to placebo, 100 μg ZYC101a or 200 μg ZYC101a. Treatment was administered three times at three-week intervals as intramuscular injections. After six months, the patients underwent Loop electrosurgical excision procedure (LEEP) surgery and the cervical tissue was evaluated.

At the DNA Vaccines 2002 conference in Edinburgh, UK (23–25 October 2002), Hedley presented preliminary results that showed that, in the study population as a whole, lesions were resolved in 43% versus 27% of women (ZYC101a versus placebo; $p = 0.12$), whereas in women <25 years of age, the lesions resolved in 70% versus 23% of women (ZYC101a versus placebo, $p < 0.01$). The results are encouraging, says Tyring. He believes that 'the success of a therapeutic vaccine will stimulate other people to work on this area with more intensity'.

There is a great need for a medical alternative to currently available treatment options. At present, women with high-grade cervical dysplasia undergo surgery to remove the diseased tissue. However, surgical methods, such as LEEP, do not always clear the disease, and they can lead to complications during pregnancy. This is a crucial problem because more and more women between 12 and 25 years of age present with the disease, relates Hedley.

Future work

Zycos now wants to confirm these data in younger women and find out why the regression of lesions was not significant in women over 25 years of age. 'There should not be a magic cut-off at 25', says Hedley. 'Perhaps we can see some efficacy in those older women as well, potentially by changing the route of injection.'

Hedley and colleagues also want to explore the efficacy of the drug in other indications. Although cervical dysplasia is mainly associated with HPV-16 and HPV-18, 20 different HPV subtypes were present at baseline in the study population. ZYC101a cross-reacted with most of these subtypes.

This result suggests that the agent, or a similar formulation, could be beneficial in treating genital warts, anal dysplasia and even cervical cancer. 'I think what we are seeing is the tip of the iceberg', says Hedley. 'HPV causes so many [conditions] that once you have this initial efficacy, this really opens up a large possibility for you.'

References

1 American Social Health Association (1998) *Sexually Transmitted Diseases in America: How Many Cases and at What Cost?* Kaiser Family Foundation

2 Sheets, E.E. *et al.* Immunotherapy of human cervical high-grade cervical intraepithelial neoplasia with microparticle delivered HPV 16 E7 plasmid DNA. *Am. J. Obstet. Gynecol.* (in press)

New immunotherapy triple strategy for AML

Kathryn Senior, freelance writer

A patient's own blood cells could be manipulated to 'mop up' residual malignant cells that might remain after standard treatments for acute myeloid leukaemia (AML), according to a study published in late October 2002 [1]. Edward Ball's group at the UCSD Cancer Center (University of California at San Diego, CA, USA; http://cancer.ucsd.edu/) treated the AML cells of patients to turn them into antigen-presenting cells and expanded T cells with anti-AML specificity during *in vitro* culture. 'The basic idea was to make the AML cells more visible to the patient's immune system, and to boost specific immunity to AML cells at the same time', explains Ball.

Immune response important in AML recovery

AML is a difficult disease to treat. Standard therapy is usually high-dose chemotherapy, with haematopoietic stem cell transplantation (HSCT). It has been known for some time that the immune system is important in the eradication of residual leukaemia after HSCT. Ironically, the removal of T cells from the allogeneic graft, done to reduce graft-versus-host-disease (GVHD), is associated with an increased incidence of leukaemia recurrence, although patients who experience GVHD tend to have a reduced risk of relapse. 'Attempts have been made to enhance

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the anti-leukaemic effect without risking massive GVHD, but this has proved very difficult', says Ball. The group at UCSD reasoned that it must be possible to use the patient's own blood cells to bring about a therapeutic immune response. 'Theoretically, the blood of a patient at presentation contains all the elements necessary to induce that immune response', adds Ball.

Manipulating the blood cells of AML patients

Rui-Kun Zhong, Ball and colleagues obtained blood from 12 AML patients and cultured the white cells from each sample. Typically, the samples contained 92.3% AML blasts and 3.4% CD3+ T cells. These were cultured *in vitro* with interleukin-4 (IL-4) and recombinant granuloctye–monocyte colony-stimulating factor (Fig. 1). Recombinant IL-2 was added on day 8. On day 21, the culture conditions were changed to anti-CD3–anti-CD28 monoclonal antibodies and IL-2. By day 42, the amount of T cells in the culture had expanded 354-fold. Cytotoxic T-lymphocyte assays demonstrated that the T cells produced had a significant killing effect against autologous leukaemia cells and AML cell lines, but did not kill cells of other lineages [1].

'We originally started this study to investigate the possibility that T-cells with anti-AML specificity could be expanded from the blood of patients with active disease, but we have also managed to develop a strategy of 3-stage sequential modulation of growth factors to induce the patients own T cells to attack AML cells', explains Ball. The first phase is the initiation of AML blast differentiation into dendritic cells, the second is the priming of T cells, and the third stage is the expansion of the primed T cells, ready for transfer back into the patient. However, this important next step has yet to be done. Work is under way to refine and scale up the procedure for clinical application. 'This may require substitution of some of the culture ingredients with products that can be used clinically', comments Ball. This is expected to take up to a year, because of the need to comply with complex regulatory issues.

Further studies

Stan Riddell (Fred Hutchinson Cancer Research Center, Seattle WA, USA; http://www.fhcrc.org/) is also cautious about the time schedule, as he thinks that further preclinical work is still necessary. 'Others have shown that inducing differentiation of AML blasts to a dendritic cell like phenotype can provide stimulator cells that elicit effector T cells that are cytotoxic for AML cells. Ball and colleagues have streamlined the approach to culturing the cells by adding steps that promote T cell expansion but important questions remain about the nature of the effector cells that are generated and what antigens are being recognised', comments Riddell. He points out that the responses seen cannot be entirely classic major histocompatibility complex (MHC)-restricted responses, because MHC mismatched leukemia lines are killed very efficiently. 'The authors provide some data that the response requires class I antigens on the target cell based on blocking with an anti class I antibody but this blocking occurs even if the target is not class I matched', he notes. It will be interesting, adds Riddell, to see whether it is possible to generate T cell clones that can be more carefully and adequately characterized. 'This could prove very interesting because the approach could provide T cells for molecular identification of the target antigens', he continues.

T cell therapy is still in its infancy

T-cell therapy for AML and chromic myeloid leukaemia (CML) is a new field that is currently generating exciting results. Riddell reports that several other groups are looking at defined tumor

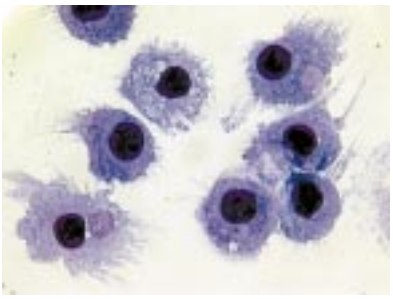

Figure 1. Acute myeloid leukaemia cells showing dendritic morphology after 8–10 days culture with interleukin-4 and recombinant granuloctye–monocyte colony-stimulating factor. Figure courtesy of Edward Ball at the UCSD Cancer Centre (http://www.cancer.ucsd.edu)

associated antigens. In November 2002, Jeff Molldrem (Department of Bone Marrow Transplantation, MD Anderson Cancer Center, University of Texas, Houston, TX, USA; http://www.mdanderson.org/) published a study in which his group identified proteinase-3 as a potential target in AML and CML [2]. Hans Staus (Department of Immunology, Hammersmith Hospital Imperial College School of Medicine, London, UK; http://www.med.ic.ac.uk) claims to have exciting data showing that T cells specific for the antigen WT-1 can eliminate human AML in immunodeficient mice. Edus Warren, an immunologist colleague of Riddell's at the Fred Hutchinson Cancer Research Center, and Els Goulmy (Leiden University Medical Centre, the Netherlands; http://www.lumc.nl/) have identified T cell responses to minor histocompatibility antigens that are expressed on leukaemic cells but have limited expression on nonhematopoietic cells in allogeneic stem cell transplant recipients [3,4]. Riddell considers that identification of such antigens might make it possible to augment the graft versus leukemia effect without GVHD, and data from both groups in a few patients treated for post-transplant relapse in Phase I

trials of T cell therapy suggest that this is feasible. But he stresses the need not to forget the preliminary stage of all of this research: 'A much larger number of candidate minor antigens must be identified to allow this approach to be tested more broadly', he concludes.

References

1 Zhong, R.K. *et al.* (2002) Sequential modulation of growth factors: a novel strategy for adoptive immunotherapy of acute myeloid leukaemia. *Biol. Blood Marrow Transplant.* 8, 557–568

2 Molldrem, J.J. *et al.* [2002] Overexpressed differentiation antigens as targets of graft-versus-leukemia reactions. *Curr. Opin. Hematol.* 6, 503–508

3 Warren, E.H. *et al.* (2002) Feasibility of using genetic linkage analysis to identify the genes encoding T cell-defined minor histocompatibility antigens. *Tissue Antigens* 4, 293–303

4 Mutis, T. *et al.* (2002) Generation of minor histocompatibility antigen HA-1-specific cytotoxic T cells restricted by nonself HLA molecules: a potential strategy to treat relapsed leukemia after HLA-mismatched stem cell transplantation. *Blood* 100, 547–552

Targeting cell migration

Stephani Sutherland, freelance writer

There are many ways in which cells migrate around the body to carry out essential functions, such as targeted immune reactions, metastatic invasion and angiogenesis. In spite of the diversity of cell type involved, they all use the same basic machinery for motility: the actin cytoskeleton and its associated proteins. Scientists are beginning to develop new ways to identify and study these proteins. Researchers at the University of Illinois in Chicago (http://www.uic.edu/index.html/), for example, have recently identified a compound that stops cells in their tracks in a wound-healing assay [1].

Gabriel Fenteany, lead author of the study, says that the work represents a shift toward therapeutic drugs that target cell motility. But finding an inhibitory compound is only the first step in elucidating the still-mysterious workings of cell movement, and in the even more distant process of developing drugs to exploit it.

Screening for mobility-affecting compounds

The researchers used a screening assay that they report is inexpensive, easy to use, and gives unambiguous results. The assay uses standard 384-well tissue culture plates; a 'wound' is drawn across the confluent cells, and the effects of various compounds on the wound size are then examined. By using Madin-Darby Canine Kidney (MDCK) cells, which maintain a smooth wound edge and migrate as a sheet, Fenteany says that they have eliminated the ambiguity that comes with individually motile cells. The assay enables the screening of 1000 compounds per day, which Fenteany says is 'getting into the realm of respectable high-throughput'. Although that might be high throughput for an individual in an academic lab, there are also commercial operations that screen motility-affecting compounds, such as Automated Cell in Pittsburgh, PA, USA (http://www.automatedcell.com).

Instead of starting with an individual protein, manipulating it, and then measuring its effects on the actin system and motility, Fenteany has taken a different approach: to find compounds that affect motility and work backward to the proteins involved. The newly identified compound, dubbed UIC-1005 (Fig. 1), is similar in structure to a class of antibiotics but has no antibacterial activity. Although both types of compound contain an oxazolidinone ring, says Fenteany, 'it's what's attached to it, and where, that makes all the difference'.

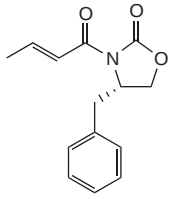

Figure 1. Structure of the compound UIC-1005. Figure provided by Gabriel Fenteany (University of Illinois in Chicago; http://www.uic.edu).

Compound identification

Justin Yarrow, working in the lab of Tim Mitchison at Harvard University Medical School, MA, USA (http://www.hms.harvard.edu/), has used a similar assay to find small molecules that affect motility [2]. Although he agrees that Fenteany's assay is unambiguous, he suggests that it might be an oversimplification to call UIC-1005 a specific inhibitor of cell movement. 'When you're asking for a binary read-out, you have to be asking a very specific question.' One caveat that Yarrow says comes with the assay is that there is a multitude of molecules, from transcription factors to membrane trafficking proteins, that could be affected upstream of actin proteins. 'There are probably billions of compounds that can inhibit wound

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