Università degli Studi di Padova

Corso di Laurea in

BIOTECNOLOGIE

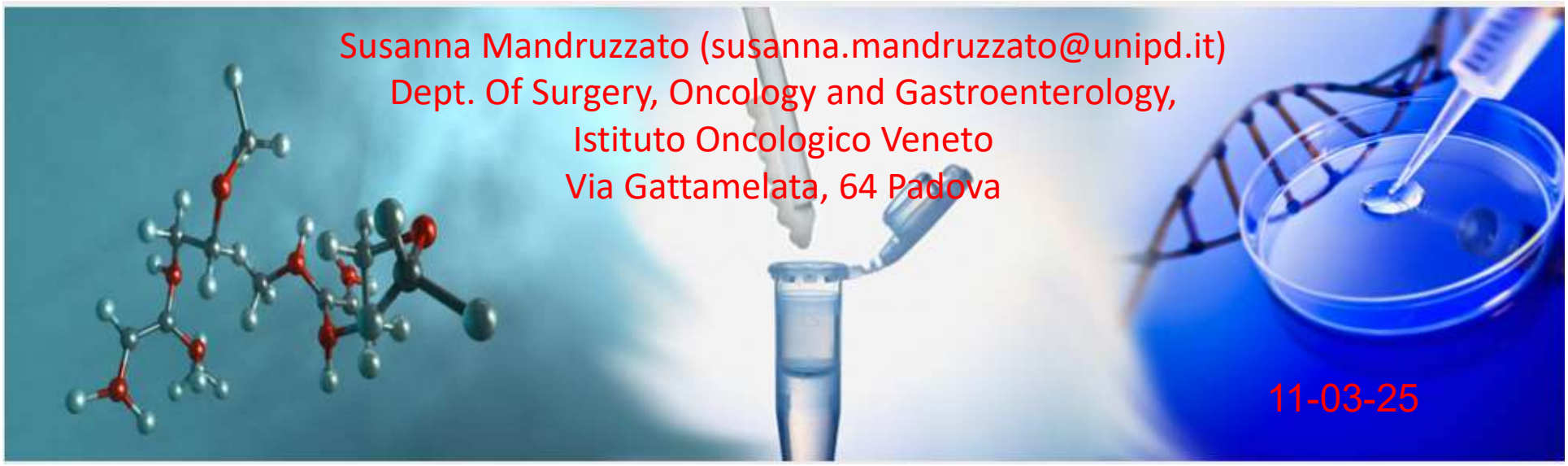
Piano di studi Farmaceutico

Anno Accademico 2045-2025

Insegnamento di

Immunologia Farmaceutica

Immunità innata: i recettori (2 parte)



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11-03-25

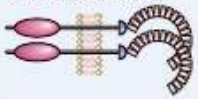
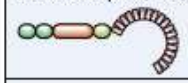
L'immunità innata riconosce i DAMP ed i PAMP utilizzando:
sia recettori cellulari, distribuiti su diversi compartimenti,
sia molecole solubili presenti nel sangue e nelle secrezioni mucose.

I recettori cellulari sono noti come: RECETTORI CHE RICONOSCONO I PROFILI MOLECOLARI (*Pattern recognition receptors, PRR*) .

La localizzazione dei PRR può essere:

- Membrana citoplasmatica
- Membrana endosomiale
- Citoplasma
- Plasmatica

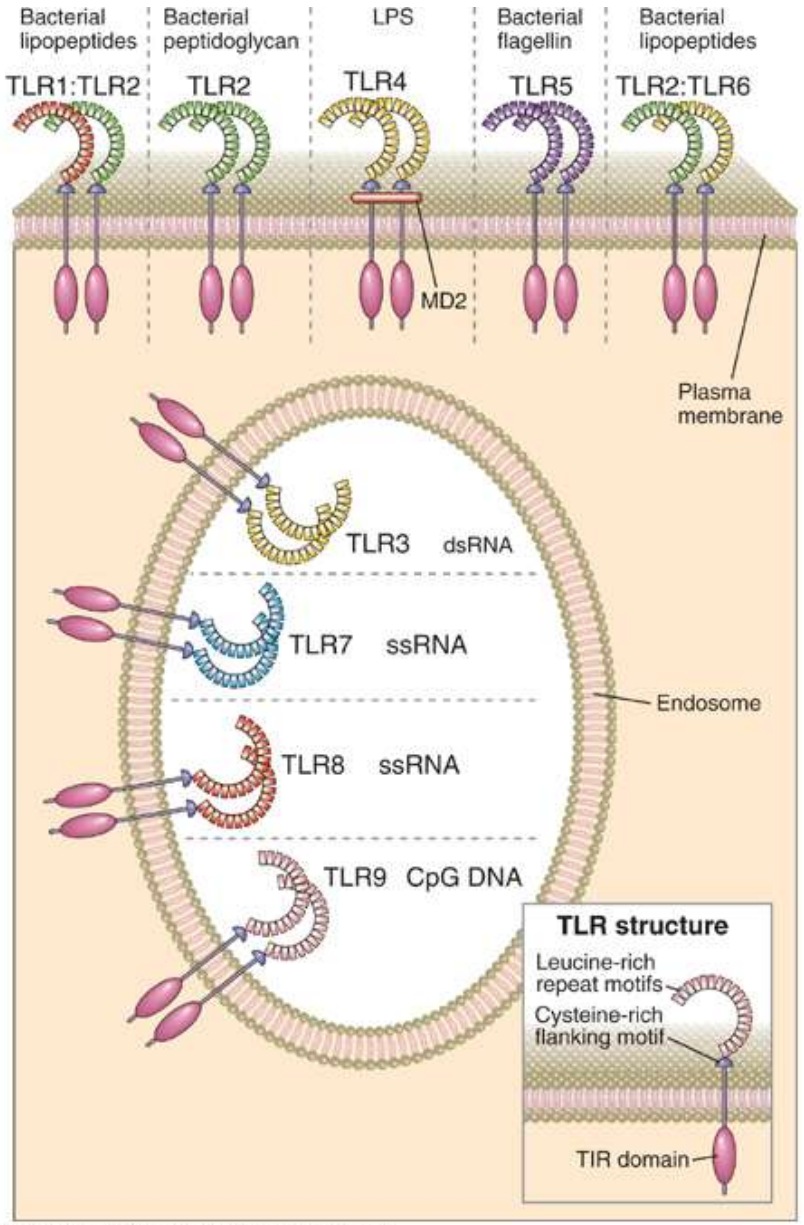
TABLE 4–3 Pattern Recognition Molecules of the Innate Immune System

Cell-Associated Pattern Recognition Receptors	Location	Specific Examples	PAMP/DAMP Ligands
Toll-like receptors (TLRs) 	Plasma membrane and endosomal membranes of dendritic cells, phagocytes, B cells endothelial cells, and many other cell types	TLRs 1-9	Various microbial molecules including bacterial LPS and peptidoglycans, viral nucleic acids
NOD-like receptors (NLRs) 	Cytoplasm of phagocytes epithelial cells, and other cells	NOD1/2 NALP family (inflammasomes)	Bacterial cell wall peptidoglycans Flagellin, muramyl dipeptide, LPS; urate crystals; products of damaged cells
RIG-like receptors (RLRs)	Cytoplasm of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
C-type lectin–like receptors 	Plasma membranes of phagocytes	Mannose receptor Dectin	Microbial surface carbohydrates with terminal mannose and fructose Glucans present in fungal cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues
Soluble Recognition Molecules	**Location**	**Specific Examples**	**PAMP Ligands**
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-Acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	C3	Microbial surfaces
Natural antibodies 	Plasma	IgM	Phosphorylcholine on bacterial membranes and apoptotic cell membranes

Structure, location and specificities of mammalian TLRs

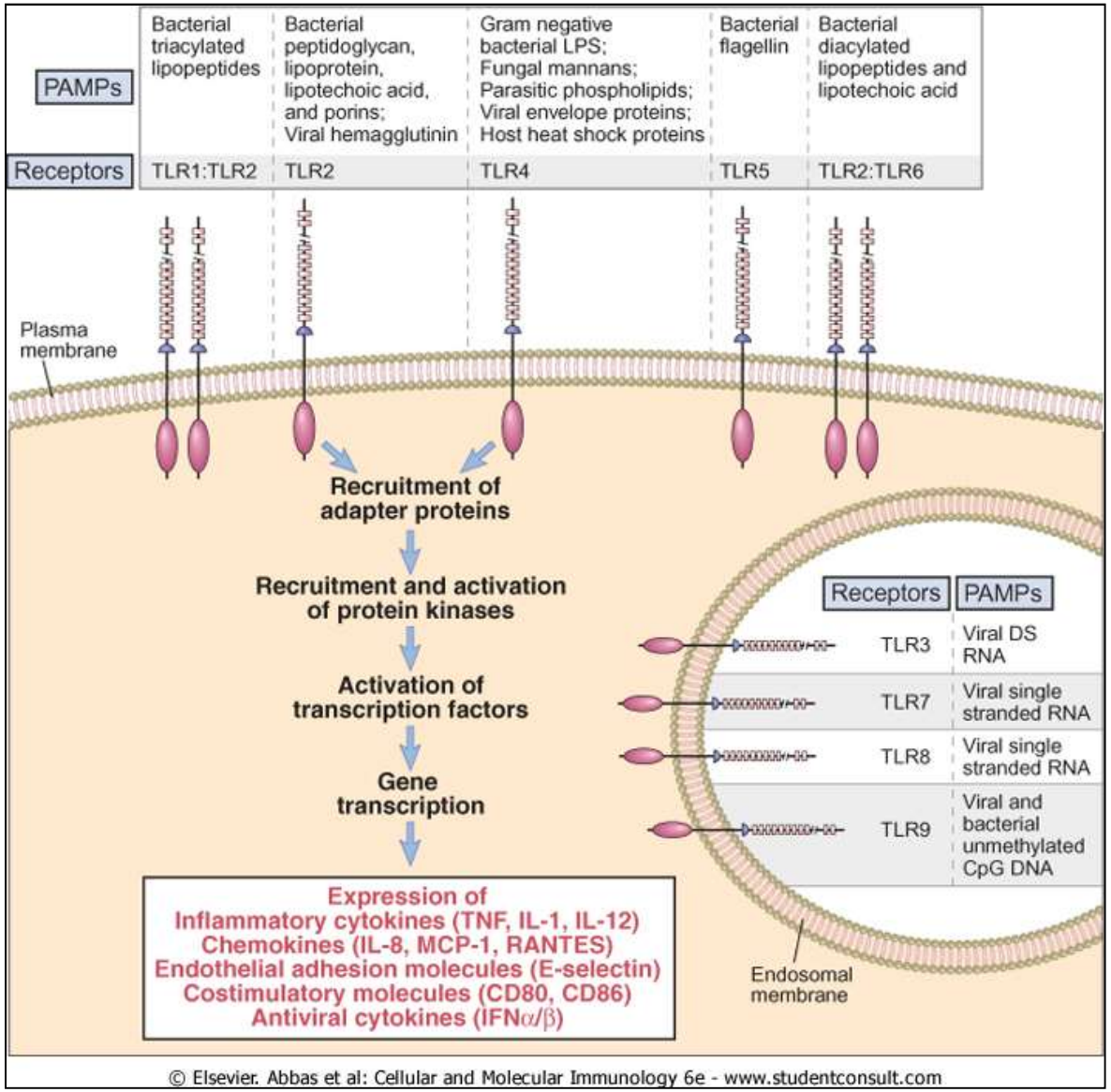
TLR 1,2,4,5 e 6 sono espressi sulla membrana citoplasmatica, dove riconoscono i PAMP presenti nell'ambienti extracellulare.

TLR 3, 7, 8 e 9 sono intracellulari, espressi prevalentemente nel RE e sulle membrane endosomiali, dove riconoscono ligandi composti da acidi nucleici.
(RNA e DNA dell'ospite non sono normalmente presenti negli endosomi)



Meccanismi di trasduzione del segnale dei TLR

Quando i TLR riconoscono i propri ligandi, essi innescano diverse cascate di trasduzione del segnale che portano all'attivazione di fattori trascrizionali i quali inducono l'espressione di geni fondamentali per le risposte infiammatoria e antivirale



Meccanismi di trasduzione del segnale dei TLRs

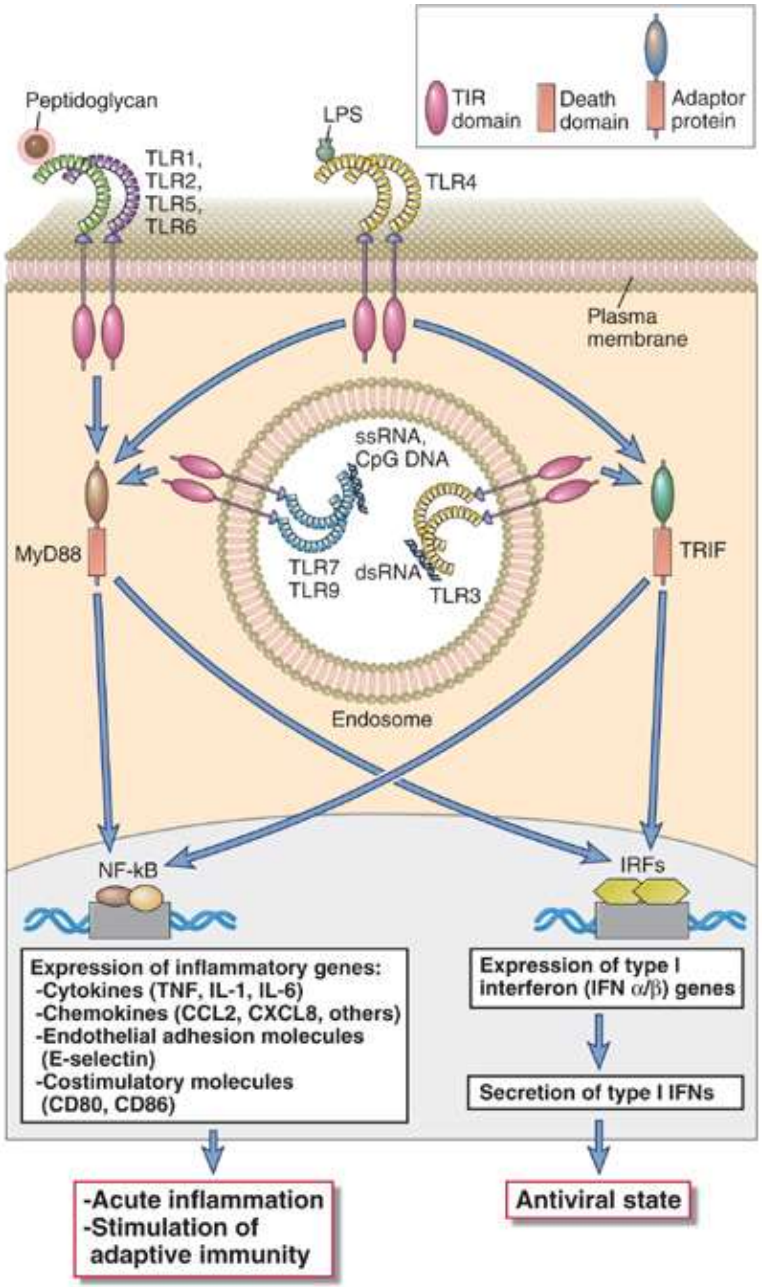
I principali fattori trascrizionali attivati dai TLR sono:

- NF-κB
- AP-1

Responsabili della maggior parte delle molecole necessarie per la risposta infiammatoria

- IRF3
- IRF7

Responsabili della risposta antivirale



RECETTORI CITOSOLICI PER I PAMP E I DAMP

Oltre ai TLR di membrana, le cellule dell'immunità innata esprimono PRR che possono riconoscere i prodotti delle infezioni o del danno cellulare a ***livello citoplasmatico***.

Le 2 classi principali sono:

- Recettori di tipo **NOD**
- Recettori di tipo **RIG**

Come I TLR, essi attivano vie di trasduzione che promuovono l'infiammazione o la produzione di IFN di tipo I

Intracellular PRRs: Present in the Cytosol of Host Cells

NOD proteins or Nucleotide-binding Oligomerization Domain

- Recognize intracellular peptidoglycan-derived structures and transduce signals
- three distinct functional domains
  - carboxy-terminal ligand-recognition domain (LRD)
  - centrally located NOD
  - amino-terminal effector-binding domain (EBD)
    - CARD domains in mammals
    - Interacts and activates RIP2 inducing NF-kB and MAP-kinase pathways

NOD Proteins

NOD-like receptors (NLRs) are a family of more than 20 different cytosolic proteins, some of which sense cytoplasmic PAMPs and DAMPS and recruit other proteins to form signaling complexes that promote inflamation.

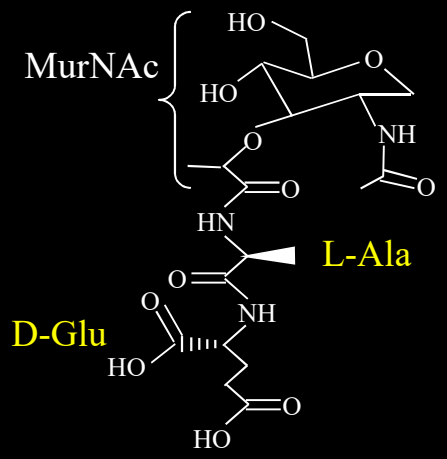
NOD 1 and NOD2 are expressed in the cytoplasm of several cell types including Mucosal epithelia cells and phagocytes, and they respond to bacterial cell wall peptoglycans.

NOD2 is particularly highly expressed in intestinal Paneth cells , where it stimulates expression of antimicrobial *defensins* in response to pathogens.

NOD1 and NOD2 are important in innate immune responses to bacterial pathogens in the gastrointestinal tract (such as *H. pylori*)

NOD1 and NOD2 motif recognition

NOD2

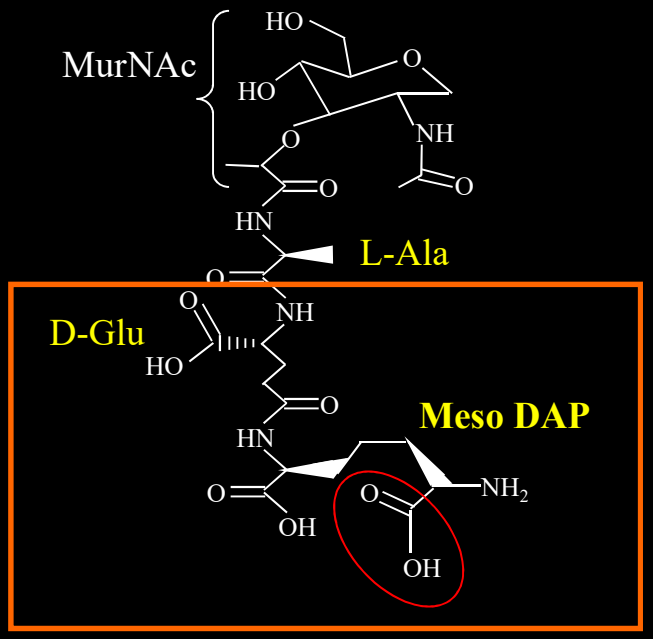


MurNAc-L-Ala-D-Glu

Minimal structure detected by NOD2

Muramyl dipeptide
(from both G- and G+ bacteria)

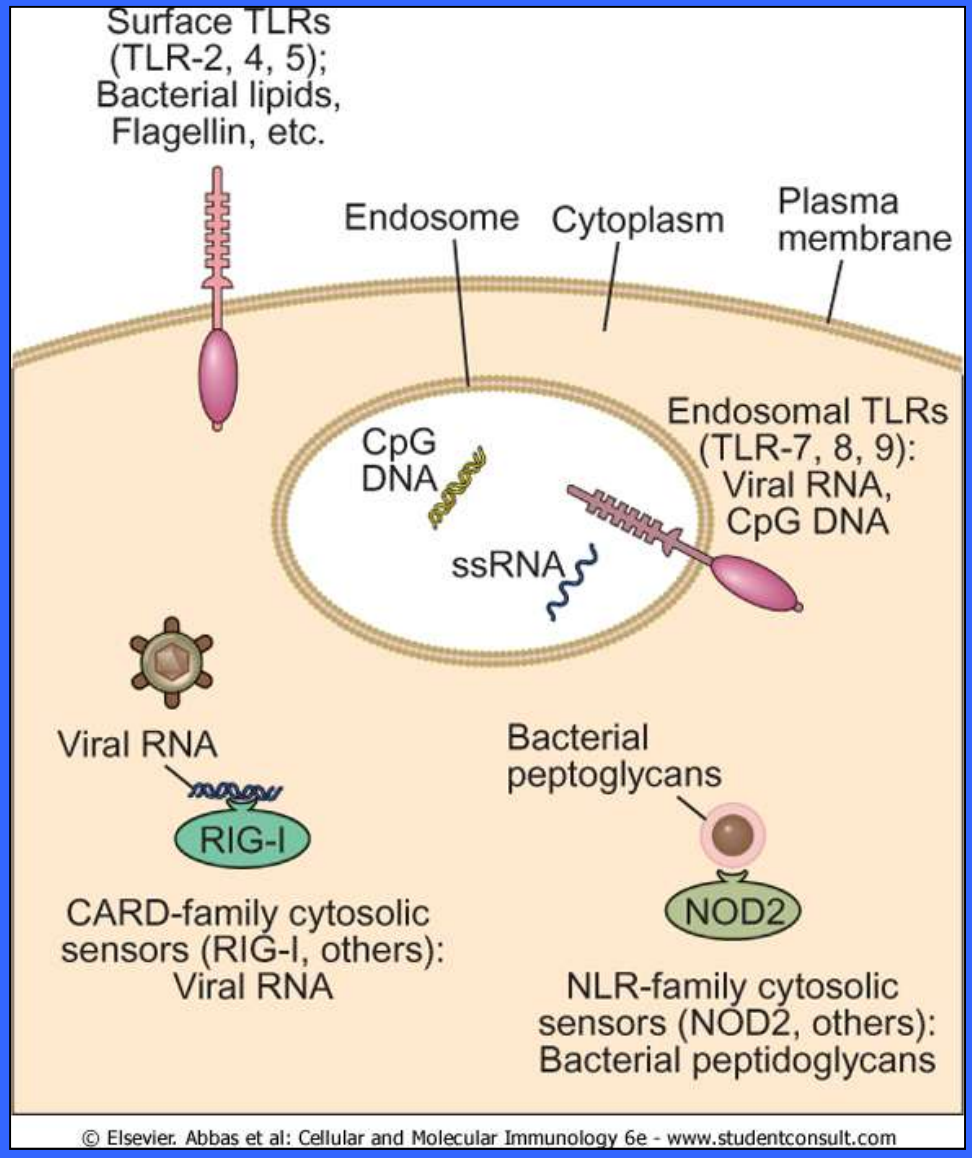
NOD1



MurNAc-L-Ala-D-Glu-mesoDAP

Minimal structure detected by NOD1

(mainly from G- bacteria)


Surface TLRs
(TLR-2, 4, 5);
Bacterial lipids,
Flagellin, etc.
Endosome
Cytoplasm
Plasma
membrane
CpG
DNA
ssRNA
Endosomal TLRs
(TLR-7, 8, 9):
Viral RNA,
CpG DNA
Viral RNA
RIG-I
CARD-family cytosolic
sensors (RIG-I, others):
Viral RNA
Bacterial
peptoglycans
NOD2
NLR-family cytosolic
sensors (NOD2, others):
Bacterial peptidoglycans
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NLRs involved in innate immunity

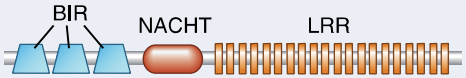
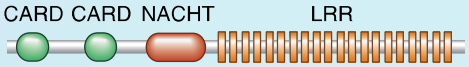
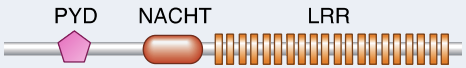
Subfamily	Examples	Typical domain structure	Activating stimuli	Function
NLRA	CIITA		IFN-γ	Class II MHC expression
NLRB	NAIP		Flagellin	Control of *Legionella pneumophila* infection
NLRC	NOD1, NOD2, NLRC3-5		DAP (NOD1)	NF-κB activation
			MDP (NOD2)	NF-κB activation, autophagy, type 1 interferon production
			Flagellin (NLRC4)	Caspase 1 activation, cell death
NLRP	NLRPs, 1-10		Extracellular ATP, alum, asbestos, bacterial toxins, silica, sodium urate, ROS, reduced cytosolic K+ (NLRP3)	Caspase 1 activation
			Lipopeptides (NLRP7)	Caspase 1 activation

FIGURE 4.4 NLRs involved in innate immunity. Members of the NLR family that perform immune functions can be assigned to one of four subfamilies: NLRA, NLRB, NLRC, and NLRP, each with a different N-terminal effector domain. NLRA, better known as CIITA, is a transcription factor that has an N-terminal transactivating (TA) domain required for class II MHC gene expression. NLRB has a baculovirus inhibition of apoptosis protein repeat (BIR) domain, of unknown function. NLRC members have an N-terminal caspase recruitment and activation domain (CARD), which is involved in caspase-1 activation. NLRP members have a pyrin (PYD) domain, which also activates caspase-1. All NLRs contain a central NOD or NACHT (NAIP, CIITA, HET-E, and TP1) domain involved in nucleotide binding, and C-terminal leucine-rich repeat domains involved in ligand recognition. Some of the principal functions, and activating ligands of NLRs, are shown. *DAP*, Diaminopimelic acid; *LRR*, leucine rich repeat; *MDP*, muramyl dipeptide; *NOD*, nucleotide oligomerization domain.

Inflammasome

- A subfamily of NLR proteins, is known as NLRP family. (Hanno un dominio Pirina)
- Humans have 14 NLRP proteins.
- The best characterized is NLRP3 (detta anche criopirina, è un sensore di danno o stress cellulare).

NLRP3 resides in an inactive form in the cytoplasm.

NLRP proteins react to infection or cellular damage through an inflammasome to induce cell death and inflammation.

Several events seem to induce NLRP3 signalling: reduced intracellular K+, generation of ROS, disruption of lysosomes by particulate or cristallin matter. Also death by nearby cells can release ATP into extracellular space.

NLRP3 signalling leads to the generation of pro-inflammatory cytokines and to cell death through formation of a complex known as INFLAMMASOME.

This leads to the trigger of autocleavage of pro-caspase-1, which releases the active caspase-1.

Inappropriate inflammasome activation is associated with various diseases (gout, …)

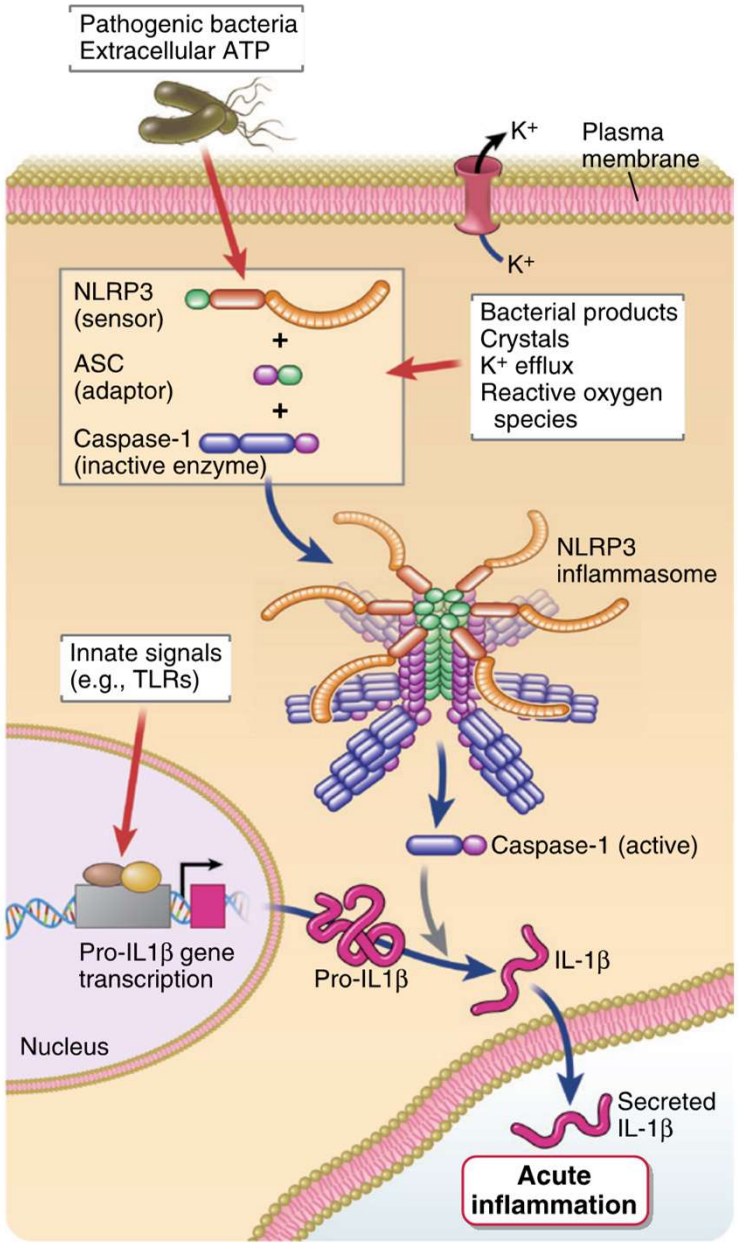


FIGURE 4.6 The inflammasome. The activation of the NLRP3 inflammasome, which processes pro–IL-1 to active IL-1, is shown. Inflammasomes with other NLRP proteins function in a similar way. Various PAMPs or DAMPs induce pro–IL-1β expression through pattern recognition receptor signaling. *ASC*, Apoptosis-associated speck-like protein containing a CARD; *IL-1*, interleukin-1.

Summary

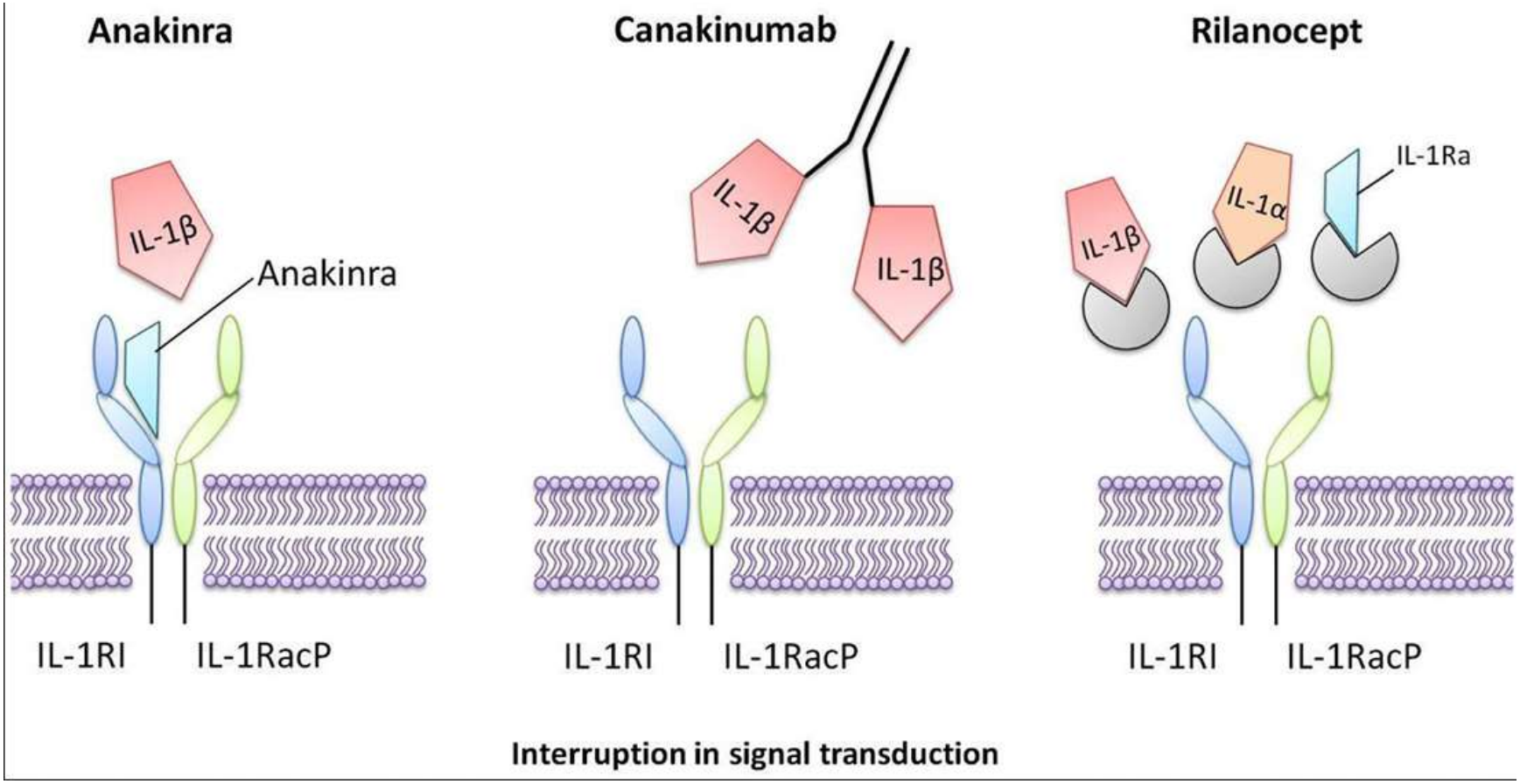
Pyrin-domain containing NLRs assemble into inflammasomes

Simulated inflammasomes activate caspase 1 that in turn generates active IL-1β

Gain of function mutations in these NLRs causes IL-1 dependent autoinflammatory syndromes.

These NLRs can "sense" microbial ligands & also somehow particles and crystals.

DRUGS antagonizing IL-1



Interleukin-1 inhibitors that are in current clinical use.

Anakinra is a recombinant form of human IL-1Ra that directly competes with IL-1 for binding to the IL-1 type I receptor, therefore blocking the biological activity of IL-1. **Canakinumab** is a human monoclonal antibody that selectively targets IL-1β. **Rilonacept** is a human dimeric fusion protein that interrupts IL-1 signaling by incorporating components of the IL-1 receptor, thus trapping and sequestering circulating IL-1.

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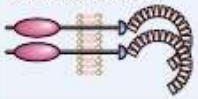
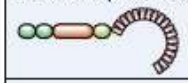
Toll-like receptors (TLRs) 	Plasma membrane and endosomal membranes of dendritic cells, phagocytes, B cells endothelial cells, and many other cell types	TLRs 1-9	Various microbial molecules including bacterial LPS and peptidoglycans, viral nucleic acids
NOD-like receptors (NLRs) 	Cytoplasm of phagocytes epithelial cells, and other cells	NOD1/2 NALP family (inflammasomes)	Bacterial cell wall peptidoglycans Flagellin, muramyl dipeptide, LPS; urate crystals; products of damaged cells
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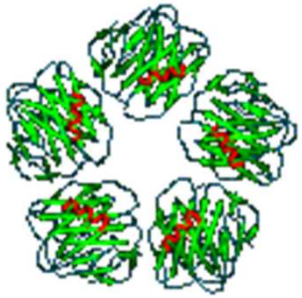
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-Acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	C3	Microbial surfaces
Natural antibodies 	Plasma	IgM	Phosphorylcholine on bacterial membranes and apoptotic cell membranes

PENTRAXIN

A superfamily of conserved proteins that are characterized by a cyclic multimeric structure.

Family of oligomeric plasma proteins all of which bind Ca^{++} ions

They are arranged in a pentagonal or rarely hexagonal cyclic symmetry



Short Pentraxins:

CRP and Serum Amyloid P-like proteins (**SAP**).

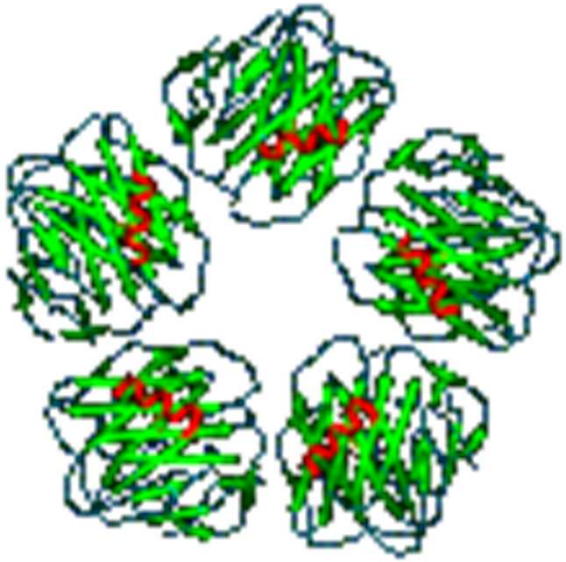
The proteins that bind phosphocholine (in bacterial and fungal polysaccharides and of most biological cell membranes) are CRP- like proteins that bind carbohydrate moieties are SAP-like proteins

Long Pentraxins:

PTX3, riconosce diverse molecole su funghi, batteri G- e G+ e sui virus.

PCR, SAP e PTX3 attivano tutte il complemento legandosi a C1q e iniziando la via classica!

They are arranged in a pentagonal or rarely hexagonal cyclic symmetry



Pentraxin family signature: HxCxS/TWxS

x : - amino acid

Ruolo delle pentraxine nella risposta infiammatoria

Le concentrazioni plasmatiche di PCR sono molto basse negli individui sani, ma possono aumentare fino a 1000 volte durante le infezioni e in risposta ad altri stimoli infiammatori.
Gli aumenti di PCR sono il risultato di un aumento della sintesi a livello epatico attivata dalle citochine IL-6 a IL-1 prodotte dai fagociti nell'ambito dell'immunità innata.
IL-1 e IL-6 inducono la sintesi a livello epatico e l'aumento di livelli plasmatici di SAP e di altre proteine non strettamente legate alle pentraxine, che nel loro insieme sono chiamate proteine di fase acuta.
PTX3 è prodotta da molti tipo cellulari, incluse cellule dendritiche, cellule endoteliali e macrofagi, in risposta ai ligandi del TLR e alle citochine proinfiammatorie, ma non è una proteina della fase acuta.
Viene anche accumulata nei granuli dei neutrofili e rilasciata quando i neutrofili muoiono. Essa riconosce anche le cellule apoptotiche e alcuni microorganismi.

Cosa sono le chemochine?

• Una famiglia di citochine chemoattrattanti, indotte da vari stimoli patogenici, citochine, GF, ecc,

- *Piccole proteine, divise in 4 gruppi: C, CC, CXC, CX3C,*
- *Orchestrano il movimento cellulare: per esempio il traffico delle cellule staminali emopoietiche, il reclutamento dei leucociti durante la risposta infiammatoria, etc.*

• Ogni recettore può rispondere a più di una chemochina, molte chemochine possono usare più di un recettore, e ogni cellula può esprimere molte chemochine e diversi recettori,

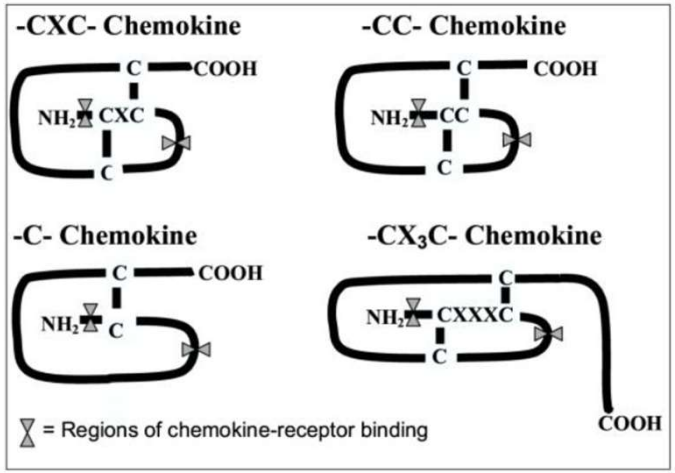
Il **profilo dell'espressione recettoriale** è determinato dalla linea cellulare e dallo stadio di differenziazione della cellula, così come da GF, Ormoni, altre citochine infiammatorie, ecc.

Famiglia	Esempi	Motivo strutturale
CC famiglia	MIP, MCP, I309, ELC, TECK	C C C C
CXC famiglia	IL-8, Gro, SDF-1, NAP-2, γIP-10	CXC C C
C famiglia	Linfotactina	C C
CX_3C famiglia	Fractalchina	CXXXC C C Dominio extracellulare

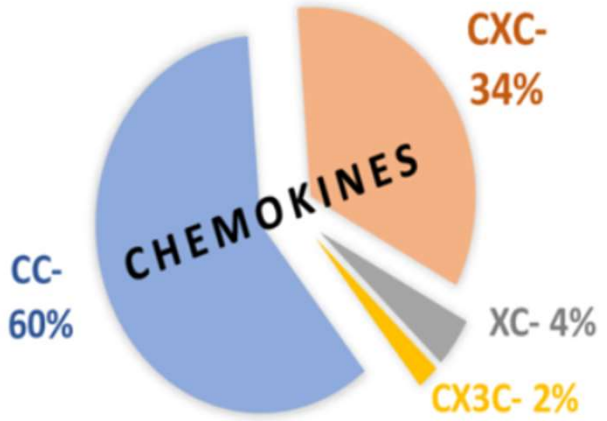
Citoplasmatico

TM

Figura 15.9 Sequenze specializzate (motif) strutturali delle quattro famiglie delle chemochine. La famiglia C, la famiglia CC e la famiglia CXC sono costituite da proteine solubili con residui di cisteina conservati (C) nelle porzioni indicate. X indica la presenza di un aminoacido qualunque tra le cisteine conservate. La famiglia CX_3C attualmente contiene un solo membro: la fractalkina. La fractalkina è una proteina transmembrana che comprende un dominio distale extracellulare contenente un motivo strutturale simile a quello delle chemochine delle altre famiglie. A questo dominio fa seguito una regione peduncolata, una regione transmembrana (TM) ed una regione citoplasmatica. Modificato da S.G. Ward e J. Westwick, *Biochem.J.* 333 (Part 3): 457-470, 1998, previa autorizzazione.

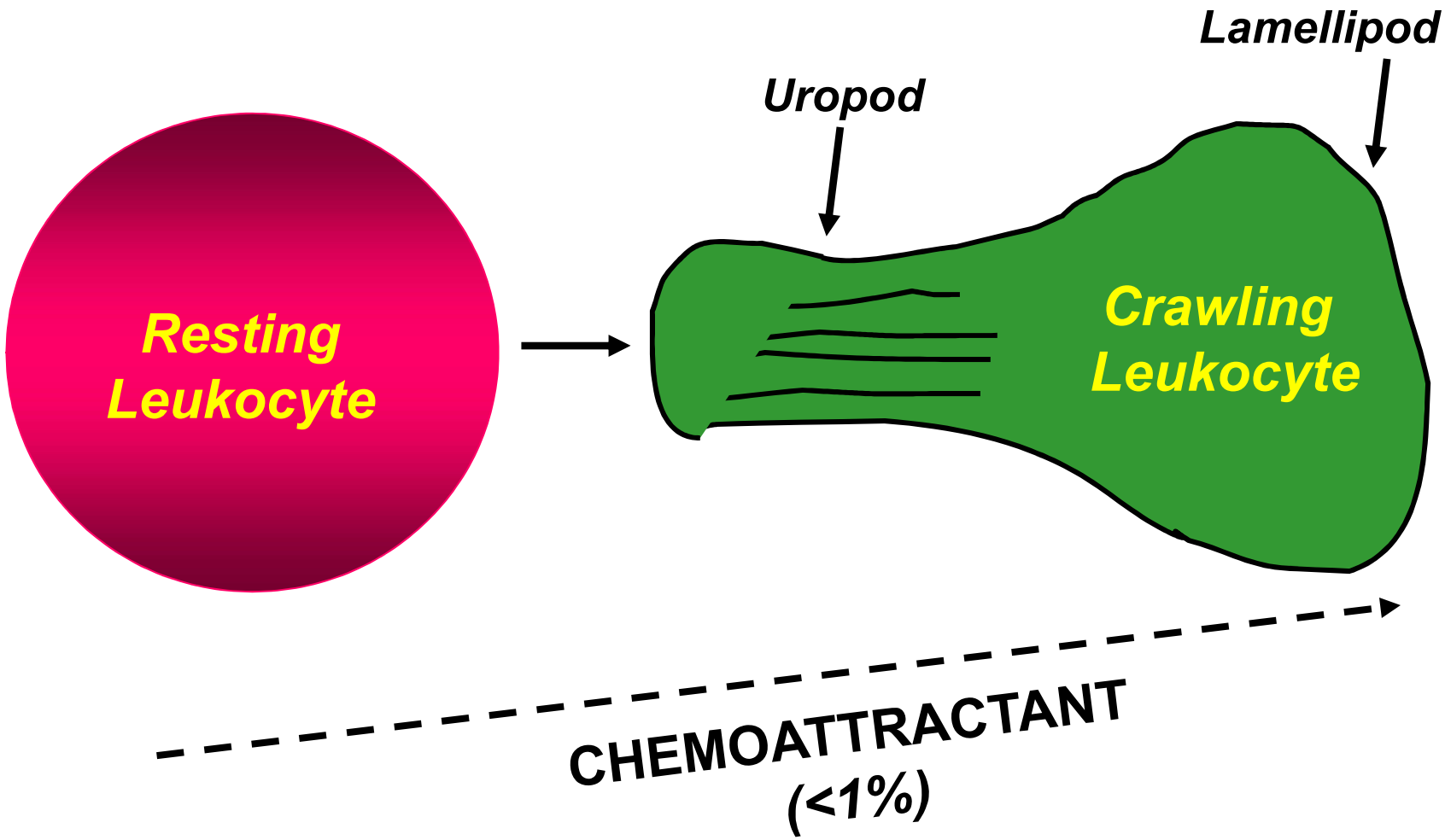


FAMILY	CHEMOKINE	RECEPTORS
CC-	CCL1, I-309	CCR8
	CCL2, MCP-1	CCR1, CCR2, CCR4
	CCL3, MIP-1α	CCR1, CCR5
	CCL4, MIP-1β	CCR1, CCR5, CCR8
	CCL5, RANTES	CCR1, CCR3, CCR5
	CCL6, -	CCR1
	CCL7, MCP3	CCR1, CCR2, CCR3, CCR5
	CCL8, MCP-2	CCR1, CCR2, CCR3, CCR5
	CCL9, -	CCR1
	CCL10, -	unknown
	CCL11, Eotaxin	CXCR3, CCR3, CCR5
	CCL12, -	CCR2
	CCL13, MCP-4	CCR1, CCR2, CCR3, CCR5
	CCL14, HCC-1	CCR1
	CCL15, HCC-2	CCR1, CCR3
	CCL16, HCC-4	CCR1
	CCL17, TARC	CCR4
	CCL18, DC-CK1	unknown
	CCL19, MIP-3β	CCR7
	CCL20, MIP-3α	CCR6
	CCL21, 6Ckine	CXCR3, CCR7
	CCL22, MDC	CCR4
	CCL23, MPIF-1	CCR1
	CCL24, Eotaxin-2	CCR3
	CCL25, TECK	CCR9
	CCL26, Eotaxin-3	CCR3, CCR10
	CCL27, CTACK	CCR10
	CCL28, MEC	CCR3, CCR10, CCR10



FAMILY	CHEMOKINE	RECEPTORS
CXC-	CXCL1, GROα	CXCR2
	CXCL2, GROβ	CXCR2
	CXCL3, GROγ	CXCR2
	CXCL4, PF4	unknown
	CXCL5, ENA-78	CXCR1, CXCR2
	CXCL6, GCP-2	CXCR1, CXCR2
	CXCL7, NAP-2	CXCR1, CXCR2
	CXCL8, IL-8	CXCR1, CXCR2
	CXCL9, Mig	CXCR3
	CXCL10, IP-10	CXCR3
	CXCL11, I-TAC	CXCR3
	CXCL12, SDF-1α/β	CXCR4
	CXCL13, BCA-1	CXCR5
	CXCL14, -	unknown
	CXCL15, -	unknown
	CXCL16, -	CXCR6
XC-	XCL1, SCM-1α	XCR1
	XCL2, SCM-1β	XCR1
CX3C-	CX3CL1, Fractalkine	CX3CR1

Leukocytes Polarize in Gradients of Chemoattractants, then Crawl to the Source



Chemokines

- Multiple families of small molecular weight cytokines (at least 60 at present time)
- Classified based on different cysteine motifs
- Involved in multiple immune functions including inflammation, cell recruitment, lymphocyte trafficking, lymphoid organ development and wound healing
- Expressed in primary and secondary lymphoid organs

• Una delle funzioni più importanti delle chemochine è quella di indurre e regolare la migrazione dei leucociti nelle varie fasi della flogosi così come dirigere il normale traffico di queste cellule nell'ambito dei tessuti linfoidi.

• Le chemochine influenzano il movimento cellulare attraverso gradienti chemotattici, la produzione di molecole di adesione e l'attivazione di funzioni cellulari.

• Possono inoltre esercitare funzioni al di fuori del sistema immune, come ad esempio la differenziazione cellulare dei tessuti

Chemokines in Inflammation

The large number of chemokines and chemokine receptors allows for a significant amount of homing specificity to be imparted by these molecules

Examples: (PARTIAL LIST)

Cell type	Chemokine Receptors	Ligands
Neutrophils	CXCR1, CXCR2	IL-8, GCP-2, Gro-α
Eosinophils	CCR1, CCR3	Eotaxin, MIP-1α, MCP-3
Monocytes	CCR1, CCR2, CCR5	MCP-1, 2, 3, 5, RANTES, MIP-1α
Naïve T	CCR7, CXCR4	SLC, SDF-1
Naïve B	CXCR5, CXCR4, CCR7	BLC, SDF-1, SLC
Th1 effector	CCR2, CCR5, CXCR3	MIP-1α, MCP1, RANTES, IP10
Th2 effector	CCR3, CCR4, CCR8	Eotaxin, MDC, TARC, I309
CD8 effector	CCR2, CCR5, CXCR3	MCP1, MIP1a, RANTES, IP10
Immature DC	CCR1,2,3,4,5,6	MCP-1, 2, 3, 5, RANTES, MIP-1α

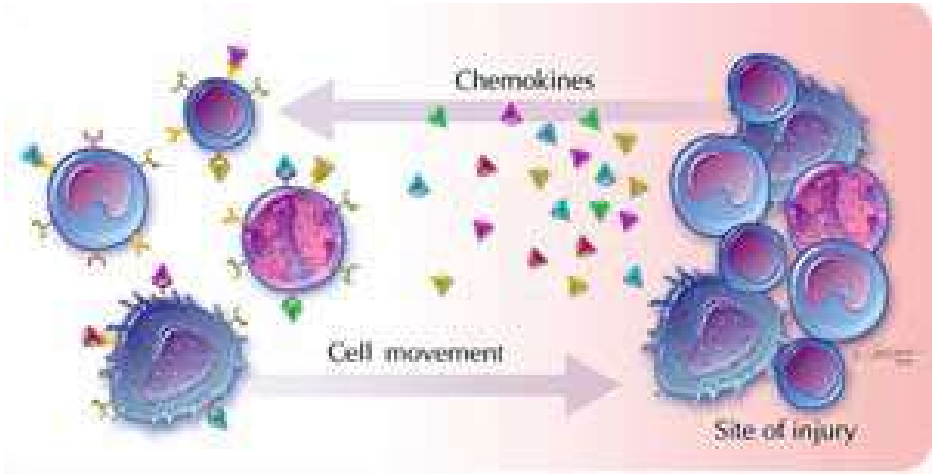
(see Zlotnik and Yoshie, Immunity 12, 121 (2000) for standardized chemokine nomenclature)

Table 3 | **Major chemokine and chemokine receptor antagonists tested clinically as cancer therapies**

Target	Tumour-promoting actions	Antagonists	Other cancer treatments used in combinations	Refs
CCL2 or CCR2	Myeloid cell chemoattraction and T cell stimulation	Carlumab (anti-CCL2 mAb), plozalizumab (anti-CCR2 mAb), PF-04136309, CCX872-B (small-molecule CCR2 antagonists) and BMS-813160 (dual CCR2/CCR5 inhibitor)	FOLFIRINOX, nab-paclitaxel–gemcitabine or ICIs	172–180
CCR4	Highly expressed on T_{reg} cells	Mogalizumab (anti-CCR4 mAb) and FLX475 (small-molecule CCR4 antagonist)	Nivolumab or pembrolizumab (anti-PD-1 mAbs) or utomilumab (4-1BB agonist)	181–186
CCR5	Immunosuppression, angiogenesis, metabolic reprogramming and stem cell stimulation	Maraviroc and vicriviroc (small-molecule CCR5 antagonists), leronlimab (anti-CCR5 mAb) and BMS-813160	Pembrolizumab or nivolumab	188–192
CXCL8 (IL-8) or CXCR1/2	Inflammation, angiogenesis and myeloid cell chemotaxis	BMS-986253 (HuMax-IL8; anti-IL-8 mAb), SX682, navarixin, AZD5069 and reparixin (small-molecule CXCR1 and/or CXCR2 antagonists)	Nivolumab or paclitaxel	193–199
CXCR4	Malignant cell proliferation, angiogenesis, and stem cell migration and chemotaxis	Plerixafor and LY2510924 (small-molecule CXCR4 antagonists), BL-8040 (small-peptide CXCR4 antagonist)	Sunitinib (multi-target TKI), gemcitabine, carboplatin–etoposide, pembrolizumab or durvalumab (anti-PD-L1 mAb)	200–212
CSF1 or CSF1R	CSF1 is a growth factor and CSF1R a lineage marker for monocytes and macrophages	Lacnotuzumab and PD-0360324 (anti-CSF1 mAbs); pexidartinib, edicotinib, ARRY-382, PLX7486 and BLZ945 (CSF1R TKIs); emactuzumab, LY3022855, cabiralizumab and AMG820 (anti-CSF1R mAbs)	Multiple trials of combinations with ICIs, chemotherapy or targeted agents	174,213–226

FOLFIRINOX, folinic acid, 5-fluorouracil, irinotecan and oxaliplatin; ICIs, immune-checkpoint inhibitors; mAb, monoclonal antibody; TKIs, tyrosine kinase inhibitors; T_{reg}, regulatory T.

Circolazione leucocitaria e migrazione nei tessuti: Molecole di adesione e chemochine

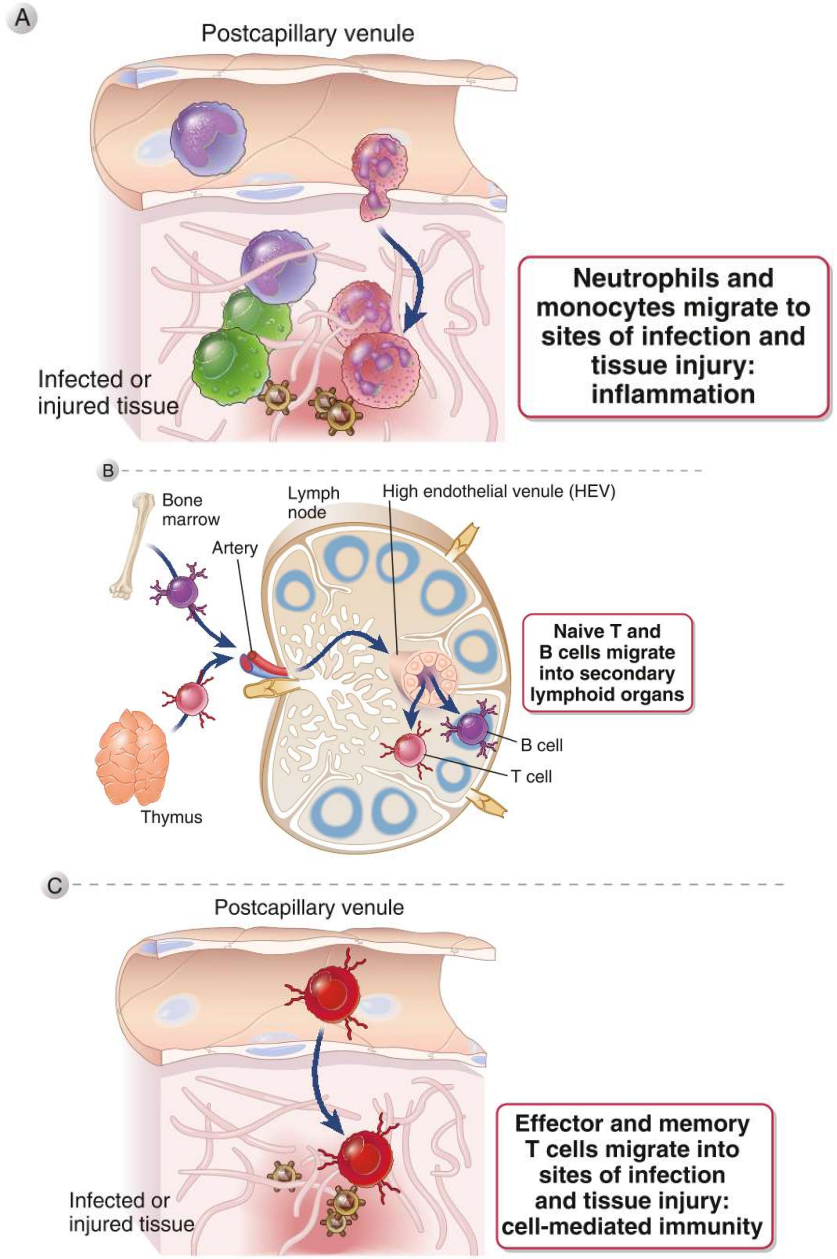


MIGRAZIONE DEI LEUCOCITI

Un adeguato direzionamento dei leucociti ai tessuti è un aspetto critico per una efficace risposta immunitaria!

Scopo della migrazione dei leucociti:

- Rilascio di PMN e monociti dalla circolazione ai siti di infezione o danno
- Rilascio di linfociti dai siti di maturazione (organi primari) agli organi linfoidi secondari in cui i linfociti riconoscono l'antigene, proliferano e differenziano
- Rilascio di linfociti effettori dagli organi linfoidi secondari ai siti di infezione



Biological activities of chemokines:

• Inflammatory cells recruitment. General rule: **CC family** attracts monocytes and lymphocytes; **CXC family** attracts granulocytes and lymphocytes (CCL2-monocytes, CCL11-eosinophils, CCL5-T effettor lymphocytes; CXCL1, CXCL2, IL-8, CXCL3-neutrophils; CXCL9, CXCL10, CXCL11-T helper 1)

• Recruitment in peripheral lymphoid tissues (CXCL13, B lymphocytes in the spleen; XCL1, T cell precursors in the thymus; CCL19, CCL21-T naive lymphoocytes and DCs in lymph nodes; SDF-1, precursors in the bone marrow)

• Development and organization of secondary lymphoid tissues (produced in response to lymphotoxins)

• Angiogenesis and wound repair (CXC family, receptors expressed on fibroblasts and endothelial cell surface as CXCR2)

• Embriogenesis (CXCR4 regulates heart and cerbellum development)

• Chemokine receptors are desensitized and internalized after binding

TABLE 3.2 Selected Chemokines and Chemokine Receptors

Chemokine	Original Name	Chemokine Receptor	Major Function
CC Chemokines			
CCL2	MCP-1	CCR2	Mixed leukocyte recruitment
CCL3	MIP-1α	CCR1, CCR5	Mixed leukocyte recruitment
CCL4	MIP-1β	CCR5	T cell, dendritic cell, monocyte, and NK recruitment; HIV coreceptor
CCL5	RANTES	CCR1, CCR3, CCR5	Mixed leukocyte recruitment
CCL11	Eotaxin	CCR3	Eosinophil, basophil, and Th2 recruitment
CCL17	TARC	CCR4	T cell recruitment
CCL19	MIP-3β/ELC	CCR7	T cell and dendritic cell migration into parafollicular zones of lymph nodes
CCL21	SLC	CCR7	T cell and dendritic cell migration into parafollicular zones of lymph nodes
CCL22	MDC	CCR4	NK cell, T cell recruitment
CCL25	TECK	CCR9	Lymphocyte recruitment into intestine
CCL27	CTACK	CCR10	T cell recruitment into skin
CXC Chemokines			
CXCL1	GROα	CXCR2	Neutrophil recruitment
CXCL8	IL-8	CXCR1, CXCR-2	Neutrophil recruitment
CXCL9	Mig	CXCR3	Effector T cell recruitment
CXCL10	IP-10	CXCR3	Effector T cell recruitment
CXCL12	SDF1	CXCR4	B cell migration into lymph nodes; plasma cell migration into bone marrow
CXCL13	BCA-1	CXCR5	B cell migration into lymph nodes and into follicles; T follicular helper cell migration into follicles
C Chemokines			
XCL1	Lymphotactin	XCR1	T cell and NK cell recruitment
CX3C Chemokines			
CX3CL1	Fractalkine	CX3CR1	T cell, NK cell, and monocyte recruitment

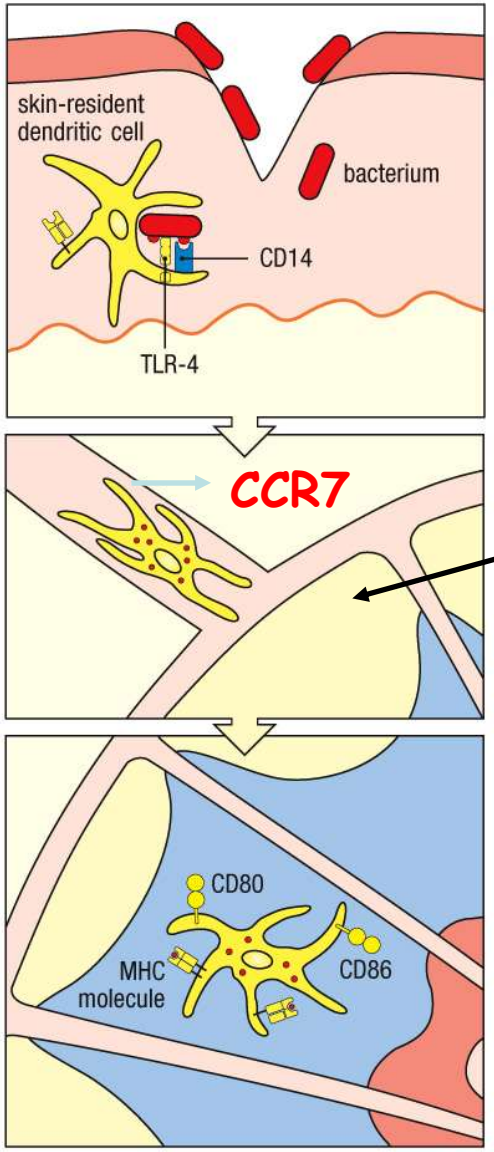
CTL, Cytotoxic T lymphocyte; *IL*, Interleukin; *NK*, natural killer cells.

Prodotte da leucociti e da altre cellule in cui la secrezione è indotta da PRR-PAMP

Da CCL1 a CCL27

Da CXCL1 A CXCL17

CHEMOKINES ARE RELEASED BY CELLS OF INNATE IMMUNITY IN RESPONSE TO SIGNALLING OF PATTERN RECOGNITION RECEPTORS (PRR)



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- Activation of innate sensors in macrophages and dendritic cells triggers changes in gene expression that have far-reaching effects on the immune response.

CCL19 and CCL21 by HEV

- These changes result in the expression/down-regulation of adhesion molecules, chemokines and chemokine receptors allowing migration and also immune cell recruitment.

Chemokines released by macrophages and dendritic cells recruit effector cells to sites of infection

Class	Chemokine	Produced by	Receptors	Cells attracted	Major effects
CXC	CXCL8 (IL-8)	Monocytes Macrophages Fibroblasts Epithelial cells Endothelial cells	CXCR1 CXCR2	Neutrophils Naive T cells	Mobilizes, activates and degranulates neutrophils Angiogenesis
	CXCL7 (PBP, β-TG, NAP-2)	Platelets	CXCR2	Neutrophils	Activates neutrophils Clot resorption Angiogenesis
	CXCL1 (GROα) CXCL2 (GROβ) CXCL3 (GROγ)	Monocytes Fibroblasts Endothelium	CXCR2	Neutrophils Naive T cells Fibroblasts	Activates neutrophils Fibroplasia Angiogenesis
CC	CCL3 (MIP-1α)	Monocytes T cells Mast cells Fibroblasts	CCR1, 3, 5	Monocytes NK and T cells Basophils Dendritic cells	Competes with HIV-1 Antiviral defense Promotes T_H1 immunity
	CCL4 (MIP-1β)	Monocytes Macrophages Neutrophils Endothelium	CCR1, 3, 5	Monocytes NK and T cells Dendritic cells	Competes with HIV-1
	CCL2 (MCP-1)	Monocytes Macrophages Fibroblasts Keratinocytes	CCR2B	Monocytes NK and T cells Basophils Dendritic cells	Activates macrophages Basophil histamine release Promotes T_H2 immunity
	CCL5 (RANTES)	T cells Endothelium Platelets	CCR1, 3, 5	Monocytes NK and T cells Basophils Eosinophils Dendritic cells	Degranulates basophils Activates T cells Chronic inflammation
CXXXC (CX_3C)	CX3CL1 (Fractalkine)	Monocytes Endothelium Microglial cells	CX_3CR1	Monocytes T cells	Leukocyte–endothelial adhesion Brain inflammation

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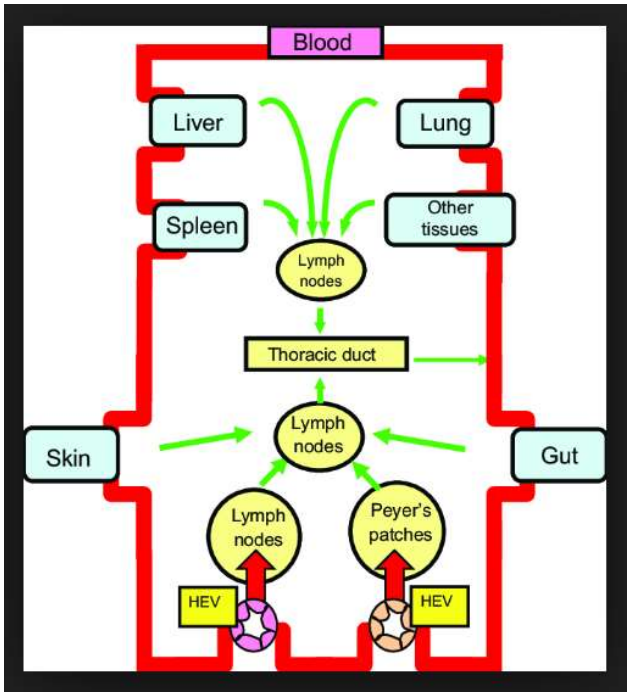
Azione biologica delle chemochine:

1. Nelle reazioni infiammatorie

- Aumentano l'adesione dei leucociti all'endotelio
- Indirizzano la migrazione attraverso i vasi sanguigni

1. Sono coinvolte nello sviluppo degli organi linfoidi e regolano il traffico dei linfociti e di altri leucociti attraverso diverse regioni degli organi linfoidi secondari

2. Sono necessarie per la migrazione delle DC dai siti di infezione ai linfonodi drenanti

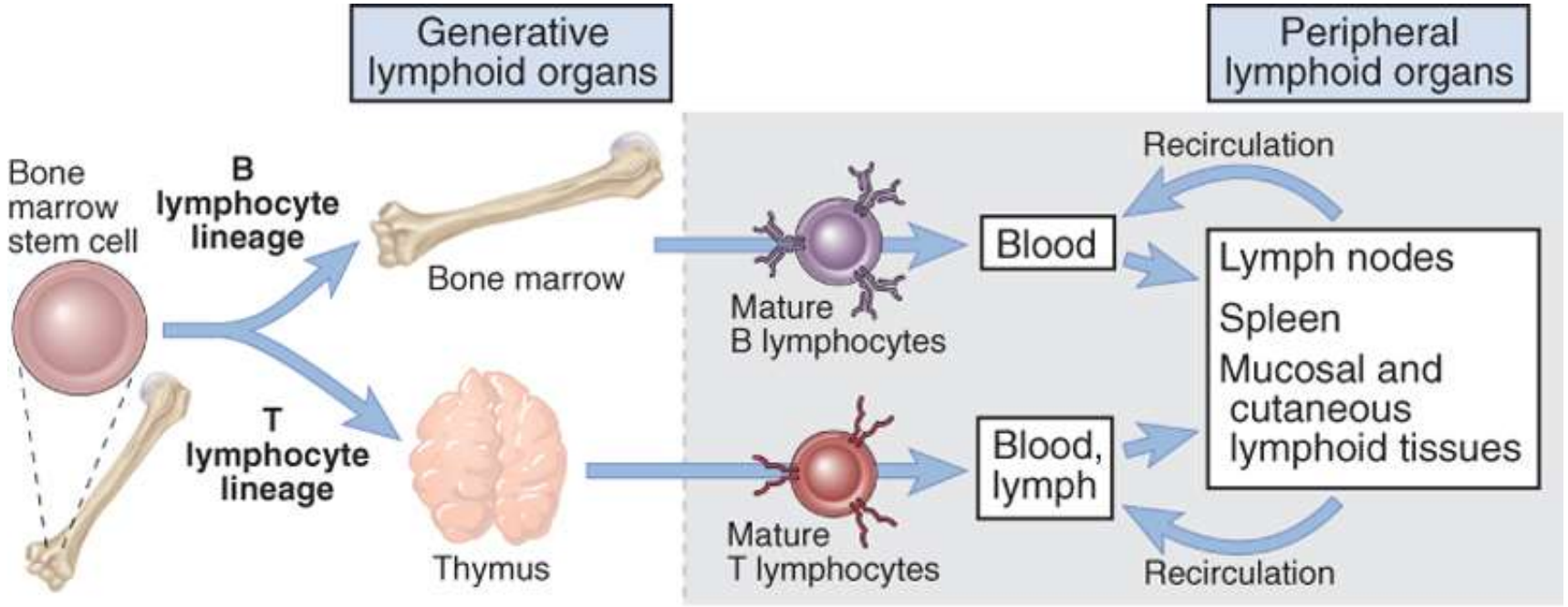


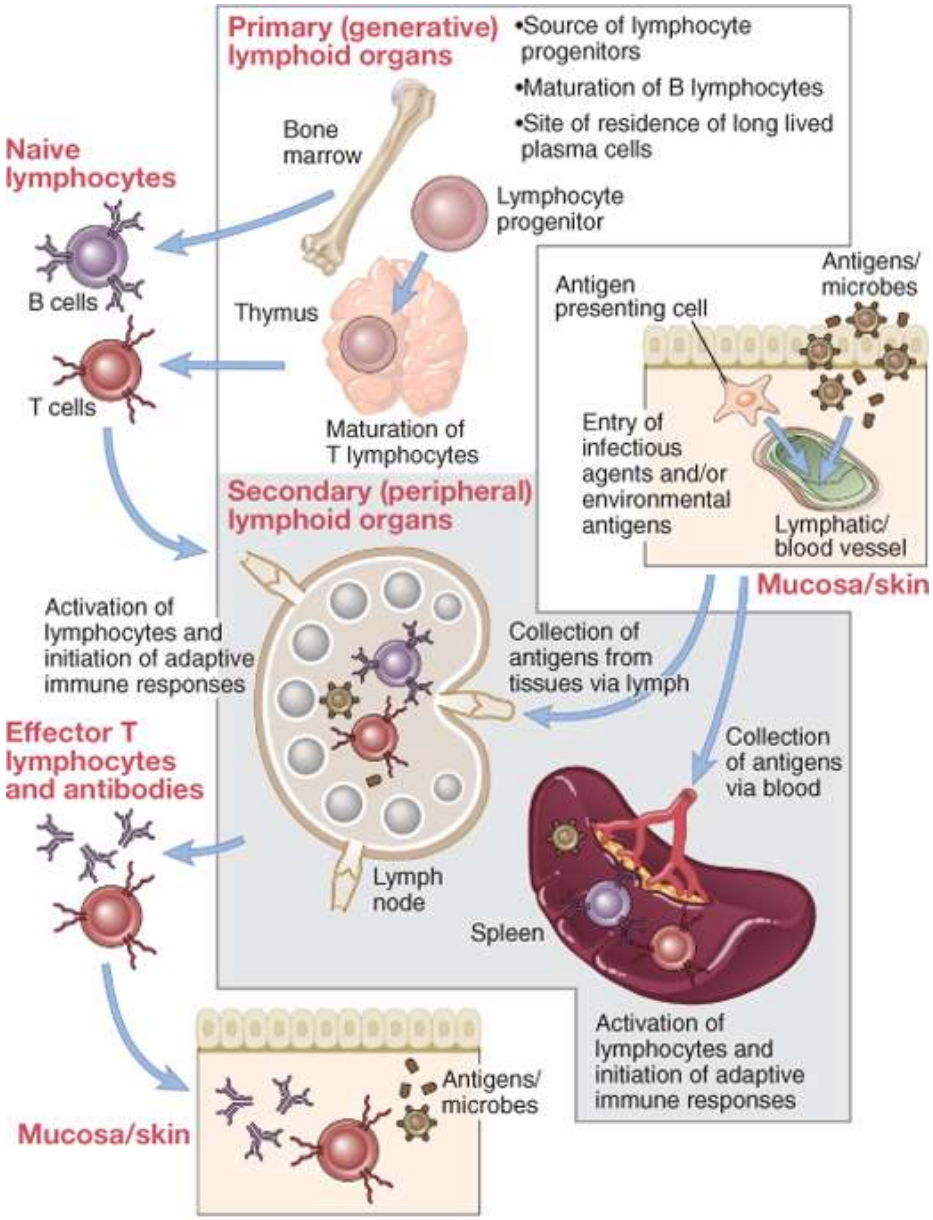
RECLUTAMENTO DEI LEUCOCITI NEI TESSUTI: MIGRAZIONE E RICIRCOLAZIONE DELLE CELLULE DEL DEL SISTEMA IMMUNITARIO

Una delle caratteristiche peculiari del SI è il movimento costante e strettamente regolato delle sue componenti cellulari che possono passare dal sangue ai tessuti e spesso ritornare in circolo.
Questo movimento assolve a 3 funzioni principali:

1. Indirizzamento delle cellule mieloidi (neutrofili e monociti) dal sangue ai focolai tissutali di infezione
2. Indirizzamento dei linfociti dai siti di maturazione (timo e midollo osseo) agli organi linfoidi secondari
3. Indirizzamento dei linfociti effettori dagli organi linfoidi secondari (sede di attivazione) ai siti di infezione

La migrazione di un leucocita dal sangue ad un determinato tessuto o verso un sito di infezione o di danno tissutale viene definita HOMING leucocitario mentre il movimento dei leucociti dal sangue ai tessuti viene definito MIGRAZIONE o RECLUTAMENTO.
La capacità dei linfociti di migrare ripetutamente in organi linfoidi secondari , risiedervi in via transitoria e tornare al sangue viene definita RICIRCOLAZIONE.





Molecole di adesione espresse dai leucociti e dalle cellule endoteliali coinvolte nel reclutamento leucocitario

L'adesione dei leucociti circolanti all'endotelio vascolare è mediato da 2 classi di molecole:

- ***SELECTINE***
- ***INTEGRINE***

e dai loro ligandi.

L'espressione di queste molecole varia tra le diverse popolazioni leucocitarie e nei vasi sanguigni presenti nei diversi distretti anatomici.

Selectine: molecole di adesione che legano carboidrati sulla membrana plasmatica; mediano lo step iniziale a bassa affinità di adesione all'endotelio delle venule post-capillari. Il dominio extracellulare è simile alle lectine di tipo C.

Integrine: molecole di superficie che mediano l'adesione cellula-cellula o cellula-matrice extracellulare. Sono tutti eterodimeri (catena α e catena β). Oltre alla funzione di molecole di adesione, sono anche in grado di trasdurre il segnale.

TABLE 3.1 Major Leukocyte-Endothelial Adhesion Molecules

Family	Molecule	Distribution	Ligand (Molecule; Cell Type)
Selec		Endothelium activated by histamine or thrombin	Sialyl Lewis X on PSGL-1 and other glycoproteins; neutrophils, mon memory)
		Endothelium activated by cytokines (TNF, IL-1)	Sialyl Lewis X (e.g., neutrophils, monocytes, T cells (effector, memory)
		Neutrophils, monocytes, T cells (naive B cells (naive)	Sialyl Lewis X/PNAd on GlyCAM-1, CD34, MadCAM-1, others; endothelium (HEV)
Integrin	LFA-1 (CD11aCD18)	Neutrophils, monocytes, T cells (naive, effector, memory), B cells (naive)	ICAM-1 (CD54), ICAM-2 (CD102); endothelium (upregulated when cytokine activated)
	Mac-1 (CD11bCD18)	Neutrophils, monocytes, dendritic cells	ICAM-1 (CD54), ICAM-2 (CD102); endothelium (upregulated when cytokine activated)
	VLA-4 (CD49aCD29)	Monocytes, T cells (naive, effector, memory)	VCAM-1 (CD106); endothelium (upregulated when cytokine activated)
	$\alpha_4\beta_7$ (CD49dCD29)	Monocytes, T cells (gut homing, naive, effector, memory), B cells (gut homing)	VCAM-1 (CD106), MadCAM-1; endothelium in gut and gut-associated lymphoid tissues

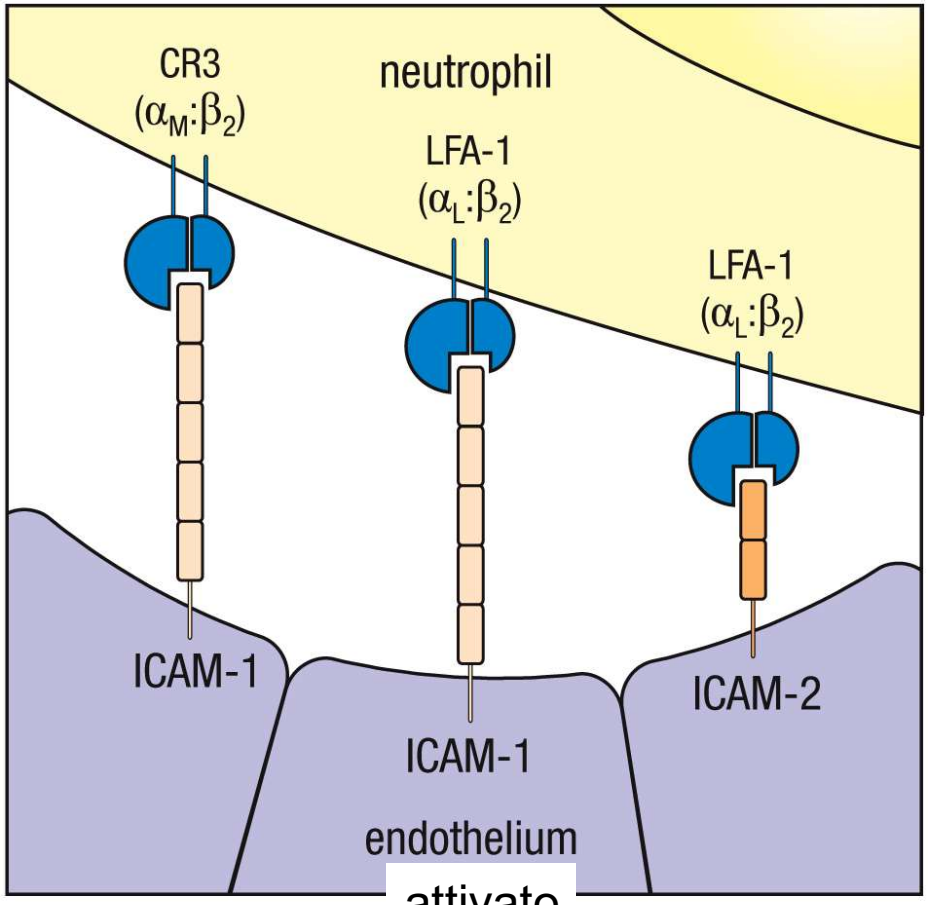
Stoccate nei granuli citoplasm. delle cellule endoteliali ed espresse sulla superficie nell'arco di 1-2 ore , dopo stimolazione con citochine o dal rilascio di istamina o trombina

Cruciale per indirizzare i In T e B ai In attreverso HEV

I leucociti esprimono molecole che legano la E la la P-selectina

CLA-1, Cutaneous lymphocyte antigen 1; *GlyCAM-1,* glycan-bearing cell adhesion molecule 1; *HEV,* high endothelial venule; *ICAM-1,* intracellular adhesion molecule 1; *IL-1,* interleukin-1; *LFA-1,* leukocyte function–associated antigen 1; *MadCAM-1,* mucosal addressin cell adhesion molecule 1; *PNAd,* peripheral node addressin; *PSGL-1,* P-selectin glycoprotein ligand 1; *TNF,* tumor necrosis factor; *VCAM-1,* vascular cell adhesion molecule 1; *VLA-4,* very late antigen 4.

Le integrine sono proteine di membrana responsabili dell'adesione intercellulare o delle cellule alla matrice extracellulare.
Sono costituite da eterodimeri tra una catena α e una β



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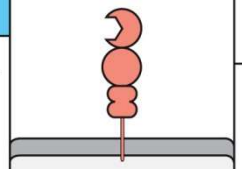
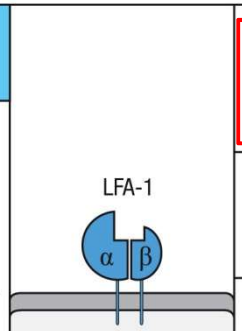
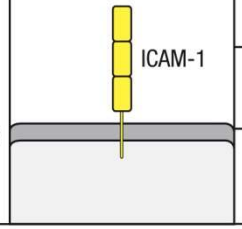
Ligandi di LFA-1:
ICAM-1
ICAM-2
ICAM-3

Selectins, integrins, addressins, CAMs

Cell-adhesion molecules (CAM) control interactions between leukocytes and endothelial cells during an inflammatory response.

		Name	Tissue distribution	Ligand
Selectins Bind carbohydrates. Initiate leukocyte–endothelial interaction		P-selectin (PADGEM, CD62P)	Activated endothelium and platelets	PSGL-1, sialyl-Lewis^x
		E-selectin (ELAM-1, CD62E)	Activated endothelium	Sialyl-Lewis^x
Integrins 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
		α_5:β_1 (VLA-5, CD49d:CD29)	Monocytes, macrophages	Fibronectin
Immunoglobulin superfamily Various roles in cell adhesion. Ligand for integrins		ICAM-1 (CD54)	Activated endothelium, activated leukocytes	LFA-1, Mac1
		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

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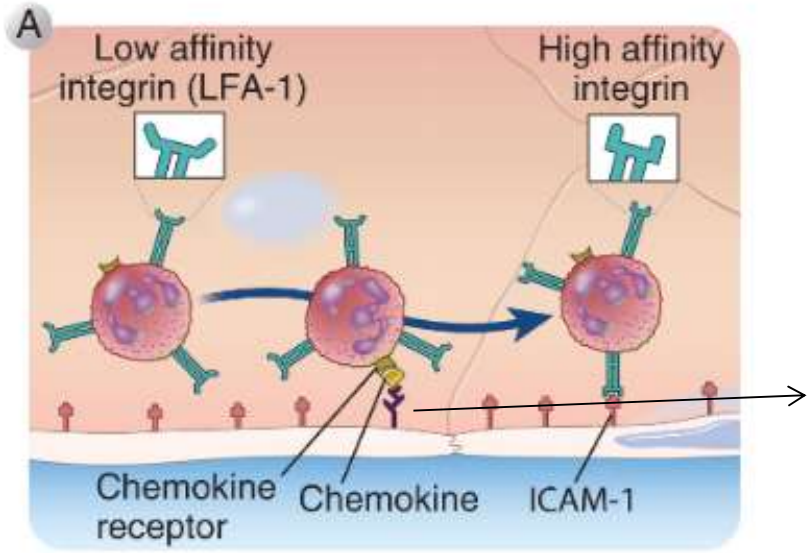
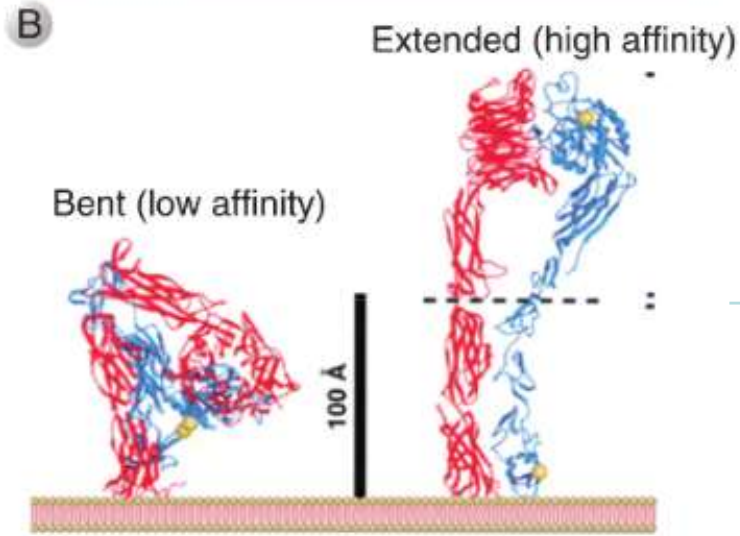
Le integrine aumentano rapidamente la loro affinità in risposta a segnali attivati dal legame delle chemochine ai rispettivi recettori

Le integrine sono espresse sui leuciti circolanti in uno stato di bassa affinità. Quando un leucocita entra in contatto con le cellule endoteliali attivate, le chemochine presenti sulla superficie vengono riconosciute da specifici recettori.



Le chemochine prodotte nel tessuto si legano ai proteoglicani Eparan solfato sulle cellule endoteliali



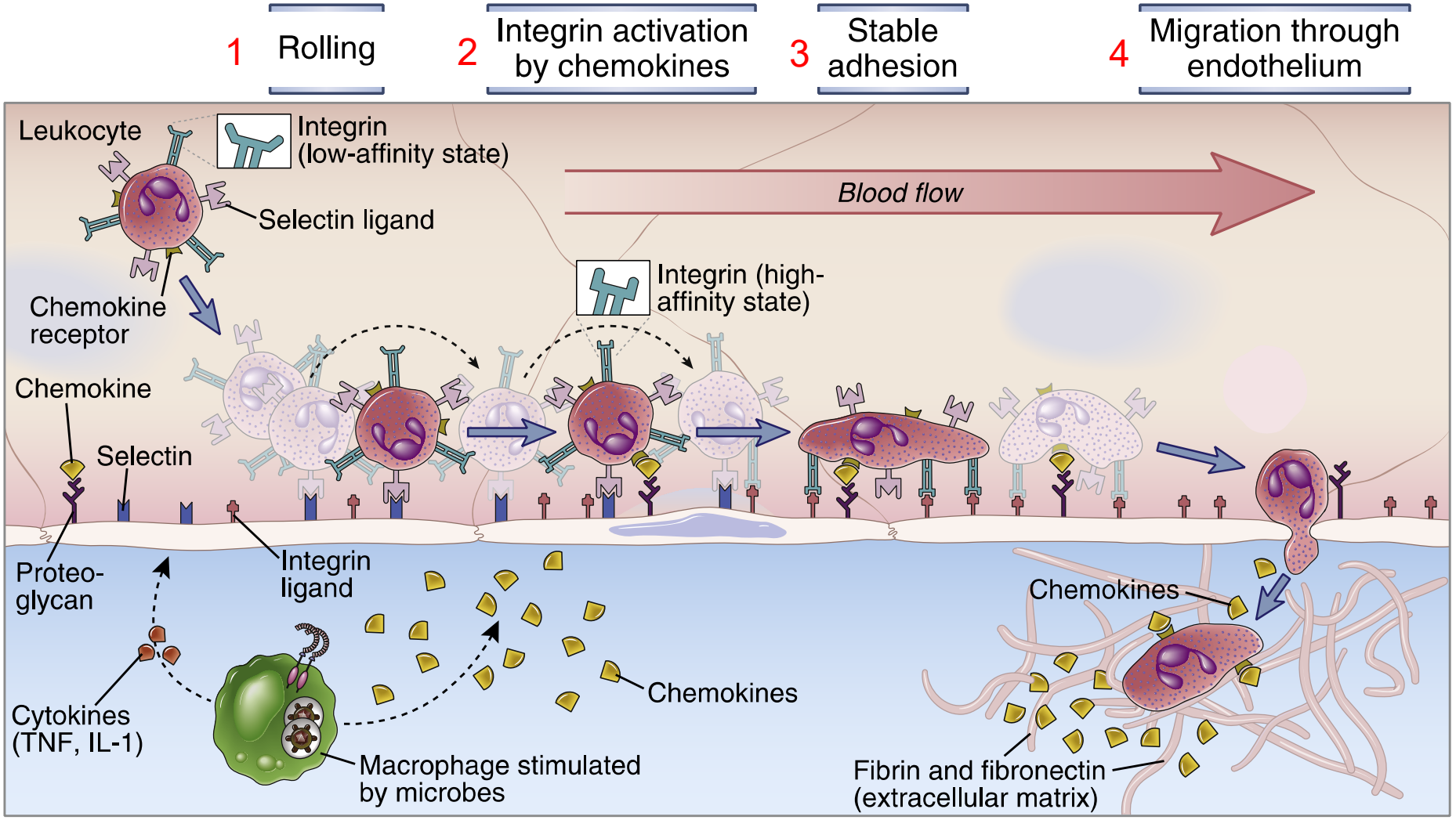
Inside-out signaling

Interazione leucociti-endotelio e reclutamento dei leucociti nei tessuti

- Il reclutamento dal sangue ai tessuti necessita l'adesione ed il passaggio dei leucociti all'endotelio delle venule postcapillari ed il passaggio nella regione extravascolare.

- Questo processo avviene attraverso tappe sequenziali orchestrate da specifiche molecole di adesione e chemochine

Ruolo delle molecole di adesione nella migrazione leucocitaria



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Neutrophils make up the first wave of cells that cross the blood vessel wall to enter an inflamed tissue

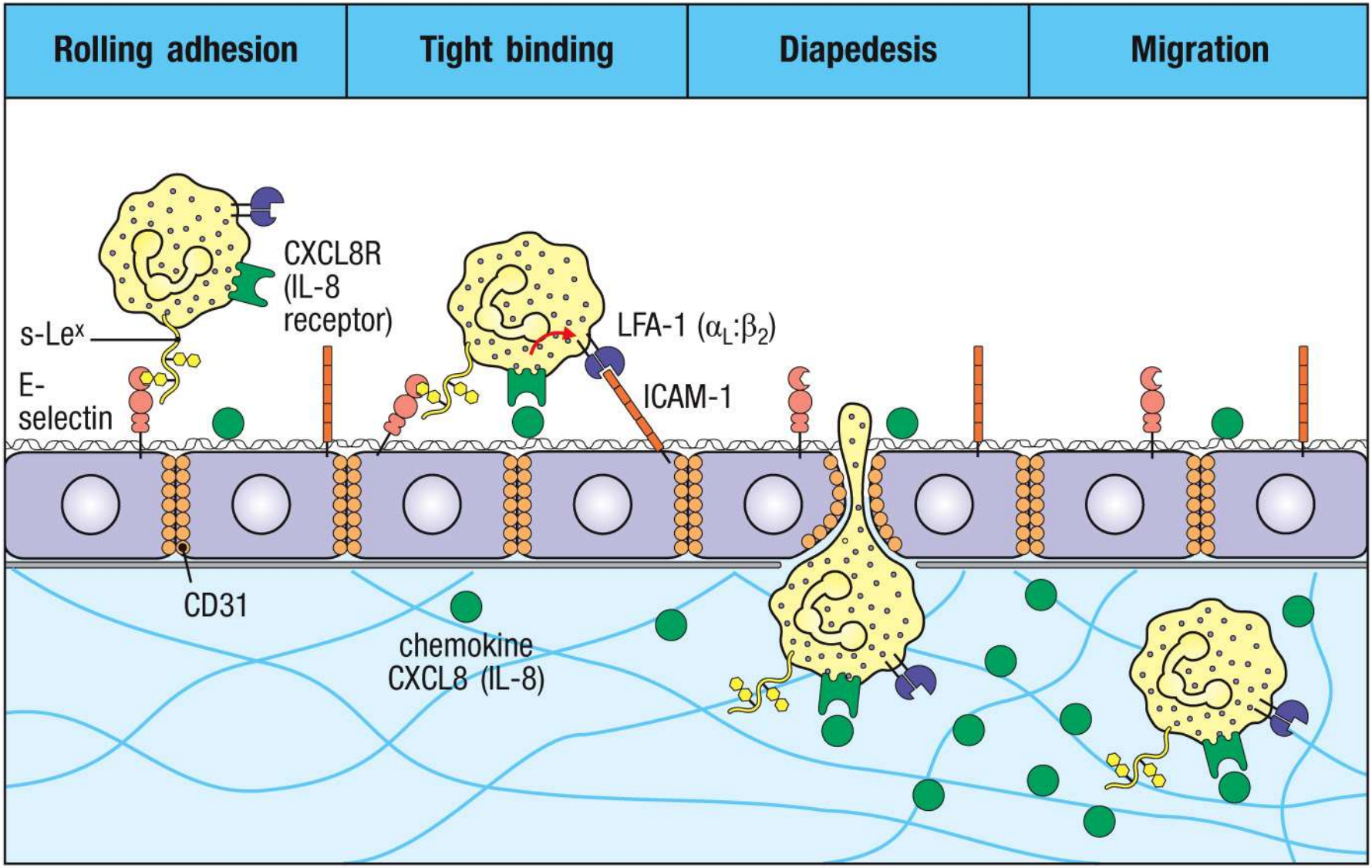
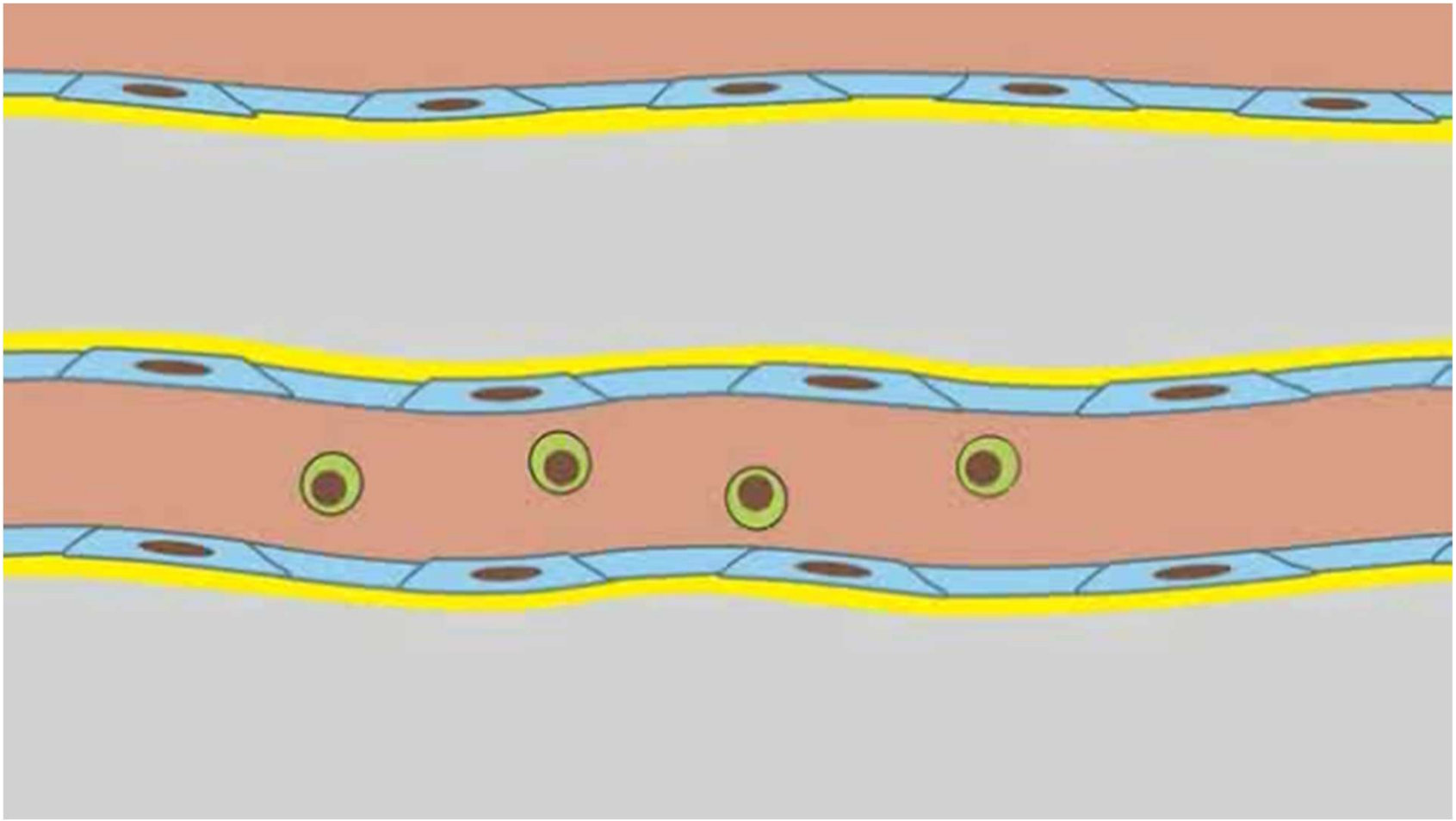
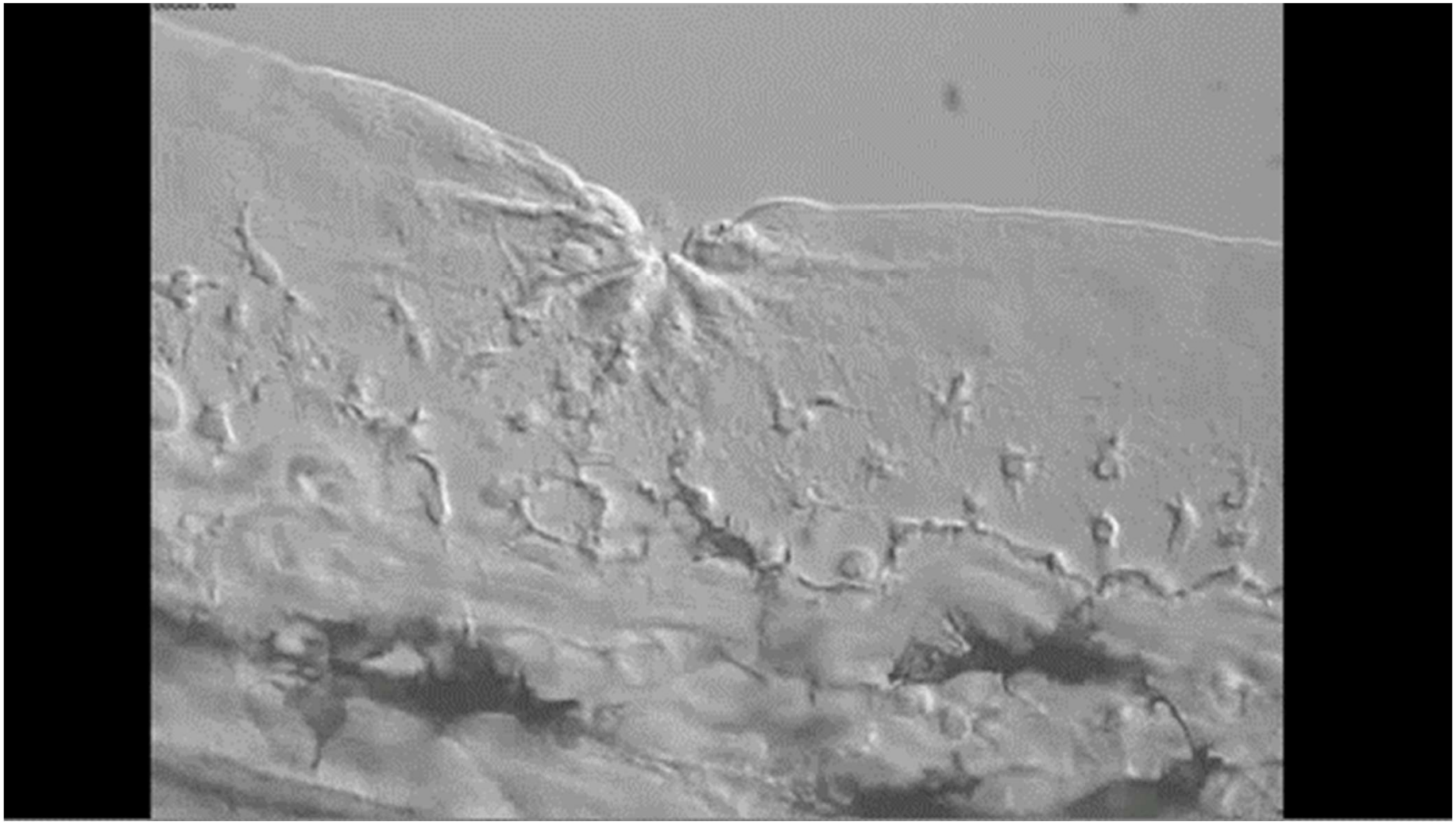


Figure 3.31 (part 2 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

LEUKOCYTE ROLLING



Lymphocyte homing



MIGRAZIONE E RICIRCOLAZIONE DEI LINFOCITI T

- LINFOCITI T NAIVE (ricircolazione linfocitaria tra sangue e organi linfoidi secondari; homing e HEV).
- LINFOCITI T EFFETTORI (verso i siti di infiammazione)
- LINFOCITI T DI MEMORIA