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Novel imaging tools for investigating the role of immune signalling in the brain

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ABSTRACT

The importance of neuro-immune interactions in both physiological and pathophysiological states cannot be overstated. As our appreciation for the neuroimmune nature of the brain and spinal cord grows, so does our need to extend the spatial and temporal resolution of our molecular analysis techniques. Current imaging technologies applied to investigate the actions of the neuroimmune system in both health and disease states have been adapted from the fields of immunology and neuroscience. While these classical techniques have provided immense insight into the function of the CNS, they are however, inherently limited. Thus, the development of innovative methods which overcome these limitations are crucial for imaging and quantifying acute and chronic neuroimmune responses. Therefore, this review aims to convey emerging novel and complementary imaging technologies in a form accessible to medical scientists engaging in neuroimmune research.

1. Introduction

1.1. What is neuroinflammation?

The neuroimmune system is an incredibly diverse and intricate system comprised of endogenous (astrocytes, endothelial cells, microglia, neurons and oligodendrocytes) and exogenous (infiltrating monocytes and T cells) immune-functioning cells and their associated signalling factors within the central nervous system (CNS) (Table 1) (Hutchinson et al., 2011). These immunocompetent cells are pivotal to both the homeostatic and disease states of the brain and spinal cord (Grace et al., 2014; Ousman and Kubes, 2012). For example, following injury or infection, glial and immune cells act to enhance the elimination of pathogens as well as facilitate repair, via processes ranging from central immune signalling and neurokine events, to gross "neuroinflammation".

Neuroinflammation occurs along a graded continuum with each insult displaying its own unique neuroinflammatory signature. While acute neuropathological insults such as ischemia or infection and chronic neurogenically mediated disorders such as hypertension, have robust neuroinflammatory responses, sub-inflammatory neuroimmune responses are now also recognized to be associated with various psychiatric disorders (Hutchinson and Watkins, 2014; Najjar et al., 2013; Wu et al., 2012). These sub-inflammatory responses do not function in a traditional inflammatory manner. Rather, this response more closely resembles the discrete and localized nature of neurotransmission and hence requires a separate terminology of neurokine or central immune signalling (Jacobsen et al., 2014; Hutchinson et al., 2011). Consequently, neuroinflammation has profound effects on both human and animal behaviour. For example, the high levels of inflammatory mediators observed during pathological neurodegenerative disease states such as Alzheimer's and Parkinson's disease cause neuronal cell death, which in turn directly affects an individual's memory, language, and mood (McGeer and McGeer, 2010; Rogers et al., 2007). In contrast, the lower level of neurokine and central immune signalling observed during psychiatric disorders such as depression do not appear to result in a gross loss of cells, instead they subtly alter neuronal function and in turn

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Table 1
Cellular targets historically employed to image, detect and "quantify" neuroinflammation.

Cell type	Phenotypic markers	Functionally derived markers	Function	Neuroimmune function
Microglia	CD11b, CD68, and IBA-1	CX3CR1	Macrophage-like behaviour	Primary immune effector cells
Astrocytes	GFAP, S100β	Aquaporin 4, EAAT, (Humans), GLAST-1, GLT (Rodents)	Contribute to CNS homeostasis	Secondary immune effector cells
Neurons	Beta III tubulin, MAP2, NeuN, Neurofilament, NSE	nNOS, Tyrosine Hydroxylase, ChAT	Chemical/electrical messengers of the brain	Secondary immune effector cells
Endothelial cells	e-selectin, ZO-1	VCAM-1	Create a semipermeable barrier surrounding the brain	Secondary immune effector cells
T Cells	CD4, CD8	FOXP3, IL-17	Adaptive immune response within the CNS	Adaptive immune response cells
Oligodendrocytes	MOG	MBE	Ensheath CNS neurons in myelin for support and protection	Limited immune capabilities

CD, cluster of differentiation; ChAT, choline acetyltransferase; EAAT, excitatory amino acid transporters; FOXP3, forkhead box P3; GFAP, glial fibrillary acidic protein, GLAST-1, glutamate aspartate transporter-1; GLT, L-glutamate transporter-1; Iba-1, ionized calcium binding adapter molecule-1; IL, interleukin; MAP2, microtubule-associated protein 2; MBP, myelin basic protein; MOG, myelin-oligodendrocyte glycoprotein; NeuN, neuronal nuclei or feminizing locus on X-3; nNOS, neuronal nitric oxide synthase; NSE, neuron specific enolase; VCAM-1, vascular cell adhesion protein; ZO-1, zona occuldens-1.

Table 2
Molecular targets historically employed to image, detect and "quantify" neuroinflammation.

Inflammatory mediator	Structure	Function	Neuroimmune Function	Examples
Cytokines/chemokines	Small 5-20 kDa proteins	Cell signalling proteins	Activate/dampen immune responses from cells	CCL2, IL-1β, TNFα
Danger associated molecular patterns (DAMPS)	Purine metabolites (ATP); DNA and RNA; protein chaperones and chromatin binding proteins	Endogenous warning molecules	Initiate and perpetuate an inflammatory response	ATP, DNA, heat shock proteins, HMGB1
Matrix Metalloproteases	Enzymatic proteases	Tissue remodelling and regulation of intracellular signalling cascades	Break down proteins	MMP-1, MMP-2
MicroRNAs	Single-stranded noncoding RNA sequences (approximately 21 nucleotides)	Regulation of gene expression	Regulate inflammatory pathways	miRNA-155
Reactive Oxygen Species	Small free oxygen radicals	Produced in response to threat; can function as neurotransmitters by influencing intracellular calcium signalling	Kill invading pathogens	NO

ATP, adenosine triphosphate; CCL2, chemokine (C-C motif) ligand 2; HMGB1, high-mobility group box 1; IL-1β, interleukin1β; miRNA, micro RNA; MMP, matrix metalloprotease; NO, nitric oxide; TNFα, tumour necrosis factor α.

behaviour (Eyre and Baune, 2012). Table 2 highlights the main inflammatory, neurokine and central immune signalling mediators implicated in the neuroimmune system.

1.2. Current limitations of viewing and "quantifying" neuroinflammation

The most recent technological advances to discern the structure and function of CNS cells have focused predominantly on neurons. For example, significant methodological improvements of viral tracing, development of fluorescent reporter transgenic animals and optogenetics measurement techniques have drastically increased our understanding of neuronanatomy and have identified many specific functional subgroups of CNS cells. However, most of these techniques are unsuitable for imaging neuroimmune cells as they are unable to address the most pressing issues within the field of neuroimmunology such as, understanding the complex organization of multicellular systems, the heterogeneity within and between immune cells and how low abundant inflammatory proteins (neurokines) alter behaviour. To address these issues, researchers in the field are left with traditional technological modalities that are fraught with difficulties. The limitations of antibodies is a particular problem for imaging central immune or neurokine signalling due to the low expression levels of inflammatory mediators, necessitating high affinity antibodies with a strong signal and long life span in order to view and quantify these cellular responses.

Antibodies, which are the most common method to visualise antigens, are at the centre of the "reproducibility crisis" within biological science as current estimates suggest approximately 50 per cent of antibodies are either unreactive towards the purported epitope or cross-react with others (Berglund et al., 2008). This problem is amplified by common chemical treatments on the collected tissues that are necessary for many immunohistochemical techniques. Specifically, the potential binding of antibodies is reduced by detergents that can denature proteins and fixatives can inadvertently mask antigen-binding sites during the protein cross-linking required to stabilize fragile tissues in order to survive throughout harsh staining conditions and subsequent washes. Thus, all the advancements that have been made in imaging and collecting the scarce signal remain confounded by these specificity issues. Furthermore, the current fluorescent compounds (fluorophores) used to visualise antibody binding, for example, fluorescein isothiocyanate (FITC), Alexafluors and green fluorescent protein, have a limited lifespan and/or spatial resolution, quench in the presence of light and emit in the same spectrum as biological