LYMPHOID ORGANS AND LYMPHOCYTE HOMING

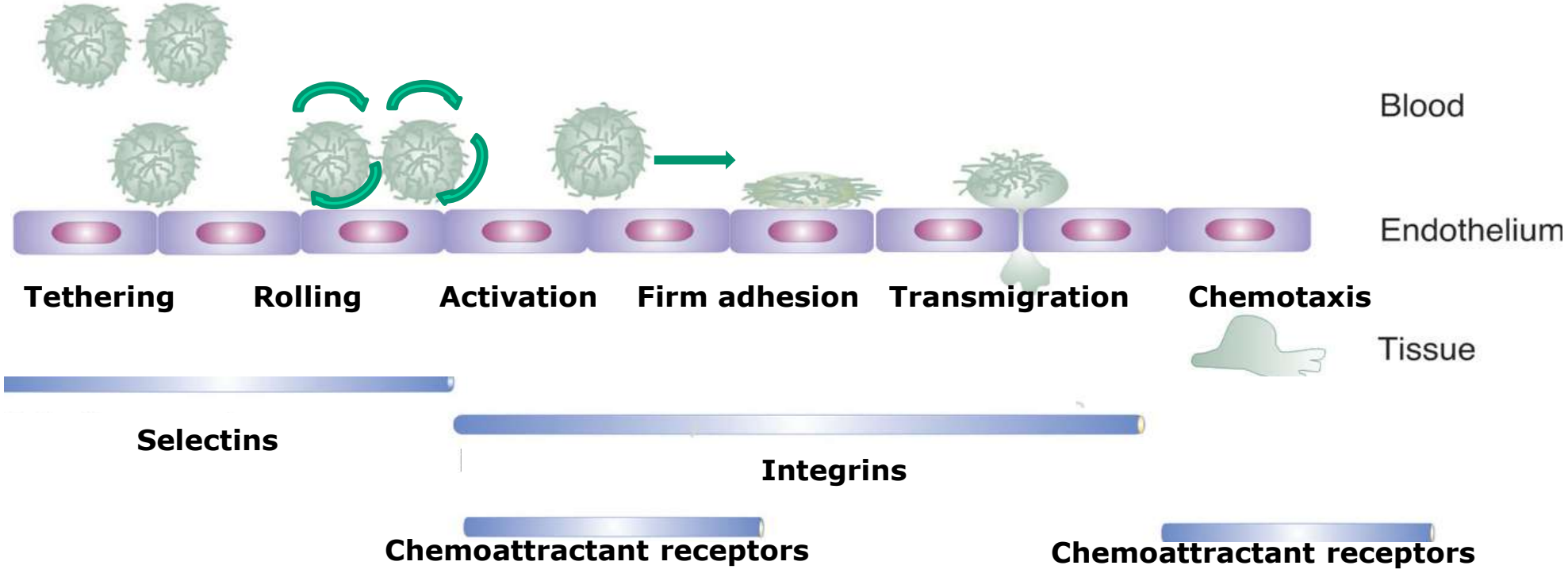
November 5th 2025

The dynamic life of lymphocytes

How is the immune system anatomically organized?

What role does cell migration play in the immune system and how?

Multistep paradigm of transendothelial migration



How is the immune system anatomically organized?

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The immune system consists of:

Cells of the adaptive/acquired compartment (**B and T lymphocytes**) and of the innate compartment with accessory and /or effector function (i.e. **mononuclear phagocytes, granulocytes, Natural Killer cells**)

Different organs distributed throughout the body that function as generation (maturation) sites, deposits or transit/information stations of immune cells

A system of **blood vessels** and **lymphatic vessels** that forms interconnections between these organs allowing their functional union

PRIMARY lymphoid organs are the maturation sites of immune cells in the absence of exogenous antigen

- **Thymus** is the site of maturation of T lymphocytes

- **Bone marrow** is the site of production of mature B lymphocytes in mammals as well as the site where all **hematopoietic cells** are generated.

Hematopietic system is responsible for the continuous production of blood circulating mature cells

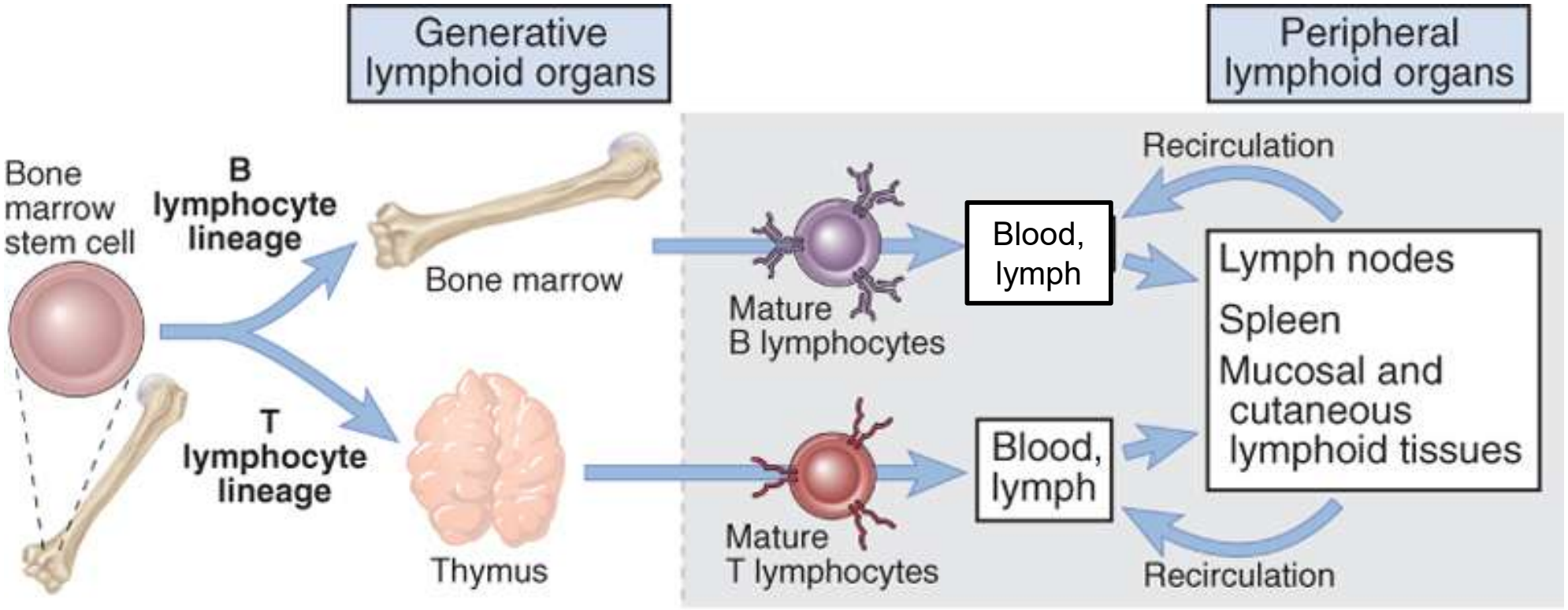
SECONDARY (or peripheral) lymphoid organs are the sites where mature lymphocytes differentiate in a manner dependent on **exogenous antigens**:

- The main function of the **lymph nodes** is to respond to the antigens introduced into the tissues connected to them through the lymphatic vessels

- The main function of the **spleen** is to respond to the blood antigens

- **MALT (Mucosal-associated Lymphoid tissue)** protects against pathogens in the most important entry points (mucosa of the gastro-intestinal tract and respiratory tract)

Differentiation of mature lymphocytes in primary lymphoid organs and recirculation in seconary lymphoid organs



Following maturation, naive (mature) T and B lymphocytes make a cyclical path from blood into lymphoid organs and reverse until recognition of the antigen.

When activated, they differentiate into effector or memory cells and change their itinerary.

What is the lymph?

The blood capillaries of many tissues have micro-leaks.

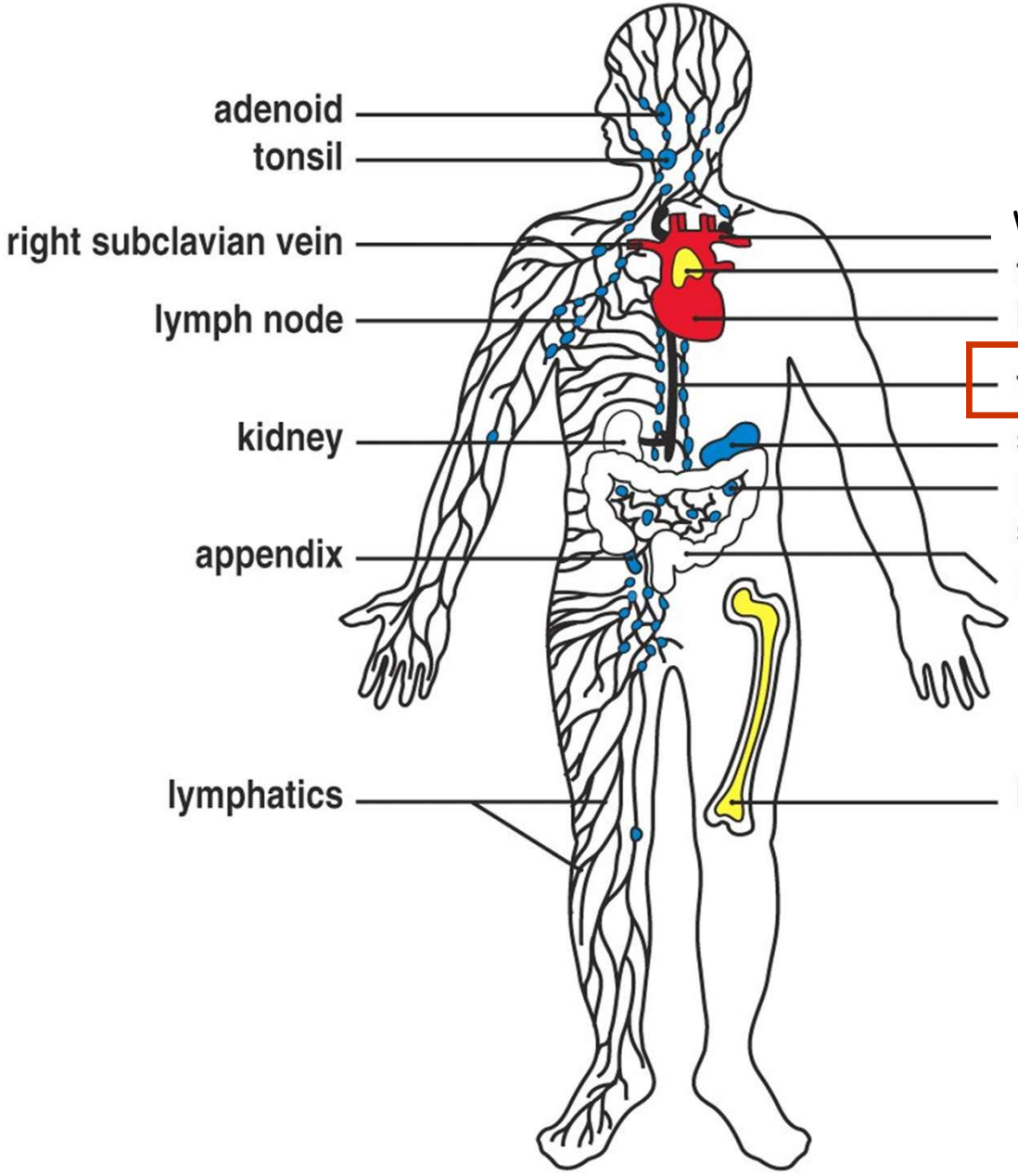
This involves the daily passage of 20 liters of protein-poor fluids into the extra-vascular space.

About 90% of these fluids are reabsorbed locally while the remaining 2 liters return to the circulation through the lymphatic vessels.

The passage of interstitial fluids (lymph) through lymph vessels of ever increasing caliber ends in the **thoracic duct**.

Eventually, the lymph spills into the bloodstream mainly through the **left subclavian vein**

Human lymphoid system



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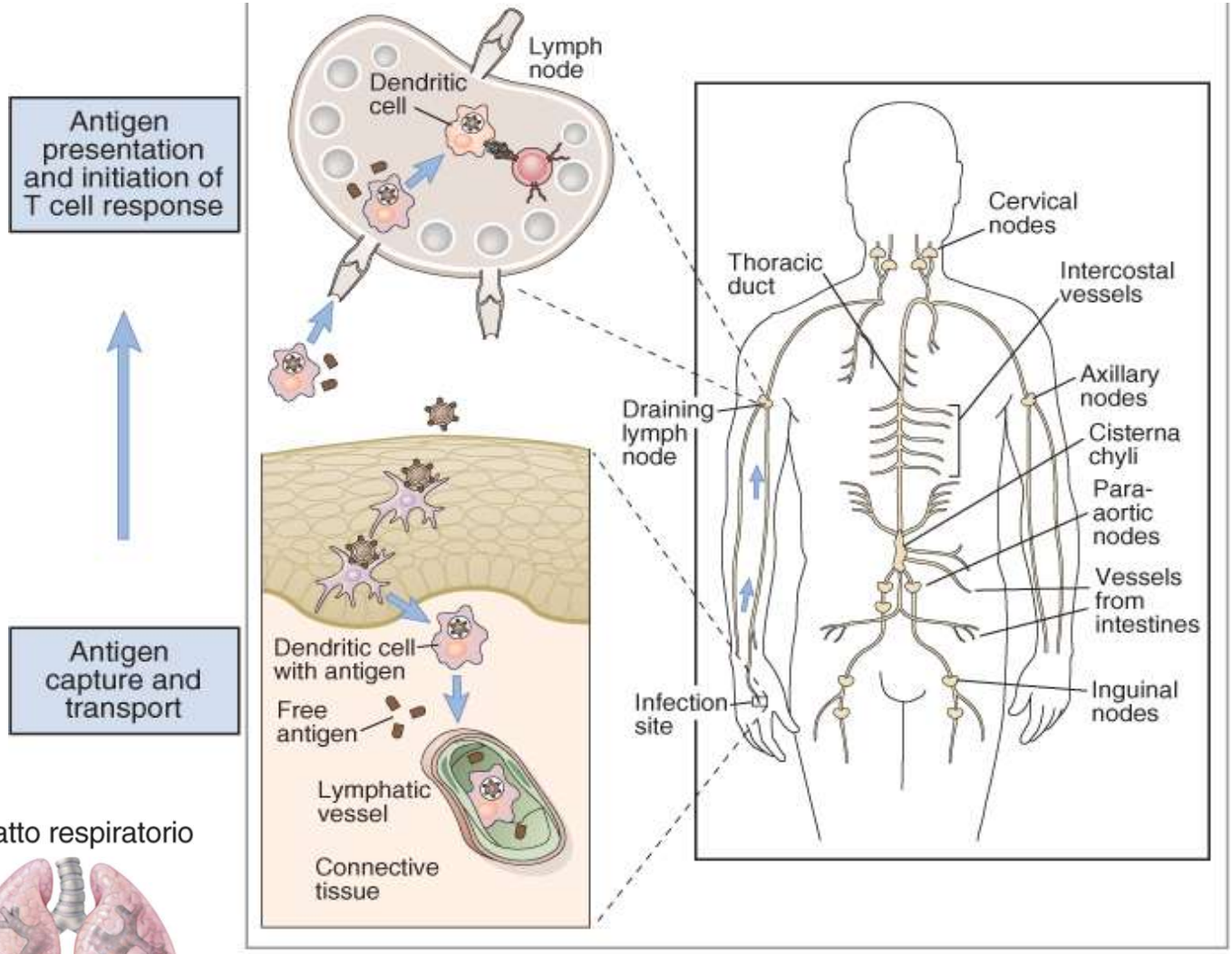
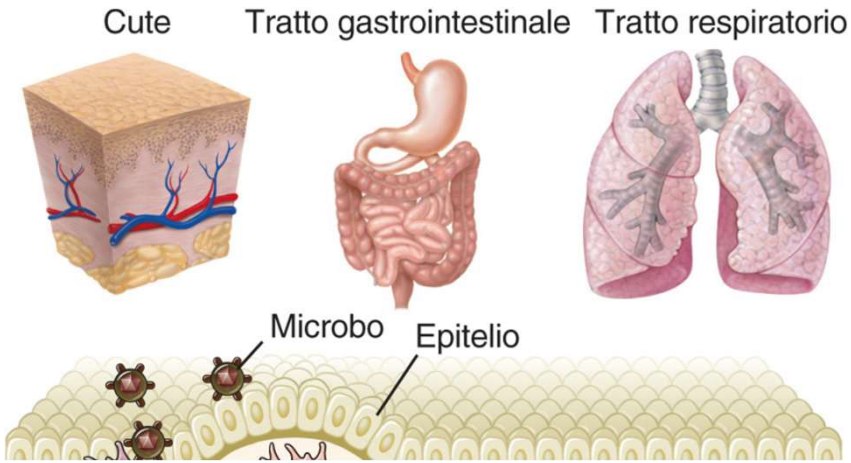
Lymph nodes are located along lymphatic vessels

THE ROUTES OF ANTIGEN ENTRANCE AND TRANSPORTATION TO THE SECONDARY LINFOID ORGANS





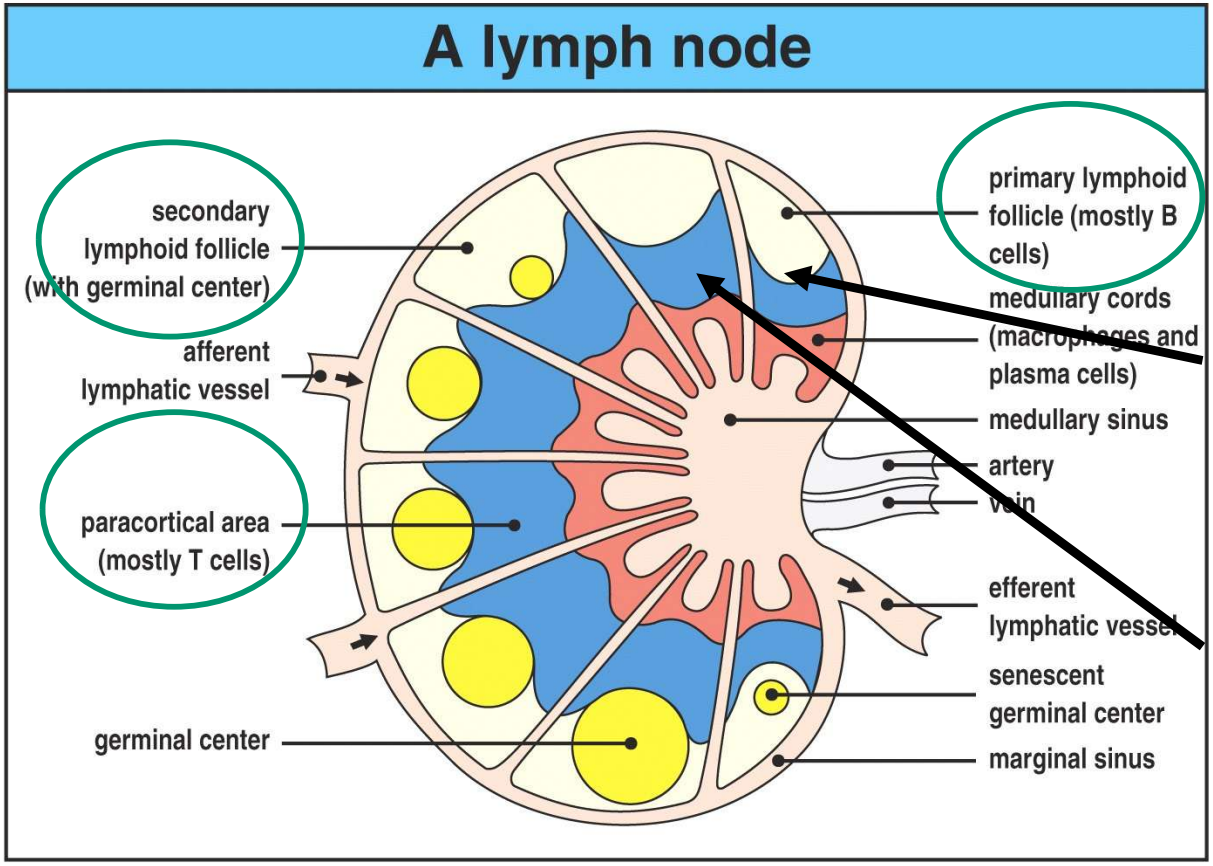
The lymphatic system

Lymph nodes are regularly found along lymphatic vessels and act as filter controllling lymph before it reaches the bloodstream.

Secondary lymphoid organs

Organization in anatomical sites

Lymph node structure



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It is coated with a capsule made up of collagen fibers.
The lymph flows into the **subcapsular sinus** carrying lymphocytes, dendritic cells and macrophages and soluble antigens
Follicles with germinal centers are present in the **cortical area** in which the B cells mature in plasma cells after activation.

T lymphocytes and dendritic cells are predominantly located in the **paracortical area**

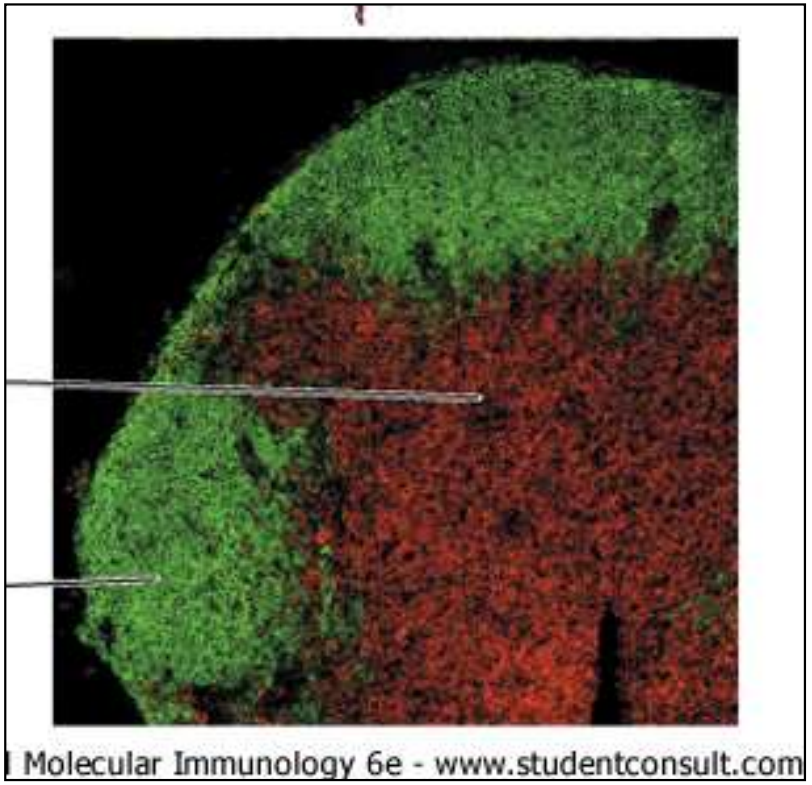
The **medullary zone** contains predominantly plasma cells and macrophages

Follicular Dendritic Cells (FDC) are stromal cells of **non**-haematopoietic origin **resident in follicles** where they have the function of 1) presenting an intact antigen to B lymphocytes to select effector and memory B cells and 2) maintaining B lymphocytes in the follicle.

The **anatomical segregation** of lymphocytes in the cortical (follicle) and paracortical areas favors the appropriate responses to the antigens because each lymphocyte population is in close contact with its own accessory cells

Red: **T cells**
(interact with DC)

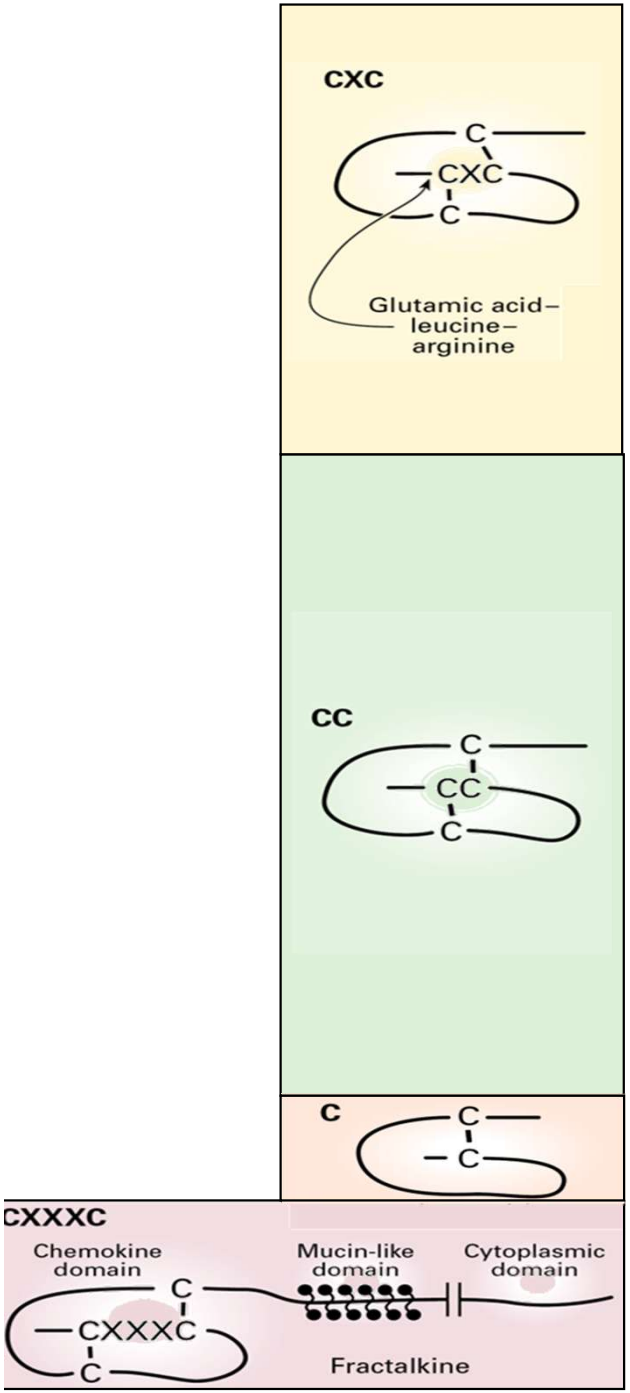
Green: **B cells**
(interact with FDC and Tfh cells



HOW IS IT OBTAINED?

A fundamental role is played by the interaction of chemoattractant molecules of the chemokine family with their receptors.

Chemokines



Chemokines are small secreted proteins with chemotactic activity.

… represent a family of molecules with high sequence homology whose structure and nomenclature depends on conserved cysteines. Depending on the motif associated with the first two cysteines, they have been classified into 4 classes

CXC o alpha

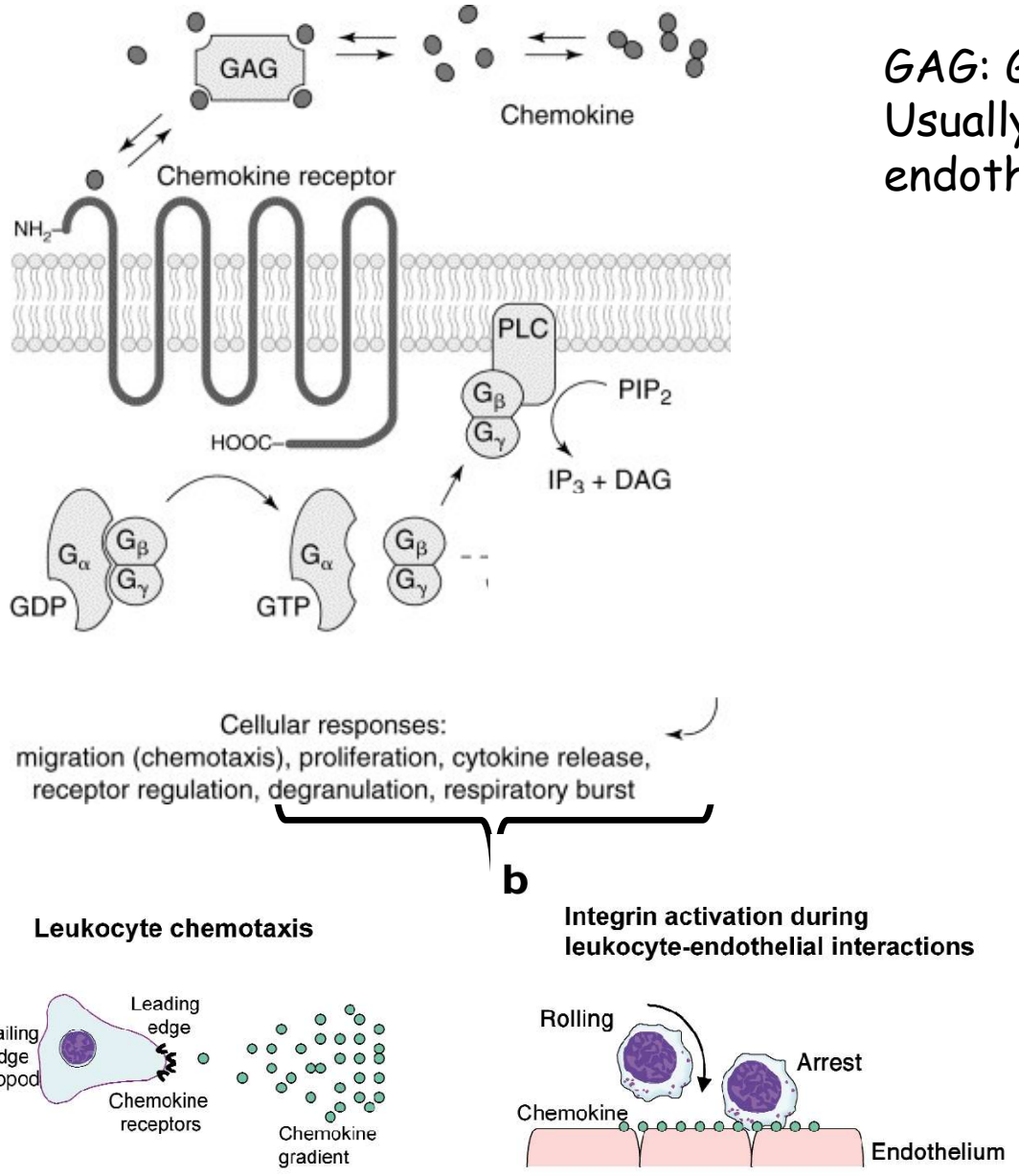
CC or beta

C or gamma

CX3C

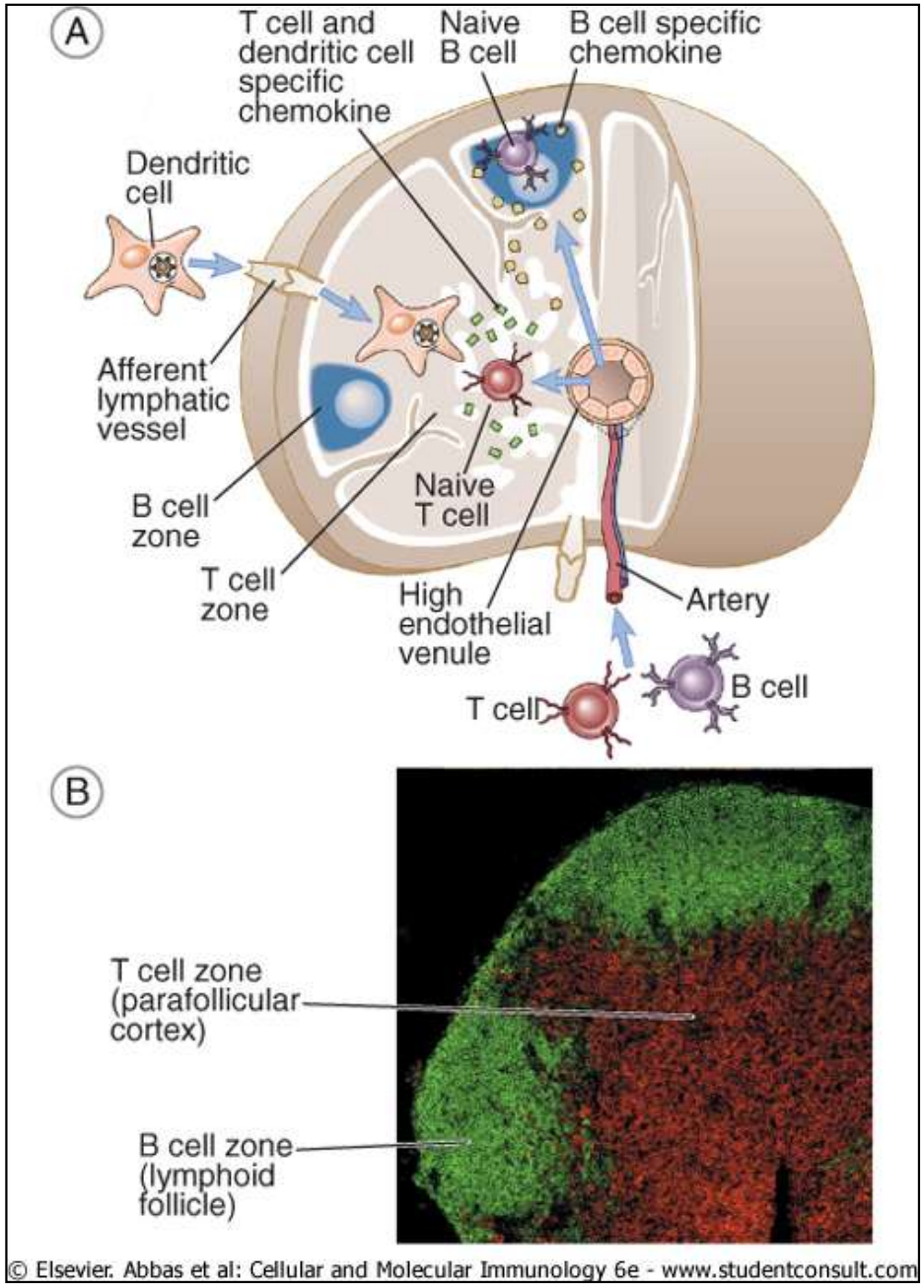
Chemokine receptors are classified based on the chemokine class they bind into: CC, CXC, CX3C and C receptors

Chemokines and chemokine receptors



GAG: Glycosaminoglycans
Usually exposed by endothelial cells

Anatomical segregation of lymphocytes



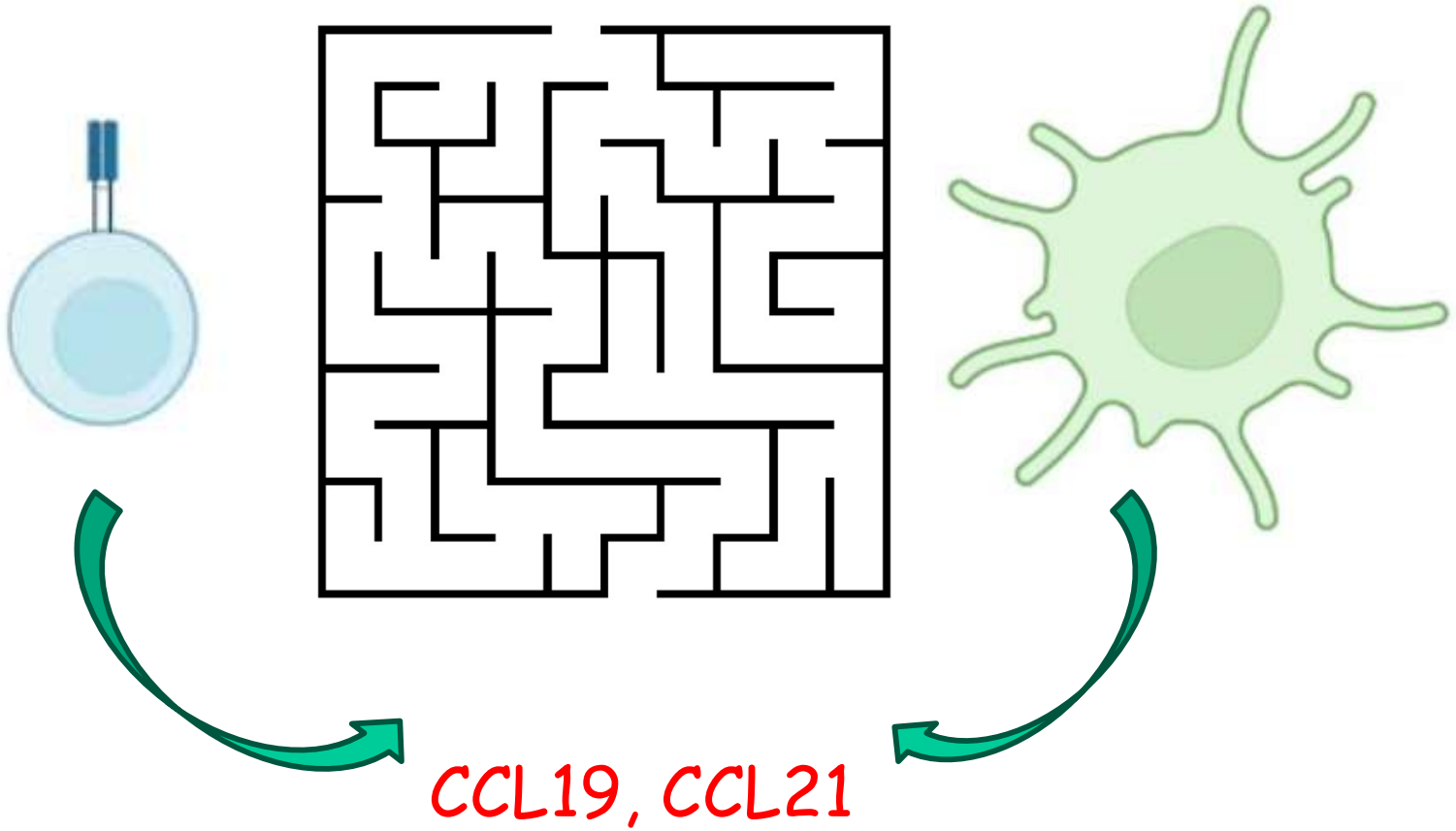
Chemokines play key roles in the segregation of lymphoid populations within lymphoid organs.

CCL19, CCL21 (produced by reticular fibroblasts) are ligands for the receptor **CCR7**, preferentially expressed by naïve T cells

…..mature dendritic cells express this receptor too.

CXCL13 (produced by follicular dendritic cells) binds **CXCR5**, a receptor expressed by naïve B cells in lymph nodes

Colocalization in chemokine-rich areas increases the likelihood of encounters between APCs and T cells

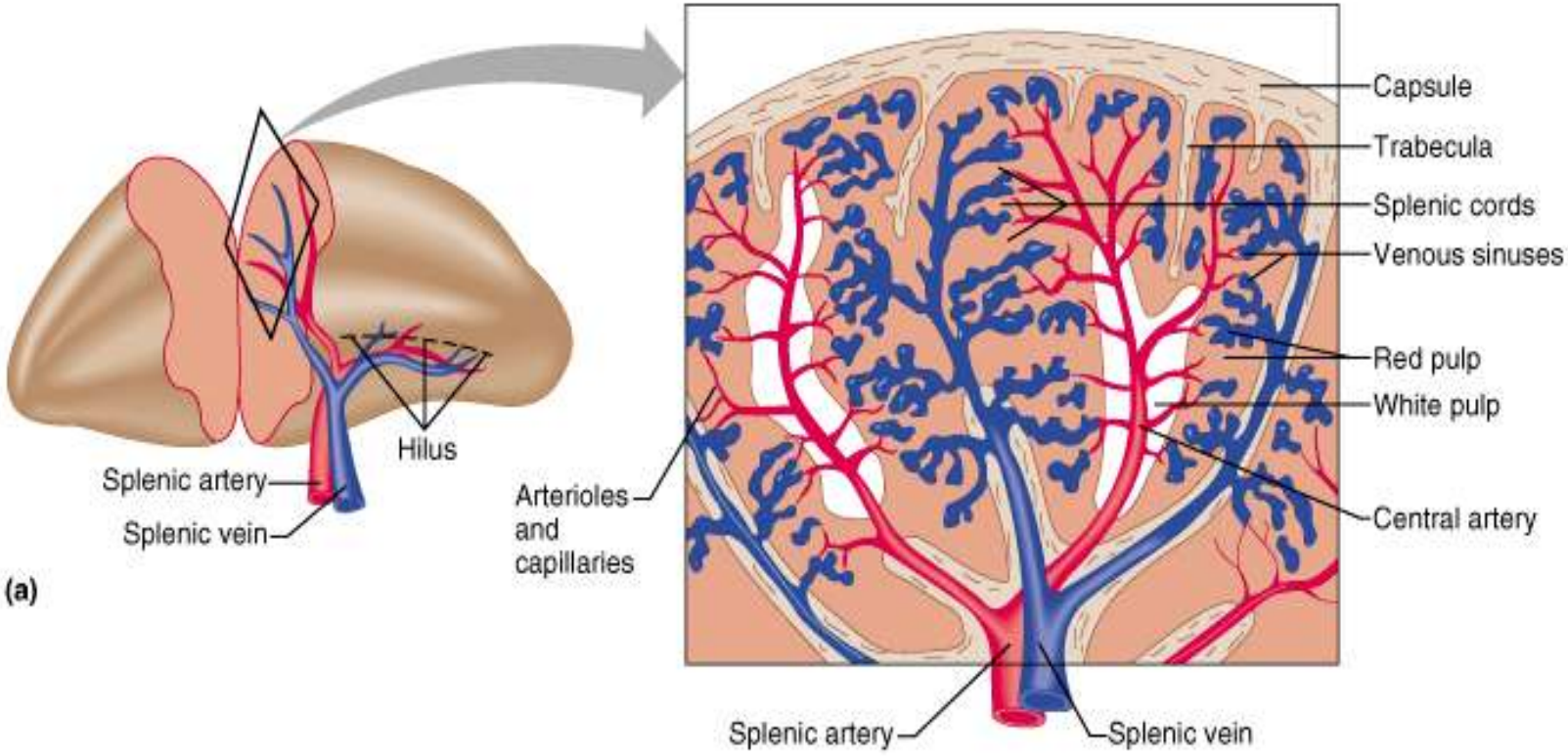


The spleen

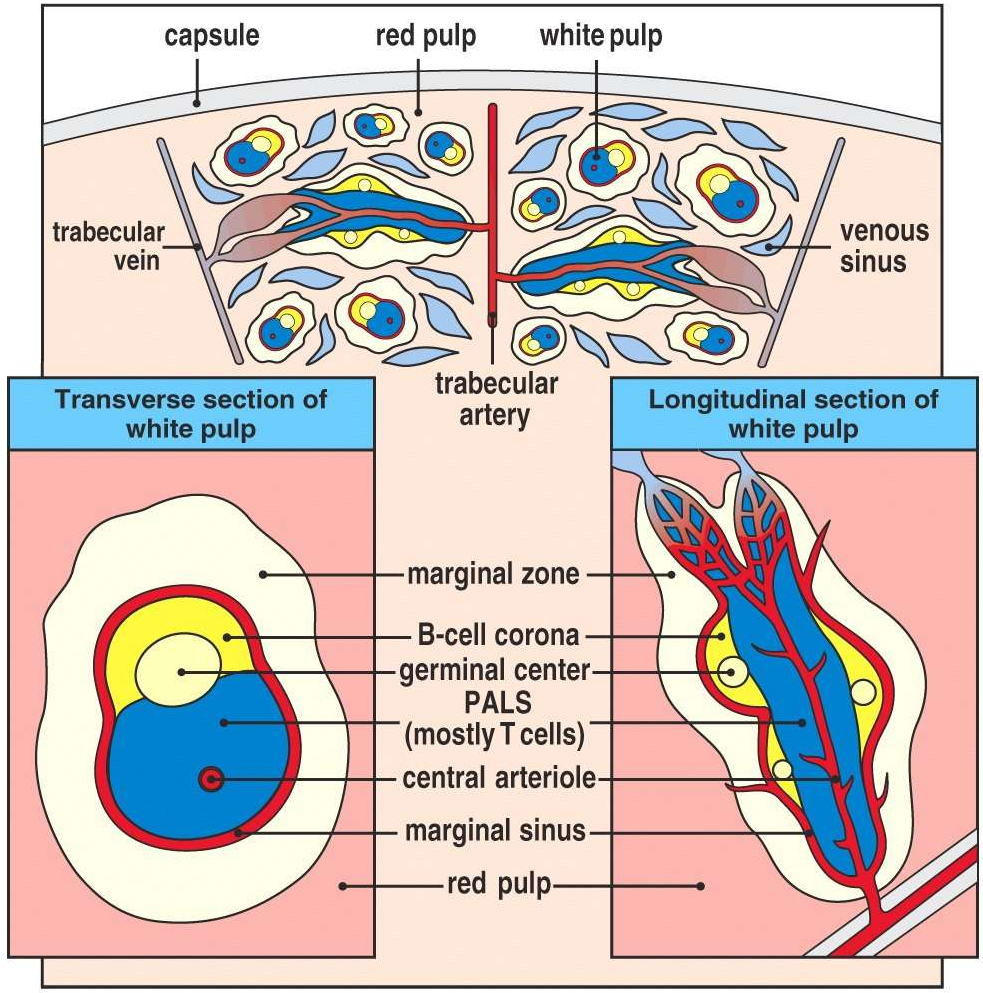
…. is composed of an external capsule of connective tissue and an internal structure divided into two parts:

Red pulp: containing venous vessels and macrophages and many red blood cells. 1) It eliminates defective red blood cells and remove debris from the blood 2) It is a reservoir of platelets and iron

White pulp: covers a small area where lymphocytes are located



White pulp function:
activate the responses to the antigens present in the blood



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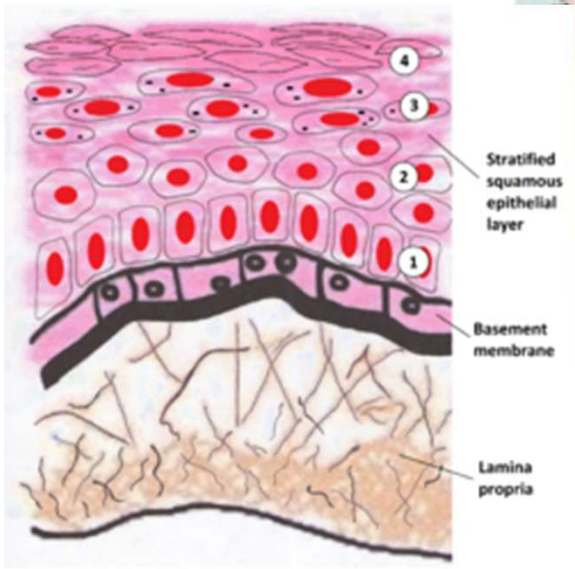
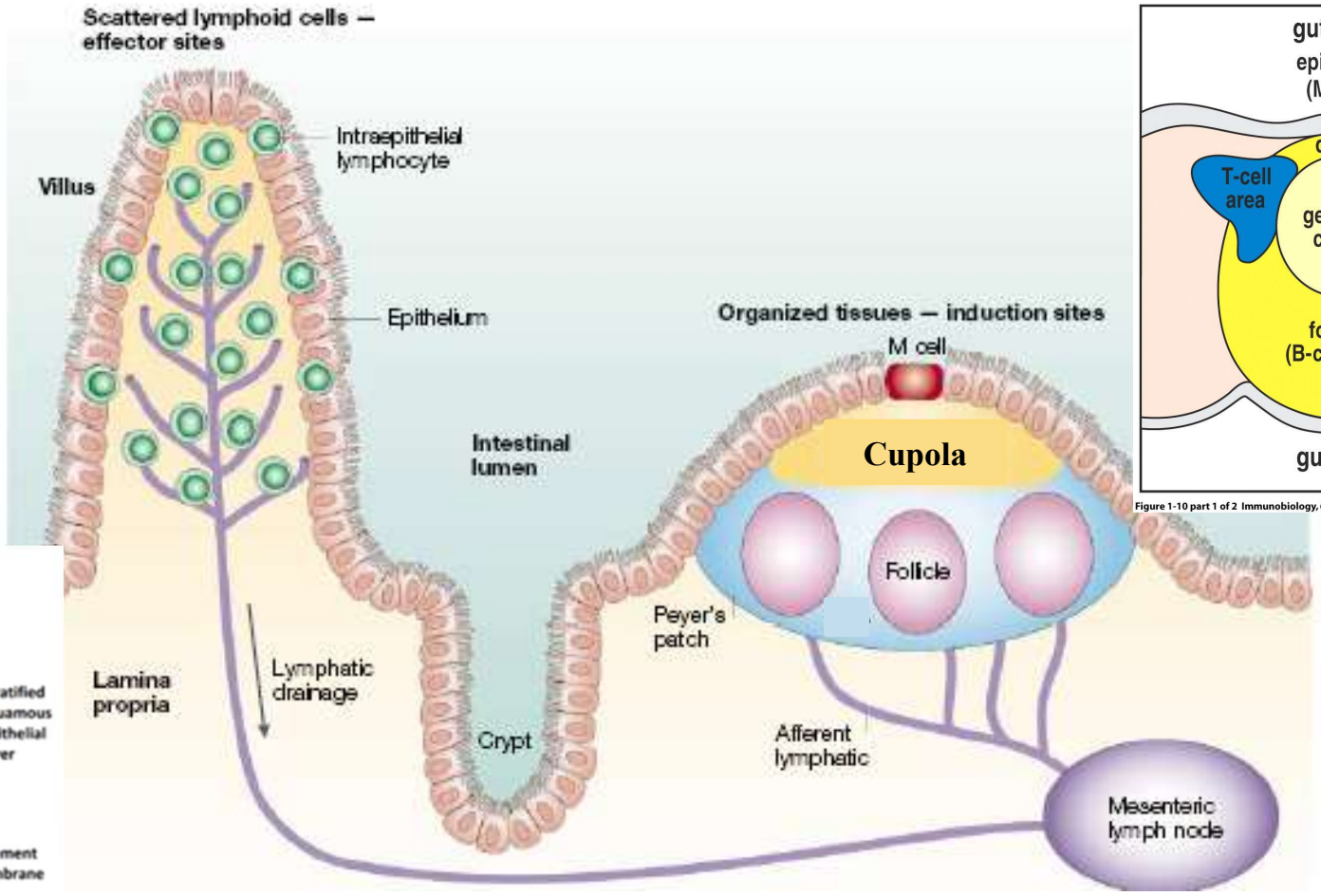
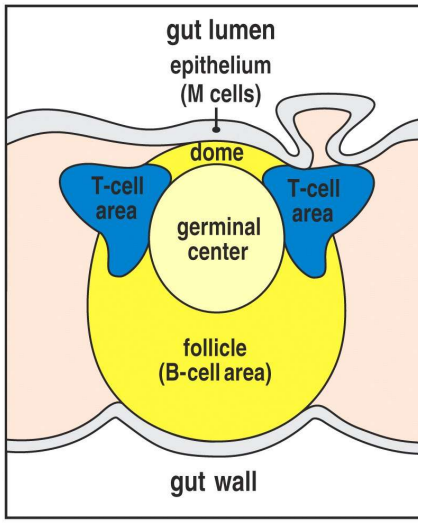
In the white pulp there is a trabecular artery from which the central arteriole originates (on their sides there are the periarteriolar lymphoid sheaths, called PALS, composed of T lymphocytes).

The B lymphocytes, with germinal centers, form a crown around the periarteriolar area. Specialized B lymphocytes are also present in the marginal area

The mucosa associated lymphoid tissue (MALT) consists of:
lymphocytes located in the epithelial layer or scattered in the lamina propria
The intestine includes also organized structures: the PEYER patches







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Organizzazione funzionale degli organi linfoidi secondari

	White pulp	**Lymphnodes**	**Peyer Patches**
Surveillance	Blood	Lymph	Intestinal content (local)
Antigen scanning zone	Marginal zone	Subcapsular zone	Follicle associated epithelium
T cell area	Periarteriolar lymphoid sheets	Paracortical zone	Interfollicular area
B cell area	Follicles	Cortical or follicular zone	Follicles e corona

Ricapitolando….

Che cosa si intende per organo linfoide secondario?

Che cosa è il dotto toracico?

Dove si trovano le cellule follicolari dendritiche?

Che funzione svolge la polpa bianca della milza?

Come fanno i leucociti circolanti ad entrare nei tessuti?

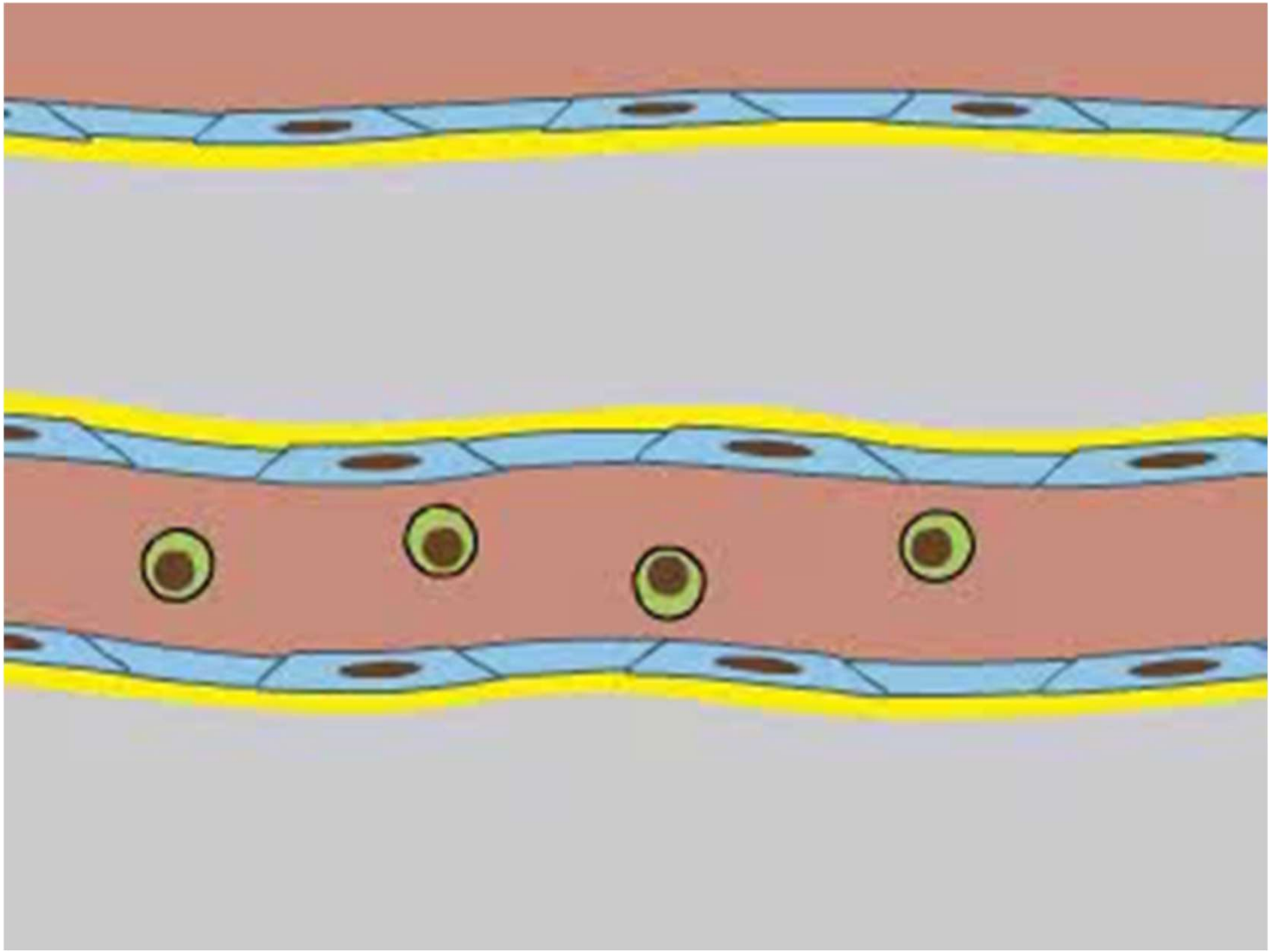
The role of migration in the immune system

- During maturation and differentiation
- In the immune surveillance that depends on constitutive and tissue-specific (or inducible) homing
- Inflammatory process regulation

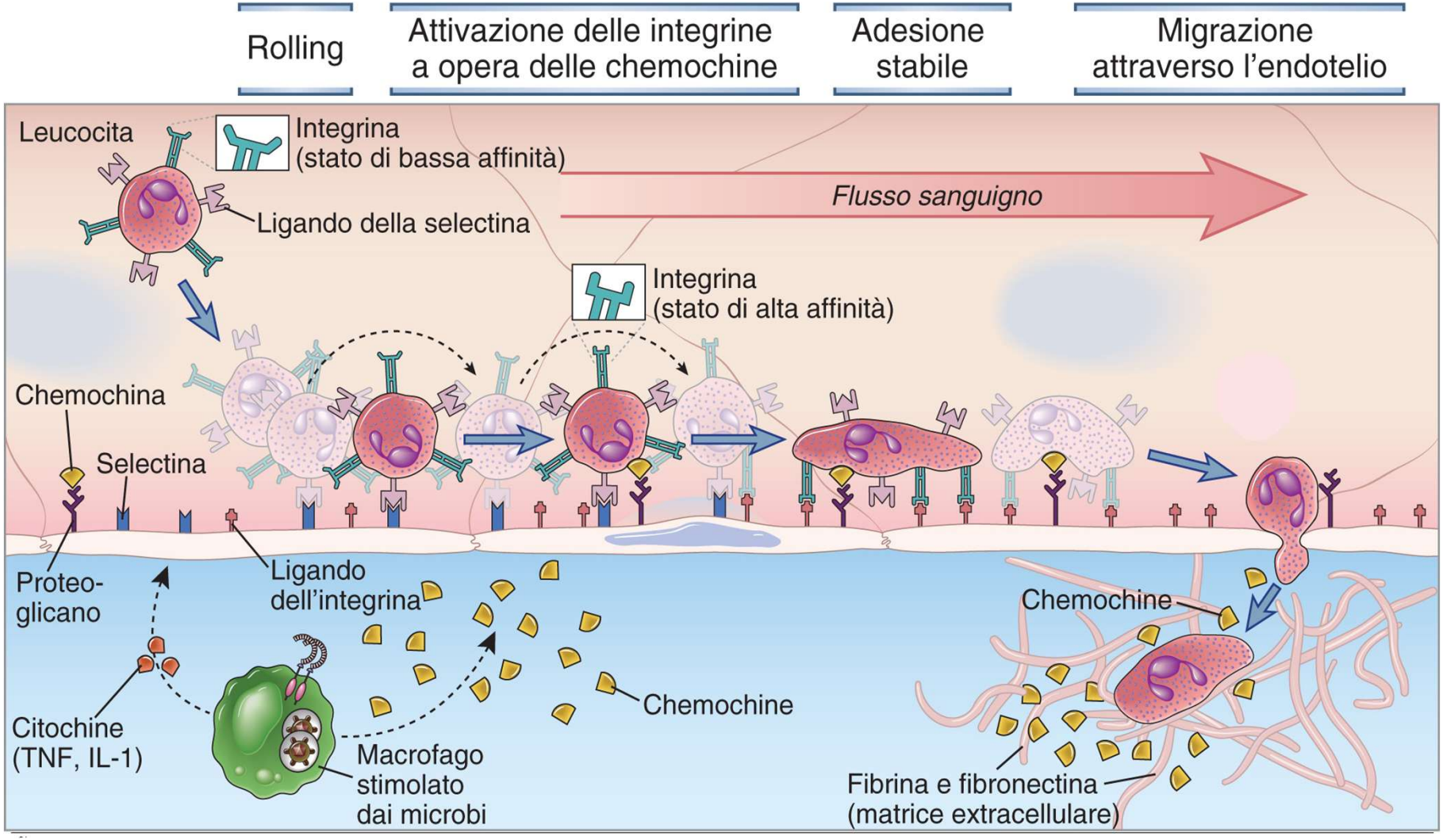
The role of migration in the immune system

- During maturation and differentiation
- In the immune surveillance that depends on constitutive and tissue-specific (or inducible) homing
- **Inflammatory process regulation**

A fundamental event for leukocyte function is the passage from the bloodstream to the tissues (extravasation)



Migrazione transendoteliale dei neutrofili durante l'infiammazione



Migration of leukocyte through endothelium depends on:

- speed of blood flux
- endothelial cell surface electric charge
- **adhesion molecules**
- **chemotactic factors**
- **Cytokines**

Intercellular adhesion molecules

The intercellular adhesion molecules are membrane molecules that allow:

Adaptive lymphocyte migration into tissues and secondary lymphoid organs (recirculation and homing)

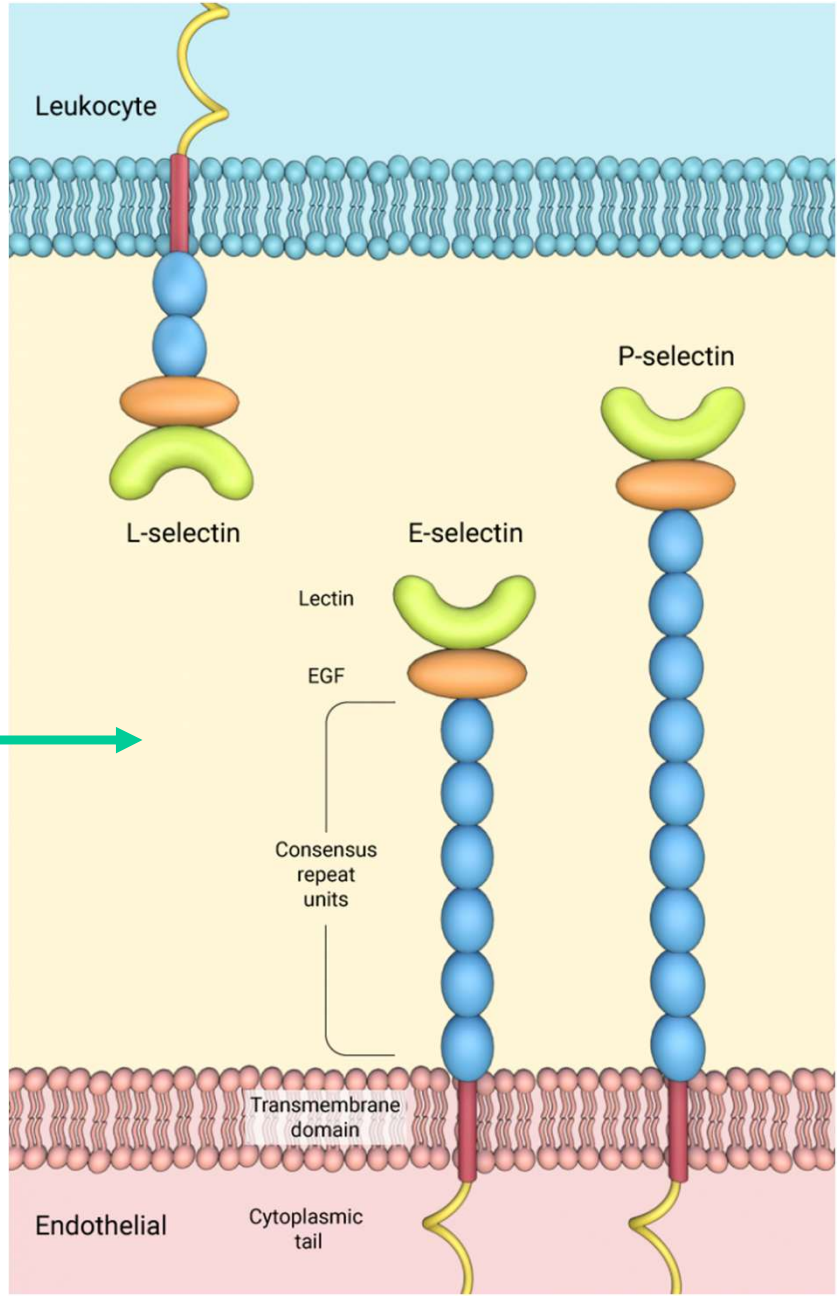
Leukocyte migration to the area of infection

Interaction of T and B lymphocytes with each other and with other cells involved in the immune response

1-Adhesion and rolling

Selectins are membrane glycoproteins, members of the family of Ca2 + dependent (C-type) lectin receptors

CCP= domini omologhi a quelli presenti nelle proteine regolatorie del complemento

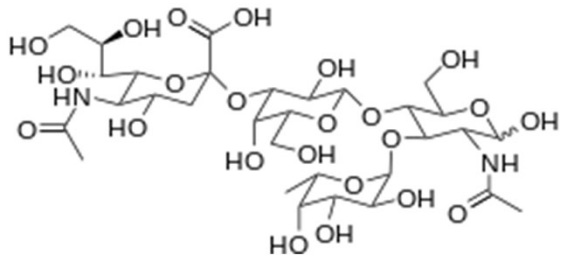


Low affinity interactions, 100 µM

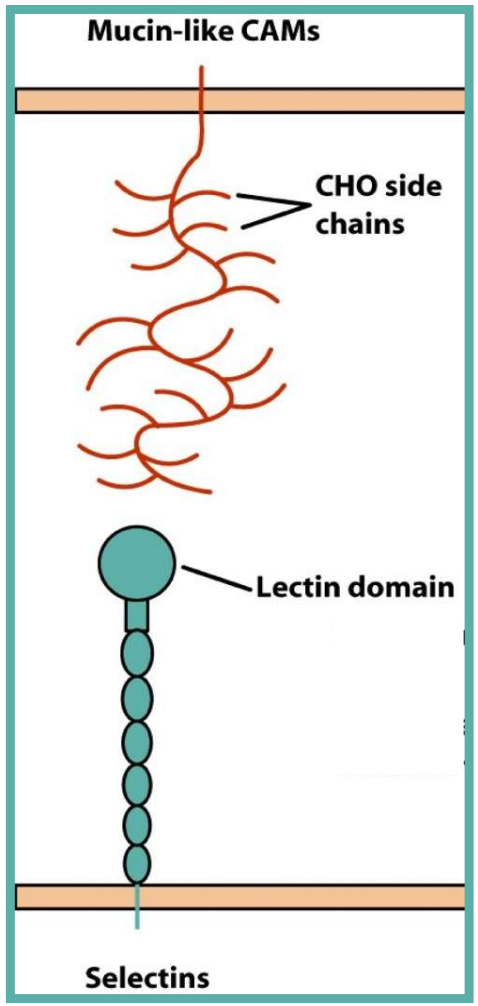
Selectin ligands

They are **membrane mucin-like** proteins highly glycosylated

Sialyl-Lewis X (sLeX) is the main recognized carbohydrate and is presented by glycoproteins



E- e P-Selectin Ligands



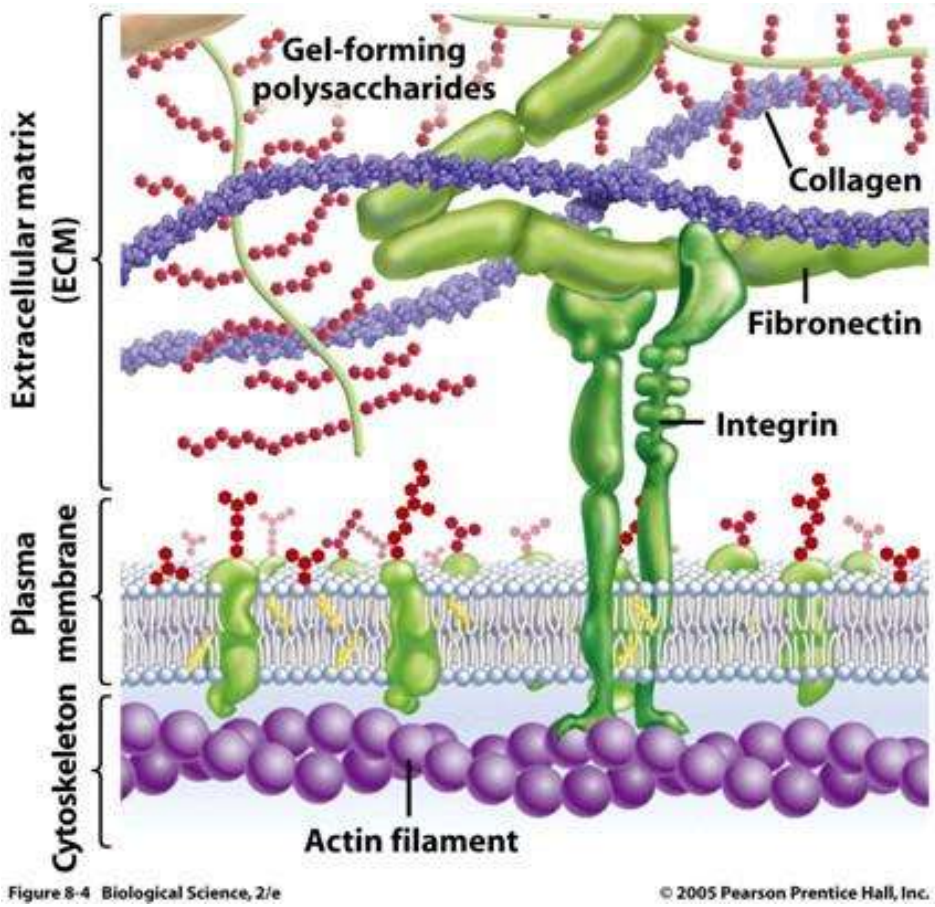
Name	Tissue distribution	Ligand
P-selectin (PADGEM, CD62P)	Activated endothelium and platelets	PSGL-1, sialyl-Lewis^x
E-selectin (ELAM-1, CD62E)	Activated endothelium	Sialyl-Lewis^x

PSGL1= P-selectin glycoprotein ligand 1. Expressed on all leukocytes.

Sialyl-Lewis X (sLeX) is the main carbohydrate recognized. It is found on numerous surface proteins expressed by granulocytes, monocytes and memory T cells.

2. Adhesion and transendothelial migration

Integrins



Various subfamilies according to the beta-chain subunits

a large family of heterodimeric transmembrane glycoproteins that attach cells to extracellular matrix proteins of the basement membrane or to ligands on other cells

Why integrins do not constitutively promote adhesion strenghtening on the endothelium layer?

Structure and affinity/avidity changes for ligands of leuckocyte integrins

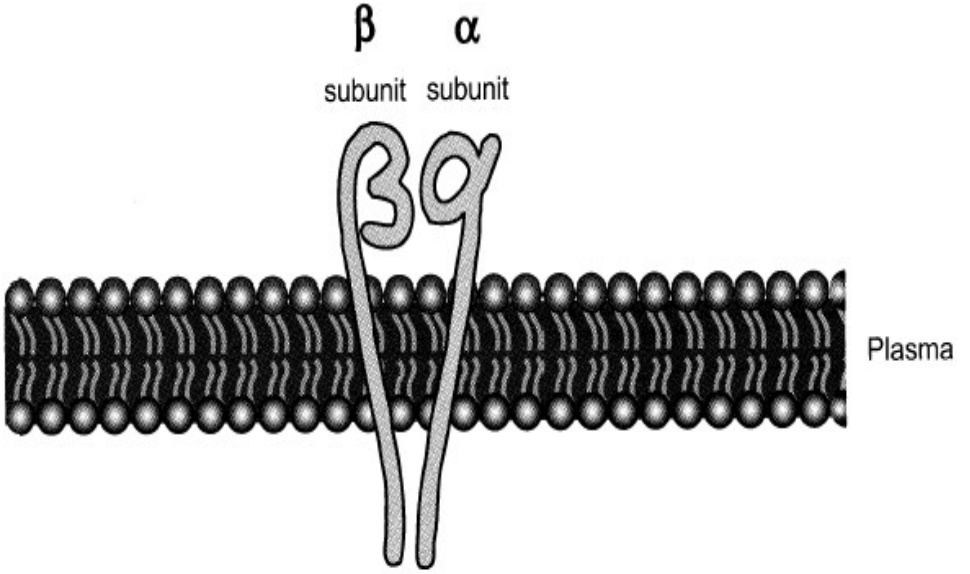
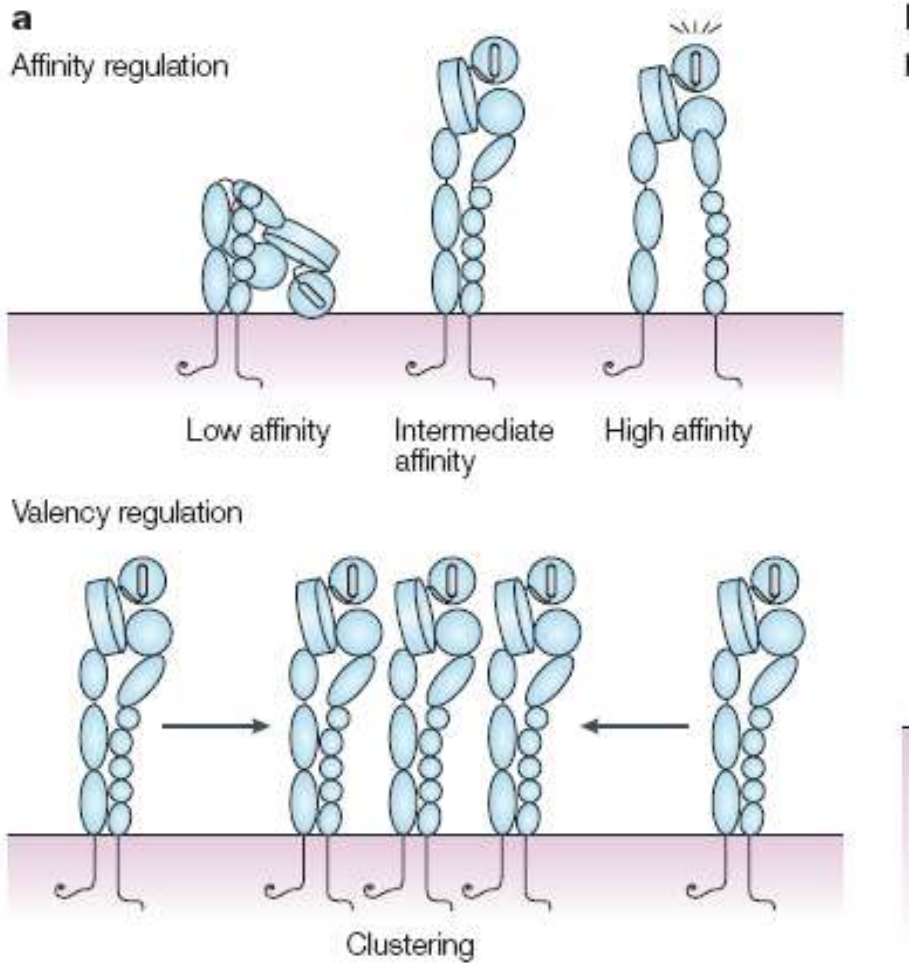
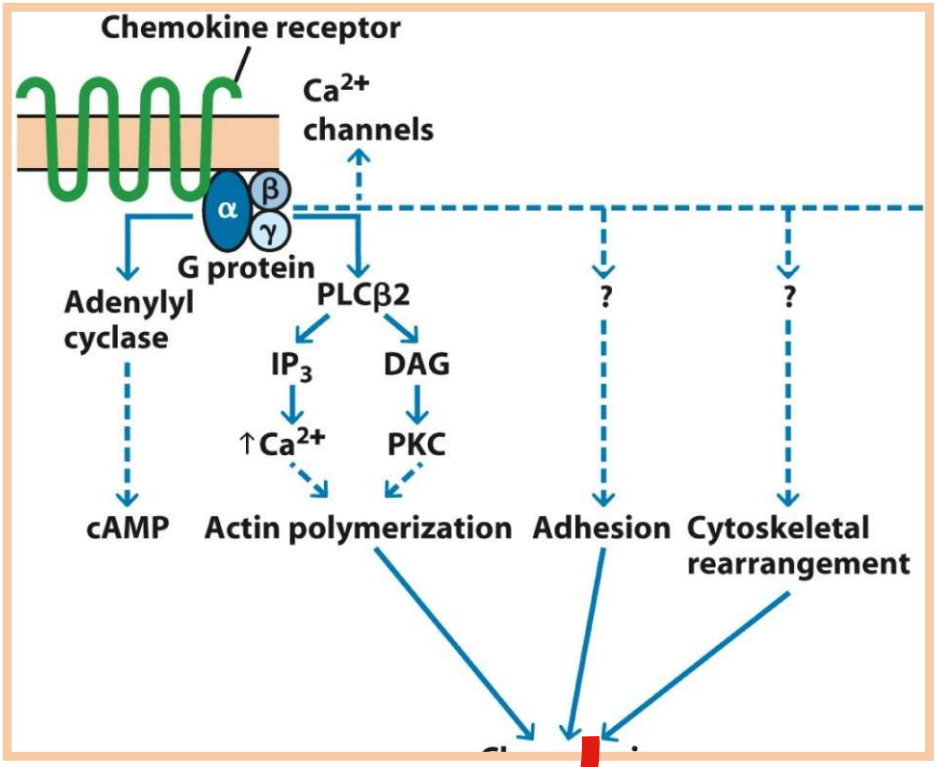


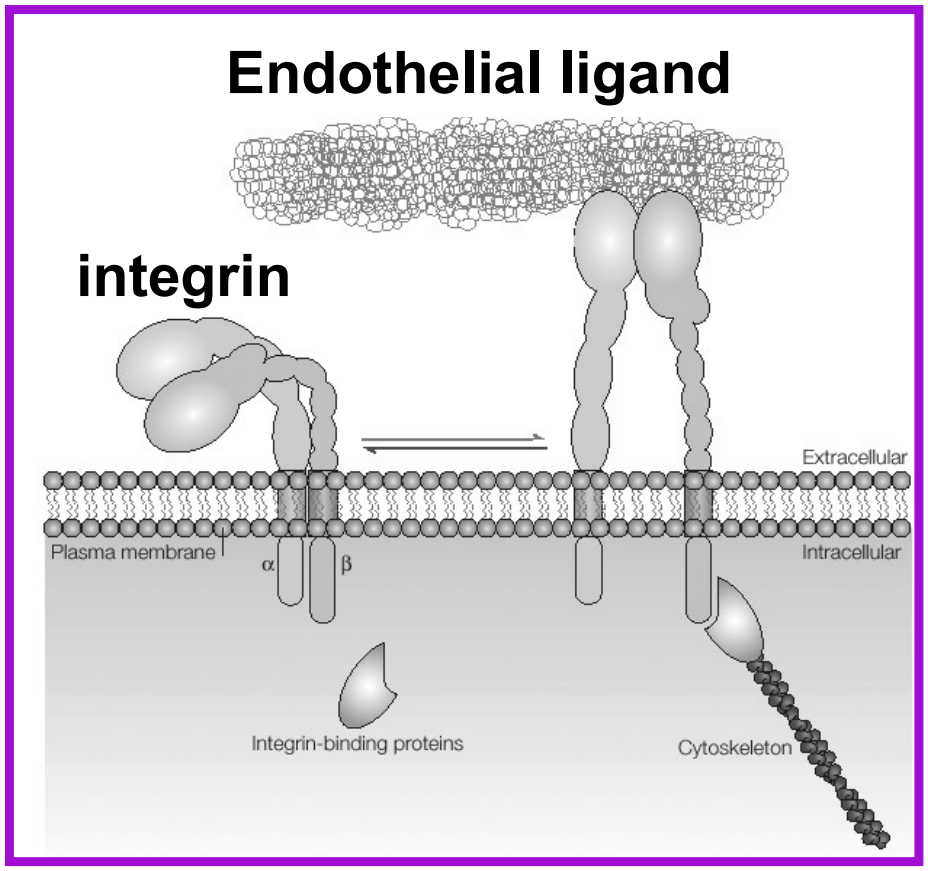
Figure 2. Schematic diagram of integrin heterodimer.



Integrins contain large (α) and small (β) subunits of sizes 120-170 kDa and 90-100 kDa, respectively.

Finding the perfect couple (ligand/receptor) affinity!





inside-out signalling

Signaling events initiated by chemokine receptor triggering converts integrin conformation into high affinity/avidity state

Alterazioni congenite della funzione leucocitaria

- **Difetti di adesione**

LAD-1: Espressione assente o deficitaria delle integrine beta-2(LFA-1, Mac-1) con conseguente deficit delle funzioni leucocitarie (infezioni ricorrenti da batteri e miceti)
(mutazione del gene che codifica per beta-2 o , in alcune varianti, mancata espressione di molecole coinvolte nell'attivazione delle beta-2)

LAD-2 Espressione deficitaria di ligandi per E- e P- Selectine
(mutazione nel gene che codifica per la fucosil-transferasi)

LAD-3 Difetto nelle vie di segnalazione che portano all'attivazione di integrine beta-2

The role of migration in the immune system

- During maturation and differentiation

- **In the immune surveillance that depends on constitutive and tissue-specific (or inducible) homing**

- Inflammatory process regulation

LYMPHOCYTE HOMING

(lymphocyte localization):

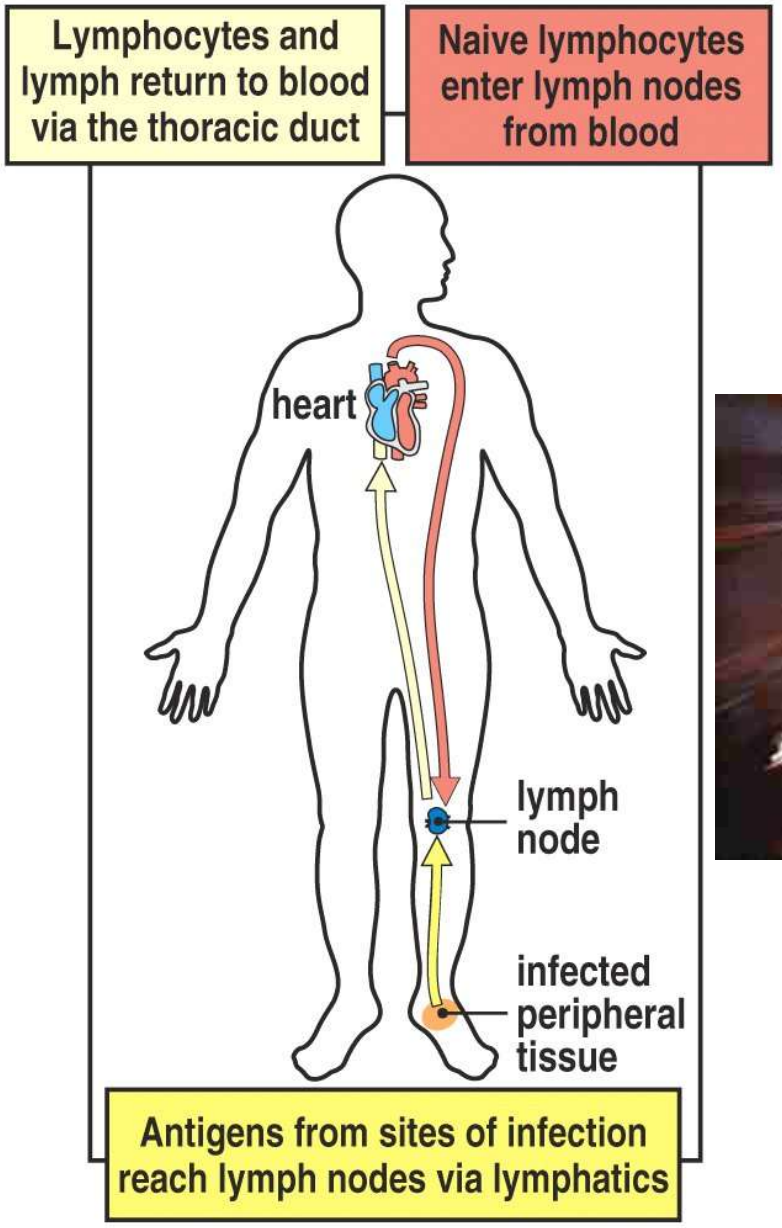
Selective migration of functionally distinct lymphocyte populations in specific body districts:

1-lymphoid organs (**constitutive homing**)

2-sites of infection/inflammation (**inducible homing**)

Lymphocytes differ from most leucocytes (which only cross the blood to participate in the inflammatory response in terminal destinations) because they **recirculate** from the blood to the lymph.

Lymphocyte recirculation



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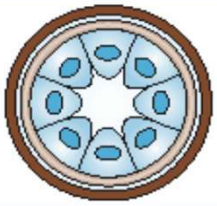
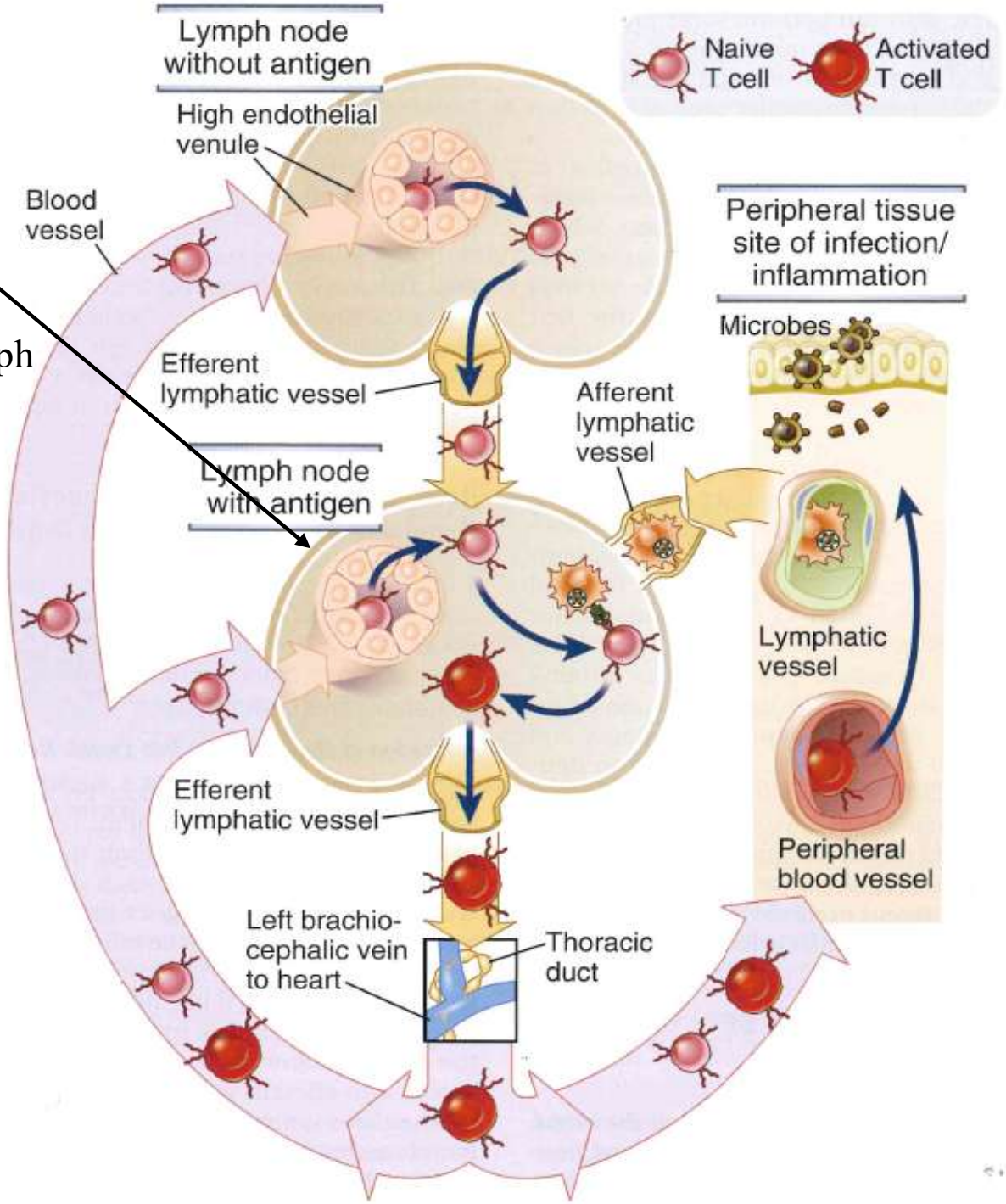
T lymphocyte recirculation



High endothelial venules(HEV)
Are located in lymph node and Payer patches but not in spleen



The molecules expressed on the membrane of the lymphocytes responsible for the recirculation process are called Homing Receptors

The molecules expressed on the endothelium are called Addressins.

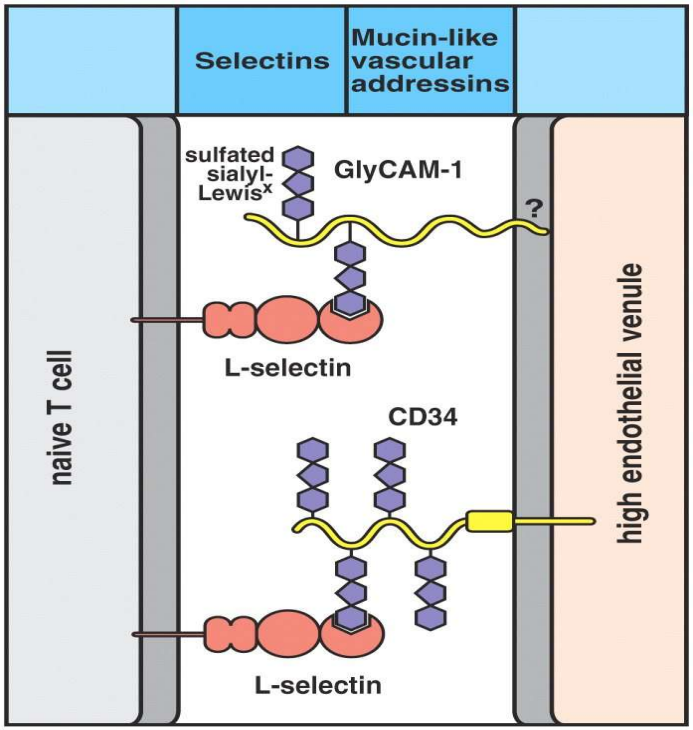
How is the site of extravasation targeted so precisely?

High endothelial venules have peculiar characteristics

	Normal venule	Peripheral lymph-node HEV	Peyer's-patch HEV
			
Endothelium	Flat	Tall and plump	Tall and plump
Basal lamina	Thin	Thick	Thick
Perivascular sheath	Scanty	Prominent	Prominent
CD31	+	+	+
ICAM2	+	+	+
ICAM1	–/+	++	++
VE-cadherin	+	+	+
Sialomucins			
Core protein	+	++	+
PNAD epitope	–	++	+
Sulphation	–	+	+
MADCAM1	–	–	+
Chemokines (CCL19, CCL21, CXCL12 and CXCL13)	–	+	+

Homing of naïve T cells

L-selectin and vascular addressins



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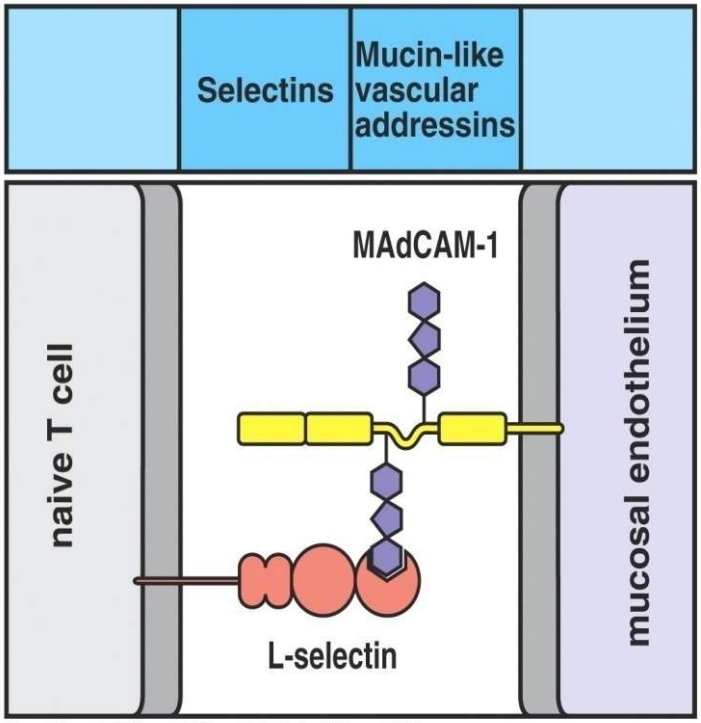

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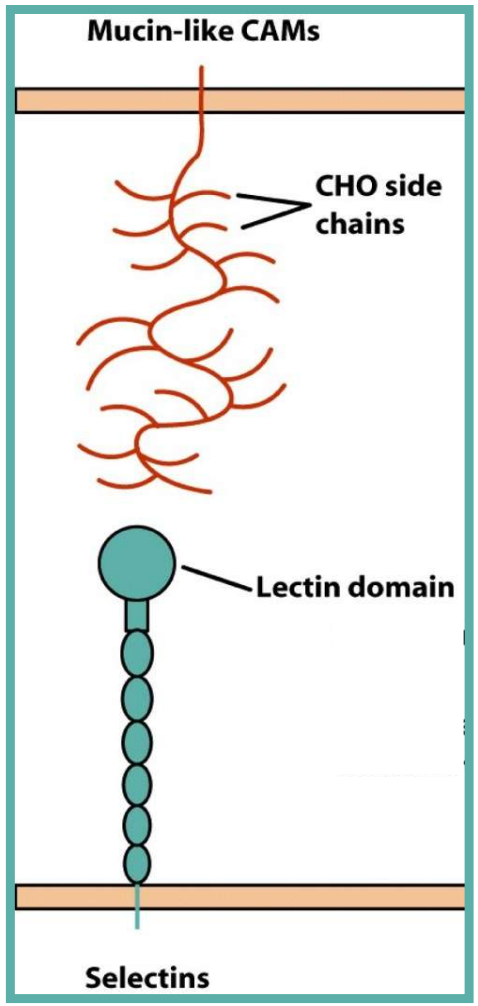
GlyCAM-1 (glycosylation-dependent cell adhesion molecule 1) e **CD34,** sono espresse a bassi livelli dagli endoteli ma sono espresse sulle HEV dei linfonodi e legano la **L-selectina**

MadCAM-1: Mucosal addressin Cell Adesion Molecule-1

Homing of effector/memory T cells

E- e P-Selectin Ligands = Addressins



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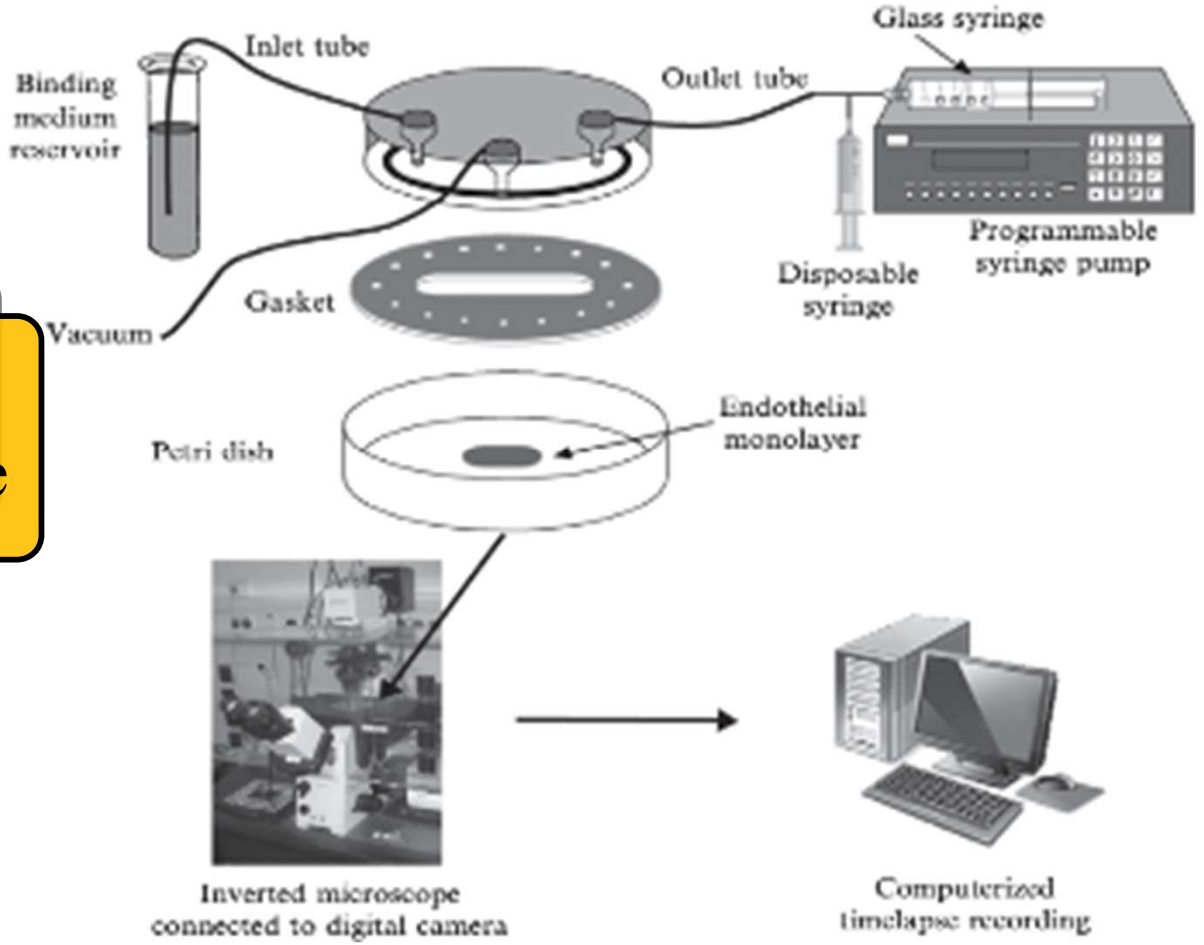
PSGL1= P-selectin glycoprotein ligand 1. Expressed on all leukocytes.

Sialyl-Lewis X (sLeX) is the main carbohydrate recognized. It is found on numerous surface proteins expressed by granulocytes, monocytes and memory T cells.

Come si può dimostrare il contributo di determinate molecole nella migrazione transendoteliale di specifici leucociti ?

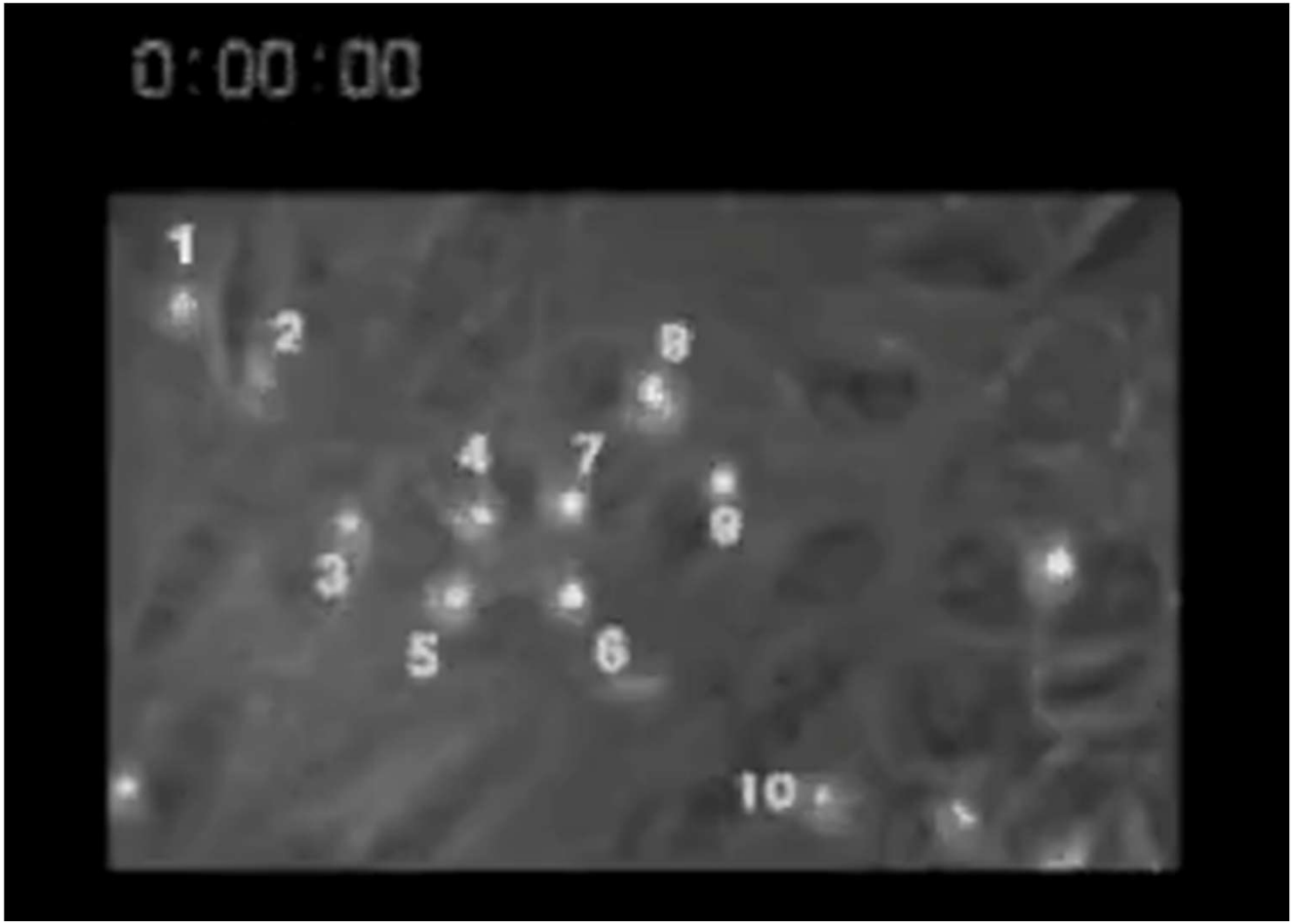
SAGGIO DI MIGRAZIONE SOTTO FLUSSO

- Analisi
  - Fase di accumulo
  - Fase di migrazione



SAGGIO DI MIGRAZIONE SOTTO FLUSSO

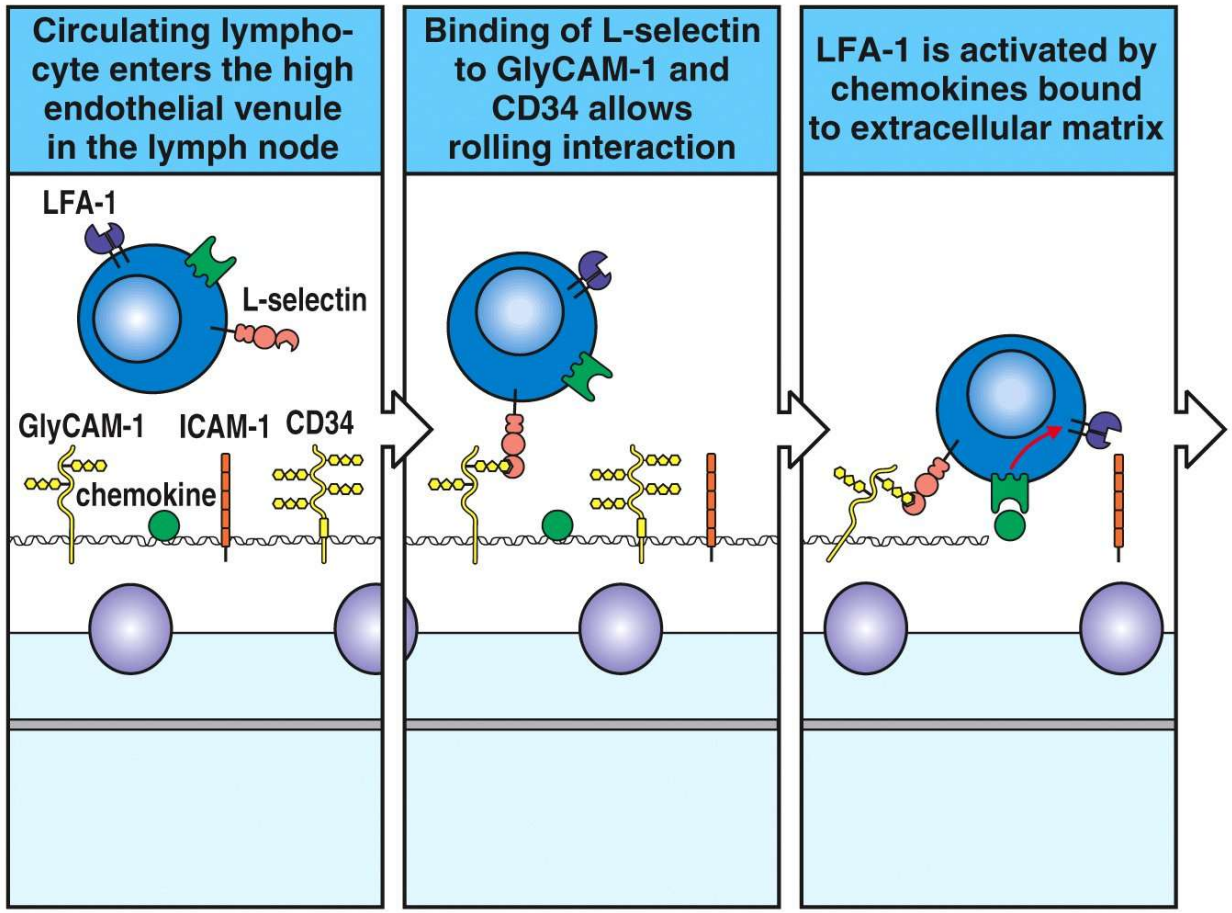
Migrazione dei linfociti attraverso l'endotelio vascolare che espone chemochine apicali



Cinamon et al., Nature Immunology, 2001

What is the contribution of selectins to transendothelial migration in secondary lymphoid organs?

Contact with endothelium and cell rolling



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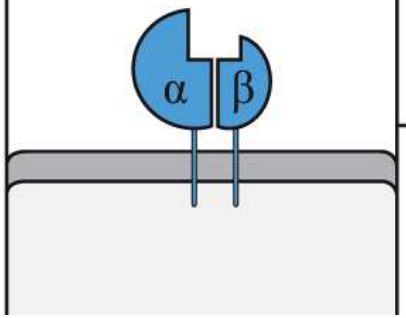
What are the integrins/ligand axex involved to transendothelial migration in secondary lymphoid organs?

Integrins of beta-2 subfamily and their ligands

Integrins		Name	Tissue distribution	Ligand
Bind to cell-adhesion molecules and extracellular matrix. Strong adhesion		α_L:β_2 (LFA-1, CD11a:CD18)	Monocytes, T cells, macrophages, neutrophils, dendritic cells, NK cells	ICAM-1, ICAM-2
		α_M:β_2 (CR3, Mac-1, CD11b:CD18)	Neutrophils, monocytes, macrophages, NK cells	ICAM-1, iC3b, fibrinogen
		α_X:β_2 (CR4, p150.95, CD11c:CD18)	Dendritic cells, macrophages, neutrophils, NK cells	iC3b

Integrin ligands involved in transendothelial migration are adhesion molecules belonging to the superfamily of immunoglobulins

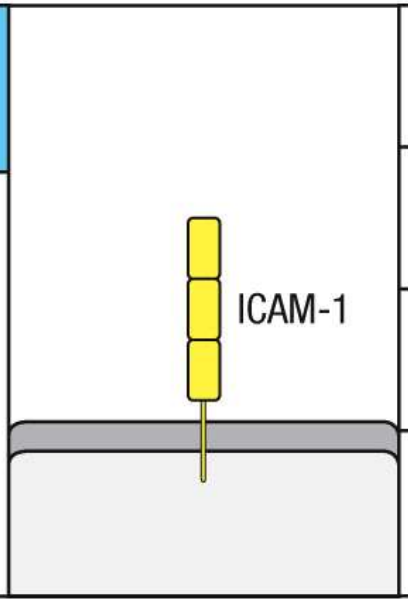
		Name	Tissue distribution	Ligand
Immunoglobulin superfamily		ICAM-1 (CD54)	Activated endothelium, activated leukocytes	LFA-1, Mac1
Various roles in cell adhesion. Ligand for integrins		ICAM-2 (CD102)	Resting endothelium, dendritic cells	LFA-1
		VCAM-1 (CD106)	Activated endothelium	VLA-4
		PECAM (CD31)	Activated leukocytes, endothelial cell–cell junctions	CD31

Figure 3.29 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Transedonthelial migration occurs on high endothelial venules (HEV)

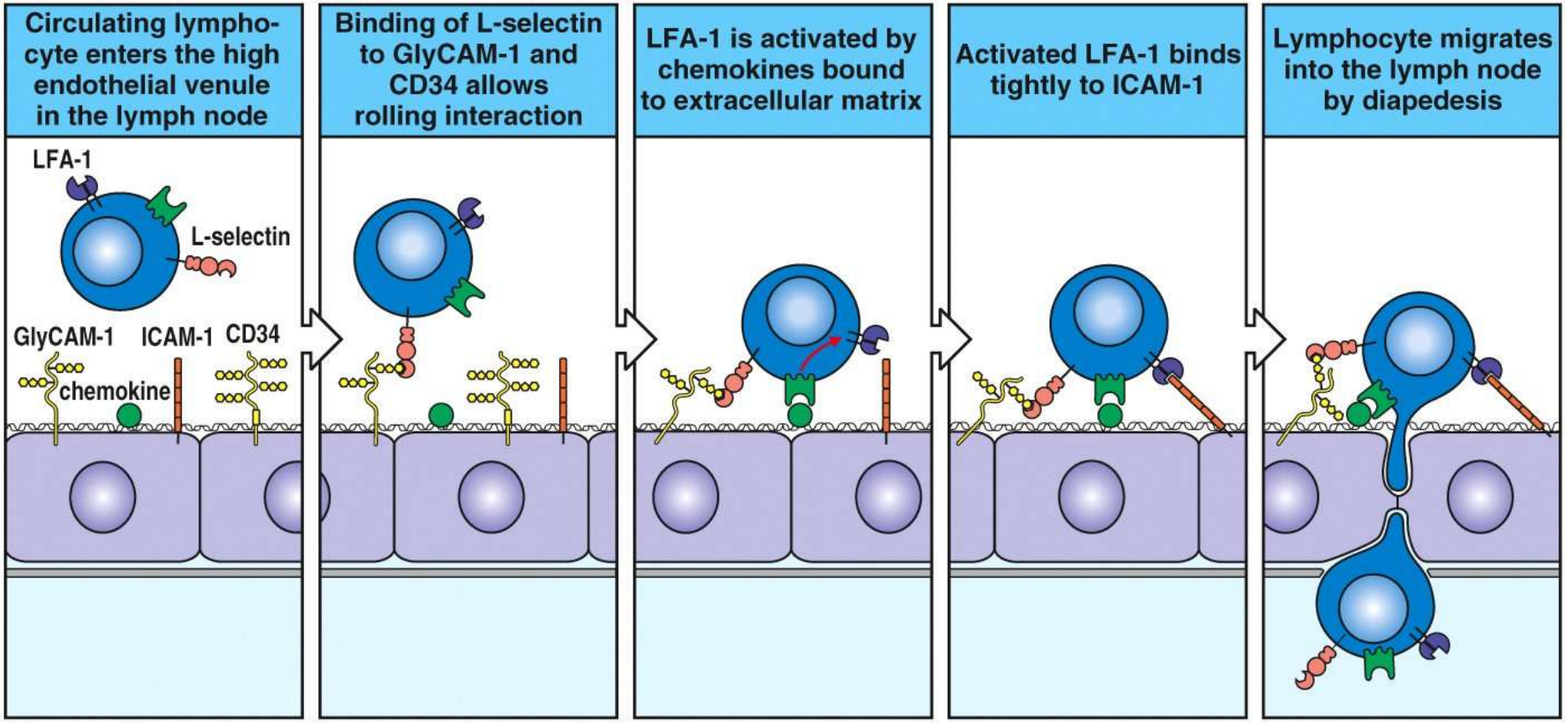
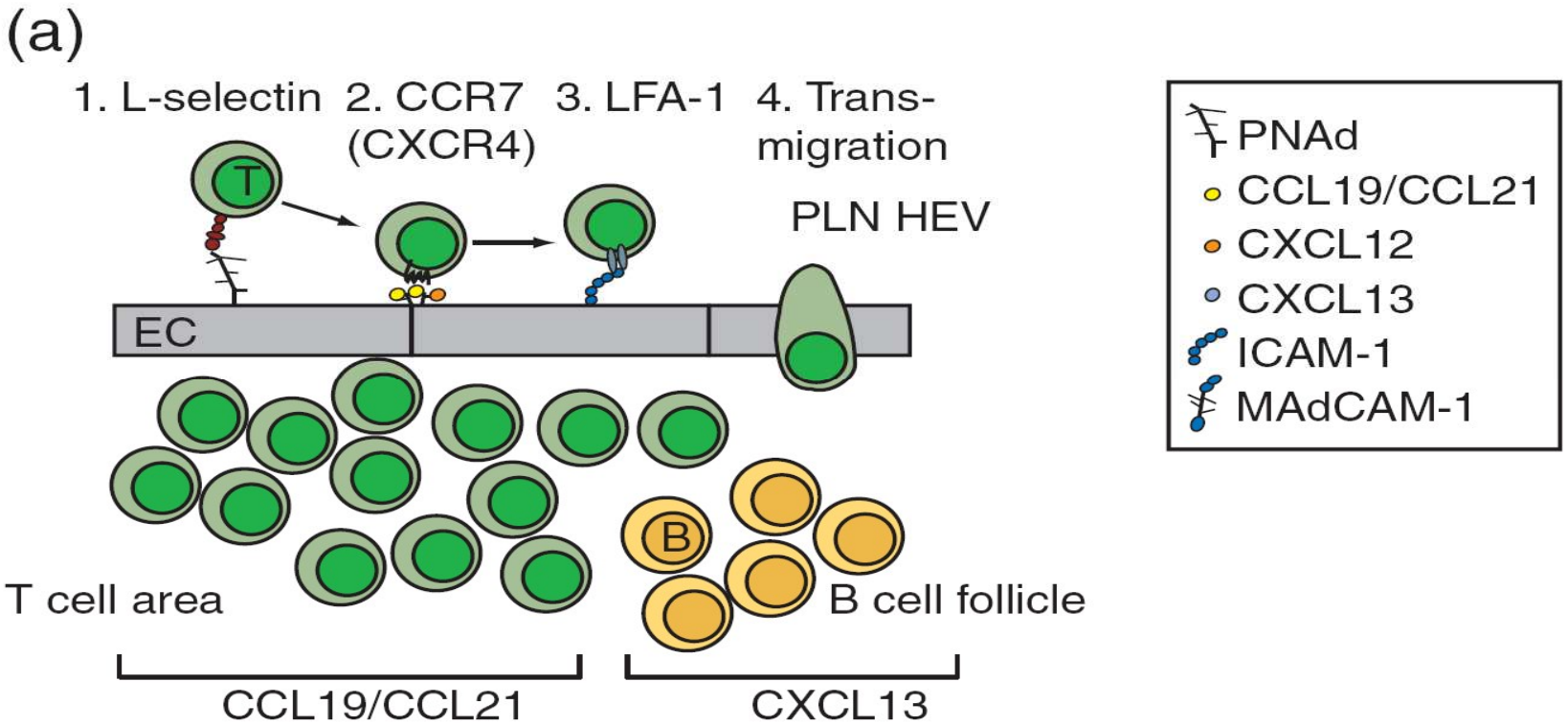
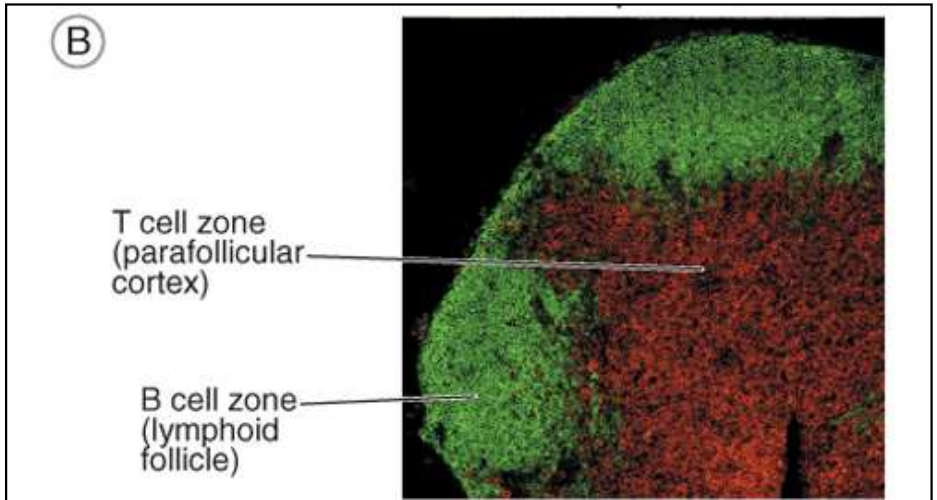


Figure 10-7 Immunobiology, 6/e. (© Garland Science 2005)

Chemokines and lymphocyte homing in secondary lymphoid organs





Lymphoid organ exit is a mechanisms controlled by sphingosine-1-phosphate

Should I stay or should I go?.....

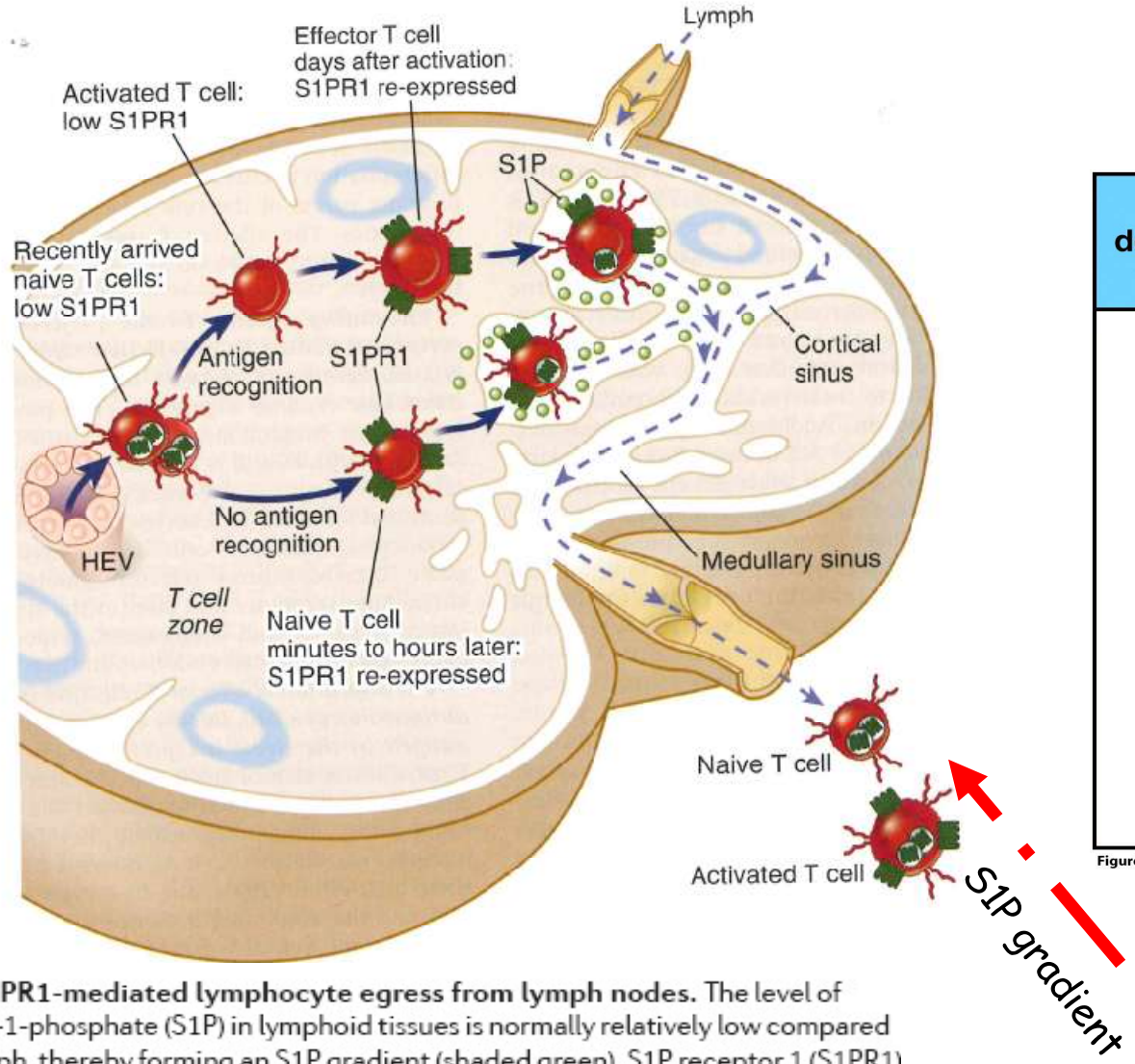


Figure 3 | **S1PR1-mediated lymphocyte egress from lymph nodes.** The level of sphingosine-1-phosphate (S1P) in lymphoid tissues is normally relatively low compared with the lymph, thereby forming an S1P gradient (shaded green). S1P receptor 1 (S1PR1) expressed on T cells is responsive to the S1P gradient and promotes T-cell egress from the lymphoid organ through the endothelial barrier into lymph. After activation of the T cell in the lymphoid organ by encountering an antigen-expressing dendritic cell or by type I interferon stimulation, S1PR1 expression is decreased. Mechanisms include

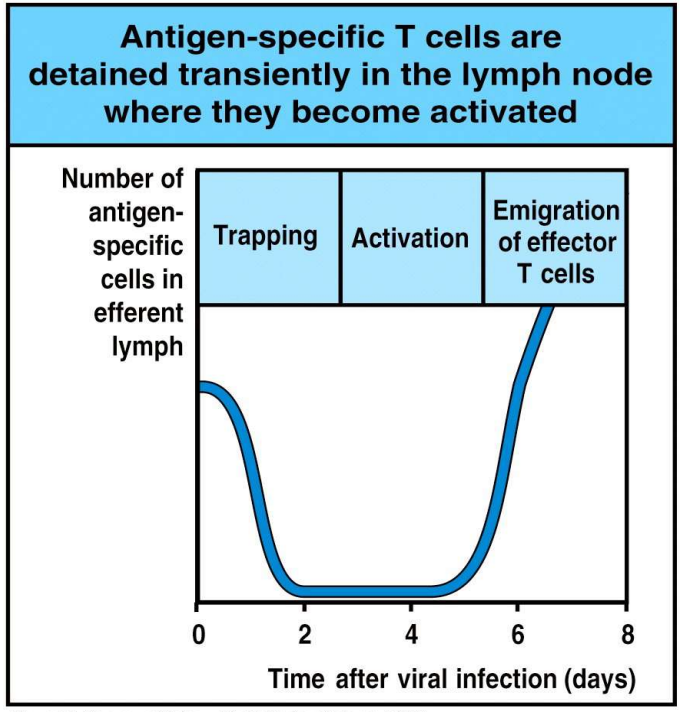
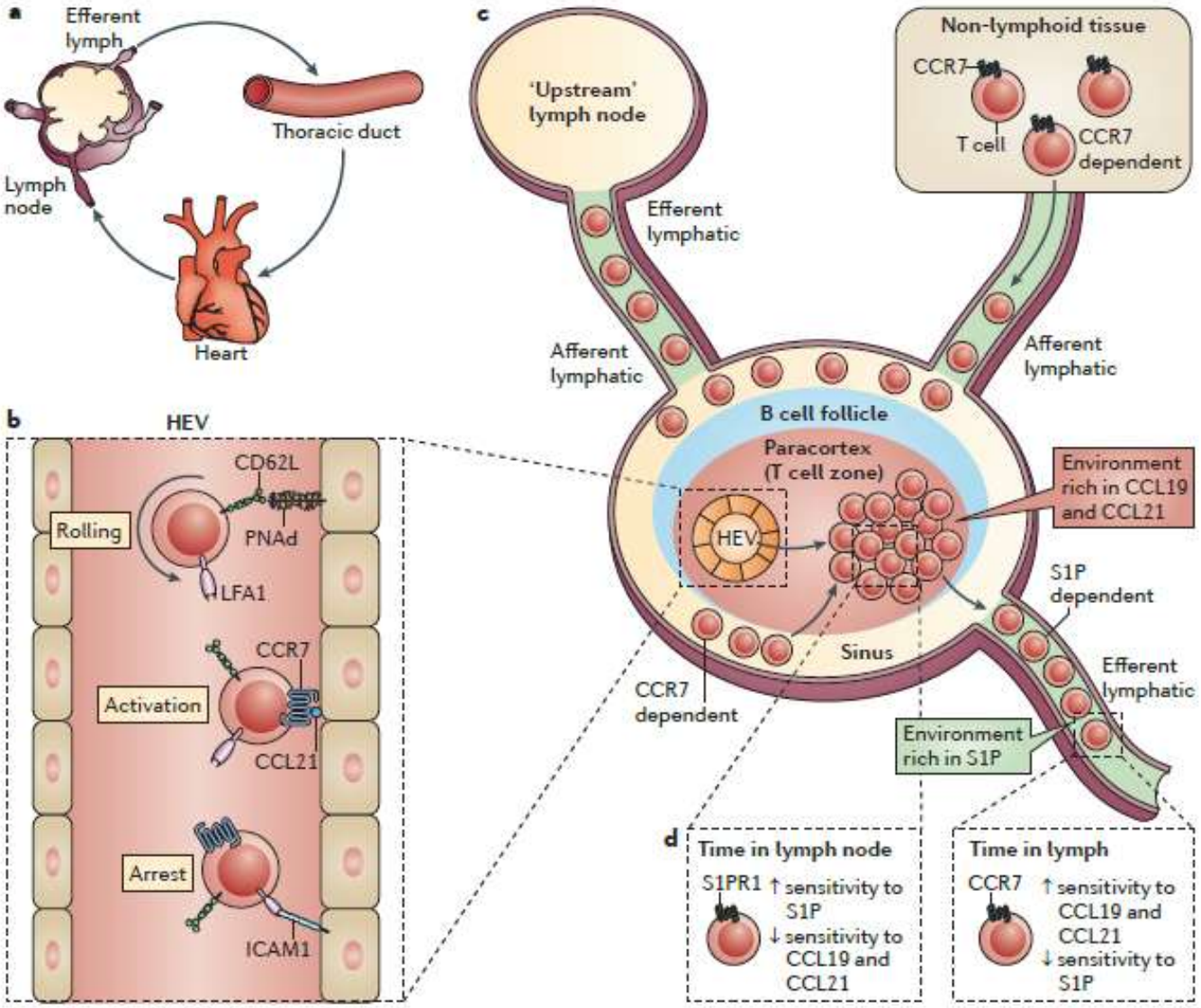


Figure 10-8 Immunobiology, 6/e. (© Garland Science 2005)

La ricircolazione linfocitaria



Effector and memory T cells

Naive T cells

PNAd: peripheral node addressins

Homing costitutivo

Tropismo per organi linfoidi secondari (linfonodi, Placche di Peyer, milza) da parte di linfociti T e B "vergini".

What happens following T cell activation?

Homing inducibile e tessuto specifico

La stimolazione antigenica "riprogramma" le proprieta' di traffico di cellule B e T "vergini" inducendo il differenziamento di cellule effettrici e memoria che

a- hanno migliori capacita' di homing per tessuti extralinfoidi rispetto alle vergini

b- sono in grado di "SELEZIONARE" tessuti e organi specifici dove dirigersi ritornando preferenzialmente nel tessuto che ne ha causato l'attivazione (cioè dove è più probabile trovare l'antigene che li ha attivati).

Homing inducibile e tessuto specifico

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Activated T lymphocytes change the surface expression of several molecules

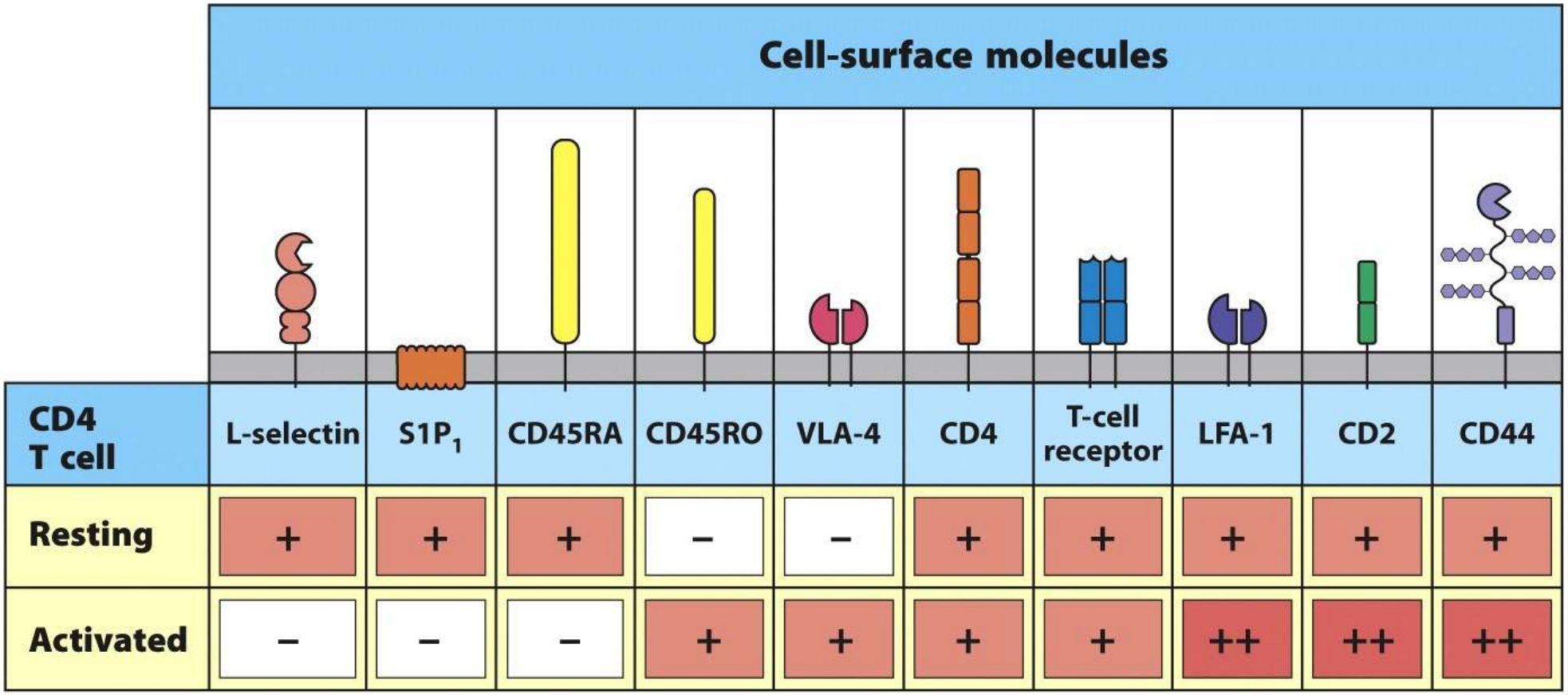

Figure 9.25 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Promotes homing to lymph nodes

Promotes exit from lymph nodes

Promotes homing to inflamed tissues

Anche il recettore CCR7 è fortemente down-modulato a seguito dell'attivazione (viene mantenuto solo da una piccola popolazione che continua a ricircolare nei linfonodi)

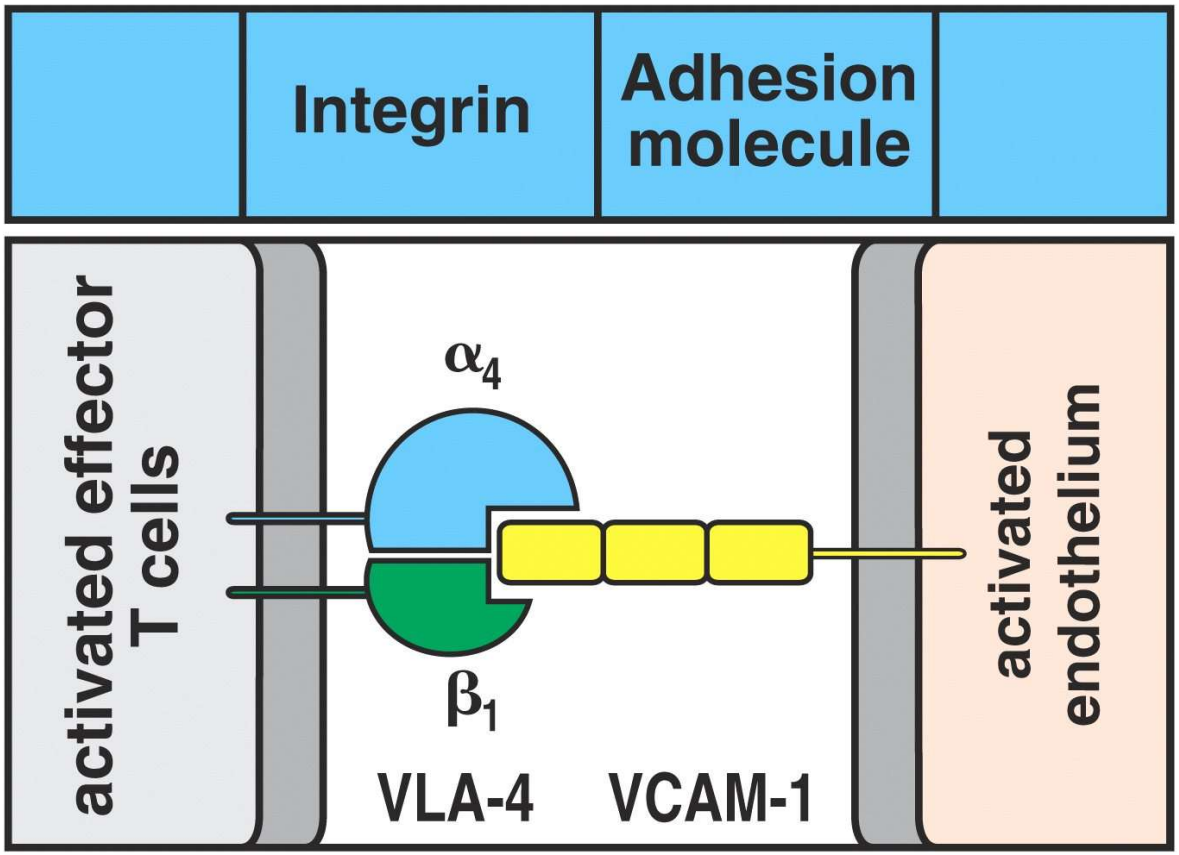

Figure 8-6 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)

Il membro più importante della sottofamiglia delle integrine beta-1 sui leucociti è **Very Late Antigen-4, VLA-4**. VLA-4 lega il suo ligando **Vascular Cell Adhesion Molecule- 1, VCAM-1** ed è primariamente responsabile dell'adesione linfocitaria all'endotelio vascolare e del loro richiamo nei tessuti.

Utilizzo di antagonisti e anticorpi bloccanti diretti contro alfa4 integrine in modelli di infiammazione cronica e autoimmunita'

Natalizumab e' un anticorpo umanizzato che blocca la funzione dell'integrina alfa4

Derivati del peptide LDV che si lega all'integrina nella sua conformazione ad alta affinita' bloccandone la funzione

E' in corso di valutazione in trial clinici che coinvolgono pazienti Affetti da Morbo di Chron, colite ulcerosa e Sclerosi Multipla