Distinct but Overlapping Sets of Gene Expression Programs Drive Macrophage and Dendritic Cell Differentiation

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1A) Introduction and Objectives

- Macrophages and dendritic cells (DCs) share a myeloid progenitor cell origin and are involved in various immune responses, but the differences in their differentiation patterns are not fully understood
- Defects in this process can lead to infections and immune disorders [1]
- The objectives of this study are to:
  - Identify the genes and functions involved in myeloid differentiation in response to three growth factors: M-CSF, GM-CSF, Flt3L
  - Characterize how the inflammatory context as modeled with different hyper-morphic NFkB mutants [2, 3] affects differentiation trajectories

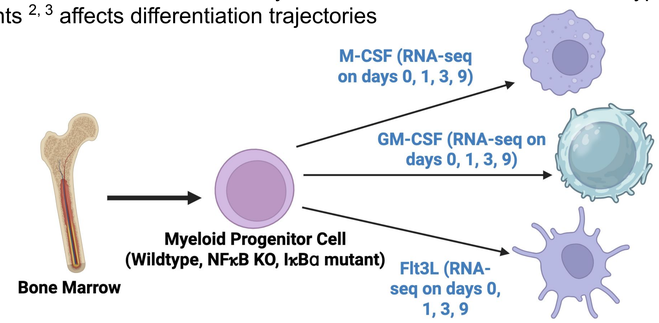

Figure 1A. Myeloid differentiation from bone marrow progenitor cells grown in the presence of one of three growth factors: M-CSF, GM-CSF or Flt3L. To chart the respective differentiation pathways, transcriptomes were profiled at indicated time points by RNA-seq.

1B) Data Analysis Workflow

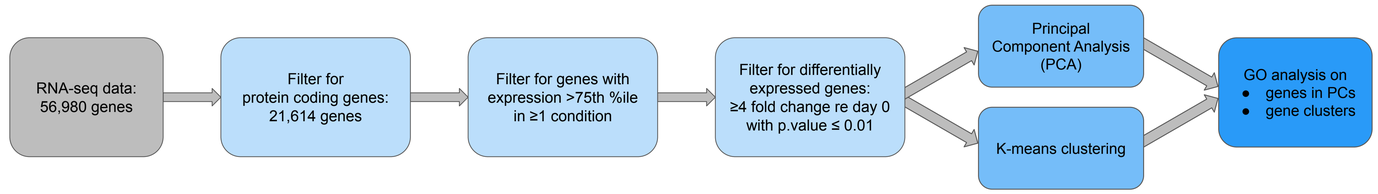

Figure 1B. Workflow for identifying gene programs involved in the differentiation of macrophages and DCs. The same workflow process was repeated when analyzing different growth factors or mutants in isolation, or when analyzing both together.

2) Growth Factors Induce Distinct Differentiation Trajectories

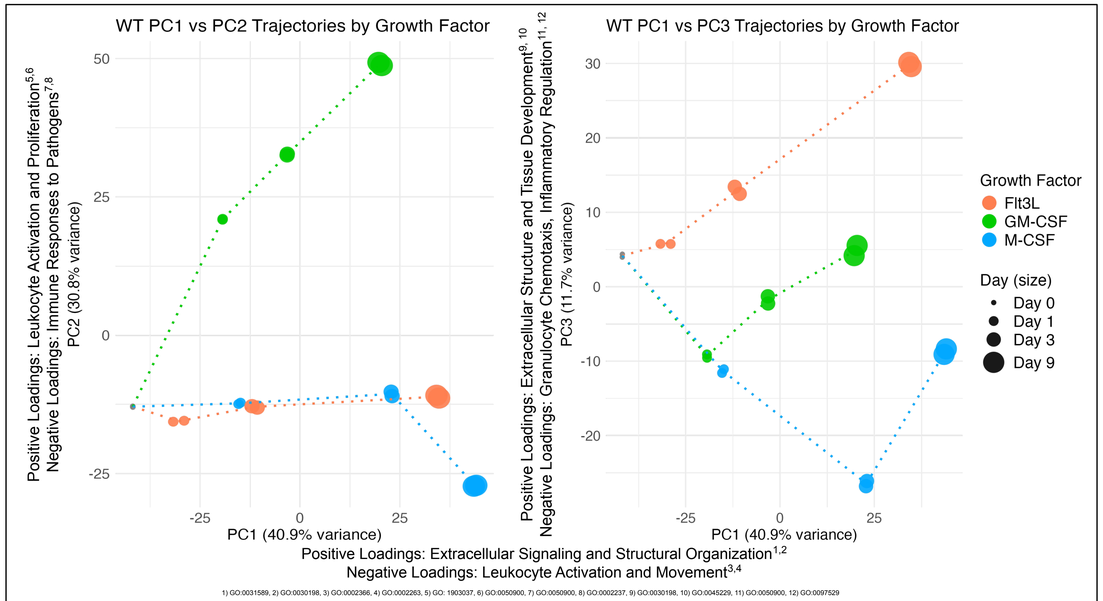

Figure 2A. Principal component analysis (PCA) shows how growth factors determine gene expression programs during WT myeloid progenitor differentiation. PC1 represents a common time-axis, PC2 is GM-CSF-specific, PC3 separates M-CSF from Flt3L.

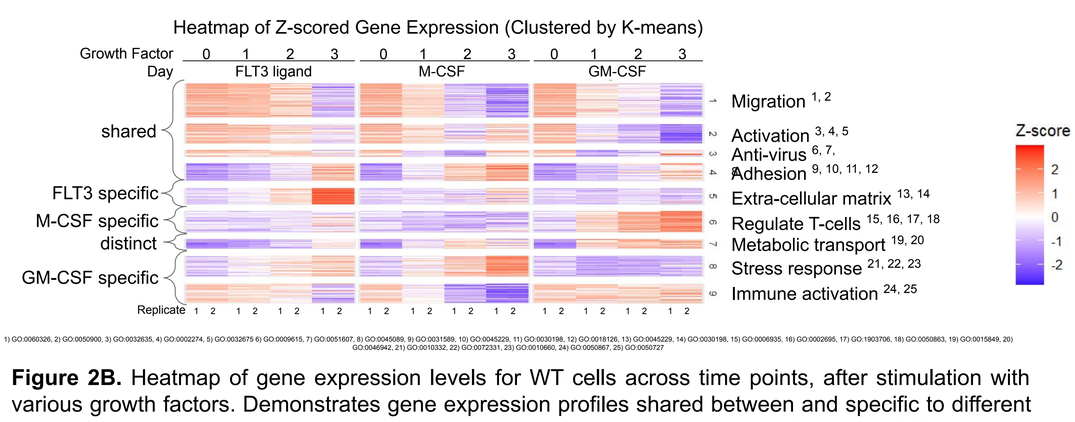

Figure 2B. Heatmap of gene expression levels for WT cells across time points, after stimulation with various growth factors. Demonstrates gene expression profiles shared between and specific to different growth factors. Includes an overview of the clusters' gene functions, and codes for the top GO terms.

3) NFκB Mutations Alter Trajectories by Affecting the Expression Dynamics of Specific Genes

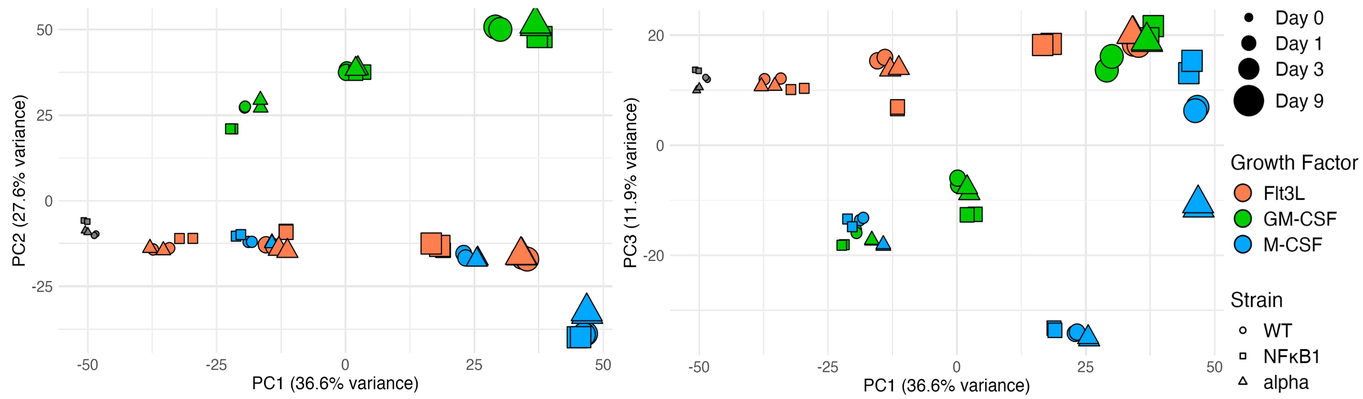

Figure 3A. PCA comparing the first principal component of all genotypes and growth factors to the second and third components, respectively.

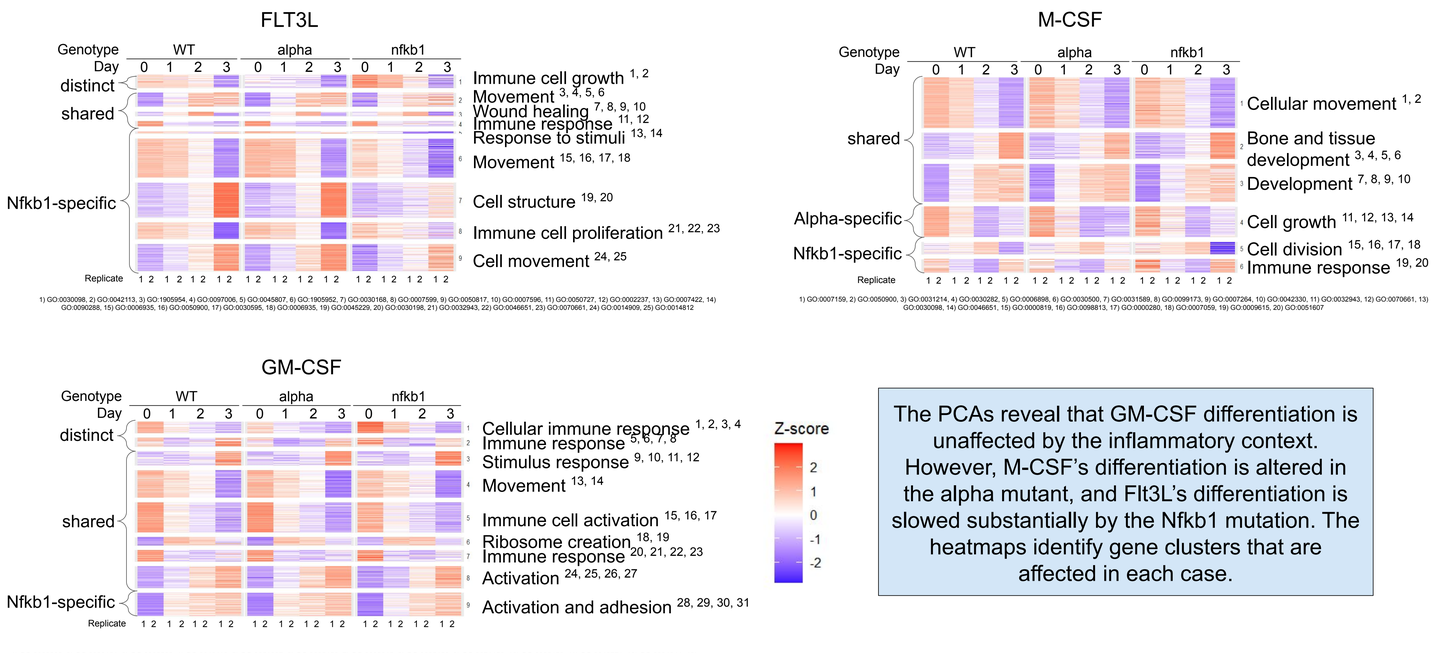

Figure 3B. Heatmaps of K-means clustered genes showing changes in gene expression levels in different mutants after stimulation with various growth factors, over the course of nine days. Shows which clusters have shared, distinct and specific gene expression patterns in different genotypes. Includes an overview of the clusters' gene functions, and codes for the top GO terms.

The PCAs reveal that GM-CSF differentiation is unaffected by the inflammatory context. However, M-CSF's differentiation is altered in the alpha mutant, and Flt3L's differentiation is slowed substantially by the Nfkb1 mutation. The heatmaps identify gene clusters that are affected in each case.

References

[1]Chen, S., Saeed, A.F., Liu, Q. *et al.* Macrophages in immunoregulation and therapeutics. *Sig Transduct Target Ther* 8, 207 (2023). https://doi.org/10.1038/s41392-023-01452-1

[2] Peng B, Ling J, Lee AJ, Wang Z, Chang Z, Jin W, Kang Y, Zhang R, Shim D, Wang H, Fleming JB, Zheng H, Sun SC, Chiao PJ. Defective feedback regulation of NF-kappaB underlies Sjogren's syndrome in mice with mutated kappaB enhancers of the IkappaBalpha promoter. Proc Natl Acad Sci U [3]A. 2010 Aug 24;107(34):15193-8. doi: 10.1073/pnas.1005533107. Epub 2010 Aug 9. PMID: 20696914; PMCID: PMC2930541.

[3] Sha, W.C., Liou, H.-C., Tuomanen, E.I., and Baltimore, D. Targeted disruption of the p50 subunit of NF-κB leads to multifocal defects in immune responses. Cell 80, 321–330 (1995). https://doi.org/10.1016/0092-8674(95)90415-8.[5]