Antibody Function & Structure

- Specifically bind to antigens in both the recognition phase (cellular receptors) and during the effector phase (synthesis and secretion) of humoral immunity
  - Serology: the study of antibodies and their reactions with antigens
    - **Antibodies present in serum, in mucosal secretions and interstitial spaces of tissues**
    - **Titer of antibodies relates to resistance to disease**

Table 3–1 Features of Antigen Binding by the Antigen-Recognizing Molecules of the Immune System

Feature	Antigen-Binding Molecule		
	Ig 	TCR 	MHC molecules* 
Antigen-binding site	Made up of three CDRs in V_H and three CDRs in V_L	Made up of three CDRs in V_α and three CDRs in V_β	Peptide-binding cleft made of $\alpha 1$ and $\alpha 2$ (class I) or $\alpha 1$ and $\beta 1$ (class II)
Nature of antigen that may be bound	Macromolecules (proteins, lipids, polysaccharides) and small chemicals	Peptide-MHC complexes	Peptides
Nature of antigenic determinants in macromolecules recognized	Linear and conformational	Linear; only 2 or 3 amino acid residues of a peptide bound to an MHC molecule	Linear; only some amino acid residues of a peptide
Affinity of antigen binding	K_d 10^{-7}–10^{-11} M; average affinity of Igs increases during immune response	K_d 10^{-5}–10^{-7} M	K_d 10^{-6} M
On–rate and off–rate	Rapid on–rate, variable off–rate	Slow on–rate; slow off–rate	Slow on–rate; very slow off–rate

*The structure and function of MHC and TCR molecules are discussed in Chapters 4 and 6, respectively.

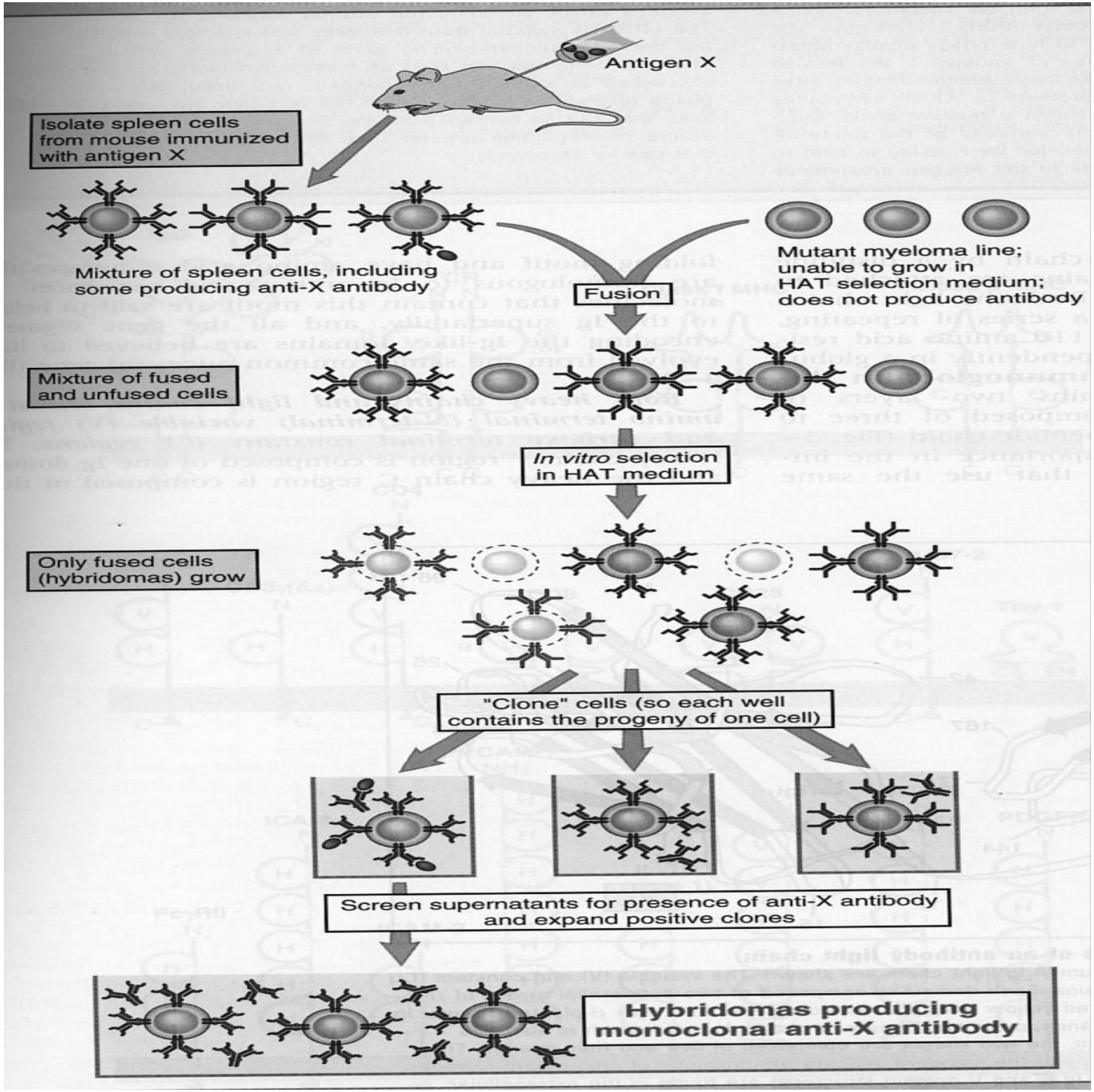
Abbreviations: CDR, complementarity-determining region; Ig, immunoglobulin; K_d, dissociation constant; MHC, major histocompatibility complex; TCR, T cell receptor; V_H, variable domain of heavy chain Ig; V_L, variable domain of light chain Ig.

- Healthy (70kg adult) produces ~ 3g of Ab's/day
  - Most of this is IgA- secreted by B cells within GI tract and respiratory tracts
- 2 discoveries crucial for elucidating the structure of antibodies:
  - Patients with multiple myeloma: (monclonal tumor of B cells) patients have high levels of Ig in blood and urine (Bence-Jones proteins)
  - Production of monoclonal antibodies: (Kohler & Milstein in 1975) immortalization of Ab producing cells by fusion of tumor cell line and specific B cell (hybridoma)

Monoclonal Antibodies

- Requires cultured myeloma cell lines that grow in normal culture medium but will not grow in a selective media because they lack genes (mutated) required for DNA synthesis. By fusing normal cells to these mutated tumor cells the necessary genes are supplied by the normal cell (B cells) and allow the "hybrid" to grow in the selective media.
- Genes from myeloma cell make "normal B cell" immortal
  - Normal cells synthesize purine nucleotides and thymidylate by a ***de novo*** pathway requiring tetrahydrofolate
  - Aminopterin-treated cells (inhibitor of folate enzymes) use a **salvage pathway** to make purines and thymidine kinase (TK) makes thymidyalate
  - Thus aminopterin treated cells grow normally– Mutated tumor cells are unable to grow in HAT media

- HAT media contains
  - **Hypoxanthine: mutated myeloma cells cannot use hypoxanthine to make purines in the salvage pathway because they are mutated in the HGPRT gene (only mutated tumor cells)**
  - **Aminopterin: stops the action of tetrahydrofolate and cells cannot make purines (both myeloma and normal B cells)**
  - **Thymidine: supplies nucleotide base in media (for both myeloma and normal B cell)**
- Unfused myeloma cells will die because they do not have the gene products to replicate DNA– normal B cells will die becaue they are not immortal– only fused cells will live in HAT medium (immortal genes from myeloma and salvage pathway genes from normal B cell)


Antigen X
Isolate spleen cells from mouse immunized with antigen X
Mixture of spleen cells, including some producing anti-X antibody
Mutant myeloma line; unable to grow in HAT selection medium; does not produce antibody
Fusion
Mixture of fused and unfused cells
In vitro selection in HAT medium
Only fused cells (hybridomas) grow
"Clone" cells (so each well contains the progeny of one cell)
Screen supernatants for presence of anti-X antibody and expand positive clones
Hybridomas producing monoclonal anti-X antibody

- All Ig molecules share the same basic structural characteristics but display great variability in the regions that bind antigens
  - Ag binding occurs at the N-terminal end of the combination of the H and L chains (variable ends)
  - Constant regions have identical (nearly identical- there may be **allotypic** differences [i.e., differences that may be as small as 1 AA change) AA sequences
    - Variability may be as great as 10^7-10^9 different Ab molecules in each individual

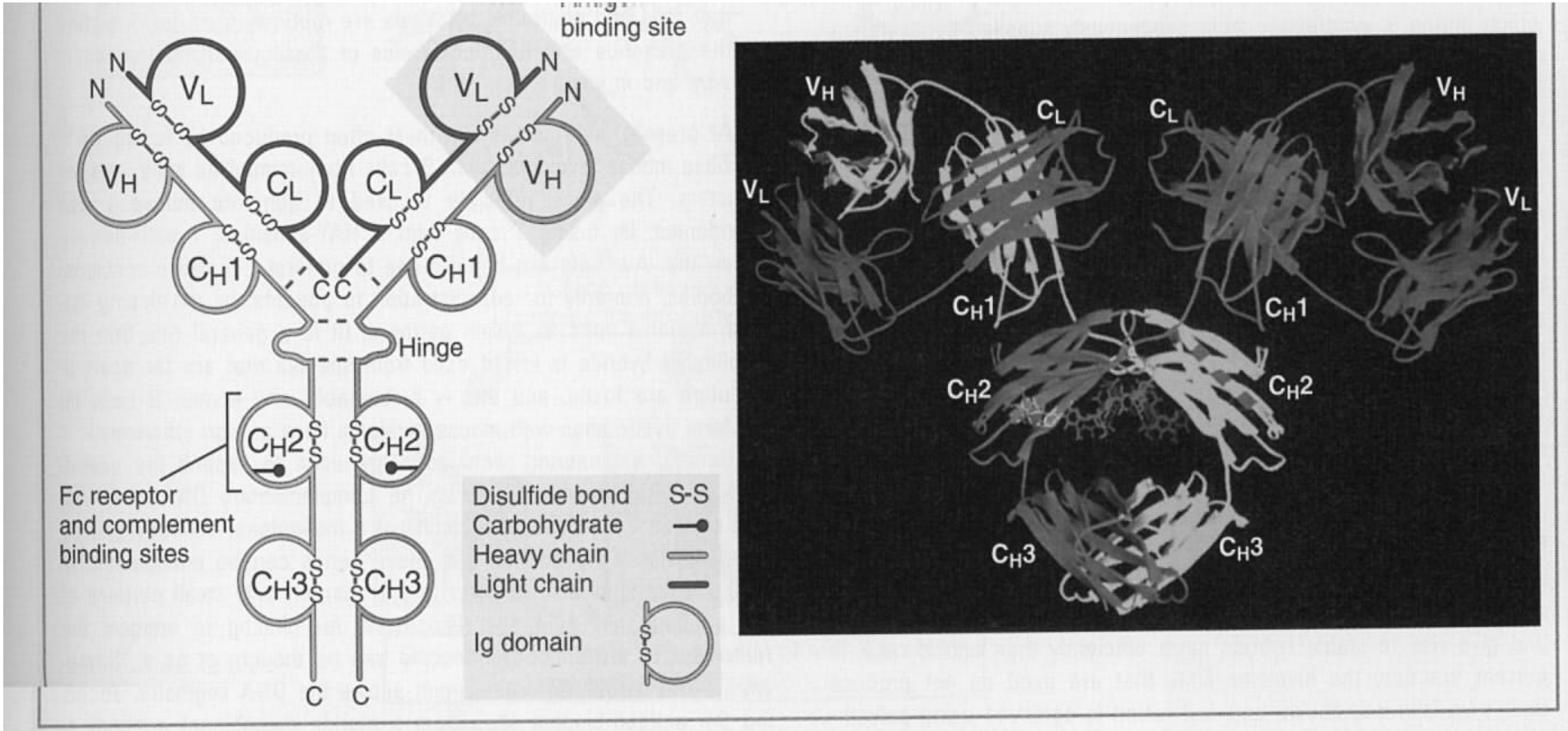
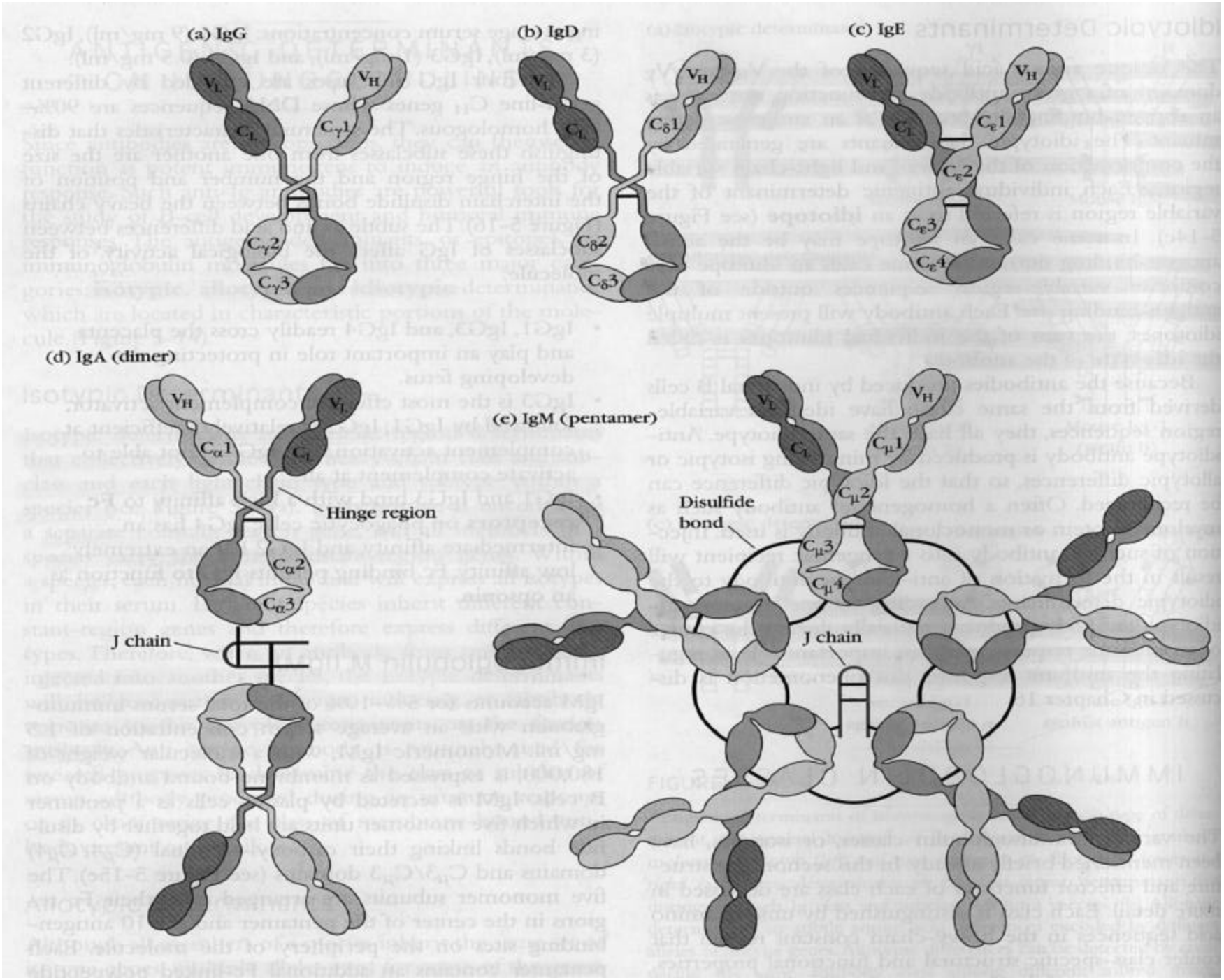


Figure 3–1 Structure of an antibody molecule.

A. Schematic diagram of an Ig molecule. In this drawing of an IgG molecule, the antigen-binding sites are formed by the juxtaposition of variable light chain (V_L) and variable heavy chain (V_H) domains. The locations of complement and Fc receptor–binding sites within the heavy chain constant regions are approximations. Fc, fragment, crystallizable; Ig, immunoglobulin.

B. Structure of a human IgG molecule as revealed by x-ray crystallography. In this ribbon diagram of a secreted IgG molecule, the light chain polypeptides are depicted in red, the heavy chain polypeptides in magenta and yellow, and the carbohydrates in blue and green. Note that each light chain is folded into two Ig domains and each heavy chain into four Ig domains (see Fig. 3–2 for a more detailed view of an Ig domain). The first variable Ig domain of each light chain (V_L) pairs with the first variable Ig domain of each heavy chain (V_H) to form an antigen-binding site. The carbohydrates are attached to the second constant region of each heavy chain (C_H2) and occupy a space between these domains. The red squares on the yellow C_H2 indicate positions involved in the activation of complement. Ig, immunoglobulin. (Courtesy of Dr. A Edmundson, Oklahoma Medical Research Foundation, Oklahoma City, OK; reproduced with permission from the cover of Immunology Today 16, 1995, Feb. Copyright Elsevier Science, Ltd.)

- Composed of 2 H and 2 L peptide chains
  - Each has a Constant and Variable region (H_C, H_V, L_C and L_V)- loop domains –S—S- bonds within regions
  - Constant H chains represent the 5 classes (**isotypes**) of Ig molecules
    - IgG(4 subclasses) , IgA (2 subclasses) , IgM, IgE, IgD (ã á ì å ä)
  - Constant L chains (2 classes)- **one or the other not both**
    - Lambda (**ë**) **[40% in humans]** and Kappa (**ê**) **[60% in humans]**
  - Variable regions come from different gene sequences, and within the variable region there are **hypervariable** regions (the hypervariable region is also called **complementarity-determining regions= CDR**– this sequence is complementary to the 3-D structure of the antigen). There are special genetic mechanisms that generate this hypervariablilty, especially in CRD3 region)


(a) IgG
(b) IgD
(c) IgE
(d) IgA (dimer)
(e) IgM (pentamer)
Hinge region
J chain
Disulfide bond
J chain

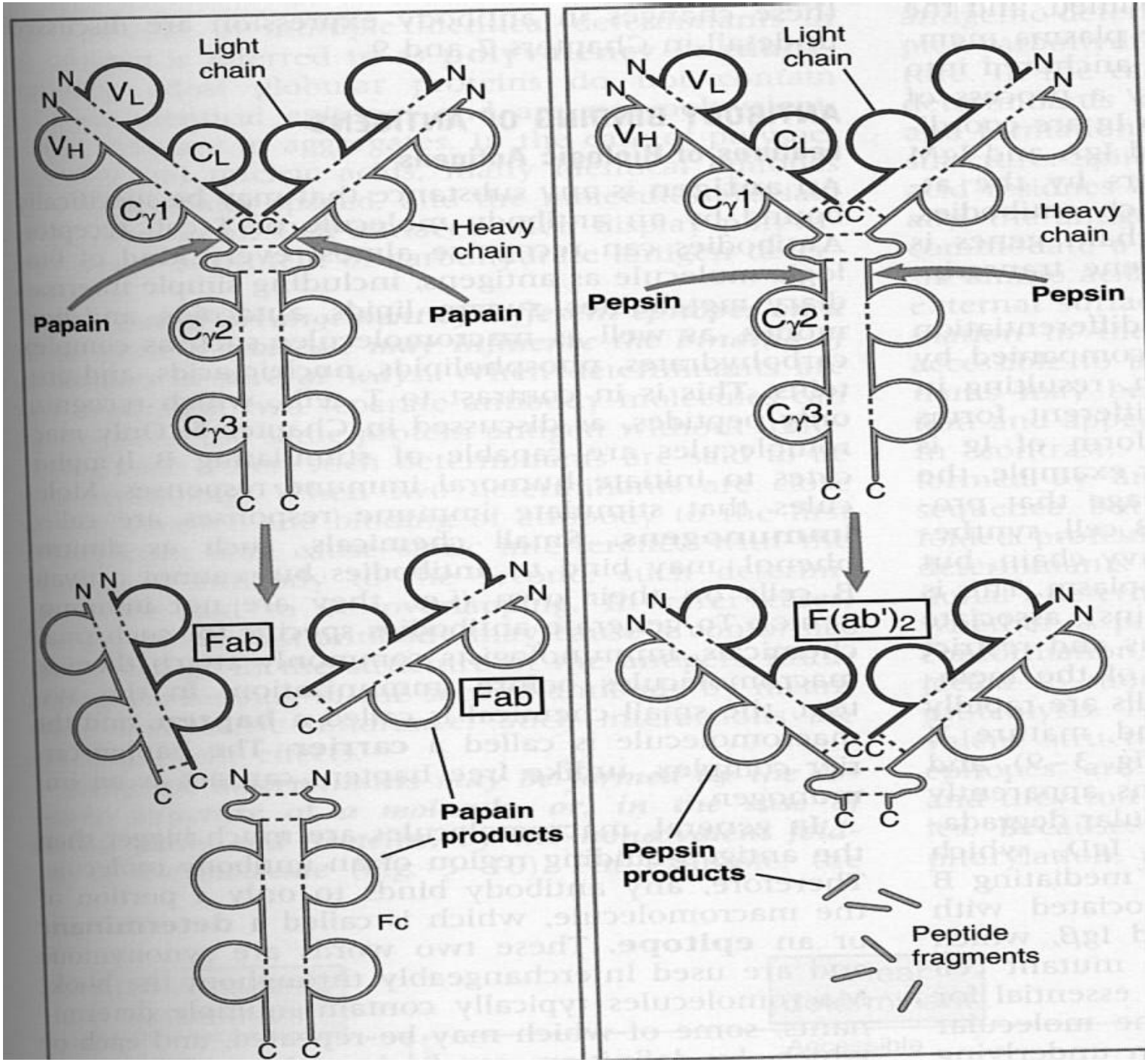
- Different classes of Ig's have different functions (differences in ½ life and tissue distribution):
  - IgM: 1st Ig made in response to Ag, Activate C' very well because of pentameric structure (J-chain)
  - IgG: 2nd Ig made in response to Ag, activates C' well, crosses placenta, has greater avidity for Ag than IgM, binds to Fc receptor of Mè
  - IgA: dimer, crosses epithelial cell barriers (mucous membranes and gut), has J-chain and SC protein
  - IgE: has extra domain (C4) that is able to bind to Fc receptor on mast cell

TABLE 5-4

PROPERTIES AND BIOLOGICAL ACTIVITIES* OF CLASSES AND SUBCLASSES OF HUMAN SERUM IMMUNOGLOBULINS

PROPERTY/ACTIVITY	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM‡	IgE	IgD
Molecular weight†	150,000	150,000	150,000	150,000	150,000–600,000	150,000–600,000	900,000	190,000	150,000
Heavy-chain component	$\gamma 1$	$\gamma 2$	$\gamma 3$	$\gamma 4$	$\alpha 1$	$\alpha 2$	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/–	++	–	–	–	+++	–	–
Crosses placenta	+	+/–	+	+	–	–	–	–	–
Present on membrane of mature B cells	–	–	–	–	–	–	+	–	+
Binds to macrophage Fc receptors	++	+/–	++	+	–	–	+	–	–
Present in secretions	–	–	–	–	++	++	+	–	–
Induces mast-cell degranulation	–	–	–	–	–	–	–	+	–

- Antibody molecules can be cleaved by papain or pepsin to form fragments that maintain specific functions—
  - Papain– cleaves to give 2 fragments of **Fab** and one **Fc**= Fab can bind, but not cross-link antigen; Fc cannot bind complement without Ag binding 1st
  - Pepsin– cleaves to give a **F(ab')$_2$** fragment= can bind and cross-link antigens and cell receptors
- **Antibodies can undergo**
  - **Affinity maturation**: somatic mutations of variable region
  - **Isotype switching-** give different set of effector functions


Light chain
V_L
V_H
C_L
$C_\gamma1$
CC
Heavy chain
Papain
Papain
$C_\gamma2$
$C_\gamma3$
N
C
Fab
Fab
Papain products
Fc
Light chain
V_L
V_H
C_L
$C_\gamma1$
CC
Heavy chain
Pepsin
Pepsin
$C_\gamma2$
$C_\gamma3$
$F(ab')_2$
Pepsin products
Peptide fragments

- Two forms of Ig
  - Membrane associated
    - Membrane associated stays on membrane and acts as antigen receptor
    - Interacts with Ag, but does not have transmembrane activity to induce activation of signal transduction processes
    - Must associate with the complete B-cell Receptor (BCR) with includes the membrane Ig plus a disulfide linked heterodimer composed of Ig-á and Ig-â (2 molecules of this heterodimer associate with one of Ig). Heterodimer has long cytoplasmic tails that help activate signal transduction molecules.
  - Secreted Ig
    - Has effector functions

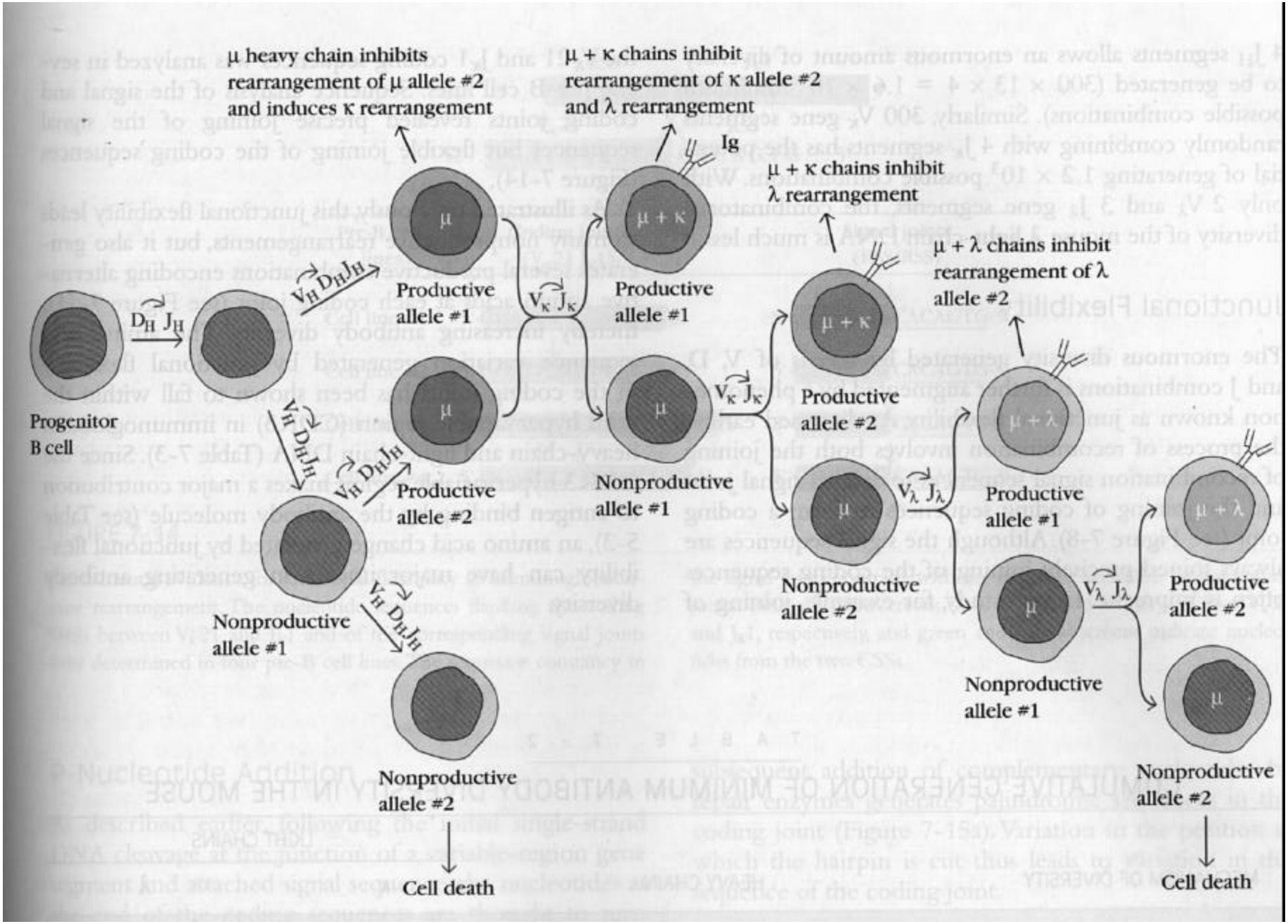
TABLE 5–5

IMMUNE BENEFITS OF BREAST MILK

COMPONENT	ACTION
	WHITE BLOOD CELLS
B cells	Give rise to antibodies targeted against specific microbes.
Macrophages	Kill microbes outright in the baby's gut, produce lysozyme and activate other components of the immune system.
Neutrophils	May act as phagocytes, ingesting bacteria in baby's digestive system.
T cells	Kill infected cells directly or send out chemical messages to mobilize other defenses. They proliferate in the presence of organisms that cause serious illness in infants. They also manufacture compounds that can strengthen a child's own immune response.
	MOLECULES
Antibodies of secretory IgA class	Bind to microbes in baby's digestive tract and thereby prevent them from passing through walls of the gut into body's tissues.
B_{12} binding protein	Reduces amount of vitamin B_{12}, which bacteria need in order to grow.
Bifidus factor	Promotes growth of *Lactobacillus bifidus*, a harmless bacterium, in baby's gut. Growth of such nonpathogenic bacteria helps to crowd out dangerous varieties.
Fatty acids	Disrupt membranes surrounding certain viruses and destroy them.
Fibronectin	Increases antimicrobial activity of macrophages; helps to repair tissues that have been damaged by immune reactions in baby's gut.
Hormones and growth factors	Stimulate baby's digestive tract to mature more quickly. Once the initially "leaky" membranes lining the gut mature, infants become less vulnerable to microorganisms.
Interferon (IFN-γ)	Enhances antimicrobial activity of immune cells.
Lactoferrin	Binds to iron, a mineral many bacteria need to survive. By reducing the available amount of iron, lactoferrin thwarts growth of pathogenic bacteria.
Lysozyme	Kills bacteria by disrupting their cell walls.
Mucins	Adhere to bacteria and viruses, thus keeping such microorganisms from attaching to mucosal surfaces.
Oligosaccharides	Bind to microorganisms and bar them from attaching to mucosal surfaces.

- Clinical uses of monoclonal Antibodies
  - Diagnostic/imaging & therapeutic
    - Detect pregnancy, microorganisms, blood levels of drugs, matching histocompatibility antigens, detecting antigens shed by tumors
    - detecting tumors by radiolabeling Ig to locate tumor and metastatic cells
    - Immunotoxins- lethal toxins bound to Ig to specifically target tumor (ricin, *Shigella* toxin, and diphtheria toxin: all inhibit protein synthesis) [each toxin has a binding portion and an effector portion. If binding portion left off effector portion cannot get into cells and is harmless. Binding portion substituted by Ig that gives specificity to toxin)
    - Catalytic Monoclonal Antibodies (**Abzymes**)- use for dissolving blood clots, cleave viral proteins

- Each B cell has two alleles for Ig genes, one derived from each parent
- To prevent the maturing B cell from becoming "confused" by having too many specificities, one allele is activated at a time (random)
  - Allelic Exclusion
- If the 1st allele does not make a productive protein, then the other allele becomes activated. If this fails the cell dies


μ heavy chain inhibits rearrangement of μ allele #2 and induces κ rearrangement
μ + κ chains inhibit rearrangement of κ allele #2 and λ rearrangement
Ig
μ + κ chains inhibit λ rearrangement
μ + λ chains inhibit rearrangement of λ allele #2
Progenitor B cell
Productive allele #1
Productive allele #2
Nonproductive allele #1
Nonproductive allele #2
Cell death

Organization of Ig Genes

- **To explain diversity of Immune reactivity**
  - **Germ-Line Theory= germline contributed by germ cells (sperm and egg) contains a large repertoire of Ig genes. This theory assumes that 15% of the genome is dedicated to just the immune response.**
  - **Somatic Variation Theory= genome contains a small number of Ig genes from which a large number of Ig specificities are generated by somatic cells by mutation or recombination**

Neither Theory Answered all Questions!

- **3rd Theory= Two-gene model (Dryer & Bennett, 1965)**
  - **Two genes encode a single Ig heavy or light chain (one for V and other for C regions)**
  - **Proposed that there were hundreds or thousands of V-region genes carried in the germ line but only single copies of C-region genes.**
  - **Combined two previous theories with both germ-line genes and somatic mutations**
- **In 1976 theory, Tonegawa found 1st evidence that separate genes coded for these two regions (Nobel)**

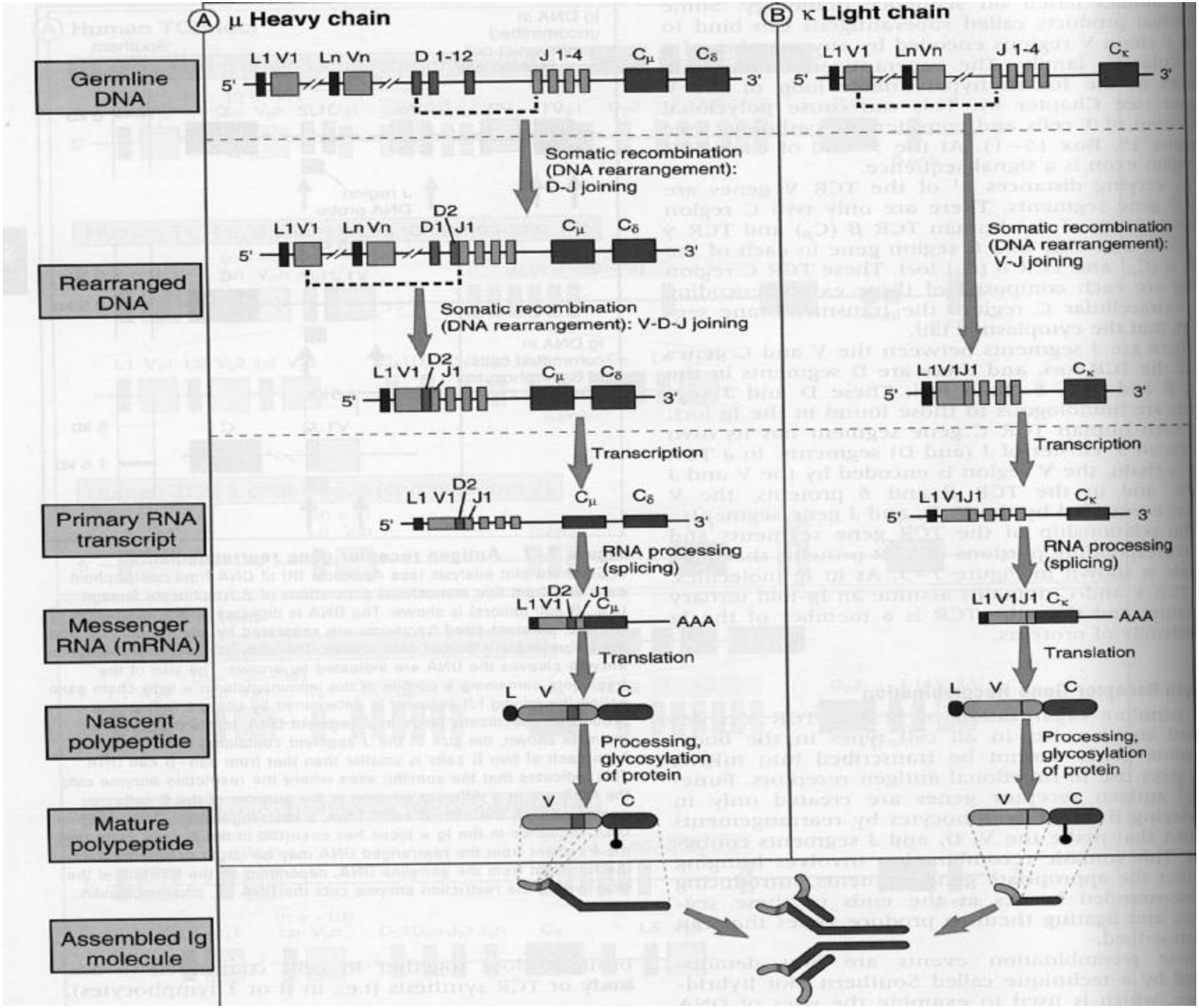
- Tonegawa-
  - Used restriction endonucleases to cleave DNA from embryonic cells and compared to adult cell DNA. Separated by size and ability to hybridize with radiolabeled mRNA probes
  - Found that in adult myeloma cell line (mature) probes labeled only one band, but in embryonic DNA two bands were radiolabeled.
    - Conclusion- in mature Ig producing cell the genes that produced Ig protein were fused, but genes in embryonic cells were separate

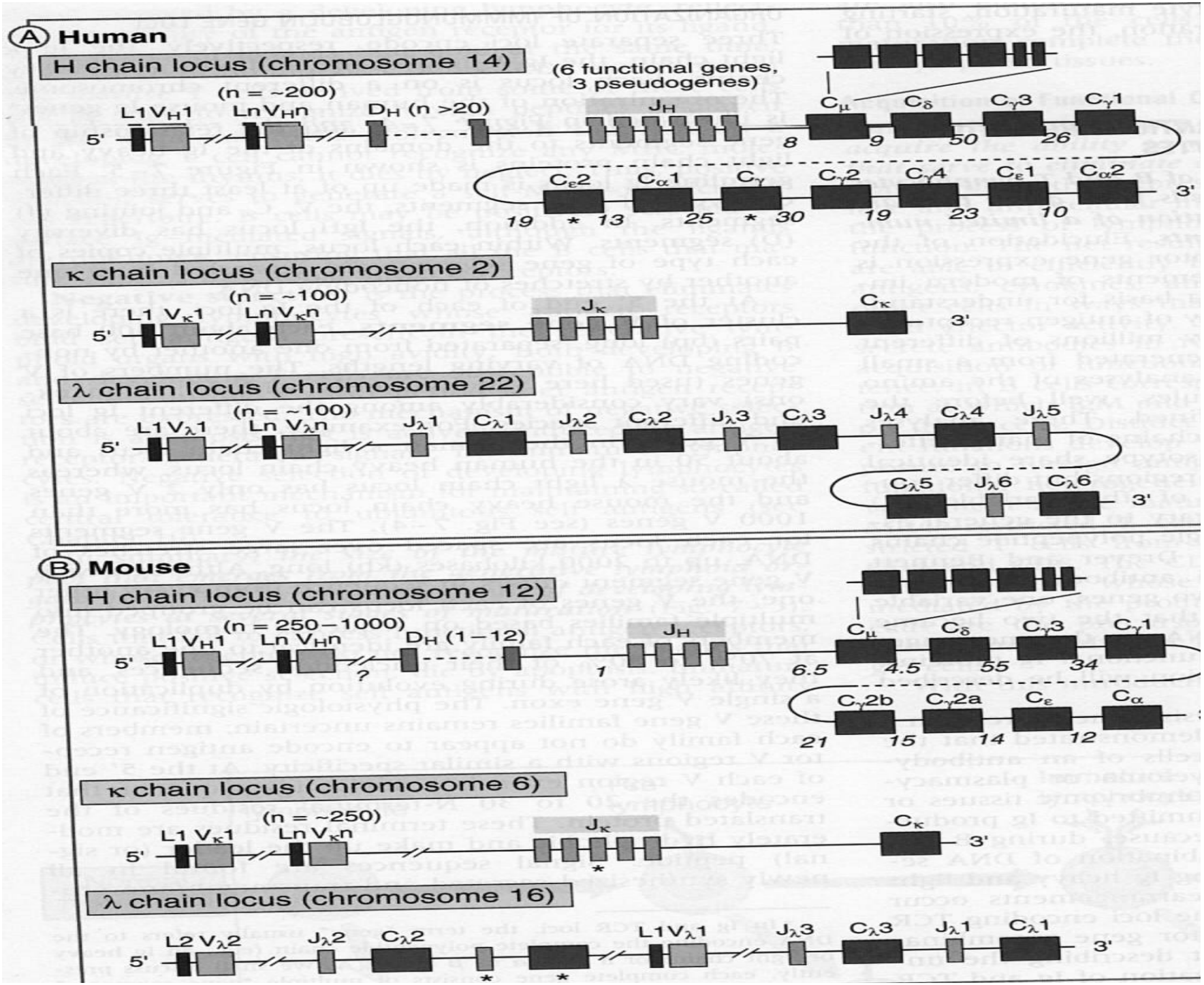
Ig Genes

- Ê and ë Light chain genes
  - Contain V, J and C gene segments
  - The V and J regions encode the Ag binding region
- Heavy Chain Genes
  - Contain V, D, J, and C gene segments
  - The V, D, and J regions form the Ag binding portion

- **Lamba Light chain gene found on chromosome 22 (human)**
- **Kappa Light chain gene found on chromosome 2 (human)**
- **Heavy chain genes found on chromosome 14 (human)**

- V= variable genes
- D= diversity genes
- J= joining genes
- How these are recombined gives Ag specificity to Ig molecule


A μ Heavy chain
B κ Light chain
Germline DNA
Rearranged DNA
Primary RNA transcript
Messenger RNA (mRNA)
Nascent polypeptide
Mature polypeptide
Assembled Ig molecule
L1 V1
Ln Vn
D 1-12
J 1-4
Cμ
Cδ
Cκ
5'
3'
Somatic recombination (DNA rearrangement): D-J joining
D2
D1
J1
Somatic recombination (DNA rearrangement): V-D-J joining
Somatic recombination (DNA rearrangement): V-J joining
L1V1J1
Transcription
RNA processing (splicing)
AAA
Translation
L
V
C
Processing, glycosylation of protein


A Human
H chain locus (chromosome 14)
(n = ~200)
L1 VH1
Ln VHn
DH (n >20)
JH
(6 functional genes, 3 pseudogenes)
Cμ
Cδ
Cγ3
Cγ1
8
9
50
26
Cε2
Cα1
Cγ
Cγ2
Cγ4
Cε1
Cα2
3'
19
*
13
25
*
30
19
23
10
κ chain locus (chromosome 2)
(n = ~100)
L1 Vκ1
Ln Vκn
Jκ
Cκ
3'
λ chain locus (chromosome 22)
(n = ~100)
L1 Vλ1
Ln Vλn
Jλ1
Cλ1
Jλ2
Cλ2
Jλ3
Cλ3
Jλ4
Cλ4
Jλ5
Cλ5
Jλ6
Cλ6
3'
B Mouse
H chain locus (chromosome 12)
(n = 250–1000)
L1 VH1
Ln VHn
DH (1–12)
JH
Cμ
Cδ
Cγ3
Cγ1
6.5
4.5
55
34
Cγ2b
Cγ2a
Cε
Cα
21
15
14
12
κ chain locus (chromosome 6)
(n = ~250)
L1 Vκ1
Ln Vκn
Jκ
Cκ
3'
λ chain locus (chromosome 16)
L2 Vλ2
Jλ2
Cλ2
L1 Vλ1
Jλ3
Cλ3
Jλ1
Cλ1
3'

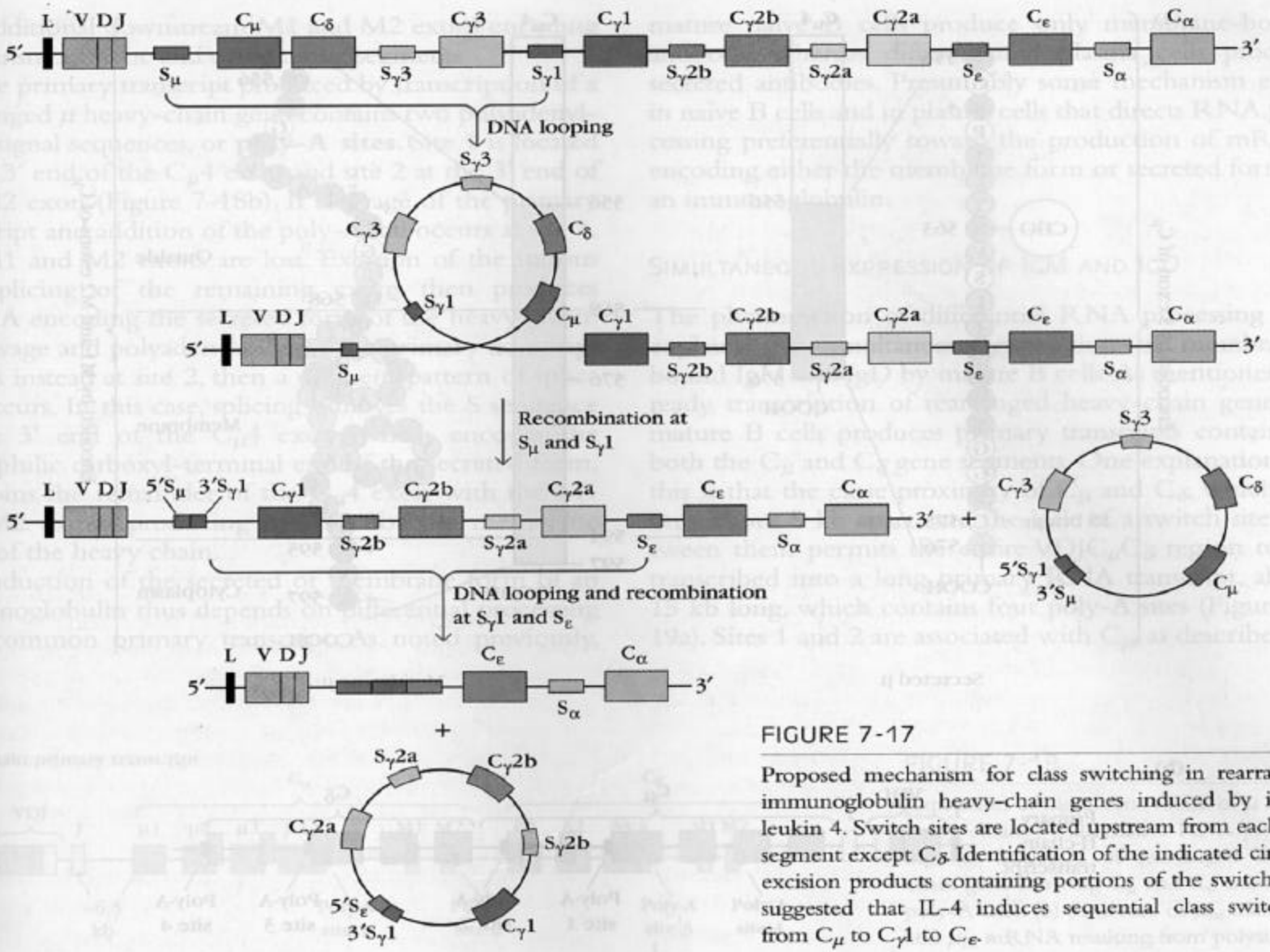


FIGURE 7-17

Proposed mechanism for class switching in rearranged immunoglobulin heavy-chain genes induced by interleukin 4. Switch sites are located upstream from each C_H segment except C_δ. Identification of the indicated circular excision products containing portions of the switch sites suggested that IL-4 induces sequential class switching from C_μ to $C_\gamma1$ to C_ε.

TABLE 7-2

CUMULATIVE GENERATION OF MINIMUM ANTIBODY DIVERSITY IN THE MOUSE

MECHANISM OF DIVERSITY	HEAVY CHAIN	LIGHT CHAINS	
		κ	λ
	ESTIMATED NUMBER OF SEGMENTS *		
Multiple germ-line gene segments:			
V	300–1000	300	2
D	13	0	0
J	4	4	3
	POSSIBLE NUMBER OF COMBINATIONS †		
Combinatorial V-J and V-D-J joining	$300 \times 13 \times 4 = 1.6 \times 10^4$	$300 \times 4 = 1.2 \times 10^3$	$2 \times 3 = 6$
Junctional flexibility	+	+	+
P-region nucleotide addition	+	+	+
N-region nucleotide addition	+	−	−
Somatic mutation	+	+	+
Combinatorial association of heavy and light chains	$>1.6 \times 10^4 \times (>1.2 \times 10^3 + >6) = \gg 1.9 \times 10^7$		