

Session 4: Molecular biology – I

Thursday 11 May, 2006. Moderators: Jim Larrick and Sachdev Sidhu

[08.00-08.30]

Alternatives to whole antibodies for human therapy

Dee Athwal

CellTech, Slough, UK

Abstract not received.

[08.30-08.50]

CDR repair: A novel approach to antibody humanization

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Current humanization methods typically involve the grafting complementary determining regions (CDRs) from a donor antibody onto a homologous human germline framework. This generally results in a dramatic loss in antigen binding affinity. The transfer of donor framework residues to the human scaffold can usually restore affinity by re-establishing a proper foundation for the CDRs; however, this approach requires insightful molecular modeling and involves the construction and analysis of many variants.

Rather than repairing the framework to accommodate the grafted CDRs, we have explored the possibility of altering the CDRs to fit the new human framework. CDRs from a donor antibody were grafted onto a human consensus variable light and variable heavy framework and displayed on phage. A large library of mutations targeted solely within the CDRs was generated and panned against antigen. Surprisingly, the selected antibody sequences were found to have specific and targeted changes within select CDR regions and their affinity for antigen was generally improved compared to that of the starting antibody.

Thus we demonstrate that CDR repair can rapidly restore high affinity binding to CDR grafted antibodies through slight modifications within the CDRs and without changes to the framework. The humanization of multiple antibodies will be discussed.

[08.50-09.10]

The multiple benefits of *in vitro* evolution for antibody discovery and development

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In vitro evolution of antibody variable regions using ribosome display is a powerful tool to improve the potency of therapeutic antibodies. The method can be used to affinity mature antibodies in a process analogous to somatic hypermutation in germinal centres *in vivo*. The benefits of using this technology will be exemplified by data from multiple *in vitro* evolution experiments. Each of these was successful in deriving therapeutic antibodies in the picomolar affinity and potency range, allowing these antibodies to progress into preclinical and clinical studies.

To extend the capabilities of the ribosome display platform we have also explored its use in the mapping of protein:protein interaction surfaces. Mapping the key residues in protein interfaces can provide extremely useful data to inform decisions during the discovery and development of antibody drugs. We have performed *in vitro* selections using ribosome display to identify the residues essential for antigen binding in a candidate therapeutic antibody. The benefits of this type of combinatorial approach to protein: protein interaction mapping will be discussed by comparison to existing mapping methods.

[09.10-09.30]

Novel ways to humanize and optimize antibodies for therapeutic applications

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EvoGenix offers a fully integrated approach to creating novel antibody products, moving from a non-human antibody to a potent product ready for administration to patients. Superhumanization™ represents a novel system for converting a mouse antibody, which would cause an immune reaction if administered to humans, into a humanized form, capable of repeat administration.

EvoGene™ protein optimisation technology, for test tube evolution of proteins provides products with higher value as drugs including greater activity and potency, greater specificity and greater stability or expression. The EvoGene™ approach can be applied not only to antibodies, but to any protein including therapeutics, diagnostics, industrial enzymes or agribiotech products.

[09.30-09.50]

Insights into antigen recognition from minimalist synthetic antibodies

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Antibodies can evolve to recognize essentially any protein with high specificity and affinity. While natural antigen binding sites utilize all 20 natural amino acids to some extent, analysis of functional antibodies reveals clear biases for or against some amino acids. Most significantly, tyrosine and serine are highly abundant in antigen binding sites in general and at antigen contact sites in particular.

As it is now possible to construct antibody-phage libraries with synthetic CDR diversity, we have used synthetic antibody libraries to investigate the roles of different chemical diversity in antigen recognition. Using a tetranomial genetic code that allows for only four amino acids (tyrosine, serine, alanine, and aspartate) we were able to generate antibodies against vascular endothelial growth factor (VEGF) that bound with high affinity and specificity. Structural and mutational analyses indicated that tyrosine was the major mediator of binding energy at the antigen binding sites of the anti-VEGF Fabs, and the results sug-

gested that it might be possible to further simplify the code for antigen recognition. To this end, we constructed Fab libraries in which CDRs were randomized with a binary genetic code that allowed for only tyrosine and serine. We envisioned that in these libraries, tyrosine might act as an effective “functional” amino acid with a large side chain that could provide significant binding contacts. In contrast, serine might act as an “auxiliary” amino acid with a small side chain that could provide space for the tyrosine side chains and also contribute to an overall hydrophilic surface.

Remarkably, naïve Fab libraries constructed with the binary code were extremely effective in generating highly specific antibodies against a wide array of antigens. Furthermore, the binary Fabs exhibited exquisite specificity in cell-based assays. The structure of one such binary Fab in complex with its antigen (human death receptor 5), revealed that the highly homogenous binding surface is dominated by tyrosine, but nonetheless, recognizes diverse chemical groups on the antigen. In ongoing studies, we are now adding back chemical and conformational diversity into the minimalist binary background in a precisely defined manner, and these highly controlled studies will enable the accurate assessment of the consequences of expanded diversity.

Our results suggest that certain amino acids possess features that increase the likelihood that they will be able to make productive contributions to binding affinity and specificity. Thus, biased libraries that favour such amino acids are likely to be much more effective in generating antibodies with high affinity and specificity. As a corollary, it seems that other amino acids are ill suited for productive contacts, and the absence or depletion of such amino acids will likely improve naïve antigen recognition. The results should aid the design and use of synthetic antibody libraries. In addition, and perhaps more importantly, the findings have significant implications for the fundamental principles and mechanisms that mediate molecular recognition at protein-protein interfaces.

[09.50-10.10]

High-throughput cloning and characterization of Fab-on-Phage library isolatesJürgen Müllberg *et al.**Dyax Corp., 300 Technology Square, Cambridge MA, 02139, USA*

The ability to rapidly identify therapeutic candidates from phage display libraries relies not just on

the ability to quickly screen the library to identify hits but also on the timely completion of the subsequent processing and characterization of those hits. Our large combinatorial Fab-on-phage display library was designed to facilitate the production of 'hits' by allowing the easy transfer of Fab-cassettes into expression vectors for production of soluble Fabs and the conversion of the Fab fragment into a full IgG. Library isolates can be handled either singly or in parallel which allows multiple Fab isolates to be easily produced for characterization. In our workflow, characterization of the expressed material generally includes an initial evaluation using a predictive cell assay. Since the majority of the cell based functional assays used cannot be performed using crude anti-

body containing culture media, but require purified reagents, we developed a high-throughput method for small scale Protein-A purification of IgGs from conditioned media, using automated liquid handlers. Here we highlight the application of our rapid recloning and characterization approach using Fab isolates specific for Tie-1, a human receptor tyrosine kinase involved in angiogenesis, and a target for tumor therapy. In this study, 131 Fab isolates specific for Tie-1 were transferred to IgG1 expression constructs, produced and evaluated using a cell binding assay. This example demonstrates that our approach allows for fast generation and characterization of fully human IgGs, an important format of therapeutic antibodies.