INFLAMMATION

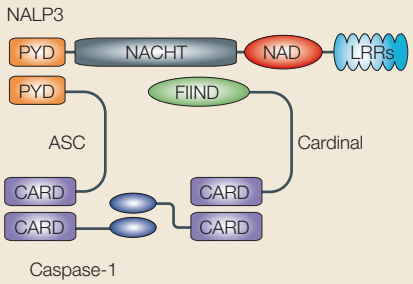
Cytokine ignition complex characterized

The genetic defect in patients with Muckle-Wells syndrome — a severe inflammatory disorder that can be treated with an interleukin-1 (IL-1) receptor antagonist — is found in the gene encoding NALP3. Now, work from Jürg Tschopp's laboratory, published in *Immunity*, has provided a molecular mechanism by which NALP3 influences the production of IL-1β.

Active IL-1β is generated by cleavage of its inactive precursor, pro-IL-1β, by caspase-1. Recently, this process has been shown to involve protein scaffolds, known as the inflammasome, generated by caspase recruitment domain (CARD)- and pyrin domain (PYD)-mediated protein–protein interactions. For example, the CARD of NALP1 is involved in recruiting caspase-5, whereas the PYD of NALP1 associates with caspase-1 through an adaptor protein, apoptosis-associated speck-like protein containing a CARD (ASC).

The CARD-containing region of NALP1 is missing in the other NALP-family members. The authors therefore investigated whether Cardinal, a protein with structural homology to the missing segment, could interact with other NALP proteins, and found that Cardinal co-immunoprecipitated with both NALP2 and NALP3. NALP2 and NALP3 were also shown to associate with ASC, and the CARD of Cardinal was shown to associate with caspase-1. These results indicate that NALP3 could form an inflammasome — consisting of ASC, Cardinal and caspase-1 — capable of processing pro-IL-1β (see figure).

The existence of a NALP3 inflammasome that can produce IL-1β, and the severe inflammatory phenotype of Muckle-Wells patients, indicate that the Muckle-Wells NALP3 mutation could result in constitutive activation of the NALP3 inflammasome. Indeed, the authors showed that monocytes isolated from a Muckle-Wells patient produced IL-1β even in the absence of stimulation, whereas monocytes from healthy individuals only produced IL-1β after activation with lipopolysaccharide. This study therefore provides the first evidence of a causative mechanism of Muckle-Wells syndrome.



The NALP3 inflammasome. NALP3 interacts with apoptosis-associated speck-like protein containing a CARD (ASC) through a pyrin domain (PYD)-mediated protein–protein interaction, and by way of its NACHT domain with the FIIND domain of Cardinal. Caspase recruitment domain (CARD)-mediated protein–protein interactions enable ASC and Cardinal to associate with caspase-1. LRRs, leucine-rich repeats; NAD, NALP-associated domain.

Karen Honey

References and links

ORIGINAL RESEARCH PAPER Agostini, L. *et al*. NALP3 forms an IL-1β-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* **20**, 319–325 (2004)

T-CELL DEVELOPMENT

From stem cell to T cell *in vitro*

Stem cells could serve as a renewable source of transplantable tissue-specific cells for the treatment of immune disorders. However, the excitement surrounding this potential has been curbed by the absence of a simple *in vitro* system that supports the differentiation of stem cells into specific lymphoid lineages. Now, Zúñiga-Pflücker and colleagues bring us one step closer to realizing this potential and describe an *in vitro* co-culture system to induce the differentiation of embryonic stem cells (ESCs) into functional T cells.

The Notch signalling pathway has a crucial role in determining whether lymphocyte progenitors become T or B cells. The authors observed that co-culturing ESCs on the stromal cell line OP9 led to the generation of B cells. However, when co-cultured on OP9 cells expressing the Notch ligand Delta-like 1 (OP9-DL1), the ESCs were induced to differentiate into T cells not B cells. These cells follow the normal programme of T-cell differentiation as seen *in vivo*, and by day 22, the cultures contained CD8 single-positive T cells. The cultures, however, did not support the development of $CD4^+$ T cells, probably because MHC class II molecules are not expressed by OP9 cells. $CD8^+$ T cells generated on OP9-DL1 cells had a diverse repertoire of expressed T-cell receptors, similar to *ex vivo* thymocytes, and underwent a coordinated expression programme of lineage-specific genes that are known to be important in T-cell development. In addition, these T cells could proliferate and produce interferon-γ in response to *in vitro* activation.

The authors next asked whether T cells generated on OP9-DL1 cells were functional *in vivo* and could reconstitute immunodeficient mice. To approach this, they isolated double-negative T-cell progenitors after 12 days in culture with OP9-DL1 cells and placed them in a lymphocyte-depleted fetal thymic organ culture, which supported the development of both $CD4^+$ and $CD8^+$ T cells. The *in vitro*-reconstituted thymic lobes were then implanted into immunodeficient mice. They found that the lymphoid organs from these mice were fully reconstituted with the donor T cells, enabling the mice to mount an effective immune response to viral infection.

Armed with this new technique, T-cell biologists are closer to realizing the therapeutic potential of ESCs.

Lucy Bird

References and links

ORIGINAL RESEARCH PAPER Schmitt, T. M. *et al*. Induction of T cell development and establishment of T cell competence from embryonic stem cells differentiated *in vitro*. *Nature Immunol*. **5**, 410–417 (2004)