Circulating Monocytes as a Predictor of Cancer Risk in Long-Term Renal Transplant Recipients

End of Grant Report (HJR01700)

Thanks to the generous support of the trustees of the Oxford Transplant Foundation, significant progress on the above project has been made over the course of the grant. This has led to exciting findings which will inform further funding applications.

We previously found that reduced monocytic HLA-DR density (mHLD-DRd) in renal transplant recipients (RTR) was associated with increased risk of developing cancer in the subsequent twelve months. The purpose of this additional work was to delineate the underlying mechanisms associated with this.

A flow cytometry panel was developed to further characterise changes in circulating monocyte phenotype associated with reduced monocytic HLA-DR density. Decreasing mHLA-DRd was associated with accumulation of monocytic myeloid-derived suppressor like cells ($CD19^-CD56^-CD3^-CD14^+CD11b^+CD33^+HLA\text{-}DR^{lo}$) both as a proportion of monocytes and absolute cell count (Figure 1).

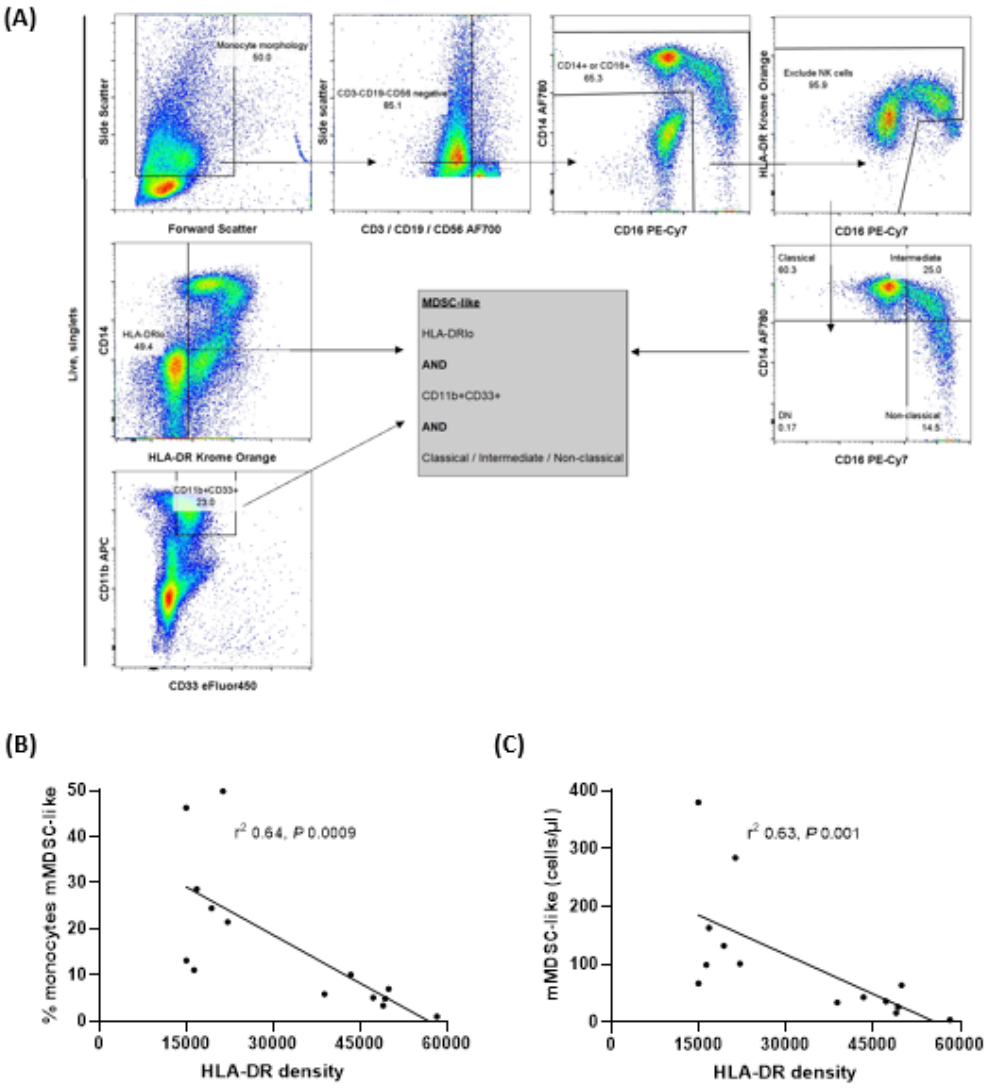

Figure 1: mHLA-DRd correlates inversely with $CD19^-CD3^-CD56^-CD14^{+/int}CD11b^+CD33^+HLA\text{-}DR^{lo}$ monocyte myeloid-derived suppressor-like cell accumulation. *(A) Example of gating strategy to delineate monocyte subpopulations. Correlation between mHLA-DRd and (B) proportion of mMDSC-like cells within the monocyte population and (C) absolute number of mMDSC-like cells. Spearman's test was used to assess goodness-of-fit (r^2) and significance.*

We found a weak correlation between monocytic HLA-DR and expression of CD86, a major costimulatory ligand (Figure 2A). Decreasing mHLA-DRd was associated with a shift from the classical ($CD14^{hi}CD16^{-}$) monocyte subset to the non-classical ($CD14^{+}CD16^{hi}$) monocyte subtype (Figure 2B). These monocytes are associated with distinct behaviours, with classical monocytes associated with a traditionally pro-inflammatory role and non-classical monocytes associated with tissue repair and resolution of inflammation. Whilst we found prednisolone use was independently associated with a reduction in mHLA-DRd (data not shown), there was no significant difference in monocyte subsets when RTR were stratified by prednisolone use as part of their immunosuppressive regime (Figure 2C).

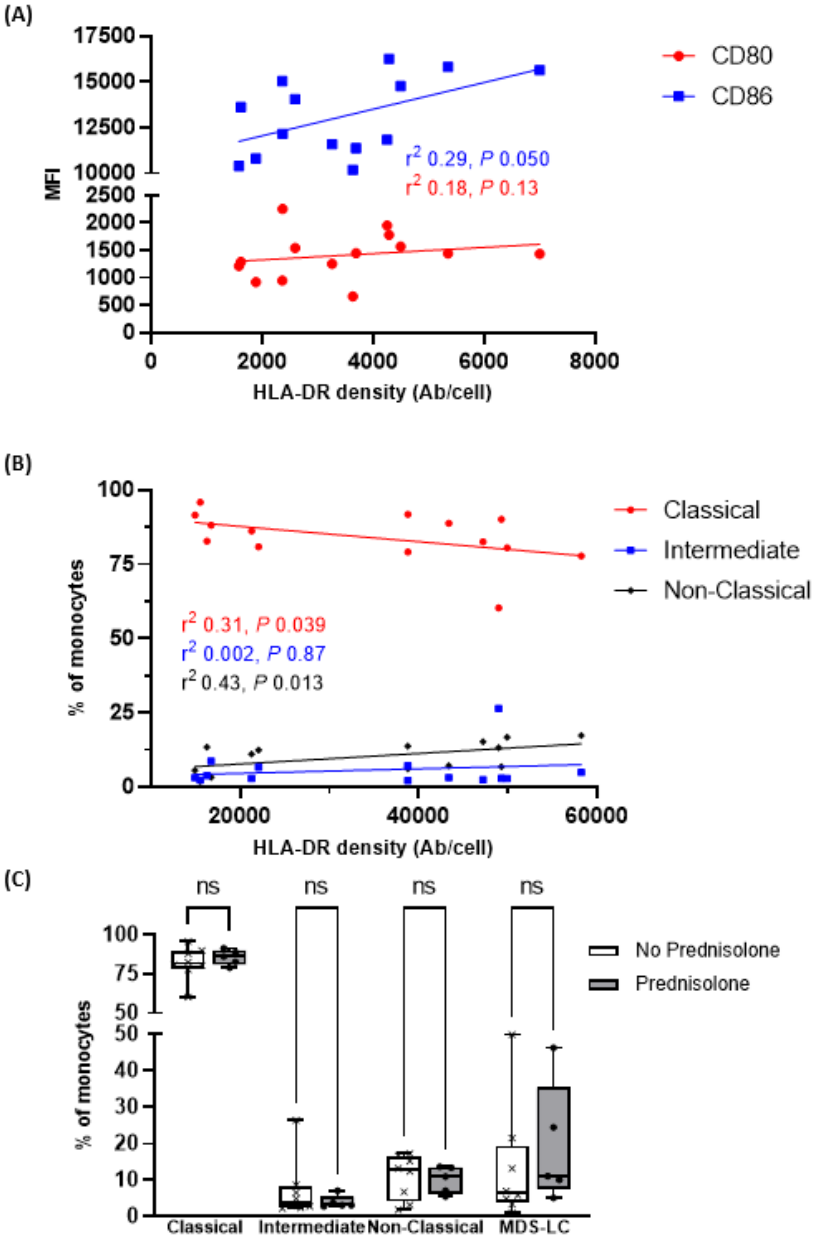

Figure 2: Correlation of mHLA-DRd and steroid use with monocyte phenotype. *(A) Correlation of mHLA-DRd with co-stimulatory receptor expression; (B) Correlation of major monocyte subpopulations with mHLA-DRd; (C) major monocyte populations stratified by corticosteroid (prednisolone) use at time of sampling. Correlations and significance are assessed using Spearman's test (n=14) for (A) and (B), whilst (C) used Mann-Witney testing of each monocyte population.*

An attempt to develop an *in vitro* assay to characterise changes in circulating monocyte response to stimulation in RTR with low and high mHLA-DRd was unsuccessful due to the constitutive expression of inflammatory cytokines at rest in these populations.

We next assessed whether these phenotypic changes corresponded with changes in resting monocyte gene expression. Monocytes from 12 RTR, matched for age and steroid use at sampling, were enriched from peripheral blood mononuclear cells (PBMC) by negative selection. Total RNA was extracted and bulk gene expression was evaluated using the 730-gene 'Human Myeloid Immunity' panel on the Nanostring nCounter platform.

Differential gene expression analysis demonstrated no significant changes in expression after correction for multiple testing. More subtle changes in gene expression were then assessed using gene set enrichment analysis using the Gene Ontology knowledgebase, which allows for detection of small but coordinated changes in expression suggestive of perturbation of cell processes or pathways (Figure 3). Monocytes from RTR demonstrating low mHLA-DRd exhibited suppression of gene sets relating to inflammatory response, chemokine receptor and cytokine signalling (Figure 3).

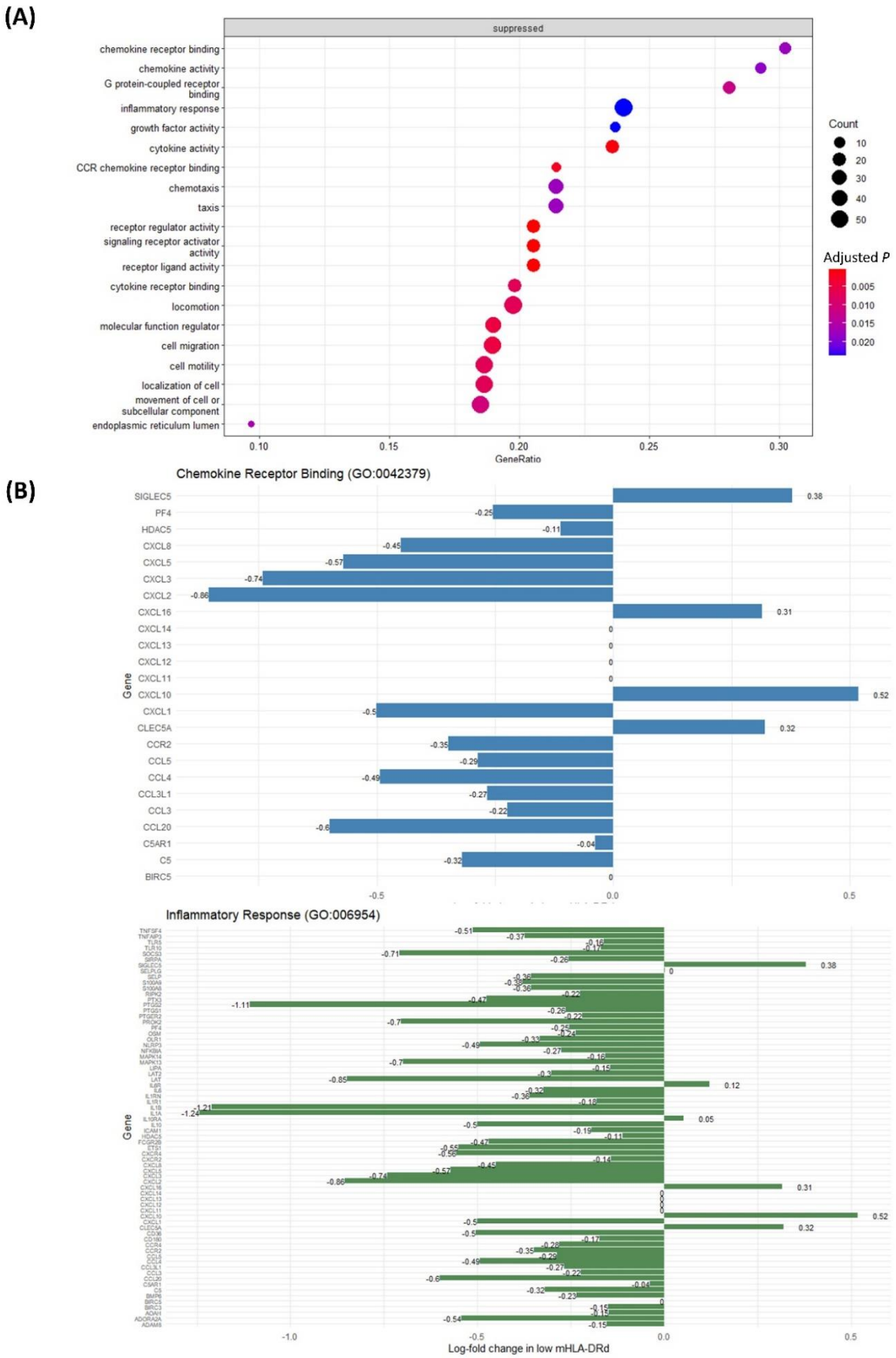

Figure 3: Monocytes from RTR with low mHLA-DRd exhibit suppression of gene sets relating to inflammatory response and chemotaxis. *(A) Pathways with significantly altered enrichment in RTR exhibiting low mHLA-DRd, using the Gene Ontology knowledge base. Pathways are listed in order of descending GeneRatio. (B) Differential expression of core genes in 'Chemokine receptor binding' and 'Inflammatory response' Gene Ontology sets.*

Conclusion and next steps

Taken together, the data elicited using the OTF grant has facilitated detailed examination of phenotypic and trancriptomic alterations associated with reduced monocytic HLA-DR density. We found reduced mHLA-DRd is a distinct immunological marker that reflects the accumulation of mMDSC-like cells within the monocyte population and dampening of pathways relating to inflammation, cytokine signalling and chemotaxis. This is clinically relevant in that it is associated with the subsequent development of malignancy.

This data generated through this grant is currently under peer review at 'Frontiers in Immunology' and is publicly available on a pre-print server, MedRxiv (https://www.medrxiv.org/content/10.1101/2022.03.23.22272699v1).

Our findings will drive further study into the mechanisms underlying this association with cancer development, which may reveal novel avenues for therapeutic manipulation. Our next step will be to evaluate how tumour-infiltrating macrophage phenotype changes with reduced mHLA-DRd.

We would like to reiterate our gratitude to the Oxford Transplant Foundation for its generous support of the work summarised here.