ANTIBODY RESPONSES

Correcting mismatched pairs

A study published recently in *Molecular Cell* provides new insight into the molecular mechanisms of antibody diversification through class-switch recombination (CSR) and somatic hypermutation (SHM) downstream of activation-induced cytidine deaminase (AID), which is required for both processes.

The most widely accepted model of the mechanism of CSR (see Further reading) is that AID deaminates cytosine (C) residues to uracil (U) residues, yielding U•guanine (G) mismatched pairs, and that this is followed by excision of the mismatched uracil residue by uracil-DNA glycosylase (UNG). However, although UNG deficiency severely impairs CSR, it does not completely abolish CSR *in vivo*.

Similar uncertainty exists about the mechanism of SHM. Neuberger and colleagues have previously proposed two phases of SHM, the first focused on C•G pairs and the second on adenine (A)•thymine (T) pairs. They suggest that AID-generated U•G pairs lead to C•G mutations and that mutations at A•T pairs arise as a result of recognition of the initial AID-generated U•G pair by mismatch-repair proteins (such as mutS homologue 2, MSH2) and error-prone polymerases. However, although deficiency in such proteins reduces A•T mutations, they are not completely abolished.

The authors therefore set out to characterize these back-up pathways of CSR and SHM. Although it has been suggested that SMUG1 (single-strand selective monofunctional uracil-DNA glycosylase 1) — which has been shown in biochemical assays to mediate uracil excision in UNG-deficient tissue extracts — could mediate the UNG-independent pathway of CSR, B cells from UNG-deficient mice that are transgenic for human SMUG1 remained impaired in their ability to mediate CSR *in vitro*. By contrast, B cells from mice lacking both MSH2 and UNG were unable to switch *in vitro* or *in vivo*, as shown by a lack of $B220^{+}IgG1^{+}$ cells after culture in the presence of both lipopolysaccharide and interleukin-4 and by the barely detectable levels of IgG and IgA in the serum of these mice, respectively.

Further analysis of the sequence of the 3′-intronic flank of rearranged V_HDJ_H genes in Peyer's patch germinal-centre B cells from mice deficient in MSH2 and UNG indicated that SHM A•T mutations were eliminated in the double-knockout animals. In addition, mutations at C•G pairs were restricted to C•G to T•A transitions, rather than the usual combination of transitions and transversions (by which C•G mutations result in either A•T or G•C). This indicates that all of the mutations occur as a result of replication over the U•G mismatch and provides evidence that either mismatch recognition by MSH2 or base excision by UNG is required for A•T mutations in the second phase of SHM. A similarly restricted pattern of C•G to T•A transition mutations was observed at the IgM switch region of germinal-centre B cells from mice deficient in MSH2 and UNG, supporting the conclusion that MSH2- and UNG-mediated processes are the two pathways that resolve the AID-generated U•G mismatches during CSR.

This study furthers our understanding of the molecular mechanisms downstream of AID that result in CSR and the second phase of SHM (that is, the introduction of mutations at A•T pairs): mismatch recognition by MSH2 backs up UNG-mediated uracil excision for CSR, whereas the reverse is true of mutations in phase two of SHM. Future studies will define further specific molecular requirements for these two processes.

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References and links

ORIGINAL RESEARCH PAPER Rada, C., Di Noia, J. M. & Neuberger, M. Mismatch recognition and uracil excision provide complementary paths to both Ig switching and the A/T-focused phase of somatic mutation. *Mol. Cell* **16**, 163–171 (2004).

FURTHER READING Chaudhuri, J. & Alt, F. W. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. *Nature Rev. Immunol.* **4**, 541–552 (2004).

IN BRIEF

INNATE IMMUNITY

Innate immunity in *Arabidopsis thaliana*: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes.

Zeidler, D. *et al. Proc. Natl Acad. Sci. USA* **101**, 15811–15816 (2004).

The recognition of pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), by Toll-like receptors is a key feature of innate immunity in vertebrates and insects. Plants can also respond to LPS, and the authors show that, similar to animal responses, this can result in the production of nitric oxide (NO). Exposure of *Arabidopsis thaliana* cells to LPS led to a rapid burst of NO production through the action of the NO synthase AtNOS1. This induced expression of an array of defence or stress-associated genes. *A. thaliana* plants lacking AtNOS1 developed disease faster and had more-severe symptoms after exposure to a bacterial pathogen. So, NO production in response to PAMPs seems to be a highly conserved immune-defence mechanism.

REGULATORY T CELLS

Role of LAG-3 in regulatory T cells.

Huang, C.-T. *et al. Immunity* **21**, 503–513 (2004).

To harness the therapeutic potential of regulatory T (T_{Reg}) cells, they need to be easily identified on the basis of cell-surface marker expression. This paper identifies the CD4-related, MHC-class-II-binding molecule LAG3 as a phenotypic marker of both natural and induced T_{Reg} cells that also has a role in their functional activity. LAG3 is highly expressed by T_{Reg} cells compared with antigen-activated effector $CD4^{+}$ T cells, and this is in contrast to other suggested markers of T_{Reg} cells such as CD25, GITR and CTLA4, which can be upregulated by activated effector T cells. Deficiency in LAG3 prevents regulatory activity, whereas forced expression of LAG3 induces suppressor activity. The regulatory mechanism of LAG3 remains to be determined, and *Lag3*-knockout mice are being studied for evidence of autoimmune defects.

EVOLUTION

Resolution of the novel immune-type receptor gene cluster in zebrafish.

Yoder, J. A. *et al. Proc. Natl Acad. Sci. USA* **101**, 15706–15711 (2004).

Previous studies have identified four families of novel immune-type receptor (NITR) genes in zebrafish. In this paper, an additional eight NITR gene families were identified in the same cluster. *nitr9* encodes a protein with a positively charged amino-acid residue in its transmembrane domain (a characteristic of activating natural-killer-cell receptors), and indeed, it was shown to have activating-receptor function. In addition, the variable domain of NITRs contains three hypervariable regions, the first and third of which are in positions that correspond to complementarity-determining region 1 (CDR1) and CDR3 in the immunoglobulin and T-cell-receptor variable domains. So, studying these molecules could lead to a greater understanding of the evolutionary transition between immunoreceptor recognition of self-receptors and of peptide-loaded self-receptors.