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What is immune privilege (not)?

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The 'immune privilege' of the central nervous system (CNS) is indispensable for damage limitation during inflammation in a sensitive organ with poor regenerative capacity. It is a longstanding notion which, over time, has acquired several misconceptions and a lack of precision in its definition. In this article, we address these issues and re-define CNS immune privilege in the light of recent data. We show how it is far from absolute, and how it varies with age and brain region. Immune privilege in the CNS is often mis-attributed wholly to the blood–brain barrier. We discuss the pivotal role of the specialization of the afferent arm of adaptive immunity in the brain, which results in a lack of cell-mediated antigen drainage to the cervical lymph nodes although soluble drainage to these nodes is well described. It is now increasingly recognized how immune privilege is maintained actively as a result of the immunoregulatory characteristics of the CNS-resident cells and their microenvironment.

Immune privilege down the ages

Privilege: 'a right, advantage, or immunity granted to or enjoyed by a person, or class of persons, beyond the common advantages of others' [1].

The concept of 'immune privilege' in the central nervous system (CNS) has a long history. That antigens trapped within the brain parenchyma evade systemic immunological recognition was shown as early as 1921 in Japan, when Shirai observed that rat sarcoma grew well when transplanted into the mouse brain parenchyma, but not when implanted subcutaneously or intramuscularly [2]. In 1923, Murphy and Sturm extended these findings by demonstrating that if recipient spleen was co-transplanted with the foreign tumour in the brain parenchyma, it inhibited the tumour growth [3]. This showed that the survival of the foreign tumour within the brain parenchyma was occurring as a result of disconnection from the systemic immune system. These were the first indications of what was later to be termed 'immunological privilege' by Billingham and Boswell [4]. Over the years, these observations have been confirmed for tissue grafts [5], bacteria [6], viruses [7] and vectors [8], which all evaded immune recognition when delivered to the brain parenchyma. Around the same time as the discovery of the immune privilege of the brain, the blood–brain barrier (BBB) was being investigated, and the two concepts grew together. This had two consequences. First, the immune privilege of the brain assumed a more absolute meaning, rendering it too strong a descriptive term for the relationship of the brain with the immune system (Box 1). Second, immune privilege was inappropriately attributed wholly to the BBB (see previous article), whereas other features, including the specialization of afferent communication from the CNS to nearby lymphatic organs and the nature of the CNS microenvironment, are much more pertinent (Box 1). In this article, we aim to update the definition of the immune privilege of the brain in the light of current evidence.

Absolute and relative immune privilege

Privilege evokes a concept of advantage gained by an individual with respect to the common advantages of others [1]. It is neither an absolute nor an immutable state. The seminal experiments described in the previous section are entirely consistent with the concept of immune privilege – they do not infer or require any qualification of absolute or partial privilege because there was no evidence in these experiments that immune privilege is absolute. The immune privilege of the brain is certainly not absolute but is relative to other organs. Also, Shirai's rat sarcoma [2] might have survived well in the brains of his mice because it was neoplastic. We now know that non-tumoral intracerebral xenografts do not survive, although their rejection is delayed [9].

Compartmentalization of immune privilege

The CNS is organized into different compartments: the parenchyma proper, the ventricles containing choroid plexus and cerebrospinal fluid (CSF), and the meninges (Figure 1). In the same 1923 paper, Murphy and Sturm had described how rejection of the foreign tumour within the brain occurred if it approached the ventricles [3]. This has also been shown to be the case for other antigens. When injected intracerebroventricularly (ICV), foreign tissue grafts were rejected [10], Bacille Calmette-Guerin (BCG) resulted in delayed-type hypersensitivity lesions in the choroid plexus [11], and influenza virus elicited humoral and cytotoxic T-cell responses [7]. As far as adaptive immunity is concerned, the privilege of the CNS is, therefore, compartmentalized, being confined to the parenchyma.

The relative immune privilege of the CNS also extends to the innate immune response [12]. Injection of lipopolysaccharide (LPS) in the skin elicited neutrophil and monocyte recruitment within 2 h. In the brain parenchyma, such an acute myelomonocytic infiltration did not occur. Monocyte recruitment was delayed to the third day after injection and only occurred with 10-fold higher doses of LPS; 100-fold doses were needed for the density of brain-infiltrating monocytes to approach that seen in skin. There is evidence of compartmentalization of 'innate immune

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Box 1. What is immune privilege (not)?

Immune privilege is:
- Relative
- Confined to CNS parenchyma
- Applicable to both adaptive and innate immunity
- Mostly a result of specialization of the afferent arm in adaptive immunity
- Active in addition to passive

Immune privilege is not:
- Absolute
- Wholly explicable by the blood–brain barrier
- Present in meninges, choroid plexus, circumventricular organs and ventricles
- Preserved after systemic immunization
- Preserved at extremes of age
- Preserved in the inflamed CNS
- Applicable to antibody production
- Wholly passive

privilege' also. In contrast to brain parenchyma, ICV injection of LPS resulted in a myelomonocytic response in the choroid plexus identical to that seen in skin [12]. Also, injection of interleukin (IL)-1β or tumour necrosis factor (TNF)-α in brain parenchyma resulted in selective neutrophilic and monocytic infiltration, respectively, whereas a mixed infiltrate was observed when either cytokine was injected into skin [13]. It is now clear that immune privilege, involving both innate and adaptive immune responses, is limited to the CNS parenchyma proper. The immune reactivity of the ventricles, choroid plexus, meninges and circumventricular organs is similar to that of the periphery.

Within the CNS parenchyma, there is evidence of further compartmentalization. When a standardized mechanical lesion was induced in murine spinal cord and cerebral cortex, larger numbers of neutrophils and macrophages were observed in spinal cord [14]. Also, the delayed neutrophil infiltration seen after intracerebral LPS challenge was restricted to white matter – it was not seen in grey matter [12].

The immune response – afferent and efferent arms compared

The apparent lack of communication between the CNS parenchyma and the peripheral immune system could be owing to privilege in the afferent or efferent arms of the immune response, or both. Intracerebral injection of BCG [15] and adenovirus [7] was ignored by the peripheral immune system as shown by T-cell and antibody response studies, suggesting that the afferent arm was deficient. By pre-immunizing animals with skin-to-skin grafting before implanting the foreign skin graft in the brain parenchyma, Medawar established that the efferent arm was relatively intact [5]. In his words, 'it is concluded that skin homografts transplanted to the brain submit to but cannot elicit an immune state'. However, one could argue that the access of the circulating immunized leukocytes to the intracerebral skin graft was facilitated by the tissue trauma sustained during implantation surgery. Therefore, Matyszak *et al.* injected BCG in the brain parenchyma of rats, and allowed the mild acute inflammatory response to subside and the blood–brain barrier to reform, before challenging the animals with BCG in adjuvant subcutaneously [6]. This resulted in a delayed-type hypersensitivity (DTH) response with bystander demyelination and axon damage, securing the hypothesis that the efferent arm was intact. These experiments showed that the afferent arm was responsible for most of the adaptive immune privilege observed in the brain.

Afferent arm

The afferent arm of the immune response involves antigen presentation to naive T cells, resulting in their priming and activation. In most tissues, antigen transport to draining lymph nodes and the spleen is crucial in generating this primary immune response, and occurs in two ways: (i) by

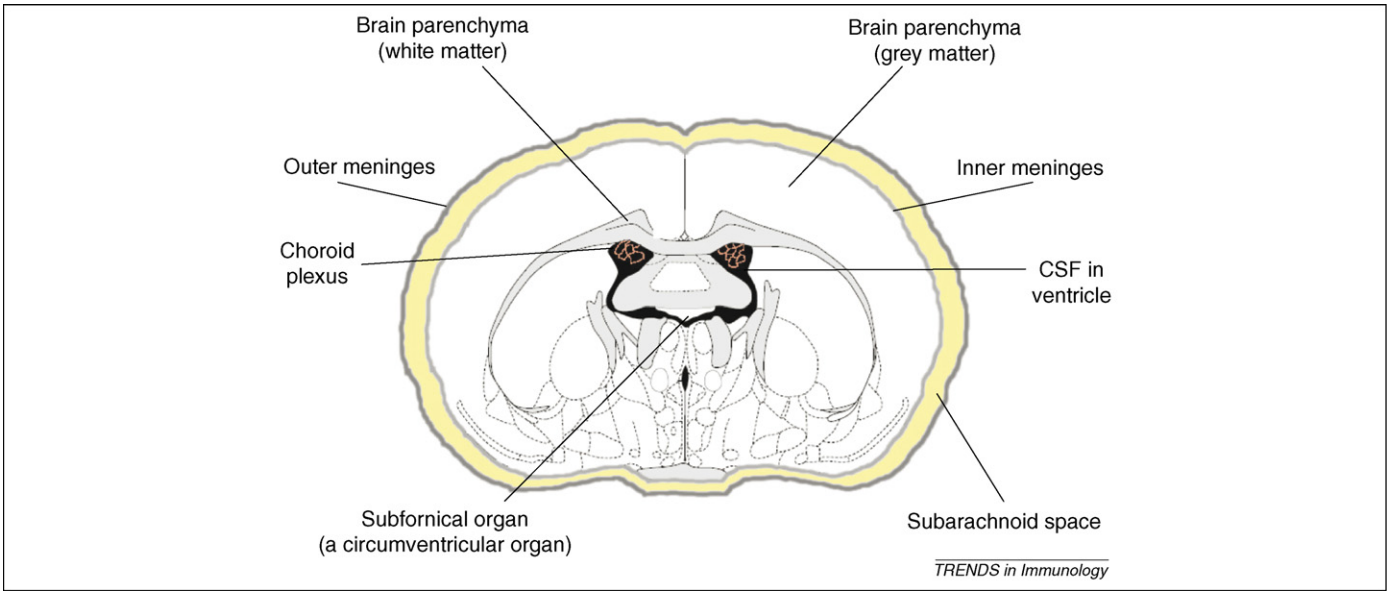

Figure 1. CNS compartments. Brain parenchyma is bathed in CSF produced by the choroid plexus, a specialized vascular organ situated in the ventricular system. CSF in the ventricles is continuous with CSF in the subarachnoid space between the inner meninges (covering the outer surface of the brain) and the outer meninges. Circumventricular organs (such as the subfornical organ) are brain regions lacking a blood–brain barrier.

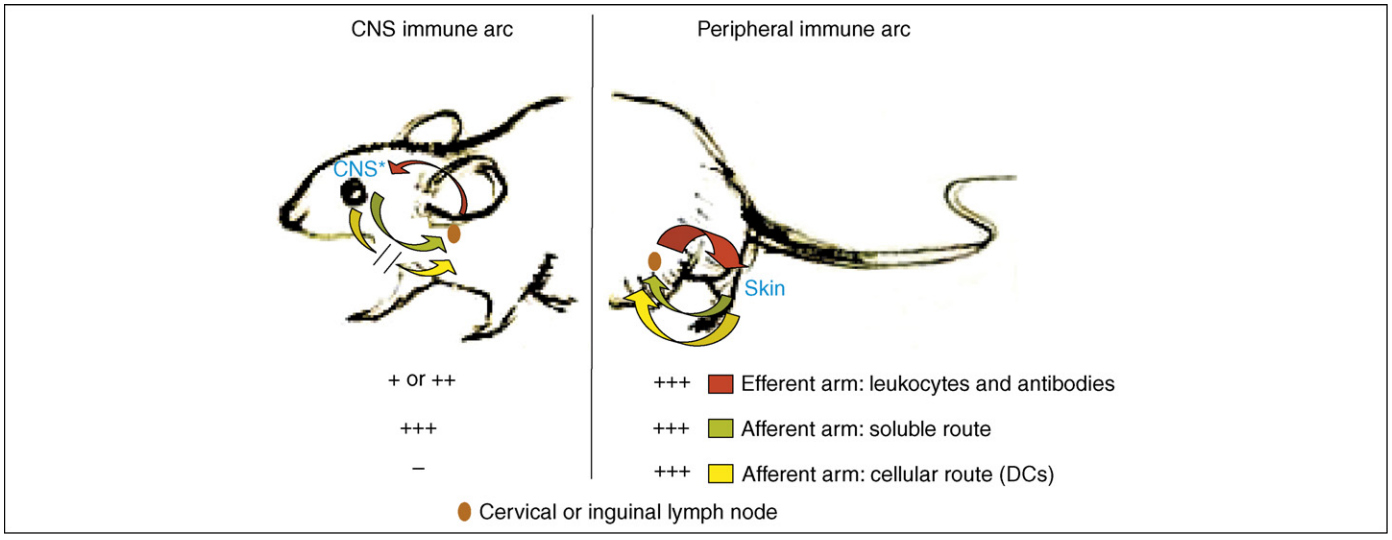

Figure 2. The immune arc in CNS parenchyma and periphery compared. An adaptive immune response is generated by the afferent arm of the adaptive immune system. Typically, antigen reaches the draining lymph nodes (cervical or inguinal lymph nodes) through soluble (green) or cellular (yellow) routes. Soluble antigen drains by bulk flow through lymphatic channels. Alternatively, cells in the tissue, including local immature DCs or other leukocytes, ingest antigen and transport it to the draining lymph nodes. Whichever way the antigen reaches the lymph nodes, it is there presented to naive T cells and B cells by professional APCs such as mature DCs. This results in a priming of antigen-specific T cells and B cells. The efferent arm of the immune response (red) involves the trafficking into the tissue of cells of the innate (neutrophils and macrophages) and adaptive (T cells and B cells activated through the afferent arm) immune systems. Note that CNS* refers only to parenchyma because the immune arc in non-parenchymal sites, such as meninges, shares similarities with peripheral tissues. Arrow widths are directly proportional to the activity of the involved arms, whereas the broken arrow (||) represents absent or non-detectable activity. +/++, reduced activity; +++, robust activity; –, absent or non-detectable activity. Adapted from a sketch by Claire Robertson at www.loobylu.com.

the emigration of professional antigen-presenting cells (APCs), termed dendritic cells (DCs), bearing antigens from the immune-challenged site to local lymph nodes, and (ii) by the drainage of soluble antigens in the lymph, representing cellular and fluid routes, respectively (Figure 2).

Cellular route

Immunohistochemical studies have failed to demonstrate the presence of cells with the immunophenotype of DCs in the uninflamed brain parenchyma or perivascular space, although they are present in the meninges and choroid plexus [16]. Indeed, the uninflamed brain cannot prime naive T cells *in situ* [17]. Once inflammation is established, DCs appear within the brain parenchyma [16], and a recent study has correlated the appearance of DCs with xenograft rejection [18].

Although macrophages bearing myelin antigens have been described within cervical lymph nodes of monkeys with experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS) patients [19], there is no reliable evidence that inflammatory cells bearing CNS antigen migrate out of the CNS parenchyma. When living DCs are injected into the brain parenchyma, they do not emigrate to local lymph nodes [20] but do following ICV injection or if excessively large numbers of cells in large volumes are injected intraparenchymally [21–23]. A crucial component for interpreting these experiments is the recognition that the injected volumes must be sufficiently small so as not to overwhelm the limited extracellular volume of the brain parenchyma [24], must avoid the ventricles and meninges, and must limit damage to the brain tissue.

Instead of migrating out of the CNS parenchyma to prime T or B cells in cervical lymph nodes, DCs accumulating in the inflamed CNS might function *in situ*. There is recent evidence that DCs isolated from the CNS of mice with proteolipid protein (PLP)-induced EAE can prime naive PLP-specific T cells *ex vivo* in the absence of PLP peptide [25]. Whether this occurs *in vivo* has yet to be shown. The presence of lymphoid follicle-like structures within the less-immune-privileged meninges has been demonstrated in mice with progressive–relapsing EAE [26] and patients with secondary progressive MS [27]. These ectopic lymphoid organs were shown to harbour a network of follicular DCs and B cells, suggesting local maintenance of B-cell responses.

Fluid route

Soluble antigen drainage from brain parenchyma occurs and is well characterized. Although the brain lacks a conventional lymphatic system, 50% of radiolabelled albumin injected in the caudate nucleus was recovered from deep cervical lymph [28]. The pathway for this drainage is along perivascular spaces of capillaries and arteries, which are in continuity with the subarachnoid space [29]. From there, fluid drains through discrete channels in the cribriform plate into lymphatics in the nasal submucosa and, thus, cervical lymph [30].

In a series of experiments, Harling-Berg *et al.* demonstrated that antigen injected within the brain parenchyma and ventricles drains to cervical lymph nodes, where it elicits an antibody response far superior to that achieved after intravenous or intralymphatic administration, yet failed to elicit a DTH or effective cytotoxic T-cell response [31]. This indicates a skewing towards B-cell and T helper 2-type responses. Soluble antigen might not even have to travel as far as the cervical lymph nodes to elicit an immune response. DCs present in the non-immune-privileged regions of the CNS, for example the CSF [32] and meninges, might take up antigen. Labelled DCs injected into the CSF reach the cervical lymph nodes, where they target B-cell areas preferentially, again indicating a

skewing towards a humoral response [20]. Furthermore, there is evidence for a tolerizing effect of intracerebral soluble antigen. For example, an ICV infusion of major basic protein (MBP) resulted in protection against MBP-induced EAE [33]. This has been substantiated by a more recent study in which cervical lymph node cells isolated from mice injected with ovalbumin in the striatum were transferred intravenously to donor mice, effectively protecting them from an ear DTH response to the same antigen [34].

Therefore, unlike other tissues, the afferent arm of the immune response in the brain lacks a cellular pathway for antigen transport, which is heavily dependent on the fluid route (Figure 2). Interestingly, a similar situation has been observed in the anterior chamber of the eye, which is another immune-privileged site [35]. Local phagocytic cells ingested fluorescent latex beads after injection in the anterior chamber of the eye and the dermis of the ear pinna. However, labelled uveal phagocytes did not migrate to regional lymph nodes, unlike their dermal counterparts. By contrast, injection of soluble fluorescent ovalbumin in the anterior chamber resulted in its appearance in the draining lymph nodes [35]. Similar experiments in the brain should reinforce the dichotomy between the cellular and fluid routes of antigen access to the peripheral immune system, which is probably the strongest determinant of immune privilege.

Efferent arm

The efferent arm of the immune response to CNS antigens is also specialized to confer a degree of immune privilege, but once again this is relative rather than absolute. The entry of monocytes, B cells and T cells into the CNS is highly regulated and has been discussed in the previous article.

Once antigen-specific T cells reach the CNS parenchyma, they face a variety of formidable challenges before they can exert their effector function. The most significant obstacle is death by apoptosis. All cells in the CNS express fas ligand (fasL), which results in the apoptosis of incoming fas-positive T cells [36], irrespective of antigen specificity [37]. If they survive, T cells need to recognize their cognate antigen in the context of MHC. However, constitutive expression of MHC is minimal in the normal CNS [38]. Once inflammation is established, upregulation of MHC occurs, although this is highly regulated in neurons [39]. T cells within the CNS parenchyma also face regulation by astrocytes, microglia and neurons. Astrocytes secrete unidentified soluble factors that inhibit T-cell proliferation and cytokine production [40] or induce regulatory T cells [41]. During inflammation, microglia express B7-H1, a homologue of the co-stimulatory molecule B7, which interacts with T-cell programmed death protein (PD)-1 and negatively regulates T-cell activation and cytokine production [42]. They also upregulate indoleamine 2,3-dioxygenase [43], resulting in a microenvironment rich in immunoregulatory tryptophan metabolites [44]. Recent data shows that neurons secrete transforming growth factor (TGF)-β and make cell–cell contact with activated T cells, converting them into regulatory T cells independent of their antigen specificity [45]. Despite this rigorous regulation, T cells can still initiate disease or contribute to pathogenicity.

Compared with T cells, B cells do not need to reach their target in the CNS parenchyma to exert their pathogenic effects. B cells in non-immune-privileged CNS regions produce antibodies, and the best testimony is the presence of unique oligoclonal bands in CSF, which are not present in serum, in a variety of inflammatory CNS disorders. We know that a germinal centre-like reaction occurs in the CNS [46], and the recently-described meningeal lymphoid neogenesis provides a putative anatomical location [26,27]. These antibodies might diffuse into the immune-privileged CNS parenchyma to exert their pathogenic effects. One of the ways in which antibodies exert their pathogenicity is by complement activation leading to lysis through terminal membrane attack complex formation. Neurons and rodent (but not human) oligodendrocytes are particularly susceptible to such lysis because they express significantly lower levels of membrane complement regulators (including CD35, CD46, CD55 and CD59) compared with other nucleated cells [47].

The effector arm of the innate immune system is also modified in the CNS, where microglia are the resident macrophages. They are of bone marrow origin and belong to the monocytic lineage, yet they have a downregulated phenotype in comparison with other tissue macrophages [38]; this is related to their location in the CNS microenvironment (Table 1). However, once inflammation is established, microglia upregulate most immunophenotypical markers depending on the inflammatory context. The exclusion of plasma proteins is involved because microglia in BBB-deficient areas display a more activated phenotype [48,49]. Neurons and astrocytes have an important role in suppressing microglial behaviour by means of cell–cell contact and the secretion of immunosuppressive factors (Box 2). Similar to macrophages elsewhere, microglia are sensitive to the effects of anti-inflammatory cytokines including TGFβ1, IL-4 and IL-10 [50]. However, TGFβ1 is produced within the naive in addition to the inflamed brain [51,52]; this cytokine is important in downregulating microglial responses, thus minimizing inflammation and brain damage, for example in prion disease [53].

Table 1. Microglial and macrophage immunophenotype compared

Molecule[a]	Resting microglia[b]	Macrophage[c]	Refs
CR3	+	++	[48,64]
FcR	+	+	[65,64]
CD68	+/−	+++	[66]
MHC-I	+	++	[67]
MHC-II	+	++	[68,67]
DC SIGN	−	+/−	[69]
CD80	−	+	[70]
CD86	−	+	[70]
CD40	−	+	[71]
LCA	+/−	++	[72]
CD4	+	++	[48]
Sialoadhesin	−	+	[49]

[a]Abbreviations: CR3, complement receptor 3; DC SIGN, dendritic cell-specific ICAM (intracellular adhesion molecule)-3 grabbing nonintegrin; FcR, Fc receptor; LCA, leukocyte common antigen.
[b]Once inflammation is established, microglia upregulate most immunophenotypical markers depending on the inflammatory context.
[c]−, no detectable expression; +, ++ and +++, increasing levels of expression.

Box 2. Regulation of microglial phenotype and function by CNS-resident cells and their products

Astrocytes

- **Unidentified soluble factors.** Sievers' group has shown that circulating monocytes and splenic macrophages can assume the ramified morphology and ion-channel characteristics of microglia after culture on astrocytic layers or exposure to astrocyte-conditioned medium [62,63]. Soluble factors have also been implicated in the astrocytic inhibition of stimulated microglial IL-12 production [61].

Neurons

- **Neuronal activity** suppresses the inducibility of MHC class II by microglia as shown by sodium-channel-blocking experiments with tetrodotoxin. This was shown to occur through electrical activity-related neurotrophin secretion by neurons, and was partly caused by agonism at the microglial p75 neurotrophin receptor [73].
- **Neuronal CD200–microglial CD200L interaction.** CD200 is expressed by neurons and its knockout resulted in spontaneous microglial activation and a worse disease outcome in EAE [74]. This occurs through microglial expression of CD200L [75].
- **Neuronal fractalkine–microglial CXCR1 interaction.** Fractalkine is a chemokine tonically released by neurons; knockout of its receptor, CX3CR1, which is expressed by microglia, resulted in an exquisite sensitivity of microglia to inflammation and resultant neurotoxicity [76].
- **Neuronal CD47–microglial signal regulatory protein (SIRP)-1α interaction.** CD47 and SIRP1α are members of the immunoglobulin superfamily, and are expressed by neurons and microglia, respectively [77,78]. Ligation of SIRP1α downregulates phagocytosis and LPS-induced TNF-α production through phosphorylation of its immunoreceptor tyrosine-based inhibition motifs (ITIMs), which, in turn, recruit and activate src homology phosphatase (SHP)1 and SHP2, thus negatively regulating cell signalling cascades by dephosphorylation [79].
- **Neuronal CD22–microglial CD45 interaction.** Neurons secrete CD22, which binds to microglial CD45, a transmembrane protein tyrosine phosphatase, and inhibits LPS-induced TNF-α production [80].
- **Various neuropeptides and neurotransmitters** including vasoactive intestinal peptide, calcitonin gene-related peptide, norepinephrine and α-melanocyte-stimulating hormone are immunosuppressive [81].

Astrocytes and neurons

- **Prostaglandins** are synthesized by both astrocytes [82] and neurons [83]. Prostaglandin E_2 downregulates inducible microglial activation and cytokine expression [84]. 15-deoxy-prostaglandin J_2, a natural peroxisome proliferator-activated receptor (PPAR)γ agonist arising from the non-enzymatic conversion of prostaglandin D_2, downregulates microglial LPS-induced nitric oxide and cytokine production, and IFNγ-induced MHC class II upregulation [85].

Immune privilege in the inflamed CNS

As mentioned in several instances earlier, the immune privilege of the CNS is severely undermined once inflammation is established. This might occur for several reasons: breakdown of the blood–brain barrier resulting in dilution of the immunosuppressive effects of the CNS microenvironment; local immunostimulatory effects of cytokines and chemokines; facilitation of antigen drainage to the periphery; the appearance of DCs; and the establishment of tertiary lymphoid tissue in the meninges.

Age-dependent effects

Relative innate immune privilege in the brain is influenced by age. For example, microglial reactivity is increased at extremes of age. Recently-established immature microglia in the developing brain are phagocytic [54], and microglia in aged rodents have an activated phenotype [55]. In both cases, they are responding to dying neuronal elements, which occurs as part of brain development or senescence. Another age-related phenomenon relates to acute neutrophilic infiltration of the brain parenchyma in response to a variety of insults, which occurs readily in juvenile rodents but not postnatally or in adulthood [56,57] – this might explain the probable susceptibility of children to head injuries or CNS infections.

Concluding remarks and future perspectives

CNS immune privilege is indispensable for damage limitation during inflammation in a sensitive organ with poor regenerative capacity. However, it is important to understand that the 'privilege' we are dealing with in the CNS does not relate to the absolute absence of immunological components but, rather, their elaborate regulation. This is similar to the concept of the BBB, which, as discussed in the previous article, is a highly regulated instead of absolute structure. Box 1 lists what CNS immune privilege is and is not. The principal determinants of immune privilege include the specialization of the afferent arm of the adaptive immune response, which is skewed away from cell-mediated towards soluble antigen drainage, and the regulated immunosuppressive microenvironment of the CNS.

Although progress has been made, we do not yet possess a thorough understanding of the molecular mechanisms underlying the induction and maintenance of CNS immune privilege. The afferent pathway is almost certainly not only about the absence of cell-mediated antigen egress; the soluble pathway might also result in active tolerization in cervical lymph nodes, which are responsible for nasal mucosal tolerance [58], although how this occurs is still unclear. T-cell egress from the CNS has just been described [59]; it would be interesting to know whether centrally generated regulatory T cells follow this route. Several molecular interactions accounting for the inhospitality of the CNS to inflammation have recently been discovered, but this is probably the tip of the iceberg. For example, triggering receptor expressed on myeloid cells (TREM)2, expressed by microglia, is essential for the phagocytosis of apoptotic neurons without eliciting inflammation [60], but its ligand is still unknown. The mysterious qualities of the astrocyte-conditioned medium [61,40,62,63,41] is another 'holy grail'. The 'window of susceptibility' to acute inflammation in juveniles is also still unexplained, as is the activated phenotype of microglia in the aged brain. The picture is far from complete. . . .

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