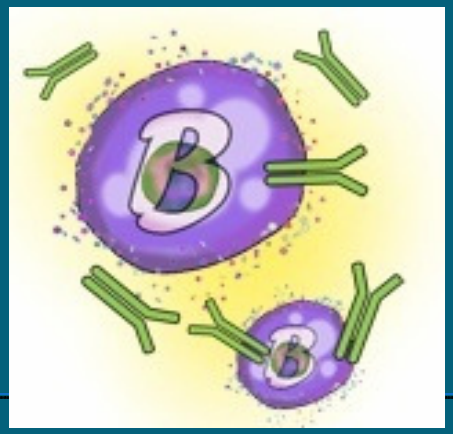
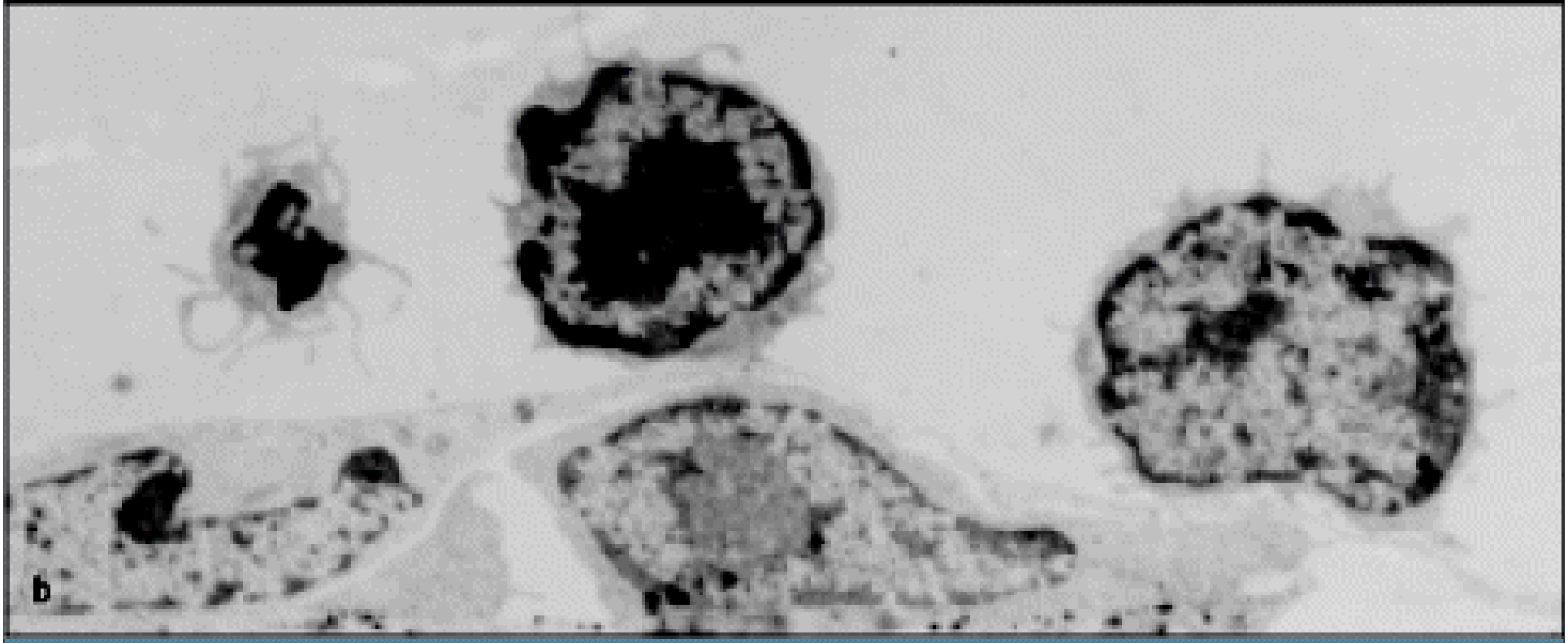
Inmunología Clínica 2010

Bioq Graciela R Svibel de Mizdraji



ONTOGENIA B

Desarrollo de Células B



- Células estromales
- IL-7
- SCF (stem cell factor) – c-Kit
- SDF-1 (stromal derived factor)

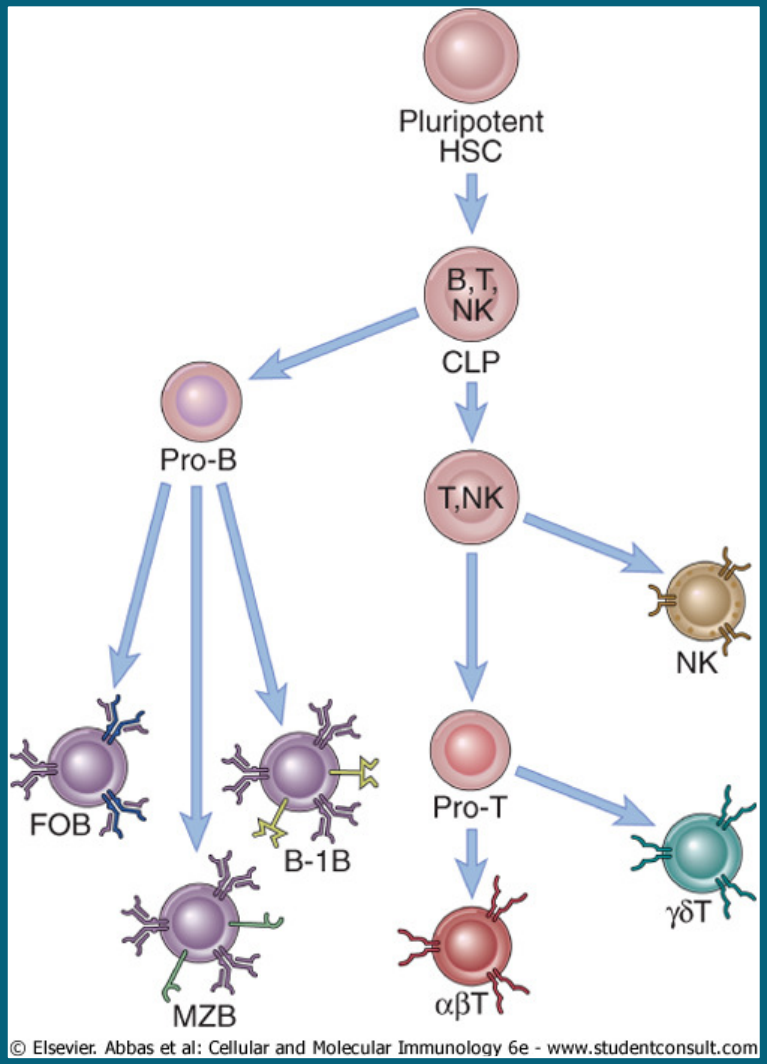
DEFICIENCIA DE FACTORES MICROOAMBIENTALES EN LA MÉDULA ÓSEA DEL RATÓN.....

Factor	CLPs	B-cell lineage				T-cell lineage			NK cells	DCs	Myeloid cells	Other defects in development	Refs
		Pre-pro-B cells	Pro-B cells	Pre-B cells	IgM* cells	ETPs	TN2 cells	T cells					
CXCL12	ND	↓↓↓	↓↓↓	ND	ND	ND	↓	↓	ND	ND	↓	Colonization of bone marrow by HSCs; colonization of gonads by PGCs; cardiogenesis; angiogenesis; neurogenesis	33,37, 40,41
IL-7	As WT*	As WT*	↓↓↓	↓↓↓	↓↓↓	ND	↓↓↓	↓↓↓	As WT	As WT	As WT	ND	64,65, 73
FLT3L	↓↓↓	↓↓↓	↓↓	↓	As WT	↓↓↓	↓↓	↓	↓↓	↓↓	↓	ND	55–57
SCF	↓↓	As WT	↓↓↓	↓↓↓	↓↓↓	ND	↓↓↓	↓↓↓	ND	ND	As WT	Erythropoiesis; mast-cell development; gastrointestinal motility; PGC development; melanoblast development	85,87
RANKL	ND	ND	ND	↓↓	↓↓	ND	ND	↓	ND	As WT	As WT	Osteoblast development; lymph-node organogenesis; tooth eruption	92,94

*B-cell differentiation potential was severely impaired. ↓, reduced by <3-fold; ↓↓, reduced by 3–10-fold; ↓↓↓, reduced >10 fold or not detectable. CLP, common lymphoid progenitor; CXCL12, CXC-chemokine ligand 12; DC, dendritic cell; ETP, early T-cell-lineage progenitor; FLT3L, fms-related tyrosine kinase 3 ligand; HSC, haematopoietic stem cell; IL-7, interleukin-7; ND, not determined; NK, natural killer; PGC, primordial germ cell; RANKL, receptor activator of nuclear factor-κB ligand; SCF, stem-cell factor; TN2, triple negative 2; WT, wild-type.

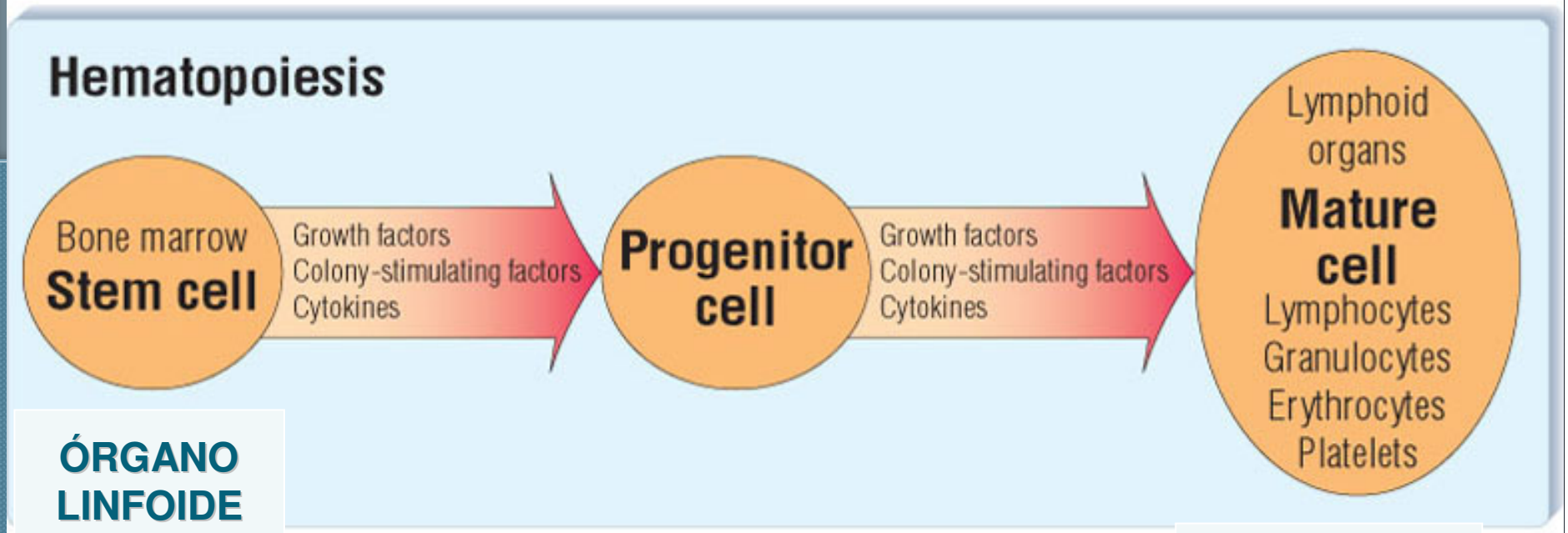
Nature Reviews Immunology 6, 107-116 (February 2006)

Hematopoyesis



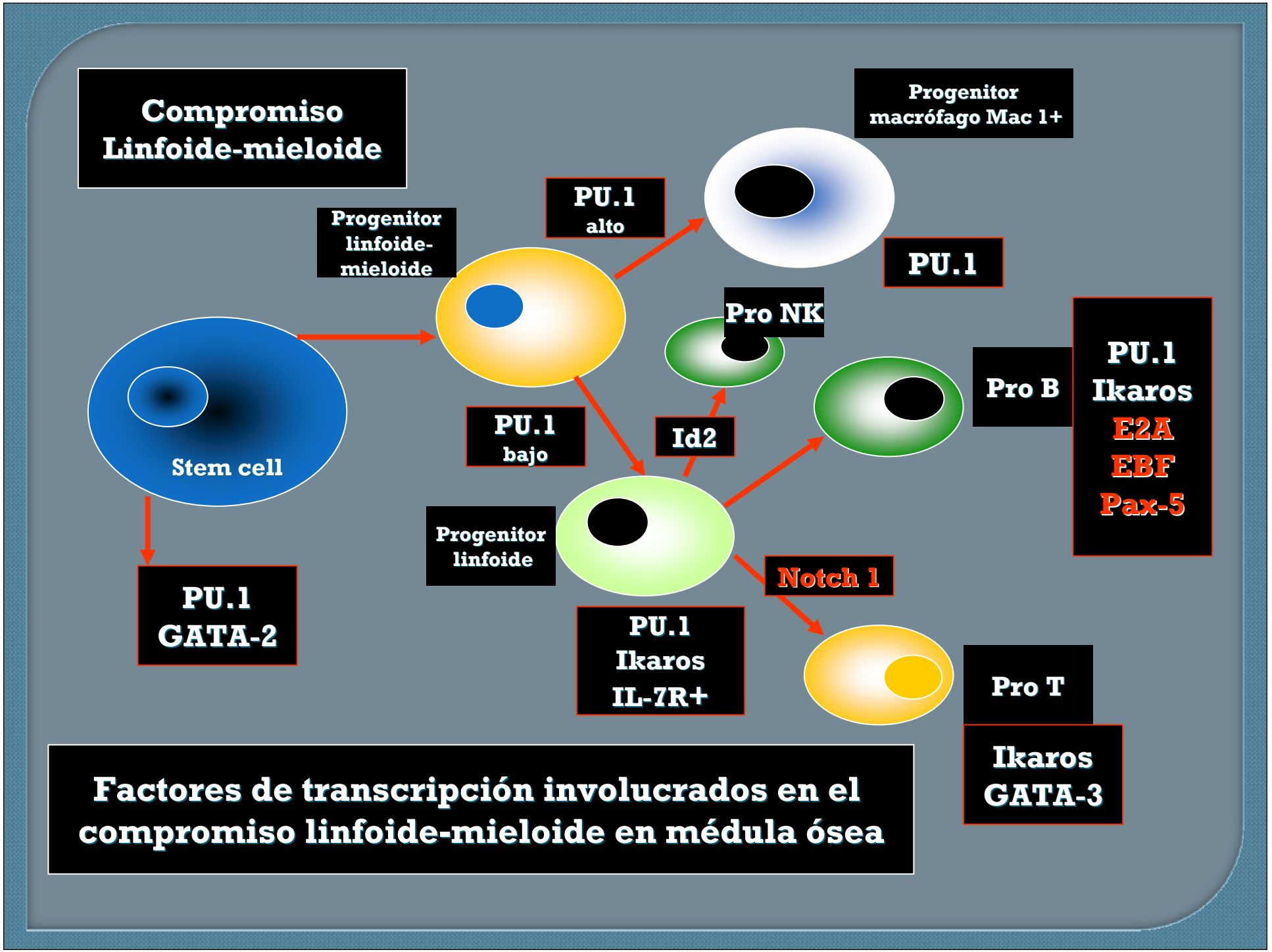
- **Durante la vida fetal, la hematopoyesis tiene lugar inicialmente en los islotes sanguíneos y después en el hígado fetal.**
- **Esta función es asumida gradualmente por la MÉDULA ÓSEA DE LOS HUESOS PLANOS, de tal manera que en la pubertad la hematopoyesis ocurre fundamentalmente en el esternón, las vértebras, los huesos ilíacos y las costillas.**
- **Todas las células sanguíneas se originan a partir de una HSC,** que se caracteriza por ser **CD34+**

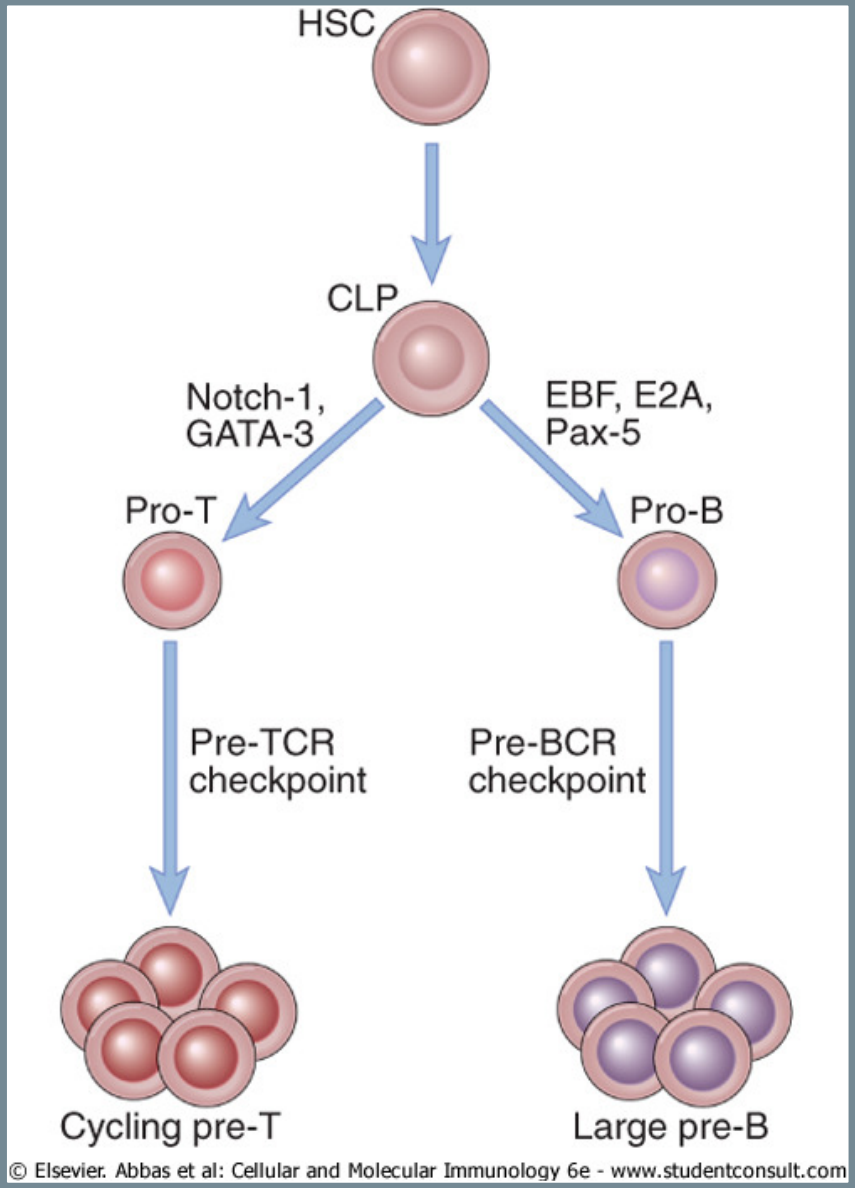
La MÉDULA ÓSEA ES EL SITIO DE ORIGEN Y DESARROLLO DE LAS CÉLULAS B.



ÓRGANO LINFOIDE PRIMARIO

ÓRGANO LINFOIDE SECUNDARIO


Compromiso Linfoide-mieloide
Progenitor macrófago Mac 1+
PU.1 alto
Progenitor linfoide-mieloide
PU.1
Pro NK
Pro B
PU.1 Ikaros E2A EBF Pax-5
Stem cell
PU.1 bajo
Id2
Progenitor linfoide
Notch 1
PU.1 GATA-2
PU.1 Ikaros IL-7R+
Pro T
Ikaros GATA-3
Factores de transcripción involucrados en el compromiso linfoide-mieloide en médula ósea


HSC
CLP
Notch-1,
GATA-3
EBF, E2A,
Pax-5
Pro-T
Pro-B
Pre-TCR
checkpoint
Pre-BCR
checkpoint
Cycling pre-T
Large pre-B
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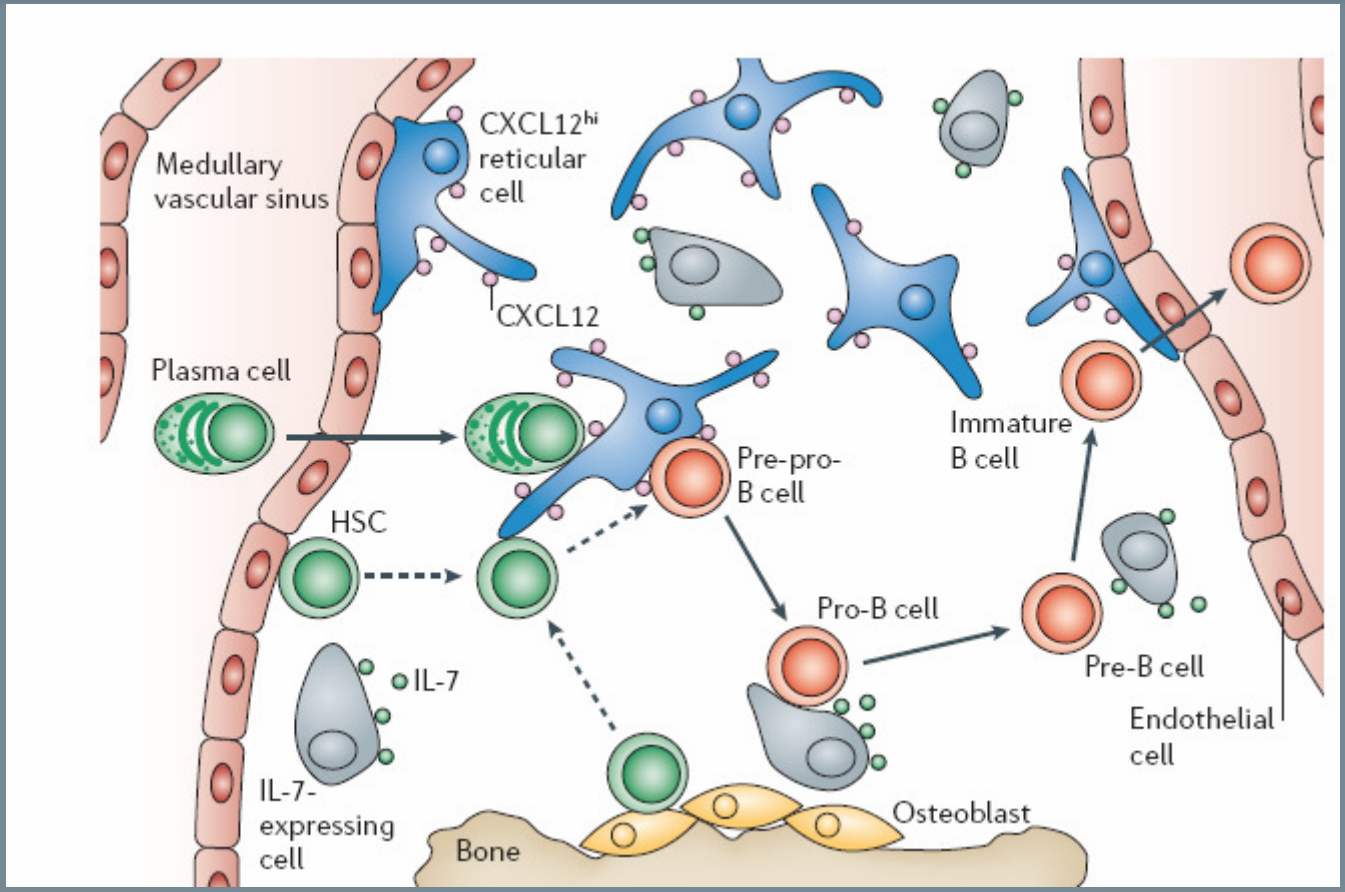
Para recordar

- **PU.1** (pertenece a la familia Ets) ha sido implicado recientemente en la determinación y compromiso temprano hacia linajes mieloides y linfoides
- **E2A** (perteneciente a la familia de factores *helix-loop-helix) y* **EBF** *(early-B cell factor)* están implicados en la iniciación de la linfopoyesis B. Estos dos factores regulan la expresión espacio temporal de las recombinasas RAG-1/RAG-2, esenciales en el proceso de recombinación V(D)J de los genes de inmunoglobulinas.
- **Pax-5** es esencial junto a E2A y EBF para lograr un total compromiso hacia el linaje B
- La señalización vía **Notch 1** permite el desarrollo temprano de células T.
- **Id2** pertenece a la familia *helix-loop-helix* y está comprometido en el desarrollo de células NK; actuaría secuestrando E2A controlando así la maduración del linaje NK.



In this model, the intermediate precursor cells between haematopoeitic stem cells (HSCS) — which are located near the **osteoblasts**, endothelial cellsor CXC-chemokine ligand 12^{hi} (CXCL12^{hi}) **reticular cells** — and pre-pro-B cells would move towards CXCL12^{hi} reticular cells. Pre-pro-B cells associate with CXCL12^{hi} reticular cells, whereas pro-B cells move away and instead adjoin interleukin-7 (IL-7)-expressing cells. Subsequently, pre-B cells leave IL-7-expressing cells.

B cells expressing cell-surface IgM exit the bone marrow and enter the blood to reach the spleen, where they mature into peripheral mature B cells.

End-stage B cells (plasma cells) again home to CXCL12^{hi} reticular cells in the bone marrow.

Nature Reviews Immunology 6, 107-116 (February 2006)

Rol de las células del estroma en el desarrollo de los linfocitos B

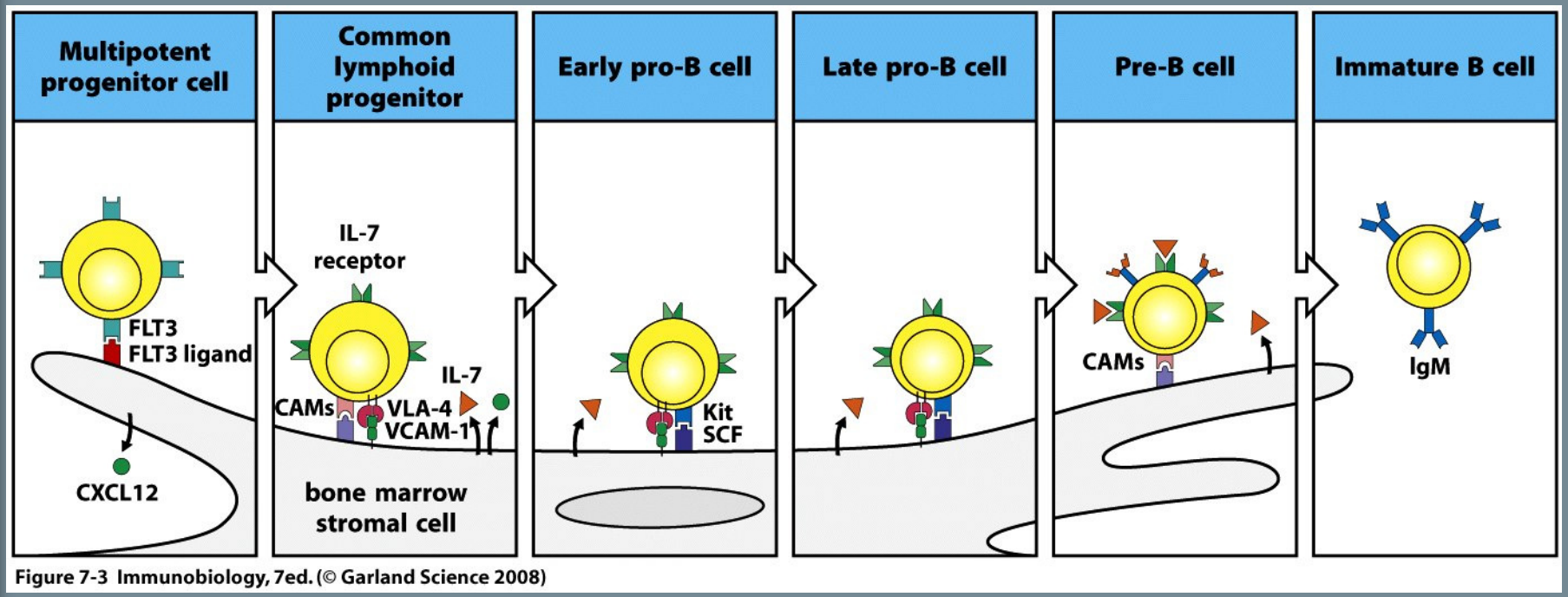
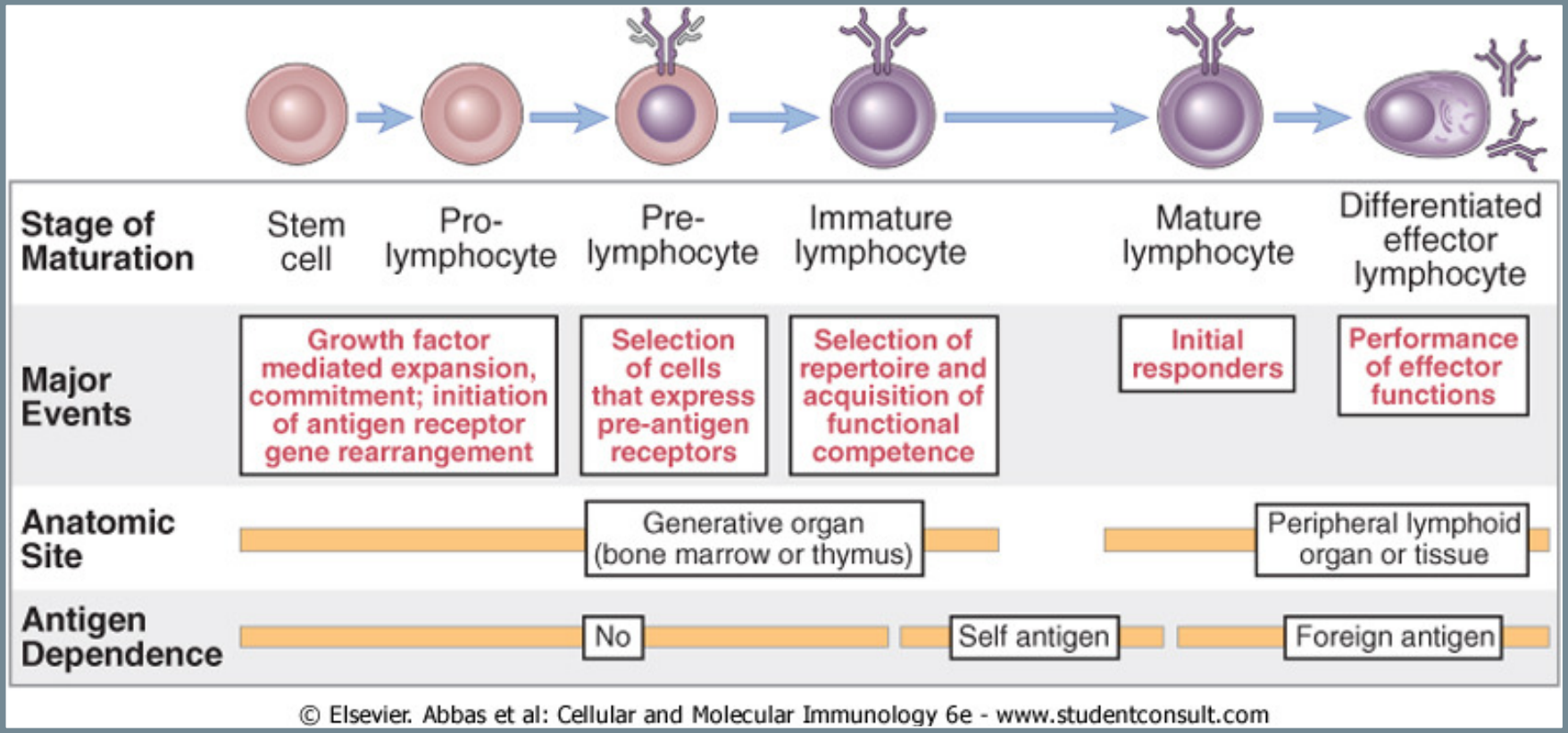
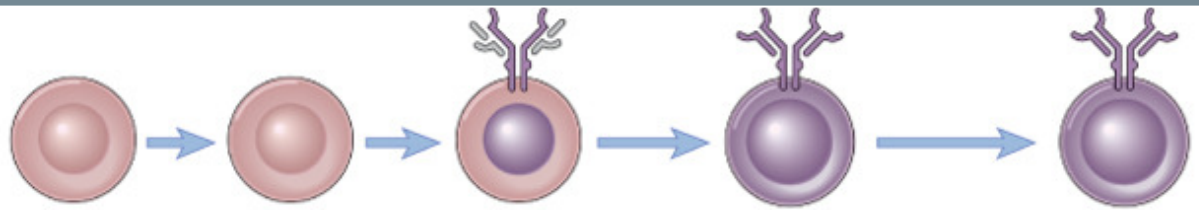


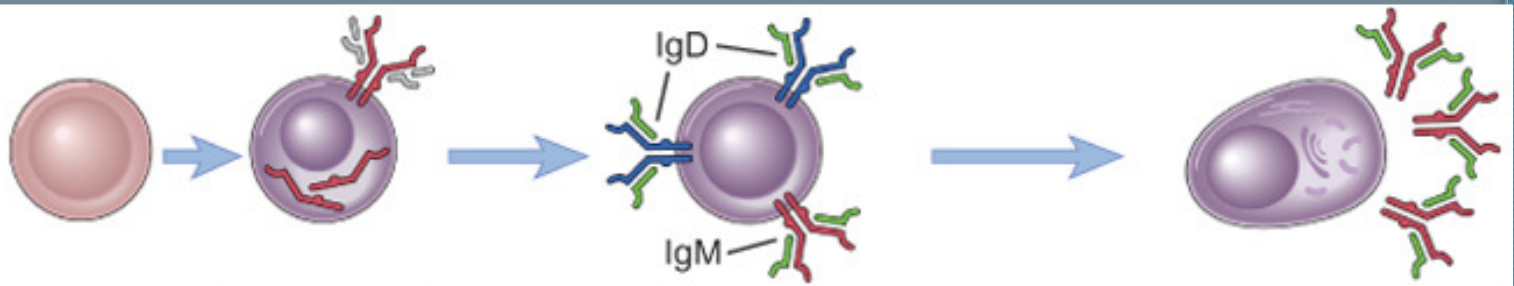
Figure 7-3 Immunobiology, 7ed. (© Garland Science 2008)

Desarrollo de la célula B

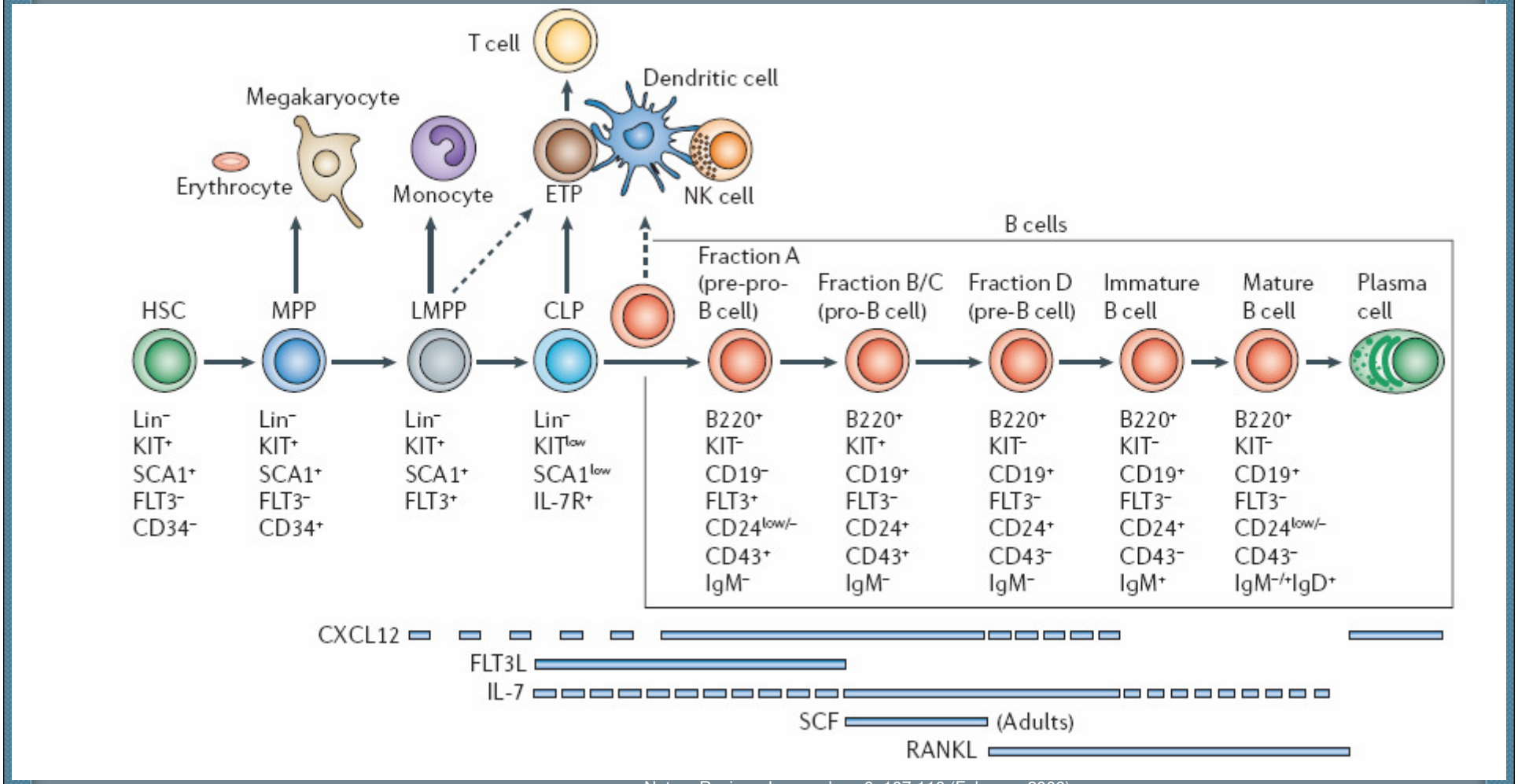




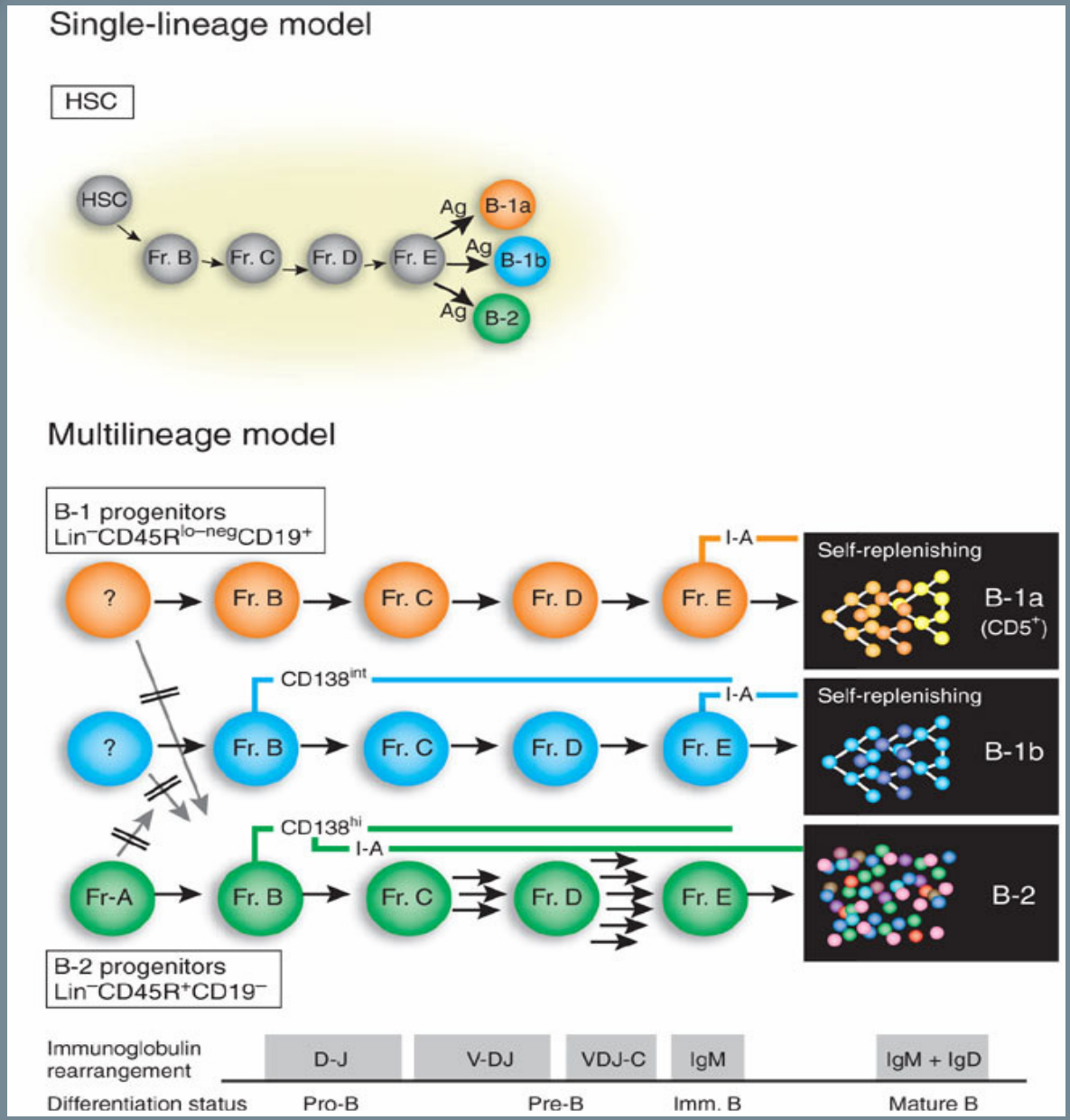
Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
Rag expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgD^+ IgM^+ $CD23^+$
Anatomic site	Bone marrow			Periphery	
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)



Stage of maturation	Stem cell	Pre-B cell	Immature B cell	Mature B cell	Activated B cell	Antibody-secreting cell
Pattern of immunoglobulin production	None	Cytoplasmic μ heavy chain and pre-B receptor	Membrane IgM	Membrane IgM, IgD	Low rate Ig secretion; heavy chain isotype switching; affinity maturation	High rate Ig secretion; reduced membrane Ig



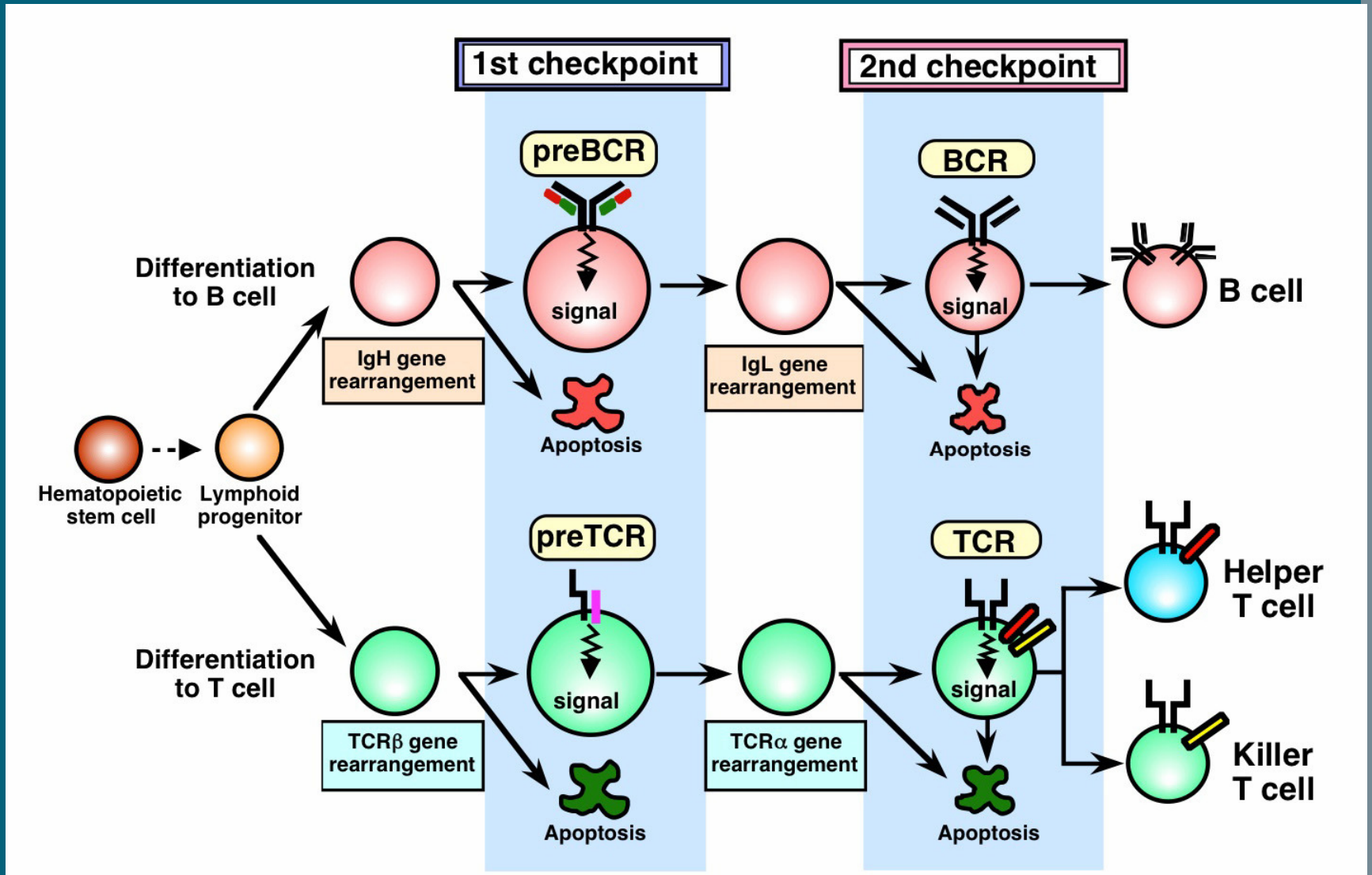
Nature Reviews Immunology 6, 107-116 (February 2006)



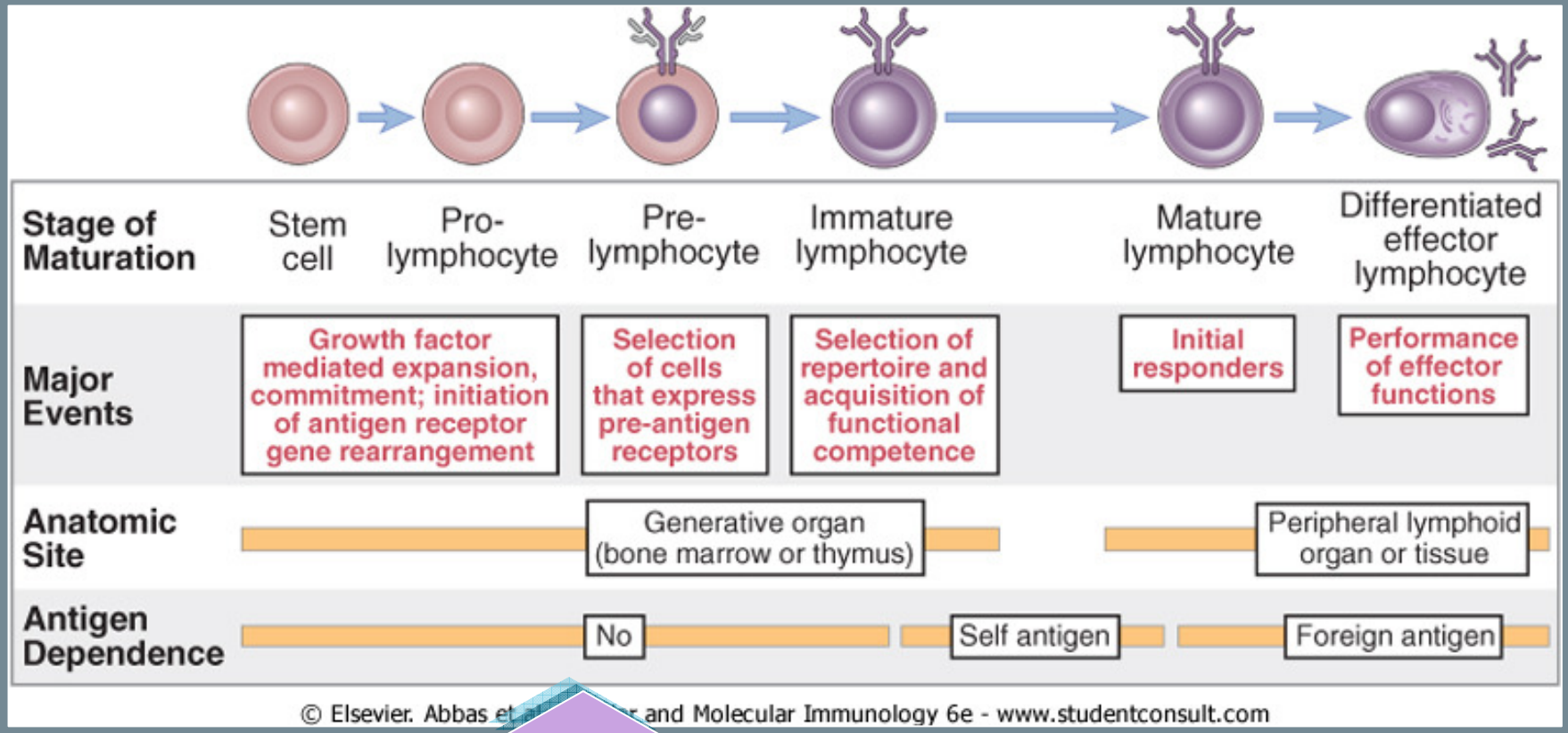
The development of three distinct B cell lineages in the layered immune system in the mouse.
The lineage commitment of B cell progenitors for each of the lineages is defined early in B cell development, before the initiation of immunoglobulin heavy-chain rearrangement. Top, previous single-lineage model, now negated by new findings presented in this issue. Bottom, multilineage model for B cell development, which best fits the present data, with the surface phenotypes now known to distinguish the progenitors for three B cell lineages. Fr. A–Fr. E represent the Hardy scheme for B cell development; gray boxes (bottom) indicate the sequential immunoglobulin heavy-chain rearrangements that occur as B cells mature through the Hardy sequence (D, diversity; J, joining; V, variable; C, constant). B-1 progenitors ($Lin^-CD45R^{lo\text{-}neg}CD19^+$) are located in a 'new' early progenitor fraction distinguished from Fr. A or Fr. B by the absence of the B220-6B2 CD45R determinant (other CD45R determinants are expressed). B-2 progenitors ($Lin^-CD45R^+CD19^-$) are located in the 'classical' Hardy Fr. A. The developmental pathways for each of the lineages is further distinguished by the expression of CD138 and the stage at which I-A (major histocompatibility complex class II) is initiated[12]. The progenitors for B-1a, B-1b and B-2 are also distinguished by the time at which they appear in bone marrow[1]. Ag, antigen; B, B cell; Imm., immature.

Nature Immunology **7**, 225 - 226 (2006)

Generación de los receptores de células T y B

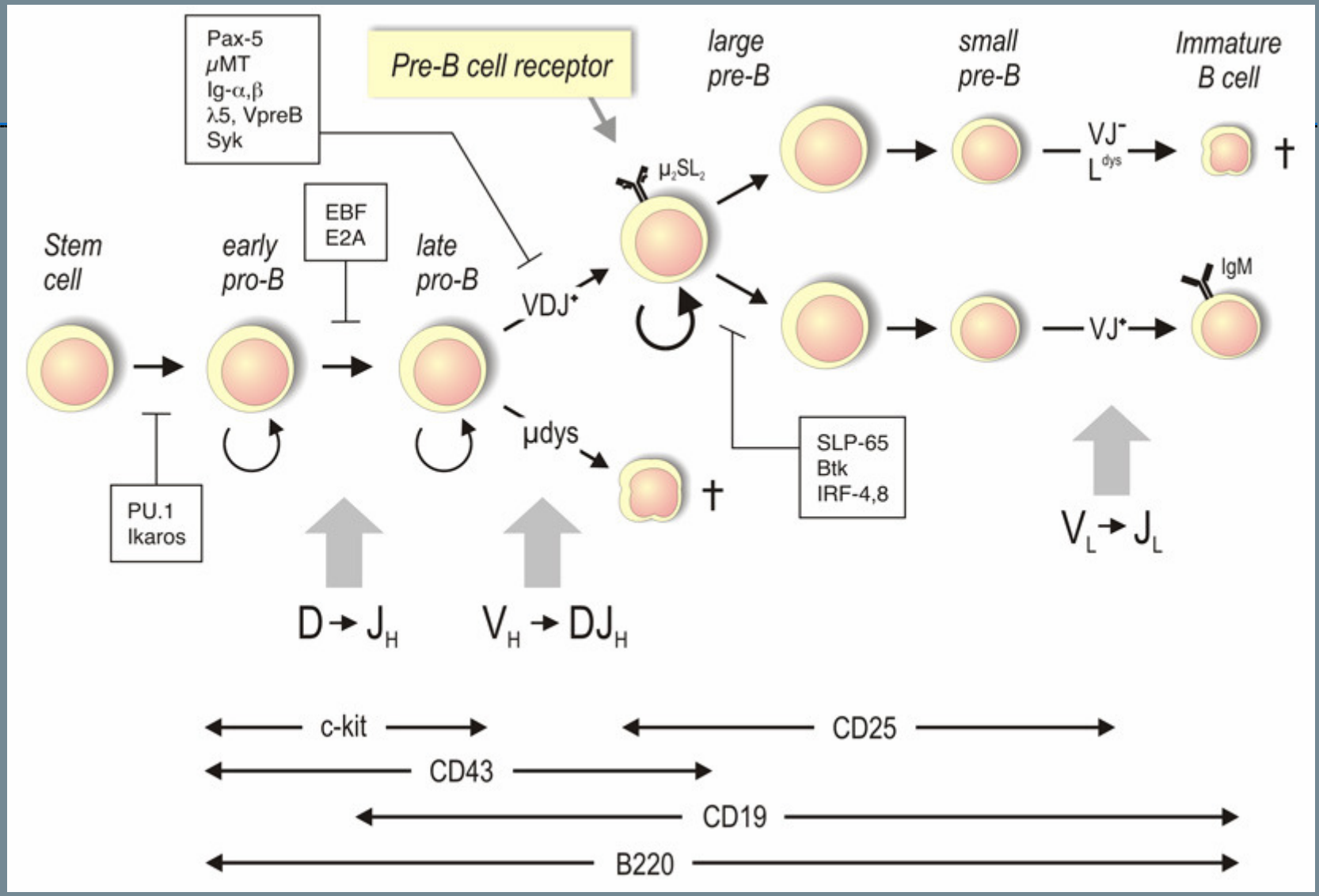


Desarrollo de la célula B



Fase independiente de antígeno

- **El factor de transcripción PU.1 junto a Ikaros son responsables de generar las células pro-B.**
- **Las proteínas E2A y EBF son importantes en la linfopoyesis B. Regulan la expresión de los genes de cadenas sustitutas λ5 y VpreB, moléculas Igα e Igβ, genes Rag-1 y Rag-2.**
- **El gen Pax-5 (cuyo producto de transcripción es BSAP: proteína activadora específica de célula B) es un regulador crítico del desarrollo de linfocitos B y actúa en forma subsecuente a E2A y EBF**


Pax-5
μMT
Ig-α,β
λ5, VpreB
Syk
Pre-B cell receptor
large pre-B
small pre-B
Immature B cell
EBF
E2A
Stem cell
early pro-B
late pro-B
VDJ+
μ2SL2
VJ-
Ldys
IgM
VJ+
μdys
SLP-65
Btk
IRF-4,8
PU.1
Ikaros
VL → JL
D → JH
VH → DJH
c-kit
CD25
CD43
CD19
B220

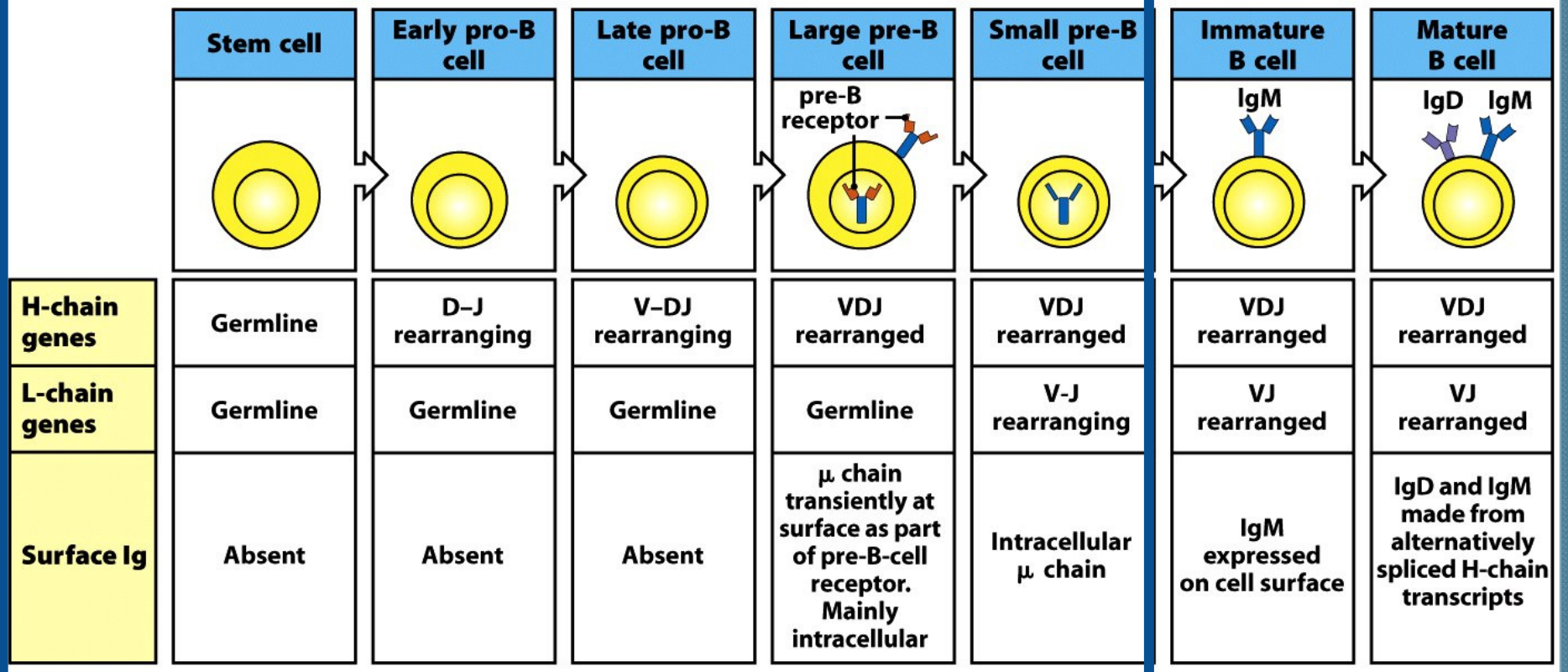
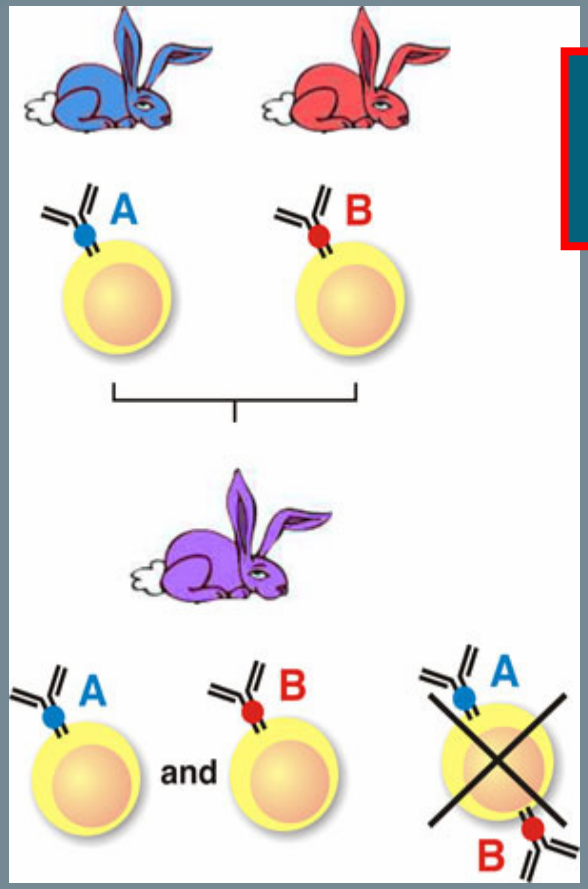
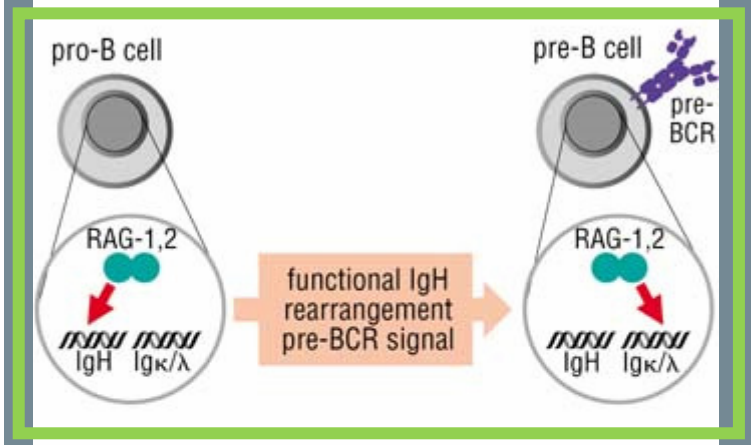
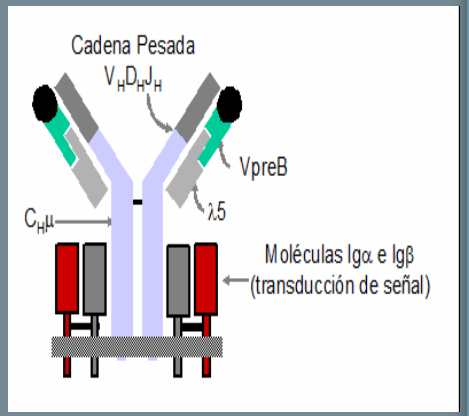


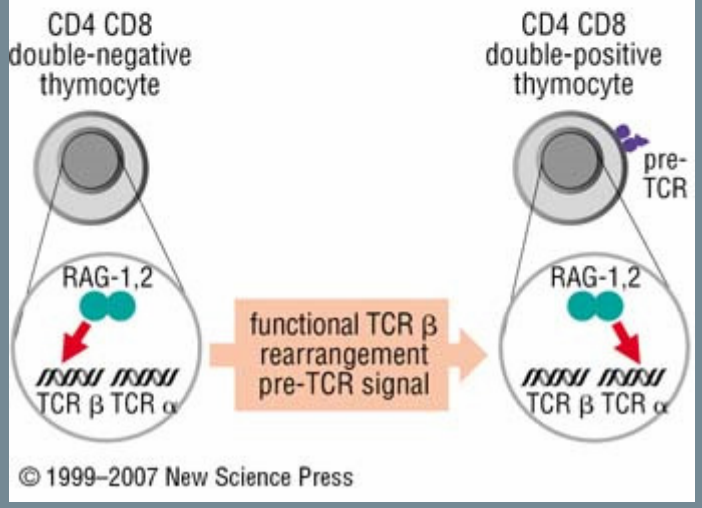
Figure 7-5 Immunobiology, 7ed. (© Garland Science 2008)

Generación del RECEPTOR

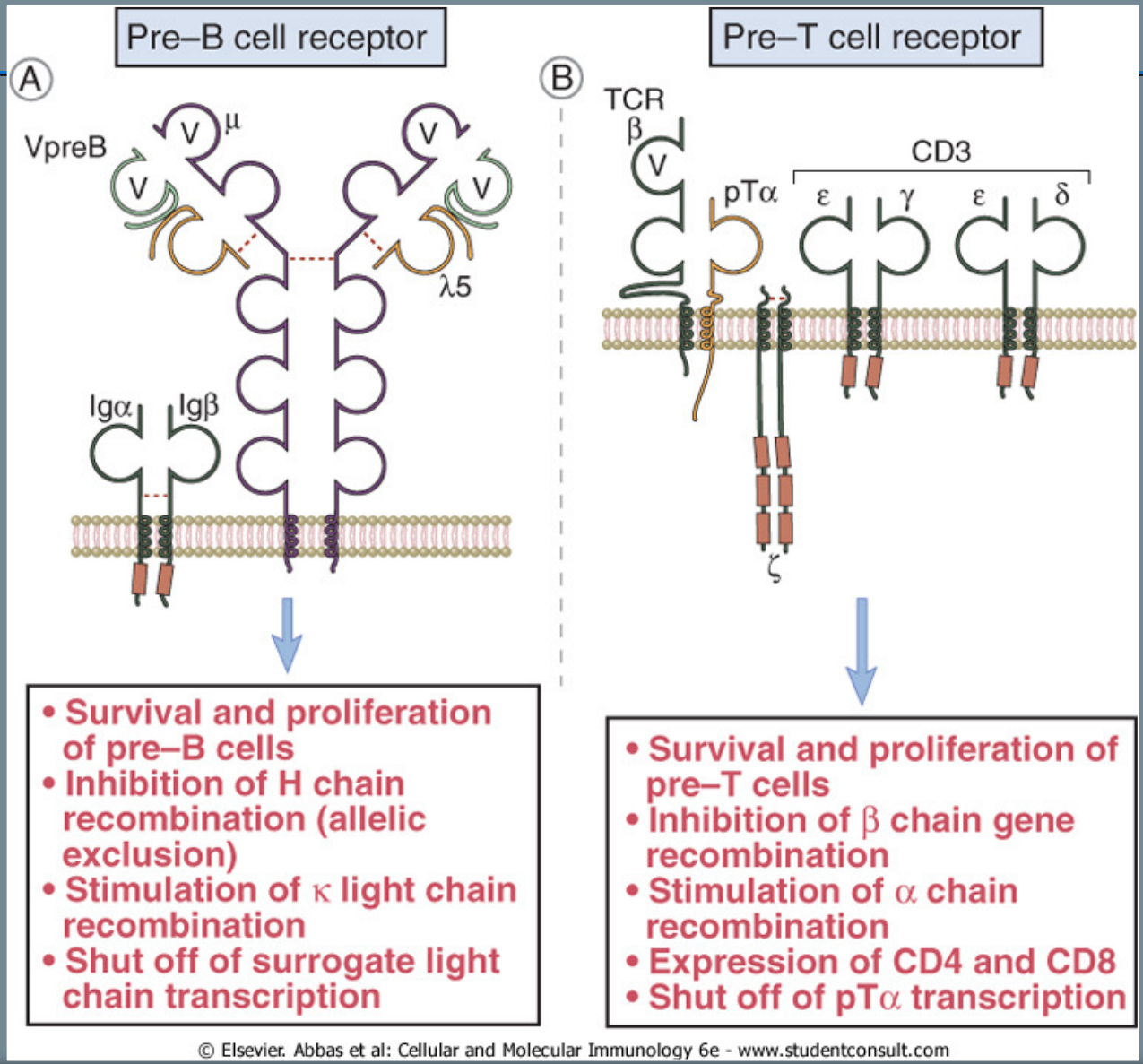








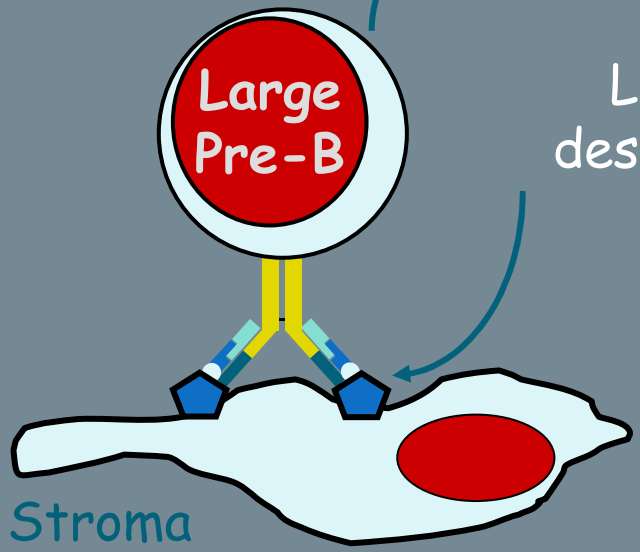
Pre-BCR



Funciones del pre-BCR

1. Suprime reordenamientos de cadena H de Ig.

2. Ingreso al ciclo celular
PROLIFERACIÓN (32-64 cél.)

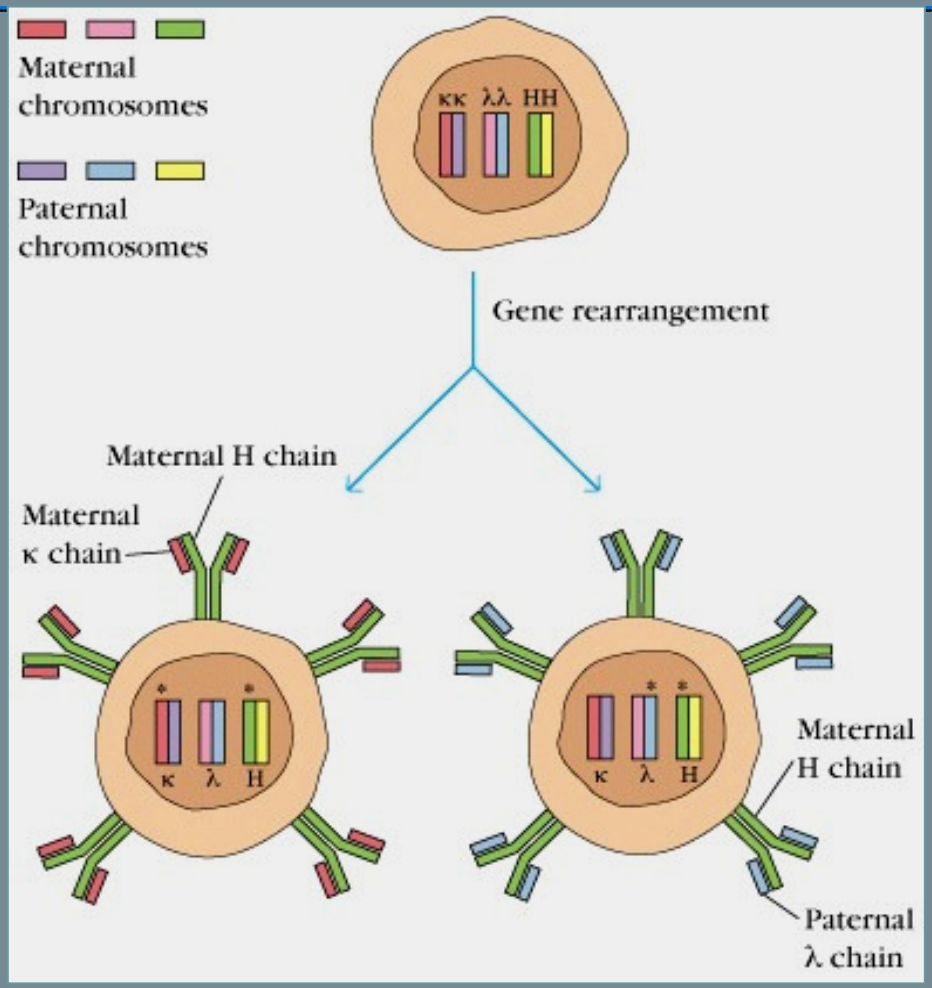


1. Asegura una única especificidad por célula

2. Expansión solo de células con pre-BCR

EXCLUSIÓN ALÉLICA

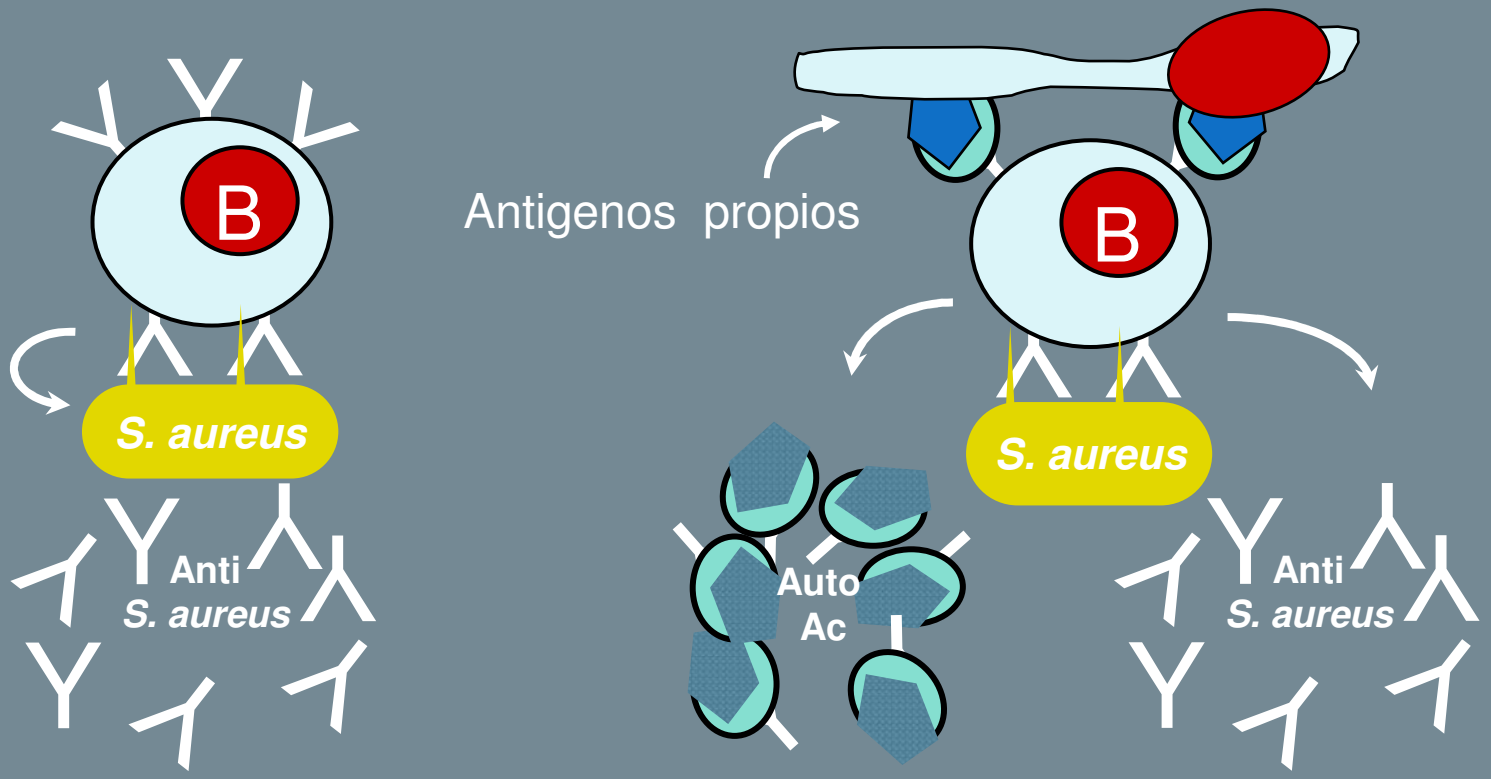
EXCLUSIÓN ALÉLICA



Cada célula expresa solamente los genes de la cadena H y L de la Ig de los cromosomas de un parental: **ESTE PROCESO ASEGURA QUE LAS CÉLULAS B TENGAN UNA ESPECIFICIDAD ANTIGÉNICA ÚNICA.** El alelo seleccionado para el reordenamiento se elige en forma aleatoria.

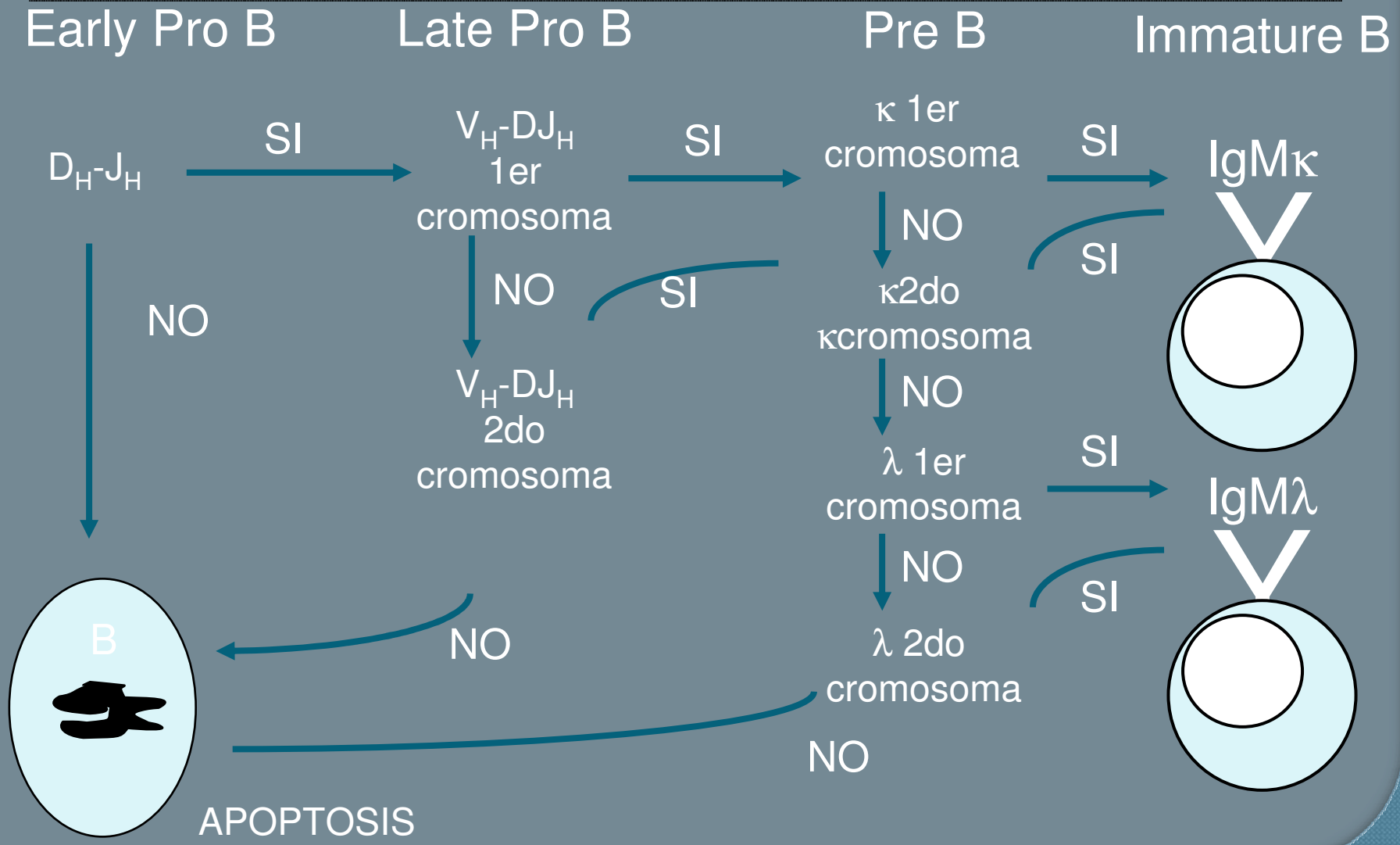
¿ Es importante la exclusion alélica ?

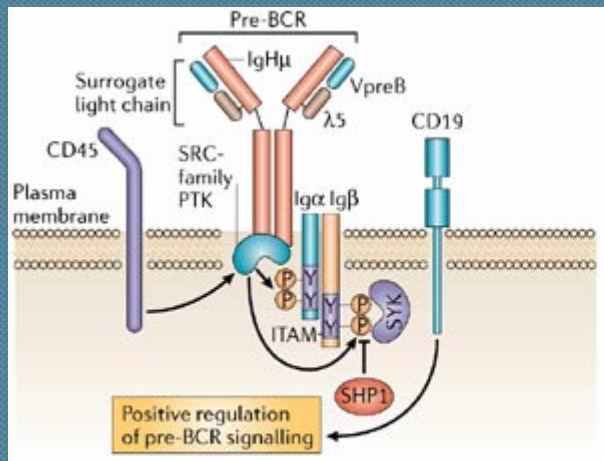
Un receptor por célula | **Dos receptores por célula**

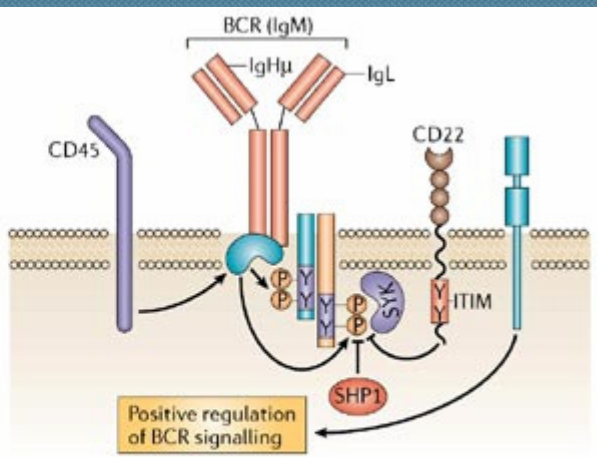


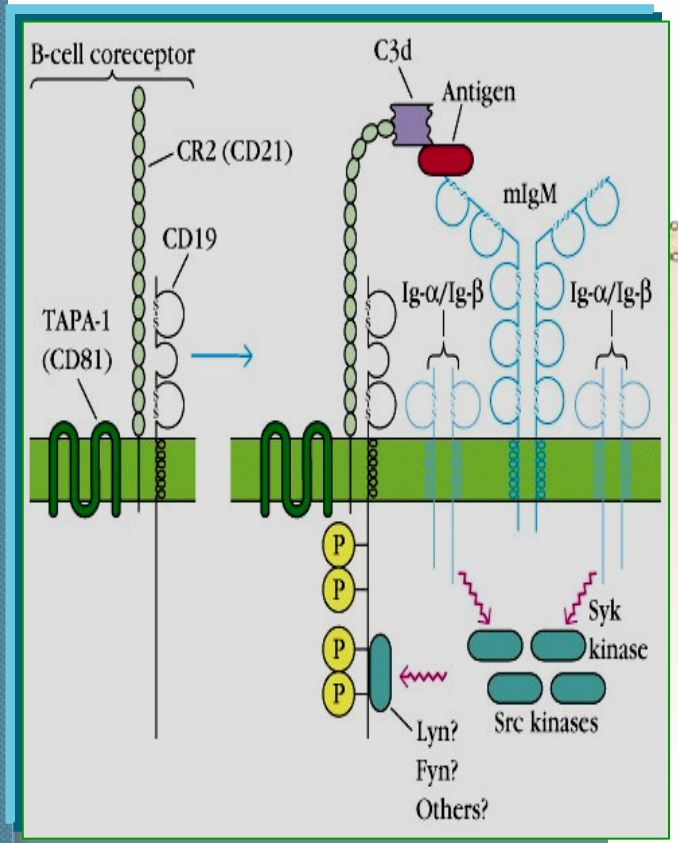
Previene la inducción de respuestas no deseadas

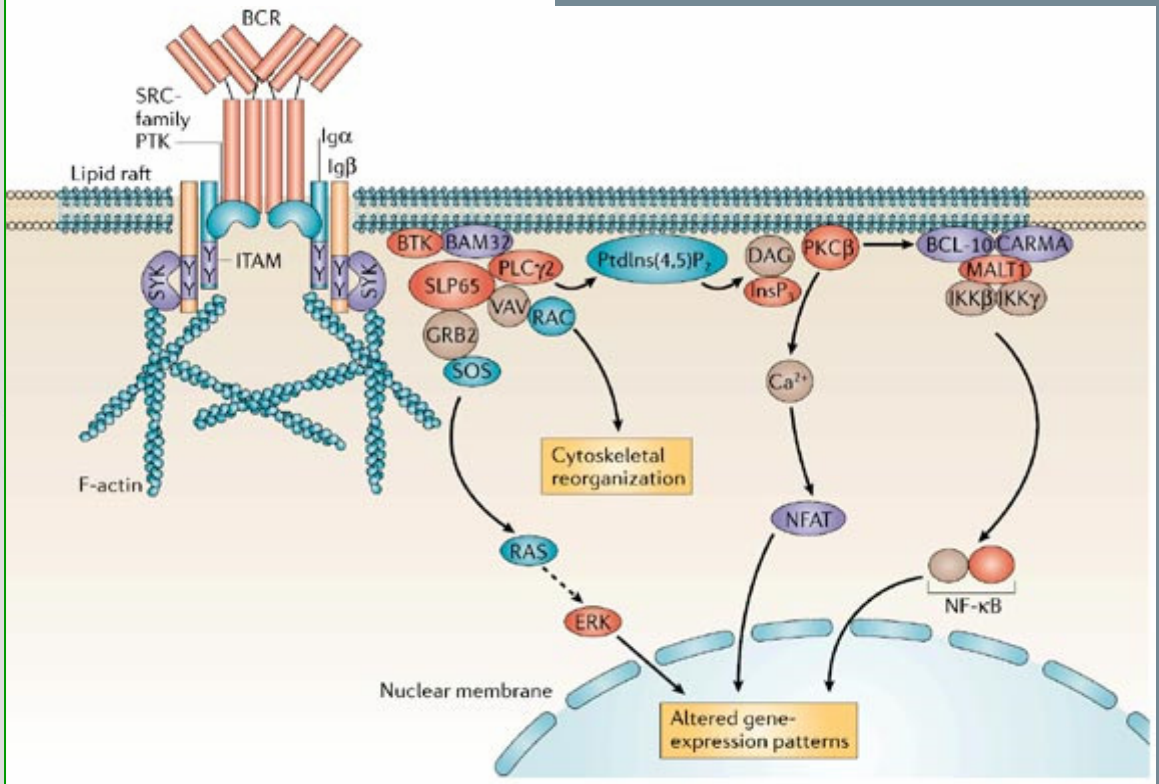
Los LB tienen varias oportunidades de lograr un reordenamiento exitoso de sus genes para Ig





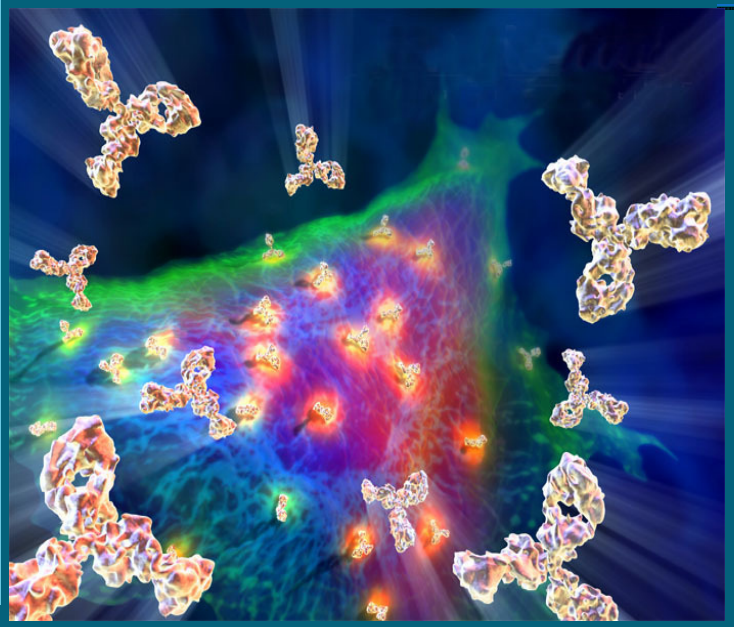




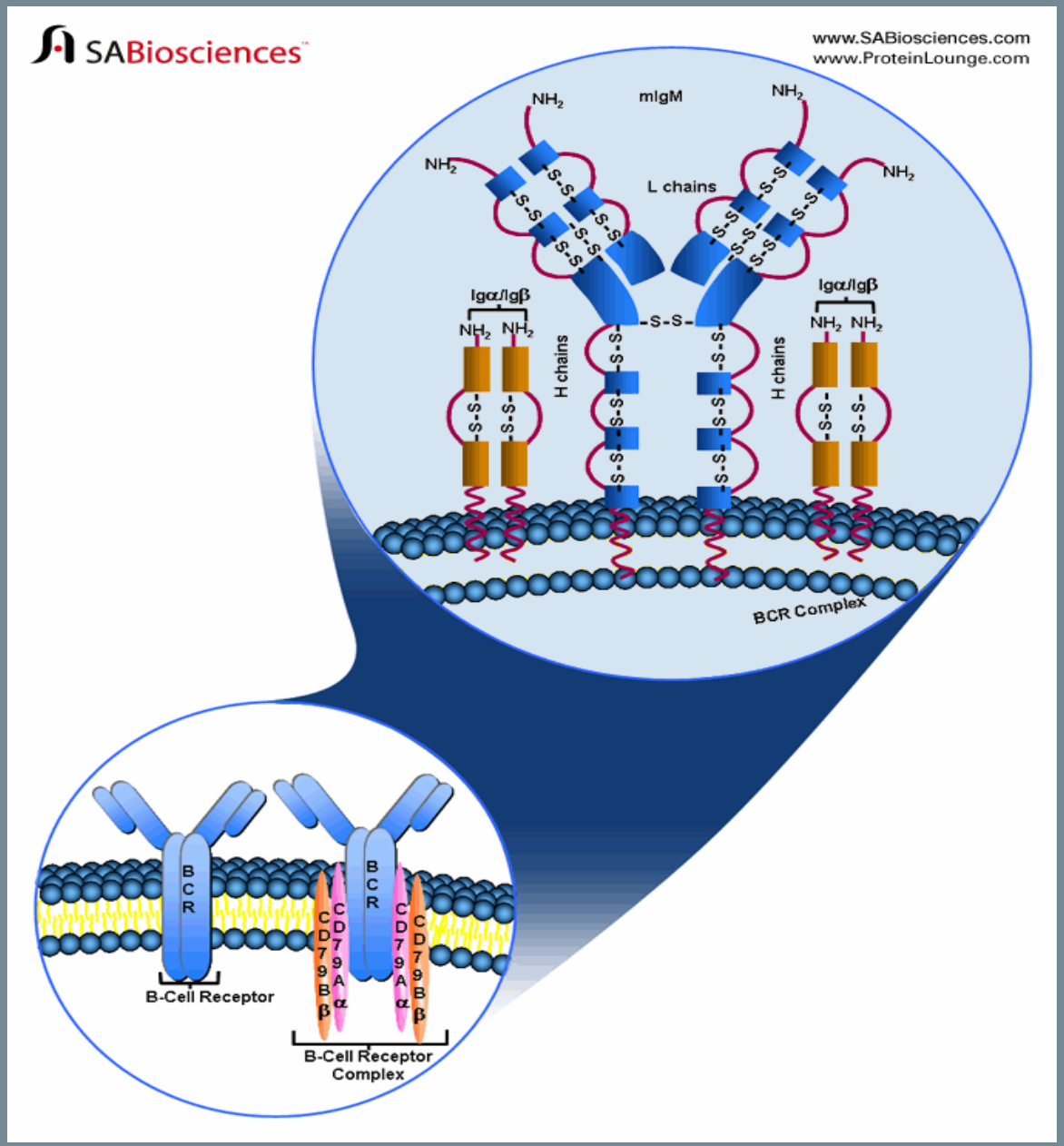


Nature Reviews Immunology 6, 283-294 (April 2006)

LA GENERACIÓN DE RECEPTORES ANTIGÉNICOS SE PRODUCE ANTES DEL INGRESO DEL ANTÍGENO, DURANTE LA MADURACIÓN DE LOS LINFOCITOS……



REPERTORIO


SABiosciences
www.SABiosciences.com
www.ProteinLounge.com
mIgM
NH2
L chains
S-S
Igα/Igβ
H chains
BCR Complex
BCR
CD79Bβ
CD79Aα
B-Cell Receptor
B-Cell Receptor Complex

Múltiples genes de la línea germinal

Organización de los genes de Inmunoglobulinas: CONFIGURACIÓN GERMINAL

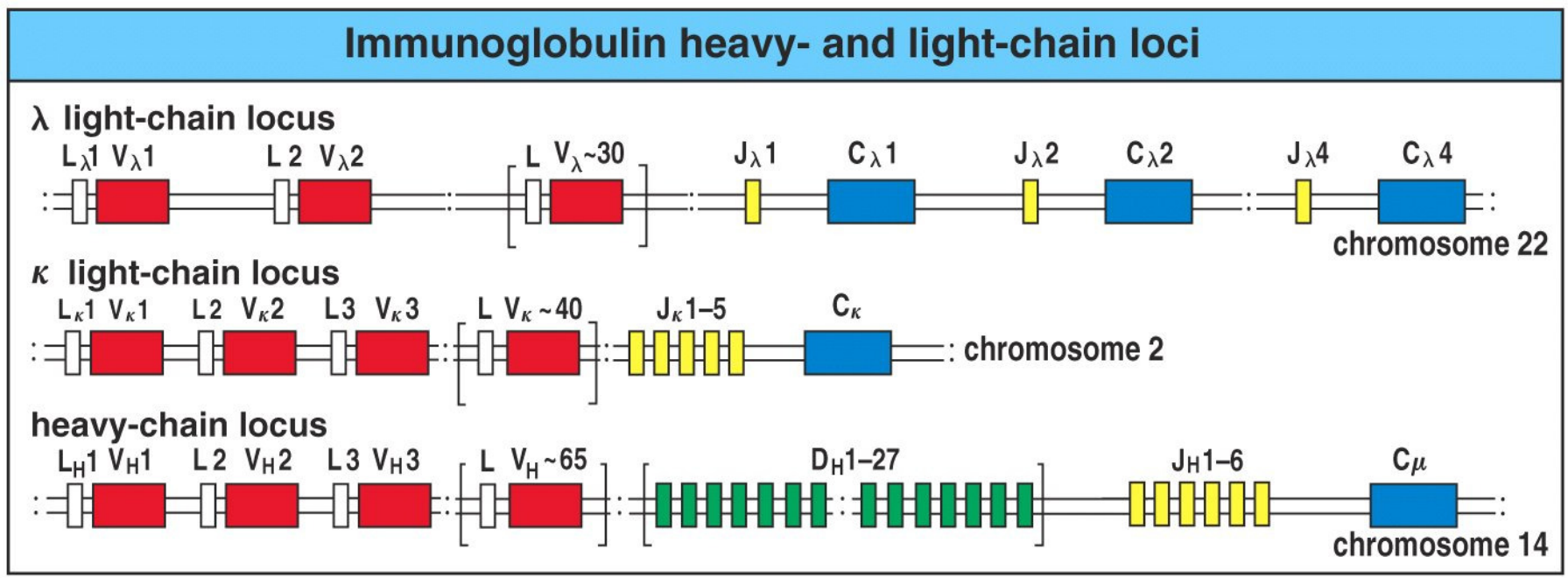
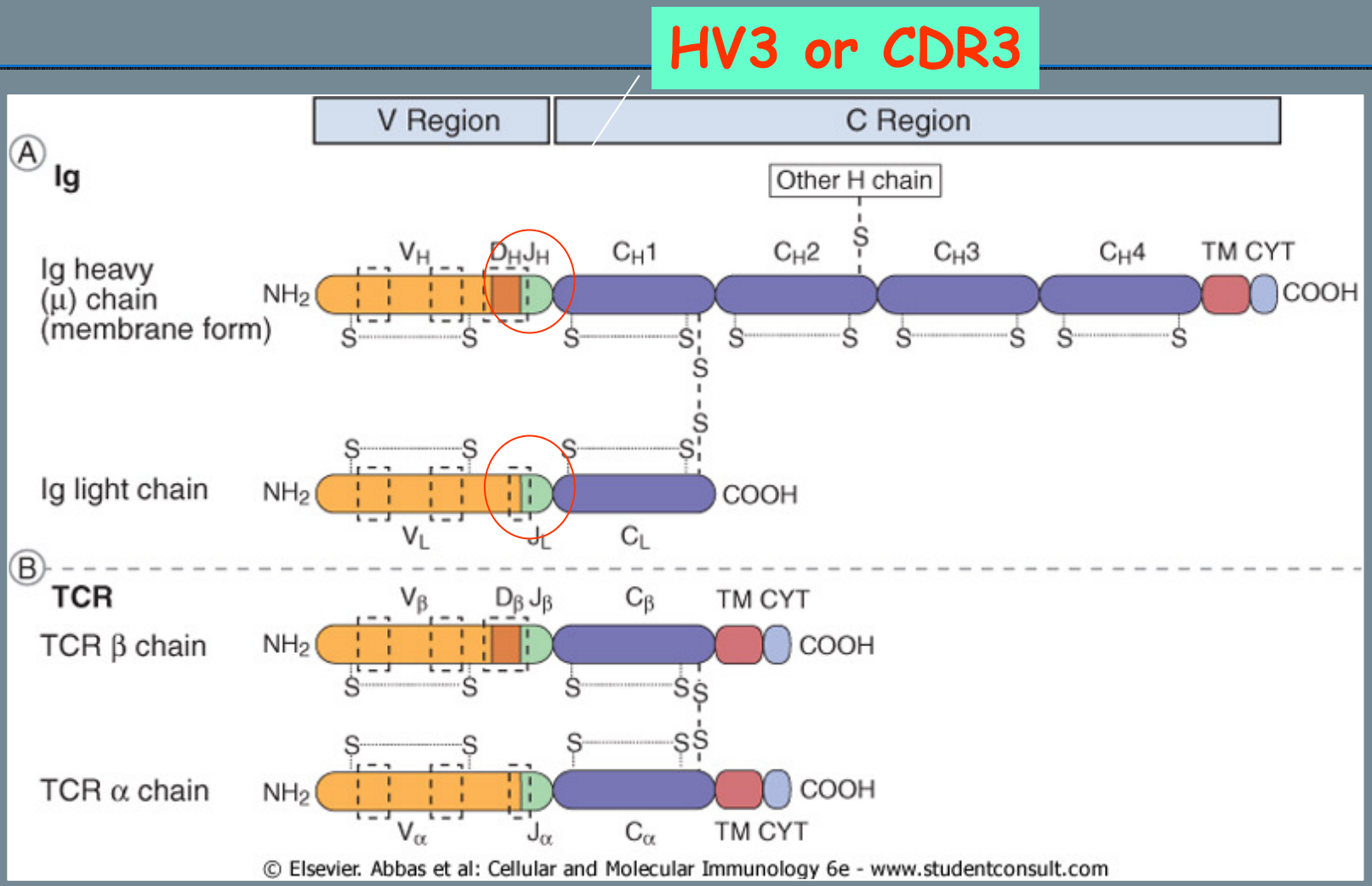


Figure 2-14 The Immune System, 2/e (© Garland Science 2005)

Dominios de los receptores



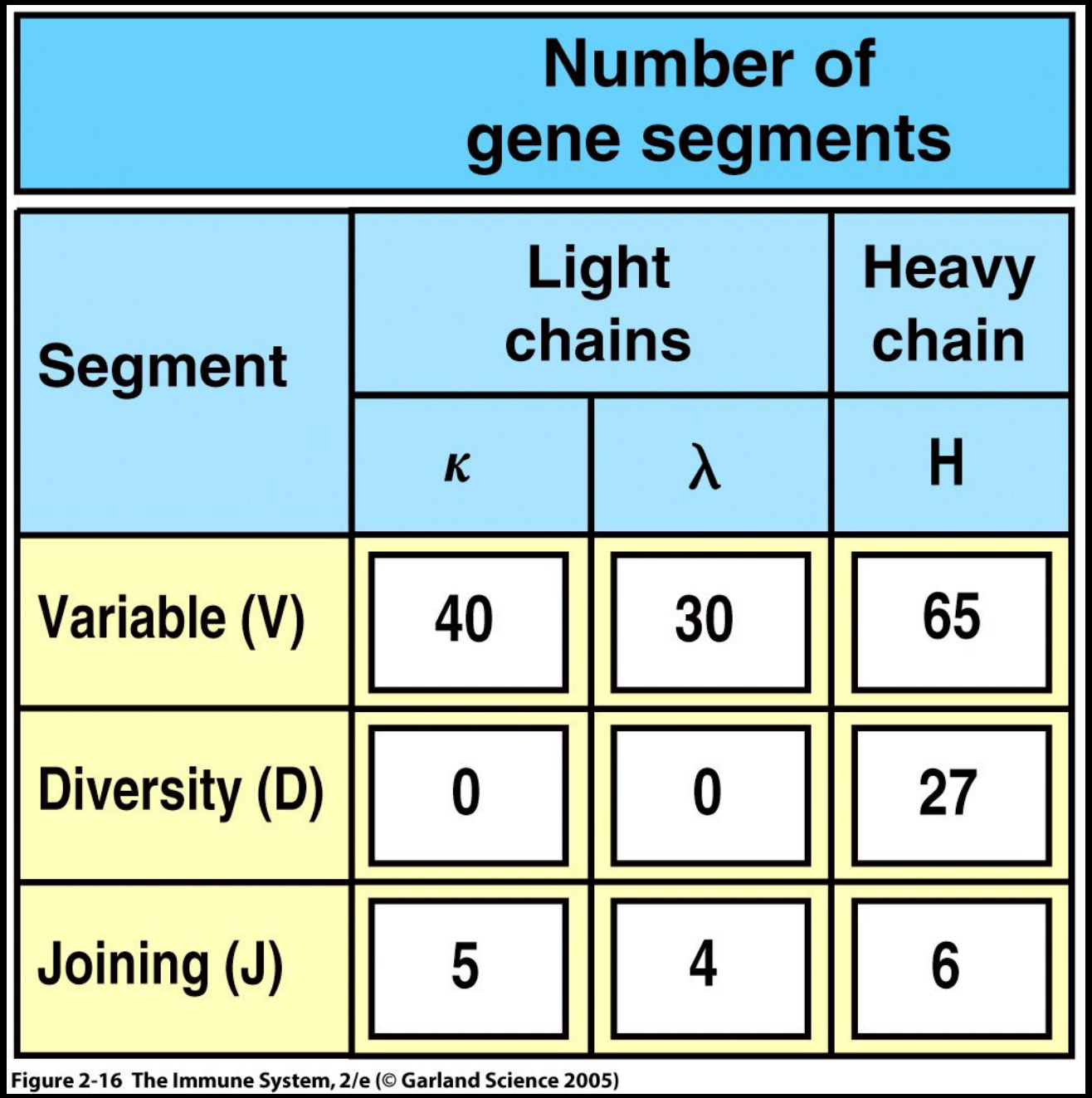


Figure 2-16 The Immune System, 2/e (© Garland Science 2005)

Mechanism	Immunoglobulin		TCR ab		TCR gd	
Mechanism	Heavy chain	k	a	b	g	d
Variable (V) segments	85	35	54	67	14	20–30
Diversity (D) segments	27	0	0	2	0	3
D segments read in all three reading frames	Rare	—	—	Often	—	Often
N region diversification	V-D, D-J	None	V-J	V-D, D-J	V-J	V-D1, D1-D2, D1-J
Joining (J) segments	6	5	61	4	5	4
Total potential repertoire with junctional diversity	$\sim 10^{11}$		$\sim 10^{16}$		$\sim 10^{18}$	

The potential number of antigen receptors with junctional diversity is much greater than the number that can be generated only by combinations of V, D, and J gene segments. Note that although the upper limit on the numbers of Ig and TCR proteins that may be expressed is very large, it is estimated that each individual contains on the order of 10^7 clones of B and T cells with distinct specificities and receptors; in other words, only a fraction of the potential repertoire may actually be expressed.

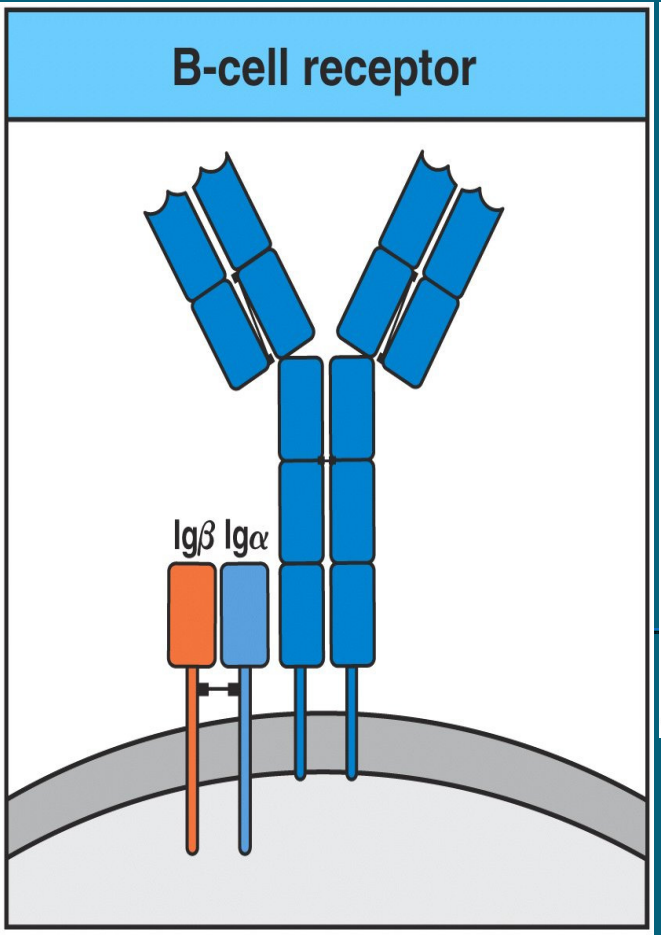


Figure 2-23 The Immune System, 2/e (© Garland Science 2005)

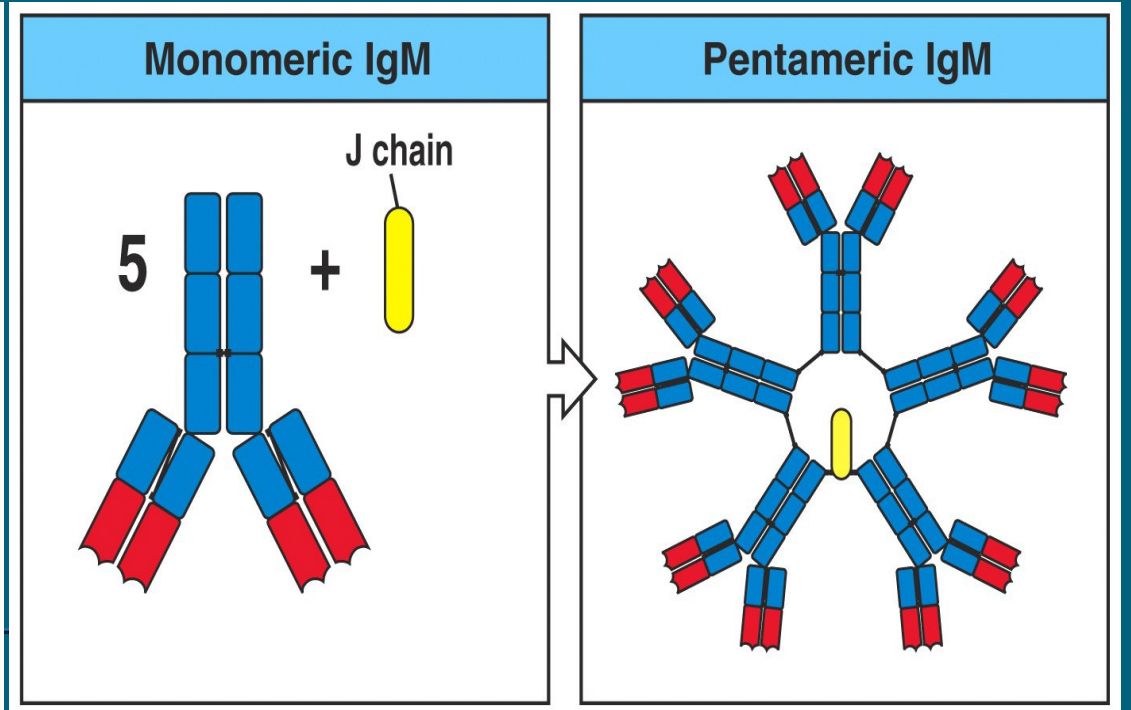


Figure 2-26 part 1 of 2 The Immune System, 2/e (© Garland Science 2005)

Diversidad combinatoria

Recombinación somática

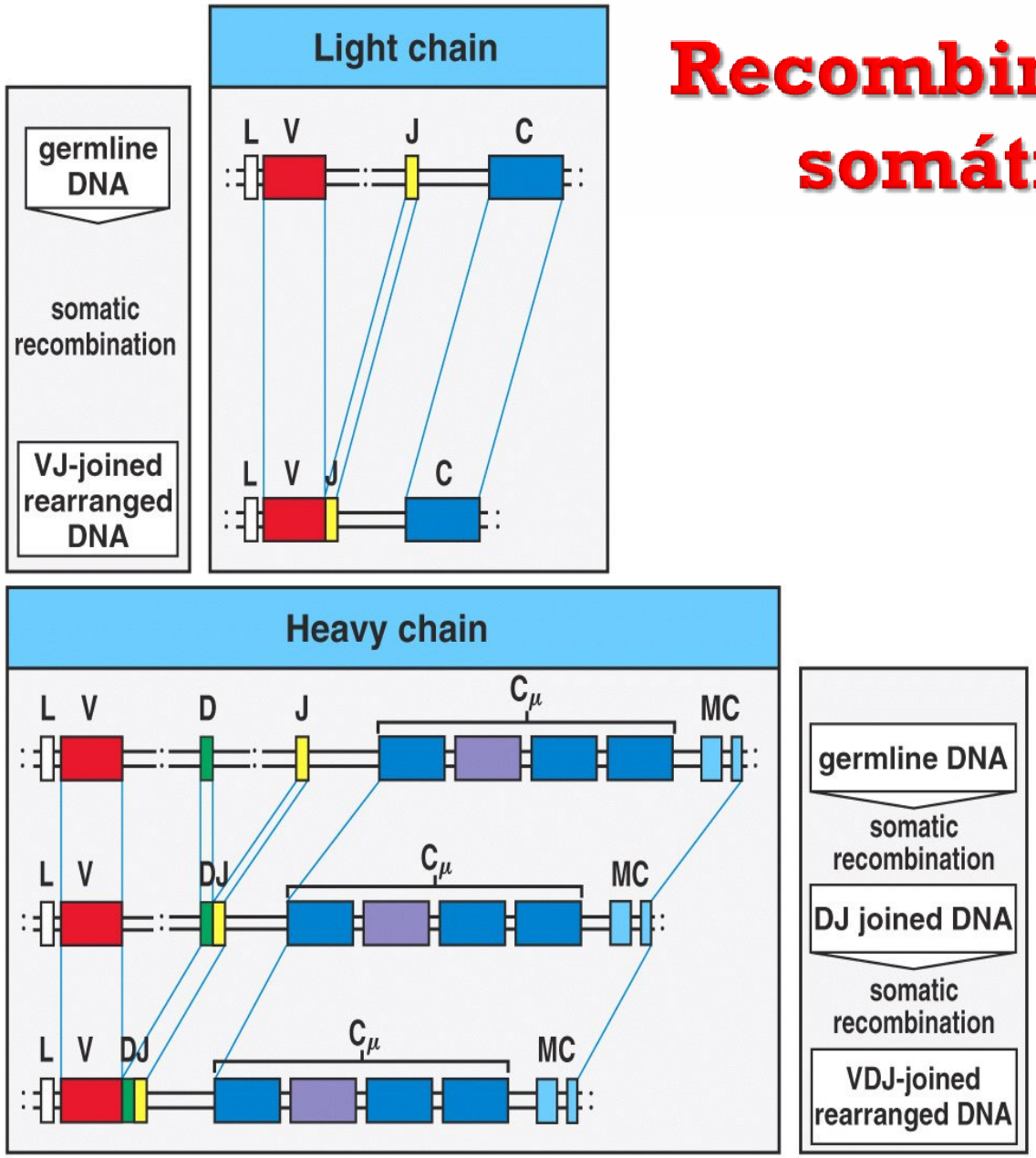
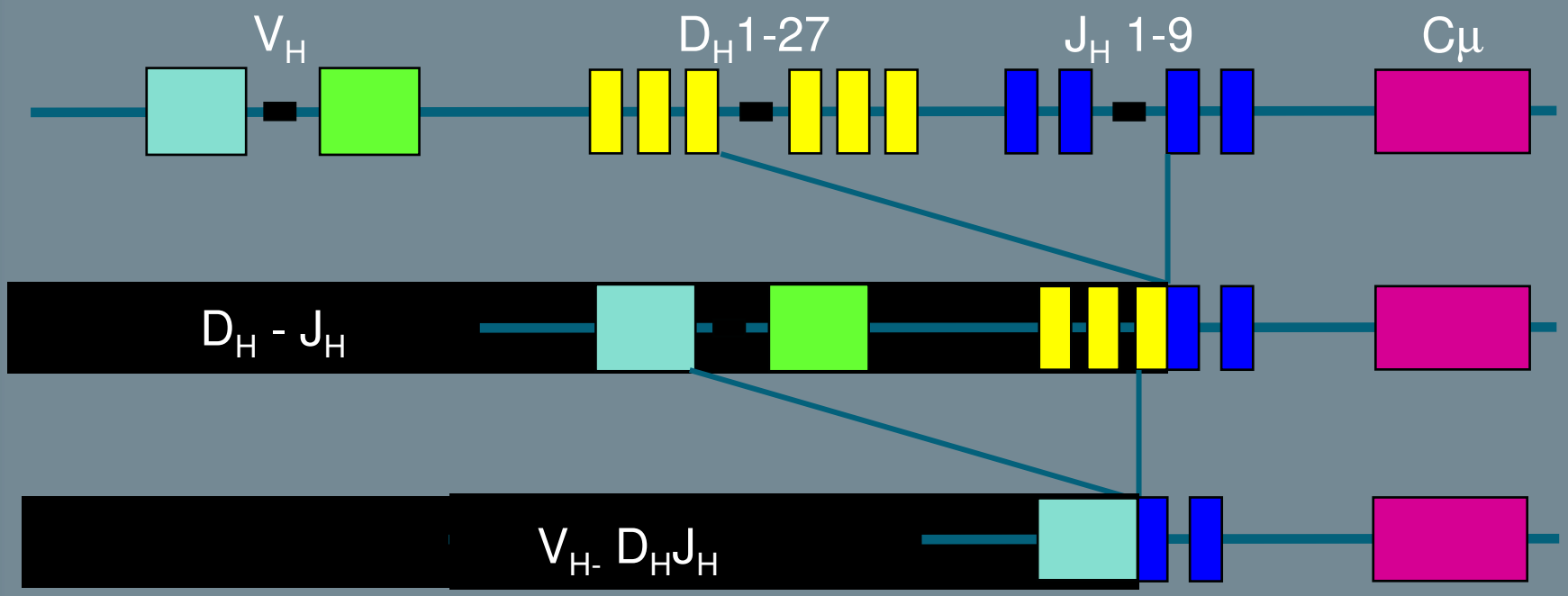


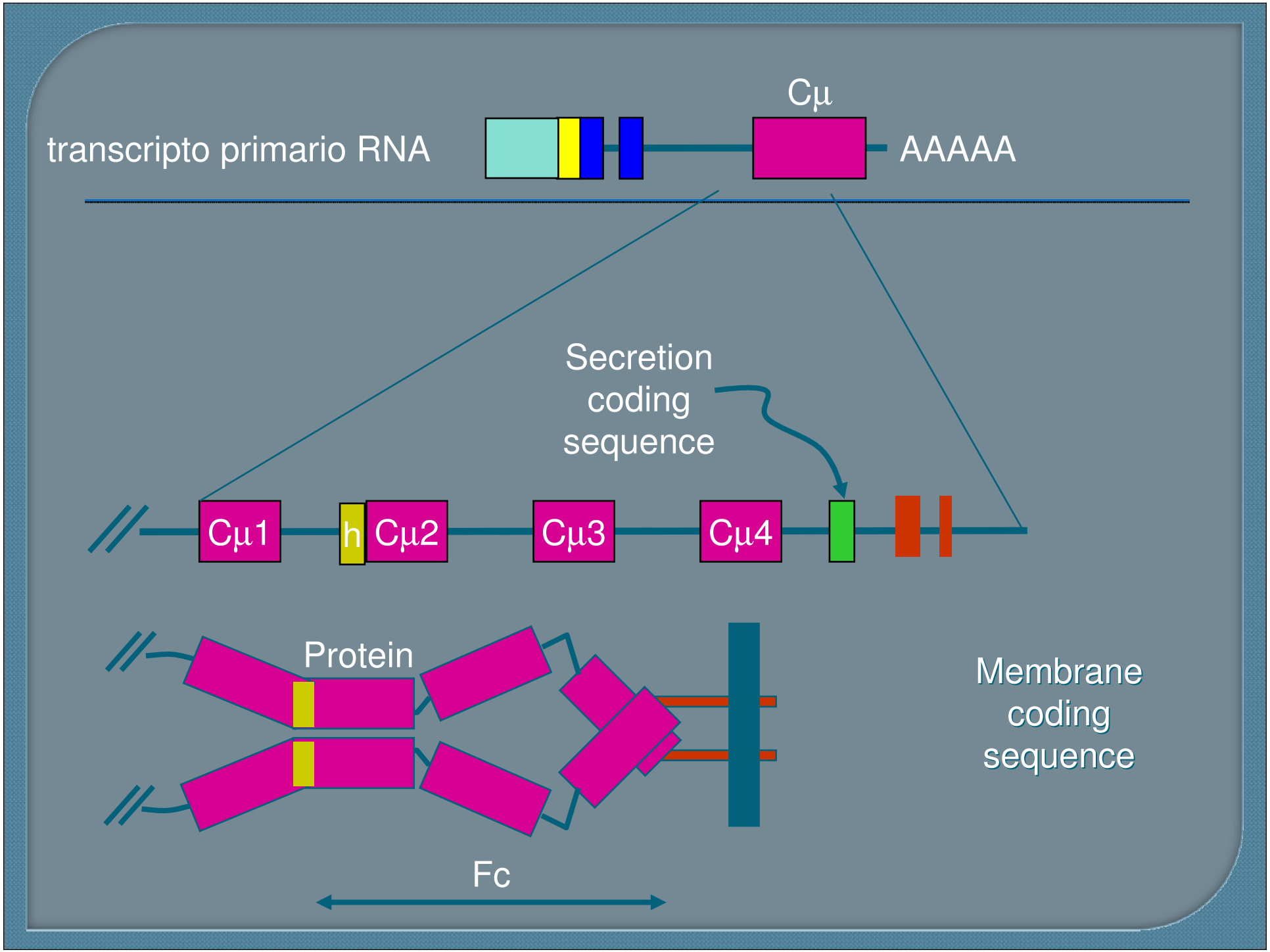
Figure 2-15 The Immune System, 2/e (© Garland Science 2005)

La asociación combinatoria de diferentes segmentos génicos V (D)J permite una amplia generación de diferentes especificidades de Ac. Cada clon de LB y su progenie expresa sólo una de estas combinaciones V(D)J (Recombinación somática).

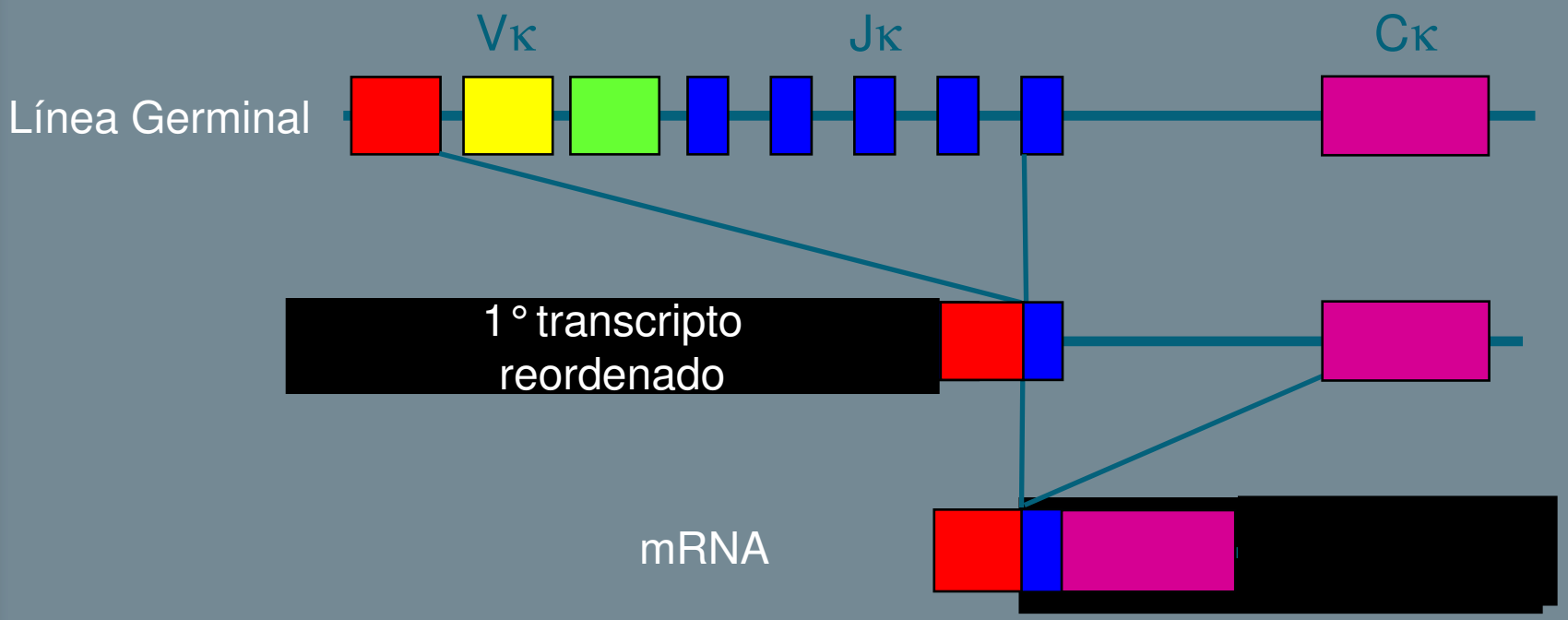
EL COMPLEJO ENZIMÁTICO QUE ACTUA EN ESTE PROCESO SE CONOCE COMO RECOMBINASA V(D)J. LOS PRODUCTOS DE LOS GENES RAG-1 Y RAG-2 FORMAN PARTE DE ESA RECOMBINASA Y SOLO SE EXPRESAN EN LINFOCITOS EN DESARROLLO.

Recombinación de la cadena pesada de Ig




Cμ
transcripto primario RNA
AAAAA
Secretion
coding
sequence
Cμ1
h
Cμ2
Cμ3
Cμ4
Protein
Membrane
coding
sequence
Fc

Recombinacion somática de cadena liviana

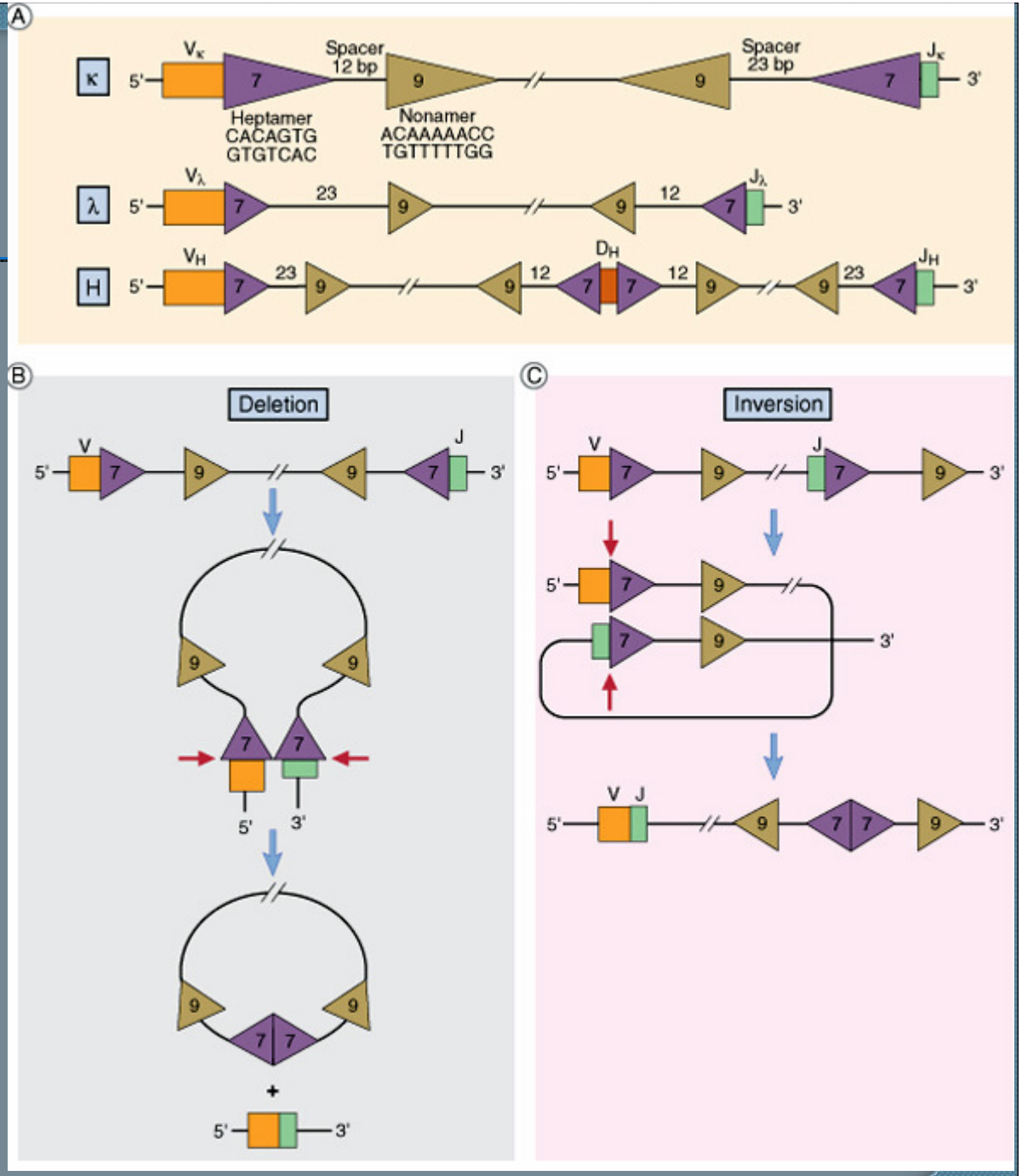


Recombinación V(D)J

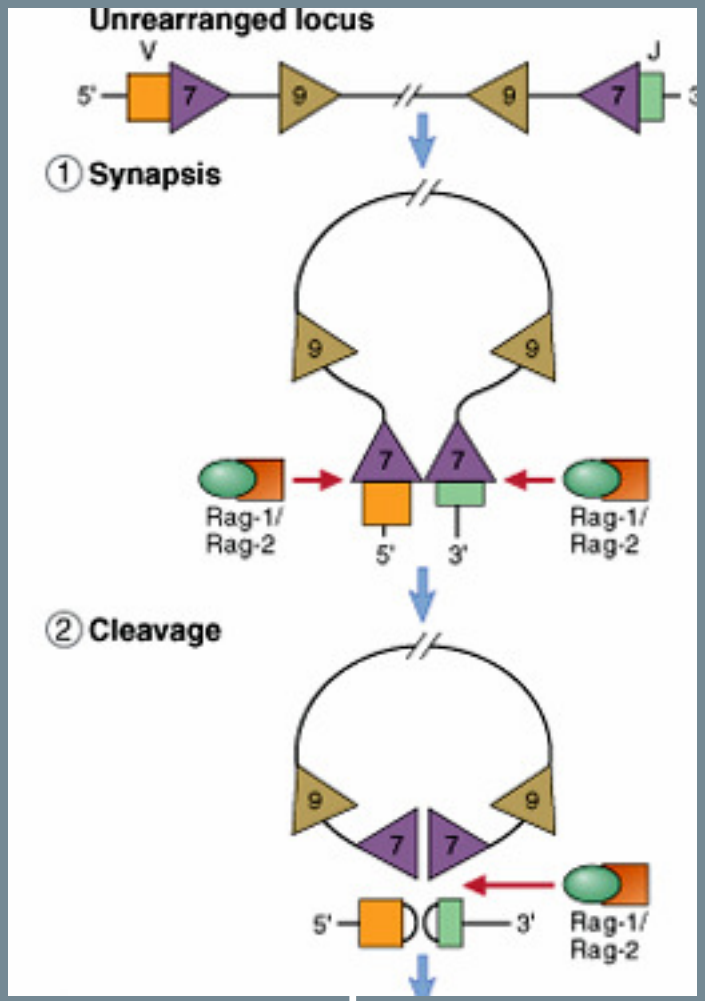
1. Recombination Signal Sequence (RSS): **Heptamer & Nonamer** => separated by 12- or 23- spacers => Recognized by **Recombinase**

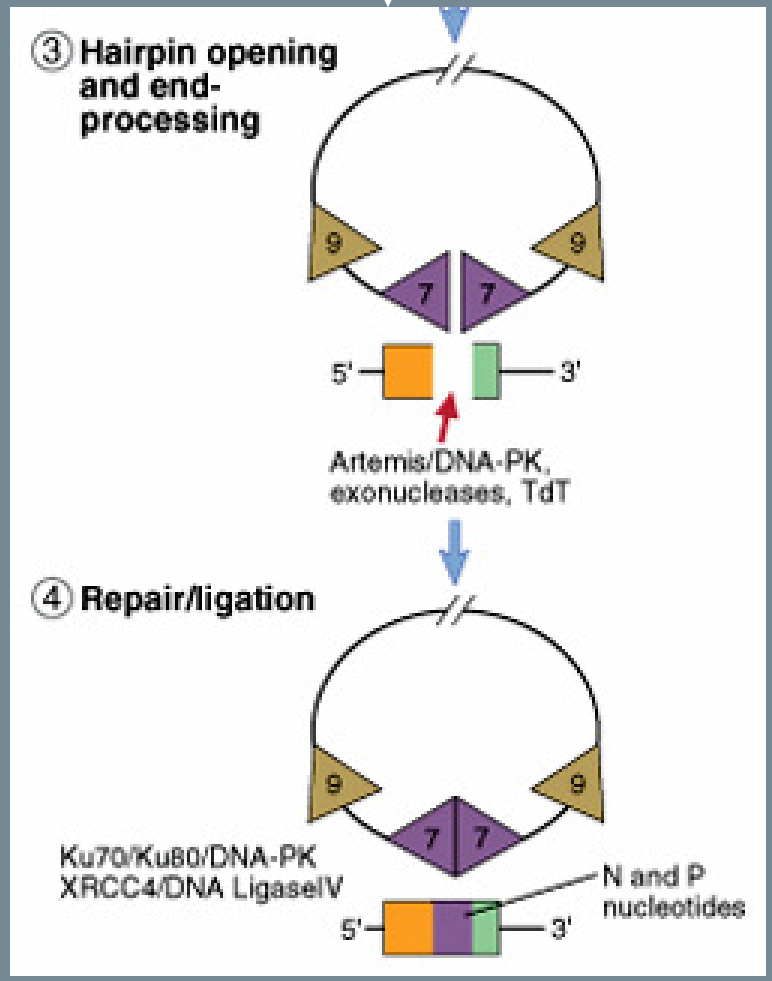
2. Deletion-VJ exons have the same orientation

3. Inversion – VJ have the different orientation



Recombinación V(D)J





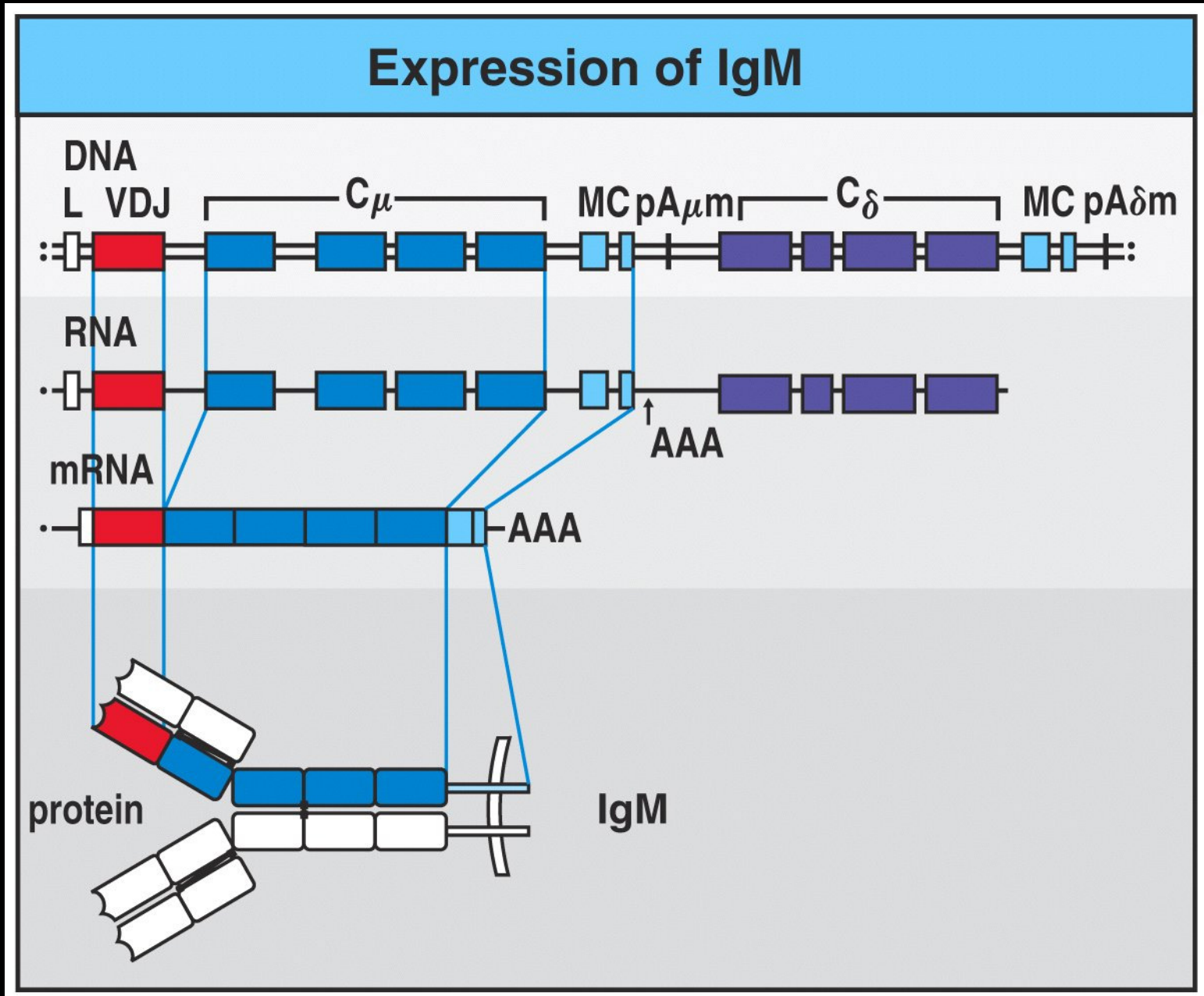


Figure 2-21 part 1 of 2 The Immune System, 2/e (© Garland Science 2005)

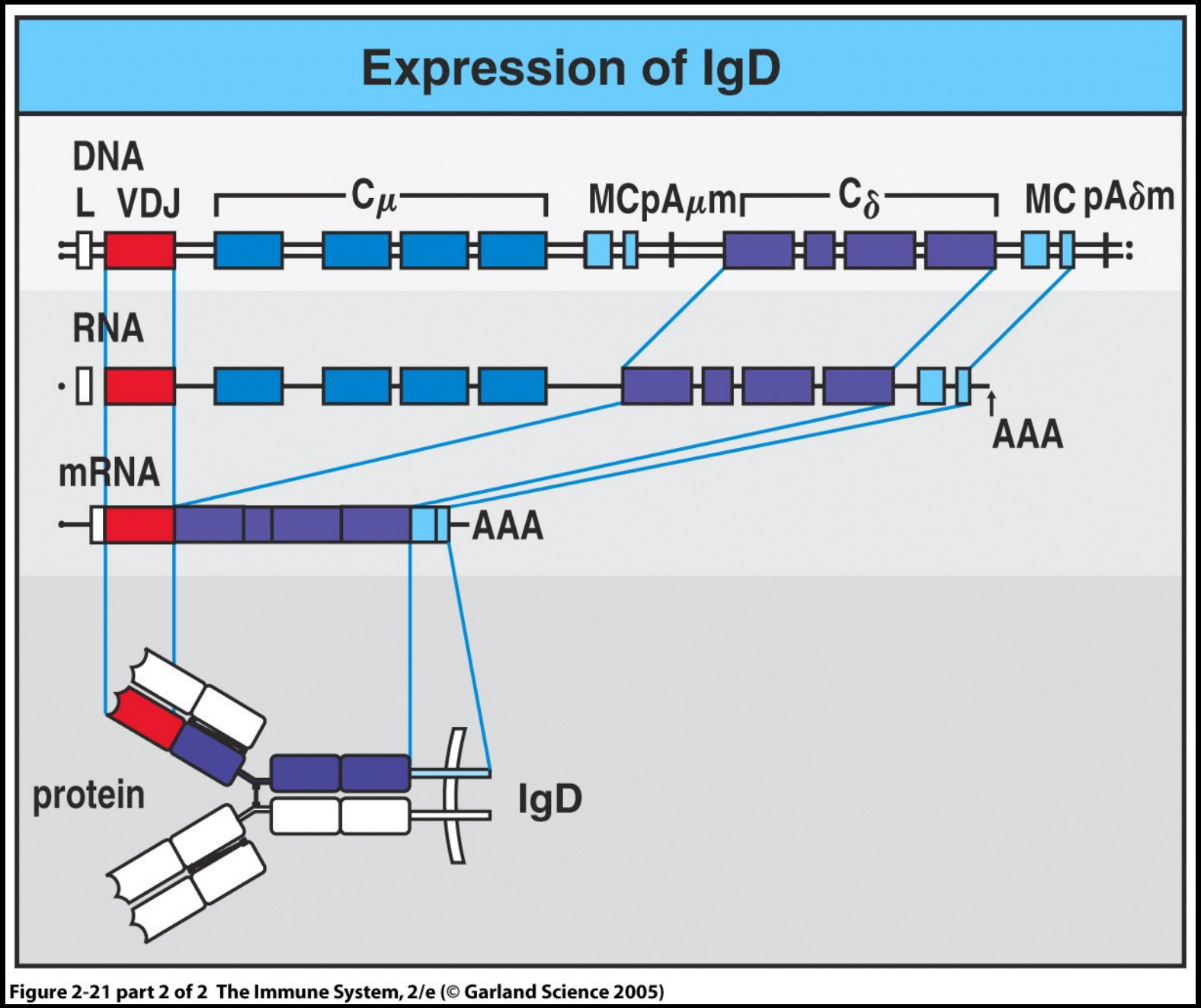


Figure 2-21 part 2 of 2 The Immune System, 2/e (© Garland Science 2005)

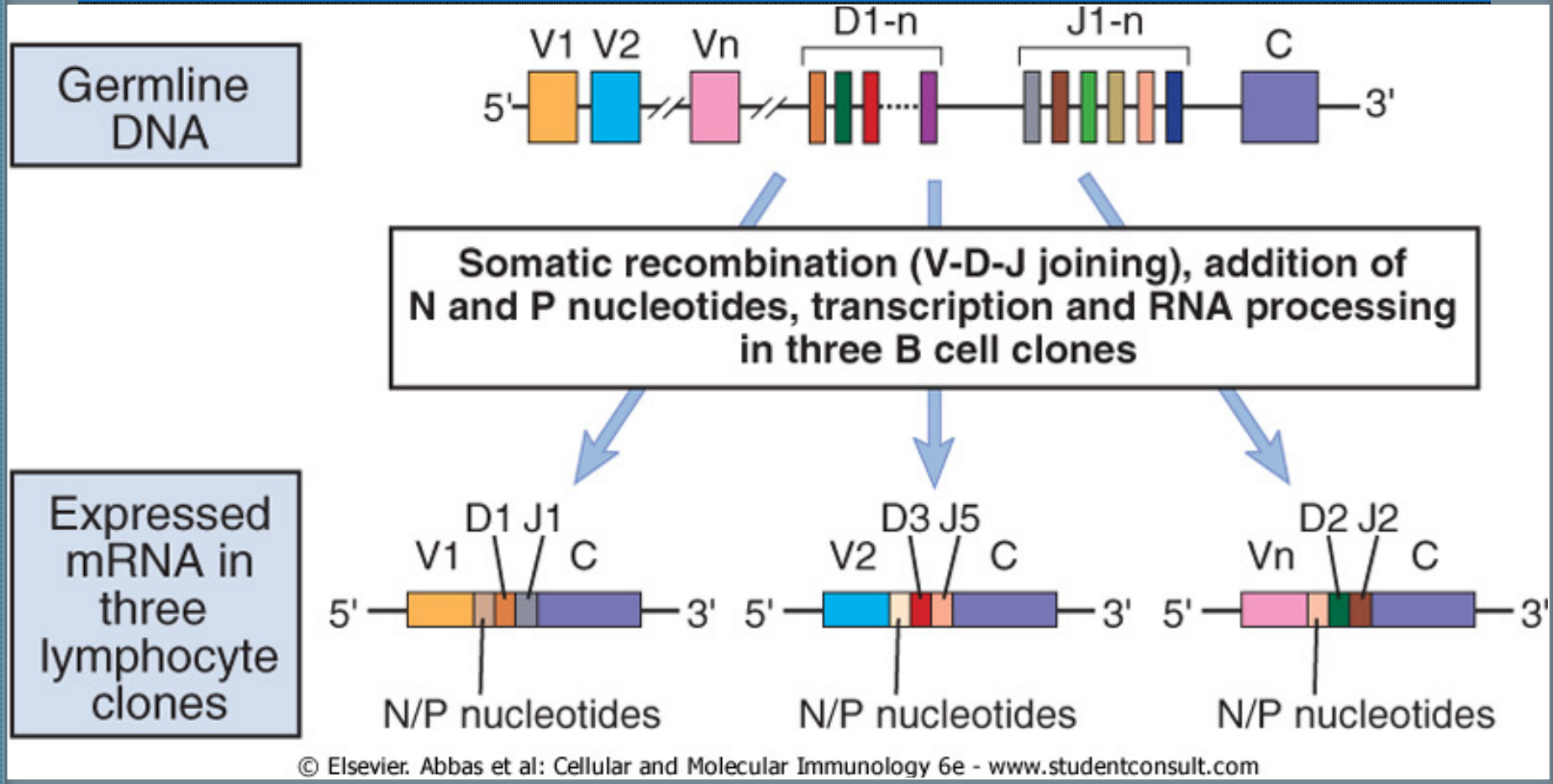
¿Es suficiente la diversidad combinatoria para generar diversidad…?

Diversidad en las uniones

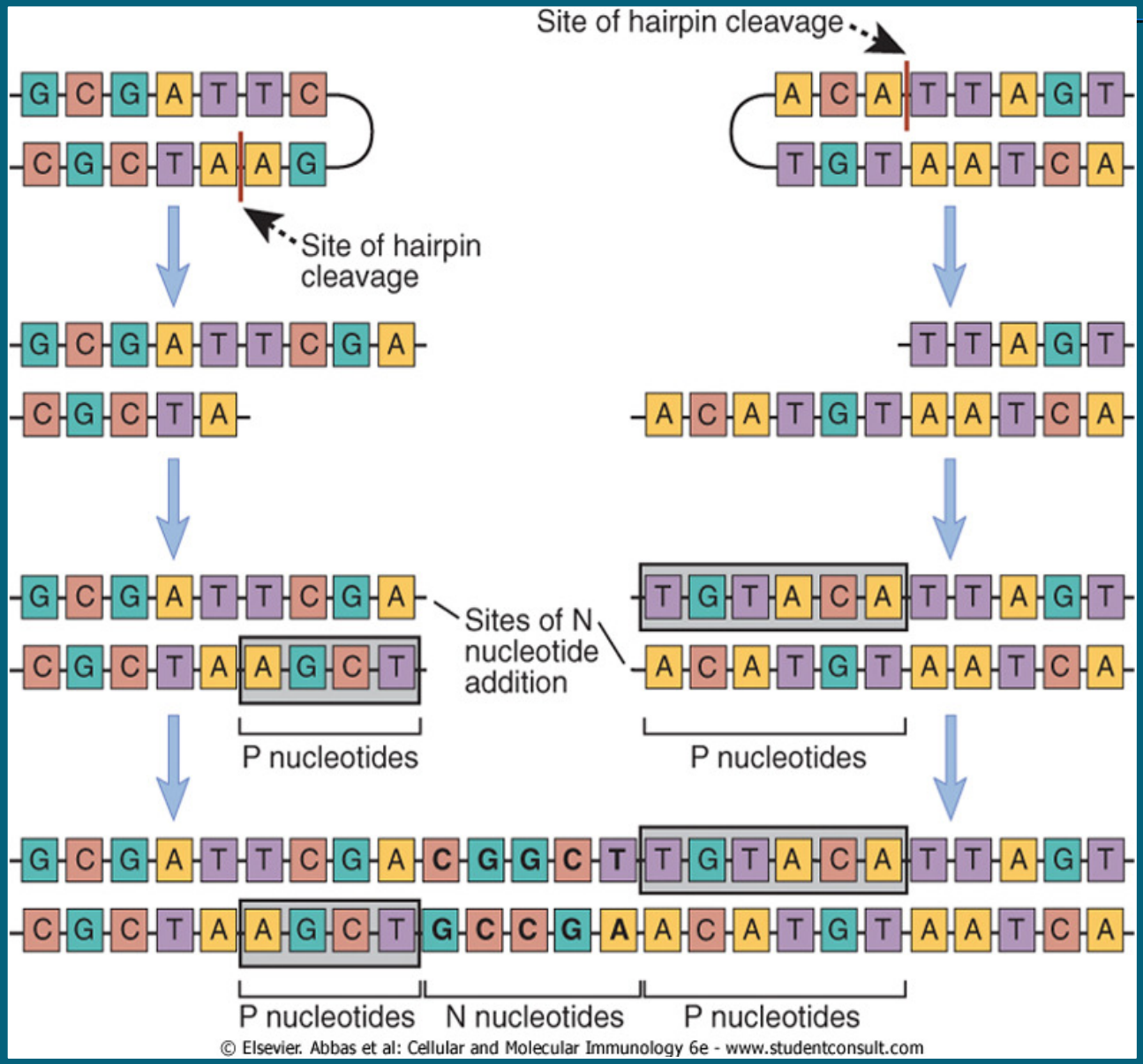
- Eliminación de nucleótidos (Nucleasas)
- Adición de nucleótidos P (ADN pol)
- Adición de nucleótidos N (TdT)

Generan la Tercera Región Hipervariable o CDR3

Diversidad del receptor: diversidad combinatoria y en las uniones



Diversidad de unión

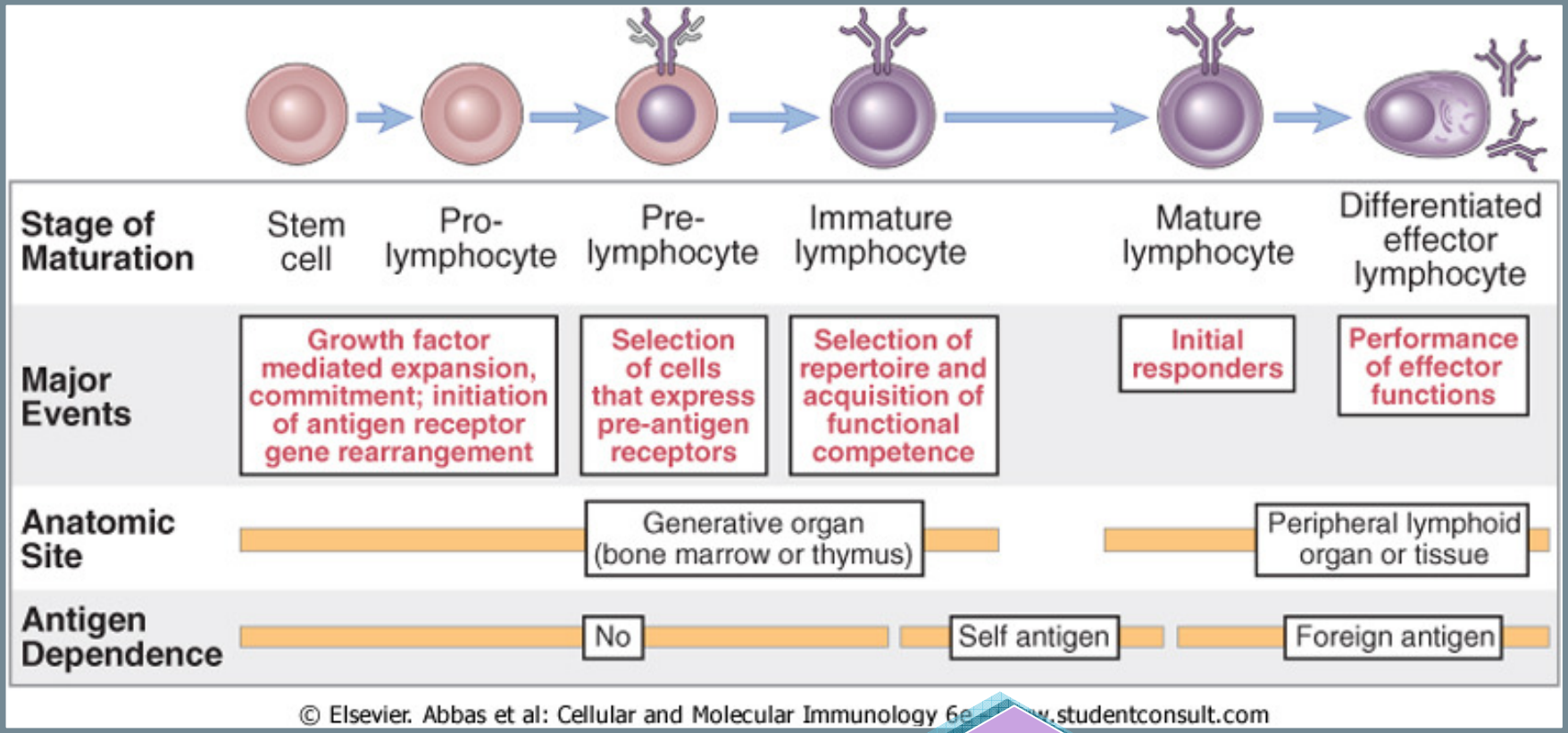


- **Es el resultado de la adición o sustracción de nucleótidos N y P, en las uniones de distintos segmentos génicos, durante el proceso de recombinación.**
- **En ambas cadenas, H y L, la diversidad del CDR3 se ve significativamente incrementada por este proceso.**

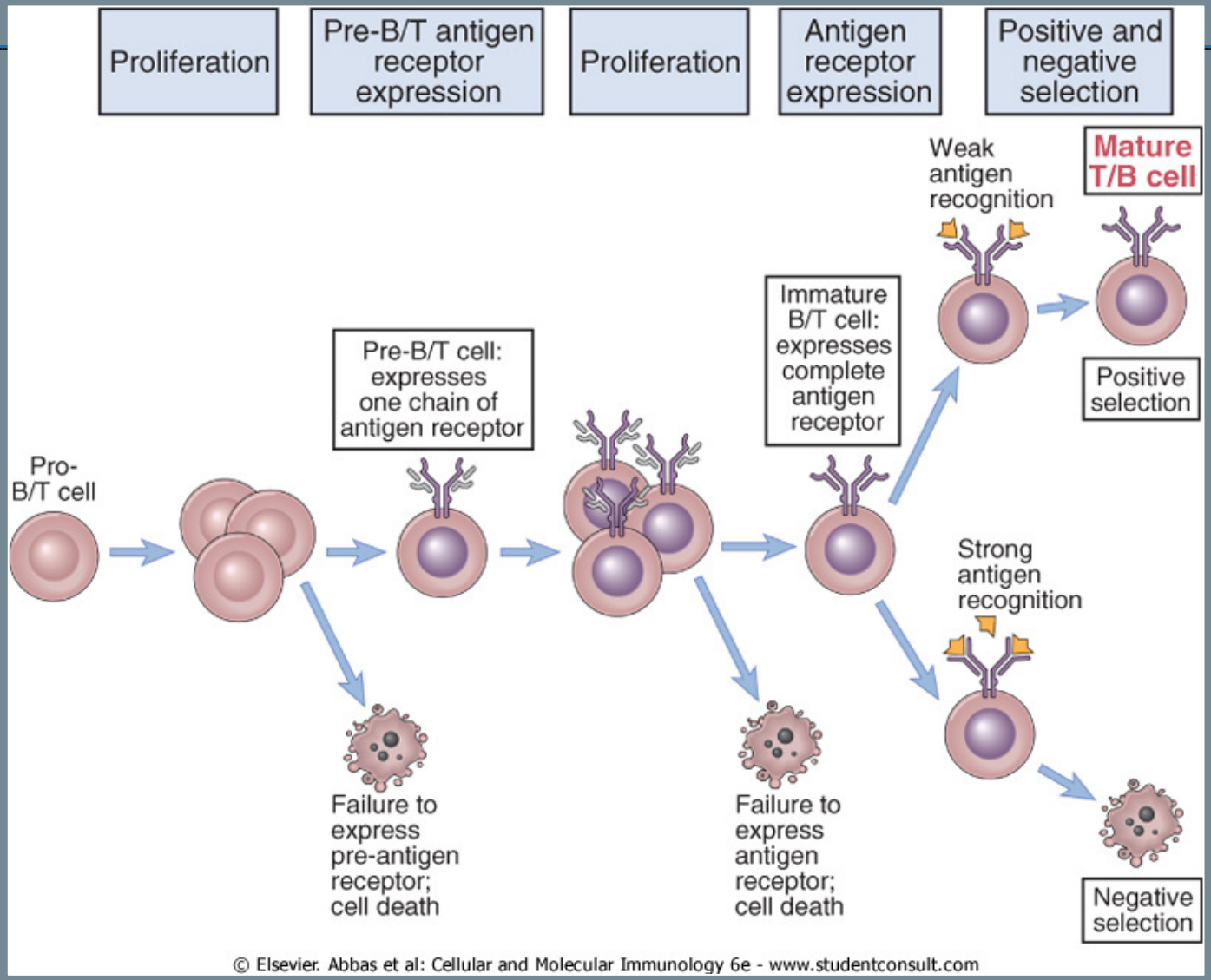
Para recordar…

- **Adición de Nucleótidos P**: denominados así porque forman secuencias palindrómicas añadidas a los extremos del gen.
- **Adición de Nucleótidos N**: denominados así porque no tienen molde que los codifique. Son añadidos por una enzima llamada DEOXINUCLEOTIDIL TRANSFERASA TERMINAL (TdT) a extremos de cadena sencilla del DNA codificante después de la rotura de la horquilla.
- **La deleción de nucleótidos** en las uniones de los segmentos génicos se lleva a cabo por exonucleasas.
- Dado que el número de nucleótidos añadidos por este proceso es aleatorio, éstos a menudo distorsionan el marco de lectura de las secuencis codificantes más allá de la unión, generando una proteína no funcional: REORDENAMIENTOS NO PRODUCTIVOS.

Desarrollo de la célula B



Check points durante la maduración



La interacción con antígenos propios selecciona linfocitos para que sobrevivan, pero elimina otros…

Existen cuatro opciones posibles para las células B inmaduras autorreactivas.....

….dependiendo de la naturaleza del antígeno al que se unen:

MOLÉCULAS PROPIAS MULTIVALENTES

- Apoptosis y eliminación de células B: **DELECIÓN CLONAL**
- Producción de un nuevo receptor**: EDICIÓN DEL RECEPTOR**

MOLÉCULAS PROPIAS SOLUBLES

- Inducción de un estado permanente de no respuesta al antígeno: **ANERGIA**

MOLÉCULAS PROPIAS DE BAJA AFINIDAD

- Una célula B que no percibe la presencia del antígeno, pues está secuestrado, en baja concentración o no reacciona con el BCR: **IGNORANCIA**

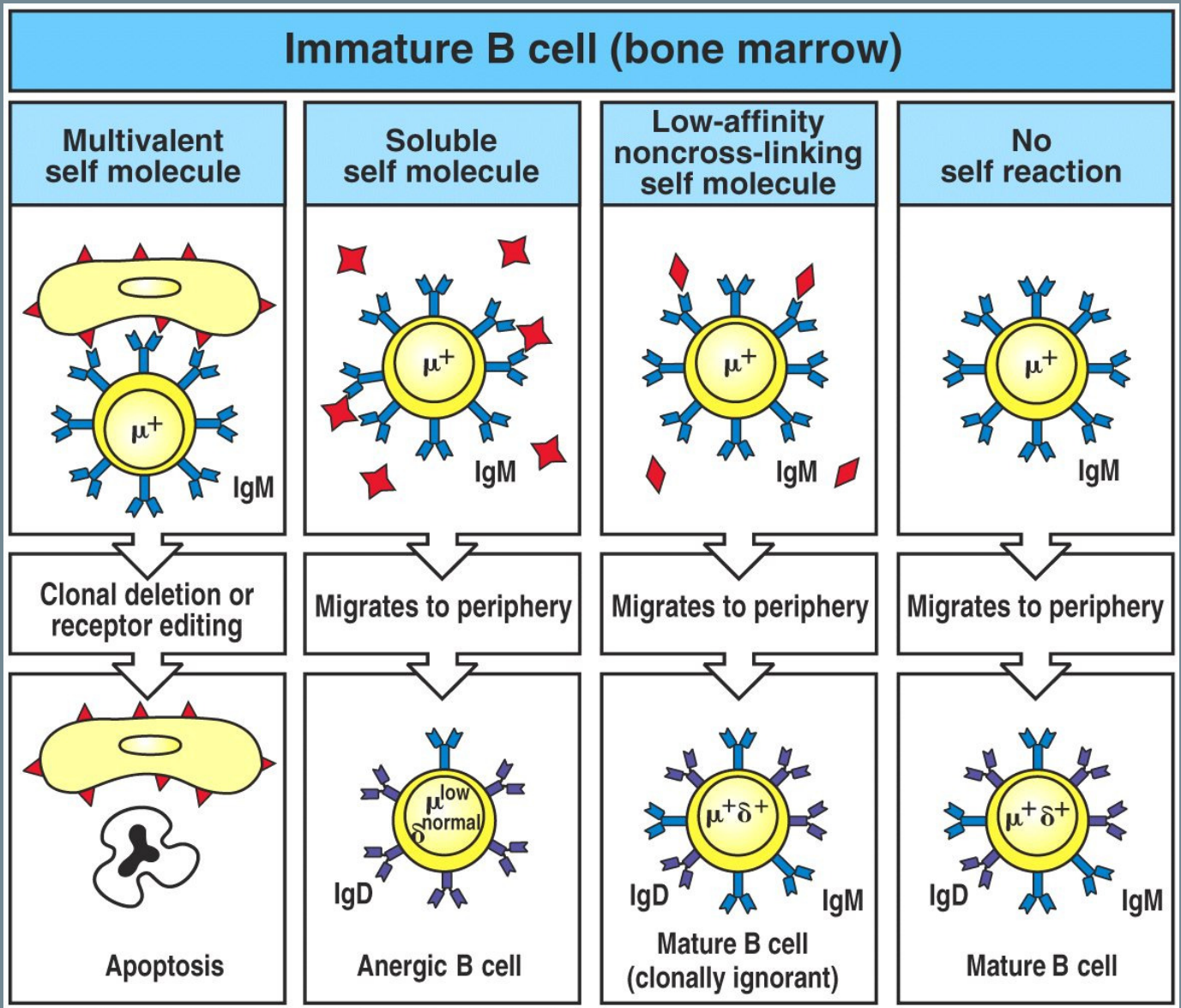
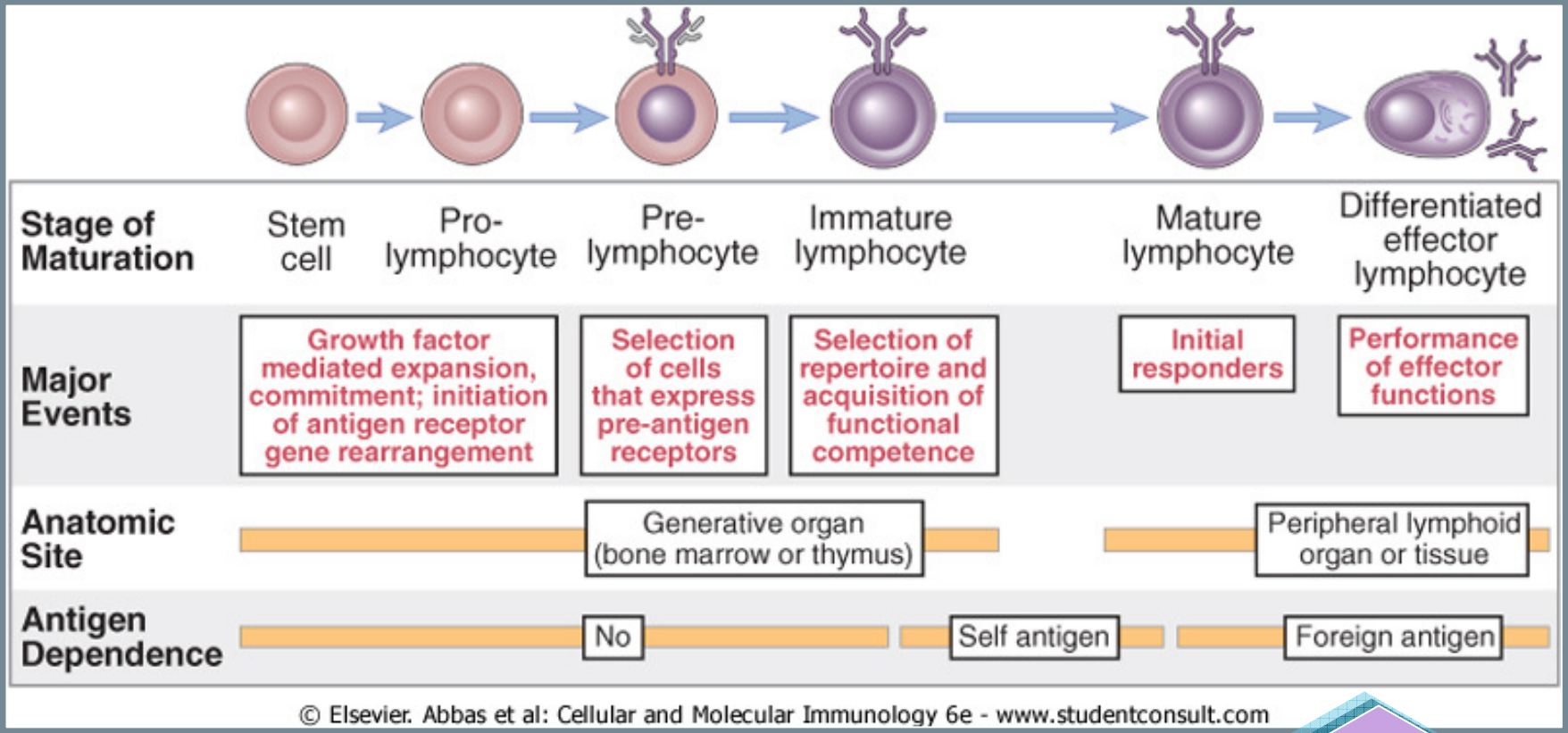
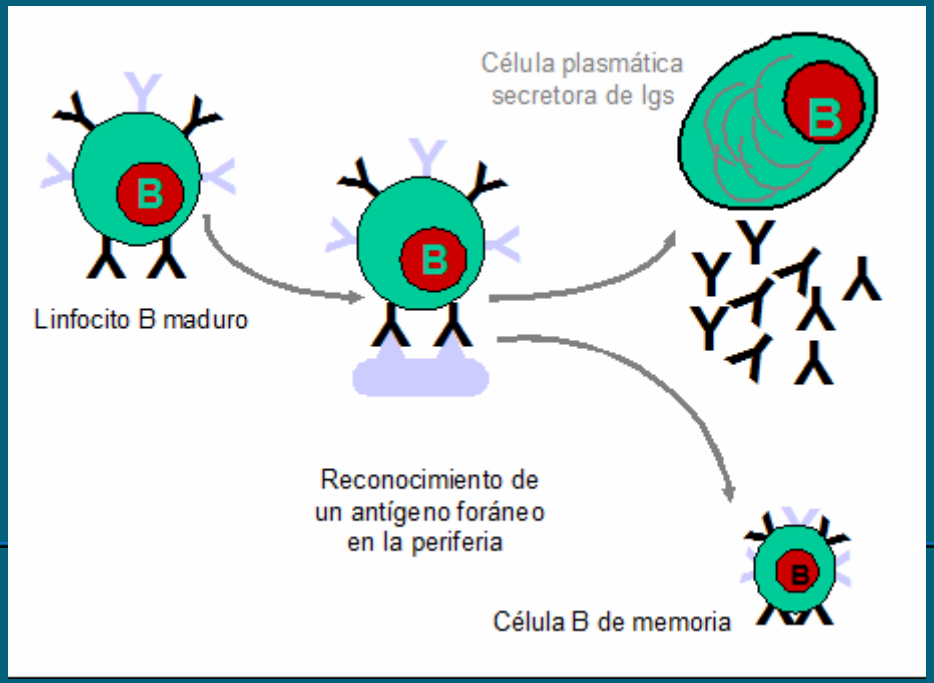


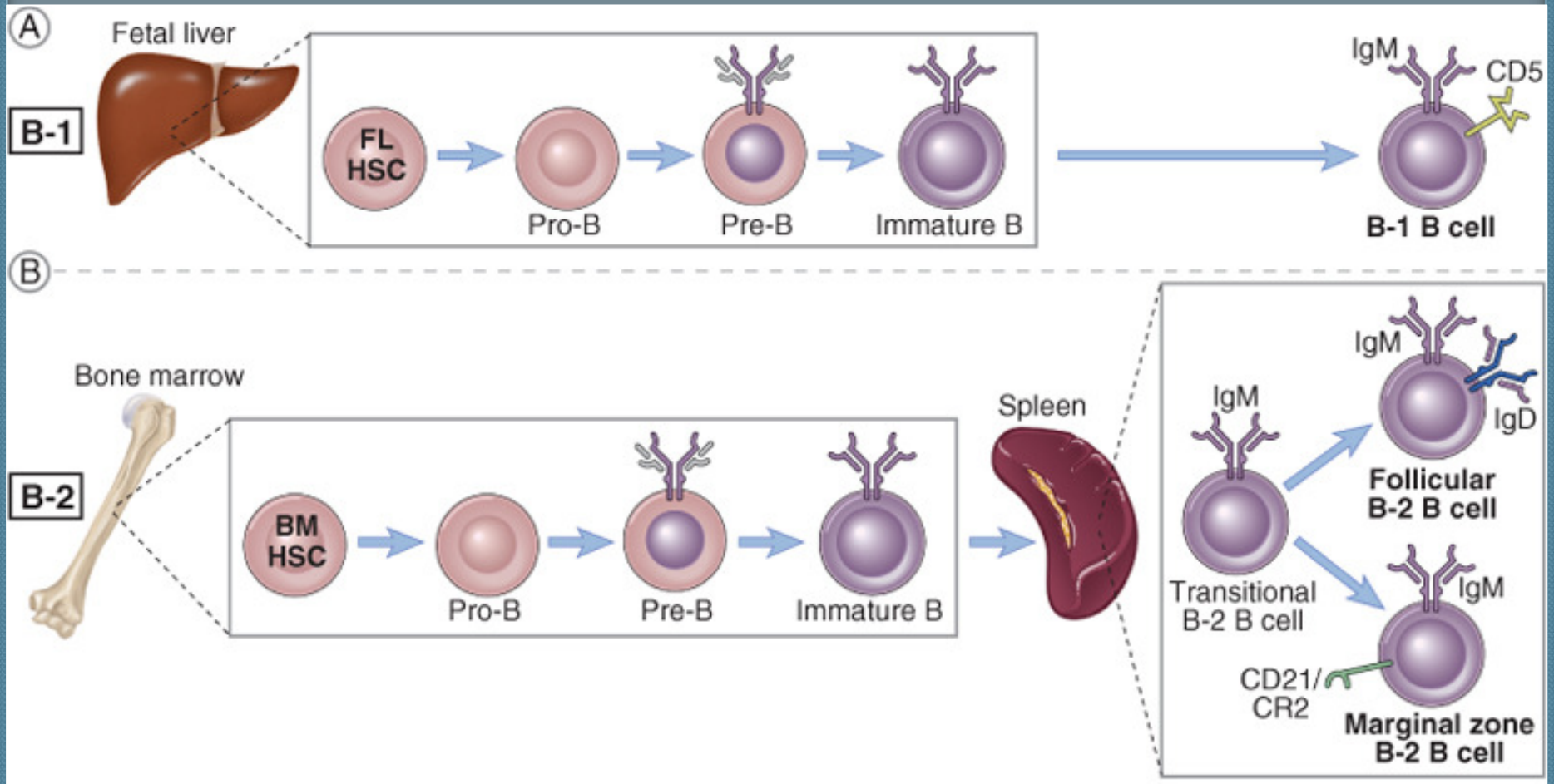
Figure 7-26 Immunobiology, 6/e. (© Garland Science 2005)

Desarrollo de la célula B





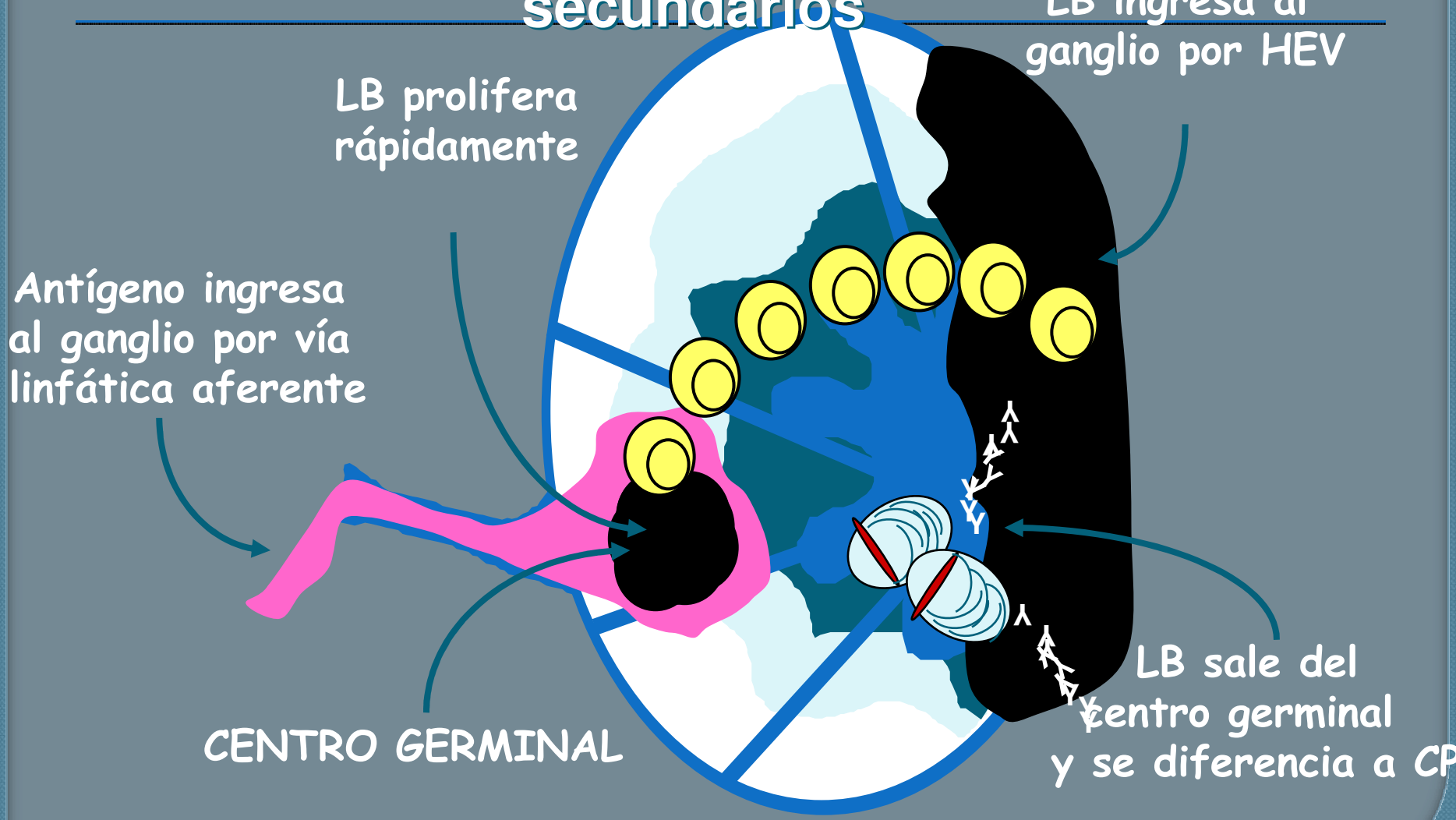
FASE DEPENDIENTE DE ANTÍGENOS EXÓGENOS



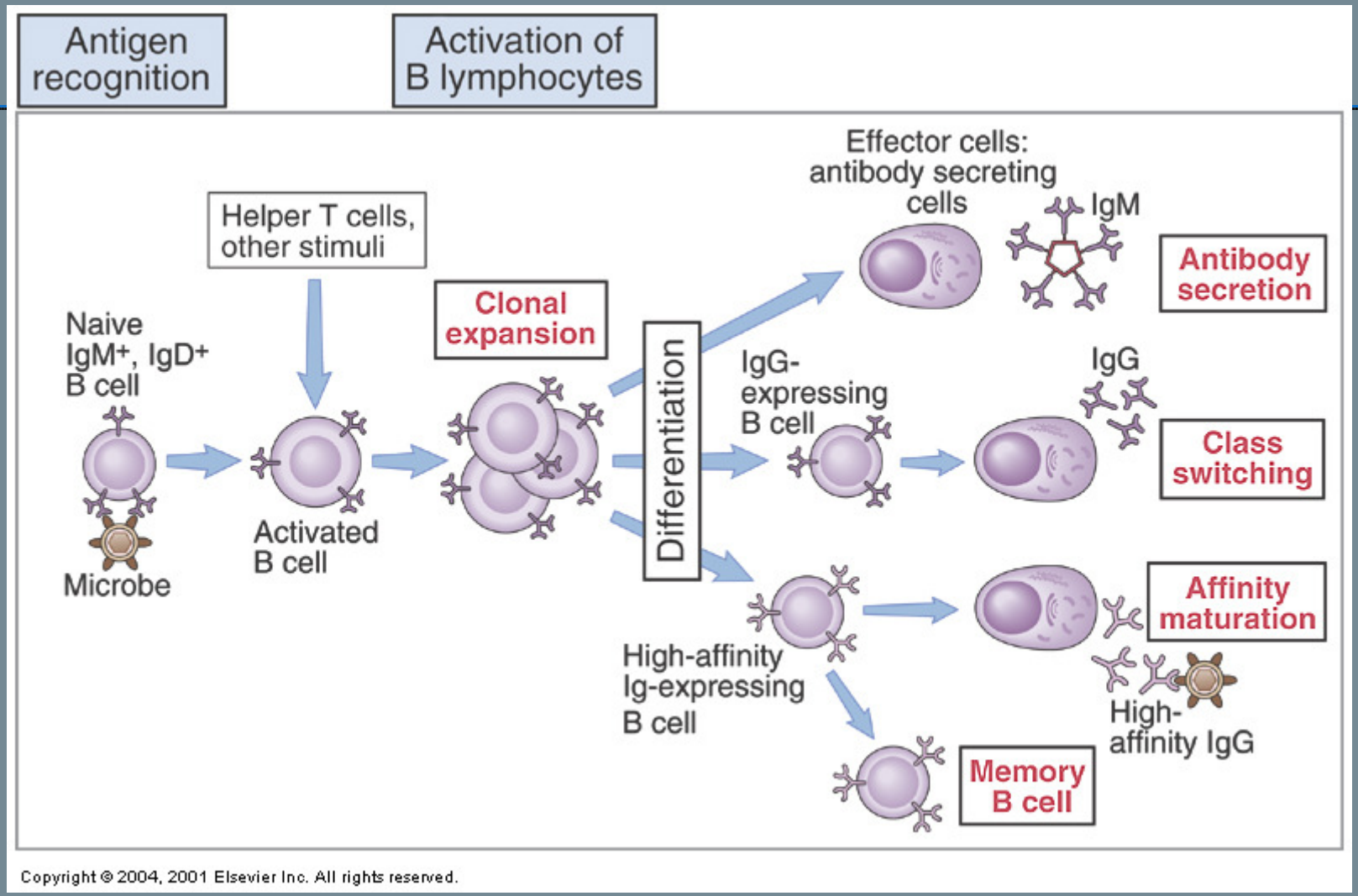
Maduración periférica de LB

- Solo un pequeño porcentaje de LB inmaduros abandona la médula ósea: aquellos que sobreviven a la inducción de tolerancia central y migran al bazo, donde culmina su maduración. Allí se encuentran como LB transicionales…
- BT1 ubicados en la vaina linfoide periarteriolar, sufren selección negativa si sus BCR reciben señales de moléculas propias. Este mecanismo de tolerancia periférica asegura que no existan LB autorreactivos.
- Los que sobreviven dan lugar a los BT2 que se encuentran en los folículos esplénicos. Para que esta subpoblación alcance el estadio de LB maduro son necesarias señales de supervivencia a través del BCR. La citocina BAFF o BlyS (factor activador de linfocitos B) estaría implicada en dicho proceso.
- BAFF es producida en forma constitutiva por Monocitos Macrófagos, DC y LT activados y se une a los LB en forma específica a través de los Rc TACI y BCMA.

LB circulantes captan antígenos extraños en órganos linfoides secundarios



Fases de la respuesta humoral frente a antígenos T dependientes


Antigen recognition
Activation of B lymphocytes
Effector cells: antibody secreting cells
IgM
Antibody secretion
Helper T cells, other stimuli
Clonal expansion
Naive IgM+, IgD+ B cell
Differentiation
IgG-expressing B cell
IgG
Class switching
Activated B cell
Microbe
Affinity maturation
High-affinity Ig-expressing B cell
High-affinity IgG
Memory B cell
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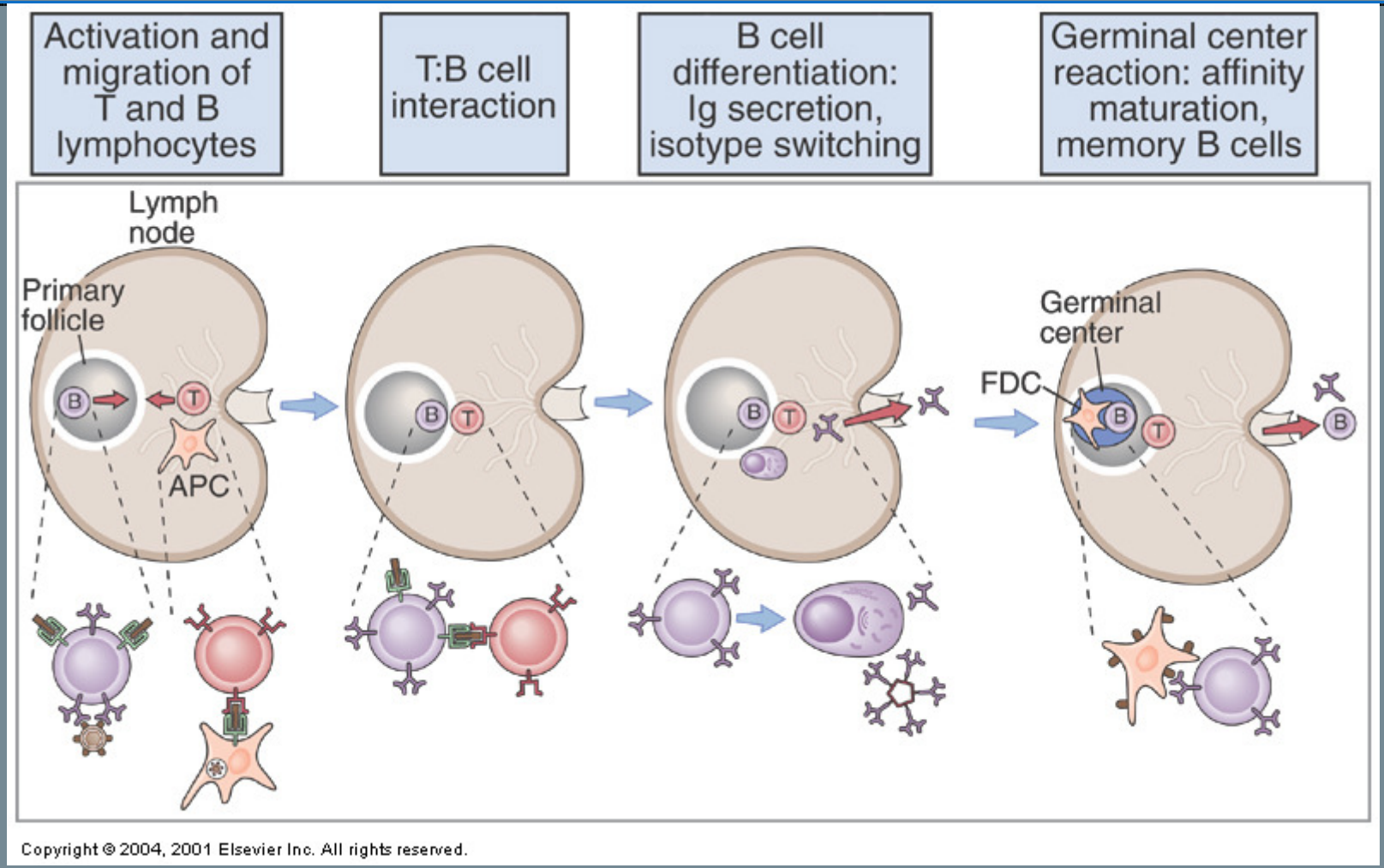
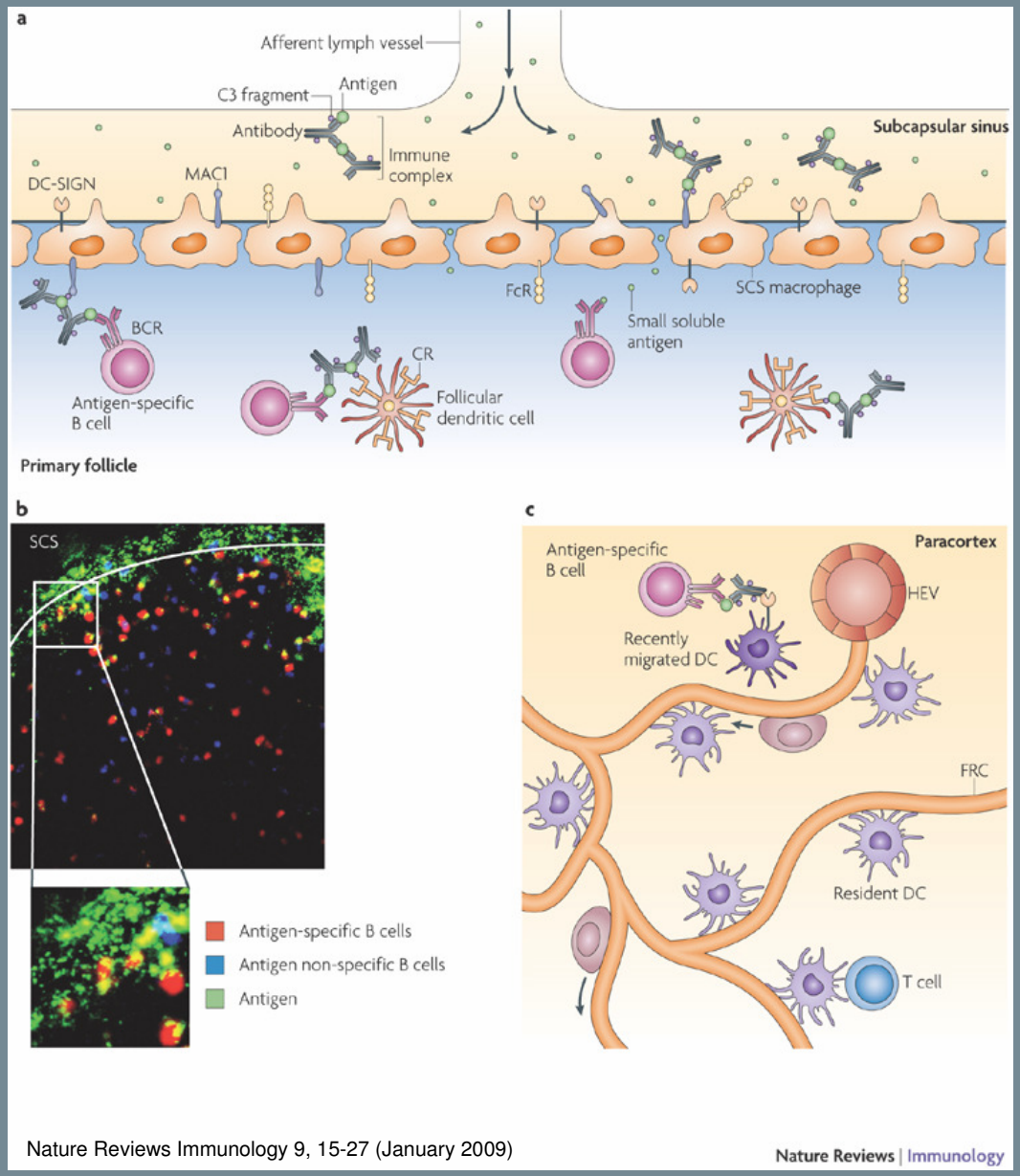
Anatomía de la respuesta inmune humoral

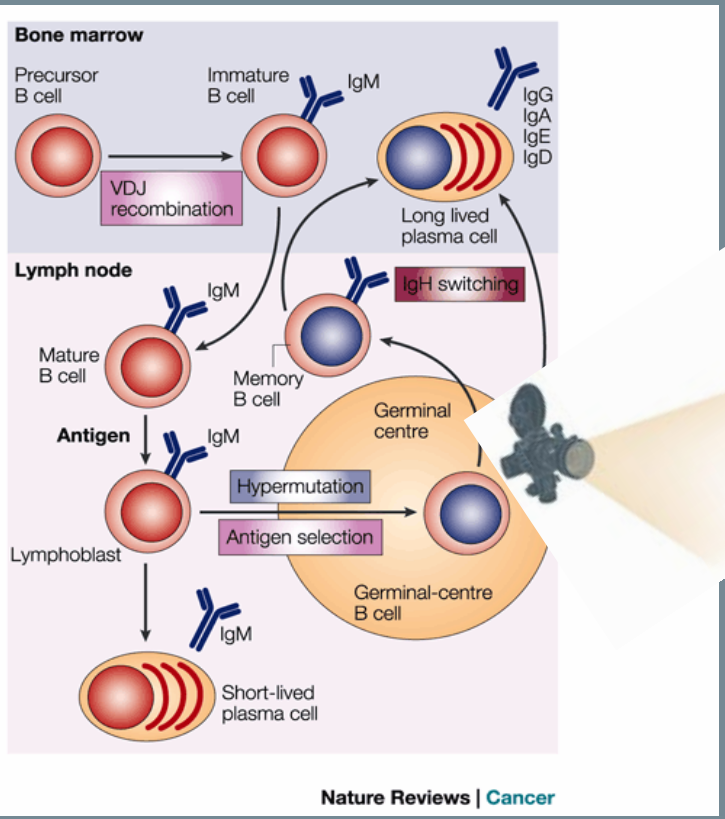


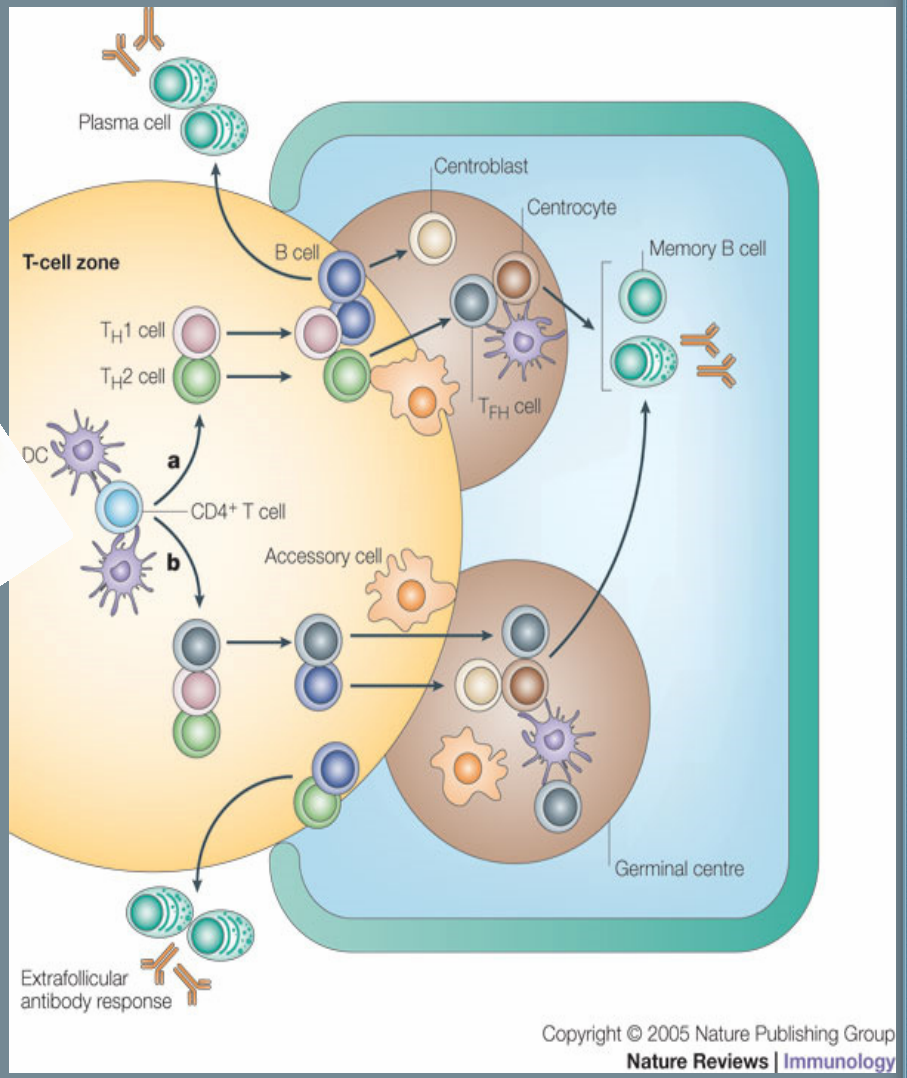


Nature Reviews Immunology 9, 15-27 (January 2009)

a | B cells in follicles have been found to encounter small soluble antigens from the lymphatic fluid as they diffuse from the subcapsular sinus (SCS) to the follicles. Large antigens, immune complexes and viruses can be presented to follicular B cells at the macrophage-rich SCS. In addition, follicular B cells may recognize antigen that is presented on the surface of follicular dendritic cells (FDCs).

c | Schematic view of the paracortex to illustrate where antigen-specific B cells encounter antigen at this site. B cells entering the lymph node can encounter unprocessed antigen on the surface of resident or recently migrated DCs, in close proximity to the high-endothelial venules (HEVs) The conduit system, which is lined with FRCs and DCs, transports low-molecular-mass components of the lymphatic fluid through the lymph node; B cells and T cells have been shown to migrate in association with the FRC network. BCR, B-cell receptor; C3, complement component 3; CR, complement receptor; DC-SIGN, DC-specific ICAM3-grabbing non-integrin; FcR, Fc receptor; ICAM3, intercellular adhesion molecule 3; MAC1, macrophage receptor 1.





Nature Reviews Immunology 5, 853-865 (November 2005)

Table 1 | **Follicular T-cell subsets**

Phenotype	Location	Function	References
$CD4^+CD25^-CD57^+$ $CD69^+CXCR5^+$	Light zone	Provide help to germinal-centre B cells by inducing AID expression, immunoglobulin class switching and immunoglobulin production; upregulate OX40 and CD40L expression on stimulation; and poor producers of interferon-γ and tumour-necrosis factor	9,18,19,22
$CD4^+CD25^-CD57^-$ $CD69^-CXCR5^+$	Outer zone	Probably provide help to germinal-centre B cells, because contain pre-formed CD40L	4,27
$CD4^+CD25^+CD57^-$ $CD69^+CXCR5^+$	Germinal centre	Suppress T_{FH}-cell help to germinal-centre B cells; and inhibit immunoglobulin production, survival and AID expression by germinal-centre B cells	29
$CD4^+CD57^-$ $CXCR5^+CRTH2^+$	Germinal centre	T_H2 cells (or precursors); produce IL-4, IL-5 and IL-13	26
$CD4^+$	Primary follicle	Undergo antigen-independent and CD40-independent localization to primary follicles; and might include $CD44^{hi}IL\text{-}7R^+$ memory T cells, which maintain secondary antibody responses	24,28
$CD4^+NK1.1^+$	Primary follicle	Function unknown; CD1.1 restricted; and present in MHC-class-II-deficient mice	30
$CD8^+$	Germinal centre	Function unknown; constitute <5% of tonsil germinal-centre T cells but 10–15% of lymph-node germinal-centre T cells	19

AID, activation-induced cytidine deaminase; CD1.1, also known as CD1d; CD40L, CD40 ligand; CRTH2, chemoattractant-receptor homologous molecule expressed by T_H2 cells; CXCR5, CXC-chemokine receptor 5; IL, interleukin; IL-7R, IL-7 receptor; NK1.1, natural-killer-cell-associated antigen 1.1; T_{FH} cell, follicular B helper T cell; T_H2 cell, T helper 2 cell.

Table 2 | **T_{FH} cells are distinct from T_H1 and T_H2 cells**

Cell type	Main immune function	Distinguishing chemoattractant receptors	Transcription factors expressed	Cytokines produced
T_{FH} cells	Help B cells	CXCR5	Possibly BCL-6	IL-10 and possibly IL-21
T_H1 cells	Provide antiviral and antibacterial immunity	CXCR3 and CCR5	T-bet	Interferon-γ
T_H2 cells	Provide antiparasite immunity	CRTH2 and CCR3	MAF and GATA3	IL-4, IL-5 and IL-13

BCL-6, B-cell lymphoma 6; CCR, CC-chemokine receptor; CRTH2, chemoattractant-receptor homologous molecule expressed by T_H2 cells; CXCR, CXC-chemokine receptor; GATA3, GATA-binding protein 3; IL, interleukin; T_{FH} cell, follicular B helper T cell; T_H cell, T helper cell.

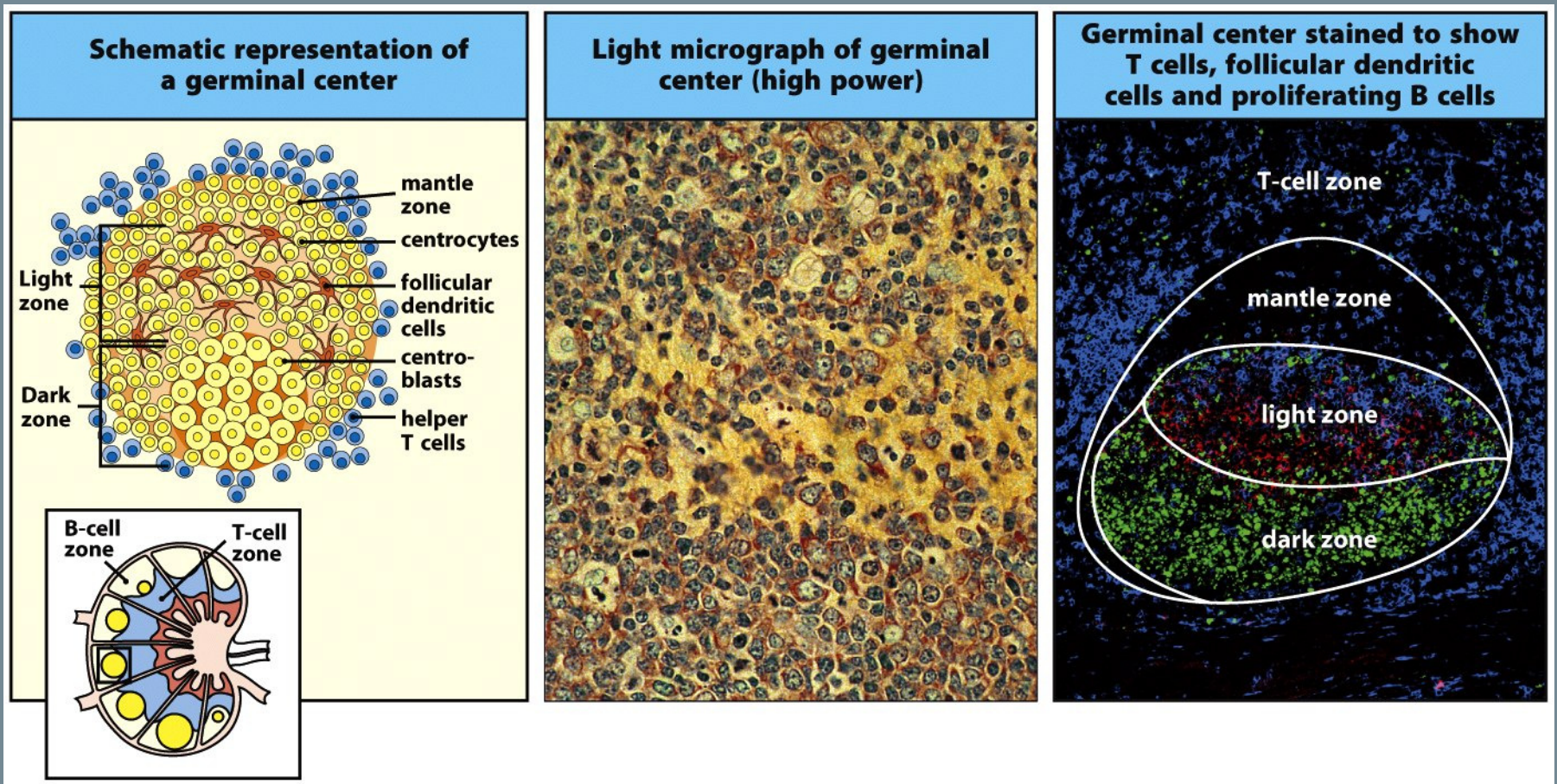
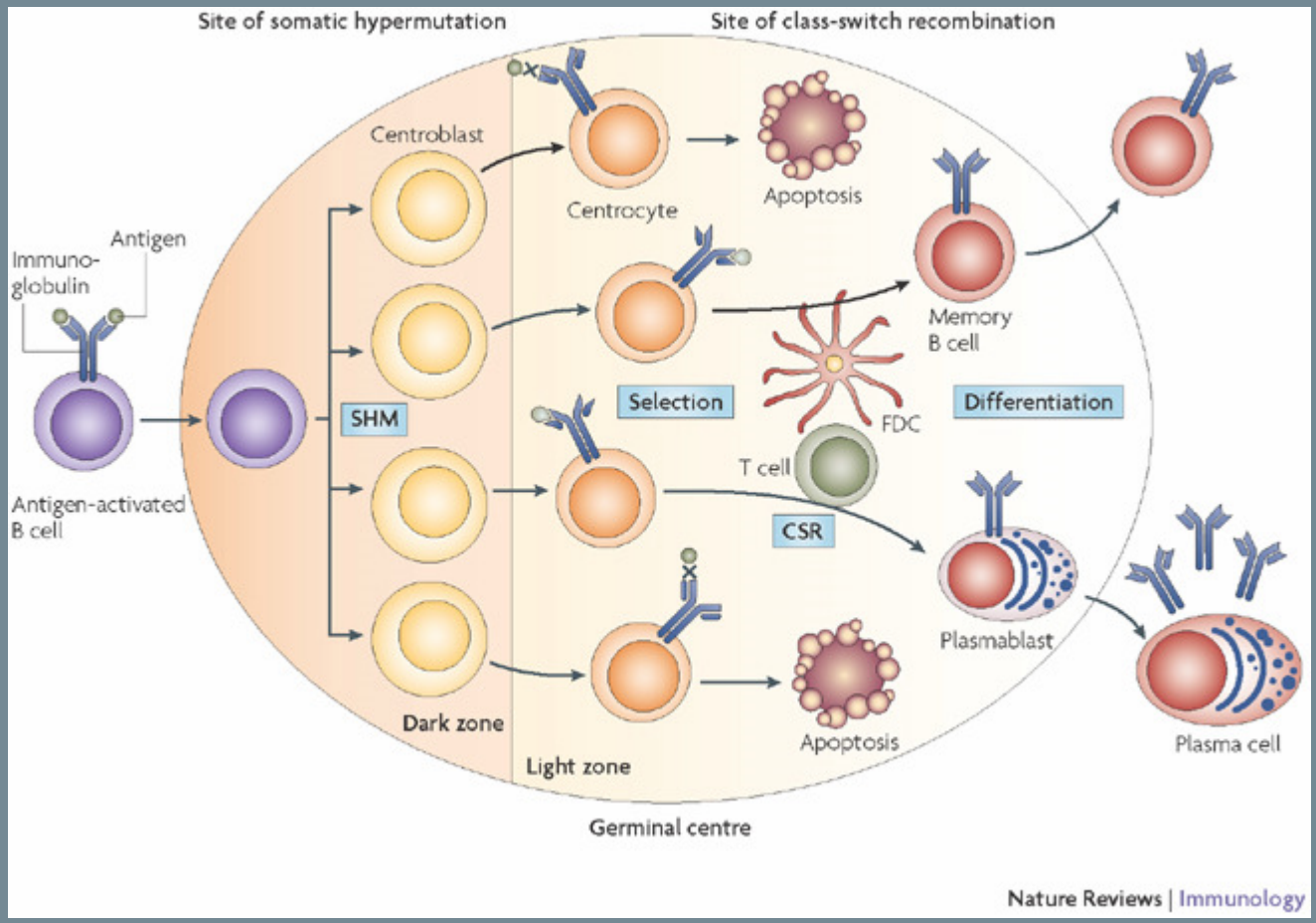


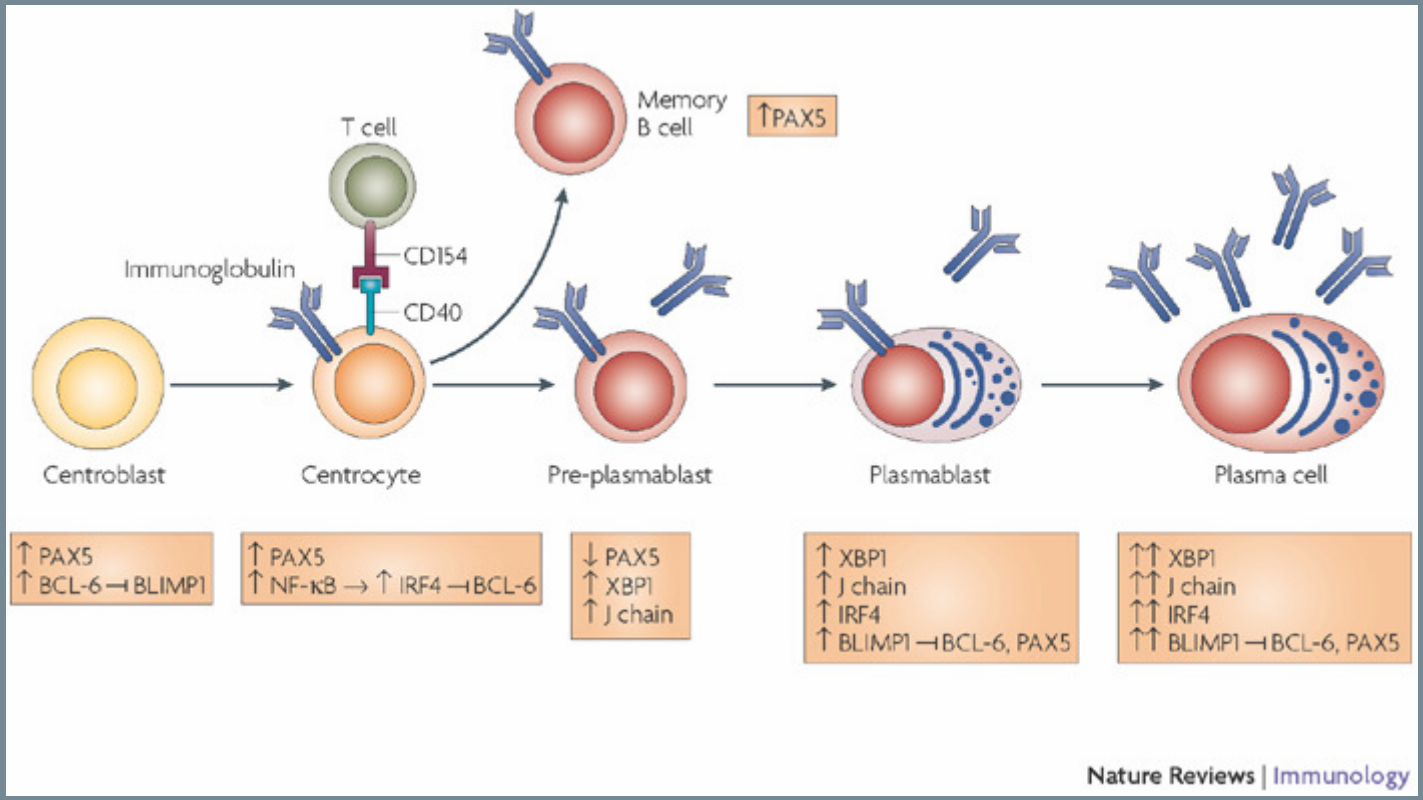
Figure 9-10 Immunobiology, 7ed. (© Garland Science 2008)

¿Qué ocurre en el centro germinal??

Nature Reviews Immunology 8, 22-33 (January 2008)



Antigen-activated B cells differentiate into centroblasts that undergo clonal expansion in the dark zone of the germinal centre. During proliferation, the process of somatic hypermutation (SHM) introduces base-pair changes into the V(D)J region of the rearranged genes encoding the immunoglobulin variable region (IgV) of the heavy chain and light chain; some of these base-pair mutations lead to a change in the amino-acid sequence. Centroblasts then differentiate into centrocytes and move to the light zone, where the modified antigen receptor, with help from immune helper cells including T cells and follicular dendritic cells (FDCs), is selected for improved binding to the immunizing antigen. Newly generated centrocytes that produce an unfavourable antibody undergo apoptosis and are removed. A subset of centrocytes undergoes immunoglobulin class-switch recombination (CSR). Cycling of centroblasts and centrocytes between dark and light zones seems to be mediated by a chemokine gradient, presumably established by stromal cells in the respective zones (not shown)[14]. Antigen-selected centrocytes eventually differentiate into memory B cells or plasma cells.

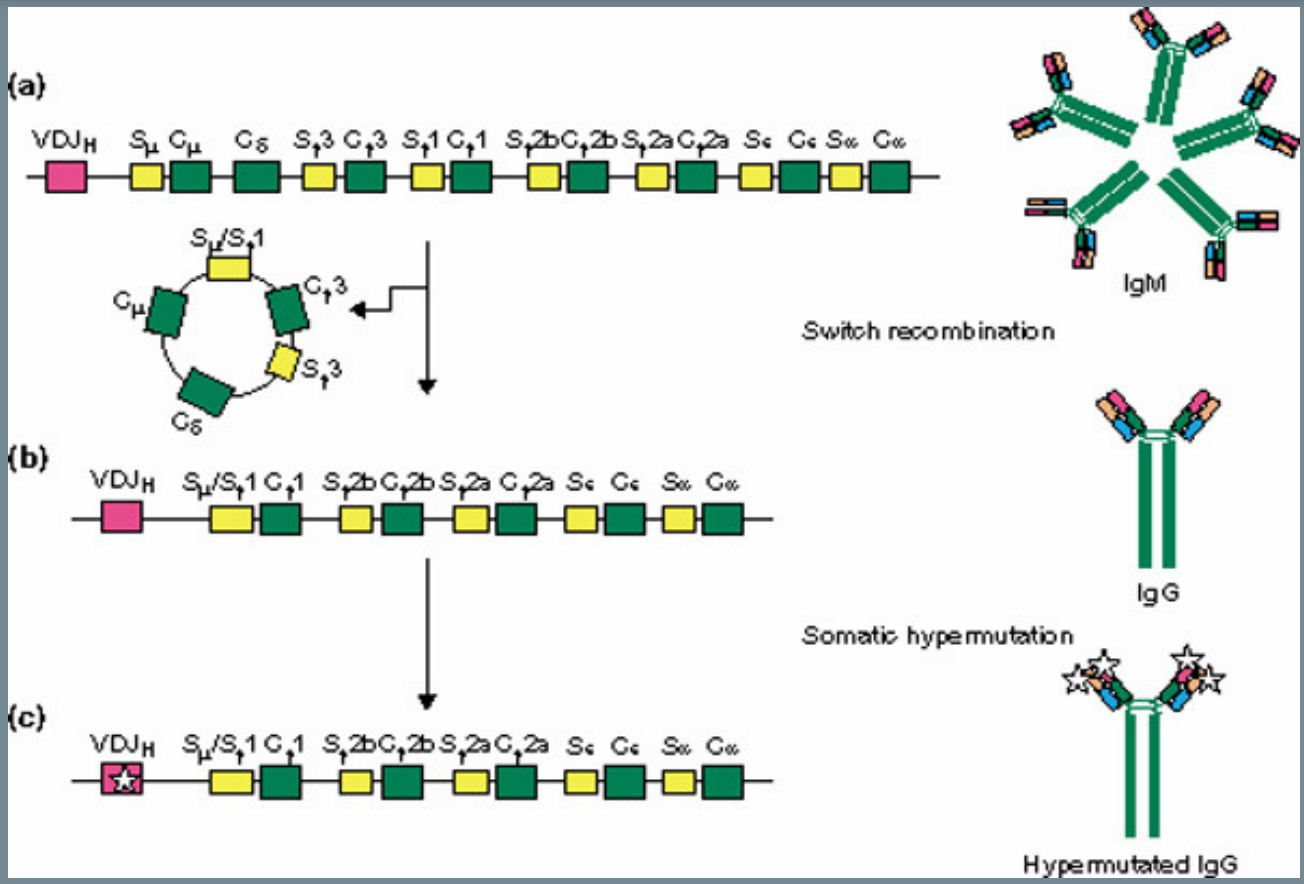

Memory
B cell
↑PAX5
T cell
CD154
Immunoglobulin
CD40
Centroblast
Centrocyte
Pre-plasmablast
Plasmablast
Plasma cell
↑ PAX5
↑ BCL-6 ⊣ BLIMP1
↑ PAX5
↑ NF-κB → ↑ IRF4 ⊣ BCL-6
↓ PAX5
↑ XBP1
↑ J chain
↑ XBP1
↑ J chain
↑ IRF4
↑ BLIMP1 ⊣ BCL-6, PAX5
↑↑ XBP1
↑↑ J chain
↑↑ IRF4
↑↑ BLIMP1 ⊣ BCL-6, PAX5
Nature Reviews | Immunology

HIPERMUTACIÓN SOMÁTICA Y MADURACIÓN DE LA AFINIDAD INCREMENTAN EL REPERTORIO DE Ig DESPUÉS DEL RECONOCIMIENTO ANTIGÉNICO

HIPERMUTACIÓN SOMÁTICA: es un proceso que está restringido a las células B en los centros germinales. Implica mutaciones puntuales e individuales que cambian un único aminoácido en las regiones V de los genes reordenados de las cadenas pesadas y livianas, con una tasa muy elevada. Este mecanismo genera anticuerpos de **mayor afinidad** a medida que progresa la respuesta inmune humoral.

El proceso de HS ocurre luego del contacto con el antígeno a diferencia de la RS que tiene lugar durante la maduración de los LB en la médula ósea.

MADURACIÓN DE LA AFINIDAD: es el proceso que conduce al incremento de afinidad de los anticuerpos por un antígeno particular como resultado de la **MUTACIÓN SOMÁTICA** en los genes de las inmunoglobulinas, seguida por una supervivencia selectiva de células B productoras de anticuerpos con alta afinidad.



Switch recombination and somatic hypermutation at the immunoglobulin heavy chain locus. **(a)** The murine heavy chain locus (left) has undergone VDJ recombination and encodes a μ heavy chain. The resulting IgM antibodies (right) are pentamers of a dimer containing two heavy and two light chains. **(b)** Class switch recombination joins a new constant region to the expressed variable (VDJ) region, resulting in synthesis of antibody of a new class. Shown is switch recombination from Cμ to Cγ1, to produce a dimeric IgG1 antibody (right). **(c)** Somatic hypermutation modifies the variable region sequences of both heavy chains (left) and light chains. Following affinity selection, hypermutated antibodies (right) have increased affinity for antigen. Stars denote mutations in the DNA (left) and protein (right). **Somatic hypermutation is shown following switch recombination, but neither process is prerequisite for the other** . VDJ, heavy chain variable region; S, switch region; C, constant region.

Maizels *Genome Biology* 2000 **1**:reviews1025.1

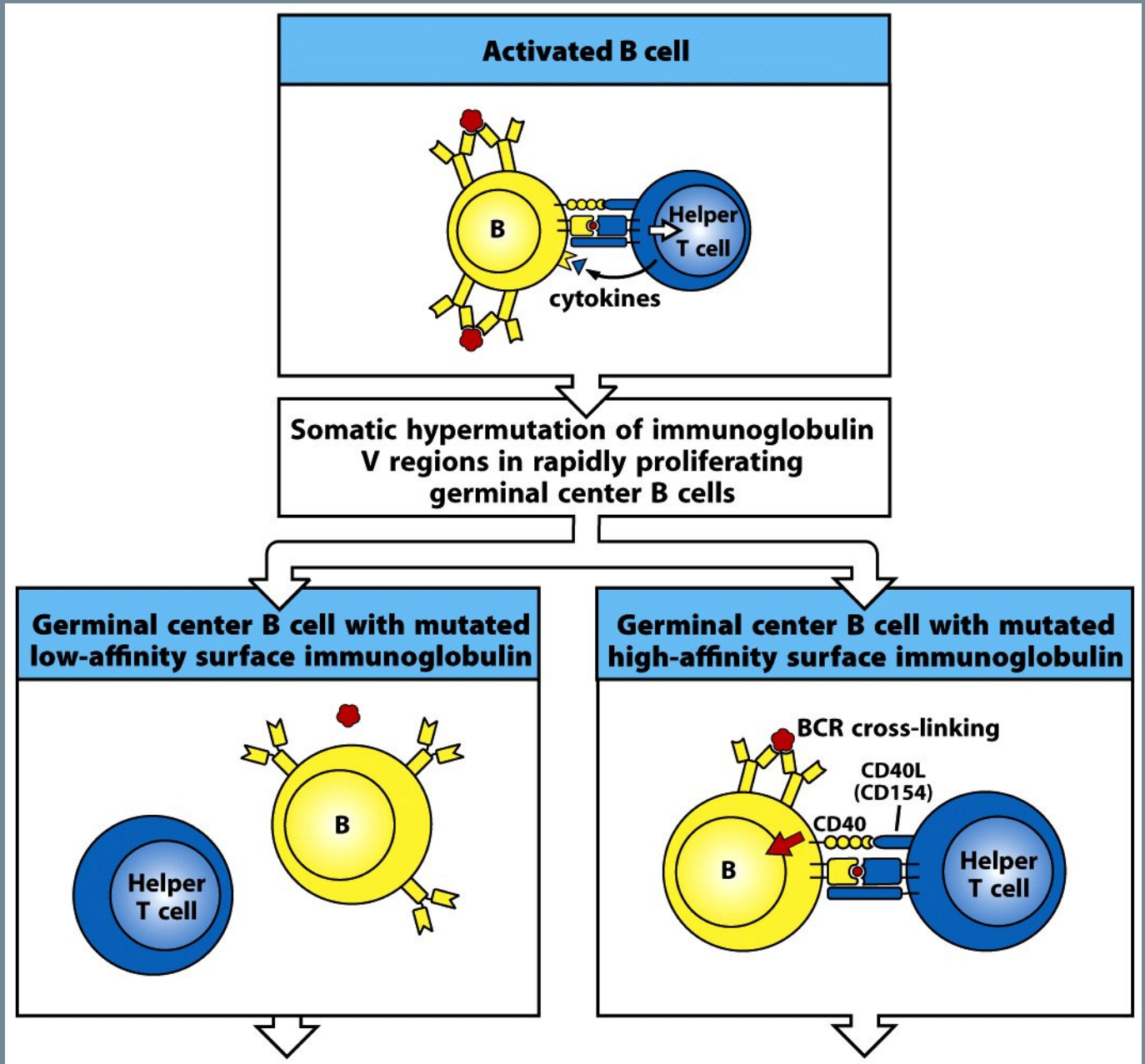


Figure 9-11 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

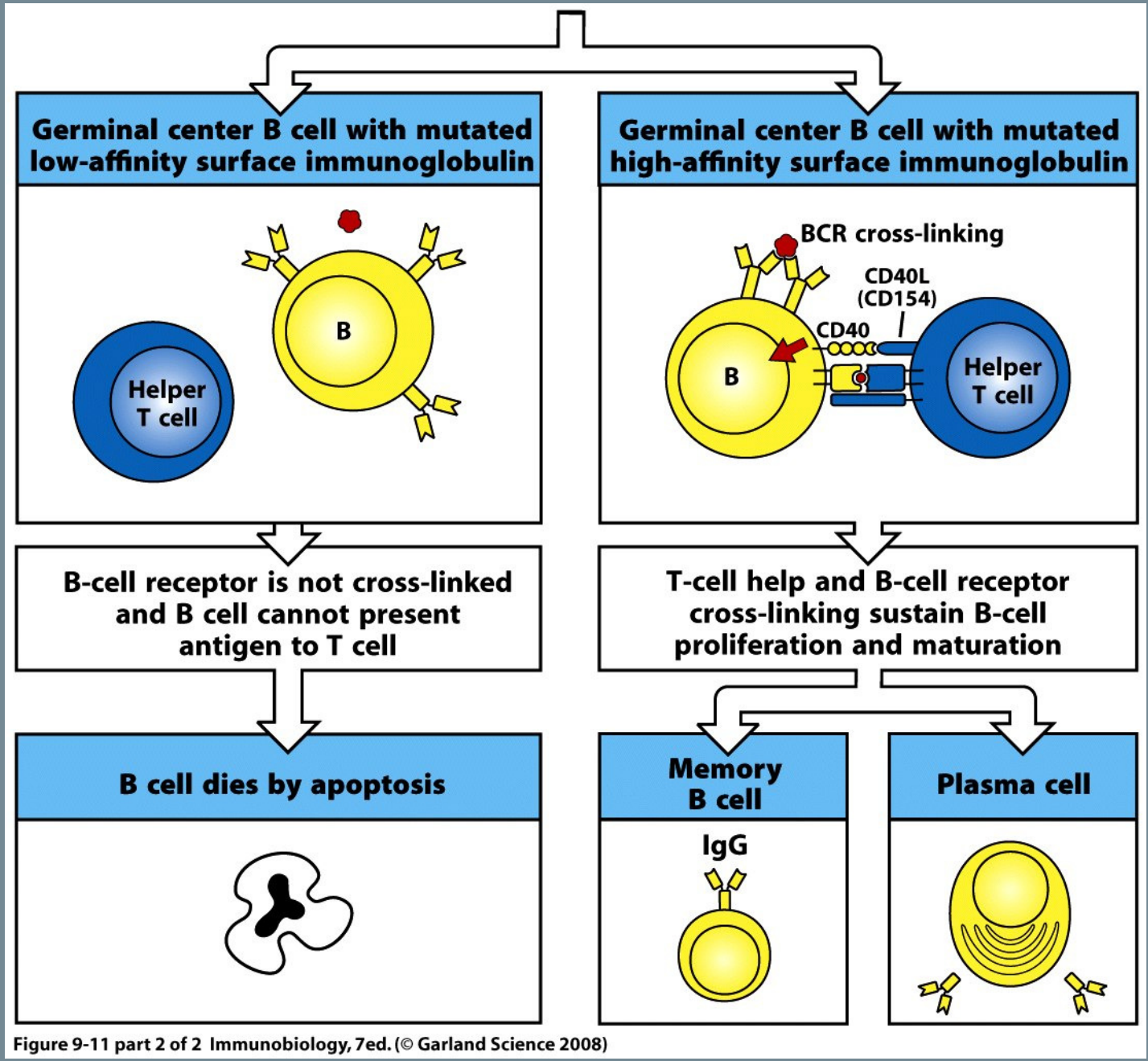


Figure 9-11 part 2 of 2 Immunobiology, 7ed. (© Garland Science 2008)

HIPERMUTACIÓN SOMÁTICA

- Los principales lugares donde tienen lugar las mutaciones somáticas son los centros germinales de los folículos linfoides secundarios en respuesta a antígenos dependientes de LTh.
- Se conocen ciertas características de las mutaciones somáticas que tienen lugar en los genes de las Ig:
- **Las mutaciones afectan principalmente a la IgG y la IgA,** en un fenómeno asociado al cambio de clase de Ig. Es presumible que las variantes somáticas sean seleccionadas por el Ag, por tener mayor afinidad que los Ac de la línea germinal.
- Hay una altísima tasa de mutación (10^{-3}·pb^{-1}·generación^{-1}, es decir, un millón de veces más que la normal) que va generando continuamente nuevas variantes de inmunoglobulinas a partir de la reordenación génica original.
- El número de mutaciones se va incrementando durante la respuesta inmune, sobre todo en la respuesta secundaria y ulteriores.
- Conforme aumenta la edad del individuo aumentan las mutaciones, lo cual parece que se debe a que existen más células B de memoria que van entrando en el proceso de hipermutación somática.
- **Las mutaciones puntuales tienden a agruparse en los exones de las regiones V y en las secuencias flanqueantes de las cadenas H y L,** y son más numerosas en las regiones hipervariables (CDR1 y CDR2).

La CITIDIN-DEAMINASA INDUCIDA POR ACTIVACIÓN (AID) juega un papel importante en la HS y switch de isotipo…..es una enzima editora de ARN y su DEFICIENCIA se asocia a SINDROME DE HIPER-IgM TIPO 2.

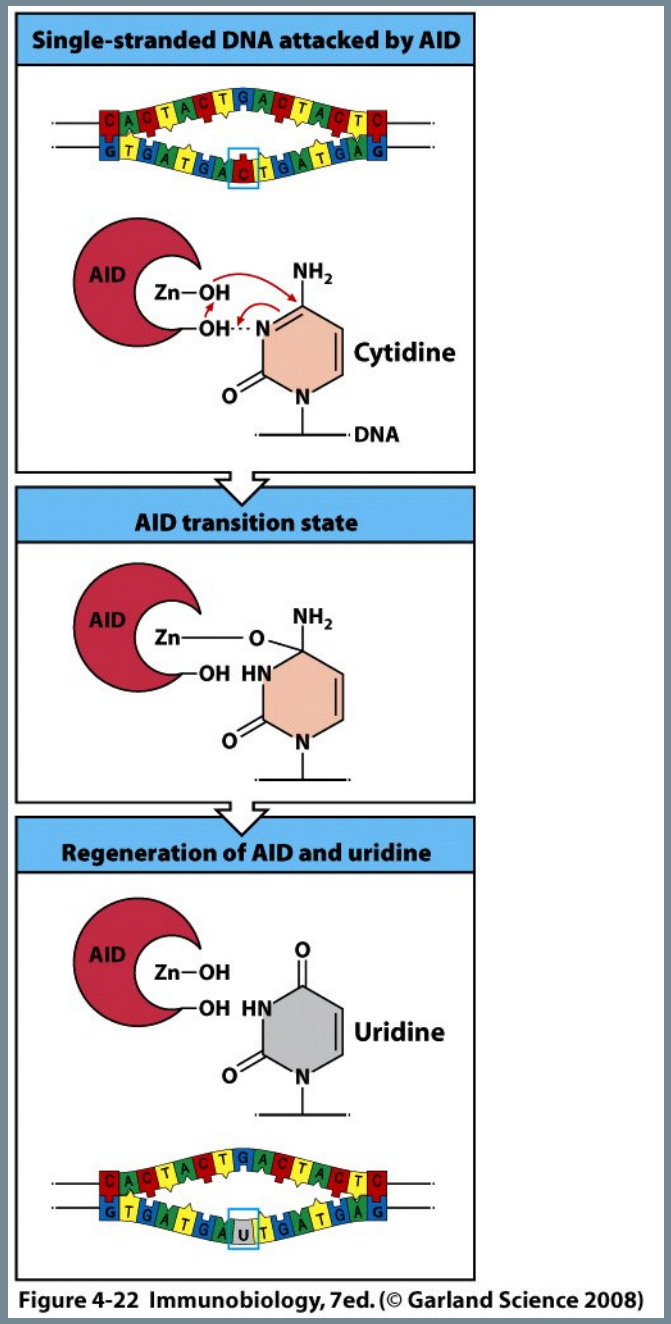


Figure 4-22 Immunobiology, 7ed. (© Garland Science 2008)

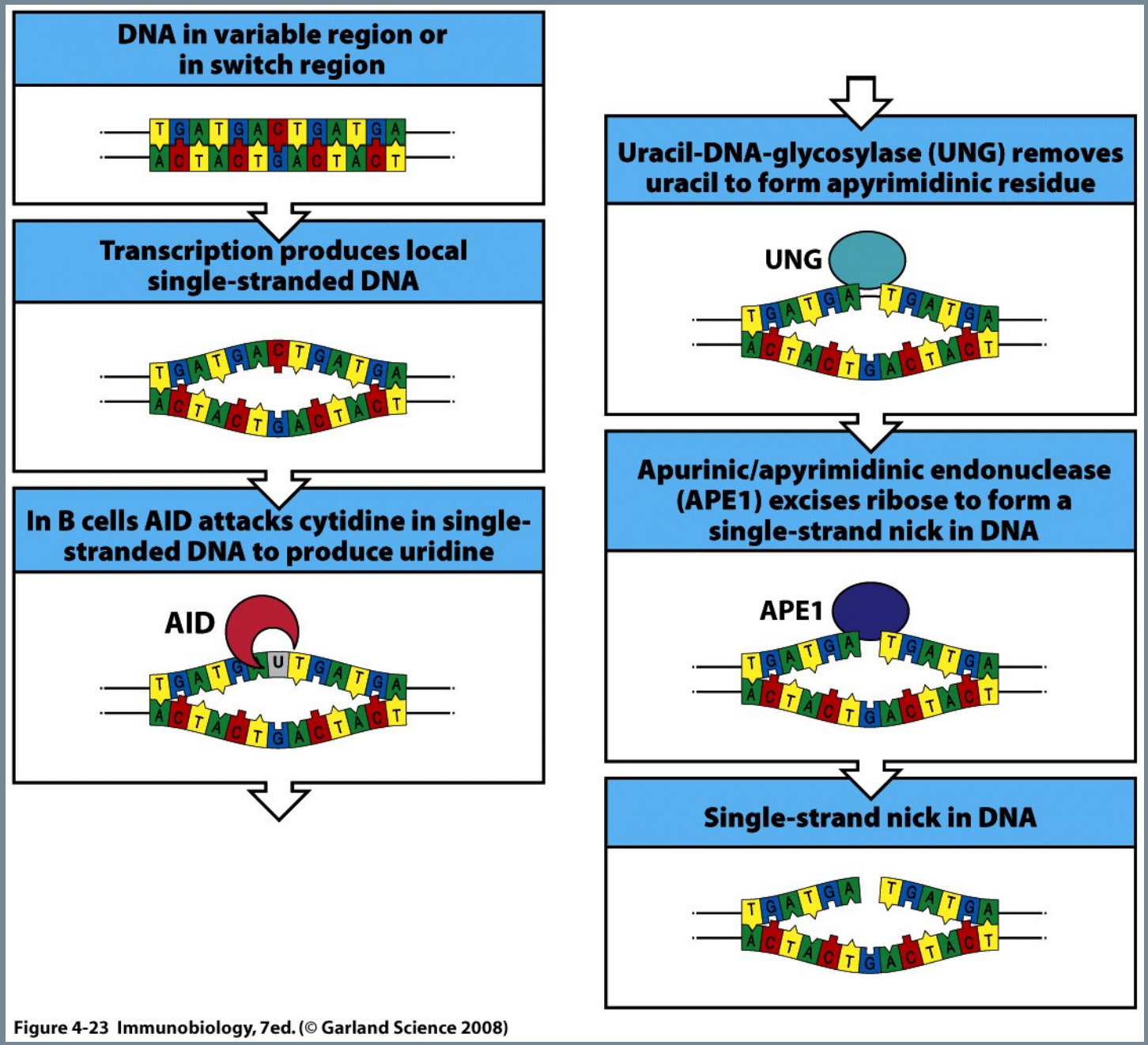


Figure 4-23 Immunobiology, 7ed. (© Garland Science 2008)

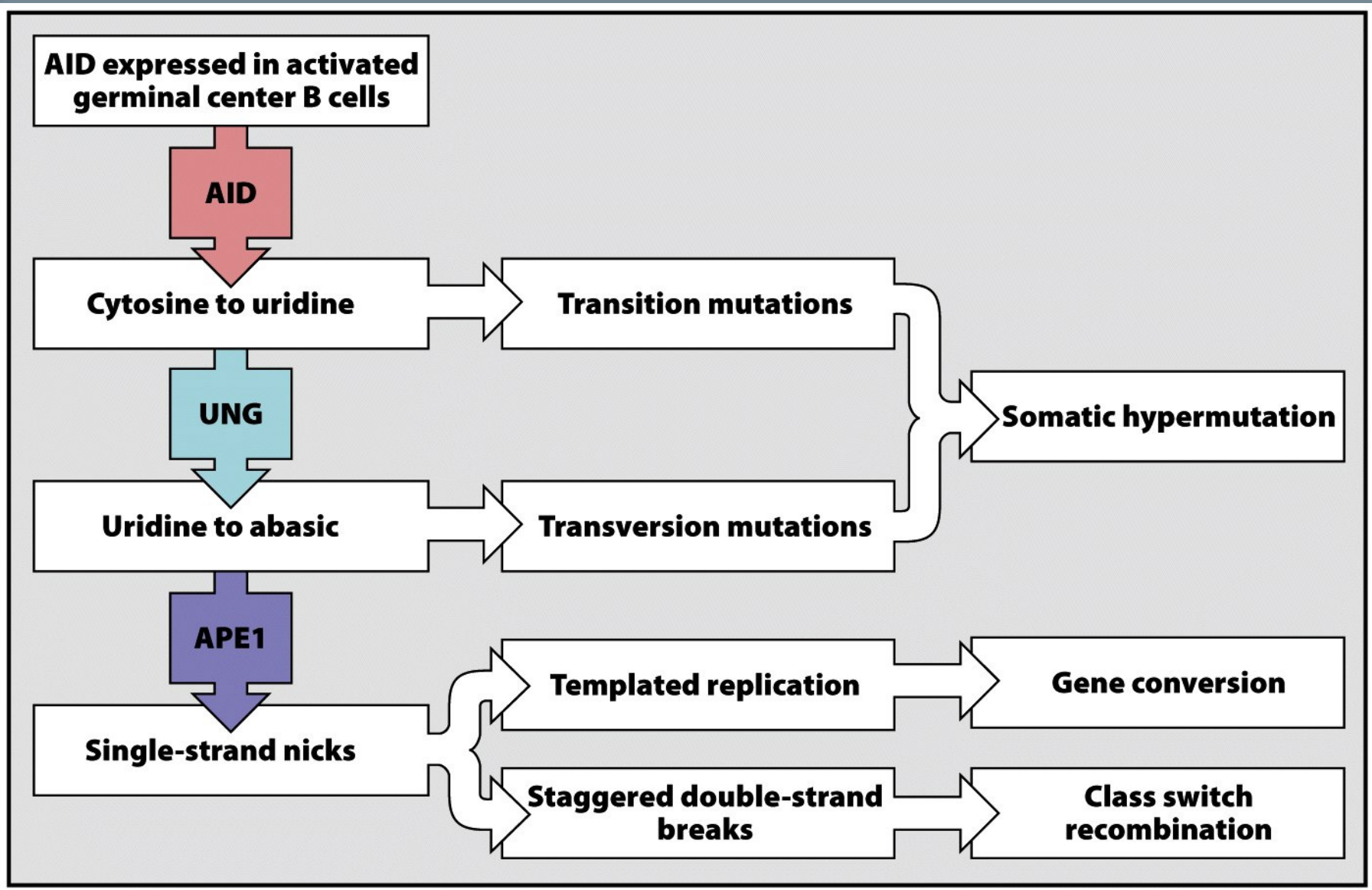


Figure 4-24 Immunobiology, 7ed. (© Garland Science 2008)

Event	Process	Nature of change	Process occurs in:	
			B cells	T cells
V-region assembly	Somatic recombination of DNA	Irreversible	Yes	Yes
Junctional diversity	Imprecise joining, N-sequence insertion in DNA	Irreversible	Yes	Yes
Transcriptional activation	Activation of promoter by proximity to the enhancer	Irreversible but regulated	Yes	Yes
Switch recombination	Somatic recombination of DNA	Irreversible	Yes	No
Somatic hypermutation	DNA point mutation	Irreversible	Yes	No
IgM, IgD expression on surface	Differential splicing of RNA	Reversible, regulated	Yes	No
Membrane vs secreted form	Differential splicing of RNA	Reversible, regulated	Yes	No

Figure 4-28 Immunobiology, 7ed. (© Garland Science 2008)

Maduración de la afinidad de las inmunoglobulinas

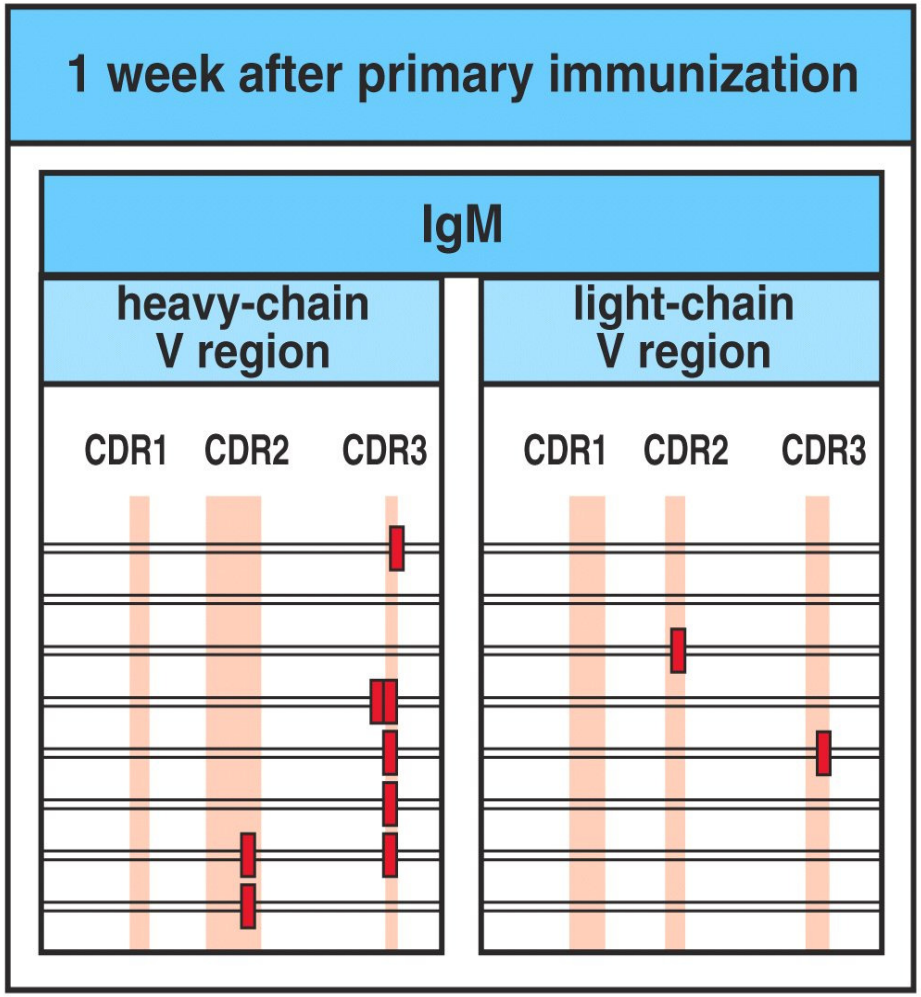


Figure 2-25 part 1 of 2 The Immune System, 2/e (© Garland Science 2005)

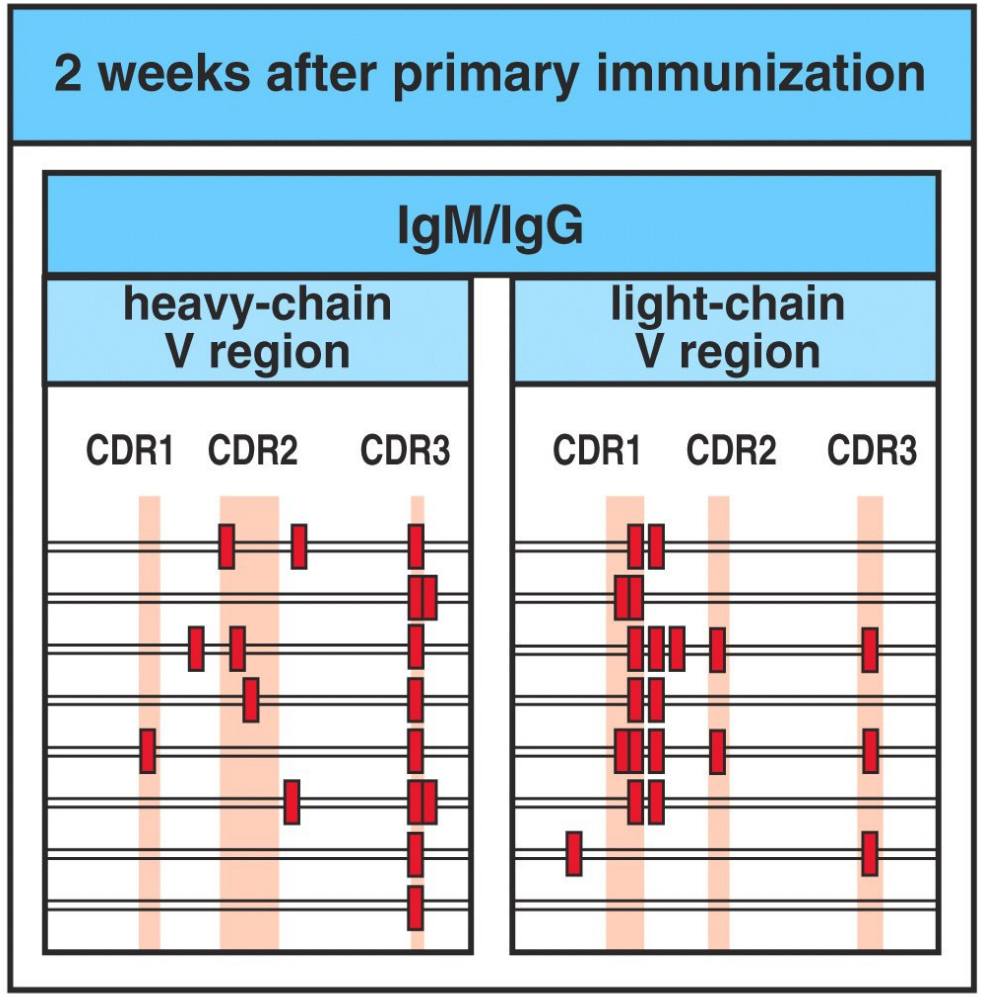
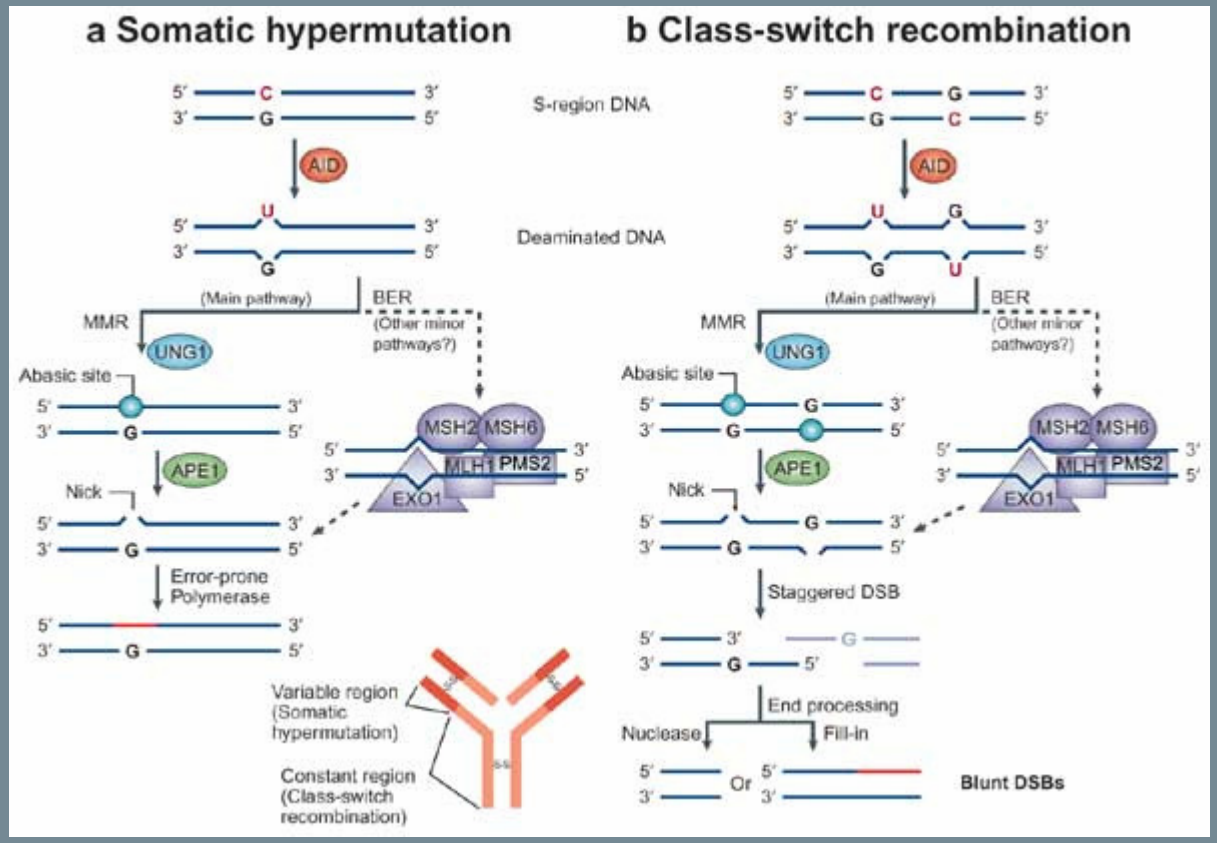
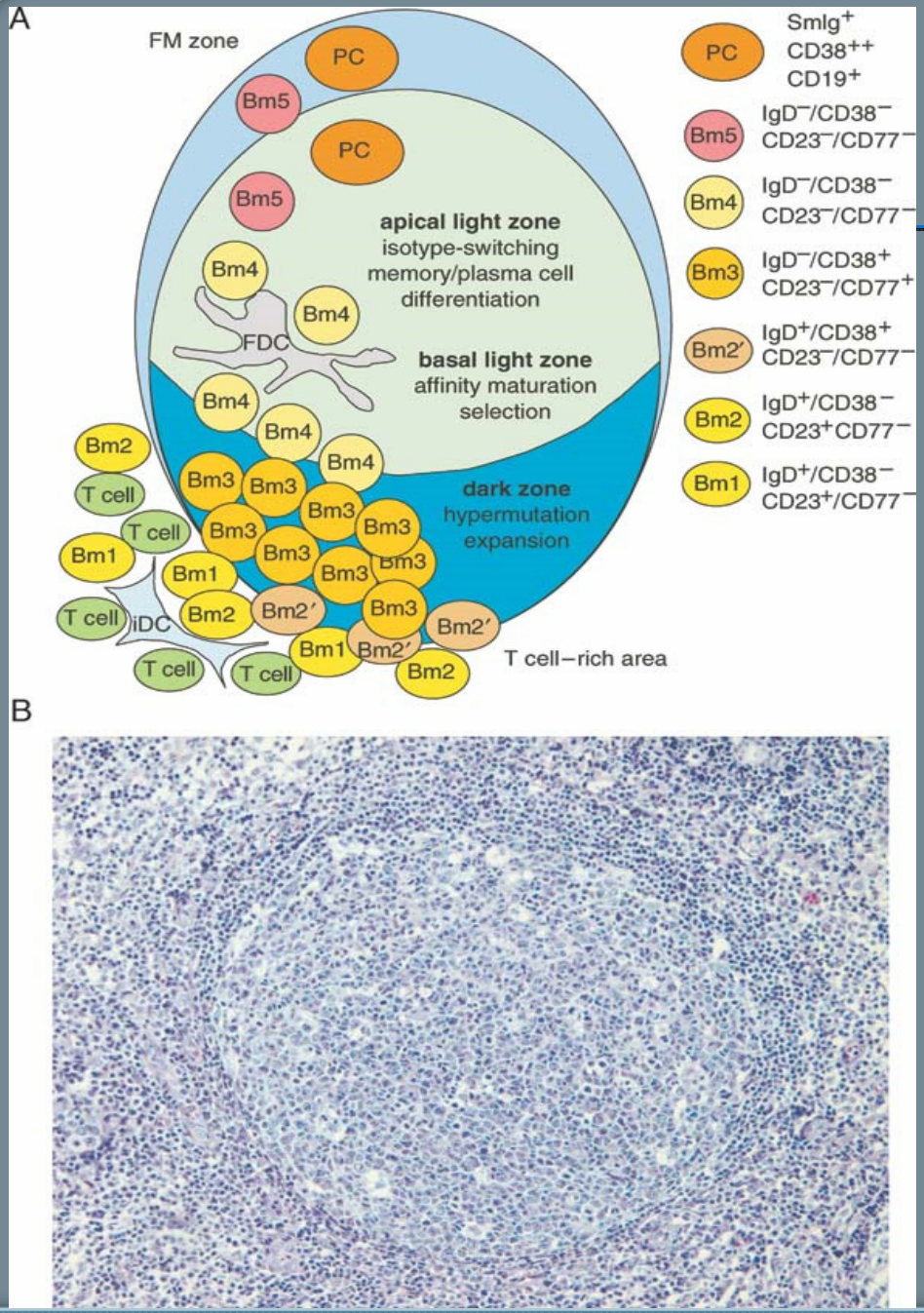


Figure 2-25 part 2 of 2 The Immune System, 2/e (© Garland Science 2005)



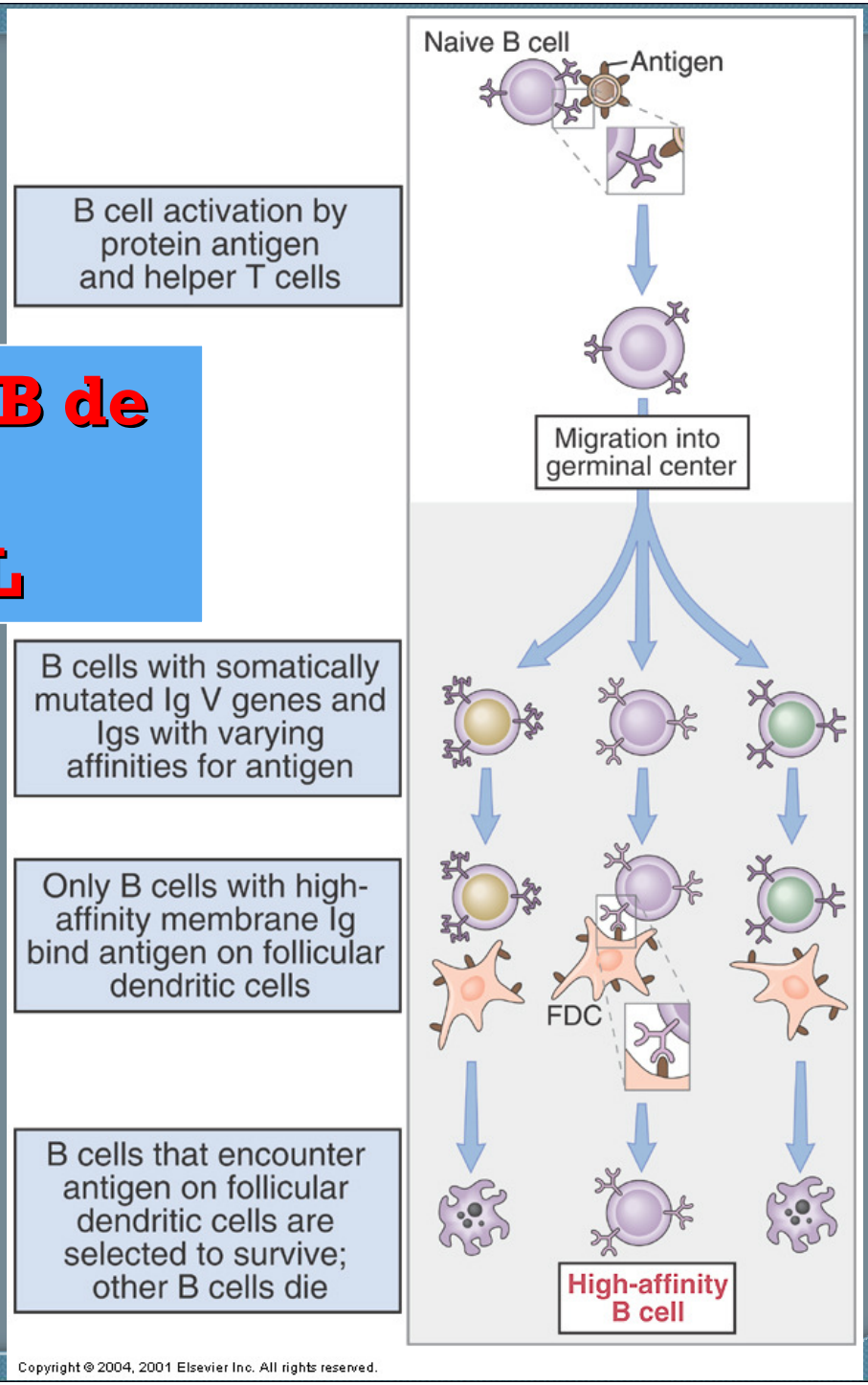
Nature Medicine **10**, 1304 - 1305 (2004)

Activation-induced cytidine deaminase (AID) deaminates cytidine residues in DNA, converting them to uridine residues. The U:G mismatch can then be processed by either uracil DNA glycosylase (UNG), a component of the base excision repair pathway, or the mismatch-repair machinery (MSH1, MSH6, EXO1, MLH1 and PMS)—resulting in gaps or nicks in DNA. (**a**) During somatic hypermutation the U:G mismatch can simply be replicated to produce a C to T mutation. Alternatively processing the nick by UNG and the mismatch repair machinery can produce an abasic site that will produce a C to A or C or T change; alternatively, a gap can be filled in by error-prone polymerases to produce mutations in nucleotides other than the targeted C. (**b**) During class-switch recombination the nicks induced by the BER pathway are thought to be generated by the following process: UNG removes the AID-introduced deoxyuridine in S-region DNA, creating an abasic site that is processed by the apurine/apyrimidine endonuclease 1 (APE1), which creates the nick. Processing of the staggered ends by unknown exonucleases or by error-prone end-filling reactions can lead to blunt double-stranded breaks that can be ligated to similarly created breaks on downstream S-region DNA to complete class-switch recombination.



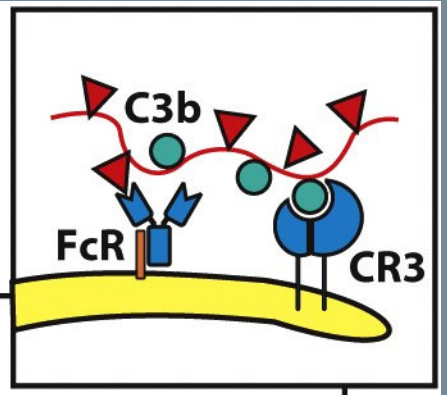
Los centrocitos expresan en su membrana Ig mutadas

Selección de células B de alta afinidad en el CENTRO GERMINAL



El **centrocito** puede unirse directamente con un antígeno que se encuentre en forma soluble o bien deberá "arrancárselo" a una CDF para endocitarlo y presentarlo al Th.
Su supervivencia está favorecida por **el aumento de expresión de moléculas anti-apoptóticas** como Bcl-xL y cFLIPL (acompleja Fas e inhibe apoptosis).
El entrecruzamiento de CD40 induce expresión de cFLIPL y preserva aquellas células rescatadas por su alta afinidad con el antígeno….

CÉLULAS DENDRÍTICAS FOLICULARES



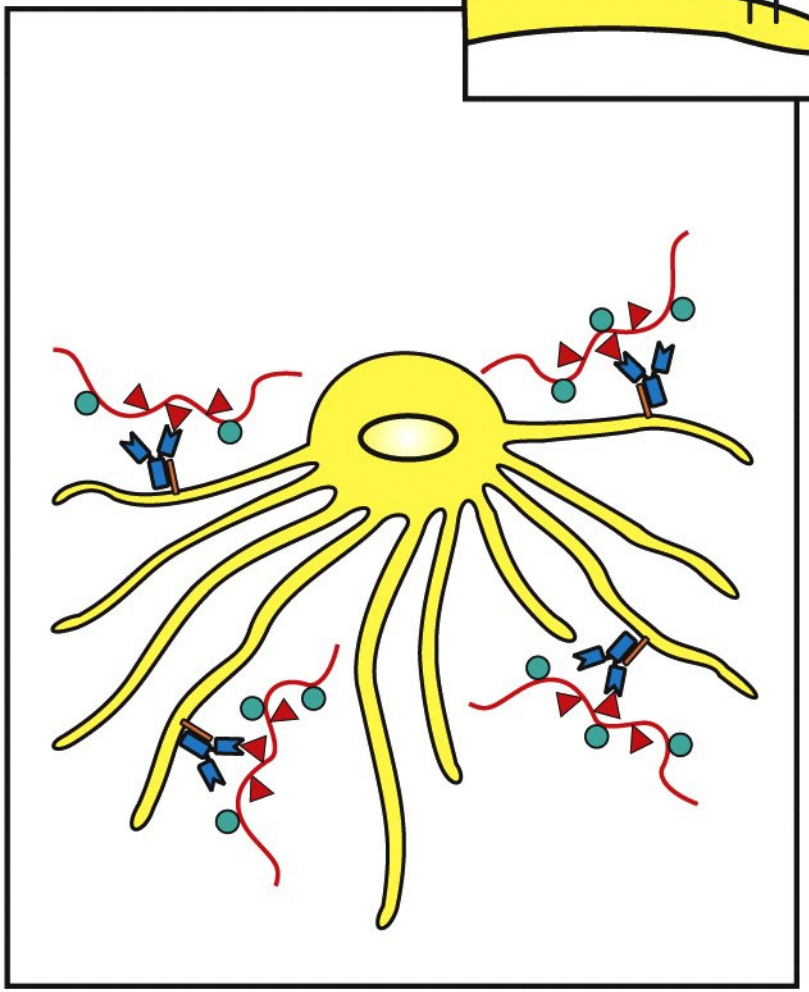
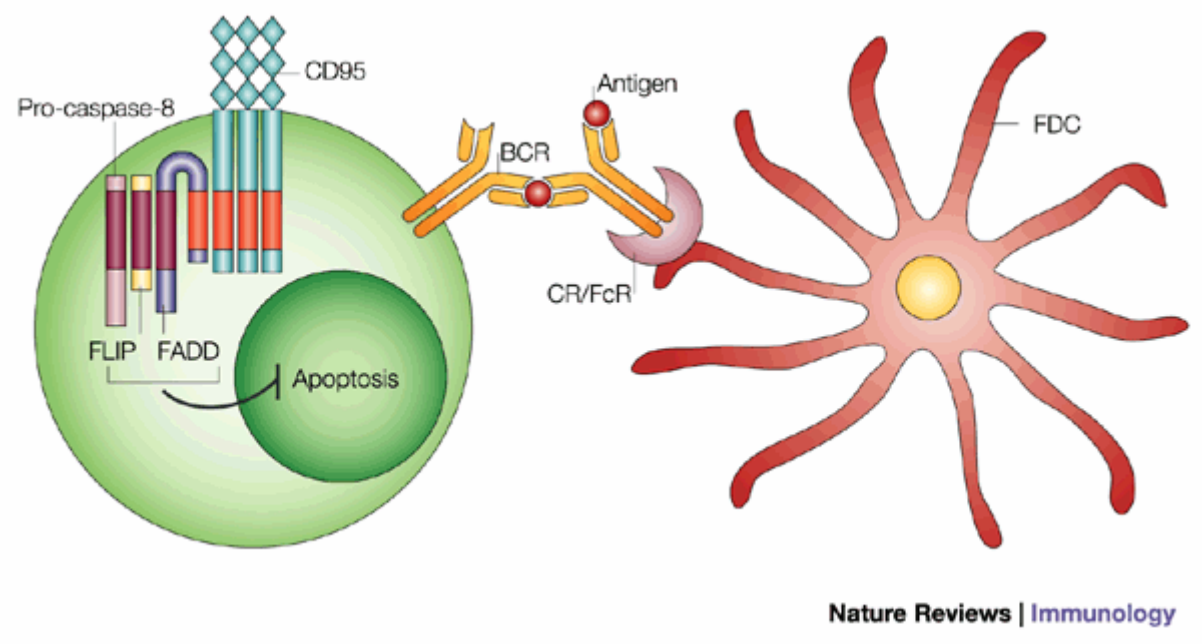


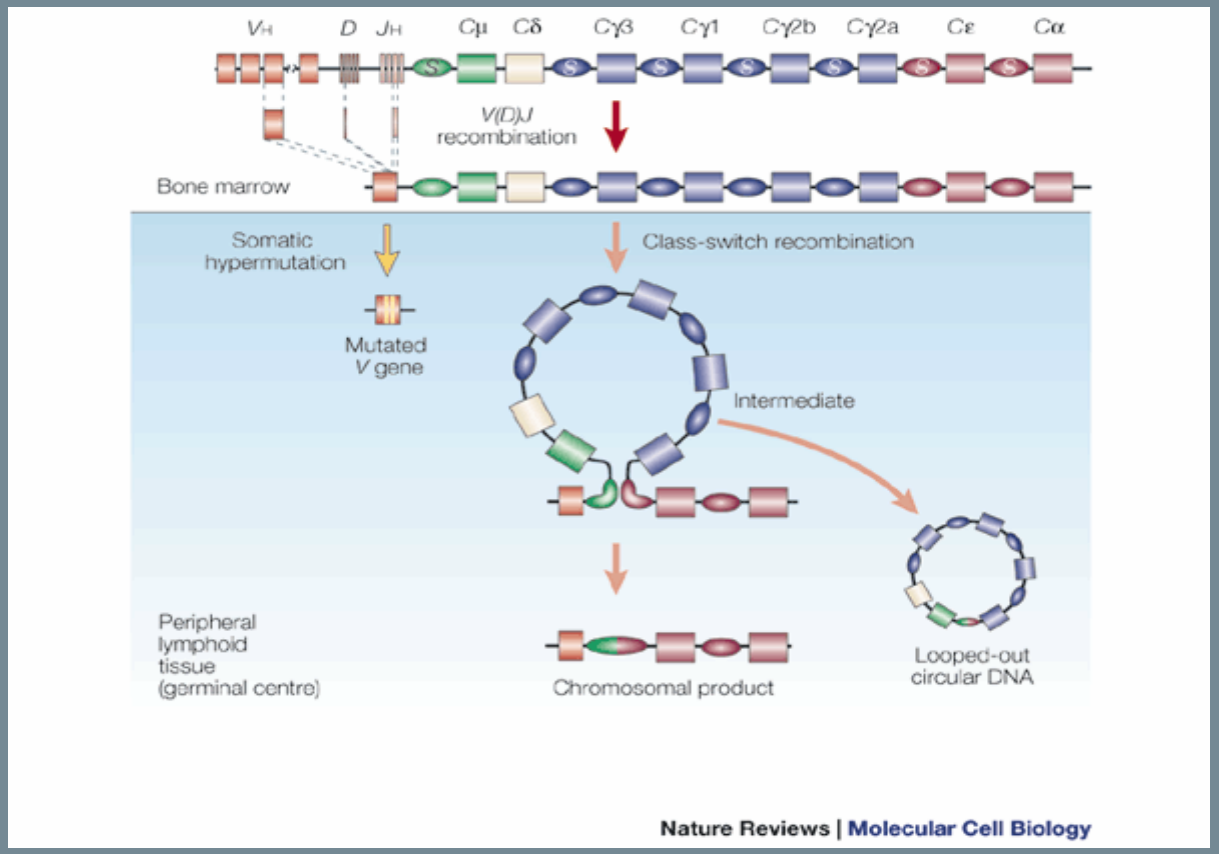
Figure 9-14 Immunobiology, 7ed. (© Garland Science 2008)



Appropriate crosslinking of the newly created B-cell receptor (BCR) by follicular dendritic cell (FDC)-associated immune complexes provides a mechanism to avert apoptosis by maintaining FLIP (FAS-associated death domain (FADD) protein-like interleukin-1 β converting enzyme inhibitory protein) in the cytoplasm of germinal-centre B cells. CR, complement receptor; FcR, Fc receptor

En el centro germinal se produce, además de la MADURACIÓN DE LA AFINIDAD DE LOS ANTICUERPOS, el SWITCH DE ISOTIPO DE INMUNOGLOBULINAS…..QUE DEPENDE DE…

- **INTERACCIÓN CD40 (LB)–CD40L ($TCD4^+$)**
- **CITOCINAS LIBERADAS POR LAS Th**


VH
D
JH
Cμ
Cδ
Cγ3
Cγ1
Cγ2b
Cγ2a
Cε
Cα
V(D)J recombination
Bone marrow
Somatic hypermutation
Class-switch recombination
Mutated V gene
Intermediate
Peripheral lymphoid tissue (germinal centre)
Chromosomal product
Looped-out circular DNA
Nature Reviews | Molecular Cell Biology

Cambio de isotipo

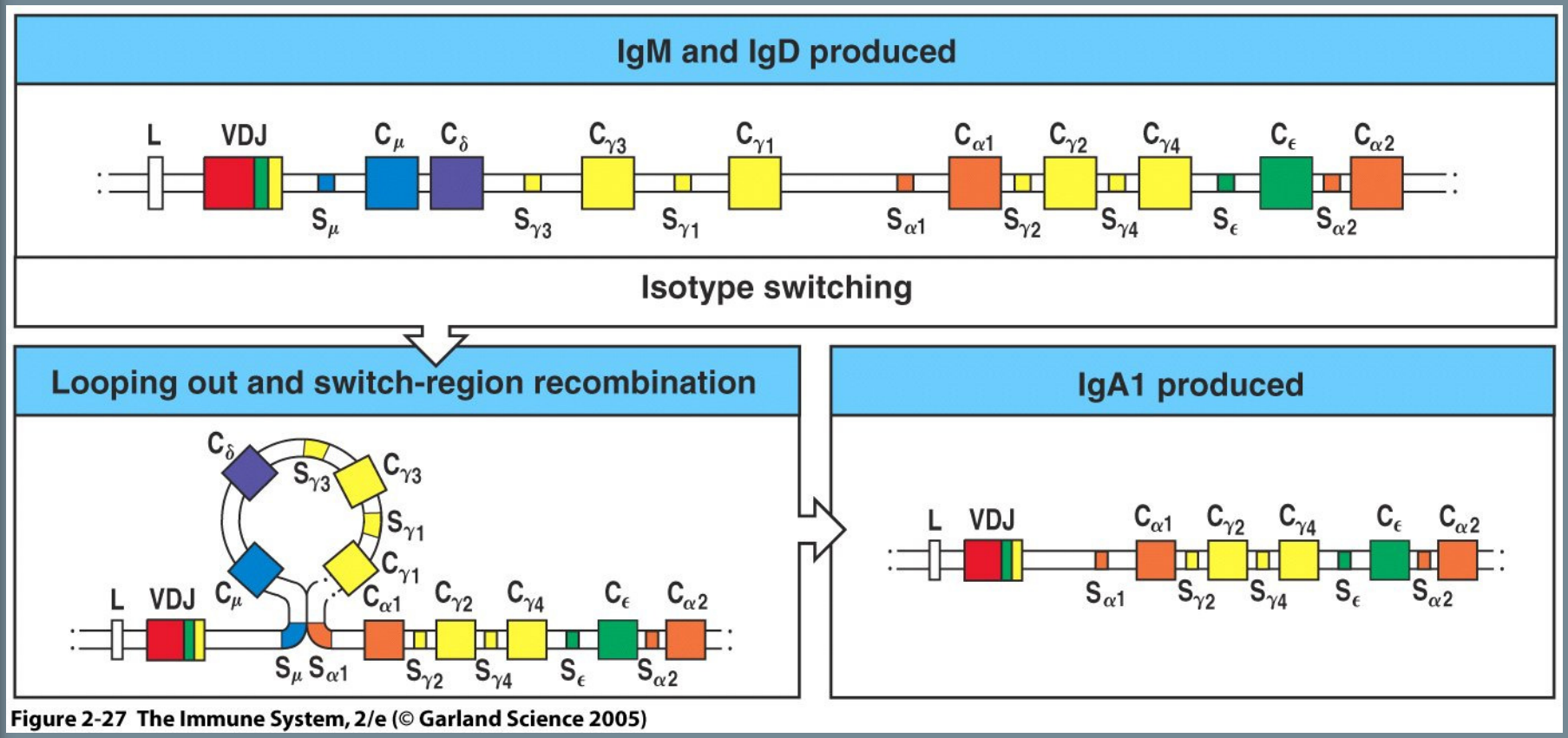


Figure 2-27 The Immune System, 2/e (© Garland Science 2005)

El switch involucra recombinación somática

- **Irreversible**
- **Usualmente con maduración de la afinidad**

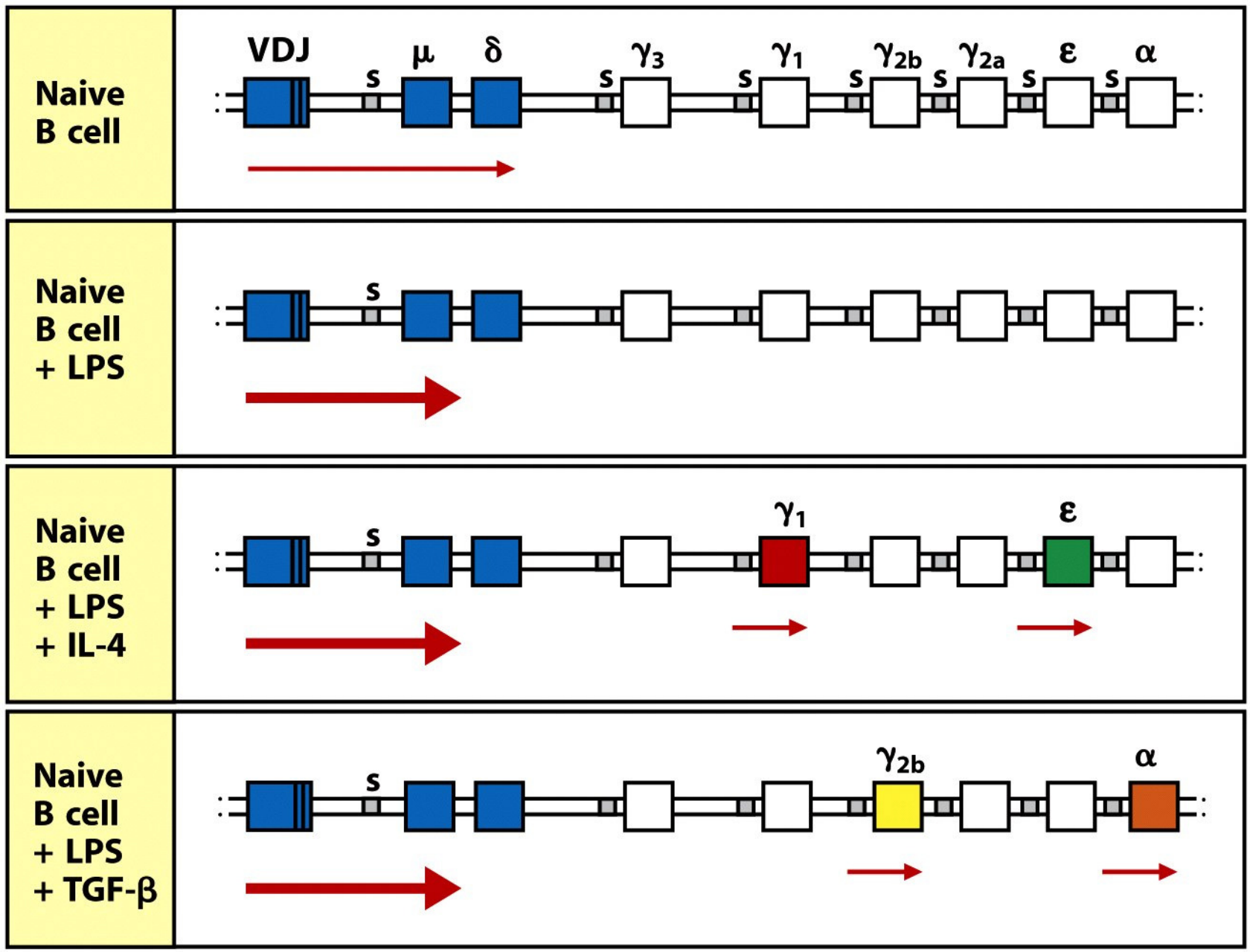


Figure 9-12 Immunobiology, 7ed. (© Garland Science 2008)

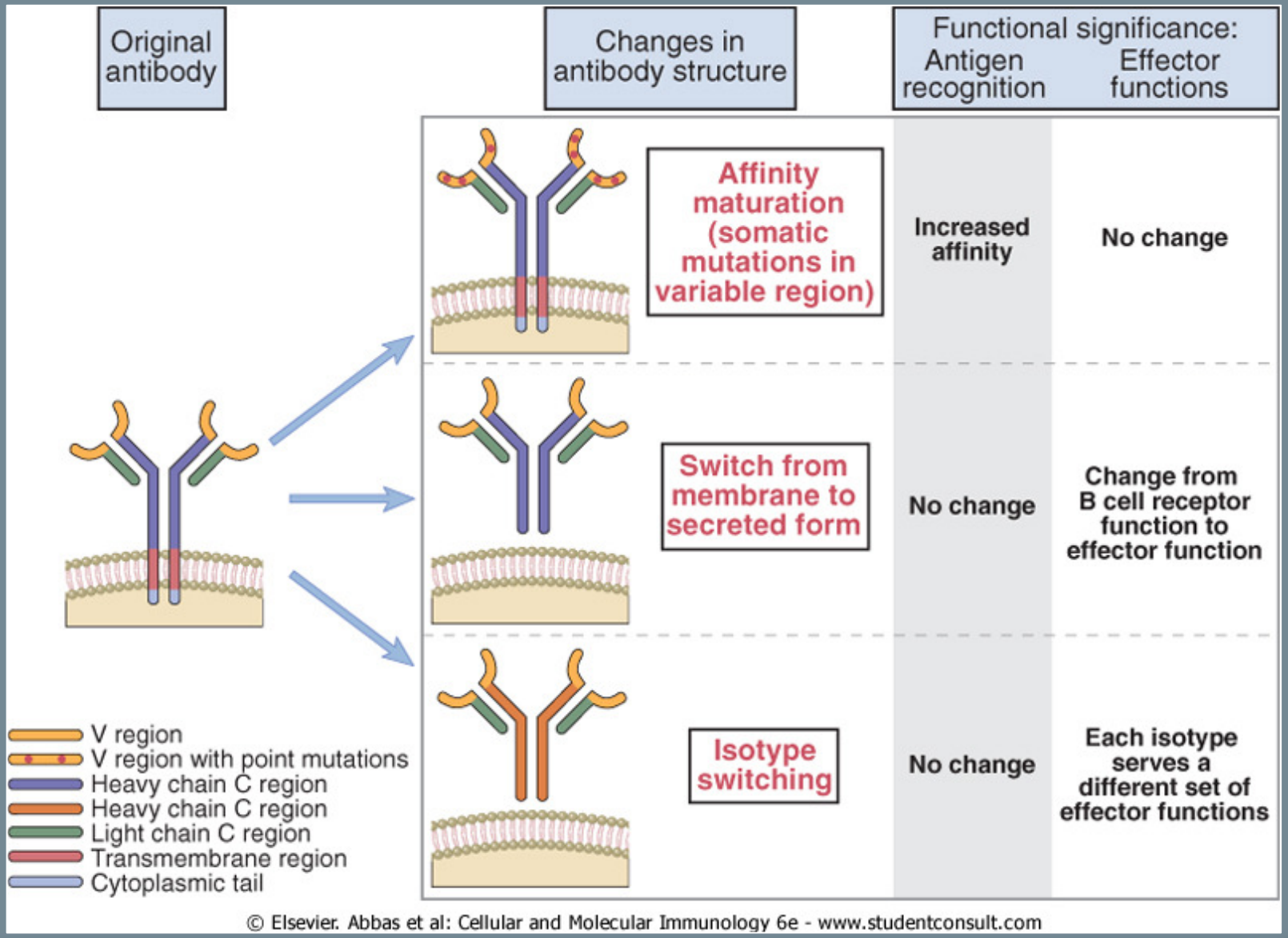
	Immunoglobulin class or subclass								
	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgE
Heavy chain	μ	δ	γ_1	γ_2	γ_3	γ_4	α_1	α_2	ϵ
Molecular weight (kDa)	970	184	146	146	165	146	160	160	188
Serum level (mean adult mg ml^{-1})	1.5	0.03	9	3	1	0.5	2.0	0.5	$5x10^{-5}$
Half-life in serum (days)	10	3	21	20	7	21	6	6	2

Figure 2-28 The Immune System, 2/e (© Garland Science 2005)

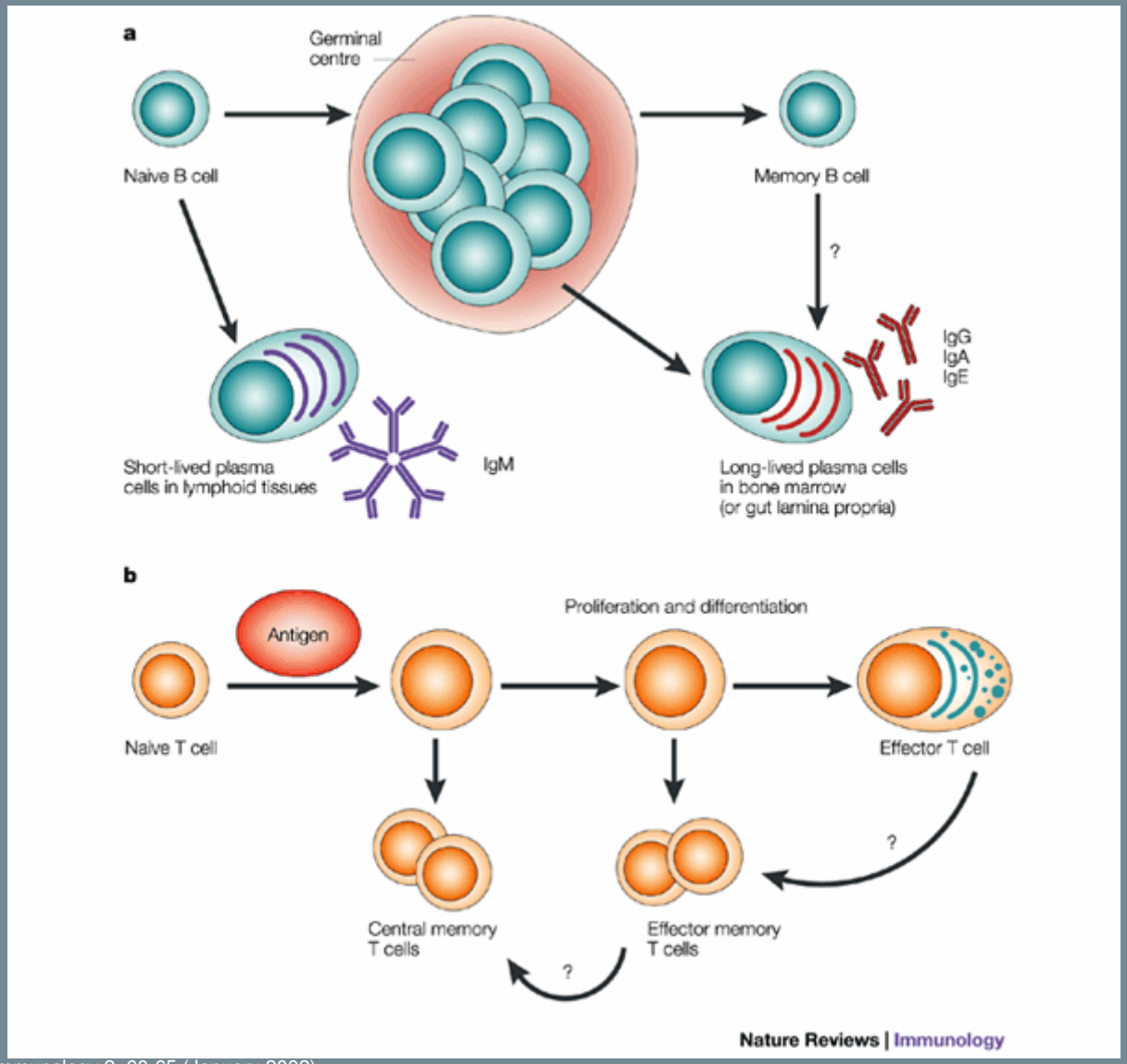
Function	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	–	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activation of complement system	+++	–	++	+	+++	–	+	–

Figure 2-29 part 1 of 2 The Immune System, 2/e (© Garland Science 2005)

Cambios en la estructura de la inmunoglobulina

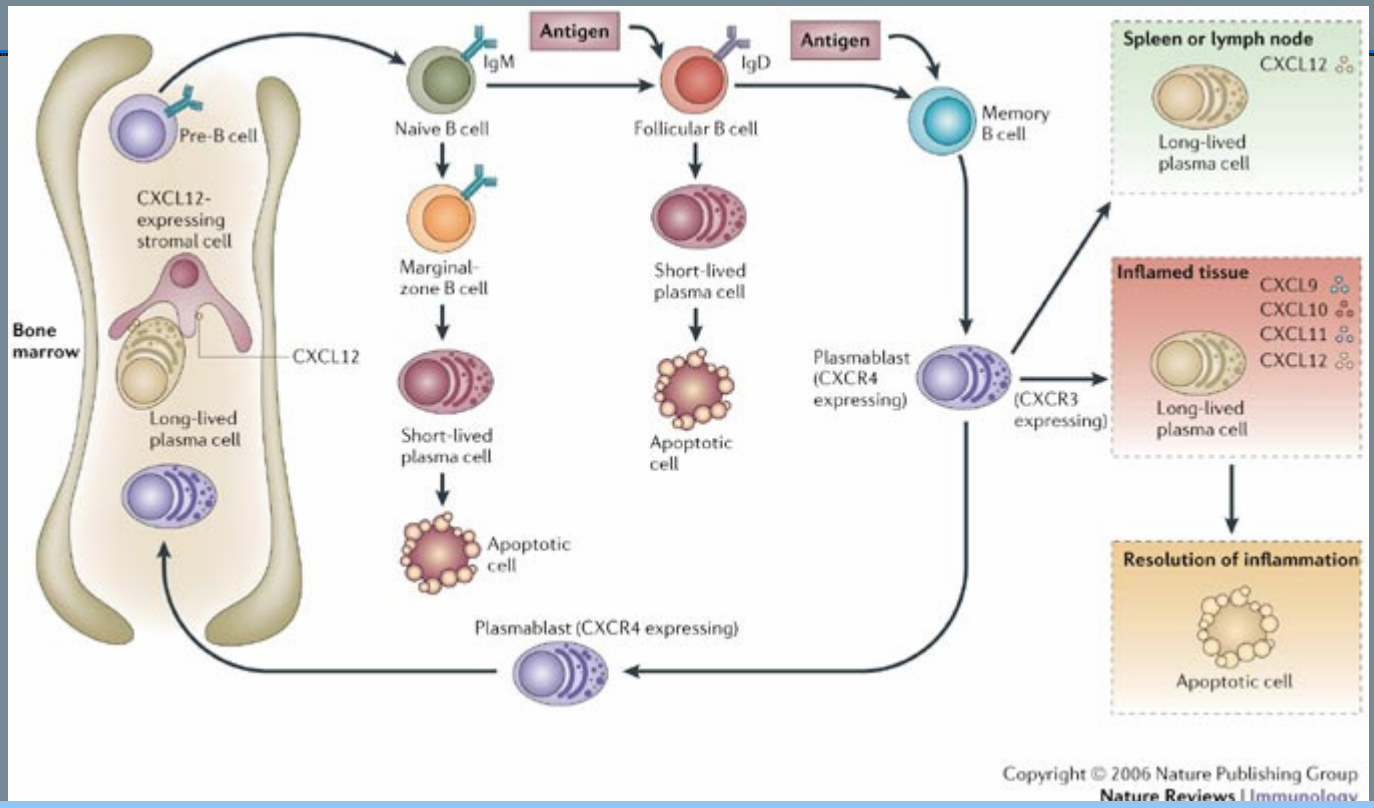


Los centrocitos que han sobrevido al proceso de selección dan lugar a los PLASMOBLASTOS que abandonan el centro germinal para generar CÉLULAS PLASMÁTICAS productoras de anticuerpos de alta afinidad y LINFOCITOS B DE MEMORIA.



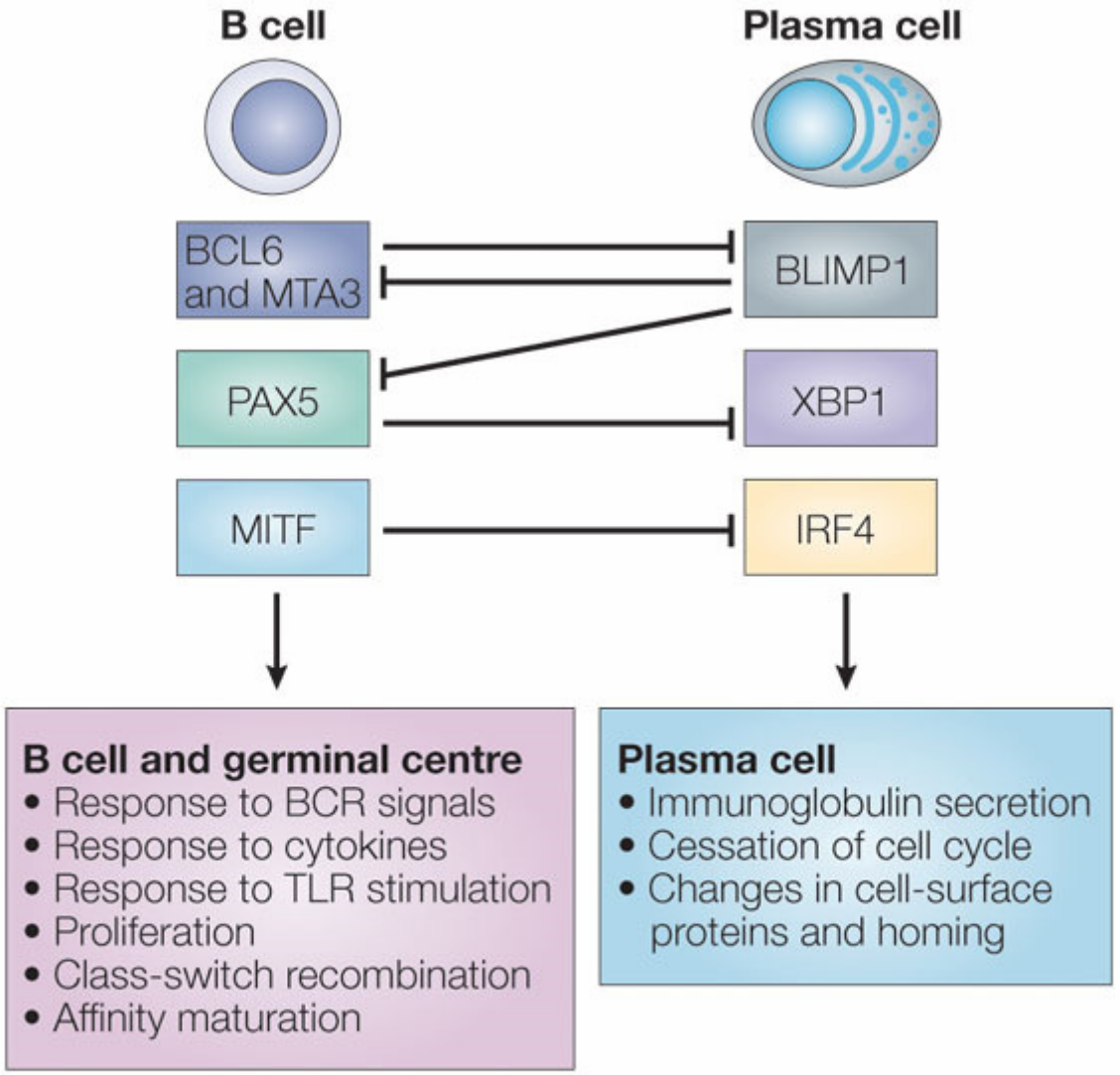
Nature Reviews Immunology 2, 60-65 (January 2002)

Ontogenia de la CÉLULA PLASMÁTICA

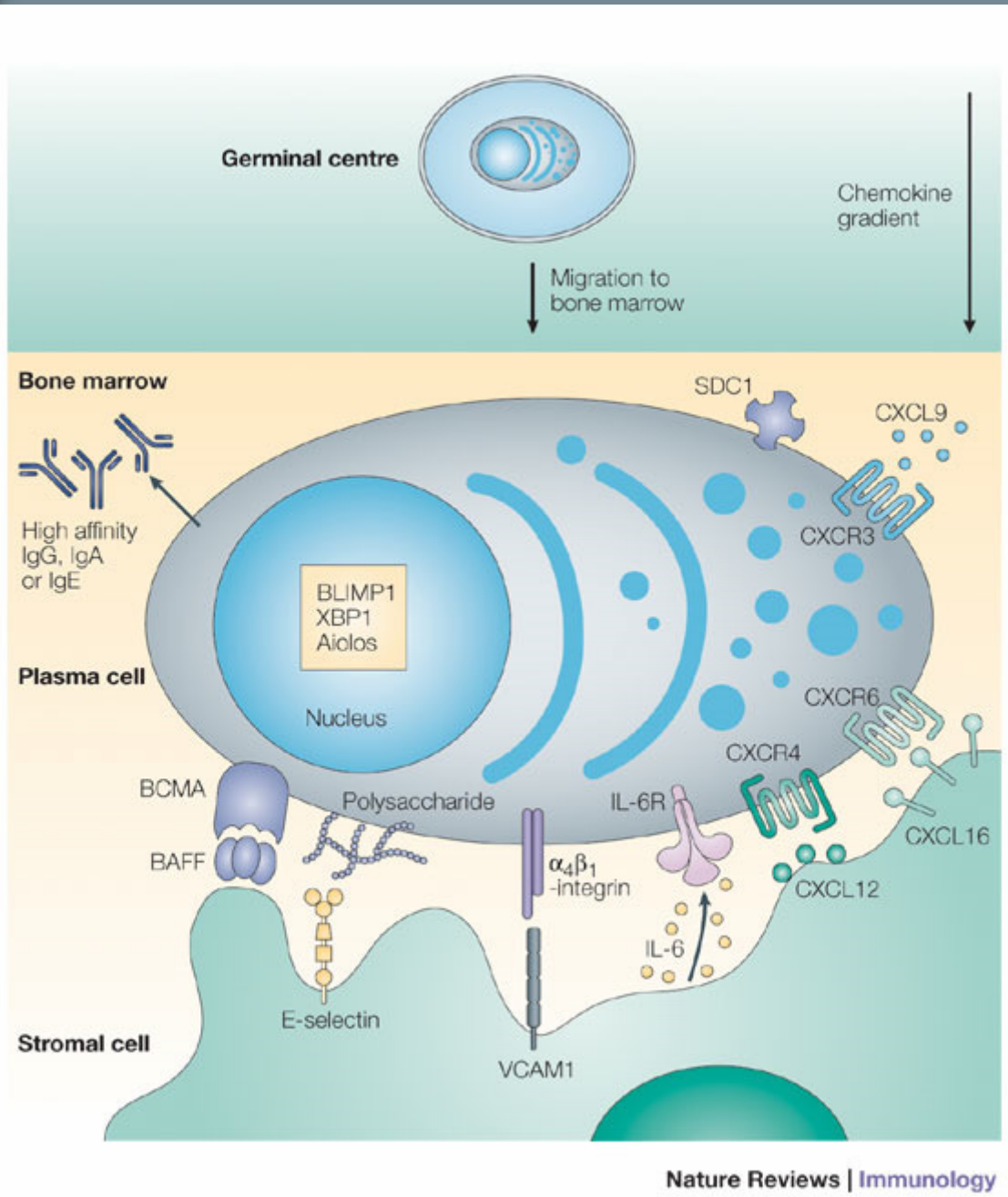


When memory B cells are activated by antigen in a T-cell-dependent manner, either they proliferate or they differentiate into plasmablasts, which secrete antibody, are migratory, and also proliferate. Plasmablasts then leave the secondary lymphoid organ (the spleen or the lymph node) and express the chemokine receptors CXC-chemokine receptor 3 (CXCR3), when activated in the presence of interferon- , and CXCR4. The chemokine that is bound by CXCR4 is CXC-chemokine ligand 12 (CXCL12), which is probably also an integral component of plasma-cell survival niches: so plasmablasts are attracted by plasma-cell survival niches. Plasma cells that reside in these survival niches are not migratory. Mobile plasmablasts that dislocate a resident plasma cell therefore have a competitive advantage. After an immobile plasma cell has been dislocated from its niche, it cannot return to a survival niche, and it dies. The successful plasmablast differentiates into a plasma cell and loses its migratory potential. This direct competition ensures that only as many plasma cells of 'old' specificities (that is, for antigens that were previously encountered) are removed from the bone marrow as plasmablasts with the 'new' specificity are recruited to the pool of memory plasma cells.

Nature Reviews Immunology 6, 741-750 (October 2006)



- Several transcription factors — BCL-6 (B-cell lymphoma 6), MTA3 (metastasis-associated 1 family, member 3), MITF (microphthalmia-associated transcription factor) and PAX5 (paired box protein 5) — repress plasmacytic development by repressing BLIMP1 (B-lymphocyte-induced maturation protein 1), XBP1 (X-box-binding protein 1) and IRF4 (interferon-regulatory factor 4). In plasma cells, BLIMP1 represses B-cell gene-expression programmes. This mutual repression prevents the unelicited formation of plasma cells in the germinal centre and prevents the reversion of plasma cells to a B-cell stage. BCR, B-cell receptor; TLR, Toll-like receptor. BCL-6, MTA3, PAX5 and MITF also regulate the expression of genes that are required for B-cell and germinal-centre functions, which are outlined in the pink box. BLIMP1, XBP1 and IRF4 induce the expression of genes that are required for plasma cells, which are outlined in the blue box.



Post-germinal-centre plasma cells, which express somatically mutated, class-switched immunoglobulin, lose expression of CXC-chemokine receptor 5 (CXCR5), facilitating their exit from the germinal centre. These cells then increase their expression of CXCR4, which helps them to home to the bone marrow, where stromal cells produce high amounts of CXC-chemokine ligand 12 (CXCL12). Endothelial-cell selectin (E-selectin) and vascular cell-adhesion molecule 1 (VCAM1) expressed at the surface of bone-marrow stromal cells are important for the retention of plasma cells in the bone marrow, through association with polysaccharides and integrins expressed at the surface of the plasma cells. Plasma cells induce the stromal cells to produce interleukin-6 (IL-6). B-cell-activating factor (BAFF), probably produced by macrophages or dendritic cells, activates the receptor B-cell maturation antigen (BCMA) and, together with IL-6, provides crucial survival signals to the plasma cells. BLIMP1, B-lymphocyte-induced maturation protein 1; IL-6R, IL-6 receptor; SDC1, syndecan; XBP1, X-box-binding protein 1.

Gracias