T-CELL DEVELOPMENT

Location, location, location

During development, thymocytes follow a particular migratory pathway in the thymus that allows them to interact with distinct populations of stromal cells, but the precise mechanisms that control intrathymic localization and migration are not well understood. Now, two studies — one focusing on the migration of early thymic immigrants from the corticomedullary region to the subcapsular region, and the other focusing on the migration of positively selected thymocytes from the cortex to the medulla — report that signalling through CC-chemokine receptor 7 (CCR7) is crucial for intrathymic migration and for the maintenance of thymic architecture.

In the first study, Misslitz and colleagues found that CCR7 is expressed mainly by double-negative (DN) thymocytes and by semi-mature and mature single-positive (SP) thymocytes. CCR7-deficient mice and plt/plt mice (which are deficient in the CCR7 ligands CCL19 and CCL21) were found to have abnormal thymic architecture. By 6–8 weeks of age, CCR7-deficient and plt/plt mice also had reduced numbers of thymocytes but normal proportions of SP and double-positive (DP) cells. By contrast, older mice had reduced numbers of DP cells, which correlated with a severe block in T-cell transition at early DN stages. Both types of mouse produced normal T cells, although fewer than wild-type mice, indicating that CCR7 or CCL19/CCL21 deficiency can be compensated in an unknown way. Next, the authors examined the anatomical localization of the DN cells and found that they accumulated at the corticomedullary junction in older mice. Misslitz and colleagues suggest a possible explanation for the worsening defect in older mice: thymocytes in young mice are derived from fetal progenitors, which enter the immature thymus when it is an unstructured organ; however, after a few weeks, progenitors enter a structured organ in which signals from CCR7 might be more crucial for migration.

In the second study, Ueno and colleagues also studied mice deficient in signals from CCR7. In support of their previous studies, they observed defective T-cell production in the neonatal period but normal peripheral T-cell numbers in adult mice. Thymic architecture was also found to be abnormal in both young and adult mice, and mature SP thymocytes accumulated in the cortex. This study shows that the defect is worse in newborn mice,

MUCOSAL IMMUNOLOGY

Recognizing the good guys

The recognition of pathogenic bacteria by Toll-like receptors (TLRs) has a crucial role in eliciting protective innate immune responses. However, it is not known how TLRs can distinguish between pathogenic and commensal bacteria — which share pathogen-associated molecular patterns such as lipopolysaccharide (LPS). In the gut, it is thought that inappropriate responses to commensal bacteria can result in inflammatory bowel diseases such as Crohn's disease. Now, new research published in *Cell* shows that TLR-mediated recognition of commensal bacteria, rather than being an unwanted side-effect, is actually important for maintaining epithelial integrity and for tissue repair.

Using oral administration of dextran sulphate sodium (DSS) as a model of intestinal injury and inflammation, Medzhitov and colleagues show that mice deficient in MyD88 — an essential adaptor molecule for TLR signalling — have increased morbidity and mortality after DSS administration compared with wild-type mice. Similar results were obtained using TLR2- or TLR4-deficient mice. Death was associated with colonic epithelial injury, severe colonic bleeding and anaemia.

The increased epithelial injury was not due to uncontrolled growth of commensal bacteria in the absence of restrictive TLR-dependent responses, because commensal depletion using antibiotics did not improve disease. Neither were there any differences in leukocyte infiltration of the colonic epithelium between wild-type and MyD88-deficient mice. Instead, they found that TLR signalling through MyD88 controls homeostasis of the intestinal epithelium. MyD88-deficient mice had increased basal levels of proliferation of colonic-crypt cells, making these cells more susceptible to damage. As a result, intestinal tissue was unable to undergo compensatory proliferation in response to epithelial injury caused by γ-irradiation. These mice also had decreased production of cytoprotective and repair factors, such as interleukin-6 and heat-shock proteins, both before and after DSS administration.

These results indicated a pathway by which TLR signalling through MyD88 can protect against the colonic epithelial injury caused by DSS. To show that commensal bacteria trigger this signalling pathway, the authors depleted wild-type mice of all detectable commensal bacteria using antibiotics and then treated them with DSS. The mice suffered severe morbidity and mortality, similar to MyD88-deficient animals. The administration of purified LPS or lipoteichoic acid — TLR ligands expressed by Gram-negative and Gram-positive bacteria, respectively — completely protected animals.

Medzhitov and colleagues therefore propose a two-step model that involves epithelial maintenance through the constitutive detection of commensal bacteria by TLRs present on intestinal epithelial cells, and epithelial repair through the detection of commensals that breach the epithelial barrier by TLRs present on underlying fibroblasts and macrophages. Because many clinical treatments that are associated with intestinal damage, such as chemotherapy, are accompanied by antibiotic treatment to prevent opportunistic infections, the discovery of this new pathway for the beneficial effects of commensal bacteria has important clinical implications.

Kirsty Minton

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and adult mice can export T cells to the periphery in the absence of signals from CCR7. The authors suggest that CCR7-dependent migration to the medulla is more important for the export of T cells from the thymus in newborns than in adult mice, and they highlight the fact that REL-B-deficient mice, which lack thymic medullas, can export functional T cells.

These two studies show that signalling through CCR7 is important for intrathymic migration of T cells, but there is clearly more to be learned.

Elaine Bell

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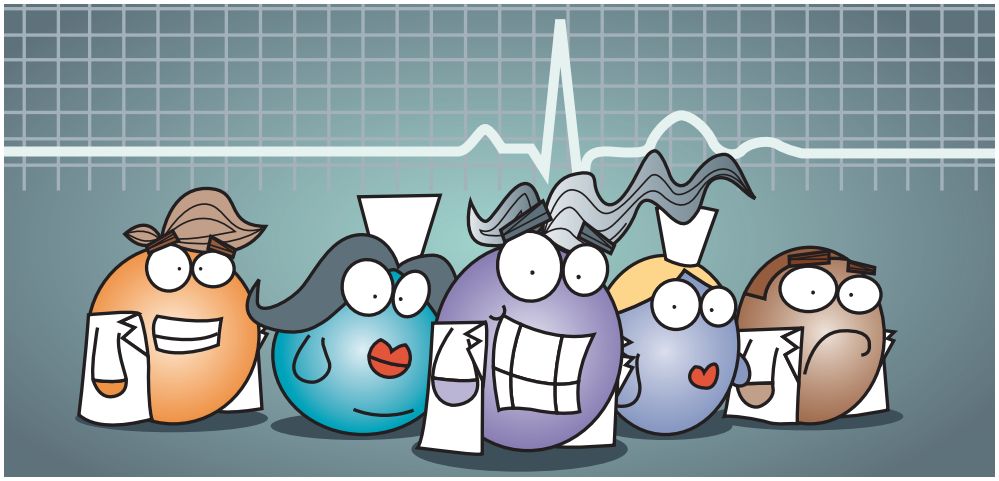

B CELLS

AID provided by RPA

Antibody diversification through somatic hypermutation (SHM) — the process by which non-templated point mutations are introduced into the variable region of immunoglobulin heavy and light chains, thereby enabling the selection of B cells that generate higher-affinity antibodies — requires the single-stranded DNA (ssDNA) deaminase known as activation-induced cytidine deaminase (AID). However, the way in which AID is targeted to sites of mutation was unclear until a paper published in *Nature* identified replication protein A (RPA) as crucial for this process.

More than half of all SHMs occur around a DNA motif known as the RGYW motif (where R denotes A or G, Y denotes C or T, and W denotes A or T), leading to the suggestion that unknown factors might target AID to these sites. To identify these putative cofactors Chaudhuri *et al.* used an *in vitro* assay for AID activity. This assay relied on the observation that although AID purified from B cells can by itself deaminate ssDNA templates, it cannot by itself deaminate an actively transcribed double-stranded DNA SHM substrate (that is, a substrate containing multiple RGYW motifs that cannot form R loops — structures generated during transcription that displace the non-template strand as ssDNA). The ability of protein fractions isolated from B cells to facilitate AID deamination of the SHM substrate led to the identification of a heat-labile complementing factor (CF) of 120–170 kDa.

In further experiments, a 30–35 kDa protein that was highly enriched in fractions containing CF activity was shown to associate with AID. Subsequent analysis identified this as the 32 kDa subunit of RPA (RPA32). RPA is heterotrimeric — consisting of a 70 kDa (RPA70) and a 14 kDa (RPA14) subunit, in addition to RPA32 — and has previously been implicated in DNA replication, recombination and repair. Confirmation of RPA as the CF was provided by the observation that recombinant human RPA containing all three subunits could replace CF in facilitating AID deamination of the actively transcribed SHM substrate. As well as complexes that contain AID, RPA and RGYW-containing DNA substrates, smaller complexes that contain only RPA and the RGYW-containing substrate were observed. Because these smaller complexes were not detected when the deamination reaction was inhibited, the authors suggest that AID-mediated deamination leads to the release of AID from the AID–RPA–DNA complex and that the DNA-bound RPA might enable the recruitment of DNA-repair proteins.

Interestingly, AID that was purified from the 293 non-lymphoid cell line ectopically expressing AID was unable to mediate deamination of the actively transcribed SHM substrate in the presence of RPA, and RPA and AID did not associate in this cell line. Because AID was found to be more highly phosphorylated in B cells than in the 293 cell line, these results indicate that the AID–RPA interaction is B-cell specific and that this specificity probably depends, at least in part, on the post-translational modification of AID.

This study identified RPA as the factor that targets AID to RGYW DNA motifs — that is, the sites of SHM — and it defined a mechanism by which SHM is a B-cell specific event. Further studies will focus on whether RPA has a similar function in class-switch recombination (which also requires AID), an outcome that the authors' initial experiments support.

Karen Honey

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