T-CELL DEVELOPMENT

Why 3D is better than 2D

Freshly isolated thymic stromal cells in monolayer cultures (TSMCs) cannot support T-cell differentiation. Until the development of the OP9-DL1 monolayer culture system — in which OP9 bone-marrow stromal cells that are stably transfected with the gene encoding the Notch ligand Delta-like 1 (DL1) can support T-cell differentiation — it was widely accepted that *in vitro* T-cell differentiation could only occur in a three-dimensional system, such as fetal thymic organ cultures (FTOCs) or reaggregate thymic organ cultures (RTOCs). So, in comparison to three-dimensional culture systems, what is it that TSMCs lack in terms of support for T-cell differentiation?

Mohtashami and Zúñiga-Pflücker set out to address this question. The authors used RT-PCR (PCR after reverse transcription of RNA) to look at gene expression by various thymic stromal cells in TSMCs and thymocyte-depleted FTOCs. Because OP9 cells cannot support T-cell differentiation but OP9-DL1 cells can, the authors focused on expression of the various Notch ligands. TSMCs failed to express mRNA transcripts encoding DL1 and DL4, whereas these transcripts were expressed in FTOCs. Moreover, TSMCs that were retrovirally transduced with the genes encoding either DL1 or DL4 regained expression of these ligands and could support T-cell differentiation, indicating that DL1 and DL4 have similar abilities to direct haematopoietic progenitor cells towards a T-cell fate. By contrast, forced expression of either DL1 or DL4 in NIH-3T3 fibroblast was not sufficient to induce and support T-cell development, indicating that signals between Notch and its ligands alone are not sufficient.

These results show that, in the absence of a three-dimensional thymic environment, TSMCs must express either DL1 or DL4 to retain the ability to support T-cell-lineage commitment.

Elaine Bell

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LYMPHOCYTE SIGNALLING

Linking the scaffold to the workforce

The ligation of cell-surface antigen receptors initiates coordinated recruitment of various signalling molecules to the membrane, to form membrane-associated signalling complexes or 'signalosomes' that control lymphocyte proliferation and differentiation. Now, two papers published in *Immunity* show that, within these signalosomes, CARMA1 (CARD–MAGUK protein 1) acts as a scaffold that coordinates the assembly of the various kinases, adaptors and other effectors that control downstream nuclear factor-κB (NF-κB) activation. Moreover, these studies indicate that phosphorylation of the CARMA1 linker domain by protein kinase C (PKC)-family members provides the missing link between activation of PKC and initiation of the downstream response.

Previous studies have shown that PKCβ (in B cells) and PKCθ (in T cells) are crucial for activation of the inhibitor-of-NF-κB kinase (IKK) complex, which controls NF-κB activation, but the downstream targets of these PKC isoforms that make the link to IKK-complex activation are unknown. However, the recent identification of the proteins CARMA1, BCL-10 and MALT1, which act downstream of PKC-family members in immunoreceptor-dependent NF-κB activation, prompted the authors to assess whether these proteins were targeted by PKC isoforms.

By western-blot analysis, Sommer *et al.* first confirmed that, after ligation of the B-cell receptor (BCR), PKCβ, CARMA1, BCL-10 and the IKK complex are recruited to immunoreceptor-containing lipid rafts. Using a PKCβ-specific inhibitor, they showed that the lipid-raft recruitment of the signalosome components was PKCβ dependent. Although some PKCβ was shown to constitutively associate with CARMA1, cell activation markedly increased this interaction. Importantly, after BCR ligation, this interaction resulted in PKCβ-mediated phosphorylation of CARMA1, but not of BCL-10. Similarly, Matsumoto *et al.* showed that CARMA1 but not BCL-10 or MALT1, was inducibly phosphorylated by PKCθ after T-cell activation through T-cell-receptor ligation and CD28 co-stimulation.

Next, both groups carried out studies to identify the sites of PKC-dependent phosphorylation in CARMA1. CARMA1 is composed of five domains: an amino-terminal caspase-recruitment domain (CARD), a coiled-coil, a PDZ domain, and a carboxy-terminal SRC homology 3 domain and guanylate kinase (GUK)-like domain. And between the coiled-coil and PDZ domains is a linker domain. By generating a series of CARMA1 mutants, both groups showed that serine residues in the linker domain are targeted by PKCβ or PKCθ. Reconstitution of cells with CARMA1 mutants that lacked these serine residues did not affect recruitment of CARMA1 to lipid rafts but impaired subsequent recruitment of BCL-10 and IKK, and inhibited NF-κB activation.

On the basis of these observations, the authors proposed that phosphorylation of the CARMA1 linker domain induced a conformational change that exposed the CARD for recruitment of BCL-10. Indeed, Sommer *et al.* showed that in its unphosphorylated (inactive) state, the linker domain of CARMA1 interacts with the CARD, blocking accessibility of BCL-10 to the CARD. Antigen-receptor-triggered PKC-dependent linker phosphorylation is required to release this inhibition, thereby allowing BCL-10 recruitment and signal propagation to the IKK complex. Consistent with this model, deletion of the linker promoted constitutive IKK-complex activation and PKC-independent NF-κB activation.

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