B-CELL DEVELOPMENT

Recipe for a B cell

Cell-fate specification of multipotent haematopoietic stem cells (HSCs) is determined by unique expression patterns of combinations of transcription factors, and for B cells, the five most important factors are PU.1, Ikaros, E2A, EBF (early B-cell factor) and PAX5 (paired box protein 5). Using a series of genetic knockout and reconstitution studies, Harinder Singh and colleagues have begun to order PU.1, EBF and PAX5 in a hierarchical regulatory network that directs an HSC through key developmental stages on the road to becoming a B cell.

First, they showed that PU.1 lies upstream of EBF in the B-cell developmental pathway and that EBF can bypass the requirement for PU.1 in early B-cell development. PU.1 is essential for both lymphoid and myeloid development and induces expression of the interleukin-7 receptor (IL-7R) by lymphoid precursors, but when PU.1-deficient fetal liver haematopoietic progenitors (FLPs) were transduced with the gene encoding EBF, IL-7-dependent proliferating cells were rapidly generated that exclusively expressed the B-cell lineage marker CD19 and not the myeloid marker MAC1. The *Ebf*-transduced cells expressed PAX5 and underwent VDJ recombination of the immunoglobulin heavy-chain loci, which are hallmarks of specified pro-B cells. Singh and colleagues identified a putative PU.1-binding site in *Ebf* that is conserved in mouse and human genes and showed that this is functional *in vitro* and *in vivo*, indicating that PU.1 directly regulates the expression of EBF. By contrast, PU.1-deficient FLPs could not be rescued by transduction of *Pax5*, indicating that the function of PAX5, as well as its expression, depends on EBF.

However, there was a fivefold reduction in the number of PU.1-deficient FLPs that could be rescued with EBF compared with PU.1. PU.1-deficient fetal

livers contained significantly fewer progenitor cells expressing the cytokine receptor FLT3 (fms-related tyrosine kinase 3), which is known to promote lymphoid versus myeloid cell fate, than did wild-type fetal livers, and FLPs expressing FLT3 were shown to have increased B-cell potential compared with those lacking FLT3. So, PU.1 is required for the generation of FLT3⁺IL-7R⁺ lymphoid progenitors, on which EBF acts, together with induced PAX5, to specify B-cell fate. Deficiency of EBF did not affect the percentage of FLT3⁺IL-7R⁺ cells, which clearly distinguishes the functions of PU.1 from those of EBF and PAX5 in B-cell development. On the basis of these results, the authors propose a hierarchical regulatory network to describe the generation of B-cell precursors from HSCs.

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References and links

ORIGINAL RESEARCH PAPER Medina, K. L. *et al.* Assembling a gene regulatory network for specification of the B cell fate. *Dev. Cell* **7**, 607–617 (2004).

IN BRIEF

T-CELL ACTIVATION

A role for the immunological synapse in lineage commitment of CD4 lymphocytes.

Maldonado, R. A. *et al.* *Nature* **431**, 527–532 (2004).

The immunological synapse forms at the T cell–antigen-presenting cell interface and is important for optimal T-cell activation. In this study, activation of naive T cells with peptide-loaded dendritic cells (DCs) induced T-cell receptor and interferon- γ receptor (IFN- γ R) co-localization at the T cell–DC interface. IFN- γ R synapse polarization occurred after activation of naive T cells from C57BL/6 mice (which are T_H1-response prone), but polarization was only partial for naive T cells from BALB/c mice (which are T_H2-response prone). Furthermore, IL-4 inhibited IFN- γ R synapse polarization, so the authors suggest that IL-4 levels may determine T-cell lineage commitment by regulating IFN- γ R migration to the synapse.

IMMUNOTHERAPY

Targeted deletion of T-cell clones using α -emitting suicide MHC tetramers.

Yuan, R. R. *et al.* *Blood* **104**, 2397–2402 (2004).

Fluorescently labelled peptide–MHC class I tetramers are used to detect peptide-specific CD8⁺ T cells. Yuan *et al.* generated tetramers composed of three peptide-bound MHC class I molecules and a radioisotope. Multimers containing the γ -emitting isotope ¹¹¹In and the LMP1 peptide from the Epstein–Barr virus latency membrane protein were used to show that radiolabelled tetramers specifically bind their cognate CD8⁺ T cells. Using LMP1 peptide–MHC multimers labelled with ²²⁵Ac (an α -emitting radioisotope), the authors were able to show specific killing of LMP1-specific CD8⁺ T cells. Specific killing of CD8⁺ T cells that recognize the listeriolysin-derived peptide LLO_{91–99} from *Listeria monocytogenes* was also shown using ²²⁵Ac-labelled LLO_{91–99}–MHC multimers. Further studies using ‘suicide’ tetramers *in vivo* could allow the generation of T-cell-selective therapies for autoimmunity.

SIGNALLING

Human Tribbles, a protein family controlling mitogen-activated protein kinase cascades.

Kiss-Toth, E. *et al.* *J. Biol. Chem.* **279**, 42703–42708 (2004).

Using an assay to identify proteins that mediate inflammatory cytokine signalling, Kiss-Toth *et al.* found a human homologue of the *Drosophila melanogaster* gene *tribbles*. Database searching identified two other homologues. Overexpression of either TRB1 or TRB3 inhibited activation of the transcription factor AP1 mediated by the mitogen-activated protein kinase kinase kinase (MAPKKK) MEKK1. Further analysis indicated that TRB3 acts downstream of MEKK1 and that TRB1 associates with the MAPKKs MEK1 and MAPKK4, whereas TRB3 interacts with MEK1 and MAPKK7. Downstream of this, low levels of TRB3 had distinct effects on the three sets of effector MAPKs: it increased both JNK and ERK activity but inhibited p38 activity. By contrast, high levels of TRB3 inhibited JNK, ERK and p38 activity. So, this study indicates that TRBs regulate MAPK activity by binding to MAPKKs.