Immunology

B lymphocytes & Antibodies

04. June 2025, Ruhr-Universität Bochum

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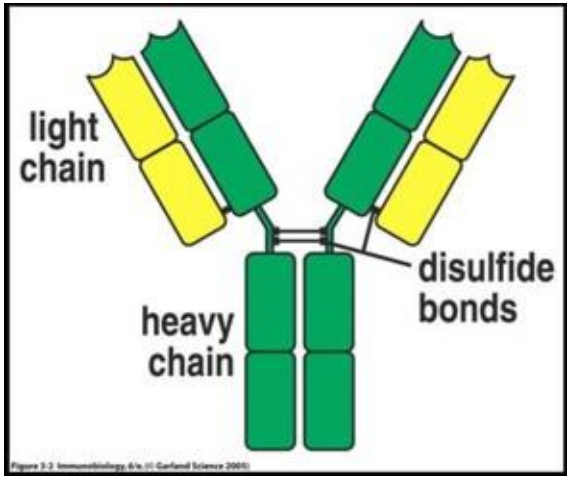
RUB

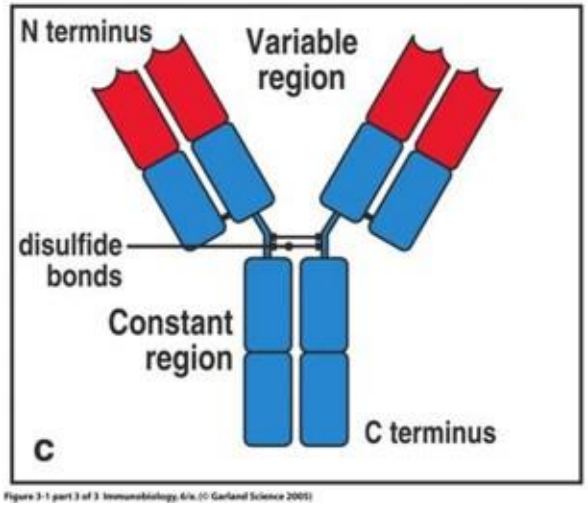
The structure of a typical antibody molecule



2 heavy chains (C_H), 4-5 domains

2 light chains (C_L), 2 domains

The chains are linked (C_H-C_H und C_H-C_L) by **disulfide bonds**.



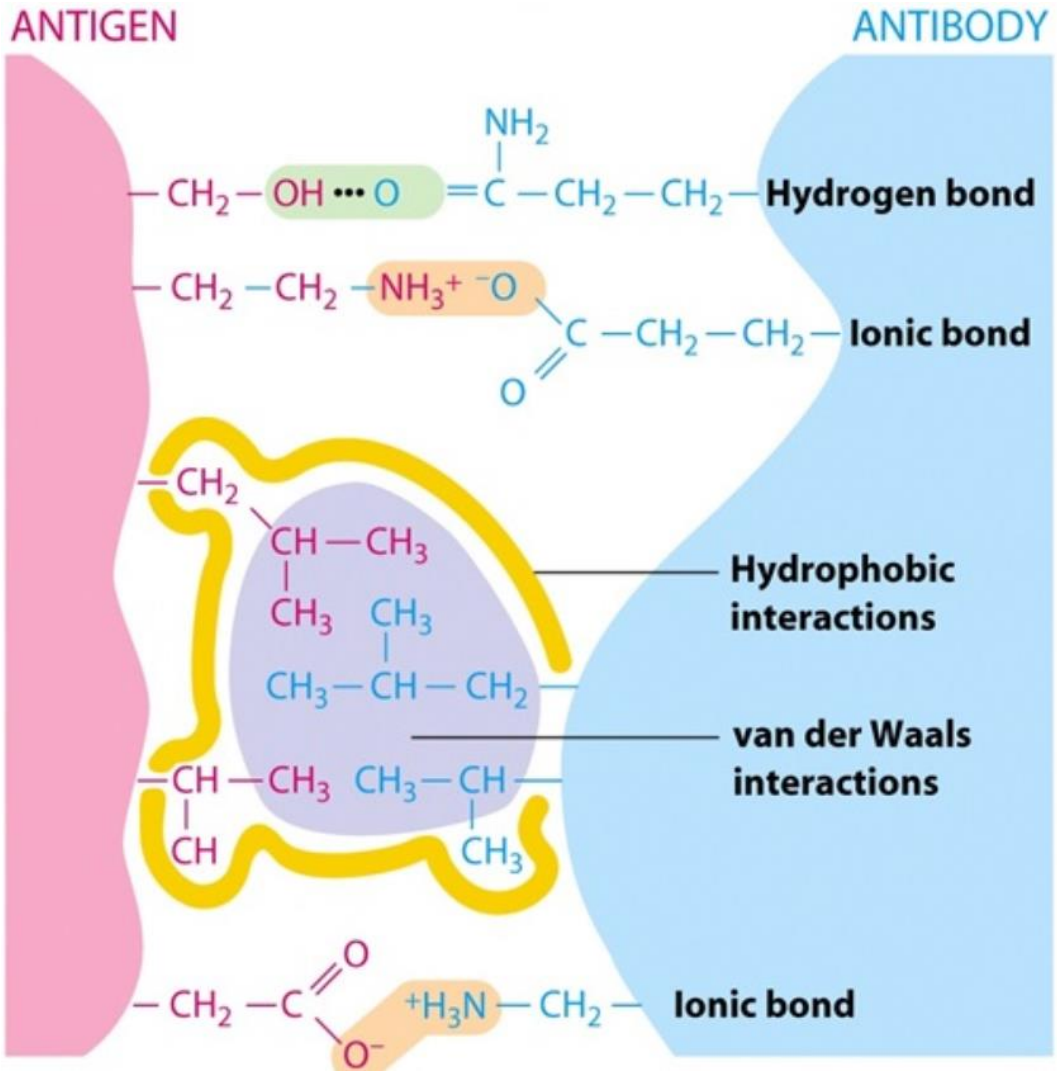
Every chain has a **variable** (N-terminal) and a **constant** (C-terminal) **domain**.

The variable domains develop the **antigen binding side** (2 per Ab).

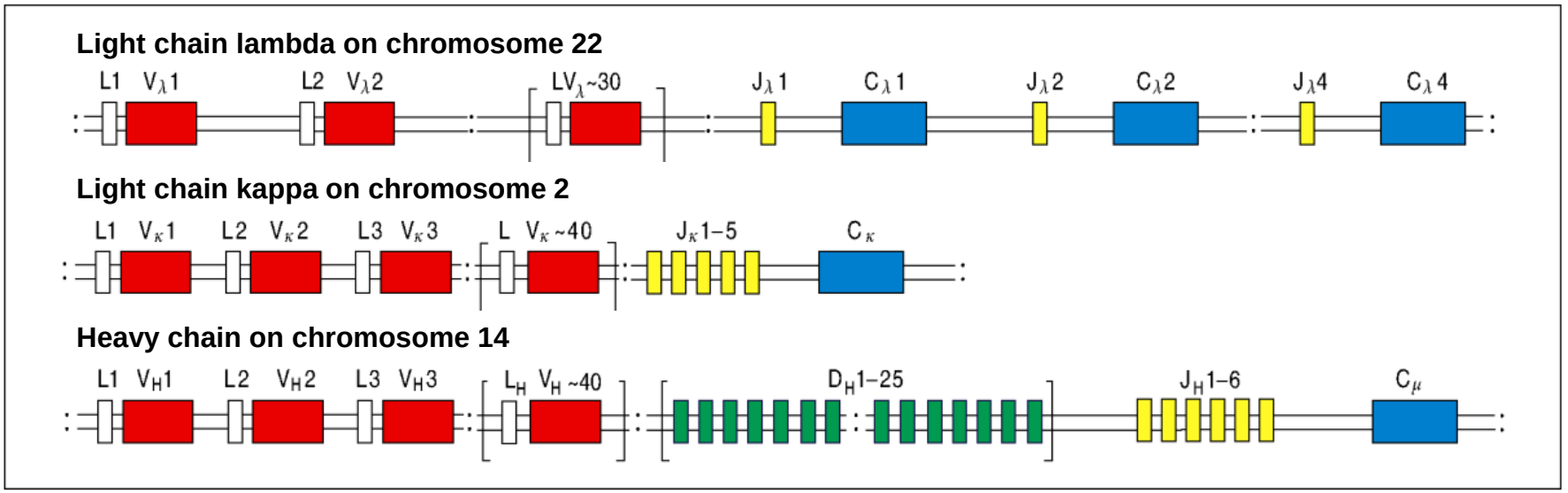
The constant domains mediate the **antibody function** (e.g. receptor binding)

The binding of an antigen

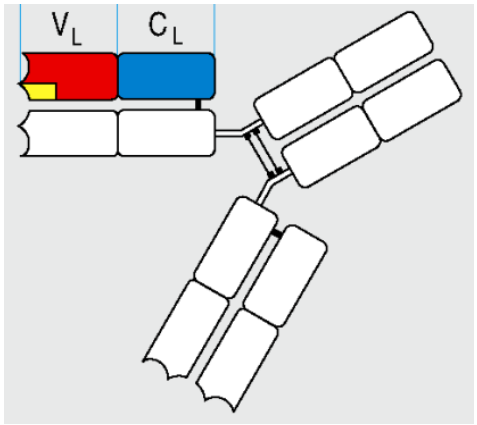
The binding of an antigen by an antibody is **never** covalent.

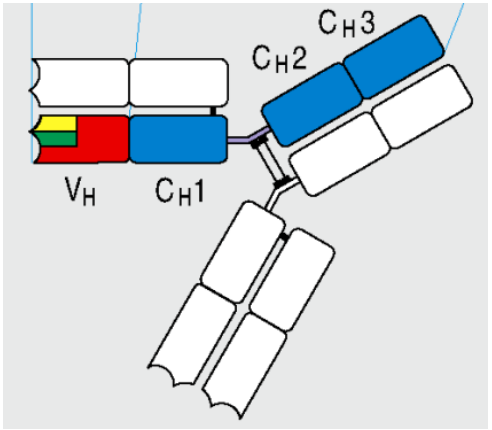


Germline configuration of genes for the heavy and light chain



Aus: Murphy, Travers, Walport, *Janeway Immunologie*, 7. Aufl. © Spektrum Akademischer Verlag 2010





Somatic recombination of the light chain

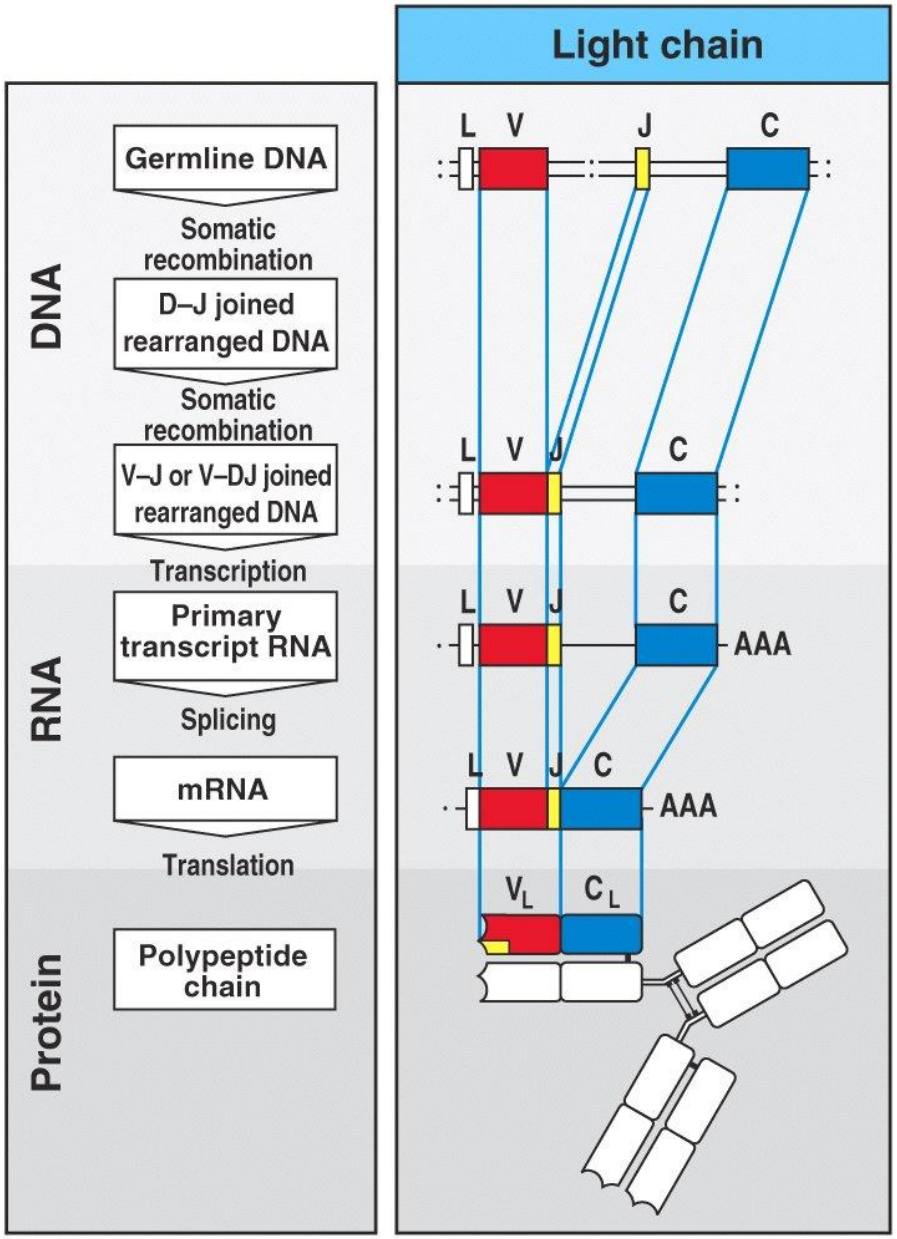


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

Combinatorial diversity:

Light chain κ: 40 V_L-segments
5 J_L-segments

40 x 5 = **200**

Combinatorial diversity:

Light chain λ: 30 V_L-segments
4 J_L-segments

30 x 4 = **120**

320 possible Light chains

Somatic recombination of the heavy chain

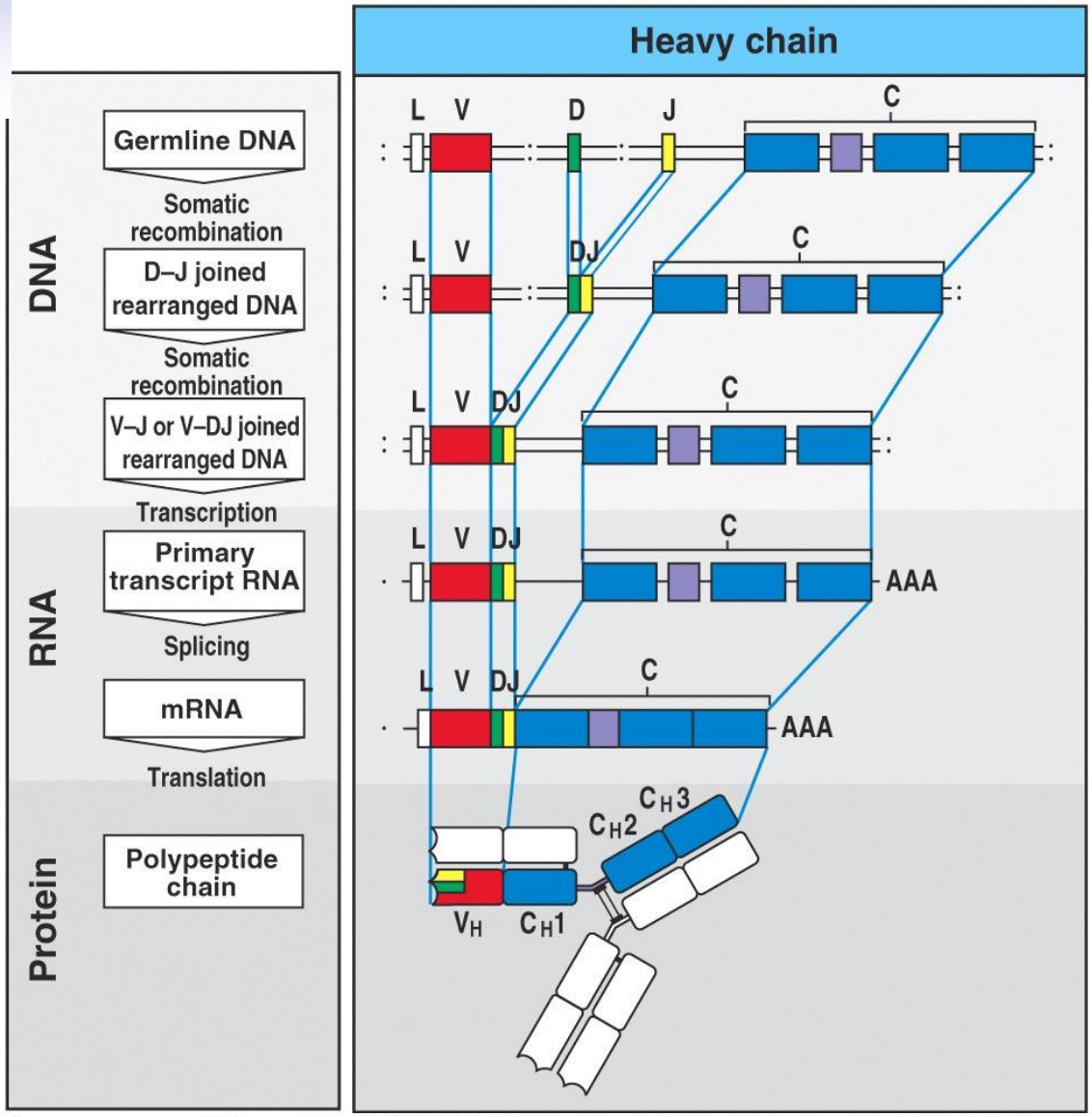


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

Combinatorial diversity:

Heavy chain:

40 V_H-segments
25 D_H-segments
6 J_H-segments

40 x 25 x 6 = **6000**

6000 possible heavy chains

The diversity of the antibody repertoire

Combinatorial diversity of antibodies:

6000 different heavy chains times

320 different light chains

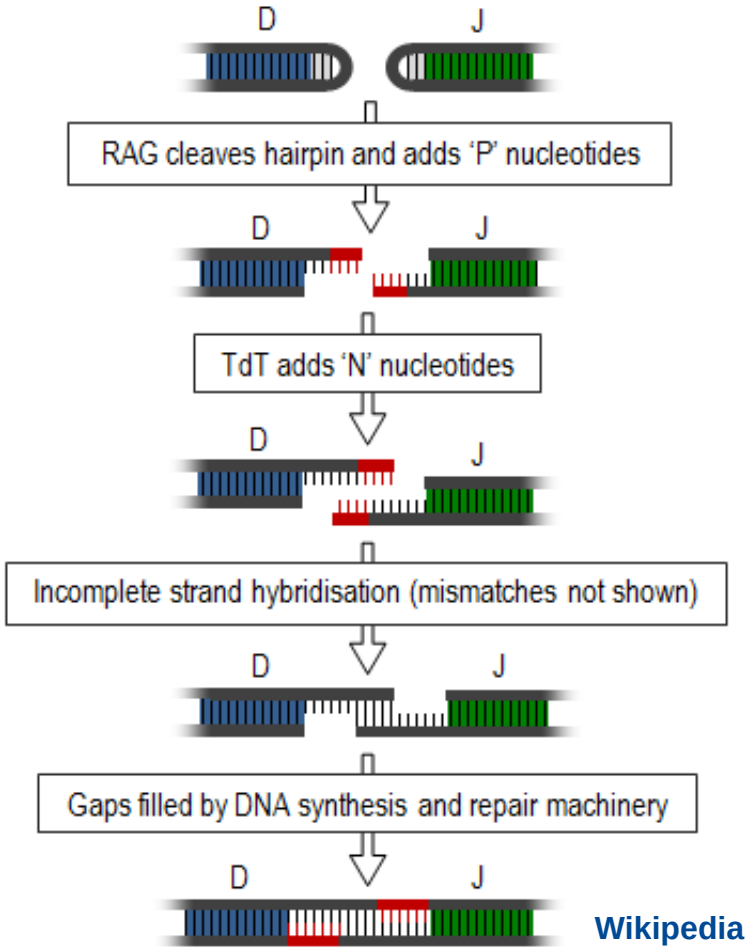
$\longrightarrow$ $1{,}9 \times 10^6$ antibodies

However total diversity is approximately 10^{18} different antibodies!
Additional mechanisms exist that further increase heterogenecity, namely:
junctional diversification, somatic hypermutation, class switching

Junctional diversity

During VDJ recombination the enzyme “recombination activating gene” (RAG1&2) induces palindromic nucleotides (1 to 4 P-nucleotides)

At the junction of the gene segments nucleotides are introduced by the enzyme “Terminal deoxynucleotidyl transferase” (2 to 10 N-nucleotides).



Wikipedia

Due to this process there is a high chance for the induction of frame shift mutation!

Somatic hypermutation

Affinity maturation of the antigen binding sites takes place in the secondary lymphatic organs (only in activated B-cells).

Mutations occur in the whole variable region of the antibody.

Mutations concentrate on so-called hot spot regions.

B cells become apoptotic when somatic hypermutation resulted in reduced binding affinity of the antibody (during affinity maturation)

Summary

Antibodies can be raised against almost every molecule

The antibody diversity is based on three different mechanisms:

1) **Combinatorial diversity**
(Somatic recombination)

2) **Junctional diversity**
(Inaccurate junction)

3) **Somatic hypermutation**
(Affinity maturing in germinal centres)

B-cell development

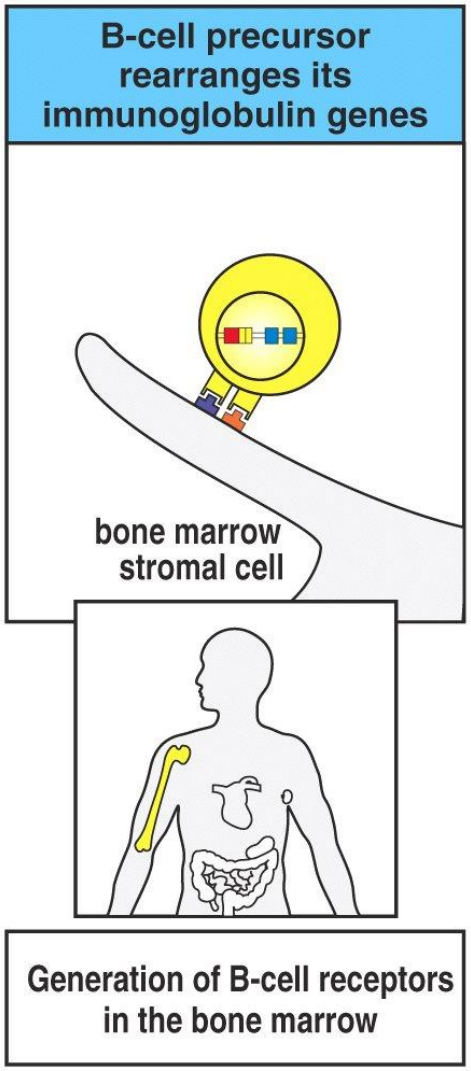


Figure 7-1 part 1 of 2 Immunobiology, 6/e. (© G

- B-cells develop continuously in the **b**one marrow, they derive from lymphatic progenitor cells
- The environment (stromal cells of the marrow) delivers the **necessary milieu** (surface molecules and cytokines) for the **development**
- The **immunoglobulin genes** are **rearranged**

B-cell development

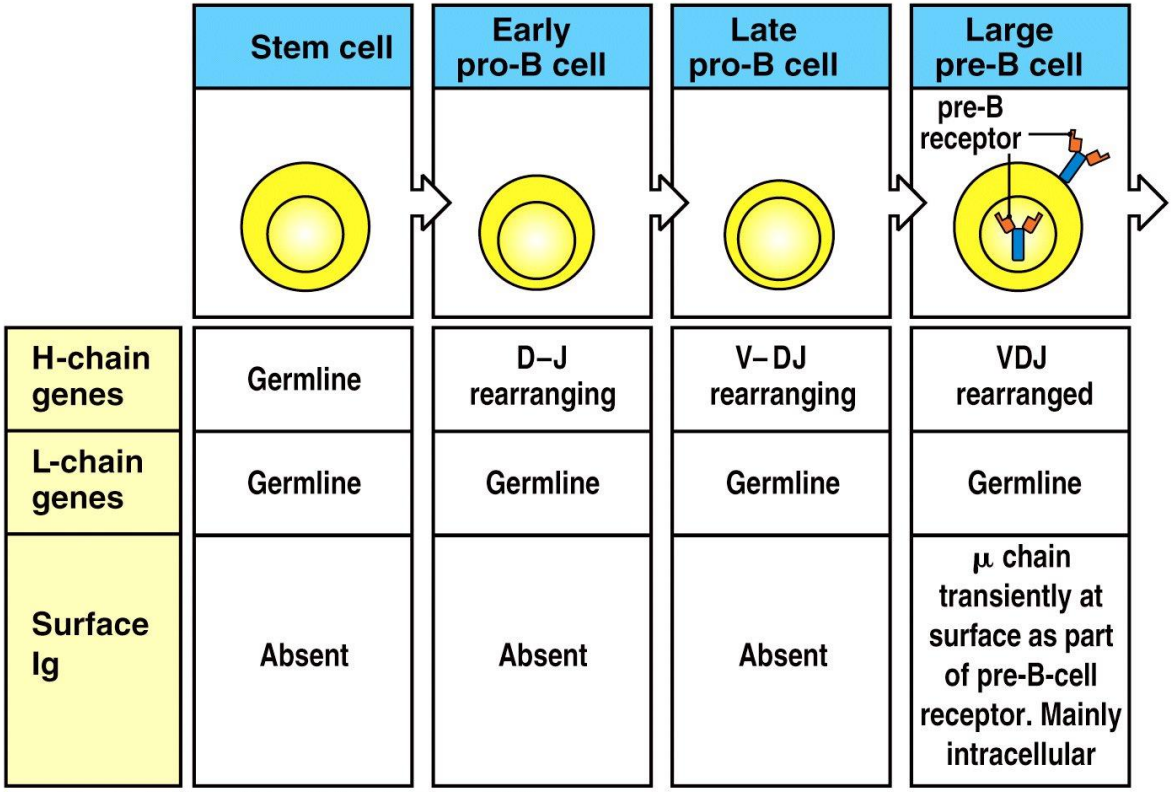


Figure 7-6 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

- The heavy chain is expressed with a surrogate L-chain to stabilize the pre-B-cell-receptor
- After successful expression of the heavy chain the pre-B-cell divides, before the rearrangement of the light chain starts

B-cell development

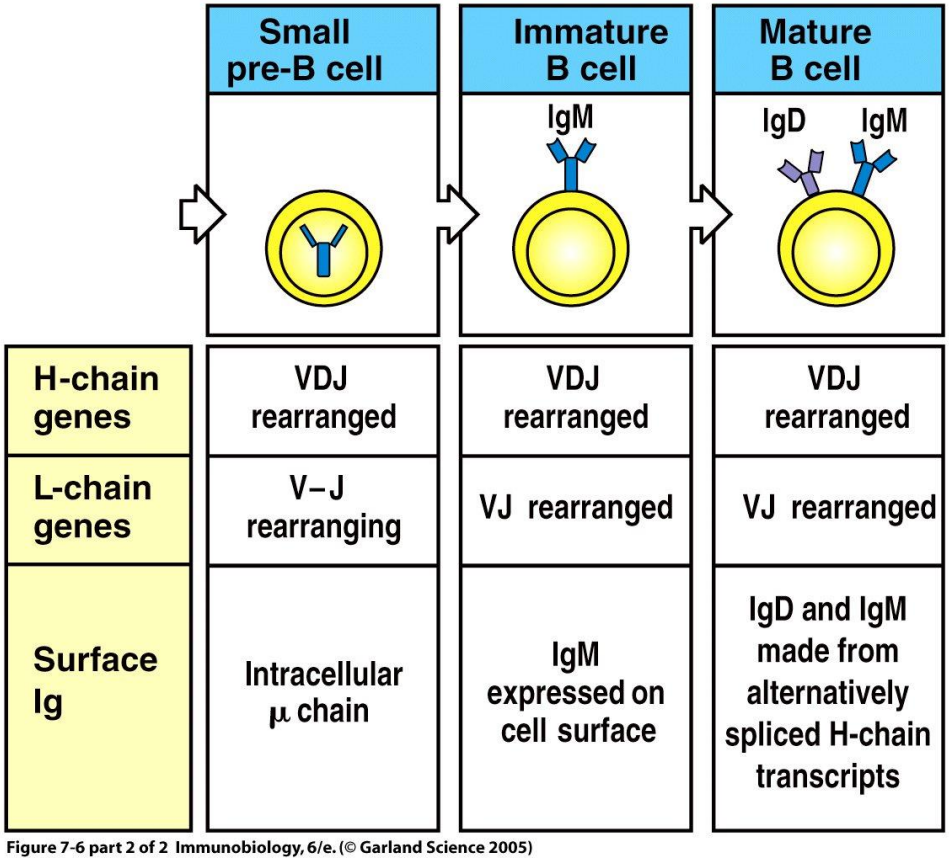
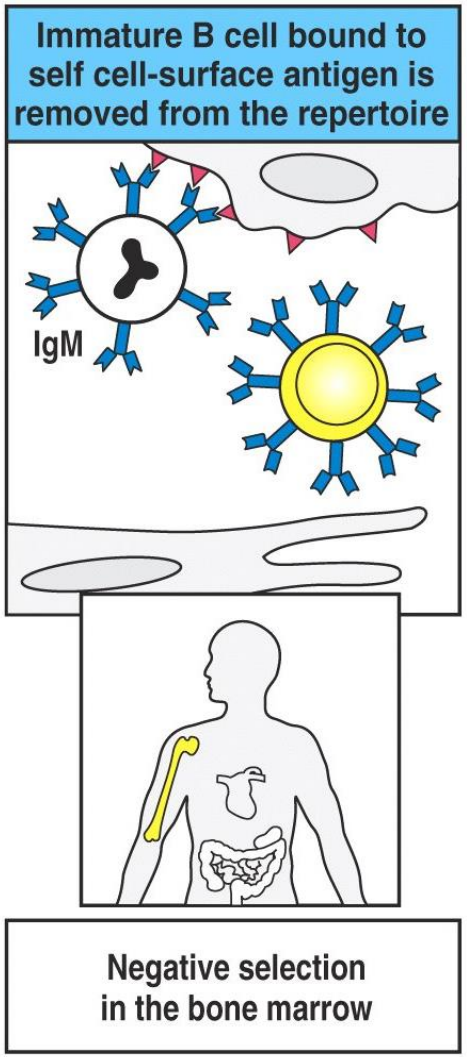


Figure 7-6 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

- Beginning of the V-J-rearrangement of the κ-locus of the light chain, in case of an unsuccessful rearrangement, the λ-Locus becomes rearranged

B-cell development



arland Science 2005)

In the bone marrow:

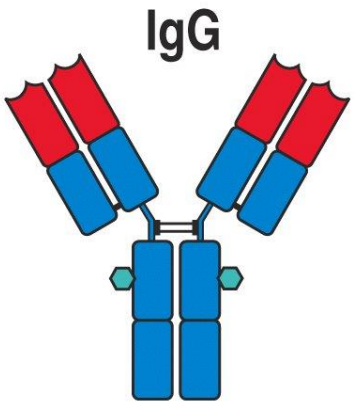
Selection on self-tolerance

- Contact to antigens in the environment of the bone marrow is leading to apoptosis or anergy in the immature B-cell

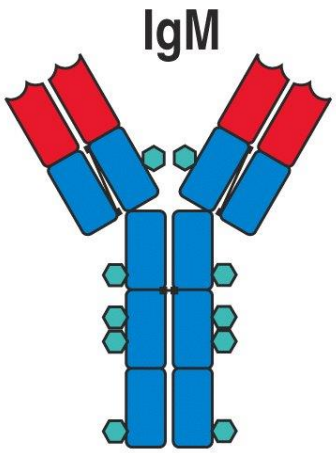
B-cell development after leaving the bone marrow

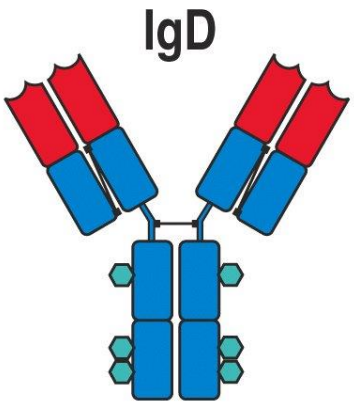
- Self-tolerant, naive B-cells leave the bone marrow.
- Naive B-cells circulate through the blood into the secondary lymphatic organs.
- If the B-cells encounter their specific antigen, they are activated and can differentiate into plasma cells or memory cells.

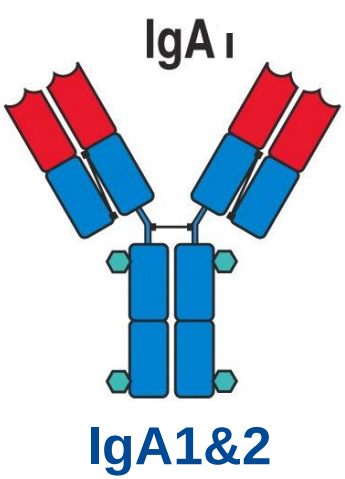
Antibody classes - Isotypes



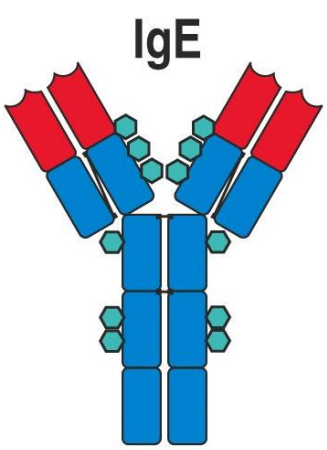
IgG1,2,3&4





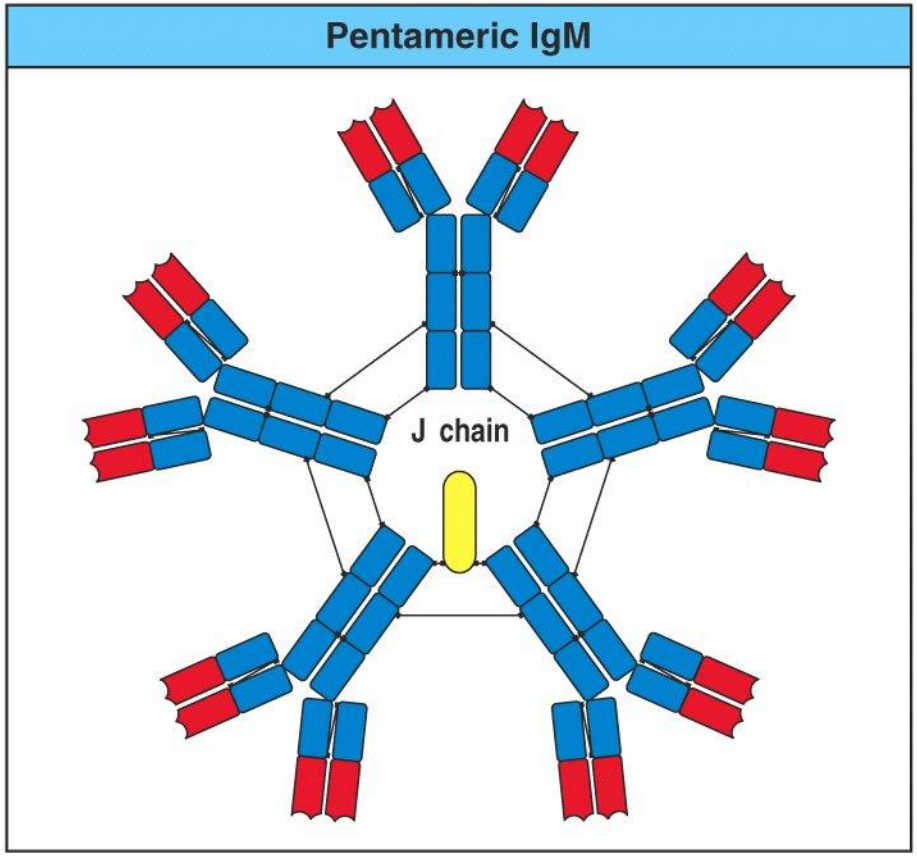


IgA1&2



Ig	IgG	IgM	IgA	IgD	IgE
Serum concentration (mg/dl)	800-1700	50-190	140-420	0.3-0.4	<0.001

Antibody classes - Isotypes



The first antibody class produced by each B cell

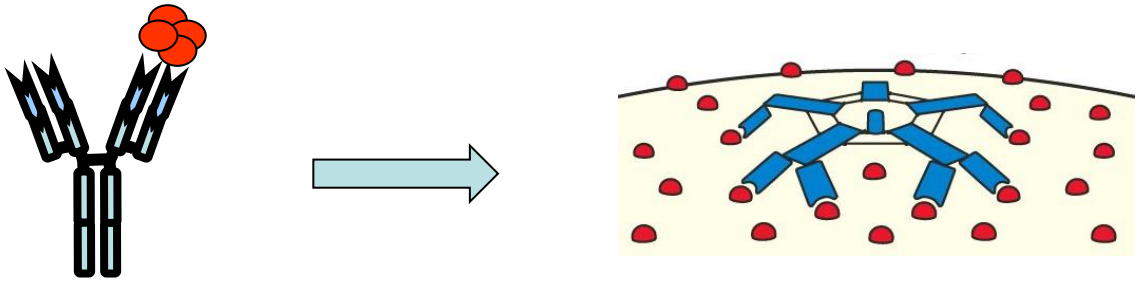
High avidity due to pentamer formation!

Affinity & Avidity

IgM compensates its low affinity with high avidity.

Affinity = Binding strength between one antigen binding side and the antigen.

Avidity = Total binding strength between more antigen binding sides and one multivalent antigen. The absolute binding strength is potentiated



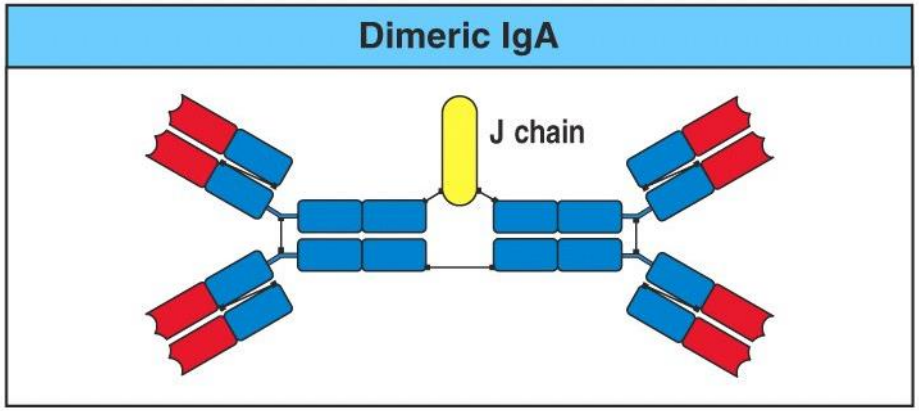
Affinity

e.g. $K_D=10^{-4}$

Avidity

e.g. $K_D=10^{-10}$

Antibody classes - Isotypes

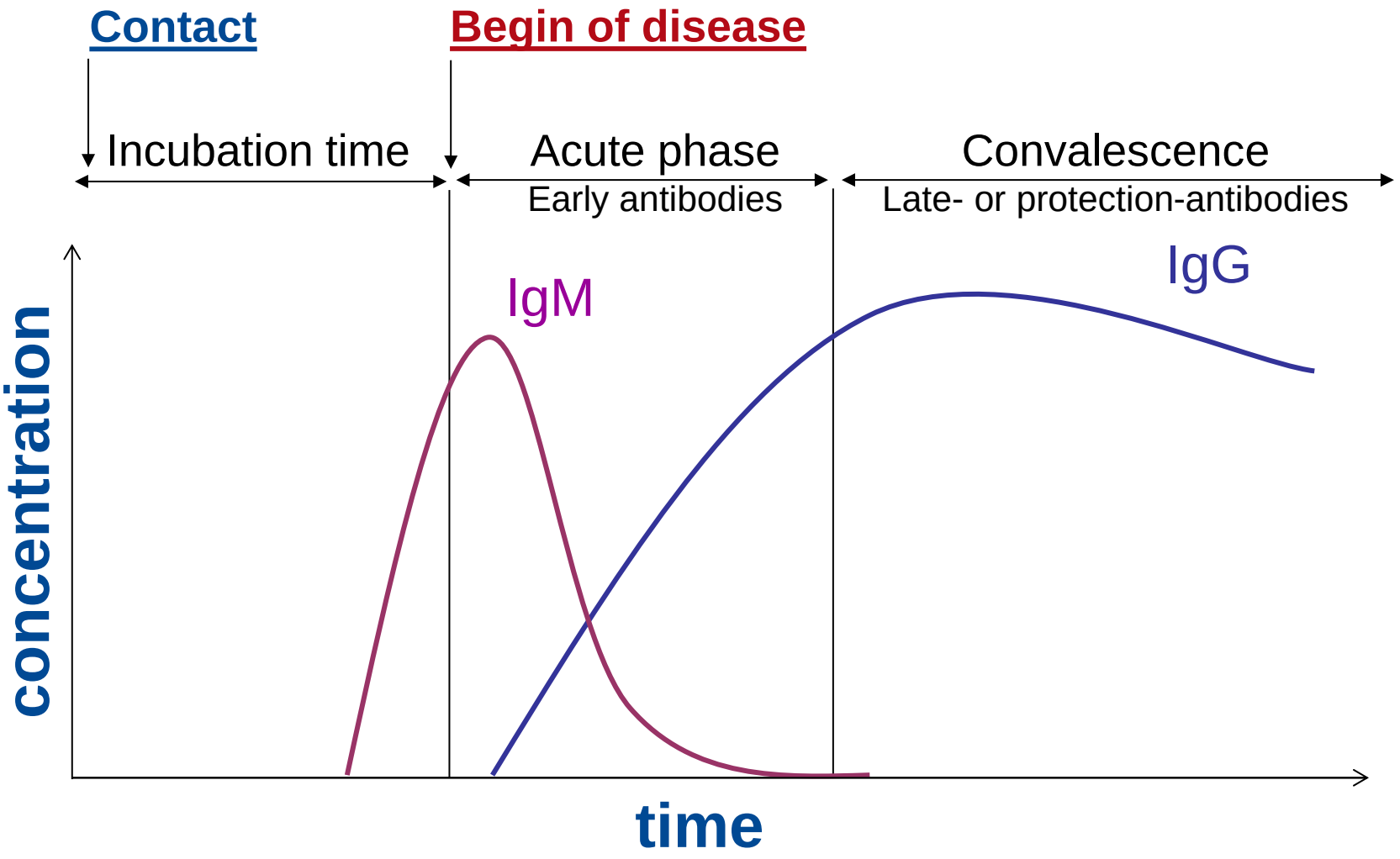


(Garland Science 2005)

IgA forms **dimers**

- Secretion of the antibody on mucosal surfaces

The serum-concentration of specific antibodies generated during infection to fight the infectious agent



Antibody classes - Isotypes

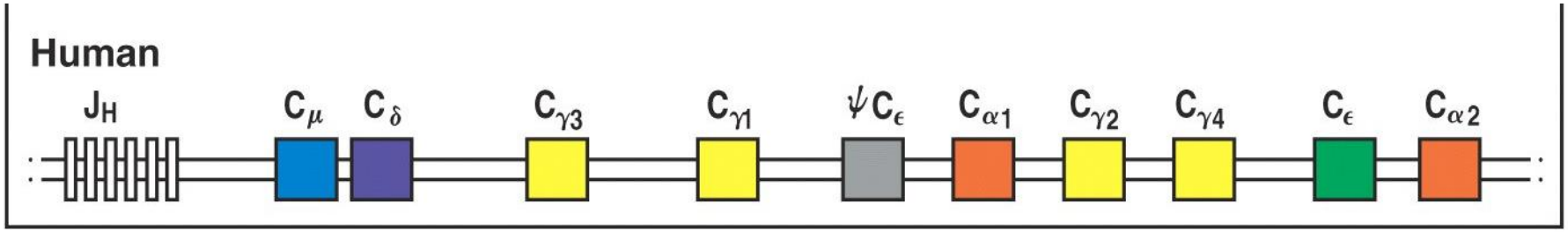


Figure 4-19 Immunobiology, 6/e. (© Garland Science 2005)

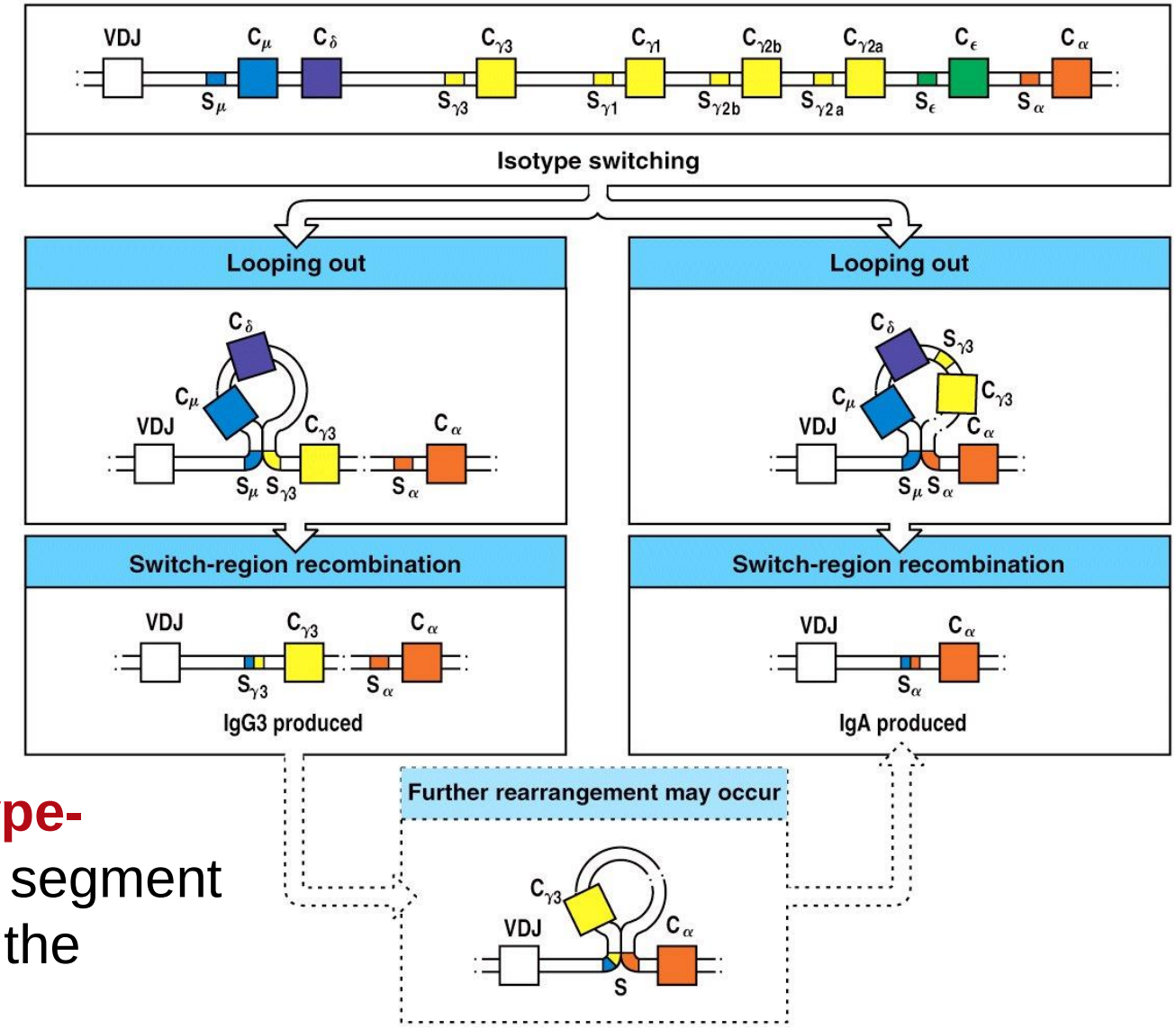
cμ = IgM, cδ = IgD, cγ = IgG, cε = IgE, cα = IgA

Ψ = Pseudogene

The expression of the genes of the constant region changes during the maturation of the B-cell =

Isotype-Switch

Antibody classes - Isotypes



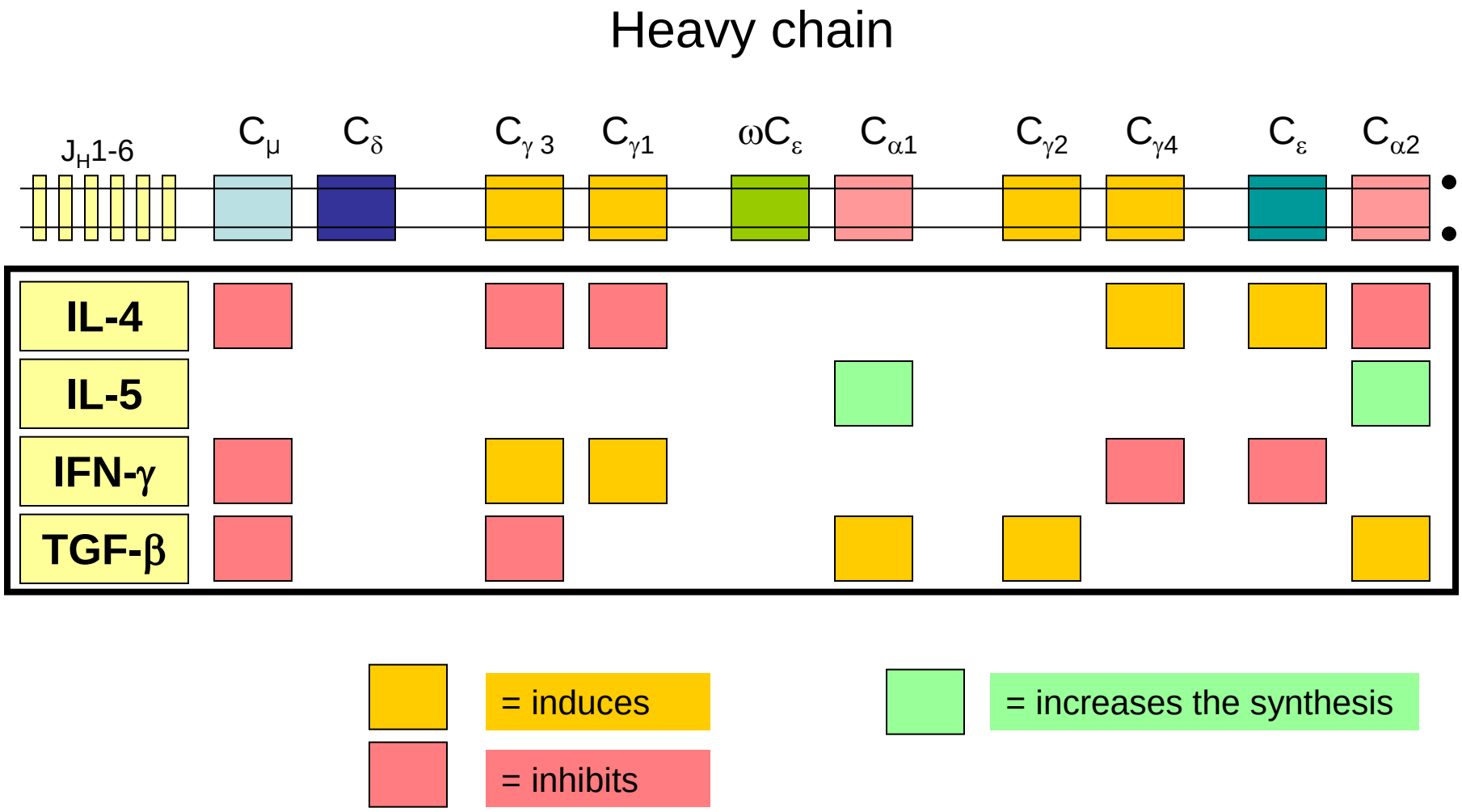
During the **Isotype-Switch** the gene segment located between the switching signal sequences is excised

→ develoment just in one direction

Antibody classes - Isotypes

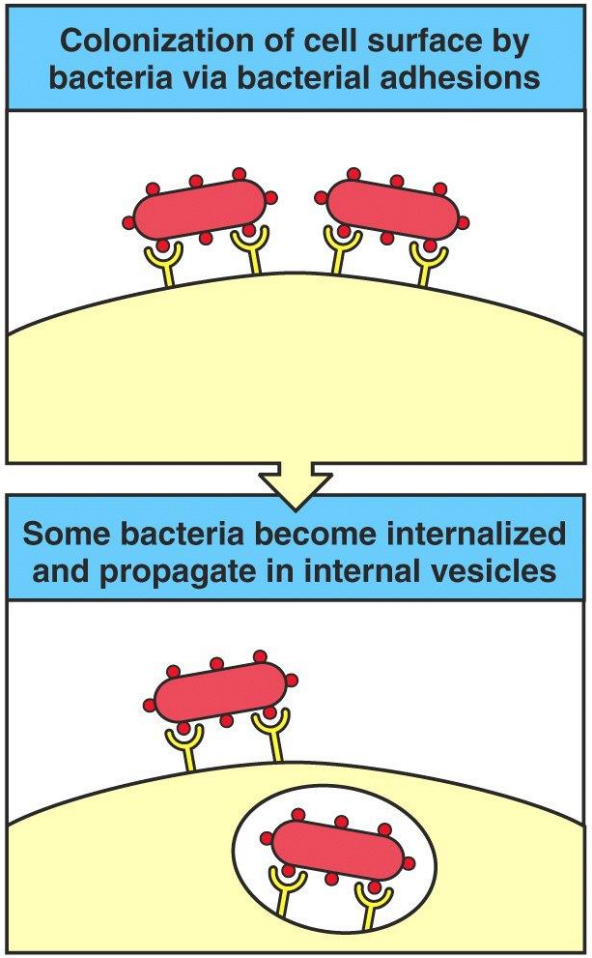
- Specific DNA-sequences (**Switch regions**) regulate the recombination
- The isotype-switch takes place in the lymph node (**germinal centre**)
- It requires a specific milieu of Cytokines, which induce transcription of enzymes involved in the switching process: **transcription factors**

Cytokines involved in isotype-switching



Antibody classes - function

1) **Neutralisation** (Viruses, bacteria, toxins): IgG and IgA



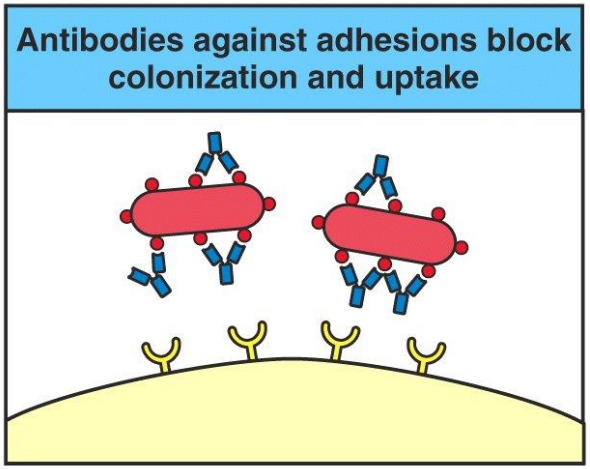


Figure 9-26 Immunobiology, 6/e. (© Garland Science 2005)

Antibody classes - function

2) Opsonisation (supports phagocytosis): IgG1 and IgG3

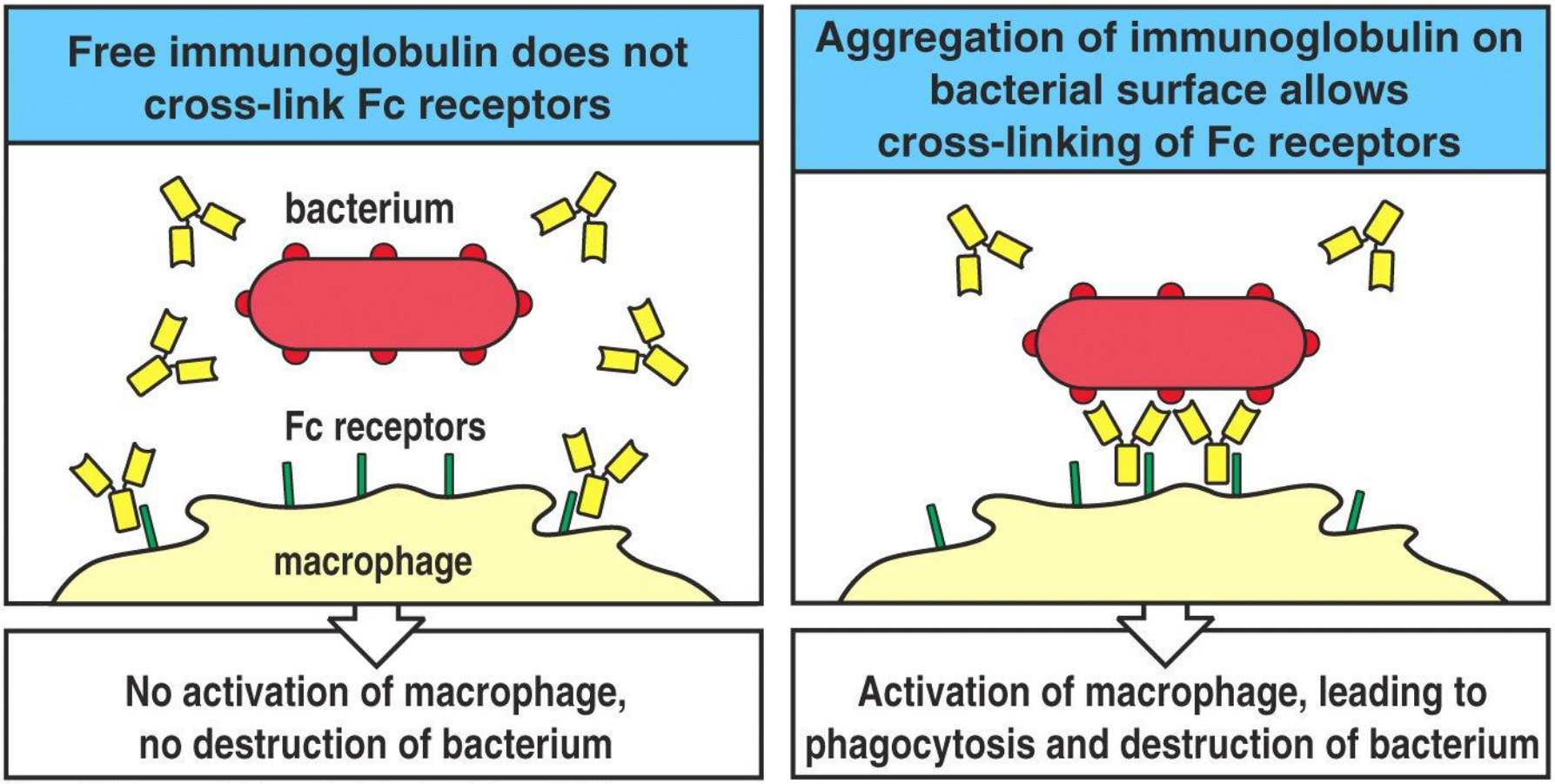
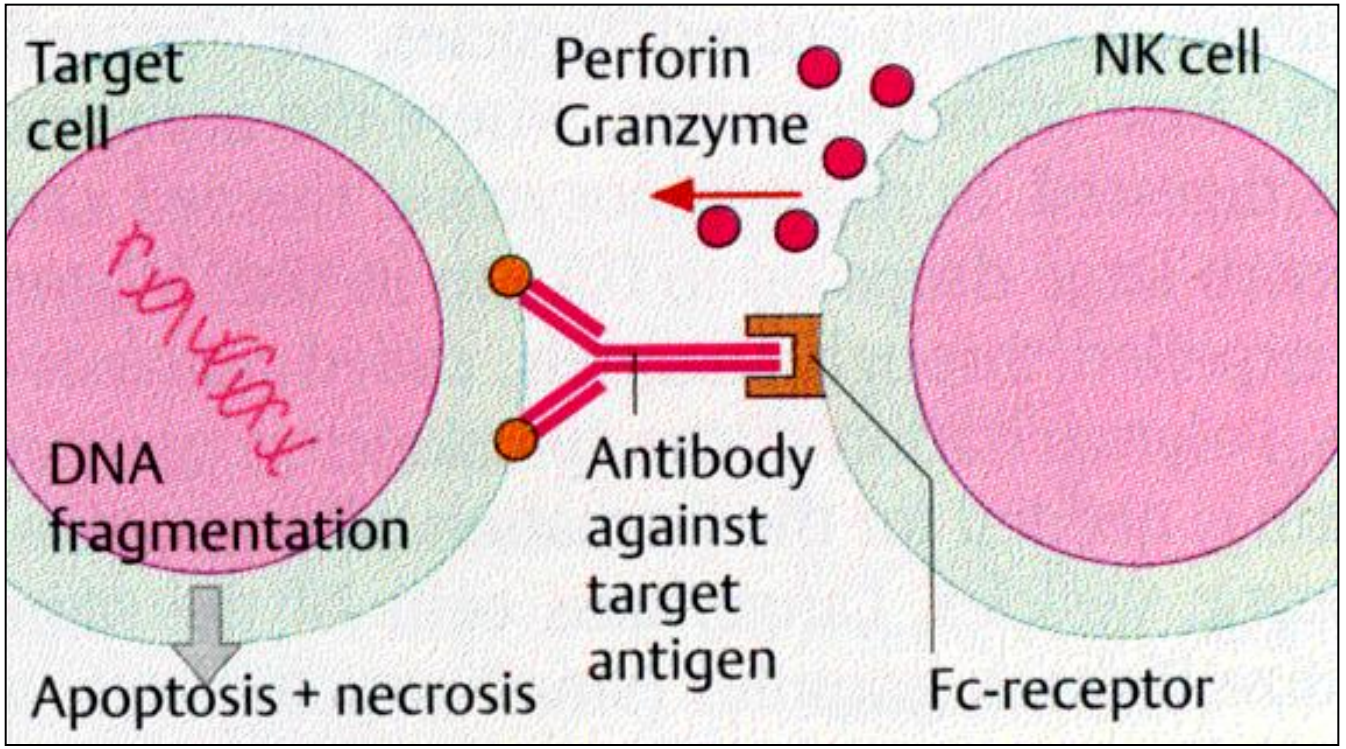


Figure 9-31 Immunobiology, 6/e. (© Garland Science 2005)

Cytolytic mechanisms of NK-cells

ADCC (antibody-dependent cellular cytotoxicity): Lysis of cells opsonized by an antibody by induction of apoptosis via secretion of granzyme and perforin



Antibody classes - function

3) Complement activation (classic way): IgG and IgM

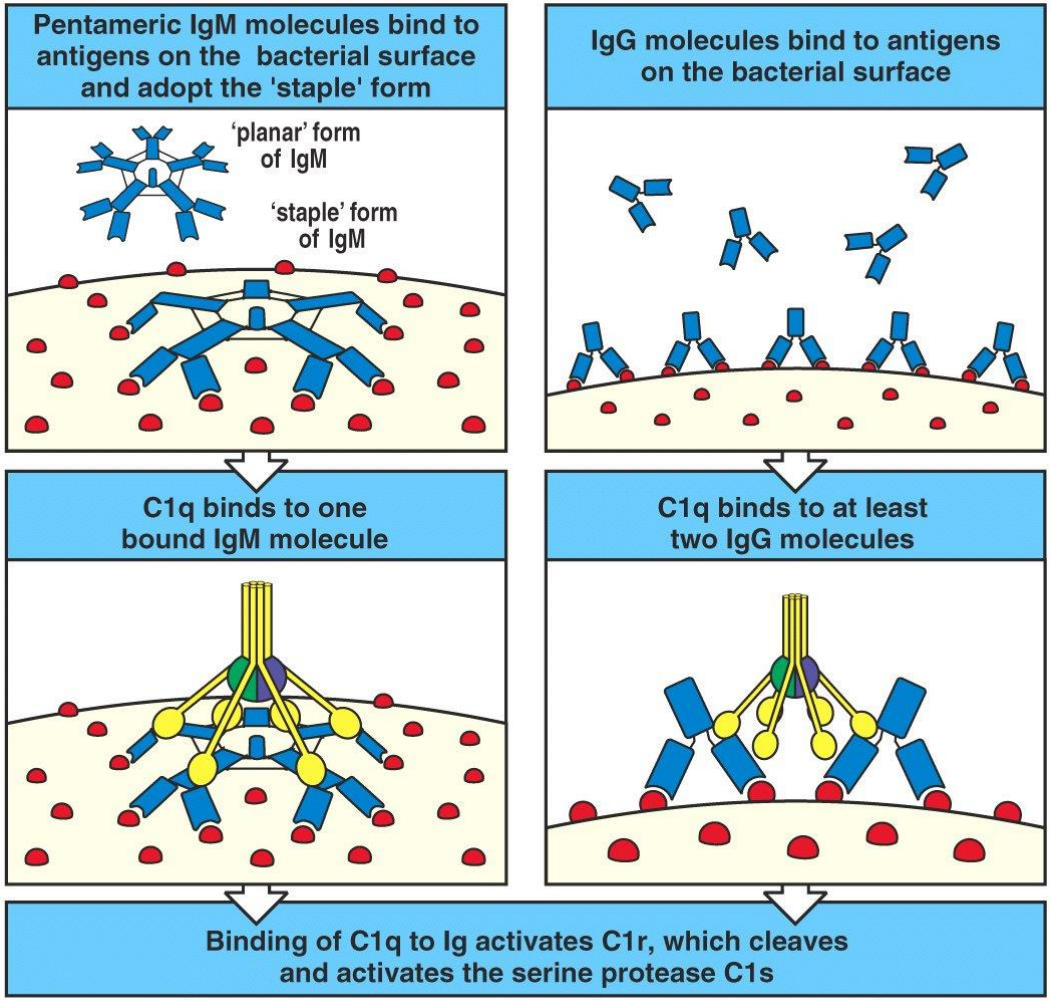


Figure 9-28 Immunobiology, 6/e. (© Garland Science 2005)

Antibody classes - function

There is a specific role for each antibody class during the immune response

Function	IgM	IgD	IgG	IgA	IgE
Neutralisation	+	-	++	++	-
Opsonisation	-	-	++	+	-
Mast cell sensitization	-	-	-	-	+++
Complement activation	+++	-	+	+	-
Transport via epithelia	+	-	-	+++	-
Transport via placenta	-	-	+++	-	-
Diffusion into the tissue	+/-	-	+++	++	+
Serum level [mg/ml]	1.5	0.04	13.5	2.1	0.005

B-cell activation

Naive B-cells need **2 signals** for activation:

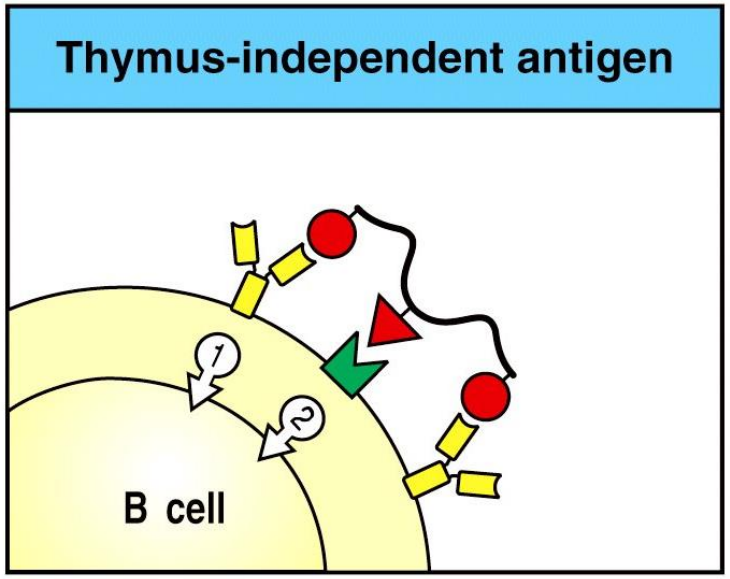
First signal: signalling through the B-cell receptor (BCR) = **Surface-Immunoglobulin**

The **second signal** can be given in two ways:

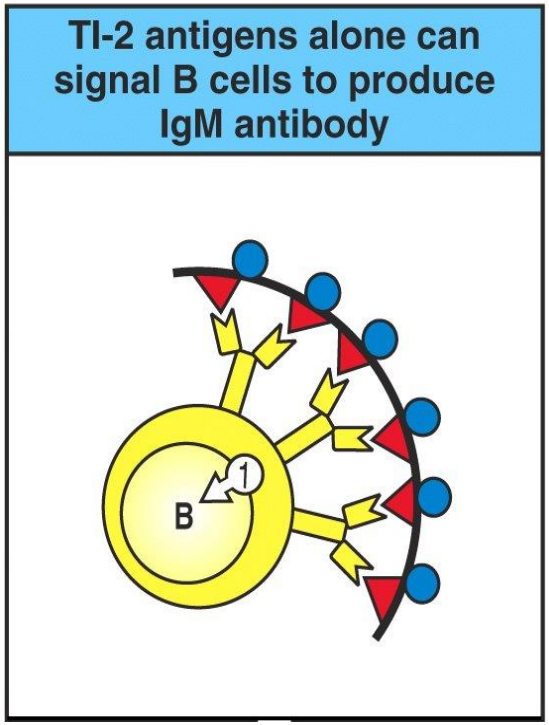
1) **Thymus-dependent** (via already activated T-helper-cells) = TD-antigens (thymus dependent)

2) **Thymus-independent** (activation without Tcell help) = TI- antigen (thymus independent)

Thymus-independent (TI)-B-cell activation



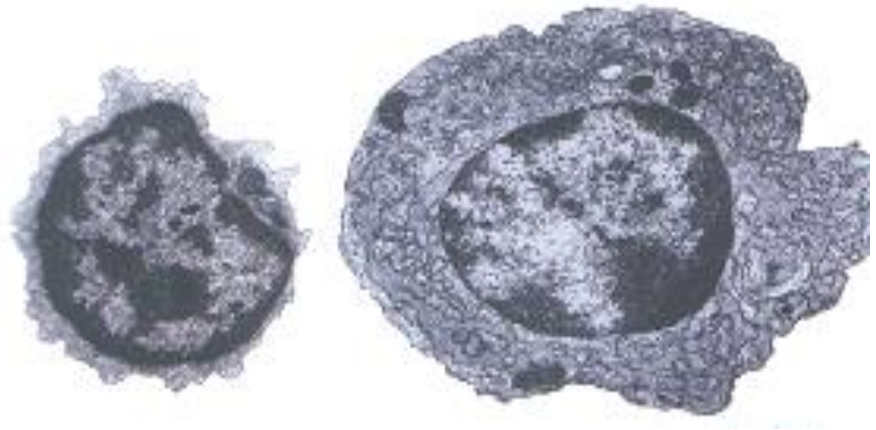
TI-1-antigens activate a further receptor on the surface, e.g. TLR-4 against LPS or a complement receptor = co-stimulatory signal



TI-2-antigens activate the B-cell by crosslinking the BCR via a large molecule containing antigens without costimulation

TI-B-cell activation

TI (1+2)-activation : B-cell → Plasma cell



- no germinal centre
- no memory cells
- no affinity maturation

Thymus-dependent-B-cell activation

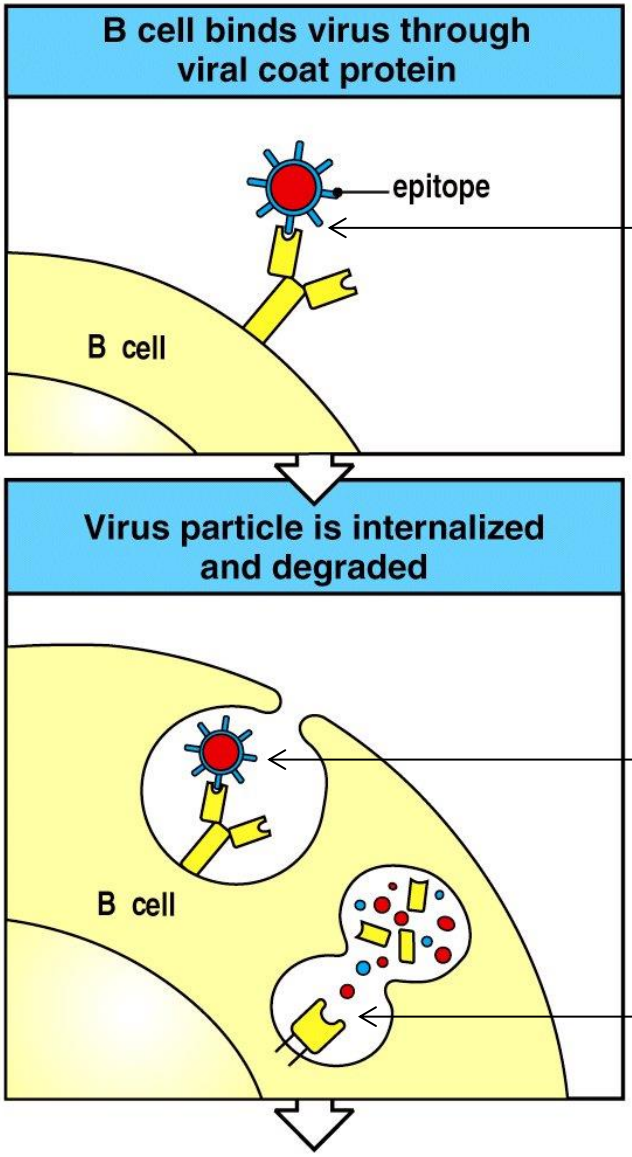


Figure 9-3 Immunobiology, 6/e. (© Garland Science 2005)

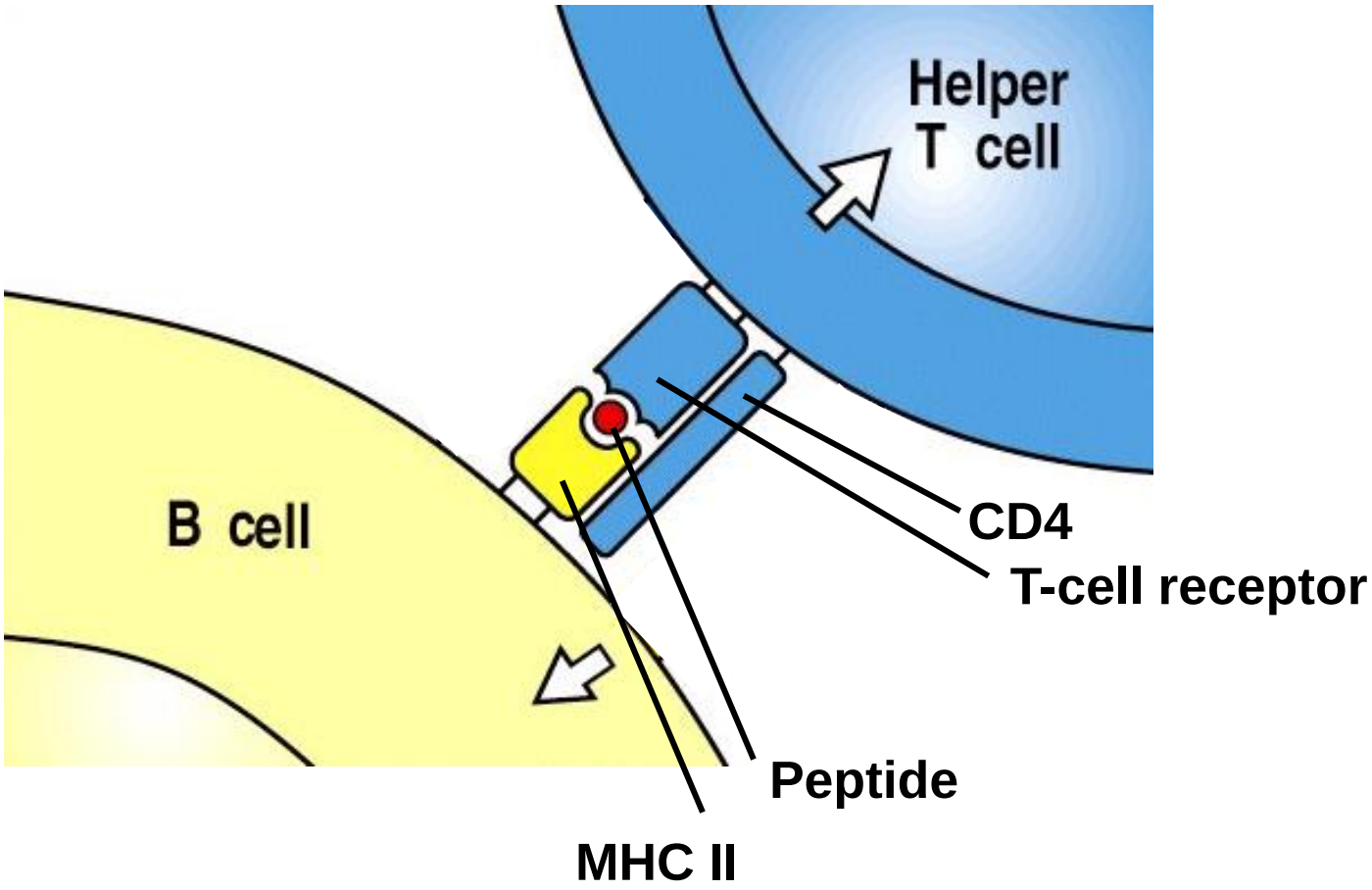
Binding of an antigen to its specific BCR

Internalization of the Antigen/BCR complex

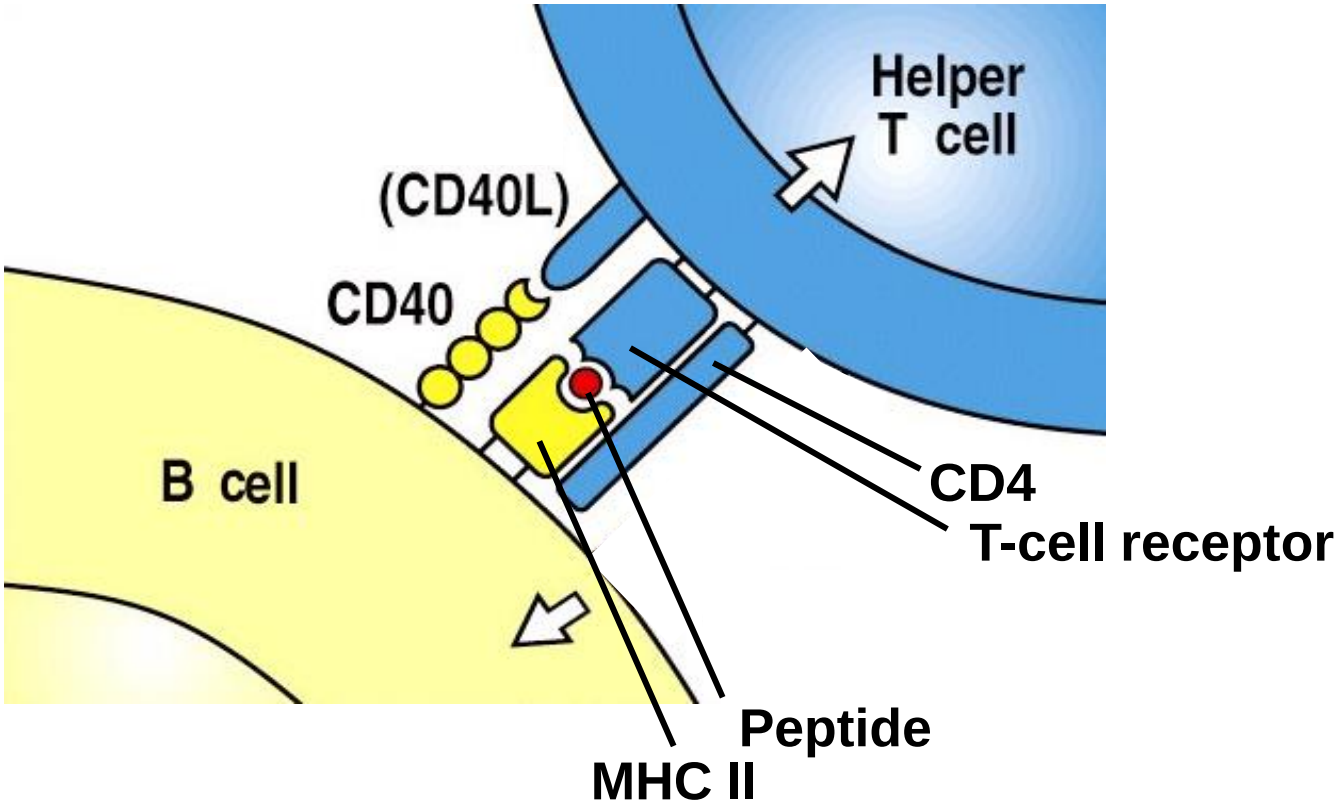
Degradation of proteins and binding to MHCII

Thymus-dependent-B-cell activation

Linked recognition = T-cell and B-cell recognise the same antigen (the B cell via the BCR; and the T-cell via the TCR)

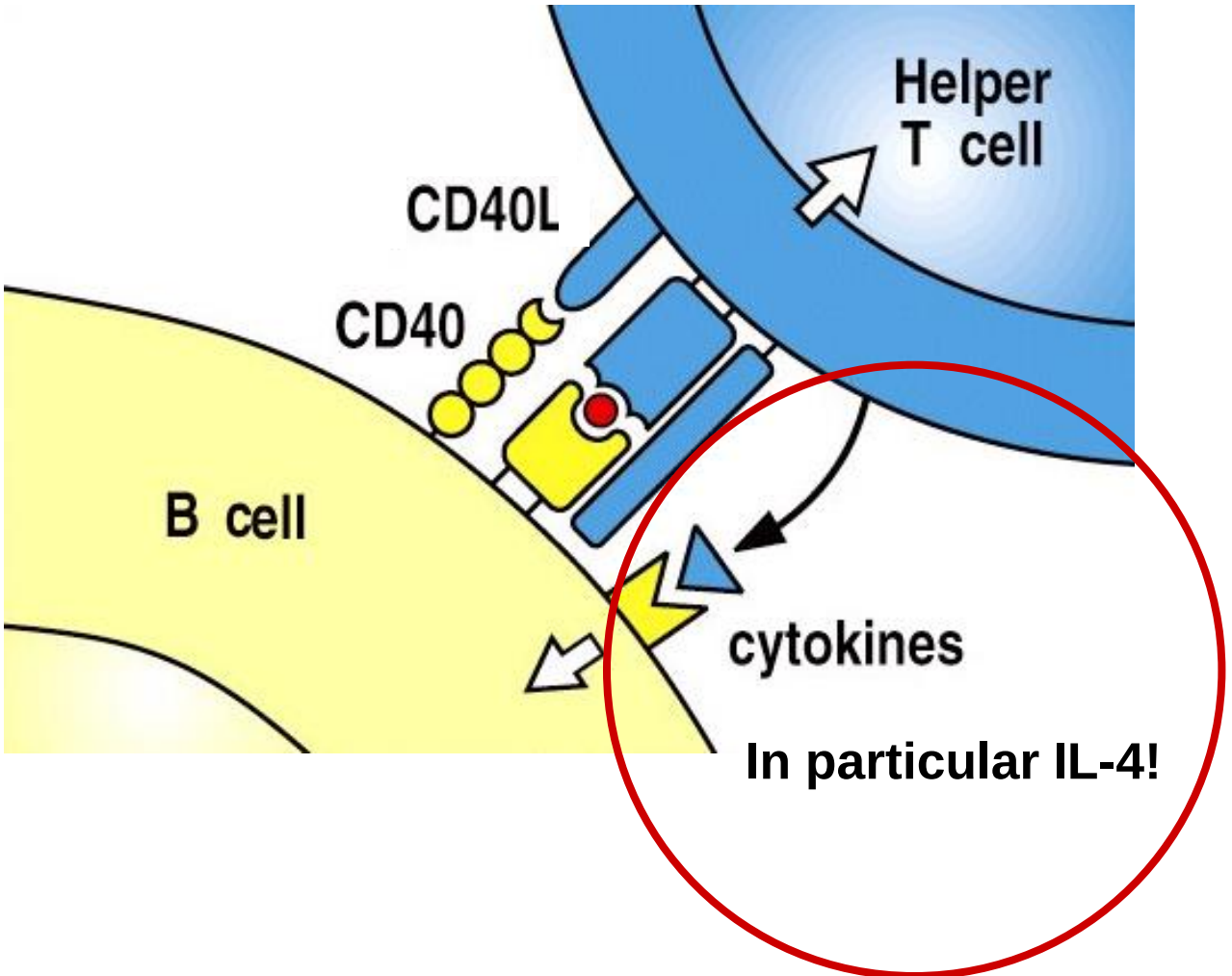


Thymus-dependent-B-cell activation



A T-cell does not express co-stimulatory signals for B cells until the cell is **activated** by a **dendritic cell!**

Thymus-dependent-B-cell activation



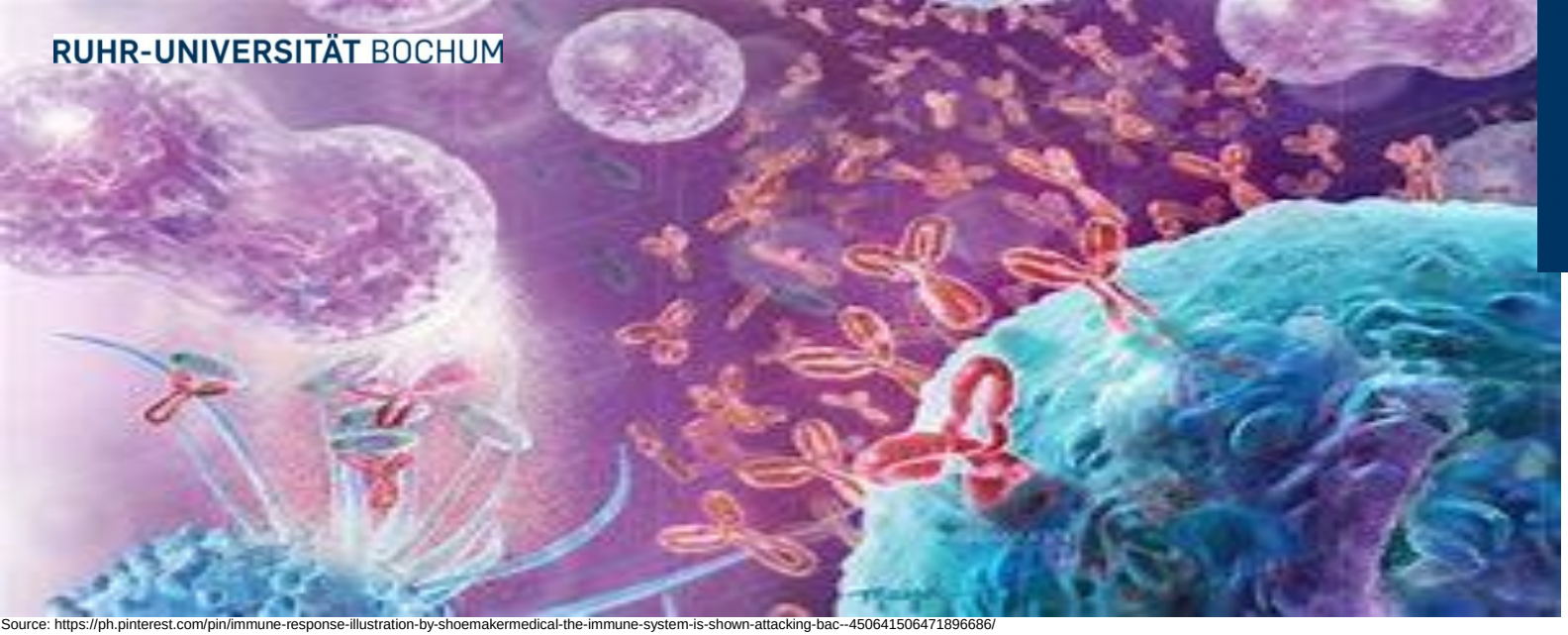
Summary: TD-B-cell activation

Signals for the B-cell activation:

1) Binding of an antigen to the BCR
   (B-cell receptor = membranous immunoglobulin)

2) Signals given by a T-cell, which recognised the MHC-II-presented peptide via TCR and the co-receptor CD4:

   - Stimulation of CD40 (B-cell) by CD40-ligand (T-cell)
   - Cytokines



Source: https://ph.pinterest.com/pin/immune-response-illustration-by-shoemakermedical-the-immune-system-is-shown-attacking-bac--450641506471896686/

B-Cells and Antibodies

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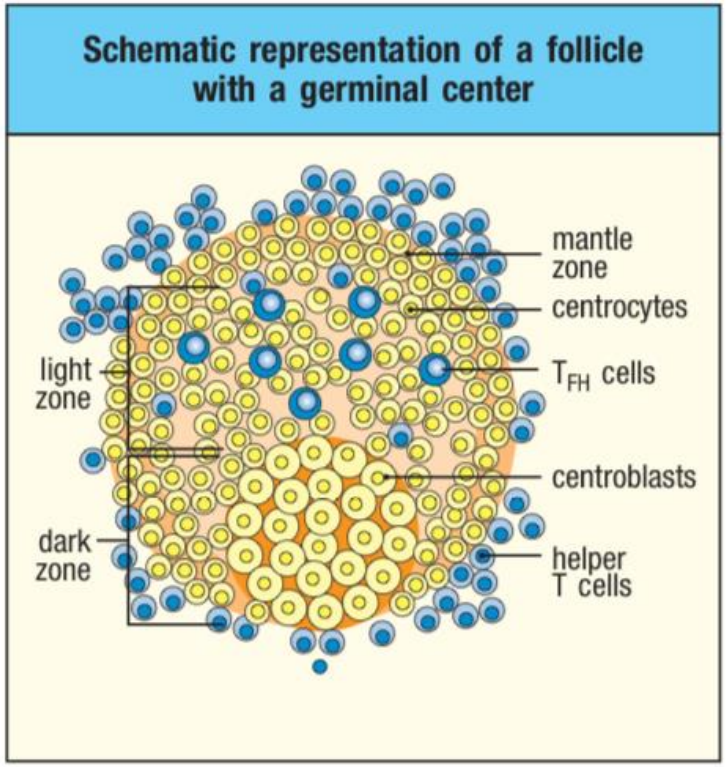
Immunology

04.06.2025

The second phase of a primary B-cell immune response

activation by TFH cells → B-cells migrate into follicles and proliferate → formation of germinal centers

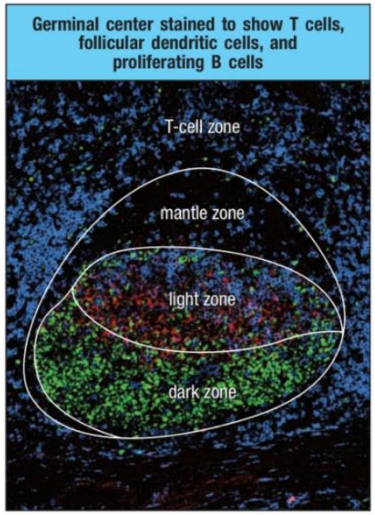
Germinal Center



Source: Murphy, Kenneth M.; Weaver, Casey (2017): Janeway's immunobiology. Unter Mitarbeit von Charles A. Janeway, Allan Mowat, Leslie J. Berg, David D. Chaplin, Paul Travers und Mark Walport. 9th edition. New York, London: GS Garland Science Taylor & Francis Group.

- ➢ Proliferating B cells, with ~ 10% antigen-specific T Cells

- ✓ Dark zone
- ✓ Light zone
- ✓ Mantle zone



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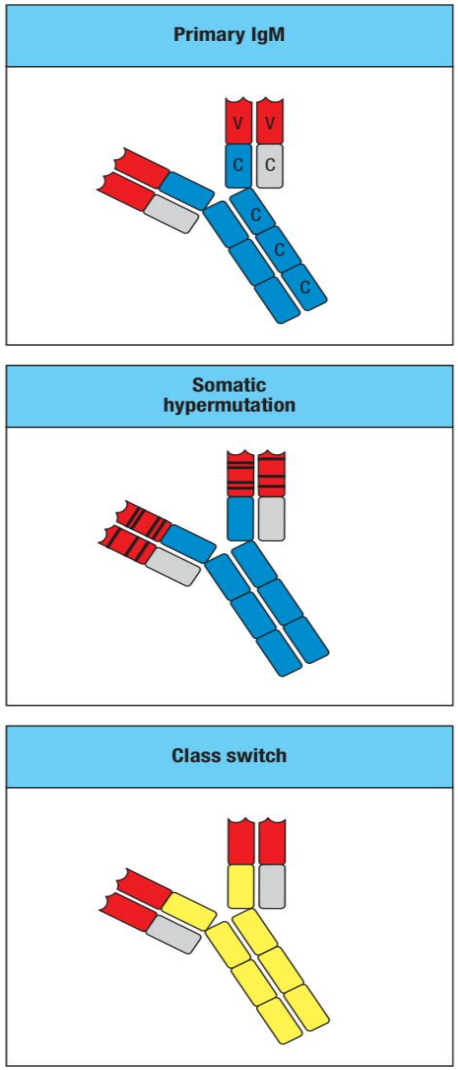
Centroblasts = proliferating antigen specific Bcells
Centrocytes = non-proliferating antigen specific Bcells

Antibody Production



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Somatic Hypermutation

→ Modifies the V region of immunoglobulin genes

Affinity Maturation

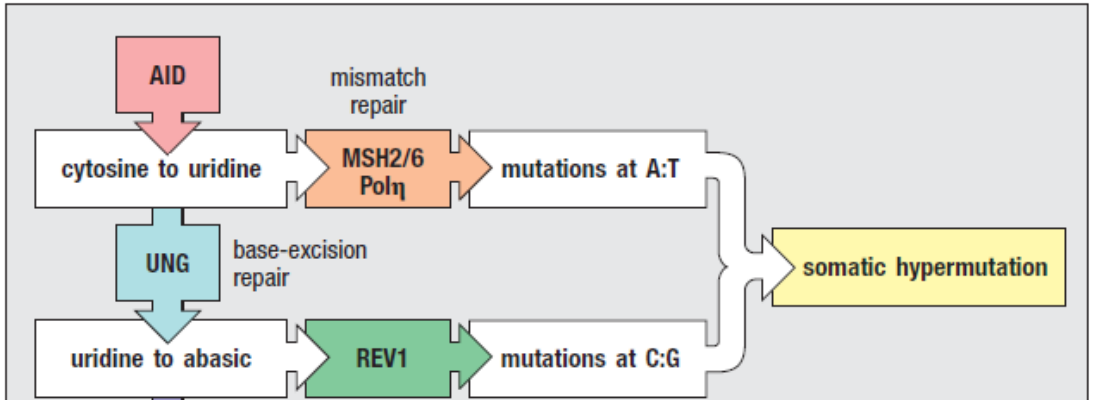
→ Selection of B Cells with high antigen affinity

Class switching

→ Production of antibodies with various effector functions

Somatic Hypermutation

- Initiation of mutations in immunoglobulin V-region genes by activation-induced cytidine desaminase (AID)
- High mutation rate in V-regions
- Negative vs. Positive Selection



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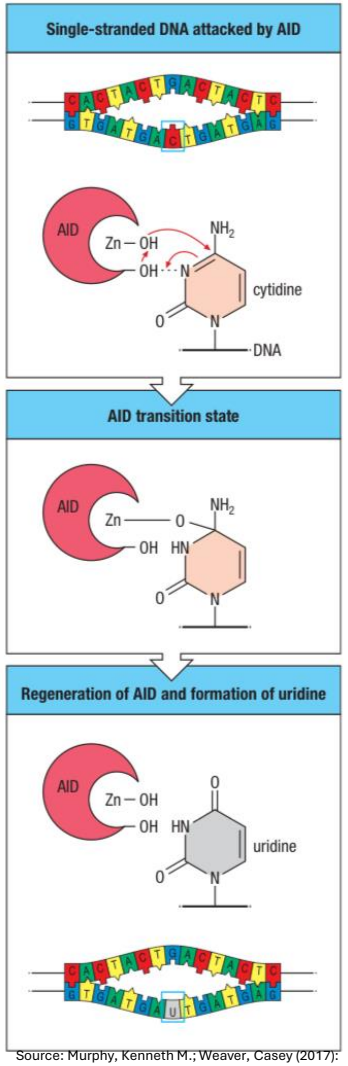

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Mismatch and base-excision repair mechanisms

Mismatch Repair Mechanism:

- Detection by MSH2 and MSH6
- Removing of uridine and neighboring nucleotides
- Repairing of DNA by error-prone DNA polymerase

Base-Excision Repair Mechanism:

- Creation of an abasic site in the DNA by uracil-DNA glycosylase (UNG)
- Insertion of random nucleotides causing mutations
- Creation of single-strand discontinuity by apurinic/apyrimidinic endonuclease 1 (APE1)

Follicular dendritic cells

- Storing of antigens in form of immune complexes on follicular dendritic cells (FDCs)

- Antigens are presented on the sufrace of the FDCs

  ⟶ Interaction with centrocytes

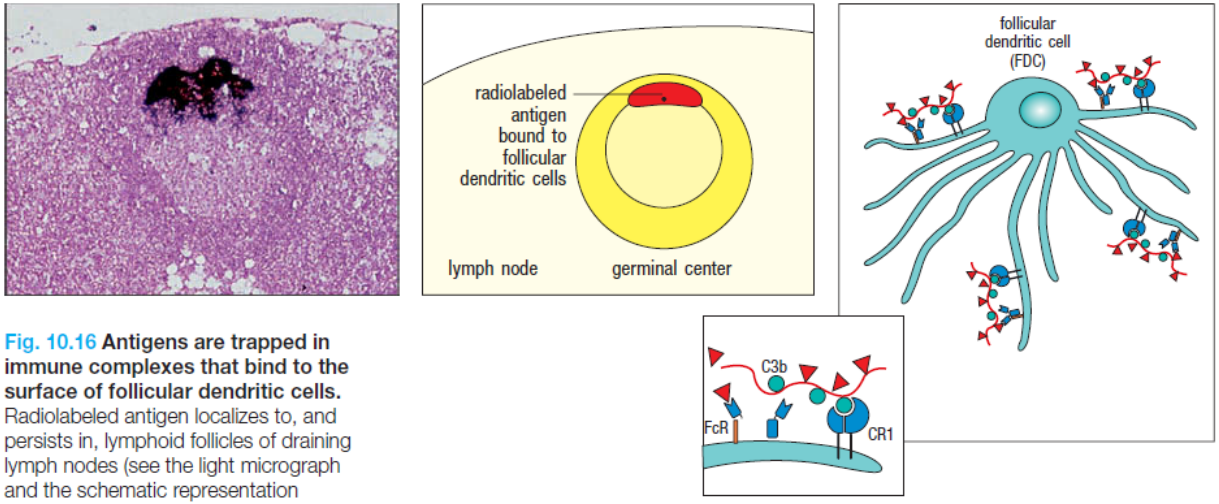

Fig. 10.16 **Antigens are trapped in immune complexes that bind to the surface of follicular dendritic cells.** Radiolabeled antigen localizes to, and persists in, lymphoid follicles of draining lymph nodes (see the light micrograph and the schematic representation (middle panel), showing a germinal center

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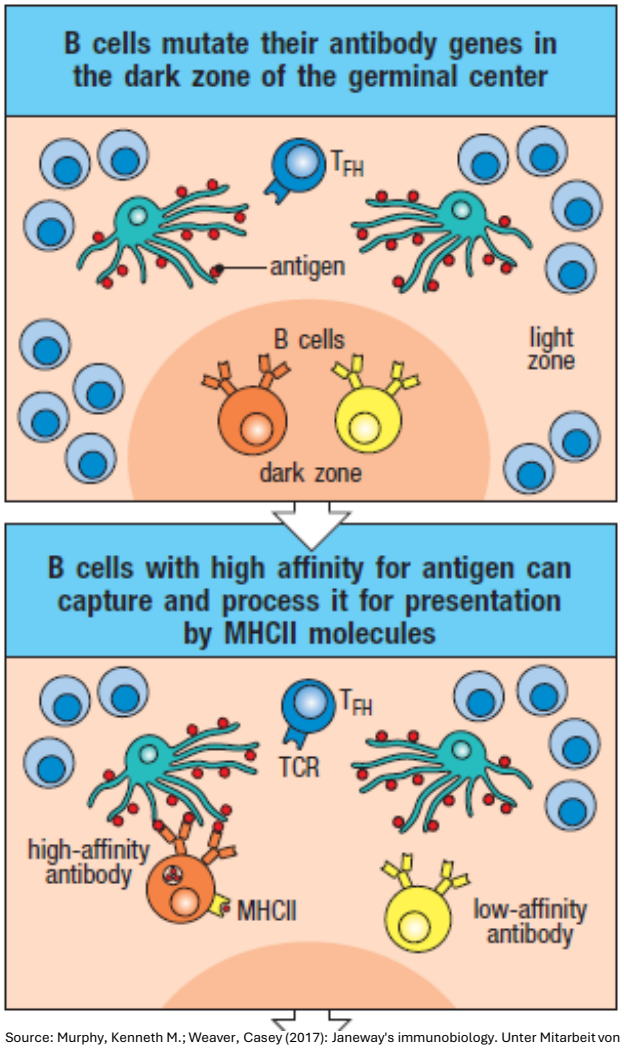
Affinity Maturation

- B cells are moving to the light zone
- Competition for antigens presented by FDCs
- Higher-affinity receptor
  - → Capturing and presenting of more antigens to TFH cells
- Recognition of antigen-derived peptides on MHCII



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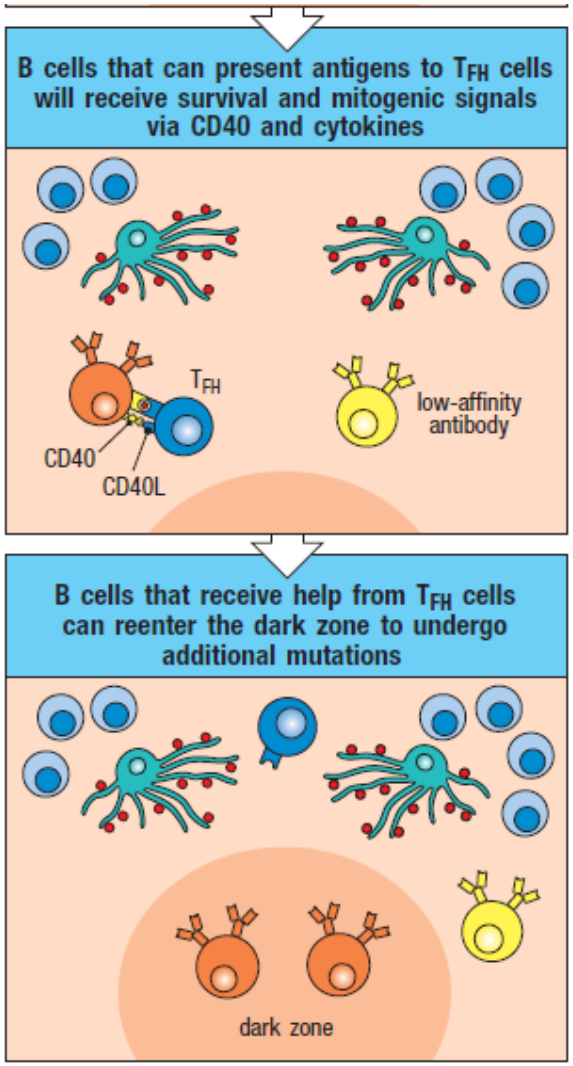
Affinity Maturation



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- Providing survival signals
  - → Ensured by CD40 signaling
- Reexpression of CXCR4 (not required) and re-enter into the dark zone
  - → **Cyclic reentry model**

Cyclic re-entry model

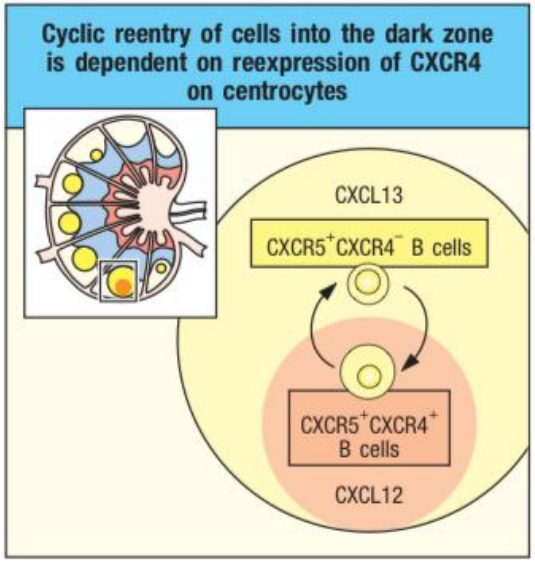
- Alternation between two zones: dark zone and light zone
- B cells mutate in the dark zone
- Mutated B cells move to the light zone
- B cells differentiate

  → Memory B cells or plasma cells

  OR

- Re-enter into the dark zone

  → Refinement of specificity and affinity



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Class Switch Mechanism

- Class Switch Recombination initiated by AID
- Switch region sequences have to be accessible to AID
- Importance of RNA exosomes and Spt5
- Recrution of AID by G-rich regions and G-quadruplexes
- Key Cytokines
  - IL-4
  - IL-5
  - IFN-γ
  - TGF-β
  - IL-21

Effect: Differentiation into Memory B Cells or Plasma Cells

Memory B Cells

- Inherit genetic changes and somatic hypermutation
- Long-living, devide slowly, secrete no antibodies
- Mucosal tissue

➡ Rapid responses

Plasma Cells

- Undergo affinity maturation and class switching
- Secretion of antibodies
- Bone marrow tissue and peripheral tissue

➡ Long-term protection

Summary

- ✓ Migration of activated B cells into lymphoid follicles
  - → Formation of germinal centers
- ✓ B cells undergo somatic hypermutation and affinity maturation
- ✓ Initiation of mutations by AID
- ✓ Repair mechanisms: Mismatch Repair Mechanism and Base-Excision Repair Mechanism
- ✓ Class Switch Recombination involves irreversible DNA recombination
- ✓ Differentiation into memory B cells or plasma cells