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OPINION

Orchestrated leukocyte recruitment to immune-privileged sites: absolute barriers versus educational gates

Ravid Shechter, Anat London and Michal Schwartz

Abstract | Complex barriers separate immune-privileged tissues from the circulation. Here, we propose that cell entry to immune-privileged sites through barriers composed of tight junction-interconnected endothelium is associated with destructive inflammation, whereas border structures comprised of fenestrated vasculature enveloped by tightly regulated epithelium serve as active and selective immune-skewing gates in the steady state. Based on emerging knowledge of the central nervous system and information from other immune-privileged sites, we propose that these sites are endowed either with absolute endothelial-based barriers and epithelial gates that enable selective and educative transfer of trafficking leukocytes or with selective epithelial gates only.

Leukocytes perform continuous immunosurveillance and are recruited, upon need, to the tissue in an immediate, direct and regulated process[1]. However, several tissues, such as the brain, eye, implanted uterus and testis, are considered immune-privileged sites. The concept of immune privilege was initially thought to reflect physical blockade of leukocyte entry mediated by restrictive barriers, but it was later accepted as an active mechanism that facilitates immune tolerance and ignorance at these sites.

Complex barriers separate immune-privileged tissues from blood-derived soluble factors, which were assumed to imply exclusion of leukocyte entry. Thus, leukocyte infiltration to immune-privileged organs was viewed as a negative result of barrier breakdown. However, leukocytes, such as neutrophils, are capable of passing through epithelial barriers *in vitro* by restructuring tight junctions[2], and trafficking across barriers occurs at non-inflamed immune-privileged sites[3], thus refuting barrier breakdown as the sole mechanism of cell entry at immune-privileged sites. Indeed, homeostatic cell trafficking at these sites is shown by the continuous leukocyte surveillance of the cerebrospinal fluid (CSF)[3], the ocular aqueous humour[4] and the straight seminiferous tubules of the testis[5,6], by the existence of maternal–fetal microchimerism[7], and by germ-cell migration across the testicular barrier during spermatogenesis[8,9]. Together, these observations encouraged us to re-visit immunological features and physiological roles of the barriers surrounding immune-privileged sites.

Here we propose that the barrier system that controls access of immune cells to immune-privileged organs is composed of either endothelial barriers and epithelial 'gates' or of selective epithelial gates only. These barriers and gates are distinguished by the junctions formed by their cellular elements (endothelial versus epithelial cells), by their anatomical location (deep parenchyma versus peripheral borders) and by their immune-skewing capacities. We propose that they serve opposite but complementary roles in the steady state; the endothelial barrier, which we propose is an absolute immunological barrier, blocks parenchymal leukocyte entry, whereas the 'permissive' epithelial gates enable selective immune cell trafficking. This does not imply that the former is impermeable to leukocyte crossing but rather that such crossing constitutes a hazard to the tissue. By contrast, the epithelial gates provide active immune-regulating mechanisms aimed at skewing immune cells towards specific effector responses, which are characterized by regulatory T (T_{Reg}) cells, T helper 2 (T_H2) cells and alternatively activated macrophages (M2 macrophages), rather than complete immune suppression.

This model acknowledges that loss of barrier control can lead to pathological leukocyte invasion and that some pathological conditions, predominantly those with inflammatory aetiology, rewrite the rules of cellular recruitment and education. Notably, much of the available evidence comes from pathological or inflammatory conditions that may not reflect the physiological situation, thus contributing to the misperception of these barriers. Here, by examining several immune-privileged sites, we focus on the underlying mechanisms that differentially operate in the two barrier systems.

The barrier systems of the CNS

The central nervous system (CNS) barrier systems include the blood–brain barrier (BBB), which encompasses deep parenchymal microvessels, and the blood–CSF barrier (BCSFB), which encases the choroid plexus, a CSF-producing structure localized within the brain ventricles (reviewed in REFS 3,10). The meninges, which cover the superficial CNS surface, connect these structures.

The BBB is an endothelial barrier comprised of low pinocytotic endothelial cells that are interconnected by tight junctions. This endothelial lining, in its abluminal aspect, is ensheathed by the glia limitans perivascularis, an astroglial endfeet structure[3,10]. With the exception of CNS capillaries, where they are fused, the in-between endothelial and parenchymal basement membranes delimit the perivascular space, which is narrow under physiological conditions. These CSF-filled perivascular spaces open towards the leptomeningeal subarachnoid space on the surface of the brain and spinal cord. Meningeal vasculature comprises a monolayer of non-fenestrated endothelium containing tight junctions but lacks direct astroglial endfeet ensheathment. Instead, the leptomeninges is separated from the parenchyma by a lining of glia limitans superficialis. Meningeal microvessels have erroneously been considered as part of the BBB, but they are now defined as the blood–leptomeningeal barrier (BLMB), which separates the leptomeningeal space from the circulation[10].

By contrast, the BCSFB is an epithelium-based structure. The main compartment of the BCSFB — the choroid plexus — is composed of a network of fenestrated capillaries that contain intracellular gaps and lack tight junctions and astroglial endfeet. In turn, the choroidal stroma is covered by a tight junction-interconnected cuboidal epithelial lining[3,10]. The choroid plexus creates a villous structure in the brain ventricular lumen, and its epithelial apical membrane has numerous microvilli and cilia. The CSF is secreted from the choroidal epithelium and circulates from the CSF-filled brain ventricles to the meningeal subarachnoid space; no CSF circulation occurs within the choroid plexus (FIG. 1).

The BBB as a true barrier. Several features of the BBB support its nature as a true barrier that restricts leukocyte migration in the steady state. These include: the production of sonic hedgehog (SHH) and the endothelial expression of its receptor, which suppresses T cell migration and production of pro-inflammatory mediators[11]; endothelial expression of interleukin-25 (IL-25), which prevents inflammatory cytokine-induced BBB collapse and increases the expression of tight junction proteins[12]; and the abluminal endothelial expression of CXC-chemokine ligand 12 (CXCL12) (this polarized expression is lost under some pathologies[13,14]), which prevents parenchymal invasion of CXC-chemokine receptor type 4 $(CXCR4)^+$ leukocytes[13,14]. In addition, the lack of selectin ligands on BBB endothelium hinders T cell tethering and rolling[10]. Thus, although the lymphoid chemokine CC-chemokine ligand 19 (CCL19) (but not CCL21) is constitutively expressed by endothelial cells in mouse and human brains[15], it is unlikely to facilitate leukocyte transmigration under steady state conditions, as the slowing down of these cells is a prerequisite for CC-chemokine receptor 7 (CCR7) engagement of CCL19.

These features do not imply that such a barrier is leukocyte-sealed under all circumstances: ample evidence indicates both T cell and myeloid cell migration across the BBB at the CNS post-capillary (but not capillary) venules under inflammatory conditions[10]. Under inflammatory conditions, the BBB loses some of its restrictive features[11–14] and facilitates T cell infiltration by luminal expression of MHC molecules and the activation of vessel-associated antigen-presenting cells (APCs)[16,17]. The inflamed BBB endothelium also upregulates the expression of selectins and integrins (the involvement of which in the multistep extravasation process is described in detail in REF. 10) and, together with the lymphoid chemokines CCL19 and CCL21, facilitates integrin-mediated arrest and subsequent transmigration of $CCR7^+CXCR3^+$ T_H1 cells[15]. Additionally, inflamed BBB endothelium exhibits increased expression of pro-inflammatory mediators (such as tumour necrosis factor (TNF), IL-1β, IL-6, IL-8 and prostaglandins) that modulate BBB permeability and the phenotype of the crossing leukocytes[18]. The inflamed BBB endothelium secretes CCL2 (REF. 19), which recruits $LY6C^{hi}$ inflammatory monocytes. Inflamed human BBB produces granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factor-β (TGFβ) and IL-6, which together skew the recruited $CD14^+$ monocytes towards a pro-inflammatory phenotype[18]. Such monocyte-derived cells secrete TGFβ1, IL-6 and IL-12p70, thereby promoting the differentiation of $CD4^+$ T cells to pathogenic T_H17 cells and interferon-γ (IFNγ)-secreting T_H1 cells[18].

BBB crossing involves the focal accumulation of immune cells between the inner-endothelial and the outer-parenchymal basement membranes, thus distending the space between them and forming structures called perivascular cuffs. The accumulation and activation of leukocytes in these cuffs is essential for their final parenchymal entry[16,17], which also necessitates glia limitans breaching by gelatinase activity, such as that mediated by matrix metalloproteinase 2 (MMP2) and MMP9 (REF. 20), and is followed by pathological clinical manifestations[20].

Despite intensive studies, there is no evidence for leukocyte-skewing towards an alternatively activated phenotype by the BBB. Moreover, there is no evidence for BBB cuffing in the non-pathological state or in cases of 'healing' immune cell infiltration[21], thus supporting the notion that the BBB can be considered as an absolute barrier that should remain sealed and that breaching of the BBB is hazardous to the tissue (FIG. 1a).

The BCSFB as a controlled gate. In contrast to the BBB, the BCSFB allows for immunosurveillance of the CSF under normal conditions through the choroid plexus, indicating the permeability of this barrier to cellular elements (reviewed in REFS 3,10). The similarity in T cell composition of ventricular and lumbar CSF supports the notion that leukocytes enter the CSF through the ventricular choroid plexus[22].

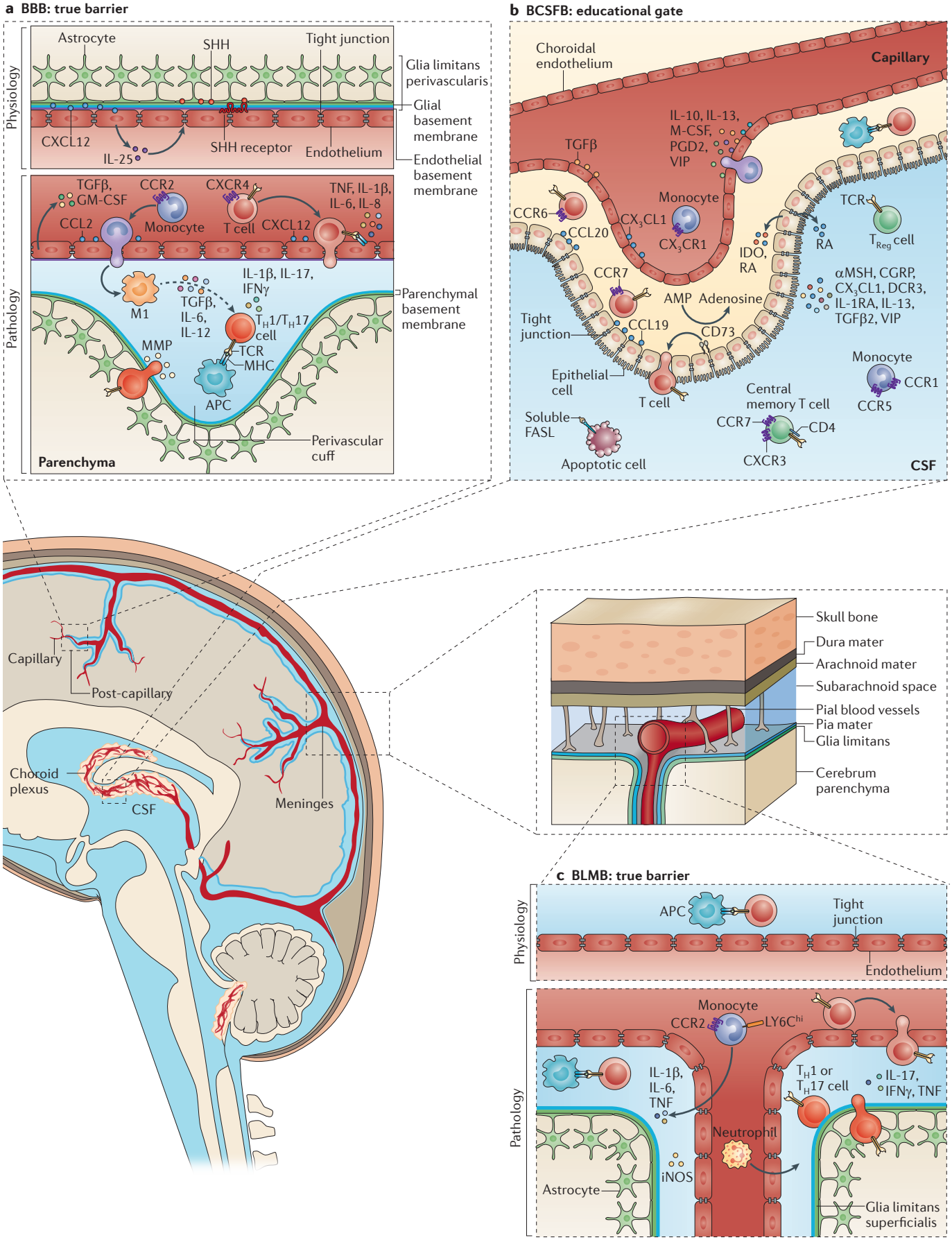
The unique cellular composition at this gate reflects its selective nature. The mouse choroid plexus is constitutively populated with $CD4^+$ effector memory T cells with a T cell receptor (TCR) repertoire specific for CNS antigens[23]. The cellular composition of the healthy human CSF is distinct from that of peripheral blood; it is almost devoid of neutrophils, but it includes a small subset of $CCR1^+CCR5^+$ monocytes[3,10] and is dominated by $CD4^+CD45RO^+CD27^+$ memory T cells that express CXCR3 and CCR7 (REF. 24), possibly responding to CCL19 expressed by choroid endothelial cells[25]. Selectin-mediated rolling and integrin-dependent adhesion on choroidal vessels may mediate the slowing down of these $CCR7^+$ lymphocytes, allowing them to sense CCL19, which further facilitates their transendothelial migration. About 40% of CSF T cells express the recent activation marker CD69 (REFS 3,10).

In addition to enabling selective recruitment, the BCSFB and the CSF bathing it are able to skew immune cells towards certain responses. Human CSF suppresses proliferation and IFNγ production by effector T cells[26] and downregulates neutrophil oxidative burst[27]. Furthermore, mouse CSF inhibits the development of cytotoxic T lymphocytes (CTLs)[28]. The underlying mechanism of immune-skewing by the CSF was initially attributed to the anti-inflammatory cytokine TGFβ[26,28] and later to numerous other potent anti-inflammatory and immunomodulatory factors, including α-melanocyte stimulating hormone (αMSH)[29], vasoactive intestinal peptide (VIP)[26], calcitonin gene-related peptide (CGRP)[30], prostaglandin D2 (PGD2)[31], IL-1 receptor antagonist (IL-1RA)[32,33] and decoy receptor 3 (REF. 34). Mouse CSF is constitutively predisposed towards a wound-healing and pro-resolving milieu, which is characterized by high levels of the T_H2 cell- and M2 macrophage-skewing cytokines IL-13 and TGFβ2 and almost undetectable levels of specific pro-inflammatory cytokines[21]. Under conditions of sterile injury[21] and subarachnoid haemorrhage[27], the CSF maintains and even increases its anti-inflammatory nature, whereas in other situations, such as in Alzheimer's disease and West syndrome[32,33], some anti-inflammatory factors are lost, although without an increase in pro-inflammatory factors.

The choroid plexus is suggested here to function as an active immunomodulatory gate, rather than an inert barrier. It is enriched with anti-inflammatory CX_3C-chemokine receptor 1 $(CX_3CR1)^{hi}$ $LY6C^{low}$ monocytes[21] and constitutively expresses the CX_3CR1 ligand, CX_3CL1 (REF. 35), which is crucial to the recruitment and survival of these monocytes. Its epithelium constitutively expresses ecto-5′-nucleotidase (also known as CD73)[36], which converts the pro-inflammatory ATP-metabolite AMP into the anti-inflammatory molecule adenosine[37] and the adenosine receptor A2AAR[38]. Furthermore, it continuously expresses the enzyme indoleamine-2,3-dioxygenase (IDO)[39] (which catalyses tryptophan synthesis, thereby inhibiting T cell activity) and metabolic enzymes of retinoic acid[40] (which skew effector T cells towards a regulatory phenotype and inhibit inducible nitric oxide synthase (iNOS) activity). The healthy choroid plexus epithelium also expresses IL-10, IL-13 and macrophage colony-stimulating factor (M-CSF)[21], and the choroidal endothelium constitutively expresses TGFβ[21]. A balance between the levels of IL-4 and IFNγ at the choroid plexus is evident in normal mice and is disrupted with ageing[23]. Surprisingly, choroid plexus anti-inflammatory milieu is increased following CNS trauma, despite the parenchymal pro-inflammatory burst[21]. This does not necessarily reflect immune tolerance but rather represents, in our view, immune-skewing towards an active immunoregulatory response.

While entering the CSF, leukocytes do not invade the parenchyma in the steady state[3]. However, in various pathological conditions, the choroid plexus serves as a permissive gate for leukocyte parenchymal entry. Epithelial expression of CD73 and adenosine receptor is involved in parenchymal infiltration by anti-inflammatory monocytes following sterile insult, and by pro-inflammatory lymphocytes in immune-mediated pathology[21,36,38]. The choroid plexus expresses the CCR6 ligand CCL20, which mediates the initial step of CNS invasion by T_H17 cells in experimental autoimmune encephalomyelitis (EAE)[41]. This study supports a two-wave model for CNS invasion in which $CCR6^+$ autoreactive T cells enter through the choroid plexus, triggering a subsequent CCR6-independent parenchymal invasion[41]. The constitutive expression of CCL20 in the choroid plexus, even in the absence of T_H17 cells, suggests that this ligand, a known T_{Reg} cell chemoattractant[42], may also facilitate normal CNS immunosurveillance. Furthermore, M2 macrophages, which are found at the lesion site following spinal cord injury,

Figure 1 | **The CNS gating system.** The figure illustrates the true barriers and educational gates of the central nervous system (CNS) and their immunological milieu. **a** | The blood–brain barrier (BBB) is a structure containing tightly connected endothelial cells that form the vessels penetrating deep into CNS parenchyma. These post-capillary venules are ensheathed by glia limitans perivascularis. Under normal conditions (top), immunological mechanisms including abluminal endothelial expression of CXC-chemokine ligand 12 (CXCL12) and interleukin-25 (IL-25) prevent leukocyte invasion and maintain barrier sealing. Under pathological conditions (bottom), a pro-inflammatory environment develops and perivascular cuffs comprised of entrapped immune cells are formed (see main text for details), which results in parenchymal invasion by pathogenic immune cells and neurological pathology. Thus, cell crossing at this barrier is hazardous to the tissue and should be avoided. **b** | The blood– cerebrospinal fluid barrier (BCSFB) is formed by the epithelial lining of the ventricular choroid plexus, which is considered the major site of CNS immunosurveillance. This gate is equipped with regulatory and pro-resolving properties (see main text for details). **c** | The blood–leptomeningeal barrier (BLMB) is formed by tight junction-interconnected endothelium that is located in the CSF-filled subarachnoid space. Beneath the pia mater, at the edge of the parenchyma, is the glia limitans superficialis. Under normal conditions (top), this structure maintains constitutive immunosurveillance as part of the BCSFB route, whereas in pathology (below), direct leukocyte extravasation with pro-inflammatory characteristics can occur (see main text for details). αMSH, α-melanocyte stimulating hormone; APC, antigen-presenting cell; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CGRP, calcitonin gene-related peptide; CX_3CL1, CX_3C-chemokine ligand 1; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; DCR3, decoy receptor 3; FASL, FAS antigen ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IDO, indoleamine-2,3-dioxygenase; IFNγ, interferon-γ; IL, interleukin; IL-1RA, IL-1 receptor antagonist; iNOS, inducible nitric oxide synthase; M1, pro-inflammatory macrophage; M-CSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; PGD2, prostaglandin D2; RA, retinoic acid; SHH, sonic hedgehog; TCR, T cell receptor; TGFβ, transforming growth factor-β; T_H, T helper; TNF, tumour necrosis factor; T_{Reg}, regulatory T; VIP, vasoactive intestinal peptide.



are derived from monocytes that enter the CNS through the choroid plexus and then traffic along the CSF and the central canal[21]. By contrast, monocytes that become classic pro-inflammatory macrophages (M1 macrophages) enter through the spinal cord leptomeninges adjacent to the site of injury[21]. The fact that functionally distinct monocyte-derived macrophages home to injured CNS parenchyma through different entry routes supports our proposal that the route taken by leukocytes affects their fate. Thus, we suggest that the BCSFB and the CSF mediate leukocyte-skewing towards an alternatively activated or regulatory phenotype, rather than facilitating inflammation, immune ignorance or tolerance, thereby establishing an active, tissue-protective immune response (FIG. 1b).

Circumventricular organs. Although the choroid plexus is considered to be the main route of cellular infiltration into the CSF, cells can also enter the CSF through the circumventricular organs (CVOs). These BCSFB structures lack endothelial tight junctions and glia limitans but contain a network of tight junction-interconnected ependymal cells (epithelial-like cells that line the CSF-filled ventricles). Although generally overlooked, the CVOs were suggested as a site for lymphocyte and macrophage entry into the CNS and CSF during EAE[43], peripheral inflammation[44], neurocysticercosis[45] and other encephalomyelitis-like disease[46]. Lymphocytes were also suggested to 'pause' at the CVOs, where they remain ready to become activated and contribute to neurological hypertension[47]. However, the phenotype of these recruited cells and the milieu they encounter at these sites are as yet unresolved.

The meningeal crossroads. Intravenously injected fluorescently labelled T cells are detected in the leptomeningeal spaces 2 hours after infusion[48], suggesting that T cell diapedesis across the endothelial-restricted BLMB may be more efficient when compared with the BBB. However, transmigration through this structure in normal rodents seems limited and was not detected by live imaging[49]. Studies have shown that learning and memory tasks are associated with increased accumulation of IL-4-producing T cells in the meninges[50], although their entry site was not determined.

By contrast, a massive infiltration of lymphocytes and myeloid cells across the BLMB occurs in various pathologies. In a meningitis model, $CD8^+$ T cells mediate the subsequent meningeal recruitment of neutrophils and monocytes, which elicit local pro-inflammatory cytokines, barrier breakdown and vascular leakage, with fatal outcomes[51]. Encephalitogenic T cells, as well as $CCR2^+LY6C^{hi}$ monocytes, accumulate in the leptomeningeal spaces in early neuroinflammatory responses[52,53]. The onset of clinical symptoms coincides with their subsequent parenchymal invasion across the glia limitans superficialis, a step requiring antigen recognition by lymphocytes[17,53]. In mice with EAE, administration of a CXCR7 inhibitor, which blocks leukocyte parenchymal entry in EAE and spares the animals from paralysis, results in cell accumulation on meningeal venules, revealing the BLMB as a primary entry site for pro-inflammatory leukocytes[13]. In a contusion model involving the presence of both M1 and M2 macrophages in the parenchyma, a specific M1 pro-inflammatory fate is observed for monocytes infiltrating via the spinal cord leptomeninges, suggesting that the BLMB instructs a pro-inflammatory commitment[21]. Meningeal infiltration is characterized by a pro-inflammatory polarization, manifested by the presence of T_H1 and T_H17 cells and pro-inflammatory $LY6C^{hi}$ monocytes, expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNF), and lack of anti-inflammatory factors[54,55] (FIG. 1c). The negligible leukocyte extravasation through this structure in the normal state and the pro-inflammatory nature of cells crossing it under other conditions position the BLMB as a true endothelial barrier that does not have immunoregulatory-skewing properties and should remain sealed.

The ocular barrier system

In a similar way to the CNS, the blood–ocular barriers are distinct in their location, structures, local milieu and immune-skewing properties. The inner blood–retinal barrier (BRB), which is located within the inner layers of the neural retina, is formed by non-fenestrated endothelium, is interconnected by tight junctions and is covered by astrocyte and Müller cell foot branches (reviewed in REF. 56). The outer BRB, which is located on the Bruch's membrane and outside the subretinal space and the outer neural retina, is formed by retinal pigmented epithelial (RPE) cells that are interconnected by tight junctions and fenestrated choriocapillaries[56]. Finally, the blood–aqueous barrier (BAqB) is located at the ciliary body, which produces the aqueous humour, and is formed by tight junctions of non-pigmented ciliary body epithelium on the side proximal to the aqueous humour and by fenestrated endothelial cells on the other side[4] (FIG. 2).

The inner BRB as a true barrier. Studies of inflammatory-mediated pathologies in the eye, such as experimental autoimmune uveitis (EAU), demonstrate the infiltration of leukocytes around inner retinal vessels soon after or coincident with disease onset. Such infiltration is associated with distortion of endothelial tight junctions and breakdown of the inner BRB, mediated by pro-inflammatory cytokines such as TNF and IL-1β[57] and reactive oxygen and nitrogen species[58]. This infiltration occasionally manifests as retinal vasculitis with cuffing, coupled with the accumulation of exudates and infiltrates between the pericytes and glia limitans in a manner reminiscent of the perivascular cuffs seen around the BBB in EAE. After autoreactive T cells cross the inner BRB endothelial basement membrane, they interact with perivascular macrophages that function as APCs, further driving the pathogenic function of either T_H1 or T_H17 cells[58,59]. Currently, there is no evidence for immunoregulatory skewing at this border. The cuffing phenomenon precedes peak clinical symptoms[58,60] and is absent under normal conditions, suggesting that this barrier should remain sealed against leukocytes (FIG. 2a).

The outer BRB — an immunoregulatory gate. The choroidal barrier is suggested as a site for retinal immunosurveillance, thereby reflecting barrier permeability to cellular crossing[61]. In contrast with the inner BRB, the outer BRB epithelium produces or is surrounded by many immunomodulatory mediators, including TGFβ, IL-10, somatostatin (SOM; also known as growth hormone release-inhibiting factor), the enzyme thrombospondin 1 (TSP1; which activates TGFβ) and the apoptotic mediators FAS antigen ligand (FASL), TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) and programmed cell death 1 ligand 1 (PDL1)[56,58,62,63]. Different subsets of T cells express different levels of FAS, with T_H1 cells expressing the highest levels[64], possibly suggesting the selective deletion of this subset by the epithelial FASL rather than general T cell elimination.

RPE cells express the inhibitory factors CTL-associated protein 2α (CTLA2α; which converts effector T cells into T_{Reg} cells), galectin 1 and PGD2 (which both inhibit lymphocyte activation and proliferation), and pigment epithelium-derived factor (PEDF; which skews myeloid cells towards a resolving phenotype)[56,58,62,63]. Furthermore, RPE cells suppress T cell activation and proliferation[62,65], as well as

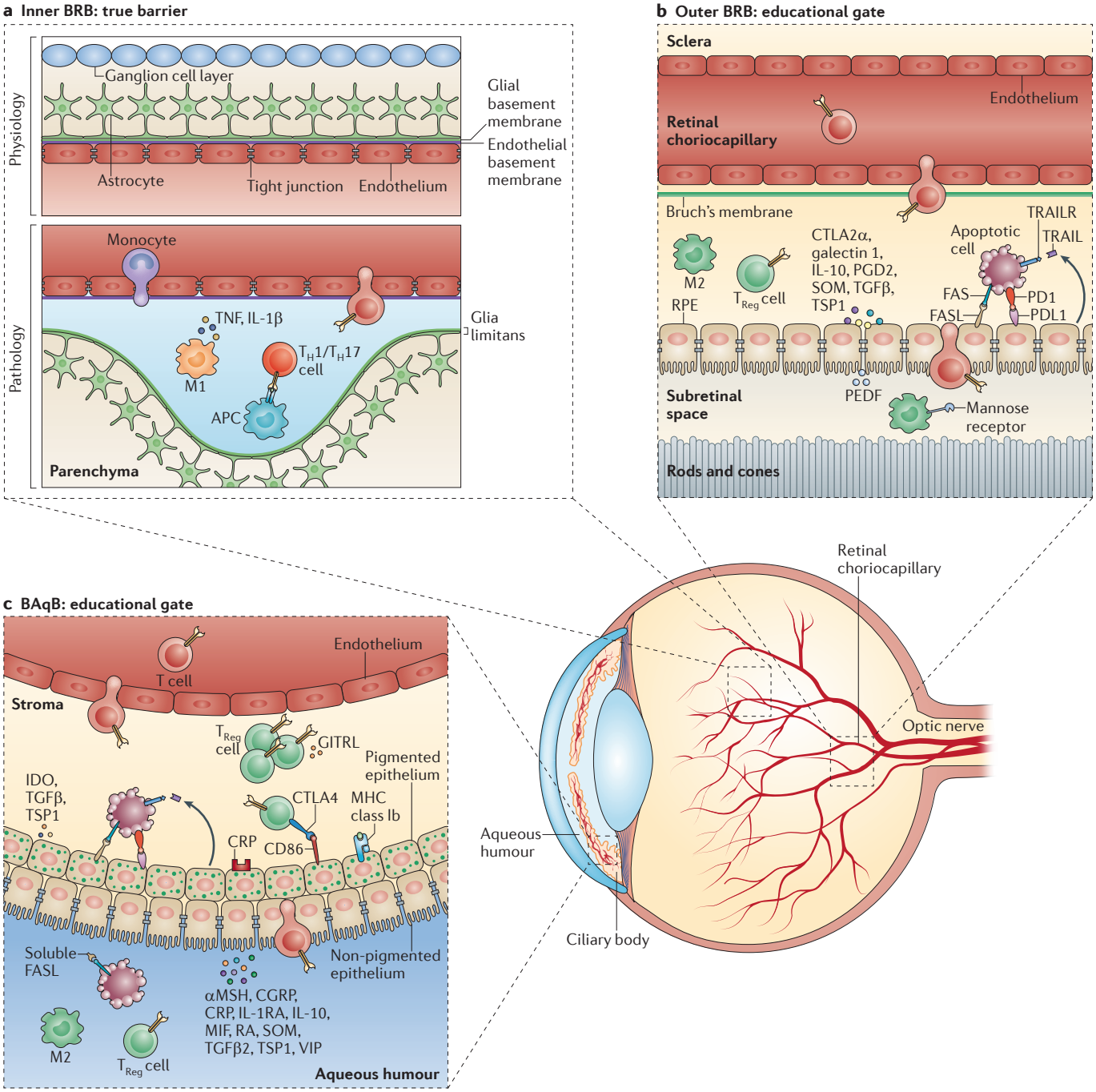


Figure 2 | **The ocular gating system.** Scheme illustrating the various ocular gate and barrier systems and their immunological milieu. **a** |The inner blood–retinal barrier (BRB), which is located within retinal parenchyma, consists of non-fenestrated endothelium interconnected by tight junctions and covered by foot branches of astrocytes. Under pathological conditions (bottom), this structure exhibits a pro-inflammatory milieu and vasculitis with cuffing (see main text for details). Cell entry at this barrier poses a hazard to the tissue and thus should be avoided. **b** | The outer BRB, which is located exterior to the neural retina, consists of tight junction-interconnected retinal pigmented epithelial cells (RPEs) and fenestrated choriocapillaries. This barrier facilitates intraocular migration by leukocytes and is equipped with immunoregulatory skewing capacities (see main text for details). **c** |The blood–aqueous barrier (BAqB) is located at the ciliary body, which produces the aqueous humour. The BAqB is formed by non-pigmented tight junction-interconnected epithelium and fenestrated endothelial cells. Leukocytes can gain access to the aqueous humour through this structure, showing that this barrier is permeable for cellular migration. This structure is the most fully characterized immunoregulatory skewing compartment of the ocular system. αMSH, α-melanocyte stimulating hormone; APC, antigen-presenting cell; CGRP, calcitonin gene-related peptide; CRP, complement regulatory protein; CTLA, cytotoxic T lymphocyte-associated protein; FASL, FAS antigen ligand; GITRL, glucocorticoid-induced TNF-receptor-related protein; IDO, indoleamine-2,3-dioxygenase; IL, interleukin; IL-1RA, IL-1 receptor antagonist; M1, pro-inflammatory macrophage; M2, alternatively activated macrophage; MIF, migration inhibitory factor; PD1, programmed cell death 1; PDL1, PD1 ligand 1; PEDF, pigment epithelium-derived factor; PGD2, prostaglandin D2; RA, retinoic acid; SOM, somatostatin; TGFβ, transforming growth factor-β; T_H, T helper; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TRAILR, TRAIL receptor; T_{Reg}, regulatory T; TSP1, thrombospondin 1; VIP, vasoactive intestinal peptide.

IFNγ, IL-2 and IL-17 production[66] *in vitro*. In line with its immunoregulatory nature, infiltrates of alternatively activated macrophages accumulate at the outer BRB during the resolution phase of EAU[58]. Thus, it is likely that the outer BRB facilitates active immune-skewing towards a regulatory and pro-resolving phenotype (FIG. 2b).

The BAqB as an immune-skewing gate. The BAqB is patrolled by leukocytes[4,61] and is suggested as a primary site for leukocyte infiltration preceding retinal inflammation[67]. In support of the BAqB as an entry route, retinal injury induces bone marrow-derived macrophage migration into the eye via the ciliary body, without damaging the BRB[61]. This suggests that this interface is not completely sealed but rather serves as a selective gate for cell trafficking.

The most extensively studied immune-skewing ocular compartments are the BAqB and the aqueous humour (reviewed in REF. 4). The epithelial and endothelial cells of the BAqB express various immunoregulatory factors, including FASL, PDL1, TRAIL, CD86 (which induces T cell anergy via CTLA4) and non-classical MHC class Ib molecules, which block CTL and natural killer (NK) cell functions[4,68]. Additionally, the BAqB expresses IDO, membrane-bound TGFβ, TSP1, glucocorticoid-induced TNF-receptor-related protein (GITRL; also known as TNFSF18), which induces local expansion of T_{Reg} cells, and various complement regulatory proteins[4]. Furthermore, the immunoregulatory nature of ciliary body cells, which are part of the BAqB, was revealed by *in vitro* studies showing that these cells suppress T cell proliferation and possibly convert effector T_H1 cells into T_{Reg} cells by direct cell contact or by soluble factor-mediated mechanisms[65,69–72].

Aqueous humour by itself promotes immune deviation. It can convert primed T cells into TGFβ-producing T_{Reg} cells *in vitro*[73]. Such immunomodulatory functions are mediated by an array of factors, including TGFβ2, IL-10, IL-1RA, retinoic acid, αMSH, VIP, CGRP, TSP1, macrophage migration inhibitory factor (MIF), SOM, soluble FASL and complement regulatory proteins[4,74,75]. Aqueous humour promotes apoptosis of inflammatory cells (lymphocytes, neutrophils and monocytes)[76] and suppresses the lineage commitment and acquisition of T_H1 and T_H17 cell effector function, while inducing conversion to forkhead box protein P3 (FOXP3)$^+$ T_{Reg} cells[77] (FIG. 2c).

Glossary

Alternatively activated macrophages
(M2 macrophages). Macrophages that are stimulated by interleukin-4 (IL-4) or IL-13 and that express arginase 1, mannose receptor CD206 and IL-4 receptor. Other factors may also drive the alternative activation of macrophages. M2 macrophages have an anti-inflammatory function and mediate wound healing.

Amniotic sac
The sac in which the fetus develops. The sac is composed of a pair of tough but thin membranes: the inner membrane (the amnion) contains the amniotic fluid and the fetus, whereas the outer layer (the chorion) is part of the placenta.

Aqueous humour
Transparent gelatinous fluid that is similar to plasma and is secreted from the non-pigmented ciliary epithelium of the eye. It circulates from behind the iris (posterior chamber), where it is formed, to the front of the iris (anterior chamber), where it drains through the trabecular meshwork into Schlemm's canal, which is a venous sinus.

Central canal
A cerebrospinal fluid-filled tube that runs along the spinal cord and is continuous with the brain ventricular system.

Choroid plexus
A microvilli-enriched epithelioid structure within the roof of each one of the brain ventricles that creates a surface area comparable to that of the blood–brain barrier; its most well-characterized function is the production of cerebrospinal fluid, a 'clear' plasma fluid ultrafiltrate.

Ciliary body
A villous structure that is located behind the iris in the eye and produces the aqueous humour. Its stroma is coated by a double layer of ciliary epithelium; the inner layer is transparent, whereas the outer one is pigmented and forms a continuous layer with the retinal pigmented epithelium.

Circumventricular organs
Structures in the brain (including the area postrema, the subfornical organ, the organum vasculosum of the lamina terminalis and the median eminence) that, owing to their neuroendocrine functions, are considered as 'windows to the brain'. They contain fenestrated endothelium, are located at strategic positions in the ventricular system and are separated from the cerebrospinal fluid by a specialized blood–cerebrospinal fluid barrier.

Decidua
The specialized endometrial stromal tissue that encases the implanted conceptus. The decidua is predominantly comprised of decidual stromal cells, which differentiate from endometrial stromal cells following embryo implantation in the mouse. The decidua also contains various types of maternal leukocytes and makes direct contact with the trophoblasts on the outer surface of the conceptus to form the maternal–fetal interface.

Ependymal cells
The ependyma is a thin epithelial layer that lines the ventricular system of the brain and the central canal of the spinal cord. Ependymal cells are specialized cuboidal epithelial cells that contain cilia on their apical surfaces, which circulate the cerebrospinal fluid.

Glia limitans
An astrocyte structure that marks the border of the central nervous system parenchyma. It is composed of the parenchymal basement membrane and astrocyte endfeet, and covers the entire surface of the brain and spinal cord on external surfaces towards the leptomeningeal space (glia limitans superficialis) and internally towards the perivascular spaces (glia limitans perivascularis).

Microchimerism
The presence within one individual of a small population of cells from another genetically distinct individual.

Meninges
Vascularized tissue membranes that envelop superficial central nervous system areas and enclose the parenchyma. The meninges are composed of three layers: the outermost dura mater (beneath the skull), the arachnoid mater and the pia mater (the innermost layer, which is proximal to the parenchyma).

Rete testis
Tubules located in the mediastinum testis that carry sperm from the seminiferous tubules to the efferent ducts, which are the initial section of the epididymis. This is the site at which sperm is concentrated and fluids are absorbed.

Seminiferous tubule
A testicular structure in which meiosis and the subsequent creation of gametes (namely spermatozoa) takes place. There are two types of tubules: convoluted tubules are located towards the lateral end, whereas straight tubules are located towards the end that will exit the testis.

Sertoli cells
Tall (columnar type) epithelial niche-forming cells, the main function of which is to nourish the developing sperm through the stages of spermatogenesis (the process of differentiation of stem cells into mature germ cells). They also consume the residual cytoplasm and engulf excess spermatozoa. The tight junctions of Sertoli cells form the blood–testis barrier, which separates the abluminal compartment of the seminiferous tubule from the blood.

Subarachnoid space
The gap between the meningeal arachnoid membrane and the innermost pia mater. This cerebrospinal fluid-filled space is traversed by blood vessels.

Tight junctions
A belt-like region of adhesion between adjacent epithelial or endothelial cells that regulates paracellular flux. Tight-junction proteins include the integral membrane proteins occludin and claudin, in association with cytoplasmic zonulaoccludin proteins.

Tolerance
A term that denotes lymphocyte non-responsiveness to antigen but implies an active process rather than passive indifference.

Trophoblasts
Specialized cells forming the outer layer of blastocytes; these cells develop to form most of the placenta, where they function in embryo implantation and the interaction with the decidualized maternal uterus.

Another well-studied phenomenon initiated at the BAqB and the aqueous humour is anterior chamber-associated immune deviation (ACAID), in which antigens in the anterior chamber are captured by APCs that migrate to the spleen, where they skew NKT cells and $CD4^+$ and $CD8^+$ T cells towards a regulatory phenotype (reviewed in REF. 4). This is suggested as a mechanism for generating peripheral tolerance to eye antigens via systemic immune skewing. Here we suggest that the same features of ACAID also achieve local immune-skewing of trafficking leukocytes within the eye, driving an alternatively activated and/or regulatory phenotype. Importantly, the immunosuppressive features of the aqueous humour are retained following lipopolysaccharide (LPS)-induced intraocular inflammation, as demonstrated by its continued ability to support tumour cell growth, ACAID induction and T cell suppression[78]. Interestingly, however, under certain inflammatory-associated pathological conditions, such as EAU, the aqueous humour loses its immunosuppressive properties; these changes are transient, and the immunosuppressive phenotype is restored after the peak of inflammation[79,80].

The immune-skewing nature of the BAqB and aqueous humour is suggested here to drive an alternatively activated and/or regulatory phenotype in trafficking leukocytes, promoting active immune regulation that is essential for preserving the integrity of this delicate tissue.

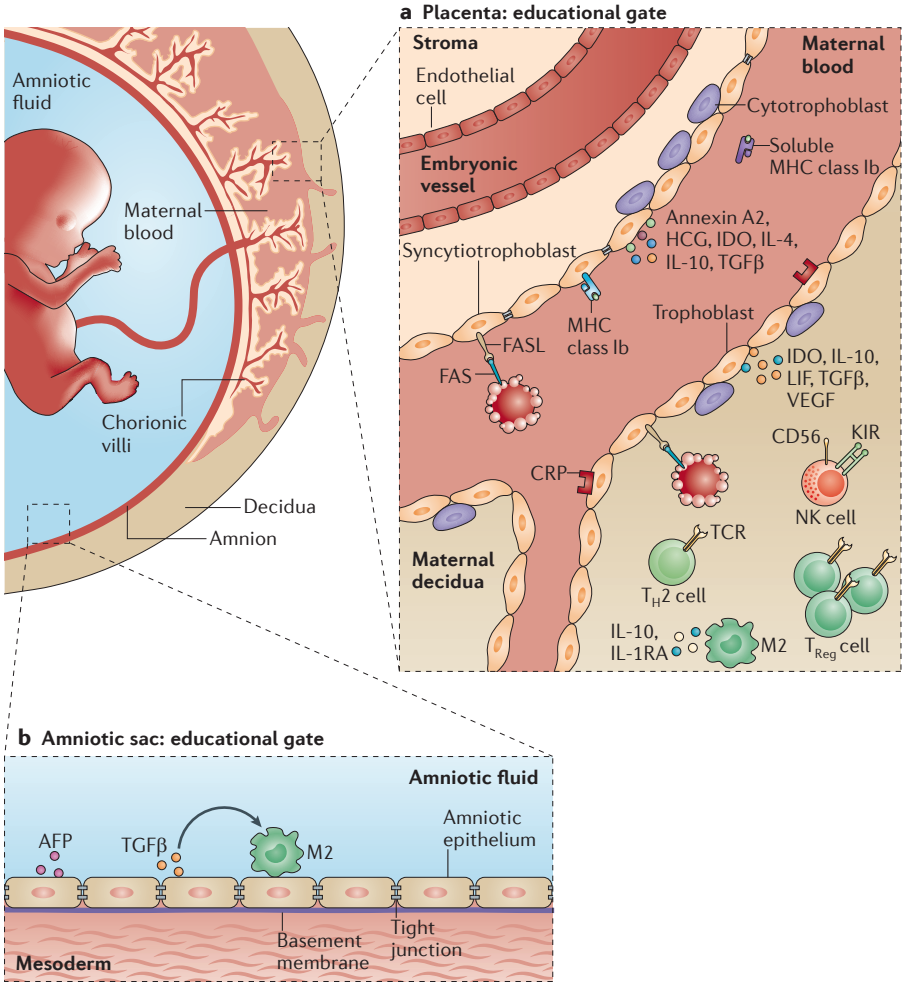


Figure 3 | **The maternal–fetal interface gating system.** Scheme illustrating the various maternal–fetal interface gates and their immunological milieu. **a** | The placenta consists of fetus-derived chorionic villi embedded in stroma ensheathed by the tightly interconnected epithelioid syncytiotrophoblasts and bathed in maternal blood supplied by the maternal epithelioid decidua. This structure is a well-studied immunoregulatory skewing compartment within the maternal–fetal interface (see main text) and is suggested to be the main gate for establishment of maternal–fetal microchimerism. **b** | The amniotic sac is composed of a tight junction-interconnected epithelial layer that secretes the amniotic fluid and is equipped with immunoregulatory capacities. AFP, α-fetoprotein; CRP, complement regulatory protein; FASL, FAS antigen ligand; HCG, human chorionic gonadotropin; IDO, indoleamine-2,3-dioxygenase; IL, interleukin; IL-1RA, IL-1 receptor antagonist; KIR, killer-cell immunoglobulin-like receptor; LIF, leukaemia inhibitory factor; M2, alternatively activated macrophage; NK, natural killer; TCR, T cell receptor; TGFβ, transforming growth factor-β; T_H, T helper; T_{Reg}, regulatory T; VEGF, vascular endothelial growth factor.

The maternal–fetal interface

Extensive research has revealed complex immunoregulatory mechanisms explaining how the maternal immune system avoids rejection of a 'semi-allograft' fetus. However, unlike the CNS and the eye, the maternal vasculature does not penetrate the fetal parenchyma, and the placenta, which is located exterior to the fetal parenchyma, serves as the basic maternal–fetal interface (reviewed in REFS 81,82).

The mammalian placenta consists of fetus-derived chorionic villi bathed in maternal blood supplied by spiral arteries in the decidua. The chorionic villi contain branched embryonic blood vessels embedded in stroma ensheathed by the syncytiotrophoblast, which is a tightly interconnected epithelioid surface layer, a fusion product of cytotrophoblast progenitors[81,82]. An additional maternal–fetal interface is the amniotic sac, which is composed of an inner single epithelial layer joined by tight junctions and secretes the amniotic fluid. Thus, this interface is solely equipped with what we have termed an epithelial gate (FIG. 3).

The decidua–trophoblast interface. Multiple immune-modulating mechanisms operate at the decidua–trophoblast interface (reviewed in REFS 81,82). The human decidua is populated by non-cytotoxic maternal uterine NK cells, which express killer-cell immunoglobulin-like receptors (KIRs) for non-classical MHC class I molecules expressed by trophoblasts, which are part of the barrier. At the decidua–trophoblast interface, decidual lymphocytes are biased towards a T_H2-type phenotype, existing T_H1 cells are low producers of IFNγ, there are no T_H17 cells, and increased proportions of $CD4^+$ and $CD8^+$ T_{Reg} cells are seen at this site compared with other tissues. The decidua also contains IL-10- and IL-1RA-expressing M2 macrophages and an array of immunomodulatory factors such as IDO, FASL and leukaemia inhibitory factor (LIF; which induces T_H2 cell polarization)[81,82].

Embryo-derived epithelial-like trophoblasts, which separate maternal and fetal circulation, have an active role in immune-skewing. These cells do not express conventional MHC class Ia molecules, thereby

avoiding $CD8^+$ T cell cytotoxic attack, but they do express membrane-bound and soluble non-classical MHC class Ib molecules (such as HLA-G and HLA-E in humans, and Qa-2 in mice[83]), which downmodulate NK and $CD8^+$ T cell responses[84,85], promote a T_H2-type phenotype[86] and induce tolerogenic dendritic cells that are associated with T_{Reg} cell generation[87,88]. Trophoblasts also express IDO, FASL and the glycoprotein annexin A2, which inhibits lymphocyte proliferation and antibody secretion[81,82]. They secrete complement regulatory proteins, T_H2-promoting cytokines such as IL-4 and IL-10 (REFS 89,90), and the hormone human chorionic gonadotropin, which is a chemoattractant for T_{Reg} cells[91]. The decidua–trophoblast interface is characterized by progesterone secretion[92] and a T_H2-type bias[93], as well as the production of soluble FASL[94,95], IL-10 and TGFβ[81,82]. The trophoblast cells circumvent antibody-mediated damage by expressing high levels of complement regulatory proteins[96] and reduce cell-mediated immunity by expressing inhibitory members of the B7 family[97]. Together, these features suggest an immune-skewing nature for this gate, which suppresses certain effector arms of the immune system — including M1 macrophages, CTLs and T_H1 cells — and activates alternative effector functions in the form of M2 and T_H2-type, regulatory and pro-resolving responses (FIG. 3a).

The phenomenon of maternal–fetal microchimerism, a regulated bidirectional trafficking event occurring at the placenta (BOX 1), suggests that the maternal–fetal interface is an active immune regulatory gate for cell trafficking rather than an inert, impermeable barrier.

The amniotic sac. Little is known about the epithelial-restricted amniotic sac. *In vitro* studies have shown that amniotic fluid mediates an immune-skewing effect: mouse amniotic fluid inhibits T cell–macrophage interactions[98], both murine and human amniotic fluid skews macrophages towards a M2 phenotype (via TGFβ)[99] and the amniotic fluid (at least in humans) inhibits graft-versus-host reactions[100]. Both human- and mouse-derived amniotic fluid express the immunosuppressive factor α-fetoprotein (AFP), which represses antibody synthesis in mice[101] and protects against preterm birth in patients with increased IL-6 levels[102] (FIG. 3b).

The testicular gating system

Sperm cells begin to be created only at puberty and express neo-antigens that are 'foreign' to the already established immune system. Self-tolerance to these cells, together with the prolonged survival of foreign grafts in the testis, shows that the testis is another immune-privileged site.

The blood–testis barrier (BTB; reviewed in REFS 103,104) is composed of a layer of tight junction-interconnected epithelial Sertoli cells that extends from the outer edge of the seminiferous tubule (the germinal compartment) towards the lumen and is covered by basement membrane and a layer of peritubular myoid cells. The interstitial space, which is a testicular parenchyma between the tubules and is located before the epithelial barrier, contains Leydig cells, various leukocytes and permeable vasculature. The testis lacks vasculature within the tubules, in which spermatogenesis occurs. Thus, the BTB falls into our category of an epithelial gate.

The epithelial Sertoli cells play an active part in immune skewing. They express FASL, PDL1, IDO, the TAM (TYRO3, AXL and MER) receptor and its ligand (which suppress innate immune responses), and complement inhibitors[103–105]. They also secrete activin A, TGFβ[106] and granzyme inhibitors (which inhibit cytotoxic activity)[107]. This immune-skewing environment is preserved by other cell types operating in the interstitial space, namely T_{Reg} cells, NK cells and mast cells, as well as testicular M2 macrophages[103,104], which express the scavenger receptor CD163, high levels of IL-10 and TGFβ, and low levels of IL-12, IL-1β and TNF. This immunoregulatory milieu is also supported by Leydig cells that synthesize testosterone, which has anti-inflammatory properties[108]. Furthermore, peritubular myoid cells express TGFβ[109], CCL2 (REF. 110), LIF[111] and activin A, which inhibit pro-inflammatory cytokine production and promote a T_H2 and M2 phenotype. Altogether, these mediators are suggested here to drive an immune-skewing milieu at the BTB gate, imposing a regulatory and alternatively activated phenotype.

Immune cells enter the testis parenchyma but are restricted to the interstitial space, where most known immune education processes occur. Leukocytes are strictly excluded from the lumen of the seminiferous tubules in the steady state, suggesting that this gate, although educative, is also absolute. However, during spermatogenesis, germ cells located in the basal compartment of the seminiferous epithelium migrate across the BTB to continue differentiation on the luminal side in a physiological migratory process that requires restructuring of tight junctions[9]. This demonstrates that the BTB at the seminiferous tubule is not an impermeable barrier. Such a physiological passage occurs following assembly of tight junctions between Sertoli cells and germ cells, enabling controlled crossing without barrier breakdown[9]. Donor spermatogonial stem cells injected into the testis of infertile animals transmigrate through the BTB between adjacent Sertoli cells, highlighting the ability of this barrier to facilitate cellular transmigration[8]. In line with this observation and in contrast with normal conditions in mammals, in which leukocytes are restricted to the interstitial space, macrophages were shown to be recruited through the BTB in healthy swans during the seasonal involution phase, in which they dispose of degenerating premature germ cells when uptake by Sertoli cells is already at capacity[112]. Such migration supports the notion that this barrier facilitates cellular crossing on demand.

Box 1 | **Maternal–fetal microchimerism**

Despite the demonstration in 1893 of fetal cells in tissues analysed from deceased mothers[115], it was still commonly accepted in the early 1900s that the placenta was an impermeable barrier, maintaining perfect separation between the fetus and the mother. The mutual exchange of genetically distinct cells or DNA between the mother and the fetus was recognized in the mid-twentieth century when placental metastasis of maternal melanoma was reported[116], and human fetal lymphocytes were identified in maternal blood[117]. These findings were the first milestones confirming that the maternal–fetal interface is not completely sealed but instead facilitates bidirectional cell trafficking, creating a physiological condition termed microchimerism (reviewed in REF. 7).

Both fetal microchimerism (maternal acquisition of fetal cells) and maternal microchimerism (acquisition of maternal cells by the fetus or infant) have been reported for B and T cells, monocytes and macrophages, natural killer cells and haematopoietic progenitor cells[118] residing in various organs[119,120]. Maternal–fetal microchimerism persists long after delivery and may have adverse or beneficial effects with respect to the health of the mother or offspring[121–126]. This phenomenon is associated with immunological changes, resulting in maternal or fetal tolerance to reciprocal antigens[127–129]. Both maternal and fetal microchimerism are suggested to be mediated via a regulated trafficking event that occurs at the placenta[130].

Although lymphocytes only cross the seminiferous epithelium of mammals under pathological conditions[113], there is evidence showing that the BTB is not completely sealed in two areas: at the tubuli recti (also known as straight seminiferous tubules) and at the rete testis (testicular structures in the terminal portion of seminiferous tubules that contain flattened tight junction-interconnected epithelial cells). Evidence exists to suggest that lymphocytes can penetrate into these structures in mice, rats, monkeys and other species[5,6]. Electron microscopy imaging in 'normal' tubuli recti and rete testes revealed lymphocytes between epithelial cells behind the basal lamina membrane, some of which are close to spermatoza[5,6]. In fact, testicular autoimmunity is primarily observed in these areas before spreading to the peripheral seminiferous tubule[114], suggesting that these structures may be the primary site for lymphocyte entry. Thus, it seems that lymphocytes may penetrate the BTB in a narrow, specialized area of the mediastinum testis, possibly reflecting immunosurveillance by this gate (FIG. 4).

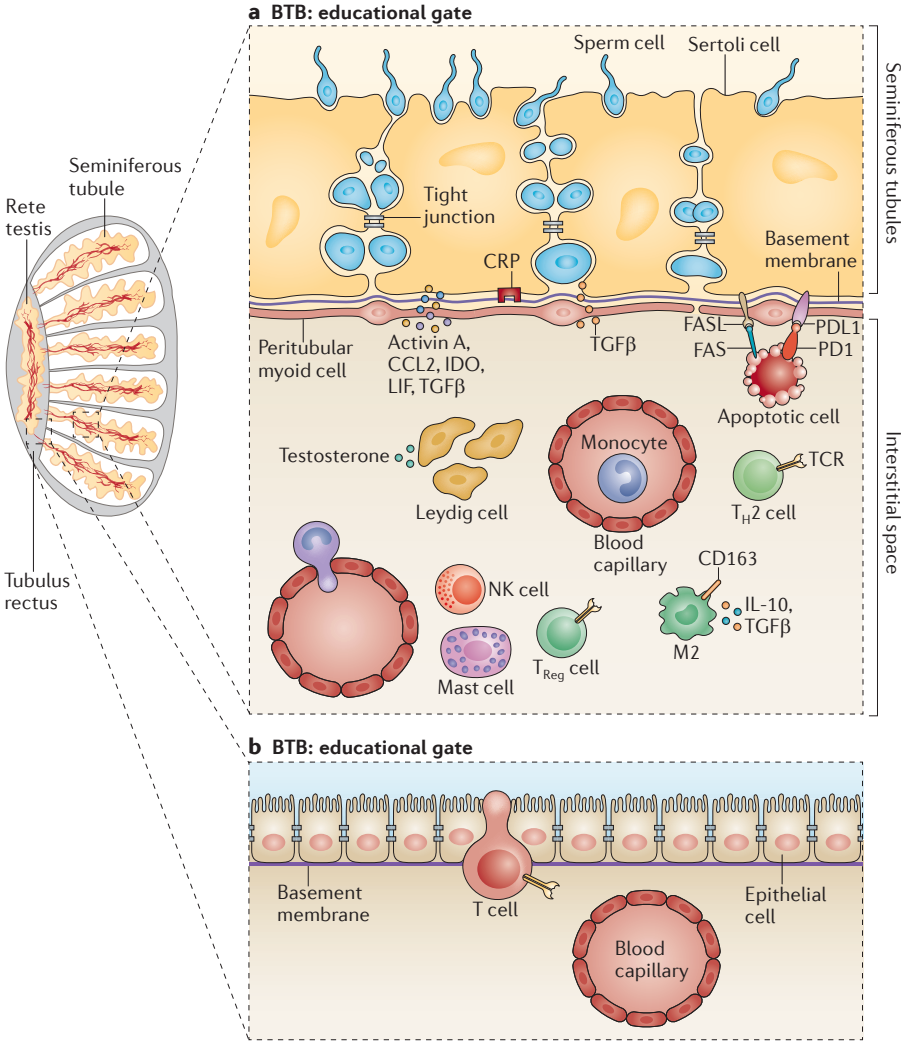


Figure 4 | **The testis gating system.** Scheme illustrating the blood–testis barrier (BTB) and its immunological milieu. **a** | The BTB at the seminiferous tubule. The testis germinal compartment is covered by a layer of epithelial Sertoli cells, which are interconnected by tight junctions. This structure is separated from the fenestrated blood vessels by the interstitial space, where multiple mechanisms of immune modulation take place (see main text for details). The BTB epithelial component is itself endowed with immunoregulatory skewing capacities and is constitutively crossed by germ cells during the process of spermatogenesis. Leukocytes are strictly excluded from the lumen of the seminiferous tubules in normal tissue, but they occasionally penetrate the BTB of tubuli recti (also known as straight seminiferous tubules). **b** |The BTB at the rete testis is lined by simple cuboidal epithelium, interconnected by tight junctions. The permeability of the BTB at this area is altered and leukocytes do cross between epithelial cells in this compartment. CCL2, CC-chemokine ligand 2; CRP, complement regulatory protein; FASL, FAS antigen ligand; IDO, indoleamine-2,3-dioxygenase; IL, interleukin; LIF, leukaemia inhibitory factor; M2, alternatively activated macrophage; NK, natural killer; PD1, programmed cell death 1; PDL1, PD1 ligand 1; TCR, T cell receptor; TGFβ, transforming growth factor-β; T_H, T helper; T_{Reg}, regulatory T.

Educational trip to immune privilege

On the basis of the accumulating evidence described above, we propose that the CNS, eye, testis and maternal–fetal interface are equipped with immunomodulatory gating and barrier systems. Our model classifies these barriers by their cellular composition: the true barriers consist of endothelial structures, such as the BBB and the inner BRB, whereas the suggested immunomodulatory gates are comprised of fused epithelial cells, such as the BCSFB, the BAqB, the outer BRB and the BTB, as well as the maternal–fetal interface.

We propose that the various mechanisms operating at the endothelial barriers aim at maintaining barrier sealing. This does not imply that cells cannot cross these barriers but rather that such migration endangers the tissue. Indeed, the pro-inflammatory phenotype of leukocytes crossing endothelial barriers highlights the fact that these barriers are designated to maintain immune cell seclusion and should remain sealed. By contrast, our model views the epithelial barriers as active immunomodulatory permissive gates rather than absolute or inert barriers. These gates facilitate regulated cellular passage and are equipped with a wide array of immune-skewing mechanisms that are selective towards certain effector subclasses with a regulatory and/or alternatively activated phenotype. In our view, these gates enable the required immunosurveillance of immune-privileged sites in a risk-limiting manner. The success of immune orchestration under normal or subclinical conditions is difficult to measure. As inflammatory-driven pathological conditions rewrite the rules of cellular recruitment and education, this immune orchestration may be broken under pathology (predominantly in conditions with inflammatory aetiology), which may lead to misunderstanding of the barriers.

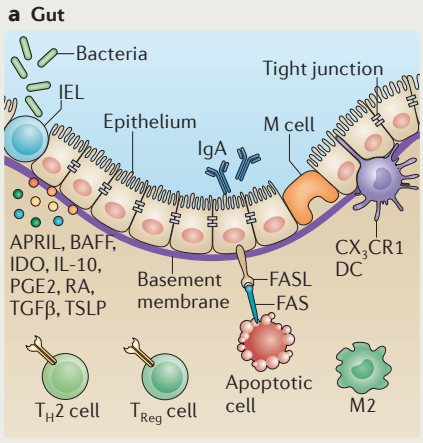
The existence of such complex barrier and gate systems suggests that the barriers do not, as was traditionally believed, facilitate complete immune cell exclusion and immune ignorance. We propose that the privilege of these organs resides not in their ability to block passive immune infiltration or to facilitate active immune tolerance or ignorance, but rather in the ability of the epithelium gate

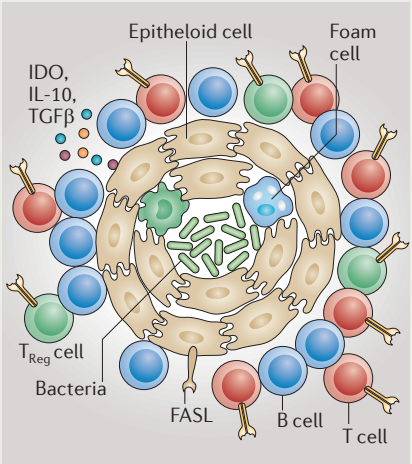
Box 2 | **Educative gates — beyond immune-privileged organs**

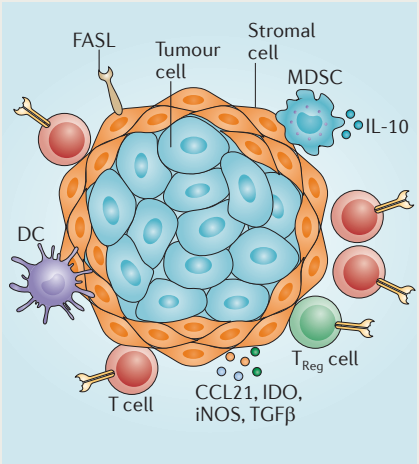
The educative gate model may be applied to other sites and conditions associated with immune privilege, such as the intestine, tumour and chronic infection. The intestine, the largest barrier bordering the outside world, has been regarded as an unconventional 'immune-privileged' site: it induces tolerance to food and commensal flora antigens rather than preventing leukocyte entry. The intestine forms a permeable barrier composed of tight junction-interconnected epithelial cells located above a basal membrane and vasculature (see the figure, part **a**). This barrier is not sealed for leukocytes, as neutrophils[131] and dendritic cells (DCs)[132] cross the epithelium upon bacterial infection. The epithelium orchestrates a local immune response (reviewed in REF. 133) by producing conditioning factors such as FAS antigen ligand (FASL), TNF-related apoptosis-inducing ligand (TRAIL), indoleamine-2,3-dioxygenase (IDO), thymic stromal lymphopoietin (TSLP)[134], prostaglandin E2 (PGE2), retinoic acid (RA), transforming growth factor-β (TGFβ), interleukin-10 (IL-10), a proliferation-inducing ligand (APRIL; also known as TNFSF13)[135] and B cell-activating factor (BAFF; also known as TNFSF13B)[136], resulting in the induction of tolerogenic DCs and the polarization of T helper 2 (T_H2) cells and regulatory T (T_{Reg}) cells, even after exposure to T_H1-inducing pathogens[134,137].

Another system in which a semi-barrier provides an immune educative site is granulomatous inflammation, which is a hallmark of certain chronic infections (see the figure, part **b**). Granulomas are highly organized structures containing pathogens, infected cells and other aggregated host cells that serve as a physical and immunological barrier that 'walls off' mycobacteria as a critical host-protective mechanism (reviewed in REF. 138). Granulomas do not eradicate the pathogen, and the associated inflammation is functionally immune privileged. Interestingly, in line with the epithelial nature of the educative gates suggested in our model, in some granulomas (such as tuberculous granulomas) macrophages undergo transformation into epithelioid cells with a tightly interdigitated cell membrane that allows them to link to adjacent cells and form a structural barrier[138]. These cells display immunoeducative properties, including expression of FASL, IDO, IL-10 and TGFβ[139,140].

Finally, on the basis of a recent report, it is possible that our model is applicable to tumours; tumour borders can form a stroma-based immune-regulating structure that includes an altered cytokine milieu enriched for TGFβ (see the figure, part **c**). This structure allows the recruitment, priming and/or skewing of various regulatory leukocytes, thereby creating local immune privilege[141]. CCL21, CC-chemokine ligand 21; CX_3CR1, CX_3C-chemokine receptor 1; IEL, intraepithelial lymphocyte; iNOS, inducible nitric oxide synthase; M cell, microfold cell; MDSC, myeloid-derived suppressor cell.







system to orchestrate active communication with the circulating immune system. We suggest that such communication is facilitated by an effector arm of the immune system in the form of alternatively activated or regulatory leukocytes, the phenotype of which is shaped by the gate.

Several potential mechanisms, possibly coexisting, may achieve immune skewing at the barriers: first, the selective recruitment, preferential survival and/or metabolic support, activation or expansion of functionally pre-committed immune cells with the desired phenotype; second, the suppression of cells with an inappropriate phenotype at the border checkpoint by deletion, anergy or active suppression by professional regulatory cells; and third, the active imposition of specific functional attributes through the induction of lineage commitment or maturation or conversion of the committed cell during the process of barrier passage. We suggest that an interplay of all of these mechanisms exists to ensure proper commitment. Moreover, we suggest that innate and adaptive mechanisms cooperate to orchestrate immunological education at the gates. The site of immune modulation is still not fully elucidated; in the CNS and eye it is clear that immunosurveillance takes place behind the barrier (in the CSF and aqueous humour), whereas in the testis and the placenta immunosurveillance has been mainly investigated at the borders but before the epithelial barrier (at Sertoli cells in the testis or at the syncytiotrophoblast in the placenta). This either reflects neglected areas of investigation or suggests that indeed the location of immunosurveillance and modulation is diverse at the different organs. Finally, the barrier model may extend beyond classical immune-privileged sites and be relevant for the immune regulation of other systems, such as the intestine, tumour and chronic infection (BOX 2).

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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