and Treasurer of Point Therapeutics, PT100 has several potential advantages. One is that it is orally bioavailable and therefore does not need to be injected. Secondly, it is inexpensive to manufacture and is thus potentially a cost-effective therapy. Third, it has a broad mechanism-of-action. 'You could get multiple beneficial effects outside of restoring your neutrophils,' he explains.

'The novelty of it is the fact that this is a new therapeutic target,' says Jaroslaw Maciejewski, Section Head of Experimental Hematology and Hematopoiesis at the Cleveland Clinic Cancer Center (http://www.clevelandclinic.org/cancer). Although clinicians are looking for better and less toxic ways to treat neutrophenia, its unclear that PT100 will prove to be as efficacious as conventional treatments. 'The effect that they've demonstrated in mice is quite a bit less than treatment with G-CSF,' says Lyman. 'Perhaps different doses or schedules will prove to be more effective.' He also suggested the possibility of evaluating the effect of PT100 in combination with Neupogen® or Neulasta®.

Other indications

Point Therapeutics released interim results from its Phase I clinical trial evaluating the effects of PT100 in patients with chemotherapy-induced neutropenia on 28 May 2003. 'It's been well tolerated and we've seen some biological effects that correlate with the preclinical animal studies,' Small concludes.

Based on data that PT100 can stimulate the production of cytokines and chemokines to promote acquired and innate immune defenses against certain cancerous tumours, the company is also initiating a clinical trial in patients with hematological malignancies this year [4]. 'In a preclinical mouse model of human B cell lymphoma, PT-100 was shown to enhance the activity of the monoclonal antibody Rituximab (Rituxan®; http://www.rituxan.com), [which] is currently used to treat non-Hodgkin's lymphoma,' says Jones. 'Because both non-Hodgkin's lymphoma and chronic lymphocytic leukaemia express the target antigen, CD20, which is recognized by Rituximab, PT100 could augment its activity in the treatment of both types of cancer.'

References

1 Jones, B. *et al.* (2003) Hematopoietic stimulation by a dipeptidyl peptidase inhibitor reveals a novel regulatory mechanism and therapeutic treatment for blood cell deficiencies. *Blood* DOI 10.1182 (epub ahead of print: http://www.bloodjournal.org)

2 Bristol, L.A. *et al.* (1995) Inhibition of CD26 enzyme activity with Pro-boro-Pro stimulates rat granulocyte/macrophage colony formation and thymocyte proliferation *in vitro*. *Blood* 85, 3602–3609

3 Whetton, A.D. and Spooner, E. (1998) Role of cytokines and extracellular matrix in the regulation of haemopoietic stem cells. *Curr. Opin. Cell Biol.* 10, 721–726

4 Jones, B. *et al.* (2003) Dipeptidyl peptidase inhibitor PT-100: An anti-tumour small molecule that amplifies immunity. *American Society of Clinical Oncology Annual Meeting 2003*, 31 May – 3 June 2003, Chicago, IL, USA (Abstract no. 860)

An everlasting flu vaccine with none of the pain

Paul D. Thacker, freelance writer

Researchers at the Wistar Institute (http://www.wistar.upenn.edu) are currently devising a prototype mouse influenza vaccine, which promises to last longer and provide increased protection against this rapidly evolving pathogen. The vaccine has three novel features: a synthetic peptide as the vaccine, a nasal delivery system and an immune response to unique protein on the virus.

The World Health Organisation (WHO; http://www.who.org) estimates that five million people are affected by influenza every year and, of those, a quarter to half a million patients die. Although a vaccine for the pathogen exists, the virus mutates rapidly, forcing researchers to constantly search and identify new strains and then ascertain which viruses to incorporate into the annual flu shot.

'Normally against influenza you use an inactivated virus,' says Winstar Institute Associate Professor, Laszlo Otvosz [1]. 'But like many other groups, we are trying to use subunit vaccines which come from proteins or peptides.'

  PII: S1359-6446(03)02798-3

Current vaccines

The influenza virus has two transmembrane proteins – haemagglutinin and neuraminidase – which have protein sections found on the outside of the virus. These ectodomains mutate rapidly in response to immune attack so new strains constantly evolve. These new strains are then identified by sequencing the extracellular portion of haemagglutinin.

To create a vaccine, virus is grown in chicken eggs and then purified. The virus is then chemically inactivated and the vaccine is standardized to a titre of the haemagglutinin. However, Otvosz and his team plan a different approach.

Creating a synthetic vaccine

The Winstar group is focusing on a third transmembrane protein, matrix protein 2 (M2). This protein is highly conserved, with an extremely short extracellular domain consisting of only 23 amino acids. Because it does not mutate and is immunogenic in mice [2], M2 has become the focus of several investigators trying to create an M2-based vaccine [3].

The Otvosz vaccine consists of a poly-(lysine-glycine) backbone for the four M2 ectodomains. Peptide vaccines are ideal because they can be made in large quantities with a high purity, but they are not generally immunogenic enough. To solve this problem, Otvosz also adds two protein fragments, which attract T-helper cells.

'When you want to elicit an antibody response you need T-helper cells involved,' says Otvosz. 'So we had to add these epitopes.' They then vaccinated the mice twice intranasally. In nature, influenza infects through the nasal passages and Otvosz says they wanted to mimic this effect.

After five weeks, blood was drawn to see if the mice had generated antibodies. 'We got a very good antibody response,' says Otvosz.

'Sometimes better than a natural infection.' He adds that the antibody was protective when mice were later infected with a live a virus. 'We saw a one log decrease in virus titre in mice that had been vaccinated versus the untreated animals.'

Drawbacks and future testing

Although the vaccine shows great promise, Otvosz says there is much more to explore. 'We know that this reduction in virus is scientifically significant, but we're not certain if it is clinically significant.' The virus they used was not lethal and in the future he would like to try a lethal strain.

'A good human vaccine is always going to involve antibodies to haemagglutinin,' says Jacqueline Katz, Chief of the Immunology and Viral Pathogens Section at the Centers for Disease Control and Prevention (http://www.cdc.gov). 'This approach using another protein is something I've always imagined as a supplement where you get increased immunity or broad immunity in a crisis situation if you don't have the vaccine.' Further, she adds that vaccines based on M2 will more likely ameliorate disease rather than providing true immunity.

Otvosz adds that he would like to test other constructs using different T-helper epitopes. 'Mice are inbred and we knew this strain would raise antibodies to this protein. So we would like to try different T-cell determinants with different mice strains.'

In the next round of studies, he is also focusing on different M2 mutants to see how mice respond. 'Unlike mice, humans are outbred and have numerous T-cell determinants. So we need to see if this will work in everybody, probably making a more promiscuous T helper epitope.'

References

1 Neirynck, S. *et al.* (1999) A universal influenza a vaccine based on the extracellular domain of the M2 protein. *Nat. Med.* 5, 1157–1163

2 Frace, A.M. *et al.* (1999) Modified M2 proteins produce heterotypic immunity against influenza A virus. *Vaccine* 17, 2237–2244

3 Slepushkin, V.A. *et al.* (1995) Protection of mice against influenza A virus challenge by vaccination with baculovirus-expressed M2 protein. *Vaccine* 13, 1399–1402