P-74; MULTI-ELEMENT ANALYSIS OF SINGLE CELLS USING A TOF-ICP-MS

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Introduction

The study of the endogenous biologically relevant elements is critical for a better understanding health and disease. Mass cytometry is a valuable tool for investigating protein expression of individual cells within heterogeneous samples. With this approach, heavy metal isotope conjugated probes – typically antibodies – label multiple cellular proteins, and TOF-ICP-MS is then used to simultaneously detect these probes on single cells. Thus, the population heterogeneity can be determined at the single cell level. However, current mass cytometry instruments focus solely on the detection of exogenous labels and therefore cannot collect the information of the endogenous elemental content of the cells.

Here, we use the Vitesse TOF-ICP-MS to determine relative levels of biologically relevant elements in a complex system of immune cells from mouse spleen. In this study, a range of major immune cell types resident in the red and white pulp of mouse spleen were identified by conventional mass cytometry concurrently with the amount of P, Ca, K and Fe that they contained. Interestingly, analysis indicated a clear difference in the elemental content of specific cells types primarily located in the two major histological zones of the spleen. These data highlight the ability of the Vitesse TOF-ICP-MS system to be utilised to analyse cellular heterogeneity and the endogenous elemental content at the single cell level.

Vitesse ICP-TOF-MS, courtesy Nu Instruments

Methods

- Single cell suspensions were prepared of mouse splenocytes and stained with lanthanide labelled antibodies specific for cell surface receptors (166Er-TIM4, 141Pr-B220, 149Sm-CD19, 156Gd-CD90.2, 152Sm-CD3, 174Yb-MHC-II, 157Lu-Ly6C, 176Yb-Ly6G, 155Gd-CD11b, 146Nd-F4/80, and 162Dy-SiglecF) along with Ir DNA intercalator and Cisplatin live/dead stains.
- These stained samples were introduced into the Vitesse TOF-ICP-MS (Nu Instruments, UK) with a single cell introduction system (Glass Expansion) such that cells individually entered the plasma and were ionised for analysis of their elemental composition as well as for the presence of the various stains.
- Data was collected and analysed in NuQuant and OMIQ. Single cells were determined by Ir DNA intercalator and cisplatin live/dead stains and immune cell populations were identified based on the pattern of lanthanide staining.

Results

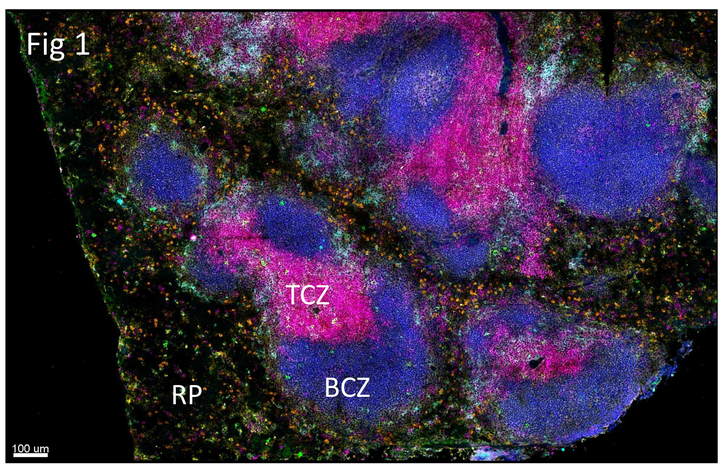

Fig 1. Specific distribution of immune cell populations within the mouse spleen. Splenic red pulp (RP) contains Neutrophils, Eosinophils, Monocytes and Red Pulp Macrophages, while T and B cells reside within the white pulp (T cell zones, TCZ, and B cell zones, BCZ, respectively). Multiparameter staining of a section of mouse spleen; CD11c (cyan), CD3 (red), B220 (blue), CD4 (purple), CD11b (orange) and MHC-II (yellow).

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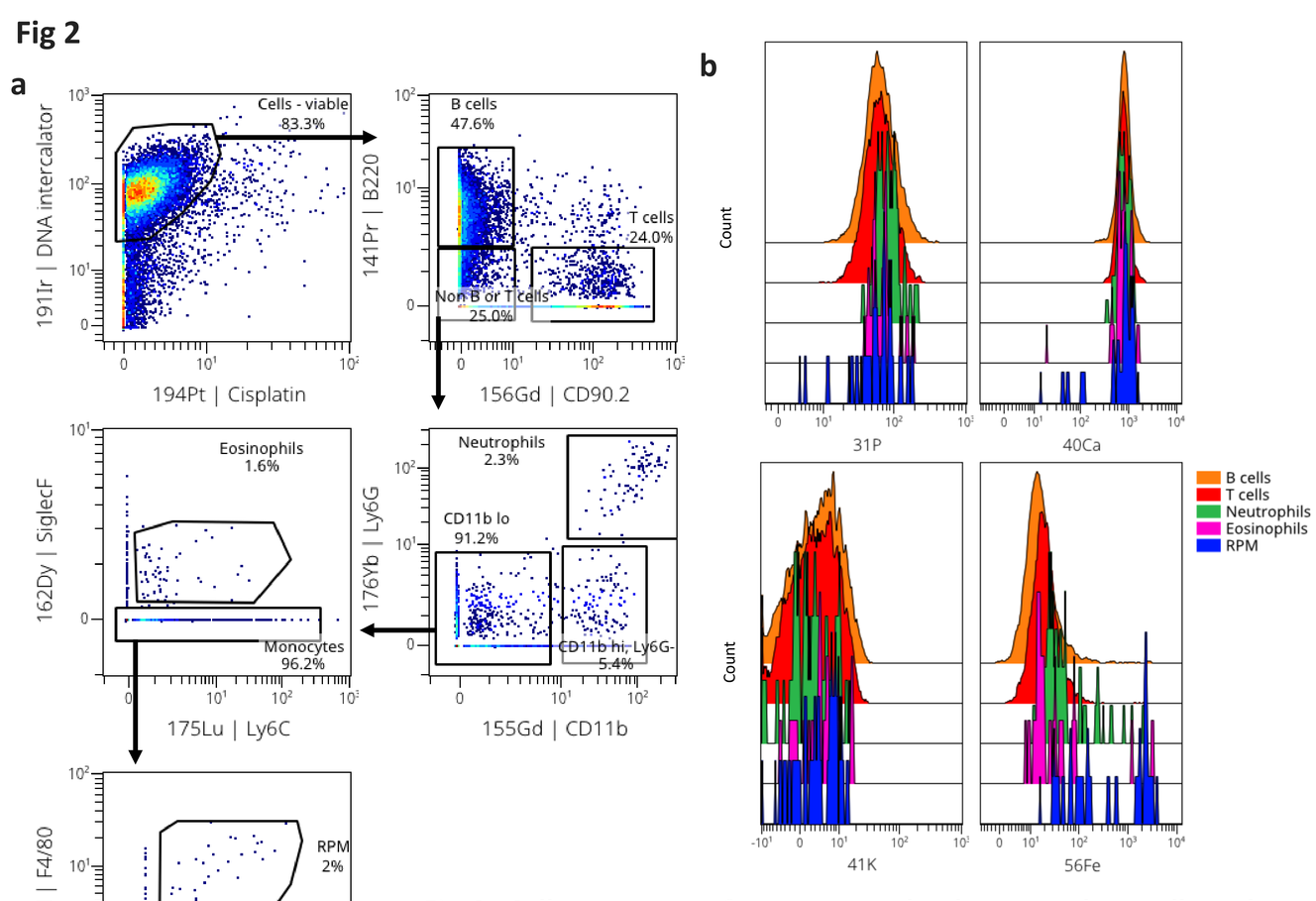

Fig 2. Cell cytometry of concatenated splenocyte data collected on Vitesse TOF-ICP-MS. a Similar to conventional flow cytometry, the B cell, T cell, Neutrophil, Eosinophil, Monocytes and Red Pulp Macrophage (RPM) populations of the spleen was determined. **b** The relative levels of 31P, 40Ca, 41K and 56Fe within the B cell, T cell, Neutrophil, Eosinophil and RPM populations.

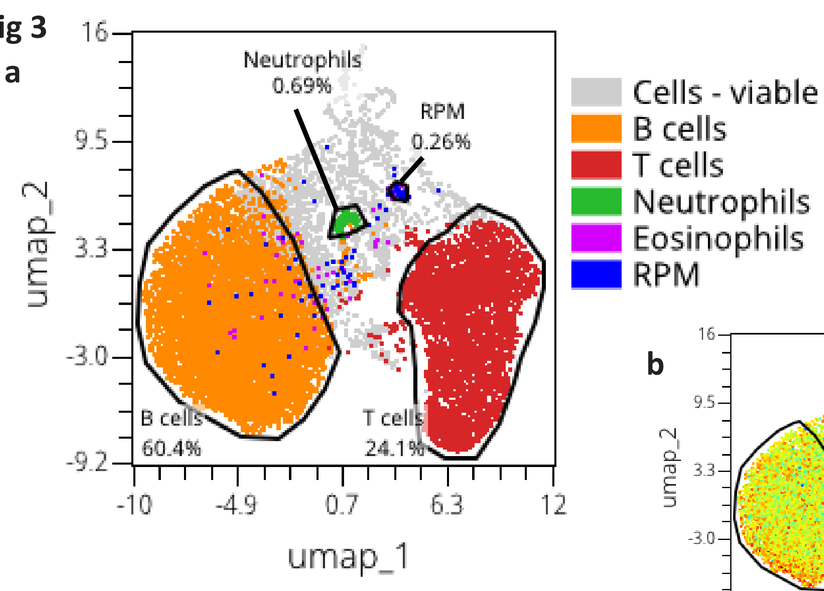

Fig 3. uMAP high dimensional data reduction reveals specific element content of splenic immune cell populations. uMAP high dimensional data reduction was perform on Vitesse cytometry data to allow unbiased analysis of the cell populations. **a** Overlaying the immune cell populations as determined in Fig 2a onto clustered uMAP data confirmed the identities of the clustered populations. **B** Overlay of 31P, 40Ca, 41K and 56Fe intensities on the uMAP populations. Compared to the B cell, T cell and Neutrophils populations, the data shows that RPMs contained a lower amount of 31P and 40Ca and higher amount of 56Fe. Similar amount of 41K was detected within all cell populations.

Conclusion

Cell cytometric analysis of Vitesse TOF-ICP-MS data allowed for the identification of various immune cell populations along with their endogenous P, Ca, K and Fe content. As expected, red pulp macrophages were shown to contain a high amount of Fe. This is consistent with the major function of red pulp macrophages of clearing dead and dying eosinophils from the circulation. Interestingly, it was also observed that the RPM also contained a lower amount of P and Ca than compared to the other immune populations.

The analysis of endogenous, biologically relevant, elements is becoming increasingly important in the study of health and disease. These data highlight the ability of Vitesse TOF-ICP-MS system to be utilised to analyse cellular heterogeneity and the endogenous elemental content of cells.

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