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REGULATORY T CELLS

Suppressing antibody production

T follicular regulatory cells (T_{FR} cells) are a recently described subset of regulatory T cells that specifically inhibits antibody production within germinal centres (GCs). Reporting in *Nature Immunology*, Sharpe and colleagues provide new insights into how T_{FR} cells exert this suppressive effect.

The GC reaction is a highly regulated process that involves the interaction of T follicular helper cells (T_{FH} cells) with cognate B cells, during which T_{FH} cells provide key signals — such as through interleukin-4 (IL-4), IL-21 and costimulatory molecules — for robust B cell activation, while the B cells provide antigenic and costimulatory signals to the T_{FH} cells. This reciprocal interaction drives class-switch recombination (CSR), somatic hypermutation and antibody production by the B cells. It is known that T_{FR} cells inhibit cytokine production by T_{FH} cells and inhibit CSR and antibody production by B cells, but the underlying mechanisms of this suppression are unknown.

To investigate how T_{FR} cells inhibit the GC reaction, the authors developed an *in vitro* suppression assay in which cognate T_{FH} cells and B cells are cultured in the presence (suppressed cultures) or absence (activated cultures) of T_{FR} cells. Although the early activation of B cells was unaffected, the addition of T_{FR} cells resulted in reduced CSR and antibody production, and this suppression occurred in a cell contact-dependent manner. These data, combined with imaging studies, suggest that T_{FR} cells physically disrupt the cognate interaction between T_{FH} cells and B cells during the GC reaction.

Next, the authors performed RNA-sequencing analysis on cells isolated from the activated and suppressed cultures. The expression of essential lineage transcription factors was unaffected, whereas the expression of the effector molecules *Il4* and *Il21* was markedly reduced in T_{FH} cells from the suppressed cultures compared with T_{FH} cells from the activated cultures. Similarly, suppressed B cells retained their GC B cell transcriptional signature but showed reduced expression of genes encoding specific effector molecules. In both cell types the suppression state did not strongly resemble anergy or exhaustion.

Further investigation showed that the expression of genes associated with numerous metabolic pathways, including glycolysis, serine biosynthesis, purine metabolism, one-carbon metabolism and the tricarboxylic acid cycle, was greatly reduced in suppressed B cells. Inhibition of glycolysis or purine metabolism in activated B cells robustly suppressed antibody production, which suggests that inhibiting these pathways can recapitulate the suppression of antibody production by T_{FR} cells.

The culture of B cells from suppressed cultures in a second culture with just T_{FH} cells showed that the B cells retained their defects in CSR, antibody production and metabolism, which suggests that B cell suppression can persist in the absence of T_{FR} cells. These B cells showed evidence of chromatin inaccessibility at genes encoding products important for B cell function, which may explain, at least in part, the durable suppressive effect of T_{FR} cells on GC B cells.

Importantly, the addition of IL-21 to the suppressed cultures blocked the suppression of B cell antibody production and metabolism by T_{FR} cells. This 'rescuing' effect of IL-21 in suppressed cultures was mediated in part by enhancing glycolysis in B cells, and the restoration of CSR and antibody production required signalling through the IL-21 receptor on B cells. Additional data suggested that IL-21 might also be able to alter T_{FR} cell metabolism and thereby reduce the suppressive capacity of this regulatory subset.

Together, these data show that T_{FR} cells induce a distinct suppressive state in T_{FH} cells and B cells to inhibit antibody production. This T_{FR} cell-mediated suppressive effect can be overcome by exogenous IL-21, providing a possible mechanism to circumvent suppression by T_{FR} cells and modulate antibody production *in vivo*.

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"This 'rescuing' effect of IL-21 in suppressed cultures was mediated in part by enhancing glycolysis in B cells"

ORIGINAL ARTICLE Sage, P. T. *et al.* Suppression by T_{FR} cells leads to durable and selective inhibition of B cell effector function. *Nat. Immunol.* http://dx.doi.org/10.1038/ni.3578 (2016)
FURTHER READING Qi, H. T follicular helper cells in space-time. *Nat. Rev. Immunol.* **16**, 612–625 (2016)