Antibody Structure and Function

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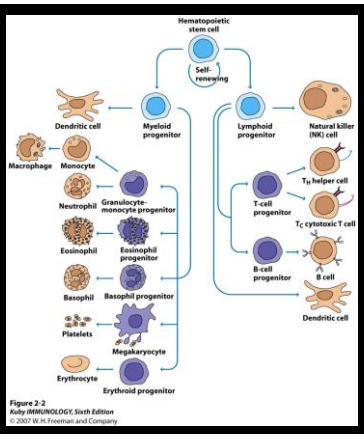
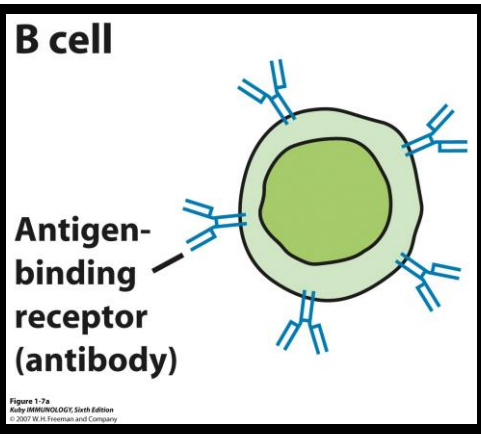
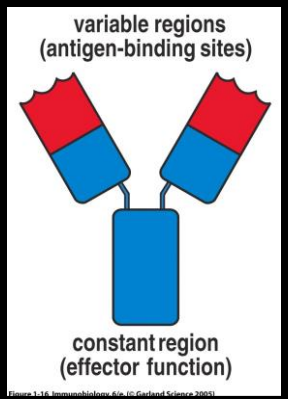
Hematopoiesis

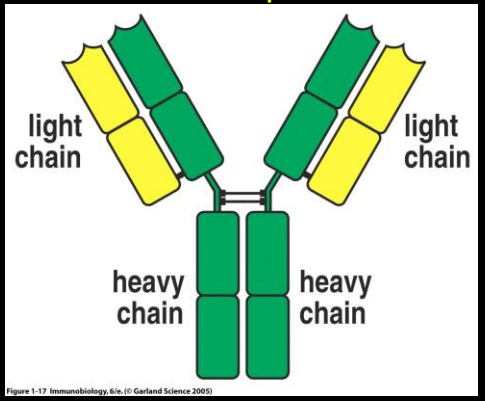




Schematic Structure of an Antibody Molecule

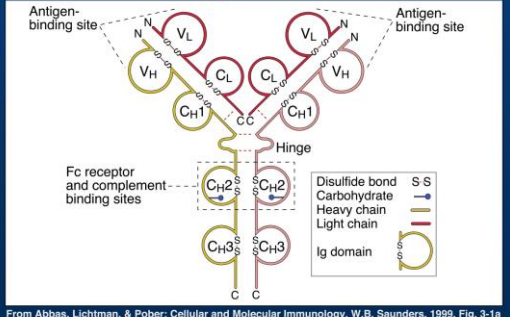


Antibodies are made up of Four Chains

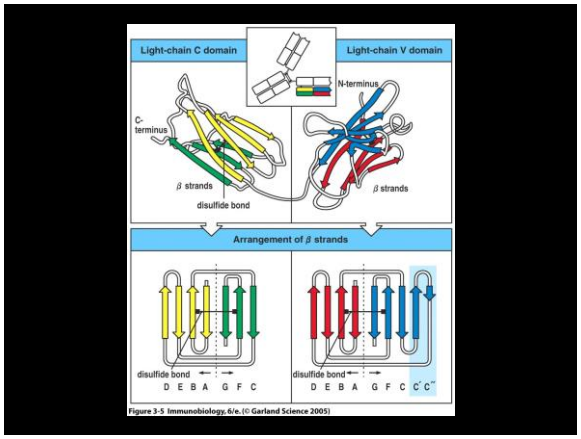


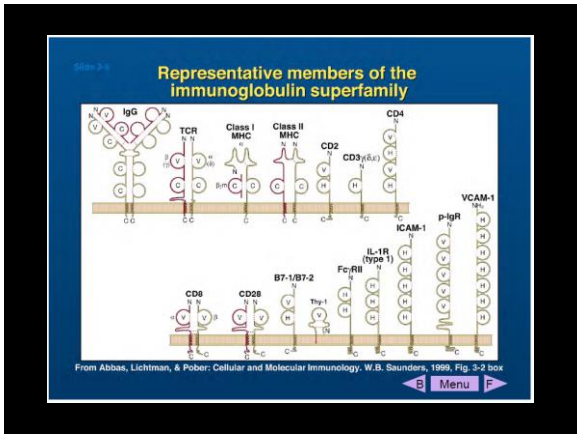
Slide 3-1

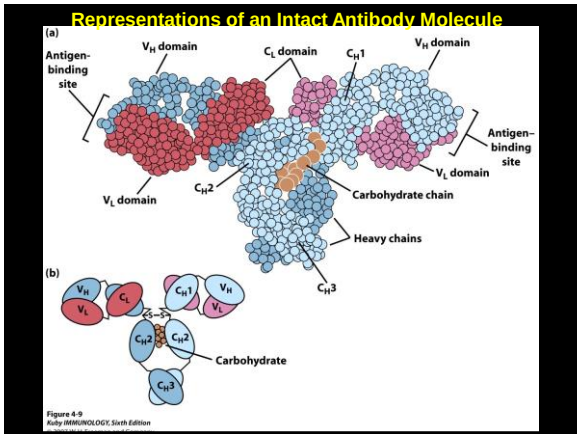
Immunoglobulin structure



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 3-1a


Light-chain C domain
Light-chain V domain
N-terminus
C-terminus
β strands
β strands
disulfide bond
Arrangement of β strands
disulfide bond
D E B A G F C
disulfide bond
D E B A G F C C′ C″
Figure 3-5 Immunobiology, 6/e. (© Garland Science 2005)


Representative members of the immunoglobulin superfamily
IgG
TCR
Class I MHC
Class II MHC
CD2
CD3
CD4
VCAM-1
p-IgR
ICAM-1
IL-1R (type 1)
FcγRII
B7-1/B7-2
CD8
CD28
Thy-1
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 3-2 box
B
Menu
F


Representations of an Intact Antibody Molecule
(a)
Antigen-binding site
VH domain
CL domain
CH1
VH domain
Antigen-binding site
VL domain
CH2
VL domain
Carbohydrate chain
Heavy chains
CH3
(b)
VH
CL
CH1
VH
VL
VL
CH2
CH2
Carbohydrate
CH3
Figure 4-9
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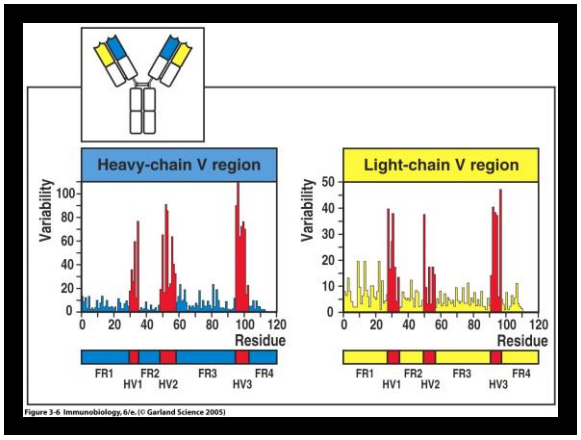


Figure 3-6 Immunobiology, 6/e. (© Garland Science 2005)

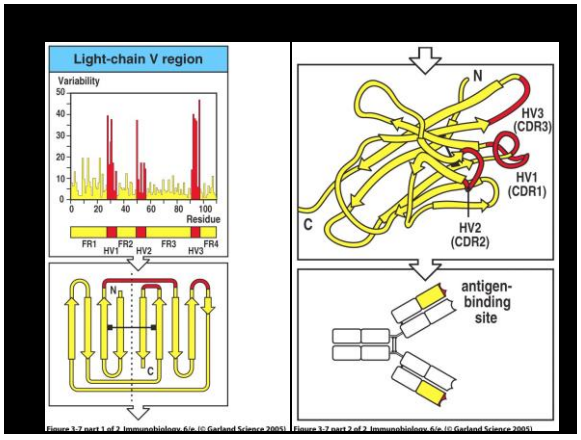
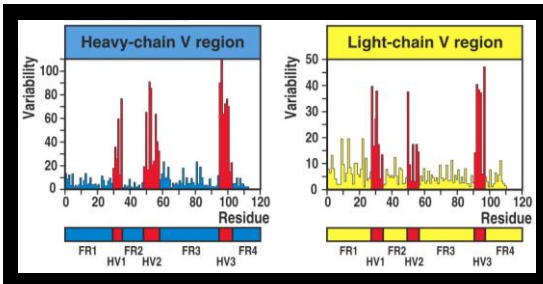


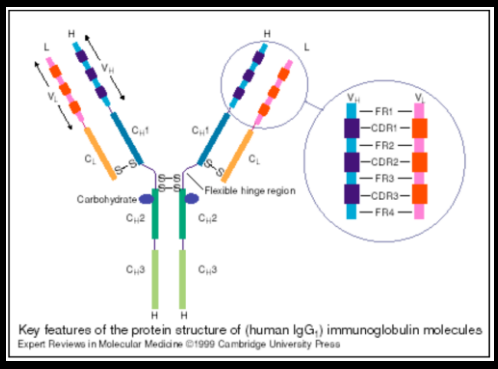
Figure 3-7 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005) Figure 3-7 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)



Variability = $\frac{\text{number of different amino acids at a given position}}{\text{frequency of the most common amino acid at given position}}$

e.g. Suppose Ser, Asp, Pro and Thr were the only a. acids found in a given location and that Ser occurred at that location 80 out of 100 times.

Then variability: $\frac{4}{0.8}$ =5



Key features of the protein structure of (human IgG_1) immunoglobulin molecules

Expert Reviews in Molecular Medicine ©1999 Cambridge University Press

Computer simulation of an antibody and influenza virus antigen

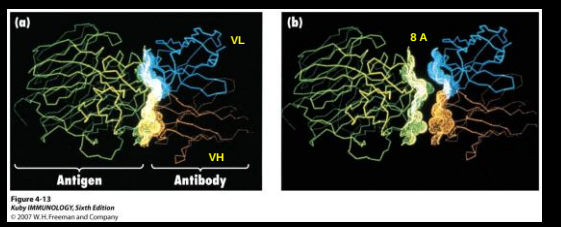


Figure 4-13
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The T cell receptor resembles a Fab fragment of an antibody molecule

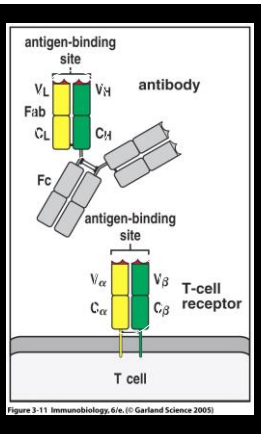

Figure 3-11 Immunobiology, 6/e. (© Garland Science 2005)

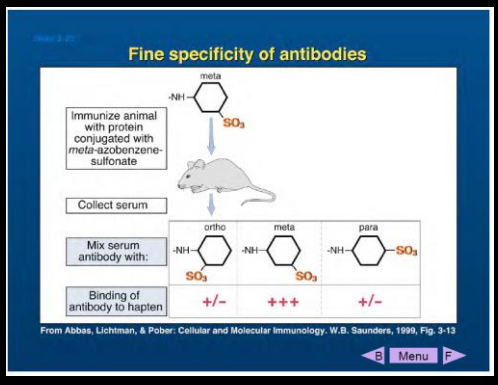

TABLE 5-1 Chromosomal locations of immunoglobulin genes in human and mouse

Gene	CHROMOSOME	
	Human	Mouse
λ Light chain	22	16
κ Light chain	2	6
Heavy chain	14	12

Table 5-1
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MAN

Ig Classes (Isotypes)		Subclasses
IgG	γ	G1 G2 G3 G4
IgM	μ	
IgE	ε	
IgA	α	A1 A2
IgD	δ	

MOUSE

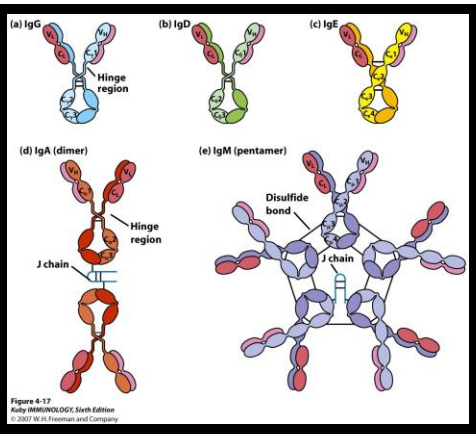
IgG	G1 G2a G2b G3
IgM	
IgA	
IGE	
IgD	

All light chains have 2 Isotypes :kappa and lambda

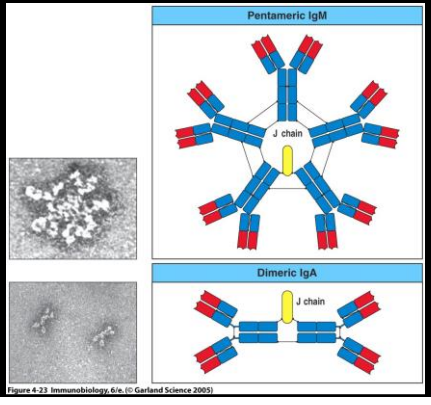
In man the k:λ ratio is 2:1

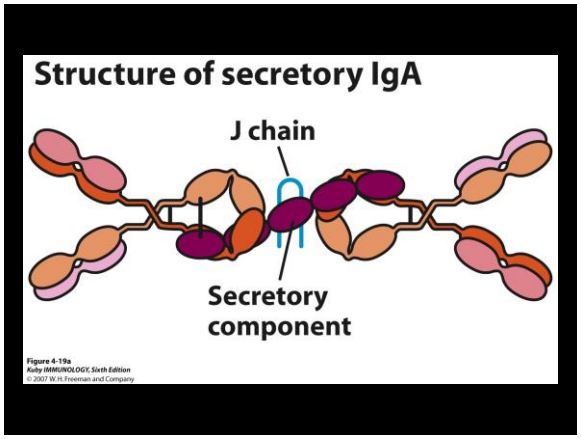
In mice: 20:1

In cattle: 1:20



IgM and IgA molecules can form multimers




Structure of secretory IgA
J chain
Secretory component
Figure 4-19a
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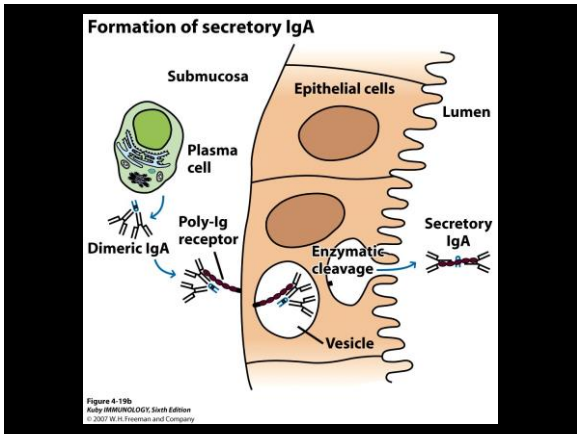
Formation of secretory IgA
Submucosa
Epithelial cells
Lumen
Plasma cell
Poly-Ig receptor
Dimeric IgA
Enzymatic cleavage
Secretory IgA
Vesicle
Figure 4-19b
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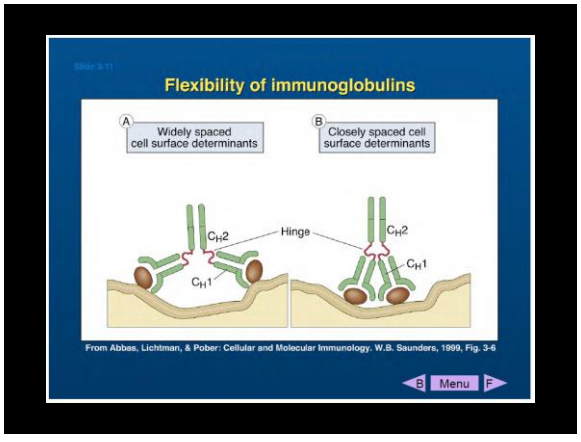

Flexibility of immunoglobulins
A
Widely spaced cell surface determinants
B
Closely spaced cell surface determinants
C_H2
Hinge
C_H1
C_H2
C_H1
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 3-6
B
Menu
F

Antibody arms are joined by a flexible hinge

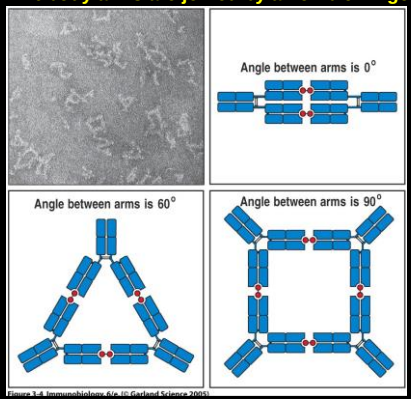


Figure 3-4 Immunobiology, 6/e. (© Garland Science 2005)

	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 weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg ml^{-1})	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
Classical pathway of complement activation	++	+	+++	–	+++	–	–	–	–
Alternative pathway of complement activation	–	–	–	–	–	+	–	–	–
Placental transfer	+++	+	++	–/+	–	–	–	–	–
Binding to macrophage and phagocyte Fc receptors	+	–	+	–/+	–	+	+	–	+
High-affinity binding to mast cells and basophils	–	–	–	–	–	–	–	–	+++
Reactivity with staphylococcal Protein A	+	+	–/+	+	–	–	–	–	–

Figure 4-17 Immunobiology, 6/e. (© Garland Science 2005)

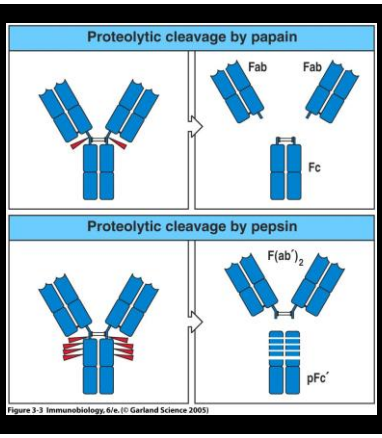
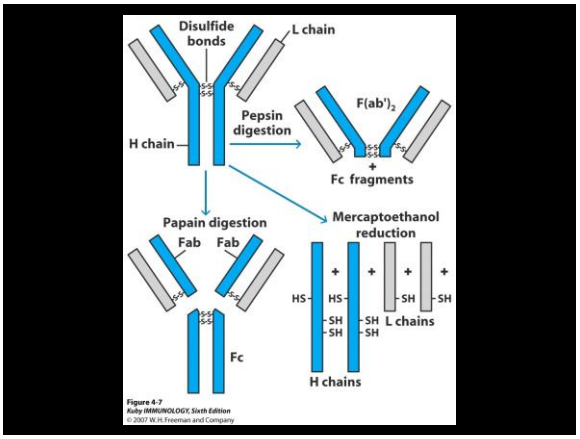


Figure 3-3 Immunobiology, 6/e. (© Garland Science 2005)


Disulfide bonds
L chain
H chain
Pepsin digestion
$F(ab')_2$
Fc fragments
Papain digestion
Fab
Fab
Fc
Mercaptoethanol reduction
HS
SH
L chains
H chains
Figure 4-7
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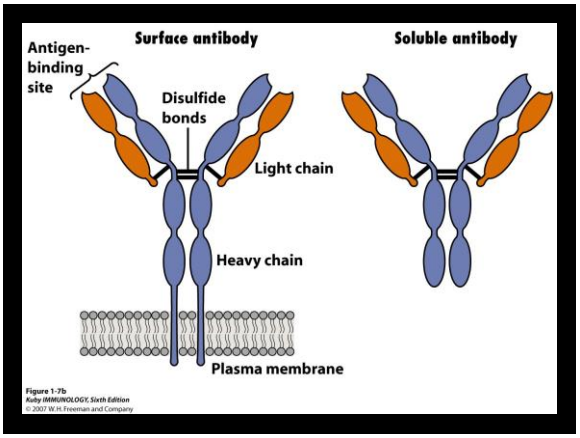
Surface antibody
Soluble antibody
Antigen-binding site
Disulfide bonds
Light chain
Heavy chain
Plasma membrane
Figure 1-7b
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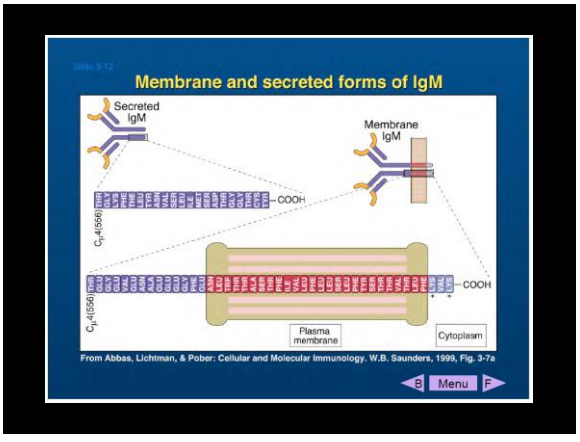
Membrane and secreted forms of IgM
Secreted IgM
Membrane IgM
COOH
C_H4 (556)
C_H4 (556)
COOH
Plasma membrane
Cytoplasm
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 3-7a
B
Menu
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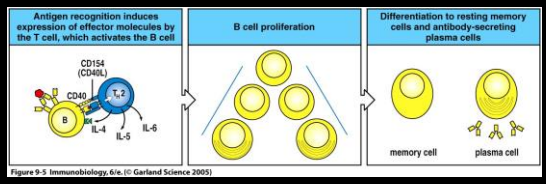


Figure 9-5 Immunobiology, 6/e. (© Garland Science 2005)

B Cells Originate from a Lymphoid Progenitor in the Bone Marrow

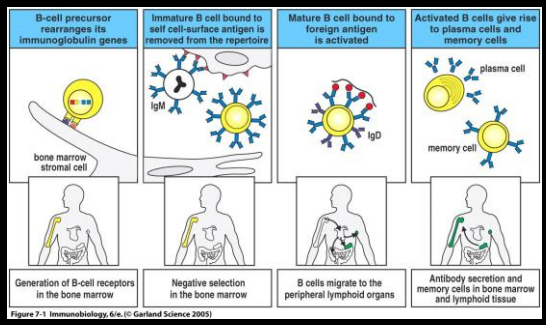


Figure 7-1 Immunobiology, 6/e. (© Garland Science 2005)

The B cell receptor complex

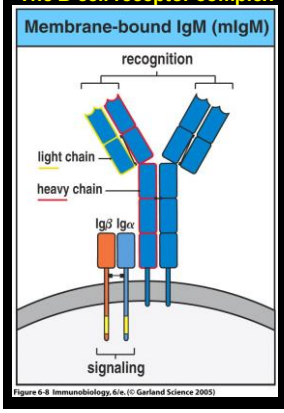


Figure 6-8 Immunobiology, 6/e. (© Garland Science 2005)

The B cell receptor complex is made up of cell-surface immunoglobulin and invariant proteins Ig α and Ig β.

The Ig α and Ig β each have a single **immunoreceptor tyrosine based activation motif (ITAM) in their cytoplasmic tails** that enables them to signal when the B cell-receptor binds antigen.

Canonical ITAM sequence is:

YXX [L/I] X_{6-9}YXX [L/I]

Clustering of antigen receptors and phosphorylation of ITAMs by receptor associated Src-family tyrosine kinases Blk,Fyn or Lyn.

Once ITAMs are phosphorylated they attract the protein tyrosine kinase Syk. Until Syk is bound to the phosphorylated ITAMs it is enzymatically inactive. To become active it itself must become phosphorylated—thought to occur by transphosphorylation mediated by Syk itself or Src kinases.

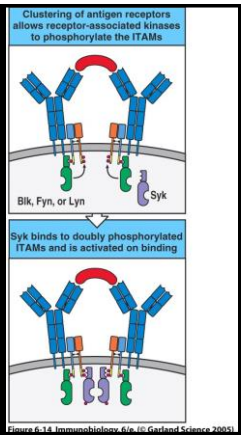


Figure 6-14 Immunobiology, 6/e. (© Garland Science 2005)

Role of cytokines in regulating Ig isotype expression

Cytokines	IgM	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN-γ	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF-β	Inhibits	Inhibits		Induces			Induces

Figure 9-7 Immunobiology, 6/e. (© Garland Science 2005)

Antibodies participate in host defense

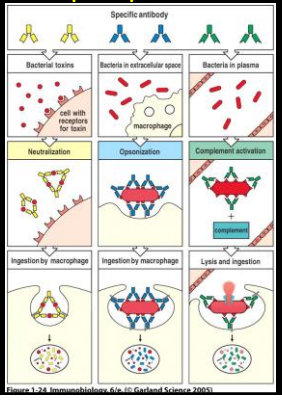


Figure 1-24 Immunobiology, 6/e. (© Garland Science 2005)

Neutralization of toxins by IgG antibody protects cells

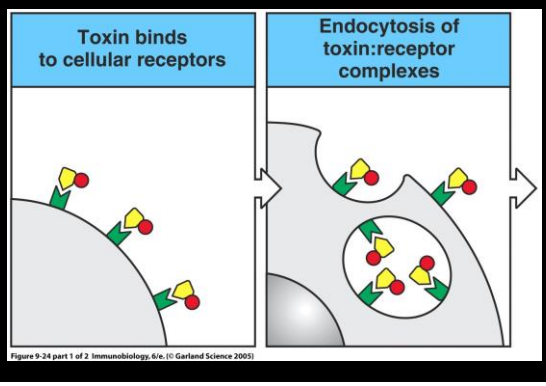


Figure 9-24 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

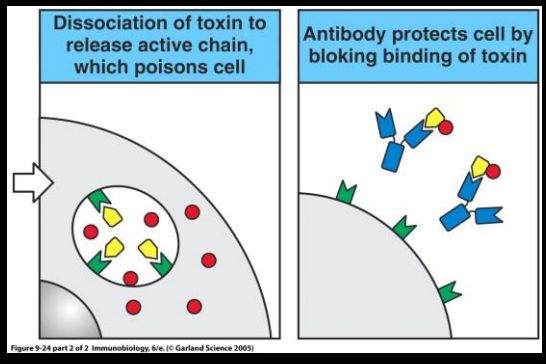


Figure 9-24 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Disease	Organism	Toxin	Effects *in vivo*
Tetanus	*Clostridium tetani*	Tetanus toxin	Blocks inhibitory neuron action leading to chronic muscle contraction
Diphtheria	*Corynebacterium diptheriae*	Diphtheria toxin	Inhibits protein synthesis leading to epithelial-cell damage, and myocarditis
Gas Gangrene	*Clostridium perfringens*	Clostridial-α toxin	Phospholipase leading to cell death
Cholera	*Vibrio cholerae*	Cholera toxin	Activates adenylate cyclase, elevates cAMP in cells, leading to changes in intestinal epithelial cells that cause loss of water and electrolytes
Anthrax	*Bacillus anthracis*	Anthrax toxic complex	Increases vascular permeability leading to edema, hemorrhage and circulatory collapse
Botulism	*Clostridium botulinum*	Botulinus toxin	Blocks release of acetylcholine leading to paralysis
Whooping cough	*Bordetella pertussis*	Pertussis toxin	ADP-ribosylation of G proteins leading to lymphocytosis
		Tracheal cytotoxin	Inhibits cilia and causes epithelial-cell loss
Scarlet fever	*Streptococcus pyogenes*	Erythrogenic toxin	Vasodilation leading to scarlet-fever rash
		Leukocidin streptolysins	Kills 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)

The ingestion of particulate matter is called phagocytosis.

The coating of an organism by molecules that facilitate its uptake and destruction by phagocytes is called opsonization

Fc and complement receptors on phagocytes trigger the uptake and degradation of antibody coated bacteria

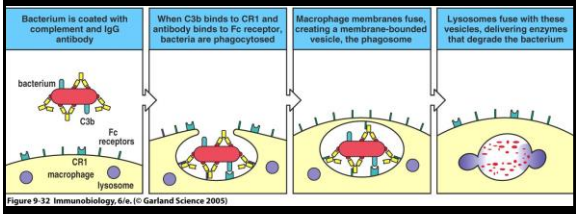


Figure 9-32 Immunobiology, 6/e. (© Garland Science 2005)

Antibodies can prevent the attachment of bacteria to cell surfaces

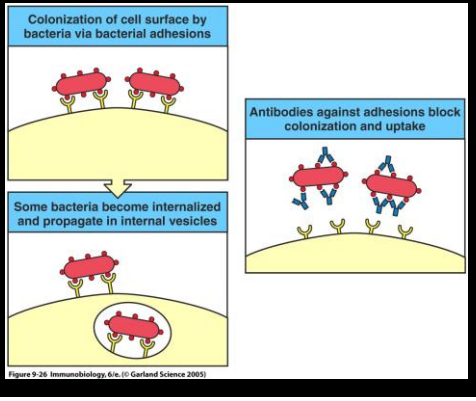


Figure 9-26 Immunobiology, 6/e. (© Garland Science 2005)

Viral infection of cells can be blocked by neutralizing antibodies

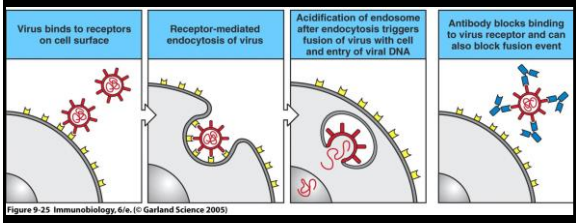


Figure 9-25 Immunobiology, 6/e. (© Garland Science 2005)

Antibody coated target cells can be killed by NK cells via ADCC

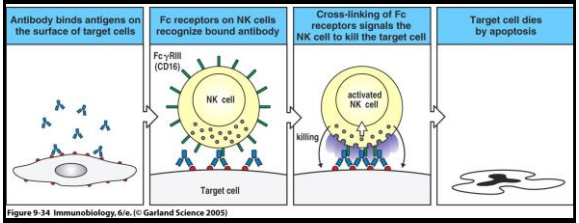
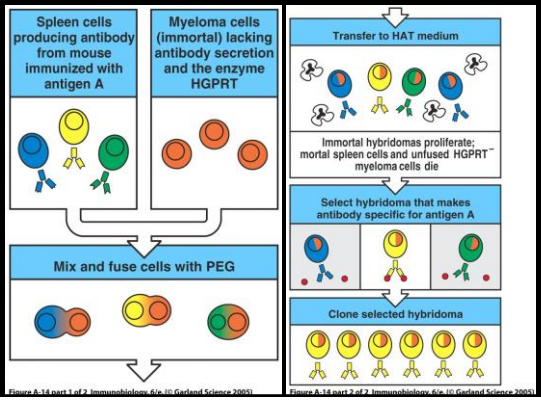
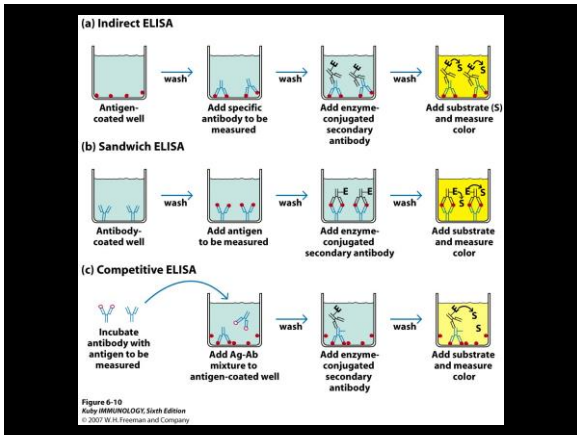


Figure 9-34 Immunobiology, 6/e. (© Garland Science 2005)

Generation of monoclonal antibodies




(a) Indirect ELISA
Antigen-coated well
wash
Add specific antibody to be measured
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate (S) and measure color
(b) Sandwich ELISA
Antibody-coated well
wash
Add antigen to be measured
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate and measure color
(c) Competitive ELISA
Incubate antibody with antigen to be measured
Add Ag-Ab mixture to antigen-coated well
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate and measure color
Figure 6-10
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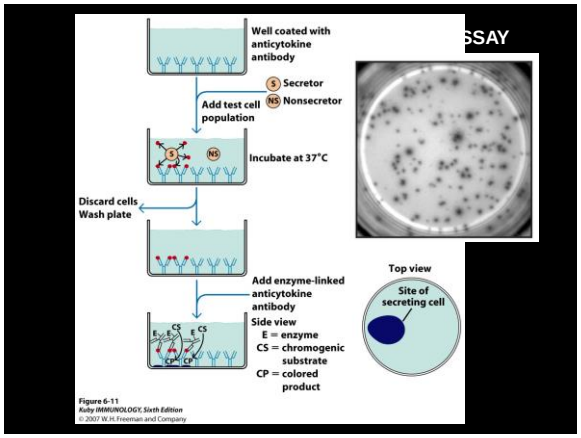
Well coated with anticytokine antibody
S Secretor
NS Nonsecretor
Add test cell population
Incubate at 37°C
Discard cells
Wash plate
Add enzyme-linked anticytokine antibody
Side view
E = enzyme
CS = chromogenic substrate
CP = colored product
Top view
Site of secreting cell
Figure 6-11
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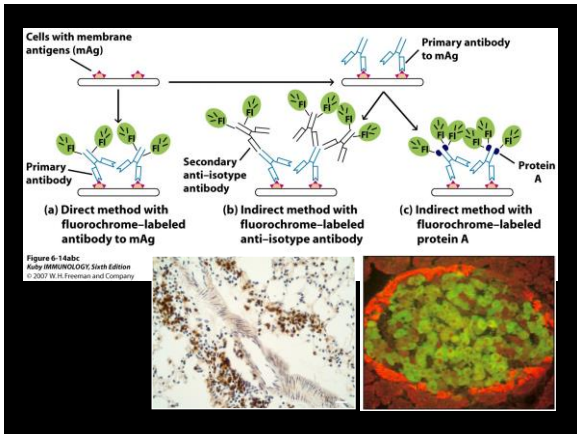
Cells with membrane antigens (mAg)
Primary antibody to mAg
Primary antibody
Secondary anti-isotype antibody
Protein A
(a) Direct method with fluorochrome-labeled antibody to mAg
(b) Indirect method with fluorochrome-labeled anti-isotype antibody
(c) Indirect method with fluorochrome-labeled protein A
Figure 6-14abc
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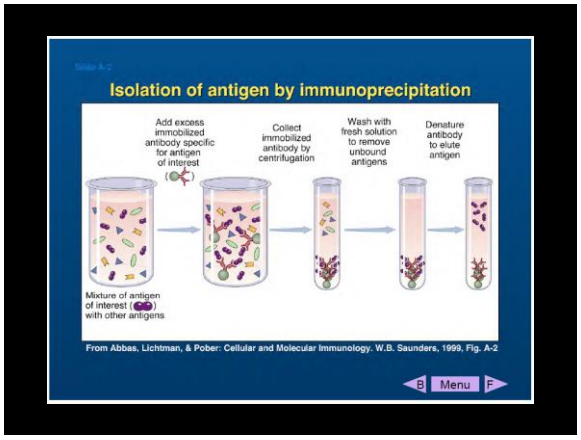
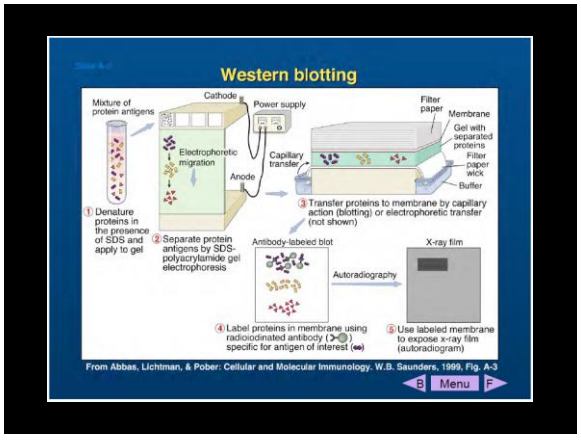
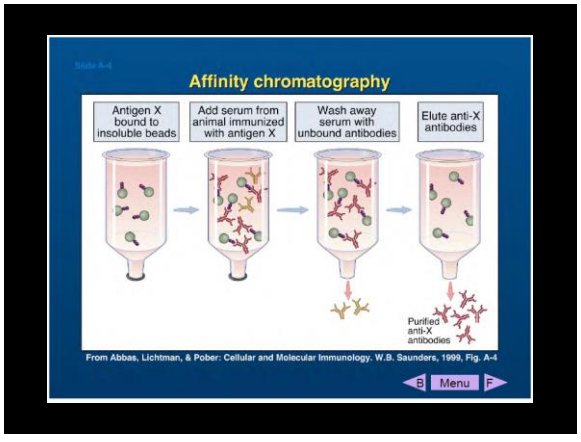
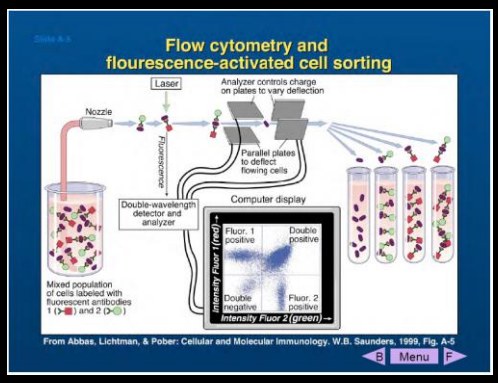
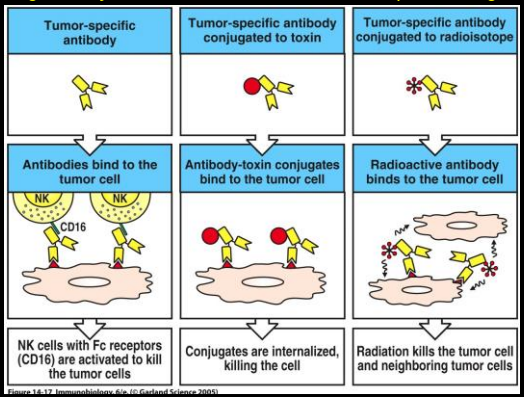

Isolation of antigen by immunoprecipitation
Add excess immobilized antibody specific for antigen of interest
Collect immobilized antibody by centrifugation
Wash with fresh solution to remove unbound antigens
Denature antibody to elute antigen
Mixture of antigen of interest ( ) with other antigens
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. A-2
B Menu F


Western blotting
Mixture of protein antigens
Cathode
Power supply
Filter paper
Membrane
Gel with separated proteins
Filter paper wick
Buffer
Electrophoretic migration
Capillary transfer
Anode
1 Denature proteins in the presence of SDS and apply to gel
2 Separate protein antigens by SDS-polyacrylamide gel electrophoresis
3 Transfer proteins to membrane by capillary action (blotting) or electrophoretic transfer (not shown)
Antibody-labeled blot
X-ray film
Autoradiography
4 Label proteins in membrane using radioiodinated antibody ( ) specific for antigen of interest ( )
5 Use labeled membrane to expose x-ray film (autoradiogram)
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. A-3
B Menu F


Affinity chromatography
Antigen X bound to insoluble beads
Add serum from animal immunized with antigen X
Wash away serum with unbound antibodies
Elute anti-X antibodies
Purified anti-X antibodies
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. A-4
B Menu F



Recognition by monoclonal antibodies of tumor-specific antigens



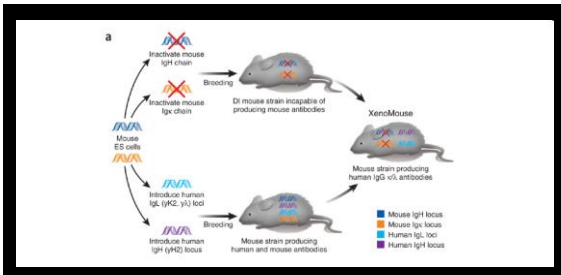
XenoMouseTM Technology (Abgenix Inc.)

A potentially rapid approach to developing therapeutic monoclonal antibodies for the treatment of cancer.

Series of strains of mice in which the endogenous murine immunoglobulin heavy chain and kappa light chain have been inactivated and the majority of the corresponding human immunoglobulin loci have been introduced as transgenes.

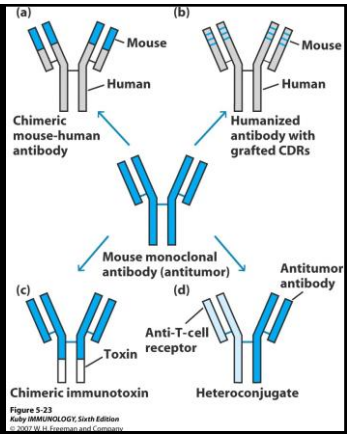
When antigenically challenged the XenoMice produce human rather than mouse antibodies. High affinity monoclonal antibodies can be generated offering the potential for rapid progress to clinical trials.

Abgenix's anti-EGFR is a fully human antibody which can inhibit many different tumors (growth and progression) in preclinical and clinical studies has received FDA approval for colorectal cancer.



Panitumumab represents the first fully human antibody developed from XenoMouse technology to be approved by a regulatory agency. This has been an important milestone in validating XenoMouse strains as well as other human immunoglobulin-producing mouse technologies as sources for therapeutic antibodies.
The path from initiation of XenoMouse technology development to regulatory approval took ~15 years, including 6 years for mouse strains derivation and mAb development and 6.5 years of clinical development.

Antibodies engineered by recombinant DNA technology



FDA Approved Monoclonal Antibodies

Antibody	Brand name	Type	Target	Approved Treatments
Abciximab	ReoPro	chimeric	inhibition of glycoprotein IIb/IIIa	cardiovascular disease
Adalimumab	Humira	human	inhibition of TNF-α signalling	inflammatory diseases
Alemtuzumab	Campath	humanized	CD52	chronic lymphocytic leukemia
Basiliximab	Simulect	chimeric	IL-2 receptor α	transplant rejection
Bevacizumab	Avastin	humanized	vascular endothelial growth factor	colorectal cancer
Cetuximab	Erbitux	chimeric	epidermal growth factor receptor	colorectal cancer
Daclizumab	Zenapax	humanized	IL-2 receptor α	transplant rejection
Eculizumab	Soliris	humanized	complement system protein C5	inflammatory diseases
Efalizumab	Raptiva	humanized	CD11a	inflammatory diseases (psoriasis)
Ibritumomab-tiuxetan	Zevalin	murine	CD20	Non-Hodgkin lymphoma
Infliximab	Remicade	chimeric	inhibition of TNF-α signaling	inflammatory diseases (auto-immune)
Muromonab-CD3	Orthoclone OKT3	murine	T cell CD3 receptor	transplant rejection
Natalizumab	Tysabri	humanized	T cell VLA4 receptor	multiple sclerosis
Omalizumab	Xolair	humanized	IgE	inflammatory diseases (asthma)
Palivizumab	Synagis	humanized	an epitope of the F protein of RSV	RSV infection
Panitumumab	Vectibix	human	epidermal growth factor receptor	colorectal cancer
Ranibizumab	Lucentis	humanized	vascular endothelial growth factor	macular degeneration
Gemtuzumab-ozogamicin	Mylotarg	humanized	CD33	acute myelogenous leukemia
Rituximab	Rituxan, Mabthera	chimeric	CD20	Non-Hodgkin lymphoma
Tositumomab	Bexxar	murine	CD20	Non-Hodgkin lymphoma
Trastuzumab	Herceptin	humanized	ErbB2/HER2/EGFR	breast cancer

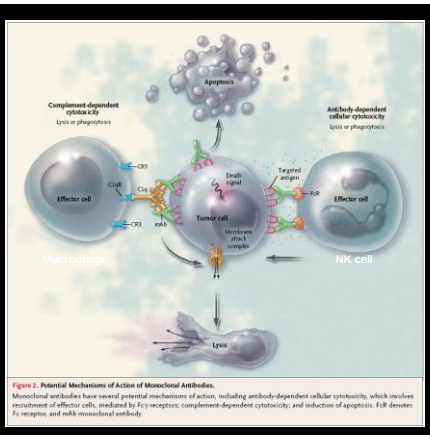
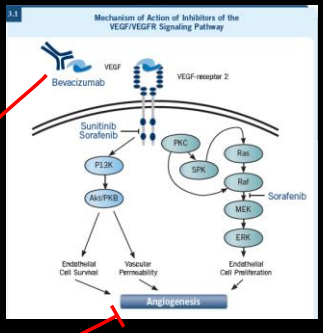


Figure 2. Potential Mechanisms of Action of Monoclonal Antibodies.
Monoclonal antibodies have several potential mechanisms of action, including antibody-dependent cellular cytotoxicity, which involves recruitment of effector cells, mediated by Fcγ receptors; complement-dependent cytotoxicity; and induction of apoptosis. FcR denotes Fc receptor, and mAb monoclonal antibody.

Rituximab also called Rituxan (IDEC Pharmaceuticals/Genentech) is the first monoclonal antibody approved for the treatment of cancer. This antibody is directed against CD20 molecule. It is effective as a single agent in patients with relapsed or refractory low grade or follicular non-Hodgkin's lymphoma.

It is also in use as combination therapy with chemotherapy, IFN-α2a and radioimmunotherapy (bound to beta emitting radioisotope 90 yttrium).



Herceptin (Genetech) is another monoclonal approved for human use. Herceptin (also called anti-Her2/neu) is against the Her2/neu oncogene which encodes a protein tyrosine kinase which is overexpressed in about 30% of breast cancers.