Chapter 8

The Development and Survival of Lymphocytes
淋巴細胞的發育與存活

陳炳宏副教授

KMU生物科技學系

第一教學大樓N1020/1023 (分機: 2676)

bhchen@kmu.edu.tw

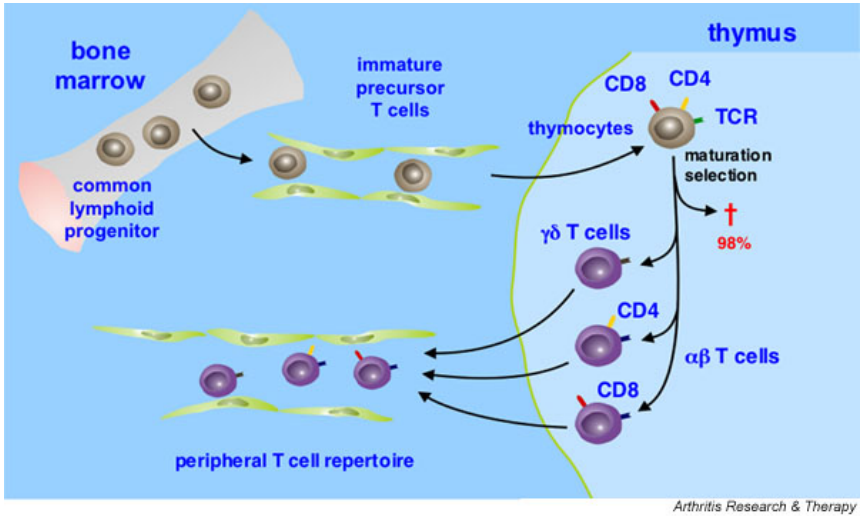
http://allergy.kmu.edu.tw

Learning objectives

- How lymphocytes are generated and selected in bone marrow and thymus?
- How lymphocyte development is controlled?
- How anti-self lymphocytes are selected and rendered unresponsive towards self antigens?
- How properly selected lymphocytes survive and mature in peripheral lymphoid tissues?

Lymphopoiesis

- Production of new lymphocytes
- Places – central lymphoid tissues
  - B cells – liver (fetus), bone marrow (adults)
  - T cells – thymus
    - T precursors migrate here from bone marrow
    - Thymocytes → T cells that are located in the thymus



Most of this chapter is about the maturation/development of lymphocytes in the *absence* of foreign antigen (from pre-lymphocyte to mature naïve lymphocyte):

"productive"
= protein is made & functional

1. Gene rearrangements
2. Testing whether the rearrangements are "productive" and the receptor is functional (and replacing nonproductive rearrangements if possible)
3. Testing the specificity of the receptor to provide assurance that it is not anti-self (tolerance)
4. T cells need to be MHC-restricted (bind to self MHC) but NOT anti-self (bind to self molecules). This seems to be somewhat contradictory and introduces a technically difficult problem for T cells

Most lymphocyte die before solving all these problems

Development of B and T lymphocytes

1. During their development (either in BM or thymus), B & T cells **MUST** receive some sort of signal ("+" or "-") in order to continue on their development.
2. By default, developing lymphocytes that receive no signal will **DIE** before becoming mature!!!

The ***intensity*** of the signal determines the fate of developing lymphocytes

(+) selection → survival

- Weakly interacts with self-Ag will survive

(-) selection → death

- Strongly interacts with self-Ag will die

Development of All Blood Cells Arises from a Pluripotent Hematopoietic Stem Cell

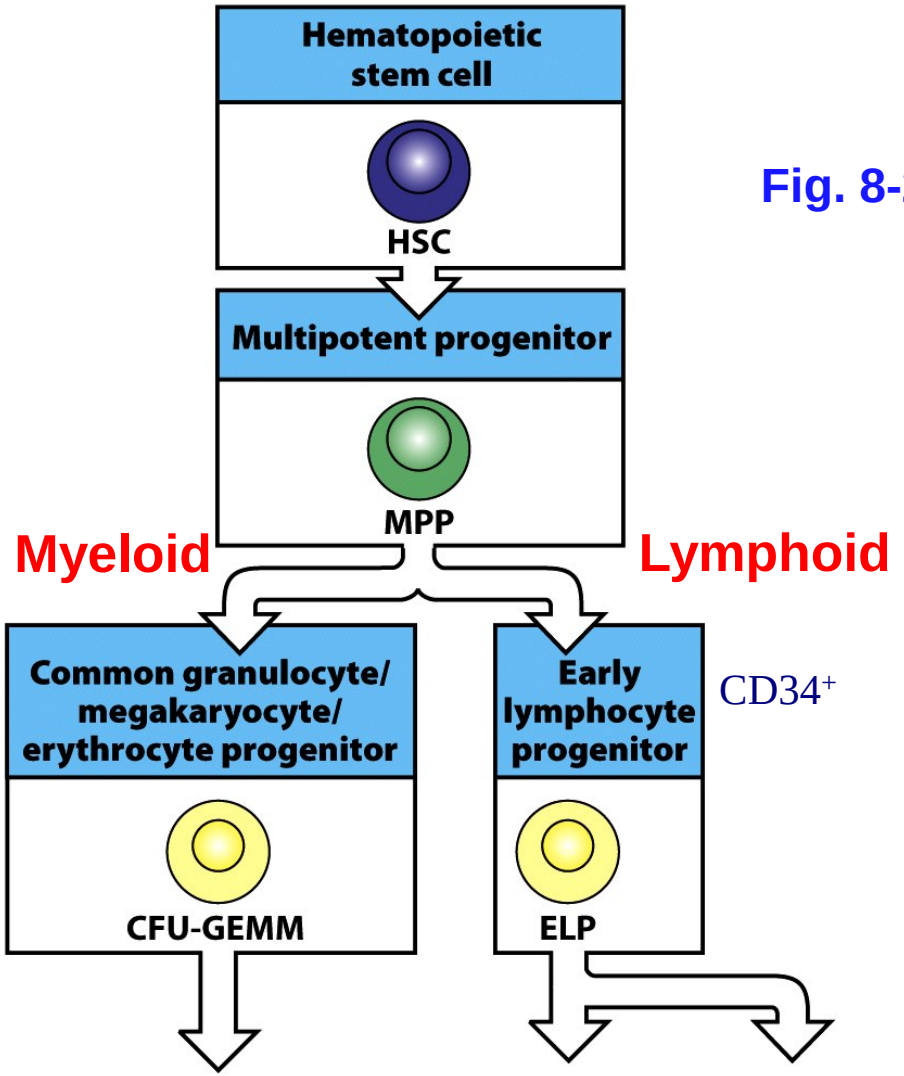


Fig. 8-2

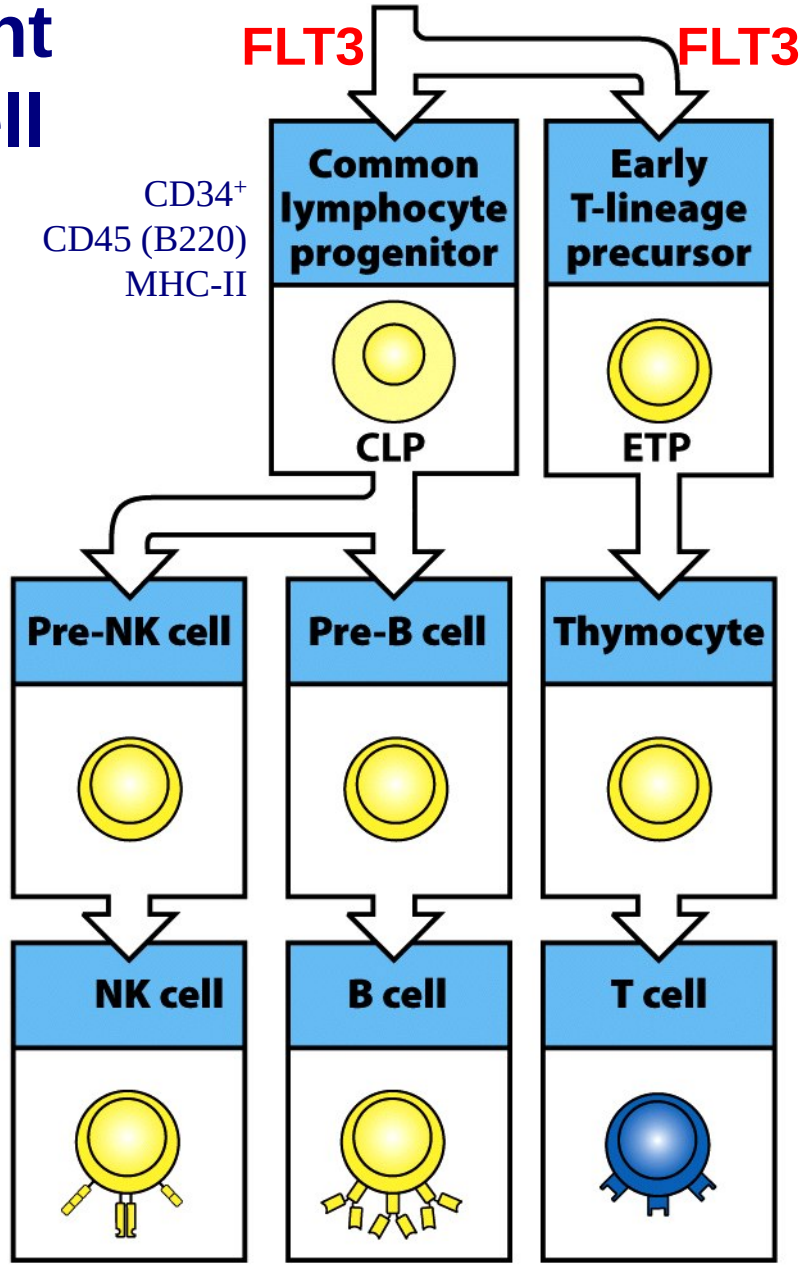


Figure 7-2 part 1 of 3 Immunobiology, 7ed. (© Garland Science 2008)

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Development of B lymphocytes

Fig. 8-1

"negative (-) selection"

Life of a B Cell

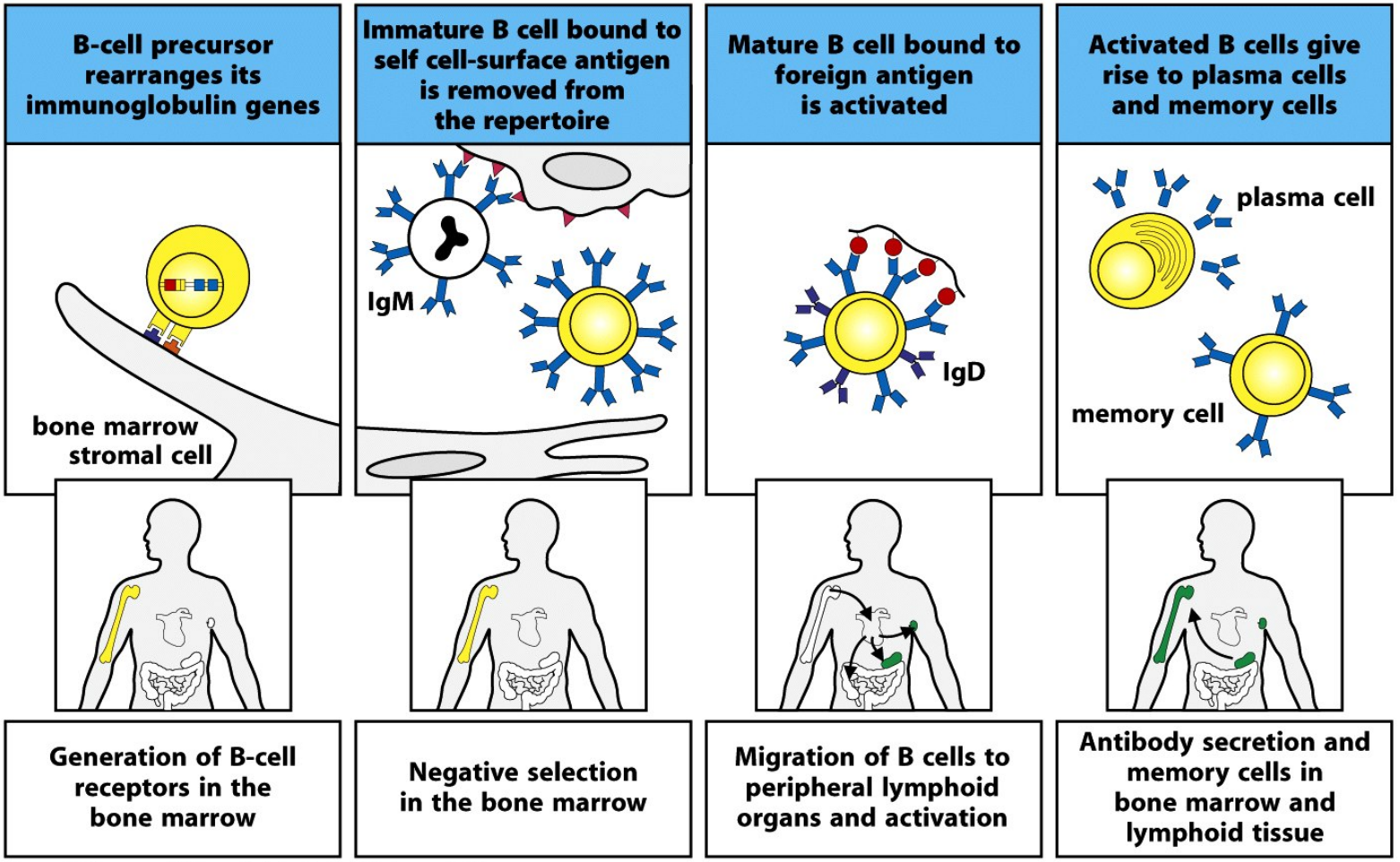


Figure 7-1 Immunobiology, 7ed. (© Garland Science 2008)

(1) In the BM, B cells rearrange their Ig genes **in the absence of antigen**.

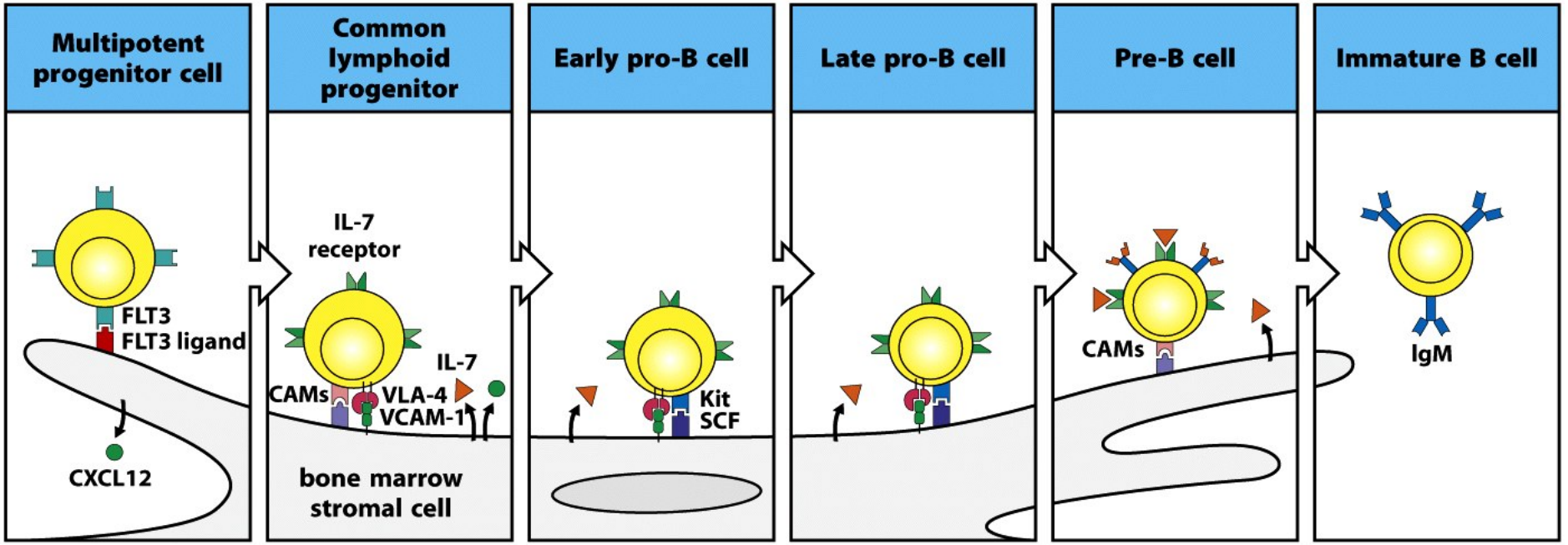
(2) Those B cells with a BCR that **bind antigen (self)** in the bone marrow ***die*** .

(3/4) B cells that do not bind antigen in the BM can leave (naïve). If they bind antigen in the periphery, they become mature and are activated (proliferate and differentiate).

(4) Activated B cells become plasma cells or memory cells.

B cell development is dependent on bone marrow stromal cells

Fig. 8-3



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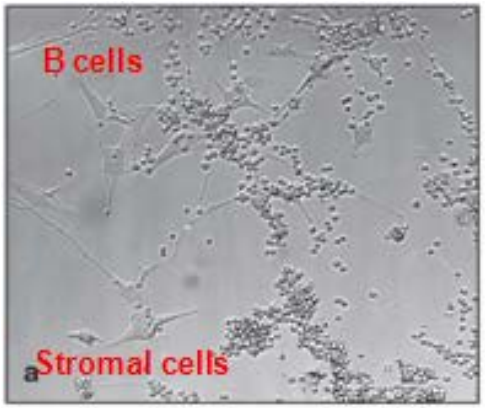
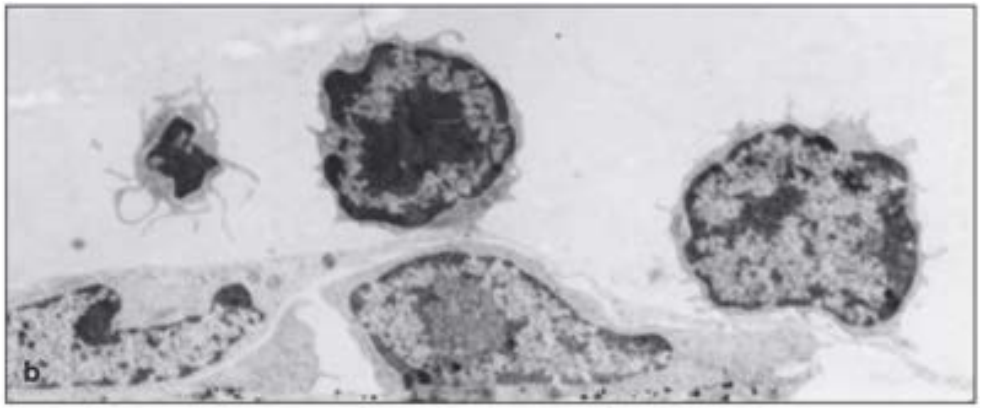


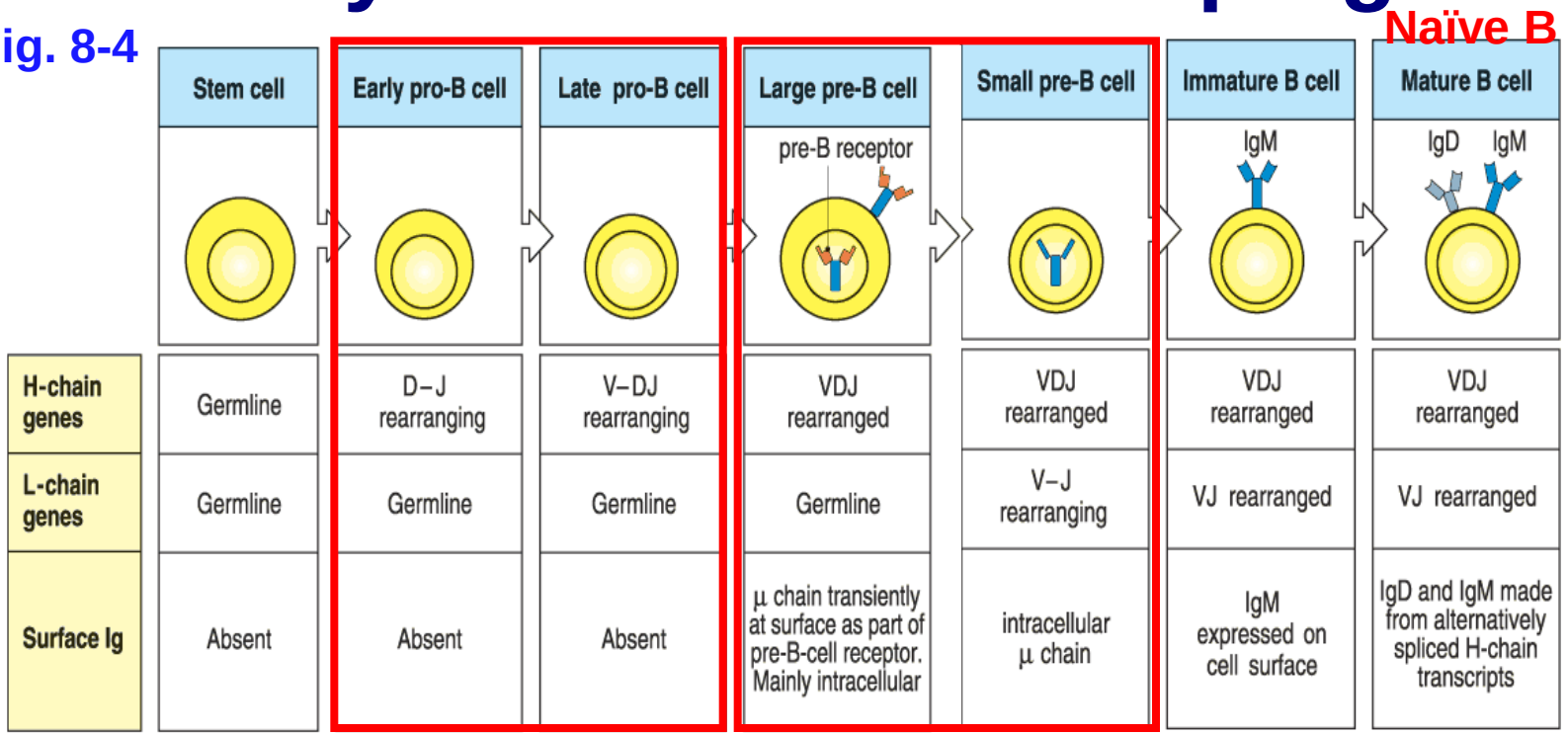


Determinants of B-cell Developmental Stages

- Expression of
  - Surface Ig chains (H-chain first, followed by L-chain)
  - Cell-surface proteins
    - Early: FLT3, Kit
    - Early to middle: IL-7 receptor (IL-7R)
    - Middle to late: CD19, BLNK, Igα, CD45 receptor (CD45-R; B220)
- 5 stages
  - Progenitor B (stem cell)
  - Pro-B (expresses IgαIgβ, CD19, CD40, CD45, MHC-II)
  - Pre-B (expresses pre-B receptor)
  - Immature B (expresses IgM)
  - Mature B (expresses IgM/D)

Ig gene rearrangements occur in an orderly fashion in developing B cells

Fig. 8-4



Pro-B: Heavy chain is being rearranged
Pre-B: Heavy chain is rearranged; Light chain is being rearranged
Immature B: Both heavy and light chained are rearranged (IgM expressed)
Mature B: Both IgM/IgD expressed

Cell surface proteins expression during B-cell development

Fig. 8-5

Genes for BCR heavy chain is the 1st to undergo rearrangement!!

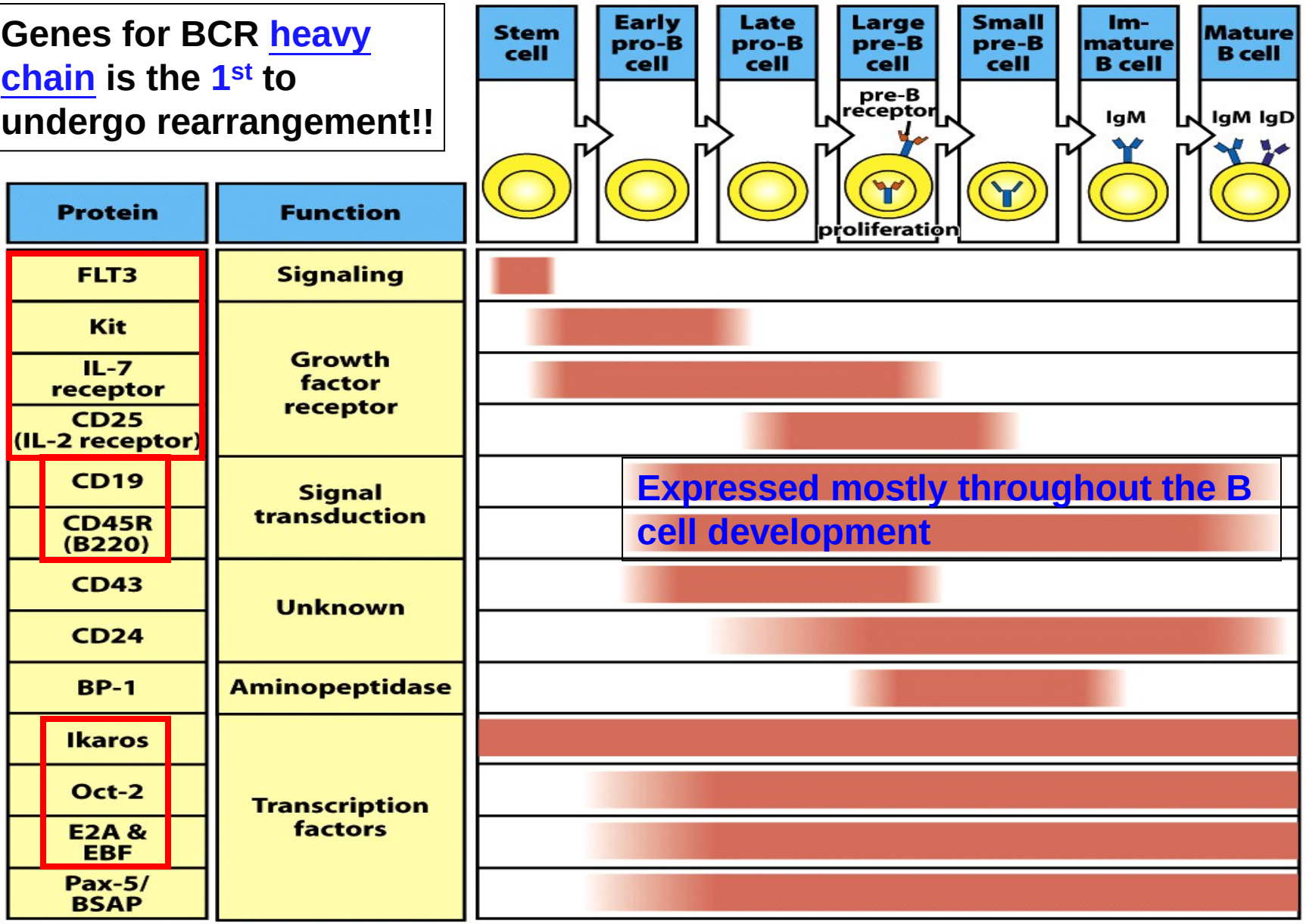


Figure 7-6 Immunobiology, 7ed. (© Garland Science 2008)

Productive rearrangement of Ig gene is followed by surface protein expression in developing B cells

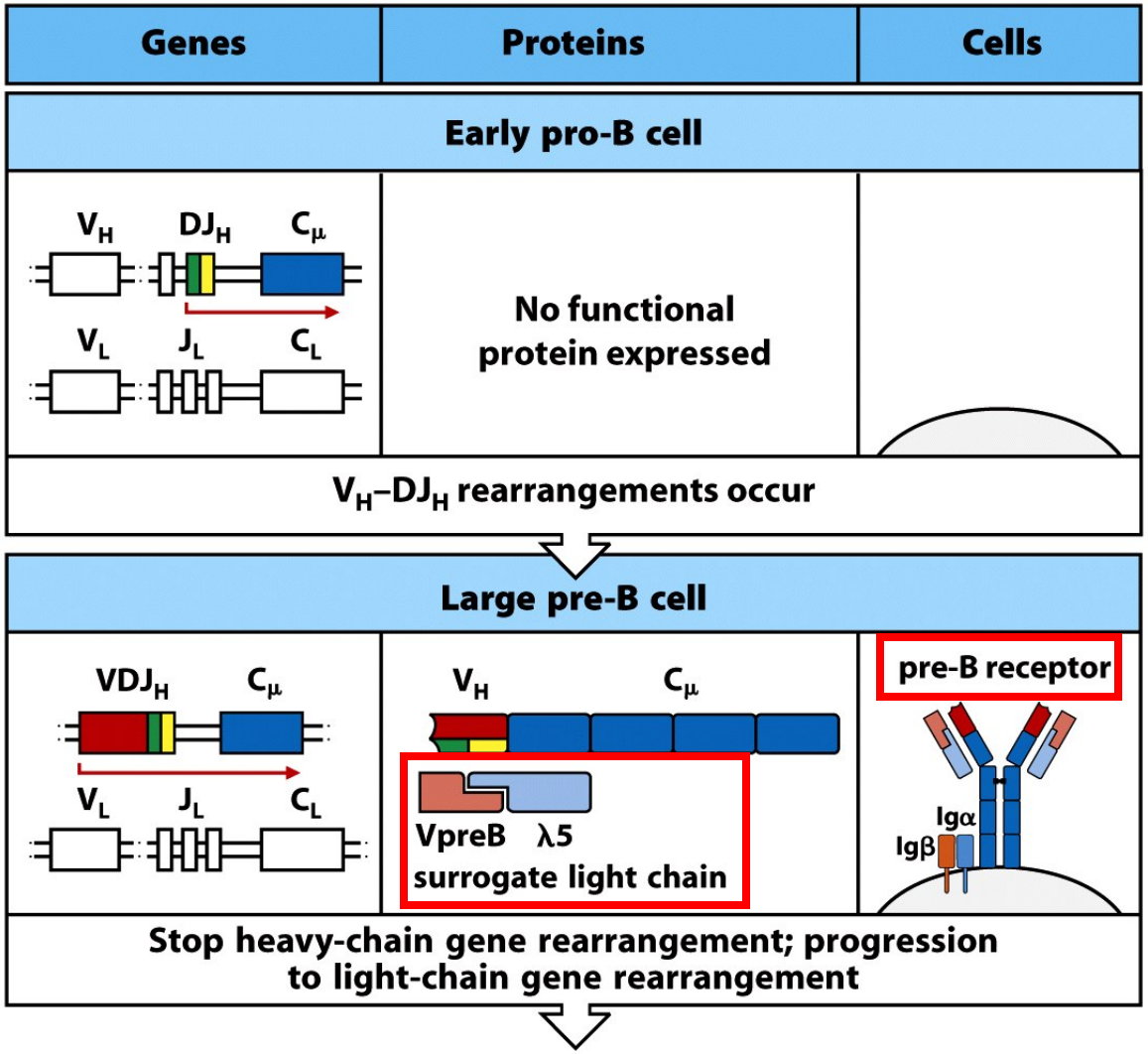


Figure 7-7 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

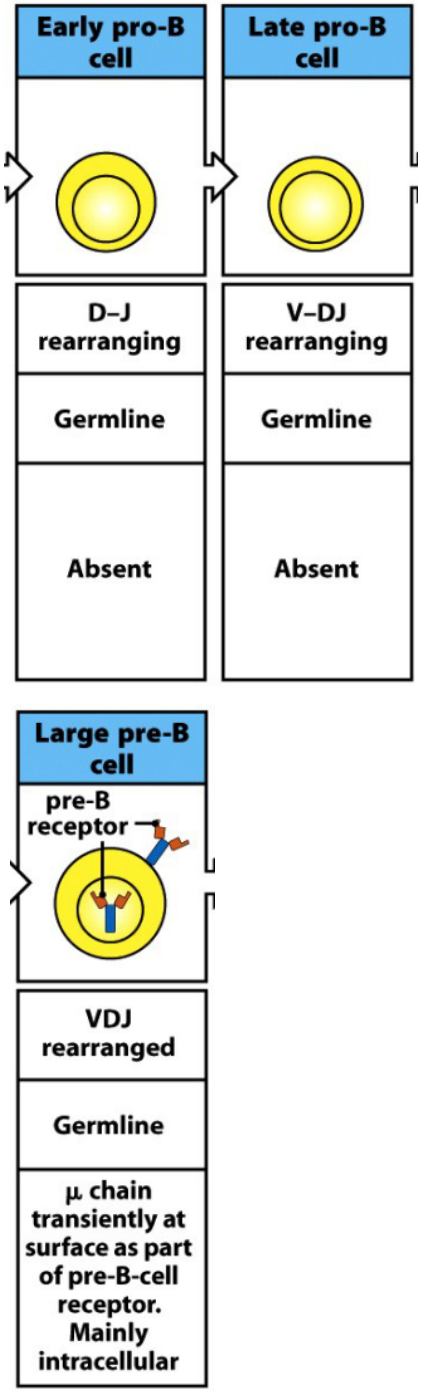
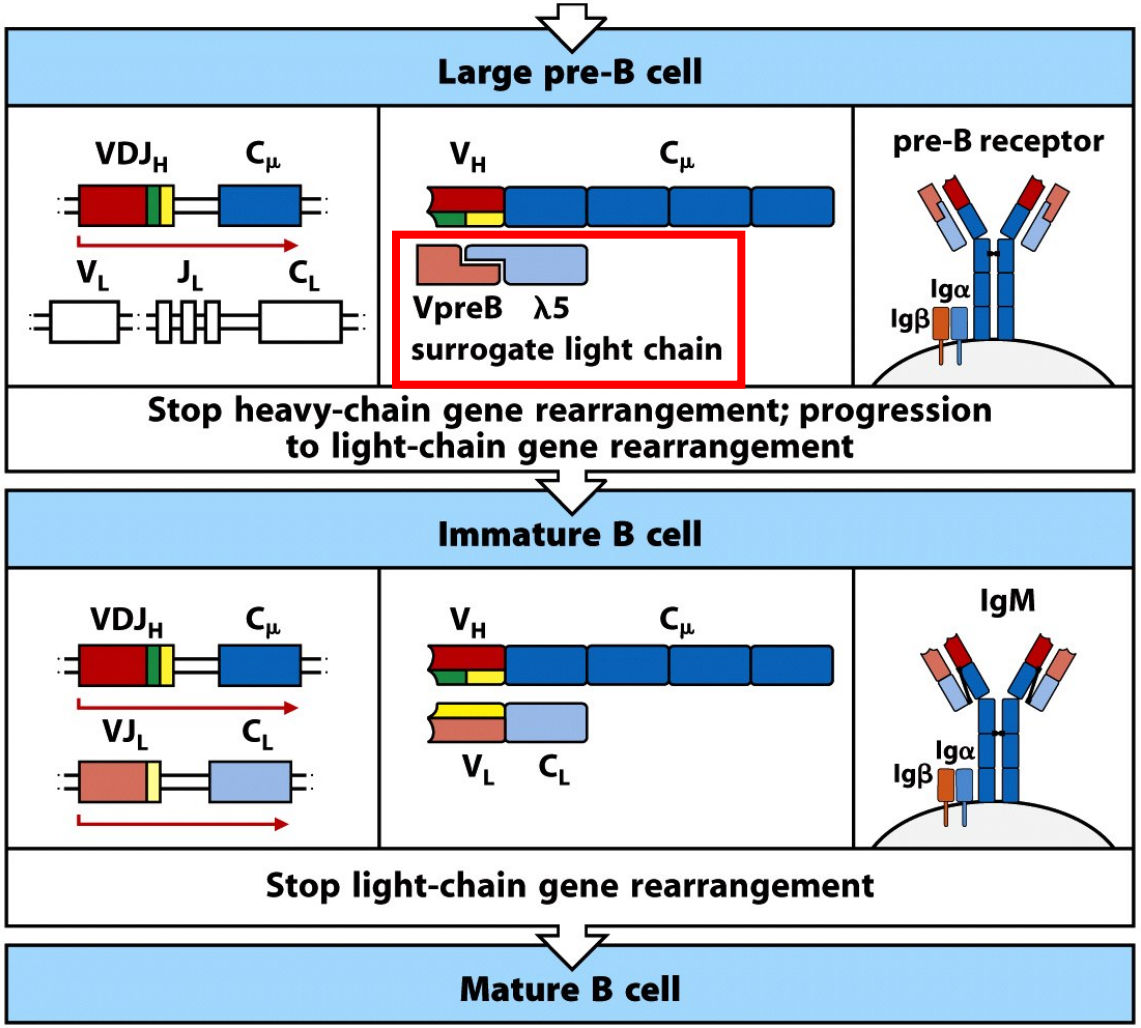


Fig. 8-6

Productive rearrangement of Ig gene is followed by surface protein expression in developing B cells



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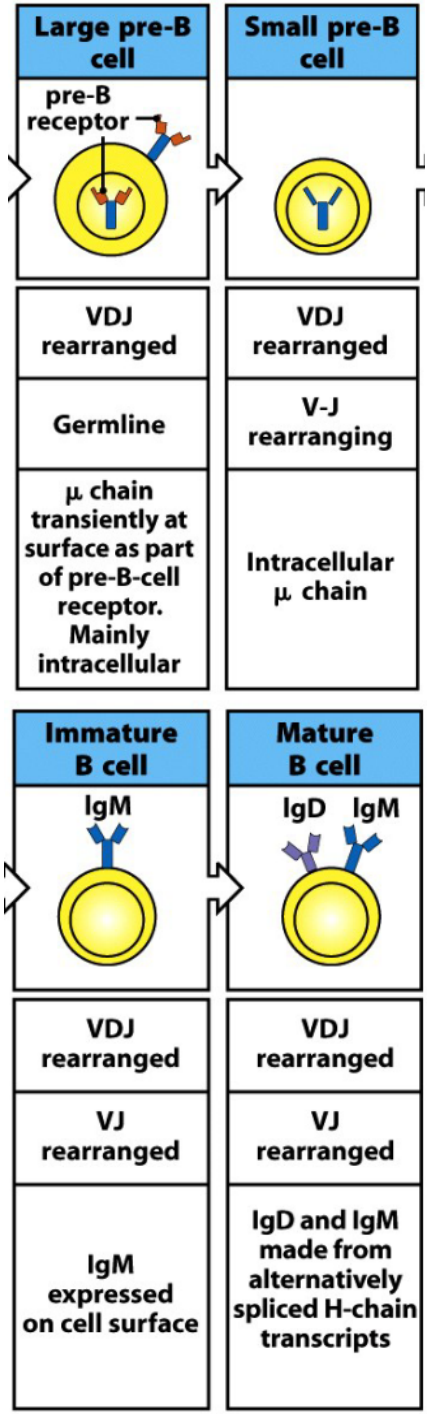


Fig. 8-7

Signaling of the pre-B-cell receptor

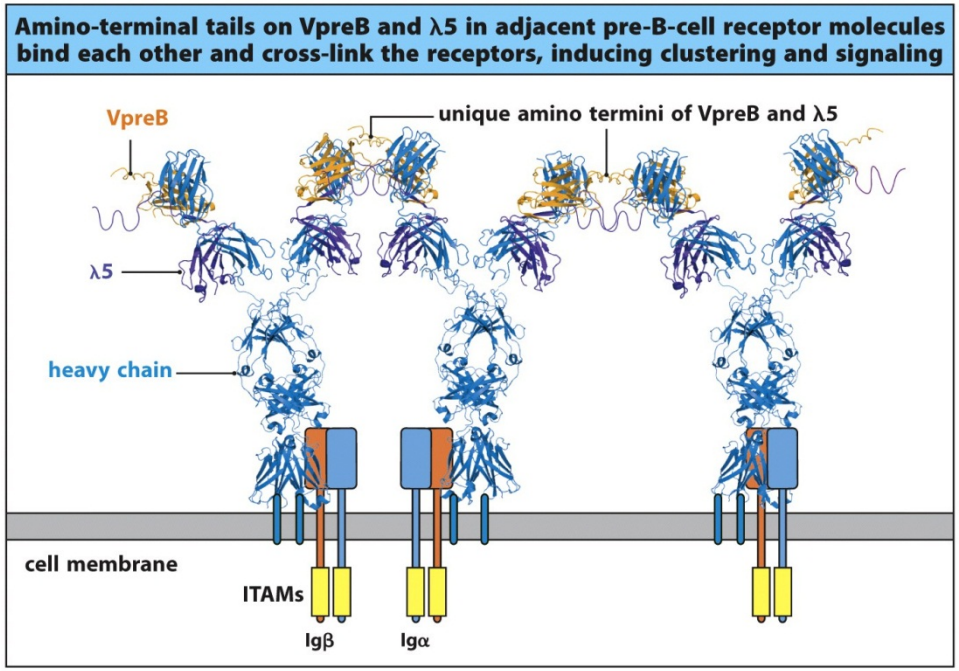
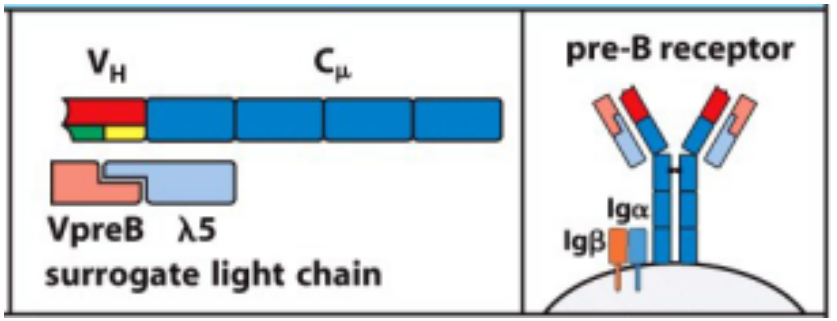


Figure 8.7 Janeway's Immunobiology, 8ed. (© Garland Science 2012)



- If successful rearrangements from **BOTH** heavy-chain alleles occur, then it's possible to generate BCR with **different Ag specificities** on a single B cell → contradictory to Burnet's theory!
- **Allelic exclusion (等位基因排斥)**:

  N-termini of VpreB/λ5 from adjacent pre-BCRs spontaneously dimerize, causing ligation of BCRs → signaling to terminate the 2nd H-chain rearrangement

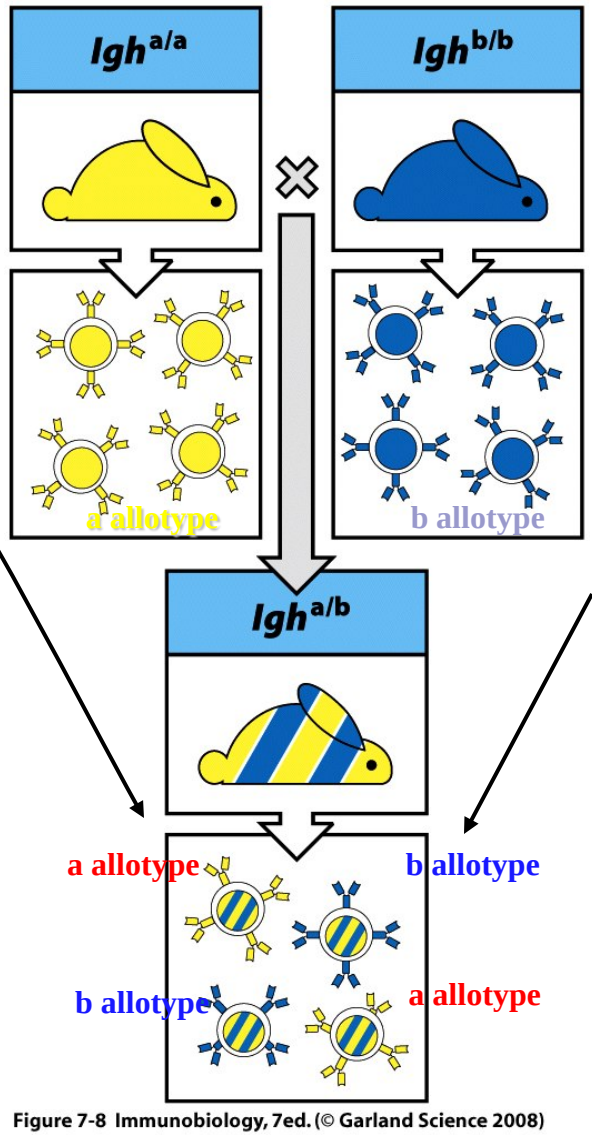
Allelic exclusion.

The expression of ONLY one of two co-dominant alleles in any given cell (important for clonal selection)

All the Abs made by one B cell (or TCR by T cell) are ***identical*** even though each cell has the genes to make up to **8** different Abs.

In theory, either of 2 H chains could pair with any of 2 κ and 2 λ L chains. (2 x 2 x 2 = 8 Ig combinations)

Fig. 8-8



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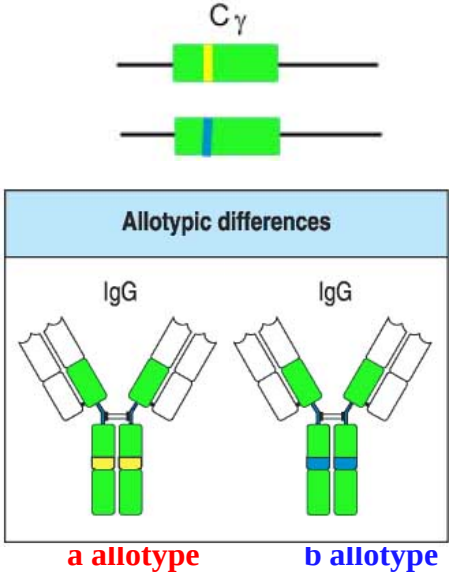


fig. 4.24

Allelic exclusion provides that there is **one specificity per B cell** (this is most efficient for clonal selection)

2 IgH loci (a or b), but each B cell only expresses either IgHa or IgHb, but not BOTH!!

How is allelic exclusion achieved?
By stepwise, orderly gene rearrangements with testing for success at each step

Non-productive L-chain gene rearrangements could be rescued by multiple attempts at gene rearrangements

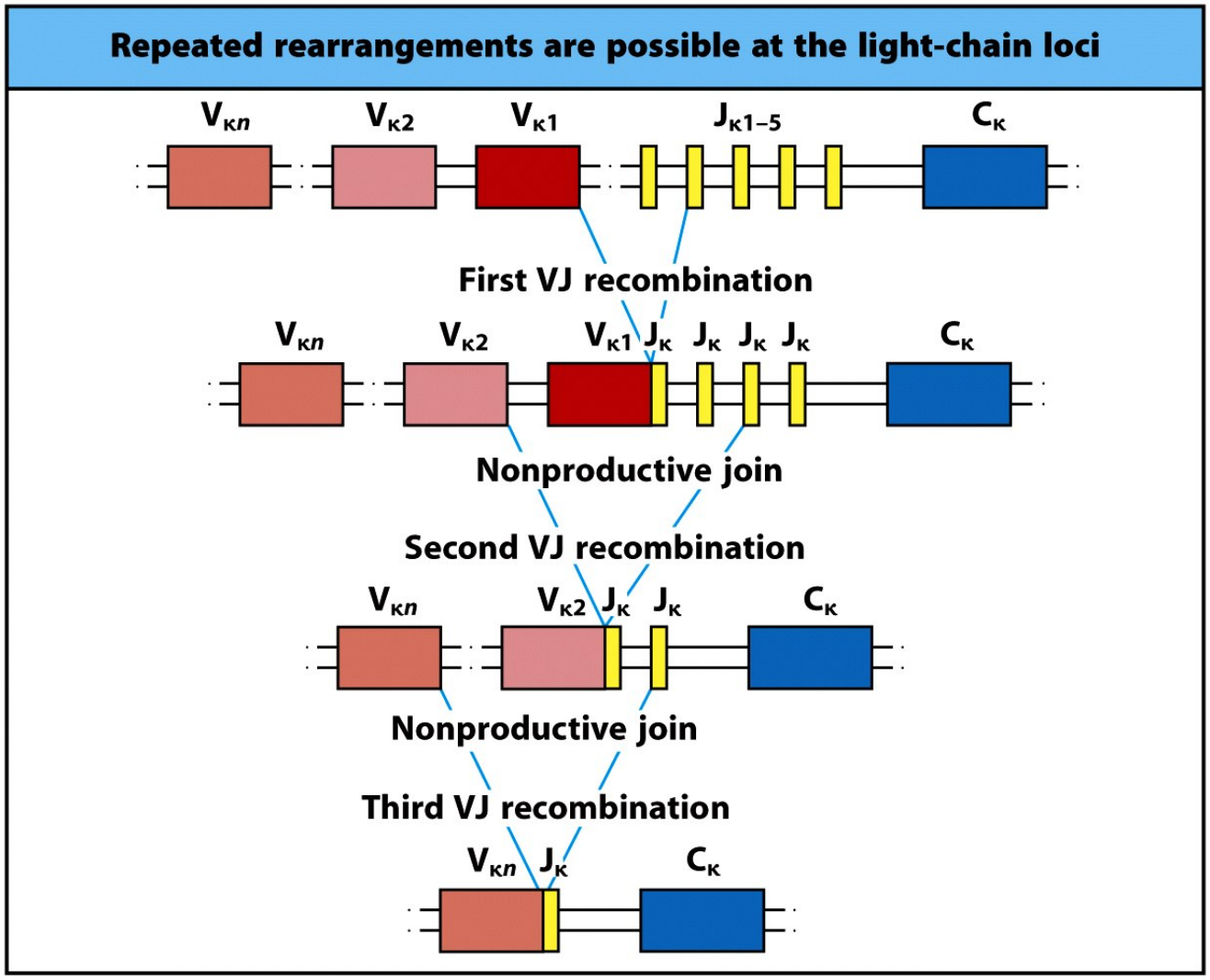


Figure 7-9 Immunobiology, 7ed. (© Garland Science 2008)

Fig. 8-9

The maximum number of attempts is equal to the number of V or J gene segments (which ever is fewer).

Usually, less than the maximum are possible. (see Fig 7-18 for why this cell had only 3 tries, but not 5).

Fig. 8-10

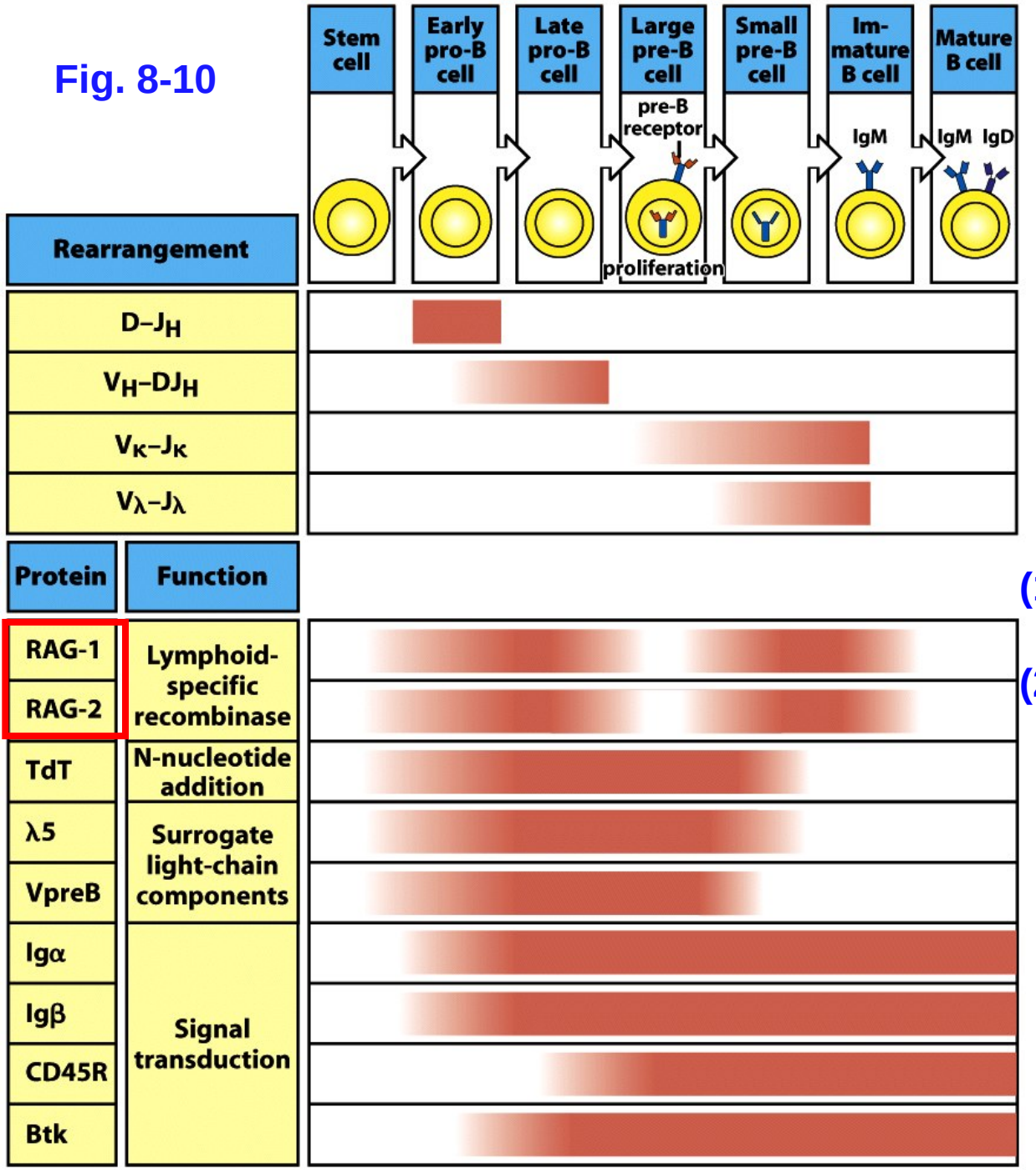


Figure 7-10 Immunobiology, 7ed. (© Garland Science 2008)

Temporal expression of crucial cellular proteins during B-cell development

(1) To make productive H & L chains

(2) To rescue anti-self B cells by '<u>receptor editing</u>" on its <u>L chain</u> (but not H chain)

Gene rearrangements in B cells occur in

Fig. 8-11

an orderly process

Total chances: 2 x 2 x 2 = 8 times

these are diploid organisms with two heavy chain loci, two κ loci and two λ loci.

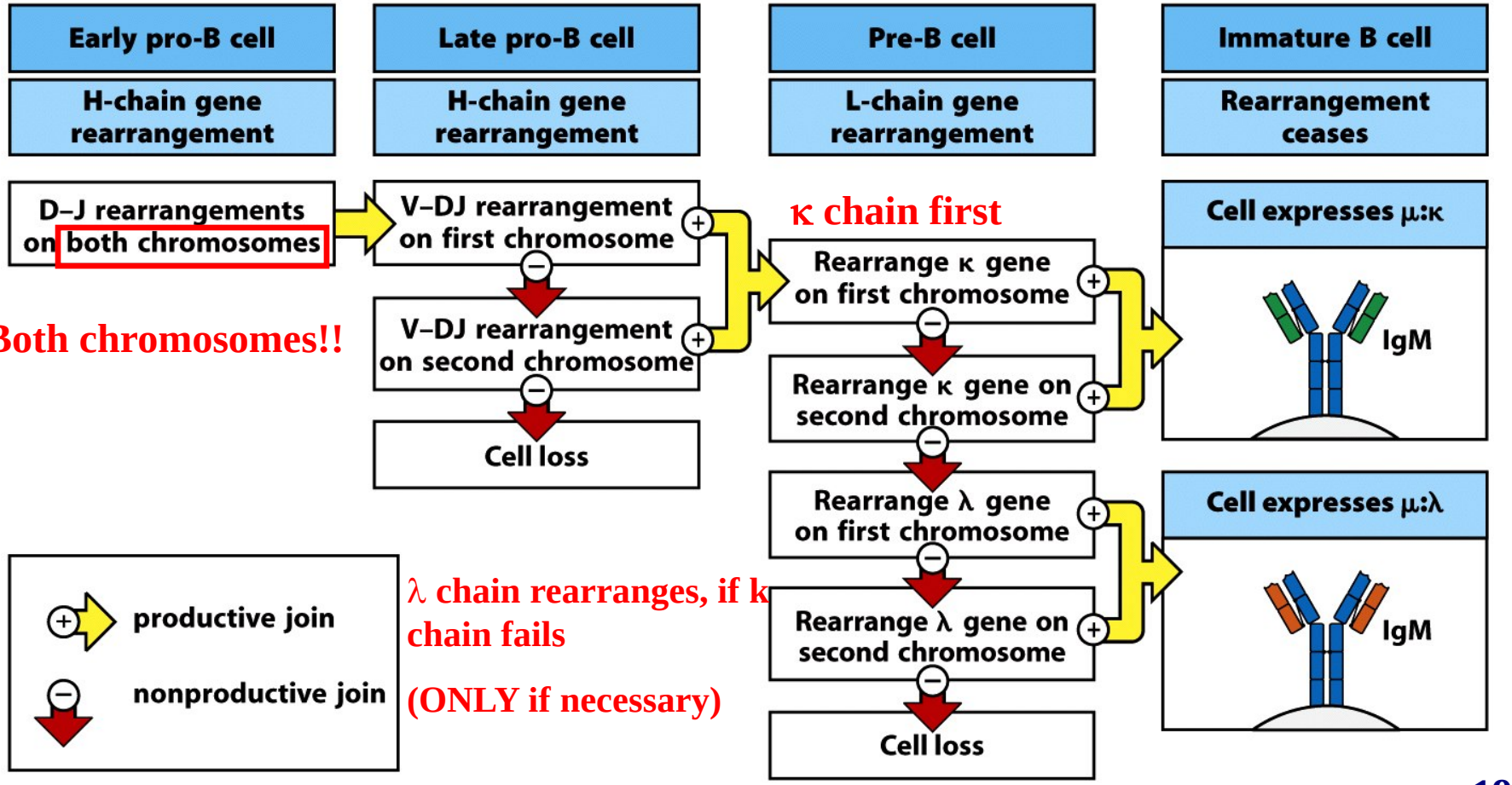


Figure 7-11 Immunobiology, 7ed. (© Garland Science 2008)

What happens next after the successful gene rearrangements in BCR?

Test for **self-reactivity**

- Will these immature B cells react to self-antigens?

(1) If not, they would migrate to periphery and become mature B cells

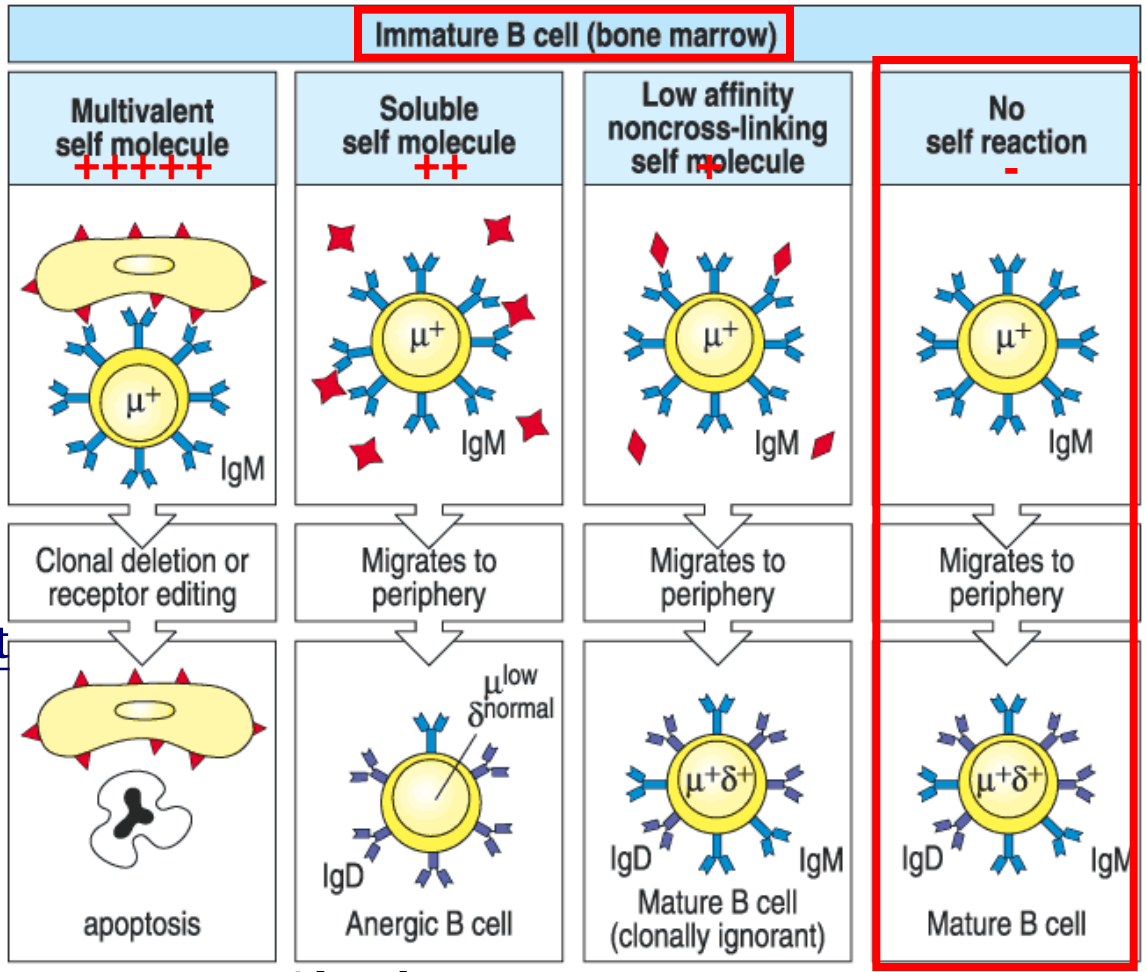
(2) If so, they MUST NOT be released into the periphery!!!

→ Otherwise, this will cause detrimental anti-self immune response

If self-active, immature B cells can be rendered inactive or apoptotic

Fig. 8-12

Signal intensity →



Maintenance of tolerance requires the persistence of antigen because self-antigens are always present but foreign antigens are transient

Clonal deletion (most important)

Alive but not functional

Ag binding is too weak to get a response (least important)

These are the cells that constitute a normal immune response

Before clonal deletion of an anti-self B cell, the cell can attempt receptor editing of the light chain

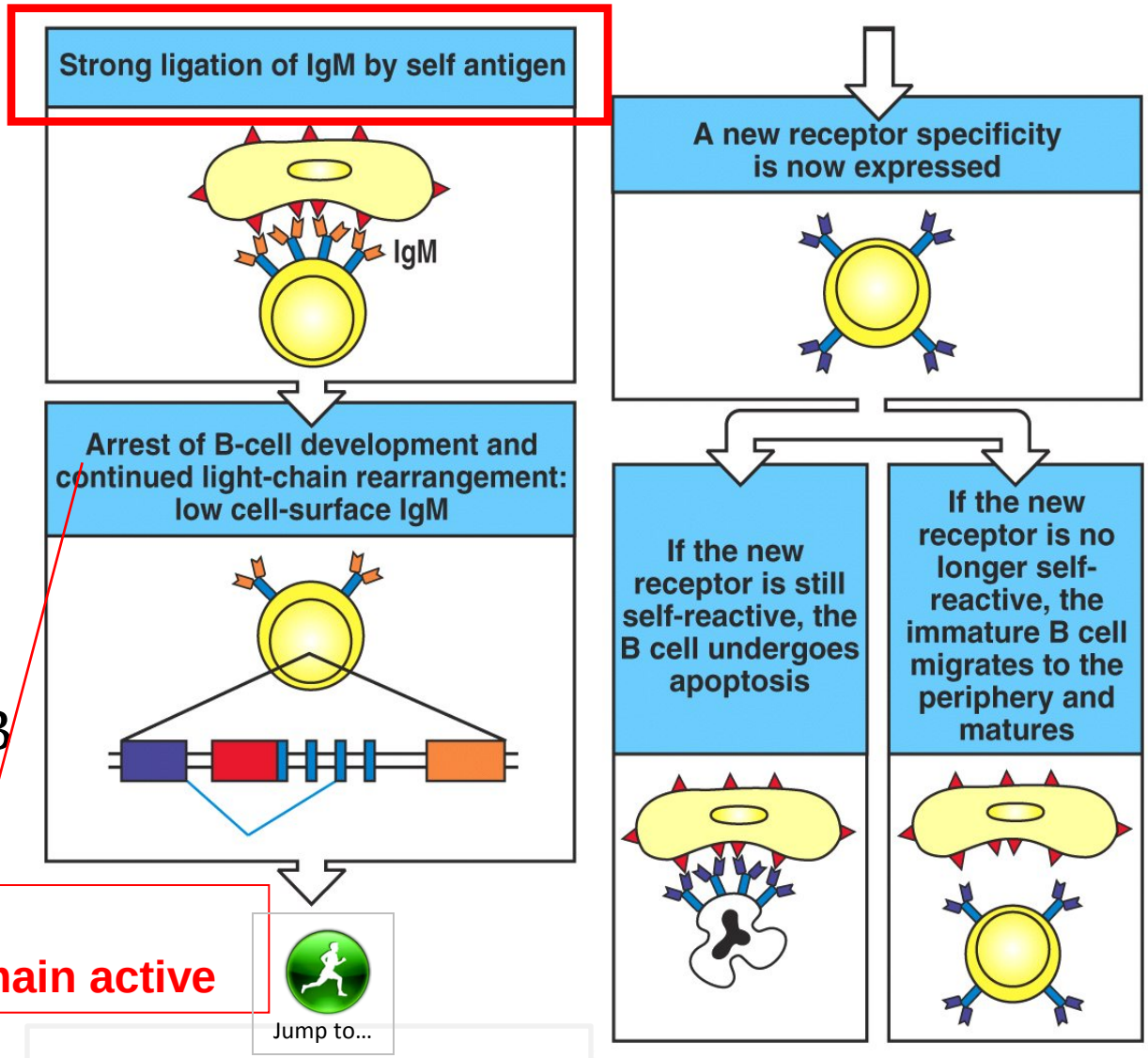
Fig. 8-13

So, **light chain** can

1. use repeated rearrangements (Fig 7.9) to make functional receptor;

and

2. receptor edit. (to avoid clonal deletion of anti-self specific B cells)



Developmentally arrested;
but RAG recombinases remain active

Jump to...

Receptor Editing in B & T Cells

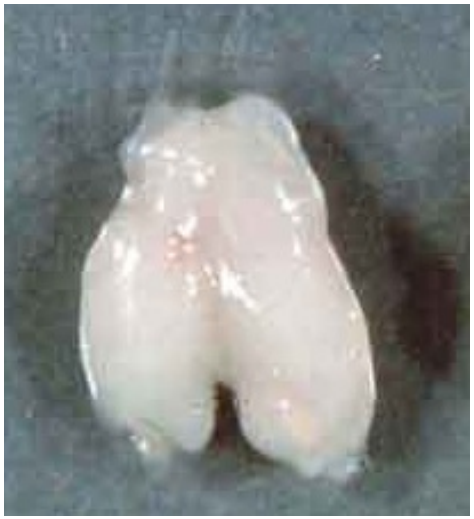
Fig. 8-13

Immature B cell "edits" ***light chain*** if it binds strongly to self-antigen (gets negative selection signal). This could rescue the cell from clonal deletion (i.e., death). (needs signal to edit)

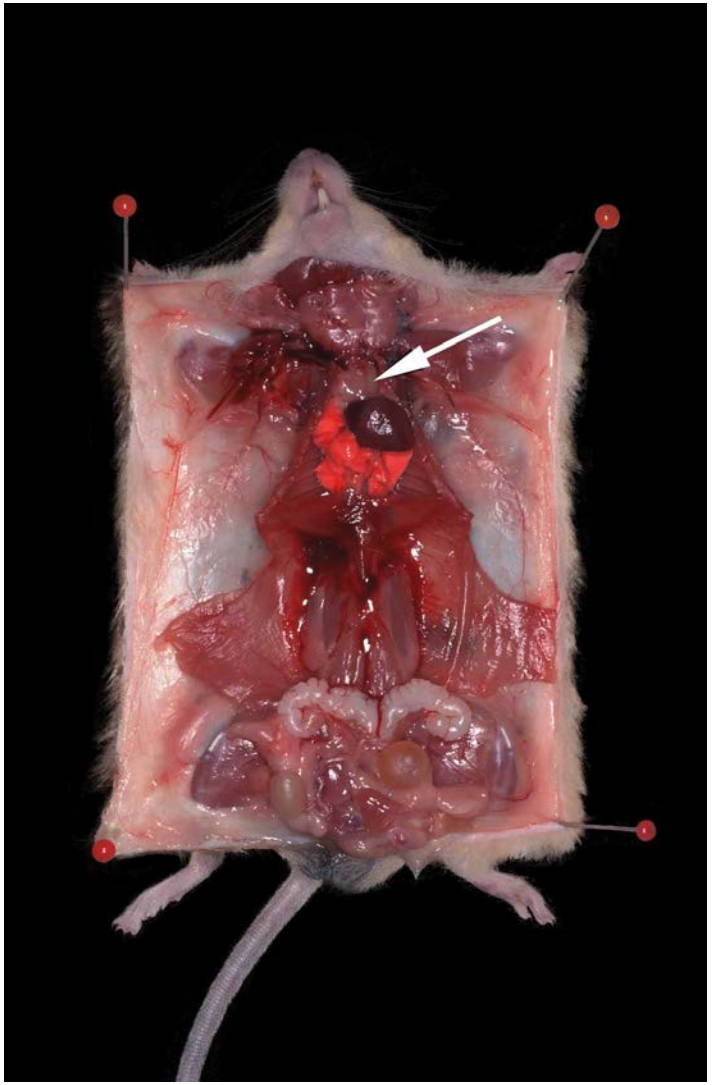
Immature T cell ***continues*** to rearrange **α chain** until it gets positive selection signal. (will eventually die if it does not receive positive selection in a few days). (needs signal to stop "editing")

Development of T lymphocytes

Anatomy of the thymus



Thymus and pancreas glands of animals are sometimes termed “sweetbreads”.
Their texture is firmer than the brain.



Pre-T → Thymus
Mouse (D11 in pregnancy), Human (W8/9 in pregnancy)

Fig. 8-14

Life of a T Cell

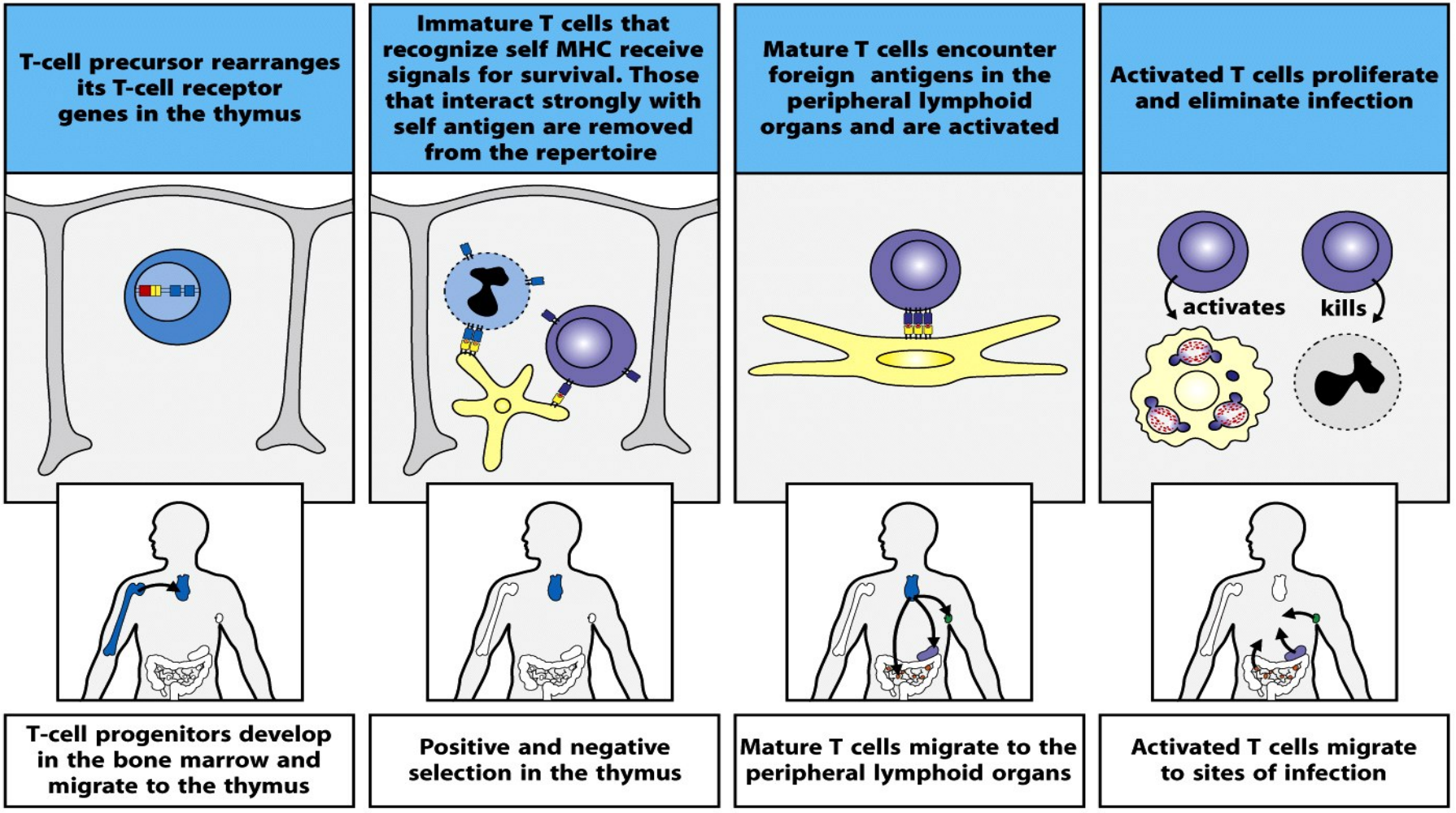


Figure 7-14 Immunobiology, 7ed. (©

(-) selection is clonal deletion!!!

In older individuals there is little thymus function and most T cells come from division of mature T cells in the periphery (c.f. new B cell are continually generated in the bone marrow)

- CTLs and T_H1 migrate to site of inflammation;
- most T_H2 cells remain in peripheral lymphoid organs

Thymic stroma: cortical/medullary epithelial cells that provide a microenvironment for T cell development

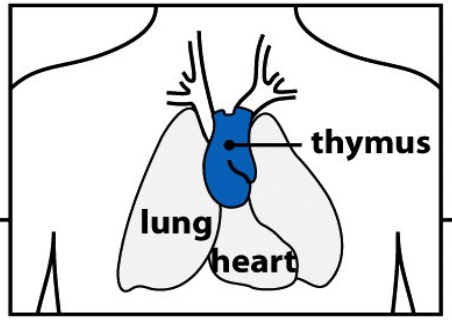
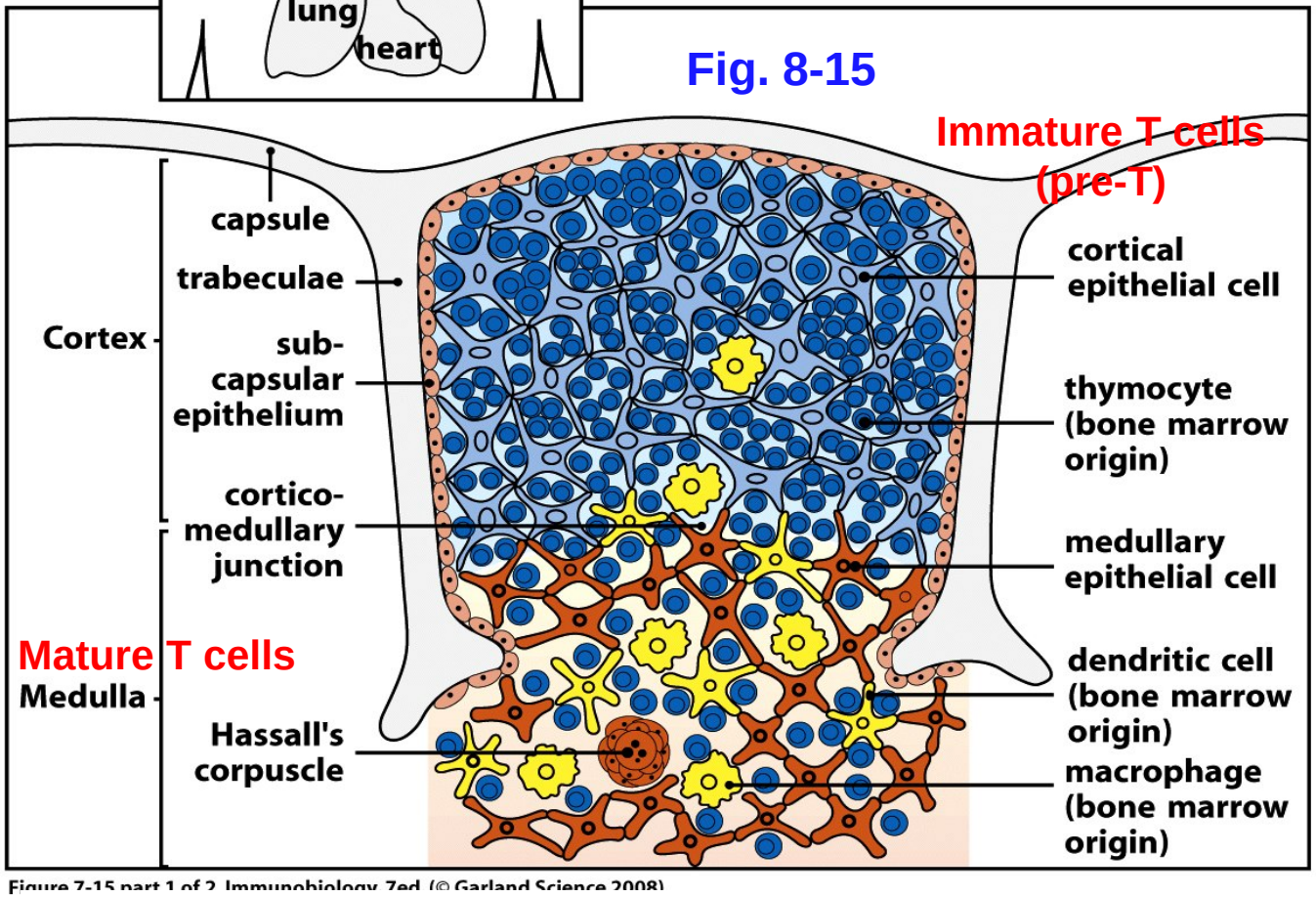


Fig. 8-15



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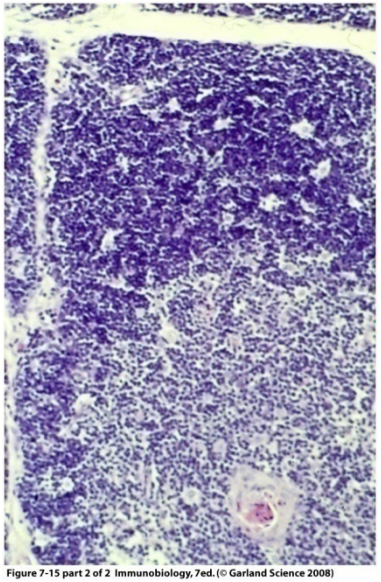

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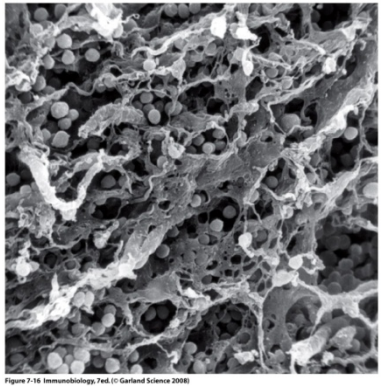

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T cells in the thymus are called **thymocytes**

Nude mice, DiGeorge's syndrome are thymic deficient (athymic) and have no T cells. Neonatally thymectomized mice are T cell deficient.

Fig. 8-17

Thymus is required for T cell development

***scid* mice:**

RAG genes defects

scid mice have a functional thymus but **cannot rearrange Ig and TCR genes** so they cannot make Ig or TCR and thus have **no B or T cells**

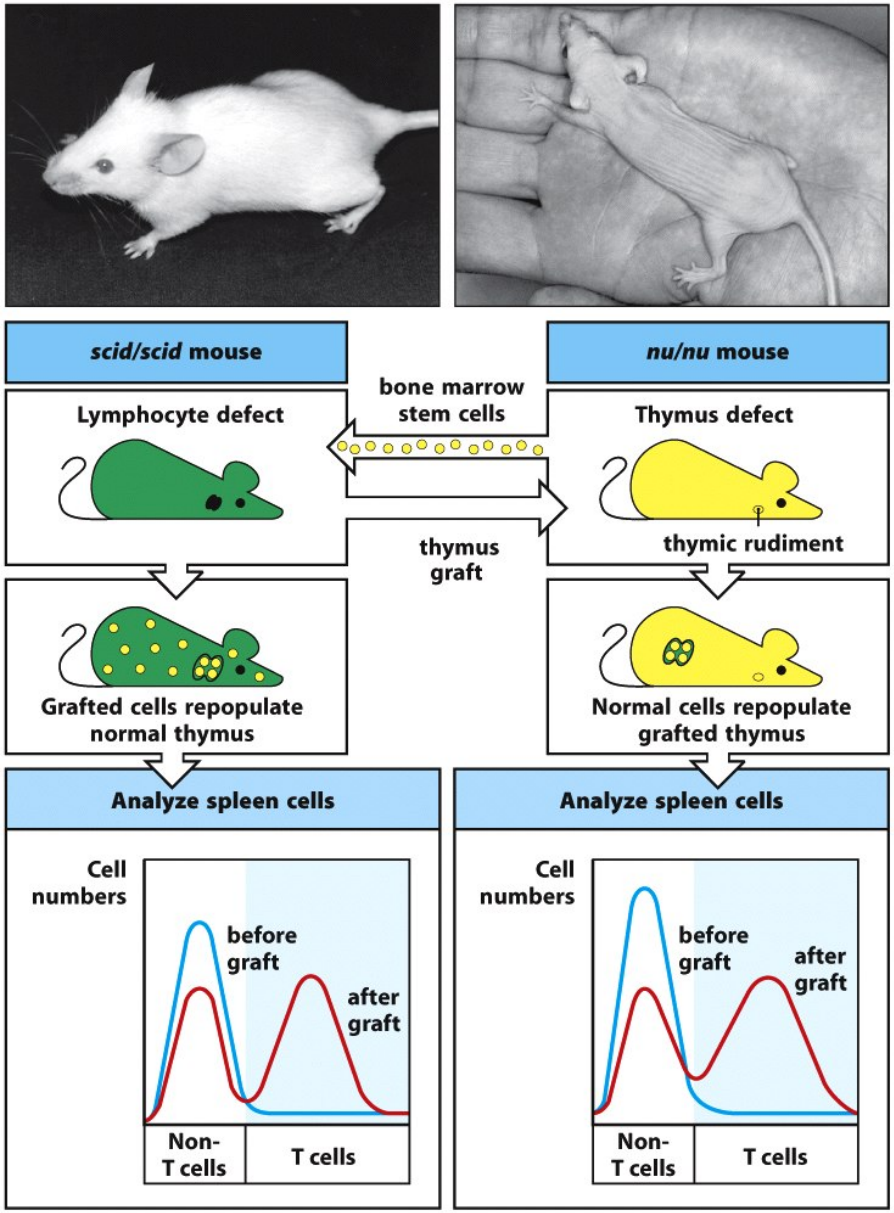

Figure 7-17 Immunobiology, 7ed. (© Garland Science 2008)

***Nude* mice:**

Thymus epithelia defects

Lymphocytes in nude mice have everything needed to rearrange Ig and TCR gene but **do not have a functional thymus** for T cell development

Apoptotic T lymphocytes can be mostly found in the thymic cortex

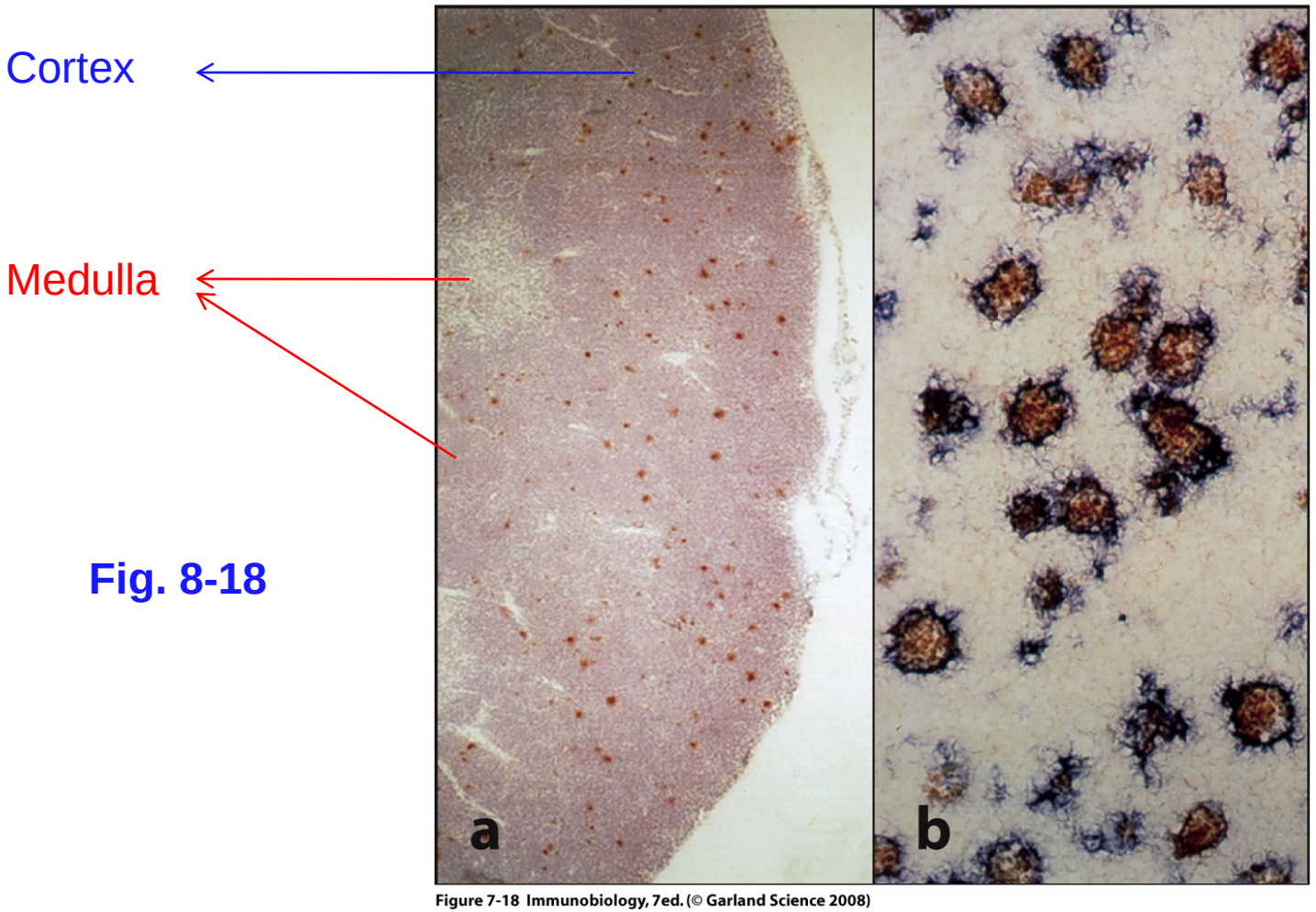

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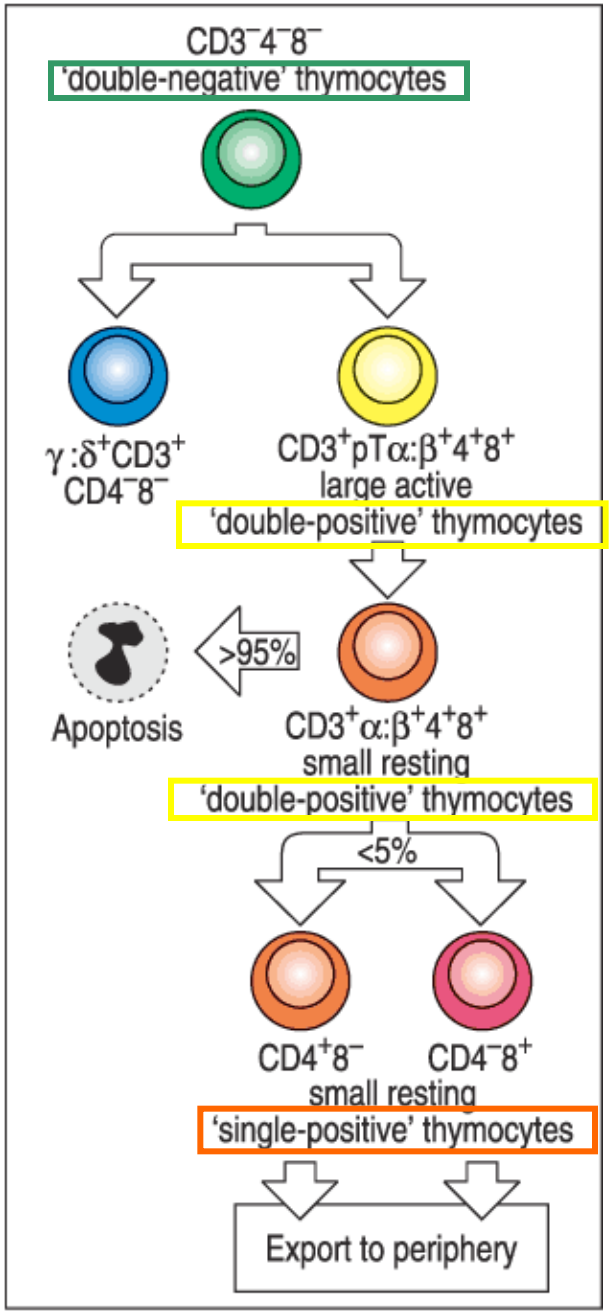
Fig. 8-18

Cortical **macrophages** engulfing apoptotic T cells

Thymocyte differentiation correlates with expression of surface markers

Fig. 8-19



Double (-), CD3 (-), TCR (-)

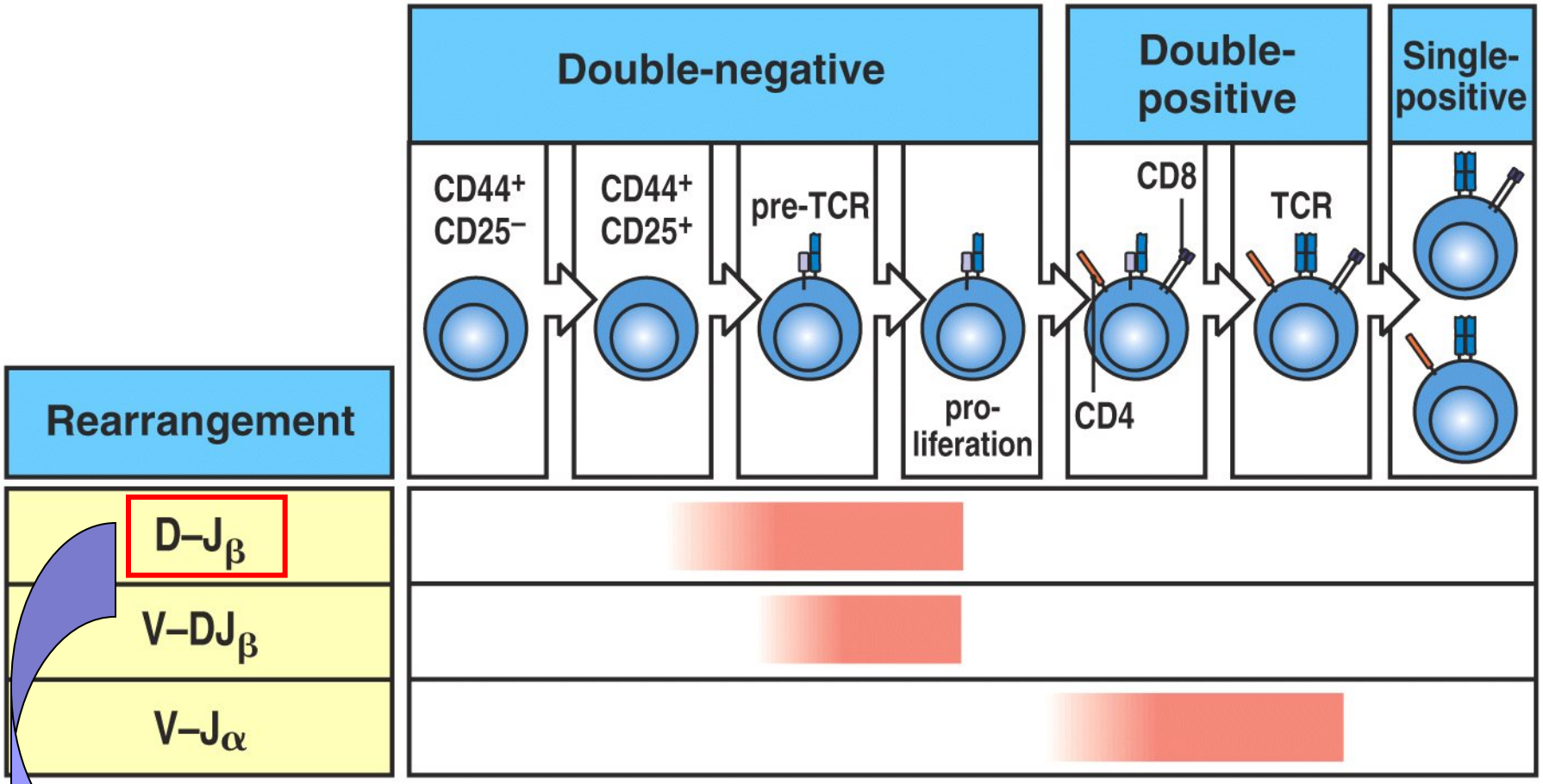
Double (+), CD3 (+), Pre TCR (β+pre-Tα)

Double (+), CD3 (+), TCR (α/β +)

Single (+), CD3 (+), TCR (α/β +)

Fig. 8-20

Developmental stages of the α:β T cell



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Genes for TCR β subunit is the first to undergo rearrangement!!

Fig. 8-20

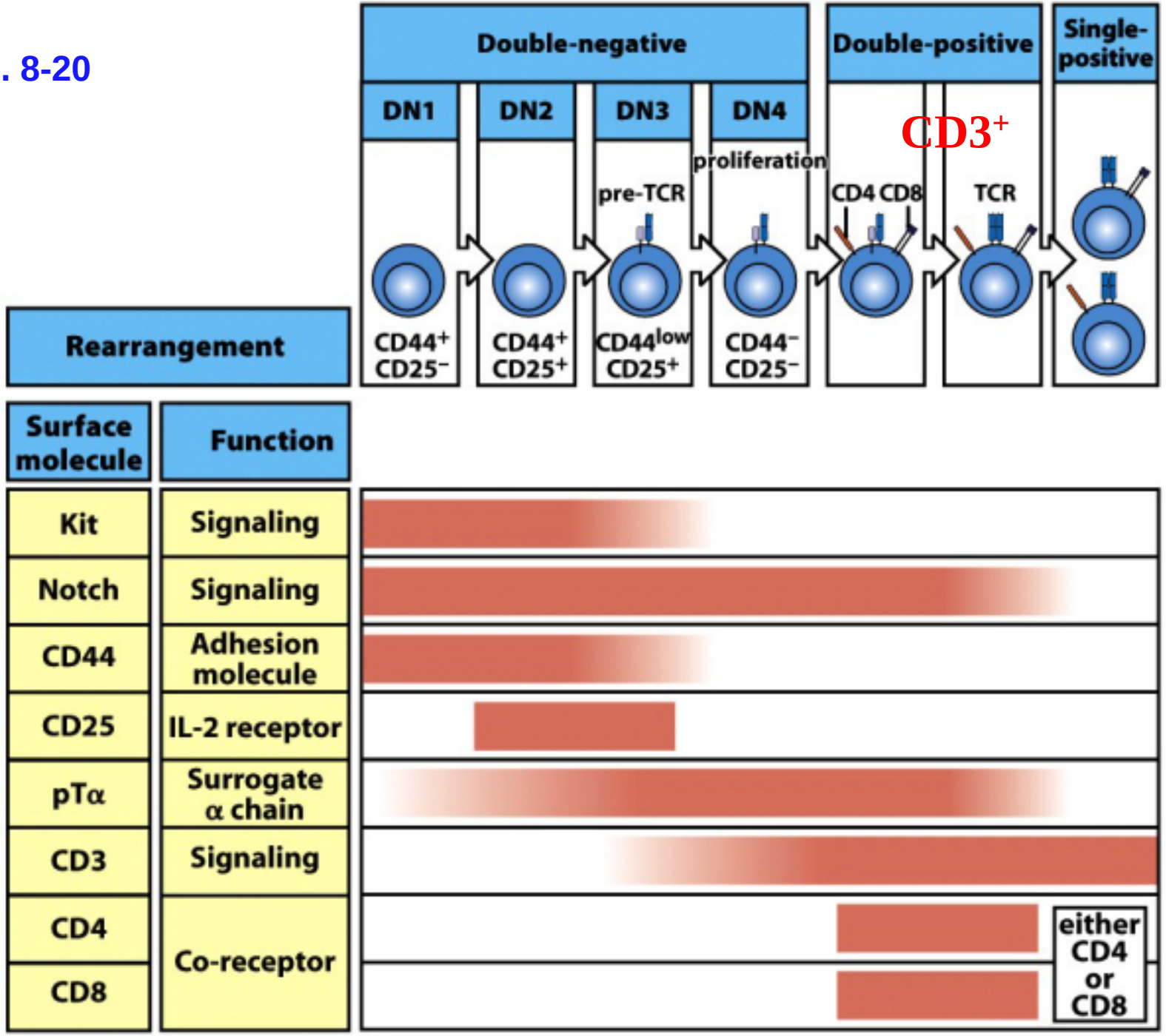

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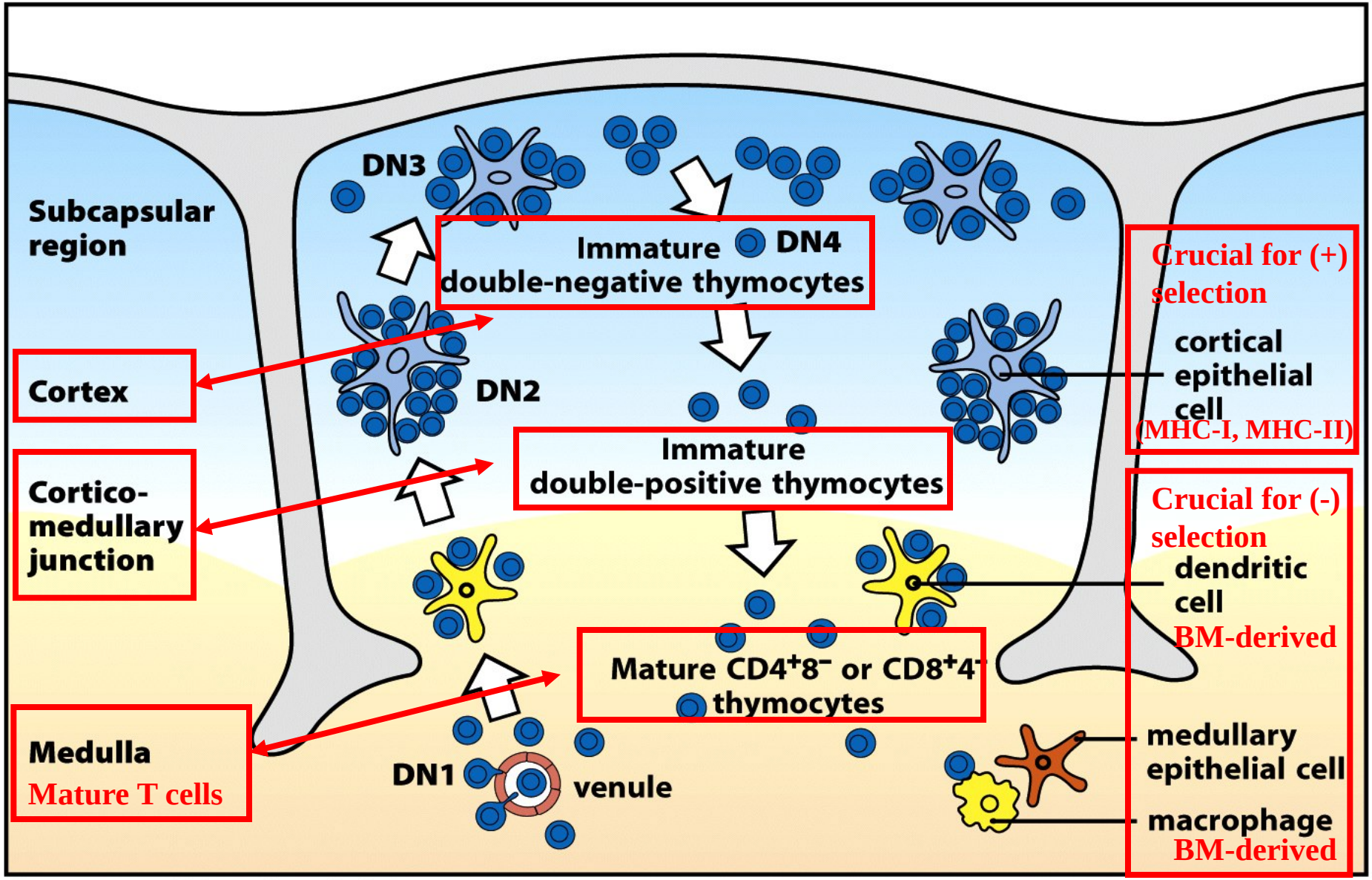
Fig. 8-21

Different developmental stages → different locations in the thymus



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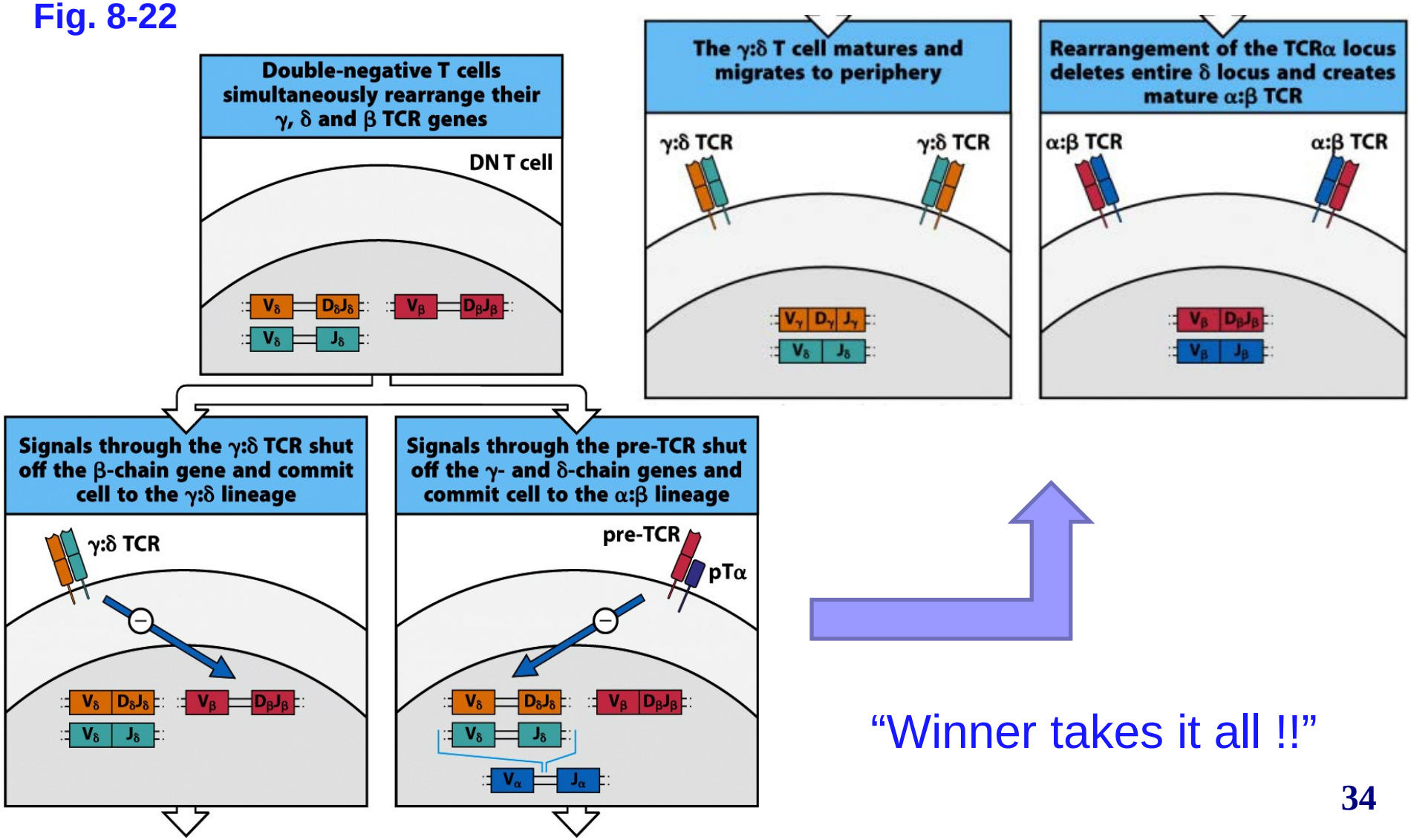
Signals via pre-TCR or γ:δ chain will determine fate of the T cell

Fig. 8-22



"Winner takes it all !!"

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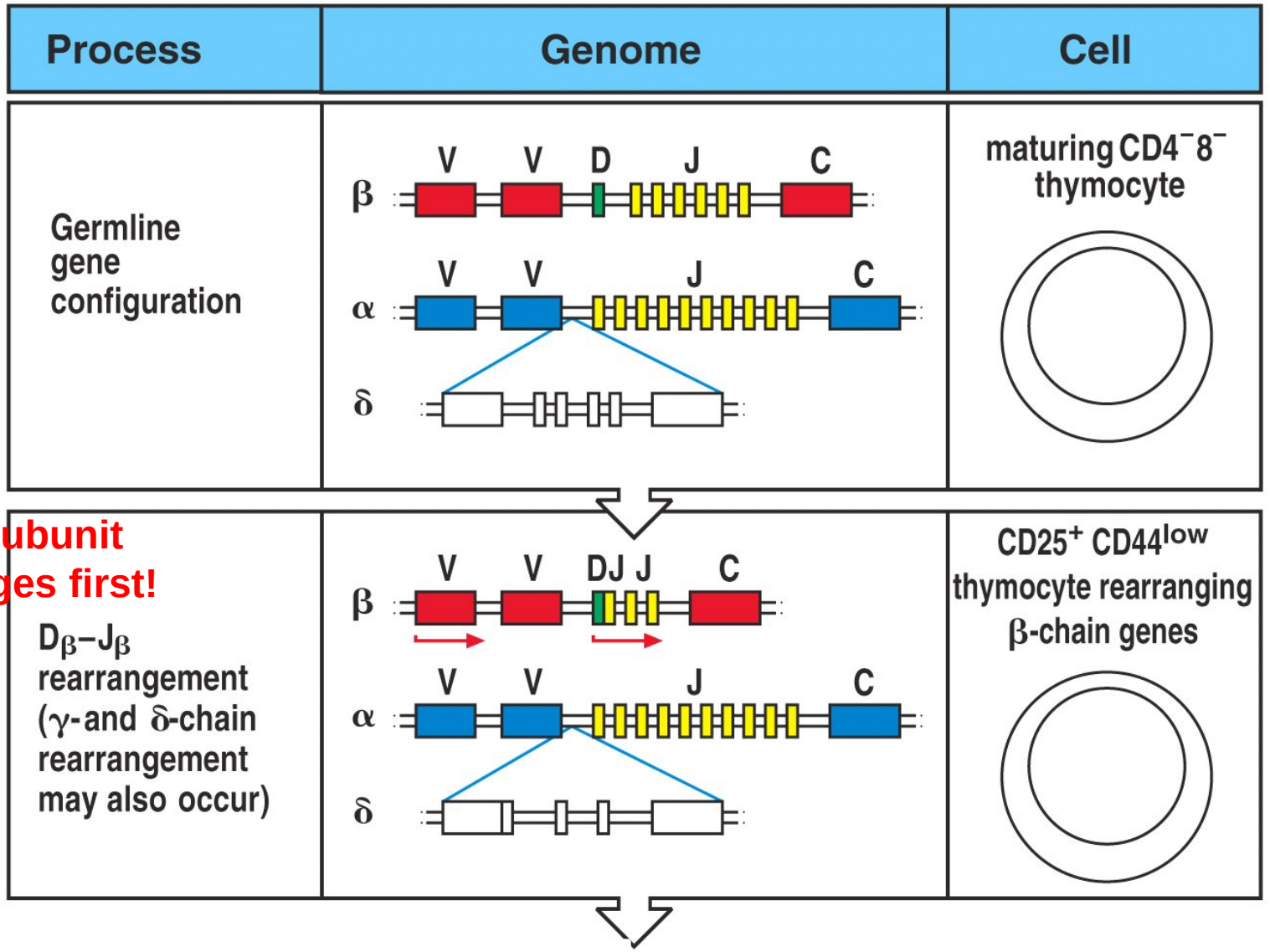
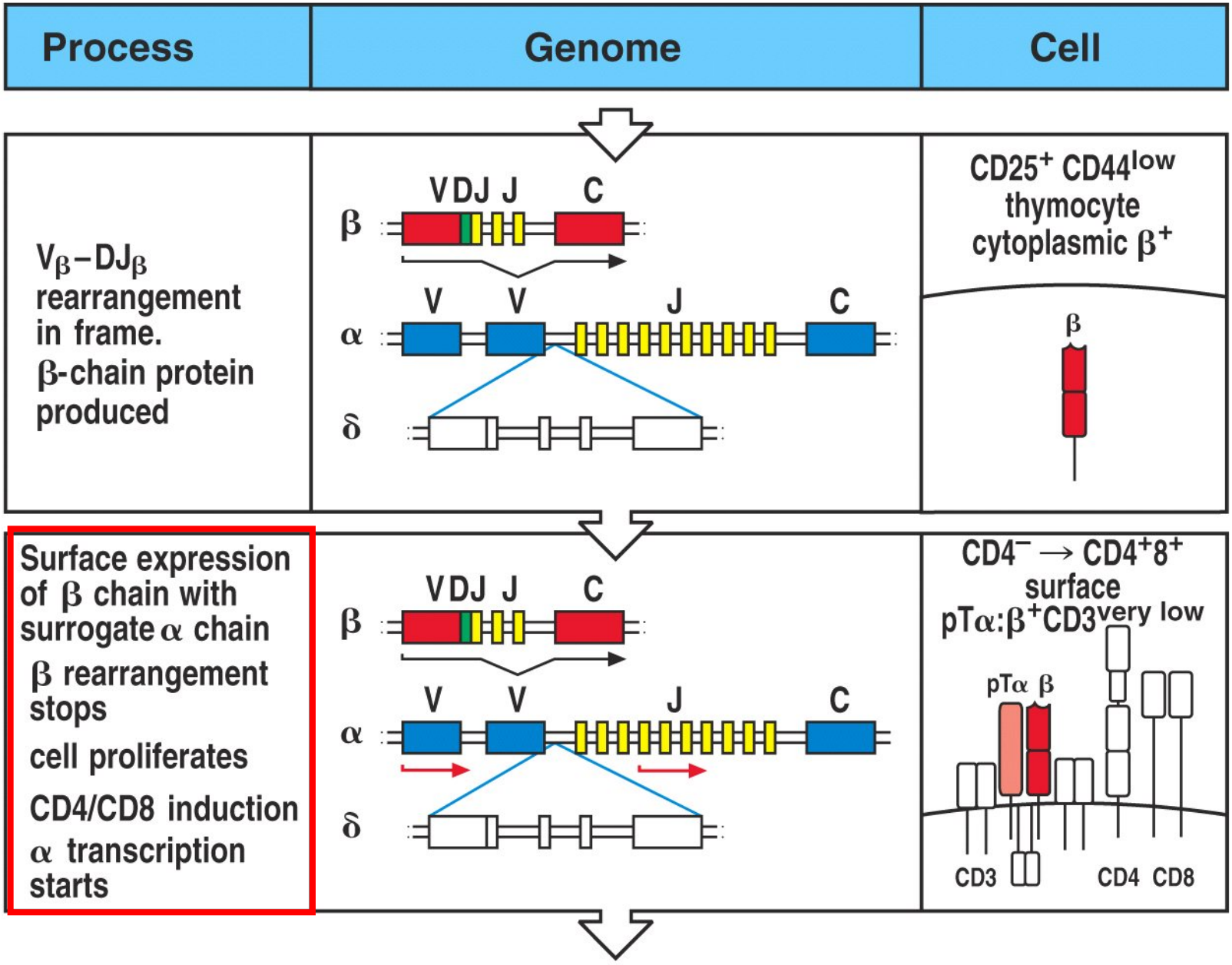
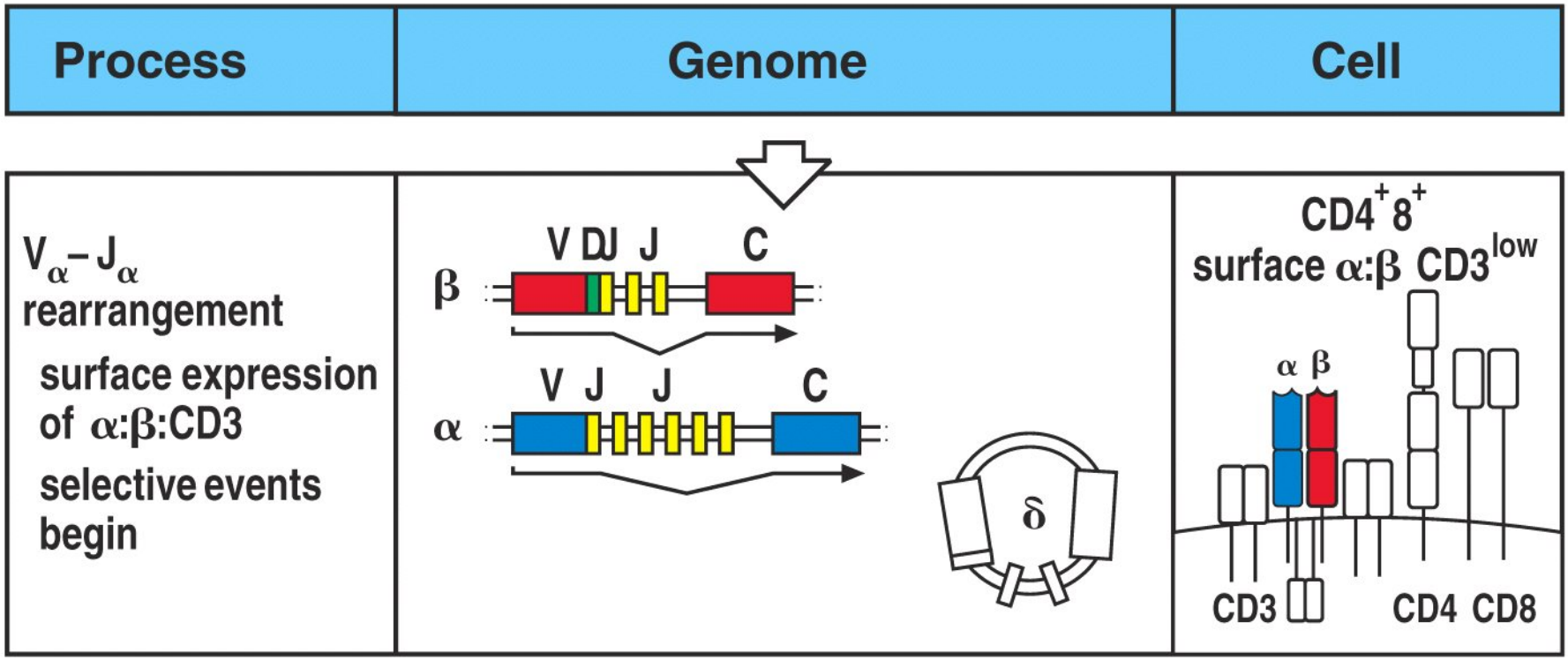
The sequential rearrangements at the TCR α and β loci are similar to the Ig rearrangements

Fig. 8-25




Process
Genome
Cell
V_β–DJ_β rearrangement in frame. β-chain protein produced
VDJ J C
β
V V J C
α
δ
CD25+ CD44low thymocyte cytoplasmic β+
β
Surface expression of β chain with surrogate α chain
β rearrangement stops
cell proliferates
CD4/CD8 induction
α transcription starts
VDJ J C
β
V V J C
α
δ
CD4− → CD4+8+ surface pTα:β+CD3very low
pTα β
CD3
CD4 CD8



1. Immature T cell ***continues*** to rearrange α chain (termed "**receptor editing**") until it gets positive (+) selection signal. (by weakly binding to self-Ag)

2. Immature T cell will eventually die if it does not receive positive selection in a few days.

→ So, signal is required to **stop receptor editing**

Fig. 8-26

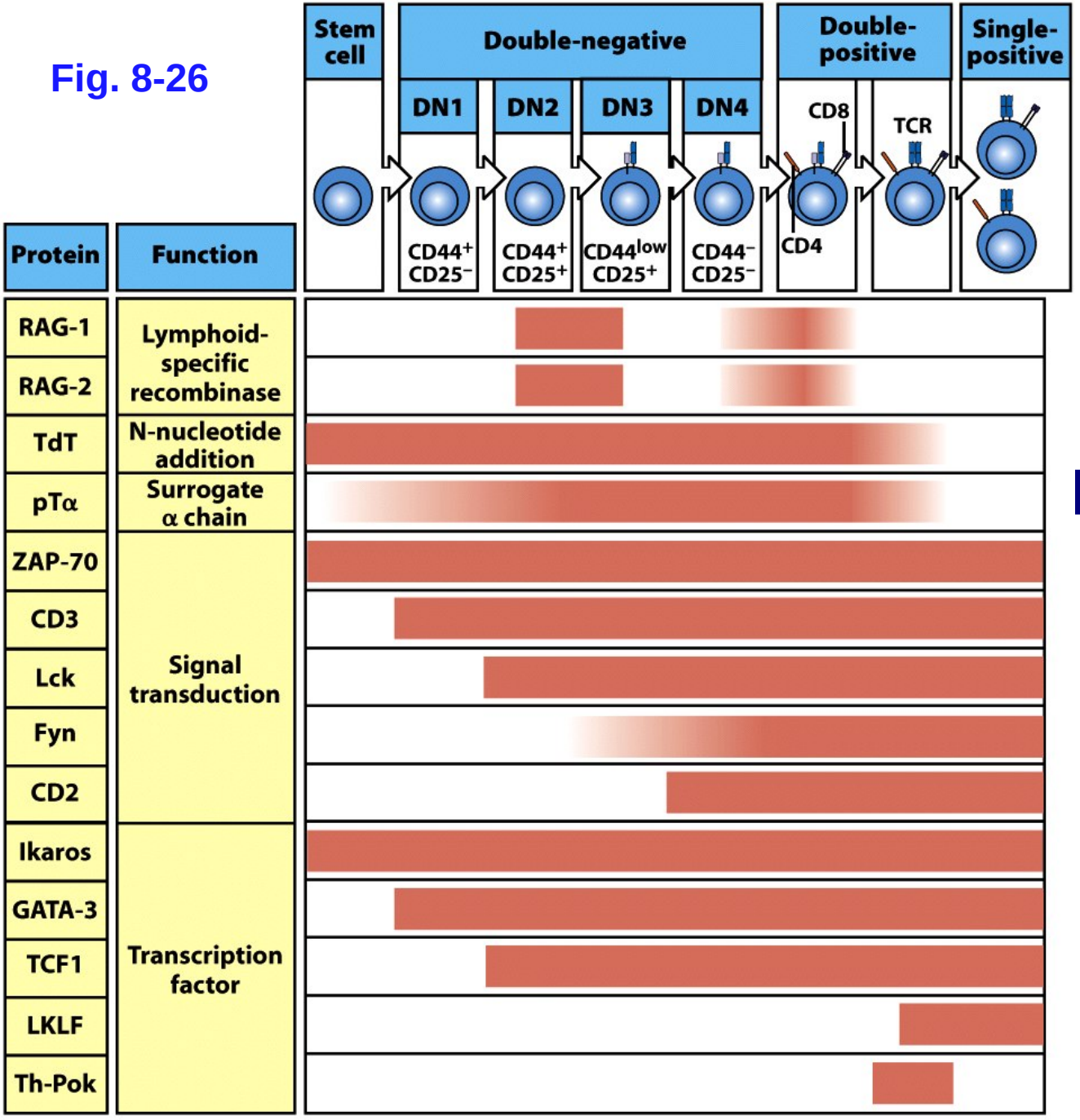


Figure 7-25 Immunobiology, 7ed. (© Garland Science 2008)

Temporal expression of crucial cellular proteins during T-cell development

T-cell α-chain genes can undergo multiple rearrangements to rescue non-productive VJ gene segments

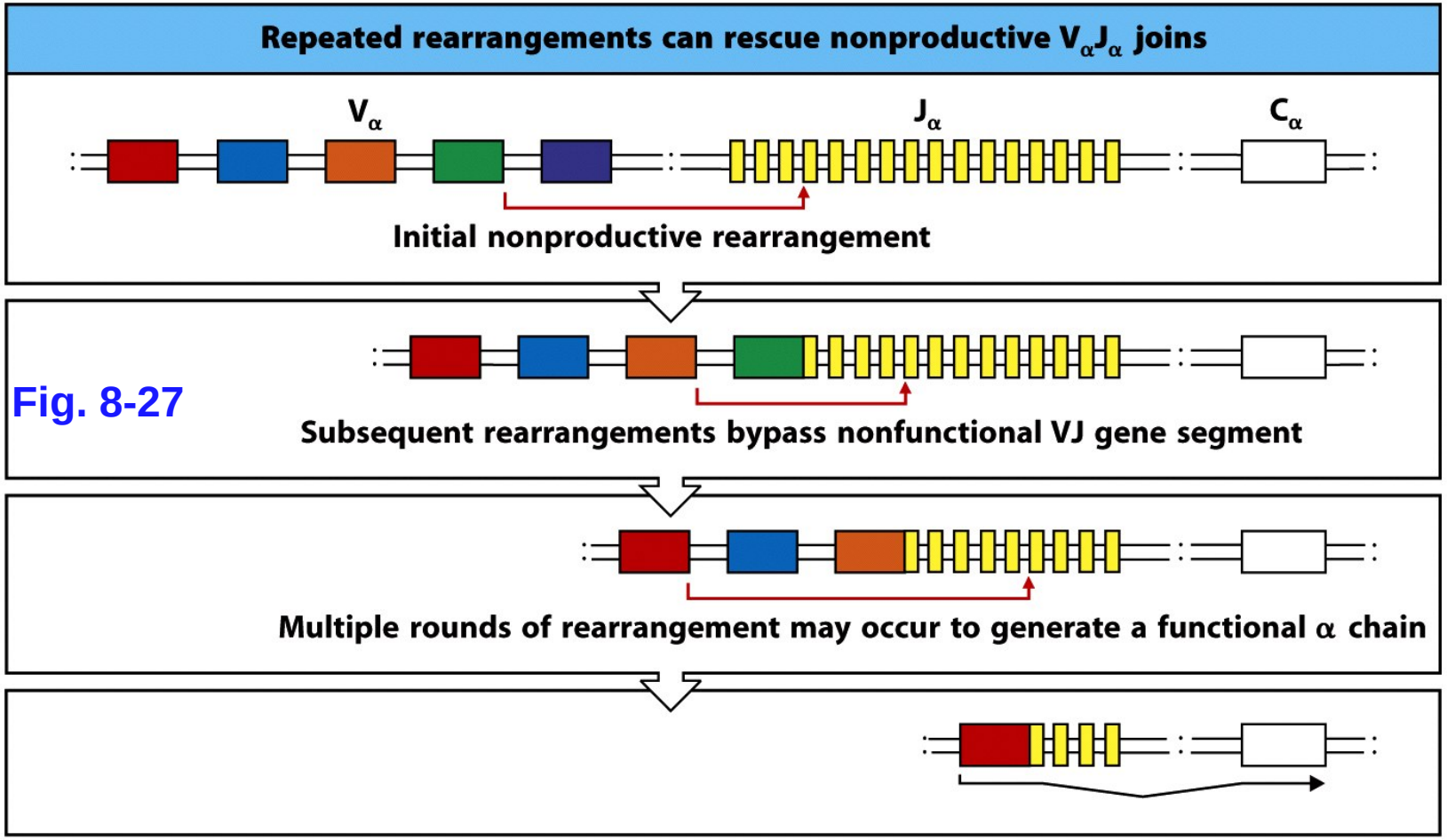


Fig. 8-27

Figure 7-26 Immunobiology, 7ed. (© Garland Science 2008)

In a TCR α locus, there are about 70 V gene segments and 60 J segments.

This provides for many attempts at a productive rearrangement.

Rearrangements stop when there is positive selection. (when TCR can bind to self-MHC molecule)

What happens next after the successful gene rearrangements in TCR?

Test for **self MHC-restriction (+)** and **self-reactivity (-)**
→ If so, those T cells MUST NOT be released into the periphery!!!
→ Otherwise, will cause detrimental anti-self immune response

T cells require (+) and (-) selections
(+): thymic cortex epithelial → test for self MHC-restriction
(-): thymic medulla APCs → test for self-Ag binding

Positive and negative selection of T cells

Positive selection (MHC restriction) is "learned"

(in the thymus)

Fig. 8-28

BM-derived cells (including T cells) express **both** MHC^a **and** MHC^b on their surfaces

Thymus epithelial cells (and all other tissues) express MHC^a

Thymus epithelial cells (and all other tissues) express MHC^b

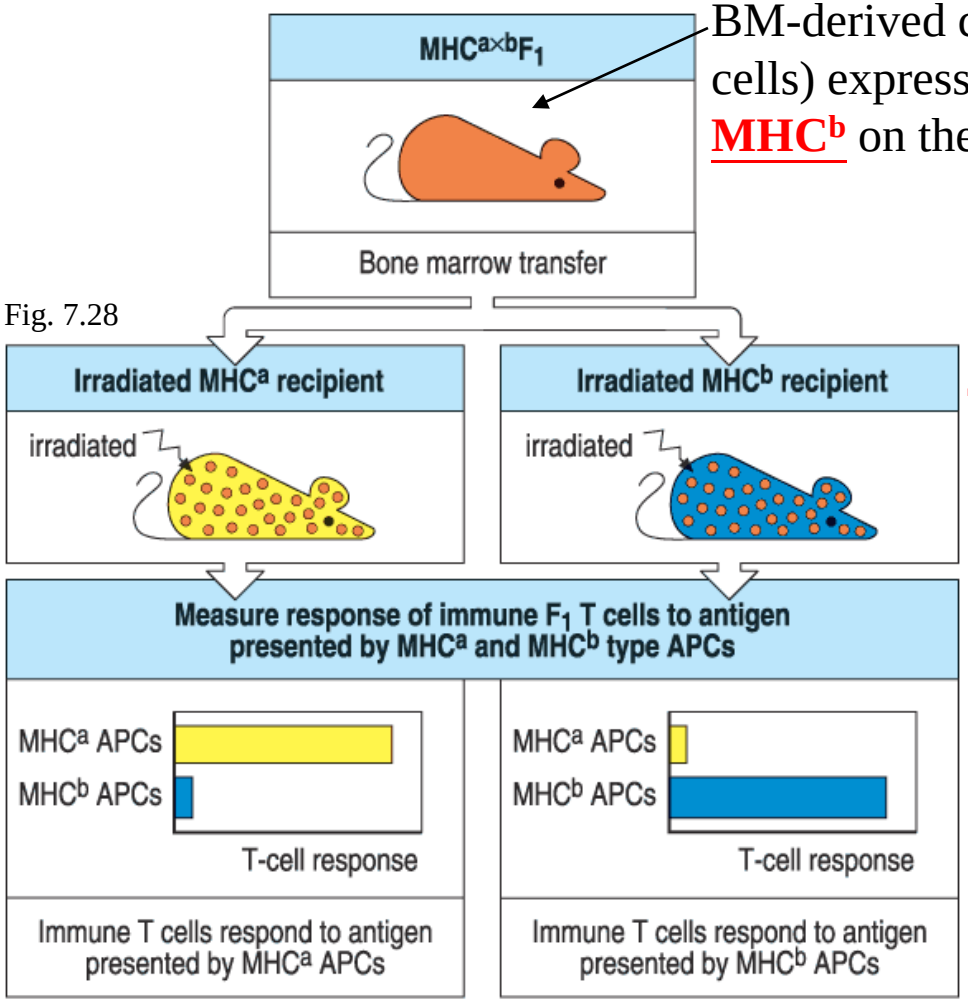


Fig. 7.28

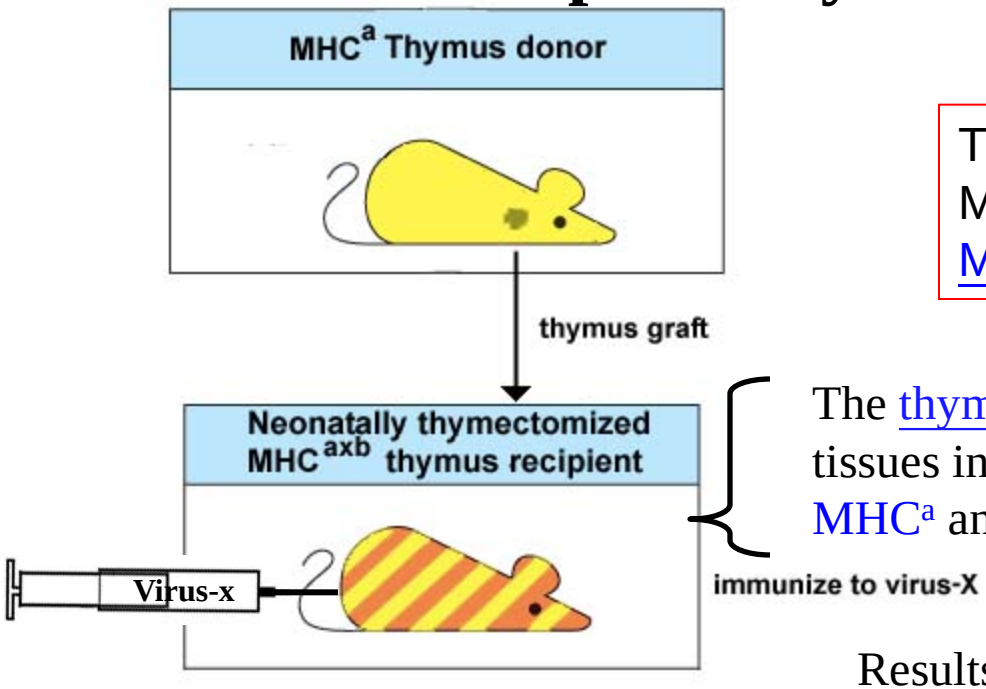
Fig 7.27 © 2001 Garland Science

These T cells have MHC^a and MHC^b on their surface but are restricted to MHC^a

These T cells have MHC^a and MHC^b on their surface but are restricted to MHC^b

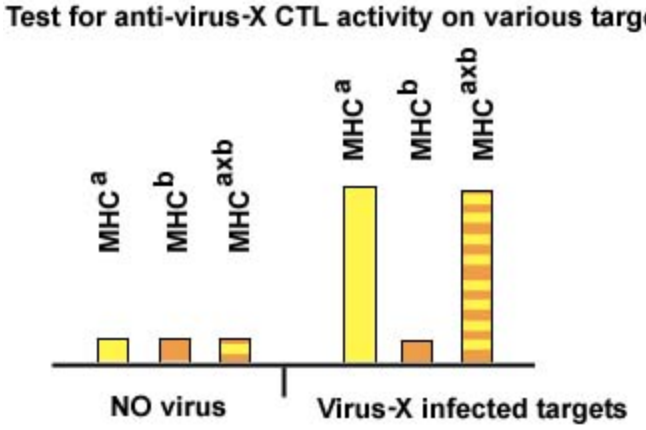
Conclusion: The MHC molecules present in the ***environment*** where T cells mature determine the MHC restriction of the mature T-cell receptor repertoire.

The environment that determines the restriction specificity is the Thymus



Thymic environment changes from MHC^{axb} to MHC^a; whereas T cells are MHC^a and MHC^b

The thymic epithelium is MHC^a. All other the tissues in this chimera, including CTL cells, are both MHC^a and MHC^b



Results

CTLs kill MHC^{axb} infected cells
CTLs kill MHC^a infected cells
CTLs cannot kill MHC^b infected cells

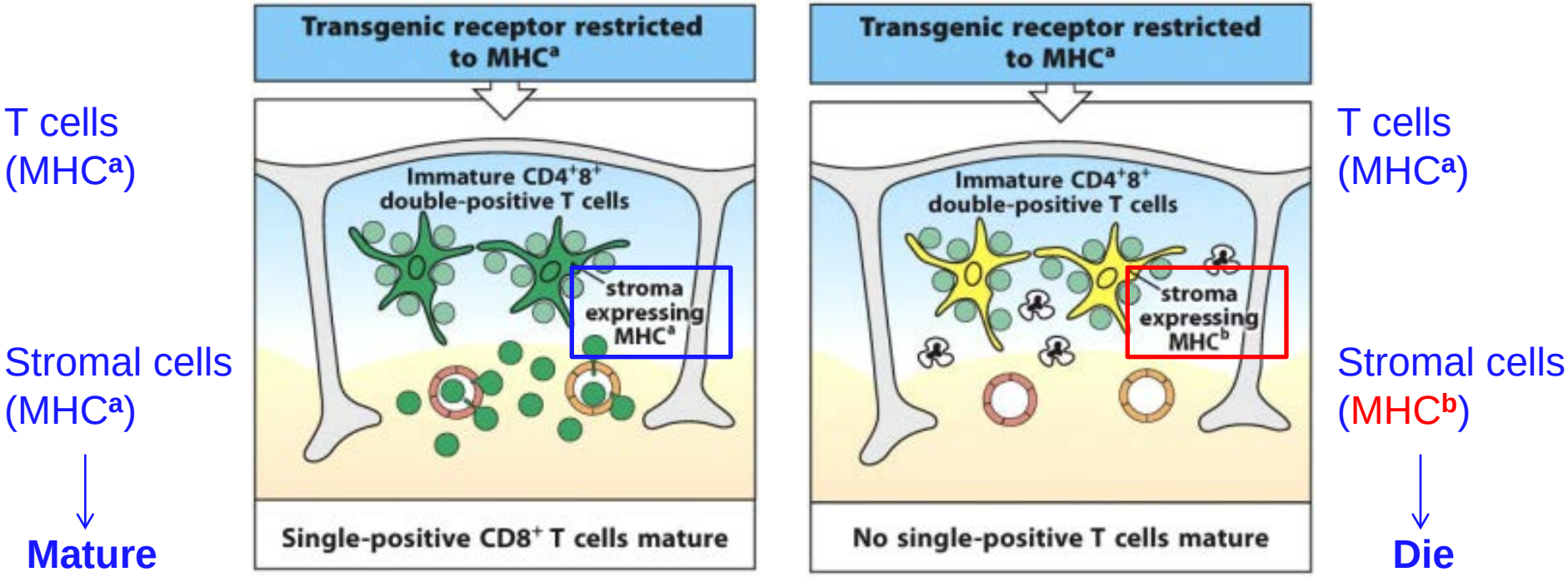
Conclusion

The CTLs are MHC^a and MHC^b but **they are restricted to MHC^a only**.

Thus, the **thymus** determines restriction specificity

Fig. 8-29

(+) Selection is demonstrated by the development of T cells expressing rearranged TCR transgenes



- Developing T cells will survive and mature ONLY if they express (or "are restricted to") the SAME MHC type as the thymus stromal cells.

The MHC molecules inducing (+) selection determine co-receptor specificity

Fig. 8-30

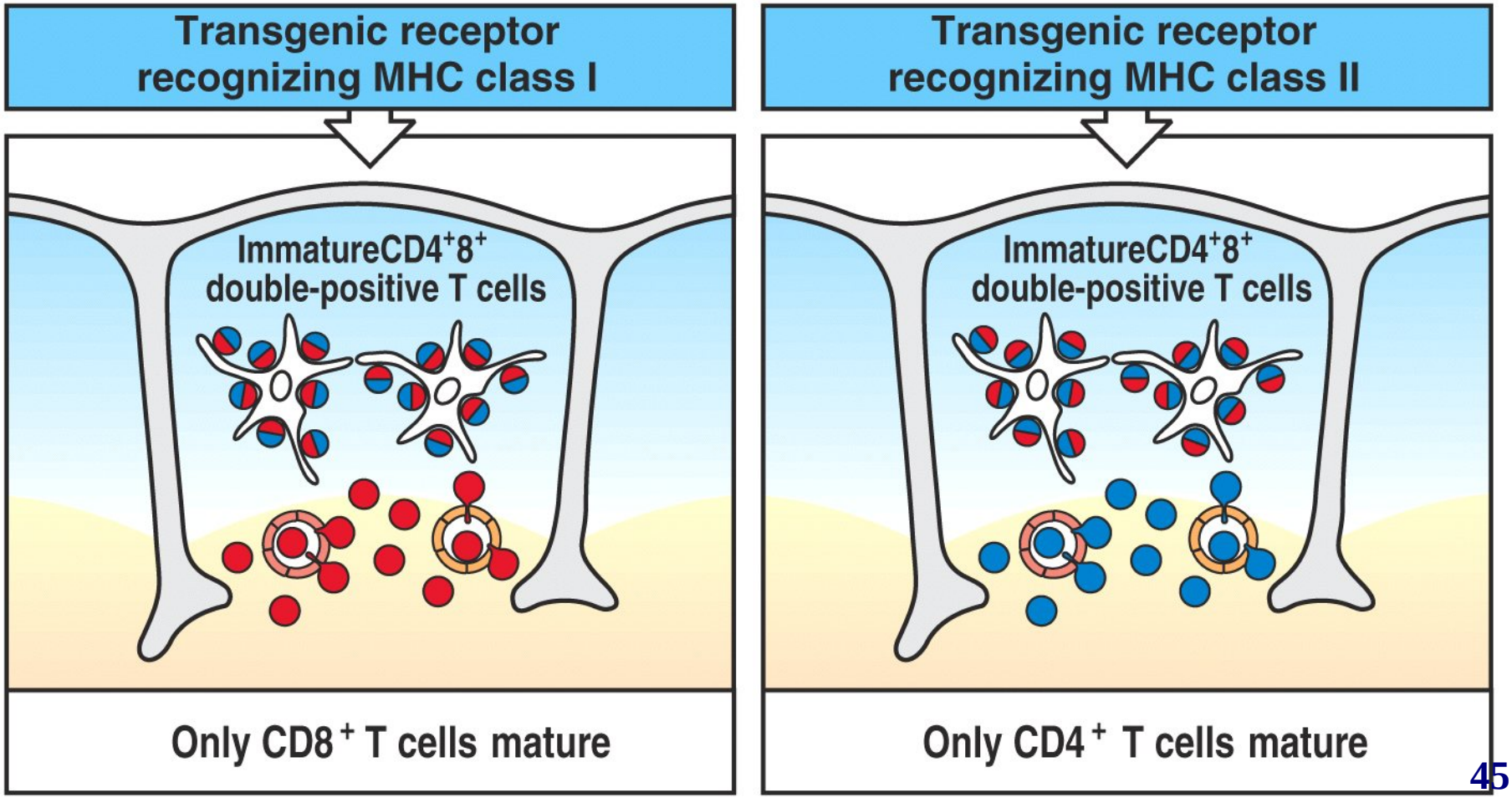
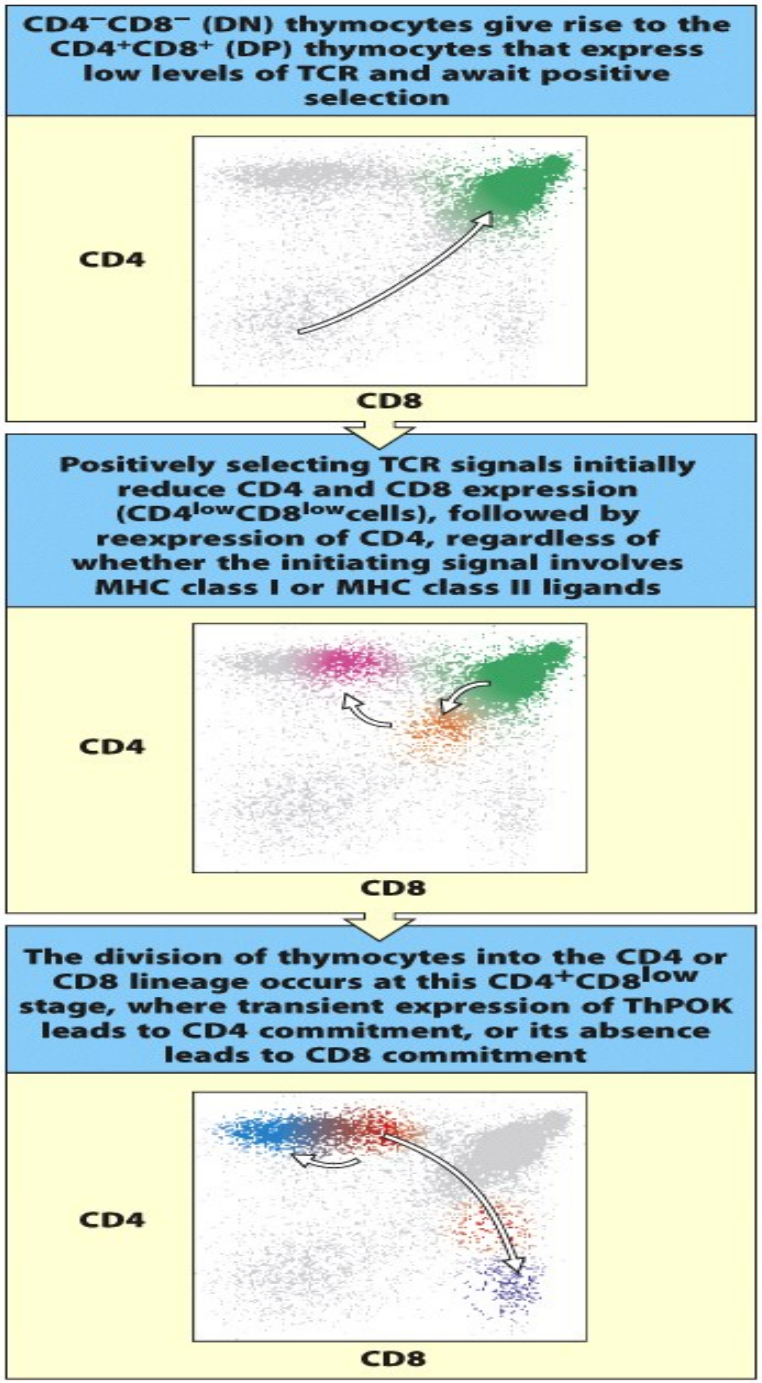
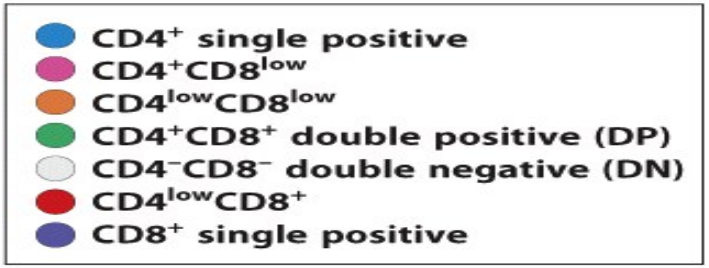


Figure 7-30 Immunobiology, 6/e. (© Garland Science 2005)

Fig. 8-31

Molecular map of T-cell development





Double-negative

↓

Double-positive

↓

Single positive

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Thymic cortical epithelial cells mediate (+) selection

Fig. 8-32

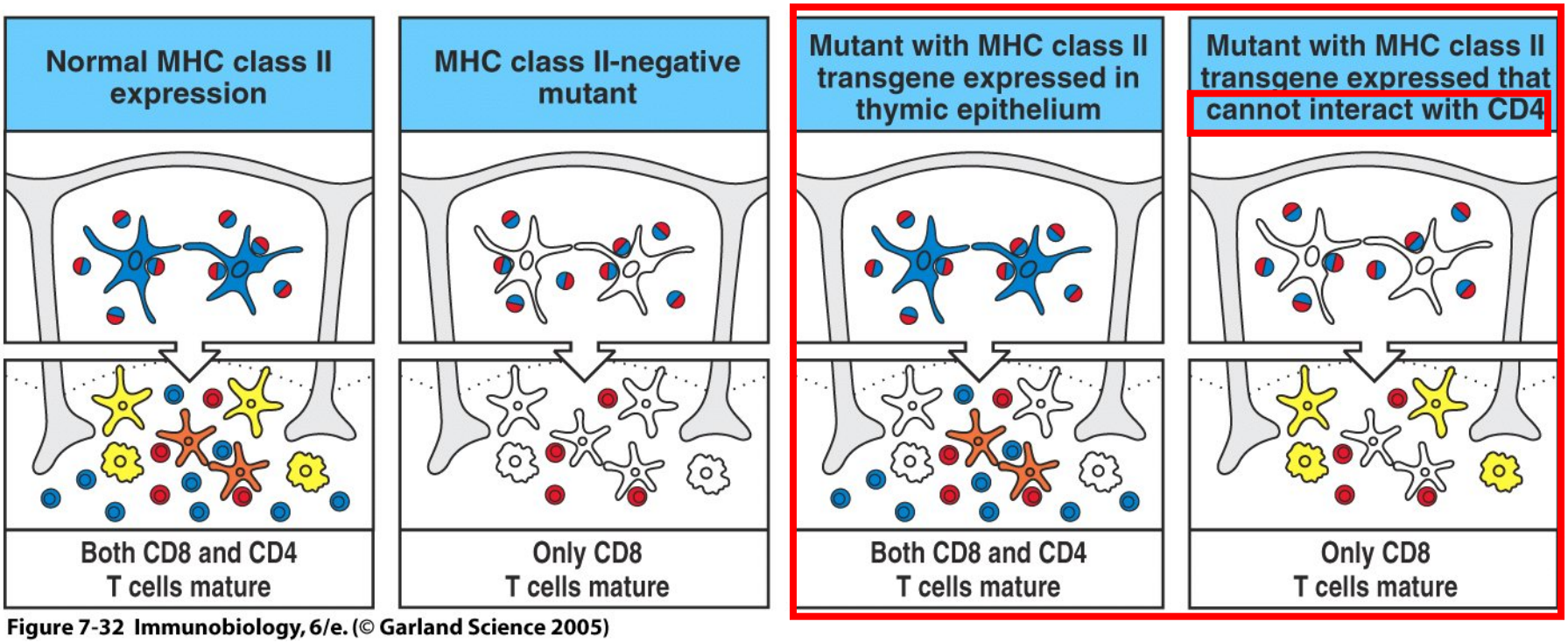


Figure 7-32 Immunobiology, 6/e. (© Garland Science 2005)

- Epithelial cells residing in the thymic cortex
  - Responsible for the interaction of MHC molecules with CD4 (or CD8) molecules → positive selection

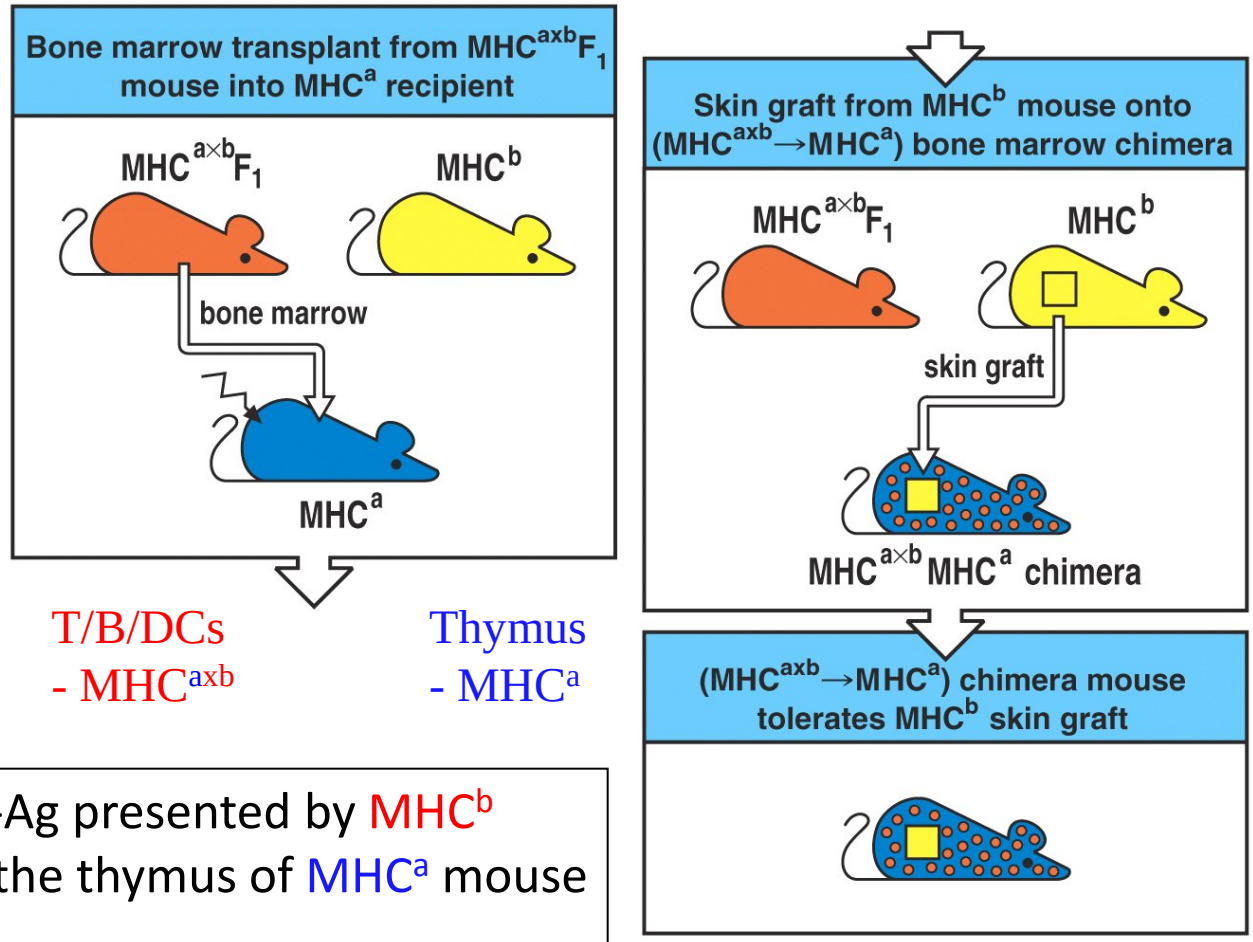
How does ‘positive selection’ affect T cells?

1. Lets cells live
2. Affects CD4 and CD8 expression
3. Affects function (CTL vs T_H)

(not TH1 or TH2, that choice comes later)

BM-derived cells mediate (-) selection of T cells in the thymus

1. Thymic epithelium mediates (+) selection (see previous slides)
2. But, BM-derived cells mediate (-) selection in the thymus

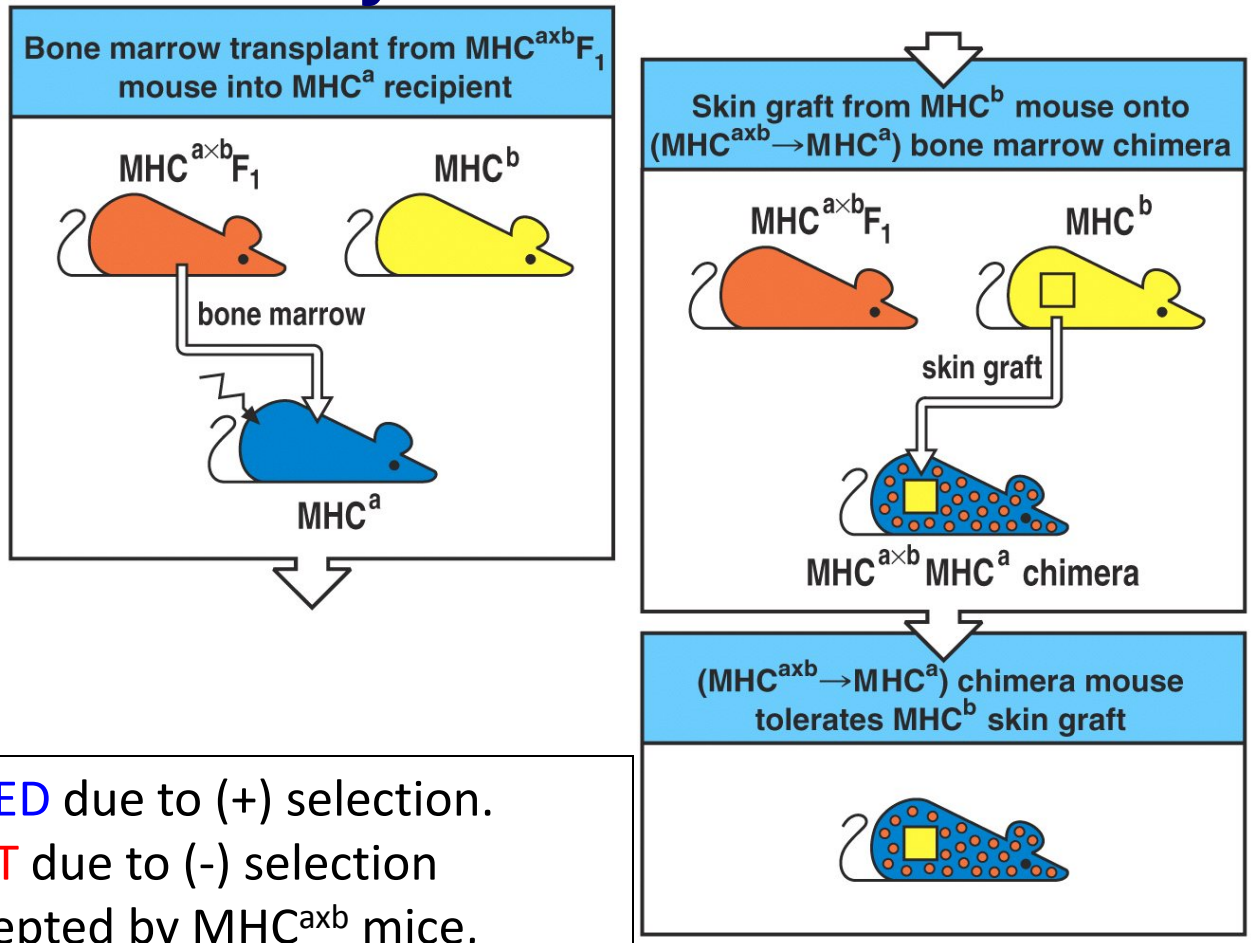


1. T cells recognizing self-Ag presented by MHC^b must be eliminated in the thymus of MHC^a mouse (via apoptosis)
2. BM was the only source for MHC^b molecules in the thymus

→ (-) selection is induced by BM-derived cells

Fig. 8-35

BM-derived cells mediate (-) selection of T cells in the thymus



1. T cells are RESTRICTED due to (+) selection.
2. T cells are TOLERANT due to (-) selection
3. MHC^b grafts are accepted by MHC^{axb} mice, because of negative selection against all thymocytes that react with self peptides and MHC^a or MHC^b.

Fig. 8-35

$MHC^a \rightarrow MHC^b$ radiation chimera cannot function without MHC^b APCs present in thymus during T cell maturation

MHC^a bone marrow into irradiated MHC^b mouse

	Bone marrow donor (T, B, DCs, M)	Recipient (irradiated)	Mice contain APC of type: (B, DCs, M)	Secondary T-cell responses to antigen presented *in vitro* by APC of type:	
				MHC^a APC	MHC^b APC
(A)	$MHC^{a\times b}$	MHC^a	$MHC^{a\times b}$	Yes	No
(B)	$MHC^{a\times b}$	MHC^b	$MHC^{a\times b}$	No	Yes
(C)	MHC^a	MHC^b	MHC^a	No	No
(D)	MHC^a	MHC^b + MHC^b APC	MHC^a + MHC^b	No	Yes

Figure 7-29 Immunobiology, 6/e. (© Garland Science 2005)

Obtained from healthy MHC^b mouse

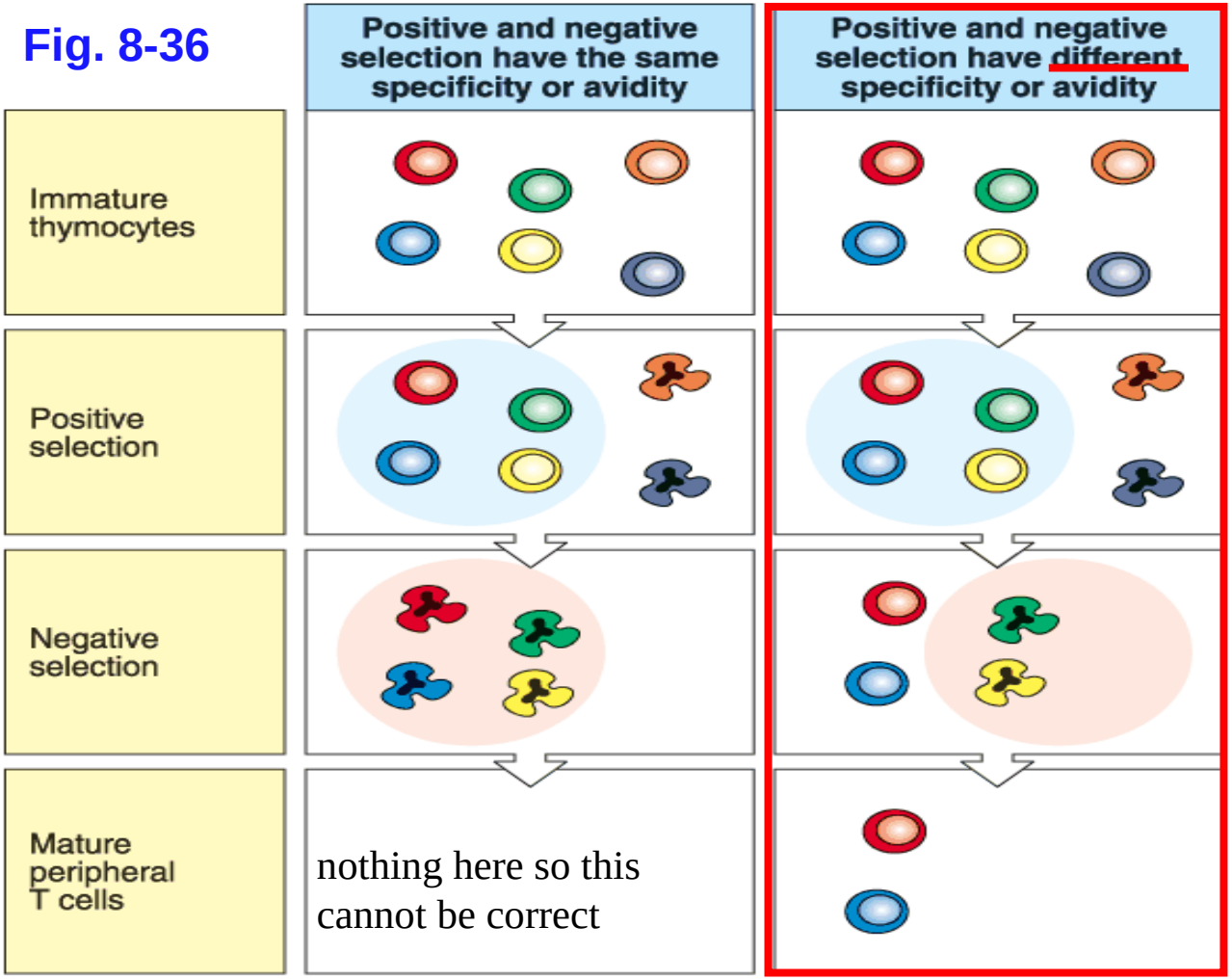
(C) is called "immune-incompetent fully allogenic chimera"

→ Thymus is MHC^b ,so T cells MHC^b-restricted .

→ However, the professional APCs (B, DC, and Macrophages) are MHC^a -restricted (BM-derived) so APCs present Ag in association with MHC^a. The T cell ***cannot*** interact with the APC and thus there are NO adaptive immune responses.

→ Interaction with APC of the same MHC restriction in thymus is also crucial!!

Fig. 8-36



Avidity hypothesis

Not differential signaling

Positive and negative selection can be successful if each is governed by different avidities (e.g., low avidity for positive selection; high avidity for negative selection)

(+) selection: Thymocytes engaging peptide-MHC complexes on thymic epithelia → survive and mature

(-) selection: Thymocytes engaging self peptide-self MHC complexes (via BM-derived APCs) in the medulla → die

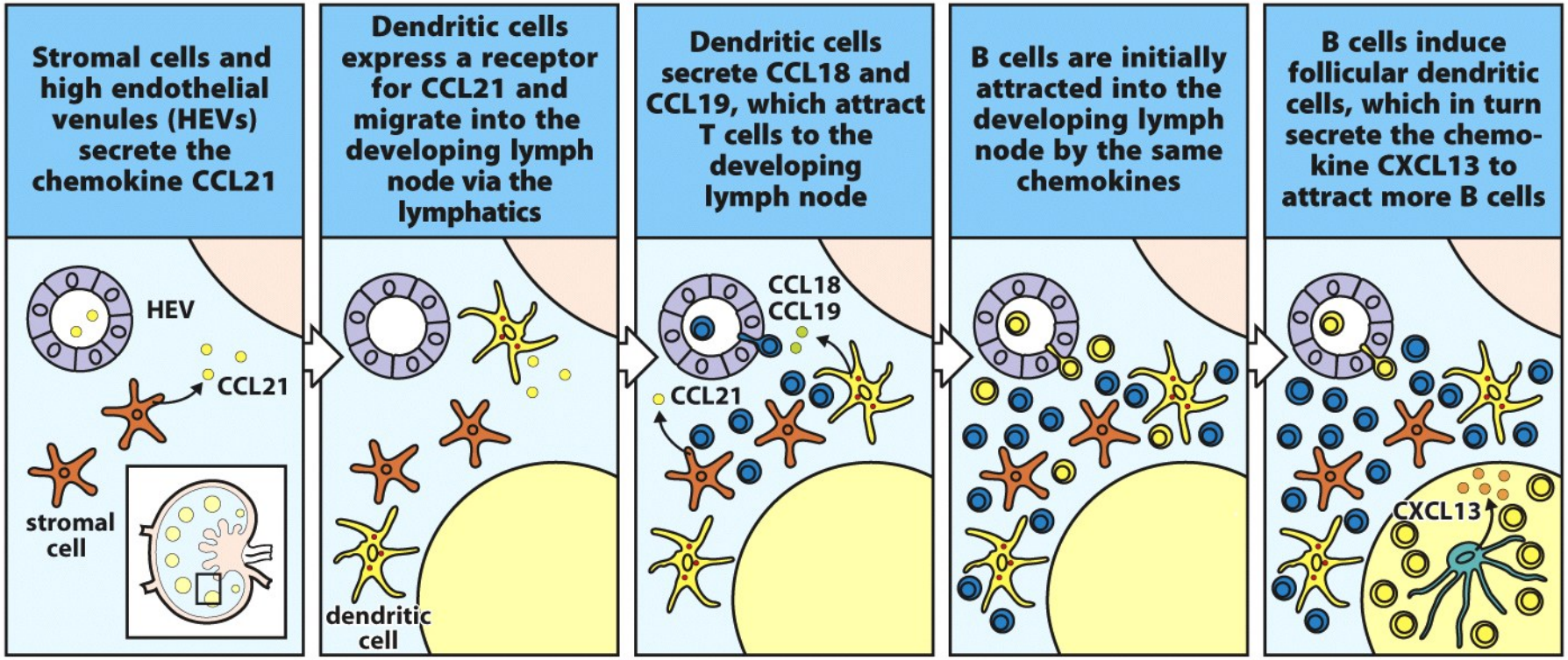
Summary of T cell selection

- Positive selection
  - T cells capable of engaging peptide:MHC on thymic epithelial will mature
  - Occurs in cortex
    - Cortex epithelial cells

- Negative selection
  - T cell which can be activated by self-peptide:MHC will die
  - Occurs in medulla
    - Bone marrow-derived cells (e.g. dendritic cells and macrphoages)

Survival of lymphocytes in the peripheral lymphoid tissues

Chemokines/lymphkines are important in orchestrating lymphoid organization



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Chemokines CCL21 and CCl19 are the two important cytokines for recruiting T cells for the formation of the T-cell zone

Stromal cells

(Interdigitating) dendritic cells

T cells

B cells

Follicular dendritic cells (FDCs)

Survival of B and T cells requires that they circulate through lymphoid organs.

1. B cell survival signal comes from the continuous circulation of B cells through lymphoid organs.
2. Without antigenic stimulation in the lymphoid organs, most B cells die shortly after leaving the bone marrow; some will survive several weeks (3-8 weeks).
3. B cell will ***not*** divide in the periphery without antigenic stimulation.
4. Memory B cells can survive for many years.

Types of B cells in the peripherals

1. Conventional B cells (B-2 cells)
   - produced after birth
   - reside in B-cell follicles

2. B-1 cells
   - produced during fetus stage
   - ~5% of all B cell populations
   - not present in B-cell follicles

3. Marginal zone B cells
   - produced after birth
   - not present in B-cell follicles

Property	B-1 cells	Conventional B-2 cells	Marginal zone B cells
When first produced	Fetus	After birth	After birth
N-regions in VDJ junctions	Few	Extensive	Yes
V-region repertoire	Restricted	Diverse	Partly restricted
Primary location	Body cavities (peritoneal, pleural)	Secondary lymphoid organs	Spleen
Mode of renewal	Self-renewing	Replaced from bone marrow	Long-lived
Spontaneous production of immunoglobulin	High	Low	Low
Isotypes secreted	IgM >> IgG	IgG > IgM	IgM > IgG
Response to carbohydrate antigen	Yes	Maybe	Yes
Response to protein antigen	Maybe	Yes	Yes
Requirement for T-cell help	No	Yes	Sometimes
Somatic hypermutation	Low to none	High	?
Memory development	Little or none	Yes	?

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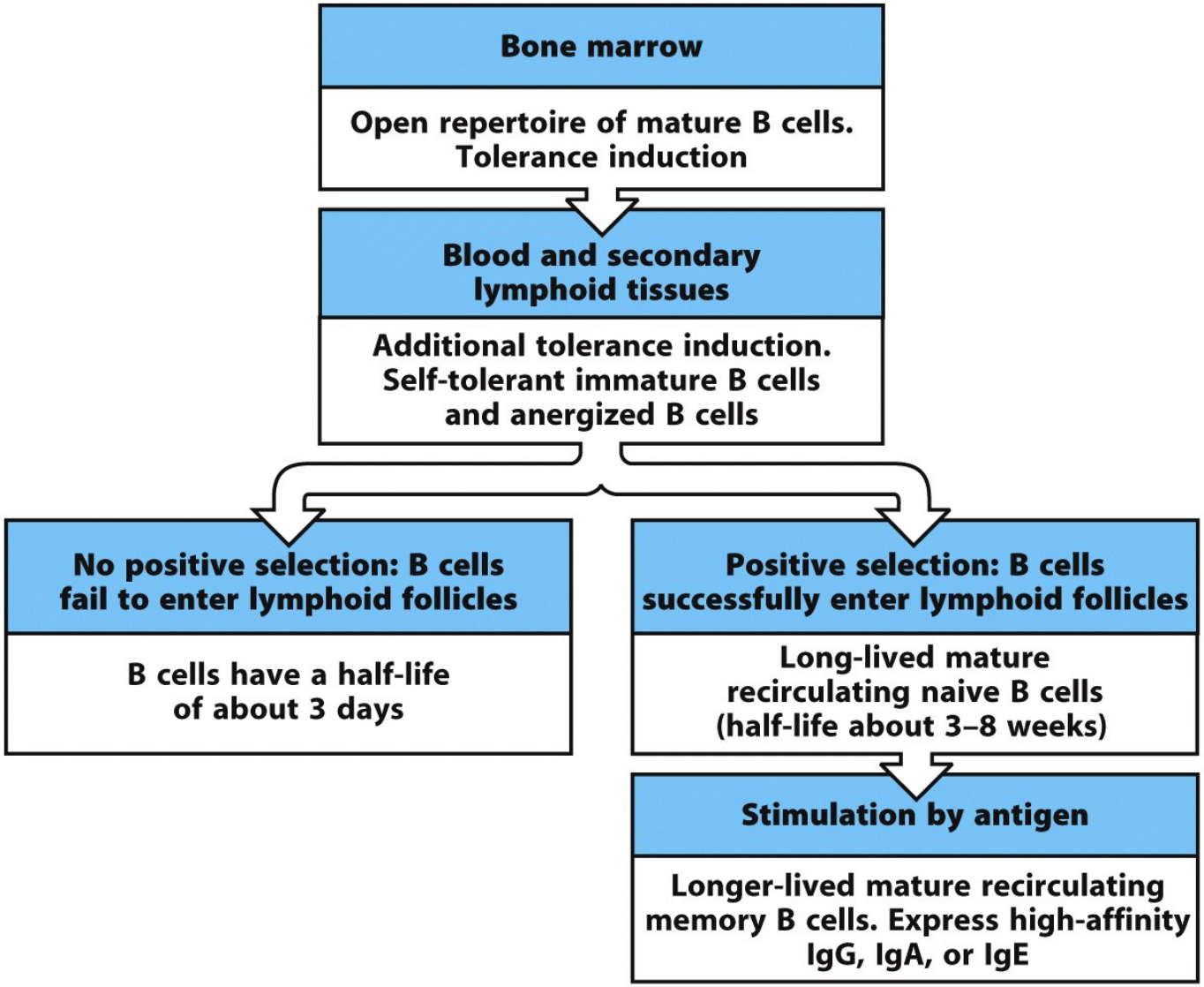
Proposed population dynamics of conventional B cells



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Survival of T cells requires that they circulate through lymphoid organs.

1. Mature naïve T cells can divide in the periphery
    - This characteristic is different from B cell !!
    - They probably get stimulation via *weak binding* to **self-peptide** and **MHC-I or –II molecule** (similar to positive selection in the thymus)
2. Memory T cells can divide without (MHC + peptide) stimulation.

Summary

- The rearrangement of antigen-receptor gene segments controls lymphocyte development.
- Interaction with self antigens selects some lymphocytes for survival but eliminates others.
- Mature lymphocytes require signals for their survival in peripheral lymphoid tissues.

End of Chapter