

La risposta Immunitaria antivirale

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Il sistema immunitario

Insieme di tessuti, cellule e molecole che proteggono l'organismo da patogeni e dal cancro

Funzioni principali:

- Riconosce ed elimina i patogeni
- Riconosce e rimuove cellule anomale come quelle tumorali
- Rimuove cellule e tessuti danneggiati o morti
- Memoria immunologica

Self e non -Self

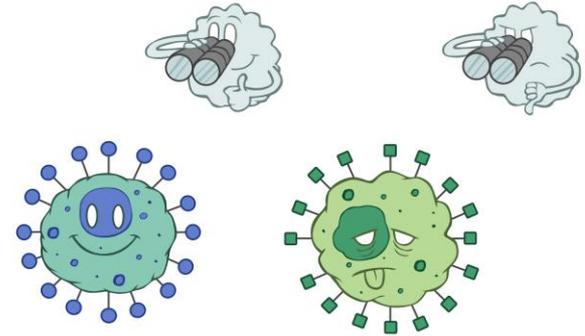
Il sistema immunitario deve distinguere tra self e non-self per proteggere l'organismo dagli agenti infettivi senza tuttavia danneggiare le proprie cellule.

Self: tutte le cellule e molecole proprie dell'organismo, che il sistema immunitario riconosce come normali e quindi non attacca.

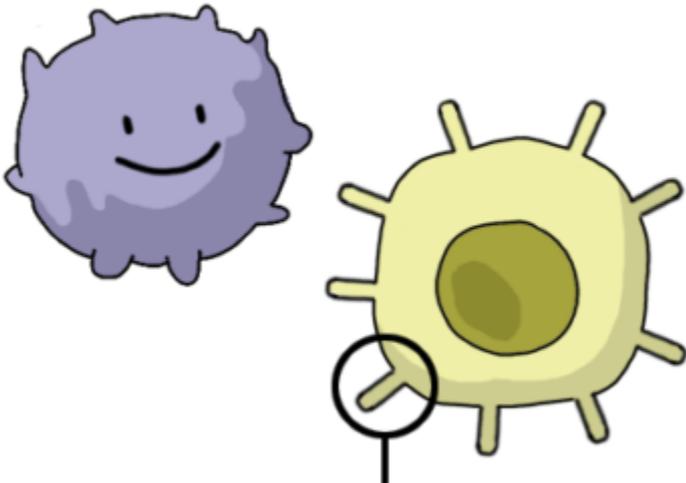
Non-self: microrganismi, virus, batteri, funghi o sostanze estranee che vengono riconosciuti come pericolosi e quindi attaccati ed eliminati.

Questo riconoscimento avviene grazie a recettori specifici delle cellule immunitarie, che individuano molecole caratteristiche dei patogeni.

Se il sistema immunitario perde la capacità di distinguere self e non-self, può attaccare i propri tessuti, causando malattie autoimmuni.

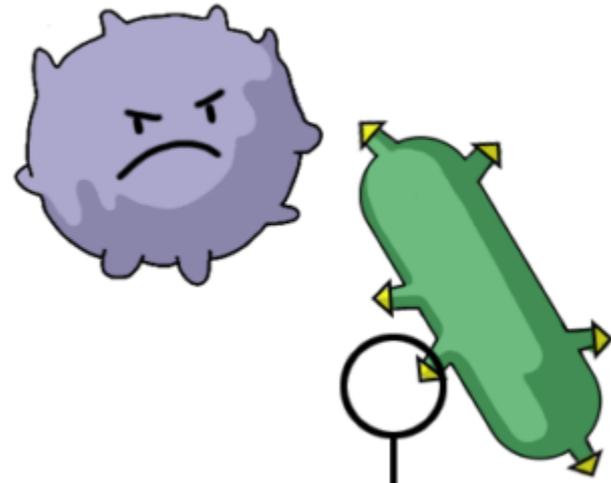


Identifying self



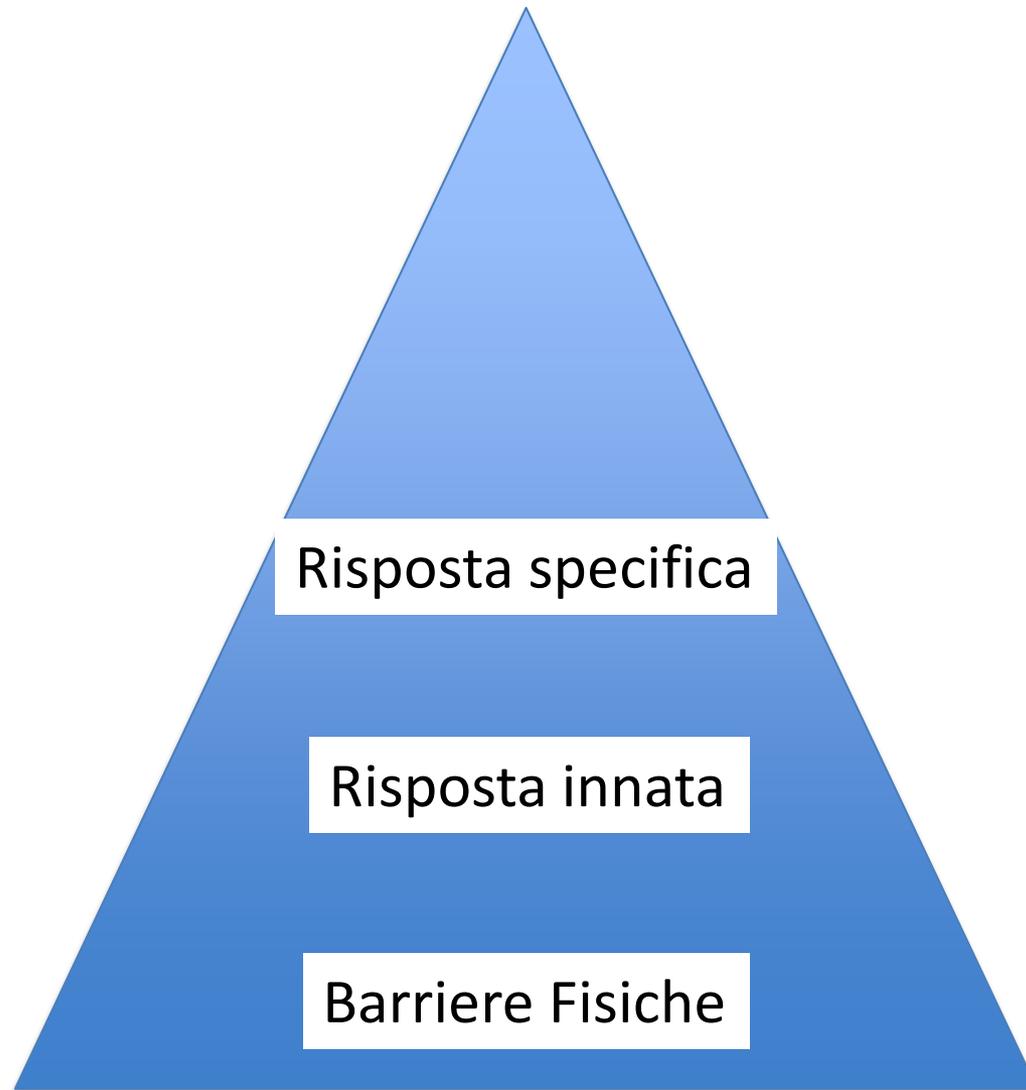
Self marker (MHC)

Identifying non-self

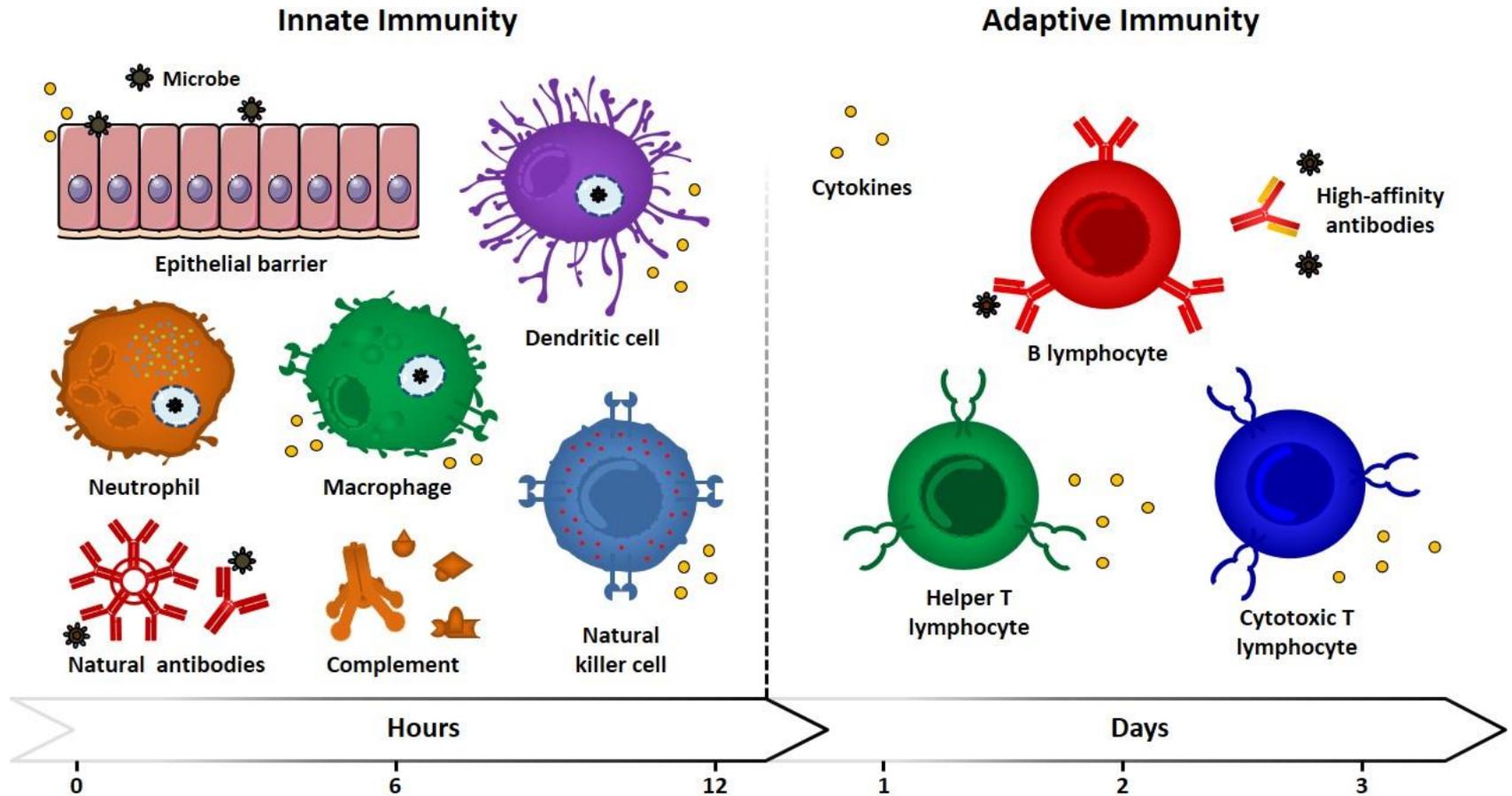


Antigen

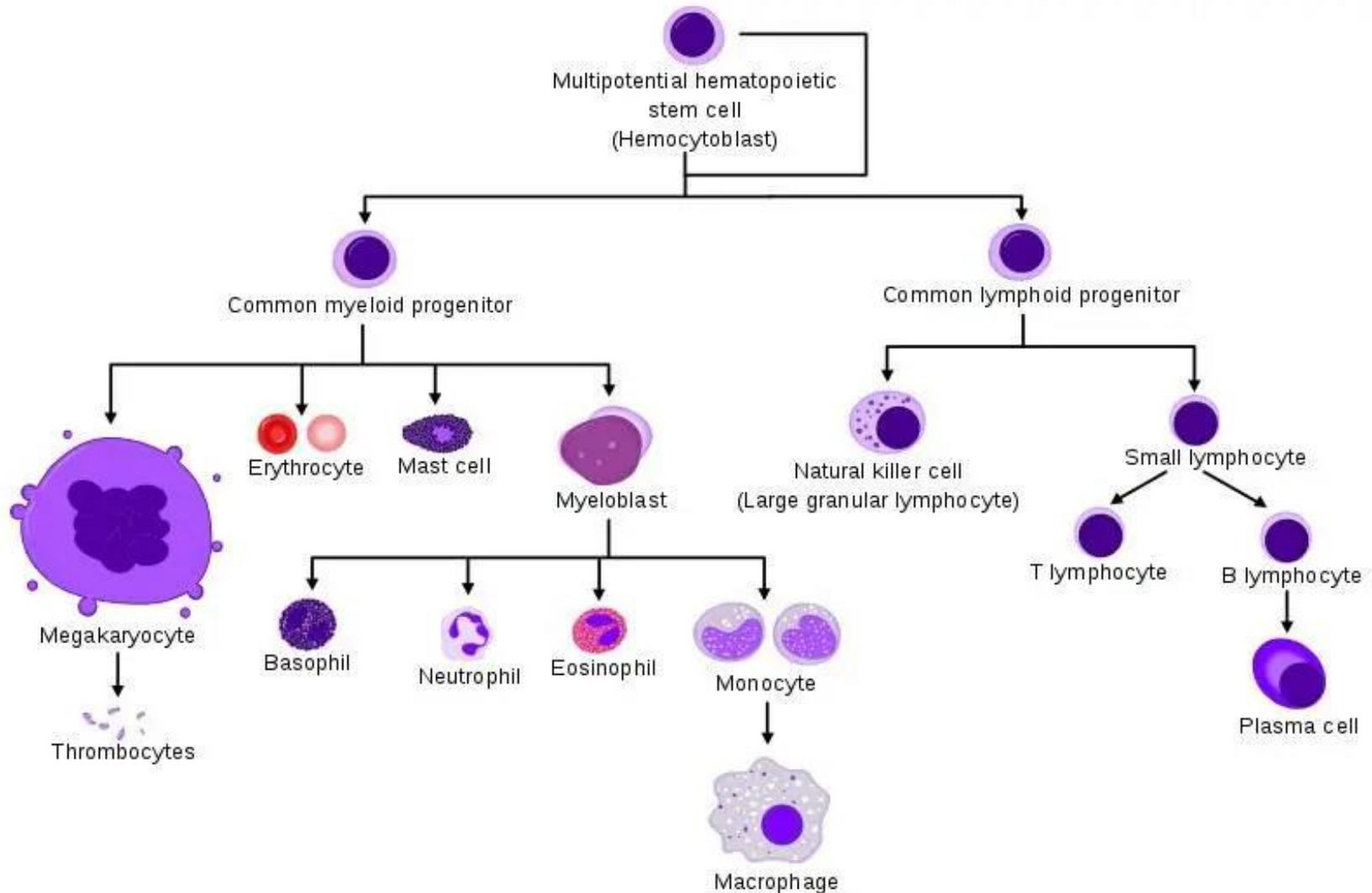
La Piramide della risposta



I due bracci dell'immunità



Da dove derivano le cellule immunitarie?



Immunita' Innata

- Presente dalla nascita
- Non richiede esposizione precedente al patogeno
- Risposta rapida
- Aspecifica
- Assenza di memoria immunologica
- Prima linea di difesa
- Riconoscimento di strutture comuni dei patogeni. Le cellule dell'immunità innata riconoscono molecole tipiche dei patogeni (PAMP).

Immunita' Innata: Barriere Fisiche

1. Pelle

Funzione: impedisce l'ingresso dei microrganismi.

Caratteristiche:

- Strato di cellule cheratinizzate molto compatte
- Desquamazione continua → rimuove i microbi
- Presenza di sebo e sudore con sostanze antimicrobiche
- pH leggermente acido che ostacola i patogeni

2. Mucose

Rivestono cavità che comunicano con l'esterno:

- apparato respiratorio
- apparato digerente
- apparato urogenitale

Funzione: intrappolare i microrganismi.

Caratteristiche:

- produzione di muco che blocca batteri e virus

Barriere Fisiche (2)

3. Ciglia (apparato respiratorio)

Funzione: eliminare particelle e patogeni spingendo il muco verso la faringe

4. Flussi meccanici di liquidi

Esempi: lacrime, saliva, urina, sudore

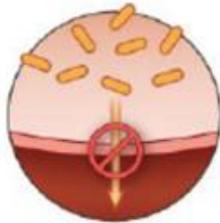
Funzione:

- Meccanica: lavano via i microrganismi dalle superfici corporee
- Chimica: Contengono enzimi che distruggono i batteri, per esempio: lisozima → rompe la parete cellulare dei batteri; lattoferrina → sottrae ferro ai batteri, impedendone la crescita; peptidi antimicrobici

Barriere fisiche (3)

PELLE

Forma una barriera quasi impenetrabile che ostacola l'ingresso di patogeni nell'organismo.



SECREZIONI ACIDE

I succhi gastrici, le secrezioni vaginali e l'urina proteggono rispettivamente il canale digerente, le vie genitali femminili e le vie urinarie dai patogeni.



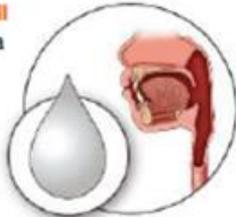
LACRIME

Le lacrime contengono sostanze chimiche antivirali e antibatteriche che eliminano i patogeni dalla zona degli occhi.



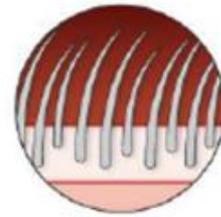
LISOZIMA E ALTRI ENZIMI

Il lisozima contenuto nella saliva e nelle lacrime e gli enzimi digestivi dell'intestino tenue uccidono molti batteri.



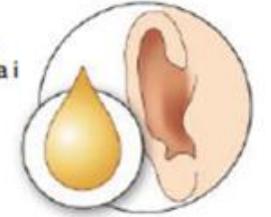
CIGLIA

I peli e le ciglia presenti sulla superficie delle vie respiratorie allontanano dai polmoni i patogeni intrappolati nel muco.

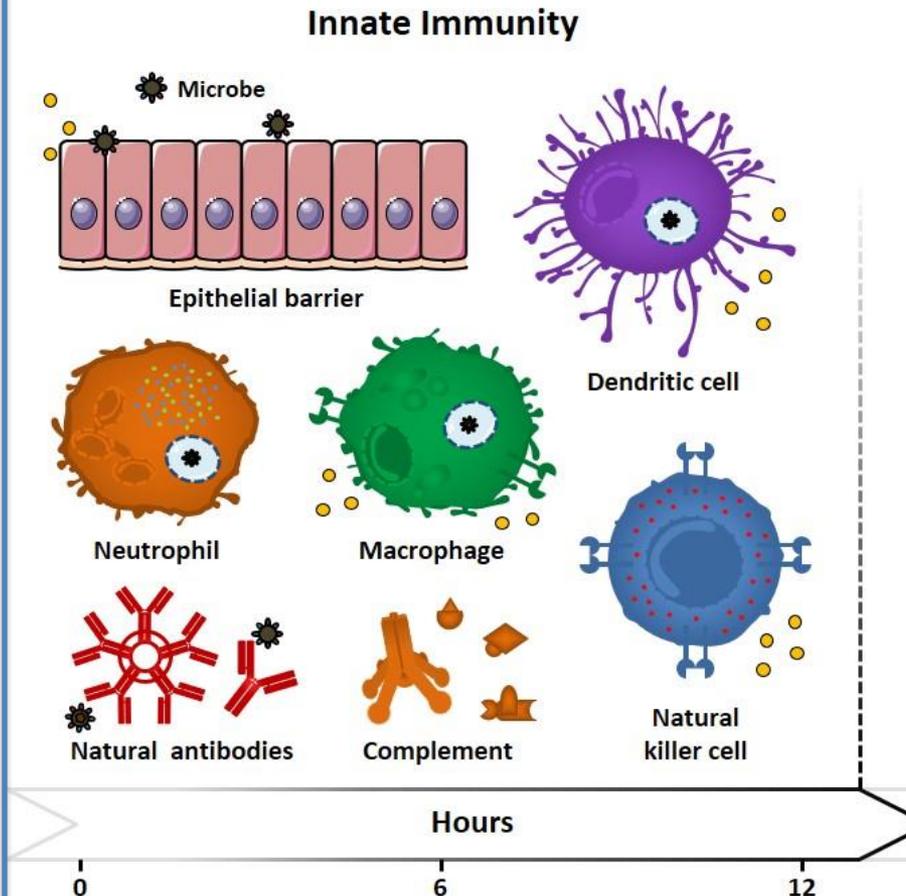


CERUME

Il cerume è una sostanza appiccicosa che intrappola i patogeni presenti nel canale uditivo.



Le cellule dell'Immunità innata



RICONOSCIMENTO DEL NON-SELF

- Come fanno a riconoscere il non Self?
Attraverso dei recettori (pattern recognition receptors, PRR) che riconoscono strutture molecolari comuni ai patogeni (e non presenti sulle cellule self).

PRR e fagocitosi

I **PRR (Pattern Recognition Receptors)** sono recettori del sistema immunitario innato che permettono alle cellule immunitarie di **riconoscere rapidamente la presenza di patogeni**. Questi recettori individuano **strutture molecolari tipiche dei microrganismi**, chiamate **PAMP (Pathogen-Associated Molecular Patterns)**, che sono comuni a molti batteri, virus e funghi.

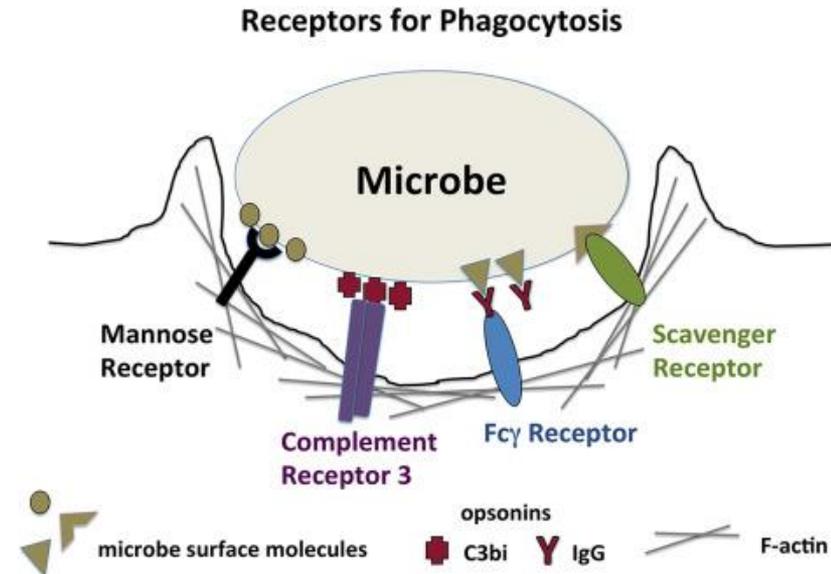
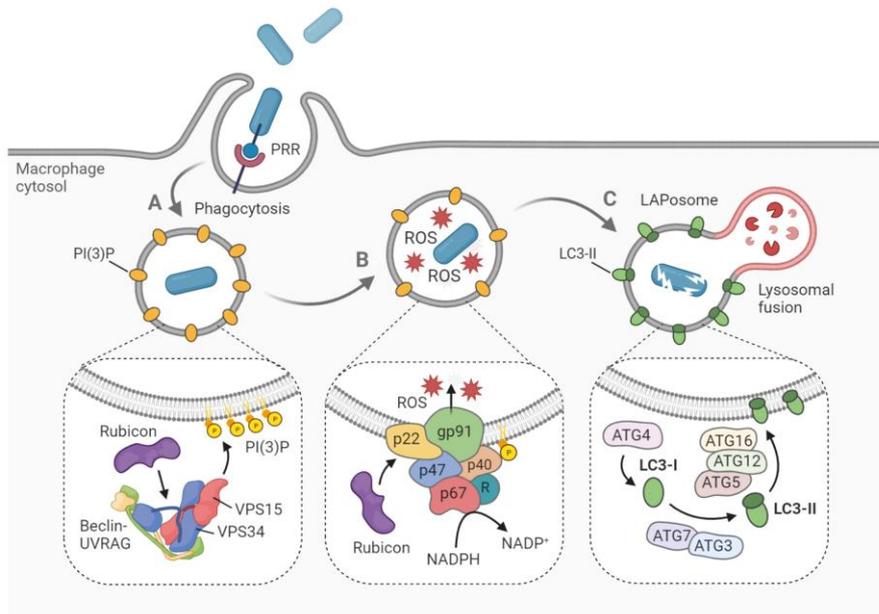
I PRR sono presenti su cellule dell'immunità innata come:

- **Macrofagi, neutrofili e cellule dendritiche**

Quando i PRR riconoscono un PAMP:

- Si **attiva la risposta immunitaria innata**
- vengono rilasciate **citochine infiammatorie**
- si stimola la **fagocitosi del patogeno**

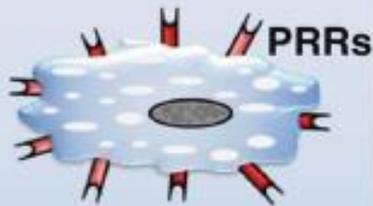
Immunità innata: PPR e fagocitosi



Il processo avviene in diverse fasi:

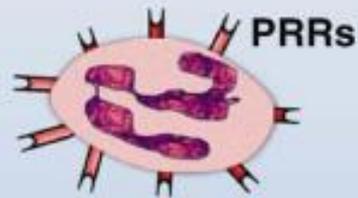
- **Riconoscimento** del patogeno tramite recettori specifici
- **Ingestione**, con formazione di una vescicola chiamata fagosoma
- **Digestione**, quando il fagosoma si fonde con i lisosomi formando il fagolisosoma
- **Distruzione** del microrganismo grazie a enzimi e sostanze tossiche

Immunità innata



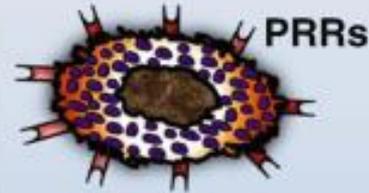
Macrophage

Macrophages, equipped with a big armamentarium of PRRs to recognize MAMPs and DAMPs, play diverse roles as phagocytic cells of the innate immune defense system including clearance of pathogens and damaged cells and assistance in tissue repair.



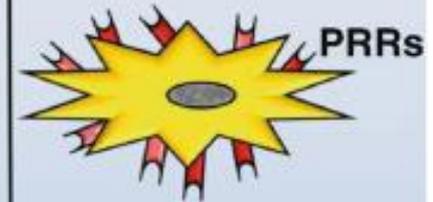
Neutrophil

Neutrophils, activated by MAMPs and DAMPs, play a fundamental role in creating an inflammatory milieu via secretion of cytokines and chemokines; the clearance of pathogens, and the release of so-called neutrophil extracellular traps (NETs).



Mast cell

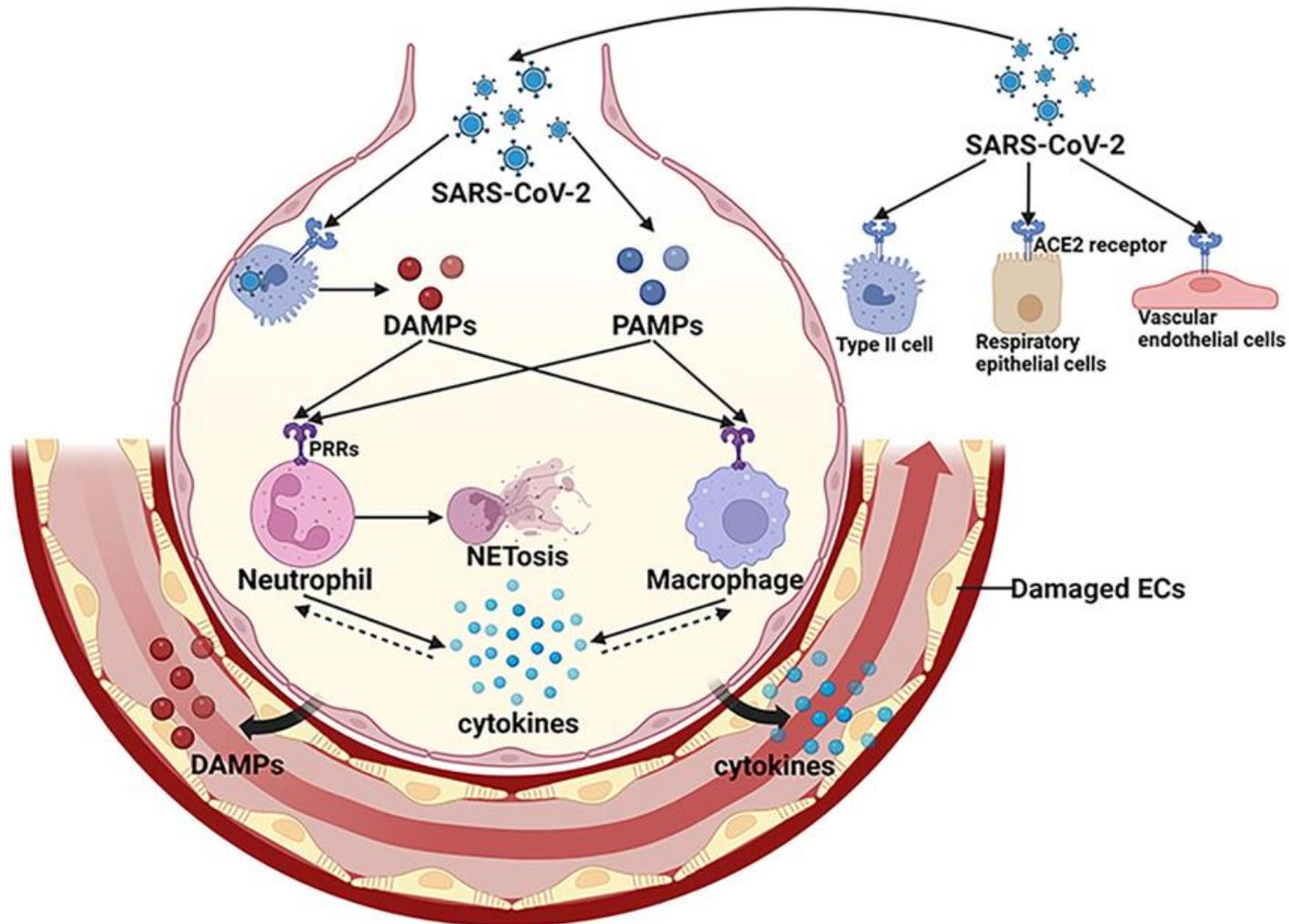
Mast cells have pleiotropic physiological functions in our body. Any type of damaging stimuli causes them to degranulate and release inflammatory mediators like histamine, proteases, cytokines, chemokines, and prostaglandins.



Dendritic cell

Dendritic cells are the professional antigen-presenting cells of the innate immune system. They possess the unique capability to initiate and expand an adaptive immune response. In fact, they can be regarded as the key players in recognizing cell stress and tissue the presence of antigen.

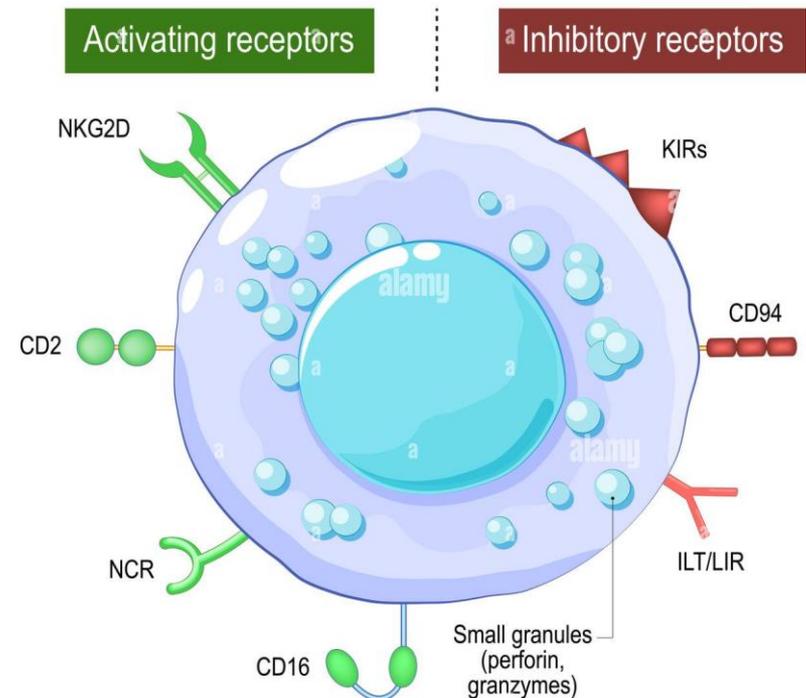
Immunità innata: citochine infiammatorie



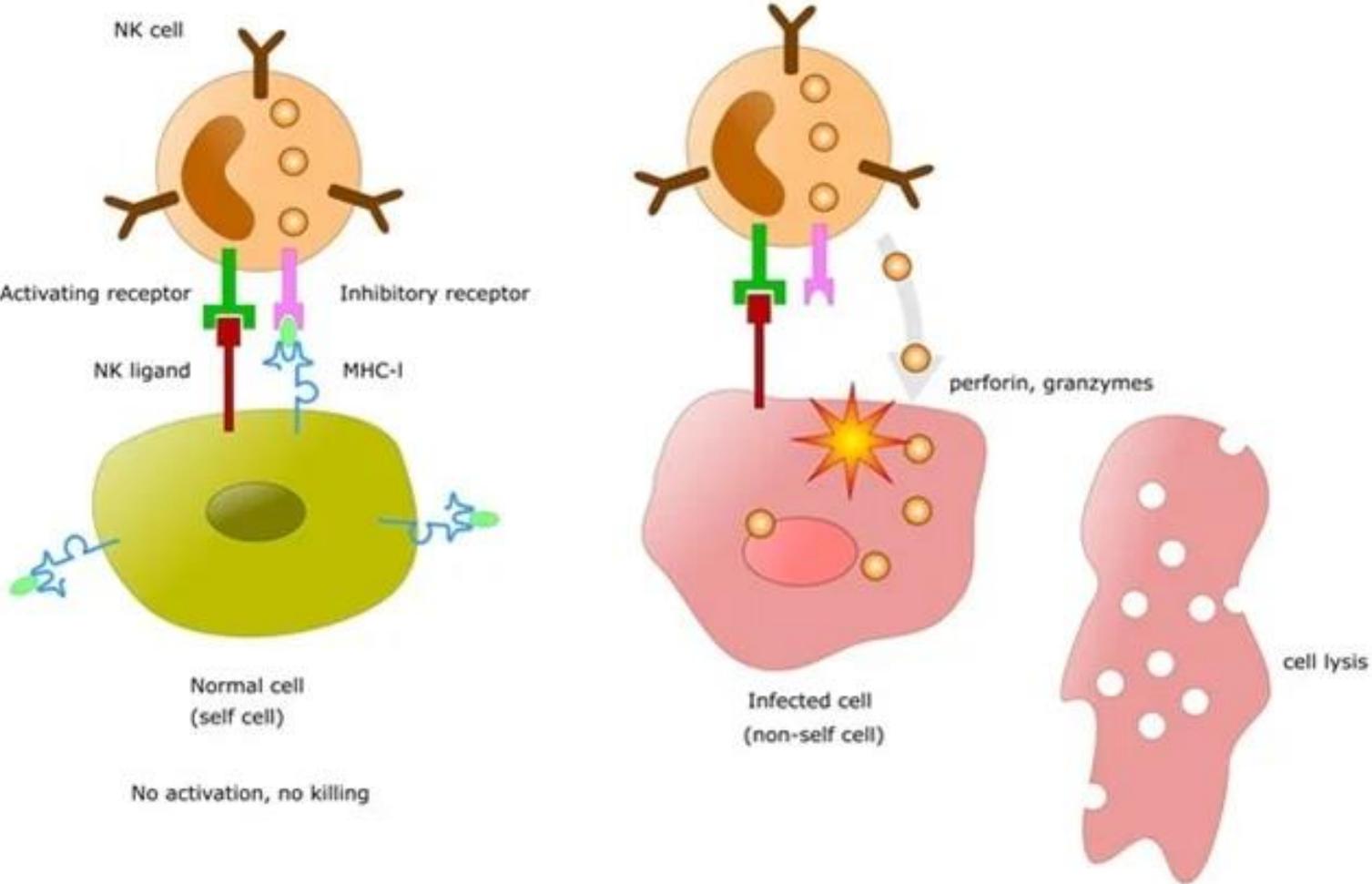
Cellule Natural Killer

Le cellule NK (Natural Killer) sono linfociti dell'immunità innata fondamentali nella difesa contro le infezioni virali. Riconoscono e uccidono rapidamente le cellule infettate da virus, attraverso la sinergia tra recettori attivatori e inibitori

Natural killer cell (NK cell receptors)

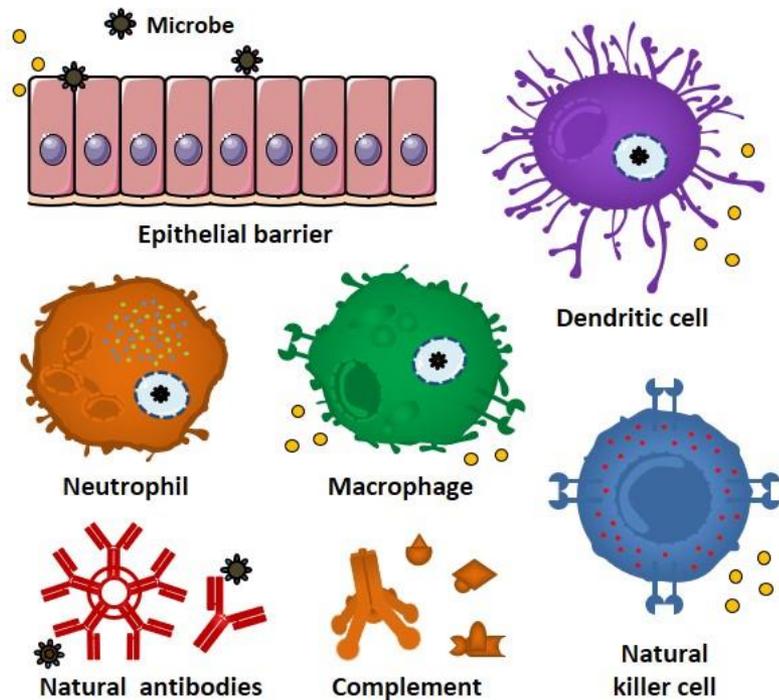


Cellule NK

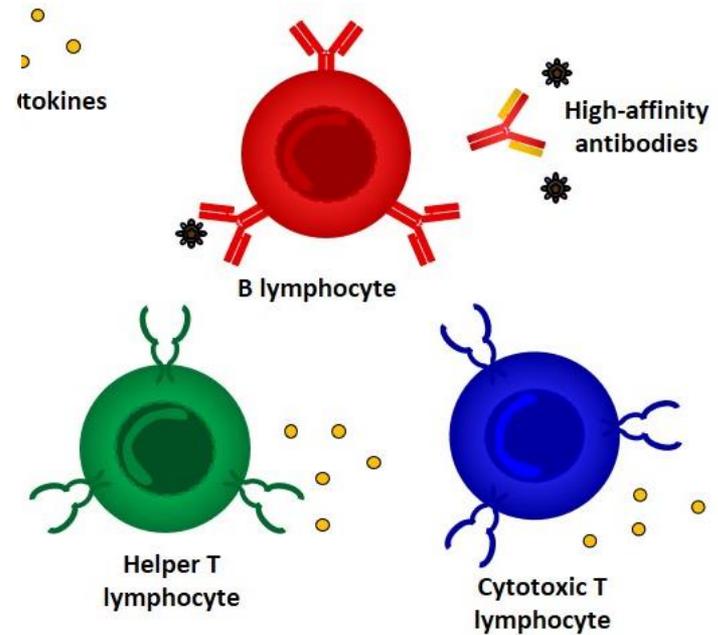


Immunità adattativa

Innate Immunity



Adaptive Immunity

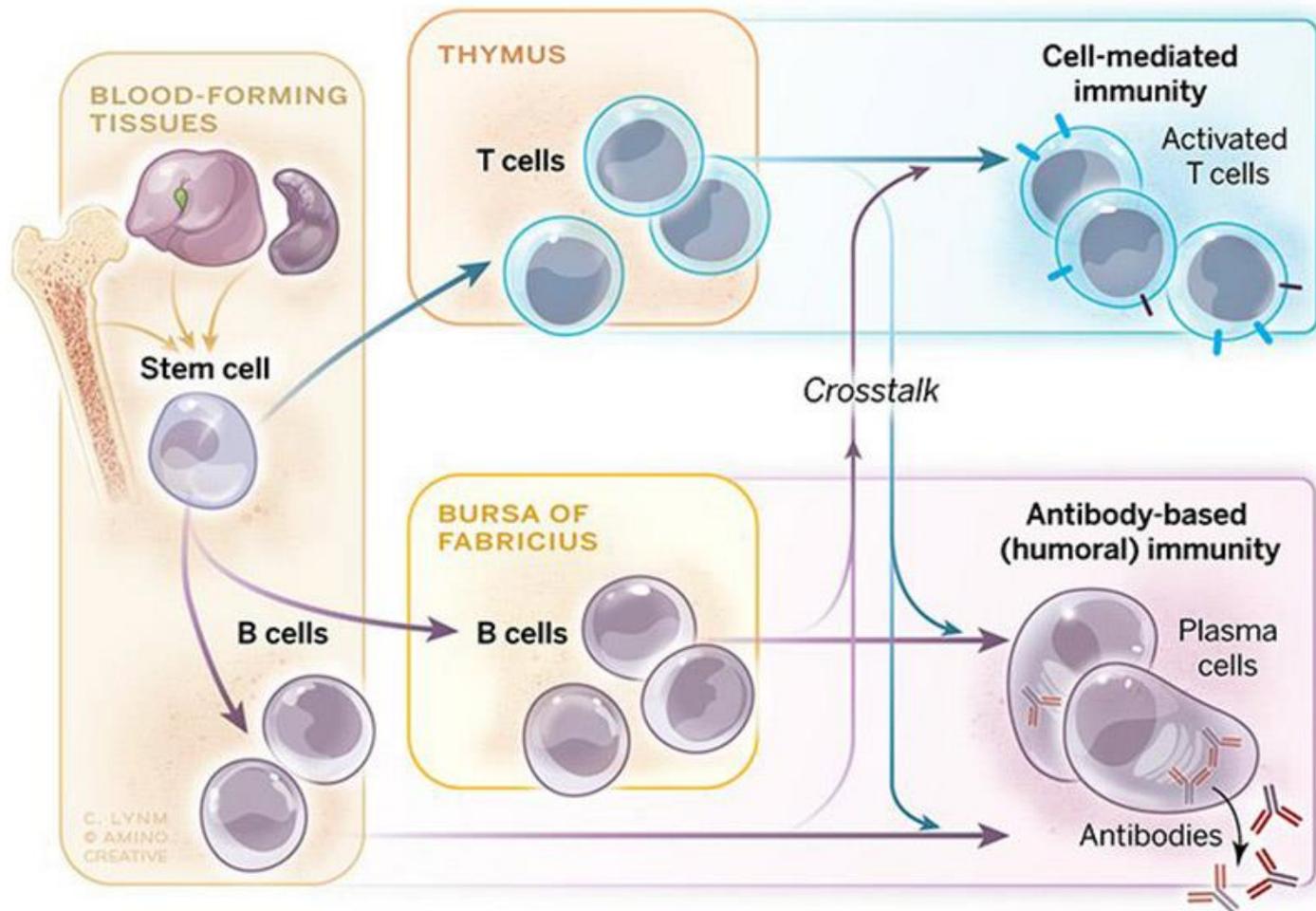


Immunità adattativa

- **Non completamente presente alla nascita** (si sviluppa con l'esposizione agli antigeni)
- **Richiede una prima esposizione al patogeno**
- **Risposta più lenta alla prima infezione** (giorni)
- **Altamente specifica** per un determinato antigene
- **Presenza di memoria immunologica**
- **Seconda linea di difesa**, interviene dopo l'immunità innata

Linfociti T e B

Two-lymphocyte lineage model of adaptive immunity



Linfociti T e B: Caratteristiche comuni

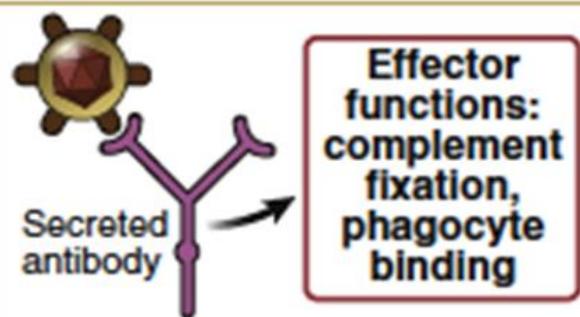
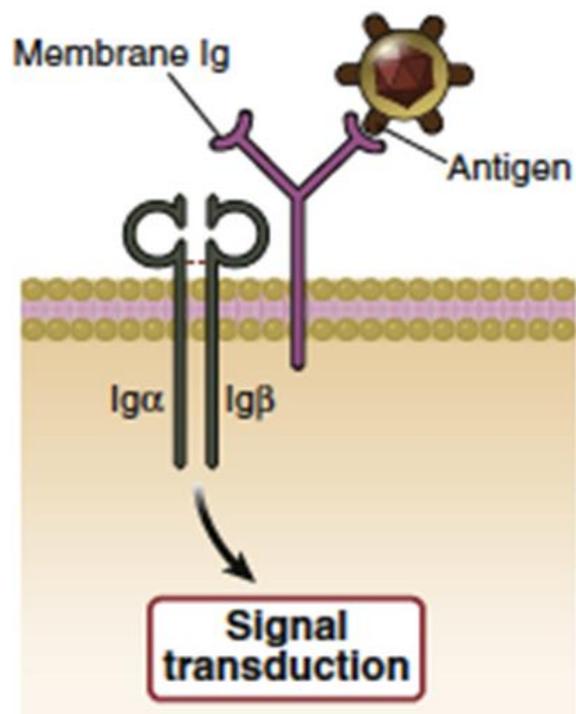
- Sono cellule dell'immunità adattativa
- Derivano da cellule staminali del midollo osseo
- Hanno recettori specifici per l'antigene (patogeni)
- Partecipano alla difesa contro i patogeni
- Sono in grado di formare cellule della memoria immunologica
- Il numero di linfociti T e B specifici per un antigene sono pochissimi in circolo ma si possono espandere clonalmente

Linfociti T e B: differenze

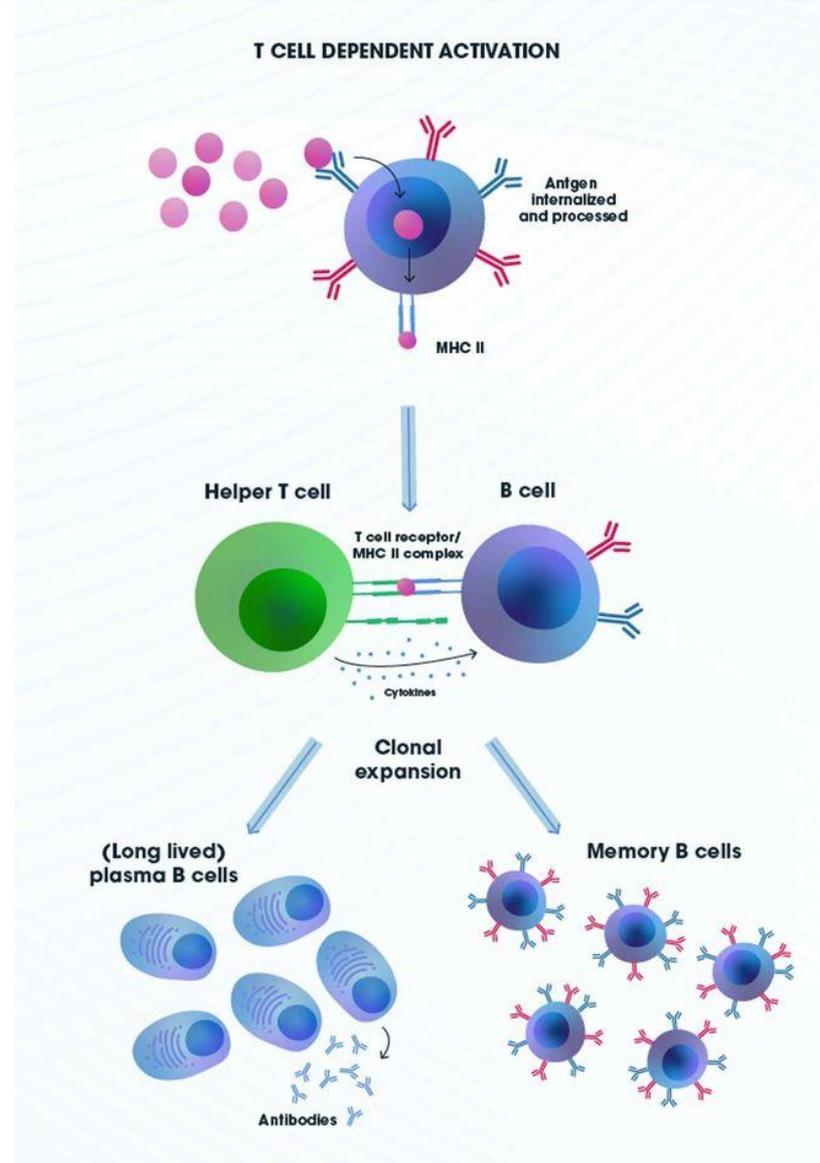
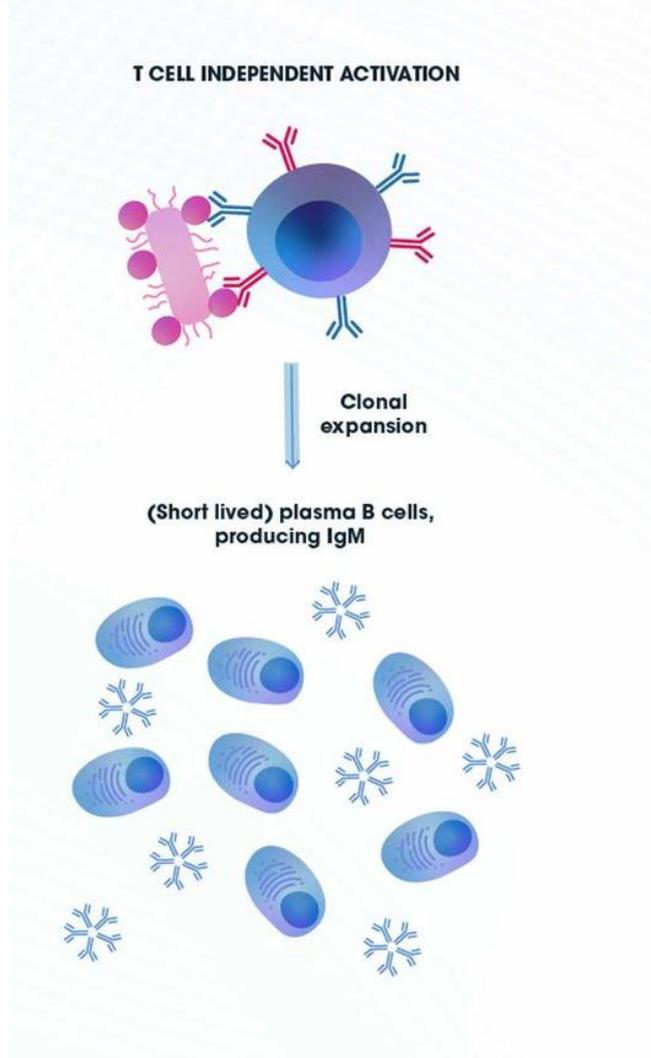
Differenze principali

Caratteristica	Linfociti B	Linfociti T
Maturazione	Midollo osseo	Timo
Tipo di immunità	Immunità umorale	Immunità cellulare
Riconoscimento antigene	Antigene libero	Antigene presentato da MHC
Recettore	BCR	TCR
Funzione principale	Produzione di anticorpi	Eliminazione cellule infette e regolazione risposta immunitaria
Tipi principali	plasmacellule, B memo 	T helper, T citotossici, T regolatori

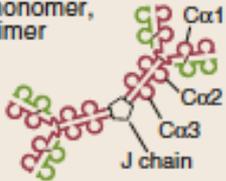
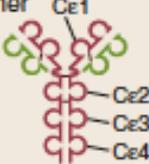
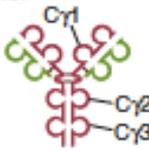
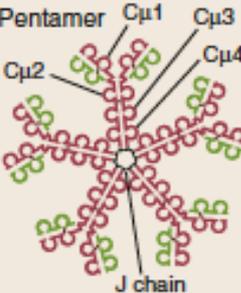
B cell receptor (antibody, Ig)



Linfociti B



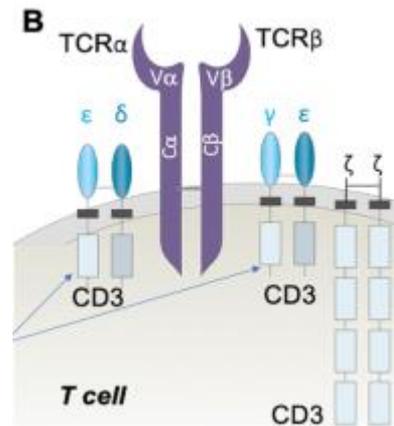
Tipi di anticorpi

Isotype of antibody	Subtypes (H chain)	Serum concentration (mg/ml)	Serum half-life (days)	Secreted form	Functions
IgA	IgA1,2 ($\alpha 1$ or $\alpha 2$)	3.5	6	Mainly dimer, also monomer, trimer 	Mucosal immunity
IgD	None (δ)	Trace	3	Monomer	Naive B cell antigen receptor
IgE	None (ϵ)	0.05	2	Monomer 	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 ($\gamma 1$, $\gamma 2$, $\gamma 3$ or $\gamma 4$)	13.5	23	Monomer 	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	Pentamer 	Naive B cell antigen receptor (monomeric form), complement activation

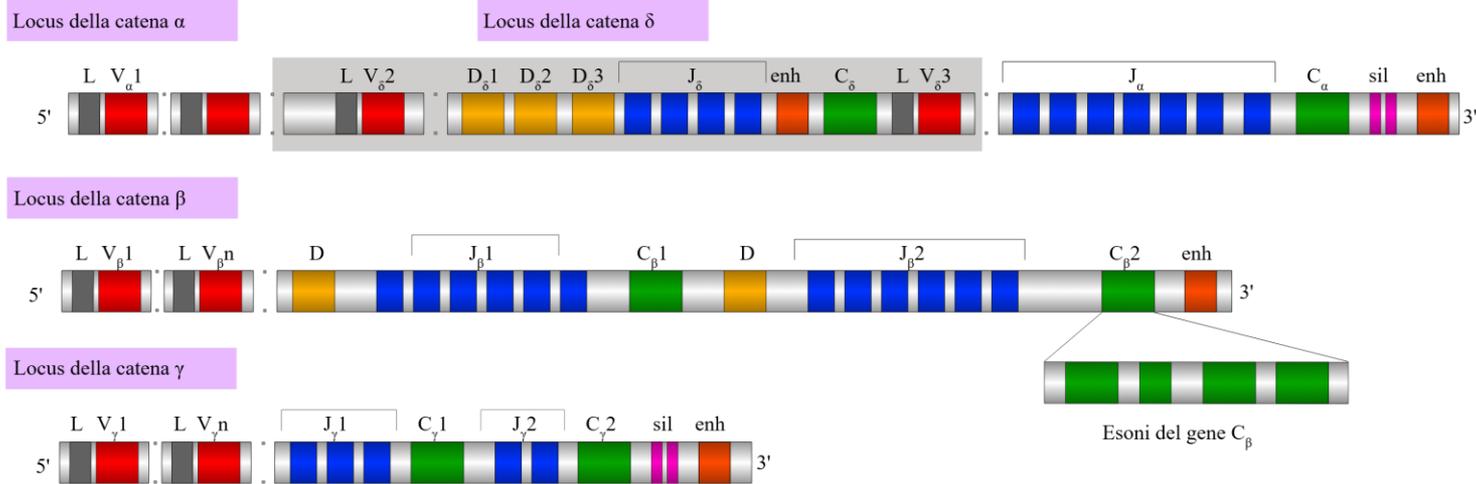
Linfociti T

Miliardi di possibili antigeni non self. Come poterli riconoscere?

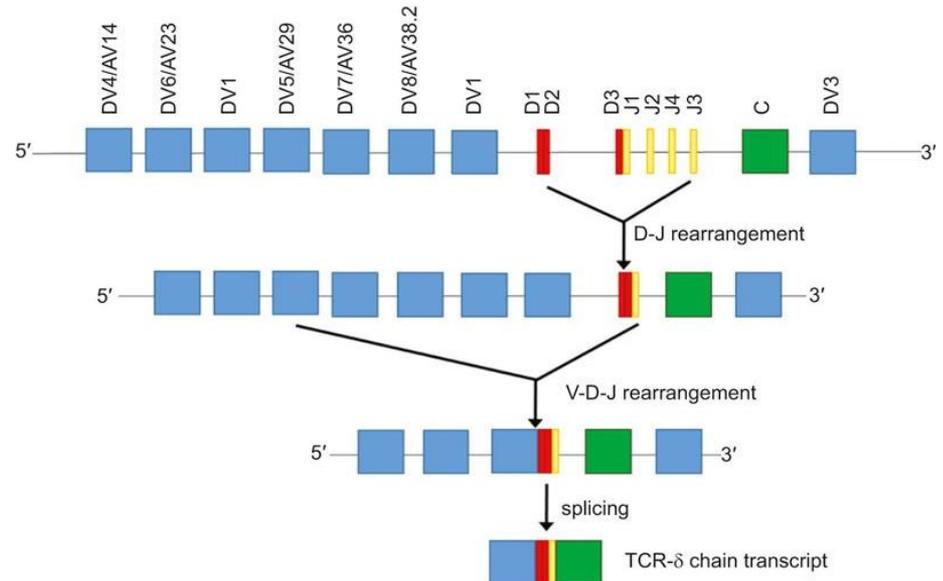
Il recettore delle cellule T (TCR) e quello delle cellule B (BCR) presenta una straordinaria diversità strutturale e funzionale, essenziale per il riconoscimento di un repertorio estremamente ampio di antigeni peptidici.



Linfociti T: enorme potenziale di riconoscimento



V-D-J recombination at *tcrd/tcrd* locus



1. Riarrangiamento genico (V(D)J recombination

Avviene durante la maturazione dei linfociti.

Segmenti genici: V (Variable) D (Diversity) J (Joining). La combinazione casuale di questi segmenti \rightarrow grande diversità

Linfociti T: enorme potenziale di riconoscimento

2. Diversità giunzionale

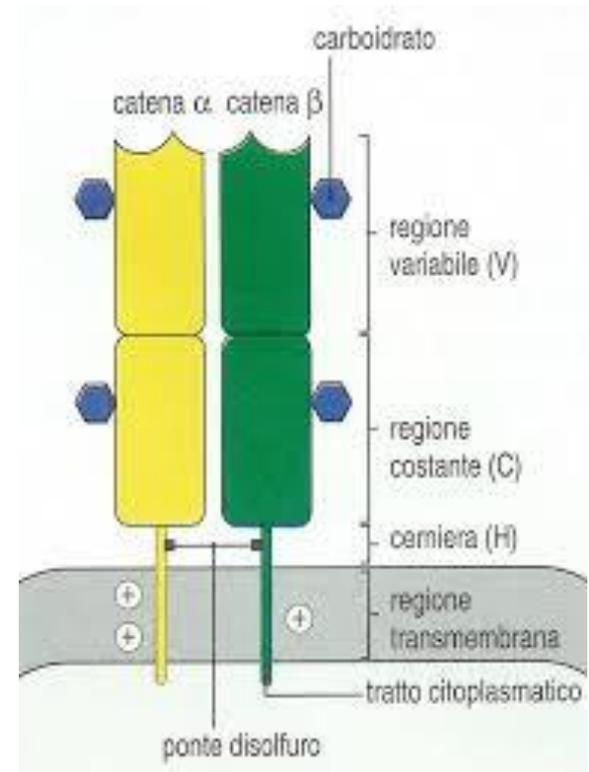
Aggiunta o rimozione casuale di nucleotidi nelle giunzioni V-D-J

Genera variabilità molto elevata (anche errori → più diversità)

3. Combinazione delle catene

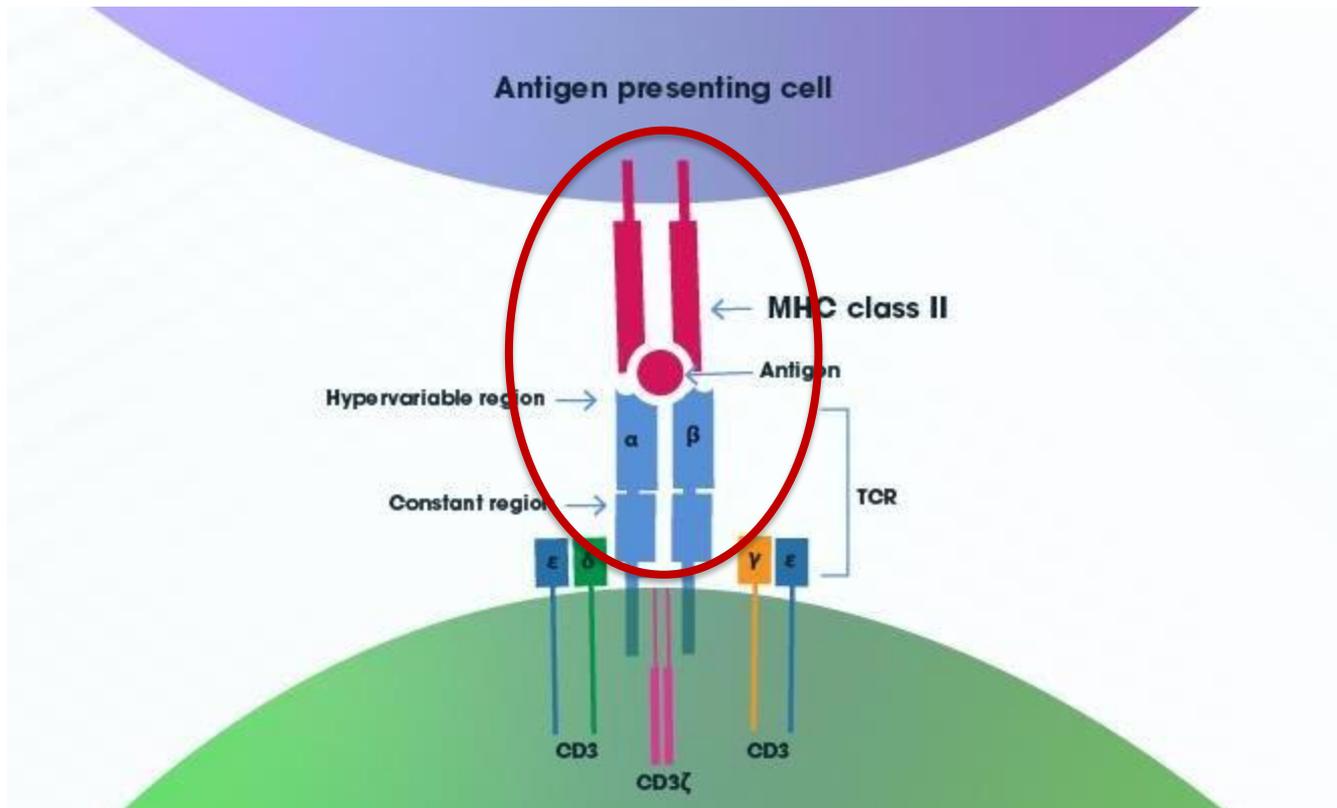
TCR: catena α + catena β

Diverse combinazioni → aumento variabilità

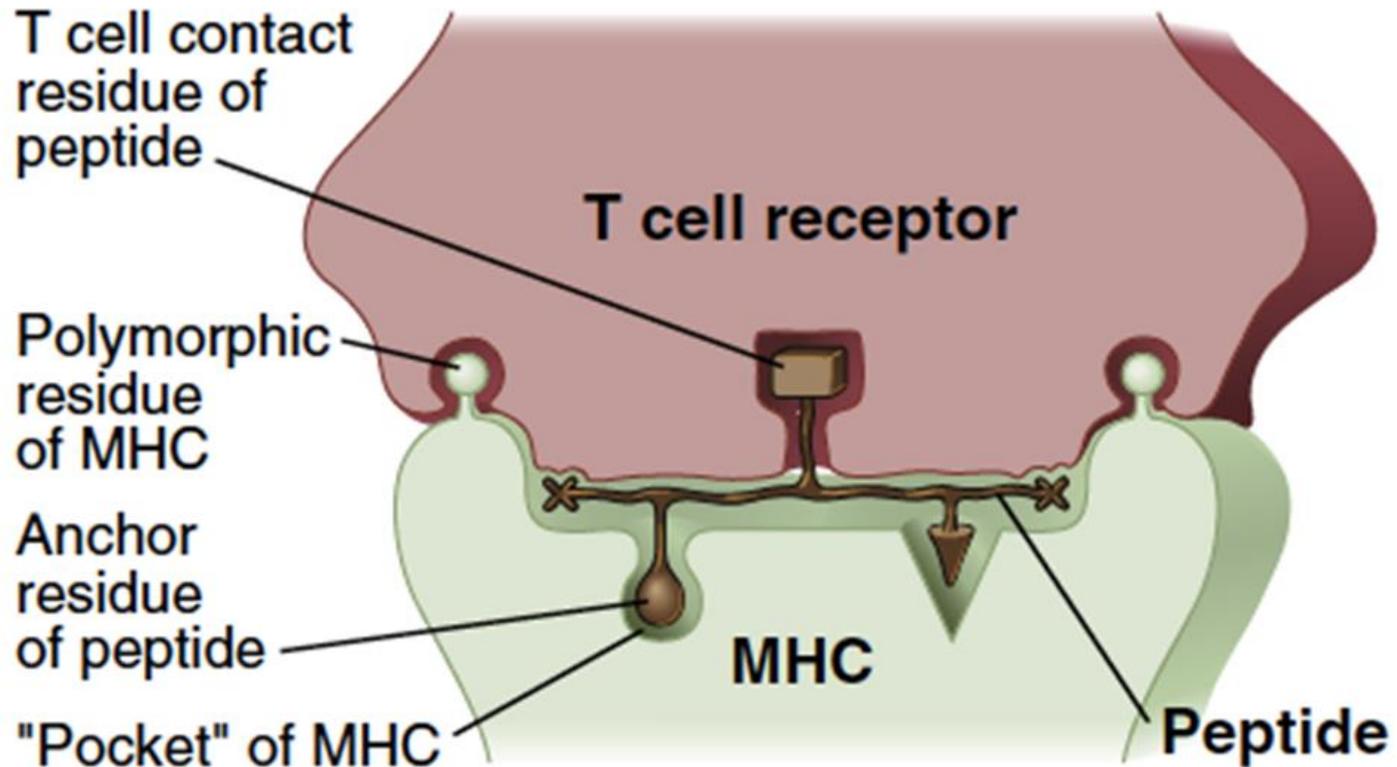


Linfociti T: meccanismo di riconoscimento

ANTIGENI: Molecole riconosciute come estranee dal sistema immunitario



Linfociti T: meccanismo di riconoscimento



1) Il TCR deve riconoscere le MHC self

2) Il TCR non deve riconoscere molecole self ma solo antigeni non self

Selezione timica

Selezione positiva:

Avviene nella **corteccia del timo**. I linfociti T immaturi vengono testati per verificare se riconoscono le molecole del **complesso maggiore di istocompatibilità (MHC)** proprie dell'organismo.

Sopravvivono solo quelli che riescono a riconoscere l'MHC (altrimenti muoiono).

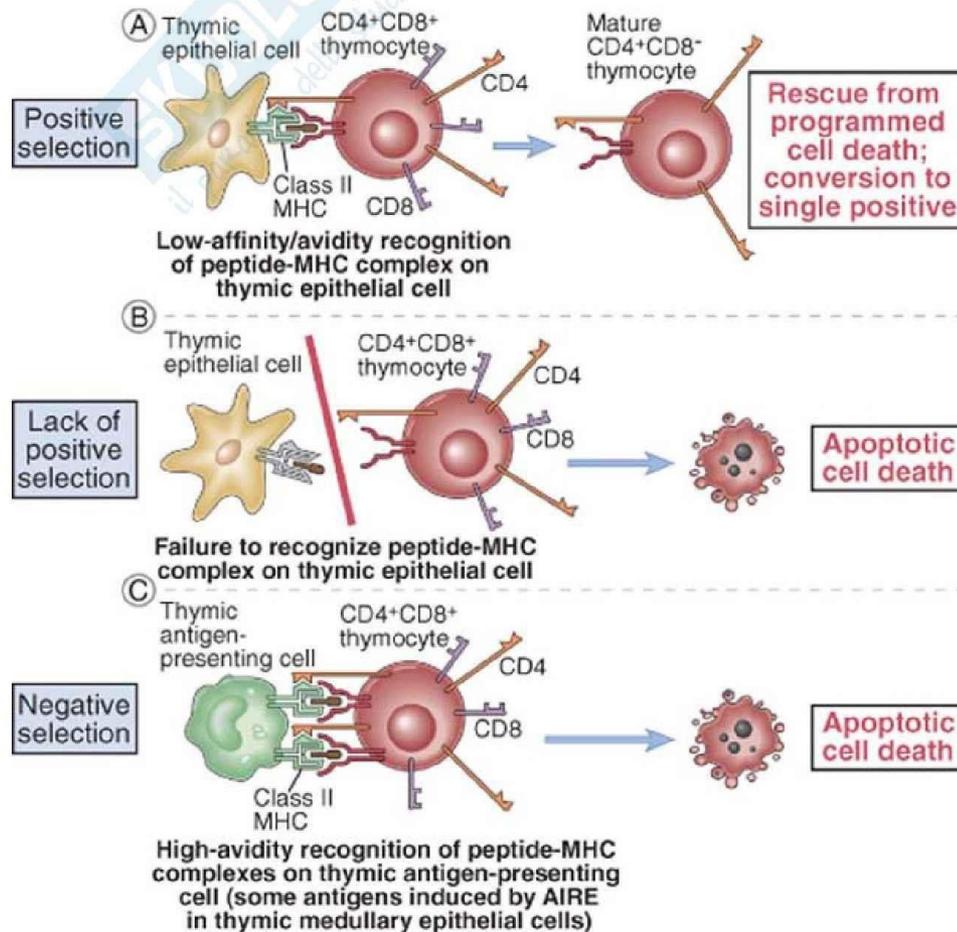
Selezione negativa:

Avviene nella **midollare del timo**. I linfociti T vengono esposti ad antigeni "self" (del proprio corpo).

Vengono eliminati quelli che reagiscono troppo fortemente, per evitare malattie autoimmuni.

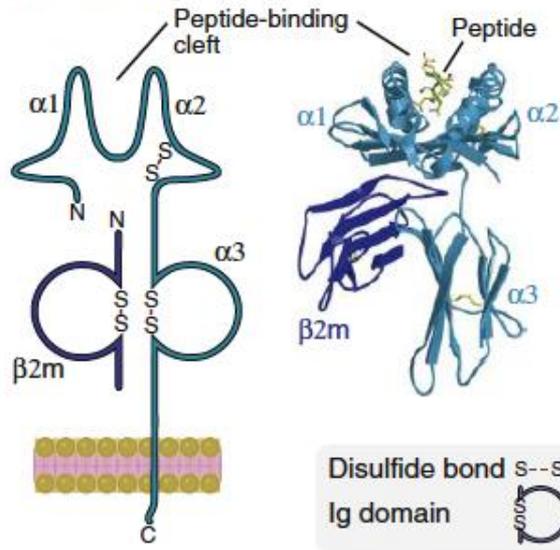
Selezione timica (2)

PROCESSI DI SELEZIONE NEL TIMO



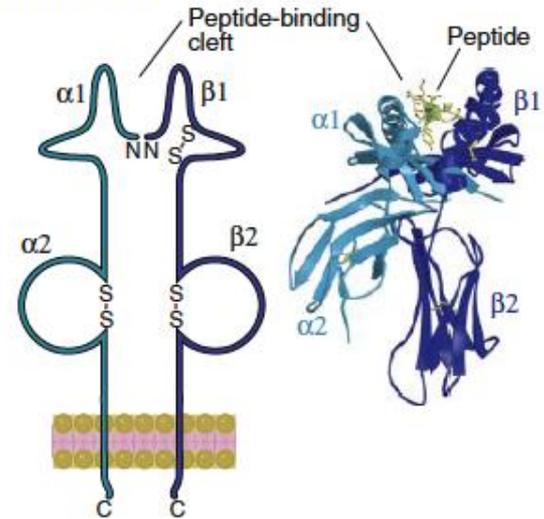
Linfociti T: meccanismo di riconoscimento

Class I MHC



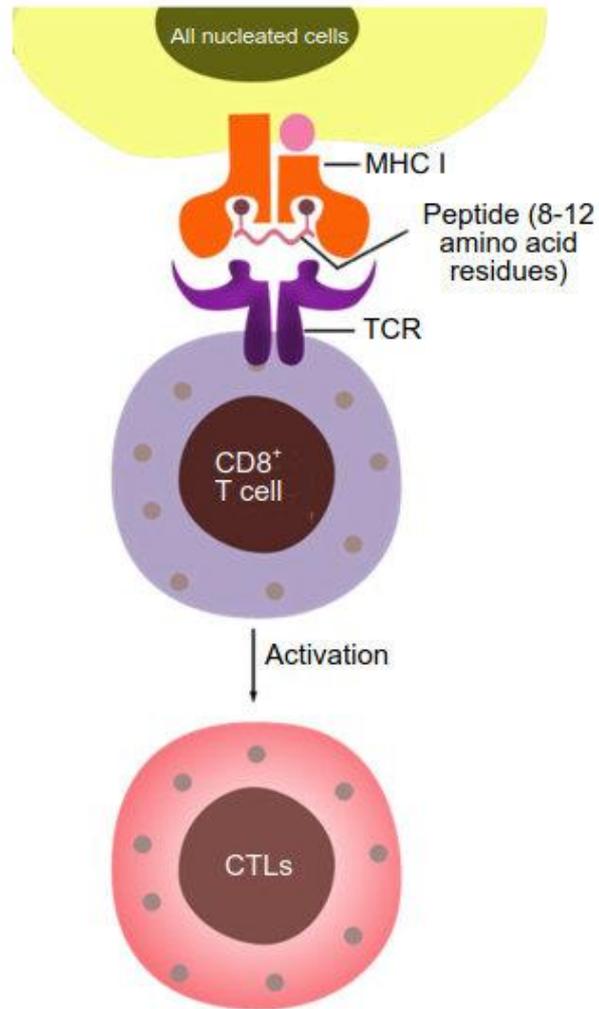
CD8+ cytotoxic T lymphocytes

Class II MHC

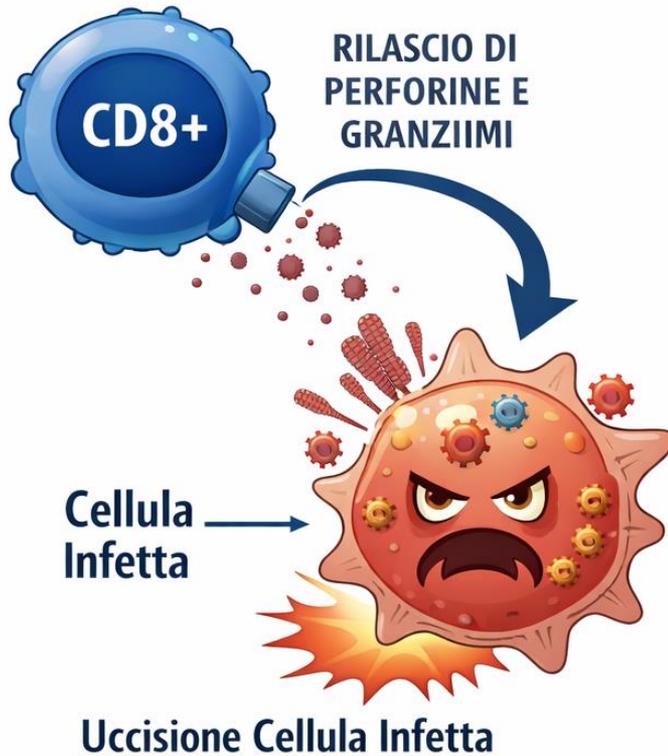


CD4+ helper T cells

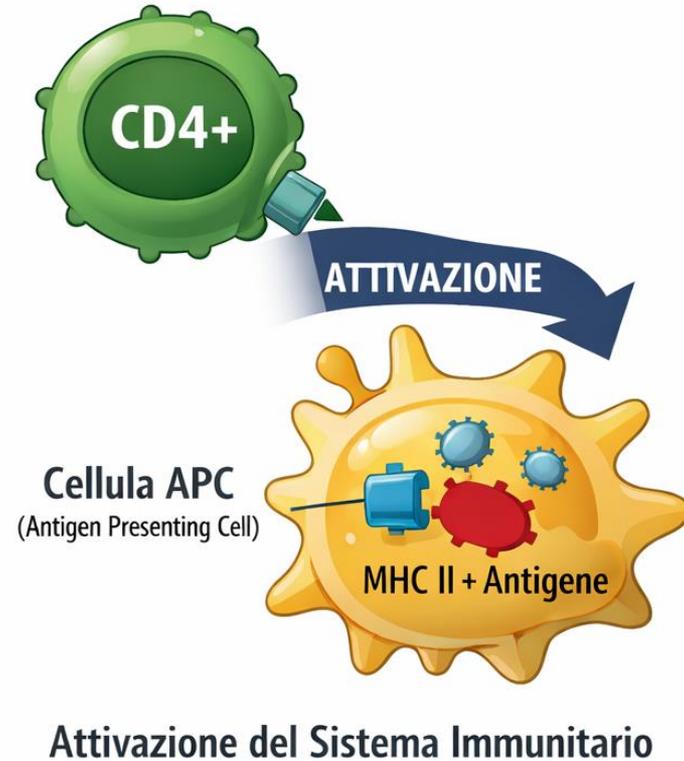
CD4 and CD8 T cells



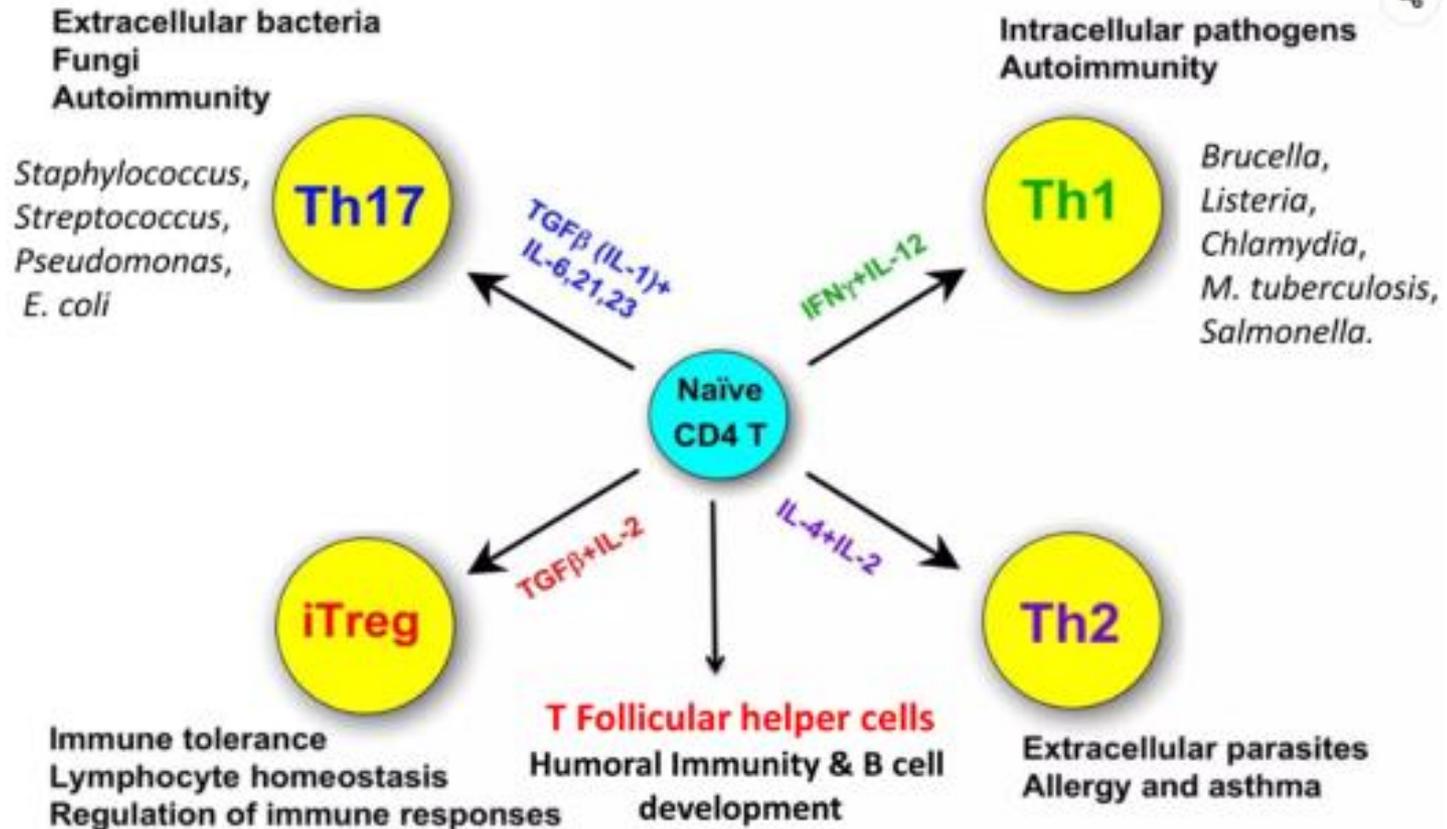
CD8+ T Citotossico



CD4+ T Helper

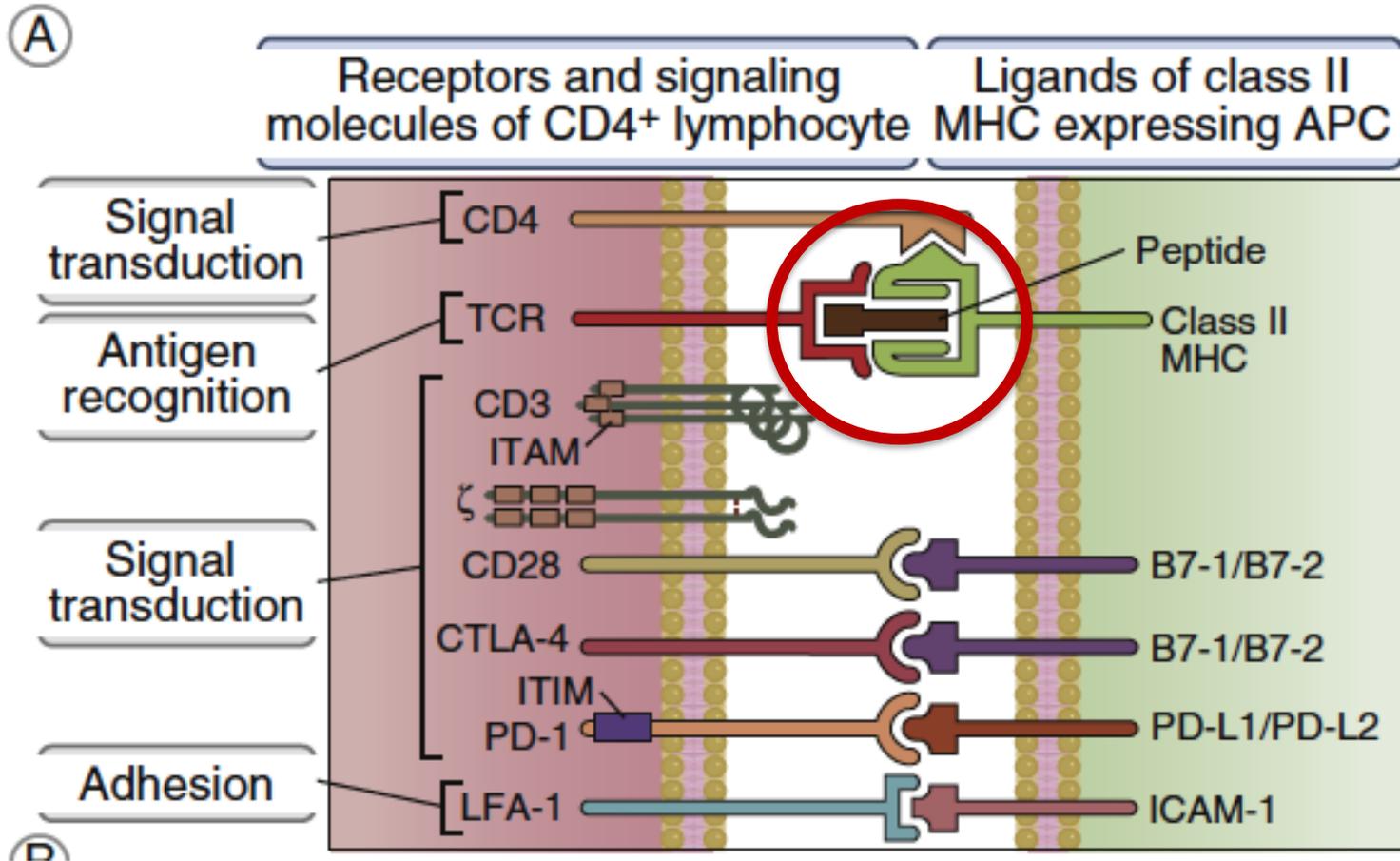


CD4+ T cells

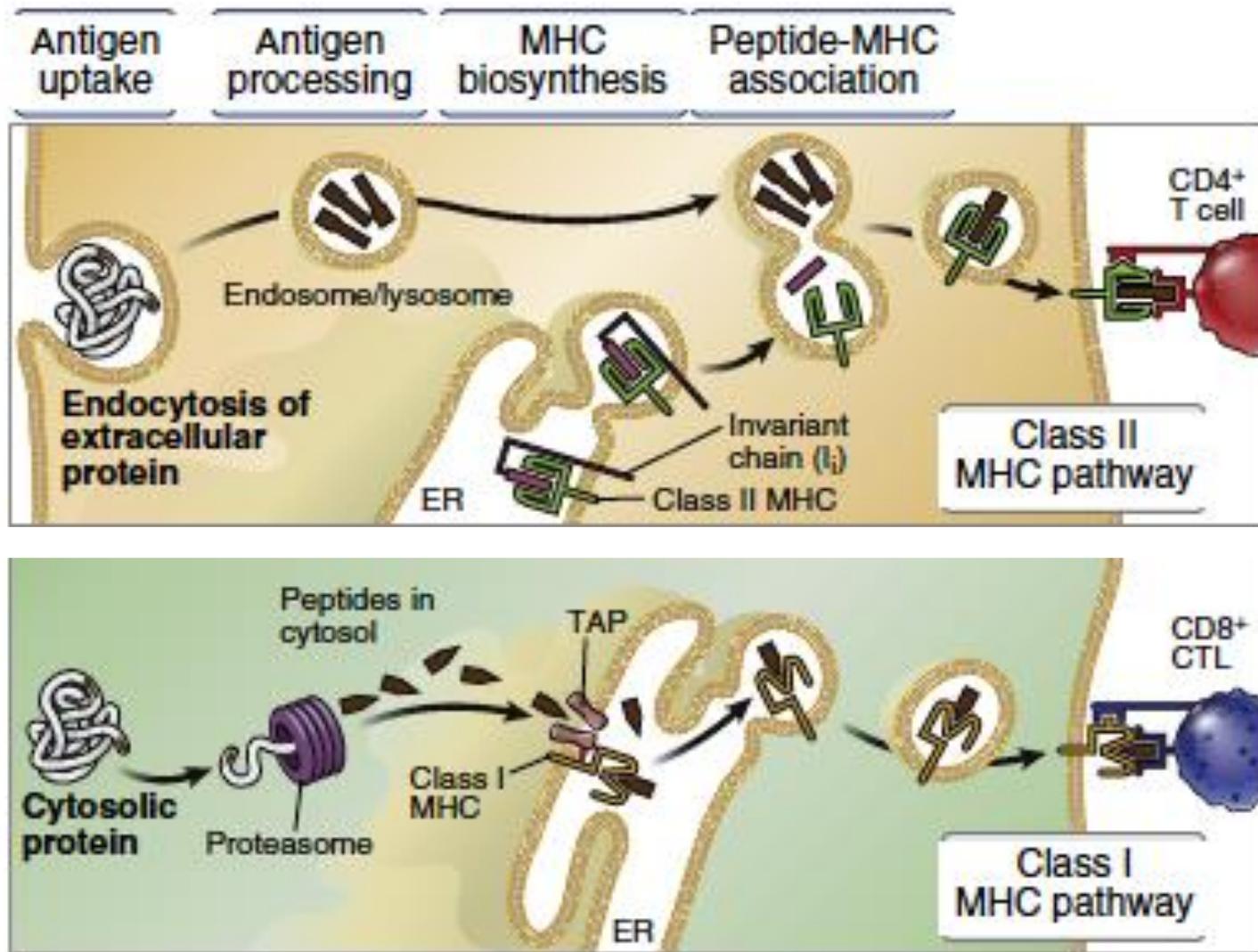


<http://www.bloodjournal.org/content/112/5> (adapted)

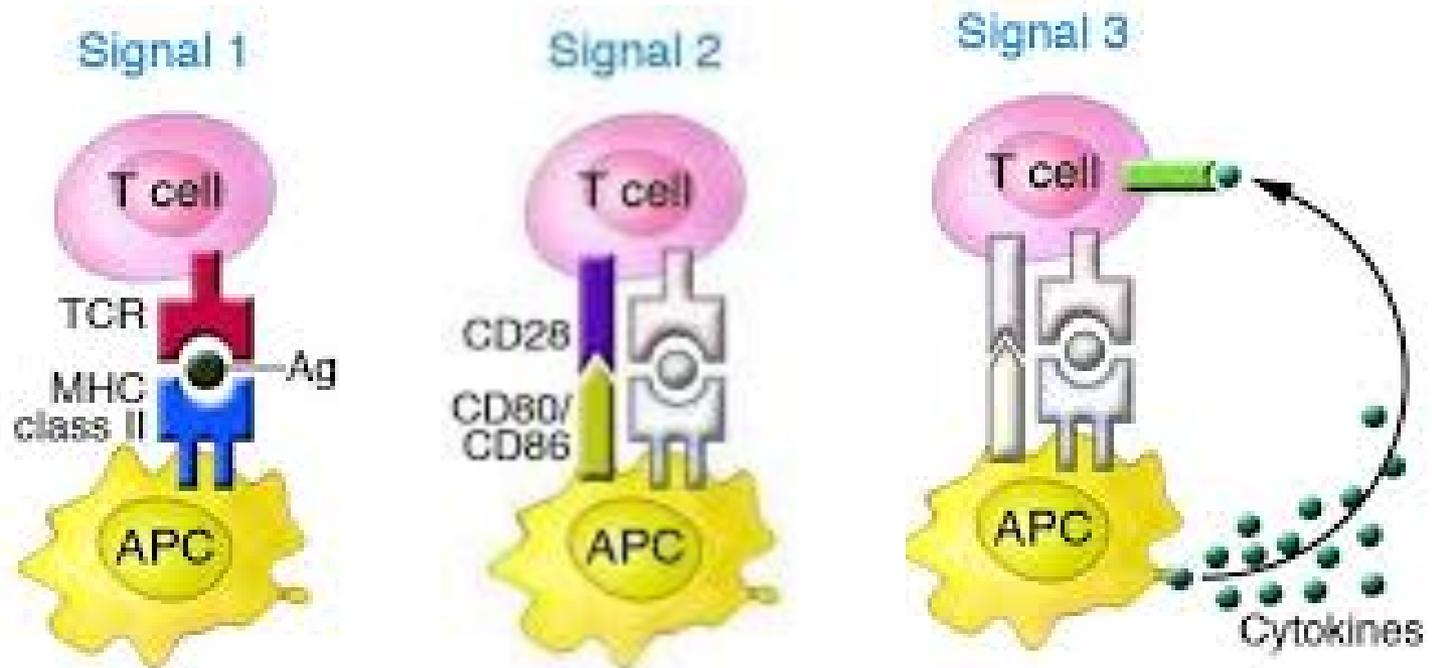
Sinapsi immunologica



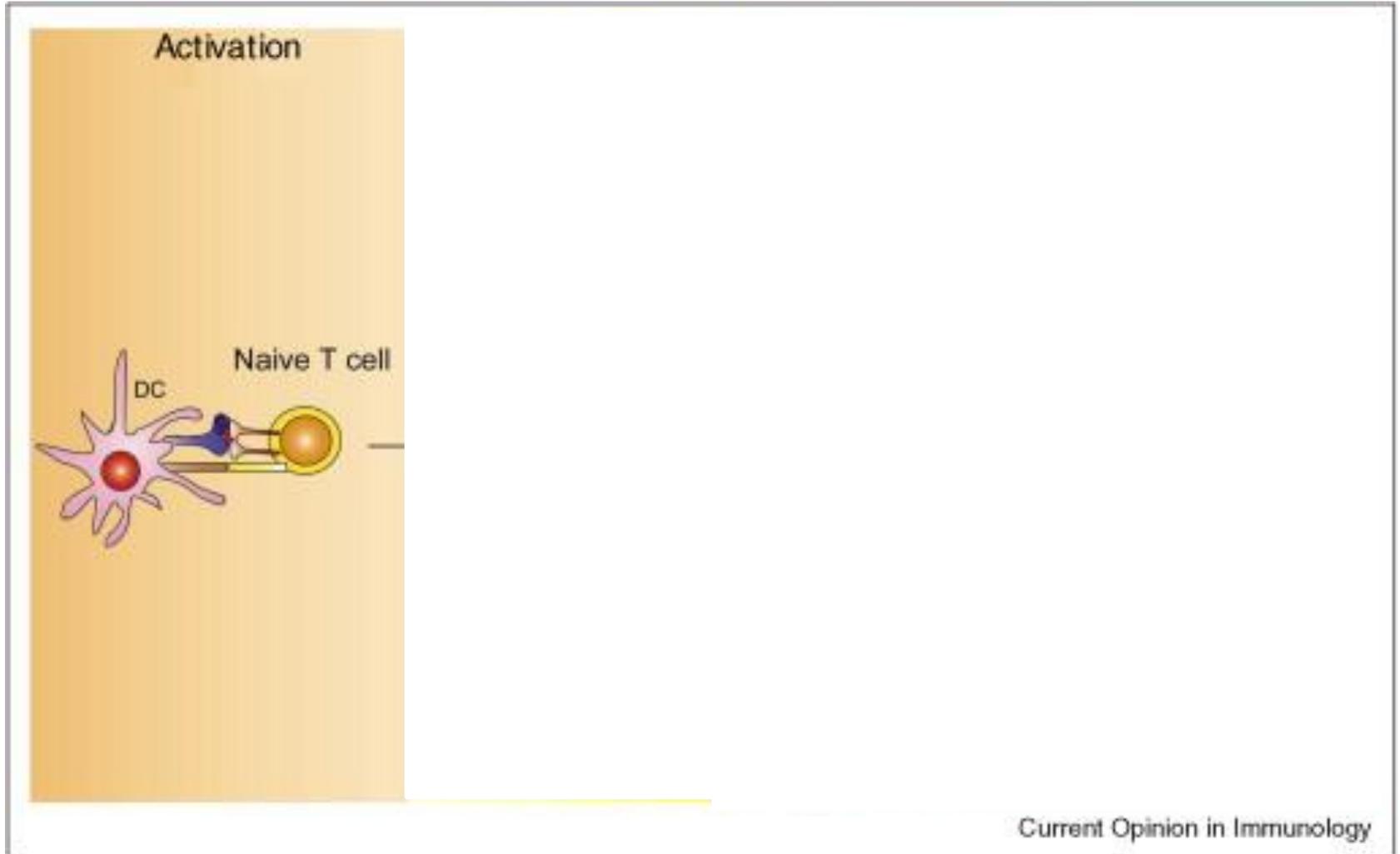
Processing and presentation of antigens



Attivazione linfociti T: 3 segnali



Differenziamento



Differenziamento

Attivazione: Riconoscimento dell'antigene (patogeno). Coinvolgimento di cellule presentanti l'antigene (APC) e attivazione

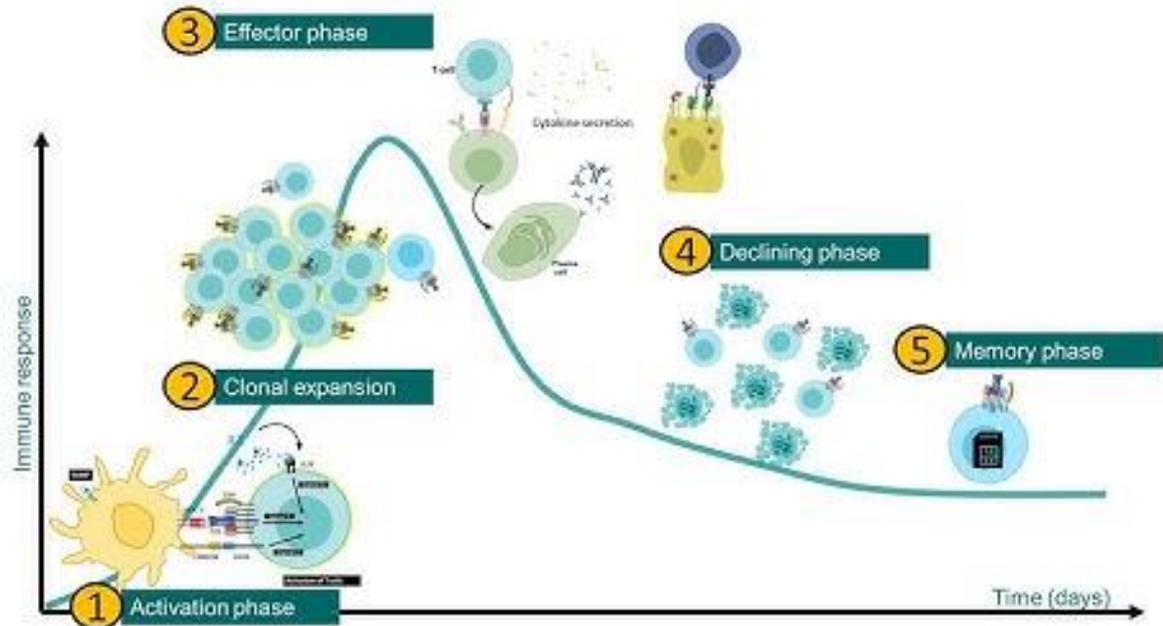
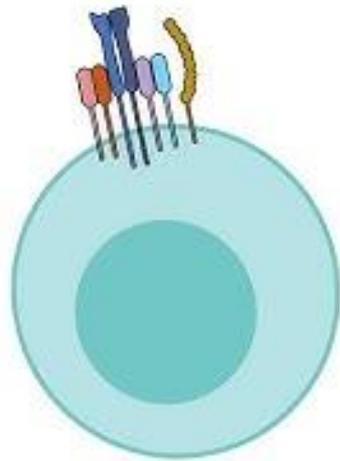
Espansione clonale: Rapida proliferazione dei linfociti attivati. Formazione di molti cloni specifici per lo stesso antigene

Differenziazione in cellule effettrici

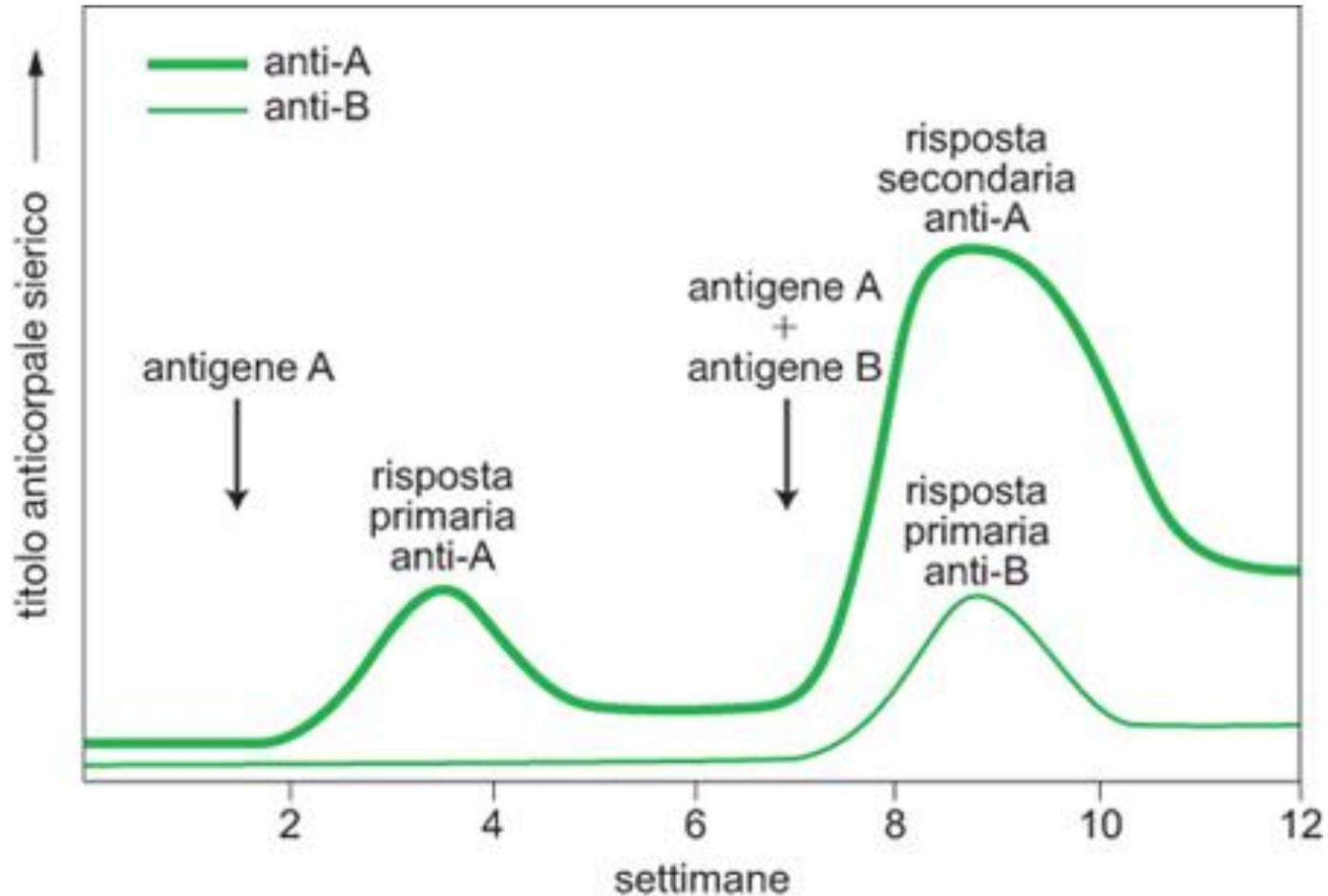
Contrazione: Eliminazione della maggior parte delle cellule effettrici. Morte programmata (apoptosi) necessaria per il ritorno all'equilibrio del sistema immunitario

Memoria: Sopravvivenza di alcuni linfociti come cellule della memoria. Risposta più rapida ed efficace a una nuova esposizione allo stesso antigene

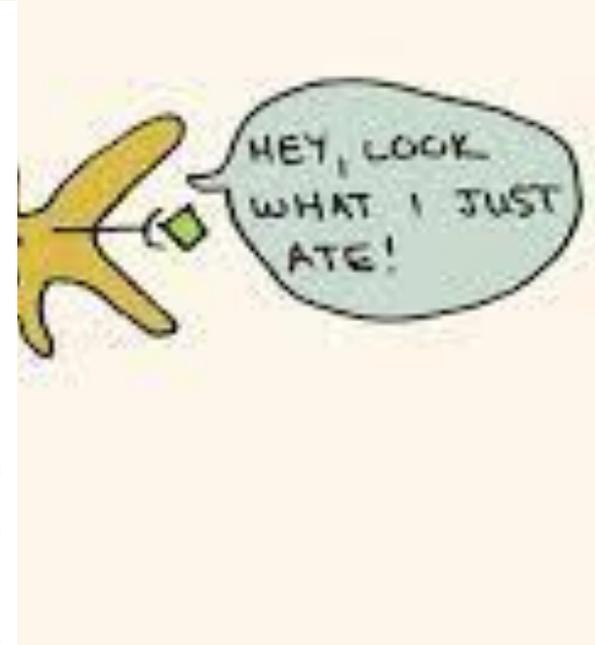
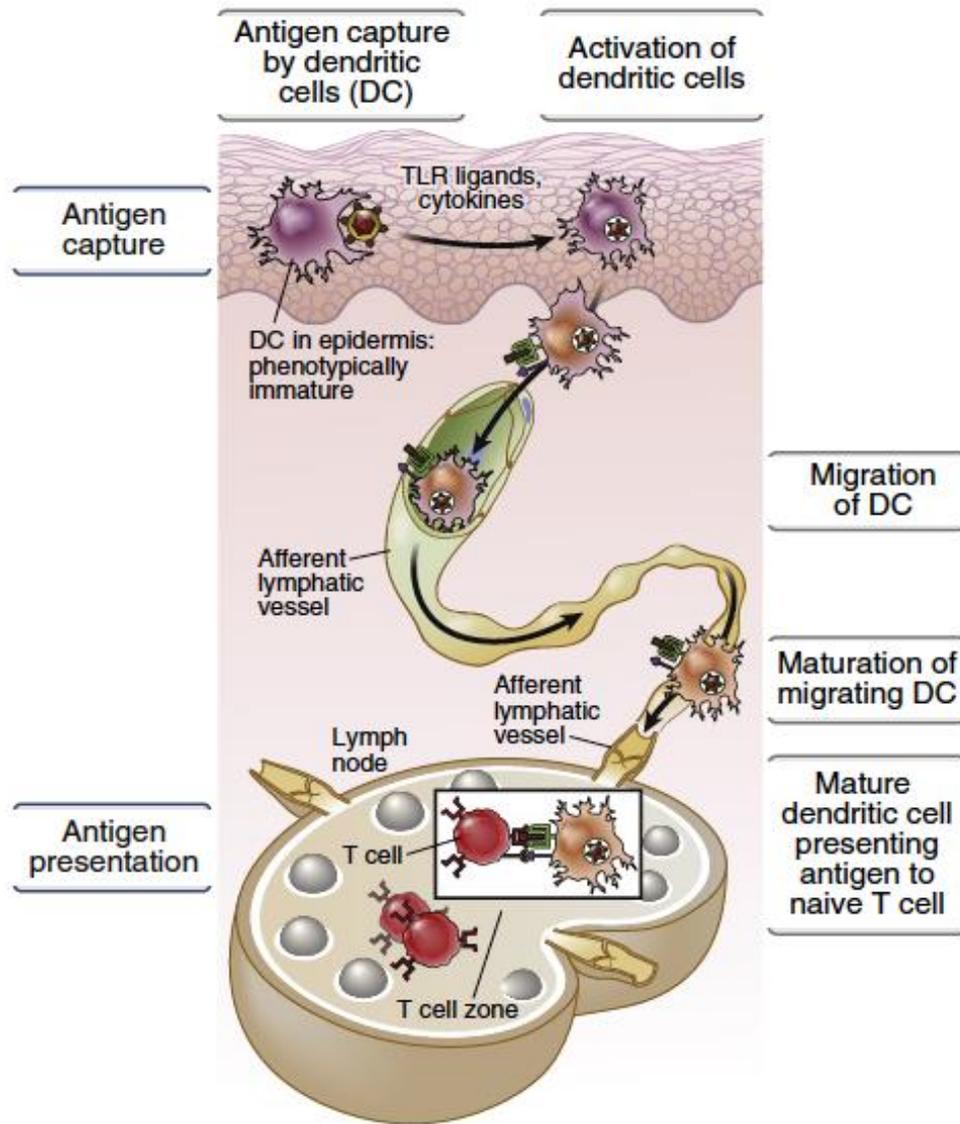
Phases of T cell mediated immune response

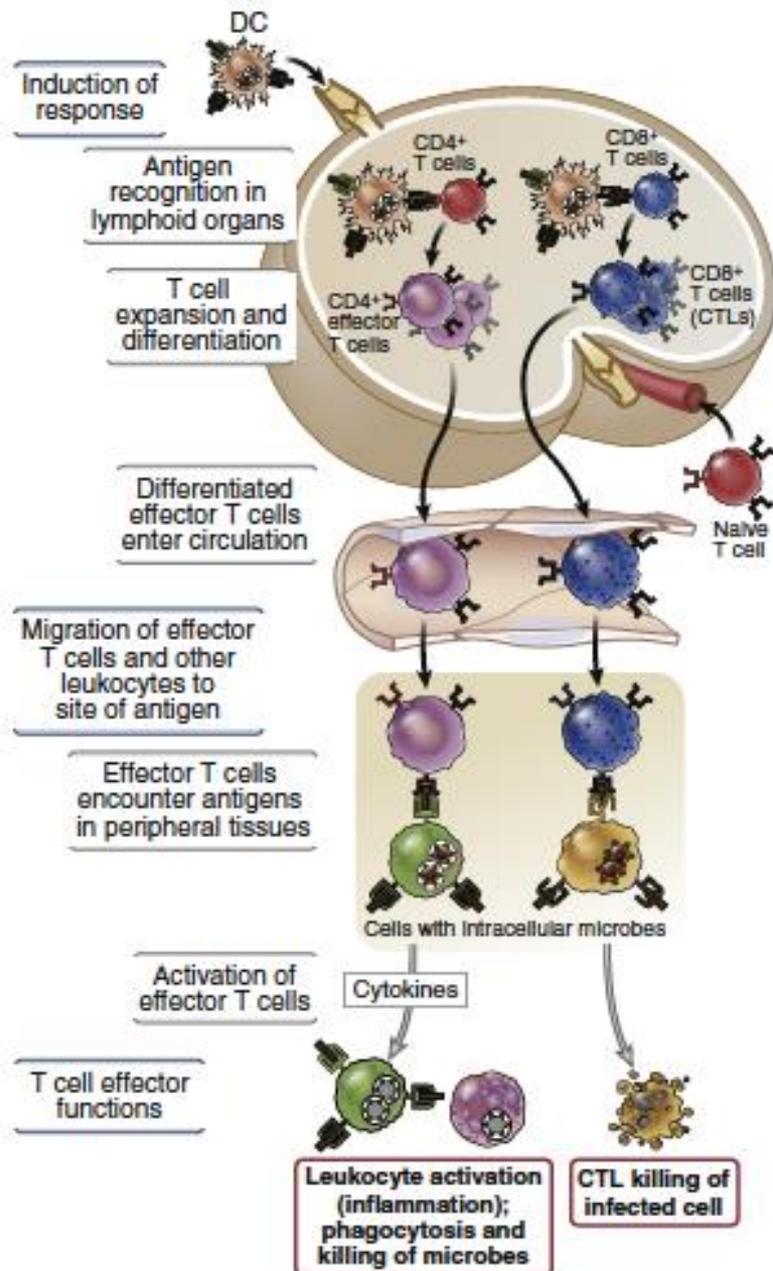


Risposta primaria e secondaria



Cattura dell'antigene nei tessuti

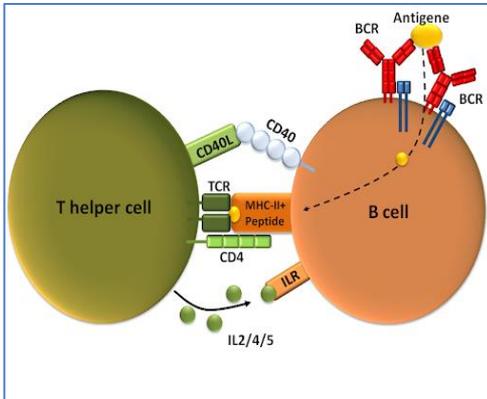




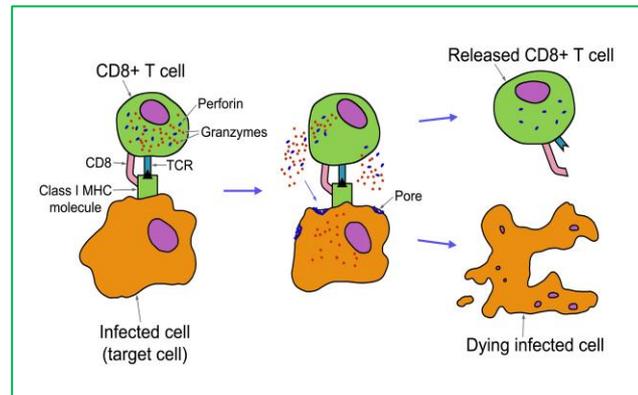
ANTIVIRAL IMMUNITY

Linfociti T: ruolo nella risposta antivirale

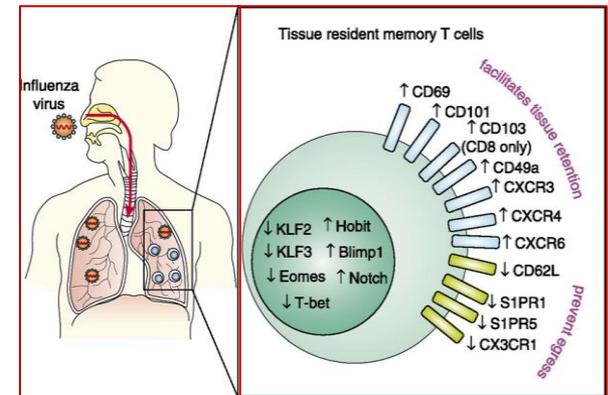
B cell help



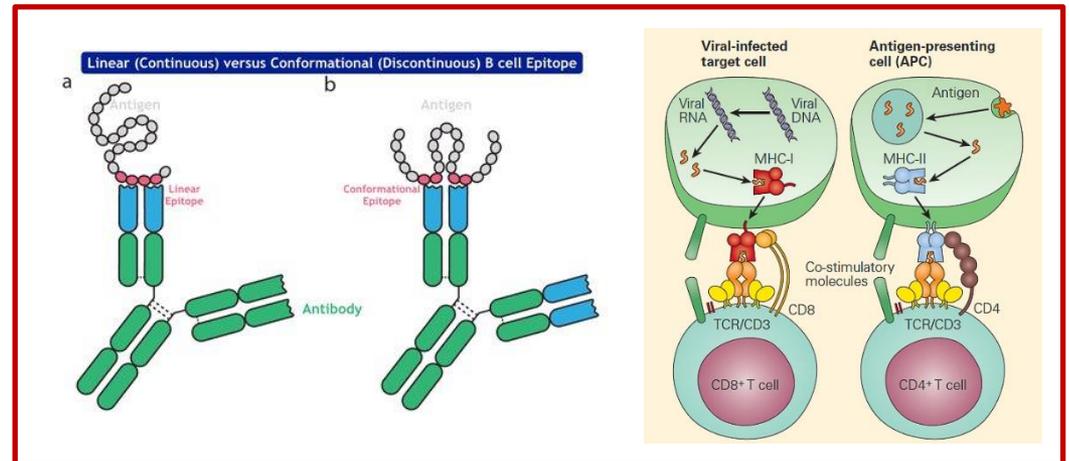
Killing of infected cells



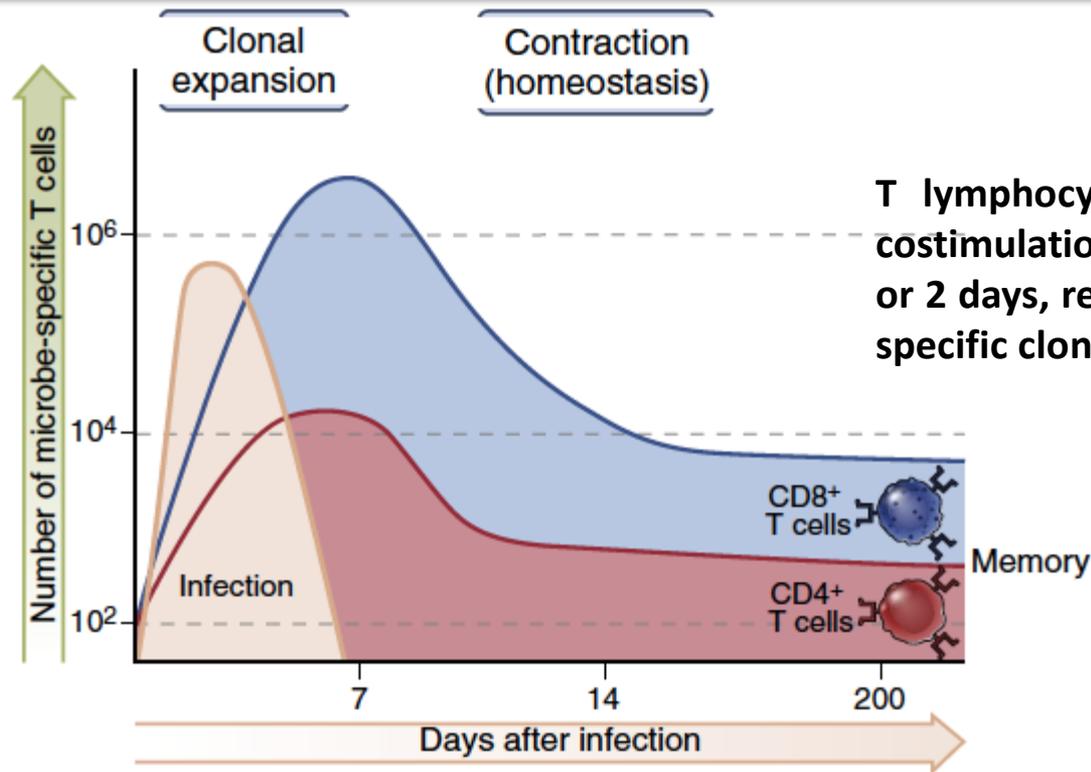
Tissue resident memory T cells



Wide cross-reactivity



Clonal Expansion



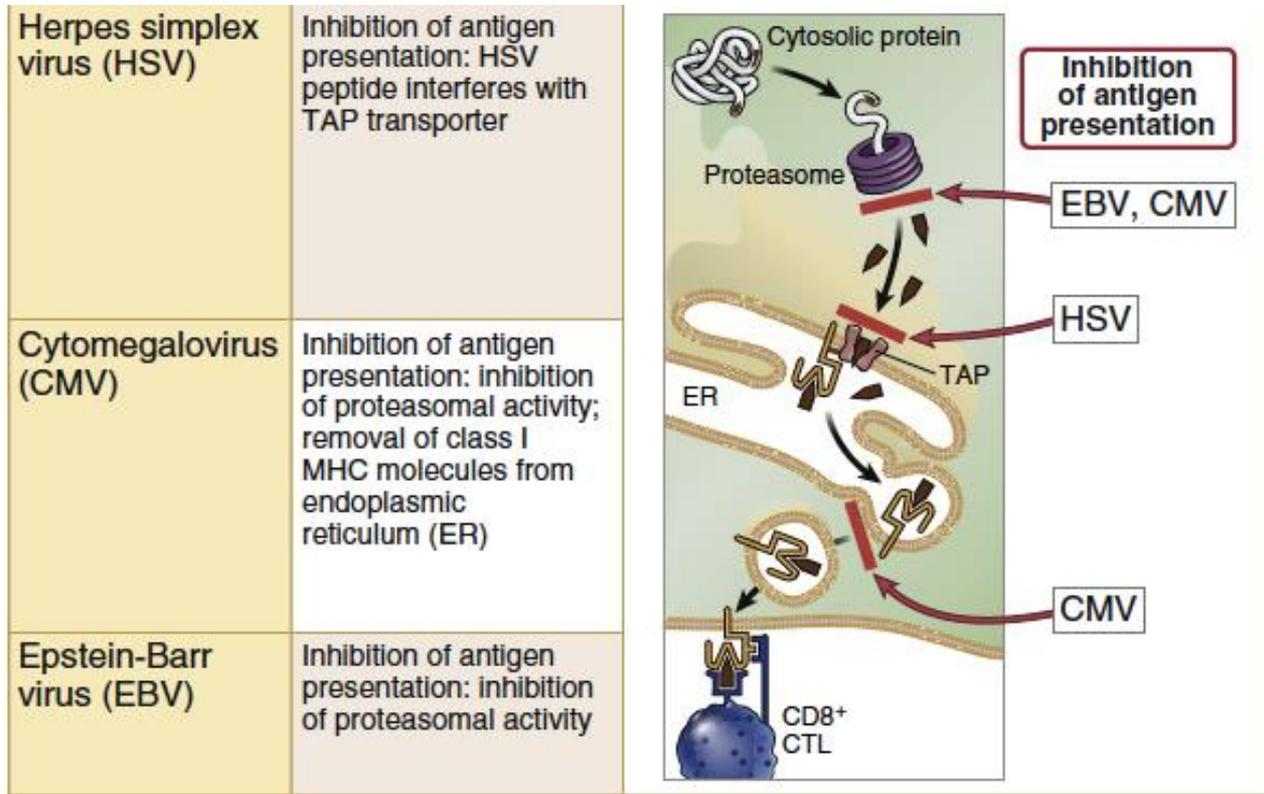
T lymphocytes activated by antigen and costimulation begin to proliferate within 1 or 2 days, resulting in expansion of antigen-specific clones

1) Anche nelle infezioni causate da microbi complessi che contengono molti antigeni proteici, la maggior parte dei cloni espansi è specifica solo per pochi **peptidi immunodominanti** di quel microrganismo, spesso meno di cinque.

2) L'espansione dei linfociti T **CD4+** è **100–1000 volte inferiore rispetto a quella dei CD8+**, probabilmente per le loro diverse funzioni. I CD8+ uccidono direttamente molte cellule infette e quindi devono essere numerosi, mentre i CD4+ attivano altre cellule tramite citochine, per cui ne bastano meno.

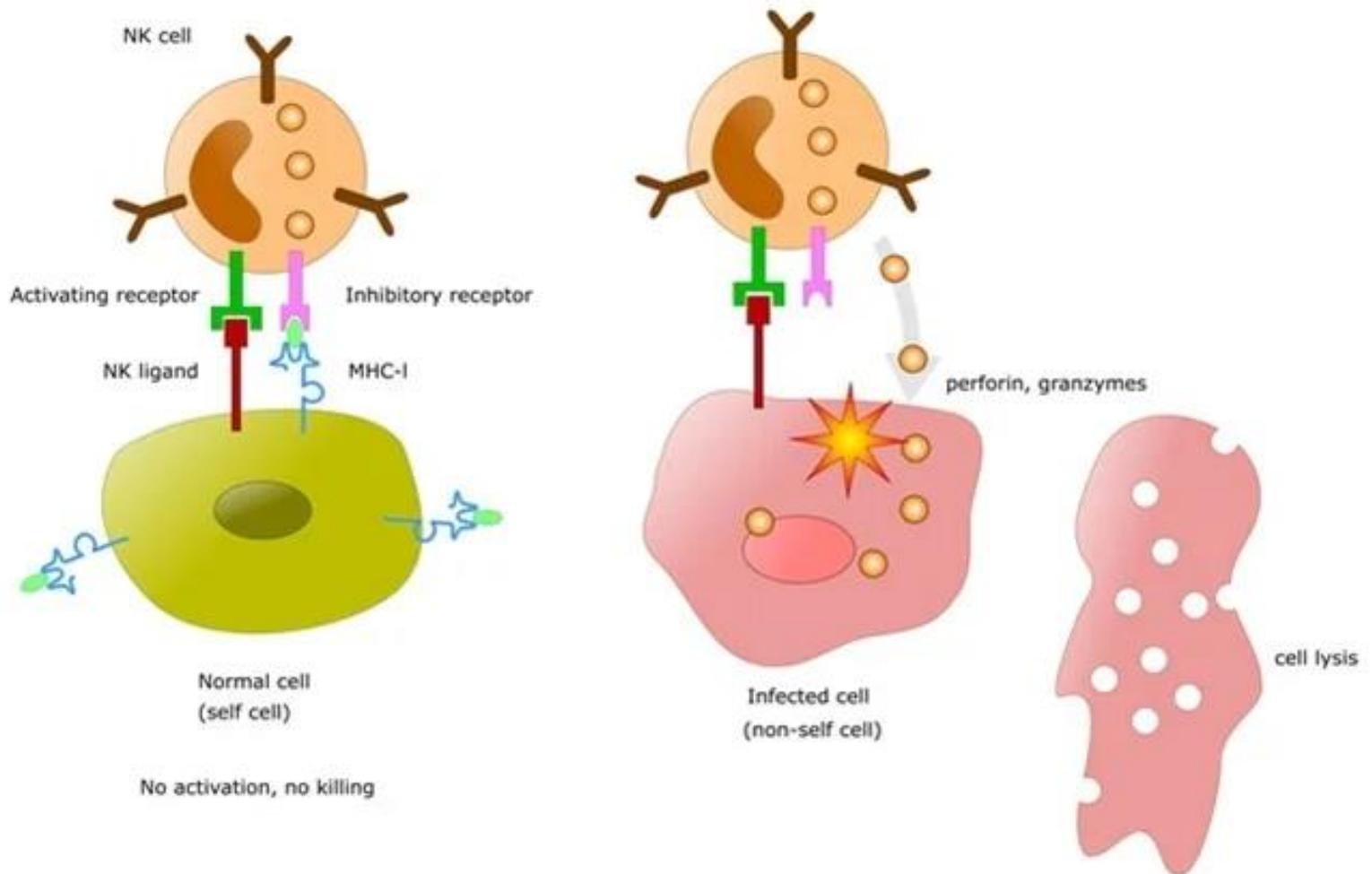
Meccanismi di escape virale

Down regolazione dell'espressione di molecole MHC



Riduzione della immunità adattativa. Ma

Cellule NK e missing self



CMV: esempio di evasione perfetta

1. Blocco dell'esposizione di MHC I

- degradazione dell'MHC I
- blocco del trasporto nel reticolo endoplasmatico
- inibizione del caricamento dei peptidi

Risultato: le cellule infette non mostrano antigeni ai linfociti T CD8+

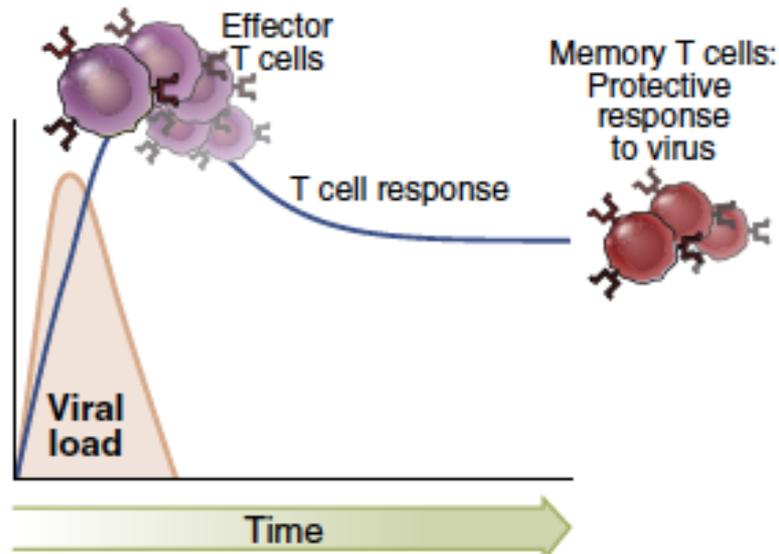
2. Mimicry per ingannare le cellule NK

Il CMV esprime proteine come: UL18, che è un omologo strutturale dell'MHC I
UL18: si lega a recettori inibitori delle cellule NK (es. LIR-1) "fa credere" alle NK che la cellula sia sana.

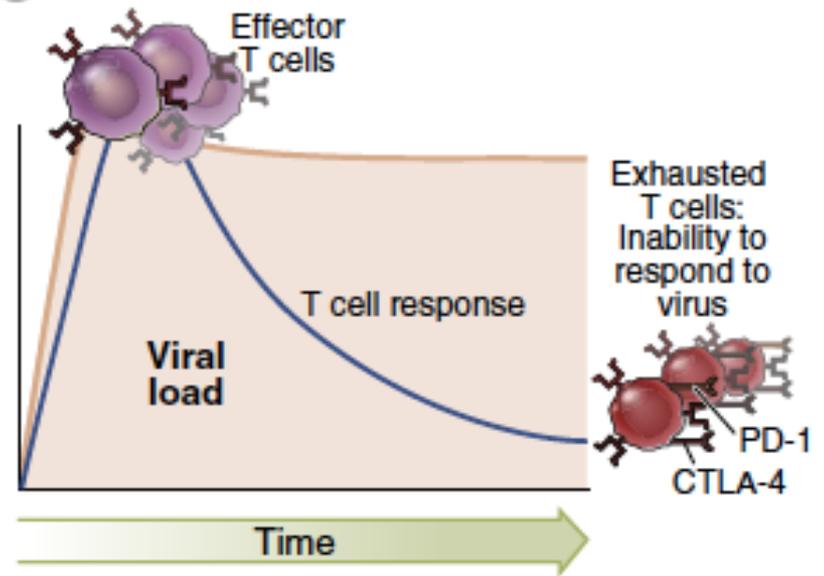
Risultato: le cellule infette non sono uccise dalle cellule NK

Infezioni virali acute e croniche

A Acute infection



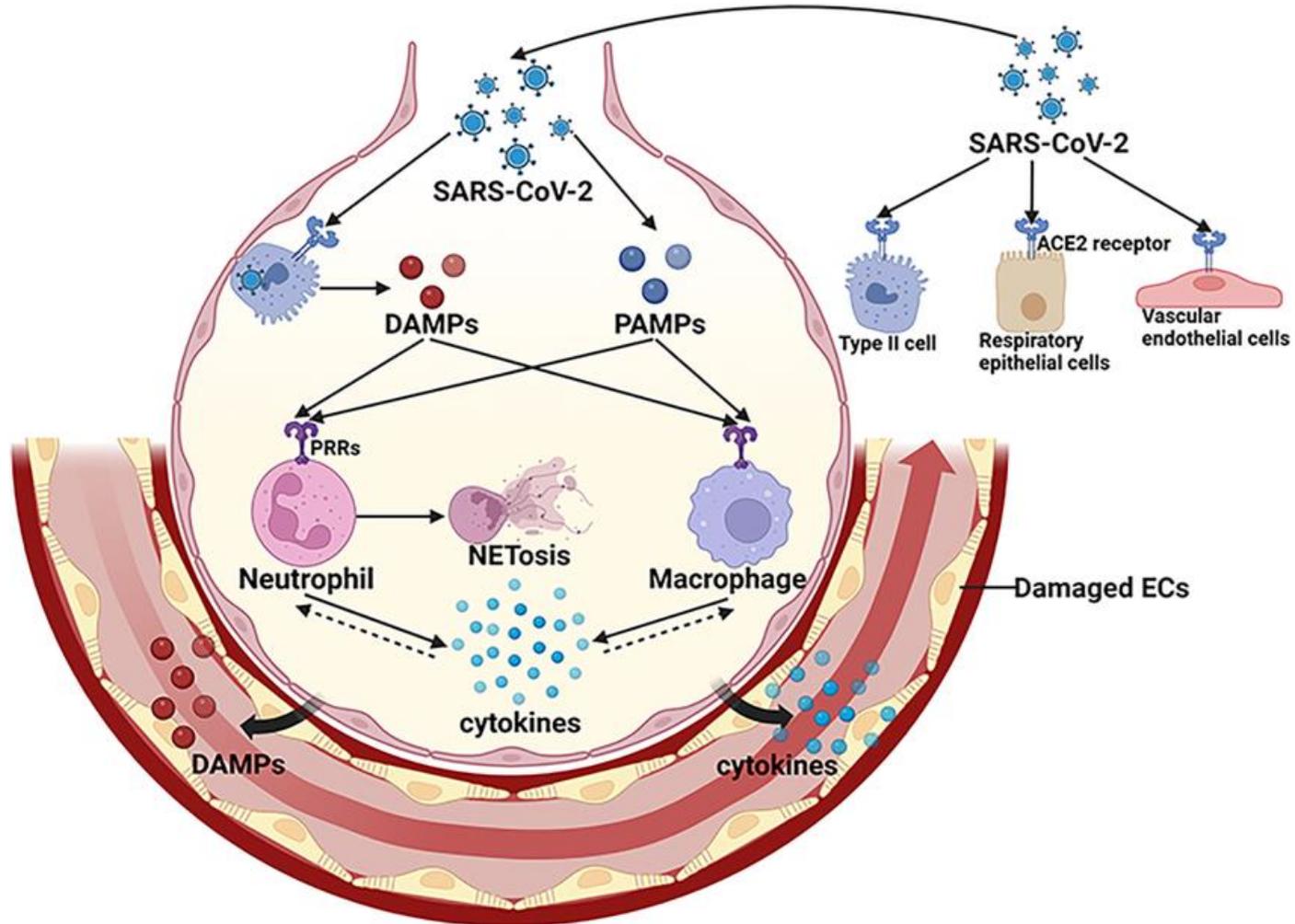
B Chronic infection



Risposta immune a infezione acuta

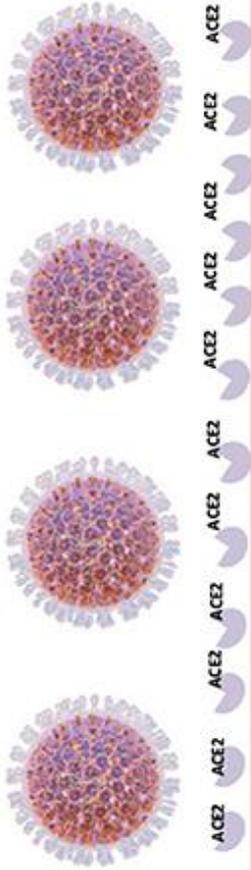
COVID-19

Infezione da SARS-CoV2: risposta innata



Tempesta citochinica

SARS-CoV-2



Epithelial lung cell

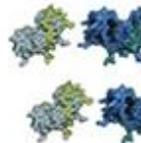
Rapid virus



Attenuation
(T cell activation)



Pro-inflammatory



Accumulation of



Multi-organ dysfunction syndrome (MODS)



Acute Respiratory Distress Syndrome (ARDS)



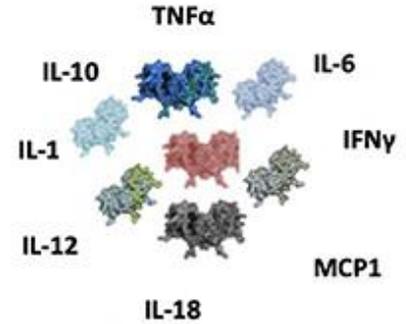
Kidney Failure



Viral myocarditis

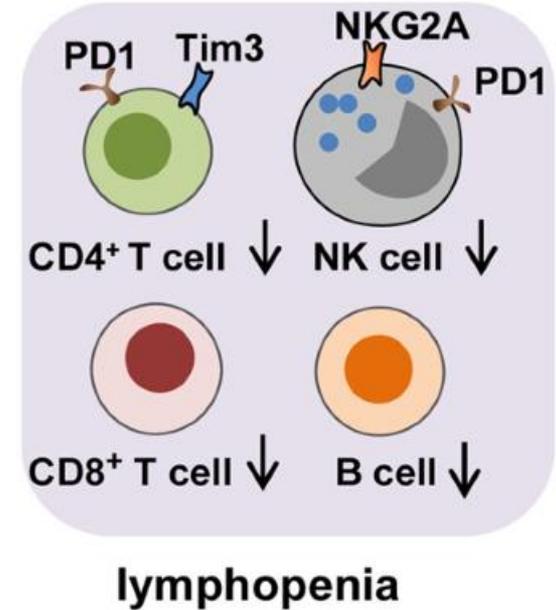
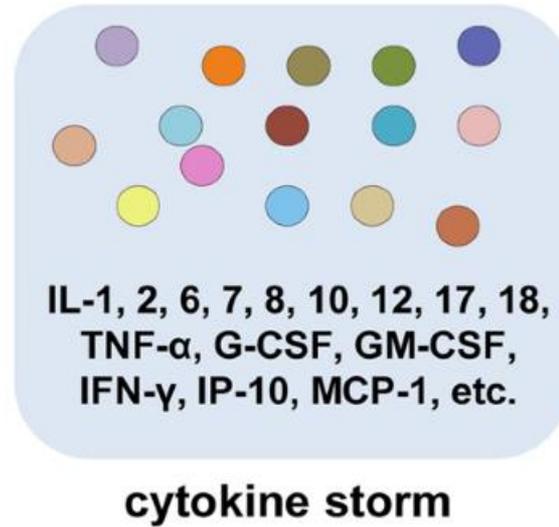
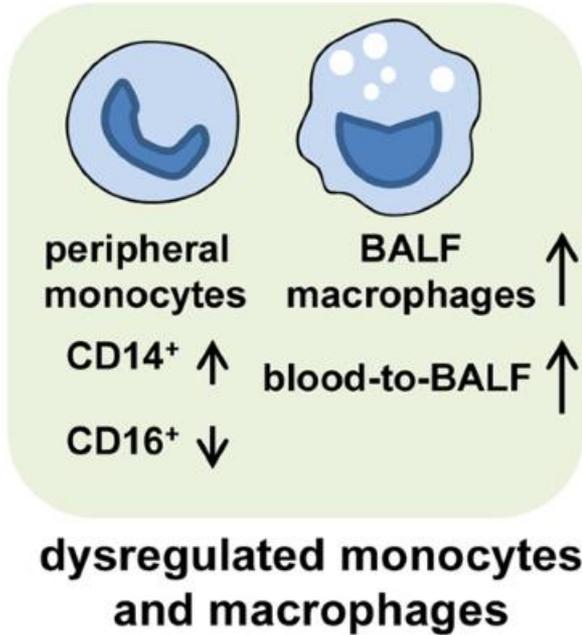


Cytokine Storm



ation

The immunopathological manifestations of severe COVID-19



Infezione da SARS-CoV2: risposta adattativa

Report

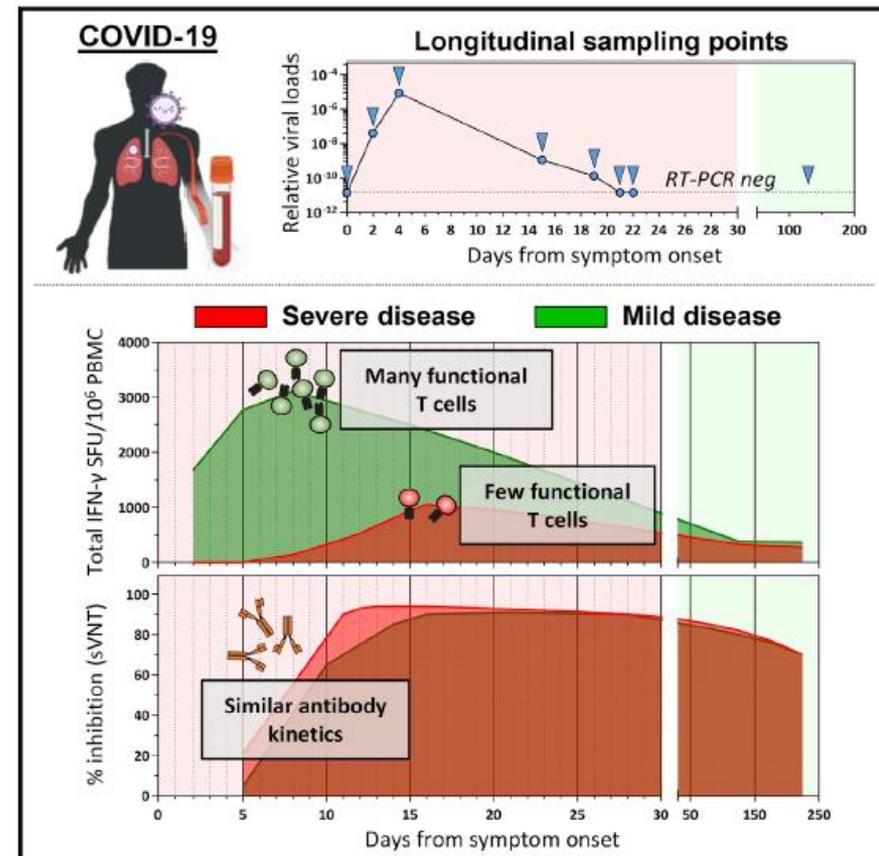
Cell Reports

Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients

Graphical Abstract

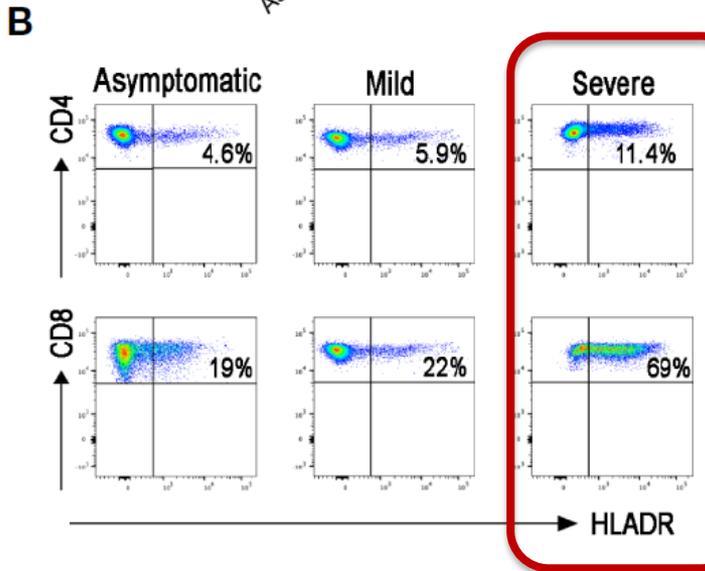
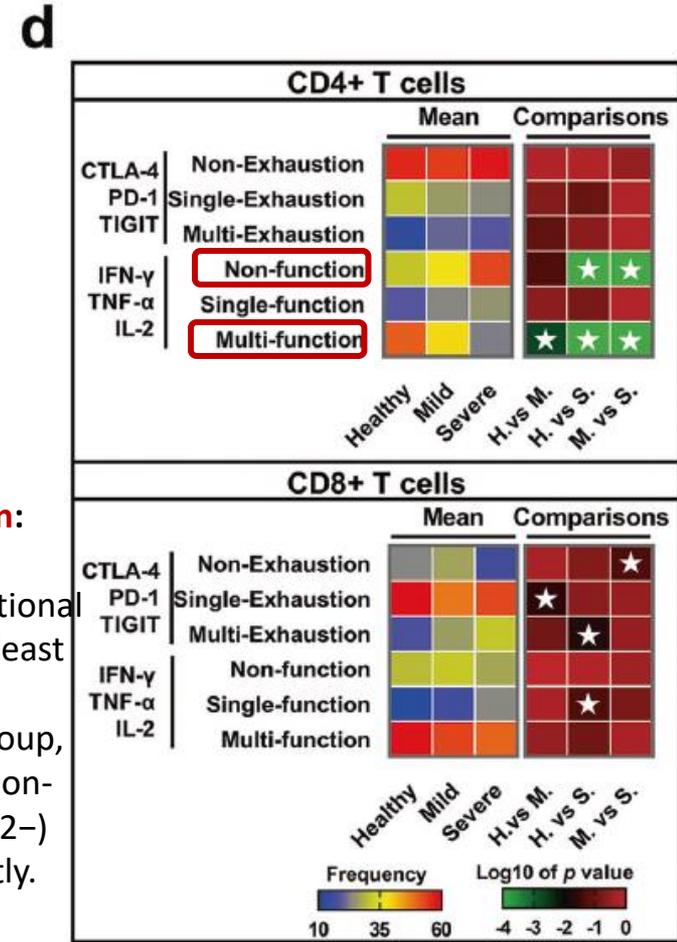
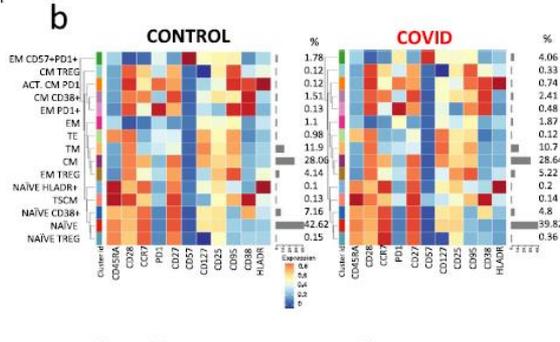
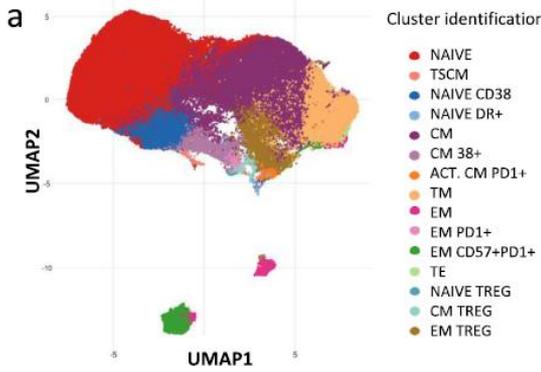
Highlights

- Longitudinal immunological analyses of COVID-19 from onset until outcome
- Early induction of SARS-CoV-2-specific T cells is associated with mild COVID-19
- Detection of functional SARS-CoV-2-specific T cells has prognostic value



Infezione severa da SARS-CoV2

Severe Infection are characterized by strong activation and exhaustion



Polyfunctional CD4 T cell participate to the protection:

The frequency of multi-functional CD4+ T cells (positive for at least two cytokines) decreased significantly in the severe group, whereas the proportion of non-functional (IFN- γ -TNF- α -IL-2-) subsets increased significantly.



Infezione da SARS-CoV2: risposta immune

Infezione lieve:

- Infiammazione controllata
- Attivazione rapida ed efficace dei linfociti T
- CD4+: buona produzione di citochine antivirali
supporto efficace per la produzione di anticorpi da parte dei linfociti B
- CD8+: eliminano rapidamente le cellule infette
- Formazione di memoria immunologica

Risultato: virus eliminato senza danni importanti

Infezione grave

- **Eccesso di citochine infiammatorie** (tempesta citochinica)
- Risposta T **ritardata o disfunzionale**
- **Riduzione dei linfociti T** (linfopenia)
- Segni di **esaurimento (exhaustion)** dei T:
 - minore capacità di uccidere
 - meno produzione di citochine efficaci
- Danno tissutale (soprattutto polmonare)

Risultato: virus + risposta immunitaria causano danno

ISOLATION AND PARTIAL CHARACTERISATION OF

A NEW VIRUS CAUSING ACUTE HEMORRHAGIC FEVER IN ZAIRE

K. M. JOHNSON
J. V. LANGE

P. A. WEBB
F. A. MURPHY

Virology Division, Center for Disease Control, Atlanta, Georgia 30333, U.S.A.

THE LANCET, MARCH 12, 1977

The distribution of the fatal pulmonary emboli in the controls was pulmonary trunk (5), main pulmonary artery (9), lobar artery (9), and segmental artery (6). In addition, in 5 patients from the control group and 1 from the heparin group, emboli found at necropsy were considered either contributory to death or an incidental finding, since death in these patients was attributed to other causes. Taking all pulmonary emboli together, 20 in the control group and 2 in the heparin group, the findings are again statistically significant.

Incidence of D.V.T.

D.V.T. was diagnosed clinically by the radioactive-fibrinogen-uptake test and at necropsy. When the 89 patients from the Basle centre, initially included in the M.C.T. analysis, were withdrawn, the significant differences observed between the control and heparin groups remained.

Treatment of D.V.T. and Pulmonary Embolism

Exclusion of the Basle data did not change the highly significant differences which were observed in the number of patients requiring treatment for D.V.T. and/or pulmonary embolism in the two groups.

Operative and Postoperative Bleeding

Exclusion of the Basle data did not influence the previously reported results¹ relating to the incidence of operative and postoperative bleeding.

Preliminary Communications

ISOLATION AND PARTIAL CHARACTERISATION OF A NEW VIRUS CAUSING ACUTE HEMORRHAGIC FEVER IN ZAIRE

K. M. JOHNSON P. A. WEBB
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An outbreak of hemorrhagic fever with an exceptionally high mortality-rate occurred in southern Sudan and northern Zaire with peak case-rates in September, 1976. A W.H.O. International Commission operated in Sudan and Zaire from October onward.^{1,2} Blood and tissue specimens from persons with hemorrhagic disease were sent to laboratories in Belgium and England, and findings from these laboratories appear in the accompanying reports.^{3,4} While these specimens were being studied, Mr E. T. W. Bowen (Microbiological Research Establishment, Porton Down) sent an aliquot of an acute blood specimen from a patient in Zaire (no. 718, patient M.E.) to the Center for Disease Control, Atlanta, for additional study.

This specimen, and all subsequent acute specimens, were inoculated into Vero (African green monkey) cells. Three days later a distinct cytopathic change (focal rounding and refractility) was evident, and an aliquot of supernatant fluid was removed for negative contrast electron microscopy.

ELECTRON MICROSCOPY OF CELL CULTURES

Carbon-coated grids were sequentially floated on droplets of

the cell-culture fluid and then on 2% sodium silicotungstate pH 7. Large numbers of filamentous virus particles were seen (fig. 1). They were approximately 100 nm in diameter and varied in length from 300 nm to more than 1500 nm. Many had terminal blebs. Particles had regular surface projections approximately 10 nm long, and when stained they were seen to have internal cross-striations indicative of a helical core structure (fig. 2). In all details, these particles were indistinguishable from Marburg virus particles studied in 1967 (isolates from Germany) and 1975 (isolate from South Africa).^{1,2-7} Two characteristics were more prominent in the 1976 Zaire isolate: there was more branching of the filamentous particles (fig. 1); and more evidence of envelope continuation beyond the ends of the more rigid internal structure (fig. 1, arrow).

Vero cells infected with the same isolate from Zaire were examined also by thin-section electron microscopy. Filamentous virus particles were found budding from the plasma membrane of cells (fig. 3), and many of the cells contained inclusion bodies. These intracytoplasmic inclusions were complex and distinct, and consisted of a finely fibrillar or granular ground substance which condensed into tubular structures. The latter had been considered to be the internal helical structure of mature virus particles. These tubules were sectioned randomly, some in cross-section, some linearly. The virus particles in

TABLE 1—COMPARISON OF RECIPROCAL I.F.A. TITRES OF MARBURG ('67, '75) AND MARBURG-LIKE ('76) VIRUS DISEASE SERA

Year of illness	Country	Human sera	Time after onset	Antigen	
				Marburg '67	no. 718 '76
1967	Germany	U	5 mo.	128	<10
		K	5 mo.	64	<8
		DO	1 mo.	64	<4
1975	Africa	MC	4 mo.	64	<4
		no. 8	+12 days	<2	16
1976	Zaire	no. 9	+12 days	<2	<2
		no. 5	1 mo.	4	160

Conclusion

The evidence that low doses of heparin prevent D.V.T. in most postoperative patients is irrefutable. The main unresolved question was whether this form of prophylaxis also reduces deaths from pulmonary embolism. This question was answered by the M.C.T.¹, and the conclusion of that trial is not affected by our reappraisal of the results: subcutaneous heparin administration before and after surgery significantly reduced the incidence of fatal pulmonary embolism, detected at necropsy. The recent article by Gruber et al.² casts doubt on the conclusion of this trial. However, as summarised by Sherry³ "... one is forced to the conclusion that there is a very high probability that the differences in the primary endpoint (fatal P.E.) between the two groups is real, and that the study should be used to influence the practice habits of the profession for preventing fatal pulmonary embolism following abdominal surgery".

Requests for reprints should be addressed to V.V.K.

REFERENCES

- International Multicentre Trial *Lancet*, 1975, ii, 45
- Gruber, U. F., Duckert, F., Fritsch, R., Töthner, J., Rem, J. *ibid.* 1977, ii, 207.
- Rem, J., Duckert, F., Fritsch, R., Gruber, U. F. *Schweiz. med. Wschr.* 1975, 105, 827.
- Communication; Rem, J. A. Inaugural dissertation, Faculty of Medicine, University of Basle, 1975, to the Co-ordinating Centre from Basle. Letter received on June 30, 1975, with comments before the publication of the M.C.T. results.
- Sherry, S. Prophylactic Therapy of Deep Vein Thrombosis and Pulmonary Embolism. D.H.E.W. publications no. (N.I.H.) 76-866, 1975, p. 229.

570

these sections were identical to those observed in the 1967 and 1975 isolates.^{8,9}

POSTMORTEM LIVER SPECIMENS

Evidence of infection was seen by light microscopy in three postmortem human liver specimens from Zaire (received in

formalin). Infection of two of these was confirmed by electron microscopy. Focal eosinophilic hepatocellular necrosis with modest inflammatory infiltration was prominent. Large eosinophilic inclusions were present in many intact hepatocytes, especially near sites of severe necrosis (fig. 4). These rather smooth and refractile inclusions were so characteristic that

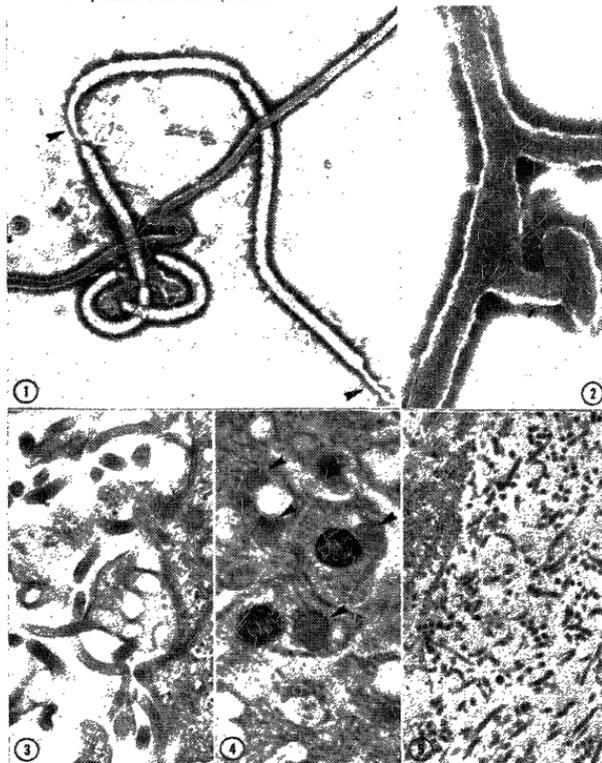


Fig. 1—Ebola virus in first Vero-cell passage after inoculation with patient's blood from Zaire (reduced from $\times 44\ 000$).

Some filamentous particles in negative contrast preparations were more than 1500 nm long; particles had a uniform diameter of 100 nm and some had ragged ends (arrows).

Fig. 2—Virus particle penetrated by negative contrast medium showing internal cross-striations (reduced from $\times 115\ 000$).

Fig. 3—Virus particles, in ultrathin section, budding from plasma membrane of Vero cell at 3 days after infection (reduced from $\times 49\ 000$).

All morphological characteristics were similar to those of Marburg virus as studied in 1967 and 1975.

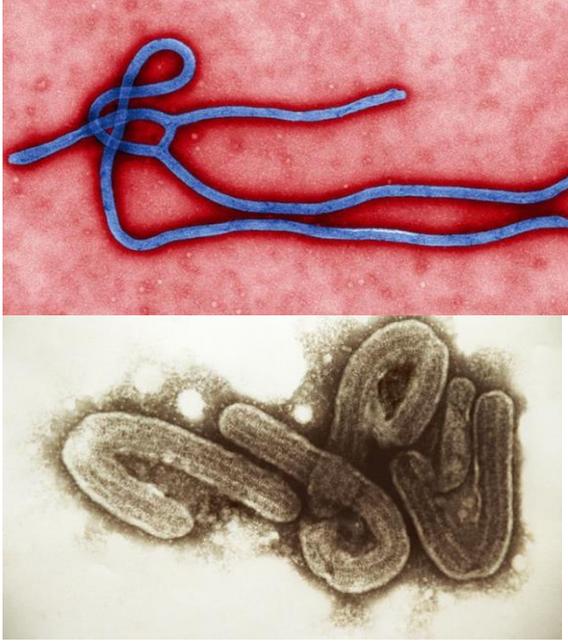
Fig. 4—Liver from fatal case in Zaire (reduced from $\times 1100$).

Large eosinophilic inclusion bodies (arrows) in many hepatocytes. Focal necrosis and inflammation. Formalin fixation, hematoxylin and eosin.

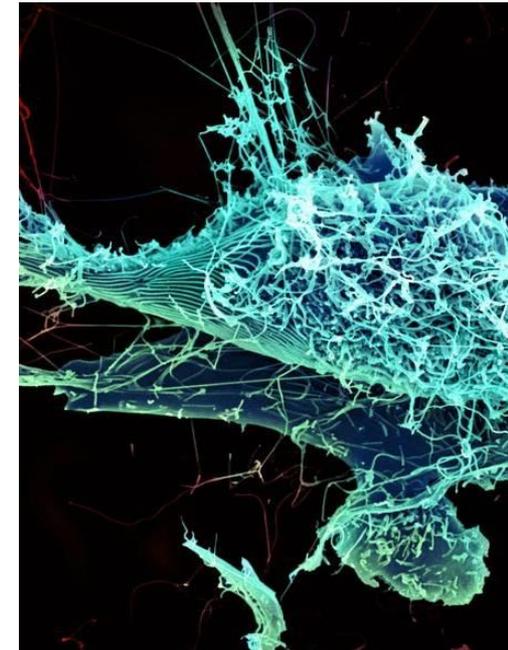
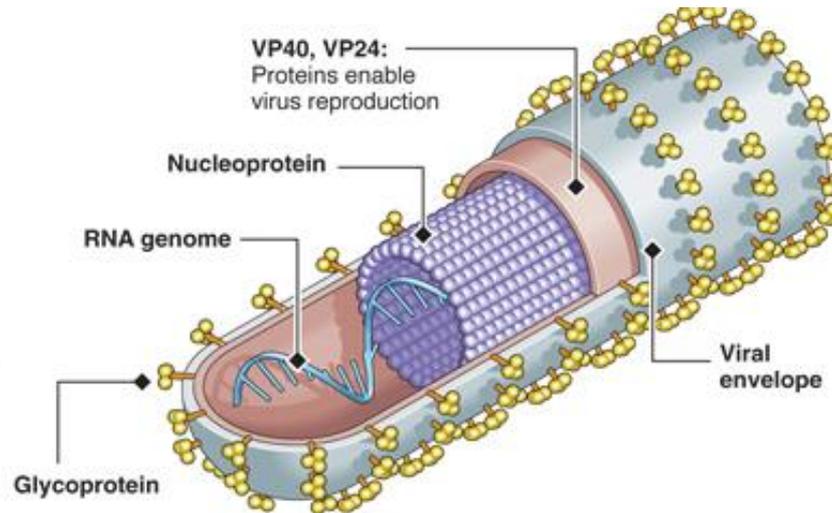
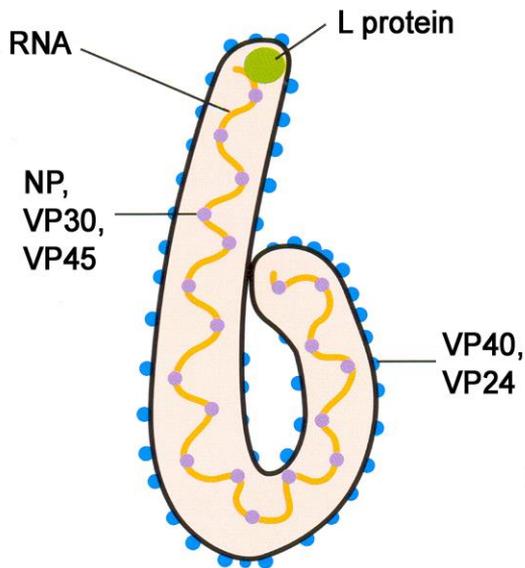
Fig. 5—Virus particles in distended extracellular space in liver from fatal case in Zaire (reduced from $\times 20\ 000$).

Despite poor tissue preservation, massive numbers of virus particles and characteristic inclusion bodies were identified by electron microscopy of two of three necropsy specimens.

Il virus



- Particella virale pleiomorfa, allungata, dotata di involucro, dimensioni: 80nm x 130-14,000nm
- Ordine: mononegavirales (ssRNA-)

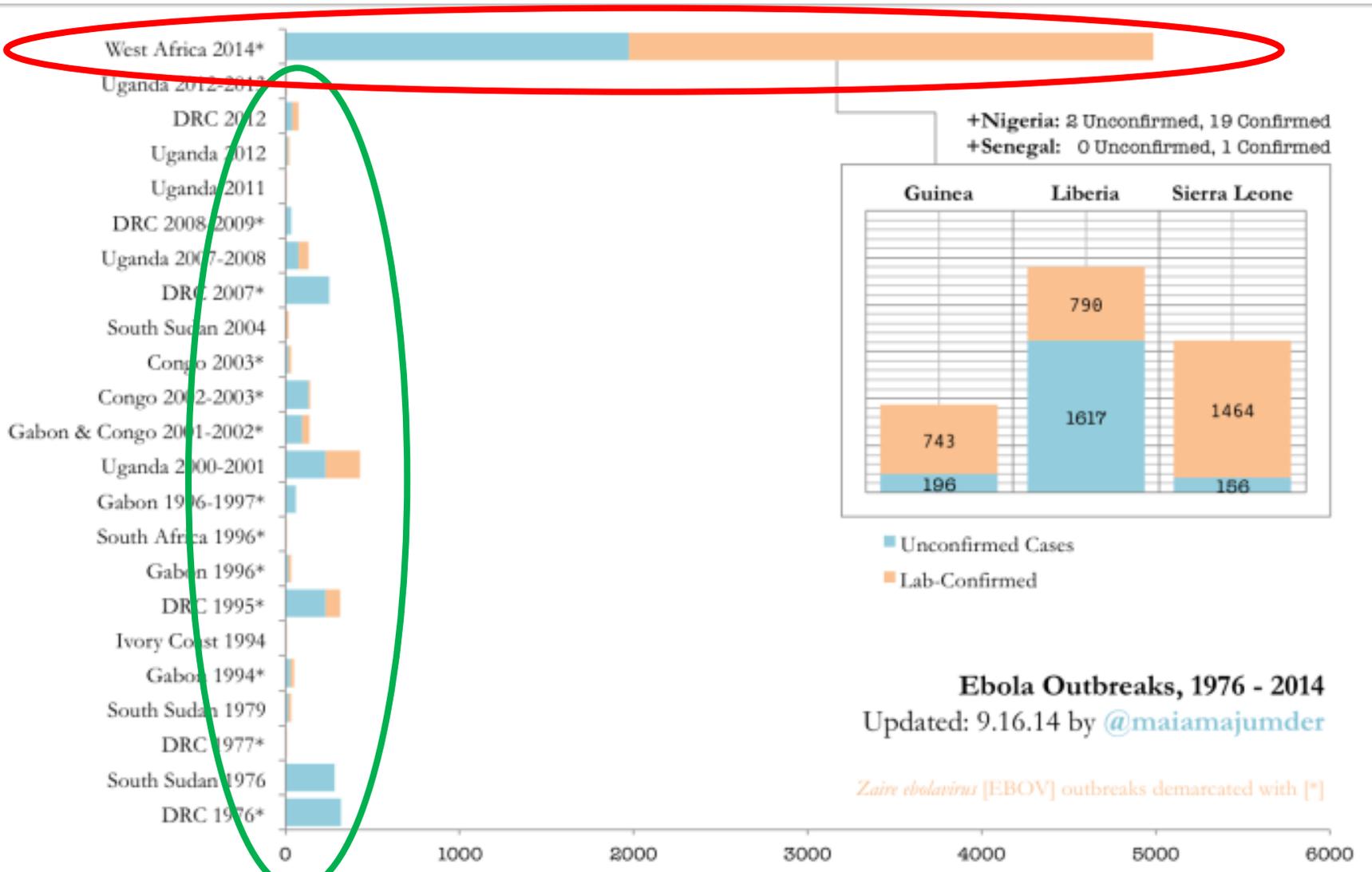


Il virus ebola, come gli altri filovirus, è un virus di gruppo di rischio 4

- Alta contagiosità
- Elevata mortalità
- Assenza di terapia specifica
- Assenza di vaccini



Epidemia di dimensioni incredibilmente massicce



Early Symptoms:



U.S. Department of
Health and Human Services
Centers for Disease
Control and Prevention

Ebola can only be spread to others after symptoms begin. Symptoms can appear from 2 to 21 days after exposure.

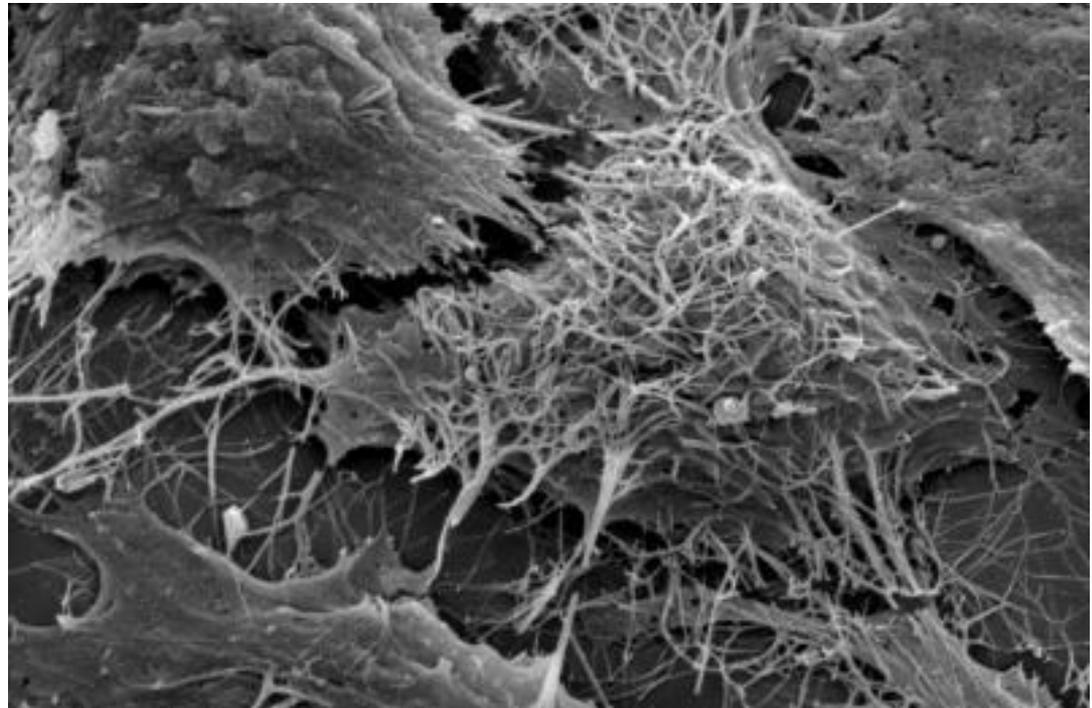
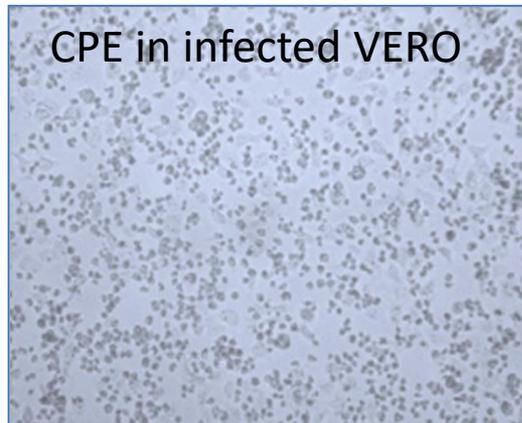
- **Fever**
- **Stomach pain**
- **Headache**
- **Unexplained bleeding or bruising**
- **Diarrhea**
- **Muscle pain**
- **Vomiting**

I segni conclamati



La Patogenesi: Ruolo diretto del virus

- **Pathway 1:** Direct damage to cells/tissues due to cytopathic virus replication

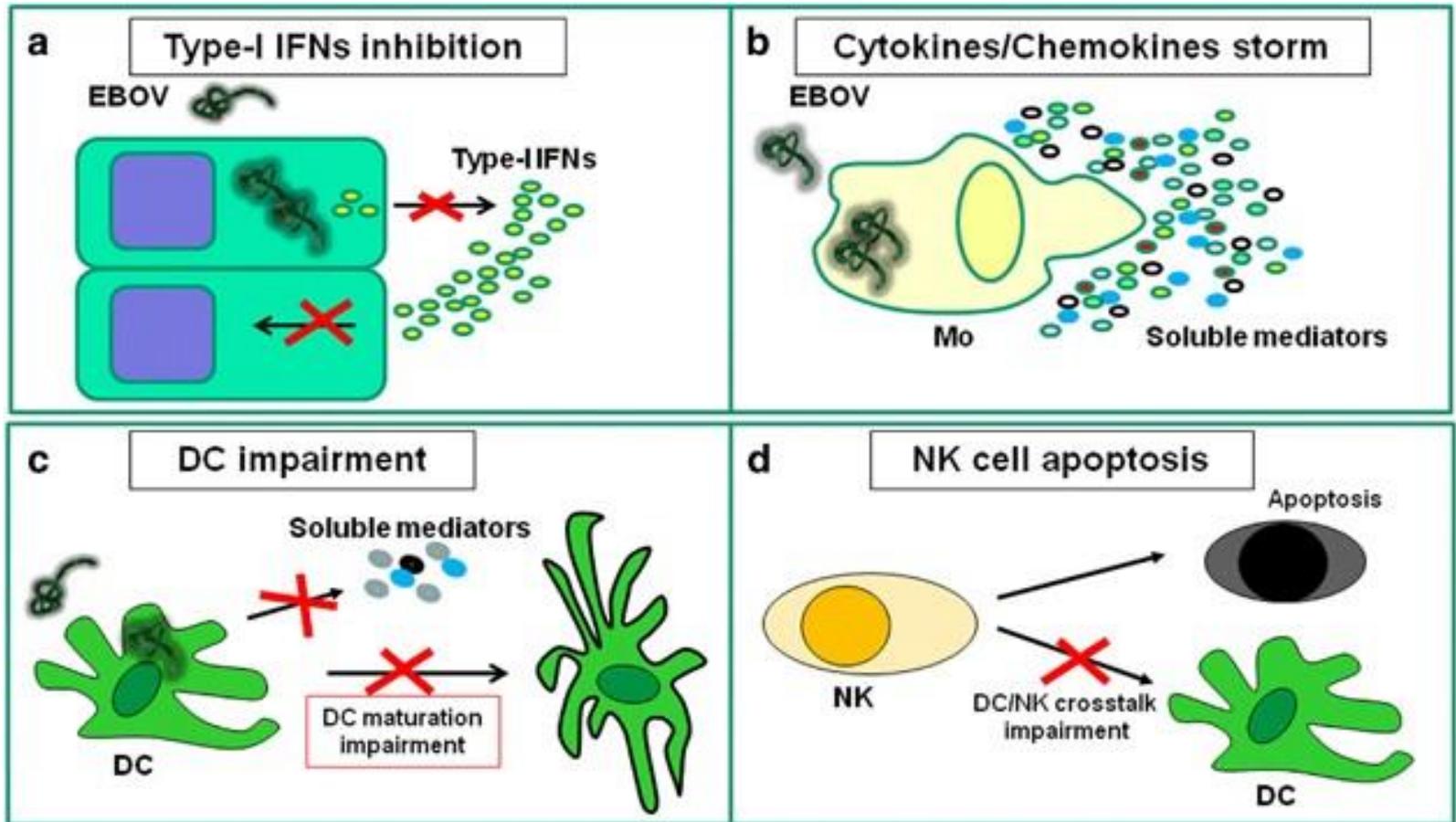


La Patogenesi: Ruolo della risposta immunitaria

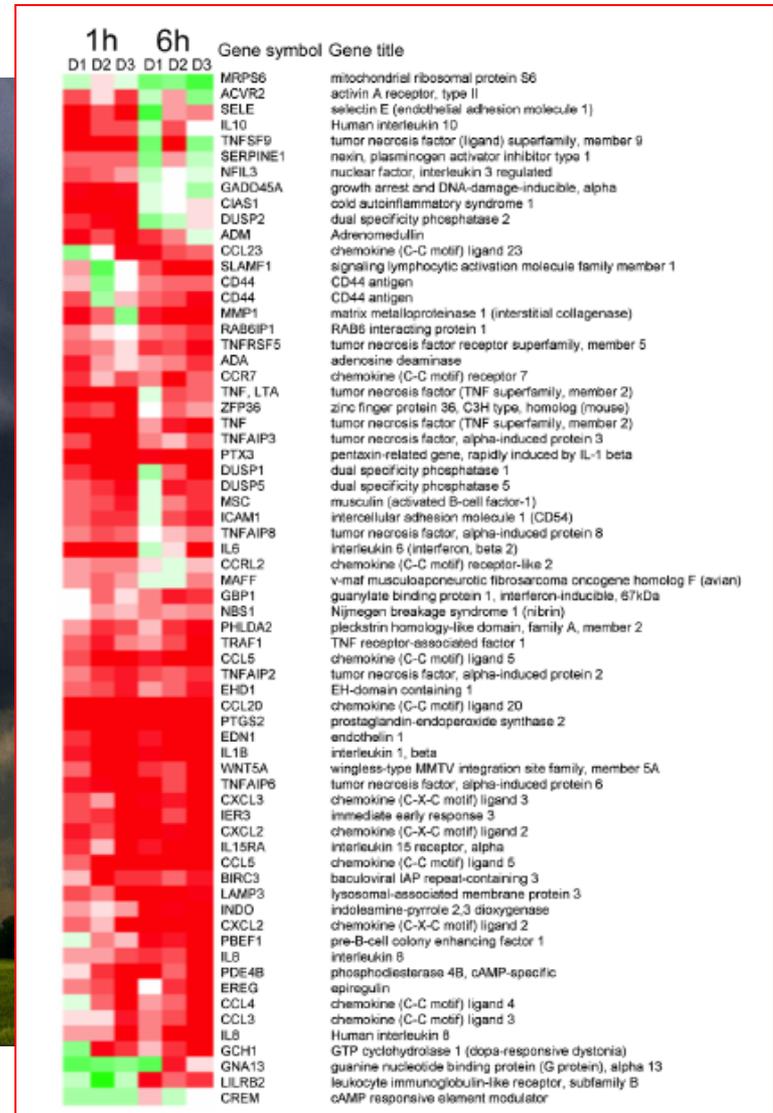
- **Pathway 2:** Alteration of innate and adaptive immune response, promoting excessive inflammatory reaction preventing an effective (protective) response



Immunità innata e Ebola



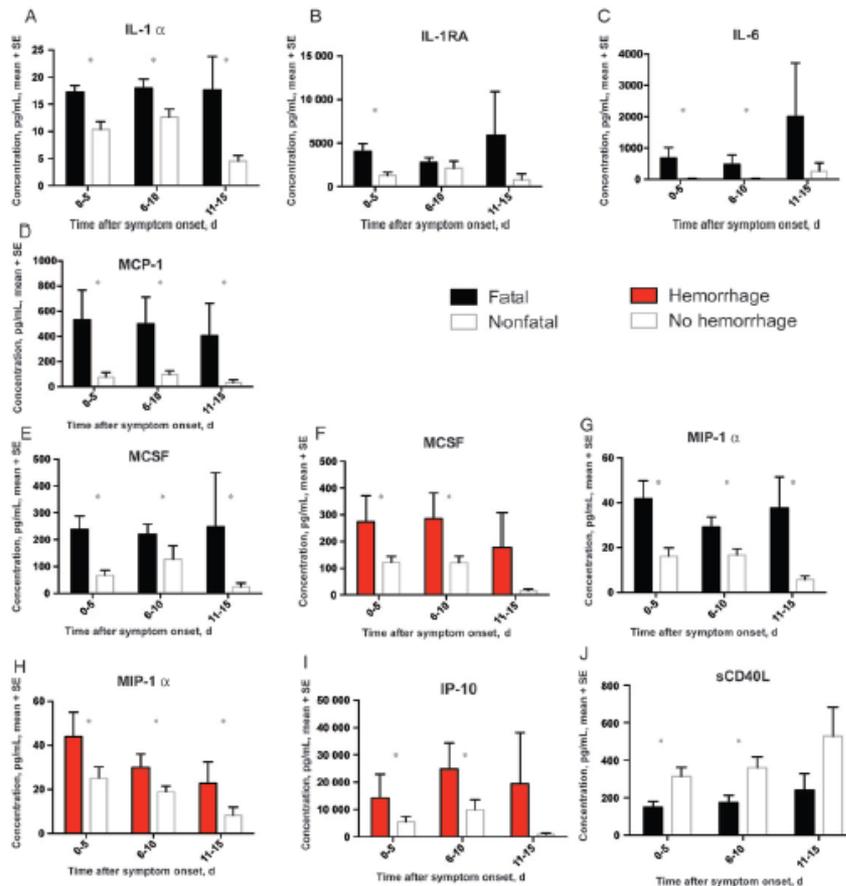
Immunità innata: Tempesta citochinica



Immunità innata: Tempesta citochinica

Ebola Hemorrhagic Fever: Novel Biomarker Correlates of Clinical Outcome

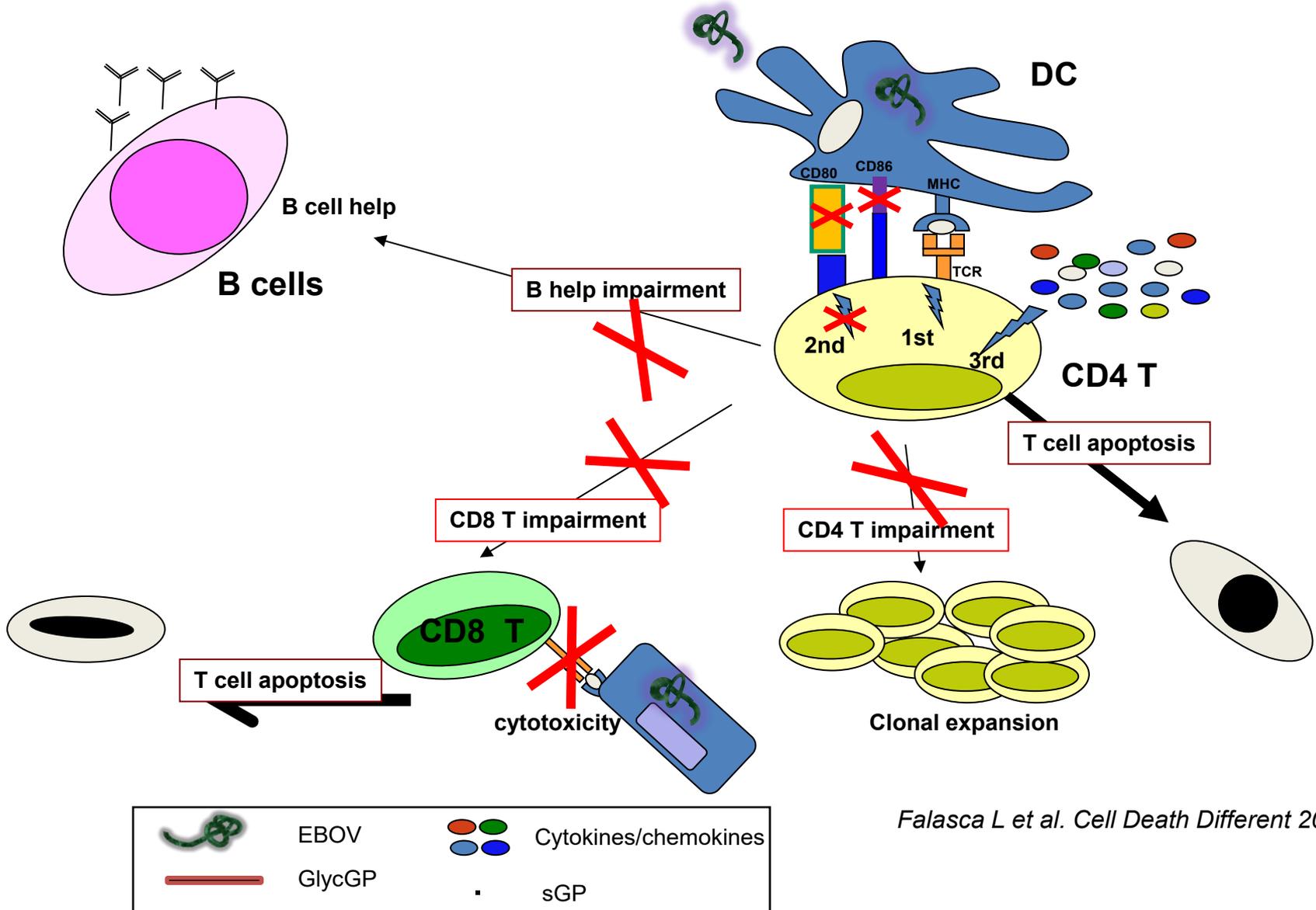
Anita K. McElroy,^{1,2} Bobbie R. Erickson,¹ Timothy D. Flietstra,¹ Pierre E. Rollin,¹ Stuart T. Nichol,¹ Jonathan S. Towner,¹ and Christina F. Spiropoulou¹



DEATH and HEMORRHAGE
Were associated with elevated levels of chemokines and cytokines

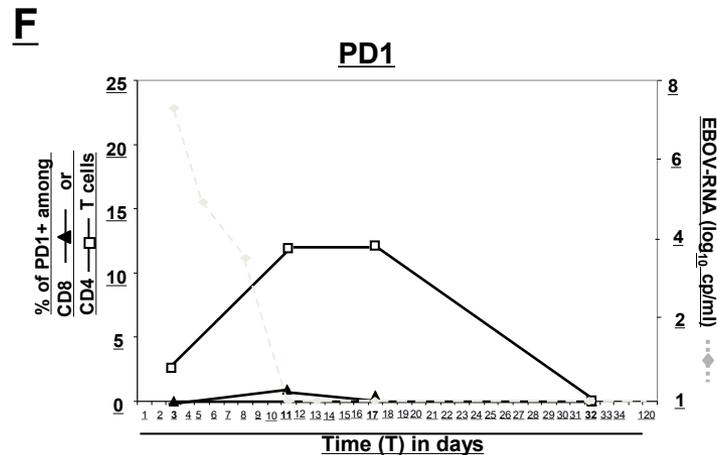
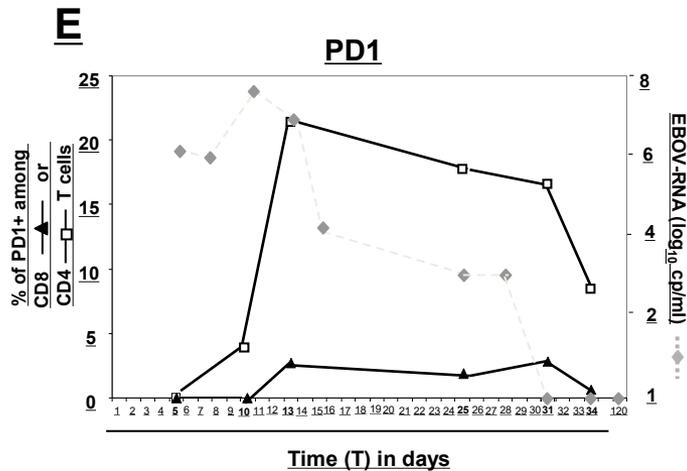
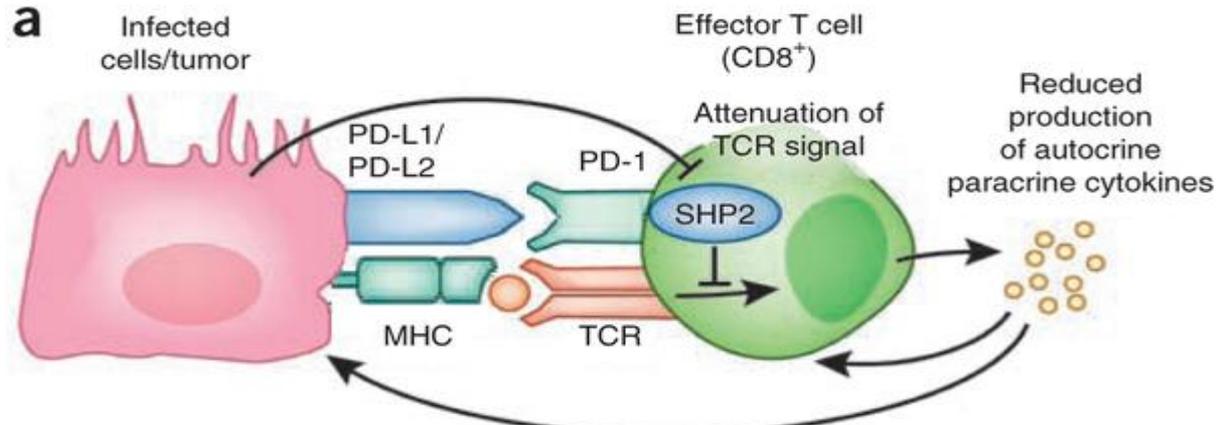
Figure 2. Cytokines and chemokines associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). * $P \leq .05$. IL-1 α , interleukin 1 α ; IL-1RA, interleukin 1 receptor antagonist; IL-6, interleukin 6; IP-10, interferon γ -inducible protein 10; MCP-1, monocyte chemoattractant protein 1; MCSF, macrophage colony-stimulating factor; sCD40L, soluble CD40 ligand; SE, standard error.

Immunità Adattativa: blocco della risposta



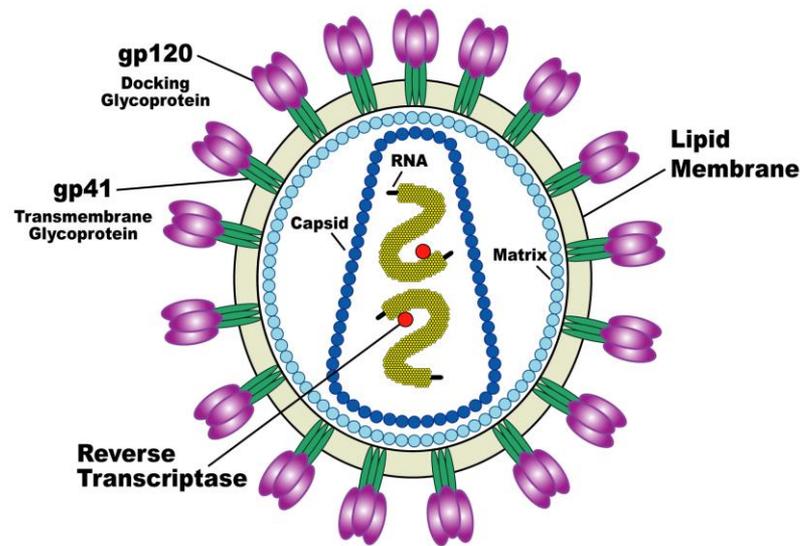
Falasca L et al. Cell Death Different 2015

PD-1 expression may be involved in T cell anergy



Quando la risposta immune fallisce

Infezione cronica: HIV



HIV & AIDS

Human **I**mmunodeficiency **V**irus

H = Human

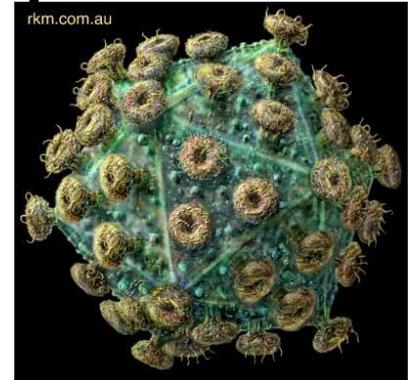
I = Immunodeficiency

V = Virus



HIV & AIDS

Acquired Immuno Deficiency Syndrome



A = Acquisita, non primitiva

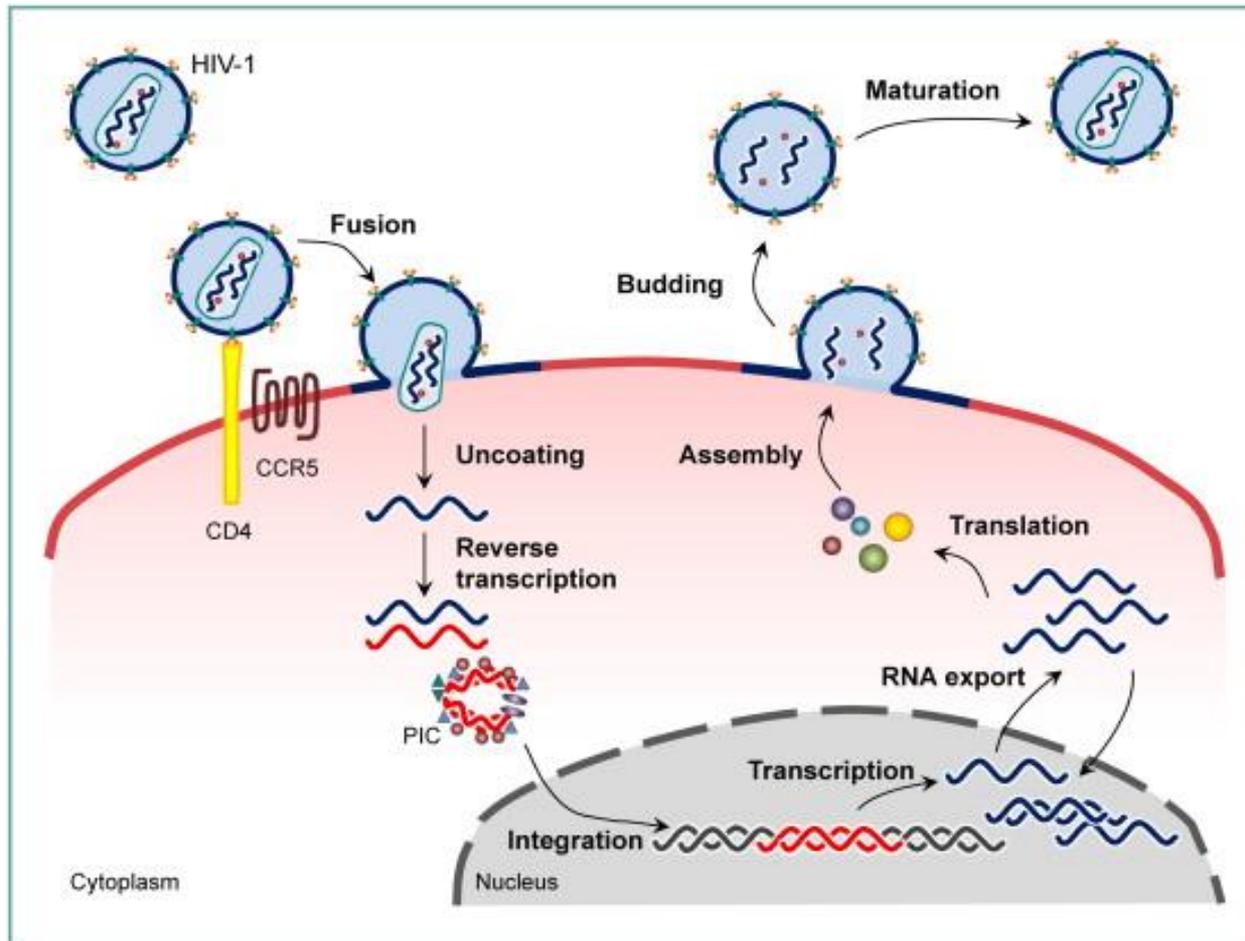
I = Indebolisce il sistema Immunitario

D = Deficienza di alcune cellule del sistema immunitario

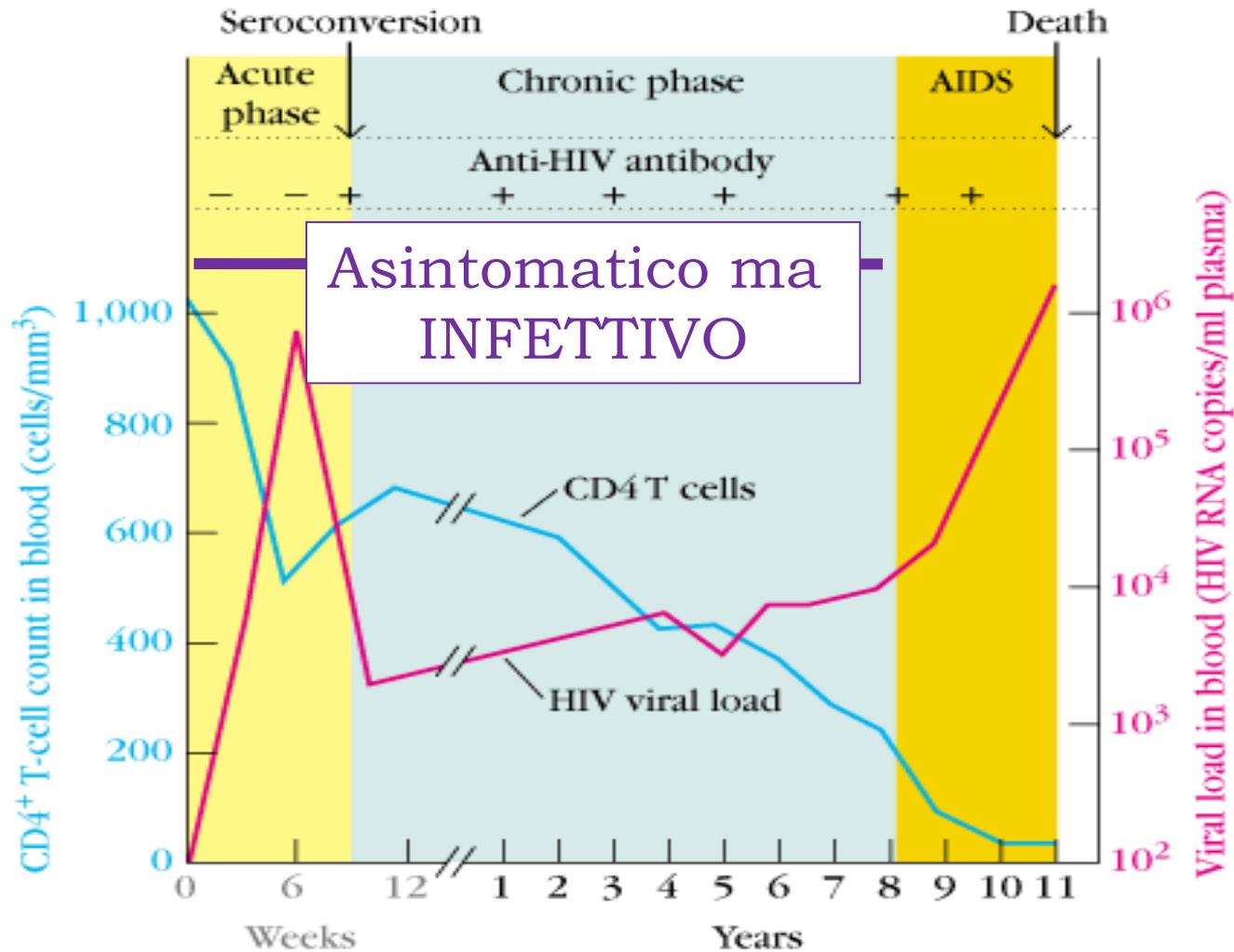
S = Sindrome, un gruppo di sintomi e differenti malattie che caratterizzano l'AIDS



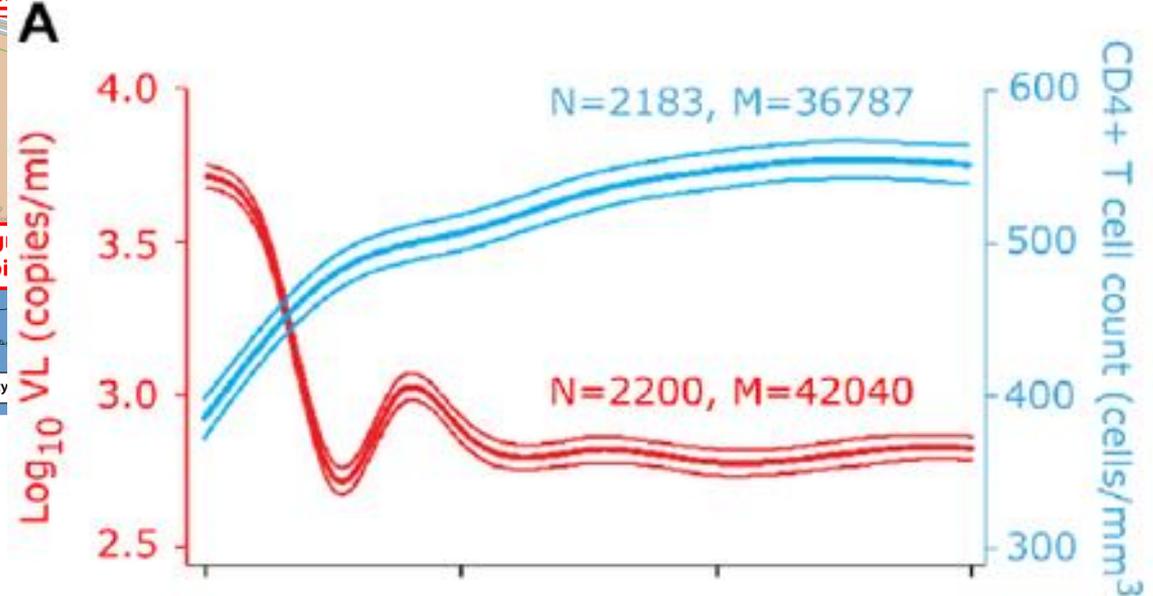
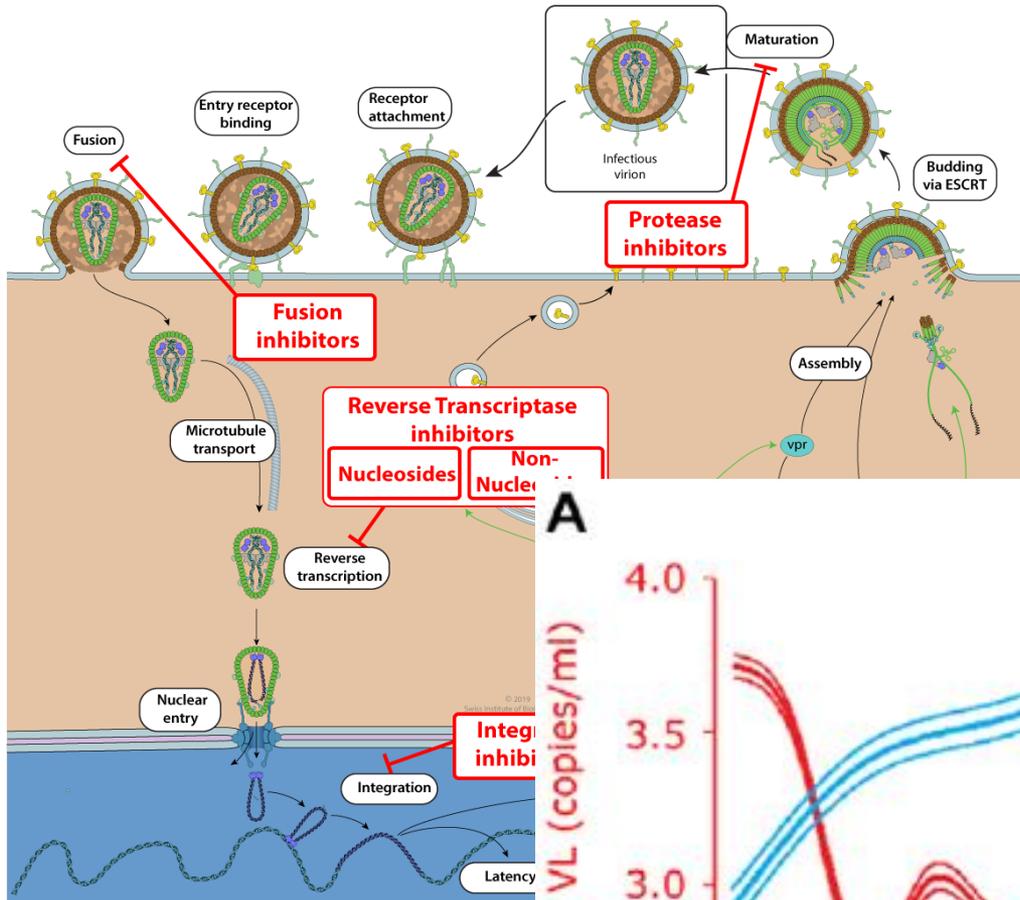
HIV infetta i linfociti T CD4



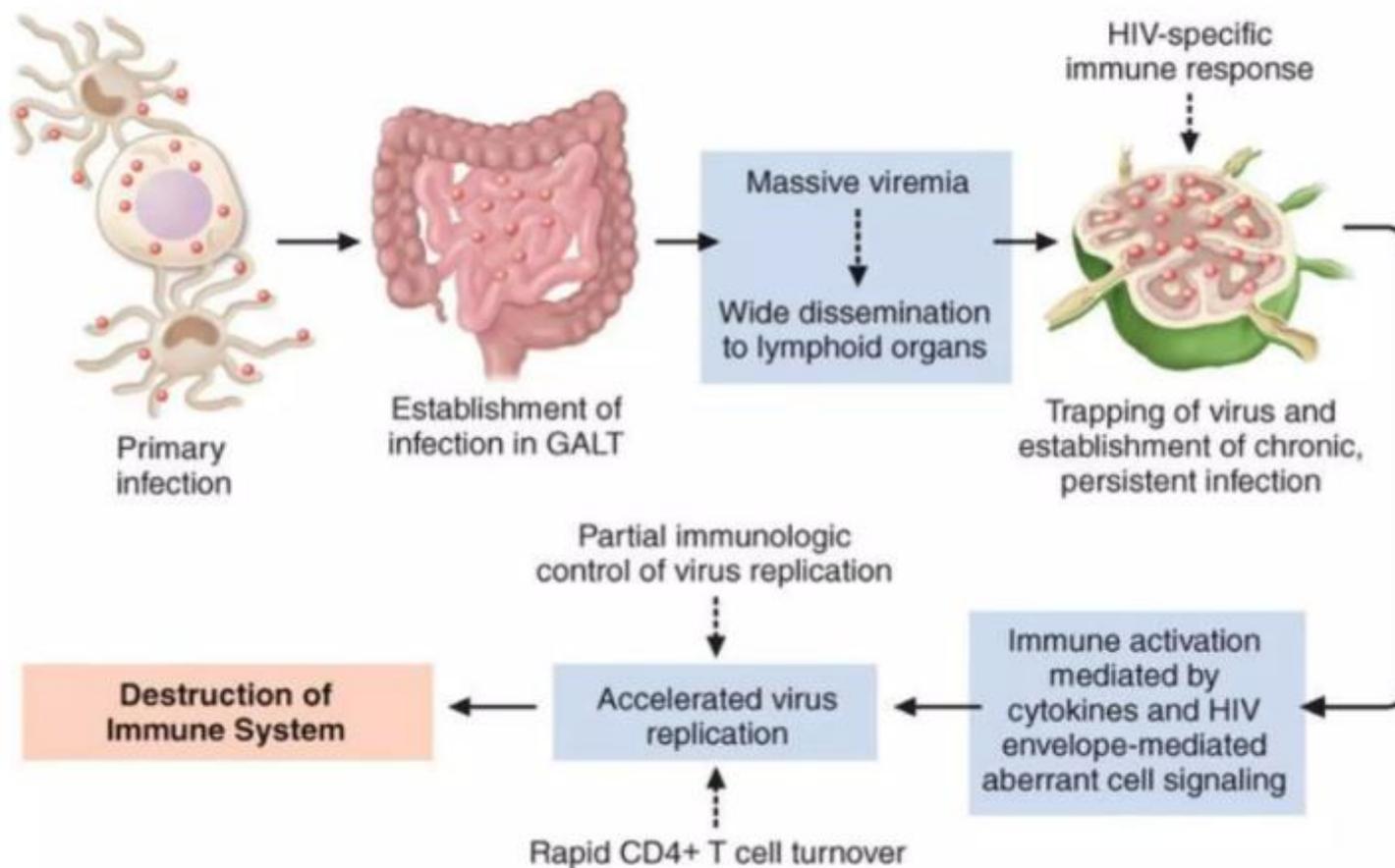
Storia naturale dell'infezione



La terapia antiretrovirale



Events that happen in an HIV infection



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*; www.accessmedicine.com

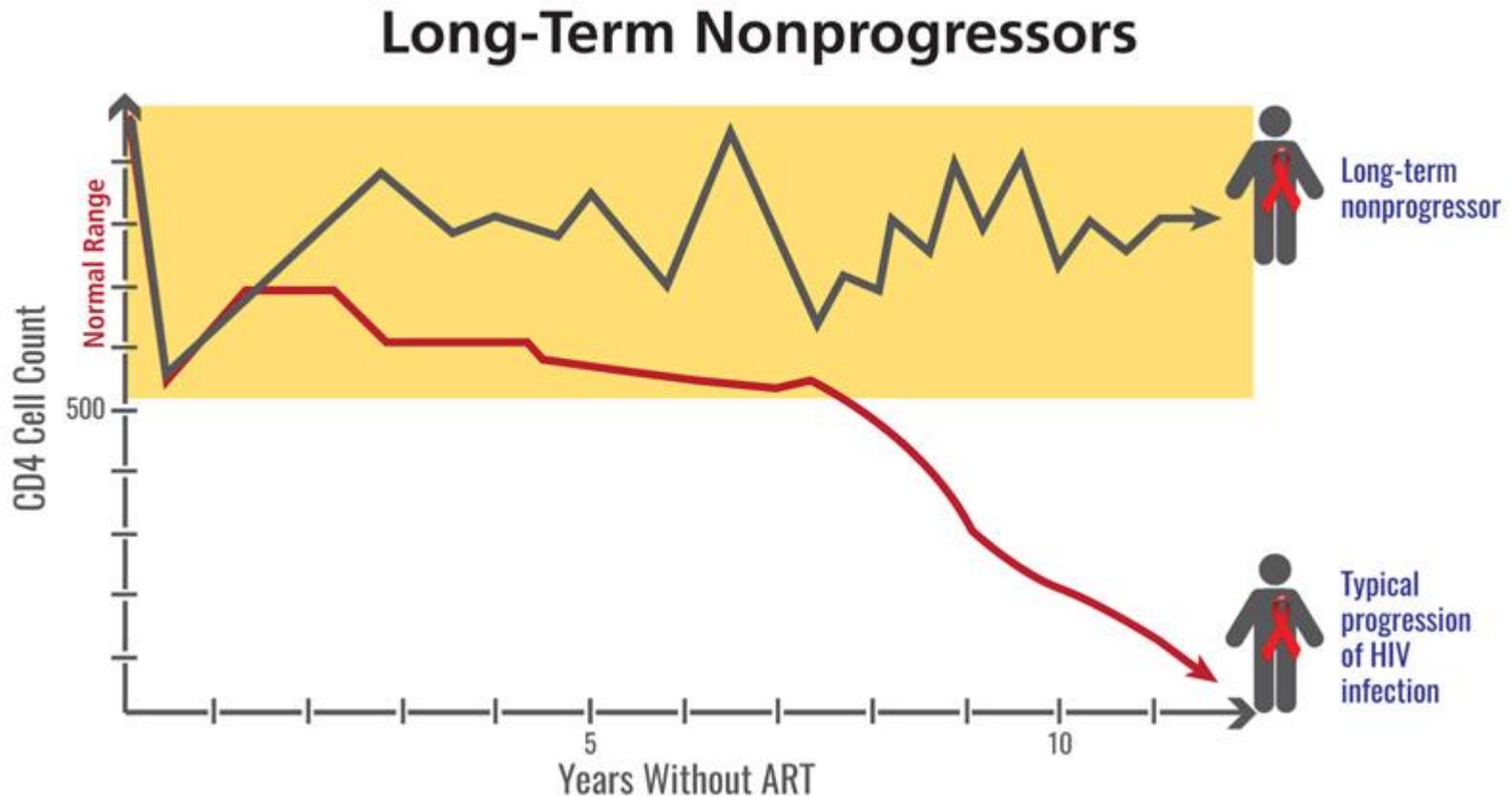
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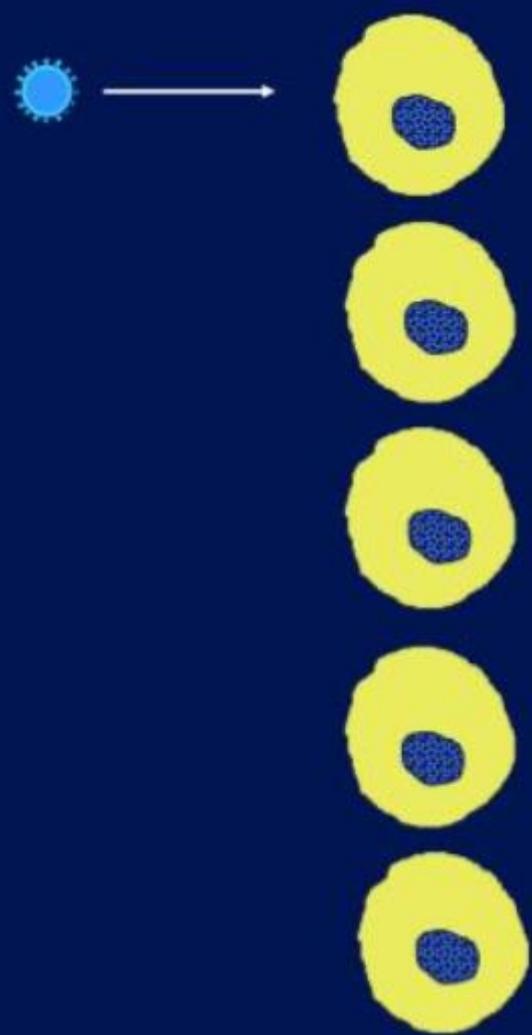
The level of HIV in the blood stream predicts subsequent survival



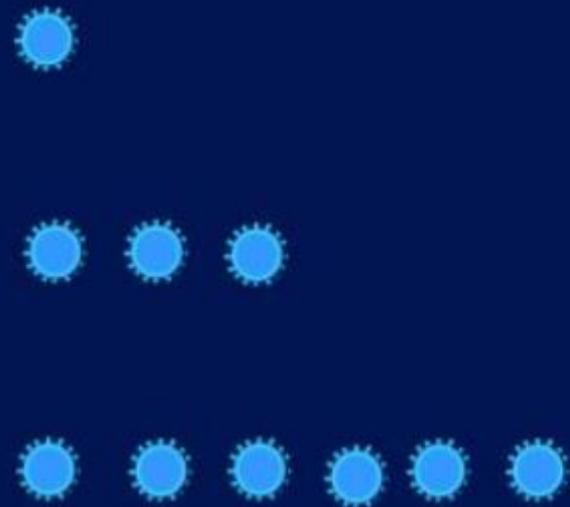
Long term non Progressors

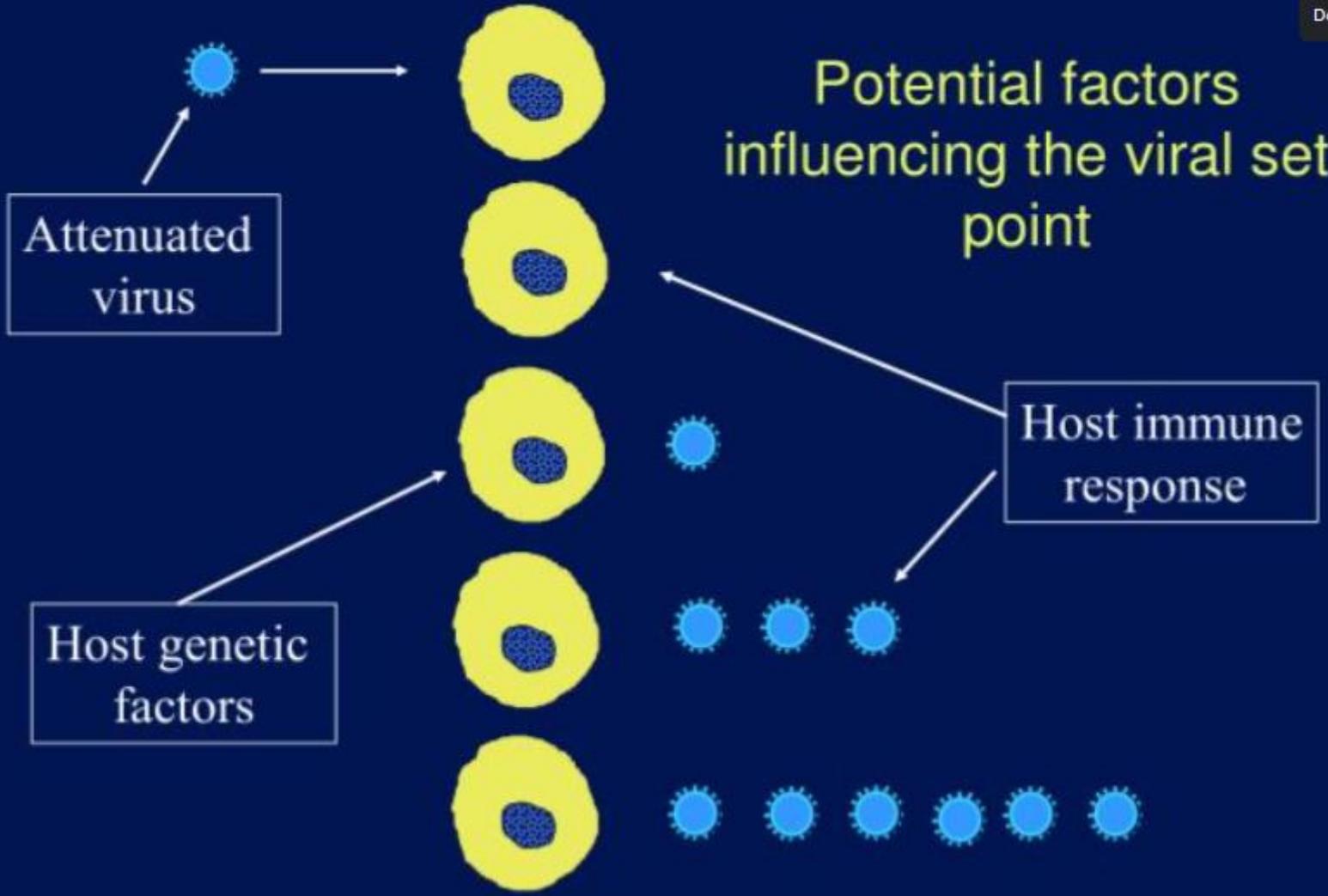
I *long-term non progressor* sono individui infettati da HIV infection che, pur non ricevendo terapia antiretrovirale, mantengono per molti anni una funzione immunitaria stabile e non sviluppano la progressione verso AIDS.

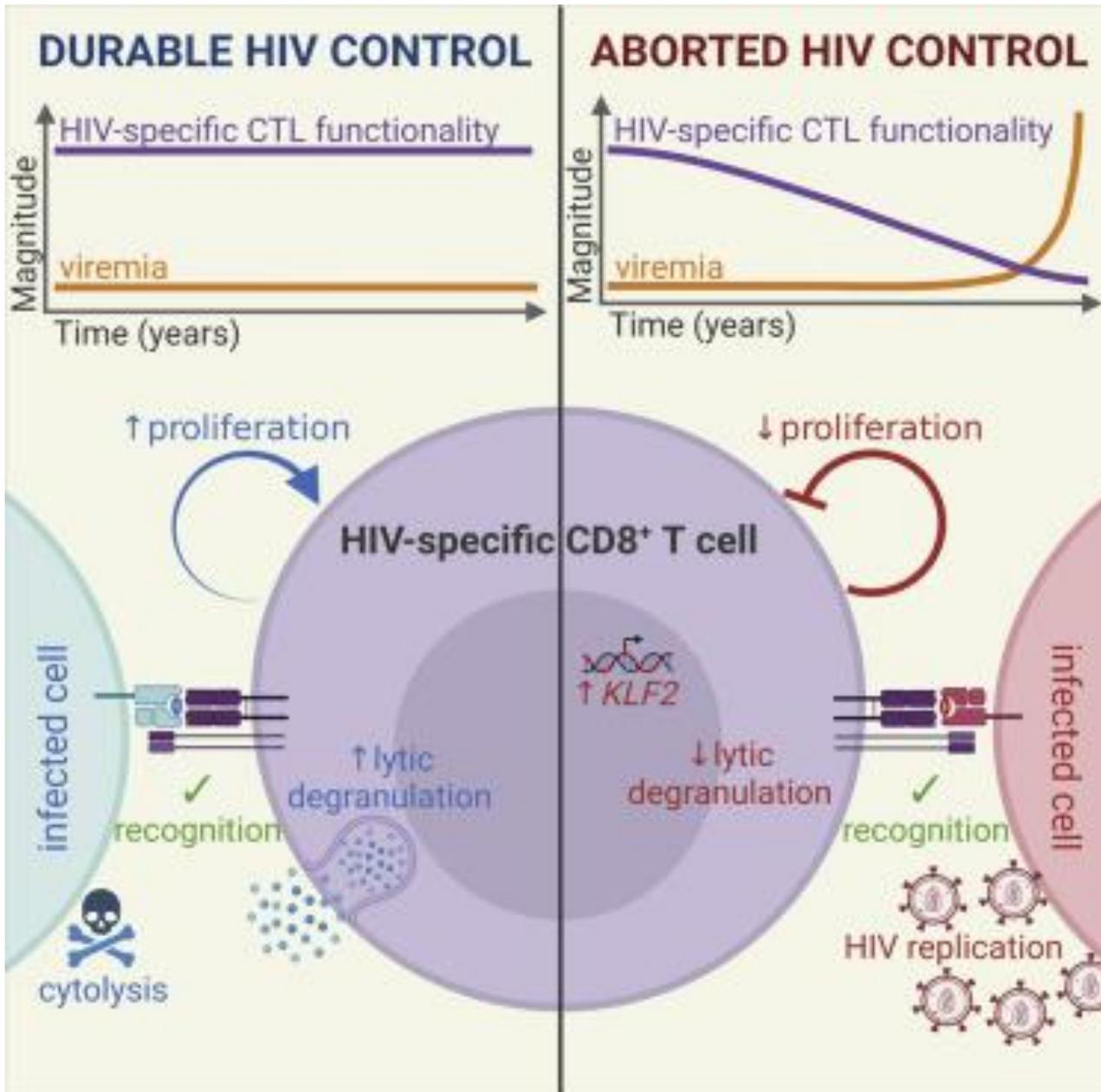




Viral set point is determined by number of viruses produced by infected cells

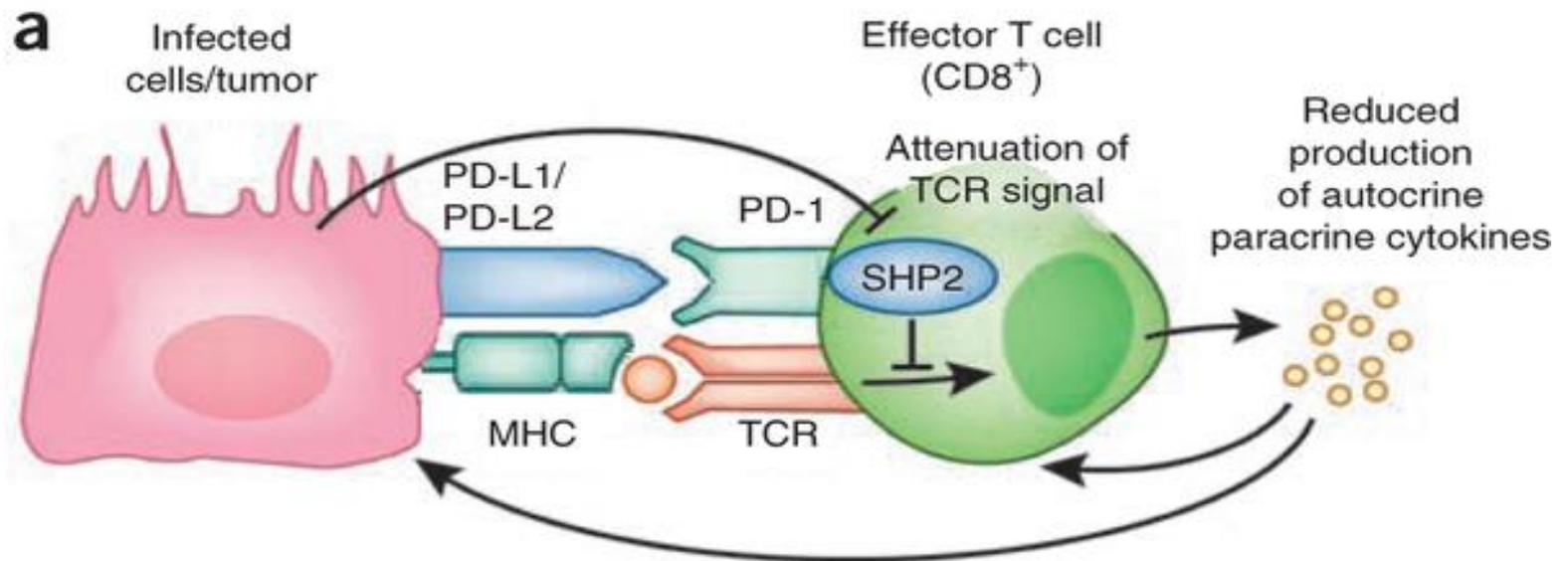


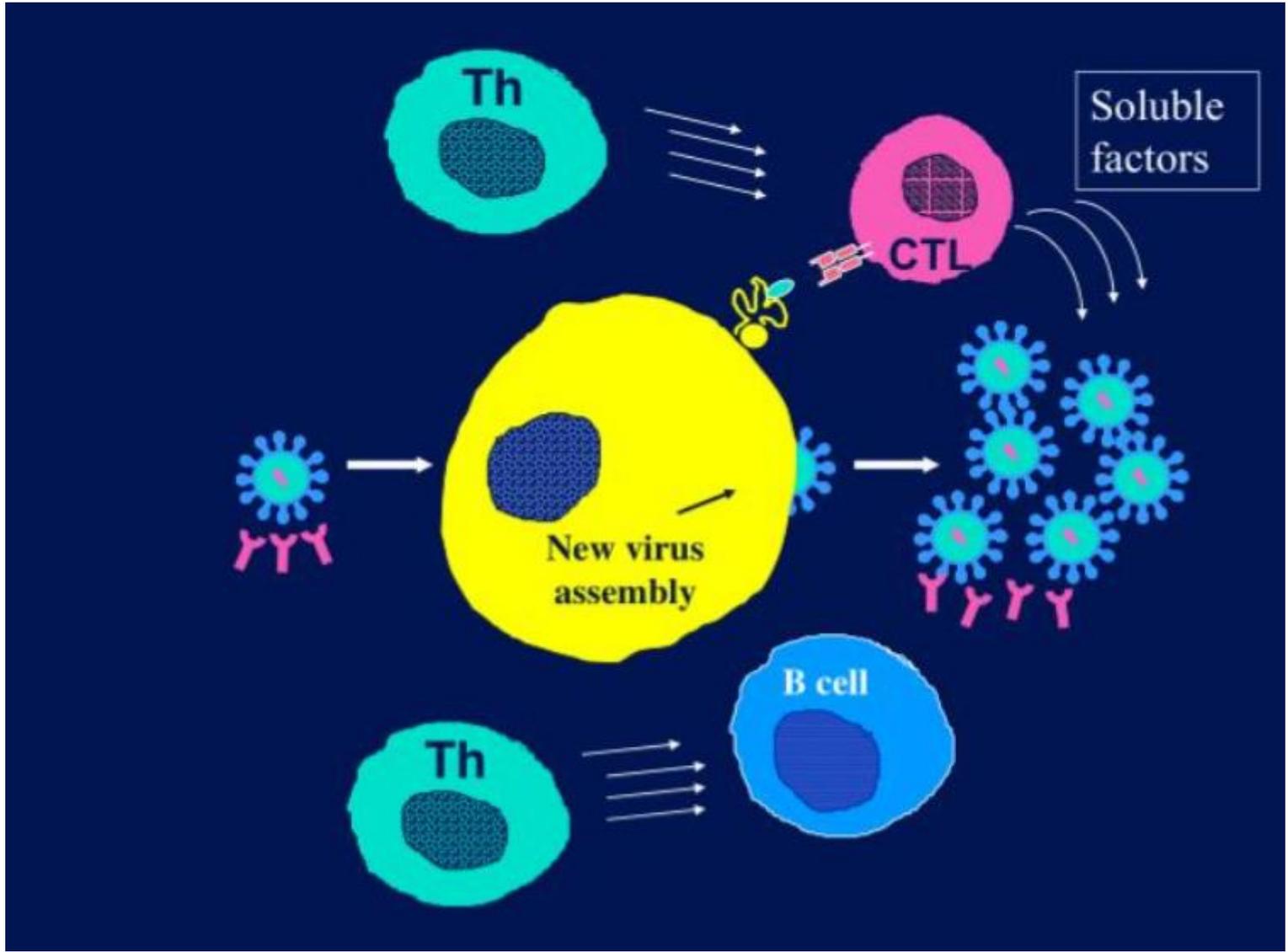




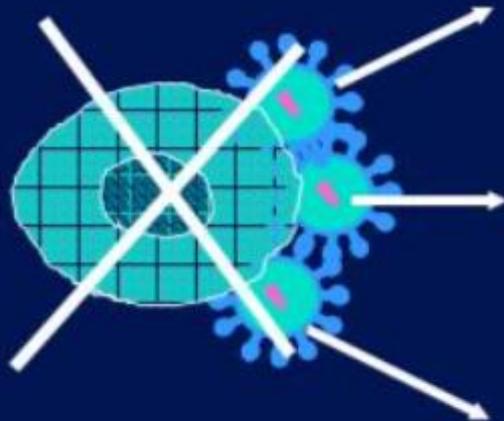
Esaurimento funzionale: PD-1

Con il progredire dell'infezione, però, l'esposizione antigenica persistente porta a exhaustion dei linfociti T, con riduzione della funzione effettrice e aumento dell'espressione di recettori inibitori (es. PD-1).

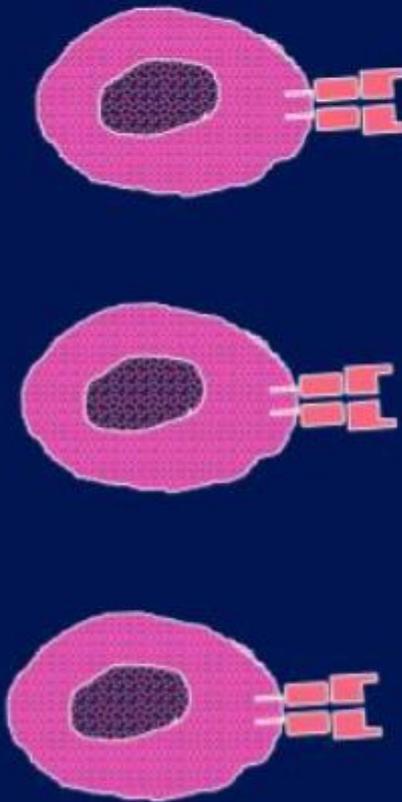




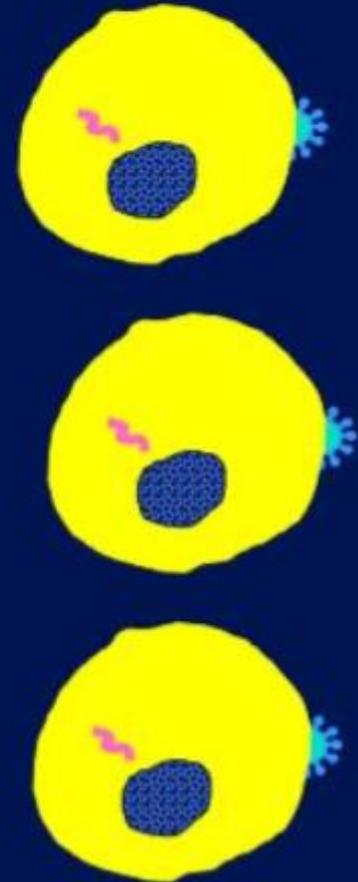
Generals
(T Helper cells)



Infantry
(CTL)

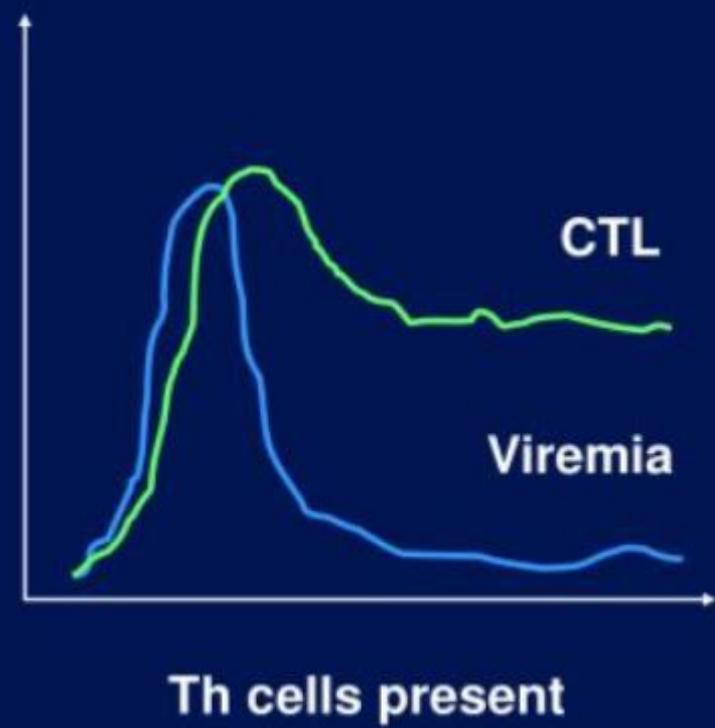
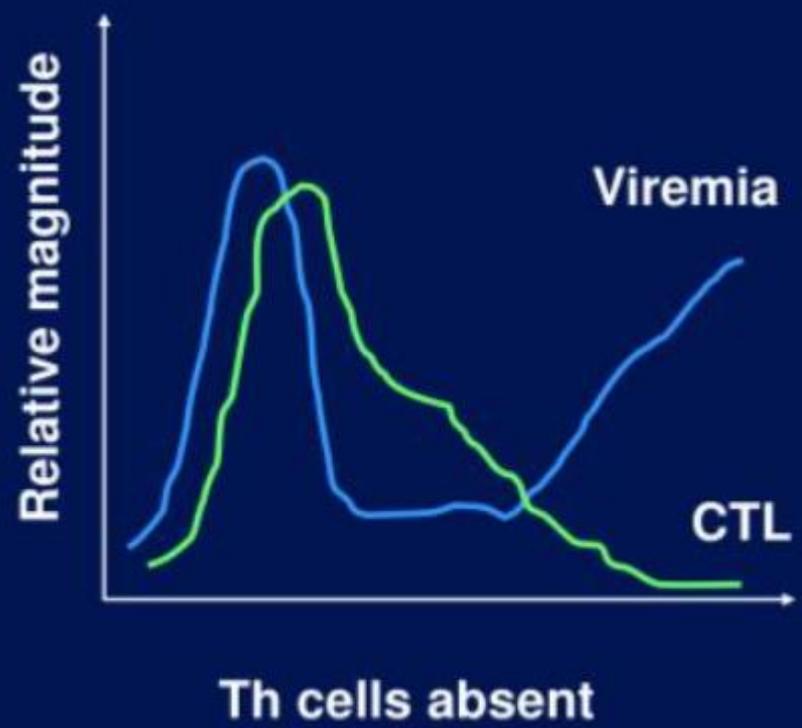


Enemy
Infected cell





Virus-Specific T Helper Cells: Essential for Maintenance of Effective CTL



**Why is HIV not controlled by
the immune system like
other chronic viral
infections?**

Mono

Chicken pox

Herpes simplex

The extreme variability of HIV over time is a major impediment to immune control, effective drug therapy and vaccine development

Viral Escape

La **trascrittasi inversa dell'HIV** è altamente imprecisa e introduce circa:

~1 errore ogni $10^4 - 10^5$ nucleotidi copiati

Circa 1 mutazione per genoma virale a ogni ciclo replicativo

- Parallelamente, l'elevata variabilità genetica dell'HIV favorisce il fenomeno del **viral escape**, mediante mutazioni negli epitopi riconosciuti dai T che permettono al virus di sfuggire al riconoscimento immunitario.
- A ciò si aggiungono la **perdita progressiva dei CD4+** helper e la formazione di **serbatoi virali latenti**, che ostacolano ulteriormente una risposta efficace. Nel complesso, l'interazione tra polifunzionalità iniziale, evasione virale e disfunzione immunitaria determina la persistenza cronica dell'infezione.

Take home message

La risposta immune efficace è il risultato di **moltissimi eventi** che devono essere **coordinati** come gli strumenti di un orchestra. Ognuno deve suonare:

- Al momento giusto
- Con il tono giusto
- Con l'intensità giusta
- Per la durata giusta

