B cells on the brain: Meningeal IgA and a novel gut-brain firewall

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Classically, the brain and its associated structures have been considered to be 'immune privileged'. However, advances over the past decade have led to a re-assessment of this assumption. Microglia are now widely accepted as the *de facto* immune cell in the brain and interest in the immune-brain relationship has grown exponentially as research reveals critical roles for these immune cells in neuronal circuit formation, brain injury and neurodegenerative diseases such as multiple sclerosis, stroke and Alzheimer's disease [1]. Moreover, it is now clear that the barriers which form the interfaces between the periphery and brain are replete with immune populations[2]. The meningeal membranes have come under particular scrutiny since the discovery that they are the home of lymphatic vessels, long thought to be absent from the brain[2]. In these regions, macrophages are the numerically dominant immune population, and have received the most attention[3]. However, recent studies have demonstrated roles for a broad range of immune cells, including T cells, γδ T cells ILCs and beyond, in regulating behaviour, inflammation and neurological diseases in the brain and at its interfaces[2, 4, 5]. Recent studies employing single-cell RNA seq, CyTOF and flow cytometry have also revealed B cell populations to be present in the meningeal membranes[2, 3], though their function and relevance for neurological health had remained poorly understood.

In the latest issue of *Nature* Clatworthy, McGavern and colleagues report for the first time a critical role for humoral immunity in the meninges, which acts as a protective barrier against microbial invasion[6]. The authors discovered that both mouse and human meninges contain both B cells and mature plasma cells at steady state, which increased in abundance in aged animals. Surprisingly, the meninges of healthy animals were found to contain significant numbers of IgA class switched plasma cells, that localized within the peri-sinus regions. Strikingly, the authors found that disruption of the intestinal barrier (where IgA plasma cells are classically enriched) increased the number of these cells in the meninges, suggesting a potential link between these two sites. Indeed, Fitzpatrick *et al* found that the IgA plasma cells in the meninges were dependent upon the presence of resident gut commensals and that developing B cells and plasma cells are seemingly 'educated' in the intestine prior to residing in the meninges. Furthermore, mice lacking IgA or with a selective loss of meningeal IgA plasma cells exhibited reduced protection against the fungus *Candida albicans* when administered into the blood stream, associated with increased fungal invasion of the brain. Together these findings suggest meningeal-resident plasma IgA cells may act as a firewall in the central nervous system to prevent entry of blood borne pathogens, and potentially translocating commensals, into the brain (Figure 1).

This work reveals a new mechanism of host protection against invasive fungal pathogens, which are a huge burden on global health (cause approximately 1.5 million global deaths every year)[7]. Invasive *C. albicans* infection is a major risk to immunocompromised individuals, and previous work has

shown that macrophages and T cell based mediated immunity is crucial to protect the brain against systemic *C. albicans* infection[8]. However, the role of B cells and IgA secretion in this context remains poorly understood, despite *C. albicans* also being a prominent commensal in the intestine with anti-inflammatory properties[9, 10]. While this study demonstrates a critical role for meningeal humoral immunity, the mechanism for how IgA itself promotes clearance of *C. albicans* from the meninges remains unclear. Recent evidence shows that within the brain microglia prevent *C. albicans* invasion of the CNS via C-type lectin receptor signalling dependent on the recruitment of neutrophils[8] (Figure 1). Furthermore, *C. albicans* is a dimorphic fungus (can grow in 'yeast' or 'hyphal' form) and these generate differing downstream immunity and pathogenesis[9]. As the authors suggest that IgA plasma cells may originate in the intestine[6], these findings raise the possibility that cross-reactive or antigen-specific IgA generated in the gut may recognise both commensal and invasive *C. albicans* (majority of which will be in yeast form)[9]. Yet, whether meningeal IgA is uniquely effective against invasive hyphal forms, possibly via boosting microglial responses, remains to be determined. Undoubtedly, this work is a big step forward in our understanding the role of invasive anti-fungal immunity.

These latest advances also significantly expand the known roles of IgA in mammalian health and disease. Indeed, IgA plasma cells are classically found within the small intestine where secretion of IgA into the lumen is controlled at the epithelial level by the Polymeric Immunoglobulin Receptor (pIgR). IgA regulates the colonization and growth of commensal microbes within the intestinal tract through immune exclusion, toxin neutralization and limiting bacterial expansion through "enchained growth"[11]. Moreover, recent studies suggest IgA may also regulate the uptake and accumulation of microbial and dietary-derived metabolites[12]. It is possible that these modes of action of IgA protection are also occurring with *C. albicans* to prevent brain colonisation. Importantly, loss of mucosal antibody secretion has been associated with enhanced susceptibility to inflammatory and metabolic disease[11].

Together with other recent advances[13], the findings of Fitzpatrick *et al* implicate IgA+ plasma cells in mediating a novel protective firewall within the meninges. This work opens up many new questions, especially concerning the origins and antigen-specificity of these recently described meningeal IgA+ plasma cells. Indeed, within the gastrointestinal tract the majority of IgA has been posited to be polyreactive, with many commensal microbes exhibiting IgA-labelling in the absence of T cell help[11]. In contrast, IgA responses against a limited repertoire of commensal bacteria, notably those known to inhabit immunostimulatory niches within the epithelium and mucosa (e.g. Segmented Filamentous Bacteria; SFB) are dependent upon T cell help that acts to induce high affinity, antigen-specific antibodies[14] (Figure 1). In this context, Fitzpatrick *et al* demonstrated the presence of IgA+ plasma cells in the meninges of otherwise naive mice was lost in T cell deficient and germ free mice, suggesting that IgA+ plasma cells present in the central nervous system are generated through T cell-dependent mechanisms and dependent upon intestinal commensal microbes[6]. Indeed, selective colonization of germ free mice with SFB, but not skin-dwelling microbes, was sufficient to restore IgA+ plasma cells within the meninges.

Strikingly, these findings raise questions about the purpose and specificity of meningeal IgA. Is meningeal-derived IgA targeted towards a limited set of immunostimulatory microbes - perhaps due to their elevated capacity to translocate the barrier, or to act as opportunistic pathogens when intestinal homeostasis is disrupted? One possibility is that commensal bacteria and fungi induce T-cell specific IgA under homeostasis, and subsequently provide protective responses upon barrier disruption and systemic translocation, or following infection with related microbes that share antigenic epitopes. In addition to IgA plasma cells, both administration of dextran sulphate sodium (DSS) and intravenous administration of *C. albicans* also led to a striking infiltration of B cells into the meninges. (Figure 1) The authors noted that IgA+ plasma cells were found to be spatially

segregated from these B cells, suggesting a degree of cellular organisation and raising the possibility that *de novo* class switching, maturation or clonal expansion may also occur locally within the meninges themselves. These findings raise further questions regarding the ontogeny of meningeal IgA response. Do IgA plasma cells develop locally within the meninges, traffic from the gut - as demonstrated previously[13], or both? Are IgA responses seen upon intestinal barrier disruption or systemic infection, the result of expansion of pre-existing, micro- or myco-biota-specific IgA+ clones, or from *de novo* responses generated locally? The authors demonstrate that 21% of meningeal BCR sequences exhibited clonal overlap with those derived from the intestine, while meningeal BCR sequences exhibit a restricted clonality and signs of clonal expansion - suggestive of potential migratory behaviour[6]. However, it remains unclear what signals control migration of gut-derived plasma cells or B cell clones to the meninges.

Together, this study also raises the question as to the role of meningeal-resident IgA+ plasma cells in other, non-infectious, diseases of the CNS or in maintaining brain health. In particular, the accumulation of IgA+ plasma cells with age could have implications for cognitive decline, behaviour or neurodegenerative disorders. Further studies are needed to dissect the fundamental biology that underpins this exciting observation and to explore the therapeutic implications of this advance.

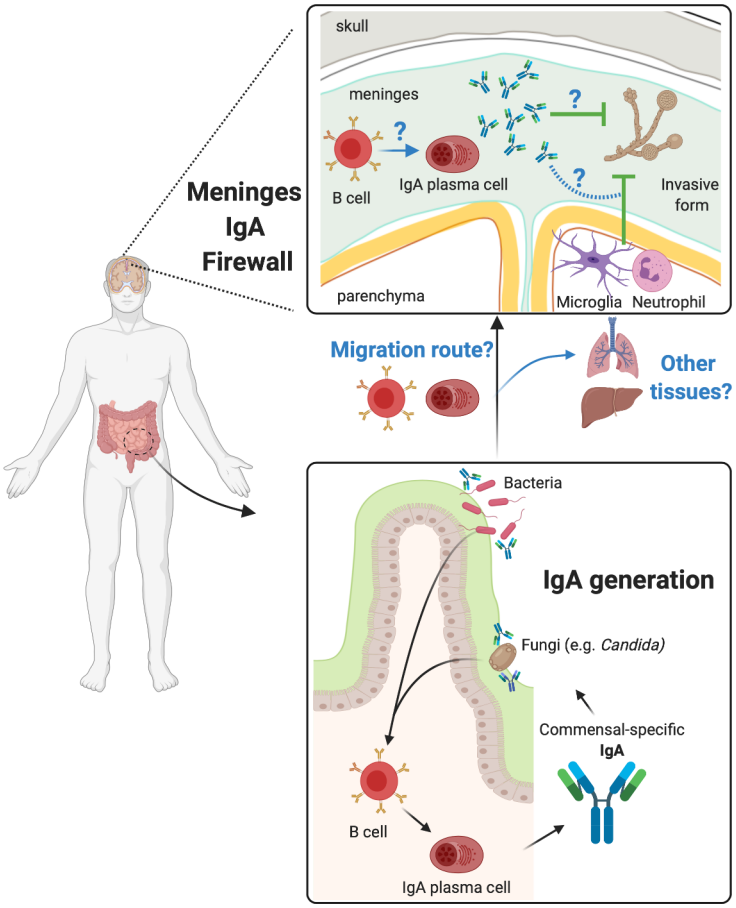

Figure 1. The Meningeal IgA firewall. Schematic depicting the findings in Fitzpatrick *et al*. B cells and plasma cells secretion of IgA in the meninges is crucial for protection against microbe invasion into the brain and CNS. Development of meningeal IgA+ Plasma Cells is dependent on commensals and requires prior 'educating' in the intestine. This work raises several key questions (highlighted on figure) that require future studies to resolve.

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References:

1. Heneka MT. Microglia take centre stage in neurodegenerative disease. *Nature reviews Immunology* 2019; **19**: 79-80.
2. Alves de Lima K, Rustenhoven J, Kipnis J. Meningeal Immunity and Its Function in Maintenance of the Central Nervous System in Health and Disease. *Annual review of immunology* 2020; **38**: 597-620.
3. Van Hove H, Martens L, Scheyltjens I*, et al.* A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. *Nat Neurosci* 2019; **22**: 1021-1035.
4. Alves de Lima K, Rustenhoven J, Da Mesquita S*, et al.* Meningeal gammadelta T cells regulate anxiety-like behavior via IL-17a signaling in neurons. *Nature immunology* 2020.
5. Mrdjen D, Pavlovic A, Hartmann FJ*, et al.* High-Dimensional Single-Cell Mapping of Central Nervous System Immune Cells Reveals Distinct Myeloid Subsets in Health, Aging, and Disease. *Immunity* 2018; **48**: 599.
6. Fitzpatrick Z, Frazer G, Ferro A. Gut-educated IgA plasma cells defend the meningeal venous sinuses. *Nature* 2020 In Press.
7. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med* 2012; **4**: 165rv113.
8. Snarr BD, Drummond RA, Lionakis MS. It's all in your head: antifungal immunity in the brain. *Curr Opin Microbiol* 2020; **58**: 41-46.
9. Paterson MJ, Oh S, Underhill DM. Host-microbe interactions: commensal fungi in the gut. *Curr Opin Microbiol* 2017; **40**: 131-137.
10. Li XV, Leonardi I, Iliev ID. Gut Mycobiota in Immunity and Inflammatory Disease. *Immunity* 2019; **50**: 1365-1379.
11. Bunker JJ, Bendelac A. IgA Responses to Microbiota. *Immunity* 2018; **49**: 211-224.
12. Uchimura Y, Fuhrer T, Li H*, et al.* Antibodies Set Boundaries Limiting Microbial Metabolite Penetration and the Resultant Mammalian Host Response. *Immunity* 2018; **49**: 545-559 e545.
13. Rojas OL, Probstel AK, Porfilio EA*, et al.* Recirculating Intestinal IgA-Producing Cells Regulate Neuroinflammation via IL-10. *Cell* 2019; **177**: 492-493.
14. Palm NW, de Zoete MR, Cullen TW*, et al.* Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* 2014; **158**: 1000-1010.