GERMINAL CENTRES

An exit strategy for B cells

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New research published in *Immunity* describes a regulatory checkpoint mediated by CBL proteins that controls the exit of B cells from germinal centres (GCs) to enter the plasma cell pool. This provides a molecular mechanism to explain how GC B cells halt the affinity maturation process, which involves cycling between light and dark zones of the GC. Understanding how the balance between affinity maturation and plasma cell differentiation is regulated has important implications for vaccine strategies to generate high-affinity antibodies.

GC B cells express higher levels of the E3 ubiquitin ligases CBL and CBL-B than naive B cells. Mice lacking both CBL and CBL-B in GC B cells had a severely impaired response to immunization with (4-hydroxy-3-nitrophenyl)acetyl (NP) in terms of the ratio of high-affinity NP-specific IgG1 antibodies to total NP-specific IgG1, which suggests that affinity maturation is impaired in these mice. The proliferation of *Cbl^{-/-}Cblb^{-/-}* GC B cells was not affected. Furthermore, DNA sequencing of GC B cells from immunized mice showed that somatic hypermutation itself is not affected by the *Cbl^{-/-}Cblb^{-/-}* mutation. Instead, wild-type GC B cells undergo stronger selection for high-affinity antibodies than do *Cbl^{-/-}Cblb^{-/-}* B cells. Whereas the ratio of high-affinity NP-specific antibodies to total NP-specific antibodies increased from 65% to 90% in wild-type mice between day 8 and day 14 after immunization, in *Cbl^{-/-}Cblb^{-/-}* mice the ratio only increased from 61% to less than 70%.

Bromodeoxyuridine labelling experiments were used to show that *Cbl^{-/-}Cblb^{-/-}* GC B cells generated 50% more plasma cells than did wild-type B cells during the same time period.

As the mutant mice had fewer GC B cells than wild-type mice, the results suggest that CBL proteins retain B cells in the GC and prevent them from differentiating to plasma cells. Further support for this idea came from inducible ablation of CBL proteins at day 7 after immunization, which resulted in the concomitant decrease in number of high-affinity GC B cells and increase in number of high-affinity plasma cells. *In vitro* stimulation of B cells showed that lack of CBL and CBL-B does not affect CD40- or B cell receptor (BCR)-induced proliferation but increases CD40-induced plasma cell differentiation.

Interferon regulatory factor 4 (IRF4) has a crucial role in plasma cell differentiation by repressing *Bcl6* and promoting *Blimp1* transcription. Wild-type GC B cells had lower levels of IRF4 in the nucleus than *Cbl^{-/-}Cblb^{-/-}* GC B cells. This was shown to be regulated by CBL-mediated ubiquitylation and degradation of IRF4 to promote continued affinity maturation of B cells in the GC, presumably until a high-affinity B cell receives sufficient signal through BCR or CD40 to downregulate CBL expression. GC B cells lacking the ubiquitin ligase activity of CBL proteins or overexpressing IRF4 had an increased propensity for plasma cell differentiation, which promoted exit from the GC and thus decreased the maturation of high-affinity GC B cells. In this manner, CBL proteins ensure that only B cells with sufficiently high affinity for antigen escape from the GC cycle to become plasma cells.

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