Human Antibodies 13 (2004) 31–32
IOS Press

Session 9: Applied technologies – I

Friday 8 October 2004. Moderators:Kathy Potter and Zdenka Jonak

[08.30–09.00]
[Keynote Lecture]
Characteristics of surface IgG expressed by a common adult human B-cell leukaemia
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Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries and generally results from the clonal expansion of IgM+IgD+CD5+ B cells. Patients with CLL fall into one of two prognostic subsets based on VH gene mutational status. The unmutated subset (36%) has worse prognosis while the mutated subset (64%) has good prognosis. The V1-69 gene segment is overutilized in the unmutated subset while the V4-34 gene segment is overutilized in the mutated subset. Expression of IgG on the surface of CLL B cells is a relatively rarer event and may represent a further subset of the disease. We sequenced V_H genes from >200 CLL patients and found that ∼6% of samples expressed IgG. V_H3 was the most frequently used V_H family, followed by V_H4. V4-34 was the most predominantly used gene segment. The D2 family was used most often and JH6 was the predominant JH gene segment. All VH genes were mutated. The nature of VH insertions or deletions had characteristics similar to healthy IgG B cells. These data are consistent with IgG expressing CLL B cells being post-germinal center memory cells. While the nature of the stimulating antigen(s) is unknown, certain viruses and bacteria have been shown to induce V4-34 expressing B cells to secrete antibody.

[09.00–09.30]
Exploiting monoclonal and recombinant antibodies for the development of surface plasmon resonance-based sensor assays for bacteria, drugs and tumour markers
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Specific assays for the detection of bacteria, drugs and tumour-associated markers are of key importance in environmental and clinical analysis. We have developed a number of monoclonal and recombinant antibodies and antibody derivatives and incorporated them into conventional and surface plasmon resonance-based assay formats. The research has focused on Listeria-associated marker proteins, including internalin B. Using the Vaughan library a scFv was generated which recognises the GW repeat region involved in anchoring the protein to the cell membrane. Novel assay formats that will be described may provide a generic approach for the detection of a range of bacteria and the development and optimisation of such assays will be discussed.

Assays have also been generated for drugs of abuse, including morphine and amphetamines. The characterisation of these assays and their incorporation into several sensor formats will be described. Preliminary studies on the development of recombinant antibodies to tumour – associated antigens in prostrate cancer will be outlined.

ISSN 1093-2607/04/$17.00

[09.30–09.50]
Establishment of a new high yielding bio-manufacturing platform using a human cell line
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The market requirements for biopharmaceutical products are safety, quality, time to market and economical manufacture. DSM Biologics and Crucell have joined their combined strengths and expertise to develop and offer the market a mammalian cell line platform technology, PER.C6$^{\mathrm{TM}}$, capable to meet these market requirements.

Having chosen this new expression platform, the three main methods of manufacturing: batch, fed-batch and continuous perfusion were developed for production of a model antibody. The data show that the PER.C6$^{\mathrm{TM}}$ cell line produces biopharmaceuticals with high yields and human glycosylation profiles. This presentation will discuss data highlighting the performance of the PER.C6$^{\mathrm{TM}}$ platform technology for different types of manufacturing processes.

[09.50–10.10]
Engineering of *Escherichia coli* to improve the purification of periplasmic Fab' fragments: Changing the pI of the chromosomally encoded PhoS/PstS protein
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Abstract: We use *E. coli* as a host for the heterologous expression of Fab' fragments. The scale and speed at which it can be cultured can result in the rapid generation of large quantities of protein product. However, in order to achieve low costs of production a simple and robust purification process is also required.

Ion exchange purification of Fab' fragments from the periplasm of *E. coli* can result in the co-purification of *E. coli* host proteins with a similar functional pI: such as the periplasmic phosphate binding protein, PhoS/PstS. In such circumstances an additional chromatographic step is required to separate the Fab' from PhoS. Here we use protein engineering to change the functional pI of the chromosomally encoded PhoS/PstS to effect its non-purification with Fab' fragments, enabling the removal of an entire chromatographic step. The strategy employs use of a markerless chromosomal recombination technique. This exemplifies the strategy of the modification of the proteins of production hosts with the aim of simplifying the production of heterologous proteins.

[10.10-10.30]
Monoclonal antibodies produced by muscle after plasmid injection and electroporation
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Abstract: Antibodies are useful for the treatment of a variety of diseases. We here demonstrate that mouse muscle produced monoclonal antibodies (mAb) after a single injection of immunoglobulin genes as plasmid DNA. In vivo electroporation of muscle greatly enhanced antibody production. For chimeric antibodies, levels of 50–200 ng mAb/ml serum were obtained but levels declined after 7–14 days due to an immune response against the xenogeneic parts of the antibody. By contrast, fully mouse antibodies persisted in serum for at least 7 months. mAb produced by the muscle had correct structure, specificity and biological effector functions. The findings were extended to a larger animal, the sheep, in which mAb serum levels of 30–50 ng/ml were obtained. Sustained levels of serum mAb, induced by single injection of Ig genes and electroporation of muscle cells, may offer significant advantages in treatment of human diseases.