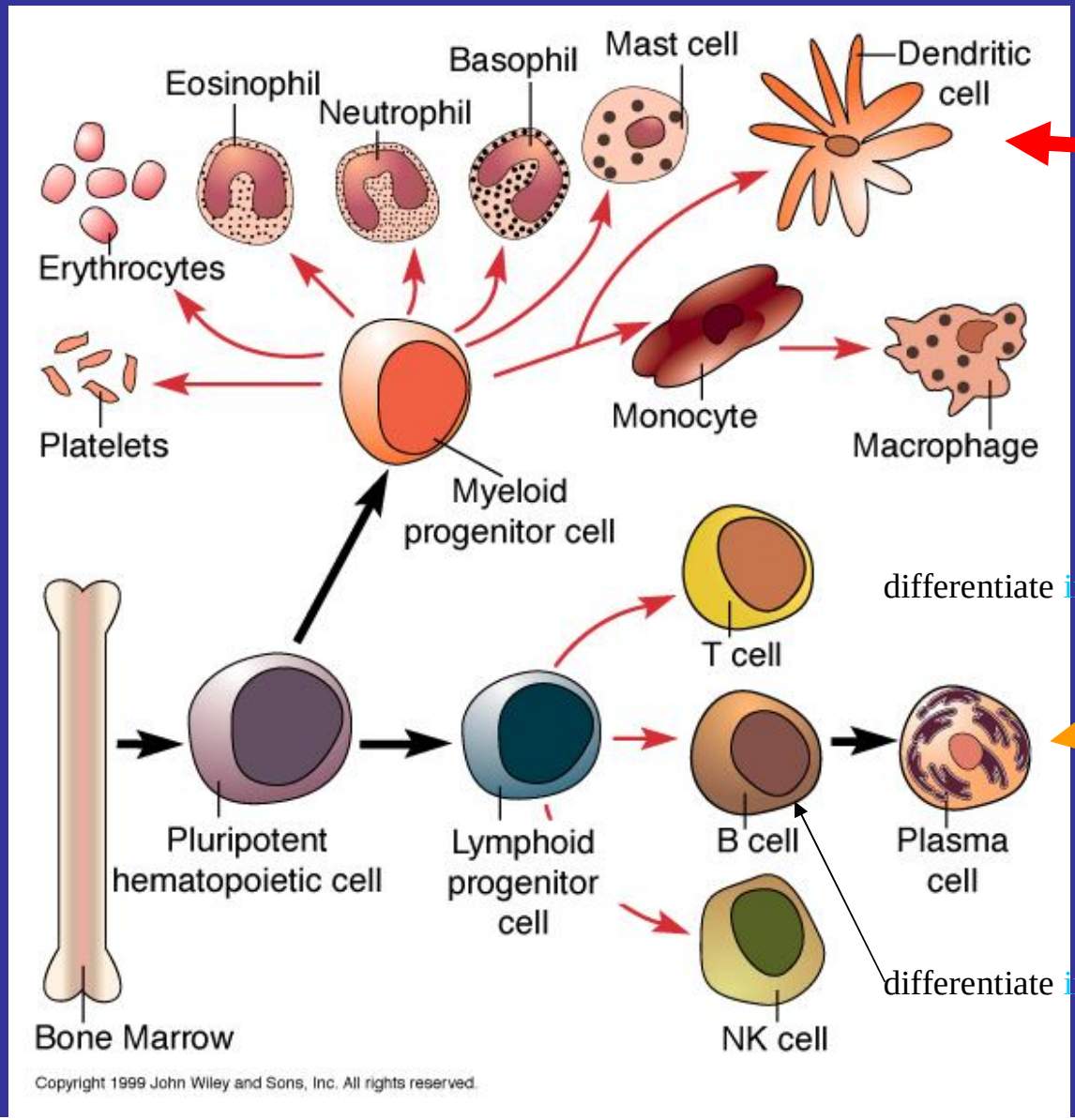
INMUNOLOGIA MOLECULAR

	HUMORAL	CELULAR
INMUNIDAD NATURAL (sin memoria)	IgM	Macrófagos Células NK Neutrófilos
INMUNIDAD ADAPTATIVA (con memoria)	IgG	Linfocitos CD4+ (helper) Linfocitos CD8+ (CTL)

Pathways of differentiation of a pluripotent hematopoietic stem cell of the bone marrow



Stem cells

ANTIGENOS

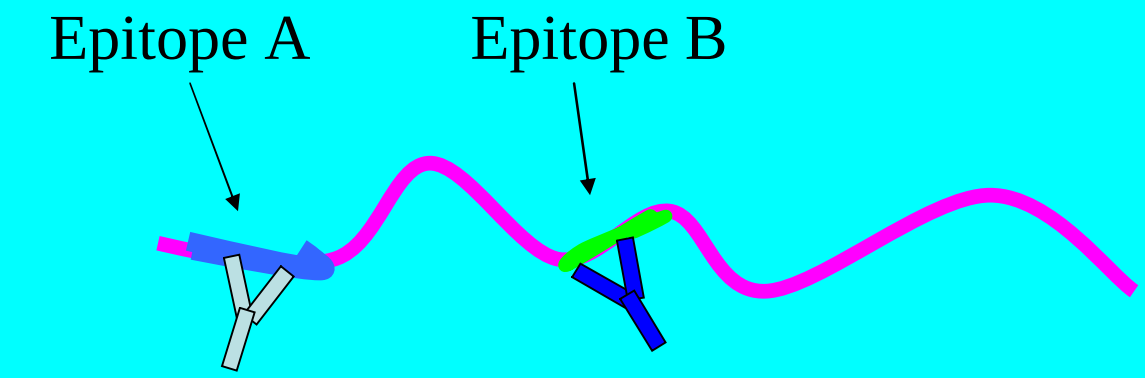
Moléculas reconocidas por receptores en células B (B cell receptor) o T (T cell receptor).

Hapteno : antígeno que para ser reconocido debe ser unido a un carrier (hidratos de carbono)

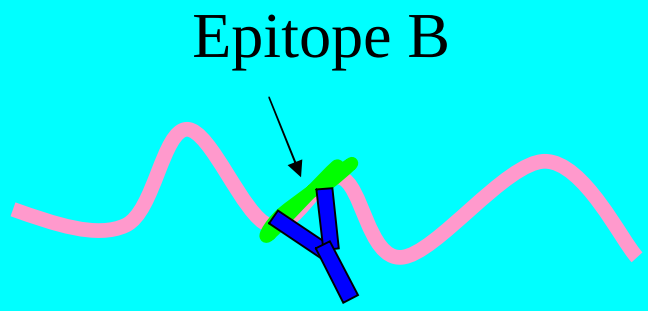
How many environmental antigens are we exposed to during our lifetime?

The immune system responds to hundreds of thousands of foreign antigens introduced from the environment

Examples of antigen, immunogen and epitopes



Antigen



Immunogen

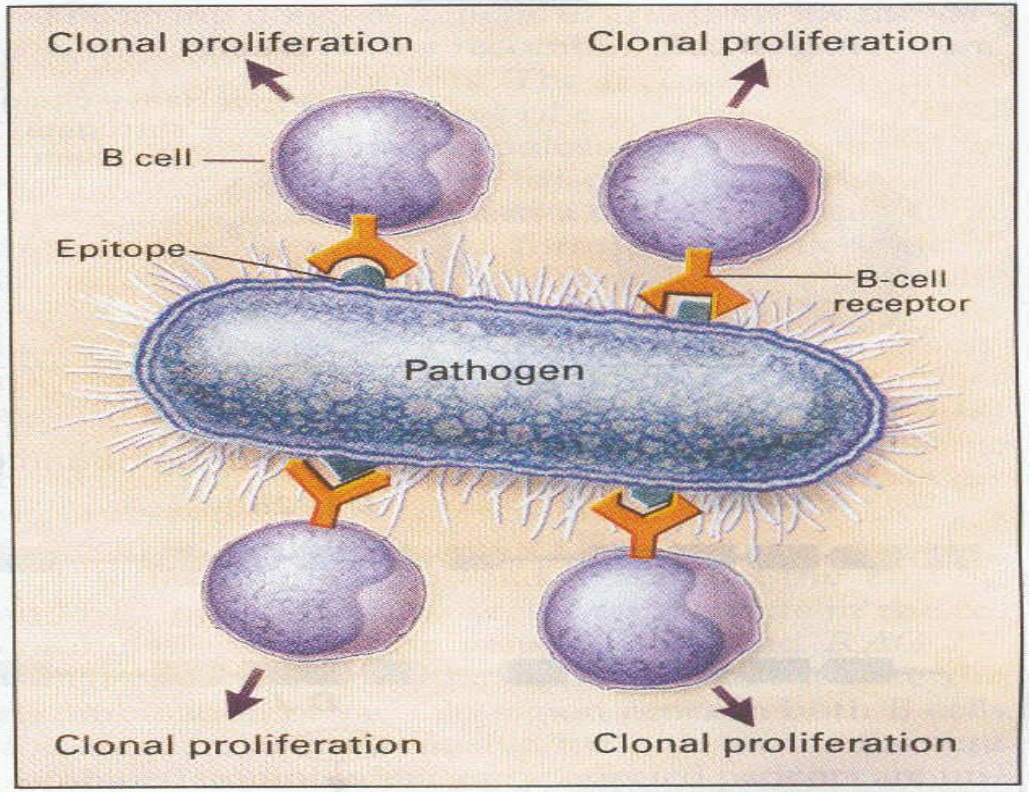
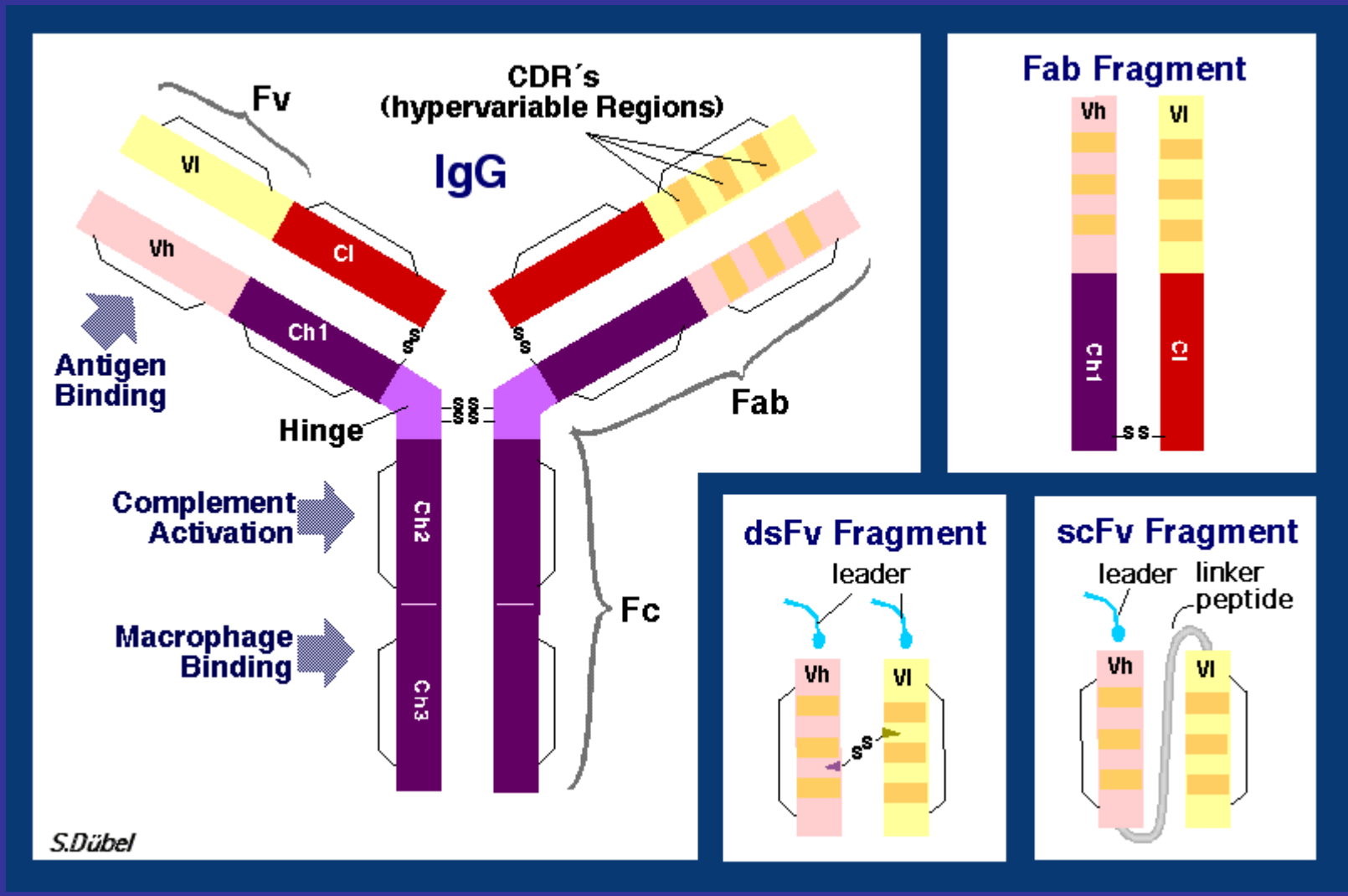
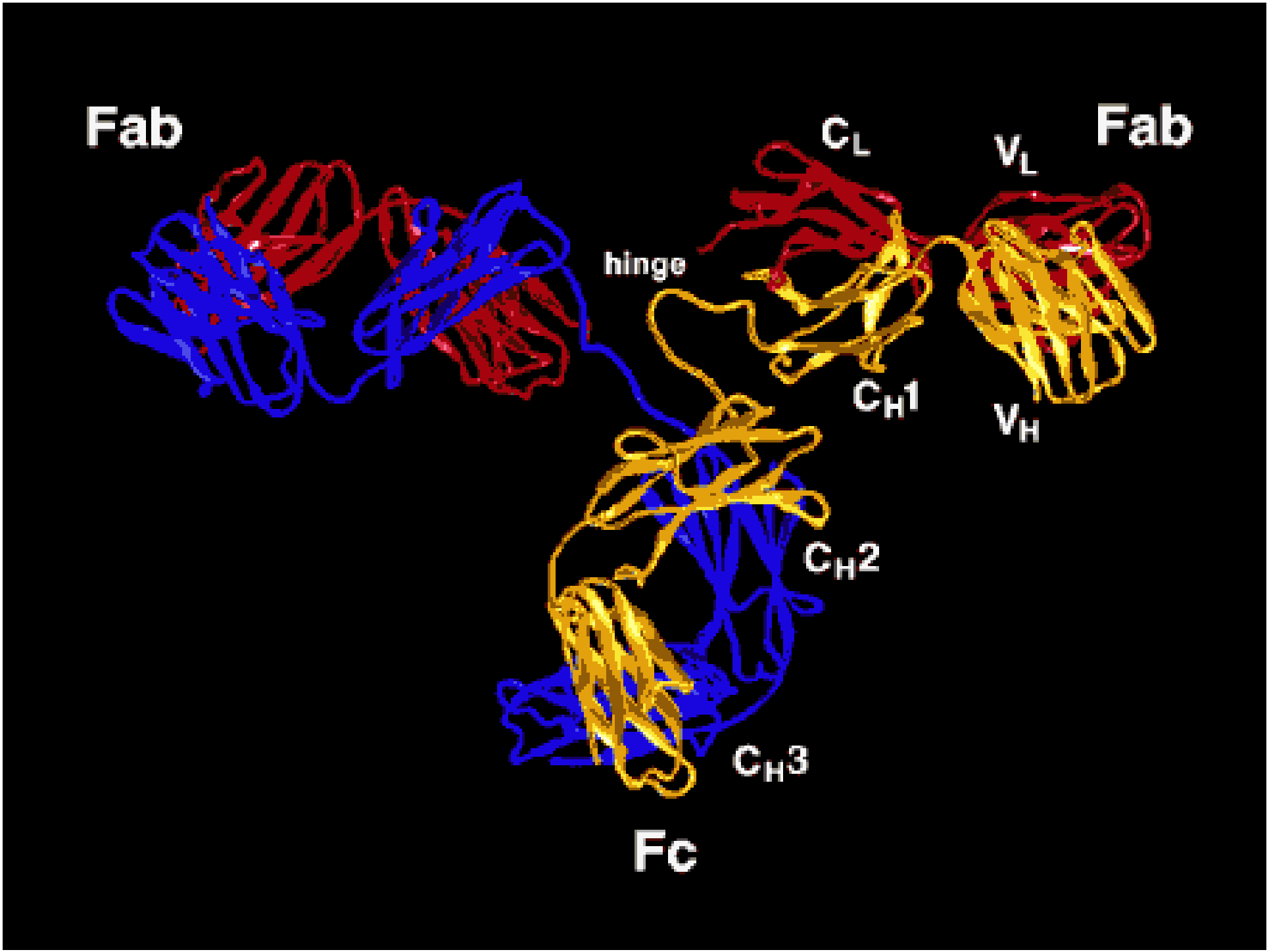


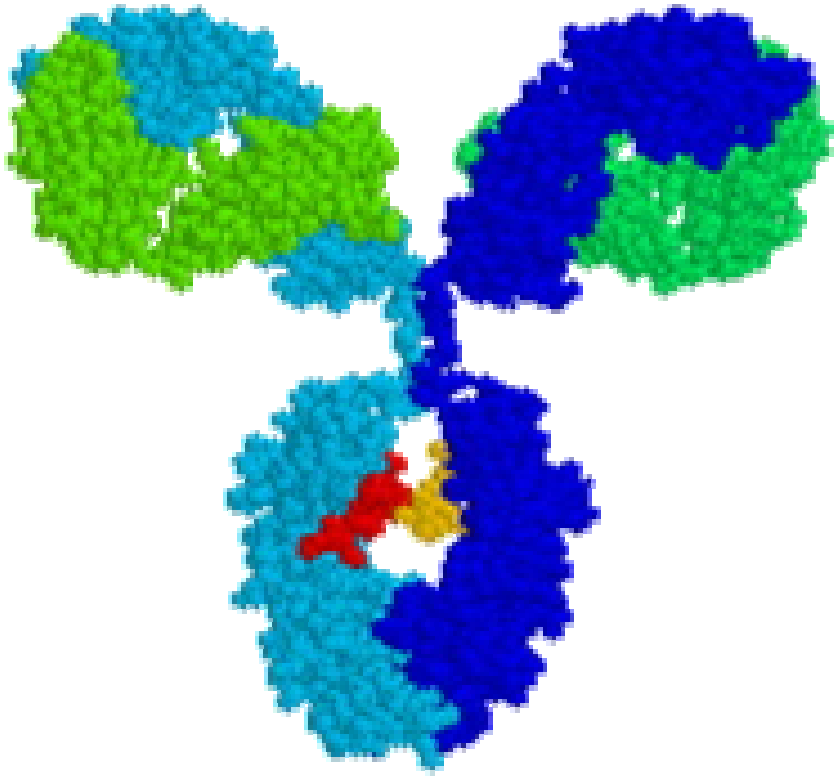
Figure 6. Recognition of Epitopes by B Cells.

Using the antibody molecule as its receptor, the B cell recognizes epitopes on the surface of the antigen. If it is stimulated by this contact, the B cell proliferates, and the resulting clones can secrete antibody whose specificity is the same as that of the cell-surface receptor that bound the epitope. Responses usually involve several different clones of lymphocytes and are therefore referred to as polyclonal. Although not shown here, for each epitope there may be several different lymphocyte clones with different B-cell receptors, each of which recognizes the epitope in a slightly different way and therefore with a different binding strength (affinity).

ANTICUERPOS

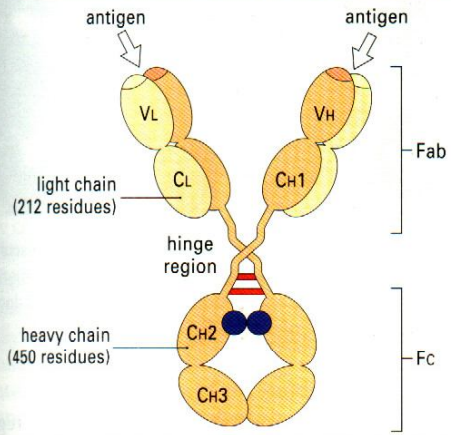

Fv
CDR´s
(hypervariable Regions)
IgG
Vl
Cl
Vh
Ch1
Antigen
Binding
Hinge
Fab
Complement
Activation
Ch2
Macrophage
Binding
Ch3
Fc
S.Dübel
Fab Fragment
Vh
Vl
Ch1
Cl
s s
dsFv Fragment
leader
Vh
Vl
s s
scFv Fragment
leader
linker
peptide
Vh
Vl


Fab
Fab
C_L
V_L
hinge
C_H1
V_H
C_H2
C_H3
Fc

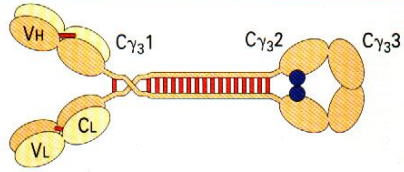


Structural characteristics of various human immunoglobulins

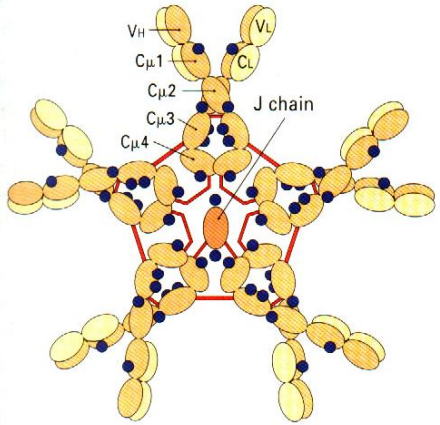
1. IgG1



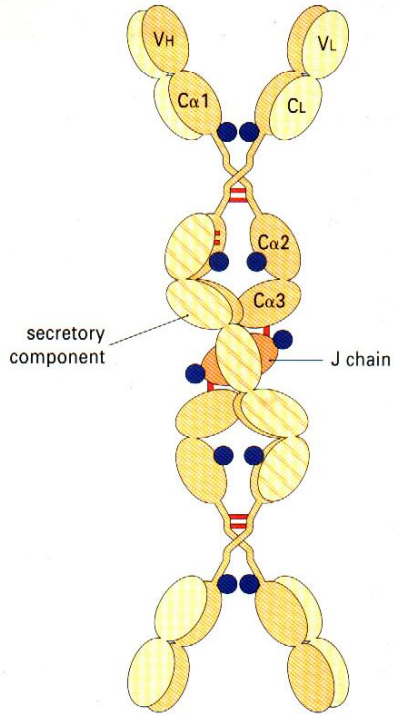
2. IgG3



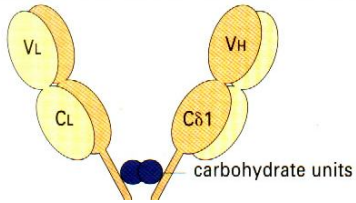
3. IgM



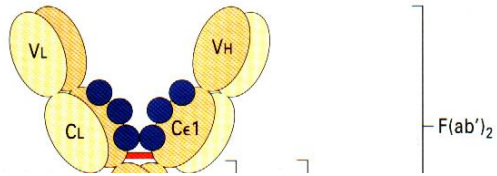
4. IgA (sIgA)



5. IgD



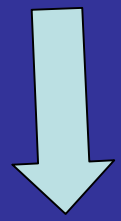
6. IgE



RECEPTORES ANTIGENICOS

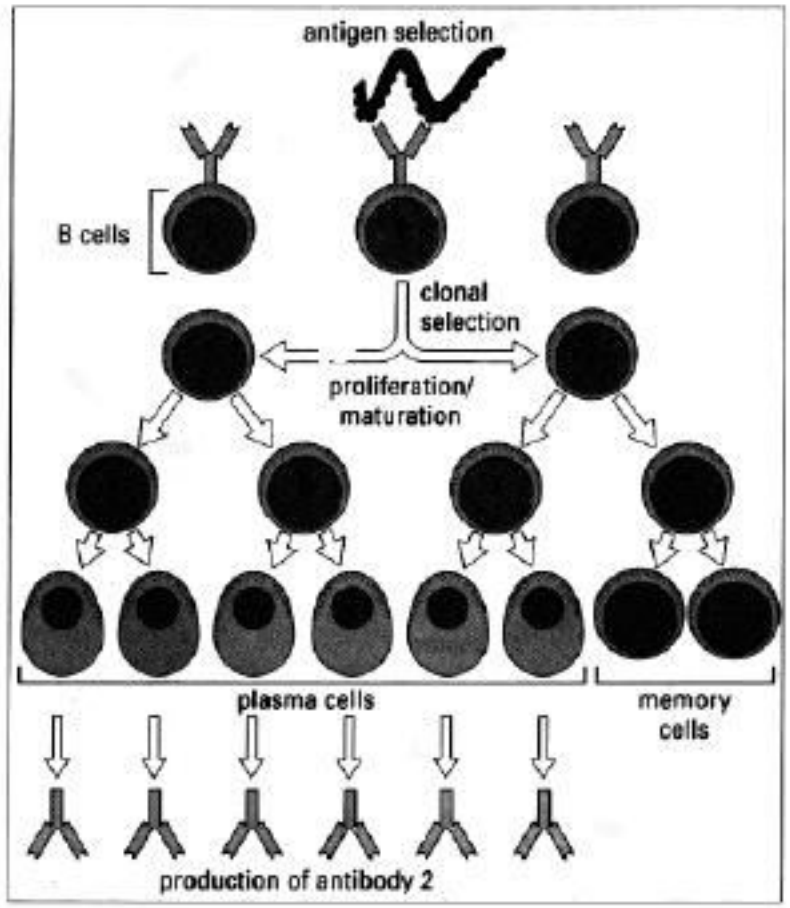
- **B cell receptor (linfocitos B) = reconoce Ag nativo**
- **T cell receptor (linfocitos T) = reconoce Ag procesado**

ANTIGENO CON MULTIPLES EPITOPES



SUERO CON MULTIPLES ANTICUERPOS

Only B-cells with a complementary antibody receptor proliferate and mature.



The B-lymphocytes expressing antibody receptor with the best fit to the **epitope** (antibody-binding domain on the antigen) are the ones that proliferate and give rise to antibody in serum

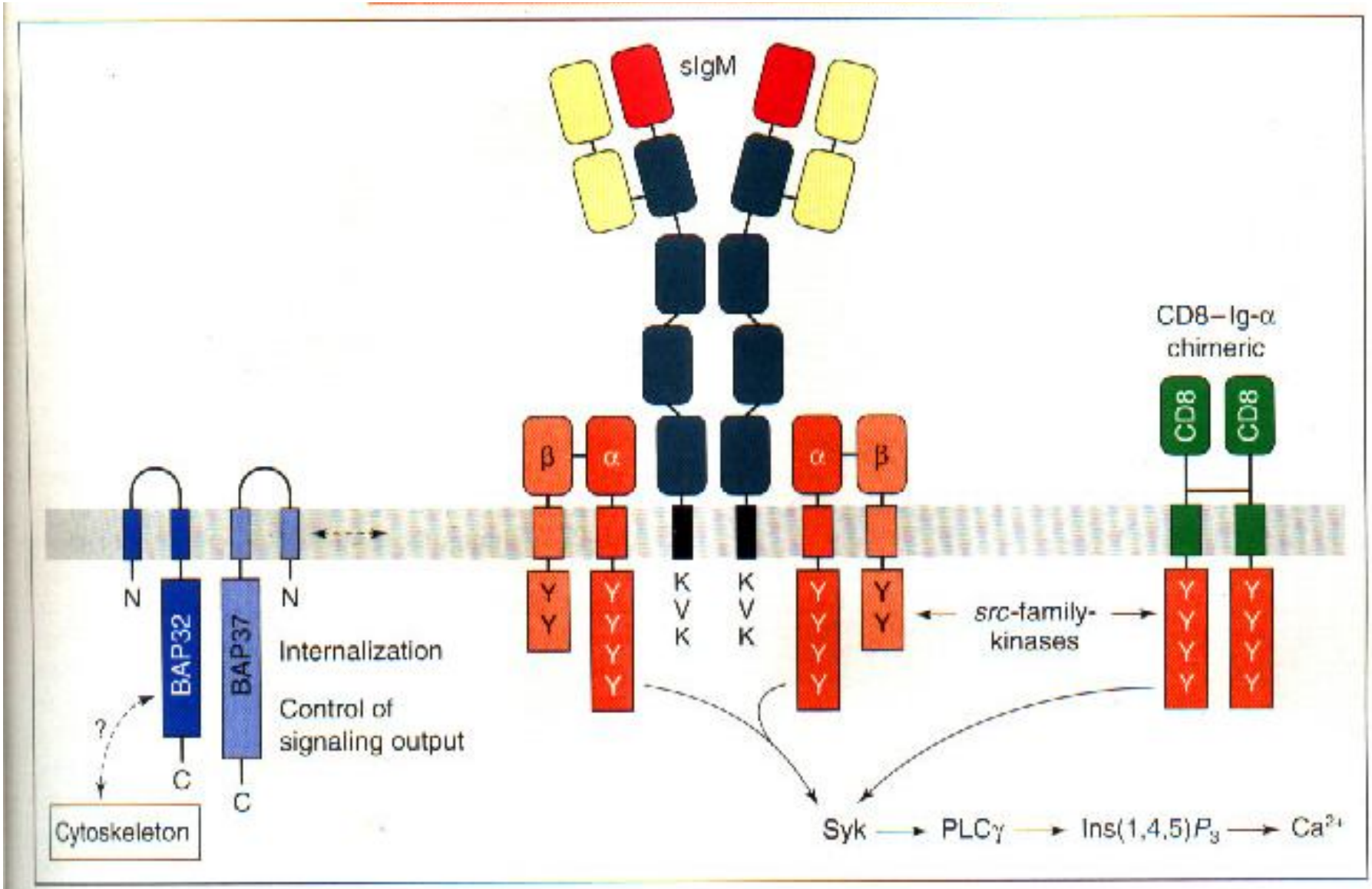
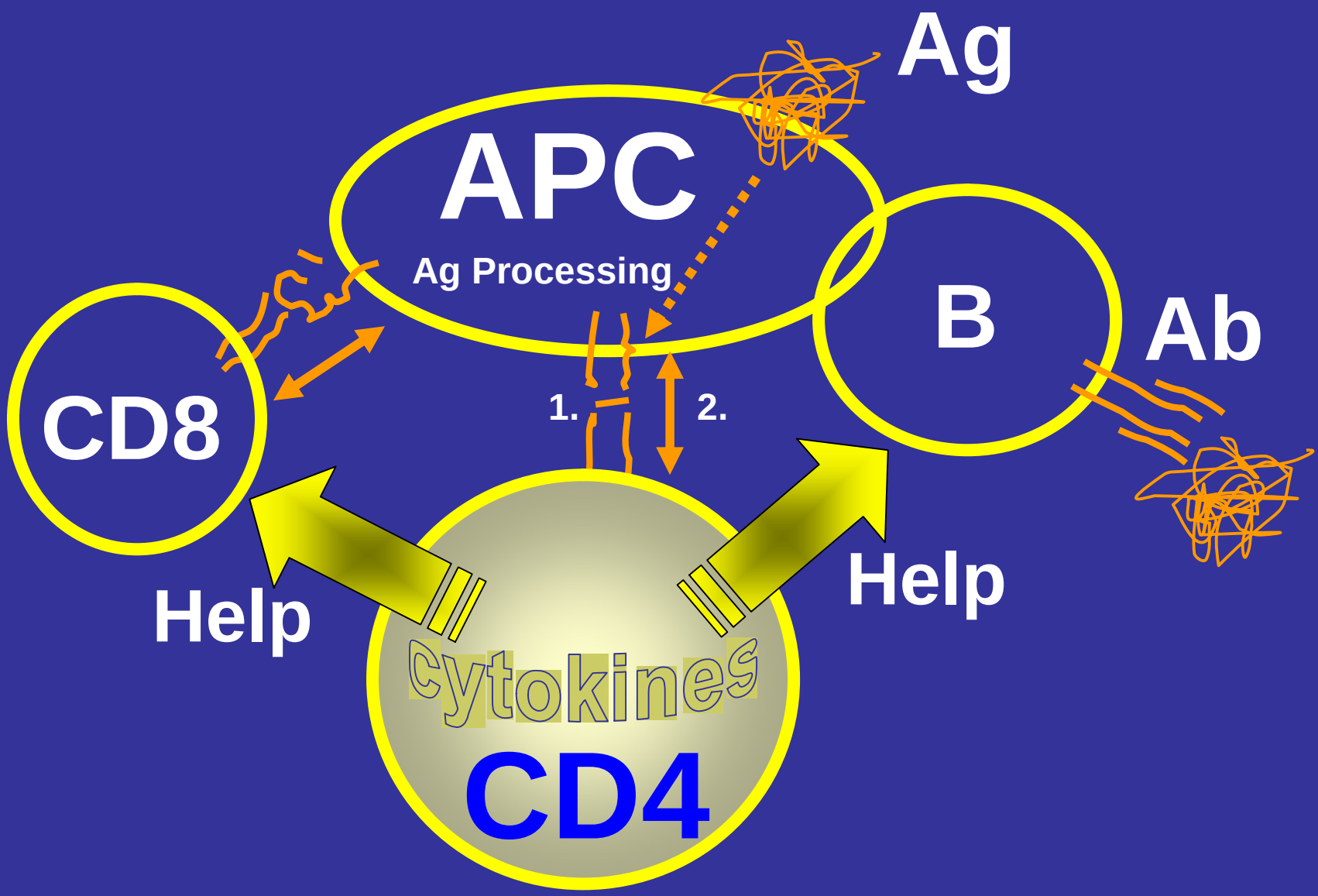
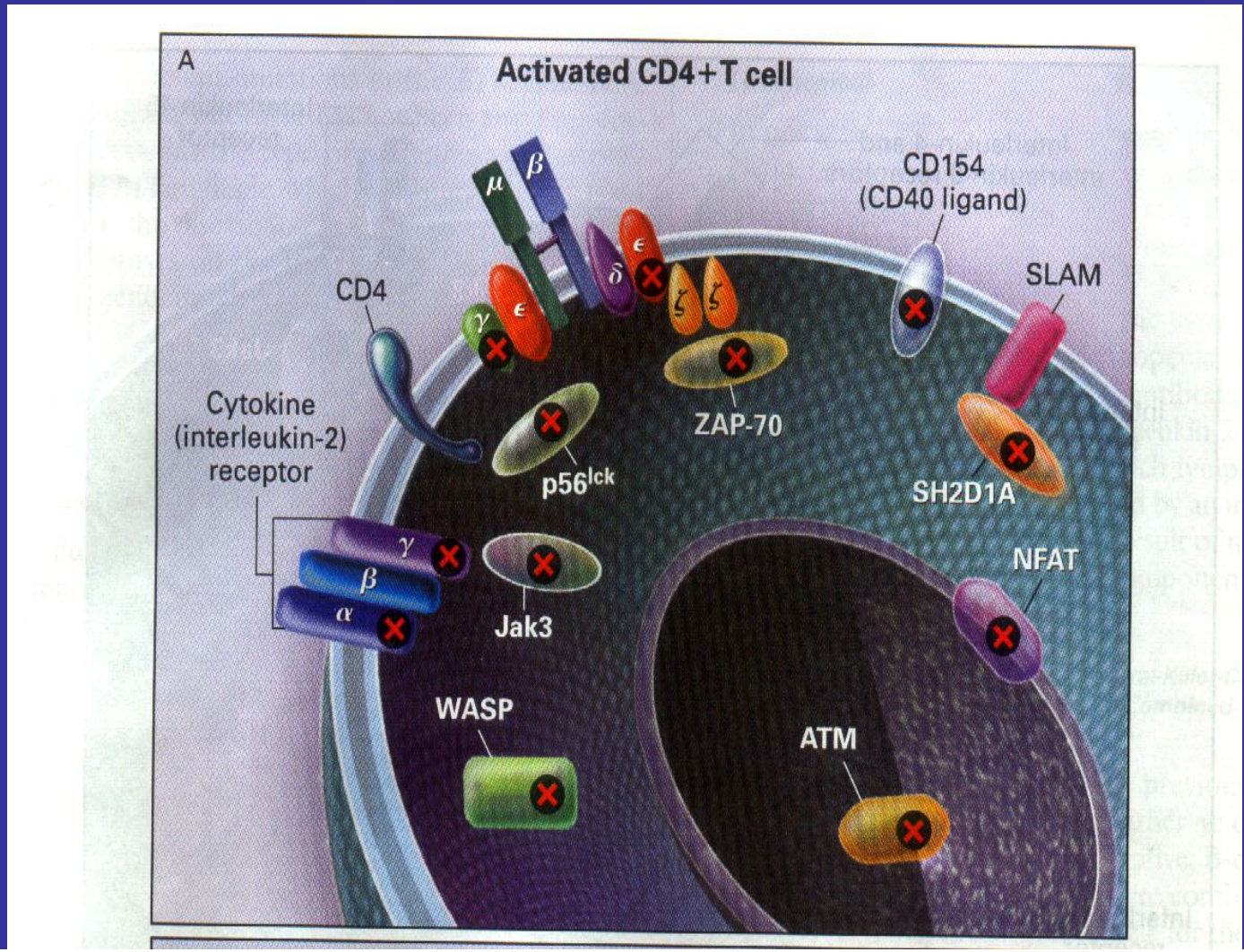


Fig. 1. *Structural model of the B-cell antigen receptor complex (BCR), the chimeric CD8–Ig-α molecule and the BCR-associated proteins BAP32 and BAP37. BAPs and the surface (s)IgM molecule interact with each other in the membrane via their transmembrane domains. BAPs may control activation of the BCR and its association with cytoskeletal elements. Upon crosslinking of the BCR or CD8–Ig-α chimeric receptor, src-family kinases are activated, tyrosine residues (Y) in the immunoreceptor tyrosine-based activation motif (ITAM) are phosphorylated and the spleen tyrosine kinase Syk is recruited to the activated receptors. These events induce activation of phospholipase Cγ (PLCγ), production of inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] and Ca^{2+} release.*

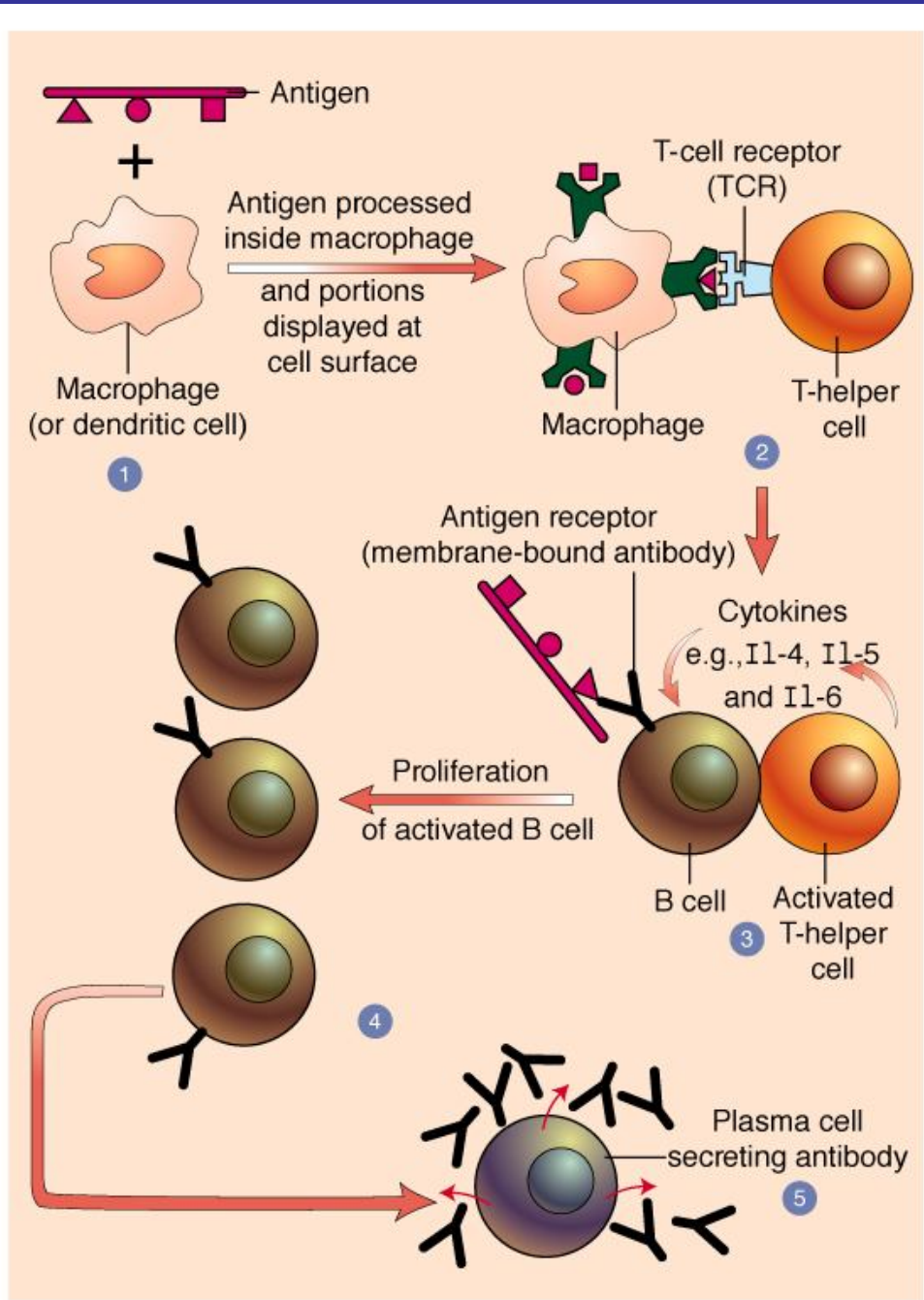
The Immune Response:



LINFOCITO CD4 ACTIVADO



Role of T-Helper cells in antibody formation.



LINFOCITO B EN REPOSO

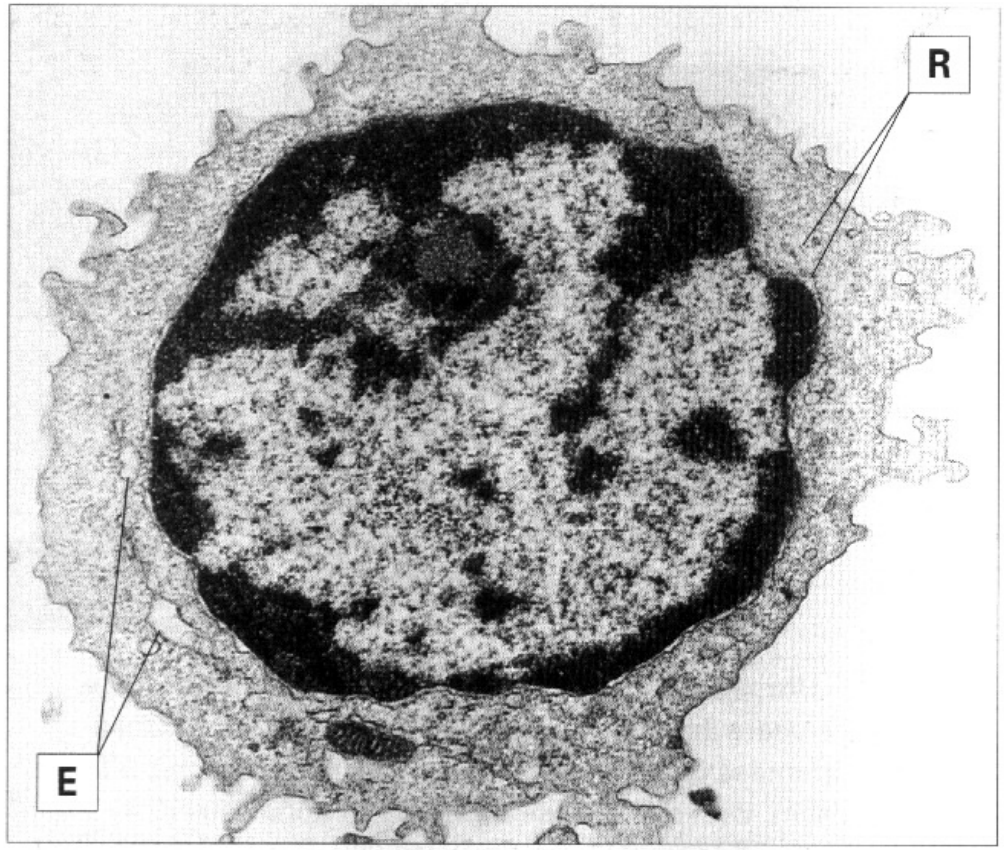


Fig. 2.27 Ultrastructure of resting B cells. These cells have no Gall body or granules. Scattered ribosomes (R) and isolated strands of rough endoplasmic reticulum (E) are seen in the cytoplasm. Development of the Golgi-lysosomal system in the B cell occurs on activation. ×11 500.

LINFOBLASTOS B

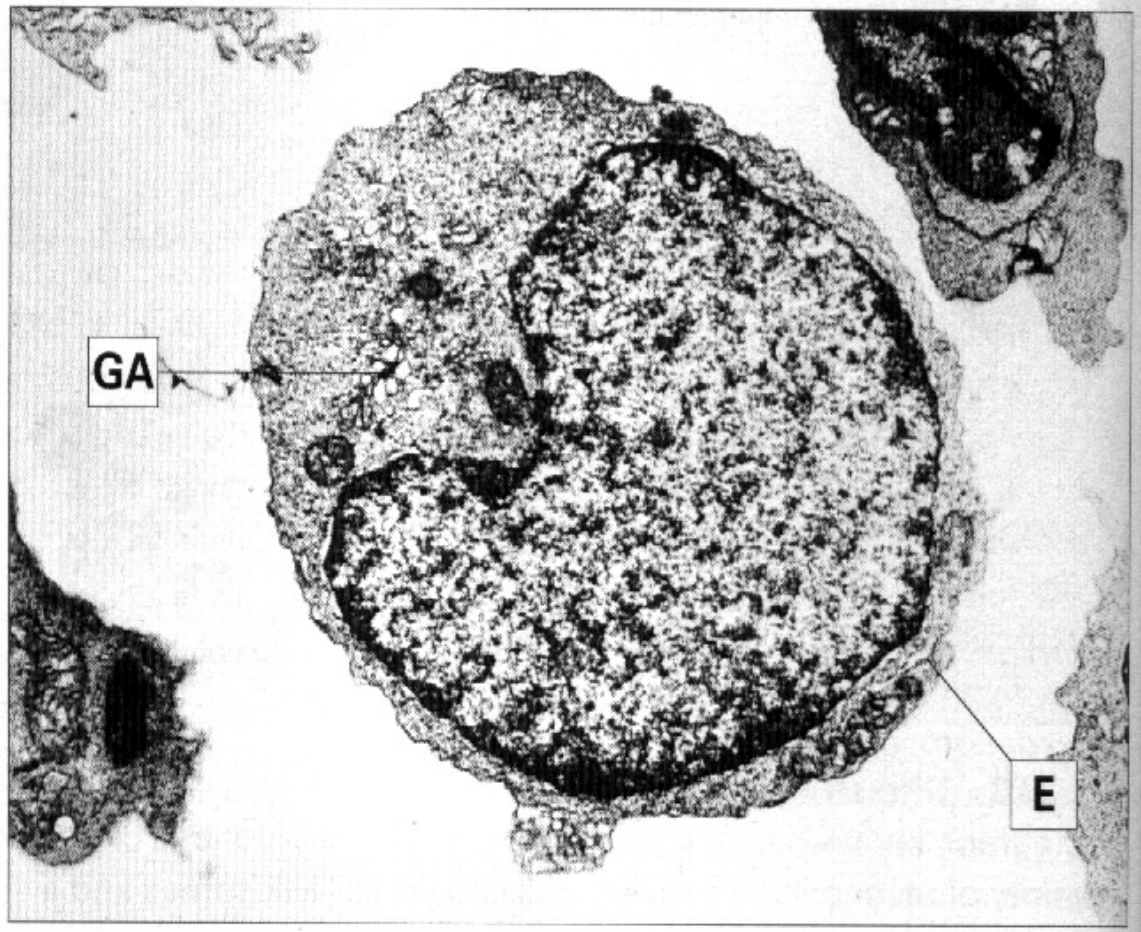


Fig. 2.28 Ultrastructure of B-cell blasts. The main feature of activated B cells is the development of the machinery for immunoglobulin synthesis. This includes rough endoplasmic reticulum (E), free polyribosomes and the Golgi apparatus (GA), which is involved in glycosylation of the immunoglobulins. ×7500.

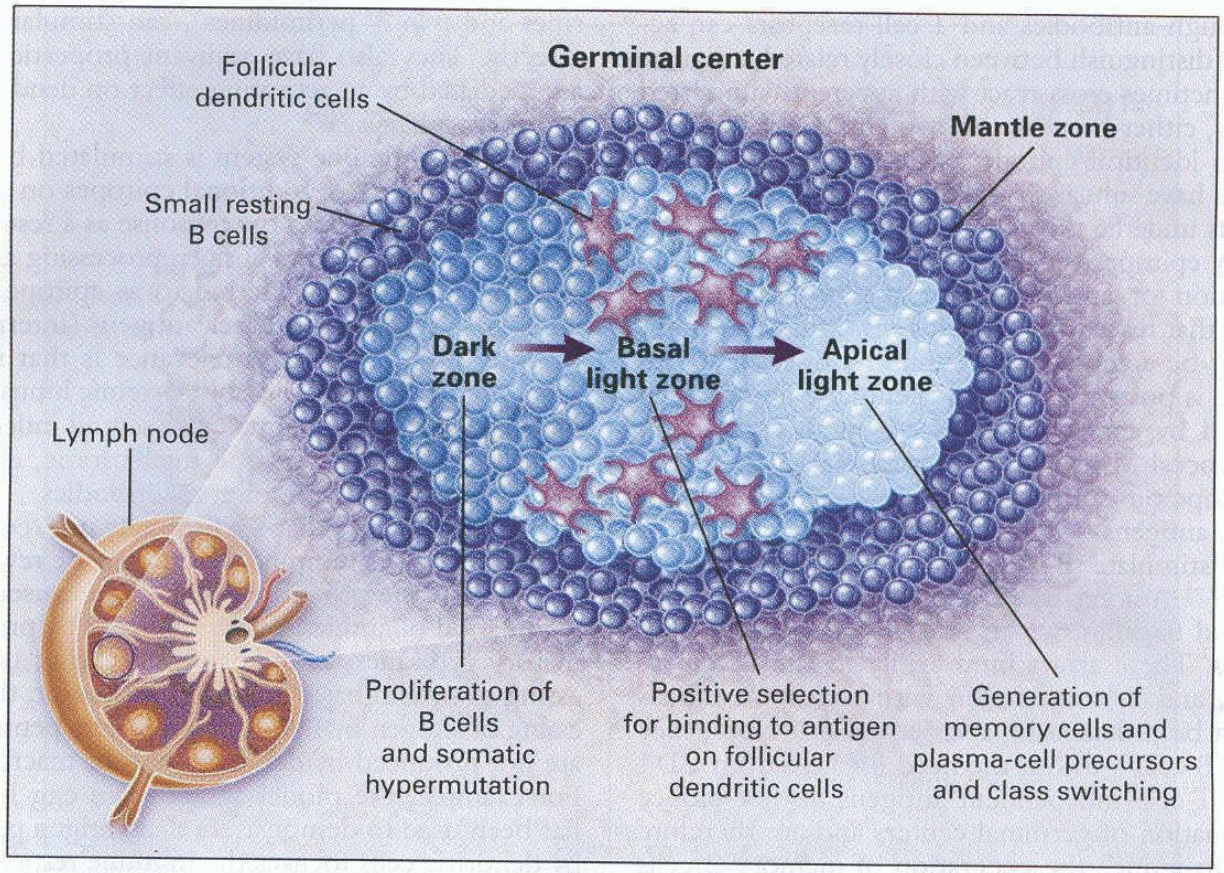
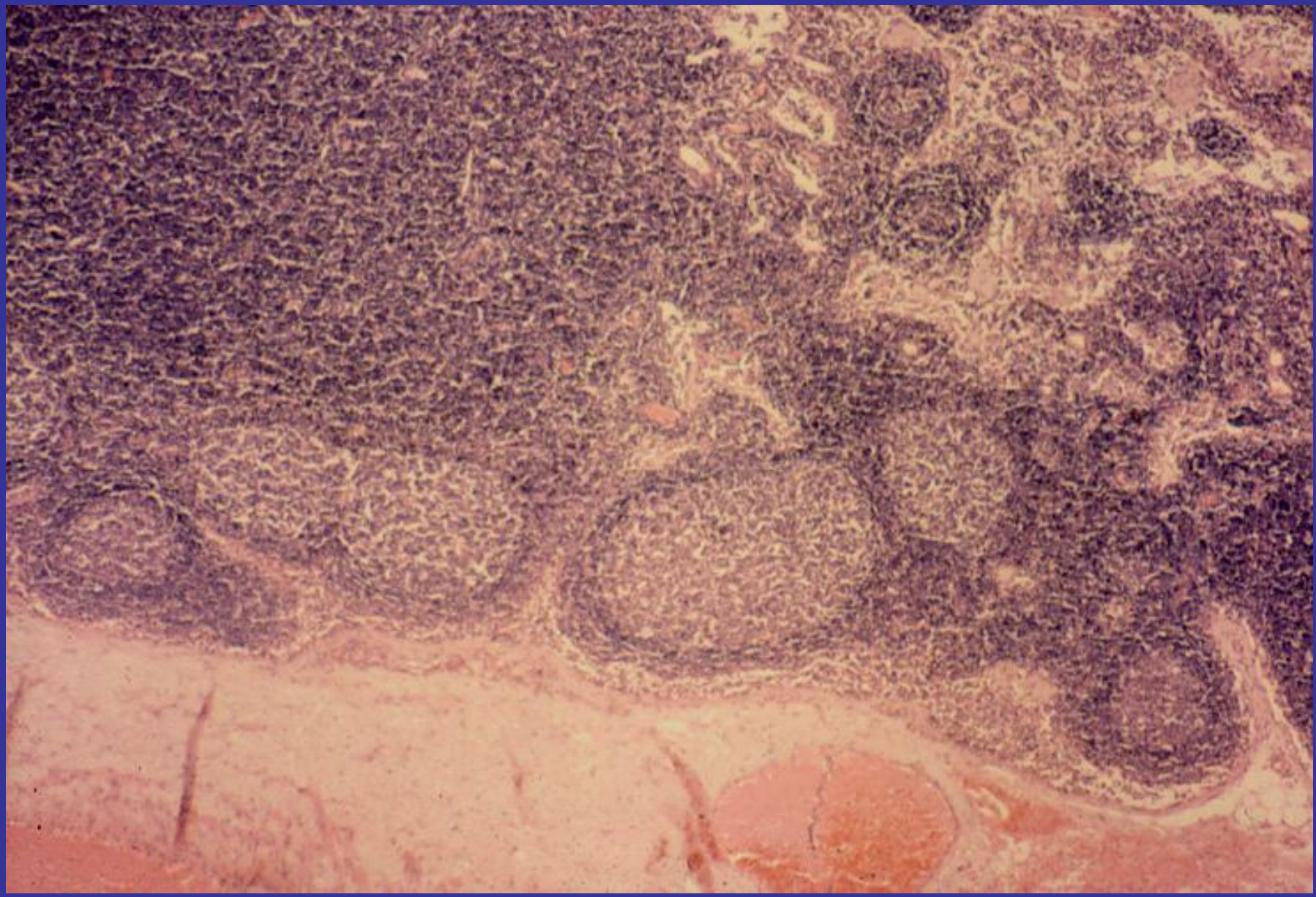


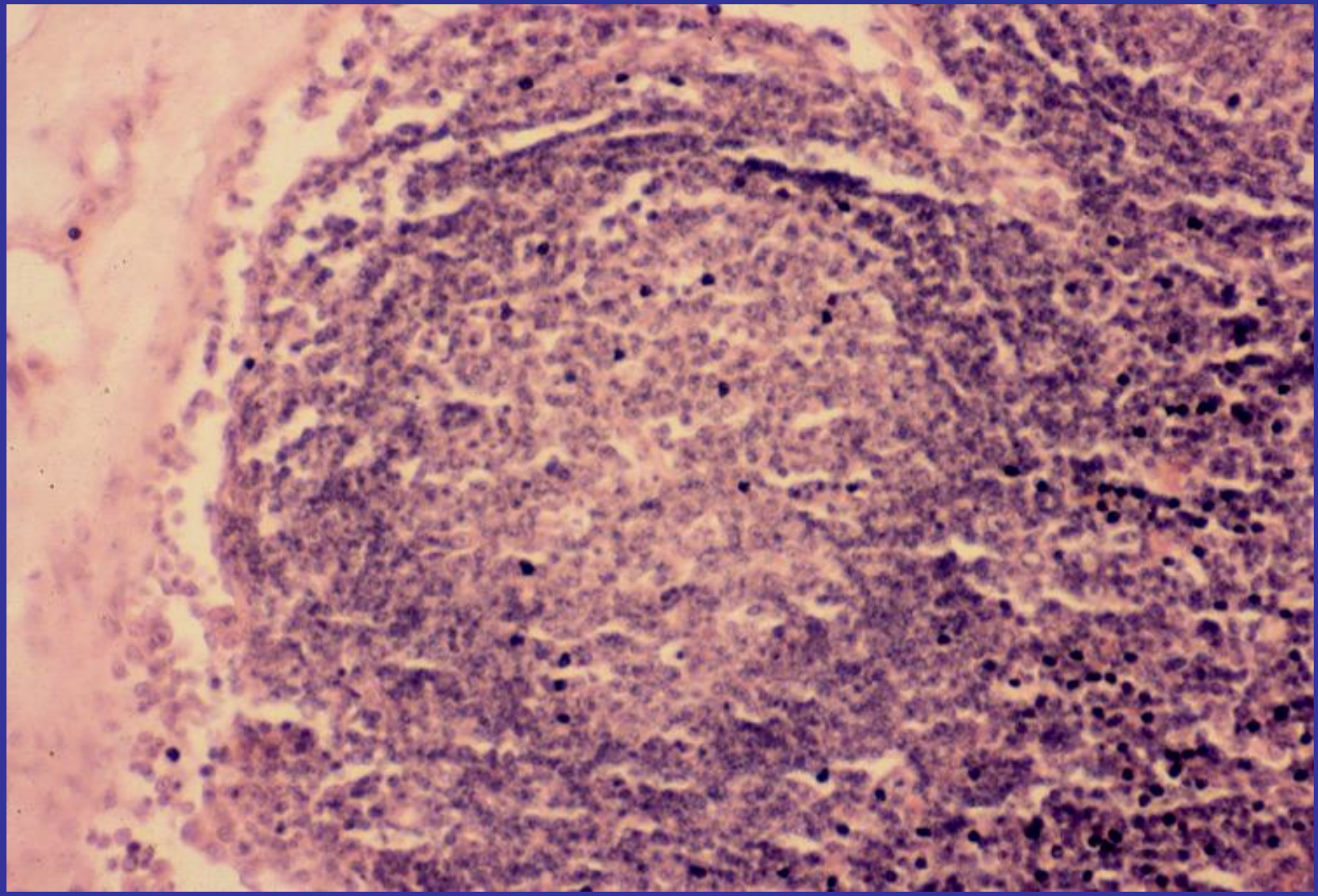
Figure 8. The Germinal Center.

During the initiation of the acquired immune response, germinal centers form in the secondary lymphoid tissues in order to create a microenvironment where all the necessary antigen-specific and innate antigen-presenting cells can interact. Several cytokines, such as interleukin-2, 4, 6, and 10 and transforming growth factor β, and various cell-surface molecules, including CD40, CD19, CD21, and B7, are critically important for these interactions. Antigen-stimulated proliferation of B cells occurs in the dark zone and is accompanied by the fine-tuning of specificity resulting from somatic hypermutation of the immunoglobulin variable-region genes. On reaching the basal light zone, high-affinity antigen-specific B cells are positively selected as a result of their interaction with antigen–antibody complexes on the surface of follicular dendritic cells. B cells that are not positively selected undergo apoptosis and are phagocytosed by tingible-body macrophages. The positively selected cells migrate to the apical light zone, where proliferation continues, class switching occurs, and memory cells and plasma-cell precursors are generated.

Lymph node



Germinal center in lymph node



PLASMOCITO

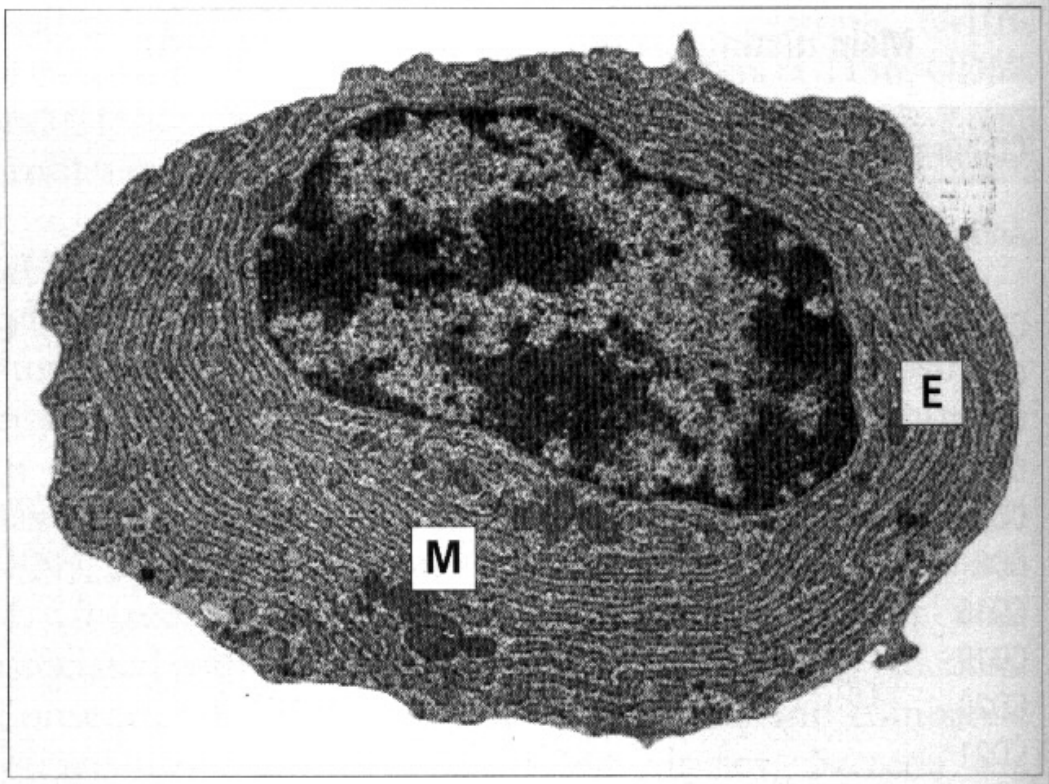


Fig. 2.33 Ultrastructure of the plasma cell. The plasma cell is characterized by parallel arrays of rough endoplasmic reticulum (E). In mature cells, these cisternae become dilated with immunoglobulins. Mitochondria (M) are also seen. ×5000. (Adapted from Zucker-Franklin D, Greaves MF, Grossi CE, *et al. Atlas of Blood Cells: Function and Pathology*. Vol II. 2nd edn. Milan: EE Ermes, Philadelphia: Lea and Febiger, 1988.)

PLASMOCITO APOPTOTICO

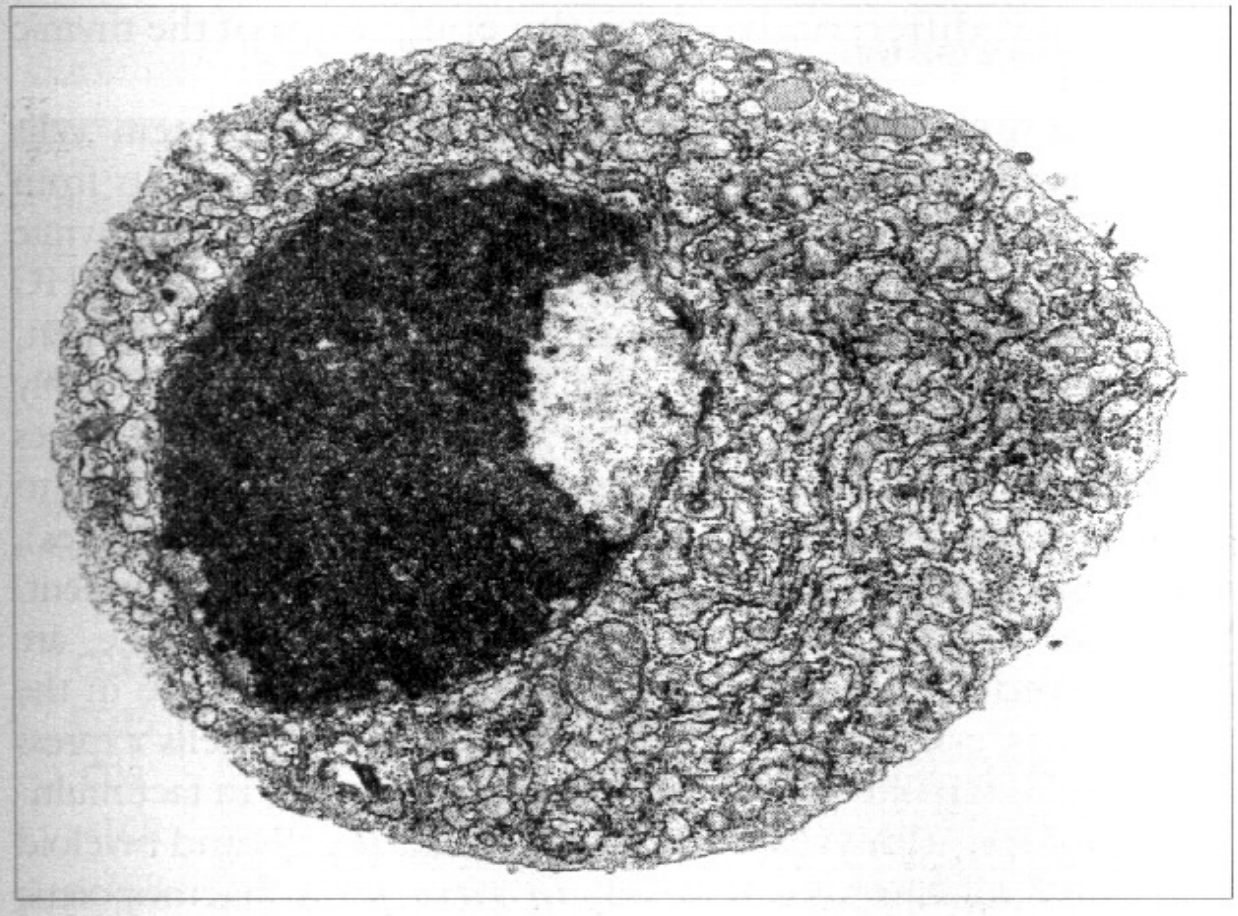
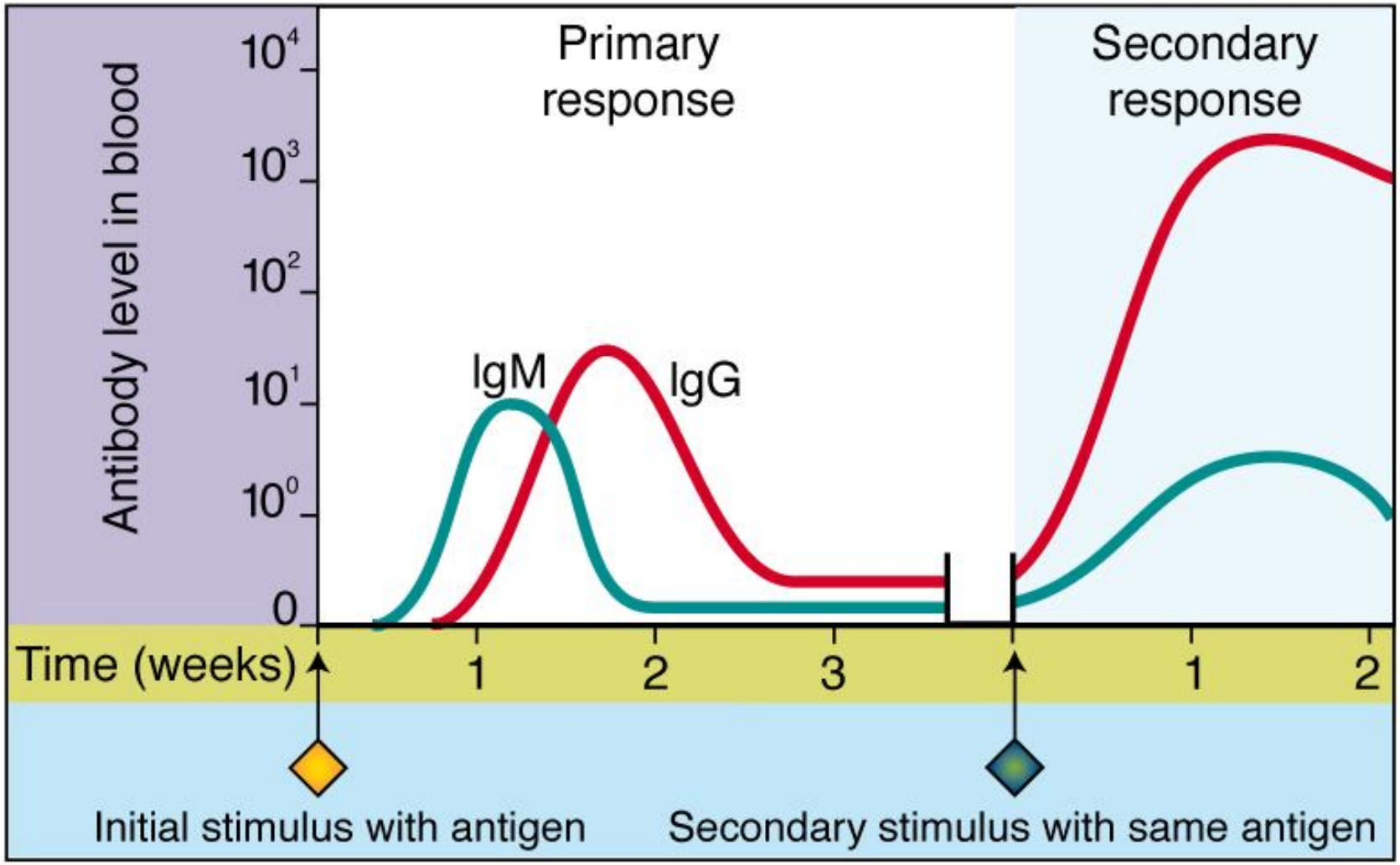


Fig. 2.35 Plasma cell death by apoptosis. Plasma cells are shortlived and die by apoptosis (cell suicide). Note the nuclear chromatin changes, which are characteristic of apoptosis. ×5000.

Primary and secondary antibody responses.



¿Cómo se produce la gran diversidad de anticuerpos?

Susumu Tonegawa

Premio Nobel 1987

Producción de cadena κ

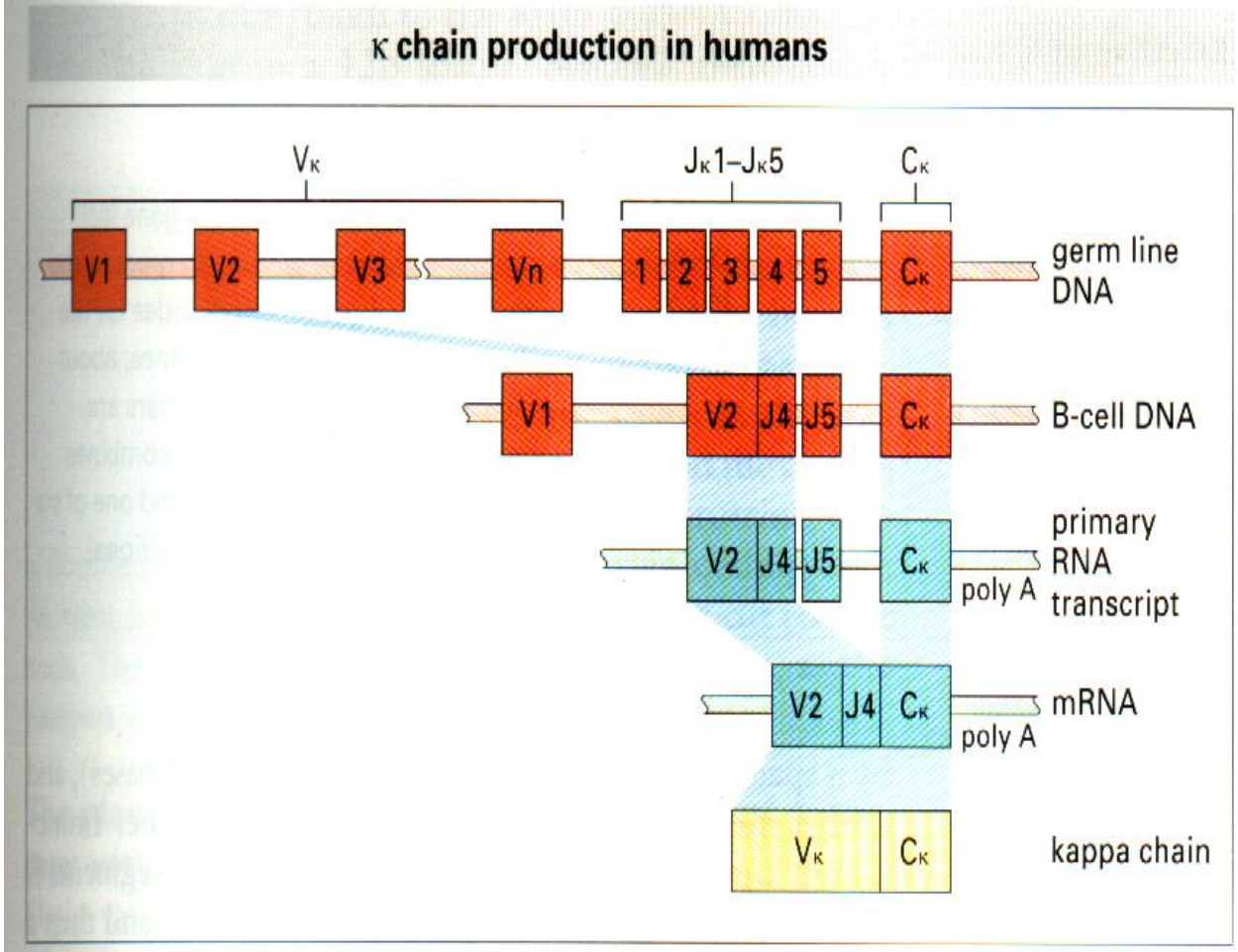


Fig. 4.29 During differentiation of the pre-B cell one of several Vκ genes on the germ line DNA (V1–Vn) is recombined and apposed to a Jκ segment (Jκ1–Jκ5). The B cell transcribes a segment of DNA into a primary RNA transcript that contains a long intervening sequence of additional J segments and introns. This transcript is processed into mRNA by splicing the exons together, and is translated by ribosomes into kappa (κ) chains; B-cell DNA is coloured light brown; RNA is coloured green; and immunoglobulin peptides are coloured yellow. The rearrangement illustrated is only one of the many possible recombinations.

GENES CODIFICANTES PARA INMUNOGLOBULINAS

- Cluster IGH: cadena H; crom.14;V,D,J,C.
- Cluster IGK: cadena K; crom.2; V,J,C.
- Cluster IGL(λ): cadena λ; crom. 22; V,J,C.

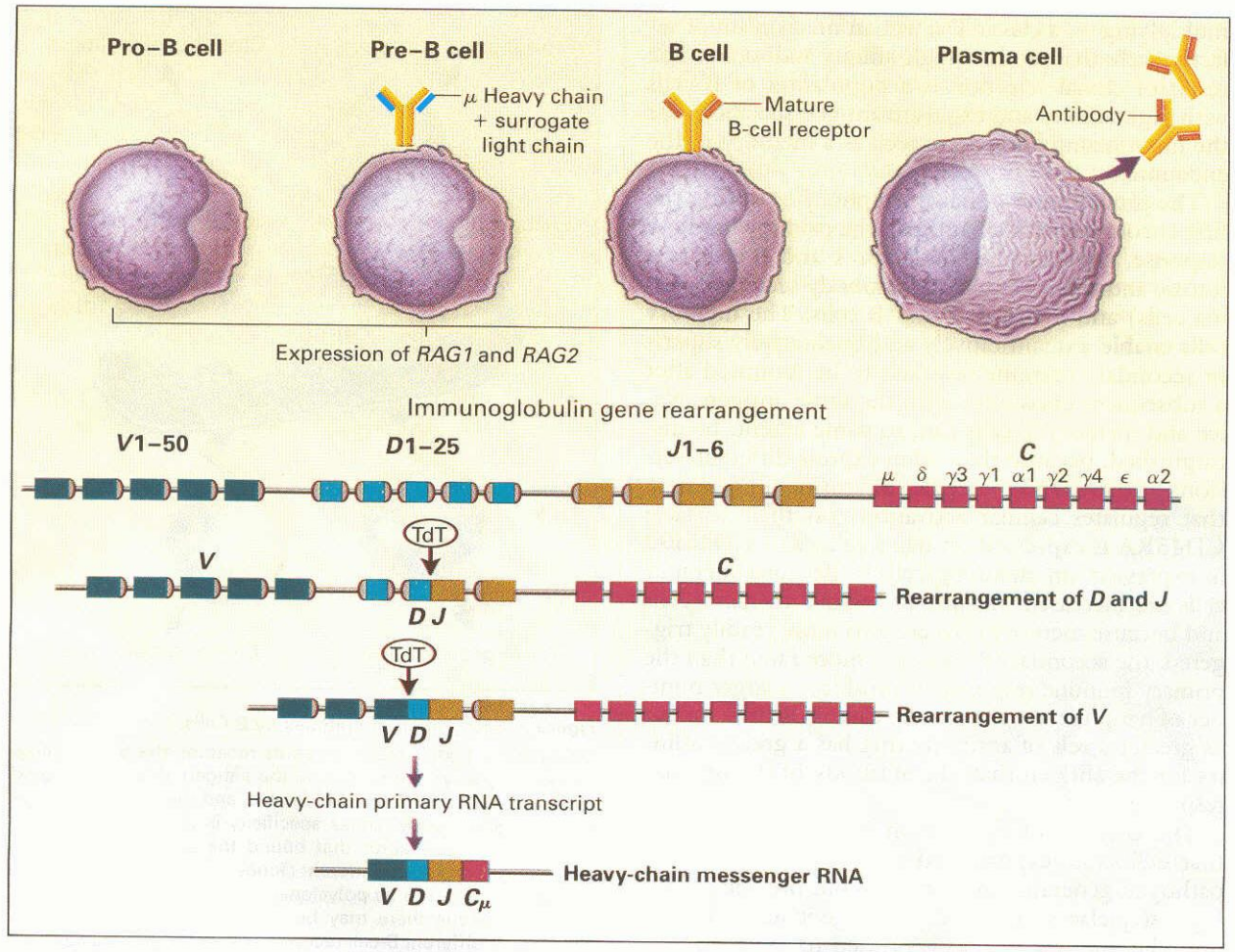


Figure 5. Diversity of Antigen Receptors.

The enormously diverse specificities of the antigen receptors are produced by gene rearrangements during the early developmental stages of the lymphocyte. The events involved in generating a coding sequence for the immunoglobulin heavy chain are shown. Early in B-cell development, pro–B cells mature into pre–B cells, at which stages they express the recombination-activating genes *RAG1* and *RAG2*. The recombinases encoded by these genes mediate the random rearrangement of 1 of 25 diversity (*D*) gene segments next to any 1 of 6 joining (*J*) gene segments. This is followed by the rearrangement of any 1 of 50 variable (*V*) gene segments next to the already rearranged *DJ* segment. Different B cells will rearrange a different segment in each pool, thereby creating one level of diversity. Further diversity is brought about by splicing inaccuracies and by the incorporation of nucleotides mediated by the enzyme terminal deoxyribonucleotidyltransferase (TdT). The heavy-chain primary RNA transcript is processed into messenger RNA (mRNA), with splicing of the rearranged *VDJ* segment next to the constant (*C*) region gene. This mRNA will encode a heavy chain that appears on the surface of the pre–B cell together with the surrogate light chain, which is encoded by genes that do not undergo rearrangement. As the pre–B cell continues to mature, the immunoglobulin light-chain genes undergo rearrangement; the resulting light chain replaces the surrogate light chain, and thereby produces a mature IgM B-cell receptor on the cell surface. The B-cell receptors at this stage also usually include IgD antibodies with the same specificity as the IgM molecule, produced by alternative splicing of the rearranged *VDJ* to either the C_μ or the C_δ gene. The expression of *RAG1* and *RAG2* is then switched off. After encountering an antigen, and in the presence of costimulatory signals, the B cell further differentiates into a plasma cell, which secretes high levels of the specific antibody (or into a memory B cell). The same general principles regarding the rearrangement process apply to the generation of α/β and γ/δ T-cell receptors. The gene segments in the figure are not drawn to scale.

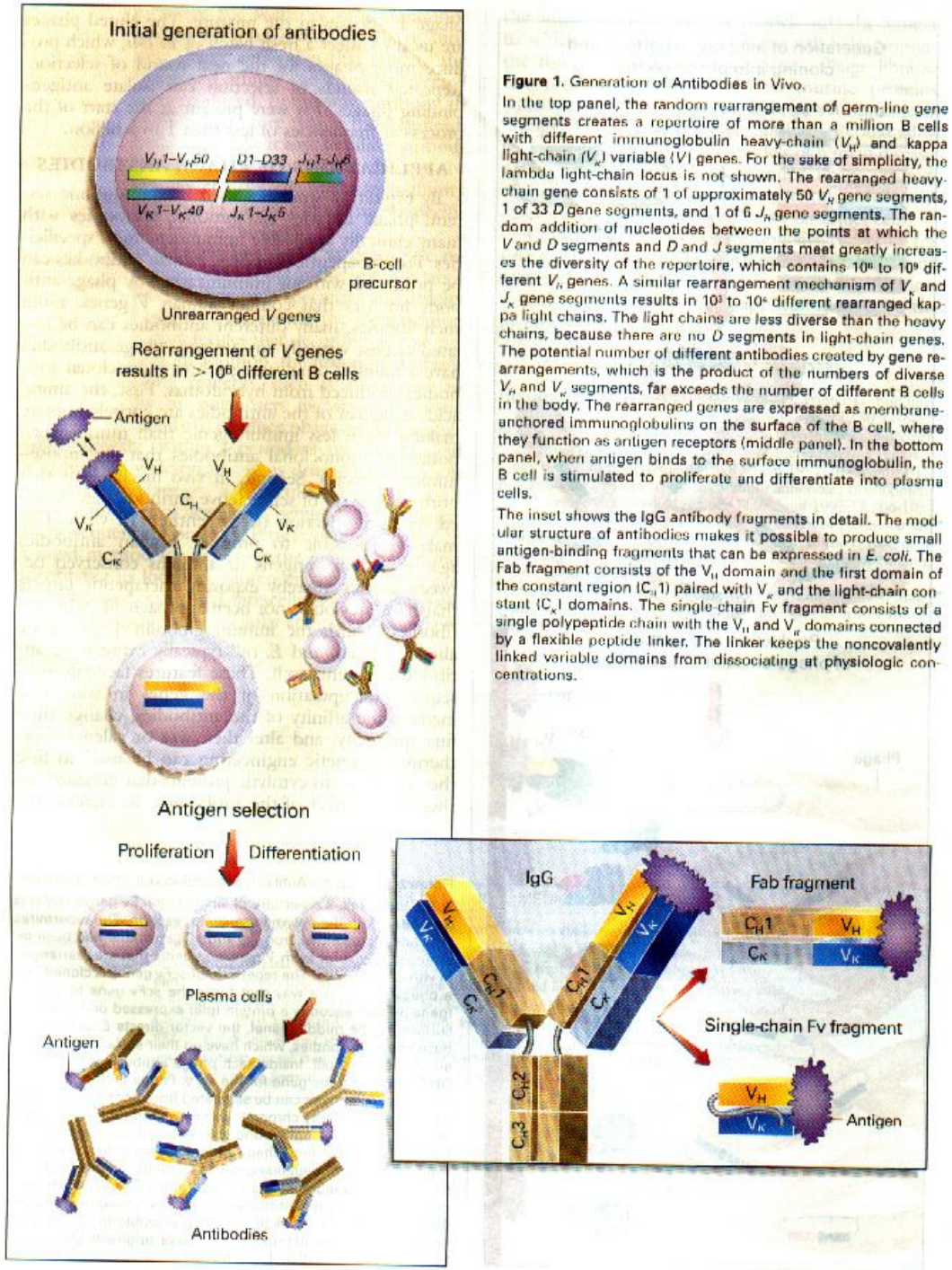
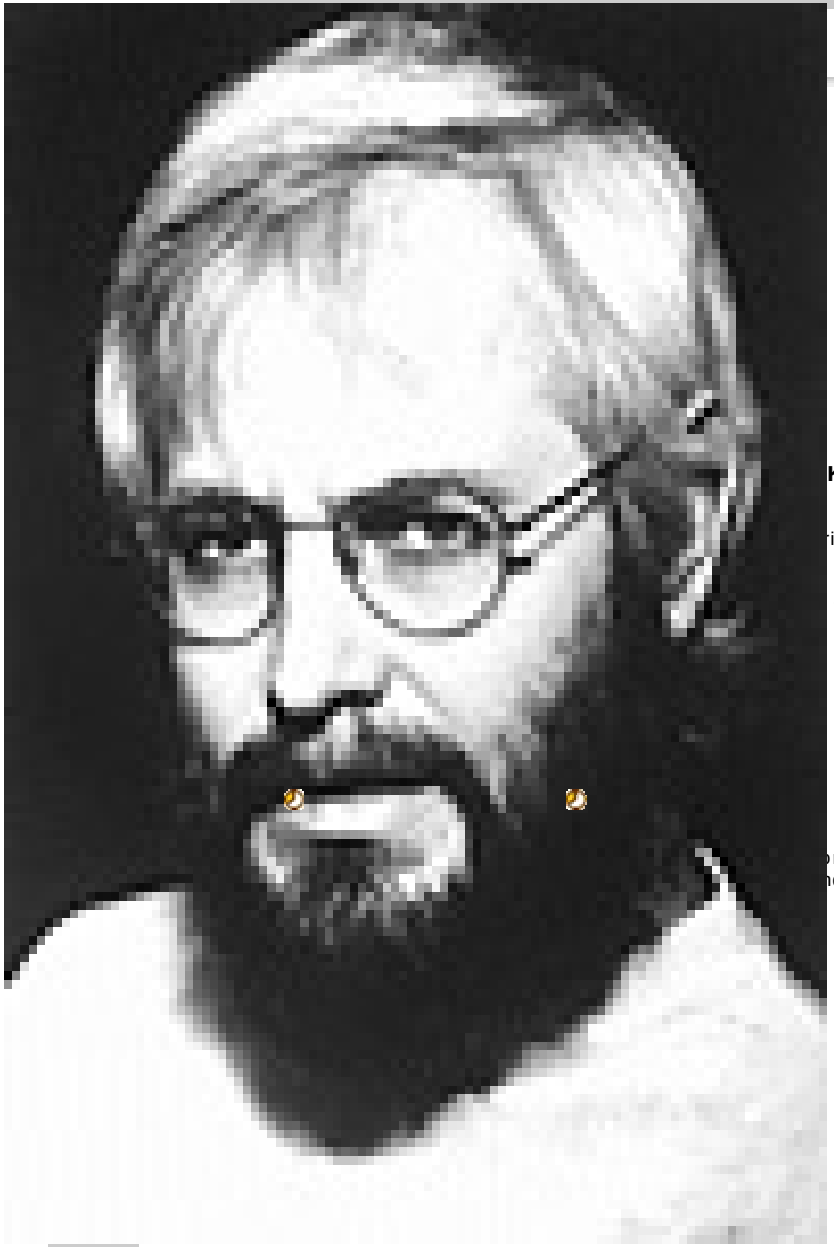


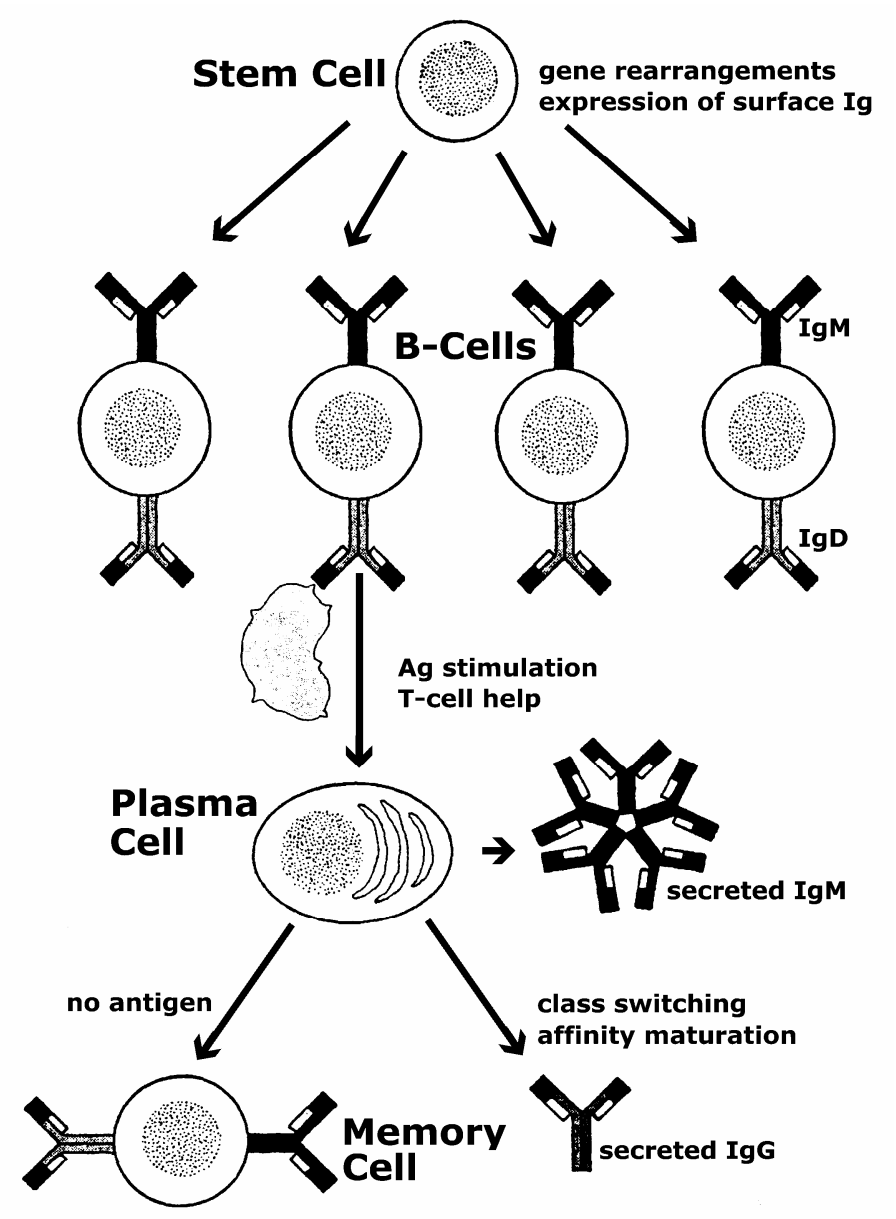
Figure 1. Generation of Antibodies in Vivo.

In the top panel, the random rearrangement of germ-line gene segments creates a repertoire of more than a million B cells with different immunoglobulin heavy-chain (V_H) and kappa light-chain (V_K) variable (V) genes. For the sake of simplicity, the lambda light-chain locus is not shown. The rearranged heavy-chain gene consists of 1 of approximately 50 V_H gene segments, 1 of 33 *D* gene segments, and 1 of 6 J_H gene segments. The random addition of nucleotides between the points at which the *V* and *D* segments and *D* and *J* segments meet greatly increases the diversity of the repertoire, which contains 10^6 to 10^9 different V_H genes. A similar rearrangement mechanism of V_K and J_K gene segments results in 10^3 to 10^4 different rearranged kappa light chains. The light chains are less diverse than the heavy chains, because there are no *D* segments in light-chain genes. The potential number of different antibodies created by gene rearrangements, which is the product of the numbers of diverse V_H and V_K segments, far exceeds the number of different B cells in the body. The rearranged genes are expressed as membrane-anchored immunoglobulins on the surface of the B cell, where they function as antigen receptors (middle panel). In the bottom panel, when antigen binds to the surface immunoglobulin, the B cell is stimulated to proliferate and differentiate into plasma cells.

The inset shows the IgG antibody fragments in detail. The modular structure of antibodies makes it possible to produce small antigen-binding fragments that can be expressed in *E. coli*. The Fab fragment consists of the V_H domain and the first domain of the constant region (C_H1) paired with V_K and the light-chain constant (C_K) domains. The single-chain Fv fragment consists of a single polypeptide chain with the V_H and V_K domains connected by a flexible peptide linker. The linker keeps the noncovalently linked variable domains from dissociating at physiologic concentrations.

ANTICUERPOS MONOCLONALES

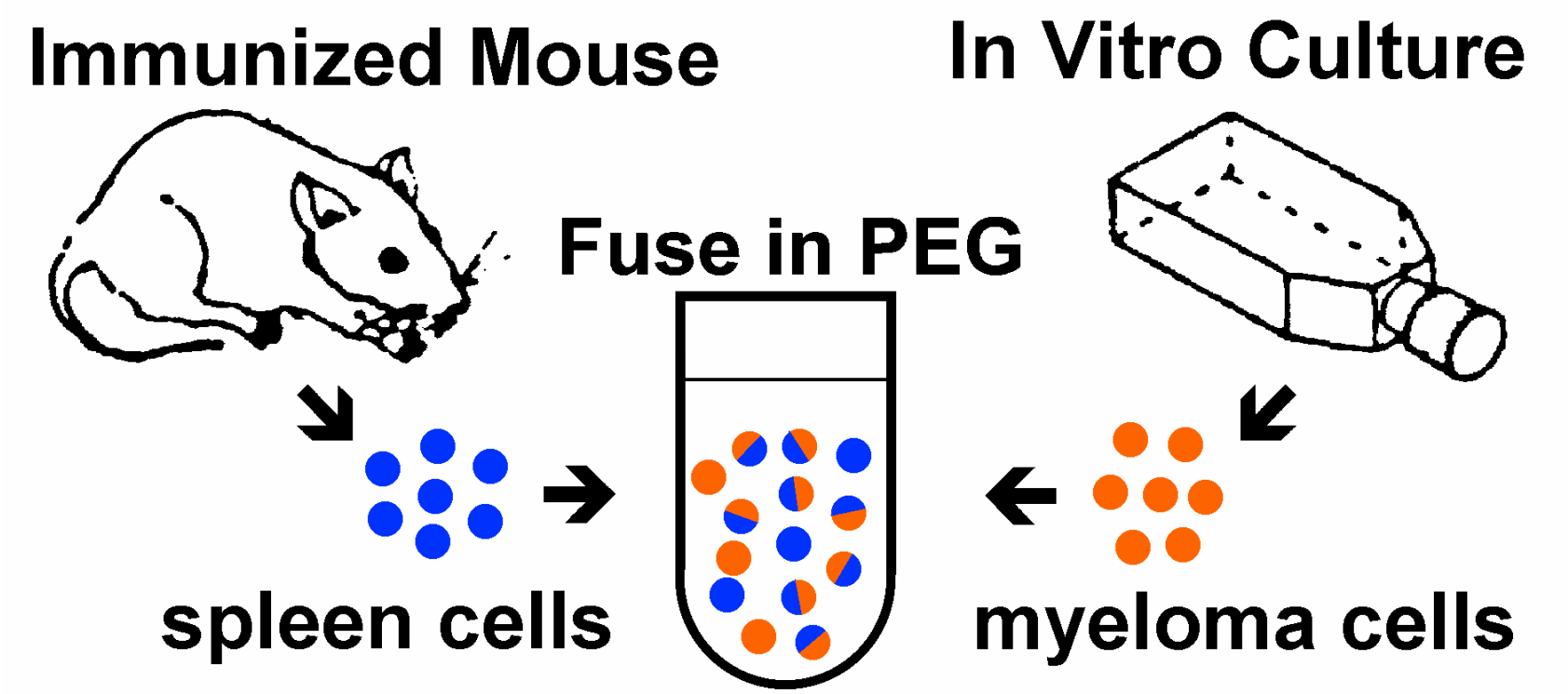




- antibodies produced by differentiated B-cells
- recombination results in B-cell lineages producing distinct antibodies
- serum contains antibodies representing many lineages
- difficult to isolate B-cells of defined specificity
- cannot grow B-cells in culture

Kohler and Milstein (1975)

- fused B-cells with myeloma cells → stable hybridoma
- hybridoma secretes **monoclonal antibody**



- immunized mouse making desired antibodies
- boost 3-4 days before fusion
- remove spleen and harvest cells
- mix with myeloma cells in presence of fusogenic agent

Balb/c Mouse
↓
MOPC 21 (tumor)
↓
P3K (cell line)
↓
P3-X63Ag8 (HGPRT-)
↓
X63-Ag8.653 (IgG-)

HAT Medium:

- **Hypoxanthine (purine salvage)**
- **Aminopterin (DHFR inhibitor)**
- **Thymidine (pyrimidine salvage)**

Nucleotide Metabolism:

- **salvage and de novo pathways**
- **HGPRT essential for purine salvage**
- **DHFR essential for de novo synthesis**

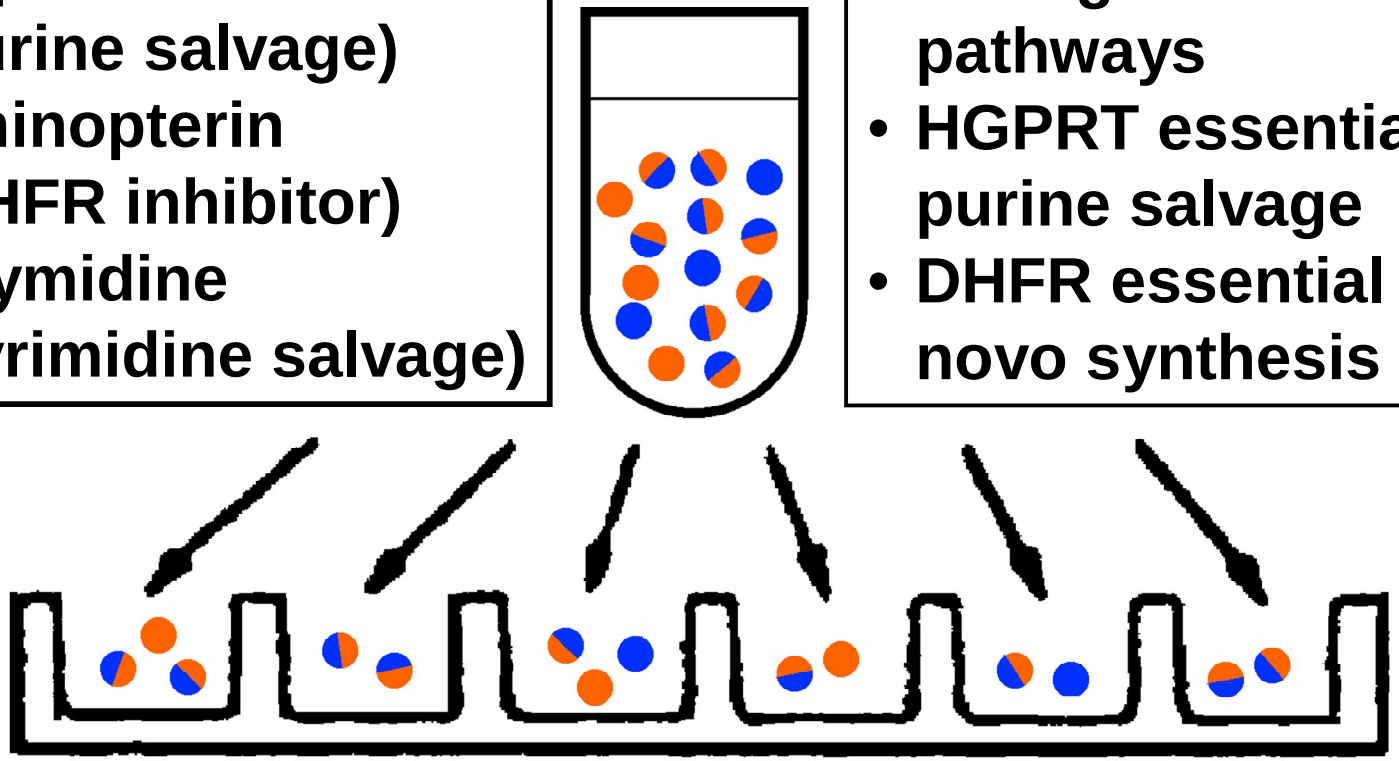
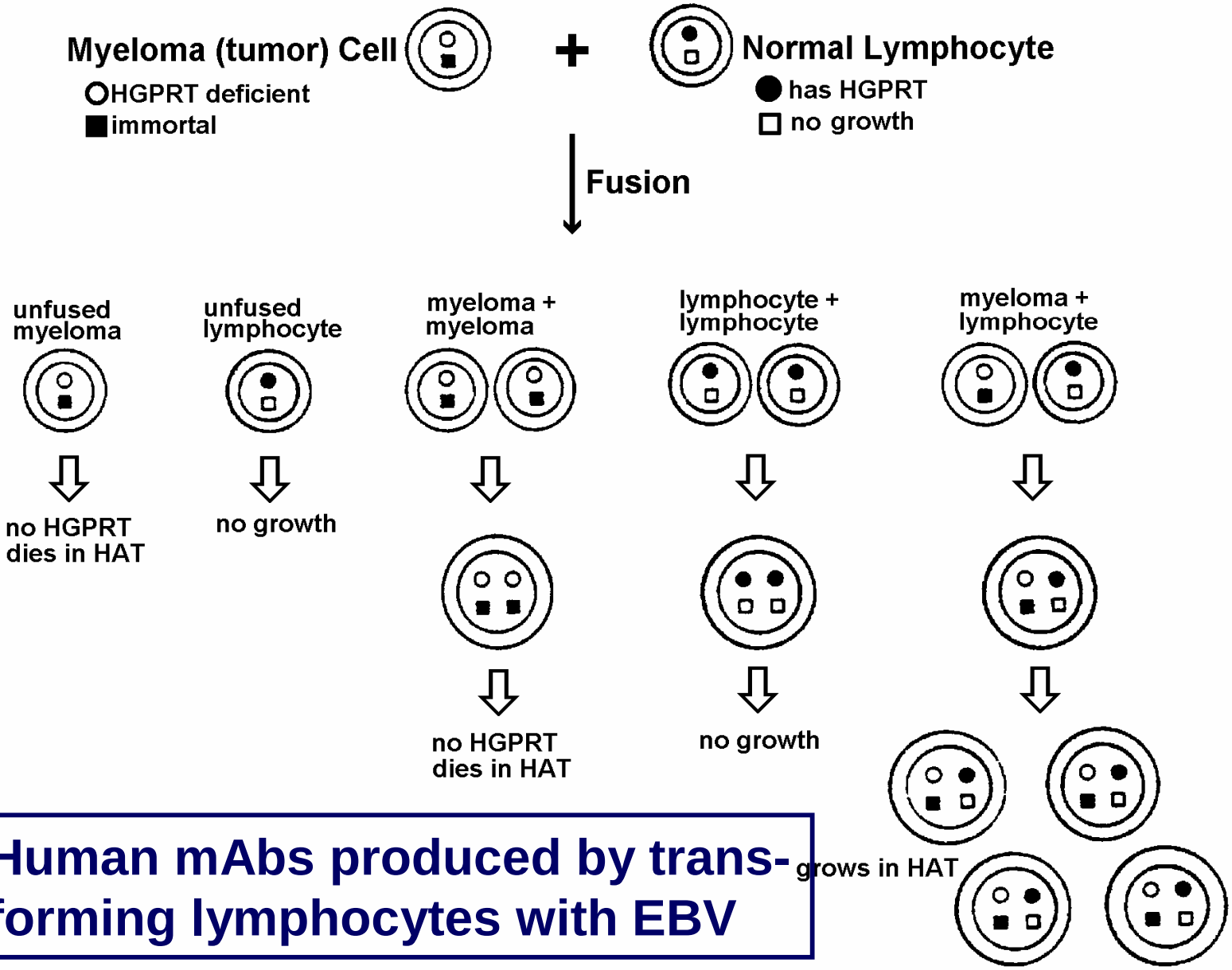


Plate in HAT Medium

HGPRT = hypoxanthine-guanine phosphoribosyl transferase
DHFR = dihydrofolate reductase

Selection in HAT Medium



Human mAbs produced by trans-forming lymphocytes with EBV

Screening/Cloning

⇩ HAT Selection

Screening
(assay Ab)

⇩ Clone Positives

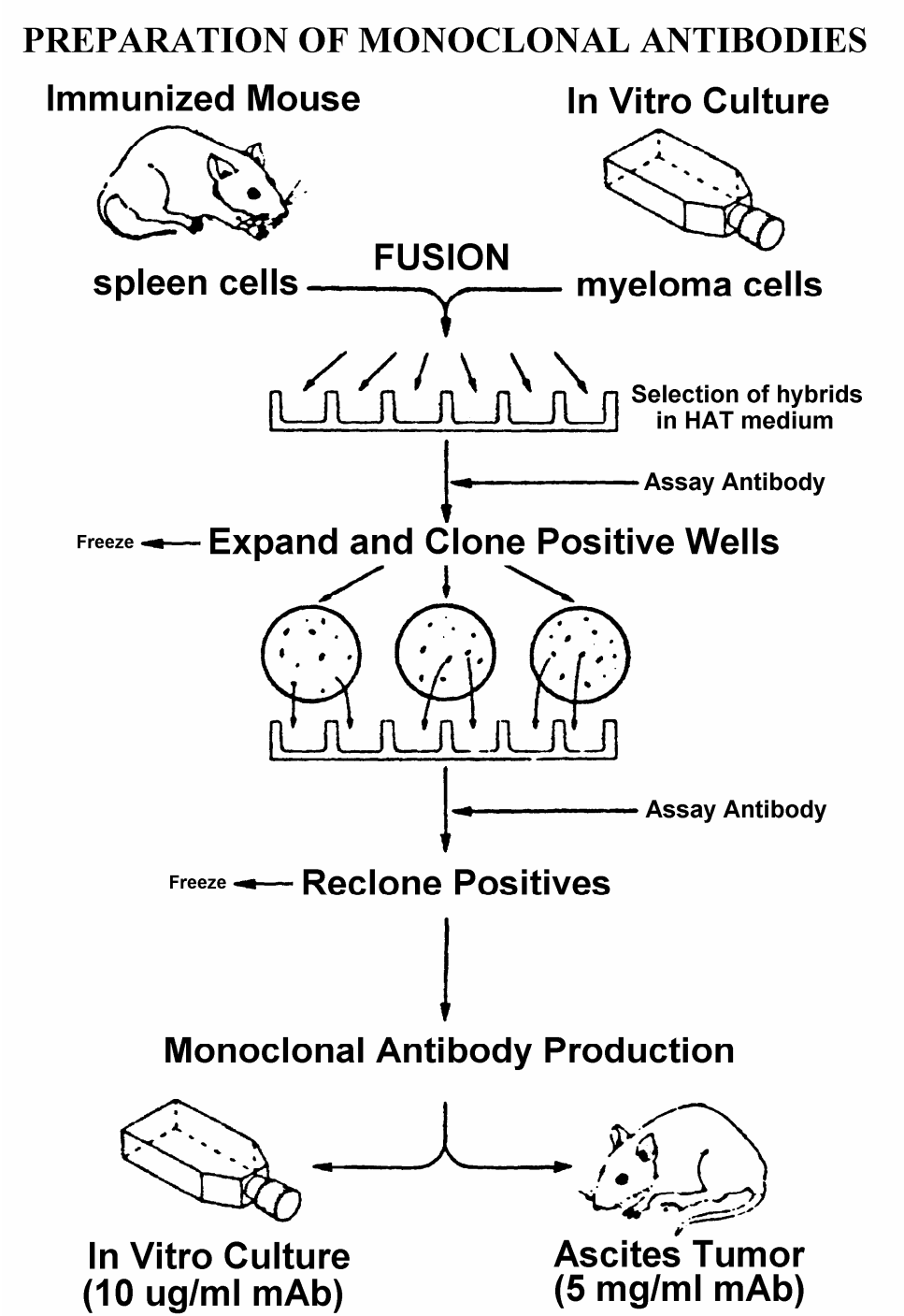
Re-assay

⇩

Re-Clone and Characterize Positives

Cloning Methods

- soft agar
- limiting dilution



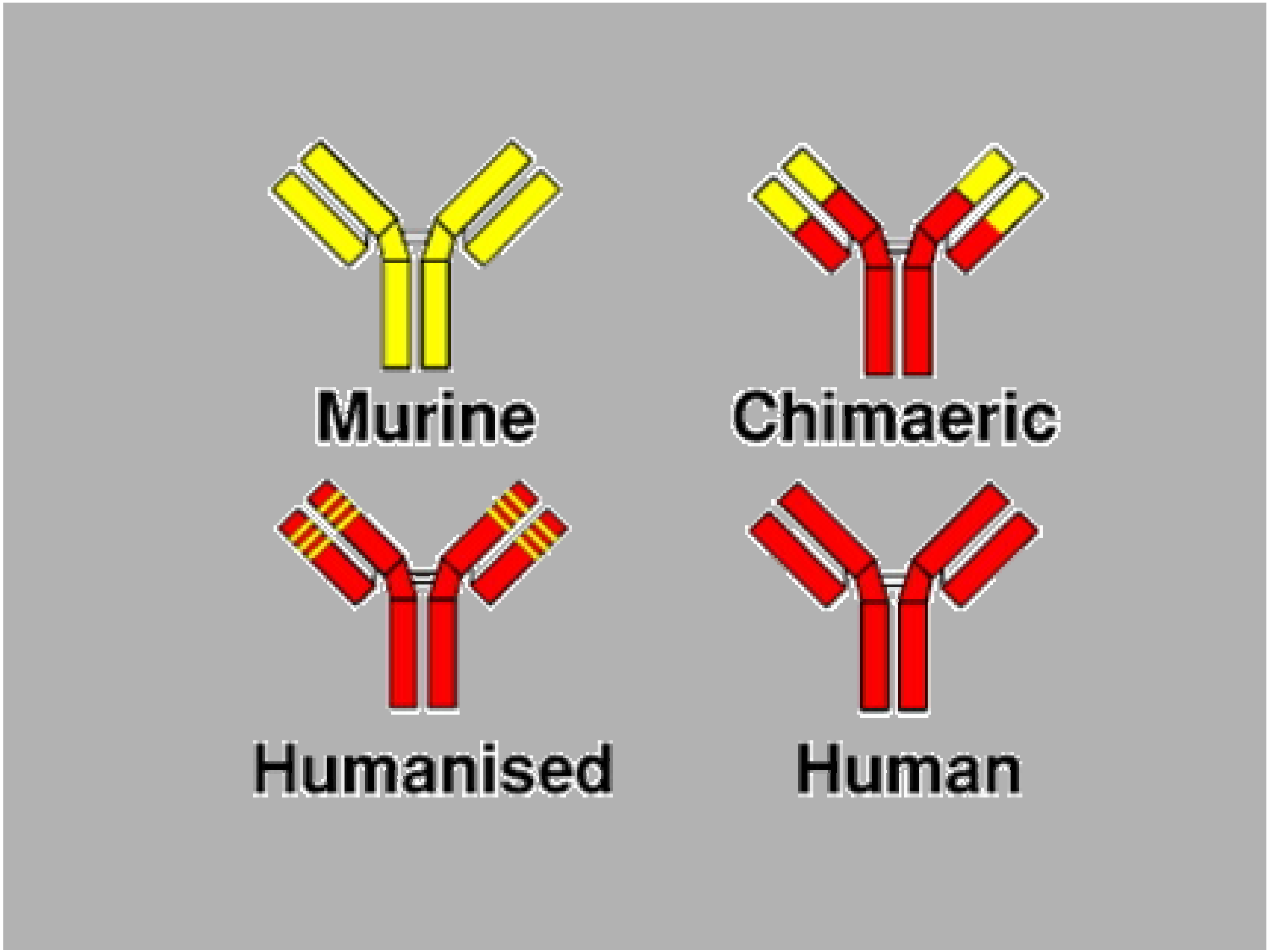
Production

- produce mAbs in vitro or with ascites
- harvest culture media (supernatant)
  - in vitro material is less concentrated and contains bovine serum
- ascites are tumors grown in peritoneal cavity
  - ascites fluid and sera contain high [mAb]
  - minor contamination with mouse IgG

HAMA ("human anti-mouse antibodies").

Humanized Monoclonal Antibodies

- Mouse monoclonal antibodies have been genetically engineered to replace all of the antibody molecule with human counterparts except the hypervariable regions directly involved with antigen binding.
- Humanized monoclonal antibodies are currently be tested in human clinical trials.


Murine
Chimaeric
Humanised
Human

RECEPTORES ANTIGENICOS

- **T cell receptor (linfocitos T) = reconoce Ag procesado**
- **B cell receptor (linfocitos B) = reconoce Ag nativo**

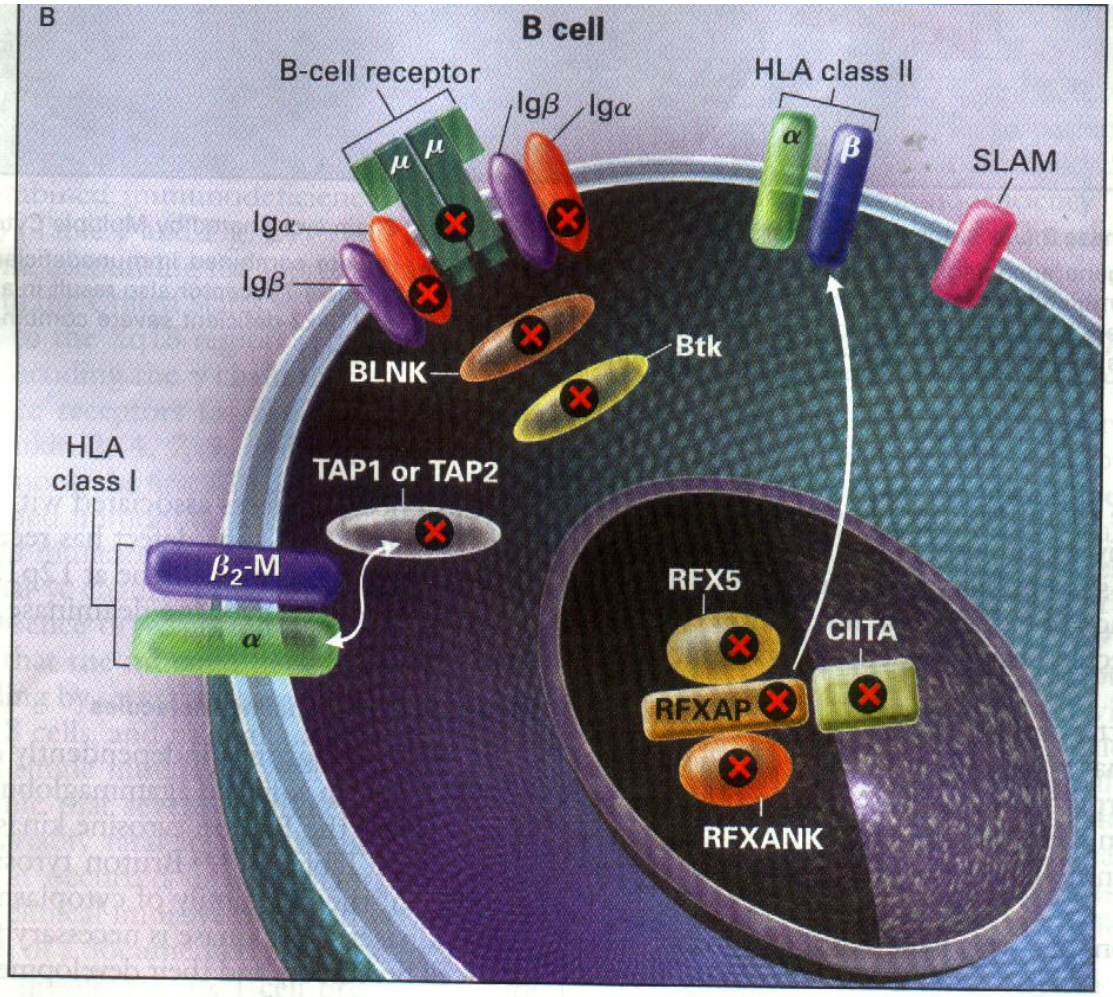


Figure 3. Locations of Mutant Proteins in CD4+ T Cells (Panel A) and B Cells (Panel B) Identified in Primary Immunodeficiency Diseases.

Each mutant protein is identified by a red X. ZAP-70 denotes zeta-associated protein 70; SLAM signaling lymphocyte activation molecule; SH2D1A SLAM-associated protein; ATM ataxia telangiectasia mutation; NFAT nuclear factor of activated T cells; Jak3 Janus kinase 3; WASP Wiskott–Aldrich syndrome protein; TAP1 and TAP2 transporter associated with antigen processing 1 and 2, respectively; Btk Bruton tyrosine kinase; BLNK B-cell linker adapter protein; β_2-M beta$_2$-microglobulin; and RFX, RFXAP, and CIITA transcription factors.

MECANISMO DE ACCION DE LOS ANTICUERPOS

Via Complemento

ADCC (antibody-dependent-cellular-cytotoxicity

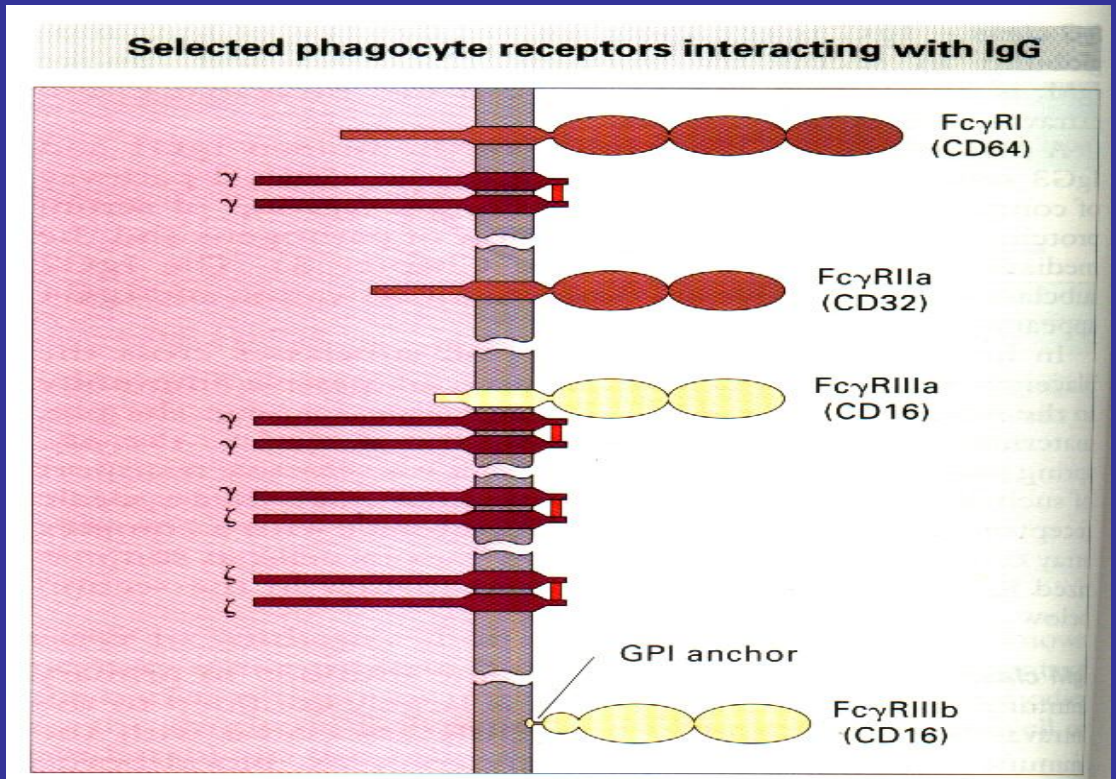


Fig. 4.22 The human Fcγ receptor structures shown are those for FcγRI (expressed by monocytes), FcγRIIa (expressed by monocytes and neutrophils), FcγRIIIa (expressed by monocytes and attached as a normal transmembrane protein) and FcγRIIIb (expressed by neutrophils and attached by a phosphatidyl inositol glycan [GPI] membrane anchor). Each receptor belongs to the immunoglobulin superfamily and expresses two or three extracellular immunoglobulin-like domains. Several of the receptors are now known to exist as complexes with various disulphide-linked subunits. FcγRI and FcγRIIIa both associate with dimers of the γ chain originally described as part of the high-affinity FcεRI complex (see *Fig 4.23*). FcγRIIIa has also been shown to associate with dimers of the ζ chain found in the TCR–CD3 complex. In the case of FcγRIIIa these subunits can associate as either homodimers (γ–γ or ζ–ζ) or as heterodimers (γ–ζ). They appear to be essential for surface expression and signal transduction. In FcγRI interactions, the receptor appears to bind a structural motif centred around Leu 235 in the C_H2 domain, present in IgG1, IgG3 and IgG4.

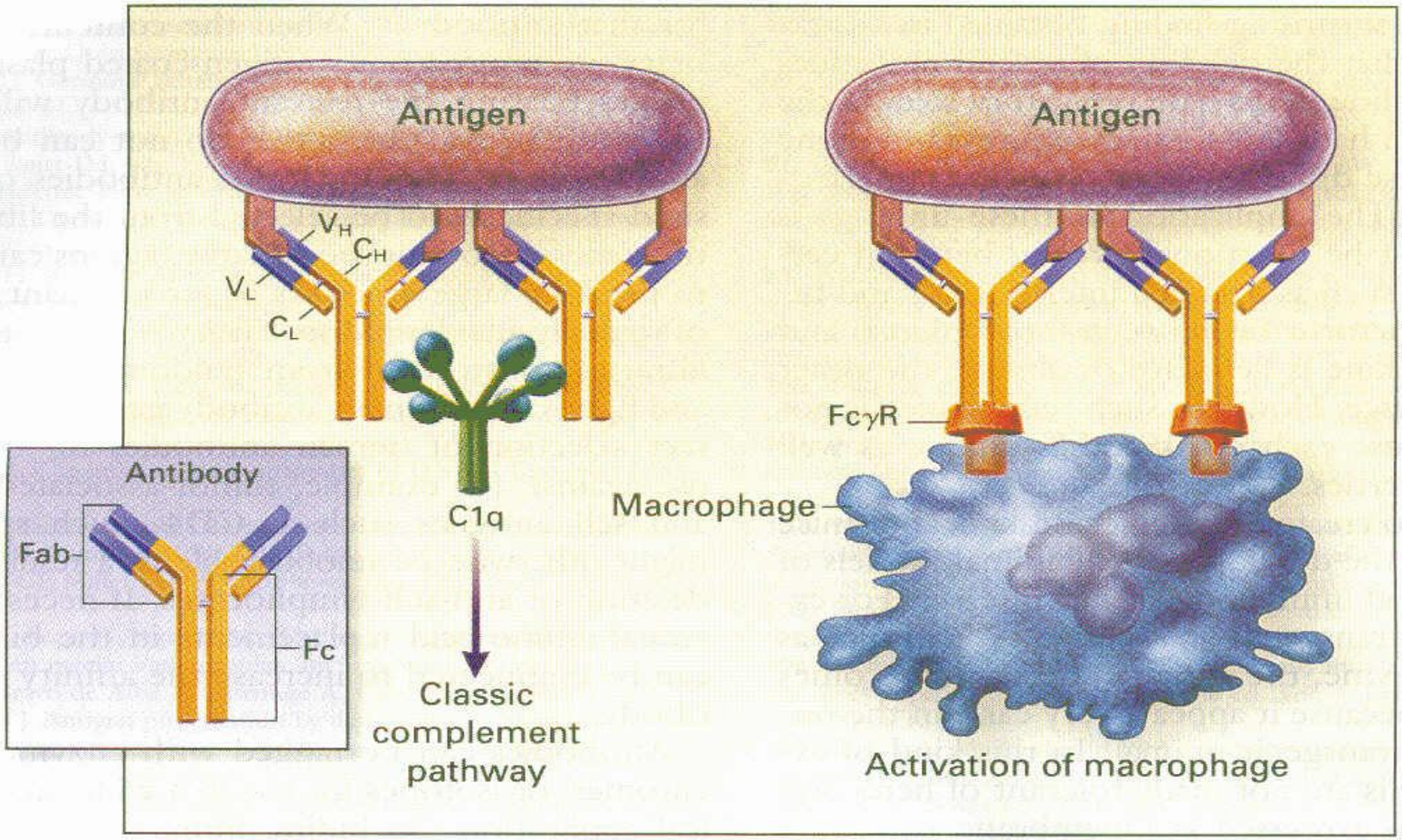


Figure 11. Role of Antibodies.

Antibodies rarely act in isolation. Their usual role is to focus components of the innate immune system on the pathogen, and the activation of these destructive forces normally requires coordinating events that occur after Fab heavy- and light-chain variable regions (V_H and V_L) of the antibody are bound to antigen, leading to the display of multiple exposed Fc regions. The figure shows two examples of this process: the activation of the classic complement pathway after binding of C1q to Fc, and the activation of phagocytosis after the cross-linking of Fc receptors and binding of the FcγR on the macrophage.

- **COMPLEMENTO**

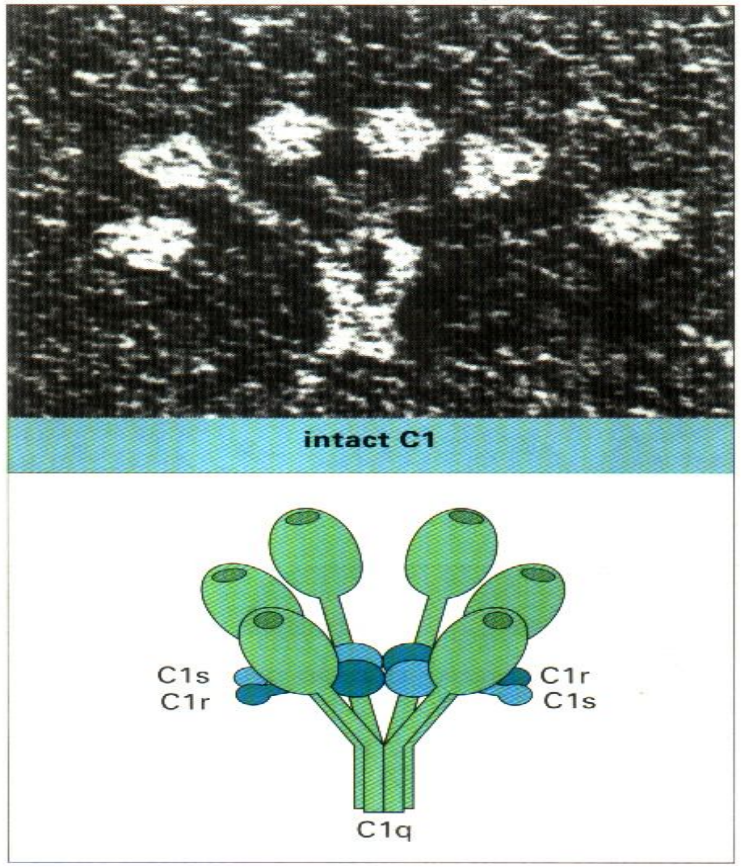


Fig. 3.21 Electronmicrograph of a human C1q molecule demonstrates six subunits. Each subunit contains three polypeptide chains, giving 18 in the whole molecule. The receptors for the Fc regions of IgG and IgM are in the globular heads. The connecting stalks contain regions of triple helix and the central core region contains collagen-like triple helix. The lower panel shows a model of intact C1 with two C1r and two C1s proenzymes positioned within the ring. The catalytic heads of C1r and C1s are closely apposed and conformational change induced in C1q following binding to complexed immunoglobulin causes mutual activation/cleavage of each C1r unit followed by cleavage of the two C1s units. The cohesion of the entire complex is dependent on Ca^{2+}. (Electronmicrograph, reproduced by courtesy of Dr N. Hughes-Jones.)

MAC (complejo ataque complemento)

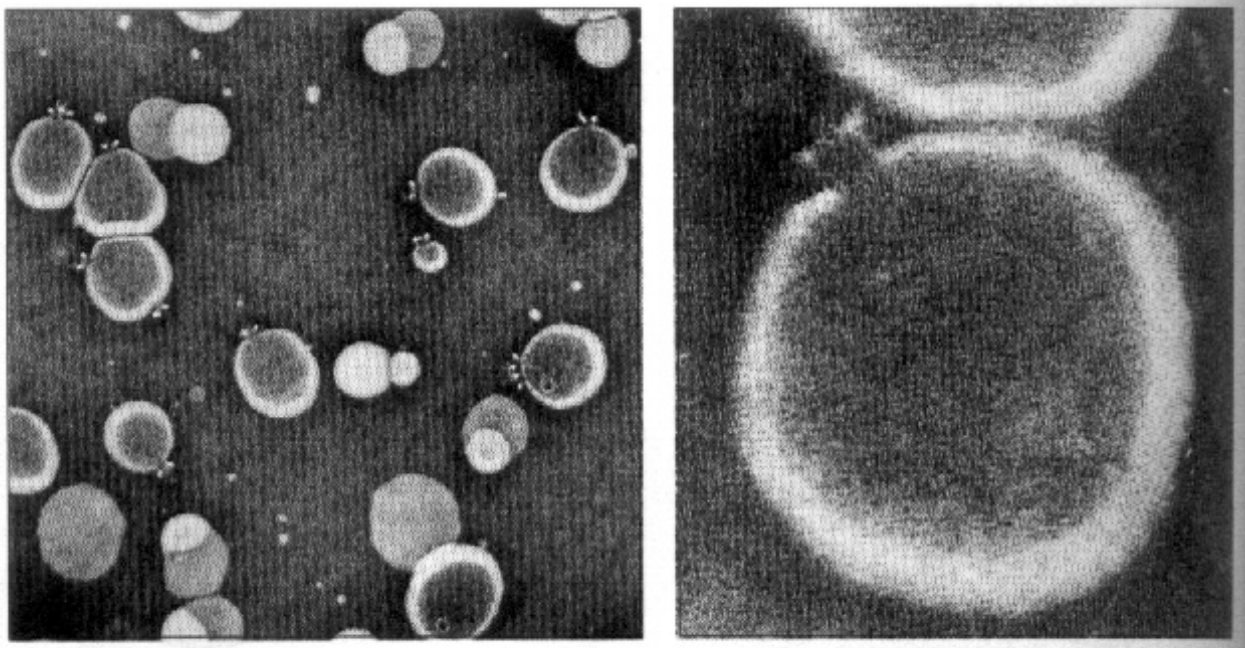


Fig. 3.19 Electronmicrographs of the membrane attack complex (MAC). The complex consists of a cylindrical pore, in which the walls of the cylinder, formed by C9, traverse the cell membrane. In these micrographs the human C5b–9 complex has been reincorporated into a lecithin liposomal membrane. ×234 000. (Courtesy of Professor J. Tranum-Jensen and Dr S. Bhakdi.)