The cellular prion protein: a player in immunological quiescence

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Despite intensive studies since the 1990s, the physiological role of the cellular prion protein (PrPc) remains elusive. Here, we present a novel concept suggesting that PrPc contributes to immunological quiescence in addition to cell protection. PrPc is highly expressed in diverse organs that by multiple means are particularly protected from inflammation, such as the brain, eye, placenta, pregnant uterus, and testes, while at the same time it is expressed in most cells of the lymphoreticular system. In this paradigm, PrPc serves two principal roles: to modulate the inflammatory potential of immune cells and to protect vulnerable parenchymal cells against noxious insults generated through inflammation. Here, we review studies of PrPc physiology in view of this concept.

Introduction

The cellular prion protein (PrPc) is known for its crucial involvement, via its scrapie isoform PrPSc, in the development of transmissible spongiform encephalopathies (TSEs), such as Creutzfeldt–Jakob disease in man and scrapie in sheep and goats. In these fatal diseases, the spontaneous or template-directed misfolding of PrPc into abnormal PrPSc subverts PrPc’s normal function and causes synaptic loss and neuronal demise (1, 2). Despite extensive investigations for three decades, PrPc remains a conundrum. What is the major physiological role of this protein and why does it exist under a plethora of isoforms and interact with so many partners? Why do Prnp knockout mice develop normally and without major phenotypic alterations, although PrPc is ubiquitous and highly conserved between species? Here, based on the current knowledge of PrPc function, we provide an overview of its potential contribution to a phenomenon generically known as immune privilege, providing a new angle to the question of the physiological role of PrPc.

During evolution of the vertebrate immune system, the potency of inflammatory responses has increased, not least during the transition from ectothermic (fish, amphibians, and reptiles) to endothermic (birds and mammals) species, dramatically increasing O2 consumption and reactive oxygen species (ROS) generation (3). In parallel with increasing immunological firepower, to combat intruding microorganisms and cancer cells, several anti-inflammatory and protective measures have evolved to shield “innocent bystander” cells from inflammatory damage. Interestingly, major areas of modern medical treatment are concerned with dampening inflammatory responses. In organs such as the eye, the brain, pregnant uterus, and testicles, inflammation can have devastating consequences. One fascinating development to protect such tissues with limited regenerative capacity is the
evolution of immune privilege. This phenomenon is established through combinations of physical barriers and circulatory adaptations, together with organ expression of potent cell surface and soluble immunomodulatory factors (4). The principal concept presented in this review is that PrP\(^C\) serves an anti-inflammatory and protective role. This explains the prevalent observation that phenotypes due to loss of PrP\(^C\) are minor if detectable at all under physiological conditions, while clearly evident under stress and particularly under inflammation in immune-privileged organs, such as the brain.

**Immune Privilege**

In the late nineteenth century, VanDooremaal and later, in the 1940s, Medawar pioneered studies that defined “immune privilege” after studying grafts that survived after being transplanted into the brain or anterior chamber of the eye (5) [reviewed in Ref. (6)]. Classically, immune-privileged sites were the central nervous system (CNS), the anterior chamber of the eye, the placenta and fetus, and testicles. Immune privilege was considered a static phenomenon, mainly resulting from anatomical and circulatory peculiarities, such as an apparent lack of lymphatic drainage. This concept was supported by the discovery of the blood–brain barrier and later the blood–testis barrier (7).

Today, our understanding of immune privilege, often referred to as immunological quiescence, is vastly extended, and may be defined as a dynamic and highly complex interplay between anatomical, physiological and immunoregulatory adaptations, which together restrict, deviate, and block inflammatory processes in the privileged tissue (8, 9). Moreover, many organs and cellular niches can obtain immune privilege, and certain elements of immune privilege have been observed in tumor growth and during chronic inflammation. Importantly, immune privilege is not a general immunosuppression, but involves tight control and often downregulation of those immune responses that potentially cause severe tissue damage. These include cytotoxic T cells, natural killer cells (NK), and pro-inflammatory cytokines.

Several cell surface and extracellular proteins play important parts in immune privilege, e.g., the immunomodulatory enzyme indolamine 2,3-dioxygenase (IDO) which causes a local depletion of L-tryptophan and thereby halts proliferation of T cells (10), the apoptosis-stimulating receptor ligand couple Fas/FasL (11), tumor necrosis factor alpha apoptosis inducing ligand (TRAIL) (12), and transforming growth factor beta (TGF-\(\beta\)) (13), amongst others. In addition, in many immune-privileged organs, cells, such as neurons, have low expression of major histocompatibility complex (MHC) class Ia molecules, which confer fundamental protection from cytotoxic T cells [reviewed in Ref. (4)]. They instead express non-classical MHC class I\(b\) molecules that downregulate NK cell activity. Novel immunomodulating proteins are constantly being discovered (14, 15), and considering the importance of monitoring and fine-tuning inflammatory responses in particularly vulnerable organs, there are probably many more to come.

Another contribution to immune privilege is active recruitment of CD4\(^+\) CD25\(^+\) Foxp3\(^+\) regulatory T cells (TREGs), which suppress the activation of other T cells, both directly and indirectly [reviewed in Ref. (16)]. TREGs exert their immunosuppressive activities in various ways, such as cell-cycle arrest via suppressive cytokines, e.g., IL-10 and TGF-\(\beta\) or cell surface expression and secretion of molecules, such as Galectin-1. They can induce apoptosis in IL-2-dependent T cells via IL-2 uptake or direct cytolysis via, e.g., granzyme. Indirectly, TREGs can also exert their effects on antigen-presenting cells, impairing their co-stimulatory or antigen-presenting activity or inducing them to remain naïve.

**Posttranslational Modifications of PrP\(^C\)**

Cellular prion protein is a 210-residue glycoprotein, encoded by a single-copy gene denoted Prnp (17, 18). It is mainly located at the outer leaflet of the plasma membrane, attached by a glycosylphosphatidylinositol (GPI) anchor (19) and exists in many forms due to variable N-glycosylation (20) and proteolytic processing (21, 22).

Mammalian PrP\(^C\) has two N-X-T sequence motifs for glycosylation (Figure 1) and most PrP\(^C\) isolated from tissues is indeed diglycosylated. The glycosylation sites of mammalian PrP\(^C\) appear invariant and they are conserved in avian, reptilian, and amphibian PrP\(^C\) sequences; glycosylation promotes trafficking of PrP\(^C\) to the plasma membrane and may increase PrP\(^C\) half-life. As a response to oxidative stress, mammalian PrP\(^C\) can be cleaved near codon 90 generating fragments PrP-N2 and PrP-C2 (22, 23). Alternatively, while passing the late-Golgi compartment, a subset of PrP\(^C\) is processed into two fragments PrP-N1 and PrP-C1 (Figure 1, C1 processing site) (24). The N-terminal PrP-N1 fragment is secreted, whereas the remaining C-terminal fragment PrP-C1 remains attached to the cell membrane. Whether it is localized in lipid rafts like full-length PrP\(^C\) remains to be shown.

PrP-C1 may also be generated on the cell membrane (23), while C-terminal shedding by ADAM10 can release full-length PrP\(^C\) (and PrP-C1) from the membrane (25, 26). The generation of truncated PrP-C1 is conserved in avian PrP\(^C\) and is likely to occur also in other vertebrates, intriguingly it is also seen in other prion family genes (PRND, SPRN) (27) and even in the structurally related zinc-transporter ZIP10 (SLC39A10) (28).

Although PrP\(^C\) processing is in principal conserved, there is evidence that the degree of cleavage and shedding is dependent on the PrP\(^C\) sequence (29). Questions remain whether cleavage and PrP-C1 specifically may influence regulation and function attributed to normal prion protein and because full-length PrP\(^C\) and PrP-C1 are not always experimentally differentiated it is often left unresolved which of the isoforms is actually responsible for the observed activity. However, the recent finding that PrP-C1 exhibited myelinotrophic activity in the peripheral nervous system strengthens the concept that all the various PrP\(^C\) molecules have physiological importance (30).

**The Intrinsically Disordered N-Terminal Domain of PrP\(^C\)**

Structural studies revealed that PrP\(^C\) is composed of an intrinsically disordered N-terminal and a structured C-terminal domain, containing three \(\alpha\)-helical regions and a short, two-stranded \(\beta\)-sheet (31–33). Classically, the activity of a protein
was linked to the ability of the polypeptide chain to adopt a stable secondary/tertiary structure. This concept was extended when it became evident that intrinsically disordered region (IDRs) and proteins can participate in a broad range of defined physiological activities and play a major role in several protein classes, including transcription factors, scaffold proteins, and signaling molecules (34–36). This ability of IDRs to interact with many different substrates may explain the observation that PrPC can flexibly engage in a variety of supramolecular complexes (see below).

Considering the evolution of PrP\(^\text{C}\), it is interesting to note that the three-dimensional structure of the globular C-terminal domain of human (121–230), chicken (121–225), turtle (121–225), and xenopus (90–222) PrP\(^\text{C}\) show extensive similarities, indicating a conserved activity (37). By contrast, the N-terminal IDR of PrP\(^\text{C}\) shows high diversity between vertebrate classes. This is in line with the observation that IDRs often evolve more rapidly than well-structured protein domains (38). Many IDRs show sequence conservation, sometimes involving particular amino-acid residues, such as leucine (L), proline (P), tyrosine (Y), and tryptophan (W) (39). The IDR of PrP\(^\text{C}\) harbors many octapeptides or nonapeptides, preceded by two hexapeptides, while in avian and reptilian PrP\(^\text{C}\) it is comprised entirely of hexapeptides, and in amphibian PrP\(^\text{C}\) only a very short pseudo-hexapeptide (ψHx) sequence is present. A highly conserved hydrophobic, alanine-rich motif (HD) at the center is characteristic for PrP\(^\text{C}\). Proteolytic cleavage of PrP\(^\text{C}\), known as α-cleavage (arrow) occurs N-terminal to the HD-motif at the boundary between the IDR domain and the globular C-terminal domain thus generating the fragments PrP-N1 and PrP-C1 of PrP\(^\text{C}\) (not shown). The globular domain consists in most PrPs of three helices (α\(_1\), α\(_2\), α\(_3\)) and a β-sheet formed by two short β-strands (β\(_1\), β\(_2\)).

Specifically, mammalian PrP\(^\text{C}\) contain glycine-rich octarepeats with conserved W, P, and histidine (H) residues in this region (18, 40–46), while a hexarepeat region with conserved P, H, and Y is found in avian (47–49) and reptile PrPCs (50). Strikingly, amphibian PrP\(^\text{C}\) is devoid of any repeats and H residues (51). Interestingly, IDRs are prevalent in virus proteins, allowing many interacting partners. Correspondingly, many proteins involved in innate immunity also carry IDRs, which may reflect the evolutionary "arms" race between invading pathogens and the host immune system. The evolutionary modifications that can be observed in the N-terminal IDR of PrP\(^\text{C}\) among terrestrial vertebrates may indeed be a relic of these evolving immune functions. The most apparent evolutionary change that has occurred first in some reptilian species and then has become the norm in avian and mammalian PrP\(^\text{C}\) are precisely spaced H residues which allow binding of divalent metal-ions, such as Cu\(^{2+}\) (52) and Zn\(^{2+}\) (53). Cu\(^{2+}\) binding will not only confer structural order to the N-terminus (54) but also by operating as a sensitive regulator of the structural state of PrP\(^\text{C}\)’s IDR it may govern protein interactions and proteolytic processing (PrP-N1/PrP-C1).
Studies, comparing wild-type PrP\(^C\) with mutated PrPs lacking the repeat region have shown that the octarepeat region is crucial for PrP\(^C\)’s neuroprotective activity. For instance against Bax induced cell death (55) or toxicity caused by ectopic expression of the PrP-like protein Doppel (Dpl) (56, 57), excitotoxic stress and PrP\(^\Delta\) toxicity (58). Interestingly, Drisaldi and co-workers demonstrated that the neuroprotective function of the repeat region is dependent on four histidine residues (57). Furthermore, the repeat region is also necessary for the PrP-mediated neuroprotection observed in models of brain ischemia (59). Similarly, the ability of PrP\(^C\) to transmit neurotoxic signaling of amyloid beta (A\(\beta\)) and other neurotoxic \(\beta\)-sheet-rich-conformers is greatly diminished in PrP\(^C\) mutants devoid of this domain (60). Further studies of the mechanisms underlying the activities of the IDR of PrP\(^C\) and its evolution, particularly in neuro-immune crosstalk appears to be an important area for future research.

Functionally, PrP\(^C\) can by virtue of its GPI anchor move between membrane subdomains (61, 62), and interact with many partners at the cell surface. These partners may include other GPI-anchored molecules like the proteoglycan Glypican-1 (63), transmembrane proteins like the neural cell-adhesion molecule, NCAM (64), the low-density-related protein LRP1 (65), the amyloid precursor protein APP (64, 66), lipid raft constituents such as caveolin (67), or src kinases (68, 69). The formation of these complexes may occur following the interaction of PrP\(^C\) with extracellular matrix components, e.g., vitronectin (70) or laminin (71) or soluble ligands such as the extracellular chaperone stress-induced phosphoprotein 1 (STI1) (72). Moreover, in lymphoid cells PrP\(^C\) has been shown to be recruited into microdomains of the membrane, the so-called immunological synapses harboring T-cell receptor components (73–76). As discussed above, many ligands appear to interact with the N-terminal IDR of PrP\(^C\) [reviewed in Ref. (77)].

Of major pathophysiological relevance is the ability of the N-terminal PrP fragment (PrP-N1) to bind to and to mediate toxic effects of A\(\beta\) oligomers (78). PrP\(^C\) may further engage into homophilic interactions or bind the two other members of the prion protein family Doppel and Shadoo (58, 68). It could be speculated that shed PrP\(^C\) or PrP-N1 can act at a distance via extracellular fluids. Indeed it has been demonstrated that soluble PrP-N1 fragments can prevent A\(\beta\)-induced toxicity and have a neuroprotective activity (79–84).

**PrP\(^C\) Pattern of Expression**

Although PrP\(^C\) is ubiquitously expressed, its main expression overlaps strikingly with the distribution of immunologically quiescent sites (Figure 2). PrP\(^C\) is abundantly present in the central and peripheral nervous system (17, 18, 85), glial cells of the CNS (86, 87), and in the testes, eye, placenta, and uterus (88, 89). PrP\(^C\) is also present in the neurovascular unit, including endothelial cells (90, 91), it may thus be one of the protagonists modulating blood–brain barrier functions (92).

Cellular prion protein is also found in microglial cells (93). Whereas its expression is associated with the inflammatory M1 producing TNF-\(\alpha\), IL-1\(\beta\), and IL-6, or the immunosuppressive M2 (producing IL-10 and TGF-\(\beta\)) phenotype of microglia has not been clarified. Notwithstanding, the observation that Prnp knockout mice produce less of the anti-inflammatory cytokine IL-10 in response to LPS-induced chronic inflammation would suggest a positive role for PrP\(^C\) in M2 microglia (94).

Cellular prion protein is abundantly expressed in neuronal and non-neuronal stem cells, including hematopoietic stem cells (HSCs) (95) and it contributes to stem-cell renewal, reviewed by Lopes and Santos (96) and Martin-Lannerée et al. (97). HSCs have been shown to co-localize with TREGs, suggesting that...
TREGs provide a “shield” conferring relative immune privilege to the HSCs (98). Interestingly, PrP\textsuperscript{C} is found at high levels in both immature HSCs (95) and TREGs (99), and probably contributes to the interplay between HSCs and TREG cells in this niche.

Differentiation of HSCs along the lymphoid (100–102) or monocytic (103) lineages maintains the expression of surface PrP\textsuperscript{C}, while during the granulocyte maturation PrP\textsuperscript{C} is downregulated (104). Within lymphoid cells, B cells express lower levels of PrP\textsuperscript{C} compared to T cells and NK cells (105), which could be linked to the observation that B cells are not repressed in immune-privileged sites. Regulatory CD4\textsuperscript{+} CD25\textsuperscript{+} T cells expressed 4.5 fold higher levels of Prnp mRNA and showed a 10-fold higher intensity of surface PrP\textsuperscript{C} than CD4\textsuperscript{+} CD25\textsuperscript{−} T cells (99). However, an attempt to identify the role of PrP\textsuperscript{C} in TREGs using Prnp knockout mice was unsuccessful since no loss-of-function phenotypes could be recognized in Tregs without PrP\textsuperscript{C} expression (99). In most immune cells, PrP\textsuperscript{C} is dynamically expressed and generally naïve immune cells contain less PrP\textsuperscript{C} than mature or stimulated immune cells, with a few exceptions (105, 106). Even expression of PrP\textsuperscript{C} in immune-privileged organs would be to protect against inflammatory processes (109, 110) and exhibit complete resistance toward prion infection with encephalomyocarditis virus variant B (EMCV-B), infection with encephalomyocarditis variant (113). For a comprehensive review of suggested physiological roles of PrPC, see Ref. (114). In addition to discriminate neuroprotection by PrP\textsuperscript{C} from immunoregulatory roles, Hu and co-workers (130) used pharmacologically selective silencing of PrP\textsuperscript{C} in lymphocytes in models of nervous system autoimmune disease. They were able to show that depletion of PrP\textsuperscript{C} in lymphocytes directly affected T-cell activation, survival, and differentiation. In the absence of PrP\textsuperscript{C} expression in lymphocytes, the severity of EAE was considerably increased. They concluded that PrP\textsuperscript{C} primarily confers neuroprotection against neuroinflammatory insult. In a different attempt to discriminate neuroprotection by PrP\textsuperscript{C} from immunoregulatory roles, Hu and co-workers (130) used pharmacologically selective silencing of PrP\textsuperscript{C} in lymphocytes in models of nervous system autoimmune disease. They were able to show that depletion of PrP\textsuperscript{C} in lymphocytes directly affected T-cell activation, survival, and differentiation. In the absence of PrP\textsuperscript{C} expression in lymphocytes, the severity of EAE was considerably increased. They concluded that lack of PrP\textsuperscript{C} in lymphocytes resulted in pro-inflammatory activities and that autoimmune brain pathologies could develop despite protective PrP\textsuperscript{C} expression in neuronal cells. Thus, under these experimental conditions, the role for PrP\textsuperscript{C} as a regulator of immunological homeostasis apparently dominated the cytoprotective role of the protein in the CNS. In Gourdain et al. (126), the authors state that their data do not exclude an important role for PrP\textsuperscript{C}, particularly in early lymphoid responses. They further discuss several possible explanations for the conflicting results in their study and the study by Hu et al. (130). The models used are complicated and obviously differ in many aspects, such as mouse strain, encephalitogenic antigens, T-cell assay protocols

### Inflammation Reveals Cytoprotective and Immunomodulatory Roles of PrP\textsuperscript{C}

Prnp knockout mice develop normally, with normal life expectancy (109, 110) and exhibit complete resistance toward prion infection. Despite a relative lack of robust and reproducible phenotypes under physiological conditions, a wide variety of roles for PrP\textsuperscript{C} have been suggested, such as in maintenance of axonal myelin (30, 111), modulating circadian rhythms (112), and neuronal excitability (113). For a comprehensive review of suggested physiological roles of PrP\textsuperscript{C}, see Ref. (114). In addition to murine models, Prnp knockout cattle have been produced (115). After extensive analysis, under physiological conditions, only minor phenotypes were observed. Similar findings have been reported from a recently discovered line of Norwegian dairy goats, carrying a nonsense mutation that renders these animals devoid of PrP\textsuperscript{C} (116, 117).

Interestingly, experiments with Prnp knockout mice involving a diverse set of inflammatory processes (Table 1), such as experimental brain ischemia, brain trauma, experimental autoimmune encephalomyelitis (EAE), experimental colitis, and, intracerebral infection with encephalomyocarditis virus variant B (EMCV-B), have revealed that in the absence of PrP\textsuperscript{C}, inflammatory damage is greatly exacerbated; reviewed by Onodera et al. (118).

Experimental autoimmune encephalomyelitis in mice, a chronic demyelinating disease of the CNS and a model of multiple sclerosis in humans, is often induced by immunization with myelin oligodendrocyte glycoprotein (MOG). Autoantigen-specific T cells of both the Th1 and Th17 phenotypes cross the BBB and are the primary immune cells recruited to the CNS where they activate microglia and attract blood monocytes and other inflammatory cells (129). Induction of EAE by MOG injection in Prnp knockout mice resulted in earlier onset, prolonged and more severe neuroinflammation than in normal mice (125). The Prnp knockout mice had persisting T-cell and monocytic/microglial infiltrates in the CNS, accompanied by demyelination and axonal drop-out in spinal cord white matter. It was concluded that PrP\textsuperscript{C} modulates T-cell-mediated neuroinflammation, with a suppressive effect on MOG-induced peripheral T-cell responses and the authors discussed whether the larger pathological lesions in mice lacking PrP\textsuperscript{C} also could be caused by increased cellular susceptibility to oxidative stress.

Gourdain and colleagues (126) conducted experiments with reciprocal bone marrow chimeras with lack of PrP\textsuperscript{C} expression in lymphoid cells or the CNS, but did not observe earlier disease onset nor increased leukocyte infiltration in the CNS in animals with Prnp knockout lymphocytes. However, they observed significantly higher pathology scores in mice lacking PrP\textsuperscript{C} expression in the brain, and concluded that PrP\textsuperscript{C} primarily confers neuroprotection against neuroinflammatory insult. In a different attempt to discriminate neuroprotection by PrP\textsuperscript{C} from immunoregulatory roles, Hu and co-workers (130) used pharmacologically selective silencing of PrP\textsuperscript{C} in lymphocytes in models of nervous system autoimmune disease. They were able to show that depletion of PrP\textsuperscript{C} in lymphocytes directly affected T-cell activation, survival, and differentiation. In the absence of PrP\textsuperscript{C} expression in lymphocytes, the severity of EAE was considerably increased. They concluded that lack of PrP\textsuperscript{C} in lymphocytes resulted in pro-inflammatory activities and that autoimmune brain pathologies could develop despite protective PrP\textsuperscript{C} expression in neuronal cells. Thus, under these experimental conditions, the role for PrP\textsuperscript{C} as a regulator of immunological homeostasis apparently dominated the cytoprotective role of the protein in the CNS. In Gourdain et al. (126), the authors state that their data do not exclude an important role for PrP\textsuperscript{C}, particularly in early lymphoid responses. They further discuss several possible explanations for the conflicting results in their study and the study by Hu et al. (130). The models used are complicated and obviously differ in many aspects, such as mouse strain, encephalitogenic antigens, T-cell assay protocols

| Insult                          | Tissue damage* | Prnp wild type | Prnp knockout | Reference |
|--------------------------------|----------------|----------------|---------------|-----------|
| Brain ischemia                 | ++             | ++++           | (69, 119–122) |
| Brain trauma                   | ++             | ++++           | (123, 124)   |
| Experimental autoimmune        | ++             | ++++           | (125, 126)   |
| Experimental colitis           | ++             | ++++           | (127)        |
| Encephalomyocarditis           | ++             | ++++           | (128)        |

*Refers to onset, duration, and severity of inflammation and magnitude of tissue damage.

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**TABLE 1 | Loss of PrP\textsuperscript{C} aggravates immunopathology in a variety of experimental settings.**
and differences in methodology for gene silencing, all factors that may have contributed to the discrepancies.

In a different model with induction of focal brain ischemia, Prnp knockout mice experience more severe tissue damage after focal brain ischemia than wild-type mice (59, 119–121). Spudich and colleagues observed 200% larger infarct volume in Prnp knockout mice. In addition, an induction of ERK1/2, STAT1, JNK1/2, and Caspase-3 activity in the Prnp knockout suggests that these signaling molecules are involved in Prnp knockout-related neuronal cell death (120). Significantly increased infarction volumes were also observed in Prnp knockout mice, in studies of permanent and transient focal cerebral ischemia (121). Moreover, they observed reduced levels of phosphorylated Akt and enhanced neuronal caspase-3 activation in Prnp knockout mice. In a rat stroke model, Shyu and colleagues revealed a time-dependent increase in PrPC levels in infarcted tissue to reach a peak 3 days post infarction and that overexpression of PrPC reduced ischemic injury (122). Mitsios and colleagues detected increased levels of PrPC in plasma from human stroke patients compared with healthy controls (131). Moreover, they found upregulation of PrPC in gray matter peri-infarcted tissue and in infarcted tissue, in 6 out of 10 patients. In a study of traumatic brain injury, Prnp knockout mice had a larger lesion volume and a breakdown of the blood–brain barrier 1 month after injury compared to wild-type mice (123). Interestingly, it has been observed that Prnp mRNA is one of two most upregulated mRNAs after induced traumatic brain injury in mice (124), supporting a protective role for PrPC. From the studies on ischemic and traumatic brain injury, the role of inflammation in the development of lesions remains to be clarified, since no inflammation-specific parameters were measured, nor infiltration of cells along the borders of the necrotic tissue were observed. Cell death also activates inflammation and further studies should include the role of cytokines and immune cells, such as locally activated or blood-derived macrophages/microglia, when evaluating how PrPC influences damage control (94).

Intracranial infection with EMCV-B resulted in similar viral titers in wild-type and Prnp knockout mice, however, mice lacking PrPC showed higher numbers of apoptotic neurons, while wild-type mice had more activation of microglial cells as well as more severe infiltration of immune cells in the hippocampal area (128), suggesting that PrPC affects the inflammatory response, while also serving a protective, anti-apoptotic role during the infection.

In a mouse model of inflammatory bowel disease, mice lacking PrPC developed a more severe colitis with markedly elevated levels of pro-inflammatory cytokines and pro-apoptotic regulatory proteins (127). Moreover, it was shown that overexpression of PrPC protected against induction of colitis. Interestingly, lack of PrPC has been shown to skew T-cell development in favor of pro-inflammatory Th1 and Th17 phenotypes (125, 130).

Taken together, these observations made under different experimental modalities demonstrate that PrPC both mediates cytoprotective signaling under inflammatory stress and has the capacity to attenuate the inflammation itself.

In summary, PrPC is highly expressed in immune-privileged organs and it serves a protective role evident most clearly under inflammatory stress and/or tissue damage. Moreover, data show that PrPC is more than a passive protector, but also dampens the inflammation itself, by modulating the activity of immune cells in an anti-inflammatory direction. The latter role of PrPC fits well into the concept of immune privilege and immune modulation. The molecular details and signaling pathways by which PrPC modulates inflammation are not yet clarified and stand out as a challenging area of future research. Below, we highlight some of the current data on signaling mediated via or influenced by PrPC presence on the cell surface.

**PrPC in Cytoprotective and Immunoregulatory Signaling**

At a molecular level, the cytoprotective activity of PrPC may depend on its capacity to engage into multimolecular complexes at the cell surface and mobilize signal transduction cascades. For a review of these signaling events in neuronal cells, see Ref. (132). With respect to the concept presented here, the molecular cascades underlying the potential contribution of PrPC to immune quiescence remain to be dissected. However, many of these probably overlap with cytoprotective signaling, which is better characterized. We will therefore elaborate somewhat on PrPC partners and effectors potentially contributing to its cytoprotective activity (Figure 3). The extracellular co-chaperone STI1, identified as a PrPC partner in 1997 through a complementary hydropathy approach (133), is a well-established inducer of PrPC-dependent signals. The STI1–PrPC interaction has been shown to protect retinal (72, 134) and hippocampal neurons (135) against chemically induced apoptosis, in both cases via cAMP-dependent protein kinase A (PKA). The neuroprotective action of this partnership is also supported by the recruitment of mTOR (136) as well as the inactivation of the GSK3β kinase (69), whose overactivity is detrimental to neurons (137). Noteworthy, STI1 can be secreted from astrocytes (138), a cell type highly contributing to immune quiescence in the CNS (92). STI1 may act in a cell-autonomous manner to favor astrocytic differentiation upon binding to PrPC (139). Whether the STI1–PrPC interaction also instigates a dialog between astrocytes and neurons deserves to be considered. Interestingly, astrocytes release STI1 in response to oxygen–glucose deprivation (140), and thereby induce neuroprotective signals through PrPC. In line with this, Lee and colleagues found that STI1 is induced in the ischemic brain and contributes to recovery via PrPC (141). The same study showed that the upregulation of STI1 promotes the recruitment of bone marrow-derived cells to the ischemic brain and thereby helps reducing brain injury. Although the full pathway of signaling events imparted by the STI1–PrPC duo in this context remains to be elucidated, a beneficial contribution of the downregulation of matrix-metallopeptidase 9 (MMP-9) transcripts and activity fostered by PrPC (142) should be proposed, since MMP-9 knockout mice are less vulnerable to ischemia than their wild-type counterparts (143), possibly because activation of MMPs during brain injury leads to increased permeability of the glia limitans (144), separating the perivascular space from the neural tissue proper and thereby opens for a higher flux of cells and solutes into the neuropil.

In addition to the mature brain, the cytoprotective action of the PrPC–STI1 interaction may operate during embryonic
GPI-anchored PrP C has the capacity to interact with multiple partners and trigger cell signaling events leading to neuroprotection and immunomodulation. The formation of PrP C-containing multimolecular complexes and subsequent mobilization of protective or immunomodulatory signals may be induced following the binding of soluble ligands, such as STI1, PrP C itself, or its N-terminal domain (PrP-N1). (A) In neurons, several targets of PrP C signaling relay its neuroprotective action, including protein kinase A, mTOR, the MAP kinases ERK1/2, and GSK3β. This action may also involve intracellular calcium mobilization. Some of these signaling cascades require the association of PrP C with the membrane protein caveolin. The participation of transmembrane partner(s) of PrP C to the signaling complexes is represented by «β».

(B) In T lymphocytes, PrP C is enriched at the immunological synapse, where it can interact with components of the T cell receptor (TCR), such as the Fyn tyrosine kinase and the zeta chain-associated protein kinase 70 (ZAP-70). These interactions may further promote the activation of downstream effectors, including NFκB, JNK, ERK, as well as elevation of intracellular calcium concentration.

FIGURE 3 | GPI-anchored PrP C has the capacity to interact with multiple partners and trigger cell signaling events leading to neuroprotection and immunomodulation. The formation of PrP C-containing multimolecular complexes and subsequent mobilization of protective or immunomodulatory signals may be induced following the binding of soluble ligands, such as STI1, PrP C itself, or its N-terminal domain (PrP-N1). (A) In neurons, several targets of PrP C signaling relay its neuroprotective action, including protein kinase A, mTOR, the MAP kinases ERK1/2, and GSK3β. This action may also involve intracellular calcium mobilization. Some of these signaling cascades require the association of PrP C with the membrane protein caveolin. The participation of transmembrane partner(s) of PrP C to the signaling complexes is represented by «β». (B) In T lymphocytes, PrP C is enriched at the immunological synapse, where it can interact with components of the T cell receptor (TCR), such as the Fyn tyrosine kinase and the zeta chain-associated protein kinase 70 (ZAP-70). These interactions may further promote the activation of downstream effectors, including NFκB, JNK, ERK, as well as elevation of intracellular calcium concentration.
was stimulated by transcription factor NFAT/AP-1, known for its function in T-cell signaling and differentiation (130). In Jurkat lymphocytes, a co-localization of PrPc and T-cell co-receptor CD3e and the lipid-raft ganglioside GM1 was observed (74, 76). Further compelling evidence of PrPc operating in immunological synapses, such as in antigen-driven interactions between T cells and DCs, have been reported (75). Interestingly, absence of PrPc in T cells and DCs had different consequences for T-cell proliferation; T cells devoid of PrPc exhibited a normal allogenic antigen response, while DCs without PrPc significantly reduced proliferation in interacting T cells, suggesting that PrPc might serve different signaling roles in the two cell types. From experiments using PrPc antibodies, the authors concluded that PrPc is a negative regulator of T-cell receptor signaling and that PrPc modulates neuroinflammation.

**PrPc and Cancer**

While immune privilege represents a physiological safeguard mechanism, it is also known to be hijacked by cancer cells to evade antitumor immunity (151). Over a decade ago, PrPc was found to be overexpressed in a breast cancer cell line that was resistant to TNFα-induced cell death (152). A correlation between PrPc expression and resistance to cytotoxic agents has now been described in various types of tumors, including breast cancer, gastric cancer, and glioblastoma [reviewed in Ref. (153)]. While the molecular mechanisms underlying the contribution of PrPc to tumor resistance are poorly understood, the disruption of the STI1 binding to PrPc was recently shown to impair glioblastoma growth (150), suggesting that cancer cells may usurp the cytoprotective activity of PrPc. A question that deserves further investigation is whether the presence of PrPc at the surface of cancer cells endows them with properties that enable them to evade the immune response.

**Future Prospects**

Although the concept presented here allows many pieces of the PrPc puzzle to fall into place, by providing principal physiological roles of PrPc in all tissues, several important questions remain to be answered. For instance, does surface-bound PrPc on proliferating immune cells interact with PrPc or a PrPc-controlled protein complex in tissues and cells, like in the blood–brain barrier tight junctions of the endothelial cells (90) and thereby sense the entrance into an immune-privileged, PrPc-enriched zone; thus contributing to “do no harm” signaling? To what extent does PrPc play part in the maintenance of stem-cell niches, in the proliferation and differentiation of cell lineages derived from the bone marrow and in modulating the development of lymphoid organs? Furthermore, the concept presented here calls for careful scrutiny of the role of PrPc in chronic inflammatory conditions, such as inflammatory bowel disease and various pathologies eliciting inflammation in the brain or other immune-privileged organs.

Besides, in all these questions, we have to examine which of the many PrPc protein/peptide forms are the actual executors of these physiological roles. From the genomic perspective, it would be of great help to understand the gene control of Prnp and whether other immunomodulators, such as TRAIL, Fas/FasL, and IDO, are part of the same expression network controlled by similar transcription factors; and whether there is genetic variation resulting in altered immune-privilege. Our proposed link between PrPc and immunological quiescence opens an exciting new avenue for the study of this protein beyond the chronic diseases of the CNS into the domain of the immune system, the reproductive system, and in senescent organs. It will encourage the study of PrPc in several inflammatory conditions and cancer.

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References

1. Prusiner SB. Prion diseases and central nervous system degeneration. *Clin Res* (1987) 35(3):177–91.
2. Aguzzi A, Calella AM. Prions: protein aggregation and infectious diseases. *Physiol Rev* (2009) 89(4):1105–52. doi:10.1152/physrev.00006.2009
3. O&n D, Medzhitov R. Evolution of inflammatory diseases. *Curr Biol* (2012) 22(17):R733–40. doi:10.1016/j.cub.2012.07.029
4. Niederkorn JY. See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat Immunol* (2006) 7(4):354–9. doi:10.1038/ni1328
5. Medawar PB. Immunity to homologous grafted skin. III. The fate of skin homographs transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br J Exp Pathol* (1948) 29(1):58–69.
6. Simpson E. A historical perspective on immunological privilege. *Immunol Rev* (2006) 213(1):12–22. doi:10.1111/j.1600-065X.2006.00434.x
7. Head JR, Neaves WB, Billingham RE. Immune privilege in the testis. I. Basic parameters of allograft survival. *Transplantation* (1983) 36(4):423–31. doi:10.1097/00007890-198310000-00014
8. Galea I, Bechmann I, Perry VH. What is immune privilege (not)? *Trends Immunol* (2007) 28(1):12–8. doi:10.1016/j.it.2006.11.004
9. Forrester JV, Xu H, Lambe T, Cornell R. Immune privilege or privileged immunity? *Muscol Immunol* (2008) 1(5):372–81. doi:10.1038/ml.2008.27
10. Dürr S, Kindler V. Implication of indolamine 2,3 dioxygenase in the tolerance toward fetuses, tumors, and allografts. *J Leukoc Biol* (2013) 93(5):681–7. doi:10.1189/jlb.0712347
11. Ferguson TA, Griffith TS. A vision of cell death: fas ligand and immune privilege 10 years later. *Immunol Rev* (2006) 213(1):228–38. doi:10.1111/j.1600-065X.2006.00430.x
12. Phillips TA, Ni J, Pan G, Ruben SM, Wei Y-F, Pace JL, et al. TRAIL (Apo-2L) and TRAIL receptors in human placentas: implications for immune privilege. *J Immunol* (1999) 162(1):6053–9.
13. WahlSM, Wei,Moutoupoulos,N. TGF-β:anmobilepurveyorofimmuneprivilege. *Immunol Rev* (2006) 213(1):213–27. doi:10.1111/j.1600-065X.2006.00437.x
14. Li MM, Mukk DD, Lee WM, Cheng CY. Connexin 43 is critical to maintain the homeostasis of the blood–testis barrier via its effects on tight
interactions between T cells and dendritic cells. *Immunol* (2006) 176(12):7254–62. doi:10.4049/immunol.176.12.7254

76. Paar C, Wurm S, Pfarr W, Soltisfließer K, Wechselberger C. Prion protein resides in membrane microclusters of the immunological synapse during lymphocyte activation. *Eur J Cell Biol* (2007) 86(5):253–64. doi:10.1016/j.ejcb.2007.03.001

77. Beland M, Roucou X. The prion protein unstructured N-terminal region is a broad-spectrum molecular sensor with diverse and contrasting potential functions. *J Neurochem* (2012) 120(6):853–68. doi:10.1111/j.1471-4159.2011.07613.x

78. Lauren J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* (2009) 457(7233):1128–32. doi:10.1038/nature07761

79. Guillot-Sestier MV, Sunych C, Druo C, Scarzello S, Checul E. The alpha-secretase-derived N-terminal product of cellular prion, N1, displays neuroprotective function in vitro and in vivo. *J Biol Chem* (2009) 284(51):35973–86. doi:10.1074/jbc.M109.051086

80. Resenberger UK, Winklhofer KF, Tatzelt J. Neuroprotective and neurotoxic signaling by the prion protein. *Top Curr Chem* (2011) 305:101–19. doi:10.1007/128.2011_160

81. Beland M, Mortal J, Barbarin A, Roucou X. PrPC homodimerization stimulates the production of PrPC cleaved fragments PrPNI and PrPC1. *J Neurosci* (2012) 32(38):13253–63. doi:10.1523/JNEUROSCI.2236-12.2012

82. Nieracki K, Choi JK, Chen S, Surevicz WK. Soluble prion protein inhibits amyloid-beta (Abeta) fibrillation and toxicity. *J Biol Chem* (2012) 287(40):33104–8. doi:10.1074/jbc.C111.406614

83. Fluharty BR, Biasini E, Stravalaci M, Sicil A, Diomede L, Balducci C, et al. An N-terminal fragment of the prion protein binds to amyloid-beta oligomers and inhibits their neurotoxicity in vivo. *J Biol Chem* (2013) 288(11):7857–66. doi:10.1074/jbc.M112.423954

84. Beland M, Bedard M, Tremblay G, Lavigne P, Roucou X. Abeta induces its own prion protein N-terminal fragment (PrPNI)-mediated neutralization in amorphous aggregates. *Neurobiol Aging* (2014) 35(7):1537–48. doi:10.1016/j.neurobiolaging.2014.02.001

85. Taraboulos A, Jendroska K, Serban D, Yang SL, DeArmond SJ, Prusiner SB. Regional mapping of prion proteins in brain. *Proc Natl Acad Sci U S A* (1992) 89(16):7620–4. doi:10.1073/pnas.89.16.7620

86. Moser M, Colello RJ, Pott U, Oesch B. Developmental expression of the prion protein gene in glial cells. *Neuron* (1995) 14(3):509–17. doi:10.1016/0896-6233(95)90307-0

87. Bertucci FR, Bourouigne DMG, Landemember MC, Martins VR, Cerchiaro G. PrPc displays an essential protective role from oxidative stress in an astrocyte cell line derived from PrPC knockout mouse. *Biochem Biophys Res Commun* (2012) 418(1):27–32. doi:10.1016/j.bbrc.2011.12.098

88. Tanji K, Saeki K, Matsumoto Y, Takeda M, Hirasawa K, Doi K, et al. Analysis of a cell line derived from PrPC knockout mice. *Intervirology* (1995) 38:309–15.

89. Johnson ML, Grazul-Bilska AT, Reynolds LP, Redmer DA. Prion (PrPC) expression in ovine uteroepithelial tissues increases after estrogen-treatment in ovarioctomized (OVX) ewes and during early pregnancy. *Reproduction* (2014) 148(1):1–10. doi:10.1530/REP-13-0548

90. Viegas P, Chavoret N, Emslen H, Perrière N, Couraud P-O, Cazaubon S. Functional expression of the prion protein PrPC by brain endothelial cells: a role in trans-endothelial migration of human monocytes. *J Cell Sci* (2006) 119(22):4634–43. doi:10.1242/jcs.03222

91. Pflanzer T, Petsch B, Andre-Dohmen B, Muller-Schiffmann A, Tischkardt S, Wegen S, et al. Cellular prion protein participates in amyloid-beta transcytosis across the blood-brain barrier. *J Cereb Blood Flow Metab* (2012) 32(4):628–32. doi:10.1177/0271204512451392

92. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* (2006) 7(1):41–53. doi:10.1038/nnr1824

93. Brown DR, Besinger A, Herms JW, Kretzschmar HA. Microglial expression of the prion protein. *Neuroreport* (1998) 9(7):1425–9. doi:10.1097/00001756-199807170-00032

94. Liu J, Zhao D, Liu C, Ding T, Yang L, Yin X, et al. Prion protein participates in the protection of mice from lipopolysaccharide infection by regulating the inflammatory process. *J Mol Neurosci* (2015) 55(1):279–87. doi:10.1007/s12031-014-0319-2

95. Balkebø et al. The cellular prion protein in immunological quiescence.

Frontiers in Immunology | www.frontiersin.org 10 September 2015 Volume 6 | Article 450
Benestad S, Austbo L, Tranulis M, Espenes A, Olsaker I. Healthy goats naturally devoid of prion protein. Vet Res (2012) 43(1):87. doi:10.1186/1297-9143-43-87

Bakkebø et al. doi:10.1038/nbt1271

Benestad S, Austbo L, Tranulis M, Espenes A, Olsaker I. Healthy goats naturally devoid of prion protein. Vet Res (2012) 43(1):87. doi:10.1186/1297-9143-43-87
The cellular prion protein in immunological quiescence

Roffe M, Beraldo FH, Bester R, Nunziante M, Bach C, Mancini G, et al. Prion protein interaction with stress-inducible protein 1 enhances neuronal protein synthesis via mTOR. *Proc Natl Acad Sci U S A* (2010) 107(29):13147–52. doi:10.1073/pnas.1000784107

Grimes CA, Jope RS. The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol* (2001) 65(4):391–426. doi:10.1016/S0079-6186(01)00011-9

Hajj GN, Arantes CP, Dias MV, Roffe M, Costa-Silva B, Lopes MH, et al. The unconventional secretion of stress-inducible protein 1 by a heterogeneous population of extracellular vesicles. *Cell Mol Life Sci* (2013) 70(17):3211–27. doi:10.1007/s00018-013-1328-y

Heraldo FH, Soares IN, Goncalves DF, Fan J, Thomas AA, Santos TG, et al. Stress-inducible phosphoprotein 1 has unique cochaperone activity during development and regulates cellular response to ischemia via the prion protein. *FASEB J* (2013) 27(9):3594–607. doi:10.1096/fj.13-232280

Lee SD, Lai TW, Lin SZ, Lin CH, Hsu YH, Li CY, et al. Role of stress-inducible protein-1 in recruitment of bone marrow derived cells into the ischemic brains. *EMBO Mol Med* (2013) 5(8):1227–46. doi:10.1002/emmm.201202258

Pradines E, Loubet D, Schneider B, Launay JM, Kellermann C, Mouillet-Richard S. CREB-dependent gene regulation by prion protein: impact on MMP-9 and beta-dystroglycan. *Cell Signal* (2008) 20(11):2050–8. doi:10.1016/j.cellsig.2008.07.016

Svedin P, Hagberg H, Savman K, Zhu C, Mallard C. Matrix metalloproteinase-9 gene knock-out protects the immature brain after cerebral hypoxia-ischemia. *J Neurosci* (2007) 27(7):1511–8. doi:10.1523/JNEUROSCI.4391-06.2007

Bechmann I, Galea I, Perry VH. What is the blood-brain barrier (not)? *Trends Immunol* (2007) 28(1):5–11. doi:10.1016/j.it.2006.11.007

Tremblay P, Bouzamondo-Bernstein E, Heinrich C, Prusiner SB, DeArmond SJ. Developmental expression of PrP in the post-implantation embryo. *Brain Res* (2007) 1139:60–7. doi:10.1016/j.brainres.2006.12.055

Tsai CL, Tsai CN, Lin CY, Chen HW, Lee YS, Chao A, et al. Secreted stress-inducible phosphoprotein 1 activates the ALK2-SMAD signaling pathways and promotes cell proliferation of ovarian cancer cells. *Cell Rep* (2012) 2(2):283–93. doi:10.1016/j.celrep.2012.07.002

Gu Z, Reynolds EM, Song J, Lei H, Feijen A, Yu L, et al. The type I serine/threonine kinase receptor ActRIIA (ALK2) is required for gastrulation of the mouse embryo. *Development* (1999) 126(11):2551–61.

Makzhami S, Passet B, Halliez S, Castille J, Moazami-Goudarzi K, Duchesne A, et al. The prion protein family: a view from the placenta. *Front Cell Dev Biol* (2014) 2:35. doi:10.3389/fcell.2014.00035

Santos TG, Silva IR, Costa-Silva B, Lepique AP, Martins VR, Lopes MH. Enhanced neural progenitor/stem cells self-renewal via the interaction of stress-inducible protein 1 with the prion protein. *Stem Cells* (2011) 29(7):1126–36. doi:10.1002/stem.664

Lopes MH, Santos TG, Rodrigues BR, Queiroz-Hazarbassanov N, Cunha IW, Wasilewska-Sampaio AP, et al. Disruption of prion protein-HOP engagement impairs glioblastoma growth and cognitive decline and improves overall survival. *Oncogene* (2014) 34(25):3305–14. doi:10.1038/onc.2014.261

Schatton T, Frank MH. Antitumor immunity and cancer stem cells. *Ann N Y Acad Sci* (2009) 1176:154–69. doi:10.1111/j.1749-6632.2009.04568.x

Diarra-Mehrpour M, Arrabal S, Jalil A, Pinson X, Gaudin C, Pietu G, et al. Prion protein prevents human breast carcinoma cell line from tumor necrosis factor alpha-induced cell death. *Cancer Res* (2004) 64(2):719–27. doi:10.1158/0008-5472.CAN-03-1735

Mehpour M, Codogno P. Prion protein: from physiology to cancer biology. *Cancer Lett* (2010) 290(1):1–23. doi:10.1016/j.canlet.2009.07.009

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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