Current Opinion in Immunology

Humoral immunity at the brain borders in homeostasis

--Manuscript Draft--

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Author Comments:	Dear Raul, here is our review, as requested. all the best. Menna

Title: Humoral immunity at the brain borders in homeostasis

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Highlights

- Adaptive meningeal immune cells pre-dominantly located around dural venous sinuses.
- Developing B cells, mature B cells and plasma cells (PC) present in dura in homeostasis.
- Stromal and endothelial cells provide chemokines and cytokines to attract and maintain dural B cells.
- Gut-educated dural plasma cells generate an IgA ‘perimeter fence’ to defend sinus wall.

Abstract

The meninges encase the brain and spinal cord and house a variety of immune cells, including developing and mature B cells, and antibody-secreting plasma cells. In homeostasis, these cells localise around the dural venous sinuses, providing a defense ‘zone’ to protect the brain and spinal cord from blood-borne pathogens. Dural plasma cells predominantly secrete IgA antibodies, and some originate from the gastrointestinal tract, with the number and antibody isotype shaped by the gut microbiome. For developing B cells arriving from the adjacent bone marrow, the dura provides a site to tolerise against central nervous system antigens. In this review, we will discuss our current understanding of meningeal humoral immunity in homeostasis.

Introduction

The brain and spinal cord, the organs of the central nervous system (CNS), are protected by bone (the skull and vertebrae) and a triple-layered membrane, the meninges. The outer meningeal layer, the dura mater, lies adjacent to bone and houses meningeal blood and lymphatic vessels and the dural venous sinuses. The arachnoid mater, adheres to the dura and is separated from the inner pia mater by the cerebrospinal fluid (CSF)-containing subarachnoid space. In the last decade, a variety of immune cells have been identified within the meninges [1, 2]. Studies are now focused on determining the extent of meningeal immune cell heterogeneity, as well as their origin and function. Their positioning of around vascular structures provides a defense 'zone' to protect the CNS from circulating pathogens, while their proximity to CNS organs enables them to influence brain and neuronal function, for example, via cytokine secretion. Here we will consider the current body of knowledge describing meningeal humoral immunity, particularly in homeostasis.

Humoral Immunity – more than antibody production

The humoral immune system forms one arm of adaptive immunity and is delivered by B cells and their antibody-secreting terminal progeny, plasma cells. B cells develop in bone marrow from common lymphoid progenitors, through pre/pro-, pro-, pre- and immature-B cell stages, where the functional integrity, and then the affinity of their antigen receptor, a surface antibody (B cell receptor (BCR)) is tested. Immature B cells with inappropriately high BCR signalling (indicative of self-reactivity), or low BCR signalling, (due to non-functional BCRs) undergo clonal deletion or BCR editing, processes that enforce tolerance against humoral autoreactivity [3]. Transitional IgM/IgD-expressing B cells emerge from the bone marrow and circulate to the spleen to complete their maturation.

At the simplest level, the main output of humoral immunity is high affinity antibodies that are important for defence against many pathogens. The process of generating these antibodies starts when an antigen engages the BCR, potentially initiating a germinal centre (GC) response [4]. Ultimately, the interaction of GC B cells and T follicular helper cells leads to somatic hypermutation and class switching from IgM to other isotypes, generating BCRs with high affinity for antigen that are expressed by memory B cells and antibody-secreting plasma cells [5, 6]. Antibodies (immunoglobulins) are potent immune effectors with varying ability to activate complement and to engage antibody receptor-expressing innate immune cells. These effector functions are determined by the nature of the heavy chain Fc domain, which differs between antibody isotypes. IgG is the dominant circulating/systemic antibody, whereas IgA predominates at mucosal surfaces where it exists largely in dimeric form, although intestinal IgG increases during gut infection and inflammation [7, 8]. Luminal IgA can neutralize intestinal pathogens and pathogen-derived toxins and enchain gut-resident microbes trapping them in the mucus layer away from the epithelium [9].

In addition to antibody generation, B cells have several important antibody-independent functions; B cells act as potent antigen presenting cells, activating CD4 T cells [10]. They are also a source of

pro-inflammatory cytokines, including interleukin (IL)-6 and GM-CSF [11-13], and have the capacity to regulate immune response, including via IL10 secretion. Indeed, the transfer of regulatory B cells can moderate murine autoimmune encephalitis (EAE), arthritis, and colitis [14, 15], and in humans regulatory B cells have been implicated in transplant tolerance and in autoimmunity [16-18].
In summary, B cells contribute to immune responses both via antibody production, but can also activate T cells and shape the nature and duration of immune responses via secretion of T cell polarising and regulating cytokines.

Meningeal B and plasma cells – phenotype and location in homeostasis

In homeostasis, meningeal B and plasma cells are most numerous in the dura mater. Analysis of mouse dural single cell suspensions have identified a variety of innate and adaptive immune cells, including B cells and plasma cells. Overall, B cells make up around 20-30% of dural immune cells in health [1, 2, 19, 20], and include CD19/B220+ IgM+ IgD+ naïve cells, as well as IgM single positive and class-switched cells. However, in most cases, the methodologies used failed exclude intravascular or skull bone marrow contaminants. In addition, immune subsets that are emmeshed with structural components may be under-represented in single cell suspensions. With these caveats, a B cell-specific sub-analysis of published scRNAseq data illustrates the extent of heterogeneity in the dural B cell compartment (**Figure 1**). While specifically assessing the humoral immune landscape in the dura under steady-state conditions, we identified CD138+ plasma cells, that surprisingly, predominantly expressed IgA as well as joining (J) chain, suggesting that they may secrete polymeric forms of IgA, with obvious similarity to intestinal plasma cells [20]. Bulk RNA sequencing of whole mouse dura confirmed that *Igha* transcripts were far more numerous than *Ighg* isotype transcripts in steady state. Importantly, IgA+CD138+ cells were also identified in human dural samples, showing that this feature of meningeal humoral immunity is conserved across species [20].

In addition to these mature and terminally differentiated B cell subsets, a number of studies have noted the presence of immature B cells in mouse dura [21-25]. Their discovery was surprising, as it flies in the face of the prevailing dogma that B cell development is limited to bone marrow. Three recent independently identified CD19+ cells that expressed Rag2 and IL7R, consistent with a developing B cell phenotype, in both neonatal and adult mice [23-25]. Single cell RNA sequencing showed pro-B to immature B cells, with no detectable common lymphoid progenitors or pre/pro-B cells, supporting the conclusion that the meninges act as a site for B cell development, but not early haematopoiesis (**Figure 2).** Developing B cells were also identified in the dura of non-human primates [23], but we await confirmation that they exist in human meninges.

One key question relating to dural immune cells that cannot be answered by techniques that generate single cell suspensions is their anatomical location. Imaging intact mouse dura revealed that both B cells and IgA plasma cells were largely localised along the walls of dural venous sinuses

[20]. Indeed, the peri-sinus region is a hotspot for immune cells more generally (**Figure 3)** [26]. Developing B cells were also noted in the sinus region in proximity to dural lymphatic vessels, with vascular endothelial cells a potential source of IL7 required for their maintenance [23]. Notably, the dural venous sinuses receive blood from the calvarial bone marrow via diploic veins, the extracranial space via emissary veins, and the dura via meningeal veins, as well as CSF from arachnoid granulations [27-29]. In addition, blood flows relatively slowly through the sinuses and the endothelium is fenestrated [30, 31]. Overall, these physiological and anatomical features place the peri-sinus immune cells in a prime position to encounter and respond to immunological information and/or challenges originating from the CNS organs and their surrounding physical membranes. In addition, the dural sinuses also present an interface for entry of circulating immune stimuli, cells or bloodborne microbes, exemplified by the observation that lymphocytes may directly exit the superior saggital sinus into the parasagittal space *in vivo* [26].

The precise origin of meningeal immune cells, and how they arrive in dura has not been completely resolved, but recent studies suggest that some immune cells, including developing B cells, may arrive directly from the adjacent bone marrow [32-34]. Brioschi *et al.* visualised developing B cells within channels extending from the calvarial and vertebral bone marrow into the dura, potentially recruited by CXCL12-producing stromal cells [24]. Consistent with the skull bone marrow acting as a source of meningeal B cells, particularly developing B cells, parabiosis studies showed that both dural and calvarial B cells largely remained of host origin, in contrast to those in the spleen. Furthermore, bone marrow chimeras generated whilst shielding the skull bone marrow during irradiation confirmed that meningeal B cells were selectively derived from local progenitors [23, 24].

Homeostatic meningeal humoral immunity changes with age and is shaped by the microbiome

Factors that determine the size and nature of the meningeal humoral compartment remain to be fully delineated, but perisinus B cells and IgA plasma cells accumulate with age [20], and an expansion of blood-derived dural IgG^+ and IgM^+ B cells and plasma cells was also observed in older mice [24]. The molecular mechanisms and environmental cues underpinning these observations are unclear, but it is evident that the gut microbiome can influence the homeostatic dural humoral immune landscape (**Figure 4**). Mice treated with broad spectrum antibiotics had lower numbers of dural IgA^+ plasma cells, which were almost absent in germ-free (GF) animals [20]. Intestinal re-colonisation of GF mice with specific pathogen-free (SPF) mouse intestinal microbiome or oral challenge of GF mice with a single pathogenic species (*Citrobacter rodentium*) resulted in the restoration of dural IgA plasma cells to SPF levels. In contrast, skin colonisation of GF mice did not increase the dural plasma cell number (**Figure 4**). Intriguingly, oral microbial re-constitution of GF mice with human gut commensals from two different donors led to the appearance of dural IgA plasma cells in both experimental groups, but dural IgG plasma cells were also numerous in one of the groups, with reduced microbial diversity and enrichment of several bacteroides species found in the intestines of

these mice [20]. In naïve homeostatic SPF mice, paired BCR sequencing of whole dural and a 1 cm sample of small intestinal showed a 20% overlap of BCRs, with more germline-like BCRs more frequently represented in small intestinal samples [20]. Overall, these data indicate that the intestinal microbiome can influence both the size of the dural humoral immune compartment, the specificity of BCRs/antibodies, and the isotype. How do immune cells get from the intestine to the dura? Presumably via the blood stream, but whether they go via the calvarial bone marrow or exit at the dural venous sinuses or meningeal vessels is not known. What is clear is that cells of intestinal origin do end up in the dura, definitively proven using intestinal photoconversion in a mouse model of stroke [35].

Meningeal B cells – functional importance?

The dura as site for central B cell tolerance: The presence of developing B cells within the dura in homeostasis raised the question of whether the meninges may act as a site to tolerise CNS antigen-specific B cells. CNS antigens such as myelin oligodendrocyte glycoprotein (MOG), a target for autoreactive T and B cells in demyelinating diseases, appear to be expressed in mouse meninges and MOG-specific B cells were under-represented in dura compared with skull or femoral bone marrow in a MOG-BCR transgenic mouse [23, 24]. Genetic deletion of *Mog* restored this self-reactive population in the dura, suggesting that local negative selection may prevent autoreactive B cells from accumulating in the meninges (**Figure 2**). Exactly how CNS antigens get to the dura and whether defective negative selection in the dura contributes to human disease remains unresolved.

Antibody-dependent functions: The peri-sinus location of IgA secreting plasma cells suggested a potential internal barrier defensive function. Indeed, deletion of meningeal plasma cells led to enhanced pathogen spread into the brain parenchyma following intravenous challenge (see accompanying review from the McGavern Lab). These data suggest a critical role for meningeal IgA in protecting the brain from bloodborne pathogens, predominantly gram-negative bacteria originating from the intestine [36].

Brioschi and colleagues also described an increase in systemic derived IgG+ B cells with age [24]. Such a change might well impact brain function and cognition, since an increase in IgG versus IgA would be predicted to be more inflammatory. IgG-opsonised local CNS or peripheral antigens would form immune complexes, with the potential to activate complement and engage FcyR-expressing cells, including macrophages, microglia, and in some contexts, neurons, astrocytes and oligodendrocytes [37]. Consistent with a deleterious effect of IgG in CNS disease, activating FcyR-deficient mice show reduced pathology and cognitive decline in a model of Alzheimer's disease [38].

Antibody independent functions: We recently showed that meningeal B cells may have anti-inflammatory functions in homeostasis and in the context of psychological stress, with CD19-deficient mice demonstrating increased meningeal myeloid cell activation and a more anxious

phenotype at steady state, as well as increased expression of IFNγ and IFNα-response genes in the dura following chronic psychosocial stress, compared with controls [39]. Meningeal B cells showed increased expression of several innate immune response genes post-stress, including *Lcn2,* which can regulate macrophage polarization, promoting an anti-inflammatory M2 phenotype and inhibiting LPS-induced IL6 and IL1β production [40] or microglial activation [41], depending on the context. Peripheral B cells in wild type stressed animals showed increased IL10 production, but whether this is mirrored in meningeal B cells remains to be determined, but local IL10 could also help dampen myeloid cell activation, for example, by acting on IL10R-expressing microglia [42]. An anti-inflammatory role for B cells in EAE, a mouse model of MS, has long been noted, with μMT mice demonstrating delayed recovery [43]. Indeed, a study from the Gommerman lab showed that gut-derived IL10-producing IgA plasma cells migrate to the brain parenchyma to inhibit CNS inflammation [44]. However, B cell depletion may both improve or exacerbate EAE depending on timing [45], illustrating that humoral immunity may also be pathogenic in CNS autoimmunity (see accompanying review from the McGavern Lab).

The cytokine output of the meningeal B and plasma cells that accumulate with age also requires investigation, as a switch from predominantly regulatory to pro-inflammatory cytokine production, could directly affect brain physiology. Of noted previously, B cells may act as an important source of TNF-α, IL-6, and IL-10 in the periphery [11, 12, 14, 15], and a change in the ratio of these cytokines in the meninges would have the potential to directly activate or regulate microglia and astrocytes, with effects on their function in both health and neurodegenerative disease [42, 46, 47].

Conclusion

B and plasma cells make up a major component of the dural immune landscape in homeostasis. Within this landscape, B cells receive cues from stromal and endothelial cells that orchestrate their position and promote their survival, while developing B cells may be exposed to CNS-specific antigens to negatively select autoreactive cells. Meanwhile, plasma cells secrete IgA antibodies, providing a ready-educated immunological shield at the CNS vascular borders and meningeal B cells may promote an anti-inflammatory immunological milieu. Work remains to delineate the extent to which many of the murine observations are re-capitulated in humans and whether this system can be therapeutically manipulated, for example to improve CNS defence or treat neuroinflammation.

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We have no competing financial interests to declare.

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Figure Legends

Figure 1: Single cell RNA sequencing studies reveal B cell heterogeneity at the CNS borders. A) UMAP of 132,985 cells from published studies of meningeal single cell RNAsequencing, coloured by cell type. B) UMAP of 24,094 B/plasma cells subsetted from panel A, coloured by B cell phenotype. C) Expression of canonical B cell marker genes on meningeal B/plasma cells. D) Summary of published single-cell RNA sequencing studies profiling murine dura, including enrichment strategy and use of intravenous CD45 antibody pre-mortem to identify extra-vascular tissue cells. Abbreviations: ILC=innate lymphoid cell; NK= natural killer cell; cDC=classical/myeloid dendritic cell; pDC=plasmacytoid dendritic cell; FO=follicular.

Figure 2: Developing B cells in the dura may be tolerized to CNS-antigens.

B cells arise from haematopoietic stem cell (HSC) precursors in the skull and vertebral bone marrow where they may develop into mature B cells. Some developing B cells migrate into the dura mater via vascular channels. CXCL12-expressing fibroblast-like cells (FLC) may attract CXCR4-expressing B cells, while endothelial cells in the sinus region express interleukin (IL)7, a survival factor for developing B cells. Developing B cells with self-reactive B cell receptors that bind central nervous system antigens such as Myelin Oligodendrocyte Glycoprotein (MOG) are negatively selected (left side of diagram). Non-autoreactive B cells survive to the mature B cell stage (right side of diagram).

Figure 3: Dural immune cells localised to the peri-sinus region. Confocal image of dural whole-mount from naïve C57BL/6 mouse. Immune cells enriched near venous sinus, indicated by CD31 (red) staining vascular endothelial cells, including B cells (B220; yellow), T cells (CD3; cyan), macrophages (F4/80;green), myeloid cells (CD11b;pink and CD11c; purple). Scale bar 100µm.

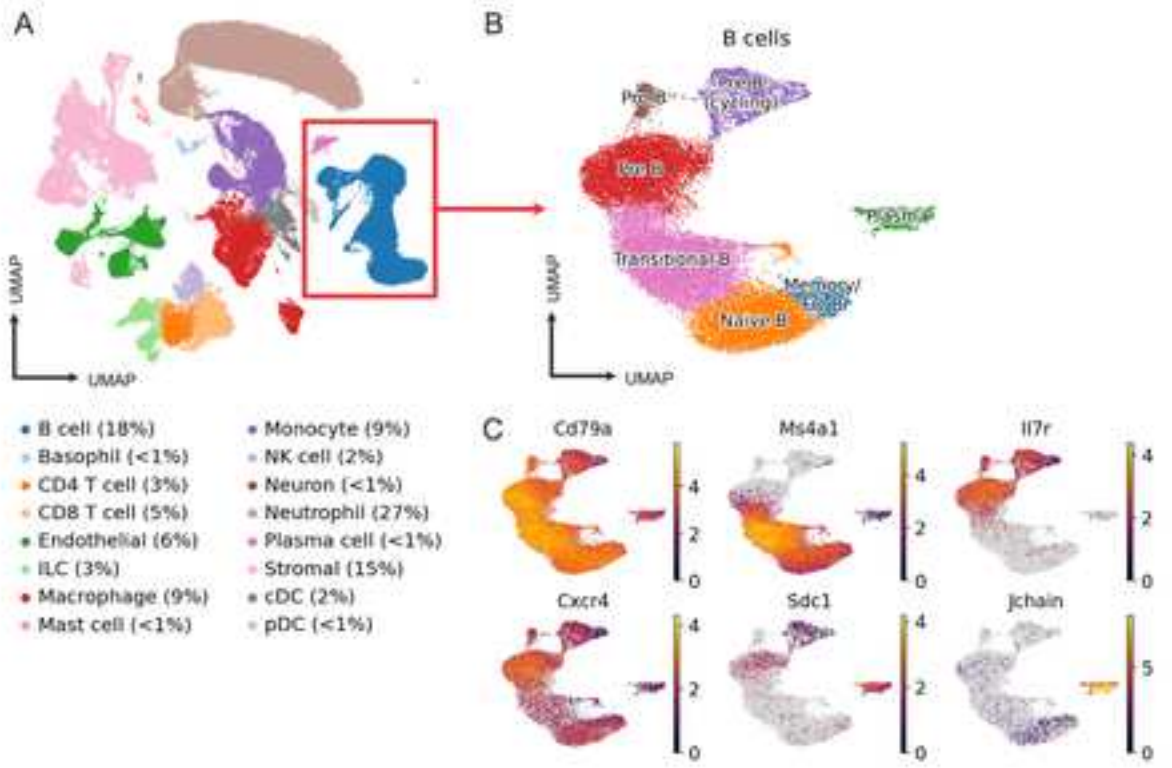
Figure 4: Intestinal microbiome determines the number and antibody isotype of dural plasma cells. In homeostasis, gut-originating IgA plasma cells are evident in the dura and are localized to the peri-sinus region (far left panel). In germ-free (GF) (or antibiotics-treated mice) dural IgA plasma cells are rarely detected (middle left panel). Gut reconstitution of GF mice with commensal microbes from SPF mice leads to restoration of dural IgA plasma cells. while re-colonisation with a bacteroides-enriched microbiome is associated with the appearance of IgG plasma cells in the dura (middle right panel). In contrast, recolonization of the skin of GF mice does not lead to restoration of IgA plasma cells in the dura (far right panel).

M.R. Clatworthy review

Competing Interests:

We have no competing financial interests to declare.

Figure 1



D.

Dataset	Method + cell n	Tissue	IV labelling	Enrichment
Van Hove et al., 2019	10x 3', 5,865 cells	Dura mater, Leptomeninges+ Brain	No	CD45+ Live cells
Brioschi et al., 2021	10x 5', 13,281 B cells	Dura mater, blood, bone marrow	No	No sort/enrich.
Farmer et al., 2021	10x 3', 16,961 cells	Brain+ Leptomeninges +Dura mater	No	Live cells
Hartlehnert et al., 2021	10x 3', 4,068 dural cells	Dura mater, spinal cord	Yes	Extravasc. CD45+ Live cells
Rustenhoven et al., 2021	10x 3', 6,815 cells	Brain, dura mater	Yes	Live CD45- CD31+/-
Schafflick et al., 2021	10x 3', 26,301 cells	Dura mater	Yes	Extravasc. Live CD45+ cells
Shemer et al., 2021	10x 3', 14,310 cells	Brain, dura mater	No	CD45+ Live cells

Figure 2

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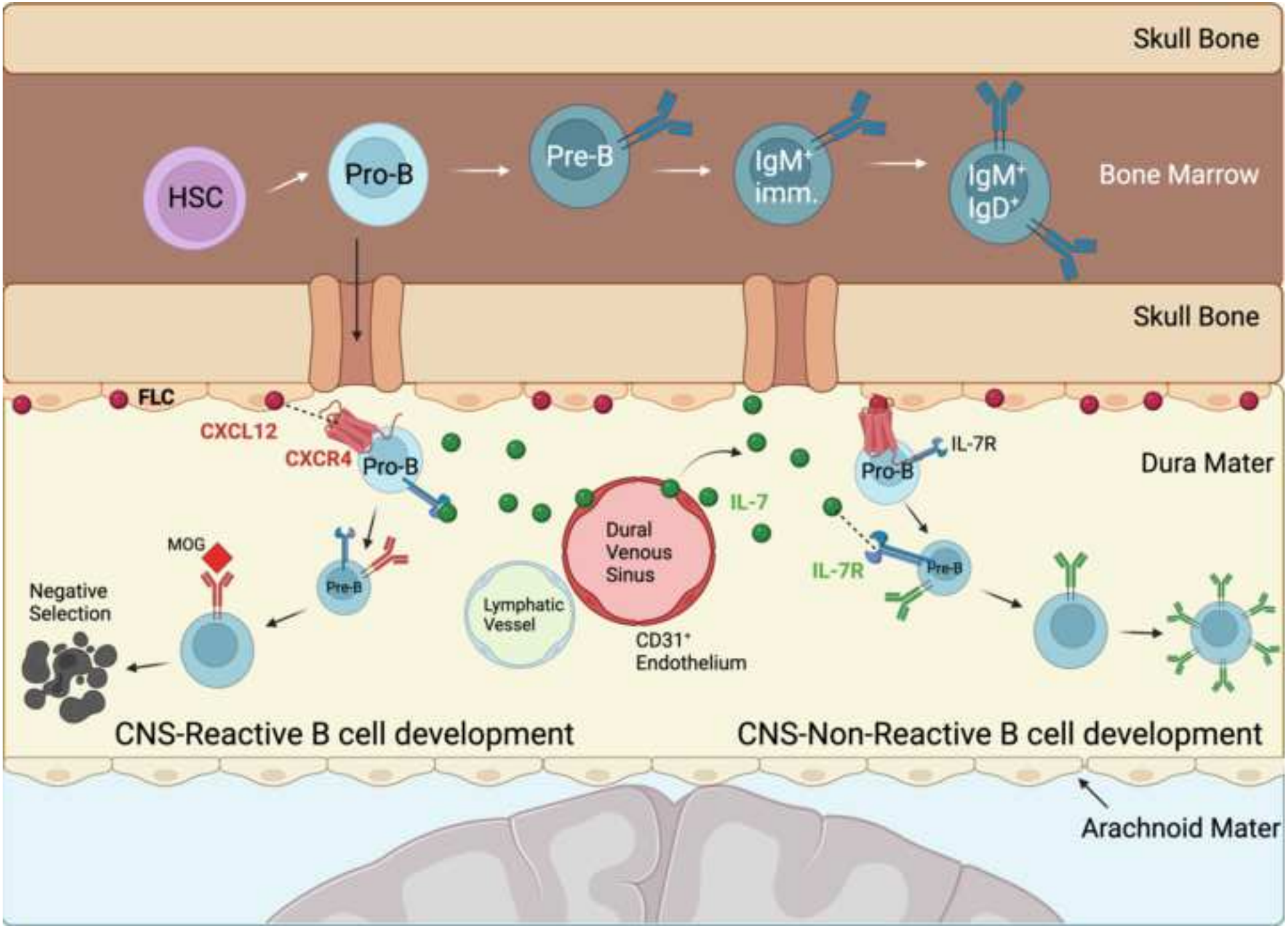

Figure 3_large

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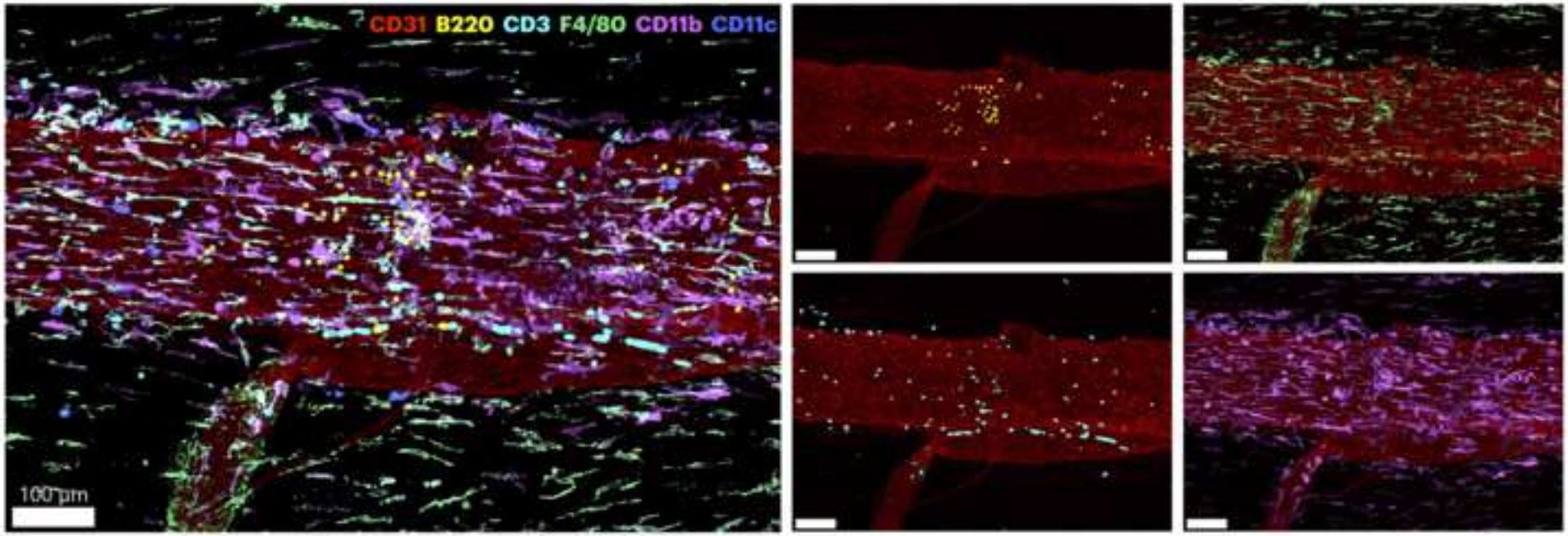


Figure 3

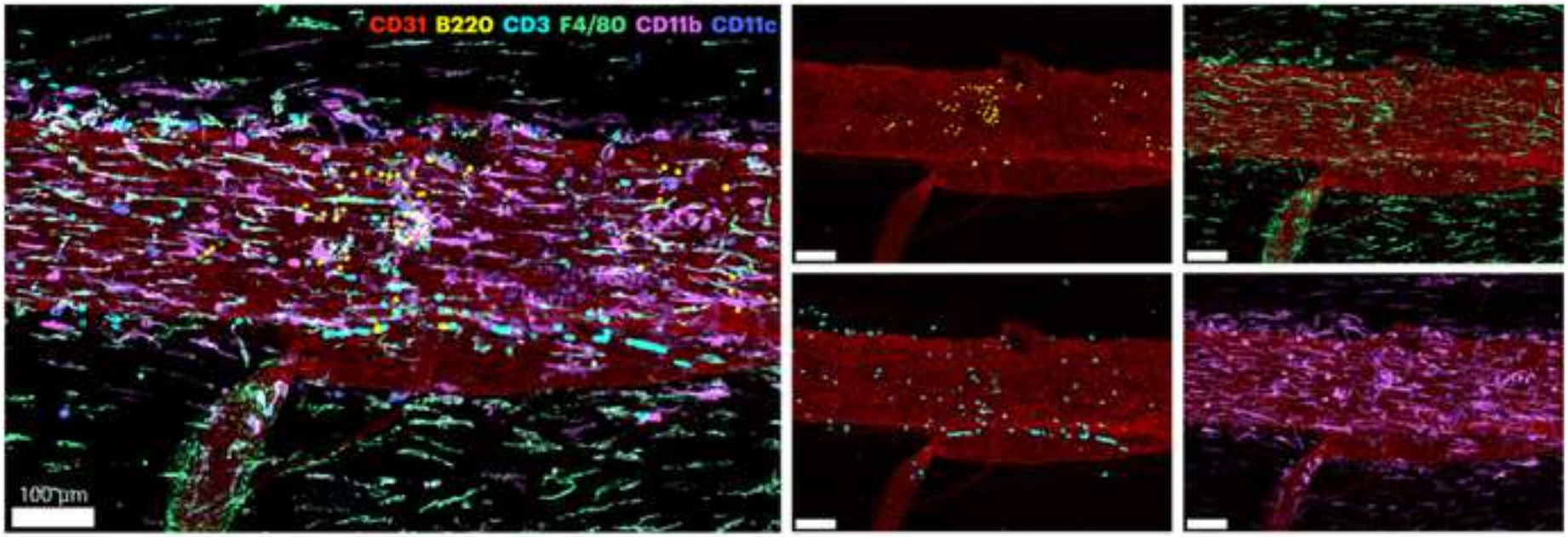
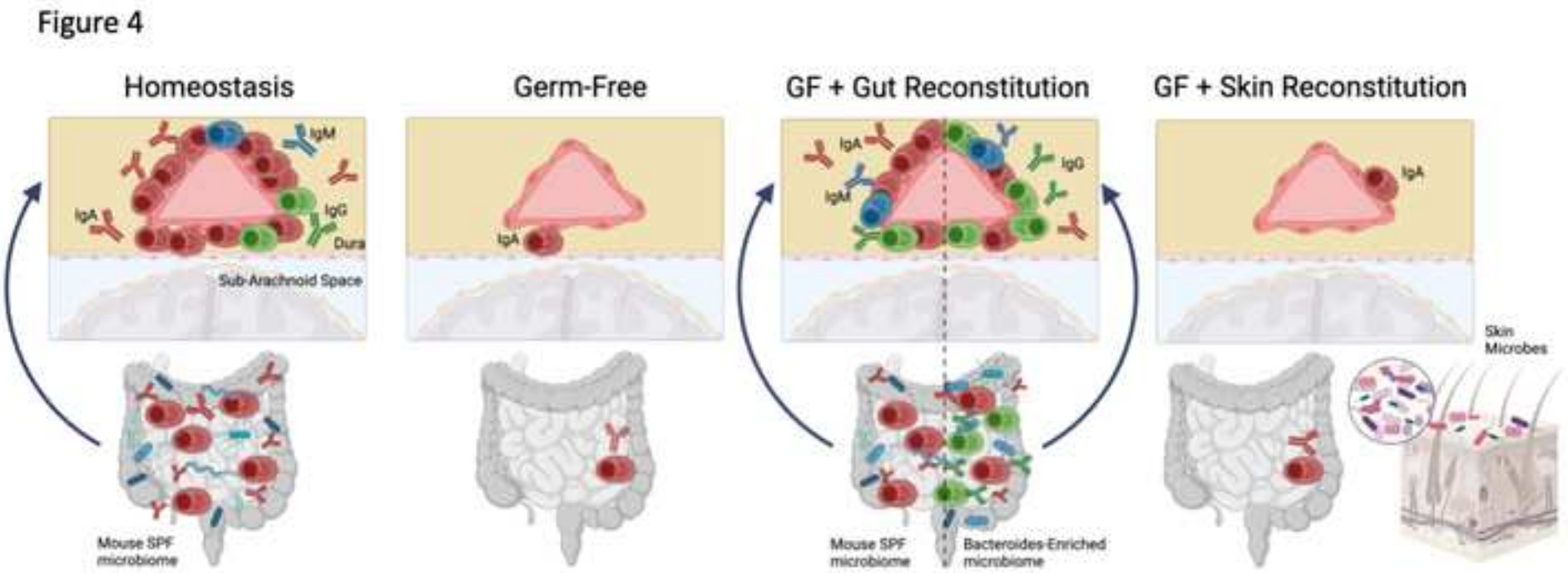


Figure 4



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Roland Liblau MD, PhD
Guest Editor
Current Opinion in Immunology

31st March 2022

Dear Dr Liblau,

Re: Posner et al. **'Humoral immunity at the brain borders in homeostasis'. (**Ms. No. COIMMU-D-22-00020)

Thank you for providing the opportunity to revise our manuscript in line with the reviewer's helpful suggestions. We have provided a detailed response below, with the reviewer's comment in black text and our response in blue.

We hope that the review article is now acceptable for publication.

Yours sincerely,

Menna

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Response to reviewer's comments:

1. You indicate that dural and peri-sinus B cells tend to accumulate with age. Would you care to speculate on any potential connection with age-related brain decline?
2. Others have shown that meningeal T cells can promote cognitive functions at steady state (see for instance Sci Immunol. 2019 Oct 11;4(40):eaay5199). I am therefore wondering (as probably other readers would) whether humoral immunity in the meninges could also contribute to the optimal functioning of the brain and spinal cord. Here again, may be a comment, even if purely speculative, would be warranted.

The reviewer raises these interesting and related points. We have now added some text paragraph and additional references (3-4, 9-11 below) along the following lines of discussion: We noted an increase in meningeal B cells and IgA plasma cells with age [1], and Brioschi and colleagues described an increase in systemic-derived IgG+ B cells [2]. How such changes would impact brain function and cognition might be two-fold; Firstly, an increase in IgG versus IgA would be predicted to be more inflammatory. IgG-opsonised local CNS or peripheral antigens would form immune complexes, with the potential to activate complement and engage FcyR-expressing cells, including macrophages, microglia,

and in some contexts, neurons, astrocytes and oligodendrocytes [3]. Consistent with a deleterious effect of IgG in CNS disease, activating FcyR-deficient mice show reduced pathology and cognitive decline in a model of Alzheimer's disease [4]. Secondly, it may be that age-associated meningeal B and plasma cells differ in their cytokine output, for example switching from predominantly regulatory to pro-inflammatory cytokine production, which may directly impact brain physiology. Of note, B cells may act as an important source of TNF-α, IL-6, and IL-10 [5-8], cytokines with known potential to activate or regulate microglia and astrocytes in health and neurodegenerative disease [9-11].

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