Next Generation Biomarkers Driving Neuroinflammation & Autoimmune Reactions in Immunotherapy

Single-cell Polyfunctional Inflammation 101

isoplexis
In this E-Book we:

- Identify the challenges in neuroinflammation, the immune cell types involved, and how it affects diseases of the CNS
- Discuss how neuroinflammation and autoimmune reactions are implicated in immunotherapy with immune related adverse events
- Identify the drivers of immune response, autoimmune reactions, and IRAEs, with polyfunctional inflammation and novel single-cell immune profiling proteomics techniques
Introduction

Neuroinflammation (NI) is a complex biological response to nervous tissue injury involving many inflammatory mediators, such as cytokines, chemokines, and many different cell types. It is well accepted that NI plays a key pathogenetic role in a number of neurological disorders and several neurodegenerative diseases.

Although the brain has long been considered an "immunologically privileged" site, the peripheral immune cells indeed are able to penetrate the blood-brain barrier (BBB) to enter brain parenchyma under both normal and pathogenic conditions.
Following neural insult, local microglia, astrocytes and infiltrated leukocytes become activated and release a myriad of pro-inflammatory cytokines, chemokines, proteinases, complement proteins, and reactive oxygen species, resulting in central neuron system (CNS) injuries. If inflammatory processes cannot be resolved by innate immune mechanisms, inflamed tissues and released proinflammatory molecules will further attract more leukocytes from peripheral blood and lead to more severe damages and disease progression (Figure 1).

One of the hallmarks of neuroinflammation is the recruitment of peripheral leukocytes and their interaction with activated microglia, however, the impact of different immune cell subsets on the outcome of CNS diseases is not fully understood and is still under investigation.

The central nervous system plays a crucial role in the regulation of immunity and inflammation. However, neuronal integrity and brain function can be severely compromised under inflammatory or autoimmune conditions. It has been long recognized that neuro-damage is often associated with neuroinflammation and activation of innate cells.
Discover, Optimize, Predict

Inflamed brain tissues release CNS-derived antigens. These antigens can be uptaken by antigen presenting cells (APCs) in the secondary lymphoid organs. APCs then present processed antigenic peptides to CD4+ T cells, which have escaped from the central tolerance to mount a robust immune response and infiltrate into the CNS. Infiltrated CD4+ T cells cause further CNS damages and lead to autoimmune diseases such as multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE).\(^\text{17}\)

**Diseases of the CNS\(^\text{18}\)**

- Alzheimer’s disease
- Parkinson’s disease
- Amyotrophic lateral sclerosis
- Traumatic brain injury
- Stroke
- Frontotemporal dementia
- Multiple sclerosis

Immunotherapies enhance Th1 and Th17 cell responses and the release of proinflammatory cytokines, such as IL-6 and IL-17, which leads to abnormal T-regulatory (Treg) cell function and humoral immunity. An altered Treg/Th17 cell axis further exacerbates autoimmune diseases. Apart from T cells, macrophages/monocytes also contribute to the development of neurodegenerative diseases\(^\text{19}\). However, the exact mechanisms by which these blocked interactions lead to a diversity of autoimmune complications remain unclear.
The role of polyfunctional cell subsets and their impacts on immune related adverse events

There is abundant evidence from patients receiving checkpoint inhibitor treatment for advanced malignancies, including metastatic cancer, especially melanoma, demonstrating that patients receiving this immunotherapy have a high risk for developing immune-related neurological adverse events\textsuperscript{20-22}. Tumor cells express inhibitory ligands PD-L1/PD-L2 on their cell surface, which interact with the inhibitory receptors, such as PD-1, on T cell surfaces to limit T cell activities. The immune checkpoint inhibitor blockade removes the inhibitory signal of T cell activation to restore host anti-tumor immune responses.

Impacts of Immune Related Adverse Events

- Neurotoxicity in cell therapy (blood cancer)
- Autoimmune disease in cancer patients after anti-PD-1 treatment
- On-target and off-tumor toxicity in T cell therapy and bispecific T cell engager therapy
- CRS in cell therapy
- Graft-versus-host disease (GVHD) in patients receiving allogeneic T cell therapy
- Crohn's & Psoriasis systems in anti-PD-1 / PD-L1
- CRS and neurotoxicity in bispecific T cell engager therapy
Although this blockade significantly improves the therapeutic effect, because of the removal of the “brake” of T cell activation, it concurrently results in the disruption of self-tolerance and eventually leads to immune-related adverse events in multiple organs, especially in the CNS\textsuperscript{23-25} (Figure 2). Retrospective studies revealed that neurotoxic adverse events were responsible for 15% of anti-PD-1/PD-L1-related fatalities\textsuperscript{26}. More strikingly, recent studies reported that as many as 40% of patients undergoing CAR T therapy will develop severe neurotoxicities in the form of a cytokine-release-associated encephalopathy\textsuperscript{27}.

Figure 2 | The stimulation of the immune system to fight cancer can lead to serious adverse effects if left unchecked. Careful balance between immune stimulation and immune repression is necessary for the minimization of side effects, such as autoimmune diseases, while fighting cancer.
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Since neurotoxicity is a complex integration of the responses of all cells present within the CNS, including the neurons, macroglia, microglia and the infiltrating leukocytes, it is critical to understand how all these cells and factors interact with each other to lead to either neuroprotective or neurotoxic inflammatory responses. We are specifically interested in the role of cytokines/chemokines released by participating cells during this complicated biological and pathological process.

The Inflammatory response is driven by the inflammatory cytokine contribution from various immune cell types, though it remains challenging to find early biomarkers of inflammatory reaction & autoimmune progression.

The initial function of acute inflammation is to recruit leukocytes from peripheral blood. The first wave of cell infiltration is dominated by neutrophils, followed by monocytes that differentiate locally into macrophages or dendritic cells.

The inflammatory response proceeds with the release of a variety of pro-inflammatory mediators, including cytokines and chemokines. The pro-inflammatory mediators released from inflamed tissues further recruit T and B cells from peripheral blood and these recruited cells interact with each other and orchestrate local inflammatory responses.
Cytokines are generally pro- or anti-inflammatory, and the balance between these determines the final outcome of an inflammatory response. For example, IL-1β, IL-8, and IFN-γ are pro-inflammatory cytokines involved in early responses and the amplification of inflammatory reactions.

Anti-inflammatory cytokines like IL-4, IL-10, and IL-13 limit inflammatory responses. The same cytokine can be secreted by different cells and the same cell can secrete different cytokines as well. Moreover, even genetically and phenotypically identical cell subsets can differ in cellular responses and secretion profiles. As our understanding of both single-cell biology and immune function evolves, it is becoming clear that a key to understanding non-genetic heterogeneity in inflammatory response lies in single-cell analysis (Figure 3).

Identifying heterogeneous functional immune cell subsets from inflamed tissues or peripheral blood is key to understanding the biomarkers of neuroinflammation and disease progression

As mentioned above, T cells bearing identical T cell receptors and phenotypes are highly heterogeneous in terms of their effector functions and cytokine profiles. It is well accepted that multiple cytokines/chemokines producing (polyfunctional) T cells at the single-cell level are the key effector cells contributing to the development of potent and durable cellular immunity against various infectious diseases, as well as cancers.
IsoPlexis’ systems identify heterogeneity and functional immune cell subsets, which correlate to early markers of progression in inflammatory disease

Efforts have been made to look at serum and body fluid levels of cytokines and other protein biomarkers to monitor inflammatory/autoimmune responses and neurotoxicity progression after cell therapies. While these approaches show some promise, they lack the single-cell resolution needed to accurately reveal the autoimmune response. Yet until recently, available technologies could not quantify multiple secreted cytokines on a single-cell level, limiting researchers’ ability to analyze the cell subsets that may influence the course of the disease (Figure 4).
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A detailed analysis of heterogeneous immune cell populations from inflamed tissues or peripheral blood is key to understanding the root causes of inflammation and disease progression and for developing therapeutic intervention (Figure 5).

IsoPlexis’ single-cell biomarkers address the gap of employing correlative immune data in drug discovery, bioprocessing, and translation to the clinic

Neuroinflammation and Immune Related Adverse Effect Biomarker Case Studies

**Figure 5** | IsoPlexis’ systems provide advanced correlative data to in vivo activity, which helps guide decisions and fills critical gaps in Drug Discovery, Bioprocessing, and Translation to the clinic.
Monocytes in neuroinflammation: Polyfunctional Inflammation and the impact of functional innate subsets on multiple sclerosis

Multiple sclerosis (MS) is a neurologic autoimmune disease characterized by myelin loss and axonal degeneration that affects more than 2.3 million people worldwide and is the leading cause of non-traumatic neurologic disability in young adults\(^{35}\). Monocytes and their associated cytokines play a critical role in the pathogenesis of MS and are thought to be one of the first immune cells to initiate and promote brain inflammation, contributing to disease progression\(^{36}\). To understand monocyte associated cytokine function in the CNS inflammation of MS patients, and to prove the hypothesis that MS patients have a deficiency in innate immune regulation leading to hyper-responsiveness to TLR2 stimulation\(^{37}\), researchers used IsoPlexis’ systems to identify a pathologically and therapeutically relevant Toll-like receptor

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*Note: Cytokines in panel are the same cytokines as those in the Polyfunctional Inflammation panel.*

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Figure 6 | Identification of enhanced MS response to TLR2 stimulation through IsoPlexis’ PII, which advances understanding of the sources of underlying pathogenesis.* Figure published as PII equivalent numeric, PSI.
T Cells in Neuroinflammation: Polyfunctional Inflammation uniquely correlates to Cytokine Release Syndrome (CRS) and Neurotoxicity (NT) in CAR T Cell Therapy, indicating potential for earlier indicator of adverse events

Given the pathogenic role of T helper 17 (Th17) cells, researchers asked whether subsets of polyfunctional T cells producing cytokines including IL-17A were associated with grade ≥3 NT. Cytokine-specific Polyfunctional Inflammation was previously uncharacterized in MS (Figure 6). These results confirmed data generated independently and suggest that the highest frequency of enhanced TLR2 responders within the MS cohort may be found among the patients with progressive forms of the disease. The ability to stratify MS patients based on monocyte functionality may be especially powerful given the observed differences in therapeutic response for those with the different forms of the disease.

**Product Polyfunctional Inflammation Component (IL-17) combined with in vivo metrics significantly correlated with grade 3+ NT**

*Figure 7 | Association between Polyfunctional Inflammation in conjunction with either CAR peak or pretreatment in vivo IL-15 levels in blood, and grade 3+ NT. CAR T cells or IL-15 levels in blood were measured by qPCR or ELISA respectively.*

*Note: Cytokines in panel are the same cytokines as those in the Polyfunctional Inflammation panel.*
computed by multiplying the percentage of polyfunctional T cells secreting a given cytokine with the average signal intensity for that cytokine. These cytokine values were analyzed in relation to outcome. Interestingly, IL-17A driven polyfunctional subsets, defined post hoc, plus CAR peak cell levels had a significant association with grade ≥3 NT (Figure 7). IL-15 levels have been shown to be positively associated with CAR T cell expansion. We investigated whether pre-CAR T cell infusion levels of IL-15, in combination with PSI, were associated with neurotoxicity. Indeed, IL-17A PSI combined with IL-15 levels at day 0 also had a statistically significant association with grade ≥3 NT, suggesting a critical role for IL-17A producing polyfunctional cell subsets in neurologic toxicities (Figure 7).
NK cells in autoimmune progression: Polyfunctional Inflammation reveals differences in mechanism for NK cell subsets responsible for proinflammatory cytokines in IBD patients

Natural Killer (NK) cells are innate immune cells and known for their crucial role in several autoimmune diseases. Killer Ig-like receptors (KIR) genes are predominantly expressed by NK cells and are one element of the receptor repertoire controlling NK cell activation, proliferation, and effector functions. KIR receptors can distinguish major histocompatibility (MHC) class I allelic variants, which allows them to detect virally infected or malignant cells. The diverse expression of KIRs has also been implicated in susceptibility to Crohn’s disease (CD), a chronic intestinal inflammatory disease. However, the exact cellular mechanism of this genetic contribution has not been elucidated. In a recent study, researchers showed that NK cells from CD patients efficiently augment antigen-specific CD4+ T cell response. Interestingly,
this augmentation is mediated by multiple soluble molecules secreted by “licensed” NK cells. Multiplexed single-cell proteomic analysis reveals that the presence of KIR2DL3 and its interaction with homozygous HLA-C1 results in NK cell cytokine reprogramming, which permits them to promote CD4+ T cell activation and Th17 differentiation ex vivo. Polyfunctional secretion analysis from thousands of single NK cells further established that “licensed” NK cells are more polarized to proinflammatory cytokine production than unlicensed NK cells, including production of IFN-γ, TNF-α, CCL-5, and MIP-1β (Figure 9). The KIR2DL3+ NK (Type 1) cell subset is responsible for enhanced proinflammatory cytokine production in Crohn’s Disease, providing potential new avenues for therapeutic intervention. This study offers a fresh biologic diagram accounting for the impact of KIR-HLA genetics on IBD and other chronic inflammatory diseases\textsuperscript{40}. 

**Figure 9** | NK cell subsets responsible for proinflammatory cytokines in IBD patients. Polyfunctional Inflammation reveals key differences in NK cell subtypes, which are polarized to proinflammatory cytokine production.
IsoPlexis’ highly-multiplexed platform for single-cell secretion analysis provides high-resolution information that helps to deepen the understanding of heterogeneous inflammatory responses and detect previously unknown functional diversities. In this E-book, we showed that the Polyfunctional Inflammation value of pre-infusion CAR T cell products significantly correlates with adverse effects, including CRS and NT. IsoPlexis’ unique single-cell biology aims to improve the understanding of cytokine mediated adverse effects in a translational fashion. With the highly-sensitive, multidimensional, single-cell analysis platform, IsoPlexis uniquely addresses current challenges in polyfunctional neuroinflammatory responses. The more single-cell functional biology is used to uncover sources of inflammatory events, the more personalized and targeted therapies will be possible in therapeutic development.
References


