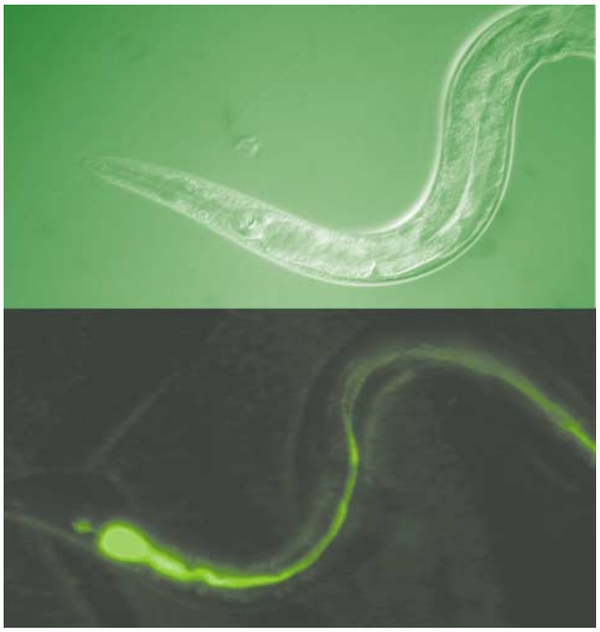
INNATE IMMUNITY

C. elegans — an innate choice?



An *esp2* mutant worm, after exposure to GFP-labelled *P. aeruginosa*. Nomarski (top) and fluorescence (bottom) images courtesy of Dennis Hyong-Kun Kim, Frederick Ausubel and Rhonda Feinbaum.

Much of what we know about innate immunity comes from studies in *Drosophila*.Two papers now show that *Caenorhabditis elegans*, another genetically tractable organism, might be a useful additional model for studying innate immunity. The results show that *C. elegans* has an inducible response to pathogen infection and that this response shares many features with innate immunity in other organisms.

Kim *et al.* assayed the progeny of mutagenized worms, which had been exposed to the bacterium *Pseudomonas aeruginosa*, for enhanced susceptibility to pathogen (esp) infection. Two mutants, *esp2* and *esp8*, that had severe phenotypes were isolated — both die much faster after exposure to *P. aeruginosa* than wild-type worms. The mutant genes were identified by phenotypic rescue — the *esp2* mutant was rescued by the gene *sek-1*, and *esp8* by *nsy-1*.

sek-1 encodes a mitogen-activated protein kinase kinase (MAPKK) homologue of mammalian MKK3/MKK6 and MKK4, and *nsy-1* encodes an orthologue of the mammalian MAPKKK ASK1. Because these kinases activate the p38 kinase family and the JNK MAPKs in mammals, the authors tested the role of p38 and JNK in the *C. elegans* defence response. The *esp2* and *esp8* mutants had markedly reduced levels of p38 MAPK activity. Moreover, the knockdown of *pmk-1*, one of two *C. elegans* p38 orthologues, by RNA interference produced a strong esp phenotype. Knockdown of *pmk-2* and a *jnk* mutation, however, produced no enhanced susceptiblity to *P. aeruginosa* infection. Together, these results show that the p38 MAPK pathway is required for innate responses to pathogen infection, which is an important discovery as this signalling pathway is also crucially required in mammals for inflammatory and innate-immune response signalling pathways.

Mallo *et al.* used an expression screen to look for *C. elegans* genes that are upregulated in response to infection by the bacterium *Serratia marcescens*. Of 7,500 cDNAs that were surveyed, several were induced more than twofold; most of these encode lectins, which function in innate immunity in other organisms. Lysozyme 1 was also upregulated. As lysozymes have been implicated in innate-immune responses, Mallo *et al.* overexpressed *lys-1* in *C. elegans* to see if this would enhance resistance to

TUMOUR IMMUNITY

Plugging the gap

Most of the tumour antigens that have been identified are abberantly expressed self-molecules that are poorly immunogenic. This realization, together with the fact that normal individuals have T cells that react against self-antigens, but are held in check by peripheral-tolerance mechanisms, has resulted in attempts to develop anti-tumour therapies that stimulate these anti-self T cells and elicit anti-tumour responses. A new study in *The Journal of Clinical Investigation* shows that stimulating the homeostatic proliferation of a polyclonal population of adoptively transferred T cells — including those that are specific for tumour self-antigens — in lymphopaenic animals can induce anti-tumour immunity.

Mice with lymphopaenia (induced by sublethal irradiation) and wild-type mice were challenged with a subcutaneous injection of melanoma cells, and tumour growth was assessed after 52 days. The lymphopaenic mice developed smaller tumours than wild-type mice. This anti-tumour effect was enhanced in a dose-dependent manner by the adoptive transfer of syngeneic lymph-node T cells before challenge with the tumour cells. *In vitro* testing of the homeostatically expanded T cells showed that they specifically recognised the melanoma tumour cells, but not unrelated colon carcinoma cells.

Next, the authors investigated whether T-cell clonal expansion *per se* was sufficient to induce the anti-tumour response or whether antigen presentation of tumour antigens was also important. The inhibition of tumour growth was more efficient in wild-type lymphopaenic mice transfused with T cells and challenged with melanoma than in lymphotoxin-α-deficient mice, which lack lymph nodes. When T cells that cannot traffic through lymph nodes (because they do not express β7-integrin or L-selectin) were transferred, they were much less effective than normal T cells at eliciting the anti-tumour response. Together, these results show that the proliferating T cells need to be exposed to tumour antigens that are presented in the lymph nodes.

When mice that had rejected the melanoma cells were rechallenged with either melanoma or colon carcinoma cells, they again rejected the melanoma, but not the colon carcinoma, tumours, which indicates that the T-cell homeostatic proliferation had resulted in the development of specific long-term anti-tumour immunity.

Could homeostatic proliferation be useful for the treatment of established tumours? Lymphopaenic mice were challenged with melanoma cells, and when the tumours reached a specific size, the mice were transfused with lymph-node cells. Tumour growth was markedly inhibited in treated mice compared with controls.

These data indicate that, in the clinic, it might be beneficial to begin tumour immunotherapy soon after the completion of chemotherapy to induce effective homeostatic T-cell proliferation.

Elaine Bell

References and links

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S. marcescens. It did, although only against a less pathogenic strain of the bacterium, possibly because this strain does not produce proteases that degrade the enzyme, whereas the more pathogenic strain does. The authors also assayed *Dbl-1* mutants for their susceptibility to *S. marcescens* infection because *Dbl-1* — a TGF-β-related gene — regulates some of the genes that were induced in the screen. *Dbl-1* mutants were extremely susceptible to *S. marcescens* infection and, surprisingly, also to infection by the *Escherichia coli* strain OP50, which *C. elegans* is often cultured on.

So, 30 years after its discovery in *Drosophila*, these studies show that *C. elegans* also has an inducible innate-immune response — the components of which are conserved in other organisms — and illustrate the ease with which it can be investigated genetically in worms.

Jane Alfred
Editor, Nature Reviews Genetics

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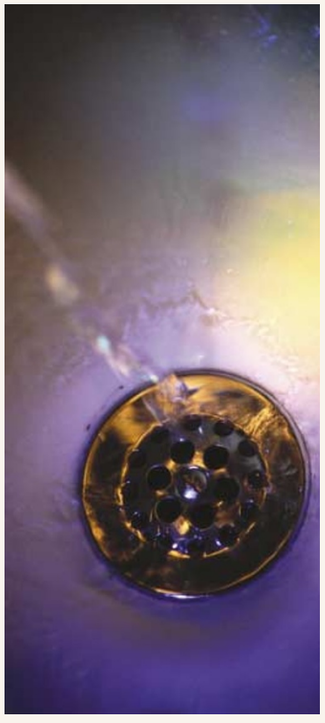

T-CELL SIGNALLING

The way of CARMA

This month, our understanding of T-cell receptor (TCR) signalling has been advanced by two studies in *Nature Immunology* that show that the scaffolding protein CARMA1 links the TCR to nuclear factor-κB (NF-κB) activation.

After the recognition of antigen by the TCR, many signalling pathways are triggered simultaneously, which leads to the activation of transcription factors, such as NF-κB, NFAT and AP1. NF-κB activation is essential for full T-cell activation, but the upstream signalling pathway is poorly defined. BCL10 — a caspase-recruitment domain (CARD)-containing protein — is known to have a role in TCR-dependent NF-κB activation, as is the novel protein kinase C PKCθ, but it is not clear how these components are linked to the TCR. Known binding partners of BCL10 include the CARD-containing subfamily of membrane-associated guanylate kinase (MAGUK) proteins, the CARMA scaffolding proteins.

Gaide *et al.* looked at the localization of CARMA1 — the only family member that is expressed in lymphocytes — in the Jurkat human T-cell line by immunoprecipitation and confocal microscopy using a polyclonal antibody. They show that CARMA1 is recruited to the TCR after stimulation with anti-CD3 antibody, together with its binding partner BCL10.

How important is the recruitment of CARMA1–BCL10 to the TCR for T-cell activation? Gaide *et al.* investigated this by generating a mutant CARMA1 protein that cannot bind to BCL10 and that functions as a dominant-negative inhibitor of CARMA1 activity. When Jurkat cells were transfected with the dominant-negative CARMA1 and stimulated with anti-CD3 antibody, NF-κB activation was inhibited markedly and interleukin-2 (IL-2) secretion was completely blocked; however, other TCR signalling pathways, such as the activation of ERK, JNK and PLCγ, and Ca^{2+} mobilization, were not affected.

Wang *et al.* isolated a mutant Jurkat clone in which anti-CD3/anti-CD28 antibody-triggered JNK and ERK signalling was preserved, but NF-κB activation was disrupted. This defect was shown to be downstream of PKCθ and upstream of IKK (inhibitor of NF-κB kinase) and to prevent IL-2 expression. The mutant cells are defective in *CARMA1* expression, and reconstitution with CARMA1 rescued the defect in both NF-κB activation and IL-2 expression. This result provides genetic evidence that CARMA1 is an essential signalling component in the TCR signalling pathway. The authors also show that CARMA1 is essential for optimal recruitment of BCL10 to the membrane and that it enhances the phosphorylation of BCL10 by PKCθ.

It has been proposed that early T-cell signalling events are initiated in membrane lipid microdomains known as rafts; so, are CARMA1 and BCL10 recruited to the rafts? Using confocal microscopy, Gaide *et al.* show that CARMA1 is enriched in rafts after cross-linking of rafts. A biochemical analysis of anti-CD3-stimulated Jurkat T cells, using detergents to isolate rafts, showed that CARMA1 is present constitutively in the raft fraction and that its concentration is increased after TCR stimulation. BCL10, which is excluded from rafts in resting cells, also translocates to rafts after stimulation. It is not clear how CARMA1 is targeted to rafts, but the authors' preliminary results indicate that CARMA1 might bind to a raft protein component, possibly through one of its protein–protein interaction domains.

Together, these papers indicate that CARMA1 has an essential and specific role in TCR-mediated NF-κB activation; it recruits BCL10 to the TCR in rafts and allows the activation of BCL10 — either directly or indirectly — by PKCθ.

Jennifer Bell

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WEB SITES

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