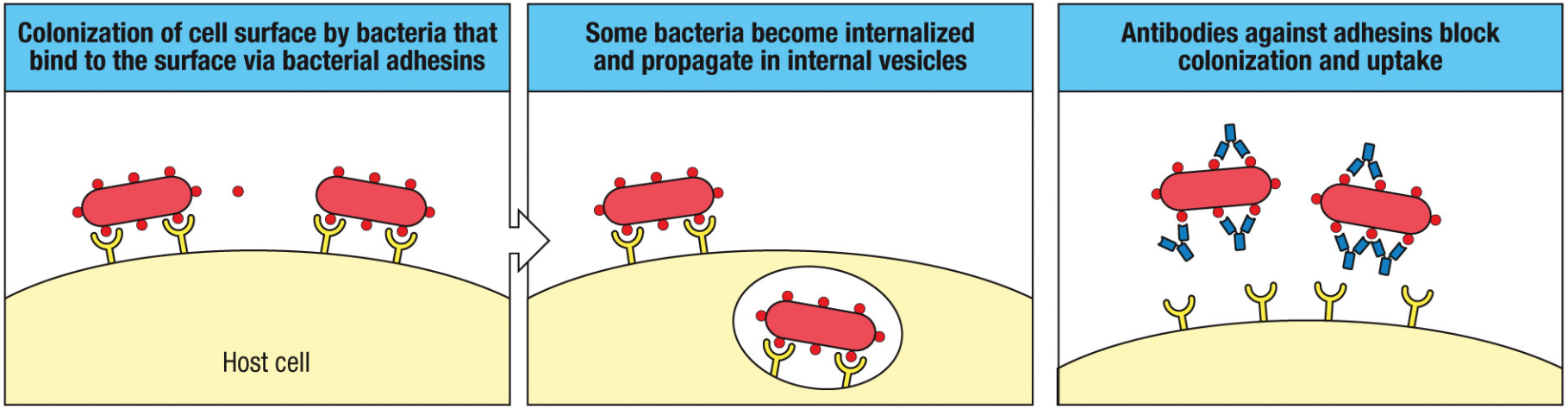
Neutralization - Prevent Binding

Disease	Organism	Toxin	Effects *in vivo*
Whooping cough	*Bordetella pertussis*	Pertussis toxin Tracheal cytotoxin	ADP-ribosylation of G proteins, leading to lymphoproliferation Inhibits cilia and causes epithelial cell loss
Scarlet fever	*Streptococcus pyogenes*	Erythrogenic toxin Leukocidin Streptolysins	Vasodilation, leading to scarlet fever rash Kill phagocytes, allowing bacterial survival
Food poisoning	*Staphylococcus aureus*	Staphylococcal enterotoxin	Acts on intestinal neurons to induce vomiting. Also a potent T-cell mitogen (SE superantigen)
Toxic-shock syndrome	*Staphylococcus aureus*	Toxic-shock syndrome toxin	Causes hypotension and skin loss. Also a potent T-cell mitogen (TSST-1 superantigen)

Neutralization - Prevent Binding

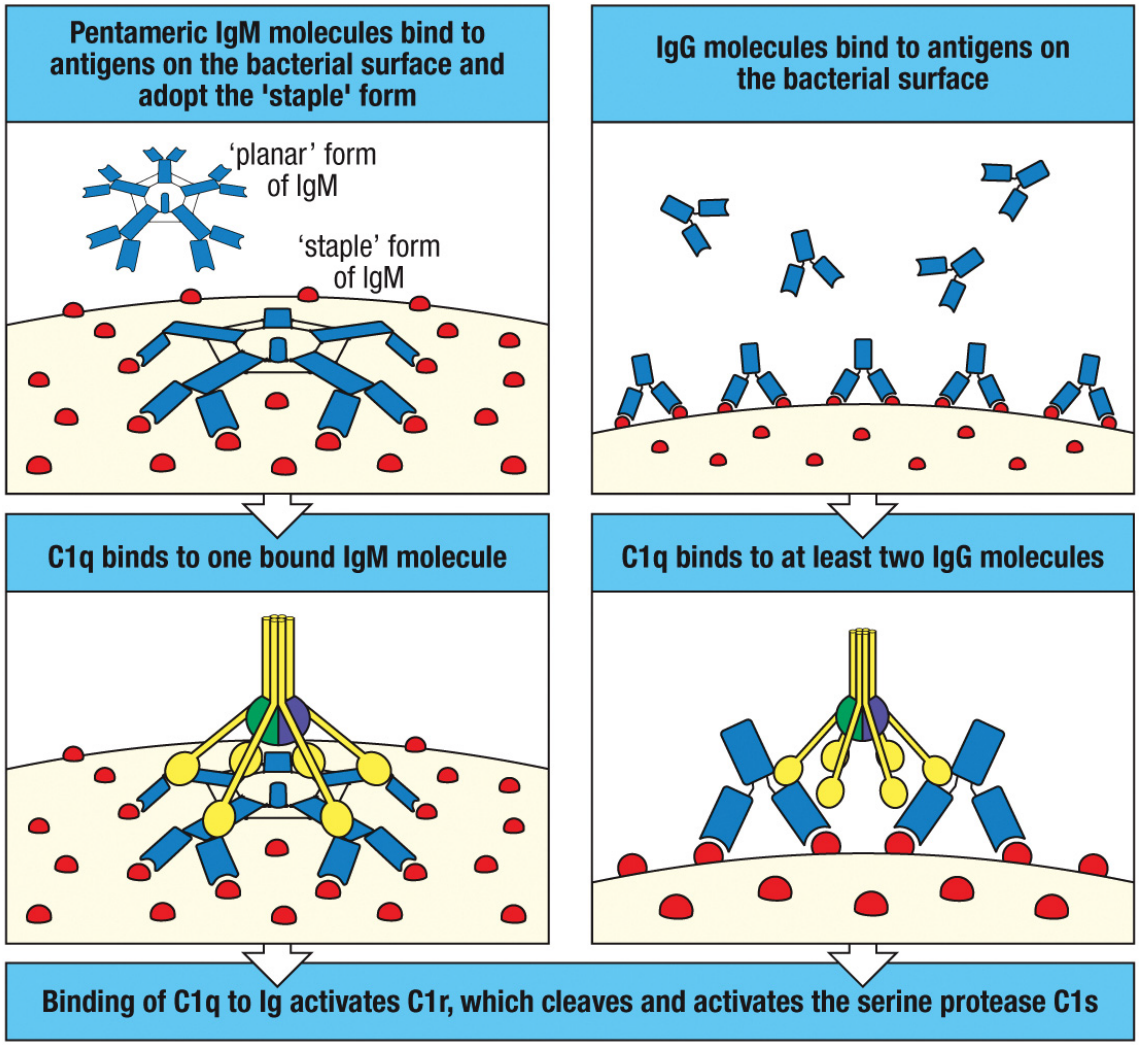


Neutralization - Prevent Binding



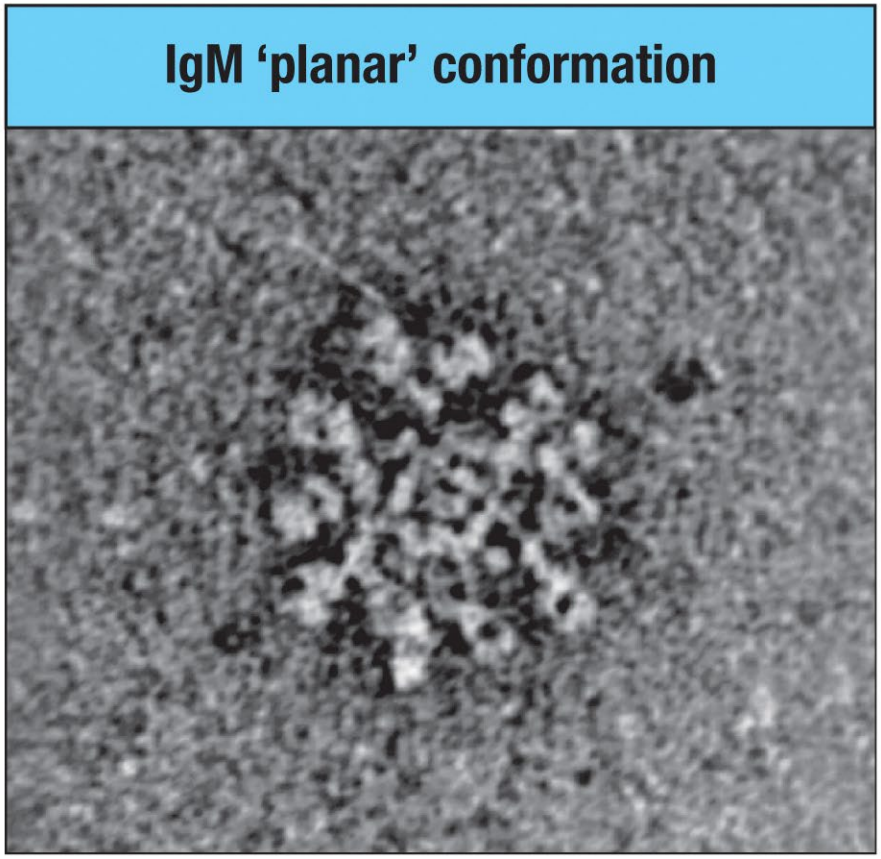
Complement Activation

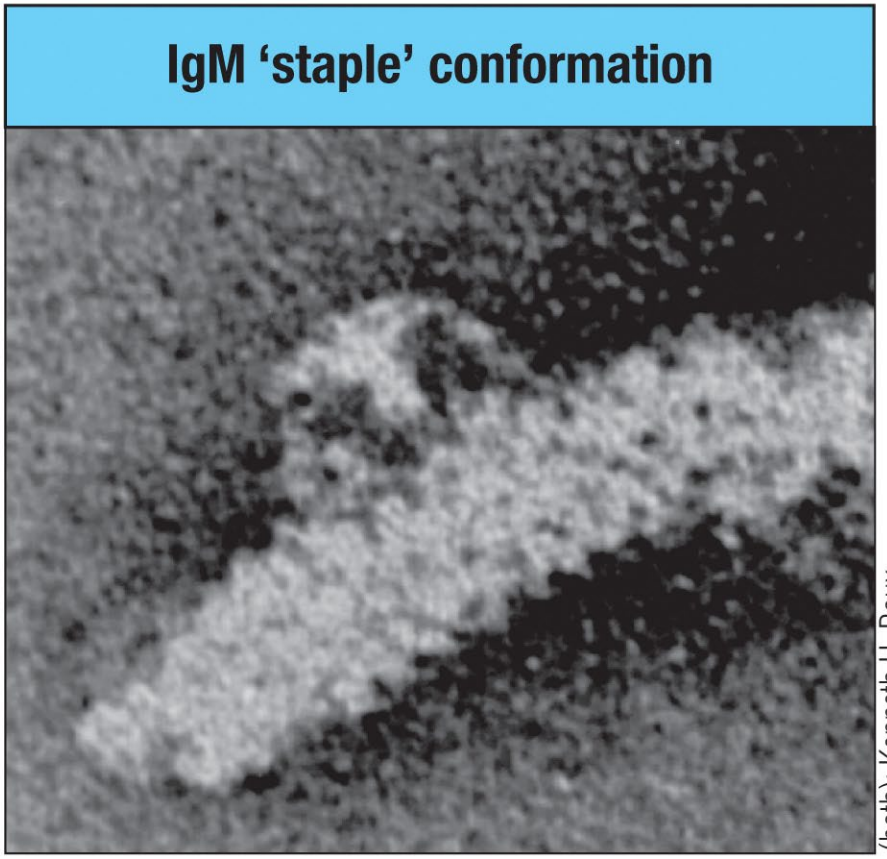
IgM=IgG>IgA,



Complement Activation

Complement is activated when antibodies bind to the pathogen surface





(both): Kenneth H. Roux

Complement Activation

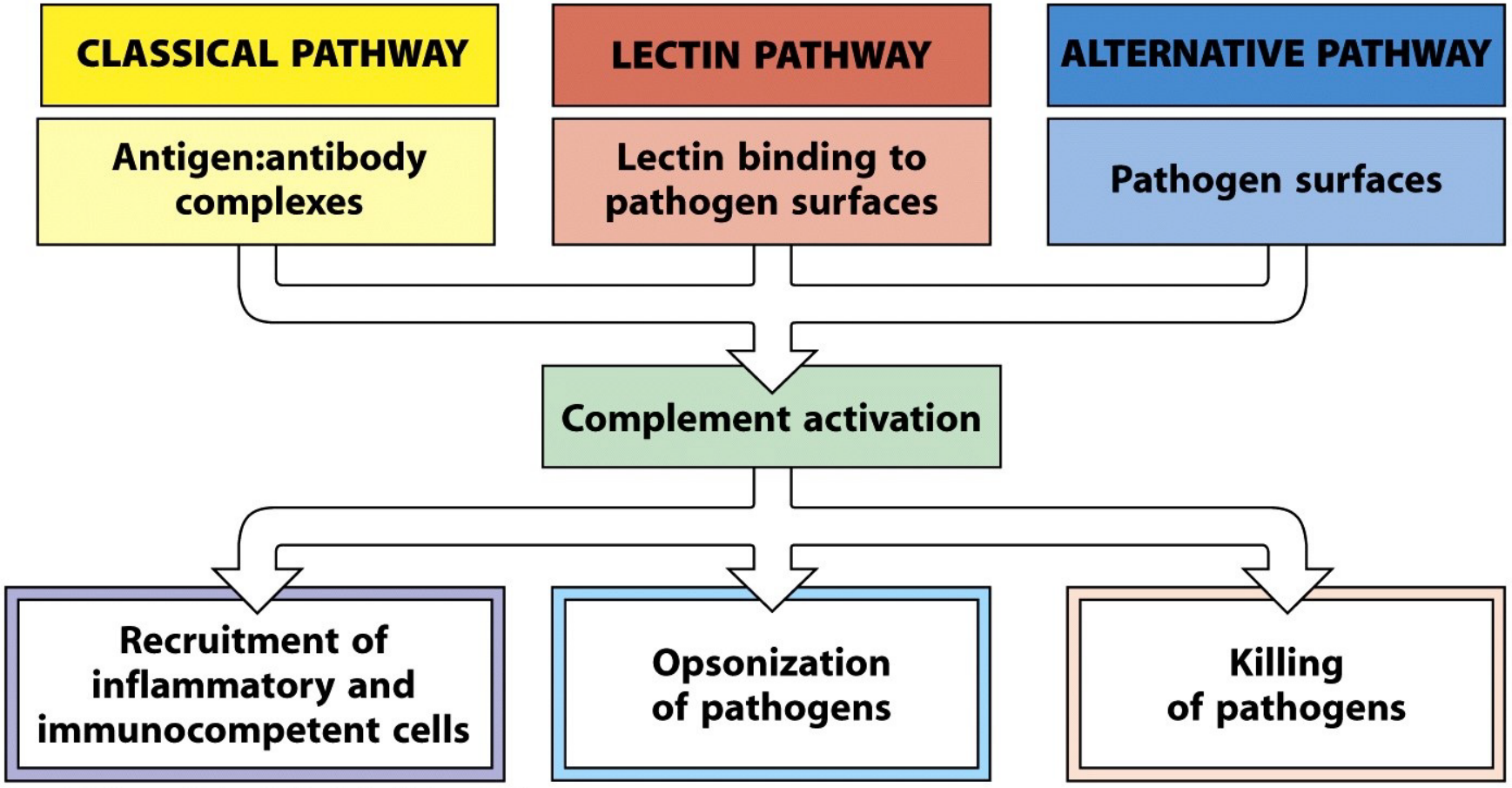
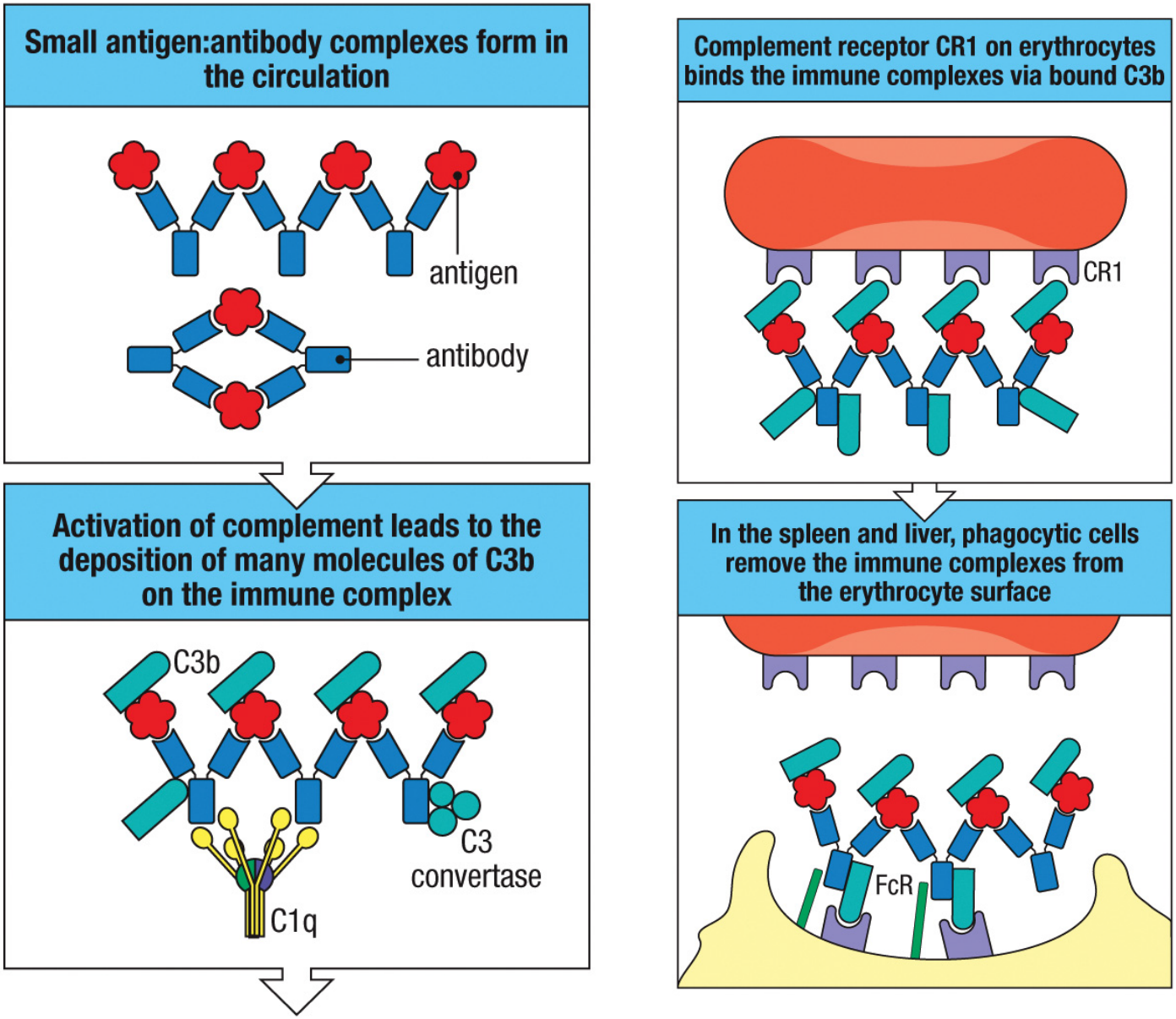


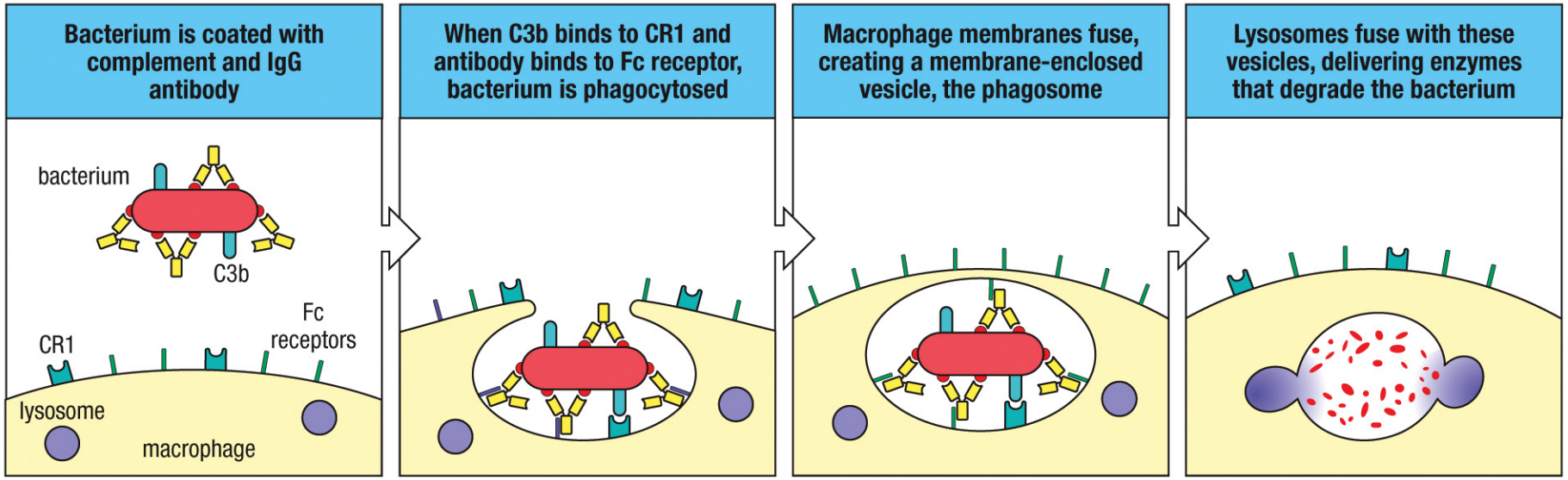
Figure 2-24 Immunobiology, 7ed. (© Garland Science 2008)

Immune Complex is Removed in the Spleen

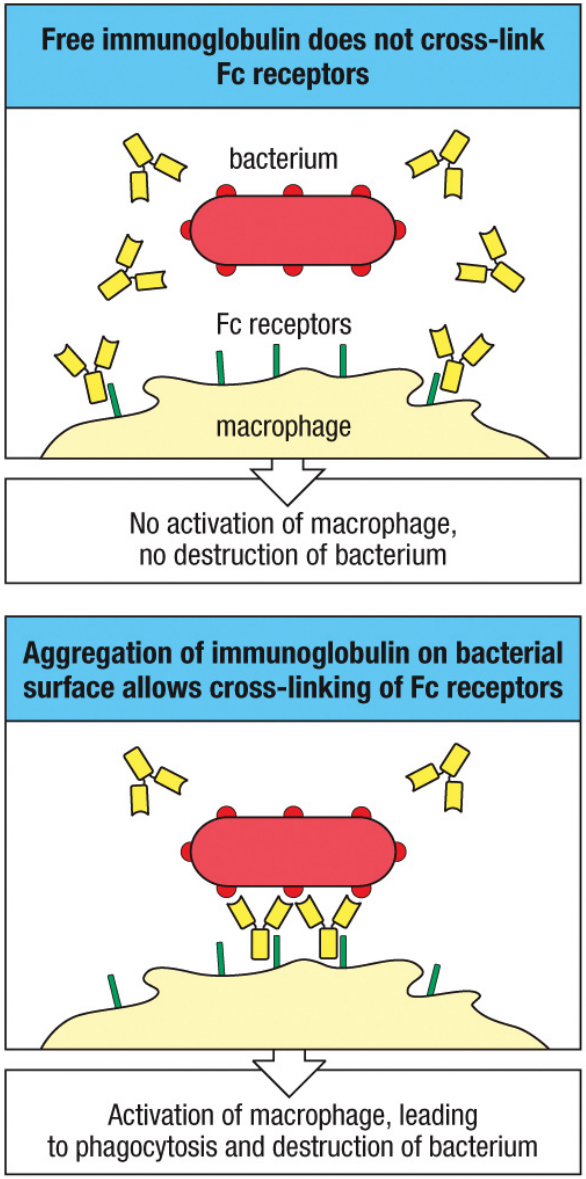


Opsonization- Enhance Phagocytosis

IgG>IgM=IgA

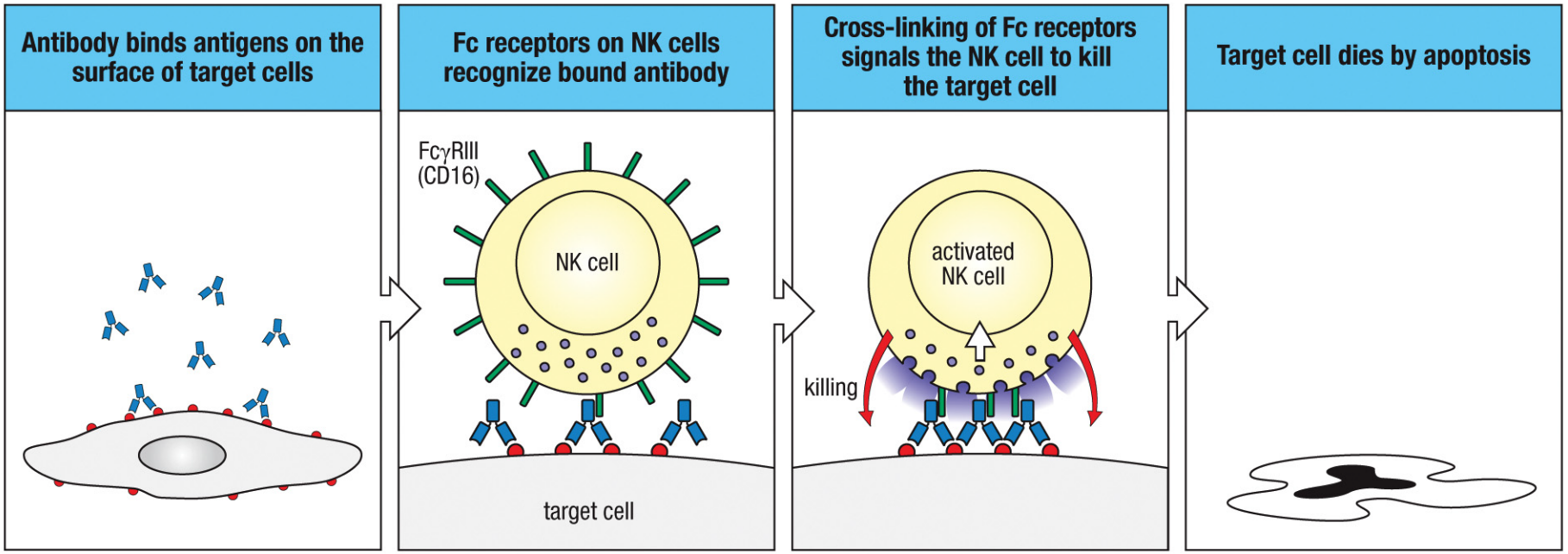


Opsonization Requires Aggregation of Ig on Bacterial Surface



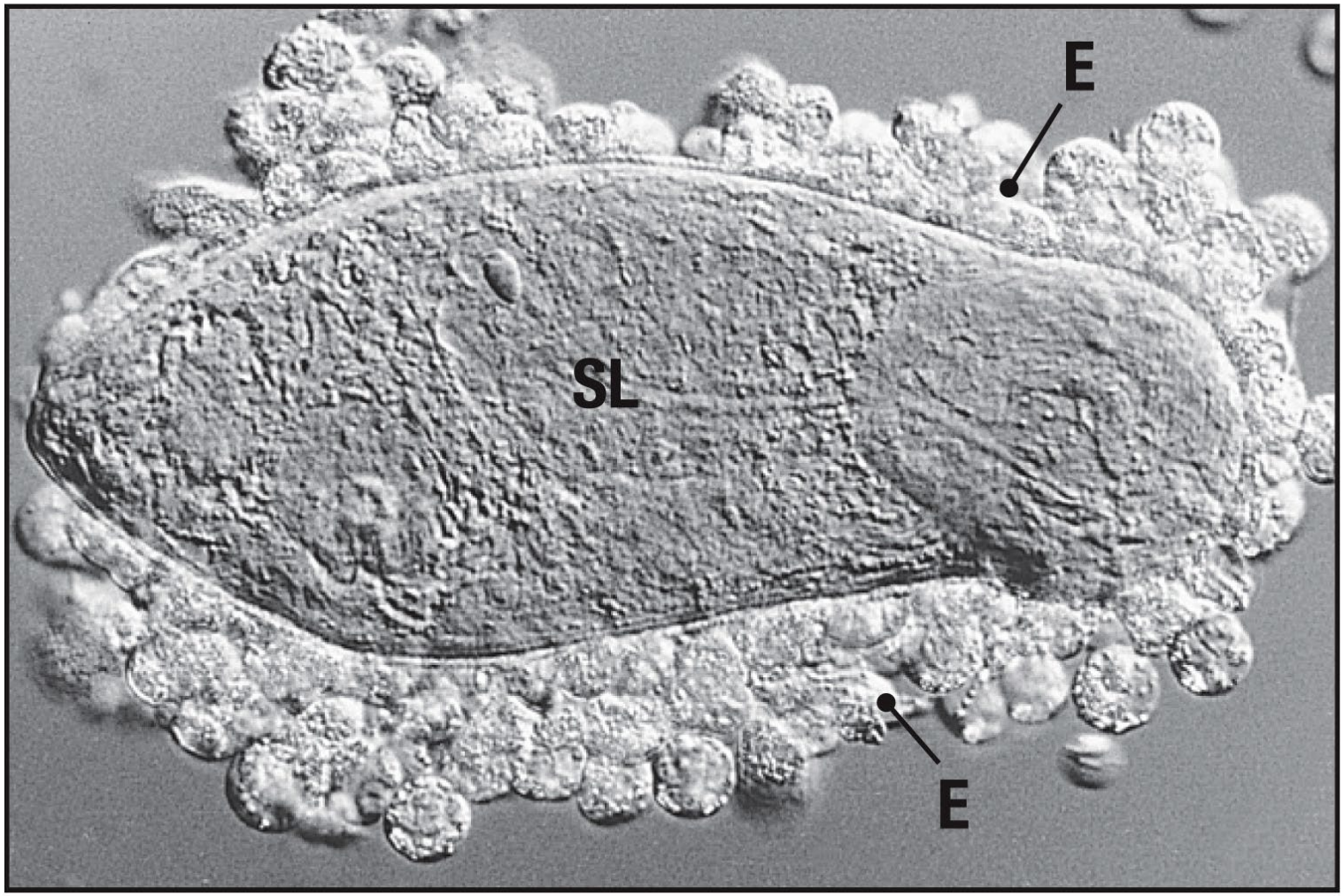
Antibody Dependent Cytotoxicity

IgG, killing of antibody coated host cells



Eosinophils Attacking a Schistosome Larva

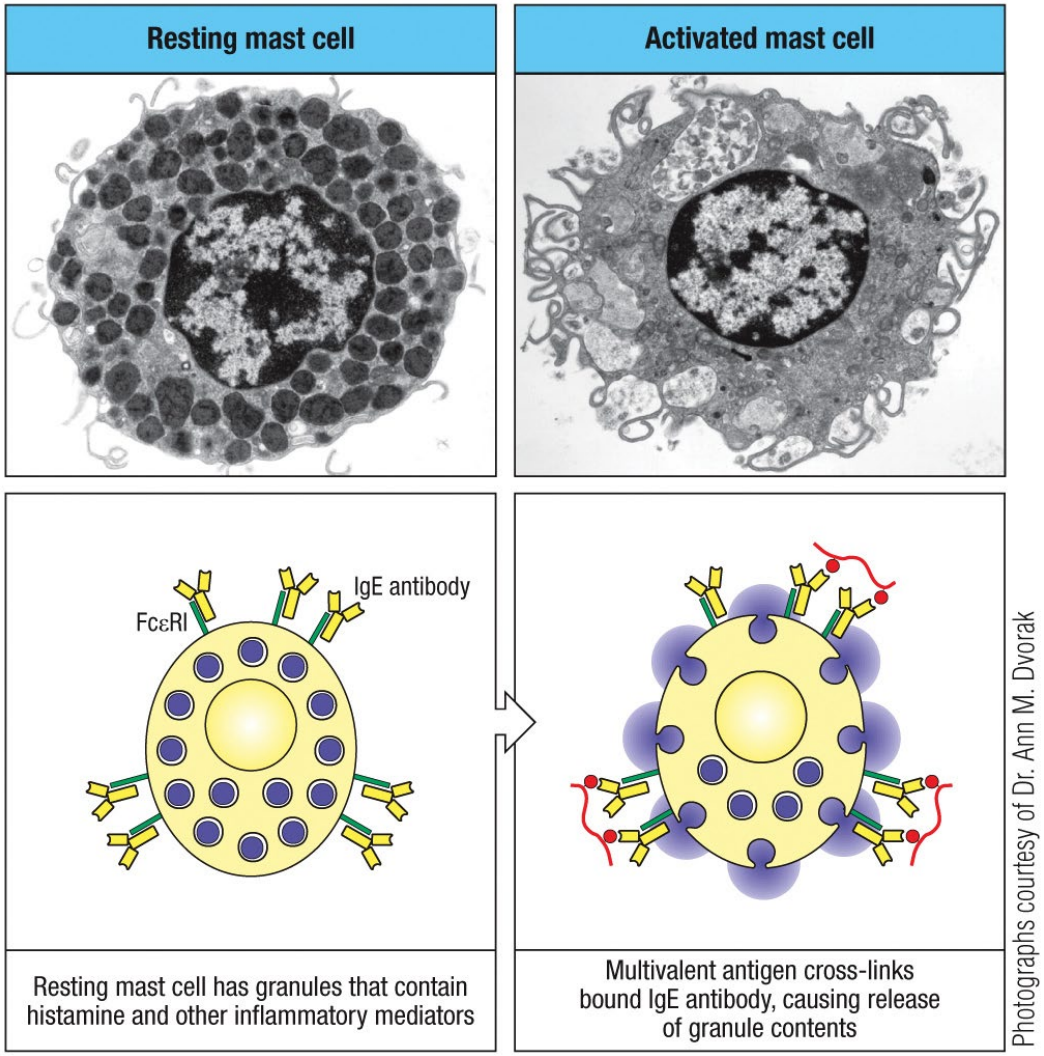
IgE, IgA and IgG



Photograph courtesy of Anthony Butterworth

Mast Cell Activation by IgE

Activates Blood Vessel; Defend against parasites; Allergy

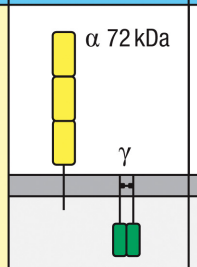
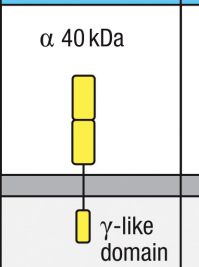
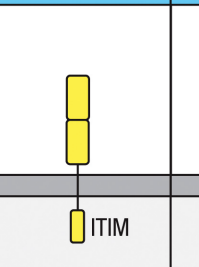
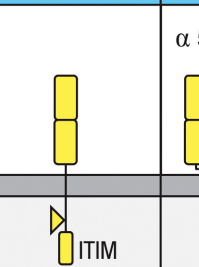
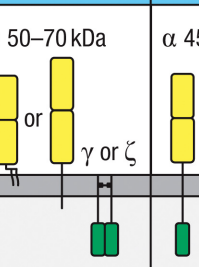
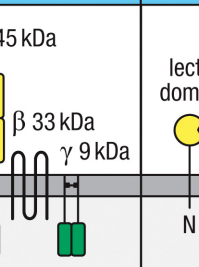
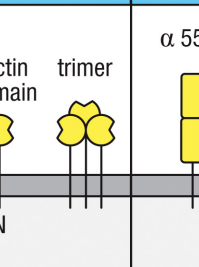
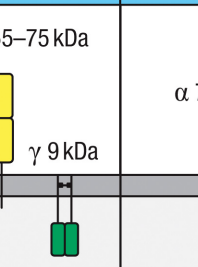
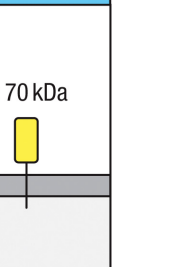


Distribution and Function of Ig Classes

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	+	–	++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Fc Receptors

Receptor	FcγRI (CD64)	FcγRII-A (CD32)	FcγRII-B2 (CD32)	FcγRII-B1 (CD32)	FcγRIII (CD16)	FcεRI	FcεRII (CD23)	FcαRI (CD89)	Fcα/μR
Structure									
Binding **Order of affinity**	IgG1 $10^8\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $2 \times 10^6\ M^{-1}$ 1) IgG1 2) IgG3=IgG2* 3) IgG4	IgG1 $2 \times 10^6\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $2 \times 10^6\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $5 \times 10^5\ M^{-1}$ IgG1=IgG3	IgE $10^{10}\ M^{-1}$	IgE $2–7 \times 10^7\ M^{-1}$ (trimer) $2–7 \times 10^6\ M^{-1}$ (monomer)	IgA1, IgA2 $10^7\ M^{-1}$ IgA1=IgA2	IgA, IgM $3 \times 10^9\ M^{-1}$ 1) IgM 2) IgA
Cell type	Macrophages Neutrophils Eosinophils	Macrophages Neutrophils Eosinophils Platelets Langerhans cells	Macrophages Neutrophils Eosinophils	B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells	Mast cells Basophils	Eosinophils B cells	Macrophages Eosinophils† Neutrophils	Macrophages B cells
Effect of ligation	Uptake Stimulation Activation of respiratory burst Induction of killing	Uptake Granule release (eosinophils)	Uptake Inhibition of stimulation	No uptake Inhibition of stimulation	Induction of killing (NK cells)	Secretion of granules	Degranulation	Uptake Induction of killing	Uptake

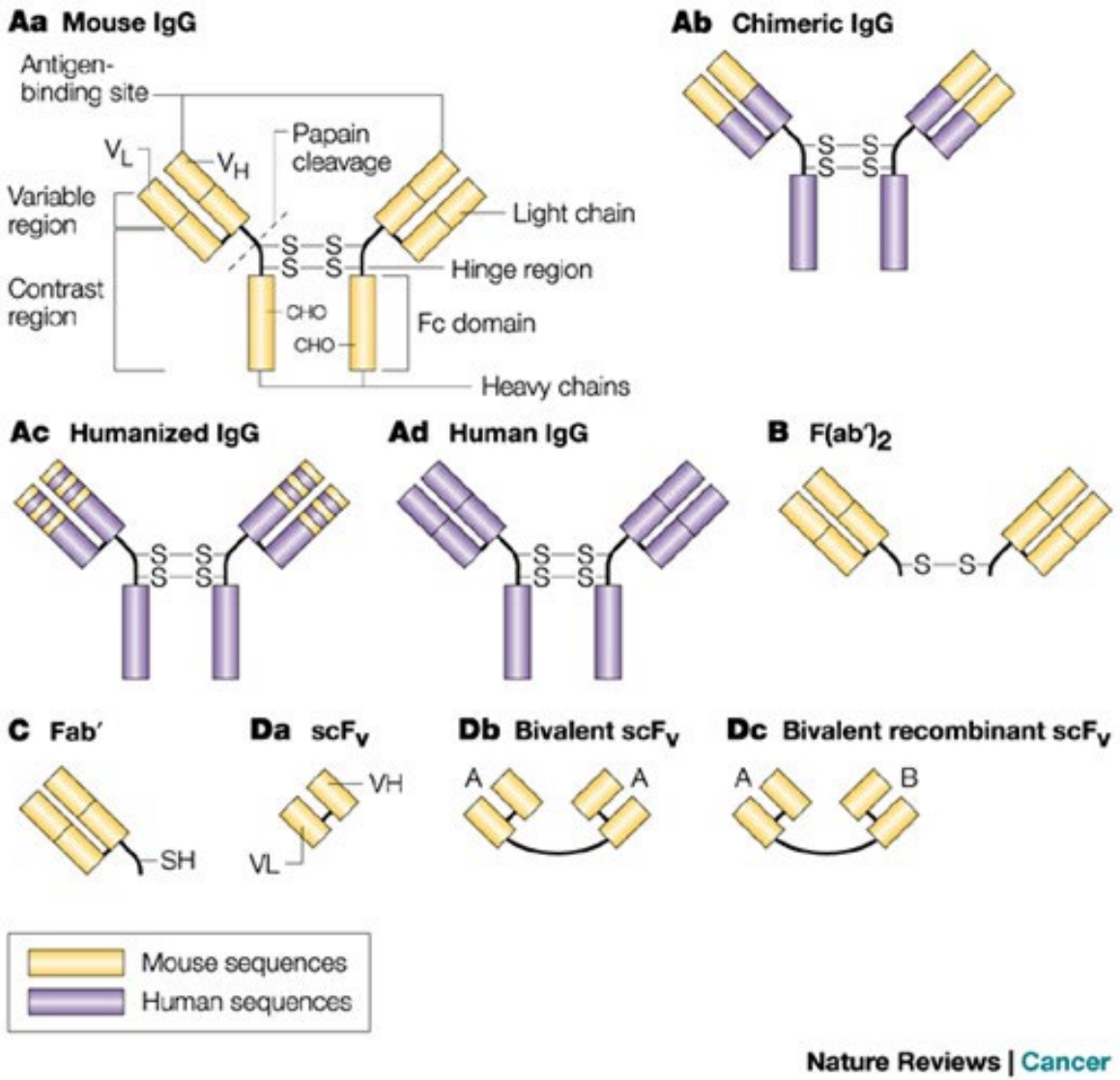
Question

- What are the three major biological functions of antibodies?
- Which type of antibody performs the most functions?

Outline

- Biological Function of antibodies
  - Neutralization
  - Opsonization
  - Complement activation
  - Mast cell activation
  - Antibody dependent toxicity
- Application of antibodies
  - Biomedical
  - Technical

Antibody in Therapy



http://www.nature.com/nrc/journal/v2/n10/fig_tab/nrc903_F2.html

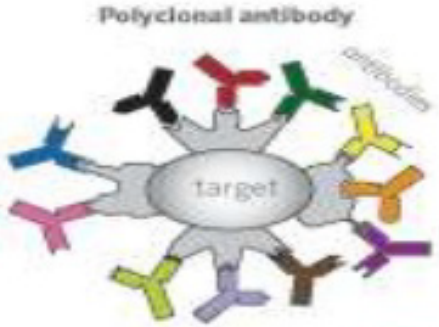
Monoclonal and Polyclonal Antibodies

Monoclonal Abs (Ig, Gama Globulin)

- Antibodies that are identical because they were produced by one type of B cell.
- Detect only one epitope on the antigen.

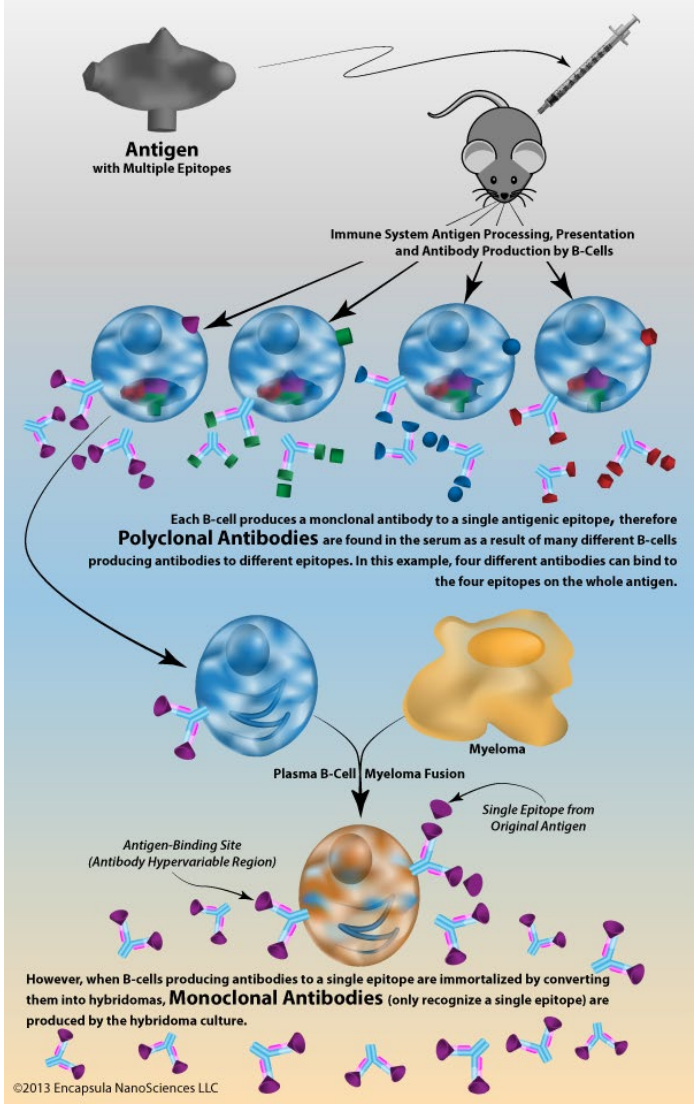
Polyclonal Abs (Ig, Gama Globulin)

- Antibodies that are non-identical because they were produced by different B cell resources.
- Detect multiple epitopes on any one antigen.



• Fairfax KA, Kallies A, Nutt SL, et al. Semin Immunol. 2008;20:49
• Radbruch A, Muehlinghaus G, Luger EO, et al. Nat Rev Immunol. 2006;6:741-750.

Production of Monoclonal Antibodies



http://www.immunosome.com/antibodies/polyclonal-and-monoclonal-antibodies/

Therapeutic Applications of Antibodies

Table 1

Antibody classification according to structure, with examples of products that are licensed or under development

mAb category	Suffix	Examples	Specificity	Reference
Chimeric	-ximab	Infliximab (Remicade®)	TNF-α	[59]
		Rituximab (Rituxan®, Mabthera®)	CD20	[60]
Humanised	-zumab	Alemtuzumab (MabCampath®)	CD52	[18]
		Tocilizumab (RoActemra®)	IL-6R	[61]
		Ocrelizumab	CD20	[62]
		Epratuzumab	CD22	[63]
		Certolizumab pegol (PEGylated Fab fragment) (Cimzia®)	TNF-α	[64]
		Otelixizumab (Aglycosyl)	CD3	[42]
		Teplizumab (Fc-mutated)	CD3	[65]
		Visilizumab (Fc-mutated)	CD3	[44]
'Fully human'	-mumab	Adalimumab (Humira®)	TNF-α	[66]
		Ofatumumab (Humax-CD20®)	CD20	[67]
		Belimumab (LymphoStat-B®)	BLyS	[68]
		Golimumab	TNF-α	[69]
Fusion proteins	-cept	Etanercept (Enbrel®)	TNF-α	[70]
		Abatacept (Orencia®)	CD80/CD86	[71]
		Atacicept	BLyS/BAFF	[72]

BAFF, B-cell activating factor; BLyS, B-lymphocyte stimulator; Fab, fragment antigen-binding; Fc, fragment crystallisable; mAb, monoclonal antibody; TNF-α, tumour necrosis factor-alpha.

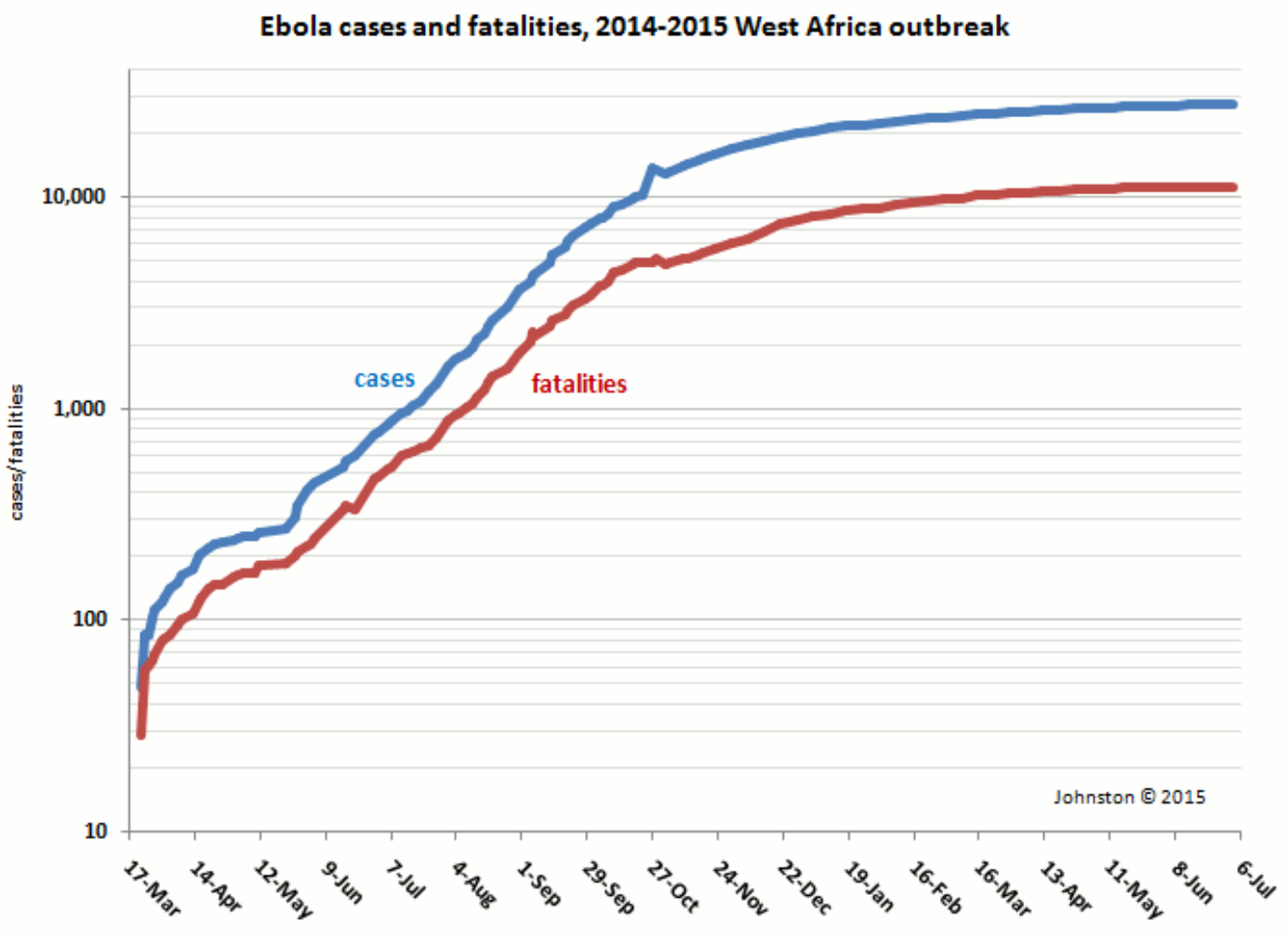
Isaacs *Arthritis Research & Therapy* 2009 **11**:225 doi:10.1186/ar2594

IgG Infusion

Intravenous Immunoglobulin (**IVIG**):
IgG antibodies extracted from the plasma of over one thousand blood donors.

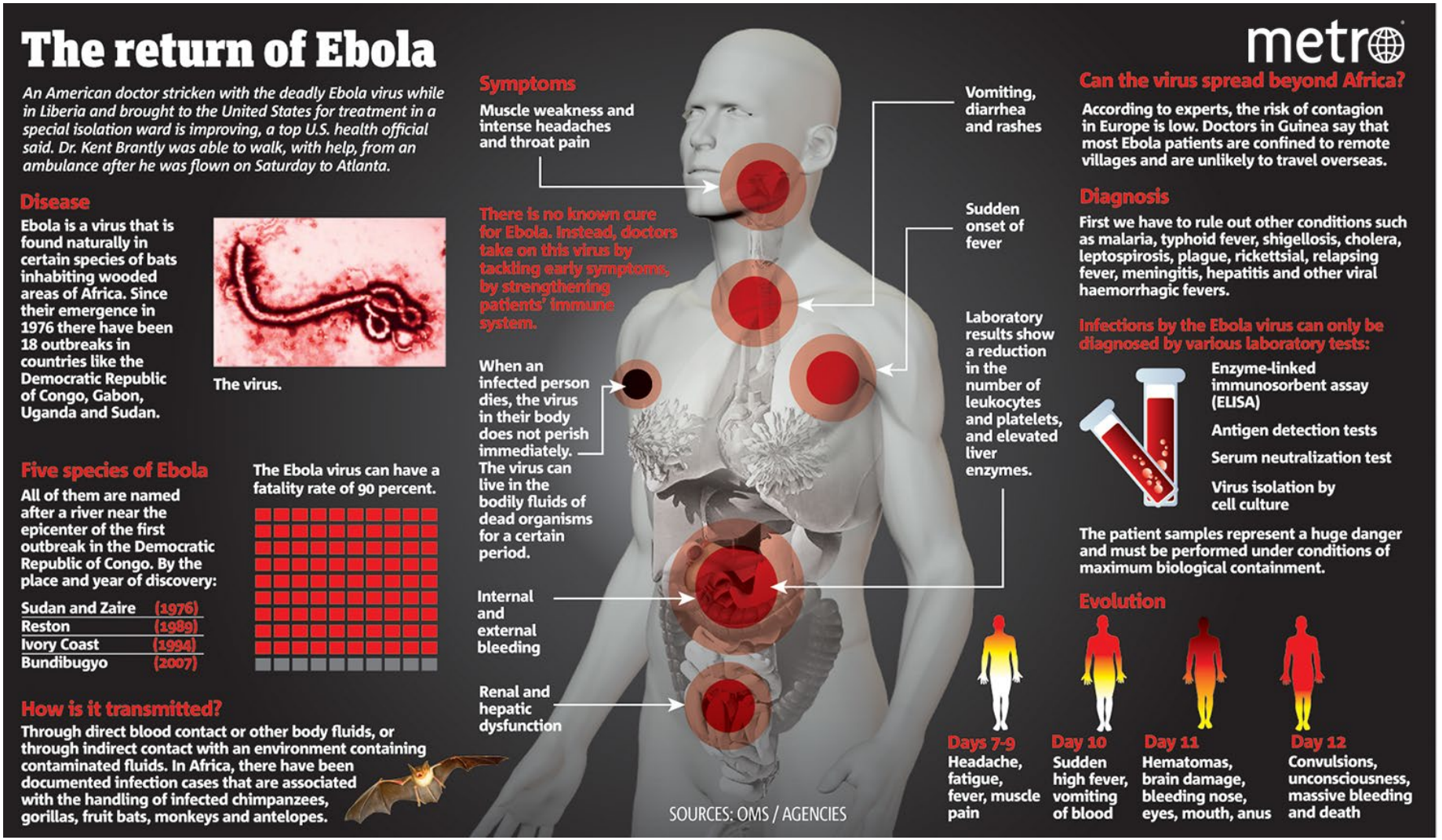
- Primary Immune deficiencies
- Acquired compromised immunity conditions
- Autoimmune diseases
- Acute infections

2014 The return of Ebola



http://www.johnstonsarchive.net/policy/westafrica-ebola.html

Ebola



http://gamisbaru.com/article/what-is-ebola-symptoms

Passive Transfer of Ebola Antibody

Volume 21, Number 3—March 2015

Dispatch

Treatment of Ebola Virus Infection with Antibodies from Reconvalescent Donors

Thomas R. Kreil✉

Author affiliation: Global Pathogen Safety, Baxter BioScience, Vienna, Austria

Suggested citation for this article

Abstract

Clinical evidence suggests that antibodies from reconvalescent donors (persons who have recovered from infection) may be effective in the treatment of Ebola virus infection. Administration of this treatment to Ebola virus–infected patients while preventing the transmission of other pathogenic viruses may be best accomplished by use of virus-inactivated reconvalescent plasma.

On This Page

Dispatch

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PDF [313 KB - 3 pgs]

Science 08/16/2019

but "we are not yet where we need to be."

Ovabrite, a U.S company in Austin, is chasing a technique that would leave the eggshell intact and sort eggs before incubation. Mass spectrometers would capture and analyze sex-specific volatile molecules that leak through the eggshell. Scientists suspect the molecules, first discovered in quail eggs, may allow parent birds to smell clues about an embryo's development and sex. But it is still a challenge to reliably detect such a faint signal from preincubation eggs, which must be refrigerated, says Ovabrite President Jonathan Hoopes.

Some predict that genetic engineering could help do away with complicated robots. Groups in Australia and Israel have used the CRISPR gene-editing technique to modify hens' sex chromosomes so that their sons carry a marker gene that makes male eggs glow under fluorescent light. That would allow hatcheries to sort out the fluorescent male eggs with a simple detector. Finding a marker that produces a strong enough signal in early embryos is a challenge, says Yehuda Elram, CEO of eggXYt (pronounced "exit") in Jerusalem. He says eggXYt has found a solution, but declined to say whether it is close to hatchery tests.

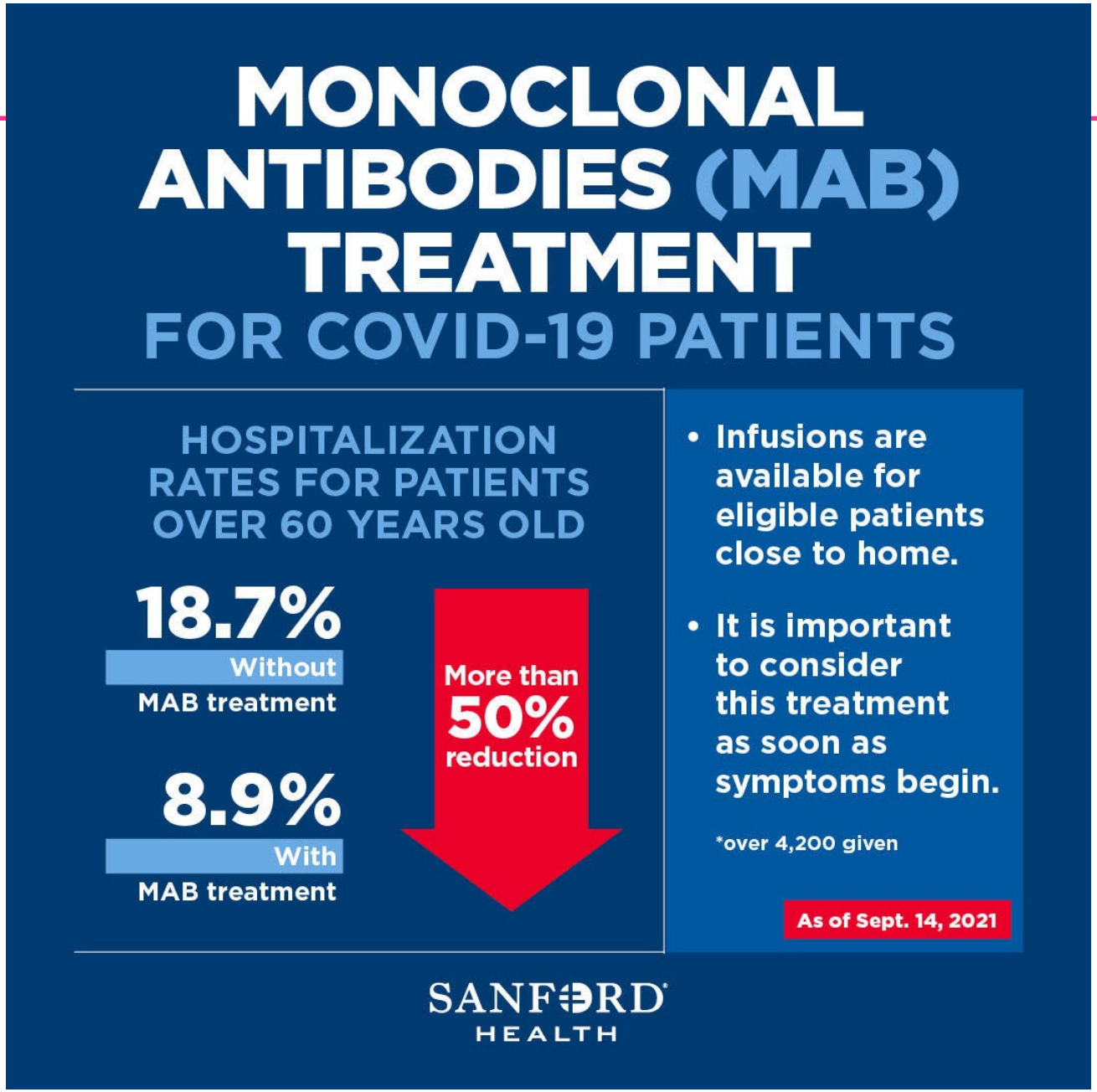
Public opposition to genetic modification in Europe means the approach is unlikely to catch on there. But Mark Tizard, a geneticist at the Australian Animal Health

A health worker puts on protective gear at an Ebola treatment center in Beni, Democratic Republic of the Congo.

INFECTIOUS DISEASES

Successful Ebola treatments promise to tame outbreak

Antibody preparations that cut the death rate dramatically will become available to all patients in Congo

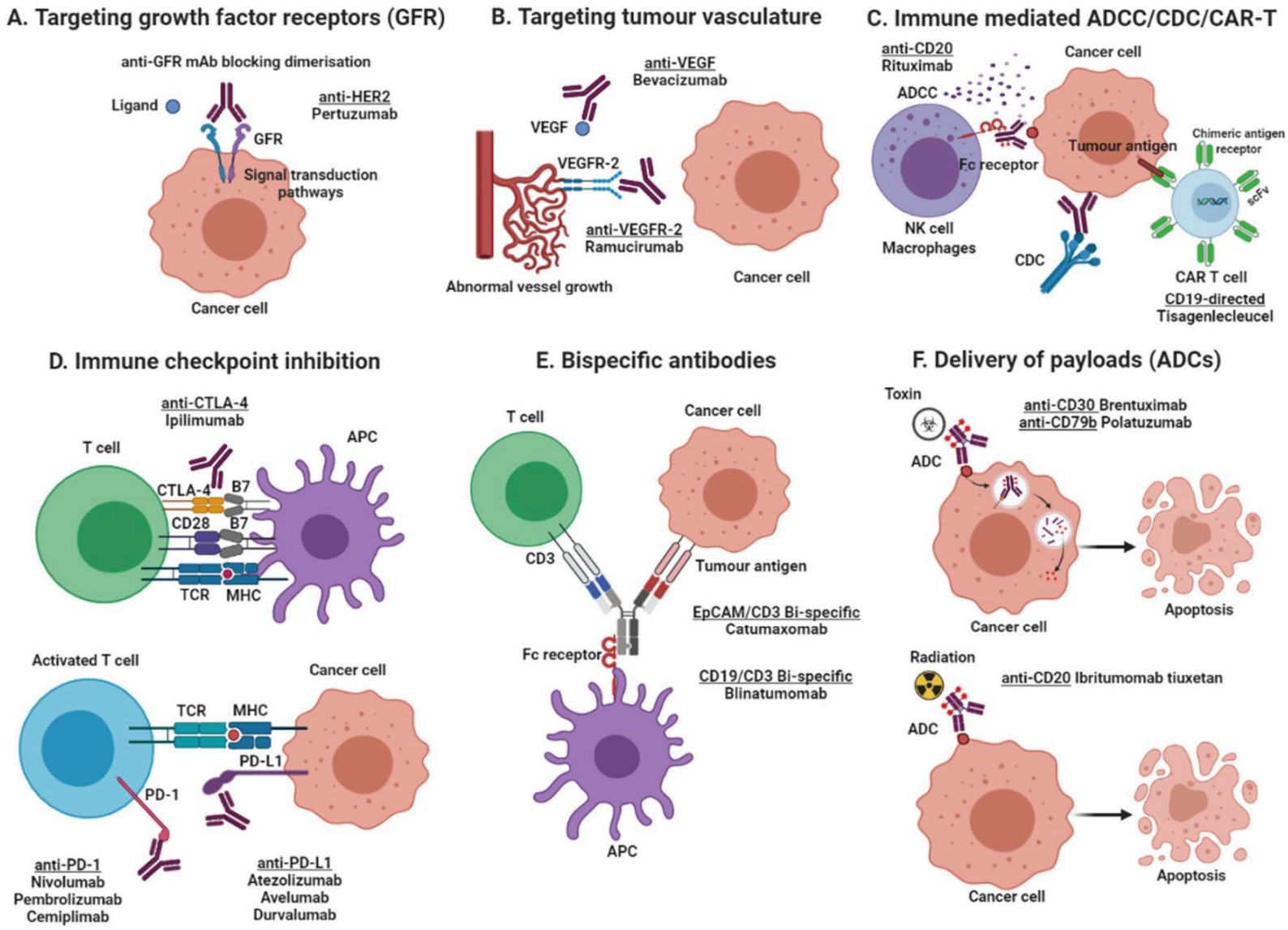

MONOCLONAL ANTIBODIES (MAB) TREATMENT
FOR COVID-19 PATIENTS
HOSPITALIZATION RATES FOR PATIENTS OVER 60 YEARS OLD
18.7%
Without MAB treatment
8.9%
With MAB treatment
More than 50% reduction
• Infusions are available for eligible patients close to home.
• It is important to consider this treatment as soon as symptoms begin.
*over 4,200 given
As of Sept. 14, 2021
SANFORD HEALTH

Antiserum-passive Immunity

Diluted venom is used to produce antibodies in animals

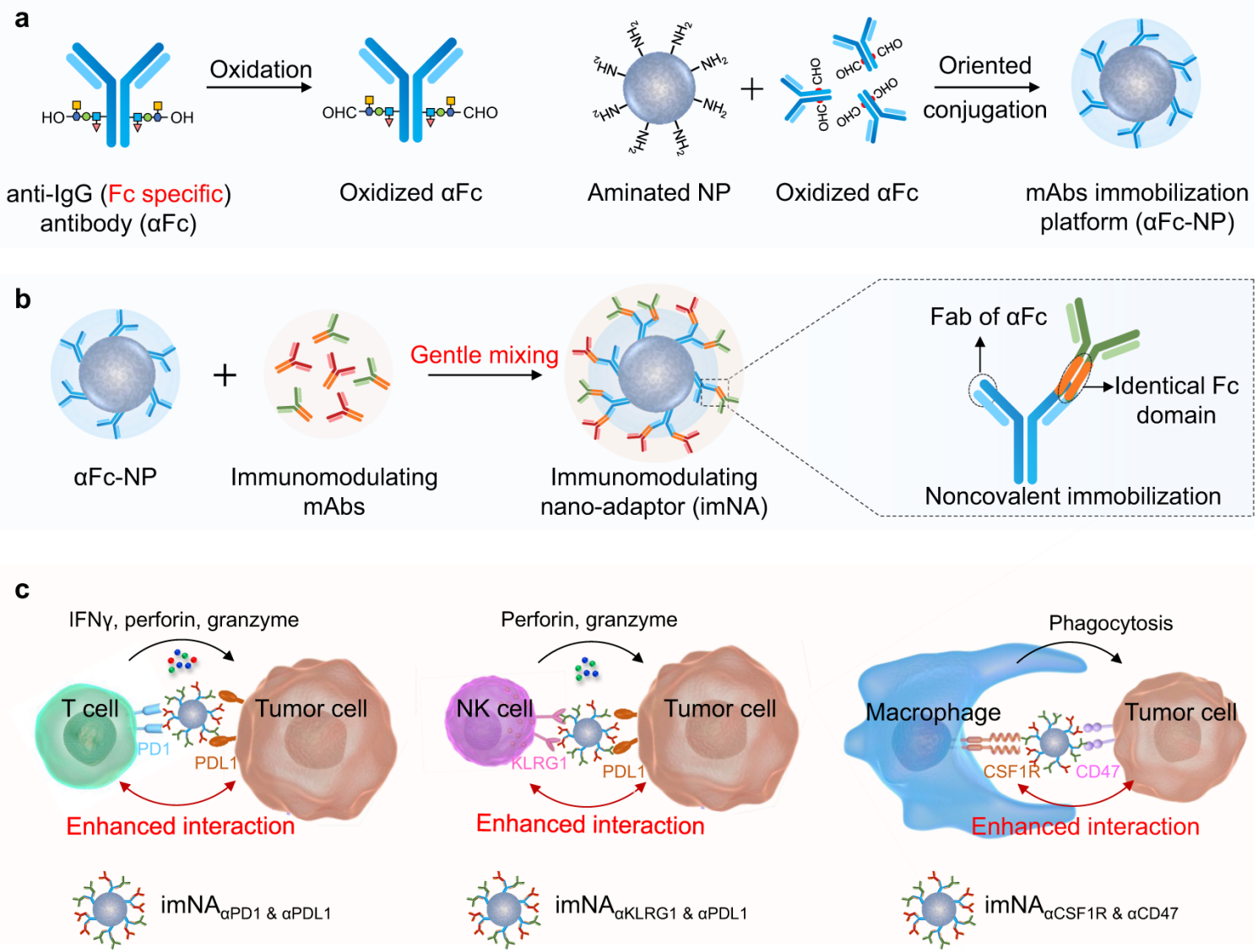
http://en.wikipedia.org/wiki/Antivenom#/media/File:Snake_Milking.jpg

Therapeutic Application of Monoclonal Antibodies



https://www.mdpi.com/2072-6694/13/8/1781/htm

Antibody-Based Cancer Immunotherapy



https://www.nature.com/articles/s41467-021-21497-6

Other Applications: Flow Cytometry

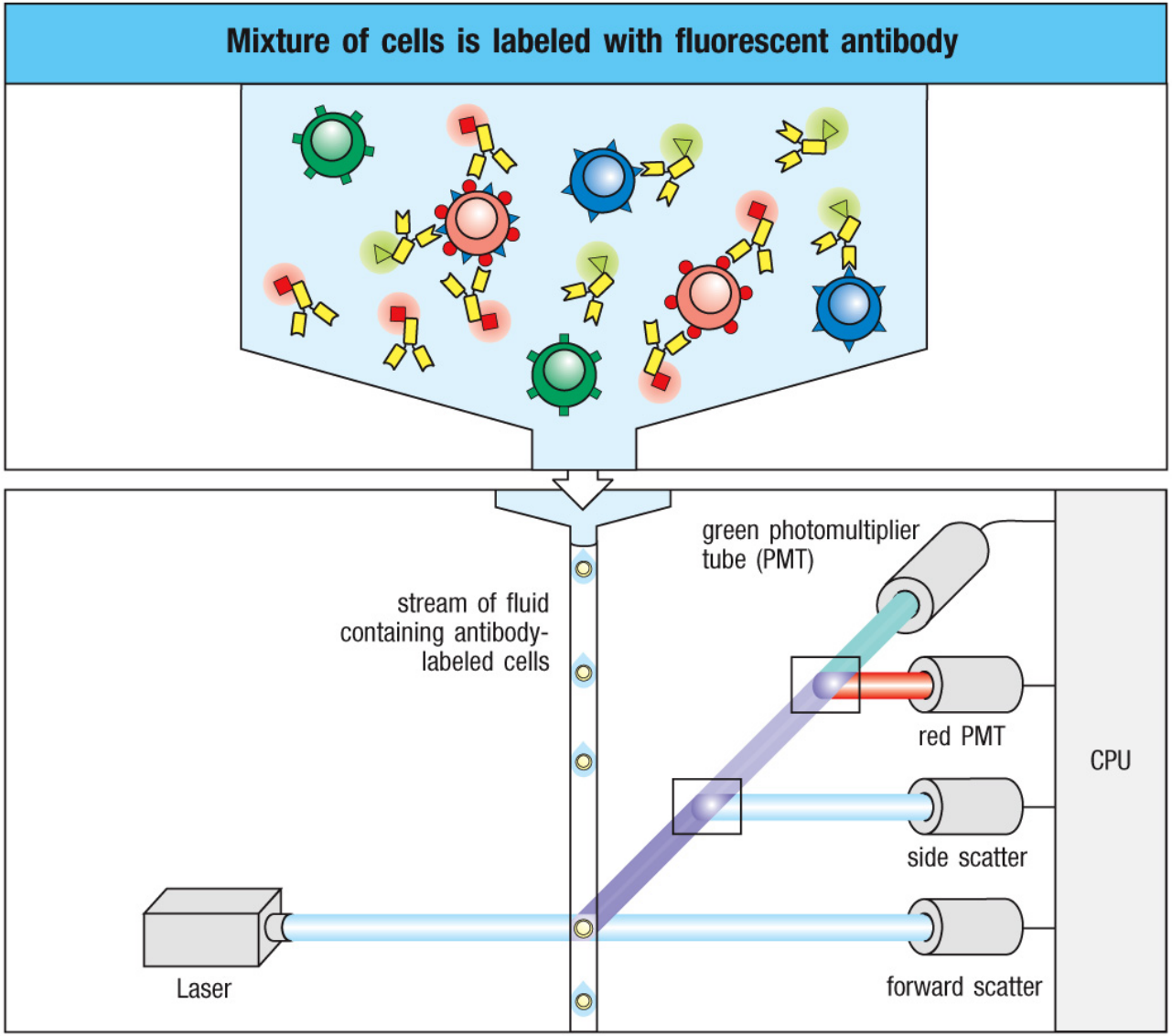


Figure A.21 (part 1 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Surface IgM and IgD

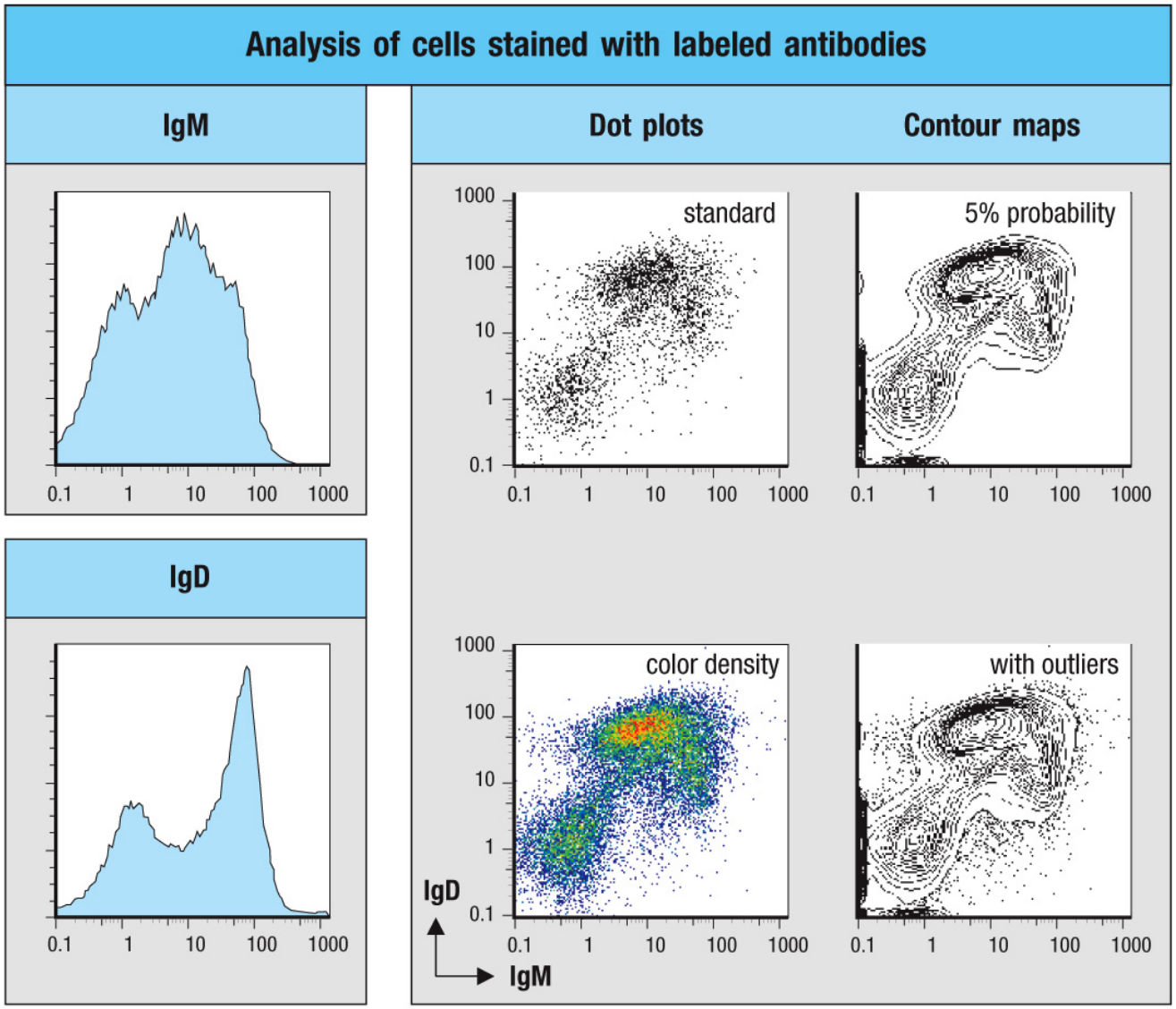
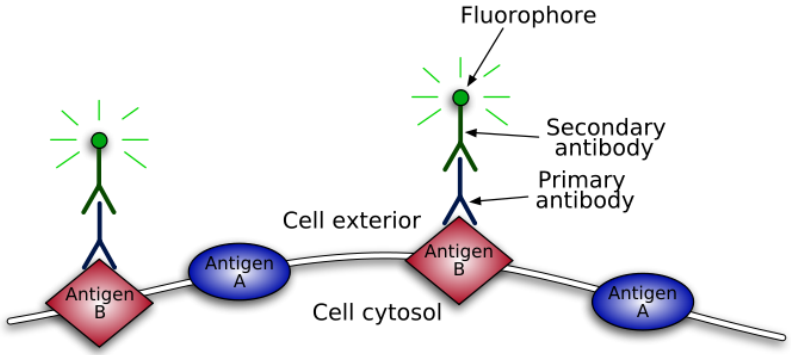
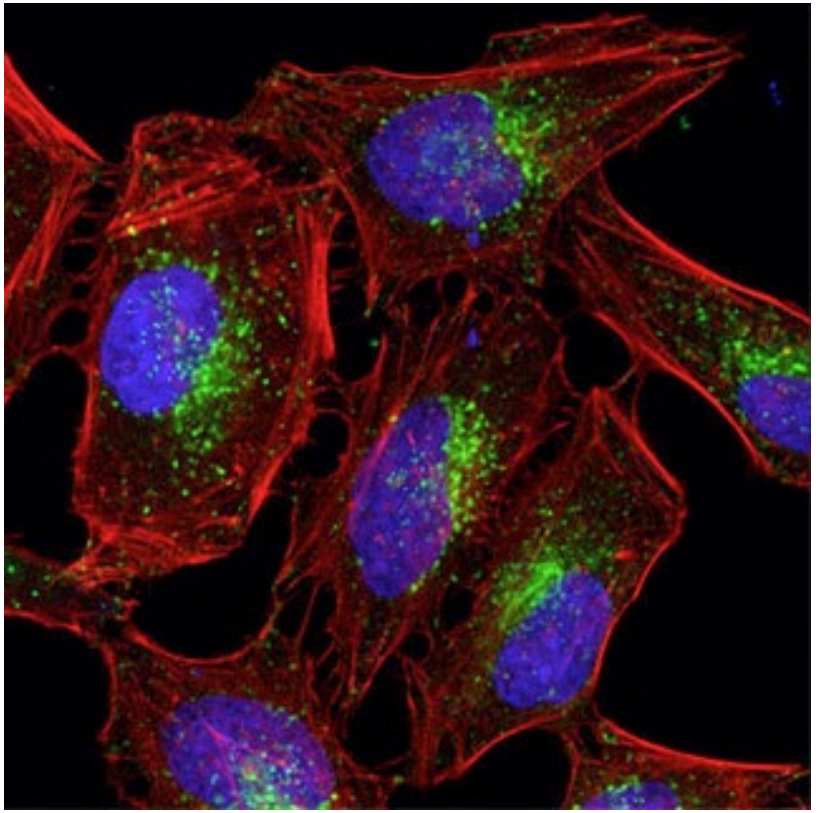


Figure A.21 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

ImmunoFluoresence





http://php.med.unsw.edu.au/cellbiology/index.php?title=File:Primary-secondary_antibody.png

http://www.cellsignal.com/common/content/content.jsp?id=apps-immunofluorescence

Question

- Is it a good idea to isolate breast cancer cells from a patient and develop that into a vaccine to protect a general population?
- A) Yes
- B) No