What is immune privilege (not)?

Ian Galea[1], Ingo Bechmann[2] & V Hugh Perry[1]

[1]CNS Inflammation Group, School of Biological Sciences, University of Southampton, SO16 7PX, UK; [2]Institute of Clinical Neuroanatomy, Johann Wolfgang Goethe-University, 60590 Frankfurt/Main, Germany.

Contents:

23 pages
1 table (end of this file)
2 boxes (end of this file)
2 figures as separate Powerpoint file

Corresponding author:

Dr Ian Galea
CNS Inflammation Group
School of Biological Sciences
Bassett Crescent East
University of Southampton
Southampton SO16 7PX
UK

Tel no - 0044 2380 594187
Fax no - 0044 2380 592711
E-mail - I.Galea@soton.ac.uk (preferred mode of contact)

The immune privilege of the central nervous system is indispensable for damage limitation during inflammation in a sensitive organ with poor regenerative capacity. It is a longstanding notion which has down the years acquired a lack of precision in its definition and a number of misconceptions. 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 brain's afferent arm in adaptive immunity, 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 actively maintained 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 has been shown as early as 1921 in Japan. Back then, Y Shirai observed that rat sarcoma grew well when transplanted into the mouse brain parenchyma, but not when implanted subcutaneously or intramuscularly [2]. Murphy and Strum extended these findings in 1923 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

shown to hold true 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 brain's "immune privilege" was being discovered, the blood-brain barrier (BBB) was under study, and the two concepts grew together. This had two consequences. First, the brain's "immune privilege" assumed a more absolute meaning, rendering it too strong a descriptive term for the brain's relationship with the immune system **(Box 1)**. Secondly, "immune privilege" was inappropriately wholly attributed to the BBB (see previous article), whereas other features such as the specialization of afferent communication from 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 brain's "immune privilege" 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 (Oxford English Dictionary). It is neither an absolute nor an immutable state. The seminal experiments described above are entirely consistent with the concept of "immune privilege" – they do not infer or require any qualification of "absolute" or "partial" privilege since there was no evidence in these experiments that immune privilege is absolute. The "immune privilege" of the brain is certainly not absolute but relative to other organs. Also, Shirai's rat sarcoma might have survived well in the brains of his mice since 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 central nervous system is organized into different compartments: the parenchyma proper, the ventricles containing choroid plexus and cerebrospinal fluid (CSF), and the outer meninges (**Figure 1**). In the same 1923 paper, Murphy and Strum had described how rejection of the foreign tumour within the brain occurred if it approached the ventricle [3]. This has now 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 in the skin elicited neutrophil and monocyte recruitment within 2 hours. 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 lipopolysaccharide (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 privilege” as well. 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 while 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 due 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, Peter 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 circulating immunized leucocytes' access to the intracerebral skin graft was facilitated by the tissue trauma sustained during implantation surgery. Therefore, Matyzak *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 naïve T cells resulting in their priming and activation. In most tissues, antigen transport to draining lymph nodes and spleen is crucial in generating such a primary immune response, and occurs in two ways: (1) by the emigration of professional antigen presenting cells (APCs), dendritic cells (DCs) bearing antigens from the immune-challenged site to local lymph nodes, and (2) by the drainage of soluble antigens in lymph, representing cellular and fluid routes respectively **(Figure 2)**.

Cellular route

Immunocytochemical studies have also 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 is incapable of priming naïve 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 rejection of xenografts [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 [21,22,23]. A critical component for the interpretation of 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.

Rather than migrating out of the CNS parenchyma to prime T or B cells in cervical lymph nodes, DCs accumulating in the inflamed CNS may do their job *in situ*. There is recent evidence that DCs isolated from the CNS of mice with proteolipid protein (PLP)-induced EAE are able to prime naïve PLP-specific T cells *ex vivo* in the absence of PLP peptide [25]. Whether this happens *in vivo* remains 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, Cserr and colleagues 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 such as the CSF [32] and meninges may take up antigen. It has been shown that labelled DCs injected into the CSF reaches the cervical lymph nodes where they preferentially target B cell areas, 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 intravenously transferred 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-priviliged” 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 labeled uveal phagocytes did not migrate to regional lymph nodes, unlike their dermal counterparts. On the other hand, injection of soluble fluorescent ovalbumin in the anterior chamber resulted in its appearance in the draining lymph nodes [35]. Similar experiments in the brain will reinforce the dichotomy between the cellular and fluid routes of antigen access to the peripheral immune system, which is likely to be 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 make it to 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 fasL which results in 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 major histocompatibility complex (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 neurones [39]. T cells within the CNS parenchyma also face regulation by astrocytes, microglia and neurones. Astrocytes secrete unidentified soluble factors which inhibit T cell proliferation and cytokine production [40] or induce regulatory T cells [41]. During inflammation, microglia express B7-H1, a homolog of the costimulatory molecule B7 which interacts with T cell 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-to-cell contact with activated T cells converting them into regulatory T cells independent of their antigen specificity [45].

Despite such rigorous regulation, T cells are still capable of initiating disease or contributing to pathogenicity.

Compared to 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 may 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 via terminal membrane attack complex formation. Neurones, as well as rodent (but not human) oligodendrocytes are particularly susceptible to such lysis since they express significantly lower levels of membrane complement regulators (such as CD35, CD46, CD55 and 59) compared to other nucleated cells [47].

The innate immune system's effector arm 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)**. Once inflammation is established however, microglia upregulate most immunophenotypical markers depending on the inflammatory context. The exclusion of plasma proteins plays a part since microglia in BBB-deficient areas display a more activated phenotype [48,49]. Neurones and astrocytes play an important role in suppressing microglial behaviour by means of cell-to-cell contact and secretion of immunosuppressive factors **(Box 2)**. Similar to macrophages elsewhere, microglia are sensitive to the effects of anti-inflammatory cytokines such as TGFβ1, IL-4 and IL-10 [50]. However TGFβ1 is produced within the naïve and inflamed brain [51,52]; this cytokine has been shown to be important in downregulation of microglial responses minimizing inflammation and thus brain damage, for instance in prion disease [53].

Immune privilege in the inflamed CNS

As mentioned in several instances above, the "immune privilege" of the CNS is severely undermined once inflammation is established. This may 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, appearance of DCs and establishment of tertiary lymphoid tissue in the meninges.

Age-dependent effects

Relative *innate* "immune privilege" in the brain is influenced by age. For instance microglial reactivity is increased at extremes of age. Recently-established immature microglia in the developing brain are phagocytic [54] and microglia in aged rodents [55] have an activated phenotype. 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 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. Yet, 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 rather than 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 may also result in active tolerization in cervical lymph nodes, which have been shown to be responsible for nasal mucosal tolerance [58], though how this happens is still unclear. Egress of T cells 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 instance, triggering receptor expressed on myeloid cells-2 (TREM2), expressed by microglia, has been found to be essential for phagocytosis of apoptotic neurones in the absence of inflammation [60], but its ligand remains 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 also remains unexplained, as does the activated phenotype of microglia in the aged brain. The picture is far from complete….

Acknowledgements

We thank the MS Society (UK) (grant reference numbers 784/03) as well as the European Union (grant references QLG3-CT-2002-00612 and LSHM-CT-2005-018637) for financial support. We would also like to thank Professor J Nicoll (University of Southampton) for helpful discussions.

Due to space limitations some of the relevant literature has not received mention. We extend our apologies to the authors concerned.

TABLE 1

Microglial and macrophage immunophenotype compared

Molecule	Resting microglia	Macrophage	References
CR3	+	++	**[48,64]**
FcR	+	+	**[65,64]**
CD68	+/-	+++	**[66]**
MHCI	+	++	**[67]**
MHCII	+	++	**[68,67]**
DC SIGN	-	+/-	**[69]**
CD80	-	+	**[70]**
CD86	-	+	**[70]**
CD40	-	+	**[71]**
LCA	+/-	++	**[72]**
CD4	+	++	**[48]**
sialoadhesin	-	+	**[49]**

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

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 as well as 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

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 astrocytic inhibition of stimulated microglial IL-12 production [61].

NEURONES

- **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 via electrical activity-related neurotrophin secretion by neurones, and was partly due to agonism at the microglial p75 neurotrophin receptor [73].
- **Neuronal CD200 – microglial CD200L interaction**. CD200 is expressed by neurones and when knocked out 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 neurones; knock-out 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 expressed by neurons and microglia respectively [77,78]. Ligation of SIRP1α has been shown to downregulate phagocytosis and LPS-induced TNFα production through phosphorylation of its ITIMs (immunoreceptor tyrosine-based inhibition motifs),

which in turn recruit and activate src homology phosphatases (SHP1 and SHP2) thus negatively regulating cell signalling cascades by dephosphorylation [79].

- **Neuronal CD22 – microglial CD45 interaction**. Neurones secrete CD22 which binds to microglial CD45, a transmembrane protein tyrosine phosphatase, and inhibits LPS-induced TNFα production [80].
- **Various neuropeptides and neurotransmitters** such as vasoactive intestinal peptide, calcitonin gene-related peptide, norepinephrine and α-melanocyte stimulating hormone have been shown to be immunosuppressive [81].

ASTROCYTES & NEURONES

- **Prostaglandins** are synthesized by both astrocytes [82] and neurones [83]. Prostaglandin E_2 downregulates inducible microglial activation and cytokine expression [84]. 15-deoxy-prostaglandin J_2, a natural PPARγ (peroxisome proliferator-activated receptor-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].

Legend to Figure 1

Central nervous system 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 outer surface of the brain and the outer meninges. Circumventricular organs are brain regions lacking a blood-brain barrier.

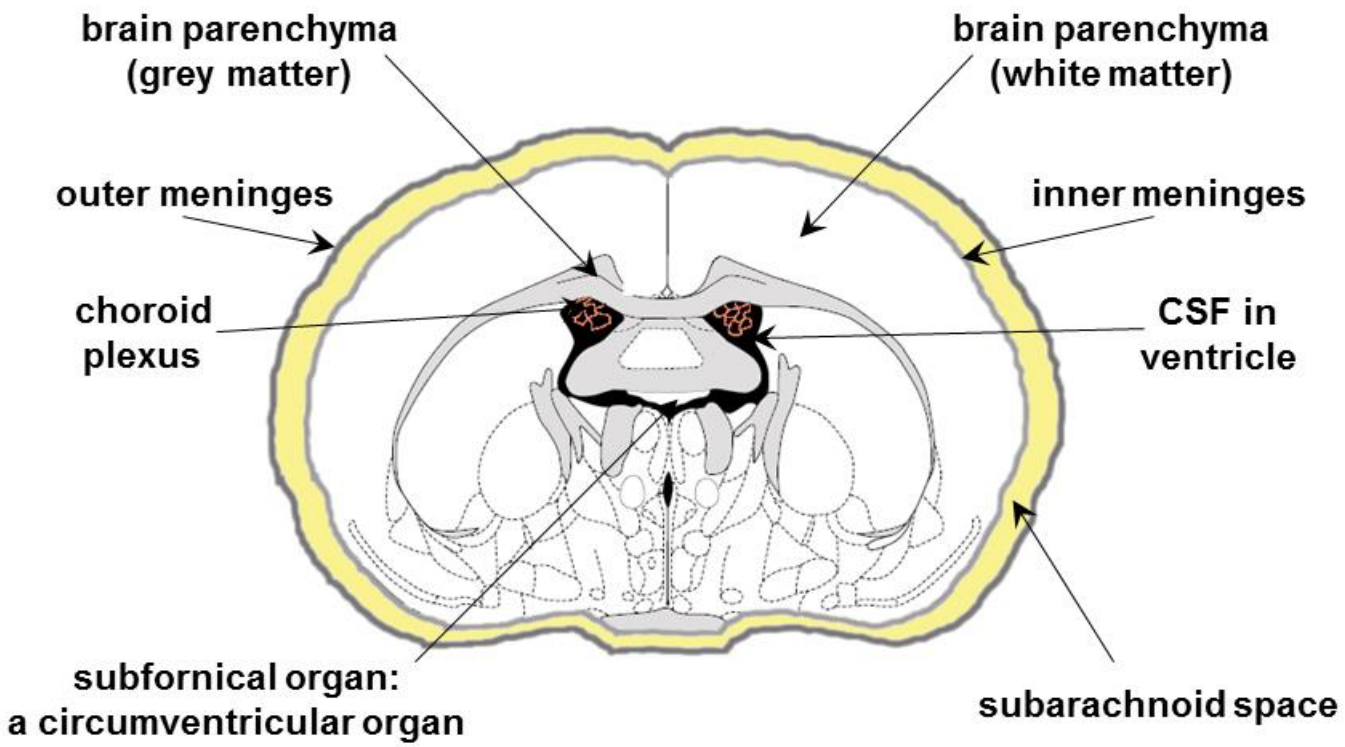

Figure 1. Central nervous system compartments

Legend to 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 DCs in the draining lymph nodes via soluble (green) or cellular (yellow) routes. Soluble antigen drains by bulk flow via lymphatic channels. Alternatively cells in the tissue such as local immature DCs or other leucocytes ingest antigen and transport it to the draining lymph nodes. Whichever way the antigen reaches the lymph nodes, it is there presented to naïve T cells and B cells by professional antigen presenting cells 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 traffic into the tissue of cells of the innate (neutrophils and macrophages) and adaptive (T cells and B cells activated via afferent arm) immune systems. Note: * CNS refers to parenchyma only since the immune arc in non-parenchymal sites such as meninges shares similarities with peripheral tissues. Width of arrows is directly proportional to activity of involved arm, while broken arrows (‖) represent absent or non-detectable activity. +/++ reduced, +++ robust, - absent or non-detectable.

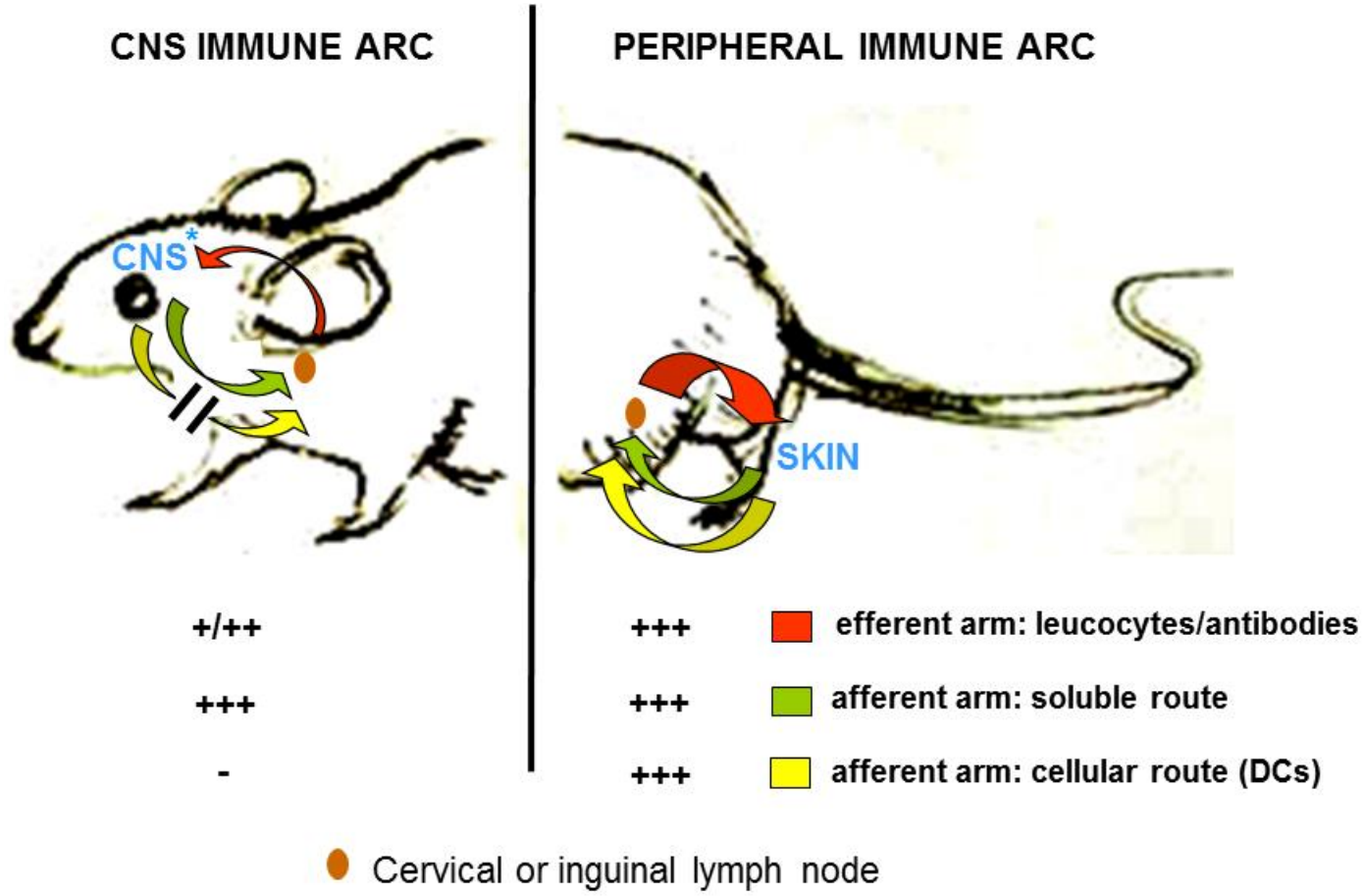

Figure 2. The immune arc in CNS parenchyma and periphery compared

References

1 Trumble, W. and Brown, L., eds (1973) *The Shorter Oxford English Dictionary*, Oxford University Press.

2 Shirai, Y. (1921) On the transplantation of the rat sarcoma in adult heterogenous animals. *Jap. Med. World* 14-15

3 Murphy, J.B. and Sturm, E. (1923) Conditions determining the transplantability of tissues in the brain. *J. Exp. Med.* 38, 183-197

4 Billingham, R.E. and Boswell, T. (1953) Studies on the problem of corneal homografts. *Proc. R. Soc. Lond. B. Biol. Sci.* 141, 392-406

5 Medawar, P.B. (1948) Immunity to homologous grafted skin. *Brit. J. Exp. Path.* 29, 58-69

6 Matyszak, M.K. and Perry, V.H. (1995) Demyelination in the central nervous system following a delayed-type hypersensitivity response to bacillus Calmette-Guerin. *Neuroscience* 64, 967-977

7 Stevenson, P.G. et al. (1997) The immunogenicity of intracerebral virus infection depends on anatomical site. *J. Virol.* 71, 145-151

8 Byrnes, A.P. et al. (1996) Immunological instability of persistent adenovirus vectors in the brain: peripheral exposure to vector leads to renewed inflammation, reduced gene expression, and demyelination. *J. Neurosci.* 16, 3045-3055

9 Finsen, B.R. et al. (1991) Leukocyte infiltration and glial reactions in xenografts of mouse brain tissue undergoing rejection in the adult rat brain. A light and electron microscopical immunocytochemical study. *J. Neuroimmunol.* 32, 159-183

10 Mason, D.W. et al. (1986) The fate of allogeneic and xenogeneic neuronal tissue transplanted into the third ventricle of rodents. *Neuroscience* 19, 685-694

11 Matyszak, M.K. and Perry, V.H. (1996) A comparison of leucocyte responses to heat-killed bacillus Calmette-Guerin in different CNS compartments. *Neuropathol. Appl. Neurobiol.* 22, 44-53

12 Andersson, P.B. et al. (1992) The acute inflammatory response to lipopolysaccharide in CNS parenchyma differs from that in other body tissues. *Neuroscience* 48, 169-186

13 Blond, D. et al. (2002) Differential induction of interleukin-1beta and tumour necrosis factor-alpha may account for specific patterns of leukocyte recruitment in the brain. *Brain Res.* 958, 89-99

14 Schnell, L. et al. (1999) Acute inflammatory responses to mechanical lesions in the CNS: differences between brain and spinal cord. *Eur. J. Neurosci.* 11, 3648-3658

15 Matyszak, M.K. and Perry, V.H. (1998) Bacillus Calmette-Guerin sequestered in the brain parenchyma escapes immune recognition. *J. Neuroimmunol.* 82, 73-80

16 Matyszak, M.K. and Perry, V.H. (1996) The potential role of dendritic cells in immune-mediated inflammatory diseases in the central nervous system. *Neuroscience* 74, 599-608

17 Mendez-Fernandez, Y.V. et al. (2005) Anatomical and cellular requirements for the activation and migration of virus-specific CD8+ T cells to the brain during Theiler's virus infection. *J. Virol.* 79, 3063-3070

18 Michel, D.C. et al. (2006) Dendritic cell recruitment following xenografting of pig fetal mesencephalic cells into the rat brain. *Exp. Neurol.* 202, 76-84

19 de Vos, A.F. et al. (2002) Transfer of central nervous system autoantigens and presentation in secondary lymphoid organs. *J. Immunol.* 169, 5415-5423

20 Hatterer, E. et al. (2006) How to drain without lymphatics? Dendritic cells migrate from the cerebrospinal fluid to the B-cell follicles of cervical lymph nodes. *Blood* 107, 806-812

21 Carson, M.J. et al. (1999) Disproportionate recruitment of CD8+ T cells into the central nervous system by professional antigen-presenting cells. *Am. J. Pathol.* 154, 481-494

22 Karman, J. et al. (2004) Initiation of immune responses in brain is promoted by local dendritic cells. *J. Immunol.* 173, 2353-2361

23 Oehmichen, M. et al. (1979) Lymphatic efflux of intracerebrally injected cells. *Acta Neuropathol. (Berl)* 45, 61-65

24 Mims, C.A. (1960) Intracerebral injections and the growth of viruses in the mouse brain. *Br. J. Exp. Pathol.* 41, 52-59

25 McMahon, E.J. et al. (2005) Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat. Med.* 11, 335-339

26 Magliozzi, R. et al. (2004) Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 148, 11-23

27 Serafini, B. et al. (2004) Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* 14, 164-174

28 Yamada, S. et al. (1991) Albumin outflow into deep cervical lymph from different regions of rabbit brain. *Am. J. Physiol.* 261, H1197-H1204

29 Ichimura, T. et al. (1991) Distribution of extracellular tracers in perivascular spaces of the rat brain. *Brain Res.* 545, 103-113

30 Kida, S. et al. (1993) CSF drains directly from the subarachnoid space into nasal lymphatics in the rat. Anatomy, histology and immunological significance. *Neuropathol. Appl. Neurobiol.* 19, 480-488

31 Harling-Berg, C.J. et al. (1999) Role of the cervical lymphatics in the Th2-type hierarchy of CNS immune regulation. *J. Neuroimmunol.* 101, 111-127

32 Pashenkov, M. et al. (2002) Recruitment of dendritic cells to the cerebrospinal fluid in bacterial neuroinfections. *J. Neuroimmunol.* 122, 106-116

33 Harling-Berg, C.J. et al. (1991) Myelin basic protein infused into cerebrospinal fluid suppresses experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 35, 45-51

34 Wenkel, H. et al. (2000) Systemic immune deviation in the brain that does not depend on the integrity of the blood-brain barrier. *J. Immunol.* 164, 5125-5131

35 Dullforce, P.A. et al. (2004) APCs in the anterior uveal tract do not migrate to draining lymph nodes. *J. Immunol.* 172, 6701-6708

36 Bechmann, I. et al. (1999) FasL (CD95L, Apo1L) is expressed in the normal rat and human brain: evidence for the existence of an immunological brain barrier. *Glia* 27, 62-74

37 Bauer, J. et al. (1998) T-cell apoptosis in inflammatory brain lesions: destruction of T cells does not depend on antigen recognition. *Am. J. Pathol.* 153, 715-724

38 Perry, V.H. (1998) A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. *J. Neuroimmunol.* 90, 113-121

39 Neumann, H. et al. (1997) Major histocompatibility complex (MHC) class I gene expression in single neurons of the central nervous system: differential regulation by interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha. *J. Exp. Med.* 185, 305-316

40 Meinl, E. et al. (1994) Multiple sclerosis. Immunomodulatory effects of human astrocytes on T cells. *Brain* 117 (Pt 6), 1323-1332

41 Trajkovic, V. et al. (2004) Astrocyte-induced regulatory T cells mitigate CNS autoimmunity. *Glia* 47, 168-179

42 Magnus, T. et al. (2005) Microglial expression of the B7 family member B7 homolog 1 confers strong immune inhibition: implications for immune responses and autoimmunity in the CNS. *J. Neurosci.* 25, 2537-2546

43 Kwidzinski, E. et al. (2005) Indolamine 2,3-dioxygenase is expressed in the CNS and down-regulates autoimmune inflammation. *FASEB J.* 19, 1347-1349

44 Platten, M. et al. (2005) Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. *Science* 310, 850-855

45 Liu, Y. et al. (2006) Neuron-mediated generation of regulatory T cells from encephalitogenic T cells suppresses EAE. *Nat. Med.* 12, 518-525

46 Corcione, A. et al. (2004) Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A* 101, 11064-11069

47 Gasque, P. et al. (2000) Complement components of the innate immune system in health and disease in the CNS. *Immunopharmacology* 49, 171-186

48 Perry, V.H. and Gordon, S. (1987) Modulation of CD4 antigen on macrophages and microglia in rat brain. *J. Exp. Med.* 166, 1138-1143

49 Perry, V.H. et al. (1992) The blood-brain barrier regulates the expression of a macrophage sialic acid-binding receptor on microglia. *J. Cell. Sci.* 101 (Pt 1), 201-207

50 O'Keefe, G.M. et al. (1999) Class II transactivator and class II MHC gene expression in microglia: modulation by the cytokines TGF-beta, IL-4, IL-13 and IL-10. *Eur. J. Immunol.* 29, 1275-1285

51 Cunningham, C. et al. (2002) Transforming growth factor beta1, the dominant cytokine in murine prion disease: influence on inflammatory cytokine synthesis and alteration of vascular extracellular matrix. *Neuropathol. Appl. Neurobiol.* 28, 107-119

52 Logan, A. et al. (1992) Enhanced expression of transforming growth factor beta 1 in the rat brain after a localized cerebral injury. *Brain Res.* 587, 216-225

53 Boche, D. et al. (2006) TGFbeta1 regulates the inflammatory response during chronic neurodegeneration. *Neurobiol. Dis.* 22, 638-650

54 Perry, V.H. (2001) Microglia in the developing and mature central nervous system. In *Glial cell development: basic principles and clinical relevance* (2nd edition) (Jessen, K.R.and Richardson, W.D., eds), pp. 75-90, Oxford University Press.

55 Perry, V.H. et al. (1993) Altered antigen expression of microglia in the aged rodent CNS. *Glia* 7, 60-67

56 Anthony, D.C. et al. (1997) Age-related effects of interleukin-1 beta on polymorphonuclear neutrophil-dependent increases in blood-brain barrier permeability in rats. *Brain* 120 (Pt 3), 435-444

57 Lawson, L.J. and Perry, V.H. (1995) The unique characteristics of inflammatory responses in mouse brain are acquired during postnatal development. *Eur. J. Neurosci.* 7, 1584-1595

58 Wolvers, D.A. et al. (1999) Intranasally induced immunological tolerance is determined by characteristics of the draining lymph nodes: studies with OVA and human cartilage gp-39. *J. Immunol.* 162, 1994-1998

59 Goldmann, J. et al. (2006) T cells traffic from brain to cervical lymph nodes via the cribroid plate and the nasal mucosa. *J. Leukoc. Biol.* 80, 797-801

60 Takahashi, K. et al. (2005) Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *J. Exp. Med.* 201, 647-657

61 Aloisi, F. et al. (1997) IL-12 production by central nervous system microglia is inhibited by astrocytes. *J. Immunol.* 159, 1604-1612

62 Schmidtmayer, J. et al. (1994) Blood monocytes and spleen macrophages differentiate into microglia-like cells on monolayers of astrocytes: membrane currents. *Glia* 12, 259-267

63 Sievers, J. et al. (1994) Blood monocytes and spleen macrophages differentiate into microglia-like cells on monolayers of astrocytes: morphology. *Glia* 12, 245-258

64 Ulvestad, E. et al. (1994) Human microglial cells have phenotypic and functional characteristics in common with both macrophages and dendritic antigen-presenting cells. *J. Leukoc. Biol.* 56, 732-740

65 Akiyama, H. and McGeer, P.L. (1990) Brain microglia constitutively express beta-2 integrins. *J. Neuroimmunol.* 30, 81-93

66 Hulette, C.M. et al. (1992) Macrophage markers in diagnostic neuropathology. *Am. J. Surg. Pathol.* 16, 493-499

67 Vass, K. and Lassmann, H. (1990) Intrathecal application of interferon gamma. Progressive appearance of MHC antigens within the rat nervous system. *Am. J. Pathol.* 137, 789-800

68 Ulvestad, E. et al. (1994) HLA class II molecules (HLA-DR, -DP, -DQ) on cells in the human CNS studied in situ and in vitro. *Immunology* 82, 535-541

69 Schwartz, A.J. et al. (2002) Distribution and immunophenotype of DC-SIGN-expressing cells in SIV-infected and uninfected macaques. *AIDS Res. Hum. Retroviruses* 18, 1021-1029

70 De Simone, R. et al. (1995) The costimulatory molecule B7 is expressed on human microglia in culture and in multiple sclerosis acute lesions. *J. Neuropathol. Exp. Neurol.* 54, 175-187

71 Gerritse, K. et al. (1996) CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. *Proc. Natl. Acad. Sci. U S A* 93, 2499-2504

72 Ford, A.L. et al. (1995) Normal adult ramified microglia separated from other central nervous system macrophages by flow cytometric sorting. Phenotypic

differences defined and direct ex vivo antigen presentation to myelin basic protein-reactive CD4+ T cells compared. *J. Immunol.* 154, 4309-4321

73 Neumann, H. et al. (1998) Neurotrophins inhibit major histocompatibility class II inducibility of microglia: involvement of the p75 neurotrophin receptor. *Proc. Natl. Acad. Sci. U. S. A.* 95, 5779-5784

74 Hoek, R.M. et al. (2000) Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 290, 1768-1771

75 Wright, G.J. et al. (2000) Lymphoid/neuronal cell surface OX2 glycoprotein recognizes a novel receptor on macrophages implicated in the control of their function. *Immunity.* 13, 233-242

76 Cardona, A.E. et al. (2006) Control of microglial neurotoxicity by the fractalkine receptor. *Nat. Neurosci.* 9, 917-924

77 Adams, S. et al. (1998) Signal-regulatory protein is selectively expressed by myeloid and neuronal cells. *J. Immunol.* 161, 1853-1859

78 Reinhold, M.I. et al. (1995) In vivo expression of alternatively spliced forms of integrin-associated protein (CD47). *J. Cell Sci.* 108 (Pt 11), 3419-3425

79 van Beek, E.M. et al. (2005) Signal regulatory proteins in the immune system. *J. Immunol.* 175, 7781-7787

80 Mott, R.T. et al. (2004) Neuronal expression of CD22: novel mechanism for inhibiting microglial proinflammatory cytokine production. *Glia* 46, 369-379

81 Niederkorn, J.Y. (2006) See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat. Immunol.* 7, 354-359

82 Murphy, S. et al. (1988) Astrocytes as eicosanoid-producing cells. *Glia* 1, 241-245

83 Vane, J.R. et al. (1998) Cyclooxygenases 1 and 2. *Annu. Rev. Pharmacol. Toxicol.* 38, 97-120

84 Levi, G. et al. (1998) Regulation of prostanoid synthesis in microglial cells and effects of prostaglandin E2 on microglial functions. *Biochimie* 80, 899-904

85 Bernardo, A. et al. (2000) Role of the peroxisome proliferator-activated receptor-gamma (PPAR-gamma) and its natural ligand 15-deoxy-Delta12, 14-prostaglandin J2 in the regulation of microglial functions. *Eur. J. Neurosci.* 12, 2215-2223