Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Single-cell multiomics in neuroinflammation

Florian Ingelfinger[1,2], Eduardo Beltrán[3,4,5], Lisa A Gerdes[3,4,5] and Burkhard Becher[1]

The central nervous system (CNS) is, more than other organs, particularly vulnerable to inflammation and immune responses must be tightly controlled in order to maintain host protection. Accordingly, neuroinflammation is an orchestrated process involving various cell types that may dramatically change their phenotypic and functional properties upon entering the CNS. Recent advances in single-cell multiomics offer the unique opportunity to resolve this cellular heterogeneity in a holistic fashion and reshape our understanding of the molecular and cellular processes during neuroinflammation. Here, we provide an overview of technical advances in single-cell multiomics and the tremendous impact on our basic understanding of neuroinflammation. We discuss insights obtained in neuroinflammatory diseases and elaborate to which extent these tool sets could be applied in a clinical setting.

Addresses
[1] Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland
[2] Department of Neurology, University Hospital Zurich, Zurich, Switzerland
[3] Institute of Clinical Neuroimmunology, University Hospital, LMU Munich, Munich, Germany
[4] Biomedical Center (BMC), Faculty of Medicine, LMU Munich, Martinsried, Germany
[5] Munich Cluster of Systems Neurology (SyNergy), Munich, Germany

Corresponding Author:
Eduardo Beltrán (Eduardo.Beltran@med.uni-muenchen.de)

Current Opinion in Immunology 2022, **76**:102180

This review comes from a themed issue on **Special Section Neuroimmunology**

Edited by **Roland Liblau** and **Isaac Chiu**

For complete overview of the section, please refer to the article collection, "Special Section Neuroimmunology (June 2022)"

Available online 21th April 2022

https://doi.org/10.1016/j.coi.2022.102180

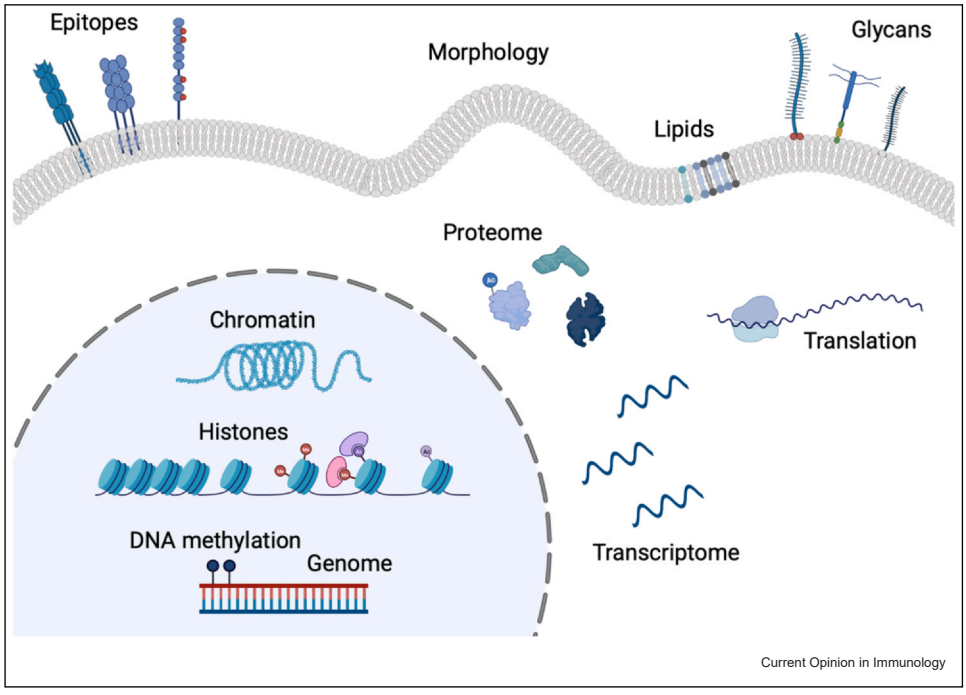
Introduction

Advances in multiomics have provided a rich selection of tools that can be applied to retrieve various cellular properties involved in heterogenous cellular networks during neuroinflammation. Multimodal single-cell technologies can, for example, simultaneously retrieve the surface proteome, the transcriptome and the chromatin accessibility of individual cells [1]. This highly parallelised approach, therefore, provides a unique opportunity to construct a reference framework of interconnected modalities across single cells that can be used to infer causality [2] or to predict one modality by measuring another one as demonstrated in individual studies before [3,4]. Accordingly, single-cell multimodal omics have been elected method of the year in 2019 [5]. Application of those tools holds great promise to tackle some of the largest unsolved challenges in basic neuroimmunology as well as provide critical insights about neuroinflammatory diseases in patients. In the following sections, we will introduce recent developments in single-cell multiomics technologies and assess to which extent these developments have already transformed the field of neuroinflammation.

Technical advances in single-cell multiomics

Flow cytometry was the first technology that could reliably quantify features among thousands of individual cells when it was introduced in 1965 [6]. The advantage of resolving cellular heterogeneity in biological systems by studying single cells during the following decades prompted omics technologies to adapt to the demand for single-cell resolution. Nowadays, single-cell RNA sequencing (scRNA-seq) has become the gold standard in single-cell profiling and is commercially available for a broad range of applications [7]. Yet, multiple additional omics technologies have reached single-cell resolution, each capturing a different modality of cellular function. For instance, scDNA-seq has been introduced to capture genome information of individual cells and has demonstrated its full potential by tracing clonal trajectories in acute myeloid leukaemia patients [8,9]. Epigenetic information of single cells has been obtained by single-cell assays for transposase-accessible chromatin sequencing [10], single-cell DNA methylation profiling [11] and the assessment of chromosome conformation [12] or histone modifications [13]. Additionally, assays detecting interactions between RNA and RNA-binding proteins and profiling of ribosomes in individual cells provided an unprecedented measure of single-cell translation [14,15]. Direct single-cell proteomics using mass spectrometry has greatly benefited from novel protocols and technical advances [16] but remains largely limited by the low protein coverage and sensitivity due to the absence of signal amplification compared to oligonucleotide-based

Figure 1



Single-cell multiomics detect an unprecedented variety of cellular features. Schematic overview of a cell with highlighted cellular components, properties or processes that can currently be captured in single-cell resolution using multiomic technologies.

technologies. Thus, proteins are currently quantified using indirect measurements derived from fluorophore-tagged, heavy metal-tagged or oligonucleotide-tagged antibodies [17]. To circumvent spill and spread errors of conventional flow cytometry while retrieving the surface proteome of a cell, Infinity Flow combines flow cytometry and machine learning. Using repetitive measurements of a common backbone panel and one variable infinity marker, Infinity Flow generates a predicted single-cell dataset of 100s of proteins [18] (Figure 1).

Albeit still at its infancy, metabolomics benefitted from exciting novel approaches to retrieve information about lipids [19] or glycans [20] in individual cells providing a promising outlook to what may be possible in the close future. Advances in multiomics did not necessarily depend on complicated experimental designs. Neural networks have been demonstrated to be able to retrieve morphological information from brightfield images of single cells that was sufficient to reliably classify the cell and reveal potential haematological alterations [21]. Similarly, morphokinetic features have been extracted from intravital microscopy to retrieve behavioural states of individual cells that could be applied to classify cells and further identify cellular states, determined by the underlying genetics and protein content, that could be associated with pathogenic inflammation [22]. All these technologies provide a fascinating outlook on the way individual cells could be characterised in the future that goes far beyond the analysis of their transcriptomes and epitopes.

Recent discoveries in basic neuroinflammation uncovered by multiomics

The role of the immune system in triggering neuroinflammation remains largely enigmatic. The use of animal models has proven to be extremely useful to identify the most important inflammatory mediators that trigger neuroinflammation in human diseases. For example, initial therapy research in multiple sclerosis (MS) has been largely driven by results obtained from experimental autoimmune encephalomyelitis (EAE). However, none of the existing models can fully recapitulate human pathology [23]. That is not only due to interspecies variability but also from the fact that EAE induction requires the use of a driver antigen (often a myelin peptide) or transgenic antigen receptors genetically engineered to target the CNS. The situation in MS is of course far more complex and the original trigger of MS remains unknown. Both in MS and EAE, the adaptive immune system, in particular, is considered to be a central mediator of neuroinflammation. EAE is a $CD4^+$ T-cell mediated autoimmune disease. However, each subset, as well as each effector and regulatory T cell phenotype plays a decisive role in the pathogenesis of EAE. Recent single-cell studies have highlighted that $CD4^+$ T cells subsets are more heterogeneous and plastic than previously anticipated [24].

Combining scRNA-seq and T cell receptor (TCR) sequencing (TCR-seq) allows to trace phenotype changes of individual T cell clones across tissues during neuroinflammation. Schnell et al. showed that non-pathogenic IL-17 producing Th cells cell can acquire an IL-23-driven encephalitogenic response characterised by a GM-CSF$^+$ (granulocyte macrophage colony-stimulating factor) IFN-γ^+ (Interferon gamma) CXCR6$^+$ (C-X-C chemokine receptor type 6) phenotype [25]. In line with this finding, using scRNA-seq and mass cytometry Rasouli et al. provide compelling evidence that GM-CSF-producing T cells are the principal trigger of neuroinflammation in EAE [26]. Hiltensperger et al. demonstrated that the phenotype of brain infiltrating encephalitogenic CD4$^+$ T cells is acquired at the priming site and maintained in the brain [27]. Encephalitogenic CXCR6$^+$ T cells reaching the CNS were predominantly primed in draining inguinal lymph nodes and infiltrated both grey and white matter, while T cells primed in the gut-draining mesenteric lymph nodes were primarily recruited to white matter [27]. Once in the CNS, encephalitogenic T cells will potentially become reactivated by encountering their self-cognate antigens. In such a scenario, inflammation per se will cause the activation of the resident myeloid cells in the brain, microglia and other CNS-associated macrophages (CAMs). Using high-dimensional single-cell multiomics analyses Amorim et al. linked the role of GM-CSF and IFN-γ in the transition of Ly6C$^+$ monocytes into mature pathogenic phagocyte subsets during neuroinflammation [28]. Giladi et al. applied index and transcriptional single-cell sorting to characterise a population of pathogenic phagocytes in EAE, which did not derive from Ly6C$^+$ monocytes but from early myeloid cell progenitors [29]. Other single-cell studies have focused on phenotypic changes in microglia and CAMs during neuroinflammation. Jordão et al. generated a comprehensive atlas of the transcriptome of the myeloid cell compartment and the role of individual subsets during neuroinflammation [30].

Unlike in EAE, the inflammatory reaction in the brain of patients with MS is dominated by CD8$^+$ T-cell infiltrates [31–33]. Using single-cell paired TCR sequencing Saligrama et al. demonstrate that expanded CD8$^+$ T cells are unresponsive to myelin in EAE but rather suppress myelin oligodendrocyte glycoprotein-specific encephalitogenic CD4$^+$ T cells [34].

Multiomics in the context of human neuroinflammation

Access to early and immunologically active human brain tissue samples is very limited. Key insights of neuroinflammatory diseases have been generated by analysing leucocytes present in the cerebrospinal fluid (CSF), which is routinely collected for diagnostic purposes at modest risk. In the recent years, few single-cell RNA-seq studies investigated CSF of patients with MS and generated an unprecedented view of the immune landscape in this leucocyte-enriched fluid filling and surrounding the brain [35–38]. By combining scRNA-seq with TCR-seq, B cell receptor (BCR) sequencing and cellular indexing epitopes, Beltrán et al. demonstrated that the earliest experimentally approachable stage of MS, prodromal MS, is characterised by a synergistic activation of the adaptive immune system, with a strong contribution of recently activated, clonally expanded CD8$^+$ T cells displaying a tissue-resident memory phenotype [39].

Even though under inflammatory conditions some CSF leucocytes adapt a tissue-resident phenotype, the CSF cell composition is altered by periodic infiltration of blood-derived immune cells. Since peripheral blood is a more accessible and targetable compartment than the CSF, an integrated analysis of CSF and blood to identify the peripheral immune cells invading the brain is crucial for preventing, monitoring and treating neuroinflammatory diseases. Combining cellular indexing of transcriptomes and epitopes sequencing (CITE-seq) on peripheral blood with spatial RNA sequencing, Kaufmann et al. identified a CNS-colonising T cell subset that could be targeted in the peripheral blood to limit its invasion of the brain and therefore prevent progression of MS [40]. Using mass cytometry Galli et al. identified a population of GM-CSF$^+$ CXCR4$^+$ CD4$^+$ T helper cells expanded in the peripheral blood of relapsing-remitting MS (RRMS) patients that was enriched in the CSF compared to peripheral blood [41]. In two integrated single cell analyses of blood and CSF, compelling evidence of cell-type diversity in the CSF was provided with a dominant T helper cytotoxic phenotype and active intrathecal interaction of T and B cells [35,38]. Of note, while T cells are always present in the CSF both, during steady-state and under inflammatory conditions, single-cell analysis studies have shown that very few B cells and in particular plasmablasts can be found in the CSF of healthy patients [38,39]. Ramesh et al. show that B cells in the CSF of patients with MS adapt an inflammatory clonally expanded memory and plasmablast phenotype but did not investigate specificity of these antibodies [38]. No evidence for constitutive Epstein–Barr Virus (EBV) transcription, or other neurotropic viruses, in B cells of patients with MS was provided [38]. Recently, a single-cell BCR repertoire sequencing study revealed that B cells of patients with MS produce antibodies that cross-bind EBV antigen EBNA1 and GlialCAM in the CNS [42]. The authors further identified CD8$^+$ T cells responding against GlialCAM [42]. These results support the long-standing theory of EBV as a trigger and most dominant environmental risk factor for MS [43]. Moreover, mass cytometry and CITE-seq analyses of monozygotic twin pairs discordant for MS revealed a dysregulated IL-2/CD25 axis in Th cells of MS twins as environmental immune perturbation that was independent of the genetic predisposition [44].

Unlike fresh material such as CSF and peripheral blood, the study of post-mortem brain tissue in single-cell resolution is only possible by single-nuclei RNA-seq (snRNA-seq). Studies using snRNA-seq on post-mortem brain samples from patients with MS and unaffected controls revealed alterations in oligodendrocytes [45] and transcriptome changes in cortical neurons and glial cells [46], which could be key for understanding the synergistic role during MS progression.

Transformative value of multiomics in clinical neuroimmunology

Multiomics offering a combination of deep, broad and ideally unbiased (hypothesis-generating) analyses is of utmost importance when it comes to clinical research fostered by several limitations related to access of precious biomaterial. This holds especially true for compartmentalised or processed tissues, such as CSF, brain biopsy or autopsy material, which in addition feature low cell numbers to harvest. At the current state, multiomics are restricted to basic research and have not entered clinical routine diagnostics due to high costs and expenditure of time.

However, applied broad profiling technologies are a prerequisite to identify novel biomarkers that will be translated from bench to bedside. In perspective, the integration of big data sets generated with this valuable tool box will provide the opportunity to support clinical decision-making processes in the close future. The transformative value of this approach into a clinical setting will be critically dependent on the low-threshold interaction between treating physicians and basic researchers. Assessment of these complex datasets needs to be flanked by machine learning algorithms to leverage and prepare the relevant data for an individualised precision diagnostic process as well as personalised treatments. This line of action is already established in oncology and enables tailored therapy with off-label treatments according to the genetic profile of the individual tumour. The landscape in clinical neuroimmunology has largely expanded during the last years, ranging from an increased variety of neuroimmunological diseases to the discovery of new disease entities and is accompanied by modification of diagnostic criteria. Most importantly, high-dimensional single-cell technologies revealed various underlying disease mechanisms having a direct impact on the choice of suitable therapy from an ever-increasing armamentarium to target autoimmune diseases. This poses an urging clinical need for per-patient specific treatments since clinical evidence confirms that a 'one-size-fits-all' approach in autoimmune diseases is not effective. Examples are immunoglobulin G (IgG) antibody-mediated diseases, such as neuromyelitis optica spectrum disorders (NMOSD, aquaporin-4-Ab) versus myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD). Single-cell transcriptome profiling comparing these two disease entities provided a predictive discrimination and might be more reliable during different disease stages compared to antibody titres themselves [47]. Whereas in NMOSD significant treatment response to B-cell-depleting therapy (rituximab, ocrelizumab) or complement inhibition is well established, treatment response in MOGAD remains limited [48]. Comprehensive *ex vivo* analyses revealed a divergent mode of action for pathogenic anti-MOG IgG antibodies and explain an impaired functional response to treatment and hence other strategies have to be pursued such as anti-IL-6 treatment [49]. Another important aspect is the yield of novel biomarkers to decipher risk factors that at best might even transform into strategies for the prevention of disease, for example, based on Lanz et al. a potential benefit of an EBV vaccination on MS risk is under debate [42,43]. 16sRNA sequencing in combination with metagenomics of the gut microbiome might be a game changer in understanding trigger factors that are accessible to intervention [50]. Intensive and multifaceted monitoring of patients or patients with a high familial risk of MS — such as monozygotic twins with discordance for MS or family member of people with MS — might lead to the establishment of interventions to avoid conversion from a prodromal phase to clinical manifest disease [39]. New insights into the complex mode of action of commonly used medications, such as steroids or interferons have been provided by a thorough analysis of the methylome leading to a better understanding of short-term and long-term treatment responses and might serve as biomarkers for individual treatment response [51].

Next steps: multiomics as a tool for routine clinics

Transition of relevant results from an 'exploratory science loop' that leverages exploratory biomarkers from complex data sets using larger cohorts to the development of emerging biomarkers into a 'fast diagnostic loop' including routine biomarkers with an assessment of clinical usefulness and applicability is the aim of multiomic approaches [52].

The long-term goal is to generate either information of screening or diagnostic value or treatment recommendations on a per-patient basis for the treating physician caring for individual patients.

Conclusions

Single-cell multiomics have clearly transformed our understanding of neuroinflammation providing a more holistic view on heterogeneous cellular networks. While current investigations have mainly interrogated transcriptomes, epitopes and epigenomes, proof-of-principle studies exploring additional modalities of cellular functions and features provide an exciting outlook on future opportunities. The choice of technologies used is often discussed heatedly. Each single cell technology has

advantages and disadvantages and often times, only the combined interrogation of the proteome, transcriptome, metabolome, etc. permits the holistic view on the cellular networks involved in neuroinflammation. The close interaction of computational scientists, immunologists and clinical neuroimmunology centres will provide the required infrastructure to introduce single-cell multiomics into routine clinics as soon as costs drop and efficient workflows are established.

Conflict of interest statement

Authors declare no conflict of interests.

Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy – ID 390857198), the Gemeinnützige Hertie Stiftung, Bavarian association and national association of the German MS society (DMSG), Dr. Leopold And Carmen Ellinger Foundation, and the association "Verein zur Therapieforschung für MS Kranke e.V.". This project has further received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No 882424, the Swiss National Science Foundation (Ambizione PZ00P3_193330 to S.M., 733 310030_170320, 310030_188450, and CRSII5_183478 to B.B.) as well as the Swiss MS Society Research grant to F.I.; F.I. received a Ph.D. fellowship from the Studienstiftung des deutschen Volkes. Figure 1 was created using Biorender.com.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Swanson E, Lord C, Reading J, Heubeck AT, Genge PC, Thomson Z, Weiss MDA, Li XJ, Savage AK, Green RR, *et al.*: **Simultaneous trimodal single-cell measurement of transcripts, epitopes, and chromatin accessibility using tea-seq**. *Elife* 2021, **10**:e63632, https://doi.org/10.7554/ELIFE.63632.
In this study, the authors present a trimodal single-cell platform capable of detecting the transcriptomes, epitopes and chromatin accessibility of thousands of cells.

2. Jackson CA, Vogel C: **New horizons in the stormy sea of multimodal single-cell data integration**. *Mol Cell* 2022, **82**:248-259.

3. Wu KE, Yost KE, Chang HY, Zou J: **BABEL enables cross-modality translation between multiomic profiles at single-cell resolution**. *Proc Natl Acad Sci USA* 2021, **118**:e2023070118, https://doi.org/10.1073/pnas.2023070118

4. • Zhou Z, Ye C, Wang J, Zhang NR: **Surface protein imputation from single cell transcriptomes by deep neural networks**. *Nat Commun* 2020, **11**:651, https://doi.org/10.1038/s41467-020-14391-0.
Multimodal single-cell technologies such as CITE-seq facilitate the simultaneous detection of epitopes in addition to the transcriptome. In this study, the authors use these datasets and establish a framework that facilitates the prediction of protein expression based on the transcriptome.

5. Method of the Year 2019: Single-cell multimodal omics. Nat Methods; 2020. https://doi.org/10.1038/s41592-019-0703-5.

6. Fulwyler MJ: **Electronic separation of biological cells by volume**. *Science* 1965, **150**:910-911, https://doi.org/10.1126/science.150.3698.910

7. Aldridge S, Teichmann SA: **Single cell transcriptomics comes of age**. *Nat Commun* 2020, **11**:4307, https://doi.org/10.1038/s41467-020-18158-5

8. Miles LA, Bowman RL, Merlinsky TR, Csete IS, Ooi AT, Durruthy-Durruthy R, Bowman M, Famulare C, Patel MA, Mendez P, *et al.*: **Single-cell mutation analysis of clonal evolution in myeloid malignancies**. *Nature* 2020, **587**:477-482, https://doi.org/10.1038/s41586-020-2864-x

9. Pellegrino M, Sciambi A, Treusch S, Durruthy-Durruthy R, Gokhale K, Jacob J, Chen TX, Geis JA, Oldham W, Matthews J, *et al.*: **High-throughput single-cell DNA sequencing of acute myeloid leukemia tumors with droplet microfluidics**. *Genome Res* 2018, **28**:1345-1352, https://doi.org/10.1101/gr.232272.117

10. Cusanovich DA, Daza R, Adey A, Pliner HA, Christiansen L, Gunderson KL, Steemers FJ, Trapnell C, Shendure J: **Multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing**. *Science* 2015, **348**:910-914, https://doi.org/10.1126/science.aab1601

11. Karemaker ID, Vermeulen M: **Single-cell DNA methylation profiling: technologies and biological applications**. *Trends Biotechnol* 2018, **36**:952-965, https://doi.org/10.1016/j.tibtech.2018.04.002

12. Ramani V, Deng X, Qiu R, Gunderson KL, Steemers FJ, Disteche CM, Noble WS, Duan Z, Shendure J: **Massively multiplex single-cell Hi-C**. *Nat Methods* 2017, **14**:263-266, https://doi.org/10.1038/nmeth.4155

13. Kaya-Okur HS, Wu SJ, Codomo CA, Pledger ES, Bryson TD, Henikoff JG, Ahmad K, Henikoff S: **CUT&Tag for efficient epigenomic profiling of small samples and single cells**. *Nat Commun* 2019, **10**:1930, https://doi.org/10.1038/s41467-019-09982-5

14. VanInsberghe M, van den Berg J, Andersson-Rolf A, Clevers H, van Oudenaarden A: **Single-cell Ribo-seq reveals cell cycle-dependent translational pausing**. *Nature* 2021, **597**:561-565, https://doi.org/10.1038/s41586-021-03887-4

15. Brannan KW, Chaim IA, Marina RJ, Yee BA, Kofman ER, Lorenz DA, Jagannatha P, Dong KD, Madrigal AA, Underwood JG, *et al.*: **Robust single-cell discovery of RNA targets of RNA-binding proteins and ribosomes**. *Nat Methods* 2021, **18**:507-519, https://doi.org/10.1038/s41592-021-01128-0

16. Petelski AA, Emmott E, Leduc A, Huffman RG, Specht H, Perlman DH, Slavov N: **Multiplexed single-cell proteomics using SCoPE2**. *Nat Protoc* 2021, **16**:5398-5425, https://doi.org/10.1038/s41596-021-00616-z

17. Galli E, Friebel E, Ingelfinger F, Unger S, Núñez NG, Becher B: **The end of omics? High dimensional single cell analysis in precision medicine**. *Eur J Immunol* 2019, **49**:212-220.

18. •• Becht E, Tolstrup D, Dutertre C-A, Ginhoux F, Newell EW, Gottardo R, Headley MB: **Infinity flow: high-throughput single-cell quantification of 100s of proteins using conventional flow cytometry and machine learning**. *SSRN Electron J* 2020, https://doi.org/10.2139/ssrn.3656603.
The authors present a framework combining traditional flow cytometry with machine learning in order to retrieve the imputed expression of hundreds of proteins in single-cell resolution.

19. Li Z, Cheng S, Lin Q, Cao W, Yang J, Zhang M, Shen A, Zhang W, Xia Y, Ma X, *et al.*: **Single-cell lipidomics with high structural specificity by mass spectrometry**. *Nat Commun* 2021, **12**:2869, https://doi.org/10.1038/s41467-021-23161-5

20. Minoshima F, Ozaki H, Odaka H, Tateno H: **Integrated analysis of glycan and RNA in single cells**. *iScience* 2021, **24**:102882, https://doi.org/10.1016/j.isci.2021.102882

21. Matek C, Krappe S, Münzenmayer C, Haferlach T, Marr C: **Highly accurate differentiation of bone marrow cell morphologies using deep neural networks on a large image data set**. *Blood* 2021, **138**:1917-1927, https://doi.org/10.1182/blood.2020010568

22. • Crainiciuc G, Palomino-Segura M, Molina-Moreno M, Sicilia J, Aragones DG, Li JLY, Madurga R, Adrover JM, Aroca-Crevillén A, Martin-Salamanca S, *et al.*: **Behavioural immune landscapes of inflammation**. *Nature* 2022, **601**:415-421.

Using a combination of spatiotemporal live cell imaging and high-dimensional analysis the authors describe behavioural single-cell landscapes of inflammation reflecting the underlying transcriptional and proteomic changes of different cell types and states.

23. Lassmann H, Bradl M: **Multiple sclerosis: experimental models and reality**. *Acta Neuropathol* 2017, **133**:223-244, https://doi.org/10.1007/s00401-016-1631-4

24. Tuzlak S, Dejean AS, Iannacone M, Quintana FJ, Waisman A, Ginhoux F, Korn T, Becher B: **Repositioning TH cell polarization from single cytokines to complex help**. *Nat Immunol* 2021, **22**:1210-1217, https://doi.org/10.1038/s41590-021-01009-w

25. Schnell A, Huang L, Singer M, Singaraju A, Barilla RM, Regan BML, Bollhagen A, Thakore PI, Dionne D, Delorey TM, *et al*.: **Stem-like intestinal Th17 cells give rise to pathogenic effector T cells during autoimmunity**. *Cell* 2021, **184**:6281-6298 e23.

26. Rasouli J, Casella G, Yoshimura S, Zhang W, Xiao D, Garifallou J, Gonzalez MV, Wiedeman A, Kus A, Mari ER, *et al*.: **A distinct GM-CSF+ T helper cell subset requires T-bet to adopt a TH1 phenotype and promote neuroinflammation**. *Sci Immunol* 2020, **5**:eaba9953, https://doi.org/10.1126/SCIIMMUNOL.ABA9953

27. •• Hiltensperger M, Beltrán E, Kant R, Tyystjärvi S, Lepennetier G, Domínguez Moreno H, Bauer IJ, Grassmann S, Jarosch S, Schober K, *et al*.: **Skin and gut imprinted helper T cell subsets exhibit distinct functional phenotypes in central nervous system autoimmunity**. *Nat Immunol* 2021, **22**:880-892, https://doi.org/10.1038/s41590-021-00948-8.
In this study, the authors demonstrate that the tissue site of priming determines the phenotype of T cells during neuroinflammation, which gives rise to divergent effector functions of these cells in the CNS.

28. Amorim A, De Feo D, Friebel E, Ingelfinger F, Anderfuhren CD, Krishnarajah S, Andreadou M, Welsh CA, Liu Z, Ginhoux F, *et al*.: **IFNγ and GM-CSF control complementary differentiation programs in the monocyte-to-phagocyte transition during neuroinflammation**. *Nat Immunol* 2022, **23**:217-228.

29. Giladi A, Wagner LK, Li H, Dörr D, Medaglia C, Paul F, Shemer A, Jung S, Yona S, Mack M, *et al*.: **Cxcl10 + monocytes define a pathogenic subset in the central nervous system during autoimmune neuroinflammation**. *Nat Immunol* 2020, **22**:880-892, https://doi.org/10.1038/s41590-020-0661-1

30. Jordão MJC, Sankowski R, Brendecke SM, Sagar, Locatelli G, Tai YH, Tay TL, Schramm E, Armbruster S, Hagemeyer N, *et al*.: **Neuroimmunology: single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation**. *Science* 2019, **363**:eaat7554, https://doi.org/10.1126/science.aat7554

31. van Nierop GP, van Luijn MM, Michels SS, Melief MJ, Janssen M, Langerak AW, Ouwendijk WJD, Hintzen RQ, Verjans GMGM: **Phenotypic and functional characterization of T cells in white matter lesions of multiple sclerosis patients**. *Acta Neuropathol* 2017, **134**:383-401, https://doi.org/10.1007/s00401-017-1744-4

32. Machado-Santos J, Saji E, Tröscher AR, Paunovic M, Liblau R, Gabriely G, Bien CG, Bauer J, Lassmann H: **The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue-resident CD8+ T lymphocytes and B cells**. *Brain* 2018, **141**:2066-2082, https://doi.org/10.1093/brain/awy151

33. Fransen NL, Hsiao CC, Van Der Poel M, Engelenburg HJ, Verdaasdonk K, Vincenten MCJ, Remmerswaal EBM, Kuhlmann T, Mason MRJ, Hamann J, *et al*.: **Tissue-resident memory T cells invade the brain parenchyma in multiple sclerosis white matter lesions**. *Brain* 2020, **143**:1714-1730, https://doi.org/10.1093/brain/awaa117

34. Saligrama N, Zhao F, Sikora MJ, Serratelli WS, Fernandes RA, Louis DM, Yao W, Ji X, Idoyaga J, Mahajan VB, *et al*.: **Opposing T cell responses in experimental autoimmune encephalomyelitis**. *Nature* 2019, **572**:481-487, https://doi.org/10.1038/s41586-019-1467-x

35. Schafflick D, Xu CA, Hartlehnert M, Cole M, Schulte-Mecklenbeck A, Lautwein T, Wolbert J, Heming M, Meuth SG, Kuhlmann T, *et al*.: **Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis**. *Nat Commun* 2020, **11**:247, https://doi.org/10.1038/s41467-019-14118-w

36. Pappalardo JL, Zhang L, Pecsok MK, Perlman K, Zografou C, Raddassi K, Abulaban A, Krishnaswamy S, Antel J, van Dijk D, *et al*.: **Transcriptomic and clonal characterization of T cells in the human central nervous system**. *Sci Immunol* 2020, **5**:eabb8786, https://doi.org/10.1126/SCIIMMUNOL.ABB8786

37. Esaulova E, Cantoni C, Shchukina I, Zaitsev K, Bucelli RC, Wu GF, Artyomov MN, Cross AH, Edelson BT: **Single-cell RNA-seq analysis of human CSF microglia and myeloid cells in neuroinflammation**. *Neurol Neuroimmunol Neuroinflam* 2020, **7**:e732, https://doi.org/10.1212/NXI.0000000000000732

38. Ramesh A, Schubert RD, Greenfield AL, Dandekar R, Loudermilk R, Sabatino JJ, Koelzer MT, Tran EB, Koshal K, Kim K, *et al*.: **A pathogenic and clonally expanded B cell transcriptome in active multiple sclerosis**. *Proc Natl Acad Sci USA* 2020, **117**:22932-22943, https://doi.org/10.1073/pnas.2008523117

39. •• Beltrán E, Gerdes LA, Hansen J, Flierl-Hecht A, Krebs S, Blum H, Ertl-Wagner B, Barkhof F, Kümpfel T, Hohlfeld R, *et al*.: **Early adaptive immune activation detected in monozygotic twins with prodromal multiple sclerosis**. *J Clin Investig* 2019, **129**:4758-4768, https://doi.org/10.1172/JCI128475.
Using single-cell transcriptomics, the authors reveal clonal and transcriptional perturbations in the cytotoxic T cell and plasmablast compartments elicited by a prodromal stage of MS.

40. Kaufmann M, Evans H, Schaupp A-L, Engler JB, Kaur G, Willing A, Kursawe N, Schubert C, Attfield KE, Fugger L, *et al*.: **Identifying CNS-colonizing T cells as potential therapeutic targets to prevent progression of multiple sclerosis**. *Med* 2021, **2**:296-312 e8.

41. Galli E, Hartmann FJ, Schreiner B, Ingelfinger F, Arvaniti E, Diebold M, Mrdjen D, van der Meer F, Krieg C, Al Nimer F, *et al*.: **GM-CSF and CXCR4 define a T helper cell signature in multiple sclerosis**. *Nat Med* 2019, **25**:1290-1300, https://doi.org/10.1038/s41591-019-0521-4

42. •• Lanz TV, Brewer RC, Ho PP, Moon J-S, Jude KM, Fernandez D, Fernandes RA, Gomez AM, Nadj G-S, Bartley CM, *et al*.: **Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM**. *Nature* 2022, **603**:321-327, https://doi.org/10.1038/s41586-022-04432-7.
In this study, the authors provide a rationale for high-affinity molecular mimicry between MS and EBV infection. They identify EBNA1-reactive B cells in patients with MS that crossreact to the CNS antigen GlialCAM.

43. Wekerle H: **Epstein-Barr virus sparks brain autoimmunity in multiple sclerosis**. *Nature* 2022, **603**:230-232, https://doi.org/10.1038/d41586-022-00382-2

44. •• Ingelfinger F, Gerdes LA, Kavaka V, Krishnarajah S, Friebel E, Galli E, Zwicky P, Furrer R, Peukert C, Dutertre C-A, *et al*.: **Twin study reveals non-heritable immune perturbations in multiple sclerosis**. *Nature* 2022, **603**:152-158, https://doi.org/10.1038/s41586-022-04419-4.
Using monozygotic twin pairs discordant for MS, the authors dissect environmental from heritable influences on the immune compartment in patients with MS and reveal perturbations in the IL-2/CD25 axis of Th cells as non-heritable immune perturbations in MS.

45. Jäkel S, Agirre E, Mendanha Falcão A, van Bruggen D, Lee KW, Knuesel I, Malhotra D, ffrench-Constant C, Williams A, Castelo-Branco G: **Altered human oligodendrocyte heterogeneity in multiple sclerosis**. *Nature* 2019, **566**:543-547, https://doi.org/10.1038/s41586-019-0903-2

46. Schirmer L, Velmeshev D, Holmqvist S, Kaufmann M, Werneburg S, Jung D, Vistnes S, Stockley JH, Young A, Steindel M, *et al*.: **Neuronal vulnerability and multilineage diversity in multiple sclerosis**. *Nature* 2019, **573**:75-82, https://doi.org/10.1038/s41586-019-1404-z

47. Liu J, Yang X, Pan J, Wei Z, Liu P, Chen M, Liu H: **Single-cell transcriptome profiling unravels distinct peripheral blood immune cell signatures of RRMS and MOG antibody-associated disease**. *Front Neurol* 2022, **12**:807646.

48. Marignier R, Hacohen Y, Cobo-Calvo A, Pröbstel AK, Aktas O, Alexopoulos H, Amato MP, Asgari N, Banwell B, Bennett J, *et al*.: **Myelin-oligodendrocyte glycoprotein antibody-associated disease**. *Lancet Neurol* 2021, **20**:762-772, https://doi.org/10.1016/S1474-4422(21)00218-0

49. Mader S, Kümpfel T, Meinl E: **Novel insights into pathophysiology and therapeutic possibilities reveal further differences between AQP4-IgG- and MOG-IgG-associated diseases**. *Curr Opin Neurol* 2020, **33**:362-371.

50. Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, Liu C, Klotz L, Stauffer U, Baranzini SE, *et al*.: **Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice**. *Proc Natl Acad Sci USA* 2017, **114**:10719-10724, https://doi.org/10.1073/pnas.1711233114

51. Gerdes LA, Janoschka C, Eveslage M, Mannig B, Wirth T, Schulte-Mecklenbeck A, Lauks S, Glau L, Gross CC, Tolosa E, *et al*.: **Immune signatures of prodromal multiple sclerosis in monozygotic twins**. *Proc Natl Acad Sci USA* 2020, **117**:21546-21556, https://doi.org/10.1073/pnas.2003339117

52. Irmisch A, Bonilla X, Chevrier S, Van Lehmann K, Singer F, Toussaint NC, Esposito C, Mena J, Milani ES, Casanova R, *et al*.: **The Tumor Profiler Study: integrated, multi-omic, functional tumor profiling for clinical decision support**. *Cancer Cell* 2021, **39**:288-293, https://doi.org/10.1016/j.ccell.2021.01.004