Role of innate immunity in Multiple Sclerosis pathogenesis requires further understanding

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 [1]. No specific diagnostic tests exist for MS and the cause of the disease is still poorly understood.

MS is a complex disease that varies dramatically between individuals, and MS patients benefit from early diagnosis and a multifaceted therapeutic approach that slows disease progression and tries to reduce the number and severity of attacks.

Characterizing critical functional subsets of innate immune cells in multiple sclerosis patients to understand novel targets and advance therapy development

• In this Application Highlight we:

• Show how IsoPlexis’ Polyfunctional Strength Index (PSI) can be used to assess cytokine secretion of single innate immune cells, i.e. monocytes, in autoimmune patients to monitor progression

• Demonstrate the value of PSI to reveal functional heterogeneity of single innate immune cells

• Show that by detecting highly dysfunctional cell subsets, PSI can lead to the unique detection of key patient differences in autoimmune progression

• Demonstrate how these differences can reveal critical therapeutic insight and novel targets

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PSI™ 
Applied to Autoimmune Progression and Precise Therapeutic Targeting
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While the role of the adaptive immune system in the pathogenesis and treatment of autoimmune disease continues to be a major research focus, more efforts are now dedicated to understanding the role of innate immunity in autoimmune diseases [2]. Monocytes and their associated cytokines play a critical role in the pathogenesis of MS and other central nervous system diseases [3]. In MS, activated monocytes are thought to be one of the first immune cells to initiate and promote brain inflammation, contributing to disease progression [4]. Monocyte-associated cytokine function is important in the earliest phases of MS pathogenesis and has a central role in the CNS inflammation that is characteristic of the disease.

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 the most. A detailed analysis of heterogeneous immune cell populations from individual patients is key to understanding the root causes of MS and for developing therapeutic intervention.

In this Application Highlight, we discuss how IsoPlexis’ systems, which are able to quantify the secretion profile and polyfunctionality of immune cells on a single cell level, were used to identify a pathologically and therapeutically relevant Toll-like receptor (TLR) previously uncharacterized in MS and to prove the hypothesis that MS patients have a deficiency in innate immune regulation leading to hyper-responsiveness to TLR2 stimulation [5].

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**IsoPlexis PSI: polyfunctionality of a sample combined with the intensity of each cell’s secreted cytokines**

![Diagram](image)

**Figure 1 | PSI (Polyfunctional Strength Index)** is defined as the percentage of polyfunctional single-cells (secreting 2 or more proteins, i.e. left panel) in a sample, multiplied by the average signal intensity of the secreted proteins from individual functional groups (middle panel) from each cell. Each cell’s strength, across 1000+ cells, is then aggregated and simplified into the readout at right. This PSI measurement provides a comprehensible visualization of the potent cell subsets, and the cytokine types driving these potent cell subsets.
PSI for assessing polyfunctional strength of dysfunctional innate immune cell subsets in MS

PSI (Polyfunctional Strength Index) is a metric of single-cell protein secretion that can be used to assess both cellular potency, and in inflammatory diseases, highly dysfunctional subsets of immune cells as well (those that attack the self rather than a disease). PSI provides an overall measurement of the functional activation of profiled immune cells (Figure 1). PSI has a variety of demonstrated uses in T cell therapy development and manufacturing, and has generated correlates with in-vivo outcomes of patients in a variety of disease and therapeutic areas [6,7]. IsoPlexis’ unique PSI readout also has the potential for similar sensitive definitions of progression and in-vivo correlates in evaluating monocytes.

Here we demonstrate how IsoPlexis’ systems can be used to help segment the heterogeneity of monocytes, and find particular monocyte subsets which secrete diverse combinations of cytokine functions that play a role in autoimmunity, specifically MS [5]. We used the 32-plex Innate Immune panel (Single-Cell Innate & Myeloid) to simultaneously detect the full spectrum of secreted cytokines associated with innate immunity, facilitating comprehensive dissection of the functional states of each single cell within the total cell population.

Figure 2 | Identification of enhanced MS response to TLR2 stimulation through IsoPlexis’ PSI, which advances understanding of the sources of underlying pathogenesis. CD14+ monocyte PSI was calculated for control (n=8) and MS patient samples (n=8) stimulated for 24 hours with P3C (TLR2 stimulation). (a) The PSI of the MS patients samples was notably higher than in the control samples; the PSI of both sample groups was dominated by polyfunctional IL-8 secretions. (b) Statistical analysis showed that the PSI of the MS patients (mean = 34) are statistically higher (p = 0.0348) than the PSI of the control samples (mean = 14). The level of PSI representing the upper threshold of control responses (based on the control IQR) is depicted by the dashed line. The percent of responses above threshold is depicted for controls and total MS patients [5]. The difference in PSI between the two groups suggests an important role of TLR2 signaling in mediating MS disease progress driven by monocytes & the early innate immune response.
PSI differentiates functional response to TLR2 stimulation of MS patients vs. controls

Most investigations into the role of innate immunity in MS have focused on response to toll-like receptor 4 (TLR4). However, using IsoPlexis’ systems, we saw a large fraction of MS patients exhibiting enhanced responsiveness to TLR2 stimulation of CD14+ monocytes by Pam3CSK4 (P3C) relative to the control population (Figure 2). Specifically, 62.5% of MS patients exhibited PSI values above the interquartile range (IQR)-derived upper threshold of responses, while none of the control responses were above this threshold.

The major difference between MS patients and controls was the higher percentage of monocytes secreting two or more cytokines among the MS cohort (Figure 3). In this study, we were also able to show that the enhanced response in MS patients is specific to TLR2 stimulation by P3C and not evident via stimulation of TLR4 on monocytes by lipopolysaccharide (LPS). While there is some increase in TLR4 responsiveness in this MS cohort, we observed a more notable enhancement in response to TLR2 stimulation.

Using PSI to stratify MS patients

Additional evaluation of this dataset allowed us to stratify the MS cohort into patients with relapsing-remitting MS (RRMS) and patients with progressive MS. One of two progressive MS patients showed enhanced response whereas four of the six RRMS patients showed enhanced response. These results confirmed data generated independently (discussed in [5]) 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 diseases. 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.
Conclusion

PSI has the potential to be a powerful discovery tool when applied to MS research, therapeutic development, and disease management:

• It reveals overall functional activation of profiled immune cells, and can be decomposed to highlight drivers of response

• Applied to phenotypically similar monocytes, it indicates that TLR2 signaling plays an important role in mediating MS disease progress

• It can be used to stratify patients and indicate disease progression for more effective immune disease treatment

References