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T-CELL ACTIVATION

Variation is the spice of life

Reliable but variable T-cell responses to antigenic stimulation within a clonal population are necessary to allow for the development of both effector and memory T-cell subsets from that population. But how is this diversity generated? Could stochastic expression of signalling proteins within the population provide this necessary variability? By combining computer modelling and single-cell analysis, Altan-Bonnet and colleagues now show that variability in the basal expression of CD8 and the phosphatase SHP1 (SRC-homology-2-domain-containing protein tyrosine phosphatase 1) within a clonal T-cell population provides phenotypic variability in a controlled manner.

The authors examined the expression levels of CD8 and SHP1 in single T-cell receptor (TCR)-transgenic $CD8^+$ T cells. They found that these proteins had a large range of expression levels across the population, with CD8 expression levels ranging by threefold and SHP1 by sixfold. Together with the predictions of the computer model, this suggested that the population would not respond homogeneously to antigenic stimulation.

The authors then analysed protein expression and TCR activation by multiparameter flow cytometry. In results that reflected the model predictions, they found that cells that expressed higher levels of CD8 molecules could be activated by fewer antigenic ligands. However, the percentage of T cells that could respond to the maximum number of ligands remained the same. This indicated that CD8 is an analogue regulator — that is, a signalling protein that fine-tunes the activation threshold.

By contrast, when the concentration of SHP1 within the population exceeded a certain threshold, the number of cells that could respond to the ligand decreased. However, the minimum number of ligands that were required for T-cell activation did not change. This indicated that SHP1 acts as a negative digital regulator — that is, a signalling protein that regulates cell responsiveness. So, the data show that stochastic expression levels of CD8 and SHP1 generate considerable phenotypic variability within a clonal T-cell population.

But T-cell responses also need to be uniform, so how is the phenotypic variability constrained? The authors found that the expression of CD8 and SHP1 is co-regulated. Therefore, any increase in SHP1 expression (which would decrease the ability of the cell to respond to its ligand) is accompanied by an increase in CD8 expression (which would decrease the number of ligands that are required for activation), thereby limiting the phenotypic variability and potentially limiting the risk of self responsiveness and autoimmune activation.

So, by combining computer modelling and multiparameter flow cytometry analysis of single cells, this study explains how stochastic expression of signalling proteins in T cells can generate controlled phenotypic variability. In addition, this study shows that the analysis of expression variability of signalling proteins can be used to study heterogeneity in signalling responses and to identify key regulators of signalling cascades.

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ORIGINAL RESEARCH PAPER Feinerman, O. *et al.* Variability and robustness in T cell activation from regulated heterogeneity in protein levels. *Science* **321**, 1081–1084 (2008)