‘Cleaning’ autologous bone marrow transplants with *E. coli* toxin

A toxin produced by *Escherichia coli* could be used to purge tumour cells from autologous stem cell grafts, possibly improving the outcome of high-dose chemotherapy in cancer patients. Autologous stem cell transplants are used for patients who do not respond to normal doses of chemotherapy and who are considered inappropriate for surgery or radiotherapy because of extensive metastases. The technique was developed by researchers at the University of Toronto (Ontario, Canada) and University of Alberta (Edmonton, Alberta, Canada), and involves harvesting the stem cell prior to chemotherapy and then reinfusing them after high-dose treatments. This technique is most commonly used for patients with breast cancer, lymphoma and multiple myeloma.

Most patients given stem cell transplants eventually relapse within months or years, probably caused, at least in part, by the reinfusion of the contaminated tumour cells in the stem cell graft. For autologous bone marrow transplants to become a frontline therapy, a safe and effective method of killing cancer cells while sparing haematopoietic stem cells is urgently required.

Mechanism of action of SLT-1

Jean Gariépy and colleagues (Department of Medical Biophysics, University of Toronto) are therefore investigating Shiga-like toxin 1 (SLT-1), which is produced by pathogenic strains of *E. coli* O157:H7 and internalized by eukaryotic cells that express the glycolipid receptor globotriaosylceramide (Gb3 or CD77) on their surface[1]. In the human hematopoietic system, CD77 expression is restricted to a subset of activated B-cells and their derived cancers. The toxin is routed to the endoplasmic reticulum (ER), where its enzymatic component (an *N*-glycosidase) migrates from the ER lumen to the cytosolic surface of the ER membrane, an anchoring surface for ribosomes. Here it excises an adenine base in the 28S rRNA, inhibiting protein synthesis and leading to apoptosis (cell death).

SLT-1 is most useful for *ex vivo* applications, as it can cause endothelial cell damage and haemolytic uremic syndrome, both of which have been observed in patients following gastrointestinal infections with SLT-1-producing strains of *E. coli*[2]. However, the toxin might prove useful *in vivo* where the compartmentalization of a tumour site, such as in the brain, could limit the damage caused by SLT-1 to other organs.

Gariépy and colleagues tested the concept by giving SCID bone marrow to severe combined immunodeficient (SCID) mice that had been seeded with the human Burkitt’s lymphoma cell line, Daudi, which is $CD77^+$. The donor bone marrow was then treated with SLT-1 in one group and compared with untreated marrow as a control group. The contaminated cells were then washed and reinfused back into the mouse[3]. ‘We found that the treated mice survived and developed a normal immune system, while all untreated mice developed tumour-induced paralysis within 50 days’, says Gariépy.

As described in a recent paper, the team took samples from patients suffering from breast cancer, lymphoma and multiple myeloma, and used flow cytometry to demonstrate whether their cells expressed the SLT-1 receptor[2]. For breast cancer, they found that 70% of 18 breast cancer cell lines studied expressed the receptor, and that eight out of ten samples aspirated from these patients by fine needle biopsy also expressed the receptor on cancerous cells. In follicular lymphoma, they also showed that 70% of samples taken from patients expressed SLT-1 on their cancerous cells. Additionally, blood samples taken from patients with multiple myeloma were treated with the toxin and analyzed using PCR to monitor the extent of residual tumour cells in their blood. Molecular analysis confirmed that a >3-log reduction in clonotypic myeloma cells could be achieved with SLT-1 pre-treatment.

Significantly, $CD34^+$ cells, which include hematopoietic progenitor cells, did not bind to SLT-1 and were insensitive to the toxin. Having satisfied two of the criteria for the toxin to be a useful purging agent (that it kills tumour cells and does not kill stem cells), these researchers are now perfecting the final criterium, that of removing all of the excess toxin before reinfusion. ‘We are trying to develop a simple washing procedure with as few steps as possible to prevent the excessive manipulation of the graft itself’, explains Gariépy.

1359-6446/00/$ – see front matter PII: S1359-6446(99)01467-1

Clinical trials

In the next 12 months, Linda Pilarski and Andrew Belch, from the University of Alberta's Department of Oncology, plan to conduct a clinical trial of the toxin in six multiple myeloma patients undergoing stem cell transplants. Stem cells will be harvested for all patients, but only some of them will be treated with the toxin. PCR will be used to check that the tumour has been successfully eradicated in the sample, and the final outcomes will be compared between those treated with toxin and those that are not. 'Myeloma patients have the worst prognosis of the three cancer groups that we have been studying for this procedure, which makes them the most suited group of patients to provide the proof-of-concept of this technique', says Gariépy.

The technology has been patented by Gariépy and the Ontario Cancer Institute. Select Therapeutics (Cambridge, MA, USA) has entered into a commercial partnership with the team and is preparing the toxin for the trial.

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A Boston T party – The highlights

With the first of many key drug discovery meetings in the year 2000 rapidly approaching, now might be a good time to reflect on one of the major meetings of 1999, IBC's *Drug Discovery Technologies '99*, which was hosted by the historic city of Boston, MA, USA (16–19 August 1999)[1]. The meeting was aggressively focused on new technologies and strategies to expedite the drug discovery process. Topics such as toxicity prediction, strategic alliances and collaborations, target discovery and validation through genomics and miniaturization, and biochip technologies were all the subject of review and discussion.

Delegate numbers exceeded 2000, and included 62 speakers and 152 exhibitors who showcased the products of more than 150 equipment, instrumentation and software suppliers. While there was a reasonable delegate attendance from other countries, those from the US dominated the event, making up 81% of the attendees. Europe provided 13% of the delegates, while Canada and the Far East totalled just 6%. The meeting was orientated towards the industrial sector of drug discovery as reflected by the fact that 93% of the delegates were from industry.

Keynote speakers

The keynote speakers succeeded in rousing the audience with their presentations. Leroy Hood (University of Washington, Seattle, WA, USA) gave a futuristic presentation, based on ideas that are already being developed, highlighting the convergence of the information technology and biotechnology disciplines, the concept of systems biology, and the need for cross-disciplinary technology development. He discussed the next generation of DNA sequencers, which will be microchannel-based and could be capable of sequencing single molecules, and highlighted the potential for making chips containing double-stranded promoter regions to capture all the active transcription factors present in a cell. Other aspects discussed included optical-fibre single nucleotide polymorphism (SNP) genotyping, and two-colour proteomic analysis using different hydrogen isotopes and gene expression analysis in single prostate cancer cells. This analysis technique uses yeasts, flies and worms to model human cancer pathways by removing the equivalent of the human tumour suppressor gene, replacing it with the normal or mutated human gene and observing the alteration of gene expression in the model organism. The University of Washington is currently setting up the Institute for Quantitative Systems Biology and is creating novel academic–industrial partnerships.

Michael Pavia (Millennium Pharmaceuticals, Cambridge, MA, USA) gave a thought-provoking presentation in which it was stressed that the pharmaceutical industry will not be sustainable unless the time from discovery to market is reduced. Pavia advocated integrating the technology and making it more comprehensive and industrialized, noting that only those who harness information appropriately will remain in the pharmaceutical race. Pavia strongly suggested that drug discovery should

1359-6446/00/$ – see front matter PII: S1359-6446(99)01465-8