APPLICATION NOTE

Applying Single-Cell Proteomics to Explore Immune Responses in Organ Transplantation

Assessing CD8+ immune responses in kidney transplant patients to reveal functional mechanisms driving humoral rejection.

In this Application Highlight we:

- Show how Bruker's platform can be applied in the field of organ transplantation to reveal key cellular differentiators between transplant recipients with a normal response versus a humoral rejection response
- Identify correlates between transplant rejection and highly polyfunctional CD8+ T cells detected using single-cell proteomics
- Reveal individual functional cytokine contributors to humoral rejection for mechanistic insight and target identification

BRUKER

Unlocking a New Layer of Functional Resolution in Organ Transplantation

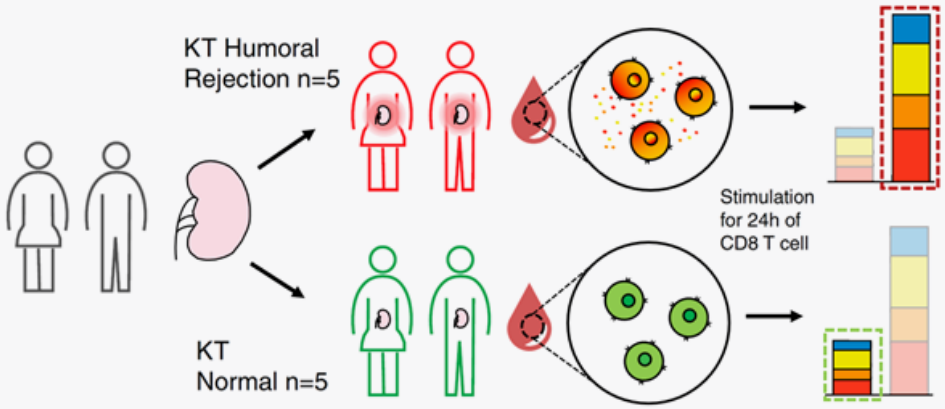

Figure 1 | Experimental Design. Patients receiving Kidney Transplants (KT) were divided into normal (green, n=5) versus humoral rejection (red, n=5) cohorts based on kidney biopsies. CD8 T cells were isolated and stimulated with plate bound anti-CD3 and soluble anti-CD28 for 24 hours. Stimulated cells were then loaded into Bruker Single-Cell Secretome chips with the Human Adaptive Immune panel to measure cellular immune response.

Improving Success Rates of Organ Transplantation Requires a Deep understanding of Transplant Immunology

Renal transplantation is a life-changing treatment for hundreds of thousands of patients suffering from end-stage kidney disease or kidney failure. Unfortunately, despite significant advances in cross matching and immunosuppression research, transplant functionality is still frequently hindered by acute and chronic graft immune rejection[1]. Organ transplantation immune rejection is mediated by a variety of dynamic cellular and humoral factors. These complexities present a substantial challenge for researchers looking to alleviate or prevent organ transplant rejection.

Improving transplant outcomes requires rigorous characterization of the immune cells involved in transplant rejection[2]. Bruker IsoCode technology can provide an unparalleled level of detail into cellular heterogeneity and functional cytokine secretion to reveal key insights about immunological mechanisms.

In this Application Highlight, we cover how the Bruker platform was used to reveal the relationship between polyfunctional CD8+ cells and humoral response in kidney transplant patients. Furthermore, a deeper dive into Bruker's single-cell proteomic data showed that the polyfunctional cells facilitated rapid production of proinflammatory cytokines and cytotoxic molecules associated with effector memory CD8+ T cells that re-express CD45RA[3].

Measuring Single-Cell Proteomics to Characterize Immune Cells in Kidney Transplant Recipients

Immune cell cytokines are a critical component in the pathophysiology surrounding immune rejection during organ transplantation. Secreted cytokines control multiple aspects of the immune system and facilitate effector, chemoattractive, stimulatory, regulatory, and inflammatory

functions. In this study, researchers chose to focus on CD8+ T cells isolated from the blood of kidney transplant (KT) recipients (Figure 1)[3]. Analysis of kidney biopsies were used to divide patients into two groups – normal biopsy versus biopsy-proven humoral rejection. CD8+ cells from the two cohorts were stimulated with plate bound anti-CD3 and soluble anti-CD28 for 24 hours to mimic physiological TCR activation. Cells were then loaded into Bruker Single-Cell Secretome Adaptive Immune IsoCode Chips that were processed using Bruker's automated system.

The IsoCode Chip design facilitates the isolation of single cells into microchambers that each contain a 32-plex proteomic barcode array to detect cytokine secretions from single cells. Bruker's platform performs automated incubation, ELISA workflows, and imaging on the chips to obtain single-cell proteomic data. Data is then imported into the IsoSpeak software suite to generate push-button advanced metrics and visualizations.

Using Polyfunctionality and Polyfunctional Strength Index to Differentiate Organ Transplant Recipient Response

The Bruker platform provides multiple metrics to characterize functional single-cell protein secretions. Percent polyfunctionality shows the percentage of cells capable of secreting significant quantities of two or more cytokines. Multiple studies have established that these polyfunctional cells exert significant physiological effects that impact *in vitro* and *in vivo* outcomes [4, 5]. In this

Revealing Potential Mechanisms for Transplant Rejection with Single-Cell Functional Proteomics

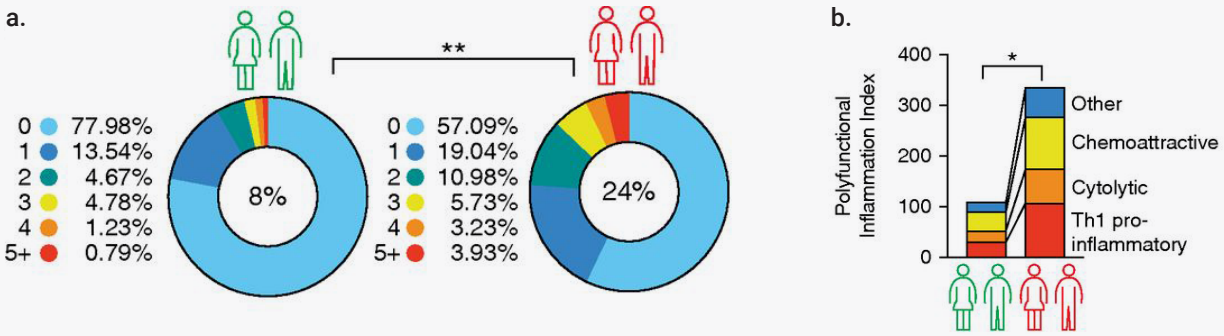

Figure 2 | a) KTs with humoral rejection had higher percentages of polyfunctional CD8+ cells than kidney transplant recipients with a normal biopsy. Comparative frequencies were 24% versus 8%, P=0.008, for humoral rejection versus normal cohorts, respectively. This suggests that polyfunctional cells are statistically associated with a higher risk of humoral rejection. b) CD8+ cells from KTs with humoral rejection displayed higher PSI than KTs with normal biopsy. PSI accounts for percent polyfunctional cells and cytokine secretion intensity to describe the overall functional potency of samples. Higher PSI indicates a greater degree of polyfunctional cytokine secretion frequency and intensity. Statistical analysis showed that CD8+ cells from KTs with humoral rejection had statistically higher PSI than CD8+ cells from normal biopsy KTs (P = 0.02). Further examination reveals that functional cytokine groups such as Th1 proinflammatory cytokines, cytolytic cytokines, and chemoattractive cytokines are all higher in humoral rejection KTs. (Figure published as Polyfunctional Strength Index equivilent, Polyfunctional Inflammation Index)

study, high CD8+ percent polyfunctionality was directly associated with humoral response KT recipients (Figure 2a)[3]. This suggests that the presence of polyfunctional cells contributes to humoral rejection by enabling increased levels of functional cytokine secretion.

The Polyfunctional Strength Index (PSI) is another powerful Bruker metric used to characterize single-cell protein secretion. PSI is calculated by multiplying percent polyfunctionality with cytokine signal intensity to provide an overall measurement of polyfunctional activity. As such, PSI is a valuable metric that summarizes multidimensional single cell data into a single, easily interpreted metric. It has shown value as a statistically significant biomarker in multiple research fields such as cancer immunotherapy, autoimmunity, and infectious diseases[6-8]. Data from this study shows that the humoral response cohort has higher CD8+ PSIs (Figure 2b)[3]. This further links polyfunctional cell activity to humoral rejection.

These results establish CD8+ percent polyfunctionality and PSI as valuable metrics associated with KT clinical outcomes. This paves the way for future single-cell research into other cell types involved in immune rejection such as CD4+ T cells, B cells, macrophages, NK cells, and endothelial cells. Using Bruker single-cell proteomic technology provides added dimensionality that can be used to further characterize cellular immune responses in organ transplantation.

Functional Differences in Cytokine Expression in Humoral Rejection

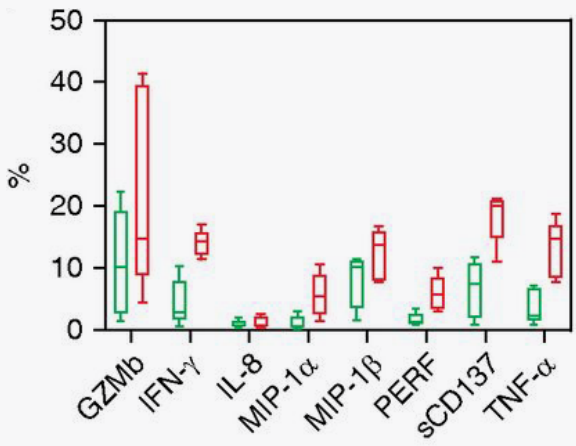

Figure 3 | Individual cytokine breakdowns show that humoral rejection KT CD8+ cells (red) secreted cytokines at greater frequencies than normal biopsy KT CD8+ cells (green). Proinflammatory cytokines (IFN-g, TNF-a, MIP-1a, and MIP-1b) and cytotoxic molecules (GZMb, PERF) are the individual cytokines responsible for a higher frequency of polyfunctional cells and elevated PSI in humoral rejection KT CD8+ cells.

Identifying the Specific Cytokines Involved in Humoral Rejection

Polyfunctionality and PSI provide aggregate measurements involving up to 32 cytokines simultaneously. However, IsoSpeak software also allows users to break down these metrics into values for individual cytokines to reveal individual proteins and explore mechanisms. This was applied to identify the cytokine culprits for humoral rejection (Figure 3)[3]. Results showed the elevation of multiple cytokines involved in proinflammation and cytotoxicity. The specific cytokines elevated in humoral response CD8+ cells suggested the involvement of effector memory CD8+ T cells that re-express CD45RA (TEMRA CD8+ T cells). This led the authors to formulate further hypotheses and experiments to test TEMRA CD8+ cells. Results established that TEMRA CD8+ T cells are dysregulated in KT recipients with humoral rejection. As such, utilizing inhibitors to reduce the migration and activation TEMRA CD8+ T cells may improve outcomes for organ transplant recipients.

Conclusion

Single cell proteomics data provides a powerful way to reveal single-cell immune cell functionality in the field of organ transplantation to investigate transplant rejection and mitigation strategies.

- Polyfunctional cells are important drivers of humoral response in transplant recipients
- PSI measurements reflect functional cytokine secretion strength and serve as a useful metric to characterize immune response to transplantation
- Identifying upregulated cytokines in cells from patients experiencing humoral response can reveal the mechanistic drivers behind immune rejection
- Comparisons between cells from recipients experiencing humoral response versus normal response can reveal specific cytokines that are drivers of immune rejection
- Exploring single cell proteomics in the context of organ transplantation can generate useful hypotheses for mechanistic understanding, patient stratification, patient monitoring, and therapy development

References

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