ANTIBODIES

Eliminating the bad guys

The existence of self-reactive antibodies is a potential problem with respect to the development of autoimmune disease. Antibodies are generated by random association of immunoglobulin variable, diversity and joining region genes, but the risk associated with generating a diverse repertoire in this manner is the development of self-reactive antibodies. It has been known for some time that there are three mechanisms by which self-reactive antibodies can be eliminated — receptor editing, deletion and anergy. But the extent of autoantibody production and the stage at which these mechanisms operate are not clear. A new study from Wardemann and colleagues now establishes the extent of autoantibody production and indicates two stages at which self reactivity is modified.

Antibodies were cloned from single B cells derived from the blood and bone marrow of healthy human donors. The B cells were phenotypically defined as pre-B cells, early

immature cells, immature B cells, new emigrant B cells or mature B cells. Two features of antibody amino-acid sequences that have previously been associated with self-reactive antibodies — a long CDR3 (complementarity-determining region 3) and positively charged amino acids in CDR3 in the immunoglobulin heavy chain — were analysed in the cloned antibodies. These features were enriched in the pre-B and early immature B cells, but were progressively lost as the B cells matured.

To assess individual antibodies for self reactivity, the antibodies were expressed *in vitro* and tested using a standard clinical ELISA for reactivity against nuclear and cytoplasmic antigens. 76% of antibodies from early immature B cells were self reactive — this figure decreased to 43.1% in immature B cells and to ~20% as recently emigrated B cells matured into mature naive B cells, showing that self-reactive antibodies are eliminated at two distinct stages of development.

INNATE IMMUNITY

TLR signalling

Toll-like receptors (TLRs) are key molecules of the innate immune system that recognize molecular patterns on microorganisms and rapidly alert the host to the presence of potentially dangerous organisms. They contain a cytoplasmic signalling domain known as TIR (Toll/interleukin-1 receptor). Signals from TLRs are transduced by the adaptor molecule MYD88, but TLR3 and TLR4 — which recognize viral and bacterial ligands, respectively — also seem capable of signalling through a MYD88-independent pathway. A second recently identified adaptor known as MYD88 adaptor-like protein (MAL) or TIR domain-containing adaptor protein (TIRAP) was initially thought to have a role in this pathway, but MAL seems to function together with MYD88. Now, two groups have identified another adaptor molecule known as TRIF (TIR-domain containing adaptor inducing interferon- β , IFN- β) as a key component of the MYD88-independent pathway.

Bruce Beutler's group used a forward-genetic approach to generate random mouse

mutants by ENU (N-ethyl-N-nitrosourea)-induced mutagenesis. Peritoneal macrophages from mutant mice were tested *in vitro* for innate immune defects by stimulation with various TLR ligands. Macrophages with the *Lps2* phenotype had a defective tumour-necrosis factor (TNF) response to lipopolysaccharide (LPS) and double-stranded RNA, which are ligands for TLR4 and TLR3. Further experiments indicated impaired formation of phosphorylated IFN regulatory factor 3 (IRF3) dimers upstream of IFN- β production in the MYD88-independent pathway, although the MYD88 pathway was intact. Even in LPS-activated macrophages from *Lps2* homozygotes, the recruitment of MYD88 and MAL, followed by the phosphorylation of ERK1/2, p38 mitogen-activated protein kinase (MAPK) and JUN N-terminal kinase (JNK) occurred as would be expected after TLR4 activation. Despite this, mice homozygous for the *Lps2* mutation were relatively resistant to the lethal effects of LPS (although less so than mice lacking TLR4), and were also hypersusceptible to cytomegalovirus infection.

The *Lps2* phenotype was then mapped, and the defect was identified as a single base-pair deletion in the *Trif* gene. Generation of compound homozygotes (*Trif*^{*Lps2/Lps2*}, *Myd88*^{*-/-*}) allowed the authors

to conclude that all LPS signals are mediated by only two signalling pathways — the Trif branch and the Myd88/Mal branch, both of which are required for full expression of LPS toxicity.

Shizuo Akira's lab generated a *Trif*-knockout mouse to assess the physiological role of Trif. Macrophages from these mice showed impaired IFN- β production in response to stimulation with the TLR3 ligand polyI:C, and LPS-induced expression of IFN-inducible genes was also reduced, indicating a defect in the MYD88-independent TLR4 signalling pathway. As in the Beutler study, defective dimerization of IRF3 was observed.

These two studies show that TRIF mediates the MYD88-independent signalling pathway. As both the mouse and human genomes are known to contain at least five TIR-domain-containing adaptors, it is probable that we have some way to go to fully understand the basis of TLR signalling and to reconcile its apparent degeneracy with different outcomes.

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References and links

ORIGINAL RESEARCH PAPERS Hoebe, K. *et al.* Identification of *Lps2* as a key transducer of MyD88-independent TIR signalling. *Nature* 23 July 2003 (DOI: 10.1038/nature01889) | Yamamoto, M. *et al.* Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. *Science* 301, 640–643 (2003)