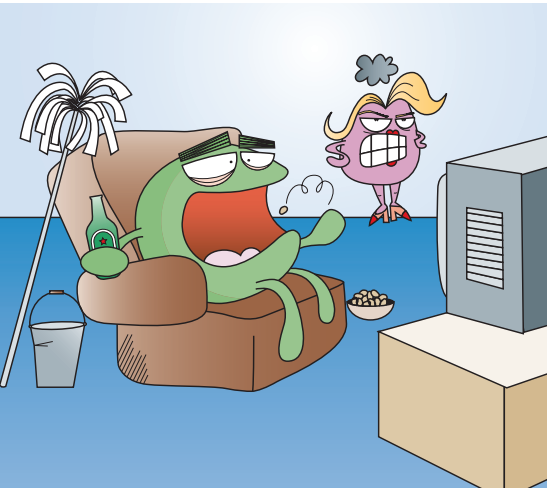
PHAGOCYTOSIS

ER fails to make a contribution



When a phagocyte encounters a pathogen, the triggering of cell-surface receptors leads to pathogen uptake in a membrane-bound vacuole (the phagosome). The simplest way to imagine the process is as an invagination of the plasma membrane, but a paper published three years ago by Desjardins and colleagues (see Further reading) concluded that the endoplasmic reticulum (ER) also contributes membrane to the phagosome. Now, Touret *et al*. have re-examined this finding and conclude from the results of various independent methods that the ER does not make a significant contribution to phagosome formation.

Using glycosylphosphatidylinositol (GPI)-linked green fluorescent protein (GFP) as a marker of the plasma membrane in a macrophage cell line phagocytosing latex beads, they showed that the plasma membrane constitutes a large proportion of the early phagosome membrane, limiting any potential contribution of the ER. By contrast, in macrophages expressing the ER marker GFP–KDEL, there was no significant overlap of this marker with phagosome markers. This observation that the ER is not a significant component of the phagosome was also made for various combinations of ER and phagosome markers and for mouse dendritic cells.

Immuno-electron microscopy of macrophage sections that were labelled with calnexin-specific antibodies could not detect the ER-resident protein calnexin on the phagosome membrane, and immunocytochemical staining could not detect the ER marker glucose 6-phosphatase in phagosomes. The possibility that the ER makes a minor contribution to the phagosome membrane by transient fusion was analysed for macrophages that had been stably transfected with the ER marker GFP–KDEL and were exposed to latex beads in the presence of high extracellular concentrations of the dye FM4-64. FM4-64 stained the plasma and phagosome membranes but did not reach the ER membrane (as shown by lack of colocalization with GFP–KDEL), even when phagocytosis was arrested by wortmannin to stabilize any transient connections that might form between the phagosome and the ER.

Biochemical assessment of ER–phagosome fusion was carried out using macrophages that had been stably transfected with the soluble ER marker avidin–KDEL and were allowed to ingest biotinylated beads. Immunostaining

B-CELL RESPONSES

What does it take to silence a B cell?

Even though there are mechanisms to eliminate developing B cells that recognize self-antigens in the bone marrow, a proportion of these cells are thought to escape elimination and enter the periphery. With their potential to contribute to autoimmune reactions, these self-reactive B cells must be kept in an anergic or tolerant state in the periphery. So, the authors of a recent report in *Nature Immunology* asked what might be required from the B-cell receptor (BCR) to maintain B-cell anergy. Using a mouse model of B-cell anergy, they showed that continuous BCR occupancy is required to ensure that self-reactive B cells remain unresponsive to encounter with additional antigens.

It had previously been suggested that, because some tissue-specific self-antigens might be encountered only rarely by patrolling B cells, a single, transient encounter with cognate self-antigen would be sufficient to induce the anergic state that is then 'memorized' for the lifetime of the B cell. But this hypothesis needed direct assessment. To this end, John Cambier and colleagues made use of immunoglobulin-transgenic mice in which B cells are specific for the hapten arsonate but crossreact with a self-antigen that induces anergy (probably single-stranded DNA). However, when these arsonate-specific B cells are cultured in the presence of high concentrations of a monovalent form of arsonate (arsonate-tyrosine, denoted ArsTyr), autoantigen is competitively dissociated, and anergy is lost.

To explore the kinetics of this reversal of anergy, the authors looked at intracellular concentrations of free calcium in the anergic B cells treated with or without ArsTyr. Anergic B cells are known to have higher basal concentrations of intracellular calcium than naive B cells, possibly because of either continuous BCR signalling or an altered physiological state that is triggered by a single exposure to self-antigen. Consistent with the former explanation, treatment of arsonate-specific B cells with ArsTyr led to a rapid reduction (within 2–4 minutes) in intracellular calcium concentrations. Washing the cells free of ArsTyr and then culturing them in the absence of ArsTyr rapidly restored intracellular calcium to the original high concentration. These findings indicate that at least some features of anergy are maintained by continued biochemical signals rather than by genetic reprogramming.

Treatment with ArsTyr also reversed other features of anergic B cells, including increased basal phosphorylation of extracellular-signal-regulated kinase (ERK) and increased expression of activation markers, such as CD80 and CD95. Importantly, the shortened lifespan that is characteristic of anergic B cells

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

References and links

ORIGINAL RESEARCH PAPER Touret, N. *et al.* Quantitative and dynamic assessment of the contribution of the ER to phagosome formation. *Cell* **123**, 157–170 (2005)
FURTHER READING Gagnon, E. *et al.* Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. *Cell* **110**, 119–131 (2002)

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

References and links

ORIGINAL RESEARCH PAPER Gauld, S. B., Benschop, R. J., Merrell, K. T. & Cambier, J. C. Maintenance of B cell anergy requires constant antigen receptor occupancy and signaling. *Nature Immunol.* **6**, 1160–1167 (2005)
FURTHER READING Wienands, J. Unraveling B cell receptor mechanics. *Nature Immunol.* **6**, 1072–1074 (2005)

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 Jα18 (denoted TCR Jα18), 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 Jα18-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 Jα18-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

References and links

ORIGINAL RESEARCH PAPER Crowe, N. Y. *et al.* Differential antitumor immunity mediated by NKT cell subsets *in vivo*. *J. Exp. Med.* **202**, 1279–1288 (2005)