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B CELL MEMORY

A second chance for antibodies

> during recall responses, germinal centres are associated with ongoing diversification of the antibody repertoire

An effective antibody response to secondary infection involves both long-lived plasma cells and memory B cells. As well as providing a quantitative back-up for antibody production by plasma cells, memory B cells have a progressive increase in the quality of antibody production during recall responses in terms of antigen-binding affinity. This study used single-cell analysis of antigen-specific B cells in primed mice to confirm that class-switched memory B cells can re-enter the germinal centre reaction to further diversify the B cell receptor (BCR) repertoire.

Using prime–boost immunization with 4-hydroxy-3-nitrophenylacetyl and keyhole limpet haemocyanin (NP–KLH), there was robust formation of germinal centre structures containing follicular dendritic cells, T follicular helper cells and class-switched antigen-specific B cells after the boost in both tissue sections and draining lymph nodes. The polyclonal relatedness of responding antigen-specific B cells from the prime and from the boost was compared by near-neighbour sequence alignment, with the conclusion that large amounts of BCR diversification occur in class-switched memory B cells. Thus, during recall responses, germinal centres are associated with ongoing diversification of the antibody repertoire. This secondary antibody diversification response did not require the persistence of primary germinal centres. In various adoptive transfer models, the authors showed that class-switched memory B cells can form *de novo* germinal centres during recall responses and that they are more efficient at doing so than IgM^+ memory B cells, which mainly seed the $CD138^+$ plasma cell compartment during secondary responses.

Single-cell transcriptional analysis of antigen-specific B cells showed that a greater frequency of class-switched memory B cells after the boost, compared with before the boost, expressed genes associated with germinal centre location, proliferation, BCR diversification and T cell–B cell contact. This germinal centre-specific transcriptional programme was induced rapidly after the boost. Further analysis of gene expression patterns in individual secondary germinal centre B cells showed that they can be sorted into four stages — indicative of progress through the germinal centre cycle — that were defined by expression of the co-stimulatory molecule CD83 and the DNA polymerase Polη. $CD83^-Polη^-$ (stage 1) and $CD83^+Polη^-$ (stage 2) cells, which lack the Polη-dependent hypermutation machinery, are proposed to be found in the germinal centre light zone. Expression of Polη by $CD83^+Polη^+$ (stage 3) cells suggests that they are dark zone cells with the potential for BCR diversification. Loss of CD83 expression by $CD83^-Polη^+$ (stage 4) cells implies that they have reduced contact with T cells but are still competent for somatic hypermutation in the germinal centre dark zone. The authors described further changes in single-cell gene expression — including in genes encoding the chemokine receptors responsible for movement between the light and dark zones — that were consistent with the cyclic progression of memory B cells through these four germinal centre stages and the re-entry of stage 4 cells into the light zone to re-initiate stage 1.

Clonal analysis of the BCR repertoire of antigen-specific B cells isolated from lymph nodes after the boost showed the presence of subclonal BCR variants that had diversified independently along multiple branches, followed by antigen-mediated selection and further diversification into separate subclonal clusters. The subclonal clusters contained individual cells at all four of the germinal centre transcriptional stages. Thus, the evolution of secondary B cell responses involves repeated cycles of antibody divergence and selection.

In summary, these results show that memory B cells undergo ongoing diversification of class-switched BCRs in secondary germinal centres, which enables a second chance at optimizing the antibody repertoire towards increased antigen binding.

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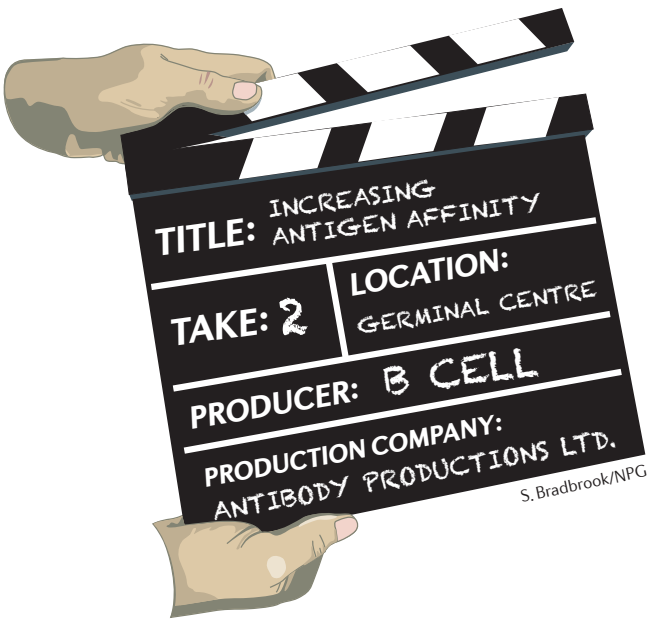

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ORIGINAL RESEARCH PAPER
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