T-CELL RESPONSES

The choice between anergy or activity

GRAIL (gene related to anergy in lymphocytes), as its name indicates, is involved in the induction of anergy in $CD4^+$ T cells. However, until now, the factors that regulate the activity of this RING-type E3 ligase (which attaches ubiquitin to proteins that are destined for degradation) were unknown. Soares *et al.* now report in *Nature Immunology* that two isoforms of the ubiquitin-specific protease otubain-1 control the expression and function of GRAIL in the induction of anergy.

Full activation of $CD4^+$ T cells requires two signals, and anergy results when a signal is received through the T-cell receptor (TCR) in the absence of a co-stimulatory signal. It is thought that GRAIL might induce anergy by ubiquitylation of membrane-associated targets that are required for T-cell activation. To investigate this system further, the authors used a yeast two-hybrid system to identify GRAIL-binding partners. Two isoforms of a gene containing an OTU domain, otubain-1 and alternative reading frame otubain-1 (otubain-1 ARF1), were shown to bind with high affinity to GRAIL and to co-precipitate with GRAIL in further assays.

What effect do these otubain isoforms have on GRAIL function in T cells? Co-expression of otubain-1 and GRAIL by mouse T-cell hybridomas resulted in the degradation of GRAIL and decreased GRAIL-mediated inhibition of interleukin-2 (IL-2) production, whereas co-expression of otubain-1 ARF1 caused an increase in GRAIL expression and enhanced inhibition of IL-2 production. Further experiments showed that the otubain-1 protein, rather than directly affecting the ubiquitylation of GRAIL, binds a deubiquitylating enzyme USP8 in a trimolecular complex with GRAIL, which regulates GRAIL ubiquitylation and degradation. Otubain-1 ARF1 binds GRAIL, not USP8, allowing USP8 to deubiquitylate polyubiquitylated GRAIL and so stabilize GRAIL function.

Next, the authors investigated the effects of overexpression of otubain-1/otubain-1 ARF1 on GRAIL function and the induction of anergy. Lethally irradiated mice were reconstituted with bone marrow from TCR-transgenic mice that had been retrovirally transduced to express one of these otubain isoforms. $CD4^+$ T cells that constitutively expressed otubain-1 showed increased IL-2 production and enhanced proliferation in response to antigen in comparison to control cells. By contrast, cells expressing otubain-1 ARF1 were functionally anergic and they responded poorly to antigen in terms of IL-2 production and proliferation.

This study shows that two isoforms of otubain-1, in conjunction with the deubiquitylating enzyme USP8, have opposing effects on the expression and function of GRAIL in the induction of anergy.

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References and links

ORIGINAL RESEARCH PAPER Soares, L. *et al.* Two isoforms of otubain 1 regulate T cell anergy via GRAIL. *Nature Immunol.* 7 December 2003 (doi:10.1038/ni1017)

VACCINES

A helping hand for CTLs

Sequences in bacterial or viral DNA known as CpG motifs are recognized by the innate immune system through Toll-like receptor 9 (TLR9) — which is selectively expressed by plasmacytoid dendritic cells (pDCs) and B cells — and lead to the stimulation of an adaptive immune response. These motifs are therefore being studied as potential new vaccine adjuvants. Hartmann and colleagues are the first to look at the ability of CpG DNA to enhance $CD8^+$ cytotoxic T-lymphocyte (CTL) responses in a human system.

They compared the adjuvant efficacy of two different types of synthetic CpG oligodeoxynucleotide (ODN) — CpG-A (otherwise known as D-type CpG ODN) and CpG-B (K-type CpG ODN), which differ in their flanking sequences — to support $CD8^+$ T-cell responses using two different peptides. When unseparated human peripheral-blood mononuclear cells (PBMCs; enriched for $CD8^+$ T cells, and also including pDCs and B cells) were stimulated with an influenza peptide, addition of CpG-A or CpG-B markedly increased the frequency of interferon-γ (IFN-γ)-producing $CD8^+$ T cells and their cytotoxic activity against a peptide-loaded target cell line. By contrast, these same T-cell parameters were only increased for stimulation with Melan-A peptide in the presence of CpG-B, and not CpG-A.

Further differences between CpG-A and CpG-B were noted in terms of the T-cell phenotype they induce. CpG-A was shown to be more potent than CpG-B in mediating the upregulation of expression of CD56 by CTLs, which correlated with increased lytic activity associated with an increase in intracellular levels of granzyme B.

Previous studies have shown that influenza-specific $CD8^+$ T cells have features of memory T cells, whereas Melan-A-specific $CD8^+$ T cells in healthy individuals are naive. Therefore, the authors suggest that CpG-B seems to be most efficient at priming naive T cells to produce CTLs, whereas CpG-A enhances memory CTL responses and lytic activity. This ties in with the fact that high levels of IFN-α — produced by pDCs in response to CpG-A but not CpG-B — promote memory-cell proliferation but have anti-proliferative effects on naive T cells. Validation of this differential activity for other sequences of CpG-A and CpG-B ODN could have important implications for use in prophylactic versus therapeutic vaccines.

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References and links

ORIGINAL RESEARCH PAPER Rothenfusser, S. *et al.* CpG-A and CpG-B oligonucleotides differentially enhance human peptide-specific primary and memory $CD8^+$ T-cell responses *in vitro*. *Blood* 20 November 2003 (doi:10.1182/blood-2003-04-1091)