CHARACTERIZATION OF CELLULAR FATES IN EPSTEIN BARR VIRUS-INFECTED CELLS.

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Epstein Barr Virus (EBV) exhibits a particular tropism to B cells where after successful infection can persist mainly in the class-switched memory B cell subsets. Soon after the viral entry, host immune responses are generated to restrict the viral progression. To counteract these antiviral defenses, EBV deploys in a sequential manner different expression programs hijacking and rewiring host cell processes to achieve a sustained latency infection. In this manner, host and pathogen enter into tit-for-tat dynamics generating a broad spectrum of cell fates. In vitro, the infection of primary B cells leads to immortalization although this process is strikingly inefficient. Our group leveraged the power of single-cell Omics to dissect the complexity of EBV-host interaction at early stages of infection leading to a characterization of distinct cellular fates. This model captured time-resolved clusters of cells previously characterized [1]but also new undescribed groups of EBV+ infected cells at early stages of infection. As validation of these results, we performed a screening of the top hits of each cluster, through a Cas9-RNP approach to knock out target genes. Using a non-essential gene for EBV infected cells as a proxy [2], and normalized FACS cell count as readout, we were able to identify genes whose loss of function could result in either a growth arrest or overgrowth and that need further analysis. It is also noteworthy that two late clusters reflect a continuum between NF-kB activation and B-cell differentiation previously described in LCLs [3]. We set out to characterize the dynamics that exist between these activated and differentiated subsets. Using ICAM-1 and CD27 as markers for activation and differentiation states, respectively, we could resemble by FACS, what was observed in the single-cell analysis. To understand the physiological relevance of each cell subpopulation, the three major subpopulations, ICAMhiCD27lo, ICAMloCD27lo, and ICAMloCD27hi, were sorted out and tracked down for several days. We observed that the ICAM1hi/CD27lo population grew most robustly, the intermediate population grew out more slowly, and the ICAM1lo/CD27hi population did not proliferate much at all after sorting. In all cases, soon after the sorting, each subpopulation trended to return to the parental distribution. Together, we are gaining valuable insights into the molecular circuitry underlying cell fate decisions that could be exploited to find a plausible therapy for EBV+ B cell lymphomas.

1. McFadden, K., et al., (2016) *Metabolic stress is a barrier to Epstein-Barr virus-mediated B-cell immortalization.* Proc Natl Acad Sci U S A. **113**(6): p. E782-90.
2. Akidil, E., et al., (2021) *Highly efficient CRISPR-Cas9-mediated gene knockout in primary human B cells for functional genetic studies of Epstein-Barr virus infection.* PLoS Pathog. **17**(4): p. e1009117.
3. SoRelle, E.D., et al., (2021) *Single-cell RNA-seq reveals transcriptomic heterogeneity mediated by host-pathogen dynamics in lymphoblastoid cell lines.* Elife. **10**.