Experiments using Ramos cells (derived from a human B-cell lymphoma) revealed that Bz-423 localizes to the mitochondria and then very rapidly boosts levels of intracellular superoxide. Rather than being cytotoxic itself, this superoxide acts as a second messenger, a signal triggering apoptosis (Fig. 1).

'This research is going to further the notion that redox signaling is abnormal in lupus', predicts Andras Perl, professor of microbiology and immunology at the State University of New York's Upstate Medical Center (http://www.upstate.edu), who has previously published in this area [2].

To learn more about how Bz-423 promotes B-cell apoptosis, the Michigan researchers pretreated cells with various agents that block known mediators of the process, and watched the effect on the actions of the compound. This revealed the key steps in its cytotoxic effects: cytochrome c release, mitochondrial depolarization and activation of caspase (Fig. 1).

Treatment implications

To explore the action of the agent in live animals, the Glick team chose a mouse model in which disease occurs because of overactive germinal center (GC) B-cells in the spleen, which have some similarity to the Ramos cells used in the *in vitro* studies. GC B-cells produce certain antibodies, which provoke kidney inflammation. Compared with controls, mice treated for 12 weeks with the benzodiazepine analogue had less kidney inflammation (in histology samples), significantly fewer and smaller GC B-cells, and increased apoptotic activity in their spleens.

These results are encouraging, but they do not mean that Bz-423 is going to be as effective in humans. 'This mouse model is largely B-cell-driven, and you cannot really translate findings from the mouse', says Perl. There are several animal models for SLE, and some agents work in one model but not in another.

Whether Bz-423 will eventually become a new drug to treat SLE in humans is anyone's guess. In any case, Glick intends to use it to 'work backwards' and identify some of the cellular defects that cause SLE. He also says that the team will soon publish results using Bz-423 in a T-cell-dominant mouse model. The researchers are also exploring the effectiveness of the compound in various animal models of cancer.

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Neural stem cells as novel drug delivery agents

Kathryn Senior, freelance writer

Neural stem cells engineered to express interleukin (IL) 12, a tumoricidal cytokine, have been shown, in mice, to track and kill gliomas as they spread through brain tissue [1]. Gliomas are currently treated by using surgical resection with adjuvant radio- or chemotherapy, which can eradicate the main tumour. However, gliomas are highly malignant and remaining cells spread quickly, setting up new tumor satellites. These are extremely difficult to destroy, even using stereotactic radiotherapy, and tumor recurrence is frequent and is associated with poor prognosis.

A stem-cell delivery system?

John Yu and colleagues at the Neurosurgical Institute of the Cedars-Sinai Medical Center (http://www.cedars-sinai.edu/mdnsi/) previously showed that gene transfer of the gene encoding IL-12 into mouse intracranial tumors using adenoviral vectors confers long-lasting immunity and a cytotoxic T-cell response [1]. The team used neural stem cells as a delivery system, hypothesizing that IL-12 secretion in the region of tumor satellites might induce a T-cell response more specifically against these problematic regions of tumor growth. 'It appears from our data that this may be the case', comments Yu.

Evan Snyder's group at Harvard Medical School (http://www.hms.harvard.edu/) first showed, more than two years ago, that neural stem cells can home in and/or track pathology in the adult mouse brain [2]. 'In our study, an oncolytic gene was expressed by the stem cells', explains Snyder, who goes on to say that his group now has a patent nearly issued for this novel approach to treating cancer. 'It is gratifying to see many people beginning to use this technique and validate our findings', he

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adds. Snyder's work was paralleled by a study showing encouraging results using mouse neural stem cells engineered to express the gene encoding IL-4 in mice with experimentally induced glioma [3].

IL-12-secreting stem cells

Yu and colleagues followed up this work and their own previous study on IL-12 by inoculating IL-12-secreting neural stem cells directly into experimentally induced gliomas. The treated mice showed significantly prolonged survival compared with controls, and developed long-term antitumor immunity. 'We found neural stem cells interspersed within the brain tumour mass and also present in small tumor islands detached from the primary tumor body', says Yu. Neural stem cells could be seen actively tracking outgrowths from the main tumor that extended deep into adjacent normal tissue [4].

Other researchers in the field welcome the results, but warn that this drug delivery strategy is still at a very early stage of development. 'More extensive analysis characterizing the effectiveness of this method for treating human glioma would be necessary to conclude whether this method is feasible for clinical use', comments Hideyuki Okano, of Keio University (http://www.keio.ac.jp/index-en.html). John Sinden, Scientific Director of Reneuron (http://www.renreuron.com), a company in the UK dedicated to researching therapeutic applications of neural stem cells, thinks that this 'is an excellent approach to glioma treatment, but not a huge step forward, since Benedetti *et al.* reported using IL4 to similar efficacy over 2 years ago' [3]. He stresses that a crucial test for neural stem cells would be to investigate whether they could successfully target and treat the gliomas seen in spontaneous tumor models.

Technique with potential

Pediatric oncologist Klaus-Michael Debatin of the German Cancer Research Center (http://www.dkfz-heidelberg.de/) agrees, but also enthuses about the potential of the technique for future exploitation. In July 2002, Debatin and colleagues reported that administering the second mitochondria-derived activator of caspase (Smac) (which sensitizes glioma cells to apoptosis), in combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) could completely eradicate established gliomas in mice [5]. 'The paper by Yu's group is really interesting. Targeting any cytotoxic approach to or into the tumor is a major challenge in the development of new treatment modalities', he comments.

Debatin views vectors or 'schlepper' vehicles directed by an intrinsic program to find tumor cells as ideal devices with which to deliver tumor-killing 'weapons' and to target diffuse metastases. 'With respect to the Smac peptide sensitizer/inducer approach, using neural stem cells as vectors may be possible, but some technical hurdles would need to be overcome', he says. 'Stem cells could certainly be used to deliver TRAIL locally, but Smac peptides are intracellular and secretory sequences would need to be added to the gene to get the peptide out of the cell to where it could work with TRAIL.'

Yu's group has recently completed a study using TRAIL protein secretion from stem cells to induce massive apoptosis in tumors and in tumor satellites. The group has also been looking ahead to clinical trials, and at ways to circumvent some of the ethical and practical problems inherent in stem cell therapy. 'The stem cells in the IL-12 study were from fetal mice. However, the use of fetal stem cells from humans is difficult, so we have developed a technique to isolate and differentiate neural stem cells from bone marrow cells', explains Yu. This technique should generate a virtually limitless supply of neural stem cells and it would obviate the immunological barriers of using stem cell lines or the ethical concerns of using fetal stem cells.

Genetics professor Mark Noble, of the University of Rochester Medical Center (http://www.urmc.rochester.edu) points out that neural stem cells used as therapeutic vehicles for brain tumours could also be modified to enable repair of the damage caused by brain tumors themselves, or by the more traditional brain cancer treatments. Snyder, for example, has previously shown that neural stem cells can repair large areas of damaged brain.

'This could happen eventually', he predicts, 'but at the moment it seems like an idealized therapy, of using transplantation of precursor cells to kill cancer cells and to replace any missing normal cells at the one and same time, is still far in the future'.

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