leading to gender separation. MIS has potential therapy for ovarian, uterine, breast and prostate cancers. Note that it has high specificity with no *N*-linked glycan problems and no toxicity issues.

Oral vaccines

Arizona Biodesign (http://www.azbio.org) is focusing on oral vaccines and is in Phase I trials for a vaccine for Norwalk Virus and Hepatitis C using tomatoes. The public perception is addressed with 3600 square feet of white seedless tomatoes, with 120 acres under glass.

Dow Agro Sciences (http://www.dowagro.com) is making oral antigens from plants. The advantages, according to the presenter, are that PMPs show decreased costs with antigen stability and the company has been doing this now for three years with no immunological responses at all. The company has many collaborations and has also shown use in the nasal delivery of antibodies.

It was interesting to hear NeoRx (http://www.neorx.com; speaker Becky Bottino), who were the first to produce monoclonal antibodies from corn but stopped because of the cost, manufacturing capacity and high investment for little return, which is a lesson for all small biotech companies. The problems included 30 months to clinical manufacture, 36 months to commercial production and only two years in stability of the corn seed itself.

Conclusions and future directions

In summing up the conference, Richard V. McCloskey of Centocor (http://www.centocor.com) explained that an antibody needs to produce at least one kilogram with no change in the production process between Phases II and III and cost should be US$10 per gram for non-purified bulk. He warned to 'watch out for the cost to the patient', have fewer production sites with efficient and rapid scale-up and multiple systems for flexibility, timing and purification. He concluded by advising that, 'you are going to want tons of the stuff!'

The future of PMPs, according to François Arcand of Medicago (St-Foy, Quebec; http://www.medicago.com), changes all the time. He forecast that, for PMPs to prosper, we are going to have to show that mammalian cells are finished and we will have to see that in next years conference program.

In conclusion, this conference, set in such a wonderful city, had presentations of fantastic and interesting science. However, let us be realistic; the actual drugs themselves are many, many moons away for those patients that will use them. It is an exciting road ahead but we are all still learning.

scFvs and beyond

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'I don't want to knock single chains, but…' was one of the leitmotifs at the *Recombinant Antibodies* and *Molecular Display* meetings (12–15 May 2003, Hyatt Regency, Boston, MA) organized by Cambridge Healthtech Institute (CHI). This statement was invariably followed by lists of problems regarding single chain antibody variable region fragments (scFv) – poor expression, aggregation, instability, difficult storage, a tendency to dimerize and a variable unknown fraction of functional antibody – and a solution to the problem. Although, there is little doubt that scFvs can have these problems, their strengths as tools for discovery should also not be underestimated. These are perhaps best represented by the seven antibody drugs Cambridge Antibody Technology (http://www.cambridgeantibody.com) currently has in clinical trials – all developed using scFv phage display.

Yeast display

Another discovery platform based on scFvs was described by Michael Feldhaus (Pacific Northwest National Lab; http://www.pnl.gov) who has created a naïve scFv yeast display library. This represents a considerable *tour de force*, particularly when one considers the low transformation efficiency of yeast, which is approximately 10,000 times less than *Escherichia coli*. The library has a diversity of just over a billion clones and has been used to select binders against a number of different targets, with affinities in the low nanomolar range.

Although one could be forgiven for thinking 'yet another display platform', yeast offer a couple of significant advantages to phage display. First, and perhaps most important, is the relative ease with which affinities can be measured. In fact, Jim Marks (UCSF; http://www.ucsf.edu) now routinely clones scFvs that have been isolated

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using phage display into yeast display vectors to measure affinities, rather than using bacterial expression. Yeast displaying scFvs can be considered as small beads with tens of thousands of scFvs correctly orientated for binding. By fluoresceinating antigen and adding it at different concentrations, these small beads bind different amounts of antigen, and become variably fluorescent as a result. Analysis of this fluorescence by flow cytometry allows affinity determinations with values within twofold of those determined by surface plasmon resonance (SPR), and well within the variability encountered whenever two different affinity-measuring methods are compared. Anyone who has struggled with scFv expression, purification and quantification for SPR, will appreciate this advantage. Second, the identification of antibodies that recognize different epitopes on a target is considerably easier with yeast display compared with SPR or ELISA, where scFv immobilization in a solid-phase often leads to inactivation. This can be accomplished by the sequential binding of antigen and fluorescently labeled second antibody to yeast displaying a scFv of interest. Yeast displaying scFvs that bind the same epitope as the labeled antibody remain unlabeled, whereas those binding different epitopes become fluorescent. This method has been used as an analysis tool for selected scFvs but could be adapted for use as a selection modality in its own right. Although effective as a naïve selection platform, with the possibility of selecting for high affinity interactions, many might find the volumes required (500ml of fresh growth culture, concentrated to 10ml per selection) daunting, even if multiplex selection is possible. This identifies it more as a powerful complementary platform to phage display, rather than one that is likely to replace it.

ScFv optimization and use

Some of the scFv problems described above were directly addressed by Dominik Escher (ESBATech; http://www.esbatech.com) who has used an antigen-independent selection system, based on yeast two-hybrid technology, to select a panel of fully human V_H and V_L domains that are highly stable and functional within the cytoplasm. This is particularly significant because the failure of intracellular antibodies owes much to the inability of these secreted proteins to fold correctly within the reducing environment of the cytoplasm, owing to the loss of their stabilizing disulfide bonds. Further analysis of these highly stable scFvs identified five (three V_L and two V_H) particularly stable human frameworks that could be combined and which tolerated the incorporation of different binding loop sequences without losing stability. Specific scFvs, selected from libraries based on these scaffolds using the yeast two-hybrid system, showed remarkably stability (e.g. 15 mins at 100°C), as well as good expression levels, suggesting that the strategy of developing stable scFv libraries that is being adopted by a number of groups might solve some of the frequently listed problems.

However, experiments by Franck Perez (Institut Curie; http://www.curie.fr) showed how careful optimization and the use of scFvs can overcome many of their apparent disadvantages, even from non-optimized libraries. Mindful of the maxim 'you get what you select for', Perez selected for scFvs (from the widely dispersed 'Griffin' library provided by Greg Winter, MRC, Cambridge, UK) using native biotinylated material, and screened directly for functionality on cells by immunofluorescence, rather than the more commonly used ELISA, which is more likely to identify scFvs recognizing denatured protein. In this way, he was able to select a high proportion of scFvs that were functional intracellularly. One of these scFvs, advertised as a 'protein conformation sensor', recognized an activated form of Rab6A, and when fused to green fluorescent protein (GFP) was able to track the activated form of Rab6A as it 'streaked' around the cell. This approach also hinted at potential artefacts associated with many commonly used GFP fusions. The same scFv-GFP fusion identified 'wriggling worms' rather than 'streaking rockets' when the cells expressed Rab6A-GFP fusion proteins, suggesting that over-expression of the YFP fusion caused the transition from 'rocket' to 'worm' and that this was not the natural state.

John Park (UCSF) described the use of antibody fragments, including scFvs, in 'immunoliposomes', in which liposomes containing cytotoxic drugs are targeted to tumor cells by antibody fragments attached to the liposome surface. Park discussed that scFvs are an ideal format for this approach, which allows the use of lower affinity ligands in highly avid particles, and can exploit specific phage display selection strategies for the property of internalization. This leads to a modular concept of targeted therapeutics development – scFvs can be interchanged to target different tumors, and the drug in the liposomes can be selected to best suit the target cell population. Impressive preclinical results were shown with Doxil® (a liposomal form of doxorubicin in widespread clinical use) targeted against the product of the HER2/c-ErbB2 oncogene, which is being developed for clinical trials.

Alternate scaffolds

Notwithstanding these successes, it cannot be denied that scFvs can be difficult to use, and a number of alternative approaches were described. V_H or V_L domains appear to be less problematic when they are separate rather than when joined together in the

scFv format. Yan Chen (Phylos; http://www.phylos.com) have used their Profusion™ technology (based on the covalent coupling of RNA to encoded protein using puromycin) to create V_H libraries and select binders with high affinities for a number of targets. One attractive feature of this selection platform, shared with ribosome display, is the ability to include affinity maturation in the selection cycle. Laurent Jespers (MRC; http://www2.mrc-lmb.cam.ac.uk) described the crystal structure, and biophysical properties, of a highly soluble refoldable V_H domain selected from a synthetic library based on a human V_H3 scaffold. These observations indicate that desirable solution properties in isolated V_H domains might be easier to achieve than previously anticipated (i.e. via camelisation). Andreas Pluckthun (Zurich University; http://www.unizh.ch) described binding ligands based on consensus ankyrin repeat sequences, comprising a 33 amino acid ßßαα structure. Proteins containing these repeats are usually involved in protein–protein interactions and have affinities for their partners in the low nanomolar range. Rather than use a natural ankyrin repeat, however, Pluckthun derived a consensus sequence based on the analysis of a large number of natural sequences (an approach previously shown to be useful to increase the stability of scFvs), and binders were rapidly selected from libraries based on this scaffold. The wisdom of this approach was shown by the high expression levels, as compared with the low expression levels obtained with a natural ankyrin repeat protein. The crystal structure of a complex showed that the binding took place where it was designed to, and the binding site strongly resembled the antibody combining site in many aspects.

This strategy of using highly stable scaffolds was also adopted by Andrew Bradbury (Los Alamos National Laboratory; http://www.lanl.gov) who described the use of an evolved form of GFP as a scaffold for binding ligands, termed 'Fluorobodies'. These intrinsically fluorescent binding ligands combine the advantages of antibodies (specific high affinity binding) with those of GFP (fluorescence, high expression and solubility) and are likely to be very useful in proteomic applications.

It is clear that the original hope that scFvs derived from scFv libraries would be able to replace antibodies in all research applications was overly optimistic. However, scFvs still have a very powerful role in the development of antibodies and antibody fragments, these being the most commonly developed protein therapeutics. The transplantation of variable regions from scFvs to immunoglobulins is a relatively straightforward procedure that preserves the specificity and often improves the affinity of the original scFvs. Stable scFvs are also likely to be useful therapeutically when short half-life or pure targeting domains are required. Given the relative ease with which antibody-based drugs can be developed and approved, scFv-related problems will continue to be tolerated in the discovery phase. However, for high-throughput applications, where robust expression and stability, coupled with affinity, are essential, alternative scaffolds, or highly stable scFvs, will have to be used.