IN THE NEWS

Setback for gene therapy

Gene therapy for the treatment of primary immunodeficiency diseases received a setback this month with the announcement that a three-year-old boy taking part in a French trial has developed leukaemia (*Reuters*).

The child, who suffers from X-linked severe combined immunodeficiency disease (X-SCID), was enrolled in a trial run by Alain Fischer at the Hôpital Necker in Paris. The therapy involves retroviral expression of the common cytokine-receptor γ-chain (γc) in haematopoietic progenitor cells of the patient, which restores signalling downstream of γc-containing receptors (see the article by A. Fischer, S. Hacein-Bey and M. Cavazzana-Calvo in the August 2002 issue of *Nature Reviews Immunology*). In this case, the virus seems to have "delivered its payload directly into a gene called *LM02*" (*Washington Post*) — modifications of this gene are linked to the development of cancers of the blood.

The French trial has been suspended pending further investigations (*The Guardian*), and scientists in the United States have recommended that similar studies proceed, but with some restrictions (*New York Times*). Related studies in the United Kingdom will continue, and Adrian Thrasher, from The Institute of Child Health, London, told us, "on current evidence from animal and human studies we still believe that the risk [of insertional mutagenesis] is very low, but we will only really be able to define this by treating more patients, and by characterizing the type and number of integration events".

Meanwhile, Californian scientists have offered a ray of hope for future gene-therapy trials — they have developed a plasmid-based method to target genes for insertion at specific sites in the genome (*Nature Biotechnology* DOI 10.1038/nbt753).

Elaine Bell

APOPTOSIS

Broader role for Bcl-2

At present, it is thought that Bcl-2 acts by protecting mitochondrial membrane integrity, so preventing the release of cytochrome *c*, the formation of the apoptosome and activation of caspase-9. However, recent work, published in *Nature*, indicates that Bcl-2 can act independently of the apoptosome to regulate caspase activation.

The fact that mammalian Bcl-2 can inhibit cell death in *Caenorhabditis elegans* — in which the mitochondrial release of apoptotic factors has not been observed — led Marsden and colleagues to question whether Bcl-2 acts solely to regulate mitochondrial pores. To investigate this, and to test whether the apoptosome is required for apoptosis regulated by the Bcl-2 family, they studied the effects of loss of Apaf1 or caspase-9 (both central components of the apoptosome), loss of Bim (an antagonist of Bcl-2) or overexpression of Bcl-2 on the survival of haematopoietic cells. As deficiency of Apaf1 or caspase-9 is embryonic lethal, they reconstituted lethally irradiated wild-type mice with fetal liver cells from the knockout or transgenic mice. *Apaf1*$^{-/-}$ or *caspase-9*$^{-/-}$ stem cells produced a normal number of lymphocytes and myeloid cells in the reconstituted mice, but reconstitution with *Bim*$^{-/-}$ or *Bcl2*-transgenic stem cells resulted in the generation of 3–5 times more splenic T and B cells. This indicates that Apaf1 and caspase-9 are not crucial for the control of apoptosis during haematopoiesis, but that Bim and Bcl-2 are.

Next, the authors investigated the role of these proteins in stress-induced apoptosis. Thymocytes, T cells and B cells from mice reconstituted with *Apaf1*$^{-/-}$ or *caspase-9*$^{-/-}$ stem cells were sensitive to stress stimuli (including γ-irradiation and dexamethasone treatment), whereas those from mice reconstituted with *Bcl2*-transgenic cells were protected against death. These results contrast with earlier work in *Apaf1*$^{-/-}$ and *caspase-9*$^{-/-}$ mice that did survive until birth, and indicate that Apaf1 and caspase-9 are not required for these death responses.

Are caspases still activated in *Apaf1*$^{-/-}$ and *caspase-9*$^{-/-}$ cells that lack the apoptosome? To answer this, Marsden *et al.* investigated whether caspase substrates are cleaved in γ-irradiated mutant thymocytes. Normal-sized cleavage products of ICAD (the inhibitor of the DNAse CAD) and PARP (poly-(ADP-ribose) polymerase) were observed, albeit at a lower level than in normal cells, indicating that caspase activity is present in these cells. Experiments with caspase antagonists showed that caspase activity is responsible for the death of the knockout cells, as apoptosis, cytochrome-*c* release and cleavage of ICAD were all inhibited by these compounds.

The authors conclude that the cell-death pathways that are controlled by Bcl-2 do not require Apaf1 or caspase-9, and that Bcl-2 can control the activity of caspases independently of the apoptosome. They speculate that, because apoptosis occurred normally or was, at most, slightly delayed by the absence of Apaf1 or caspase-9, "the apoptosome is not an essential trigger for apoptosis but is rather a machine for amplifying the caspase cascade".

Jenny Buckland

References and links

ORIGINAL RESEARCH PAPER Marsden, V. S. *et al*. Apoptosis initiated by Bcl-2-regulated caspase activation independently of the cytochrome *c*/Apaf-1/caspase-9 apoptosome. *Nature* 25 September 2002 (DOI: 10.1038/nature01101)

FURTHER READING Cory, S. & Adams, J. M. The Bcl-2 family: regulators of the cellular life-or-death switch. *Nature Rev. Cancer* **2**, 647–656 (2002)