\subsection{Mass cytometry reveals protein changes consistent with chromatin accessibility and gene expression in PsA CD14$^+$ monocytes.}

To determine the effect of chromatin accessibility and genes expression differences in CD14$^+$ monocytes between SF and PB at the protein level mass cytometry was conducted in collaboration with Dr Hussein Al-Mossawi and Dr Nicole Yager. For those samples with paired ATAC and/or qPCR expression data, intra-cellular staining for relevant cytokines was performed before and after treatment with BFA, which blocks cytokine release and enables identification of cells actively producing cytokines in absence of additional inflammatory stimuli (see Chapter \ref{ch:Mat}). Due to technical problems, only a limited number of intra-cellular cytokine staining passed QC, being TNF-$\alpha$ amongst the more relevant ones.

Mass cytometry expression of CD14$^+$ versus TNF-$\alpha$ demonstrated greater intensity of TNF$\alpha$ staining after 6h of BFA treatment in SF (Figure \ref{figure:PsA\_monocytes\_percentage\_TNFa} a blue dots bottom panel) when compared to PB CD14$^+$ monocytes in all three PsA patients (Figure \ref{figure:PsA\_monocytes\_percentage\_TNFa} a blue dots top panel). Furthermore, the percentage of TNF-$\alpha$ producing cells in each tissue was quantified as the difference of TNF-$\alpha^+$ cells before and after BFA treatment for 6h (6h minus 0h). In PB, the resulting percentage of CD14$^+$ monocytes TNF-$\alpha^+$ did not surpass the 1\%, indicating a very low increment of TNF$\alpha$ producing cells after BFA treatment. Conversely, SF CD14$^+$ cells showed a larger percentage of cells actively producing TNF-$\alpha$, ranging between 1.5 and 11.8\% (Figure \ref{figure:PsA\_monocytes\_percentage\_TNFa} b). This observation was consistent with increased chromatin accessibility nearby genes of the NF-$\kappa$B signalling pathway as well as with increased expression of a number of genes in the TLR and NOD-like signalling in SF compared to PB, which were hypothesised to result into enhanced NF$\kappa$B activation and cytokine production. Nevertheless, this trend of increased CD14$^+$ cells producing TNF-$\alpha$ in SF compared to PB did not reach significance (pval=0.25), likely due to the small sample size.

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\includegraphics[width=\textwidth]{./Results3/pdfs/PSA\_0h\_6h\_BFA\_TNFa\_mass\_cytometry\_PSA1718\_PSA1719\_PSA1607}

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\includegraphics[width=\textwidth]{./Results3/pdfs/CyTOF\_PSA1607\_PSA1718\_PSA1719\_TNFa\_percentage}

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\caption[Comparison of TNF-$\alpha$ expression in SF and PB CD14$^+$ monocytes before and after protein transport blockade with BFA using mass cytometry.]{\textbf{Comparison of TNF-$\alpha$ expression by SF and PB CD14$^+$ monocytes before and after protein transport blockade with BFA using mass cytometry.} a) For each of the three PsA patients, representation of CD14$^+$ (y-axis) versus TNF-$\alpha$ (x-axis) intensity of expression in matched SF and PB without protein transport blockade (blue dots) or after 6h treatment with BFA (orange dots). b) Percentage of CD14$^+$ monocytes producing TNF-$\alpha$ in SF and PB.For each tissue and sample shown in a, this percentage is calculated as the increment in cells producing TNF-$\alpha$ before and after protein transport blockade with BFA (6h minus 0h).}

\label{figure:PsA\_monocytes\_percentage\_TNFa}

\end{figure}

In order to validate this observation and also assess other cytokines of interest, mass cytometry was conducted in PB and SF from another ten PsA patients. This validation cohort included the patients for which scRNA-seq data was presented in this chapter (Table \ref{tab:PSA\_datasets\_per\_sample}). As previously, percentage of CD14$^+$ monocytes producing TNF-$\alpha$ , MCP-1 (protein product of \textit{CCL2} and osteopontin (protein product of \textit{SPP1}) was computed as the difference between percentage of cells producing each of the cytokines before and after BFA treatment. The percentage of CD14$^+$ monocytes producing TNF-$\alpha$ was greater in SF compared to PB (pval=0.048) (Figure \ref{figure:CyTOF\_cytokines\_validation\_cohort} a), reinforcing the results from the previous cohort. Likewise, a larger percentage of CD14$^+$ monocytes producing osteopontin and MCP-1 (pval=0.001 and pval=0.003, respectively) were also observed in SF compared to PB (Figure \ref{figure:CyTOF\_cytokines\_validation\_cohort} b and c), in line with the up-regulated expression of these two genes in SF compared to PB in bulk CD14$^+$ monocytes and in the CC-mixed scRNA-seq cluster. The percentage of cells producing cytokines in SF was particularly moderate for TNF-$\alpha$ and MCP-1, not exceeding of 1.8 and 3, respectively, for the majority of the PsA samples (Figure \ref{figure:CyTOF\_cytokines\_validation\_cohort} a and c). However, one of the patients (PSA1505) appeared to have particularly higher percentage of SF CD14$^+$ monocytes producing all three cytokines when compared to the rest of the patients in the cohort. Although no justification was found to remove this sample, the statistical significance (pval$<$0.05) in the differences found between percentage of cell producing TNF-$\alpha$, MCP-1 and osteopontin in SF versus PB remained when repeating the analysis in absence of PSA1505.

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\includegraphics[width=\textwidth]{./Results3/pdfs/CyTOF\_validation\_cohort\_TNFa\_percentage}%

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\includegraphics[width=\textwidth]{./Results3/pdfs/CyTOF\_validation\_cohort\_osteopontin\_percentage}

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\includegraphics[width=\textwidth]{./Results3/pdfs/CyTOF\_validation\_cohort\_CCL2\_zoom\_in\_percentage}

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\caption[Percentage of CD14$^+$ monocytes producing TNF-$\alpha$, osteopontin and MCP-1 in SF and PB in a validation cohort of ten PsA samples.]{\textbf{Percentage of CD14$^+$ monocytes producing TNF-$\alpha$, osteopontin and MCP-1 in SF and PB in a validation cohort of ten PsA samples.} The percentage of CD14$^+$ monocytes producing a) TNF-$\alpha$, b) osteopontin and c) MCP-1 in SF and PB are shown for each of the ten samples in a PsA cohort used to validate cytokine production by mass cytometry. In each sample and tissue, this percentage is calculated as the increment in cells producing the relevant cytokine before and after protein transport blockade with BFA (6h minus 0h). In c), a zoom in for the percentage of CD14$^+$ monocytes producing MCP-1 in all patients minus PSA1505 is included for further detail on the differences across SF and PB for these samples.}

\label{figure:CyTOF\_cytokines\_validation\_cohort}

\end{figure}

\subsubsection{\textit{CCL2}-\textit{CCR2} signalling: an example of multi-omics correlation}

The differences in percentage of CD14$^+$ monocytes producing MCP-1 between SF and PB represent an example of a putative correlation between changes observed in the chromatin accessibility landscape, bulk and scRNA-seq expression and protein production. Differential chromatin accessibility analysis between SF and PB identified a statistically significant cell type-specific SF open DAR upstream \textit{CCL2} gene (Figure \ref{figure:PsA\_10X\_qPCR\_ATAC\_CD14\_CCL2}). This SF open DAR region is annotated as enhancer according to Epigenome Roadmap chromatin segmentation and overlaps a eRNA reported by FANTOM5 in CD14$^+$ monocytes. The expression of \textit{CCL2} was shown to be significantly modulated (pval$<$0.05 and abs(mean FC)$>$1.5) between SF and PB by qPCR in CD14$^+$ monocytes only, whereas no significant changes were observed for mCD4$^+$ and mCD8$^+$ in this data from the same patients (Figure \ref{figure:PSA\_PCR\_array\_vulcano\_plots} a, b, c and Table \ref{tab:PSA\_gene\_expression\_ATAC\_overlap}). Up-regulation of \textit{CCL2} was not found in PB CD14$^+$ monocytes compared to PsA patients and healthy controls, being defined as one of the tissue-specific genes in the previous analysis (Figure \ref{figure:figure:PSA\_PCR\_array\_HC\_FC\_correlation} a). Furthermore, \textit{CCL2} was also identified by scRNA-seq as one of the up-regulated genes in the CC-mixed cluster (Figure \ref{figure:PsA\_scRNAseq\_vulcano\_plots\_mixed\_and\_IL7R\_clusters} a and \ref{figure:PsA\_scRNAseq\_qPCR\_ATAC\_correlation} a and b). Expression of \textit{CCR2}, the receptor for the chemokine MCP-1 (protein product of \textit{CCL2}), appeared up-regulated by qPCR in SF mCD4$^+$ and mCD8$^+$ cells in the same individuals, which could suggest increased chemotaxis driven by CD14$^+$ monocytes and leading to T cell infiltration in the synovium. Interestingly, in this data no significant up-regulation of \textit{CCR2} was observed in PsA PB when compared to healthy controls in any of the three cell types.

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\includegraphics[width=\textwidth]{./Results3/pdfs/ATAC\_PSA\_CD14\_UCSC\_CCL2\_track}

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\caption[Chromatin landscape in CD14$^+$ monocytes upstream the differentially expressed gene \textit{CCL2}.]{\textbf{Chromatin landscape in CD14$^+$ monocytes upstream the differentially expressed gene \textit{CCL2}.} UCSC Genome Browser view illustrating the normalised ATAC read density (y-axis) for two ATAC peaks at the promoter and upstream the \textit{CCL2} gene (x-axis) in SF and PB CD14$^+$ monocytes from three PsA patients. The ATAC peak upstream \textit{CCL2} (black rectangle) appeared as a significant DAR (FDR$<$0.01 and abs(FC)$>$1.5) in the differential analysis, being more more accessible in SF when compared to PB. Tracks are colour-coded by tissue (SF=purple and PB=turquoise). Additional epigenetic tracks of PB isolated CD14$^+$ monocytes from the Epigenome Roadmap, including chromatin segmentation map and the enhancer marks H3Kme1 and H3K27ac, are also included.}

\label{figure:PsA\_10X\_qPCR\_ATAC\_CD14\_CCL2}

\end{figure}

In addition to the mass cytometry data, collaborators in Basel have performed measurements of cytokine levels in PB and SF from same ten patients in the validation cohort. High levels of MCP-1 (approximately 1,000 pg/mL) were also reported in SF whereas this cytokine was below the lower detection level in the assay in plasma, supporting additional evidence of \textit{CCL2} up-regulated expression at the protein level (Figure \ref{figure:ELISA\_SF\_PsA}). Overall, the data here presented suggests a tissue and cell type specific dysregulation of \textit{CCL2} expression in the CC-mixed CD14$^+$ monocytes cluster that may be related to alterations in the chromatin accessibility of an enhancer in the proximity to this gene.