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Poster Session

Direct selection of synthetic antibody fragments on cell surface targets from a phage-displayed library
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The considerable heterogeneity of cell surfaces makes selection of phage-displayed antibody libraries against cell-surface antigens quite challenging. However, many proteins require the membrane environment for proper folding and stability, and as such, the ability to select phage-displayed antibody libraries against cell-surface epitopes remains crucial. We report the development of a novel method for rapidly isolating phage-displayed antibody fragments (Fabs) to cell-surface targets, using the oncogenic human epidermal growth factor receptor 2 (Her2) as a model. Our method enabled us to recover antibody fragments from a phage-displayed synthetic Fab library that bind specifically and with high affinity to Her2, as assessed by surface plasmon resonance. Flow cytometry and immunofluorescence staining demonstrates that the isolated Fabs also bind specifically to Her2 expressed on the surface of transiently transfected cells and Her2+ breast cancer cell lines. Competitive ELISAs revealed that specific Fabs bind to epitopes that are unique from that recognized by the monoclonal antibody (mAb) Herceptin, which has been one of the most successful targeted molecular therapies in the treatment of Her2+ breast cancer. However, given that the acquisition of Herceptin-resistance can significantly hamper clinical treatment of Her2+ breast cancer, there is a need for additional molecular therapies targeting Her2. Our data show that treatment of Her2+ breast cancer cell lines with specific Fabs results in anti-proliferative and pro-apoptotic activities. Together, these results suggest that our novel cell-selection approach can increase the efficiency of library selections to cell-surface targets and be used to drive the development of additional molecular therapies against aggressive cancer targets.

Hydroponic plant culture for the production a monoclonal antibody raised against MUC-1
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Plants offer an inexpensive alternative to traditional systems for the production of recombinant monoclonal antibodies. However, cultivation of transgenic plants in the field raises regulatory concerns regarding product quality and uniformity. Contained hydroponic cultivation of plants is a highly controlled production platform in which recombinant pharmaceuticals can be produced not only in the vegetative tissues of the plant, but also by the process of rhizosecretion. Rhizosecretion is potentially an appealing process as recombinant proteins can be harvested over the entire lifetime of the plant, and, most importantly, downstream processing is simplified as extraction is from simple hydroponic medium rather than complex plant tissues. Although advances have been made in the use of rhizosecretion for expression of recombinant pharmaceuticals, the principal limitation of the system has been the relatively low yields that can be obtained. Here, we present the development of a hydroponic system for tobacco plants, for the production M12, a monoclonal antibody raised against the epithelial tumour marker MUC-1. Optimization of rhizosecretion levels for M12 was obtained by manipulation of medium compounds and mechanical stimulation of roots. Initial yields were enhanced 60-fold, reaching on average 30 μg/ml/week, although a yield of 100 μg/ml has been observed, giving rise to optimism that there is further room for improvement. Further optimization is being assessed by manipulation of abiotic conditions, utilization of stabilizers and protease inhibitors. The production system developed is simple, inexpensive and potentially rapid to scale up, with the development of an automated fluid handling system. This low-tech approach addresses many regulatory concerns and would be readily transferable to low-income regions.

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Non-classical binding of a polyreactive α-type anti-idiotypic antibody to B cells

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Detailed information on the immunological relevance of α-type anti-idiotypic antibodies is lacking after more than 30 years since Jerne postulated his Idiotypic Network Theory. The B7Y33 mutant is a mouse human chimeric version of the B7 MAb, a polyreactive α-type anti-idiotypic antibody, generated against an anti-GM2 ganglioside IgM Ab1 antibody. It retained the unusual self-binding activity and multispecificity of the parental murine antibody, being able to recognize several anti-ganglioside IgM antibodies as well as non-immunoglobulin antigens. Previous work with the murine B7 MAb suggested that this antibody might have immunoregulatory properties, and therefore we investigated the possible interaction of B7Y33 with immune cells. We found that B7Y33 binds to human and murine B lymphocytes. Inhibition assays using flow cytometry indicated that this antibody is capable of binding the Fc γ receptor II (FcγRII).

The recognition of FcγRII-expressing K562, Raji and Daudi human cell lines, together with the capability of inhibiting the binding of an anti-human FcγRII antibody to these cells, suggest that B7Y33 interacts with both the FcγRIIa and FcγRIIb isoforms. We evaluated the contribution to the binding of different surface-exposed residues at the top of the heavy chain variable region (VH) CDR loops through the construction of mutants with substitutions in the three conventional VH CDRs (HCDRs) and the "HCDR4", located in the framework 3 (HFR3). In addition, we assessed the involvement of the Fc region by performing key mutations in the CH2 domain. Furthermore, chimeric hybrid molecules were obtained by combining the B7Y33 heavy chain with unrelated light chains. Our results indicate that the multispecificity and self-binding properties of B7Y33 are not linked to its recognition of B lineage cells, and that this phenomenon occurs in a non-classical way with the participation of both the variable and constant regions of the antibody. Two possible models for this interaction are proposed, with B7Y33 binding to two FcγRIIb molecules through the Fc and Fv regions, or simultaneously to FcγRIIb and another unknown antigen on B cells. The FcγRIIb has recently received great attention as an attractive target for therapies directed to B lymphocytes. The recognition of peripheral B lymphocytes from B cell chronic lymphocytic leukemia (B-CLL) patients by B7Y33 suggests its potential application for the treatment of B cell malignancies.

Anti-ricin neutralizing monoclonal antibodies

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Four anti-ricin hybridoma clones were developed and all four monoclonal antibodies (mAbs) were found to have high ricin-neutralization potency both in an *in vitro* neutralization assay and an *in vivo* antibody/ricin co-incubation assay, indicating the strong inhibition of ricin-mediated cell death. Among the four mAbs, D9 was found to be exceptionally active and further tested for pre-exposure prophylaxis and post-exposure therapy against ricin *in vivo*. Intraperitoneal (i.p.) administration of D9 at a dose as low as 5 μg per mouse protected mice not hours, but 6 weeks before i.p. toxin challenge (5 $\times$ LD50 of ricin), and rescued mice not minutes but 6 hours after i.p. poisoning (5 $\times$ LD50 of ricin). D9 also showed synergistic effects with other anti-ricin mAbs, as determined by the *in vitro* neutralization assay. Furthermore for clinical applications, D9 was humanized to minimize potential immunogenicity in humans. The humanized D9 remained its high affinity for the ricin and more importantly its ricin neutralizing capacity as well, comparable to its parental mouse D9. Thus, the humanized D9 has great potential to be developed as antibody-based therapeutic agents or antibody-gene based vaccines against ricin.

Tag-lite® is a powerful solution for the cellular screening and characterization of therapeutic antibodies targeting Receptor Tyrosine Kinases

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The development of promising new therapeutic strategies, such as monoclonal antibodies directed against Receptor Tyrosine kinase (RTK) has become a challenge in the treatment of cancers. Since the end of 1990's, biotherapeutic antibodies have represented an increasing group of FDA-approved medicines mainly in the oncology and immunology therapeutic areas. The selection of potent and selective MAbs is therefore the first critical step in the process running from drug discovery to clinical trial phases. Here we present an innovative, universal and simple assay which allows the screening and the characterization of antibodies targeting cell-surface receptors. This method is based

on the recently launched Tag-lite platform which combines HTRF detection with the fluorescent labeling of receptors using the SNAP-Tag technology. On living cells, the binding of specific antibodies to a receptor of interest is detected through a time-resolved energy transfer occurring between a HTRF donor-labeled receptor and a secondary anti-species antibody labeled with a HTRF acceptor. This assay was successfully applied to monitoring antibody binding to a large set of RTK. By performing competition experiments with natural RTK ligands, our approach determines whether the previously selected antibodies were directed or not against the orthosteric binding site. Moreover, it enables complete antibody characterization by determining its affinity (K_D) and pharmacokinetic (K_{off}) constants. We demonstrate that Tag-lite® is a powerful, easy to use and efficient technology to screen and characterize antibodies targeting RTK on living cells. This flexible and radioisotopic- free method enables the detection of different and specific binder classes, as well as the determination of their pharmocological features and functional responses.

A new cellular binding platform to measure the Fc gamma receptor (FcγR)/hIgG interaction based on the Tag-lite® technology

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Immunoglobulin G1 (IgG1) Fc receptors play a critical role in linking IgG1 antibody-mediated immune responses with cellular effector functions. Multiple FcγRs exist, which differ in ligand affinity, cellular distribution, and effector function.

The focus of this study is on CD16a, a low affinity receptor for monomeric IgG which has been exhaustively described to recruit effect cells in Fc-dependent cellular cytotoxicity events. This receptor exists under 2 different polymorphism variants differing at position 158 (Val/Phe). CD16a (Val 158) displays a higher affinity for the Fc fragment compared to CD16a (Phe 158). This difference in affinity has been directly correlated to the effector functions mediated by the ADCC (Antibody-Dependent Cellular Cytotoxicity) mechanism.

The design of new generations of improved antibodies for immunotherapy should aim at Fc optimization to increase the engagement of activating FcγR present on the surface of tumor-infiltrating effector cell populations.

With this goal in mind, we have developed a cellular binding assay to efficiently and precisely measure the binding of the different subclasses of IgG with the CD16a variants. We also evaluated the effect of antibody fucosylation on the FcγR interaction.

MabLib: display and selection of large diversity antibody libraries in mammalian cells

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In vitro display of antibody libraries has largely relied on display of antibody fragments on phage or fragments and full length antibodies on yeast. Due to limitations of transfection and cell numbers display of large complexity libraries has been limited in eukaryotic hosts. We've developed an antibody capture format for display of full length antibodies on mammalian cells. Based on this method we are able to display 1E5-1E6 full length IgG's per cell. Large diverse collections of antibodies can be displayed in this manner as essentially polyclonal pools. To select and identify individual antibodies within these pools we have developed a protocol encompassing iterative steps of; selection, amplification and deconvolution. In several test examples this process has resulted in the identification of full length IgG clones with affinities in the range of 100pM–10nM.

B-cell hybridoma coculture within EL-4 thymoma cells enhances hybridoma cell growth and monoclonal antibody production

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In this study, we have investigated a possible helper activity by the murine thymoma cell line EL4 in stimulating hybridoma cell growth (55–6 cell line) and monoclonal antibody (mAb) secretion in cultures containing both cell types.

Results obtained shown that the presence of EL-4 cells in conditions of low numbers of EL-4 cells per 55–6 cells (1 EL-4 cell per 4 55–6 cells) had a helper activity in stimulating the specific growth rate and mAb production by 55–6 cells. Both populations were seeded in coculture without previous stimulation and, therefore low constitutive CD40L and CD40 expression levels and without adding cytokines to the medium. It is therefore, very likely that the direct effect of EL-4 cells in these conditions (1:4 ratio) could be due to additional molecule(s) released to the medium for these cells, more than to a cell contact mediated for CD40

and CD40L molecules or others. We also observed a positive effect of 55–6 cells on specific EL-4 growth rate. By the contrary what observed for 55–6 cells, the increasing proportions of 55–6 cells per EL-4 cells did not decrease this effect suggesting that EL-4 cell growth is not limited by the nutrient competition between both cell types.

This fact point out that this kind of mixed cultures in an optimum relation for both populations could be interesting in the development of production processes in a cheaper way than other current processes.

Effects of CD40 activation in B-cell hybridomas engineered to overexpress CD40: Clonal variation for proliferation and monoclonal antibody production

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Vigorous growth of hybridomas and stable and high production of antibodies are the two major challenges faced by the hybridoma-based monoclonal antibody industries. Genetic modulation of CD40 dependent signalling pathways could be a route to achieve these goals of improved stability and production of antibodies. We present early results of studies of CD40 activation in human antibody-secreting B-cell hybridomas engineered to overexpress CD40. All hybridoma clones were from the same fusion event from a single mouse immunized with HIV-1 gp140 antigen. Three B-cell hybridoma clones overexpressing CD40, two of which clonally related, were activated using increasing concentrations of either CD40 ligand (CD40L) or anti-CD40 antibodies in the presence of interleukin (IL)-2, IL-4, IL-10 and lipopolysaccharide (LPS). The early results of our studies suggest clonal level differences in both proliferation and antibody production by hybridomas overexpressing CD40 and activated by either anti-CD40 or CD40L. Some clones responded positively to CD40 stimulation and showed greater proliferation, or greater antibody production. Others showed little or no effects and sometimes detrimental effects of CD40 activation. Our early results suggest that clones of hybridomas secreting antibodies to the HIV-1 gp140 antigen can be genetically engineered to overexpress CD40 and could provide a valuable resource for isolating sub-clones/cell lines with high proliferation in vitro and also higher production of antibodies. The stability of some of these hybridoma clones are currently being assessed and validated.