of isolated phagosomes with avidin-specific antibodies failed to detect association of avidin with the phagocytosed beads. Finally, the pH sensitivity of GFP (and its derivatives such as yellow fluorescent protein, YFP) was used to analyse the association of phagosomes with the ER on the basis that ER components delivered to the acidic phagosome would experience a decrease in pH. Addition of the weak base ammonia increased the fluorescence of GPI-YFP in phagosomes but had no effect on the fluorescence of GFP-KDEL, showing that this ER marker is not exposed to the acidic phagosome environment.

All of these experiments fail to provide any evidence in support of fusion of the ER with phagosomes, although the authors point out that they cannot definitively rule out the involvement of the ER in phagocytosis of particles of a different size or in phagocytosis using different cell-surface receptors.

Kirsty Minton

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was markedly extended by treatment with ArsTyr, and the inability of these cells to upregulate expression of the activation marker CD86 after stimulation with IgM-specific antibody was restored by treatment with ArsTyr.

Using pharmacological inhibitors of protein kinases that are known to be involved in BCR signalling, the authors confirmed that maintenance of the anergic B-cell phenotype depends on signals from the BCR.

One implication of these observations is that transient loss of self-antigen-mediated BCR signalling might lead to a resetting of a threshold trigger such that activation now might contribute to autoimmunity. Indeed, pretreatment of arsonate-specific B cells with ArsTyr for as little as 2–3 minutes allowed these previously unresponsive cells to respond to stimulation with IgM-specific antibody, by mobilizing calcium.

Lucy Bird

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NATURAL KILLER T CELLS

Identifying functional subsets of NKT cells

The ability of natural killer T (NKT) cells either to promote or to suppress cell-mediated immunity has been shown in various model systems in mice, but the reason for these contrasting effects is not well understood. Now, Nadine Crowe and colleagues show that there are functionally distinct subsets of NKT cells *in vivo*, which could help to explain the range of effects of NKT cells.

In previous studies, the authors showed that NKT cells derived from the liver can promote antitumour immune responses in two model systems: mice injected with the 3-methylcholanthrene-induced sarcoma cell line MCA-1, and mice injected with the melanoma cell line B16F10. Using these models, it was shown that mice that lack T-cell receptor (TCR) α -chains that contain J α 18 (denoted TCR J α 18), which are deficient in NKT cells, are more susceptible to tumour growth. In both tumour models, the ability of the NKT cells to promote antitumour responses was dependent on their production of interferon- γ .

Previous reports have shown that there are at least two phenotypically distinct subsets of NKT cells in mice and humans — CD4⁺ and CD4[–] NKT cells — and that these subsets show differential cytokine production *in vitro*. To test the idea that NKT-cell subsets are functionally distinct, as well as phenotypically distinct, the authors isolated NKT cells from the spleen, thymus and liver, then adoptively transferred these cells to TCR J α 18-deficient mice that had been injected with MCA-1. Only the liver-derived NKT cells could completely inhibit tumour growth, and this protection was found to be provided mainly by the CD4⁺ population of NKT cells. The inability of thymus-derived NKT cells to confer protection was not a consequence of their impaired survival after transfer, because they were easily detectable in the liver and other organs for at least 1 week after transfer.

Because it was possible that liver-derived NKT cells were preferentially activated in the MCA-1 model, the authors then tested various NKT-cell subsets in the B16F10 model. In this model, liver-derived NKT cells transferred to B16F10-inoculated TCR J α 18-deficient mice that were treated with the pan-NKT-cell-activating molecule α -galactosylceramide (α -GalCer) could inhibit the formation of lung metastases. Similar to the MCA-1 model, spleen- and thymus-derived NKT cells were less effective than were liver-derived NKT cells at preventing tumour growth, and the CD4⁺ subset of liver-derived NKT cells was more potent at promoting the antitumour response than was the CD4⁺ NKT-cell subset. However, these differences did not seem to result from differences in interferon- γ production, because liver-derived NKT cells that were isolated from mice deficient in interleukin-4 (IL-4) were considerably better at protecting against the formation of metastases than were their wild-type counterparts, and thymus-derived NKT cells from these mice were also protective. This indicates that IL-4 production by NKT cells could antagonize the ability of these cells to mediate tumour rejection. However, because wild-type, liver-derived NKT cells produce similar amounts of IL-4 to wild-type, thymus-derived NKT cells, it is not clear why thymus-derived cells cannot confer this protection.

This study shows that there are functionally distinct subsets of NKT cells *in vivo*, and it highlights the importance of addressing this issue in future studies and in clinical trials of α -GalCer-based therapies.

Elaine Bell

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