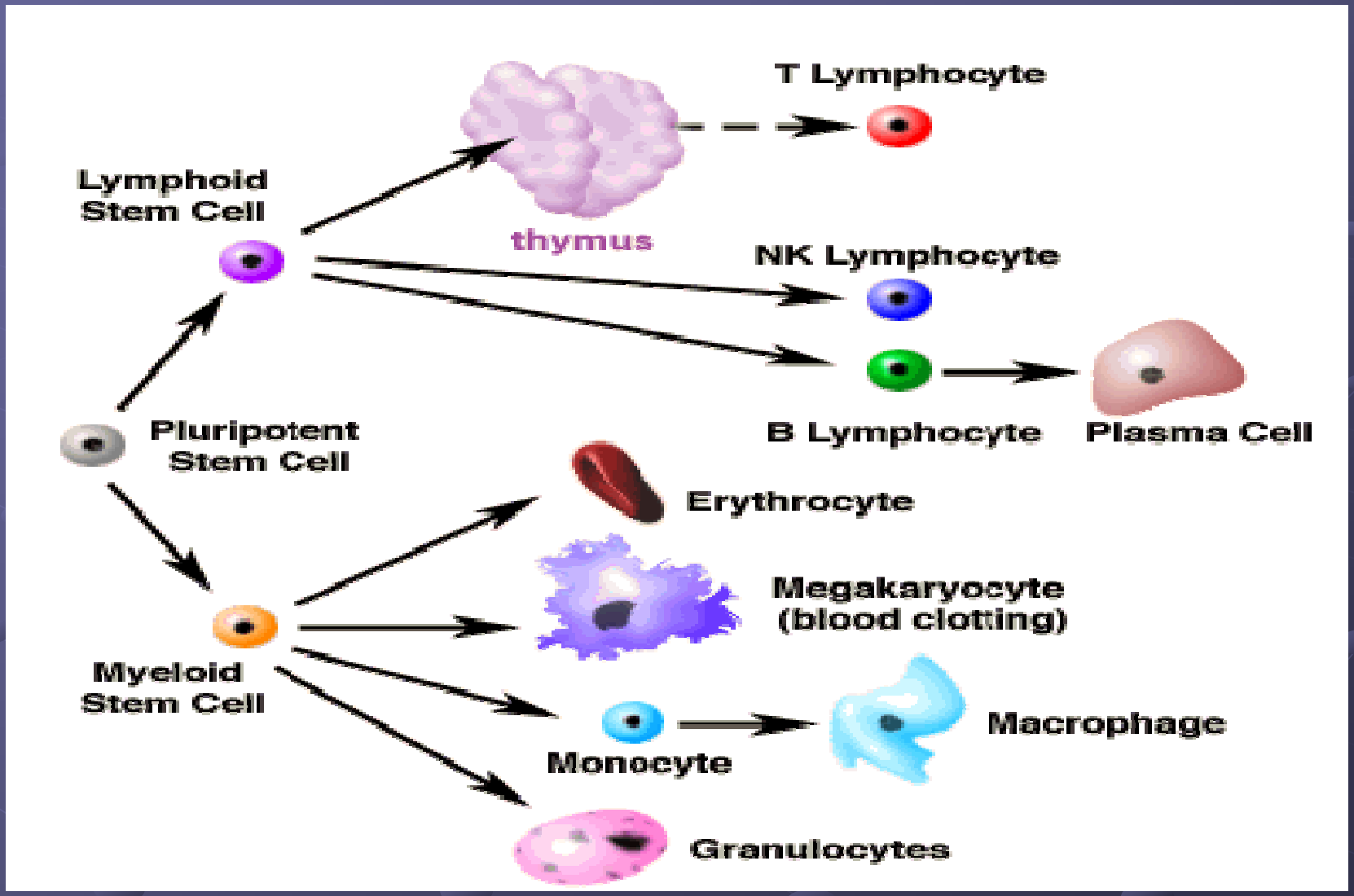
Types, production of antibodies and Antibody/antigen interaction

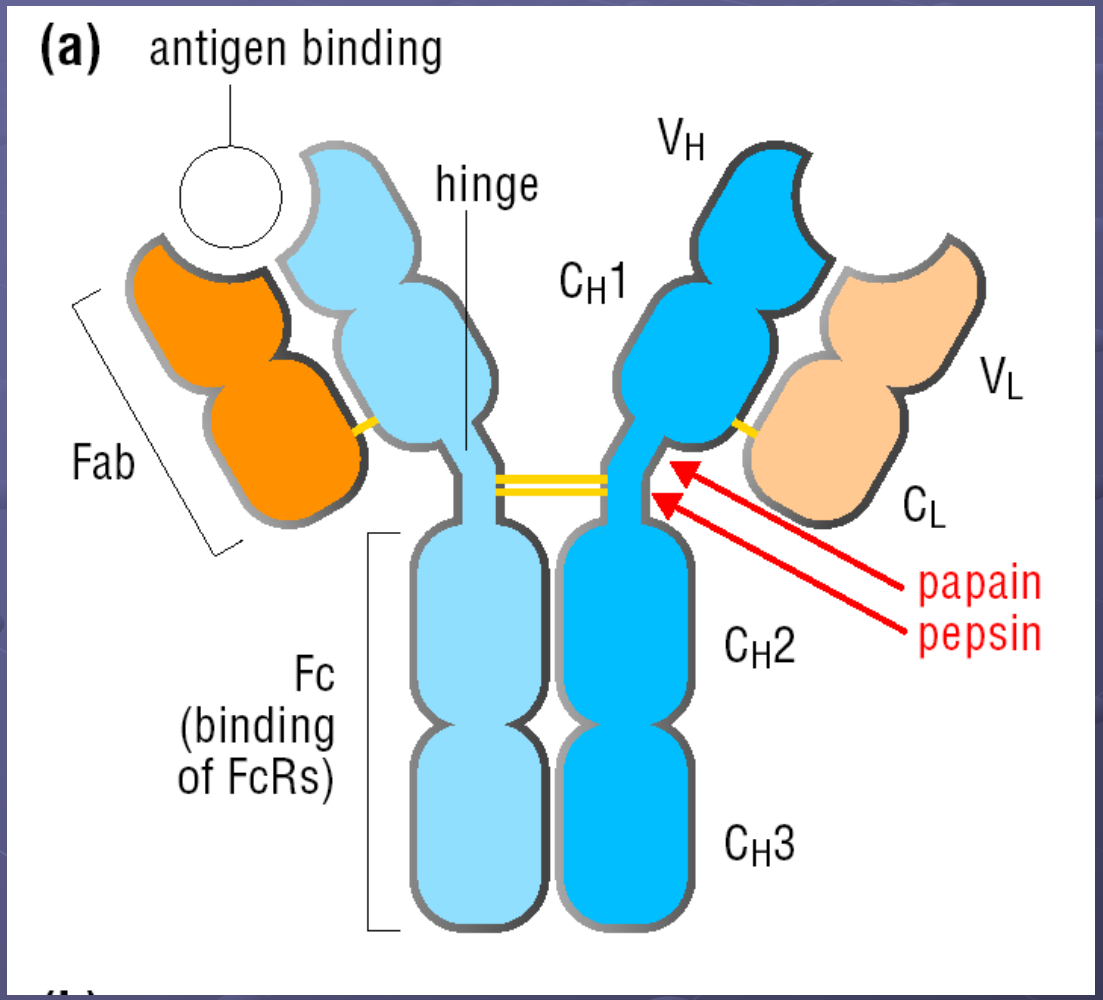
Antibodies

- Secreted by B lymphocytes
- Great diversity and specificity: >109 different antibodies; can distinguish between very similar molecules
- Tag particles for clearance/destruction
- Protect against re-infection (vaccines)

CELLS OF THE IMMUNE SYSTEM



Antibody Structure



Ig domain: 110 amino acids; globular domain used in many proteins.
Variable domains, Constant domains, Hinge.
Fab: fragment antigen binding
Fc: fragment crystallizable (effector functions)

Immunoglobulins (Ig) are glycoproteins made up of **light (L)** and **heavy(H)** polypeptide chains. The simplest antibody molecule has a Y shape and consists of four polypeptide chains:two H chains and two L chains. The four chains are linked by disulfide bonds.

L and H chains are subdivided into variable and constant regions.The regions are composed of three-dimensionally folded, repeating segments called domains. An L chain consists of one variable (VL) and one constant (CL) domain.Most H chains consist of one variable (VH) and three constant(CH) domains.(IgG and IgA have three CH domains,whereas IgM and IgE have four.)

The various regions are responsible for antigenbinding ,whereas the constant regions are responsible for various biologic functions eg, complement activation and binding to cell surface receptors.

Human Immunoglobulin Light Chain Types

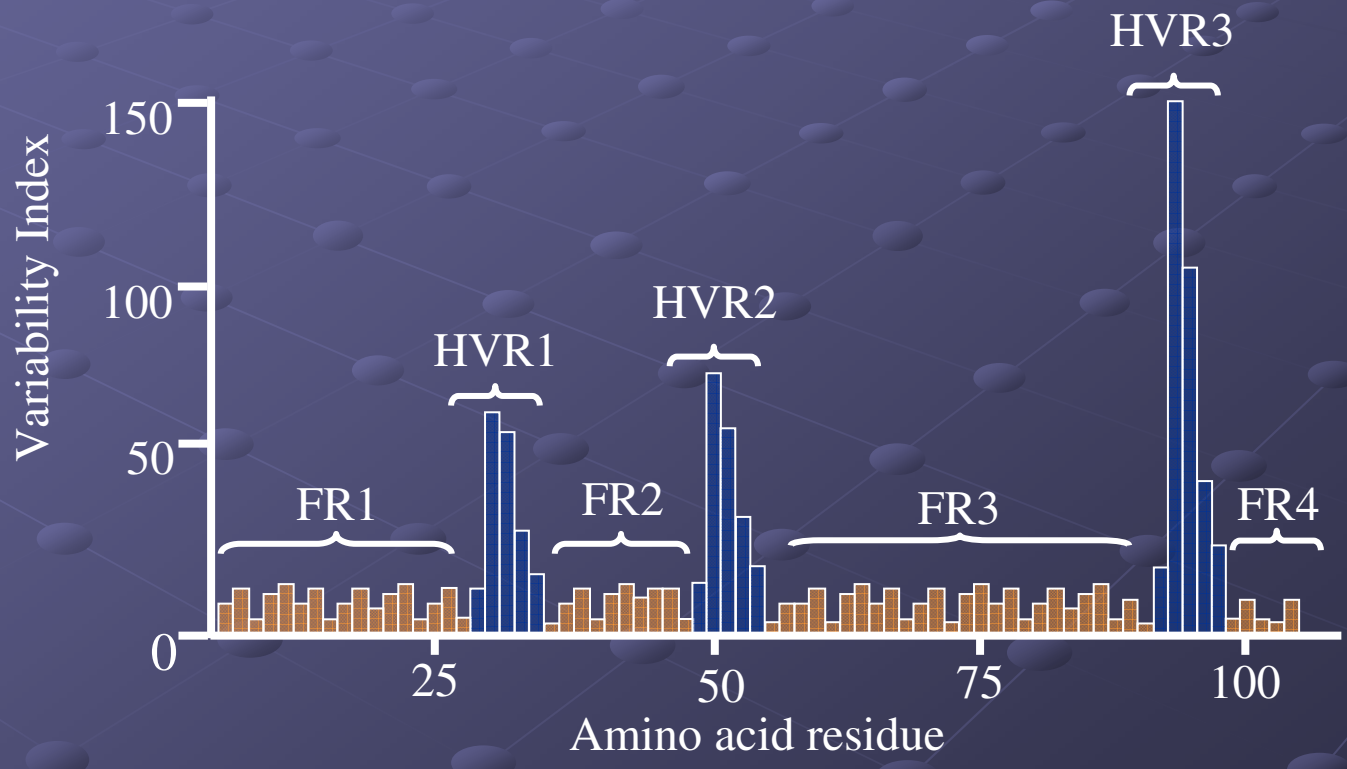
- Kappa (κ)
- Lambda (λ)

one type is found in Ig.

Structure of the Variable Region

- Hypervariable (HVR) or complimentarity determining regions (CDR)

Framework regions

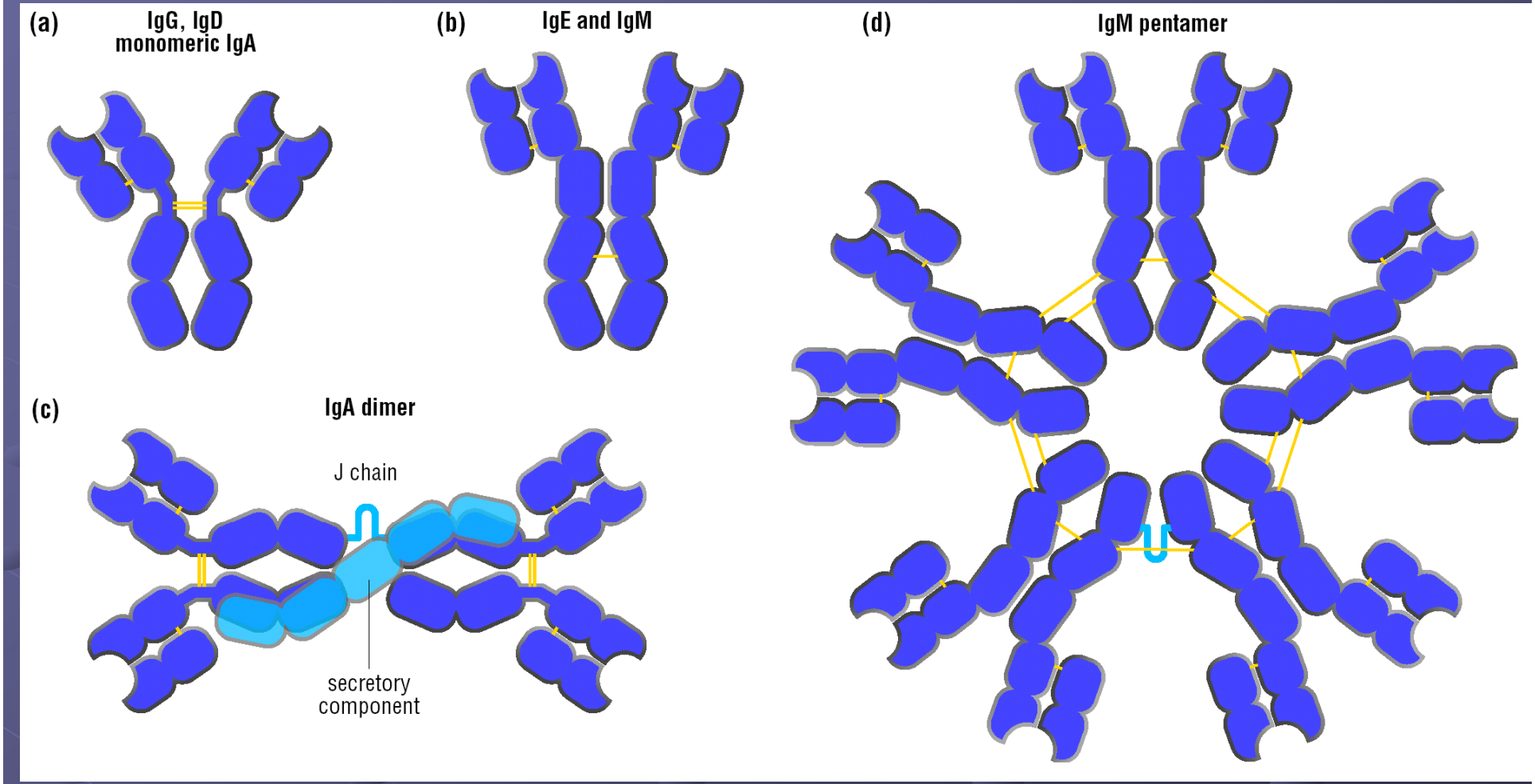


Generation of Antibody Diversity

- κ light chains: 40 Vκ x 5 Jκ = 200
- λ light chains: 30 Vλ x 4 Jλ = 120
- H chains: 40 VH x 27 DH x 6JH = 6,480
- 320 L chains x 6,480 H chains = 2.1 x 106
- Junctional diversity (addition or deletion of nucleotides at recombination sites, especially of H chain), estimated to add 3x107 fold to overall diversity.

When a B cell expands into a clone, it may switch its Ig class. When this happens, the variable region of the antibody stays the same, but the constant region changes.

Antibody Classes: Structure

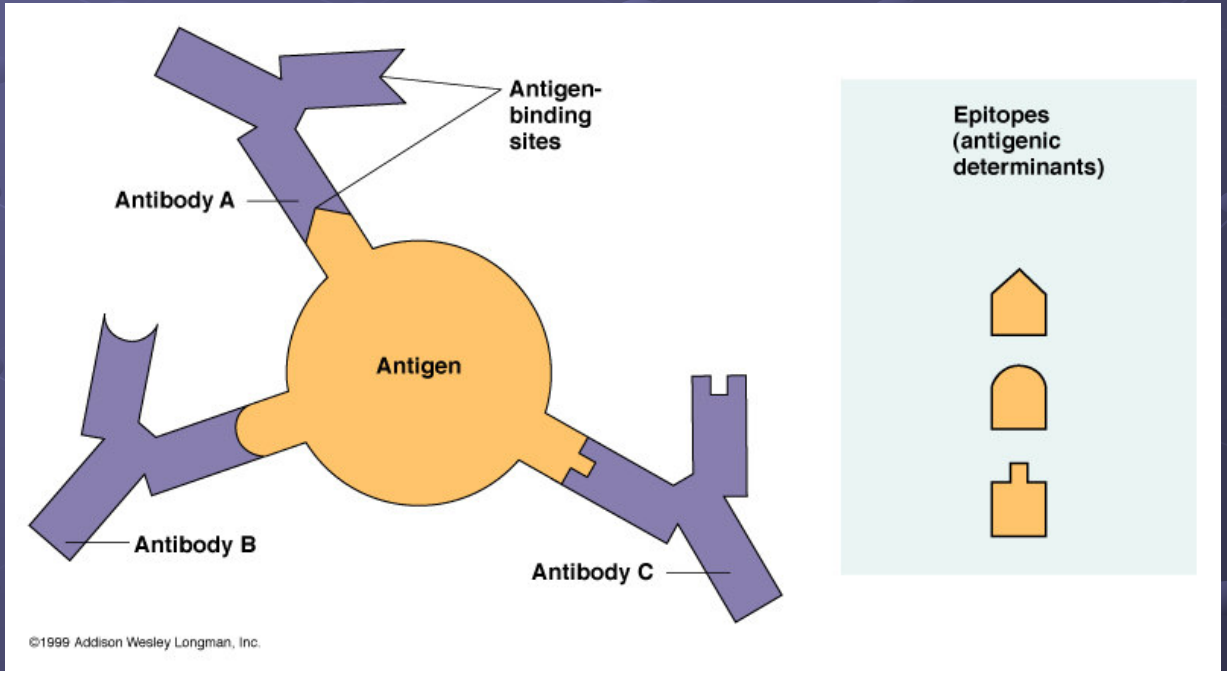


Major functional properties of antibodies

Antibody class	Major Functional properties
IgM	complement activation; antigen trapping; antigen receptor of naïve B cells
IgG	complement activation, phagocytosis, ADCC, transfer of adaptive immunity to offspring, regulation of antibody production
IgA	mucosal immunity, phagocytosis
IgE	activation of mast cells, basophils, eosinophils
IgD	antigen receptor on naïve B cells

- **Antigens**
- **Epitope:**
- **Small part of an antigen that interacts with an antibody.**
- **Any given antigen may have several epitopes.**
- **Each epitope is recognized by a different antibody.**

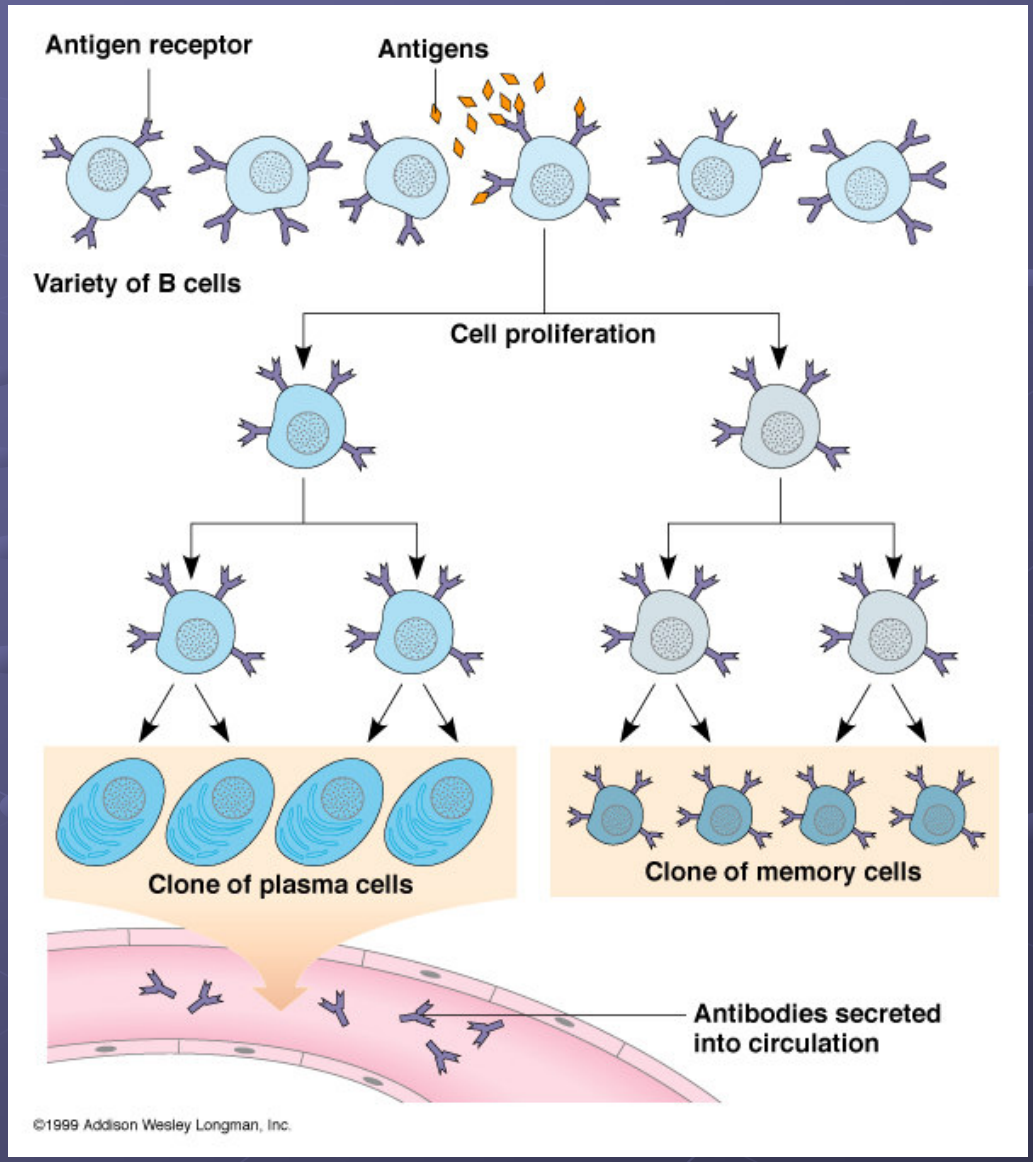
Epitopes: Antigen Regions that Interact with Antibodies



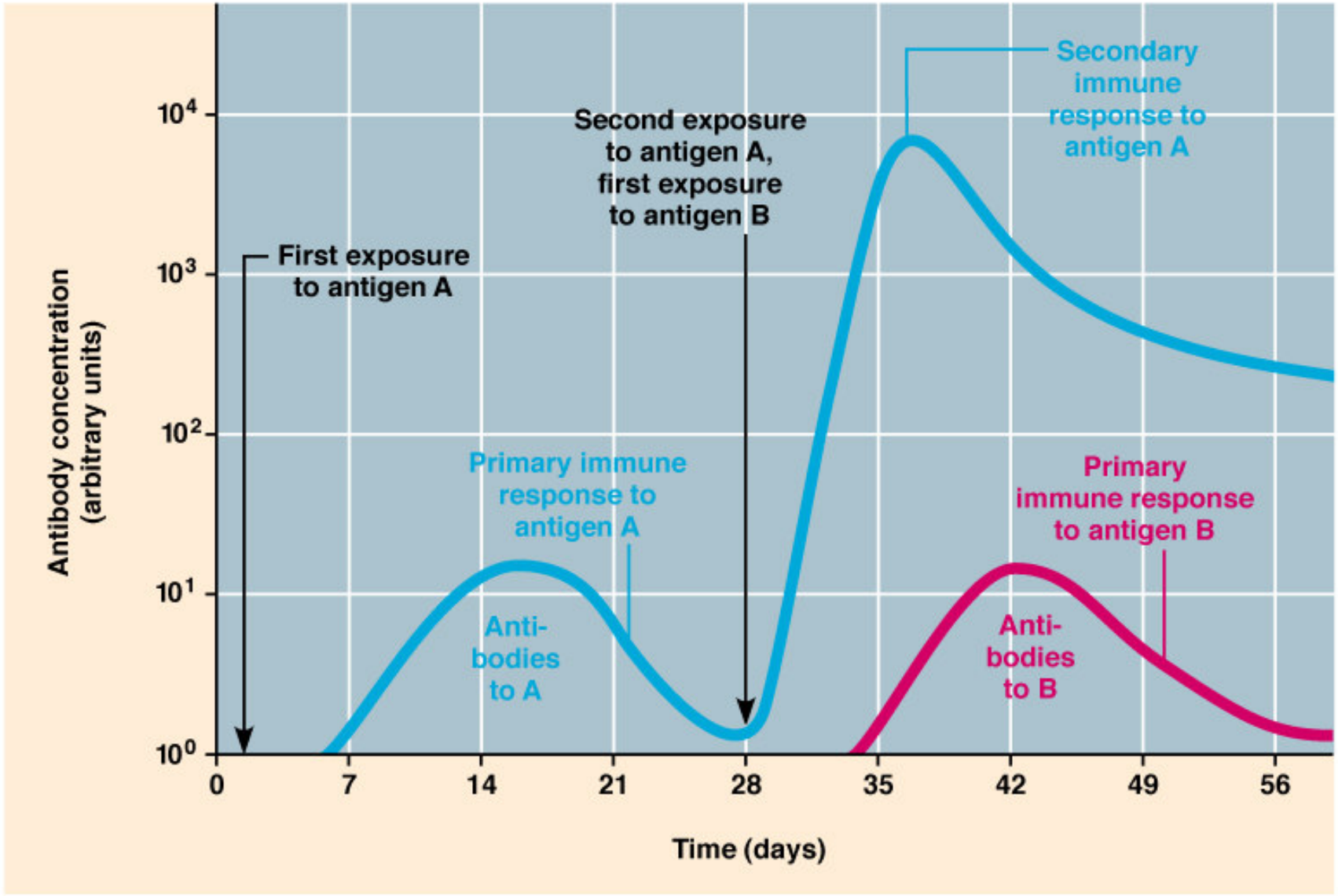
Non-covalent forces in antibody - antigen interactions

Electrostatic forces	Attraction between opposite charges
Hydrogen bonds	Hydrogens shared between electronegative atoms
Van der Waal's forces	Fluctuations in electron clouds around molecules oppositely polarise neighbouring atoms
Hydrophobic forces	Hydrophobic groups pack together to exclude water (involves Van der Waal's forces)

Clonal Selection of B Cells is Caused by Antigenic Stimulation



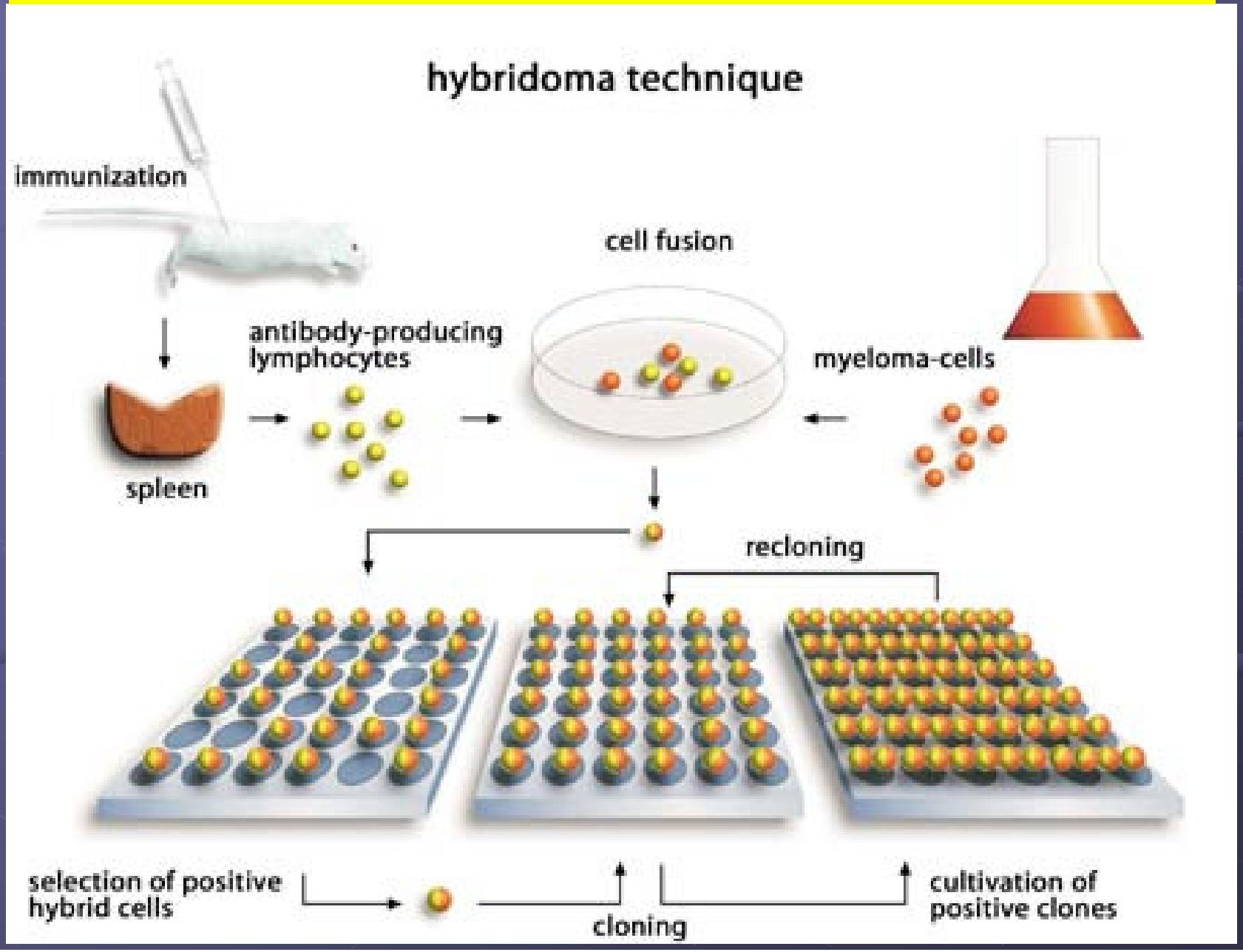
Antibody Response After Exposure to Antigen



ANTIBODIES

POLYCLONAL.	MONOCLONAL.
Derived from different B Lymphocytes cell lines	Derived from a single B cell clone
Batch to Batch variation affecting Ab reactivity & titre	mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity
NOT Powerful tools for clinical diagnostic tests	Enable the development of secure immunoassay systems.

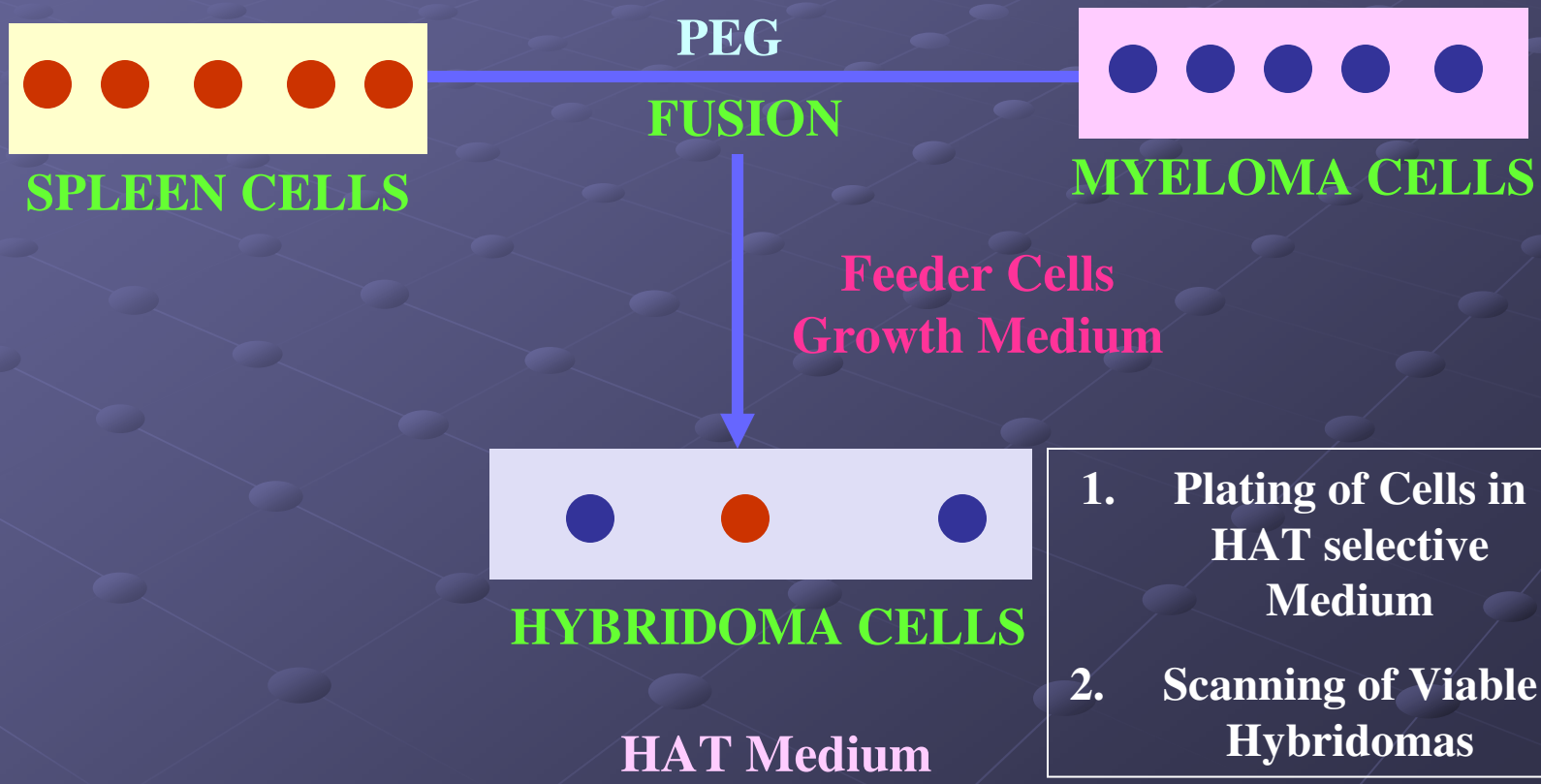
PRODUCTION OF MONOCLONAL ANTIBODY



PRODUCTION OF MONOCLONAL ANTIBODY

HYBRIDOMA TECHNOLOGY

Step 4: - Fusion of Myeloma Cells with Immune Spleen Cells
&
Selection of Hybridoma Cells



Monoclonal antibodies used in medicine

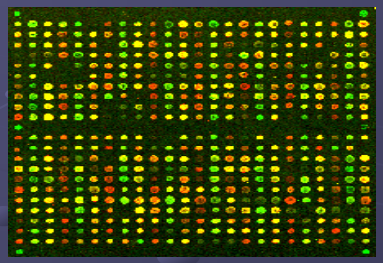
Standardized, unlimited amounts of reagents for diagnosis or therapy (human antibodies or “humanized” antibodies can be made).

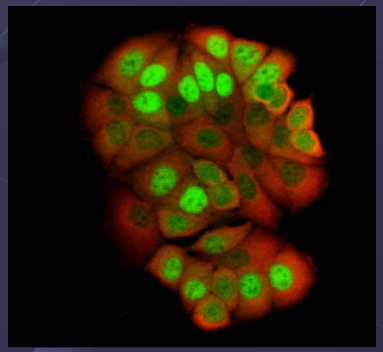
Monoclonal Antibodies Used in Therapies

monoclonal antibody	target	disease
trastuzumab	HER2	breast cancer
infliximab	TNF	rheumatoid arthritis, Crohn's disease
rituximab	CD20	non-Hodgkin's lymphoma
abciximab	GPIIb/IIIa	coronary disease
OKT3	CD3	graft rejection

Applications of Monoclonal Antibodies

- Diagnostic Applications
  Biosensors & Microarrays
- Therapeutic Applications
  Transplant rejection Muronomab-CD3
  Cardiovascular disease Abciximab
  Cancer Rituximab
  Infectious Diseases Palivizumab
  Inflammatory disease Infliximab
- Clinical Applications
  Purification of drugs, Imaging the target
- Future Applications
  Fight against Bioterrorism






thank you