IMMUNE RESPONSE

Helper T cells seek long-term partner

During the course of an immune response, antigen-specific $CD4^+$ T helper (T_H) cells are known to pair up first with dendritic cells (DCs) and later with B cells. But now, a third, longer-term partner for T_H cells has been described.

In confocal microscopy studies of mouse spleens, Kim *et al.* uncovered a new population of $CD4^+$ cells, which are not T cells (as they are $CD3^-$) or classical DCs (as they are $CD11c^-$). In contrast to DCs, which are found in the T-cell zones, these $CD3^-CD4^+$ cells are located mainly in B-cell follicles and at the interface between T-cell and B-cell areas — sites where T cells and B cells are known to collaborate.

To investigate the potential involvement of these cells in an immune response, T-cell receptor (TCR)-transgenic $CD4^+$ T cells were transferred into normal mice, which were then immunized. Soon after immunization (day 2), most of the antigen-specific T cells were associated with DCs, but by day 5, a shift in favour of interactions with $CD3^-CD4^+$ cells was evident.

In contrast to the DCs in the T-cell zone, the $CD3^-CD4^+$ cells expressed low levels of MHC class II molecules and the co-stimulatory molecules CD40, CD80 and CD86, but they expressed high levels of the tumour-necrosis factor family members OX40L and CD30L. This was intriguing as OX40 and CD30 are expressed by T cells and have been described to have co-stimulatory functions. The authors found that $CD3^-CD4^+$ cells potentiate the survival of T_H2 cells — which preferentially express OX40 and CD30 — but not T_H1 cells *in vitro*, an effect that seems to depend partially on signals from OX40. Moreover, OX40-expressing TCR-transgenic T cells had a clear survival advantage over OX40-deficient cells when they were co-transferred into mice that were immunized with a T_H2-cell polarizing antigen, with most $OX40^+$ T cells associating with $CD3^-CD4^+$ cells. Further *in vivo* studies showed that the duration of antibody responses and T_H-cell memory are impaired in the absence of OX40 signals.

So, drawing the evidence together, it seems that OX40 signals from this new type of accessory cell might be essential for maintaining T_H cells at the sites of T-cell–B-cell interaction in the later phases of antibody responses.

Jennifer Bell

References and links

ORIGINAL RESEARCH PAPER Kim, M. Y. *et al.* $CD4^+CD3^-$ accessory cells costimulate primed $CD4^+$ T cells through OX40 and CD30 at sites where T cells collaborate with B cells. *Immunity* **18**, 643–654 (2003)

WEB SITE

The MRC Centre for Immune Regulation: http://www.bham.ac.uk/mrcbcir/

B-CELL DEVELOPMENT

Cast-iron alibi

A detective will sometimes follow a hunch and investigate a suspect even if they have a cast-iron alibi. In a similar way, although early studies showed that ζ-chain-associated protein 70 (ZAP70) is expressed by T cells and natural killer cells, but not by B-cell lines, Schweighoffer and colleagues decided to test formally the role of this Syk tyrosine kinase in B-cell development. Their study, published in *Immunity*, shows surprisingly that not only is Zap70 expressed throughout B-cell development, but also it has a role in the pro-B- to pre-B-cell transition.

In the bone marrow, B cells develop through a series of developmental checkpoints: pro-B cells differentiate into pre-B cells after expression of and signalling through the pre-B-cell receptor (pre-BCR). Knockout studies have shown a role for Syk at this checkpoint, as B-cell development is partially blocked at the pro-B- to pre-B-cell transition in $Syk^{-/-}$ mice. Could the partial nature of this block result from functional redundancy between Syk and Zap70, the only other known Syk-family kinase?

To investigate this, Schweighoffer *et al.* generated chimaeras that were reconstituted with $Zap70^{-/-}Syk^{-/-}$ fetal liver cells. In these mice, pro-B cells were present at higher numbers than in chimaeras that were reconstituted with wild-type or $Zap70^{-/-}$ cells, and no pre-B cells could be detected. Therefore, in the absence of Syk and Zap70, B-cell development is completely blocked at the pre-BCR checkpoint.

Although expression of Zap70 by B cells had not been detected previously, deficiency of Zap70 exacerbated the B-cell developmental blockade that was seen in $Syk^{-/-}$ mice. So, the authors re-examined the presence of Zap70 at different stages of B-cell development, and found it to be expressed by pro-B, pre-B and splenic B cells. By radiation chimaera experiments, they also showed that the requirement for Zap70 in B-cell development is intrinsic to cells of the B-cell lineage.

Further experiments showed that the arrested B-cell development that was seen in the $Zap70^{-/-}Syk^{-/-}$ mice was due to defective pre-BCR signalling in the pro-B cells, and not due to defective synthesis or assembly of the pre-BCR. Heavy-chain allelic exclusion — a process known to require a pre-BCR signal — also failed in these cells, the first time that this has been seen in a signalling mutant.

This work shows that in B cells, functional redundancy does exist between Zap70 and Syk, as has been shown previously in T cells, and it establishes an important role for Zap70 in early B-cell development.

Jenny Buckland

References and links

ORIGINAL RESEARCH PAPER Schweighoffer, E. *et al.* Unexpected requirement for ZAP70 in pre-B-cell development and allelic exclusion. *Immunity* **18**, 523–533 (2003)

WEB SITE

Victor Tybulewicz's lab: http://www.nimr.mrc.ac.uk/immcellbiol/vtybule/