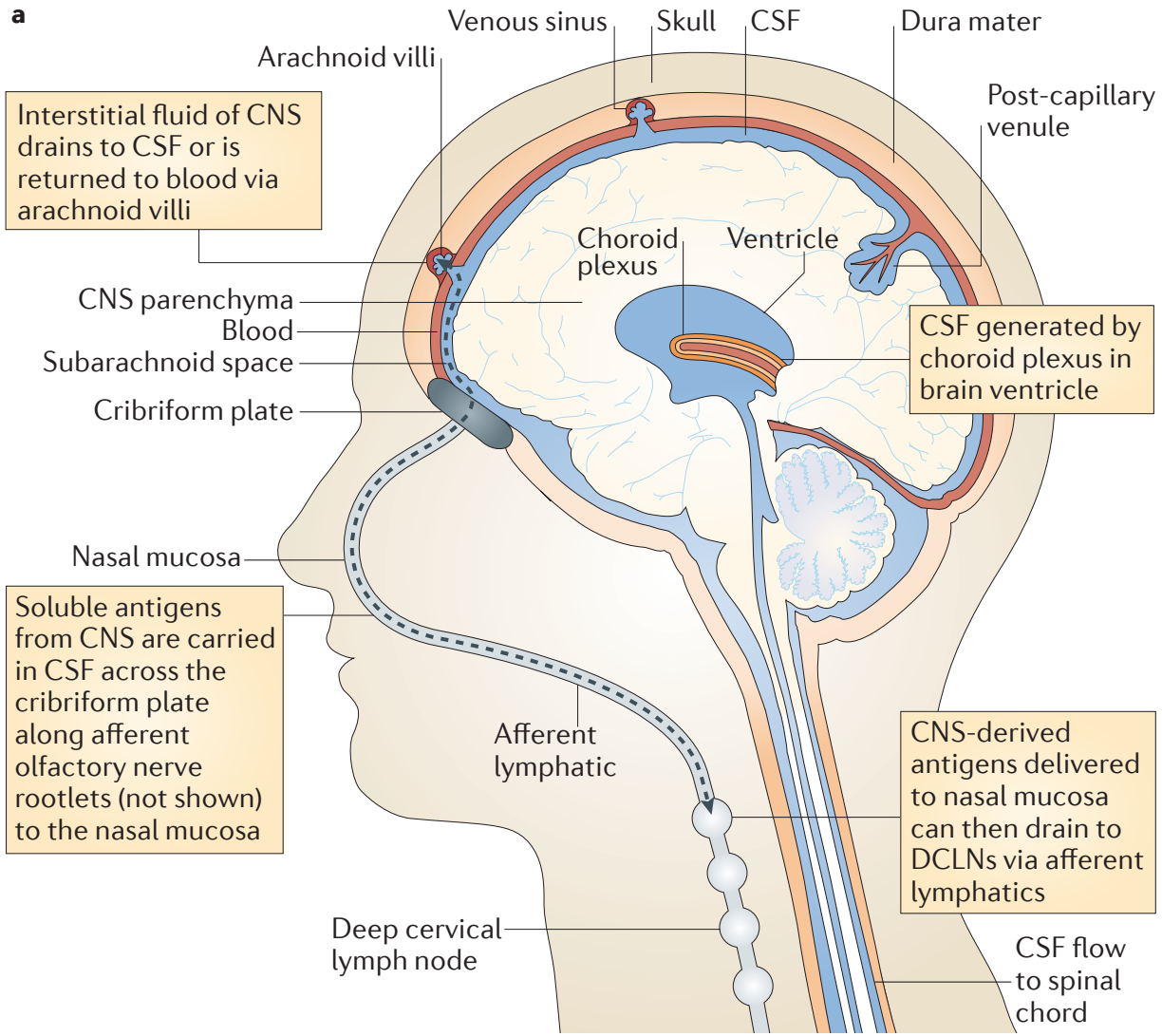
Barriers of the CNS

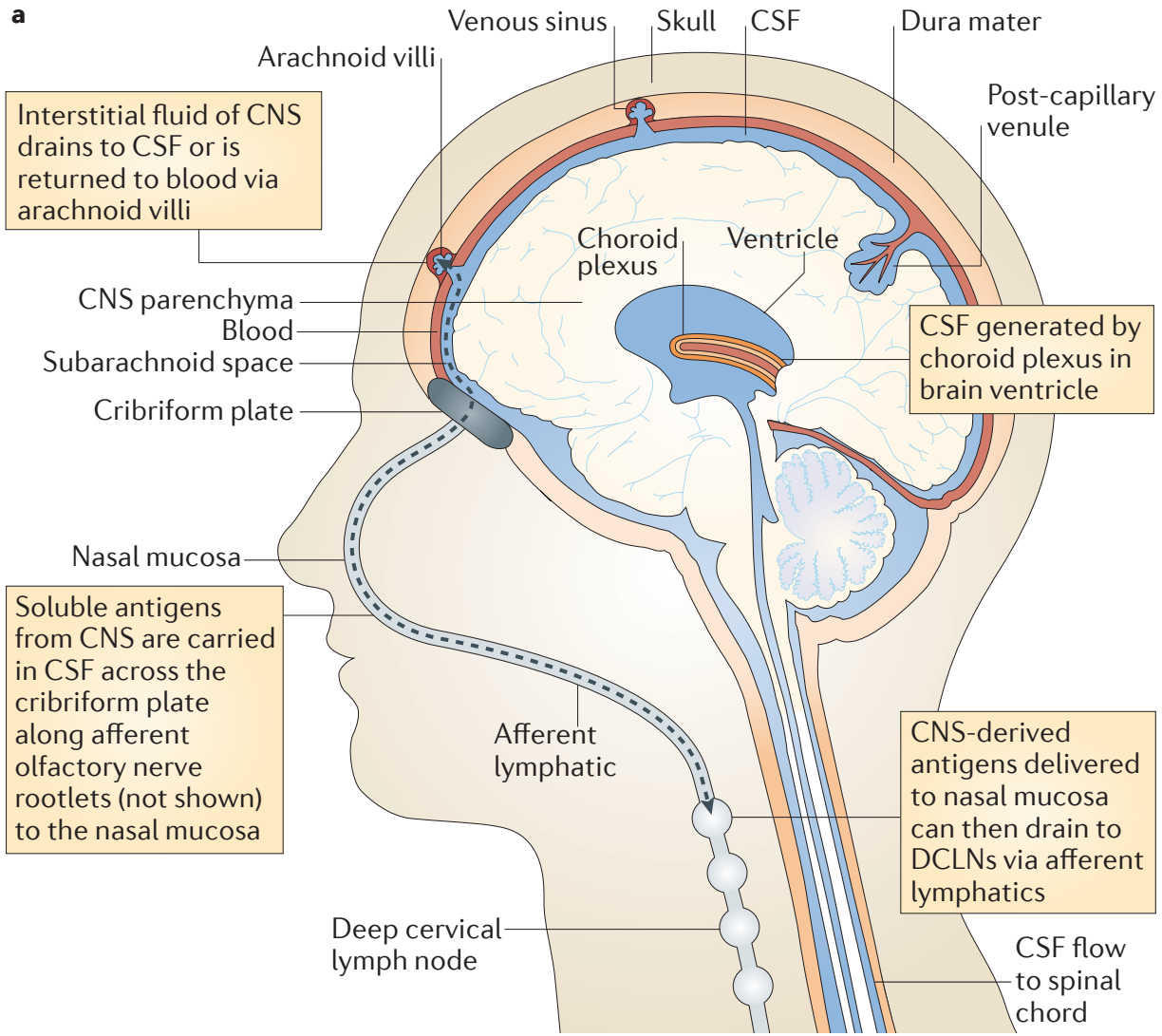
Anatomy and some definitions



The brain is a privileged site, sheltered from the systemic circulation by the Brain Barriers

Individual neurons are rarely more than 8–20 µm from a brain capillary

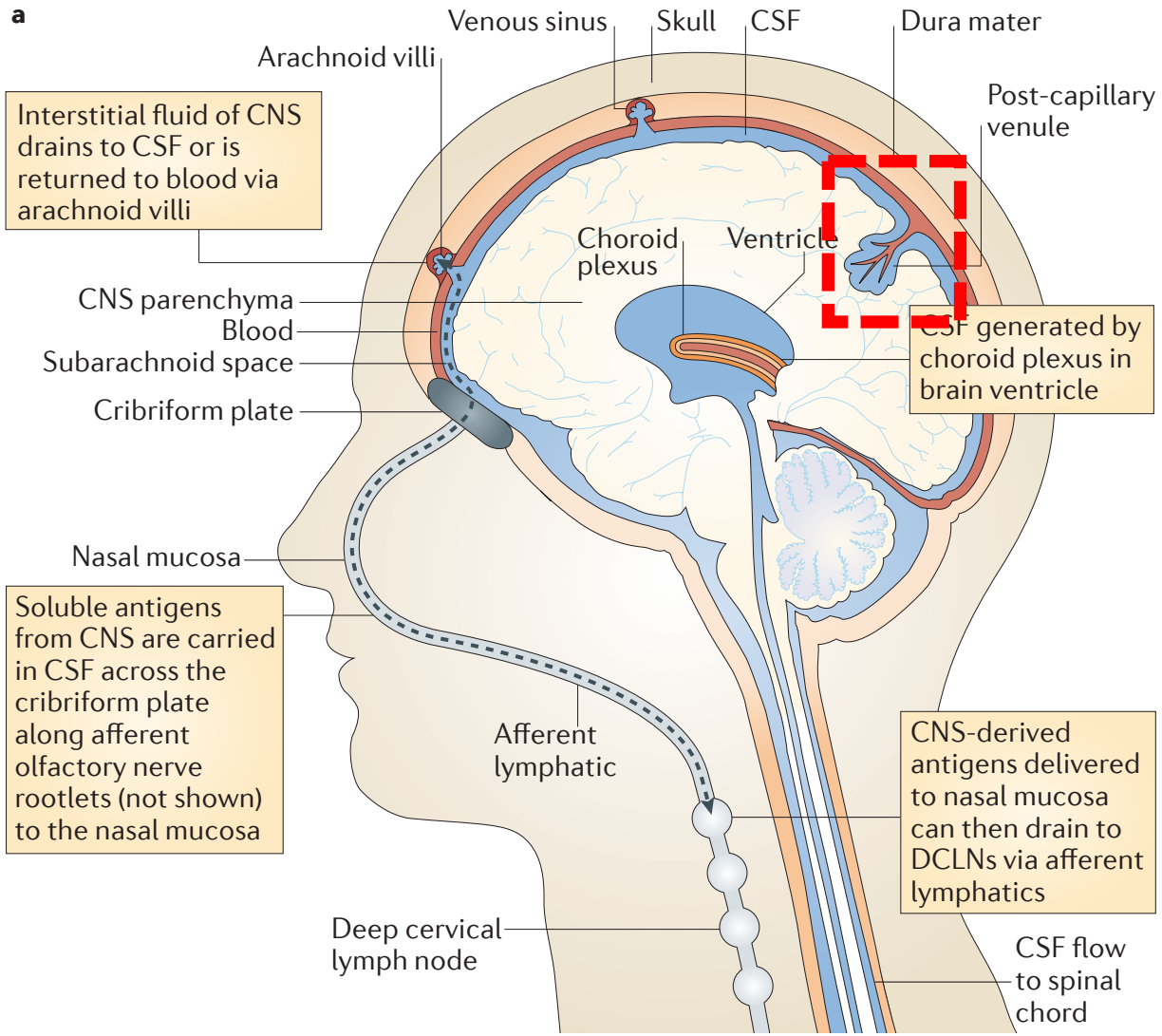
Anatomy and some definitions

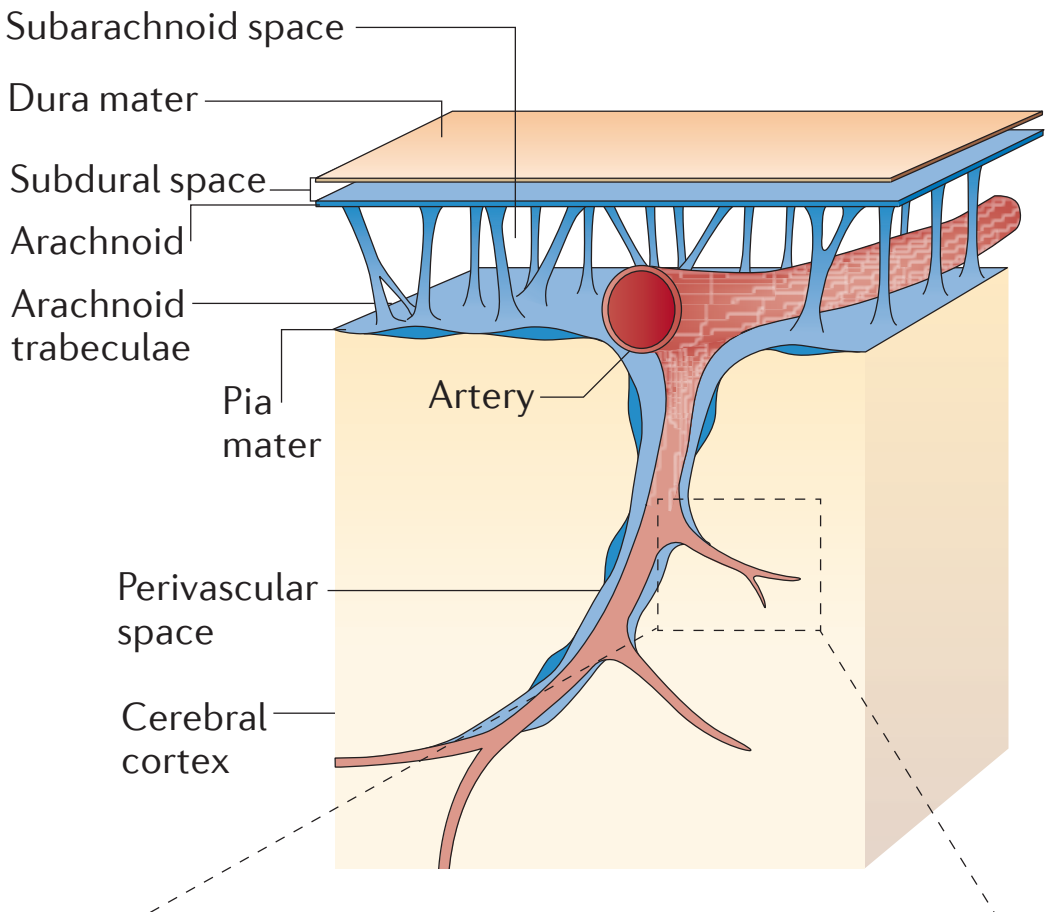


Brain barriers are composed of the:

- *endothelial blood- brain barrier (BBB)*
- *epithelial blood cerebrospinal fluid barrier (BCSFB) which protect the CNS from the changing milieu of the periphery.*

Anatomy and some definitions





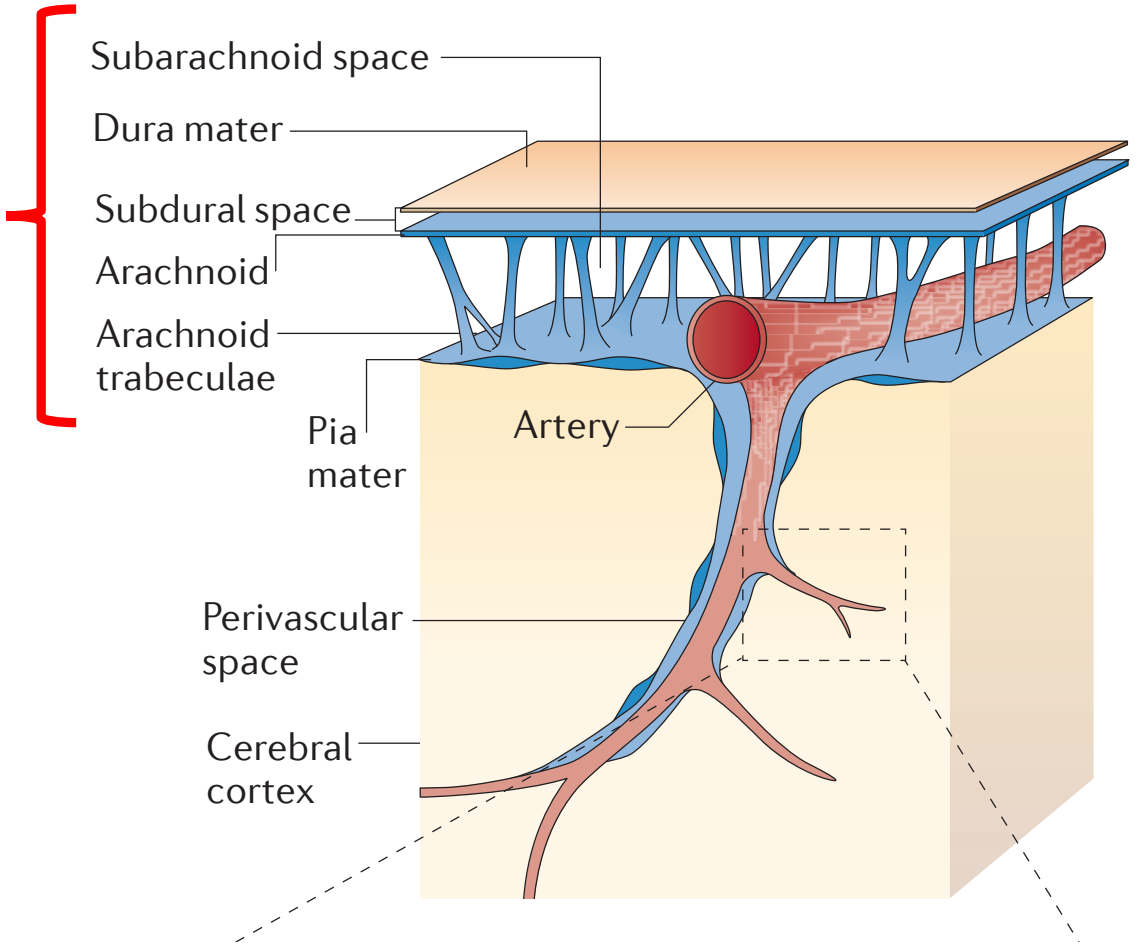
Anatomy and some definitions

Meninges: three membraned that envelope the brain and the spinal cord

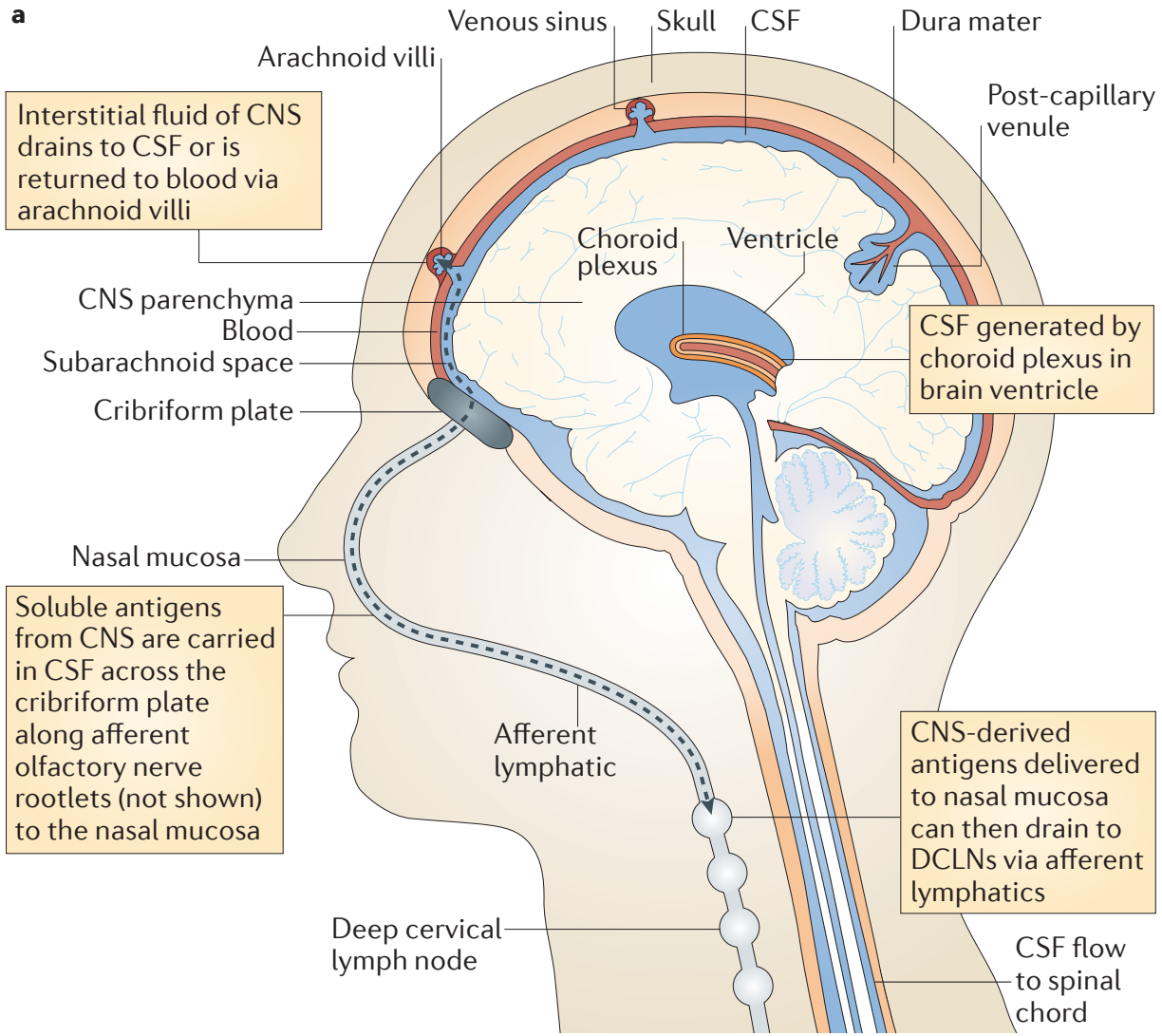
***Dura mater**: is a thick membrane made of dense connective tissue that surround the brain and the spinal cord*

Arachnoid mater is responsible for keeping in the cerebrospinal fluid

Pia mater that firmly adheres to the surface of the brain and spinal cord

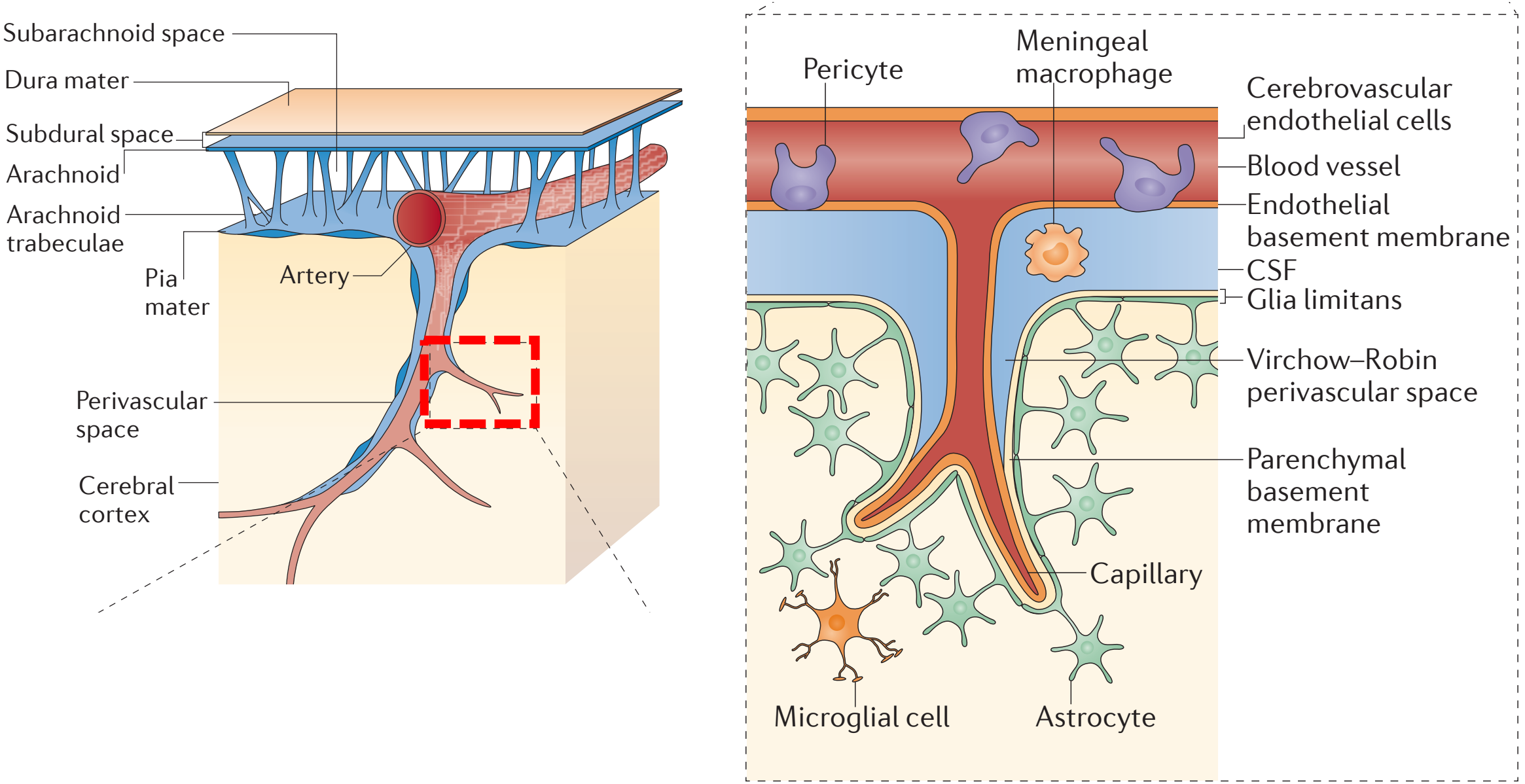


Anatomy and some definitions

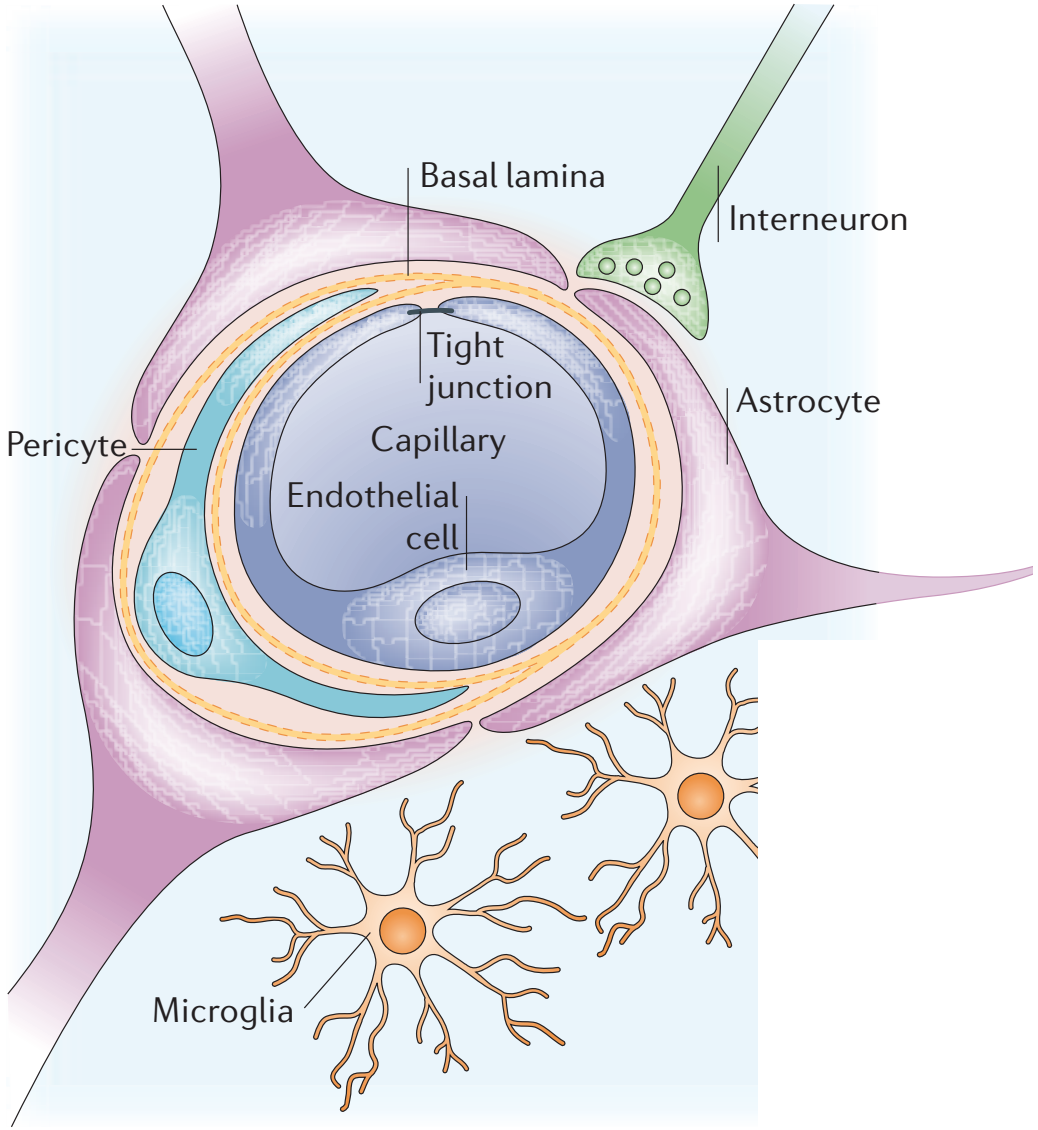


CSF: an immunologically active body fluid

The brain and spinal cord float in CSF, which provides protective padding for these delicate tissues and contributes to CNS metabolism and homeostasis.


Subarachnoid space
Dura mater
Subdural space
Arachnoid
Arachnoid trabeculae
Pia mater
Artery
Perivascular space
Cerebral cortex
Pericyte
Meningeal macrophage
Cerebrovascular endothelial cells
Blood vessel
Endothelial basement membrane
CSF
Glia limitans
Virchow–Robin perivascular space
Parenchymal basement membrane
Capillary
Microglial cell
Astrocyte

The neurovascular unit



- ***Endothelial cells***

- ***Pericytes***

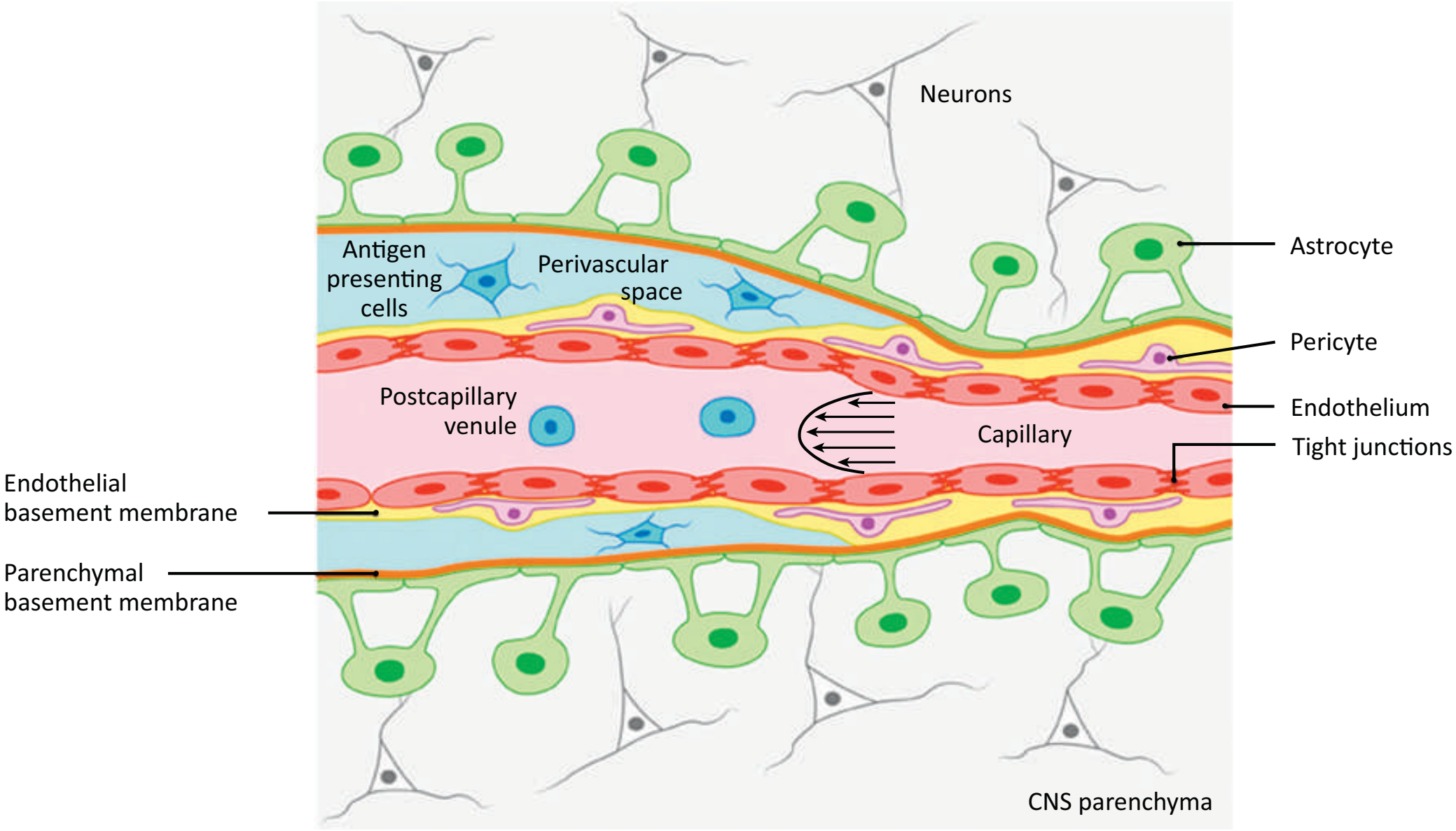
  -Regulate blood flow at the level of capillaries

  - *Induce the expression of cellular adhesion molecules in the BBB endothelium*

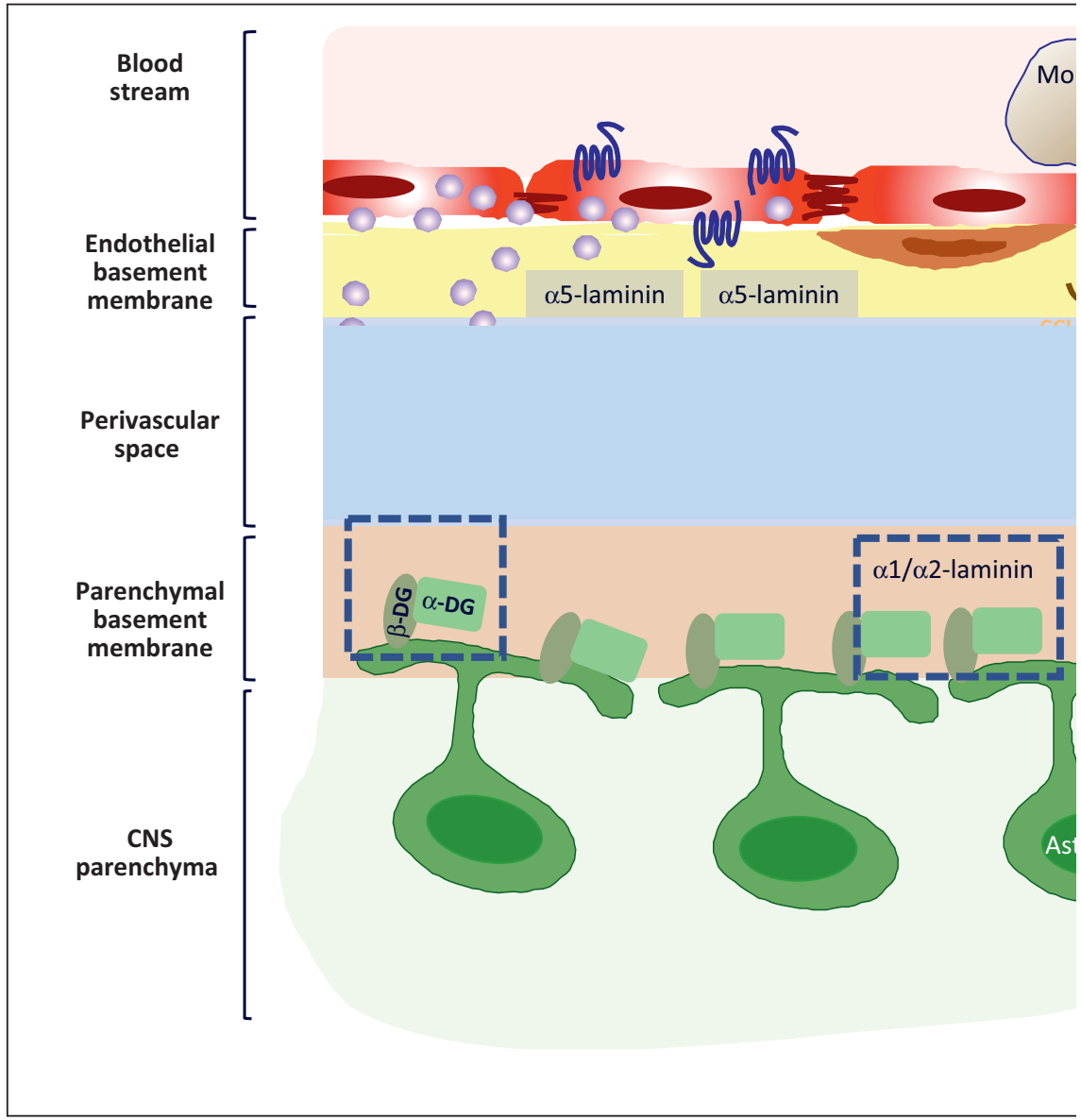
- ***Astrocytes***

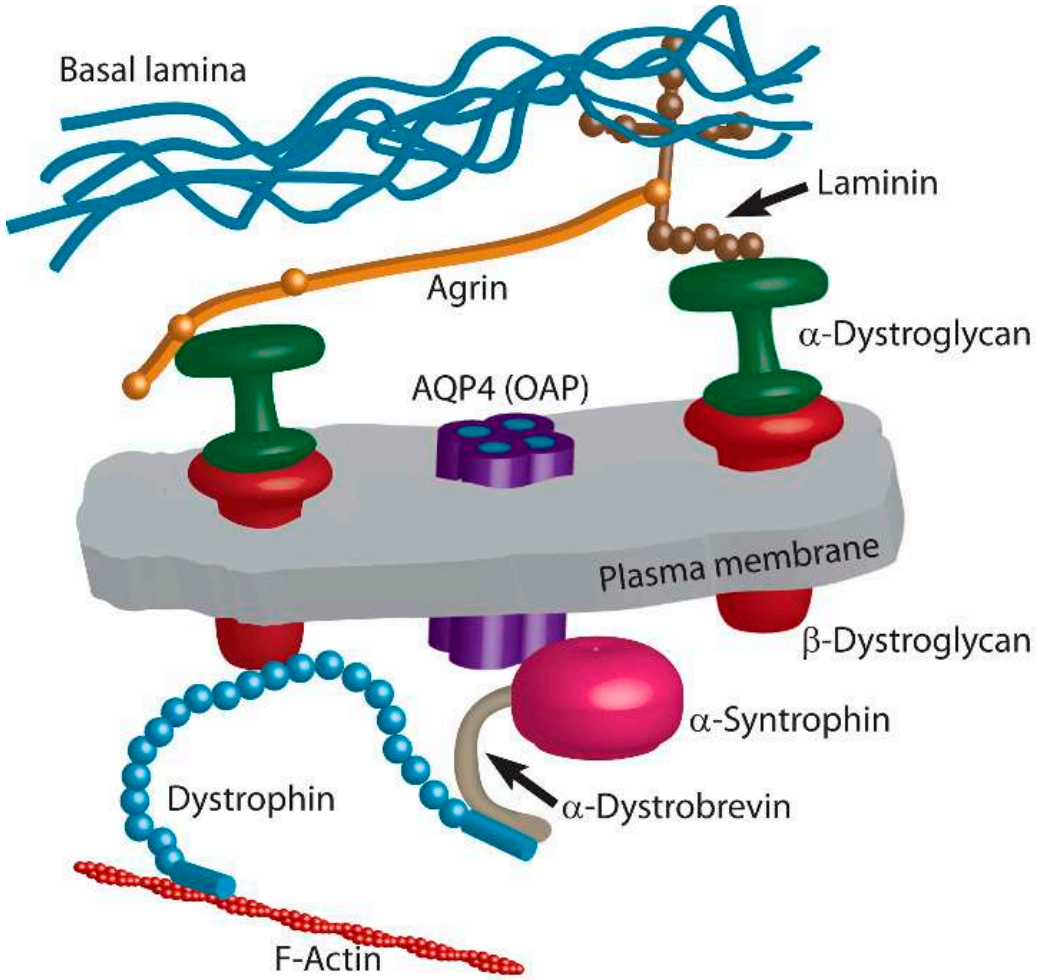
  The astrocytic endfeet and the parenchymal basement membrane form a second barrier of the CNS called glia limitans

The neurovascular unit



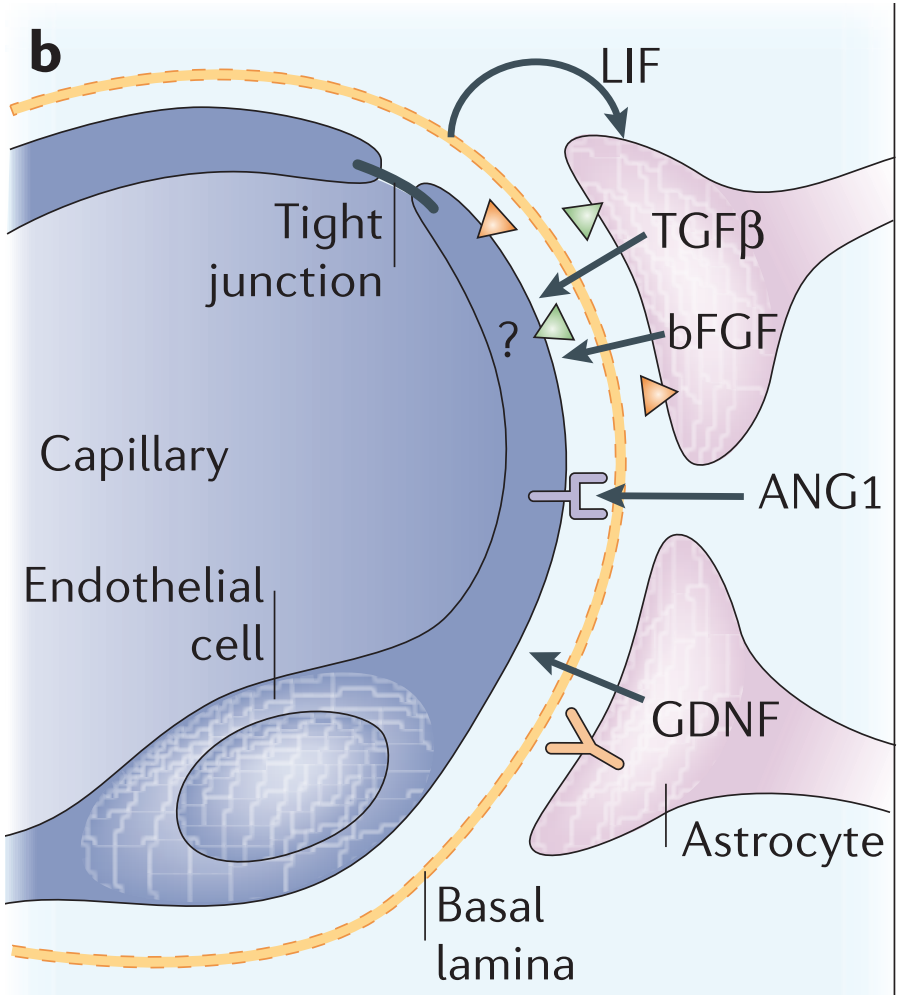
Specializations of astrocytic perivascular endfeet





AQP4, a small 30-kDa monomer, is a hydrophobic transmembrane protein with cytosolic amino and carboxy terminal ends (Verkman, 2005). The molecule spans the cell membrane 6 times, forming 5 interhelical loops

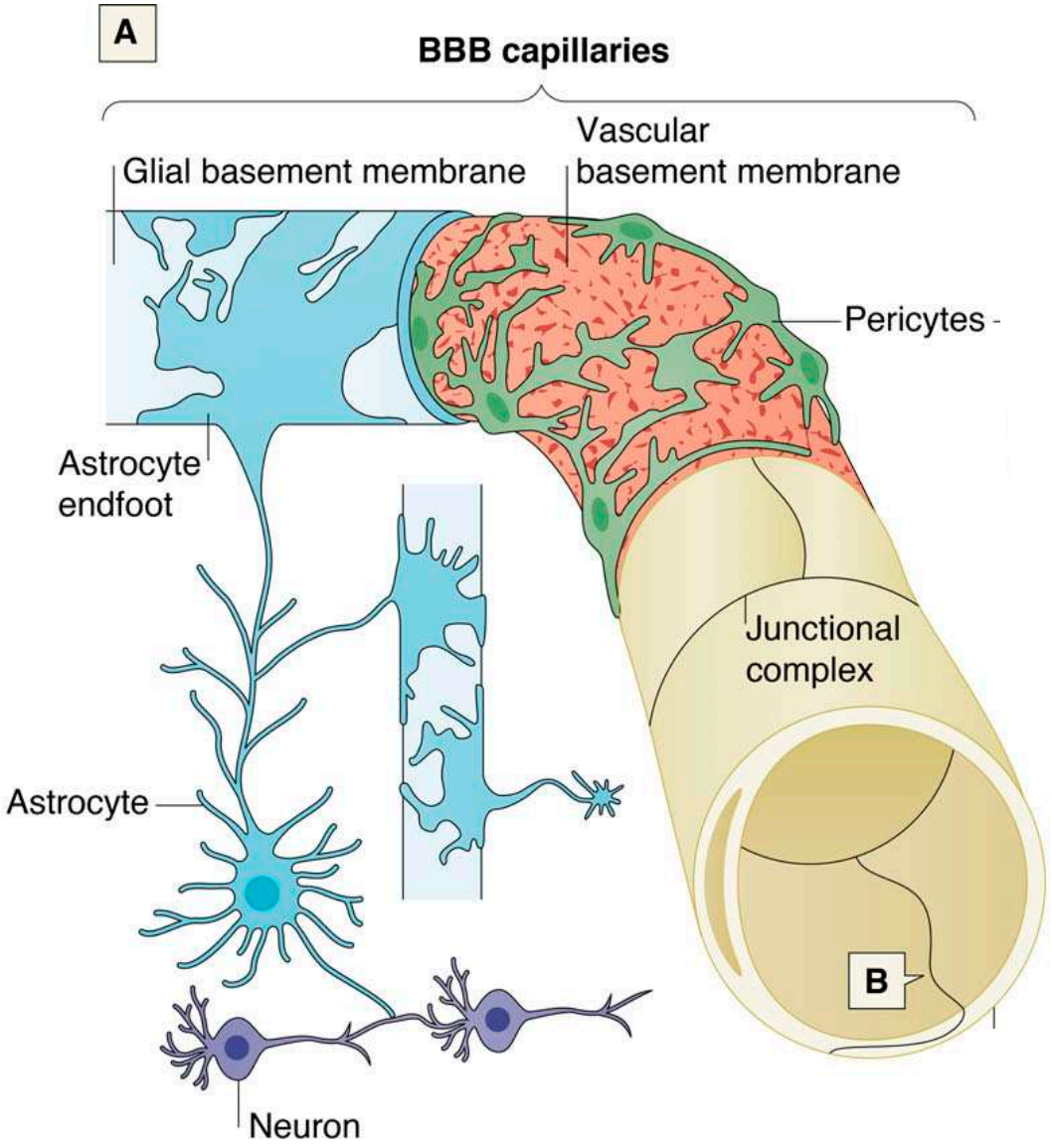
Examples of bidirectional astroglial–endothelial induction necessary to establish and maintain the BBB.



Astrocytes are able to secrete a wide range of chemical agents

Transforming growth factor-β (TGFβ)
Glial-Derived Neurotrophic Factor (GDNF)
basic Fibroblast Growth fFctor (bFGF)
angiopoetin 1 (ANG1)
acting on the endothelium-specific receptors can induce and keep the BBB phenotype.

Conversely, endothelium-derived leukaemia inhibitory factor (LIF) has been shown to induce astrocytic differentiation

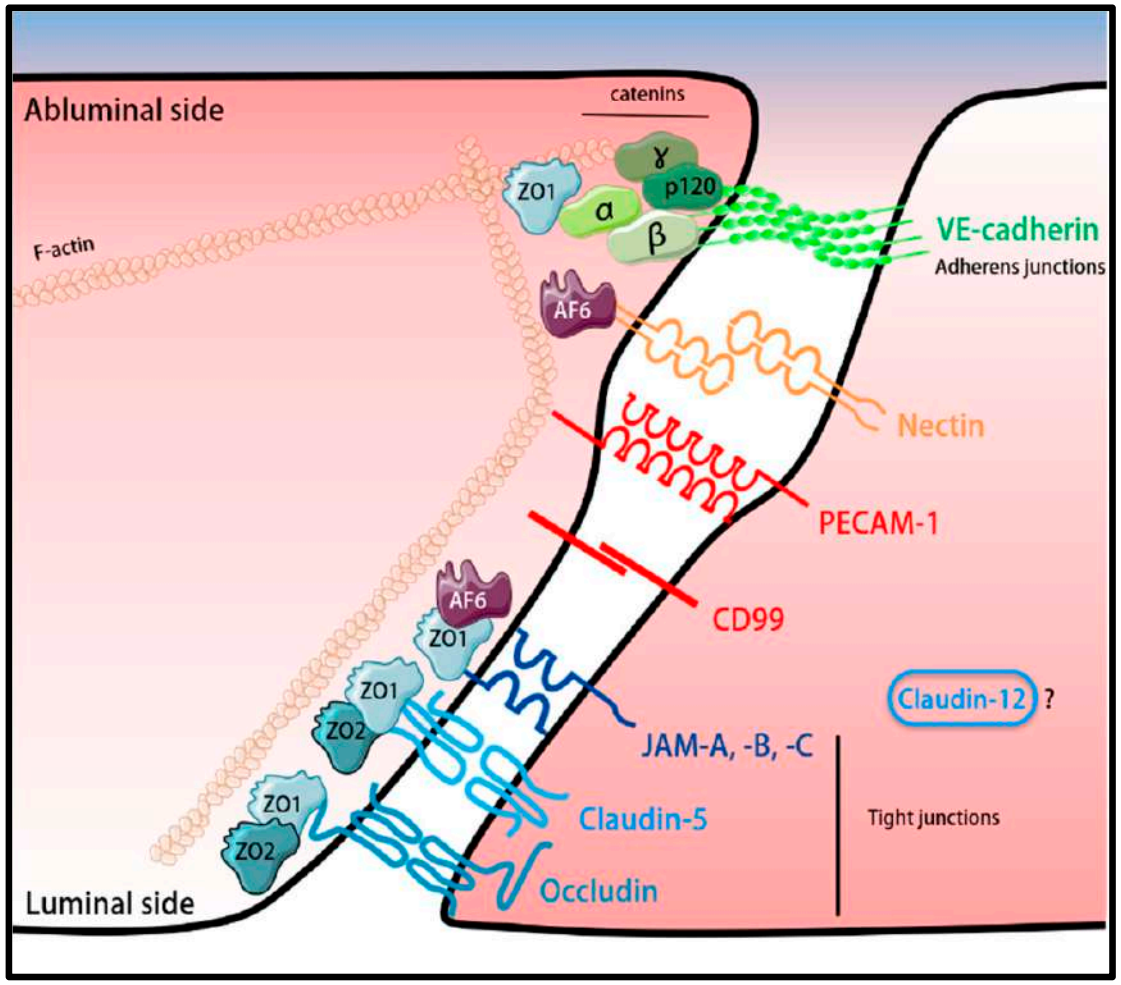


In the brain, endothelial coverage by pericytes is extremely high, with an endothelial cell/pericyte ratio between 1:1 to 3:1

Loss of pericytes impairs BBB properties of endothelial cells and loss of polarization of astrocytic endfeet

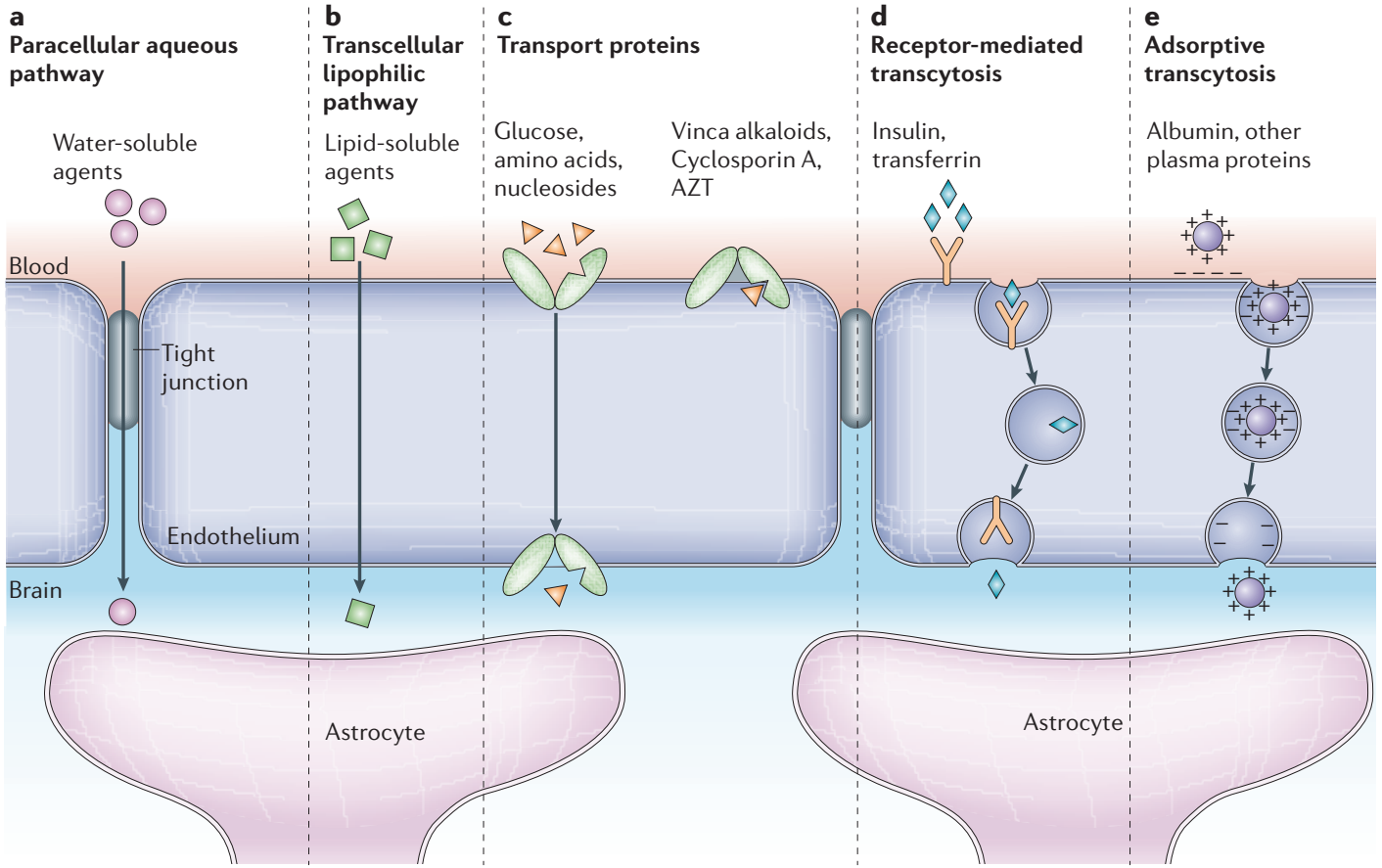
Presence of continuous and complex adherence and tight junctions that prohibit free diffusion of molecules

BBB properties at capillary and post-capillary venules
The “gate and fence” endothelial function



TJs and AJs are localized between adjacent endothelial cells are core elements actively involved in the establishment of a paracellular barrier, which limits free diffusion of ions and molecules at cell-cell junctions, adopting a “gate” function.

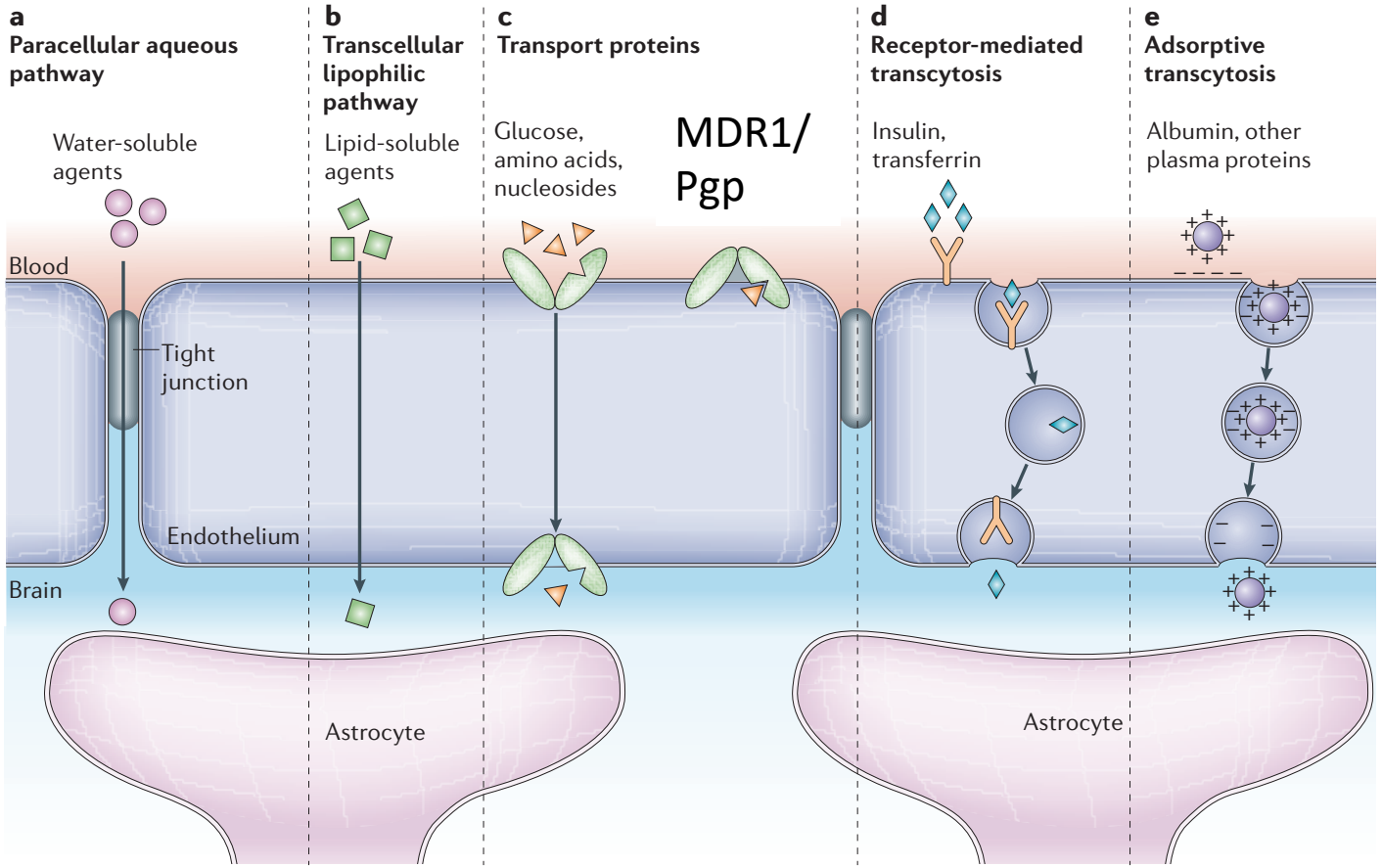
Routes of transport across the BBB



- ***The glucose transporter GLUT-1 (SLC2A1)*** *is also highly enriched in the BBB endothelium, allowing for glucose delivery to the CNS*

Only molecules with a low molecular weight (under 400–600 Da) and of positive charge can cross the BBB

Routes of transport across the BBB

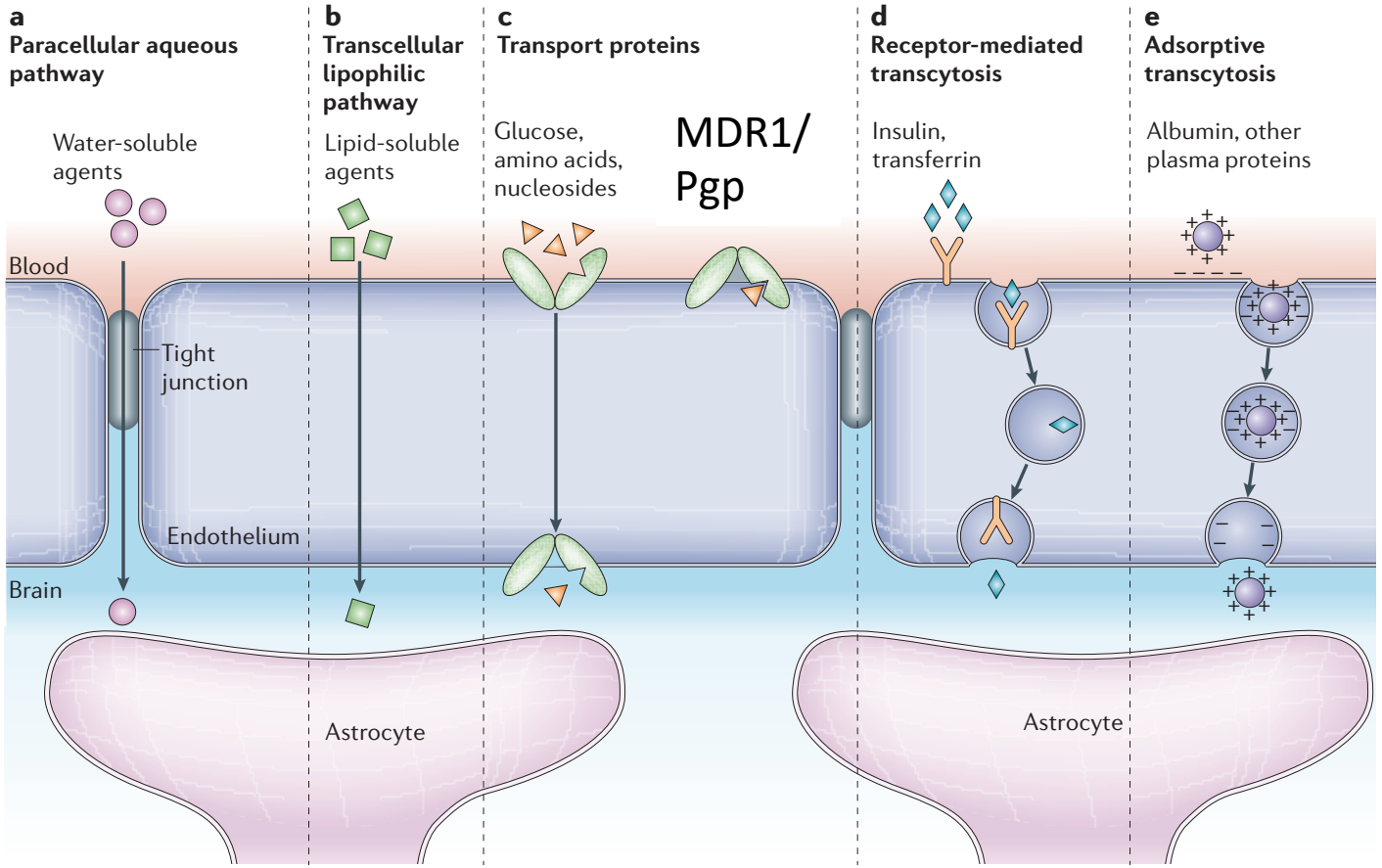


MDR1/P-glycoprotein (Pgp)

- *Some transporters are energy-dependent (for example, P-glycoprotein) and act as efflux transporters.*
- *Translocates potentially harmful lipophilic or endogenous molecules from the CNS to the blood*

Only molecules with a low molecular weight (under 400–600 Da) and of positive charge can cross the BBB

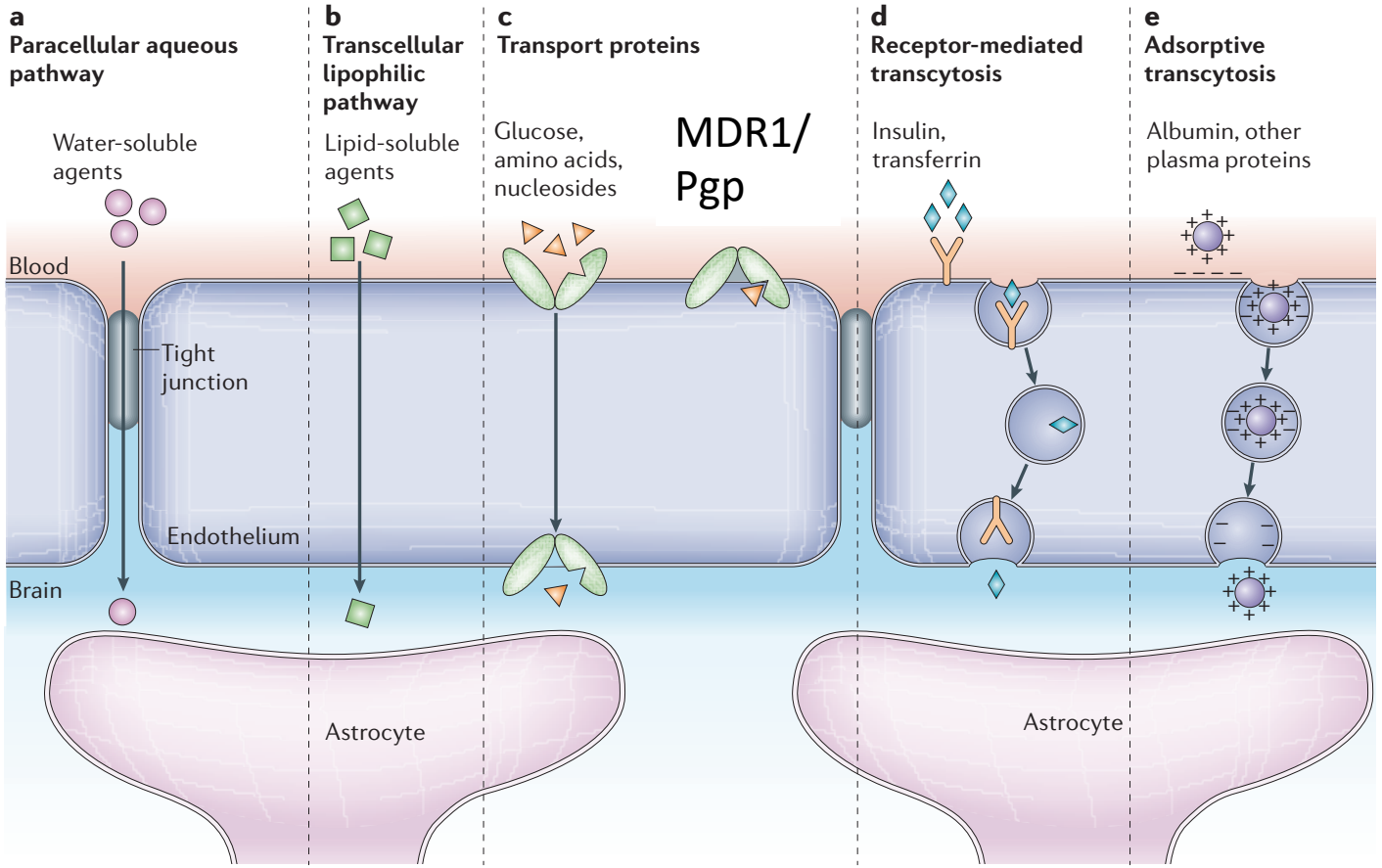
Routes of transport across the BBB



Certain proteins, such as insulin and transferrin, are taken up by specific receptor-mediated endocytosis and transcytosis

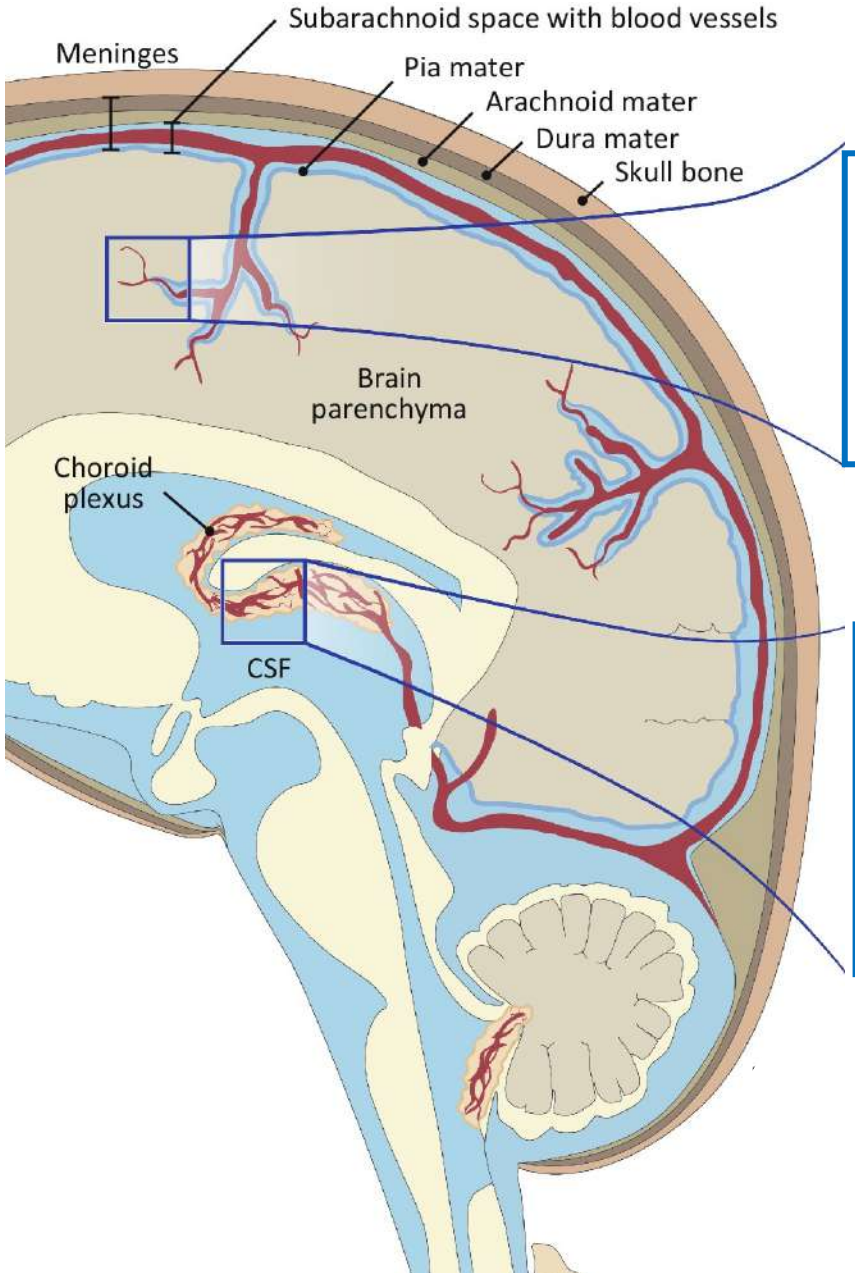
Only molecules with a low molecular weight (under 400–600 Da) and of positive charge can cross the BBB

Routes of transport across the BBB



Native plasma proteins such as albumin are poorly transported, but cationization can increase their uptake by adsorptive-mediated endocytosis and transcytosis.

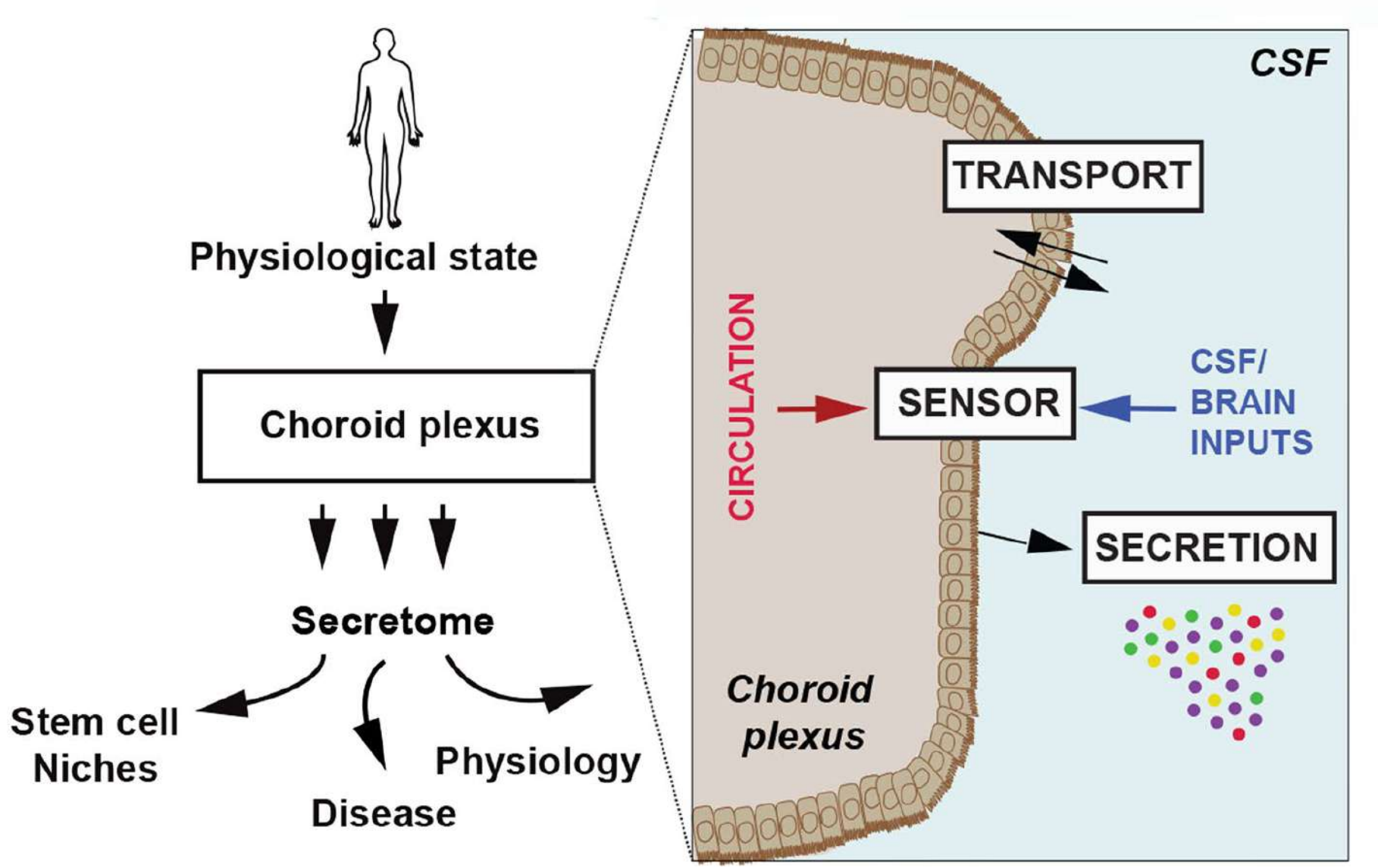
Only molecules with a low molecular weight (under 400–600 Da) and of positive charge can cross the BBB



BBB

Blood CerebroSpinal Fluid Barrier: the interface between two circulating fluids, the blood and the CSF

The choroid plexus regulates neural stem cells and brain function

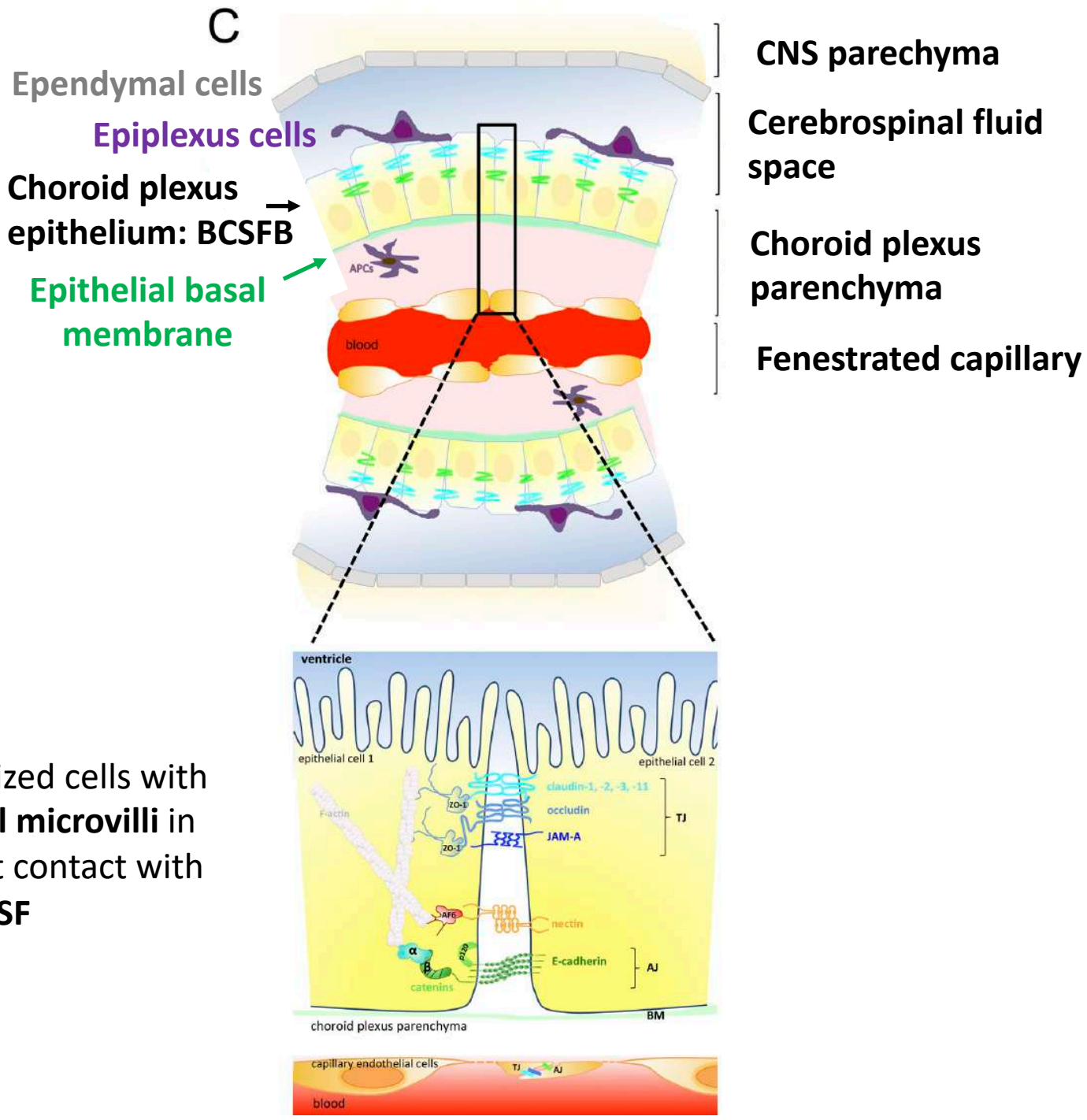


Composition of CSF

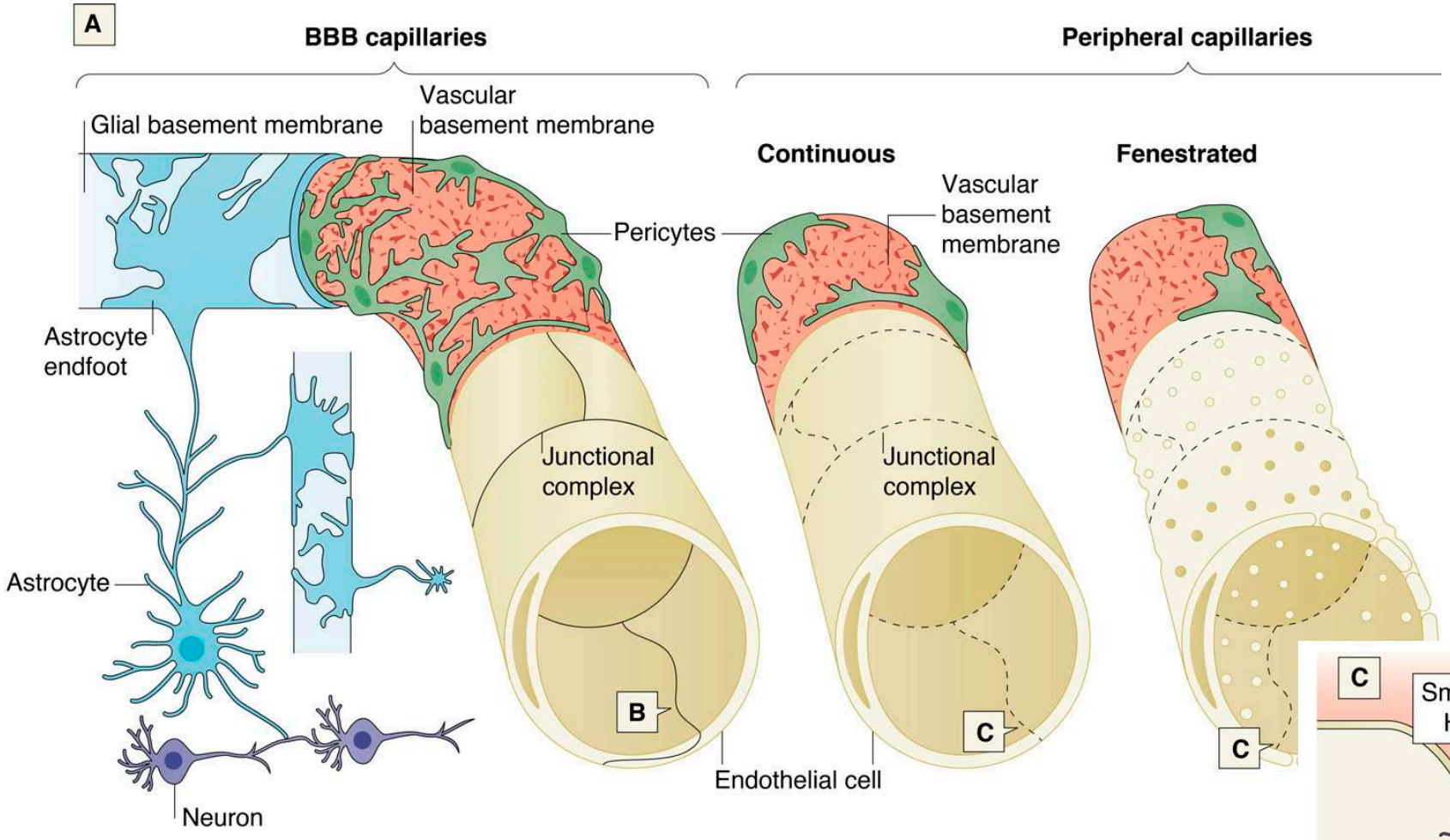
Substance	Plasma	CSF
Na^+ (mEq/l)	145.0	150.0
K^+	4.8	2.9
Ca^{++}	5.2	2.3
Mg^{++}	1.7	2.3
Cl^-	108.0	130.0
HCO_3^-	27.4	21.0
Lactate	7.9	2.6
PO_4^{---}	1.8	0.5
Protein	7000.0	20.0
Glucose	95.0	60.0

(protein and glucose expressed as mg/100 ml)

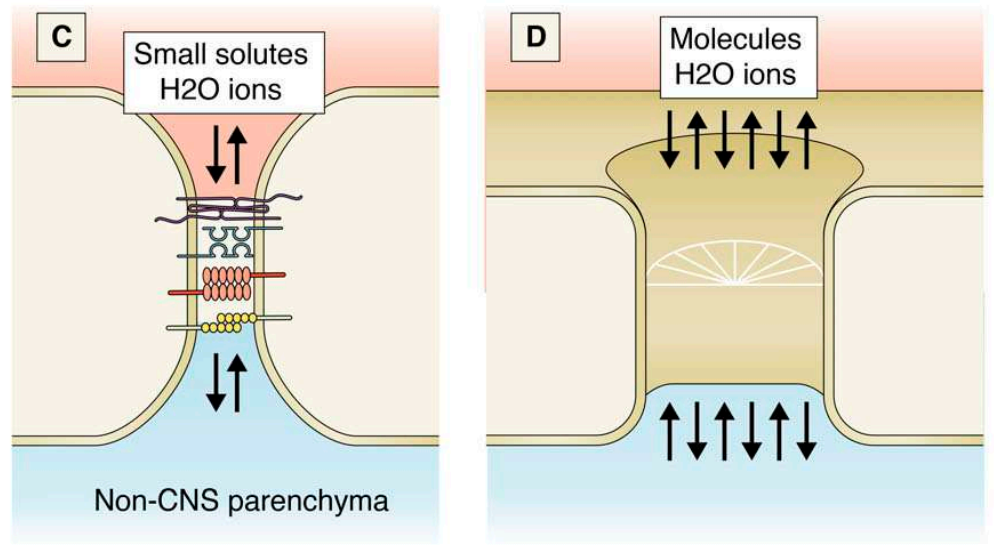
Blood
Cerebro
Spinal
Fluid
Barrier



Polarized cells with **apical microvilli** in direct contact with the **CSF**



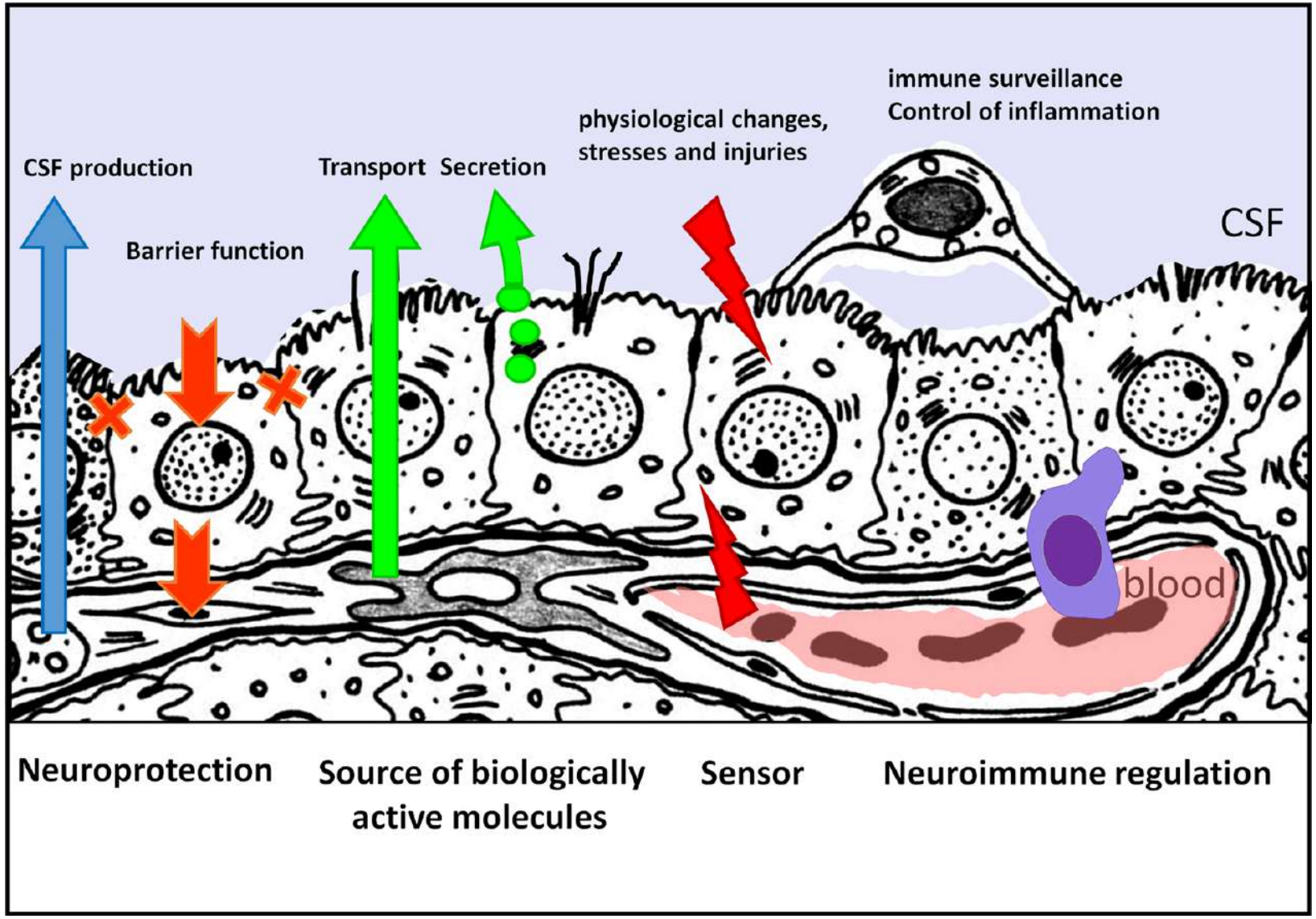
- *Fenestration: small pores raging from 70 to 100 nm in diameter*
- *Permit rapid exchange of water and larger solutes between plasma and interstitial fluid*



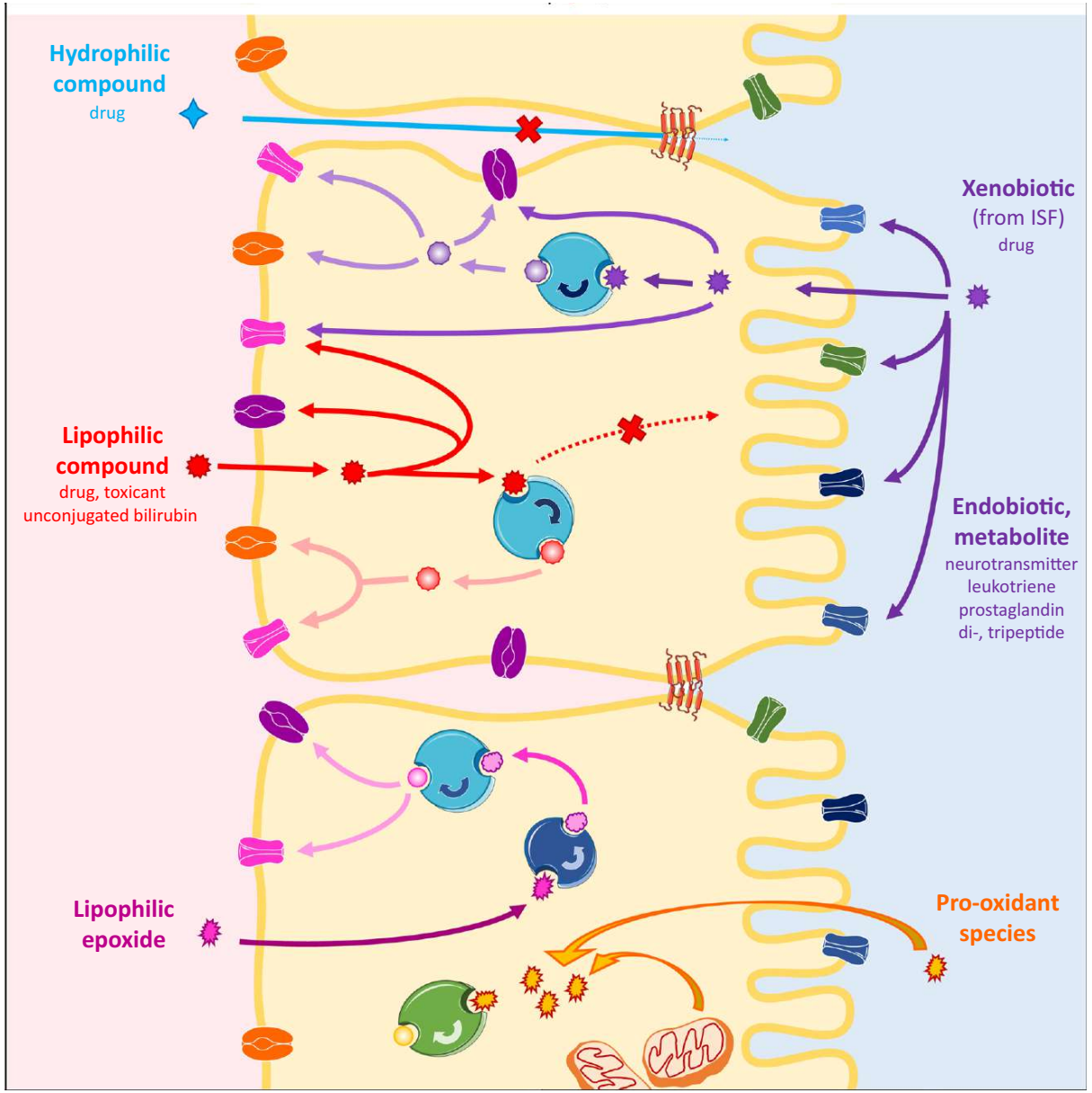
Choroid plexus functions:

The current understanding of CSF functions, go far beyond a drainage function

Overall drainage of brain metabolites diffusing into the CSF : a function that is particularly relevant to brain development and in adulthood for the maintenance of brain homeostasis and brain repair.



Molecular determinants of the neuroprotective functions in the BCSFB

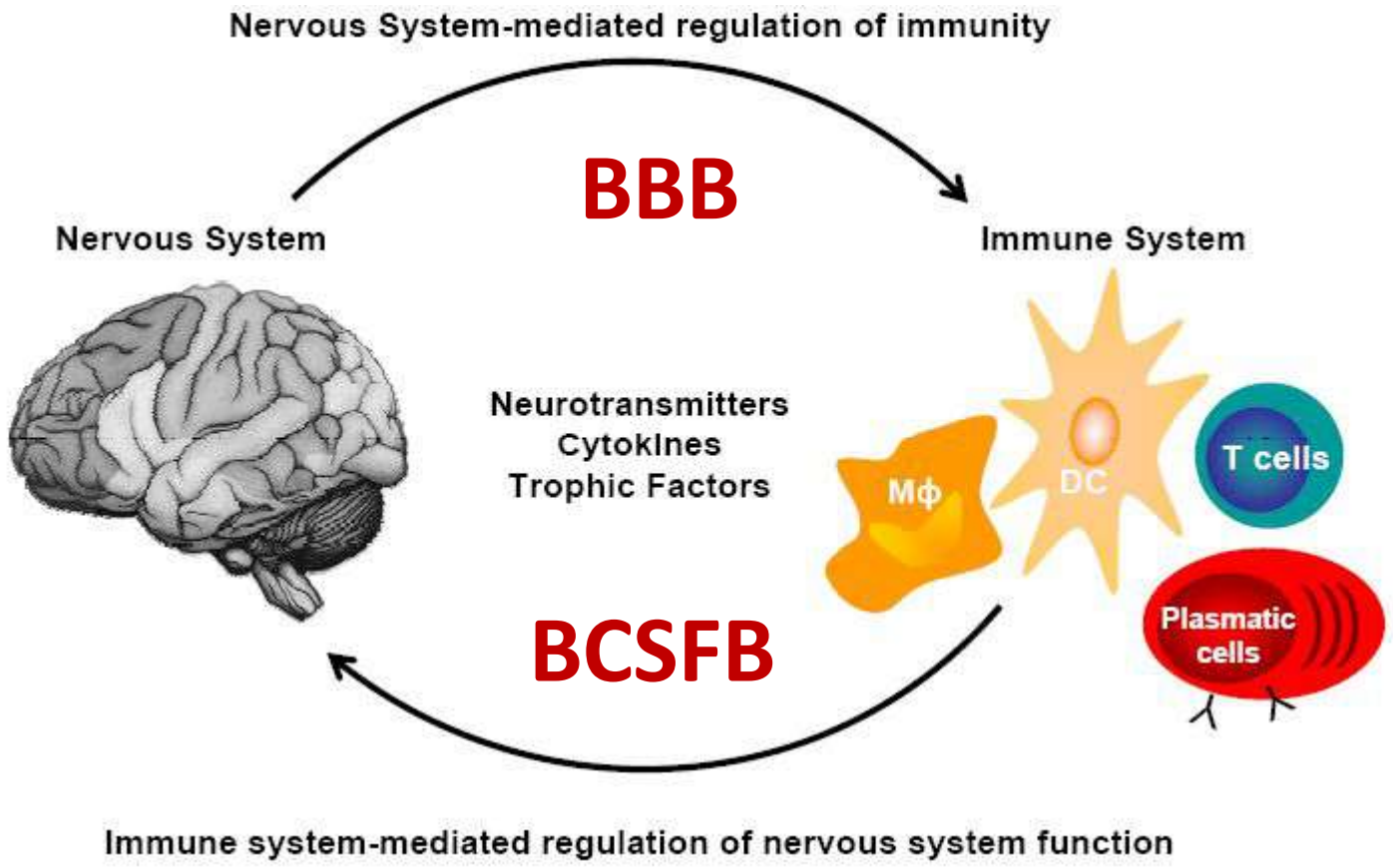


b

- Tight junction forming claudins
- Xenobiotic conjugating enzymes: GST, UGT, ST
- Epoxide hydrolase (microsomal)
- Antioxidant enzymes : catalase, GPx, SOD
- Basolateral efflux transporters
  ABCC1, ABCC4, Slco1a4
- Apical efflux transporters
  Slc22A8, Slco1a5, Slc29a4, Slc15a2 (Slc 47a1?)
- Neurotoxic molecule
- ROS and peroxide, epoxide
- Inactivated metabolite

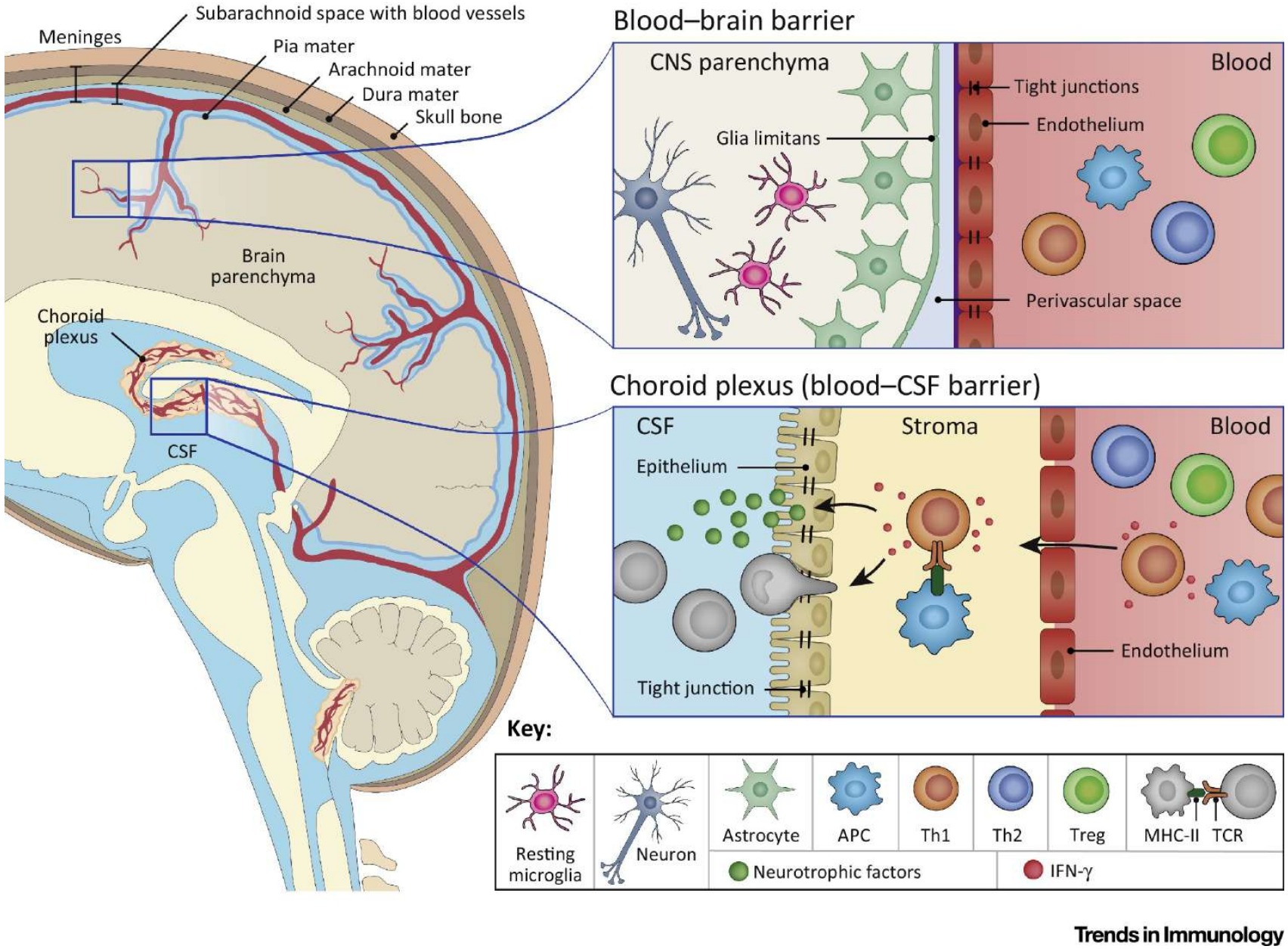
Towards understanding immunosurveillance of the CNS:

Immunesurveillance is an extensive bidirectional communication that takes place between nervous and immune system in both health and disease.

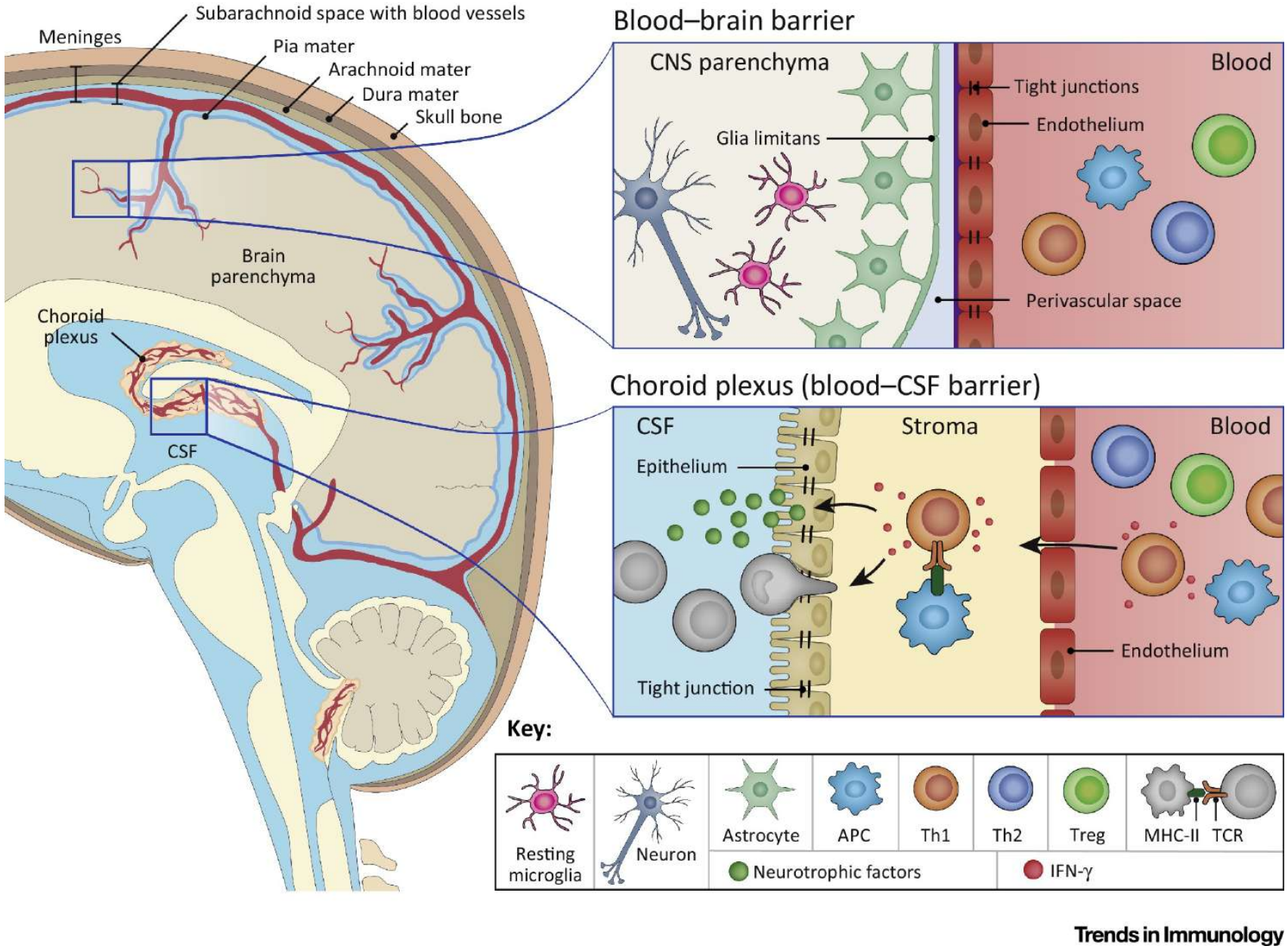


Immunosurveillance of the CNS requires the migration of circulating immune cells either across the endothelial BBB or across the epithelial BCSFB in the absence of neuroinflammation.

The same molecules, including cytokines, neurotransmitters and trophic factors, participate as mediators in both directions.

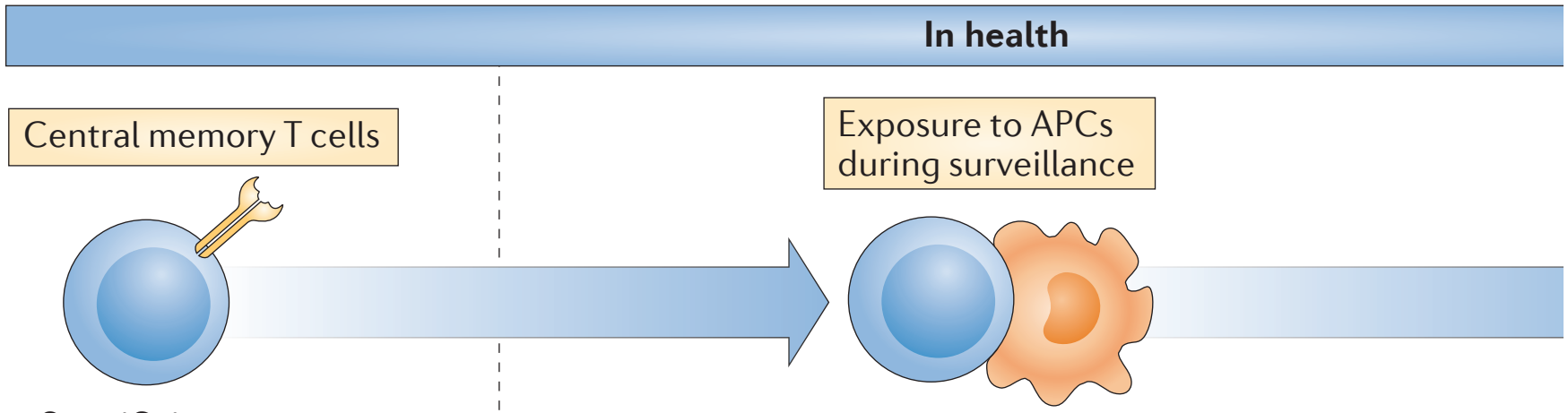


While the capillaries represent a barrier for solutes and ions, leukocyte trafficking is regulated at the level of the post-capillary venules where the endothelial cells express specific adhesion molecules



In the absence of inflammation, immune cells keep patrolling throughout the cerebrospinal fluid where they interact with nervous system cells regulating acquisition of memory, learning and behaviour

a

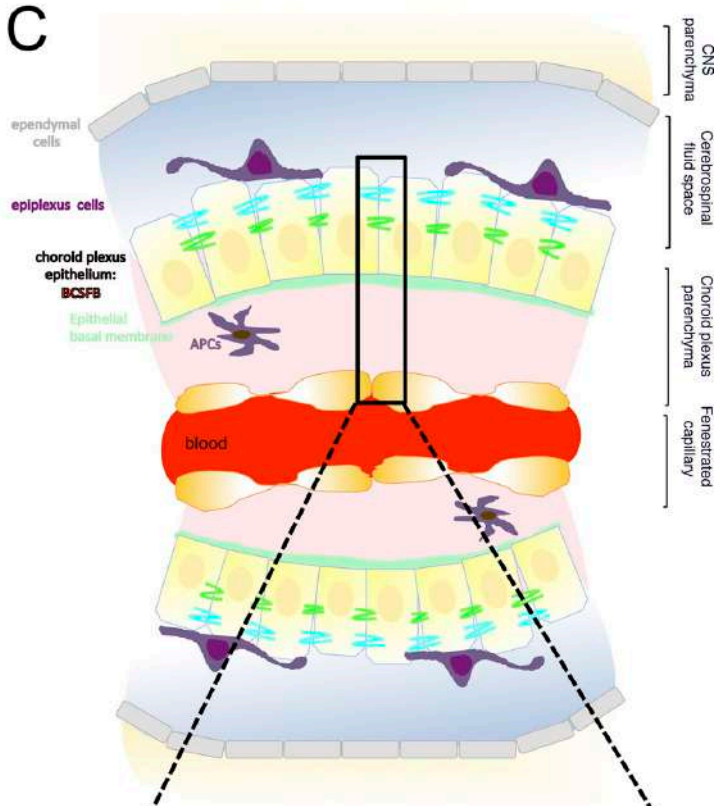


Specificity:

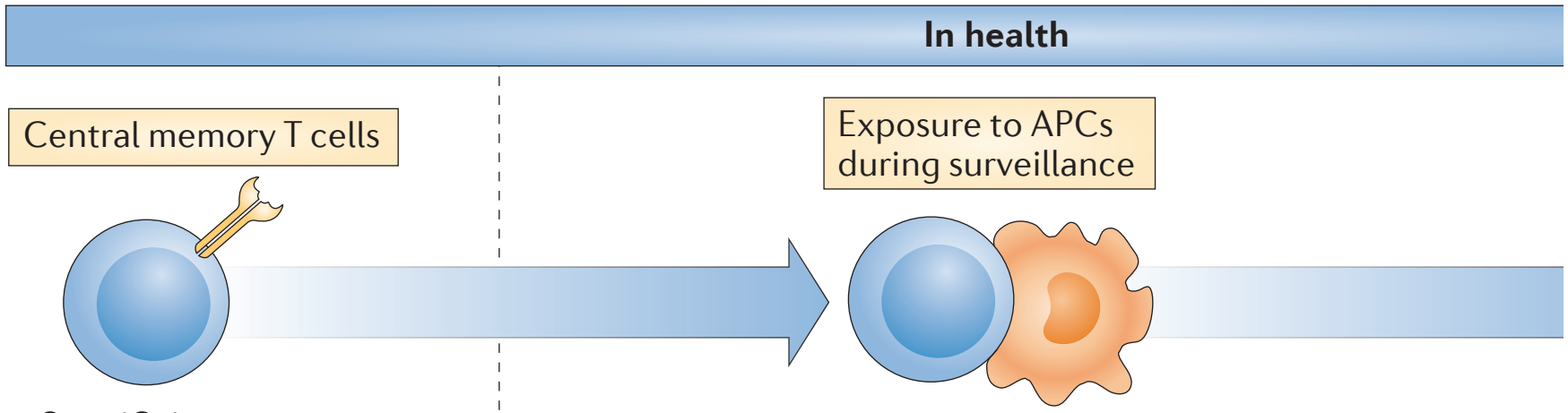
- Self antigen
- Pathogen
- Cross-reactive or degenerate for self antigen
- Dual specificity

- Choroid plexus macrophages and DCs

C



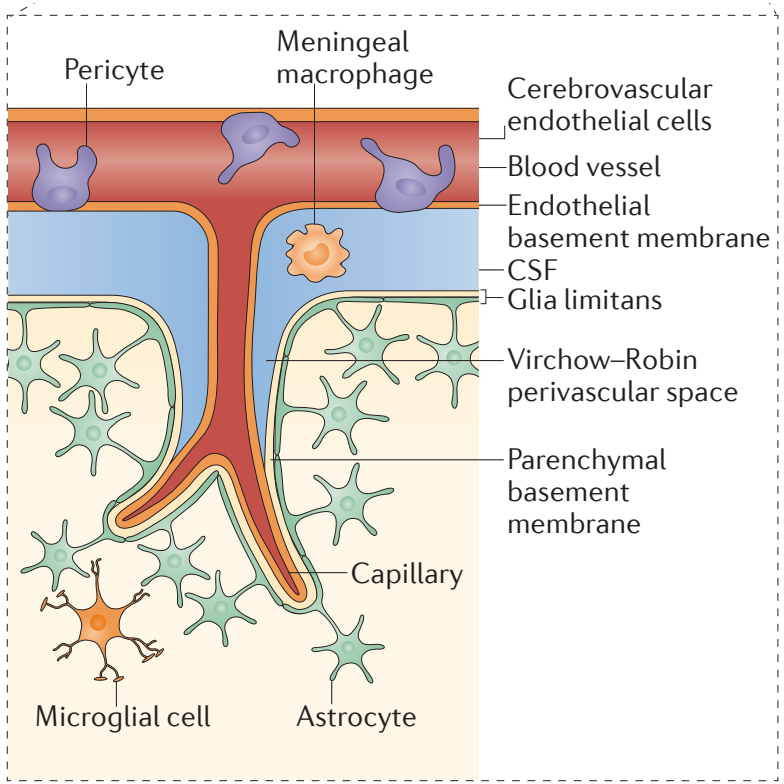
a

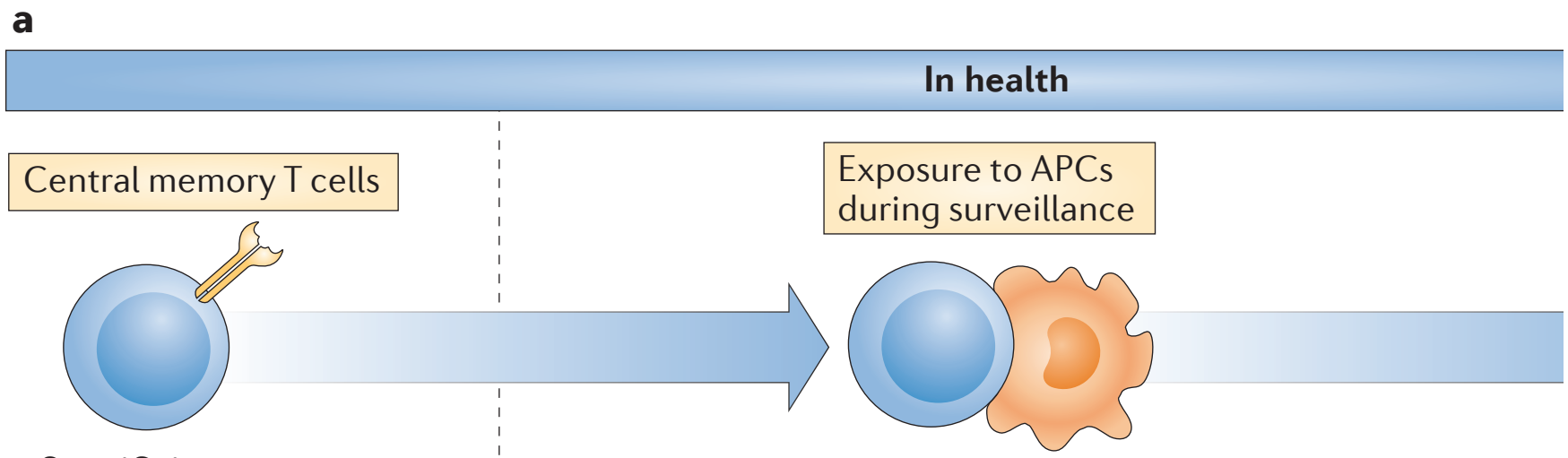


Specificity:

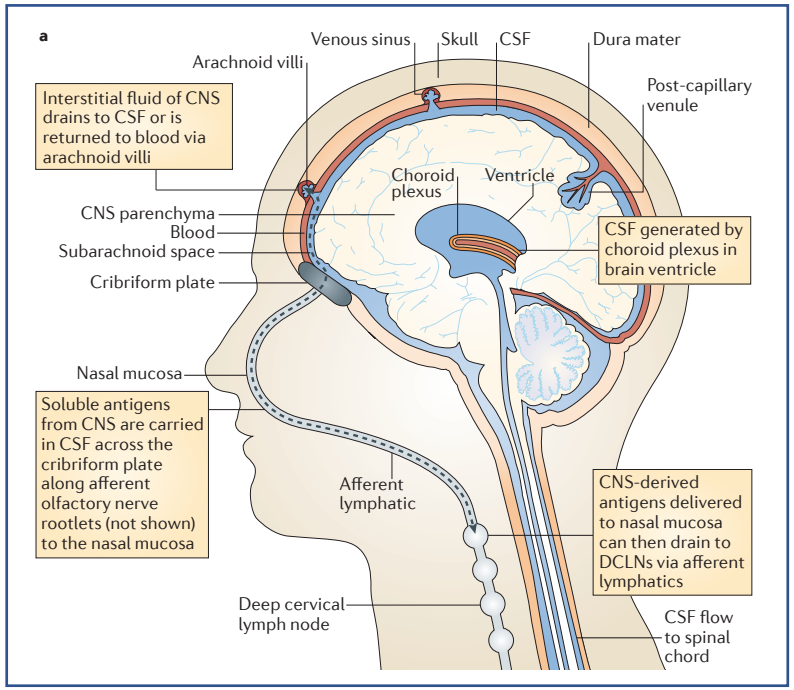
- Self antigen
- Pathogen
- Cross-reactive or degenerate for self antigen
- Dual specificity

- Choroid plexus macrophages and DCs
- Meningeal macrophages in the brain and spinal cord
- Perivascular APCs in Virchow-Robin spaces

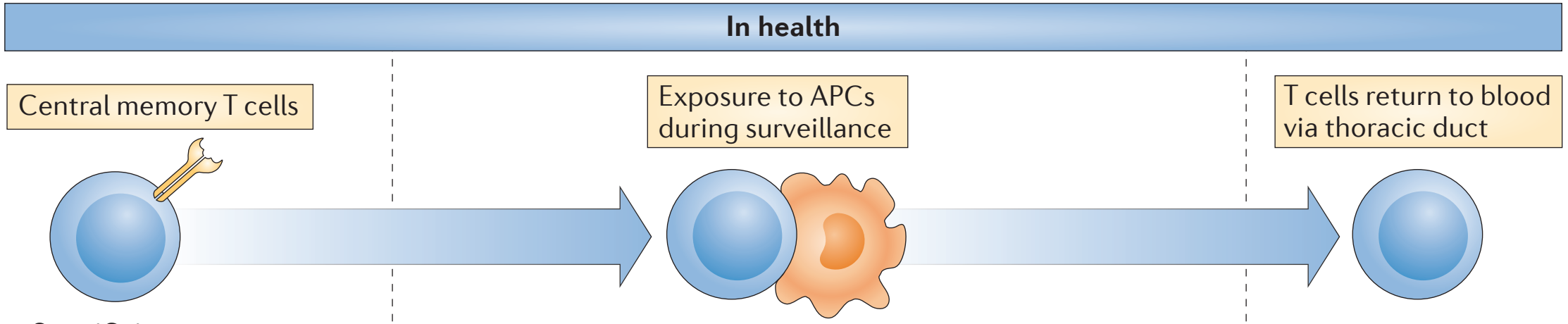




- Choroid plexus macrophages and DCs
- Meningeal macrophages in the brain and spinal cord
- Perivascular APCs in Virchow-Robin spaces
- Cervical lymph node DCs (solutes drained from CSF)



a



Specificity:

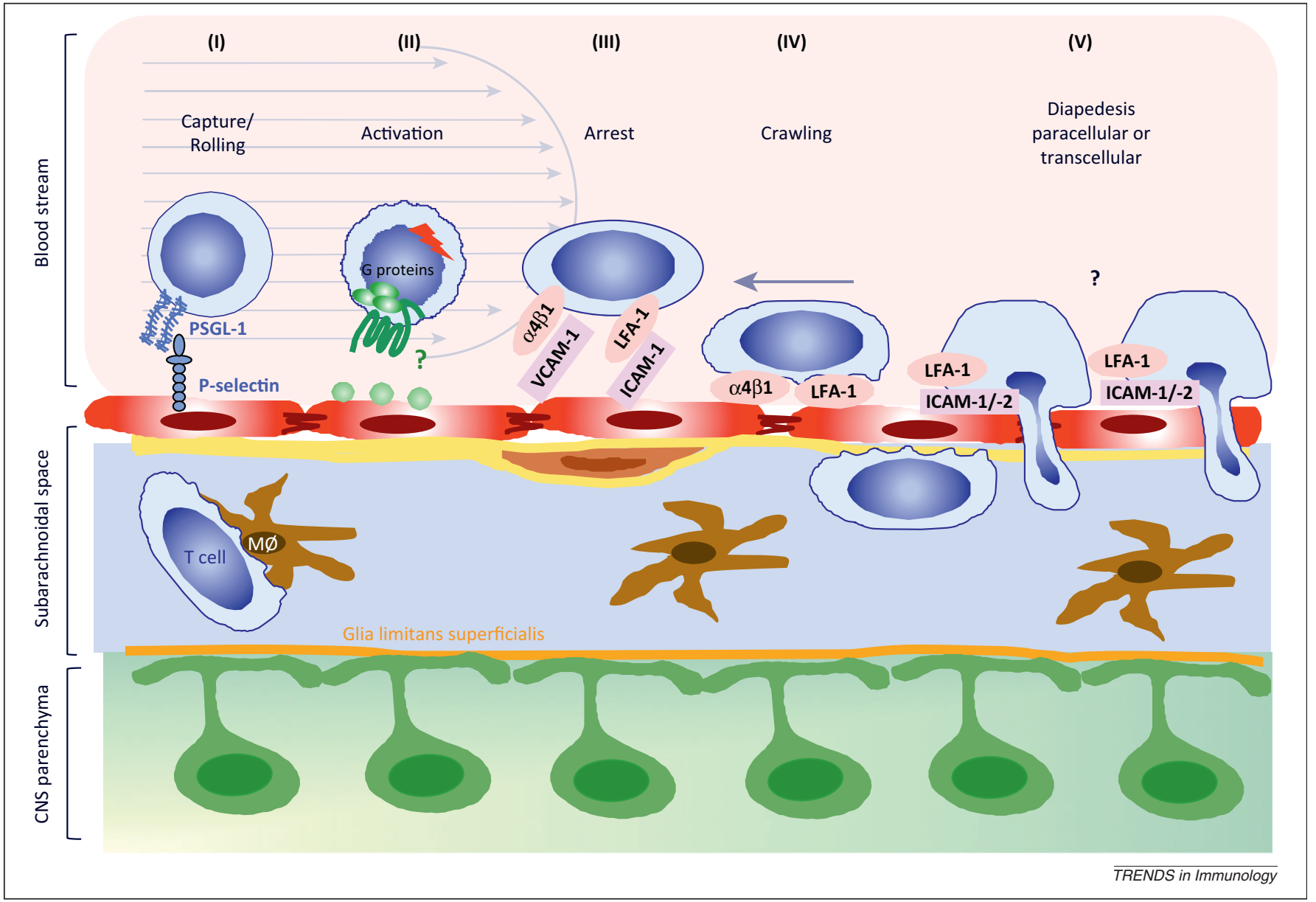
- Self antigen
- Pathogen
- Cross-reactive or degenerate for self antigen
- Dual specificity

- Choroid plexus macrophages and DCs
- Meningeal macrophages in the brain and spinal cord
- Perivascular APCs in Virchow-Robin spaces
- Cervical lymph node DCs (solutes drained from CSF)

APCs localized to these compartments will continuously be exposed to all CNS antigens and thus ensure immunosurveillance of the CNS.

When brain damage or imbalance of glial homeostasis initiate inflammatory processes, immune cells are recruited into the CNS parenchyma

Principles of the multistep extravasation of immune cells across the BBB



Principles of the multistep extravasation of immune cells during inflammation
A focus on Multiple Sclerosis

1396: Earliest Recorded Case of MS

Sister Lidwina van Schiedam fell while ice skating in 1396 and subsequently developed numerous symptoms characteristic of MS.

From Sister Lidwina to the present…

- ***1868—Jean-Martin Charcot*** *describes the disease and finds MS plaques (scars) on autopsy.*

  "Multiple sclerosis is often one of the most difficult problems in clinical medicine." (Charcot, 1894)

Jean-Martin Charcot
(1825-1893)

- ***1878—Louis Ranvier*** *describes the myelin sheath*

(the primary target of MS in the central nervous system)

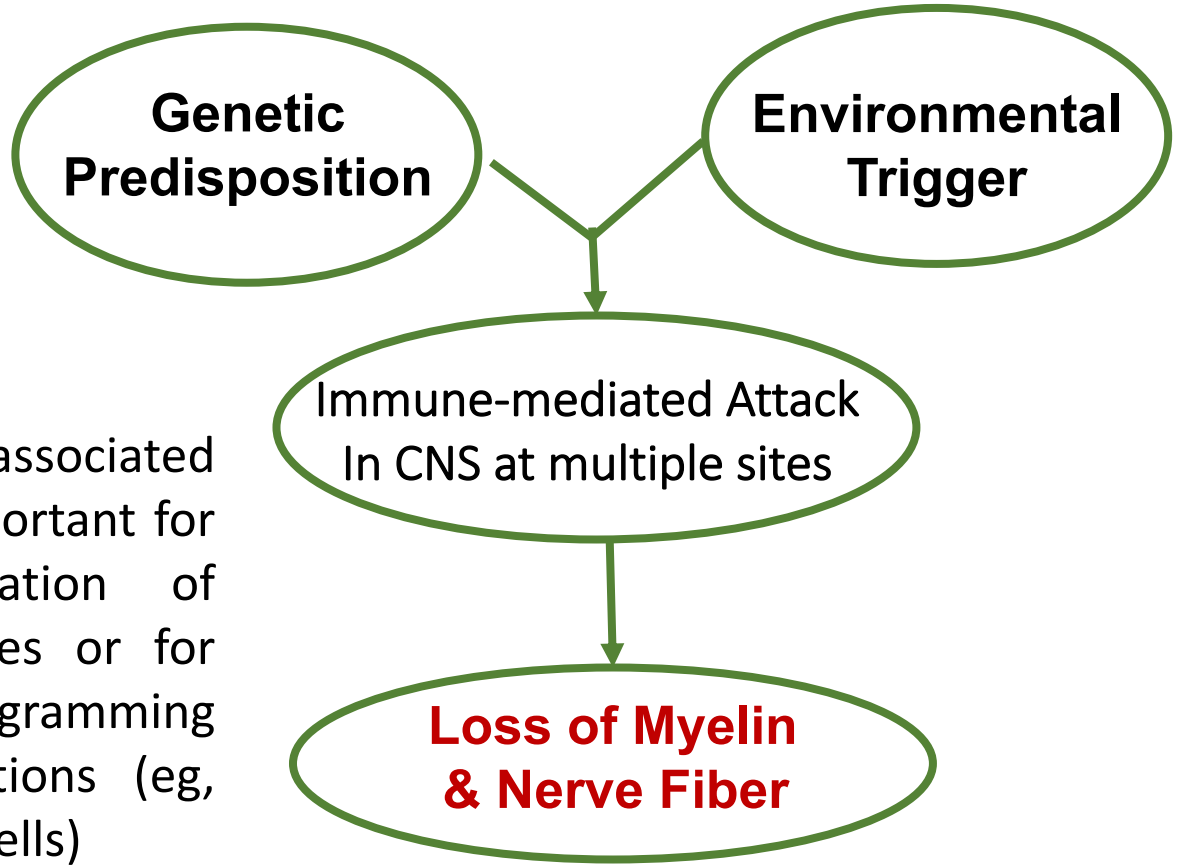
- ***1981****—1st MRI image of MS is published.*
- ***1993****—The first disease-modifying agent for MS—Betaseron—is approved in the U.S*
- *Today there are more than a dozen medications approved in the U.S. for the treatment of MS*

Louis-Antoine Ranvier
(1835 – 1922)

MS is considered an IMMUNE-MEDIATED disease caused by:

- ✓ The *DRB1*1501* allele HLA (human leukocyte antigen) that confer T- and B-cell reactivity to specific myelin protein peptides

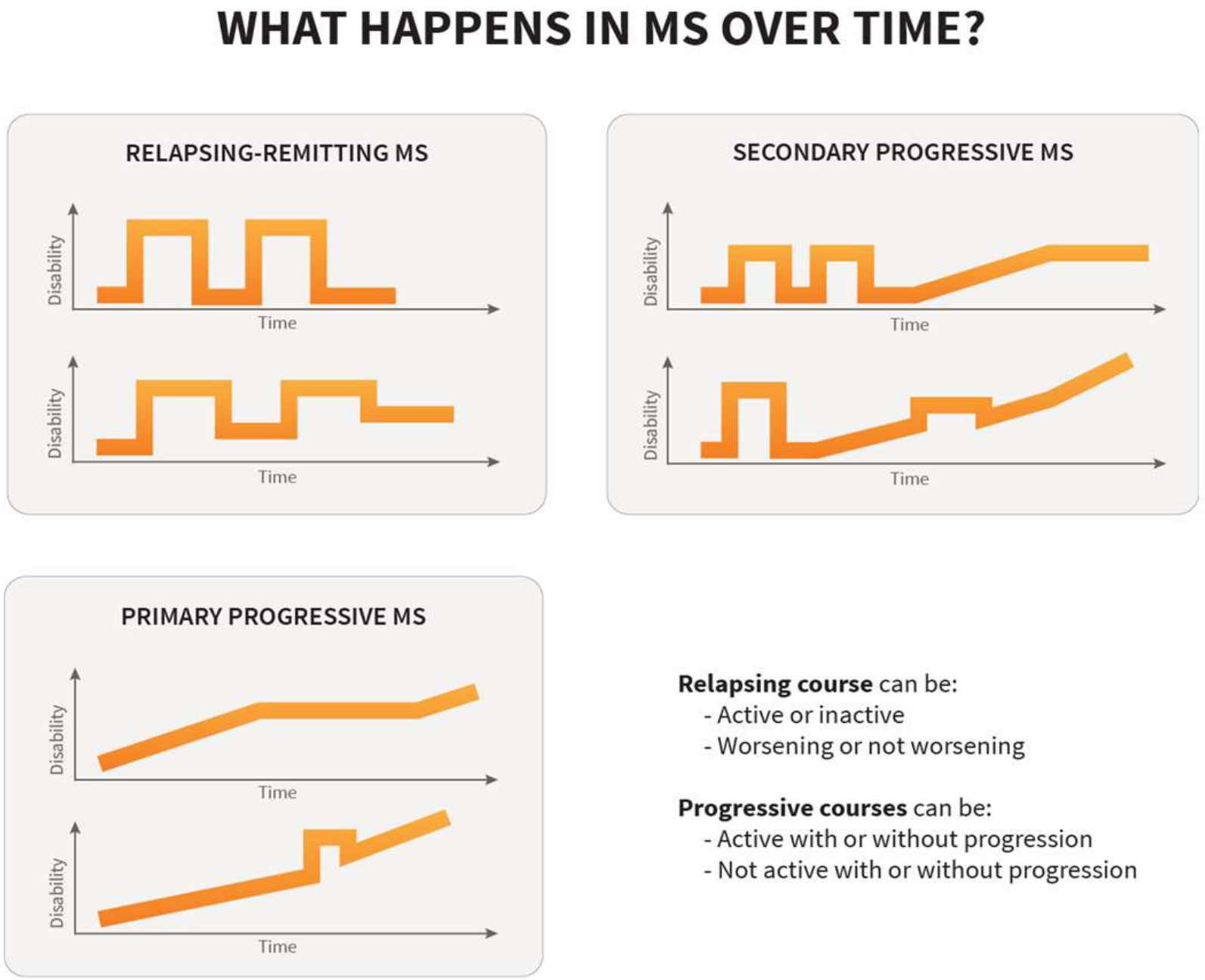
- ✓ Many of the SNPs are associated with genes that are important for either the differentiation of pathogenic T-cell species or for the modulation or reprogramming of their effector functions (eg, cytokine secretion of T cells)



- ✓ Smoking
- ✓ Obesity in adolescence
- ✓ Low vitamin D levels
- ✓ Exposure to the Epstein-Barr virus

Clinically Isolated Syndrome (CIS)

- A first neurologic event suggestive of demyelination
- Individuals with CIS are at high risk for developing clinically definite MS if the neurologic event is accompanied by multiple, clinically silent (asymptomatic) lesions on MRI typical of MS



How is the disease course treated?

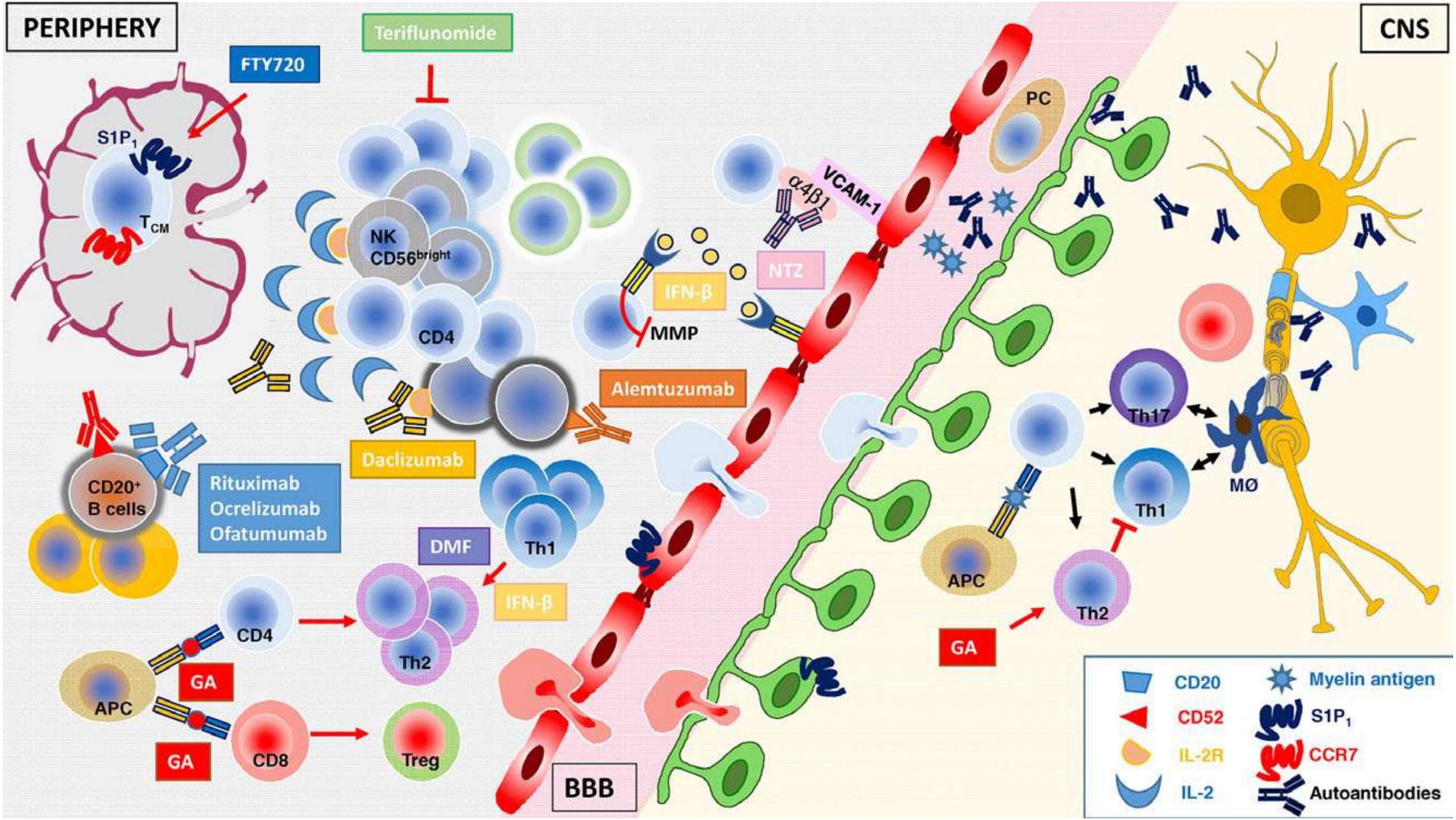
- More than a dozen disease-modifying therapies are FDA-approved for relapsing forms of MS:
  - daclizumab (Zinbryta®)
  - glatiramer acetate (Copaxone®; Glatopa®)
  - interferon beta-1a (Avonex®, Plegridy®, Rebif®)
  - interferon beta-1b (Betaseron® and Extavia®)
  - dimethyl fumarate (Tecfidera®)
  - fingolimod (Gilenya®)
  - teriflunomide (Aubagio®)
  - alemtuzumab (Lemtrada®)
  - mitoxantrone (Novantrone®)
  - natalizumab (Tysabri®)
  - ocrelizumab (Ocrevus®)

What do the disease-modifying drugs do?

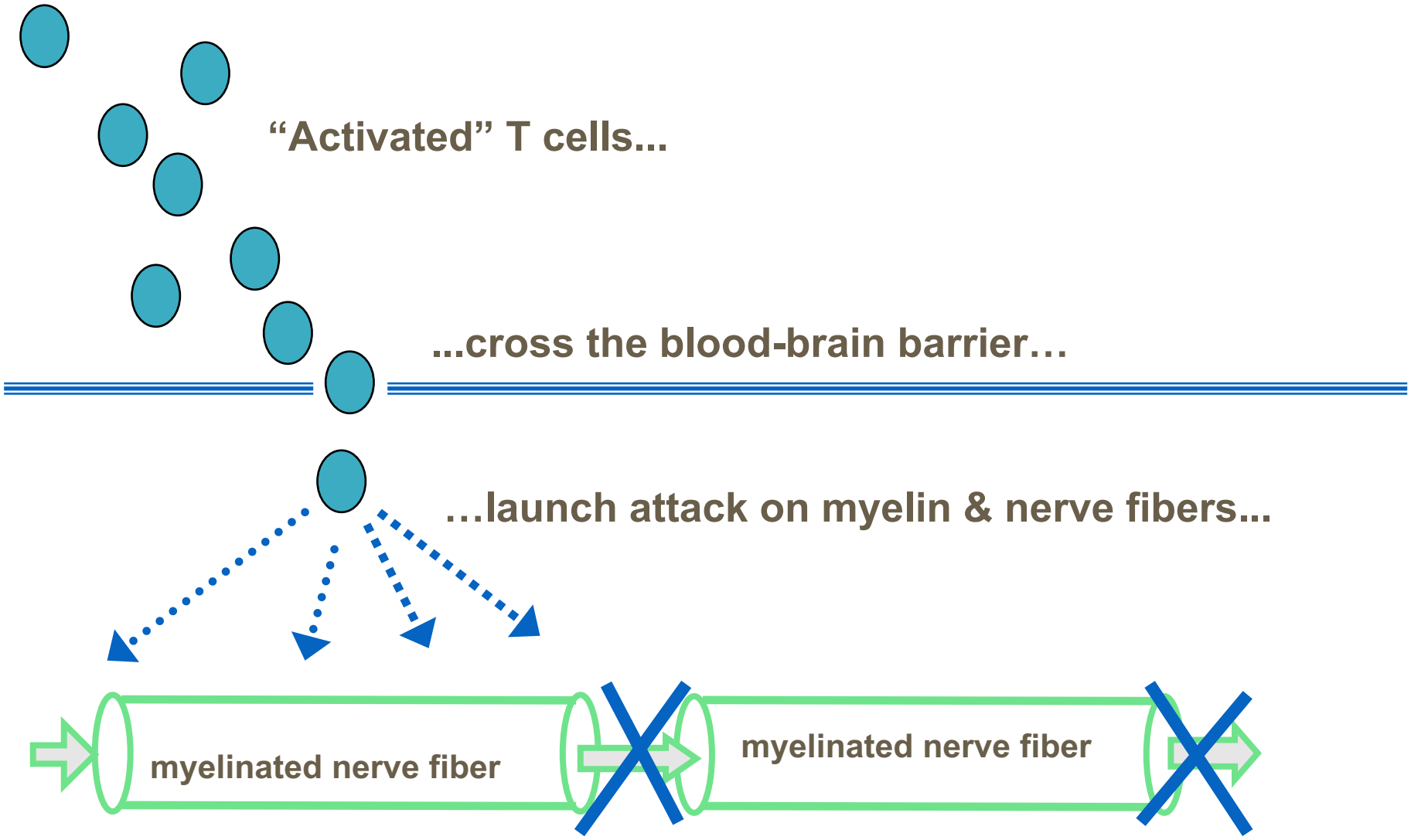
All reduce attack frequency and severity, reduce scarring on MRI, and probably slow disease progression.

These medications do not:

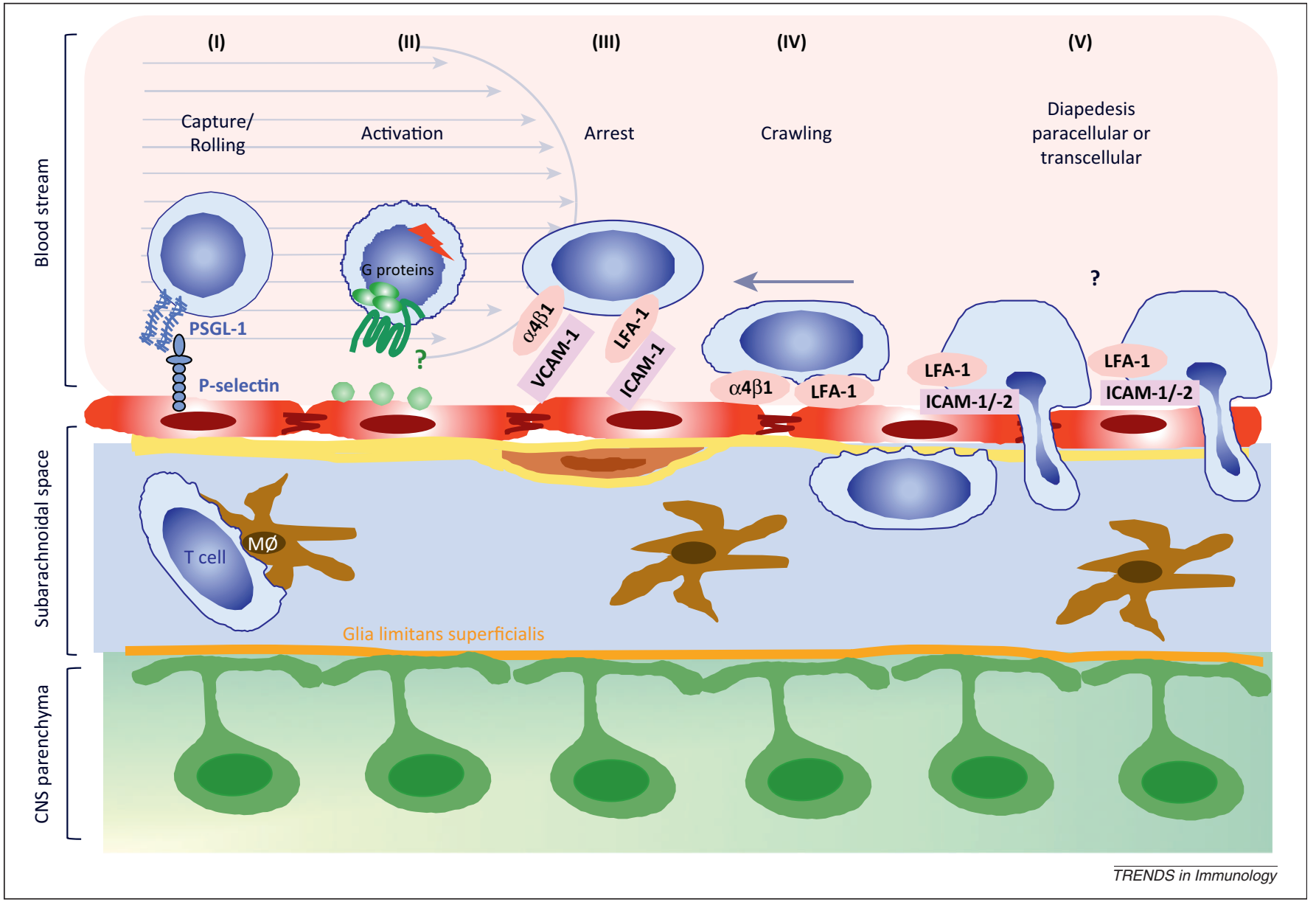
- *Cure the disease*
- *Make people feel better*
- *Alleviate symptoms*


PERIPHERY
CNS
FTY720
Teriflunomide
S1P1
TCM
NK
CD56bright
CD4
IFN-β
MMP
α4β1
VCAM-1
NTZ
PC
Alemtuzumab
Daclizumab
CD20+
B cells
Rituximab
Ocrelizumab
Ofatumumab
DMF
Th1
IFN-β
CD4
Th2
APC
GA
GA
CD8
Treg
BBB
Th17
Th1
MØ
APC
Th2
GA
CD20
CD52
IL-2R
IL-2
Myelin antigen
S1P1
CCR7
Autoantibodies

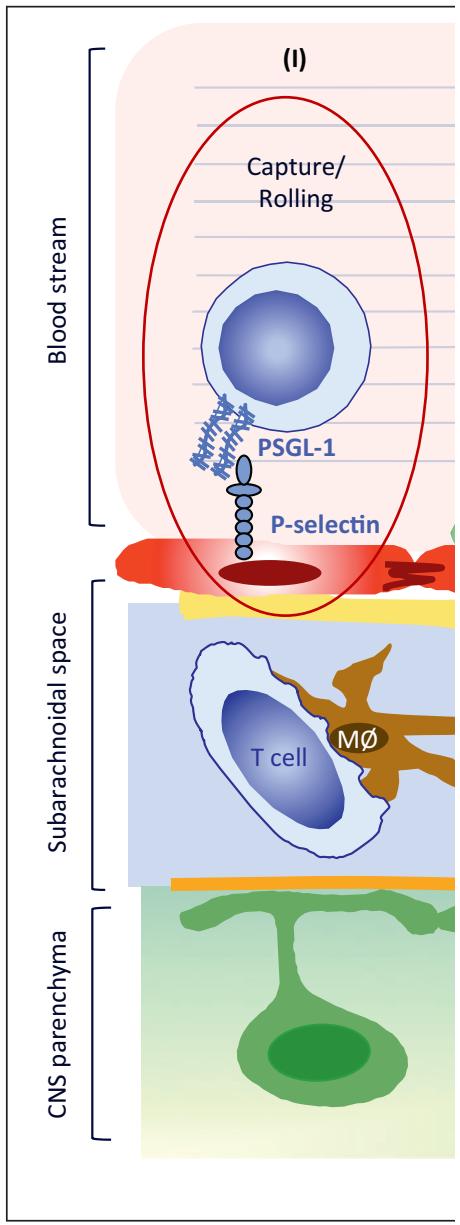
What happens in MS?



Principles of the multistep extravasation of immune cells across the BBB



Principles of the multistep extravasation of immune cells across the BBB

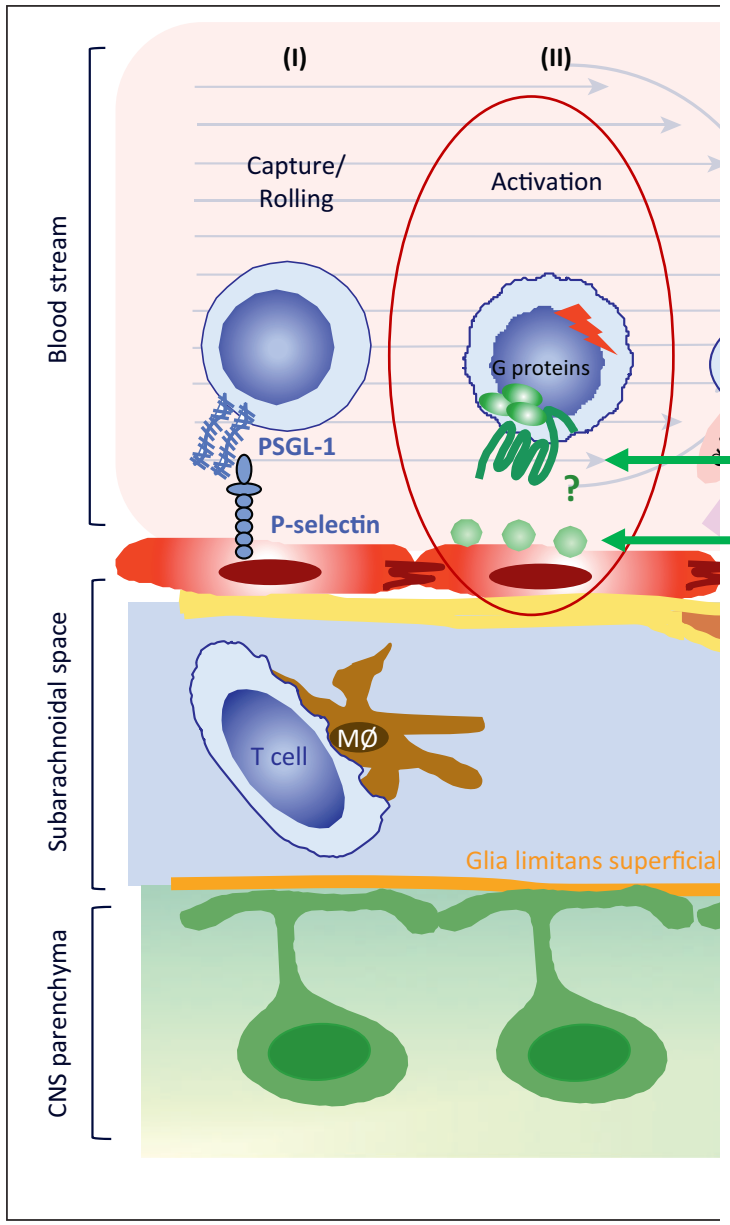


P-selectin expression is increased under inflammation

Increased numbers of circulating CD4+ T cells expressing high levels of PSGL-1 are found in MS patients

A specific polymorphism in PSGL1 associates with primary-progressive MS

Principles of the multistep extravasation of immune cells across the BBB

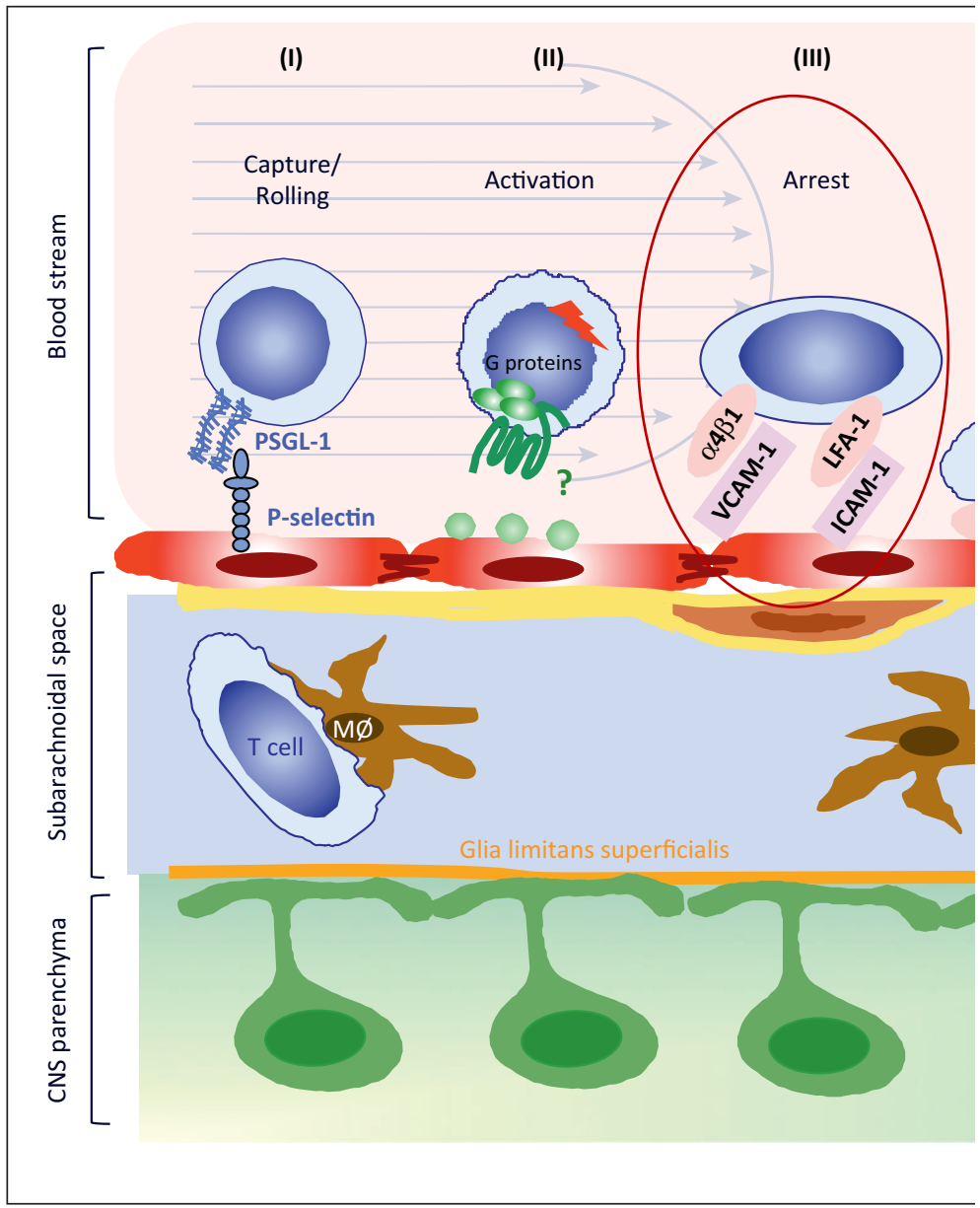


CCR7
CCL19-CCL21

CXCR3
CCL21

Stable adhesion of encephalitogenic T cells requires signaling via GPCRs present on the surface of the circulating immune cells : chemokines displayed on the luminal side of the brain endothelium are involved in T cell recruitment across the BBB

Principles of the multistep extravasation of immune cells across the BBB

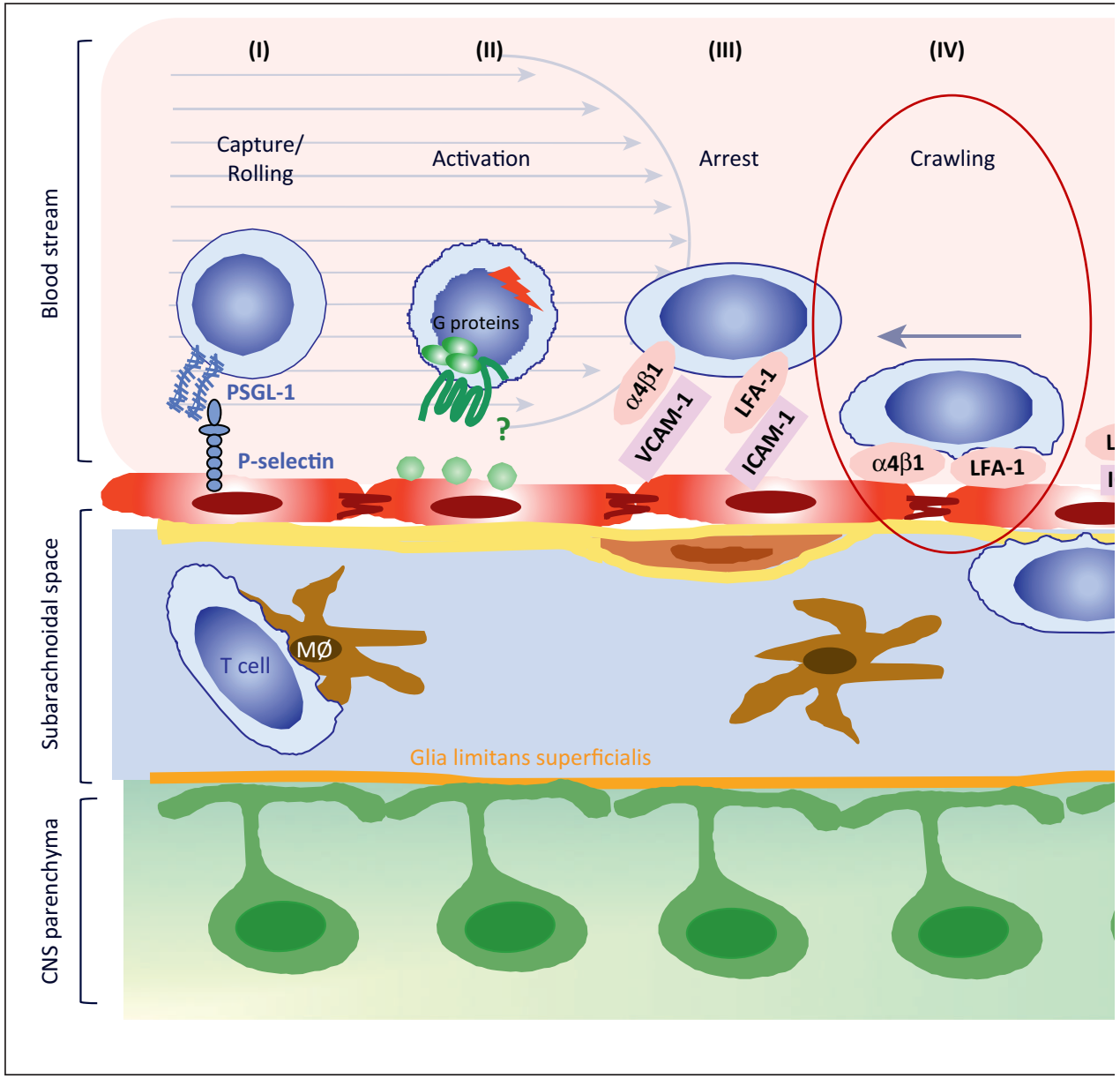


Upregulation of the integrin ligands ICAM-1 and VCAM-1 is observed on endothelial cells

In MS lesions, LFA-1+ inflammatory cells accumulate around venules with high endothelial expression of ICAM-1

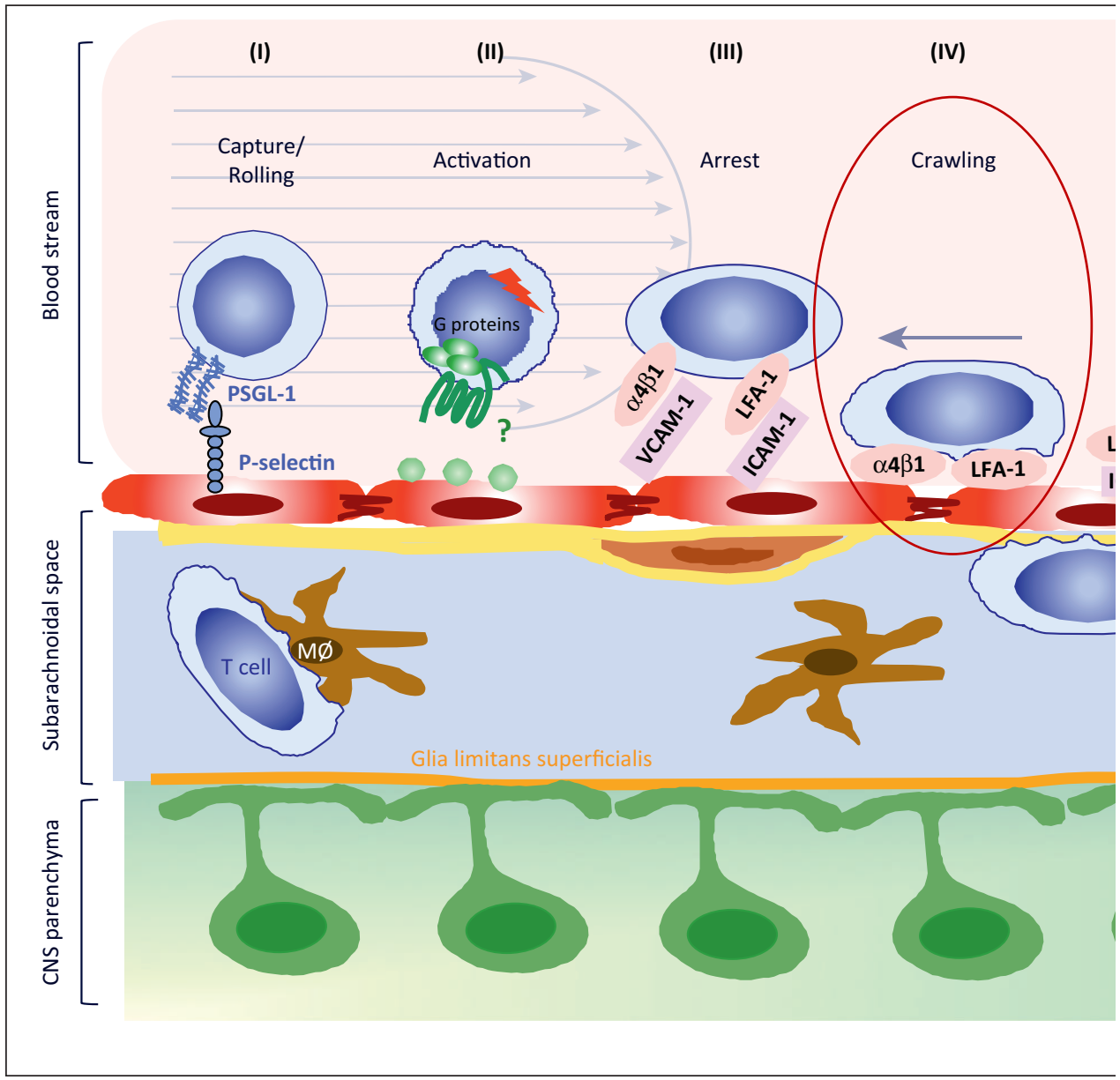
In human CNS microvessels, fibronectin could serve as an alternative ligand for a4-integrins

Principles of the multistep extravasation of immune cells across the BBB



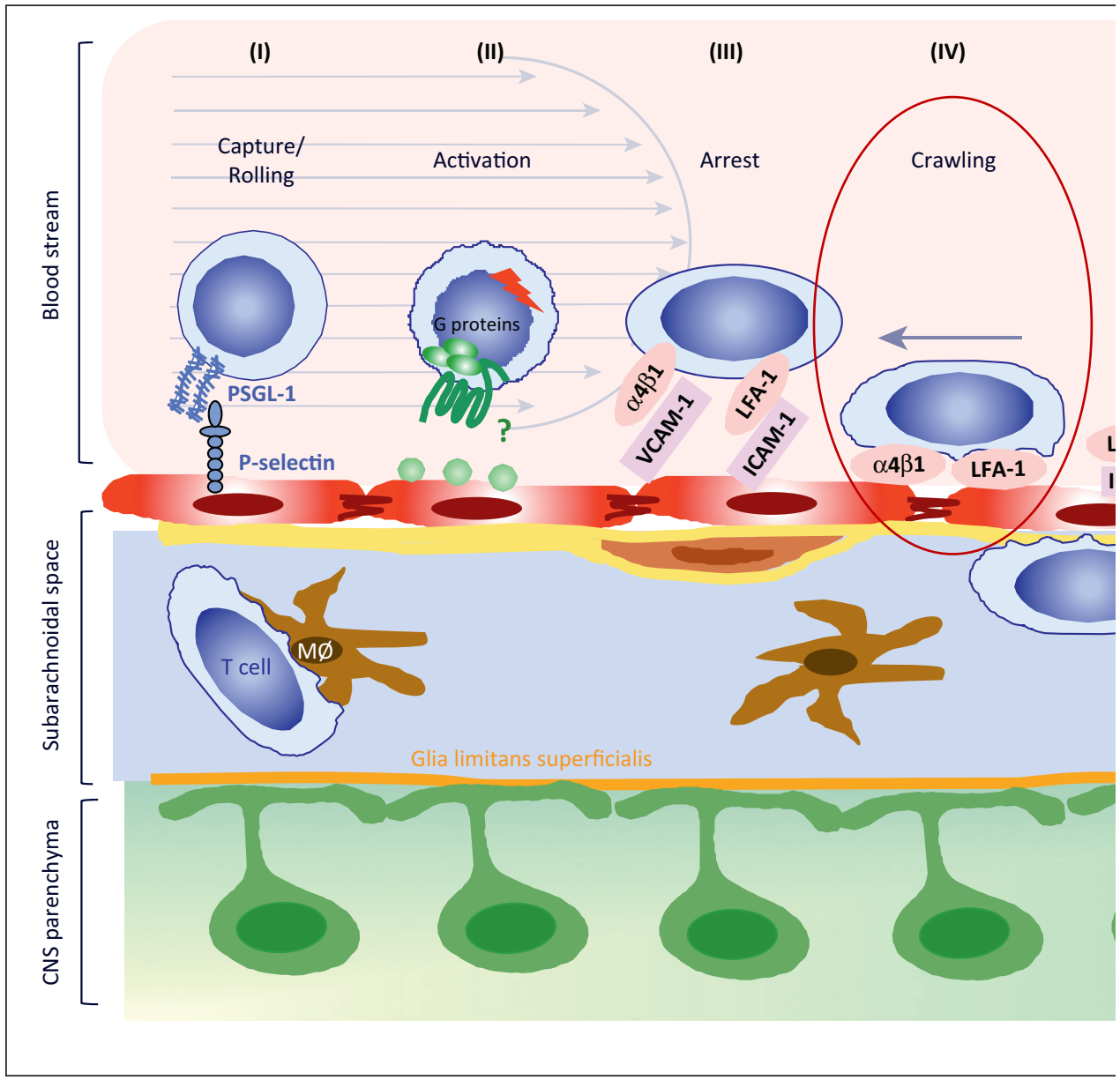
In vitro time lapse imaging techniques have more recently shown that 3 min after cytokine-stimulated arrest on primary brain endothelial cells, encephalitogenic Th1 cells polarize and begin to crawl on the surface of the brain endothelial cells, preferentially against the direction of flow, to find sites permissive for diapedesis across the endothelial barrier

Principles of the multistep extravasation of immune cells across the BBB



The necessity for the T cells to crawl long distances to find a site for diapedesis is a unique characteristic of the highly specialized barrier forming CNS endothelial cells: T cells crawl significantly longer distances on CNS endothelial cells as compared to non- barrier-forming endothelium

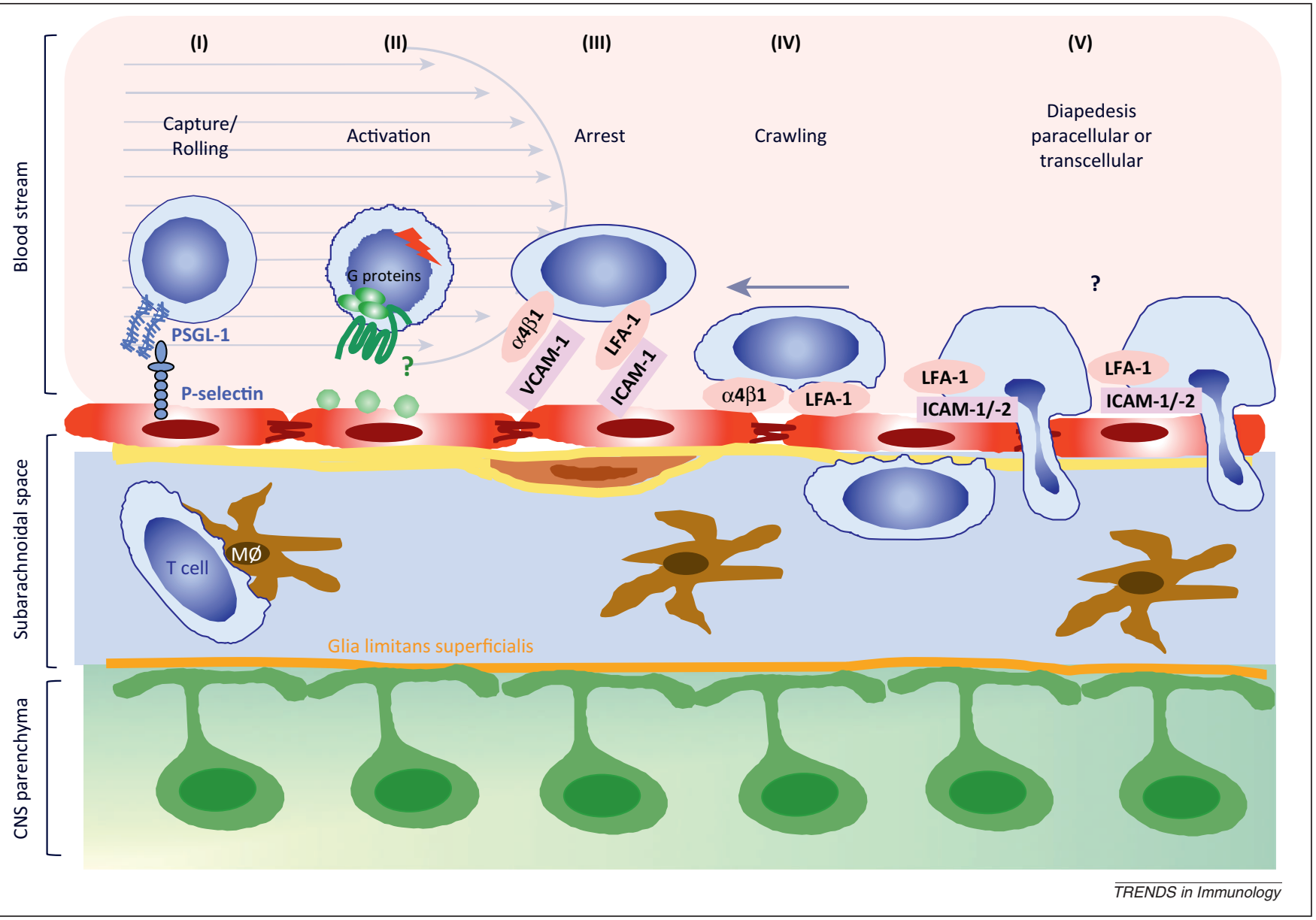
Principles of the multistep extravasation of immune cells across the BBB



T cell polarization and crawling are exclusively mediated by LFA-1 binding to endothelial ICAM-1 and ICAM-2

Therapeutic targeting of a4-integrins has been translated into the clinic, where the humanized monoclonal anti-a4-integrin antibody natalizumab has proven beneficial in the treatment of relapsing–remitting MS.

Principles of the multistep extravasation of immune cells across the BBB



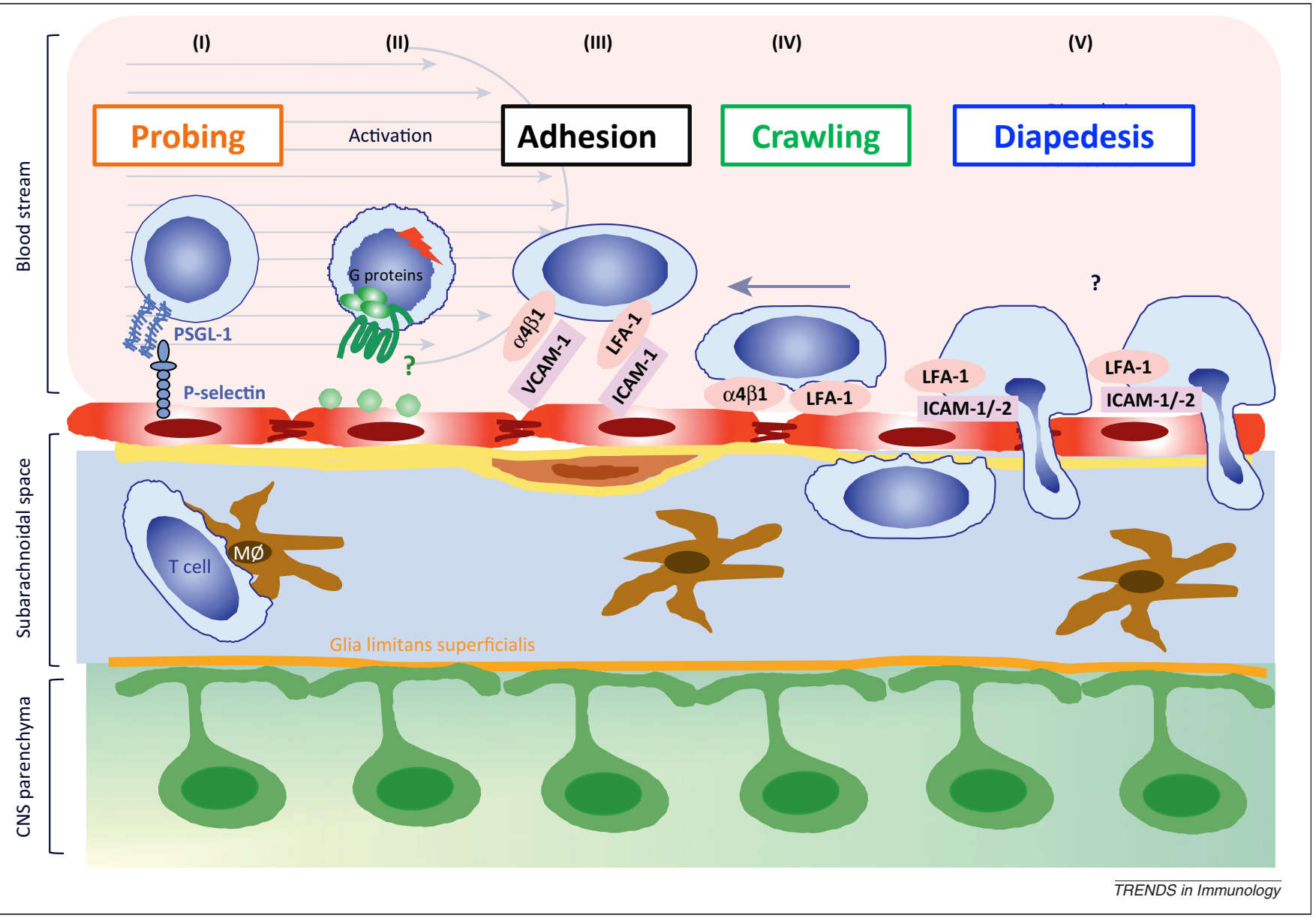
Paracellular diapedesis:

migration of cells in between endothelial or epithelial cells including transient opening of their cellular junctions.

Transcellular diapedesis:

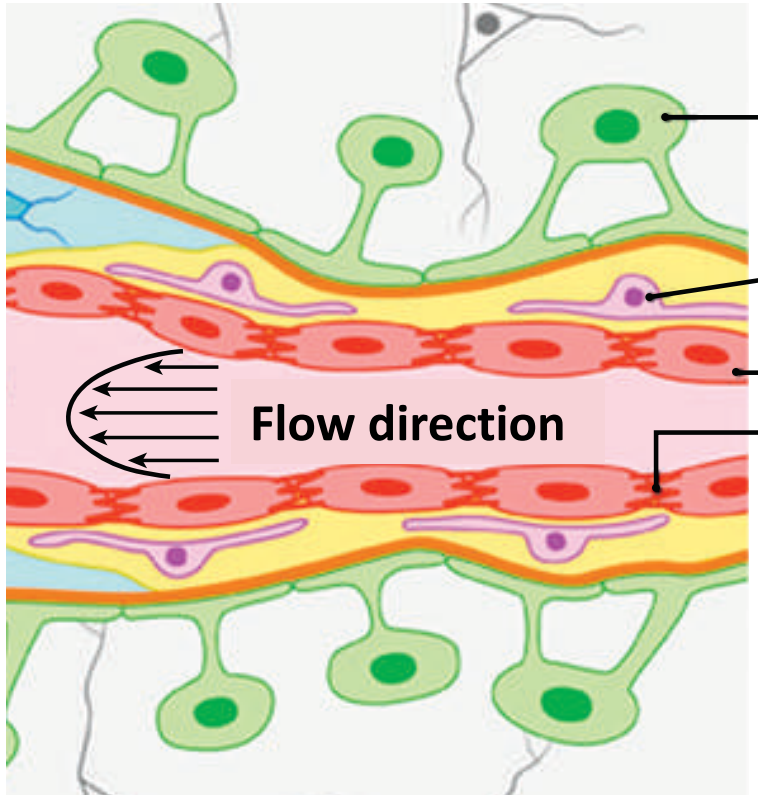
migration of cells through endothelial or epithelial cells forming a pore through the cell body.

Principles of the multistep extravasation of immune cells across the BBB

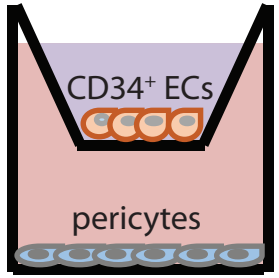


Migration of immune cells from the blood stream into the CNS parenchyma is a process only occurring during neuroinflammation and requires penetration of a second barrier, the glia limitans.

Lab on chip model to study the multi-step cascade of T cells extravasation

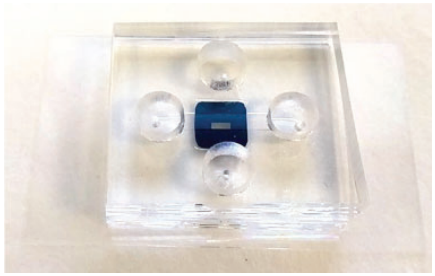


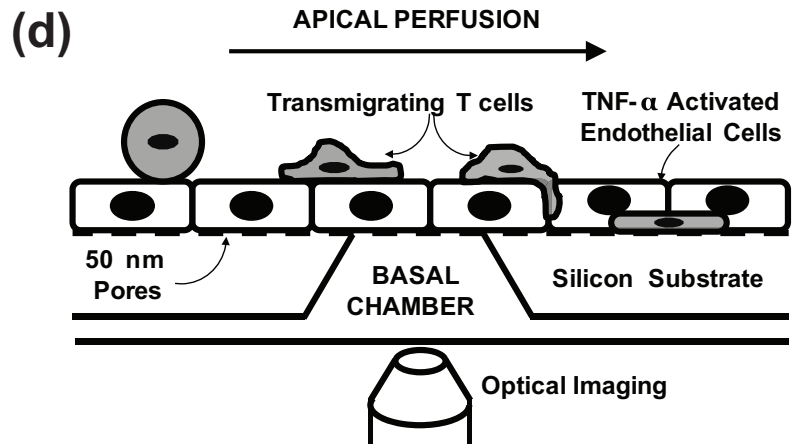
Static model

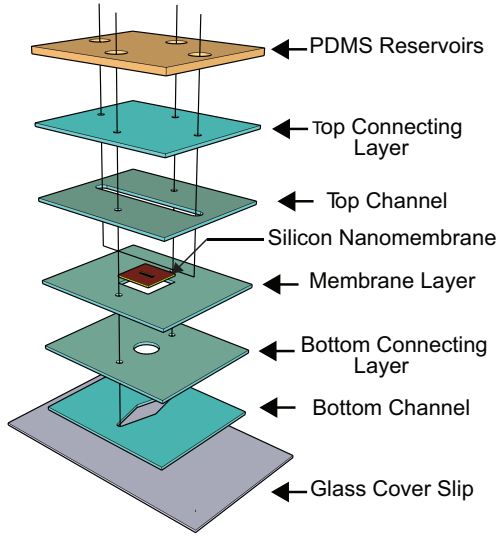


co-culture

Flow chamber model

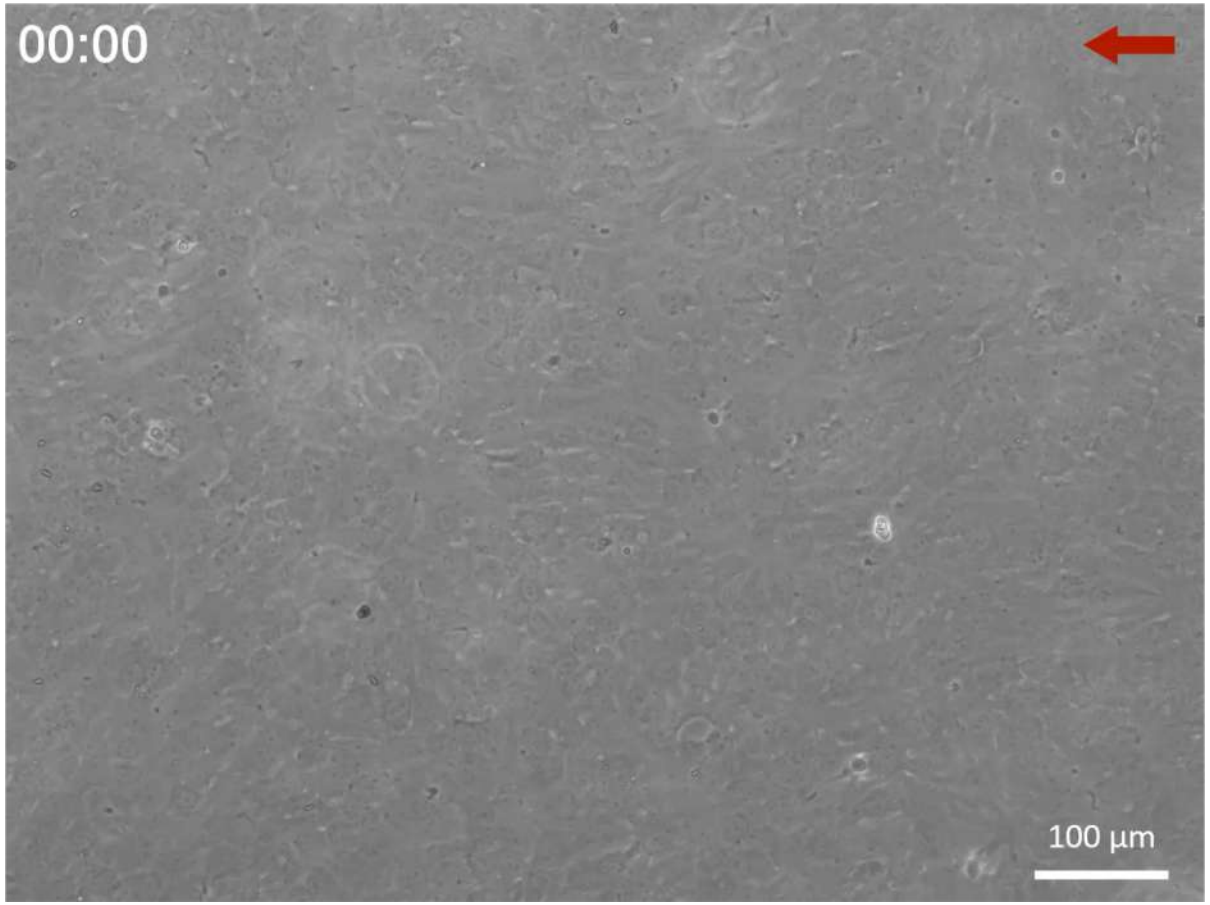




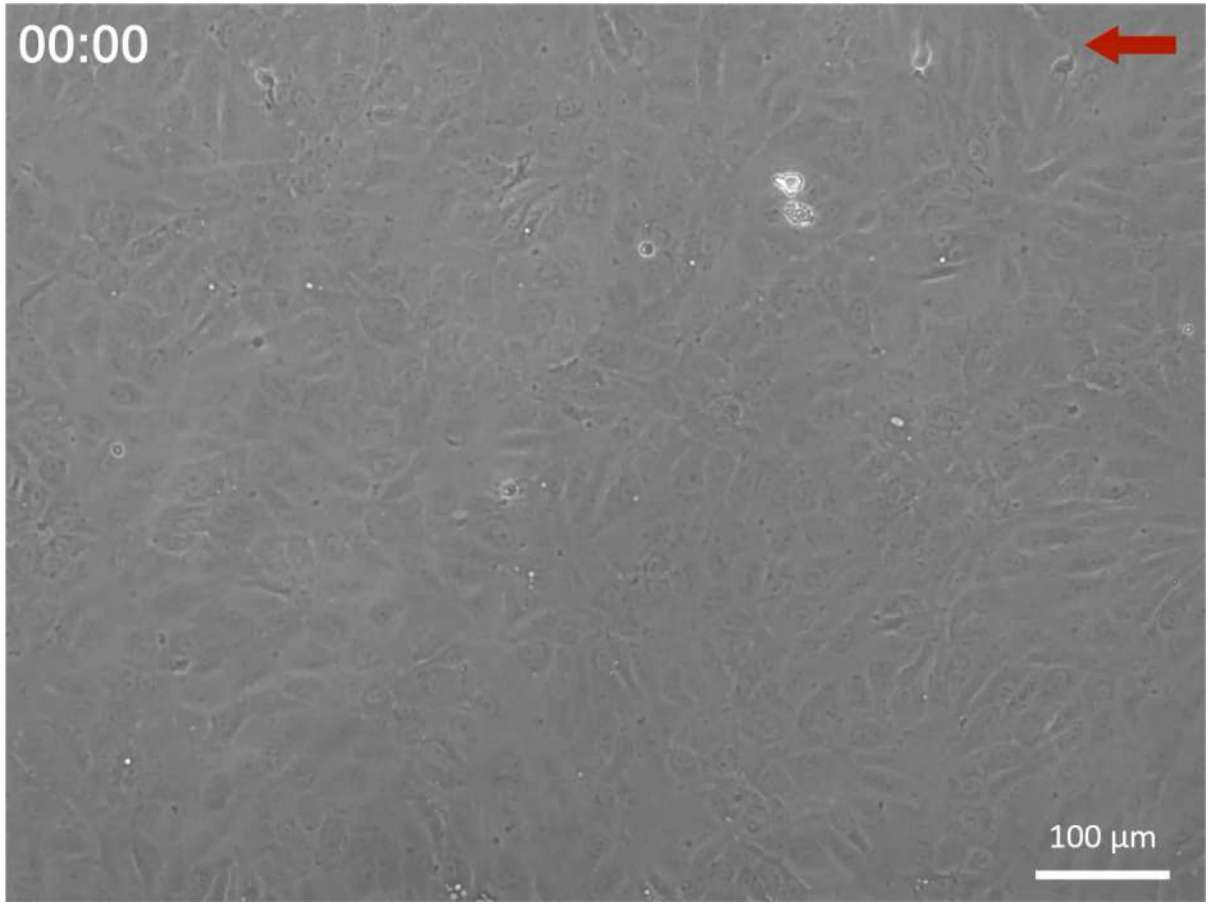


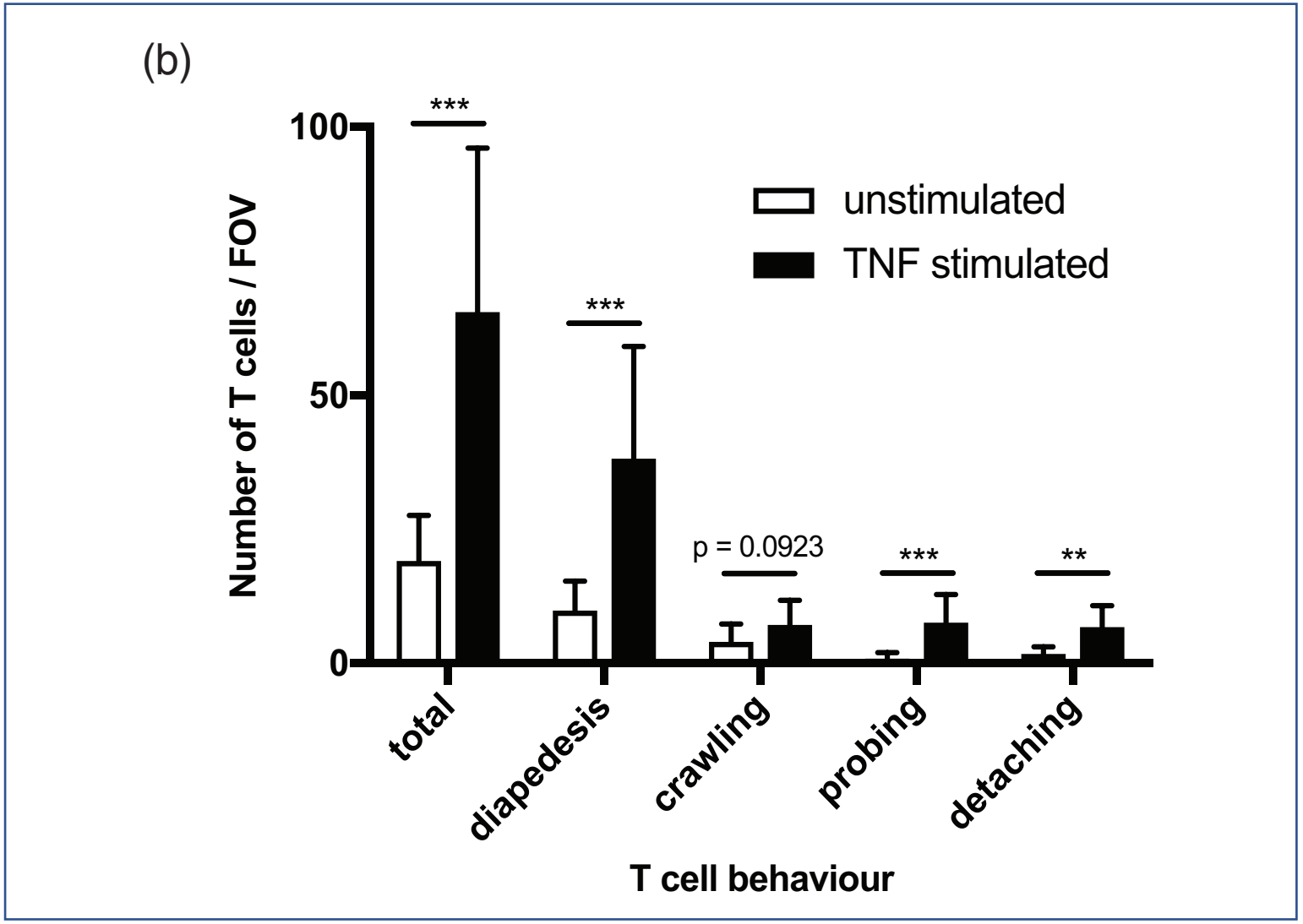
Cellular and molecular mechanism of interaction between T cells and BBB

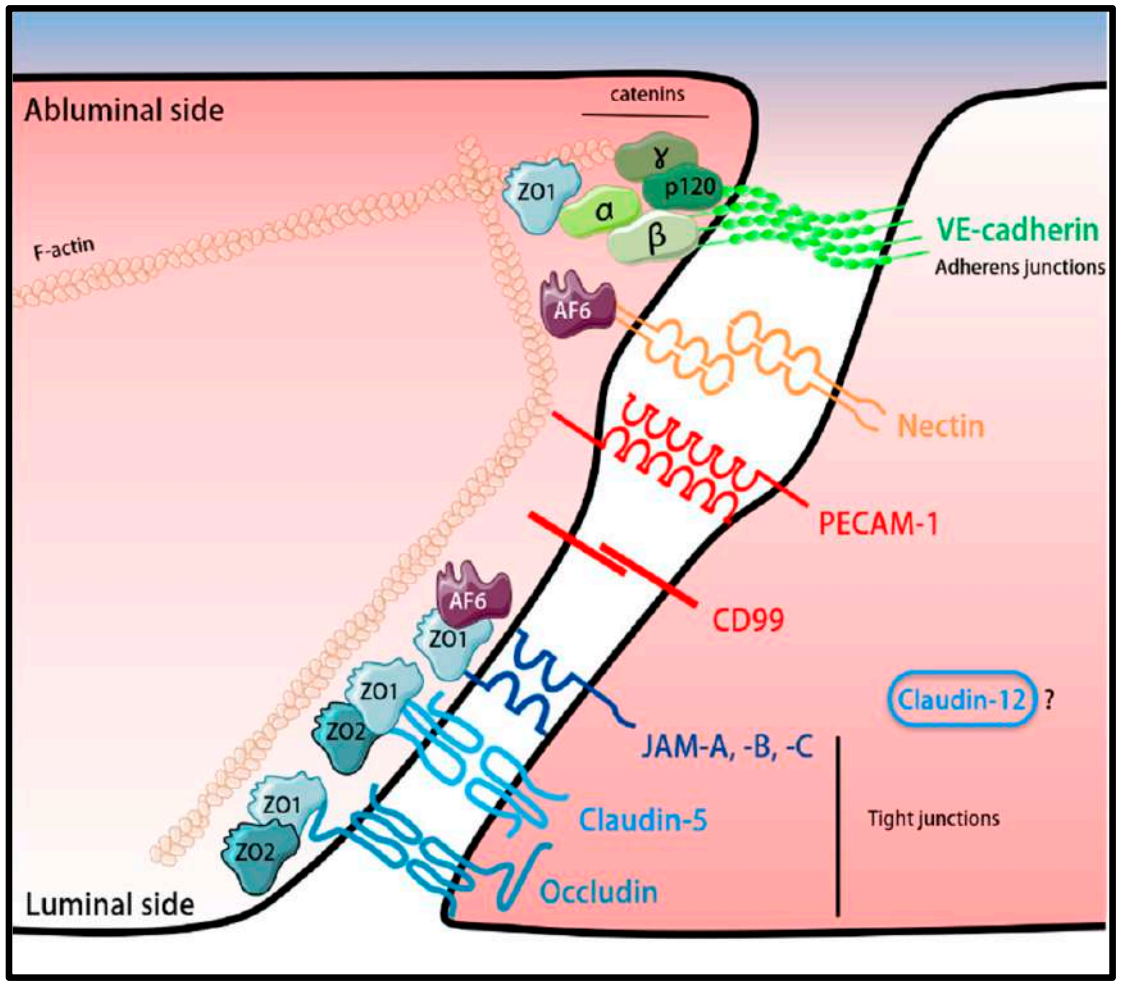
Unstimulated BBB



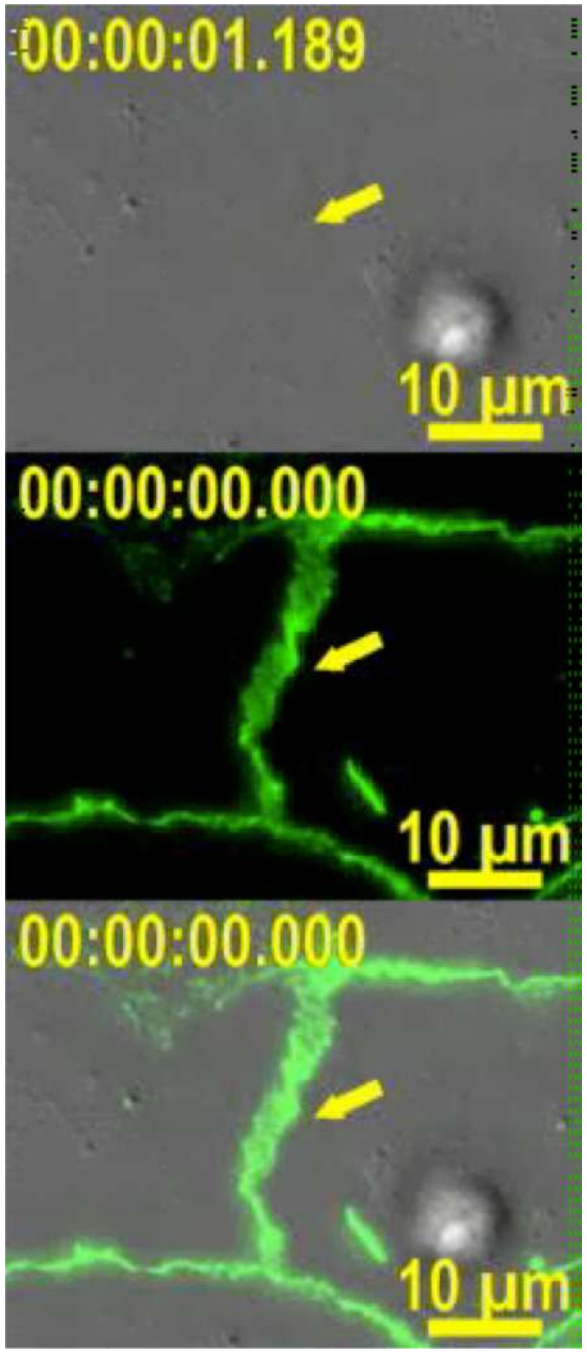
TNFα stimulated BBB



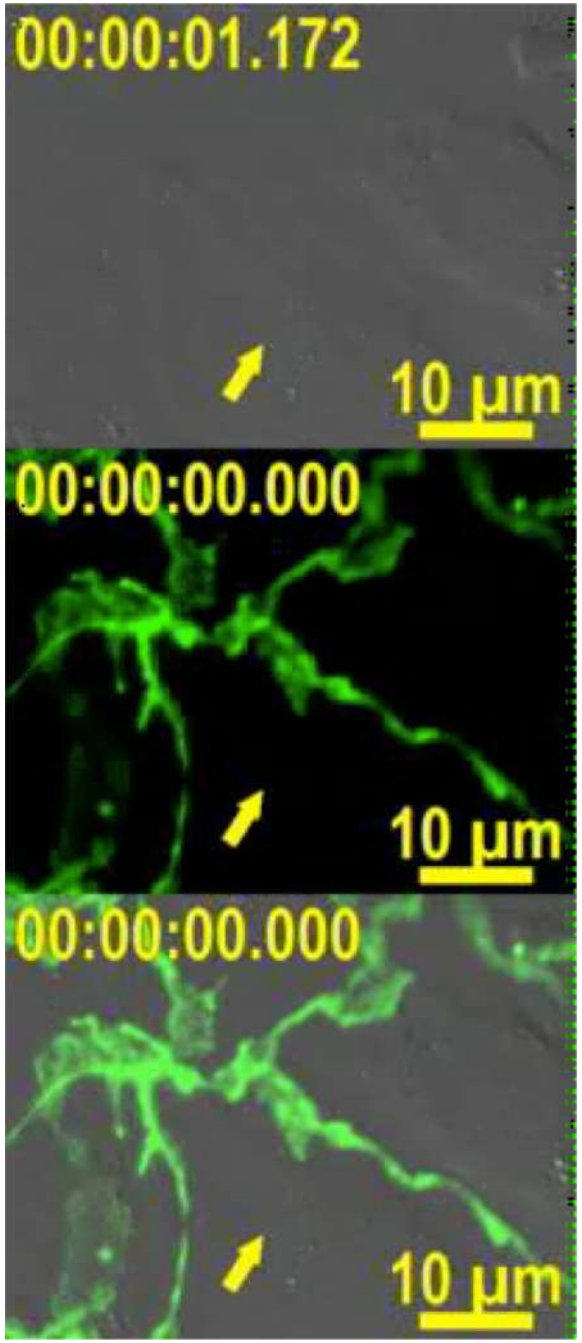

(b)
100
50
0
Number of T cells / FOV
***
***
p = 0.0923
***
**
unstimulated
TNF stimulated
total
diapedesis
crawling
probing
detaching
T cell behaviour



VE-Cadherin GFP mice



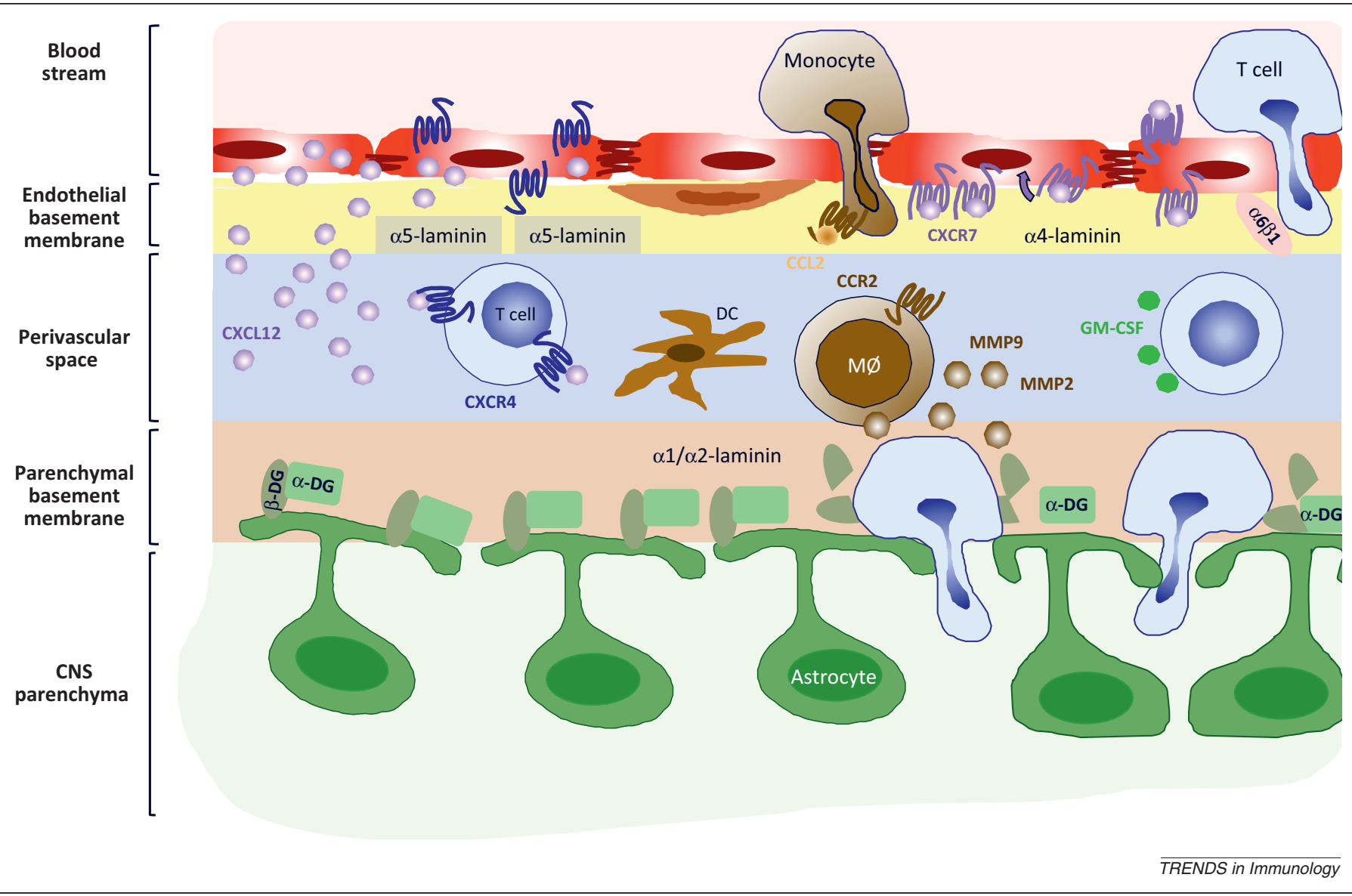
Example of paracellular diapedesis on VE-cadherin GFP-mice



Example of transcellular diapedesis on VE-cadherin GFP mice

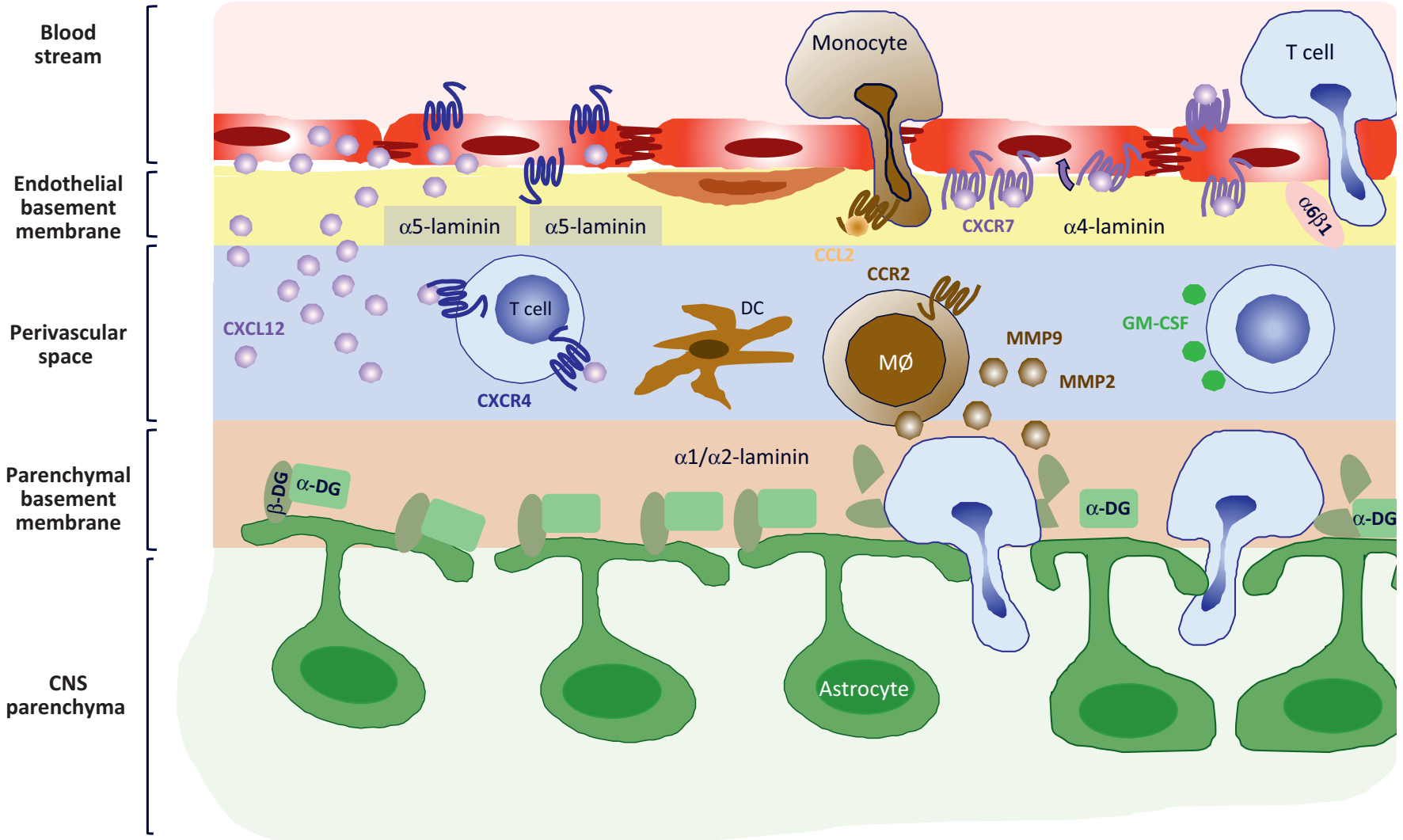
Abardier et al., 2014 EJI

Migration of immune cells into the CNS parenchyma requires penetration of a second barrier, the glia limitans



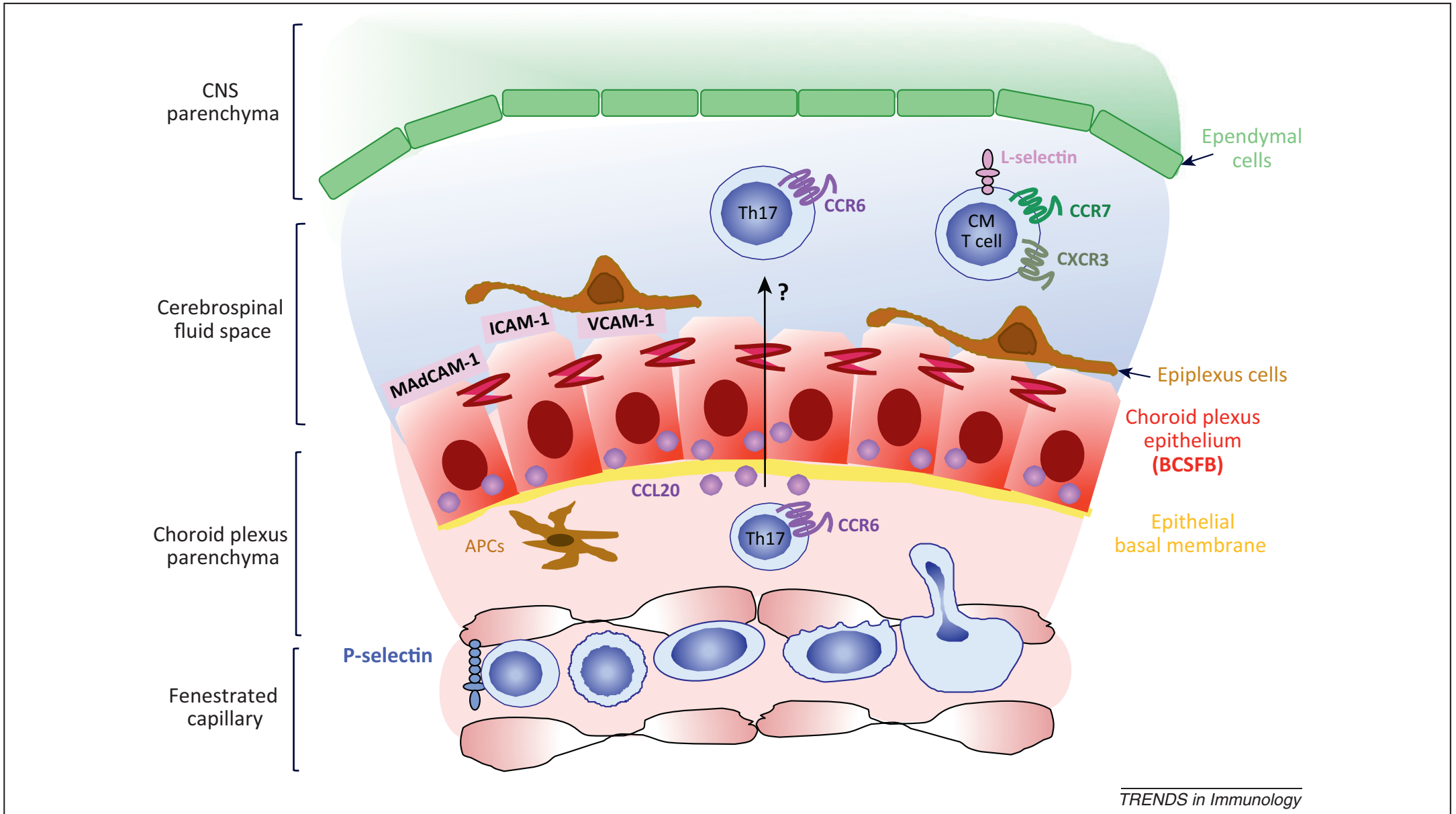
The regulatory role of MMPs might extend to modification of inflammatory chemokines released into the perivascular spaces during neuroinflammation because chemokines are physiological substrates for MMPs

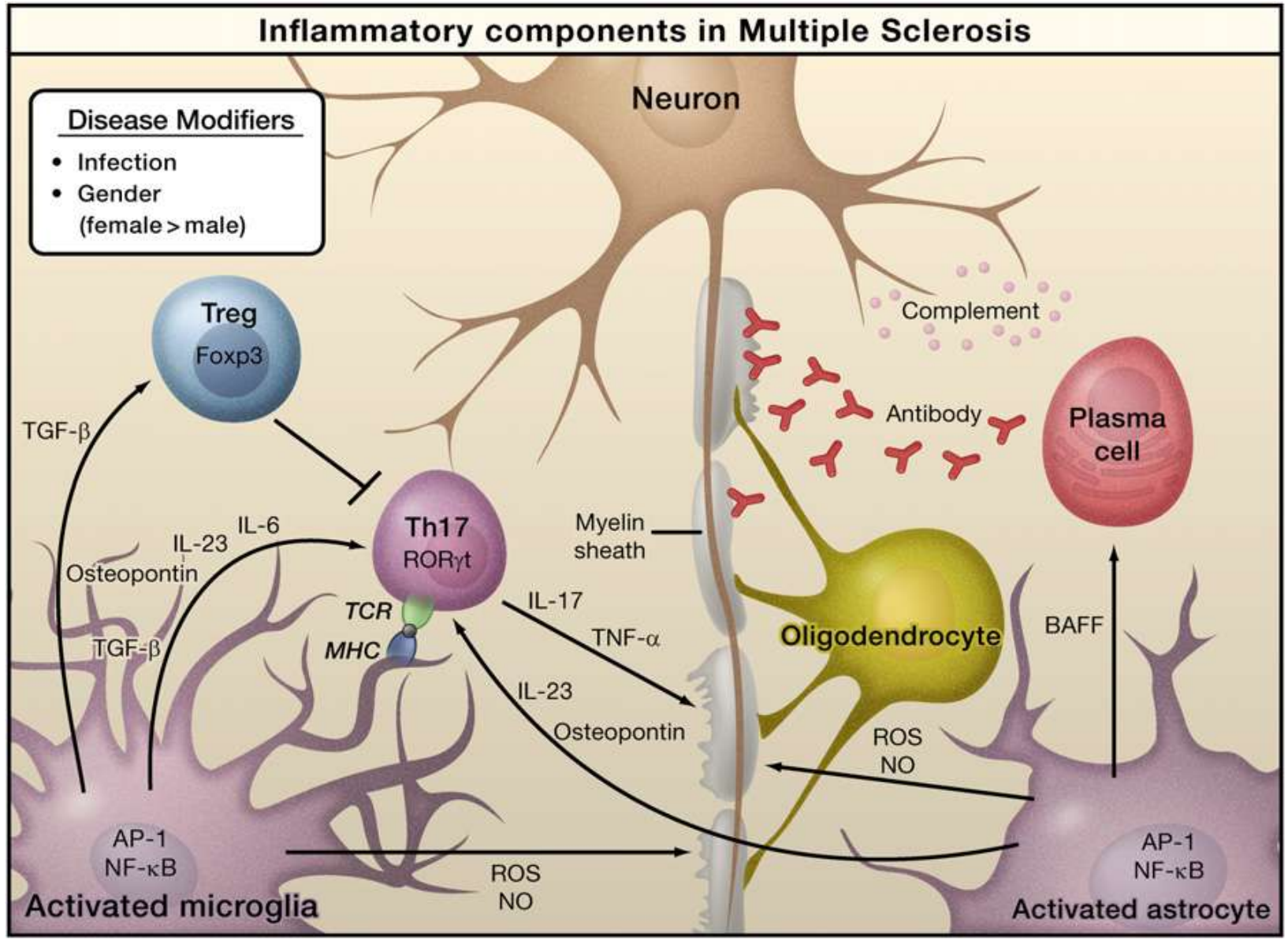
Migration of immune cells into the CNS parenchyma requires penetration of a second barrier, the glia limitans

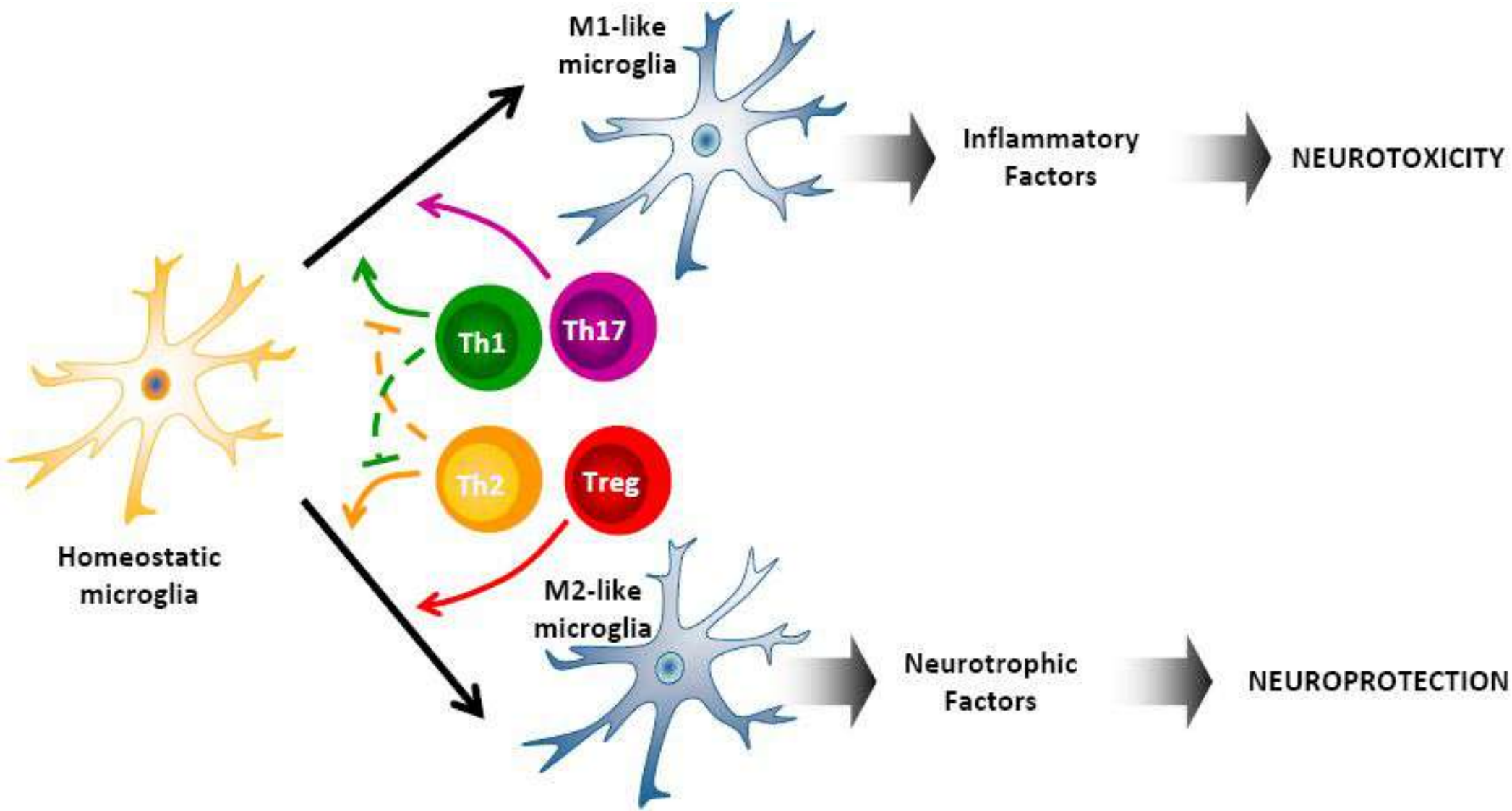


Induction of MMP-2 and MMP-9 activity is required to release immune cell migration out of the perivascular cuff, across the parenchymal basement membrane and glia limitans into the CNS parenchyma

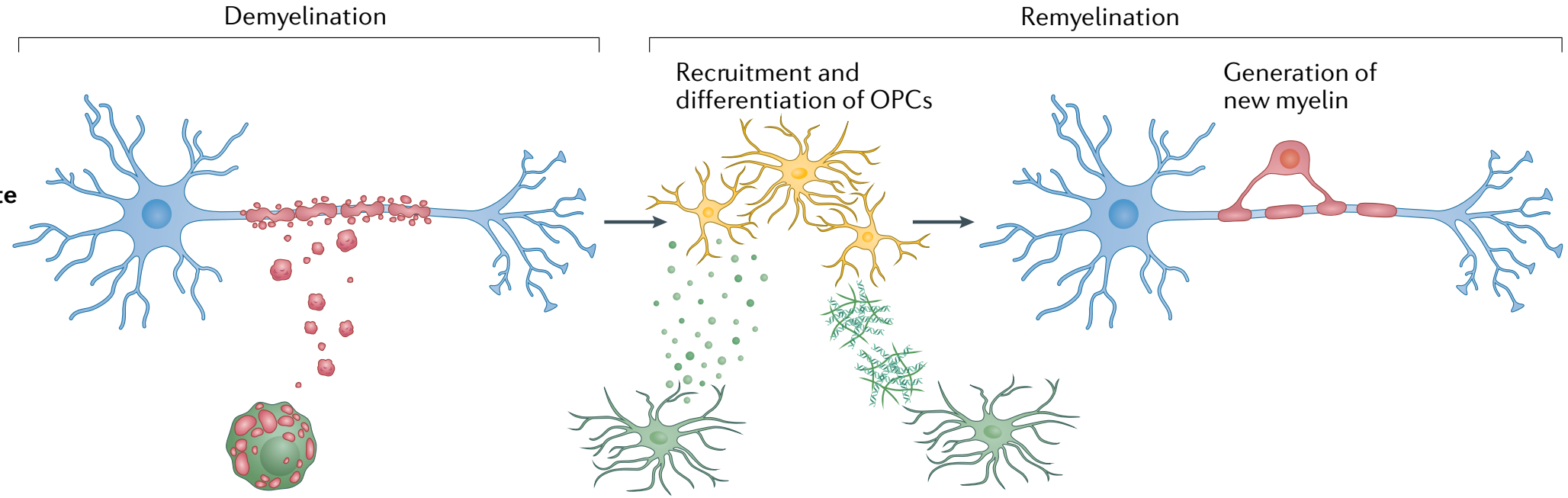
Molecular mechanisms involved in T cell migration across the epithelial blood–cerebrospinal fluid barrier (BCSFB)



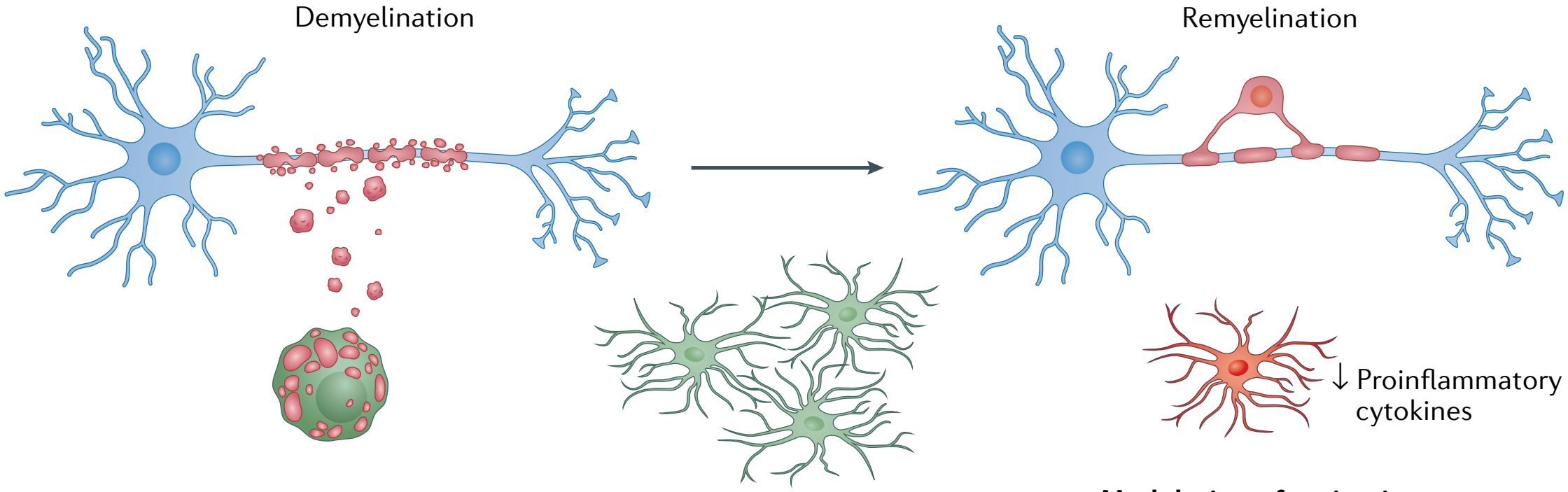

Inflammatory components in Multiple Sclerosis
Disease Modifiers
• Infection
• Gender
(female > male)
Neuron
Treg
Foxp3
TGF-β
IL-6
IL-23
Osteopontin
TGF-β
Th17
RORγt
TCR
MHC
Myelin sheath
IL-17
TNF-α
IL-23
Osteopontin
Complement
Antibody
Plasma cell
Oligodendrocyte
BAFF
ROS
NO
ROS
NO
AP-1
NF-κB
Activated microglia
AP-1
NF-κB
Activated astrocyte


M1-like
microglia
Inflammatory
Factors
NEUROTOXICITY
Th1
Th17
Th2
Treg
Homeostatic
microglia
M2-like
microglia
Neurotrophic
Factors
NEUROPROTECTION

Pro-remyelination functions of microglia



Direct and indirect effects of pro-remyelination drugs on microglia responses



Phagocytosis of myelin debris

- M-CSF
- RXR stimulation
- Glatiramer acetate
- IgM

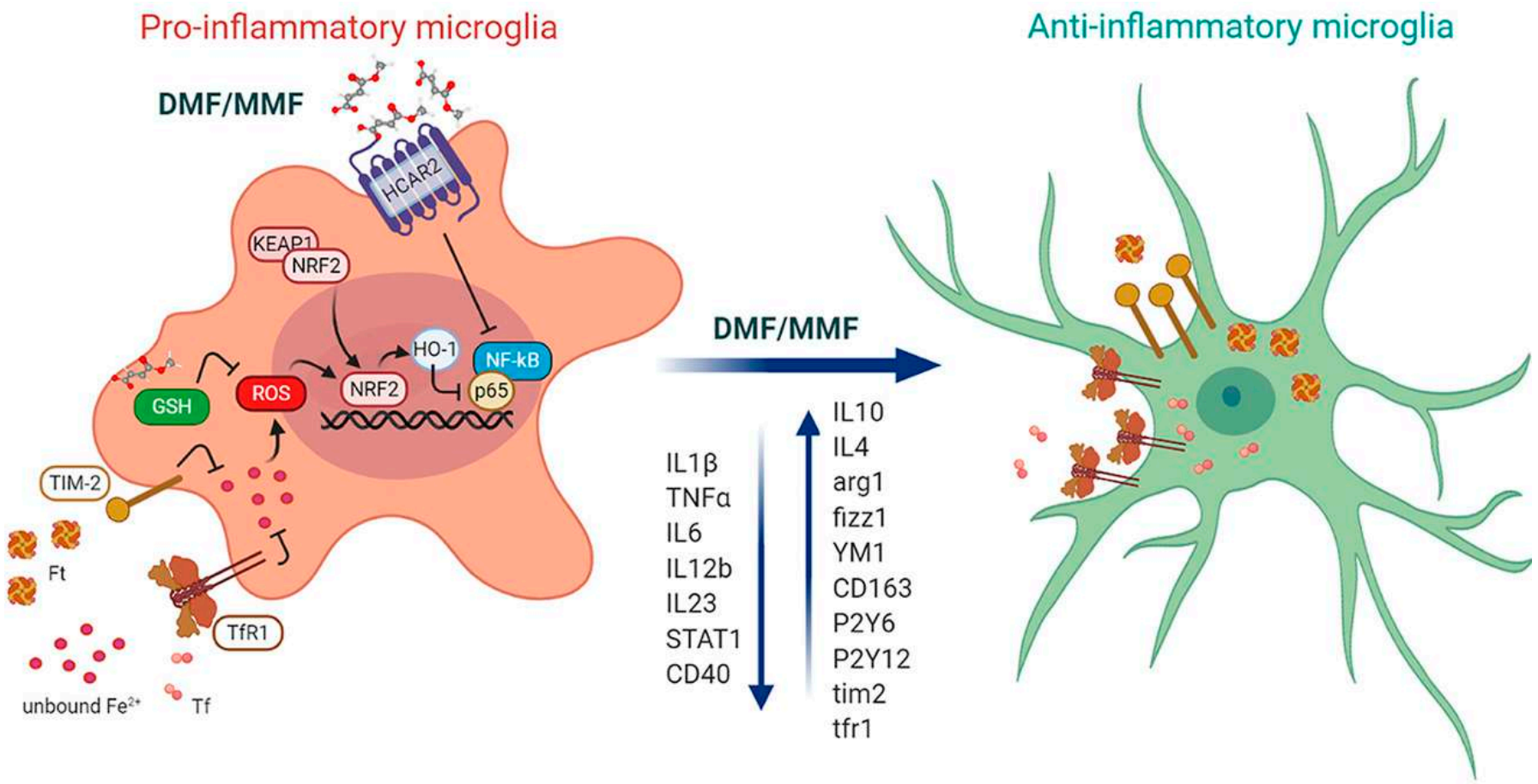
Recruitment and proliferation

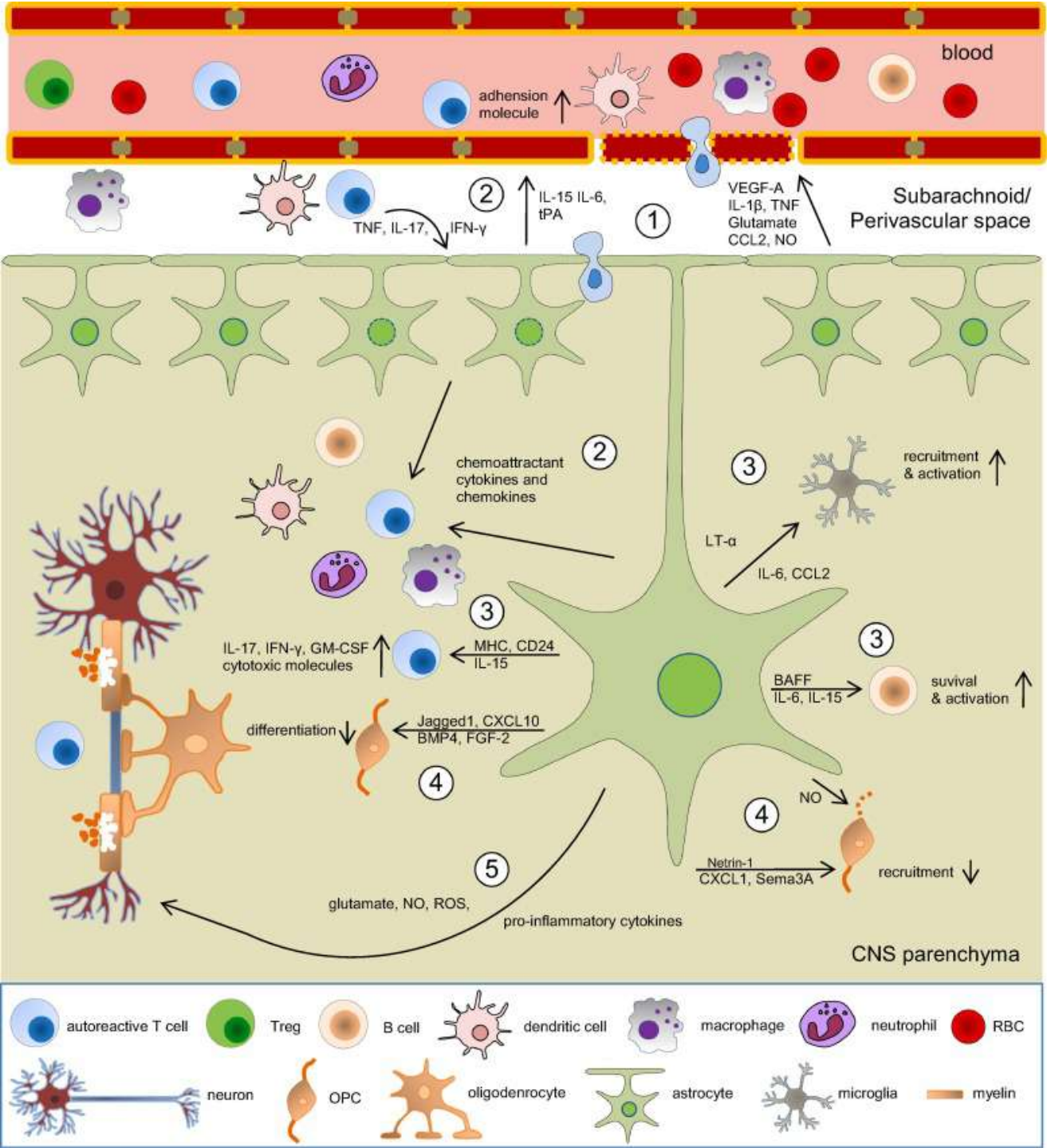
- Amphotericin B + M-CSF
- Thyroid hormone

Modulation of activation and inflammation

- Fingolimod
- Minocycline
- Quetiapine fumarate
- Clemastine

Direct and indirect effects of pro-remyelination drugs on microglia responses



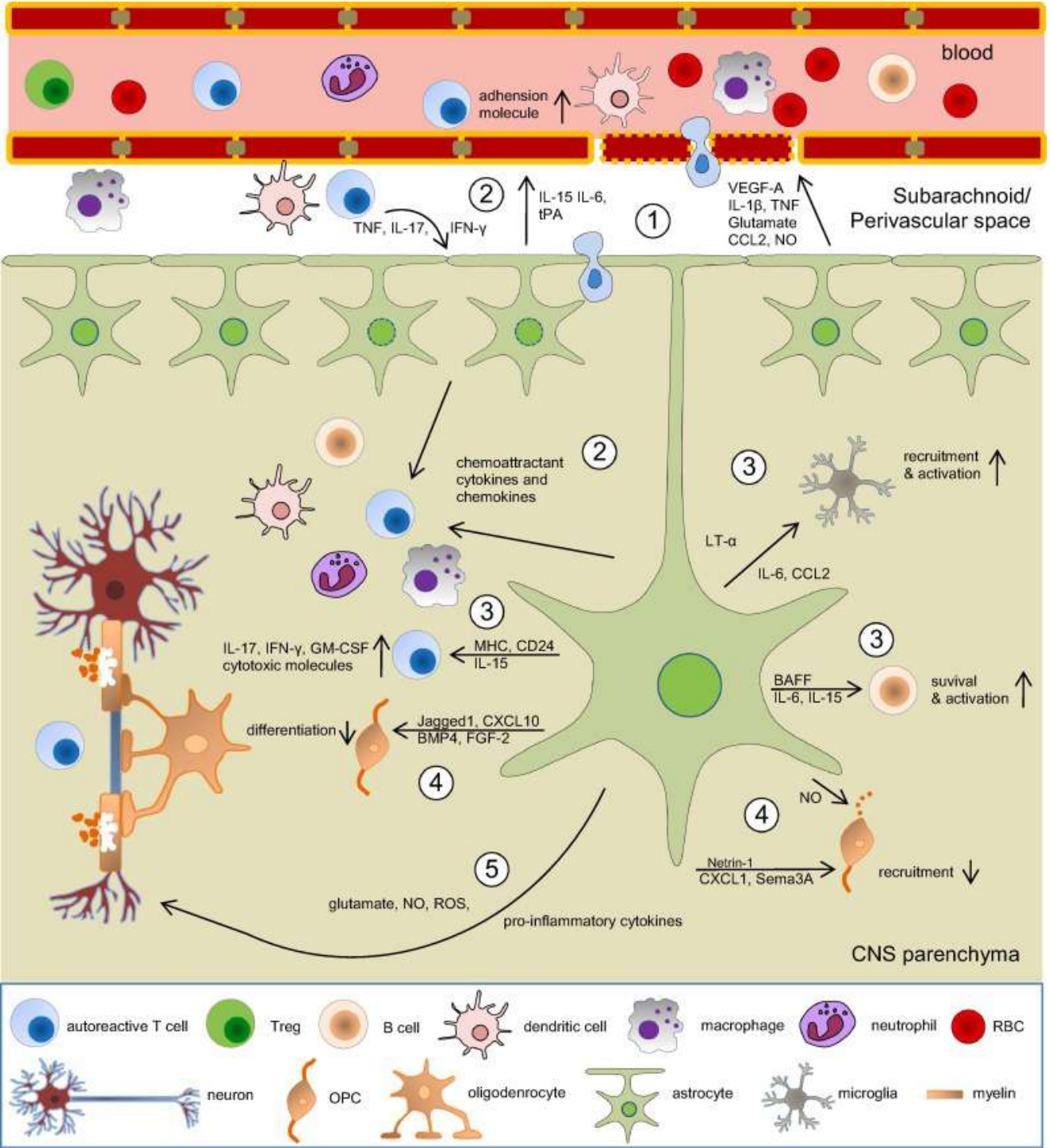


Detrimental aspects mediated by astrocytes in MS

1_ The physical barriers composed of the BBB and glia limitans are the first line of defense against immune attacks. Soluble factors released by reactive astrocytes promote apoptosis of ECs and downregulate junction proteins on their surface, causing the breakdown of the BBB.

Besides, activated astrocytes lose end feet around small vessels, leading to the disruption of glia limitans.

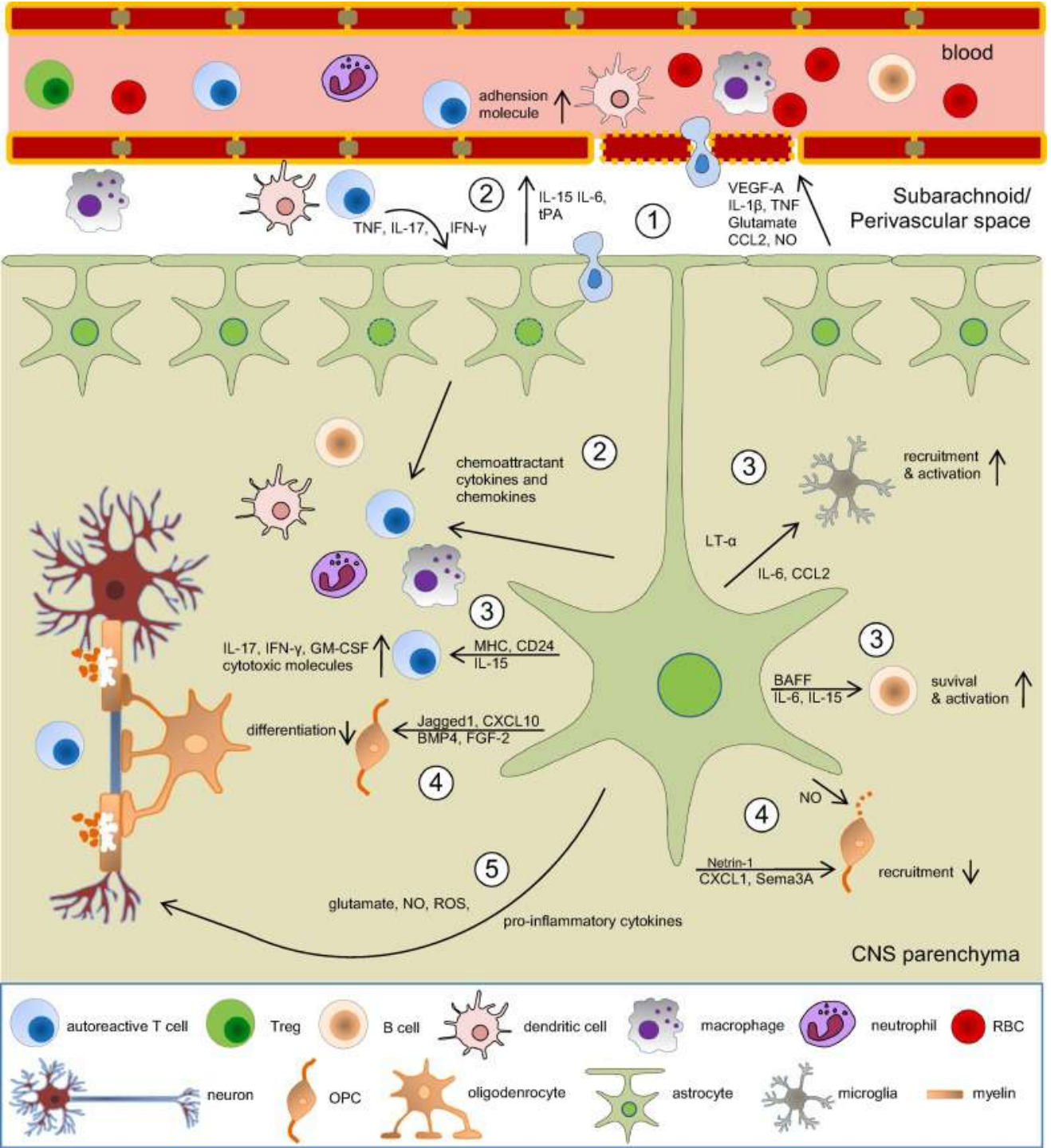
Detrimental aspects mediated by astrocytes in MS



2_Astrocytes also positively recruit leukocytes to the CNS by producing chemoattractant molecules and increasing adhesion molecules on ECs.

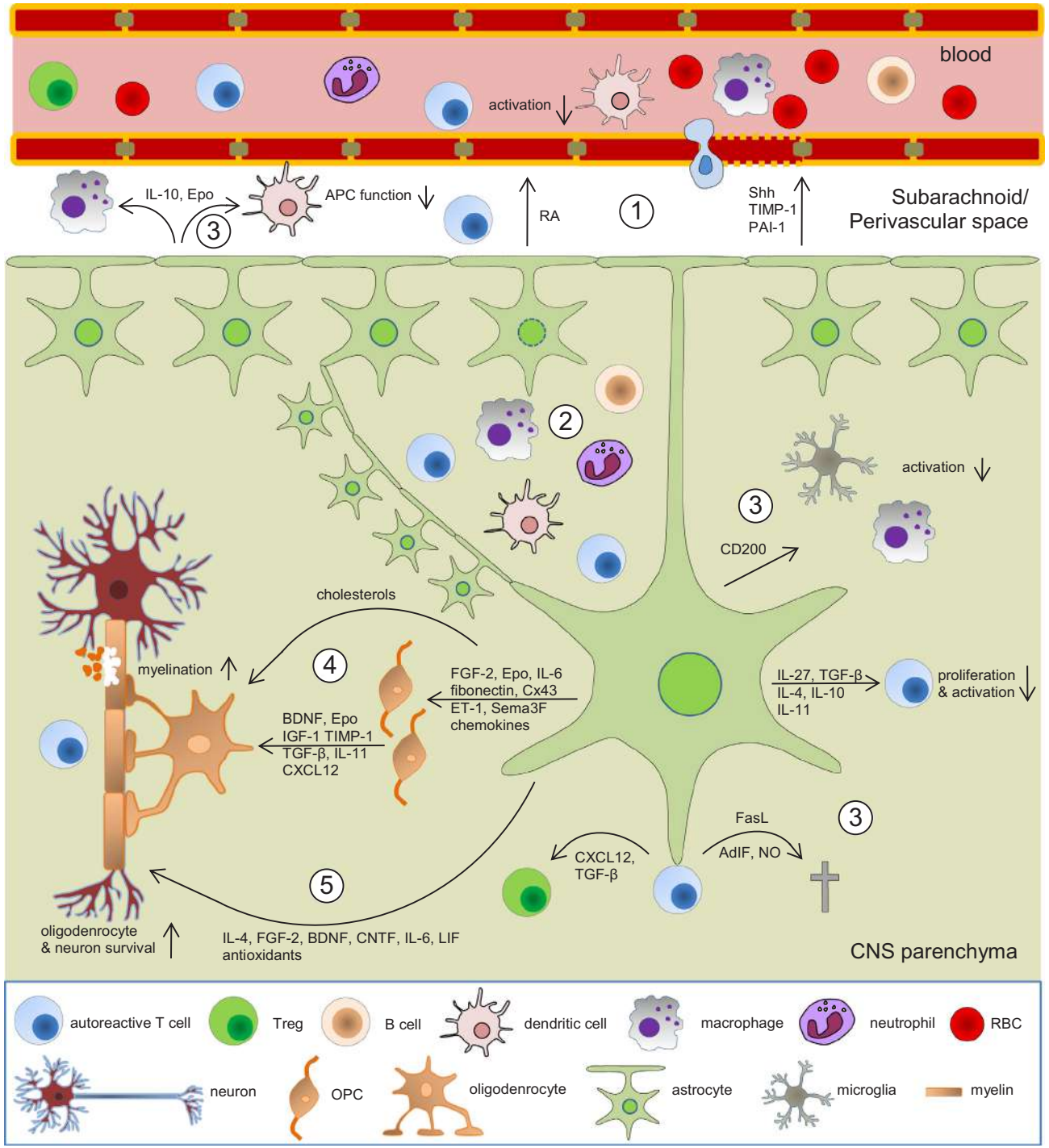
3_The recruited leukocytes and CNS-resident microglia are further activated by astrocytes to be more effective in damaging myelin and neurons.

Detrimental aspects mediated by astrocytes in MS



4_ In addition to facilitating demyelination, astrocytes inhibit remyelination by inhibiting the recruitment, differentiation and survival of OPCs.

5_ Last but not least, astrocytes aggravate the disease by directly causing axonal and neuronal damage.

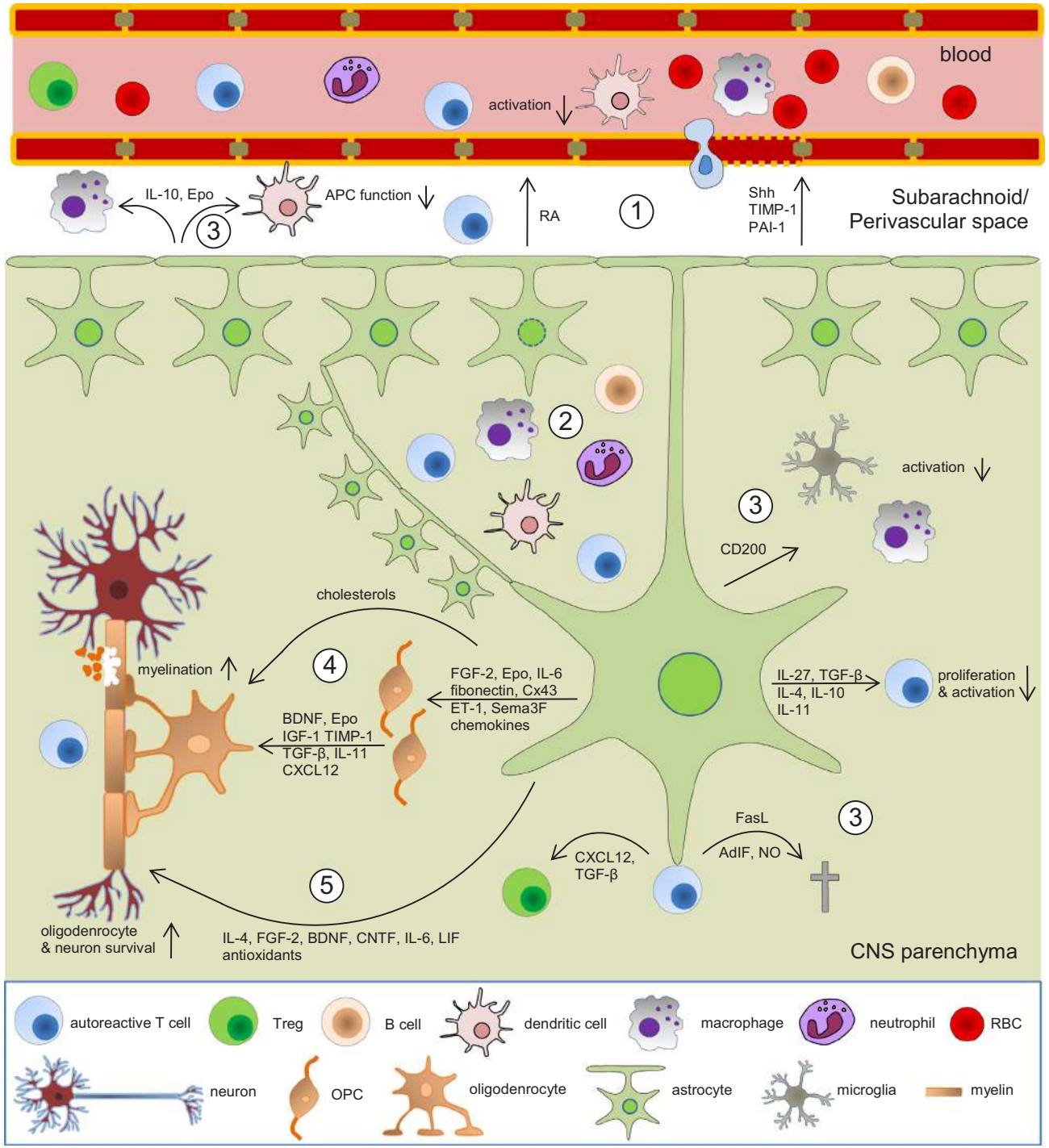


Protective aspects mediated by astrocytes in MS

1_ Astrocyte-derived factors reduce the inflammation-induced activation of ECs and preserve the expression of junction proteins, thereby reducing the adhesion of immune cells on ECs and restoring the BBB property.

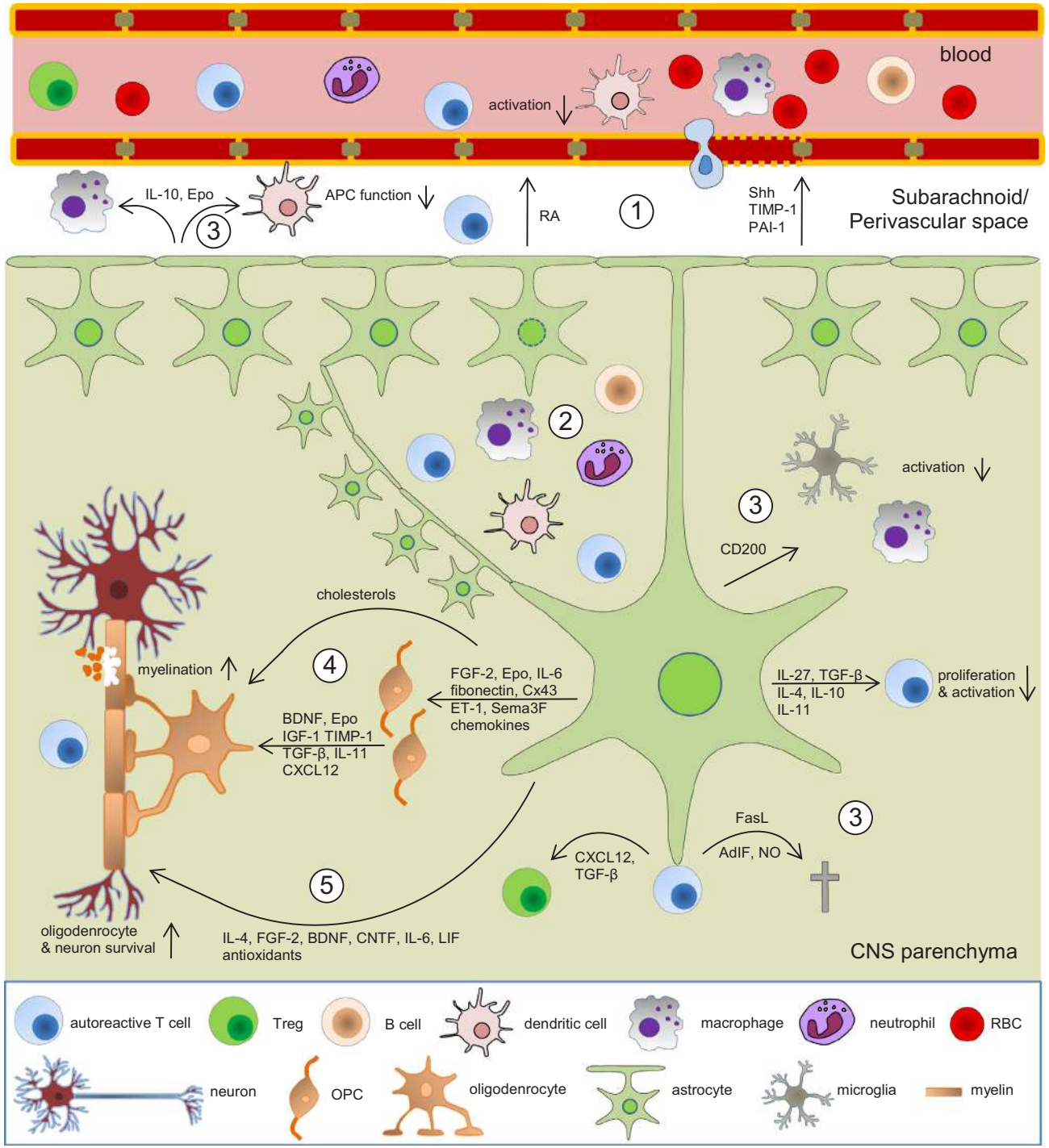
2_ At chronic lesions, astrocytes form glial scar to restrain the spread of leukocytes and inflammation.

Protective aspects mediated by astrocytes in MS



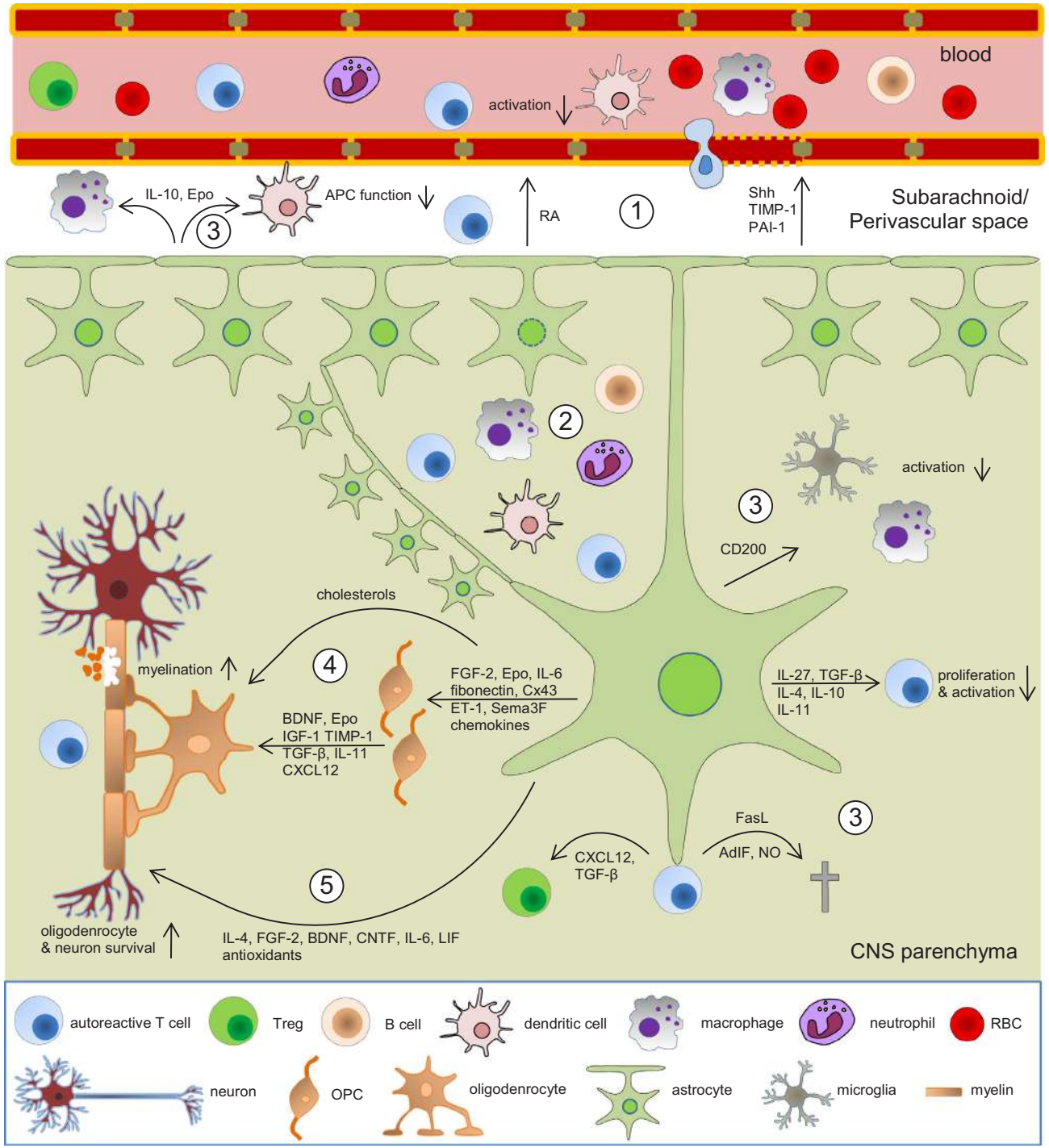
3_ In addition to limiting the invasion of leukocytes, astrocytes also restrain their activity in the CNS. Astrocytes inhibit the activity of infiltrating T cells in many ways, including polarizing autoreactive T cells to a regulatory phenotype and inducing the apoptotic elimination of encephalitogenic T cells.

Protective aspects mediated by astrocytes in MS



4_ Astrocytes have been found to promote the remyelination by facilitating the migration, proliferation and differentiation of OPCs. Besides, astrocytes enhance the myelin producing ability of oligodendrocytes by producing cholesterols.

Protective aspects mediated by astrocytes in MS



5_the neuronal function is safeguarded by astrocytes, which support the survival of oligodendrocytes and neurons by releasing neurotrophic, anti-inflammatory, and anti-oxidative factors.