Human Antibodies 15 (2006) 33–36
IOS Press

Session 7: Applied technologies

Friday 12 May, 2006. Moderators: Jim Larrick and Alois Lang

[08.30-09.00]
[Keynote Lecture]
Progress with novel therapeutic antibodies in novel transgenic models
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Abstract not received.

[09.00-09.20]
Multi-PEGylation, a new high efficiency strategy for the PEGylation of therapeutic antibody Fab fragments
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In order to confer desirable serum half life properties for therapeutic applications, Fab fragments purified from the periplasm of *E. coli* have previously been site specifically mono-PEGylated at the hinge with 40kDa of PEG. In this work we show that multi-PEGylation can be achieved with efficiencies as high as ~90% using PEG ranging in size from 20kDa to 40kDa. In this strategy the combined use of non-thiol reductants and engineered Fab can result in controlled site specific PEGylation with 1, 2 or 3 PEG molecules. Final PEGylated entities from this approach lack an interchain disulphide. We present evidence to show that serum half life, antigen affinity, functional antigen neutralisation and routes of administration are unchanged by the lack of the interchain disulphide. Data demonstrating physico-chemical stability is also presented.

[09.20-09.40]
Rapid and selective production of hybridoma cells secreting novel monoclonal antibodies based on short-term immunization
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Introduction: Hybridoma cells secreting monoclonal antibodies directed against aimed antigens are generally produced by fusing sensitized B lymphocytes and myeloma cells. The original idea for this technology was provided by Köhler and Milstein [1]. Since then, monoclonal antibodies have been widely used for many aims, for example in identifying biological phenomena, and for therapeutic and diagnostic purposes. Although the necessity for monoclonal antibodies has been increasing, Sendai virus- or poly(ethylene glycol)(PEG)-mediated fusion methods are still used for raising monoclonal antibodies against antigens of interest [1,2]. The disadvantage of these methods is that they cause non-specific fusion not only between spleen-myeloma pairs of cells, but also spleen-spleen and myeloma-myeloma pairs, resulting in lower production of the desired hybridoma cells. To address this problem, we developed a new antigen-based B-cell targeting technique, also known as the pulsed electric field (PEF) method [3-6]. The new technology consists of three critical steps. Firstly, sensitized B lymphocytes are selected by an antigen based on immunoglobulin receptors expressed on the surfaces of B lymphocytes. The antigen-selected B lymphocytes are then combined together with myeloma cells by harnessing the power of strong and specific interactions between biotin and avidin. Finally, biotin/avidin-coupled B lymphocytes and myeloma cells are selectively fused by imposing an electric field. This technology provides 5-40-fold higher efficiency that obtained by the PEG-mediated method [6]. In addition, lately, we have found that the B-cell targeting technique is also ap-

ISSN 1093-2670/06/$17.00

plicable for *in vitro* immunization. Thus, B lymphocytes after immunization *in vitro* were successfully targeted by antigen molecules. *In vitro* immunization harbors several advantages. Small amounts of antigen (1-10μg) are sufficient for immunization and the immunization process is completed in 3 to 5 days, and toxic antigens are also usable. The addition of interleukin-4 (IL-4) and lipopolysaccharide (LPS) during immunization *in vitro* may bring about an increased number of sensitized B lymphocytes and class-switching of monoclonal antibodies from IgM to IgGs [7,8].

In the present study, we focused on rapid and selective production of hybridoma cells secreting novel monoclonal antibodies using the B-cell targeting technique and short-term immunization.

Production of hybridoma cells secreting monoclonal antibodies

General method: Immunization is usually carried out using an *in vivo* method that requires 1 to 2 months. Spleen cells from an antigen-sensitized mouse after *in vivo* immunization are fused with myeloma cells mediated by PEG. The PEG-mediated fusion method causes non-specific fusion and brings about lower fusion efficiency. Only a few hybridoma cells obtained by this method secrete the aimed for monoclonal antibodies and it usually takes several months to isolate hybridoma cells secreting the desired monoclonal antibody. In some cases, this method fails to yield appropriate hybridoma cells (Figure 1, left).

Advanced method: Spleen cells from a non-immunized mouse are sensitized *in vitro* for 3 to 5 days under a humidified 5%:95% CO_2/air gas mixture at 37° [9]. Sensitized B lymphocytes are then selected by an antigen based on antigen-specific immunoglobulin receptors on B lymphocytes and attached to myeloma cells by exploiting the specificity and strength of the interaction between biotin and avidin (or streptavidin). B lymphocyte-myeloma cell complexes are then selectively fused by an electrical pulse. This advanced method brings about higer fusion efficiency, and realizes selective generation of hybridoma cells secreting novel monoclonal antibodies in a short term with predefined specificity (Figure 1, right).

Immunofluorescence analysis of B lymphocytes

Pre-selection of B lymphocytes: B lymphocytes after immunization *in vitro* were selected by a biotinylated antigen using surface immunoglobulin receptors, and specifically labeled with streptavidin-PE (or Rh) (Figure 2). When protein molecules such as ovalbumin and keyhole limpet hemocyanin were employed as antigens, specifically stained B lymphocytes were observed even after short immunization *in vitro*. In addition, B lymphocytes immunized with human insulin, one of the peptide hormones, were also pre-selected by an antigen after short-term immunization.

Expression of IgM and IgD double-positive B lymphocytes

Maturation of B lymphocytes: The coexistence of IL-4 and LPS during immunization *in vitro* facilitated expression of IgM^+ and IgD^+ B lymphocytes (Figure 3). It is known that mature B lymphocytes express both IgM and IgD on their cell surfaces [10]. LPS increases the antibody response by stimulating B lymphocytes [10] and LPS plus IL-4 enhances class-switching from IgM to IgG [11]. Our preliminary results indicate that immunization *in vitro* in the presence of IL-4 and LPS enhances class-switching of immunoglobulins. In this study, successful production of specific monoclonal antibodies against keyhole limpet hemocyanin was verified by employing the B-cell targeting technique and *in vitro* short-term immunization. In the case of human insulin, however, the monoclonal antibodies yielded showed relatively wide cross-reactivities.

In conclusion, we here demonstrate the generation of specific monoclonal antibodies against a protein molecule using a B-cell targeting technique after immunization *in vitro*. This result suggests that the combination of B-cell targeting technique and *in vitro* immunization might realize selective production of monoclonal antibodies against antigens of interest in a short term. Moreover, this technology may also be applicable for human monoclonal antibodies.

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[09.40-10.10]

Use of the ABI 8200 cellular detection system in the generation and characterisation of hybridomas

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The Antibody Technology Groups within GSK generate monoclonal antibodies against a broad range of targets for multiple applications. Identification of monoclonal antibodies with desired antigen binding properties is an important part of monoclonal antibody selection criteria. The groups at GSK have recently incorporated the ABI 8200 Cellular Detection System into the production of hybridomas. The high throughput (60 x 384 well plates overnight) and the mix and read assay nature allow automated multiple primary screening assays to be performed for each target. Examples of cell and bead-based assays for hybridoma screening and characterisation will be presented.

[10.10-10.30]

Effect of tobacco extract and temperature on the stability of the monoclonal antibody CB.Hep-1 expressed in transgenic tobacco plants

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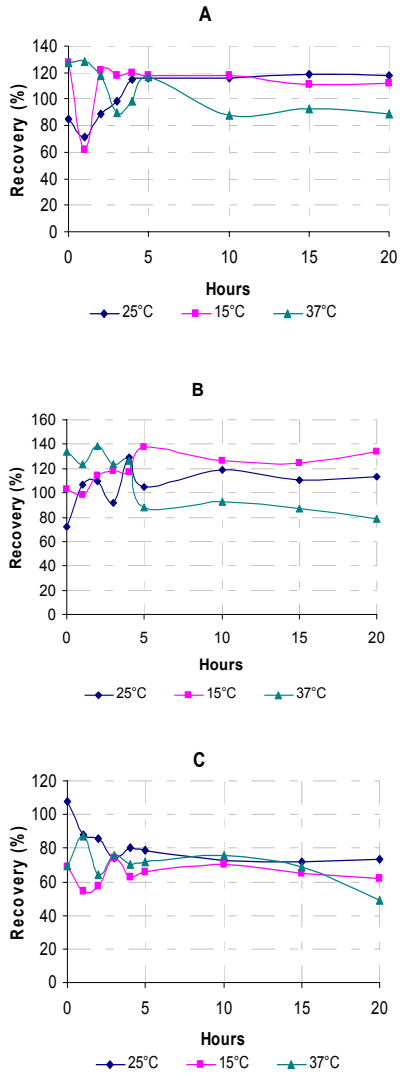
Transgenic plants are a potential system for large-scale production of proteins with pharmaceutical applications. However, little is known about the influence of plant extracts from which these proteins should be purified. In this study, the stability of the monoclonal antibody CB.Hep-1 produced in transgenic tobacco plants was analyzed at different temperatures during 20 h in a non-transgenic tobacco extract without exogenous proteolytic activity inhibitors. Additionally, to assess the contribution of the glycosylation pattern to the molecule stability, the same monoclonal antibody produced by ascitic fluid and hollow fiber bioreactor served as controls. Results indicate that incubation of the monoclonal antibody CB.Hep-1 in tobacco extract at 15°C, 25°C and 37°C did not induce significant changes in the electrophoretic pattern of the heavy and light chains of the monoclonal antibody. The monoclonal antibody antigen recognition capacity was not affected by the incubation of the molecule in these conditions but decrease in this parameter was observed at 37°C. Therefore, proteolytic degradation of the monoclonal antibody (IgG-2bk) in tobacco extract is not an important factor affecting the homogeneity of transgenic antibodies and the recovery of the purification process. Interesting, differences in the glycosylation pattern did not increase the susceptibility of this IgG-2bk molecule to the tobacco extract conditions in the evaluated temperatures.

Antigen recognition capacity measured by ELISA of the monoclonal antibody CB.Hep-1 in tobacco extract at different temperatures during 20 hours. A. mAb CB.Hep-1 produced by ascites method. B. mAb CB.Hep-1 produced by hollow fiber bioreactor. C. mAb CB.Hep-1 produced by transgenic tobacco plants. The data represented on the graphics are the percentage of recovery of the true values over the expected values of mAb CB.Hep-1 concentrations.



[10.30-10.50]

Kinetic characterization and quantification of antibodies in expression media using resonant acoustic profiling

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Resonant Acoustic Profiling (RAP™) enables real time label-free analysis of antibody interactions by measuring the change in frequency as mass binds to a resonating quartz wafer.

Antibodies or antigens are covalently immobilized or specifically captured onto the sensor surface, as the interacting partner is passed over the surface, binding is detected. Due to the direct detection of bound mass, minimal interference is observed from components commonly found in buffers and expression media.

Using this technique we analyzed hundreds of samples, taken directly from expression media, in a single automated assay. The results show that the same sensor surface can be used for the analysis of hundreds of samples with minimal user input, thus demonstrating the potential of RAP technology in the screening of antibody affinity and yield directly in expression media.