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The amazing, multipurpose antibody

Alain Beck[1] and Janice M. Reichert[2,*]

[1]Centre d'Immunologie Pierre Fabre; Saint Julien en Genevois, France; [2]Landes Bioscience; Austin, TX USA

Natural immunoglobulins, like Swiss Army™ multipurpose knives, are an integrated tool box contained within a single unit. Increasing knowledge of antibody structure and activity now allows researchers to isolate novel antibodies from designed libraries, engineer primary antibodies on a more rational basis and extend the potency of these molecular tools. The approaches can yield IgG-related structures with additional functions and specificities, such as increased cytotoxicity or dual targeting, as well as more homogeneous and stable molecules. A number of articles in this issue of *mAbs*, e.g., Igawa et al., Villa et al., Dong et al. and Fitzgerald and Lugovskoy, discuss new approaches designed to yield novel antibodies with desirable pharmaceutical properties.

The variable domain (Fv) of an antibody is responsible for interactions with antigens and dictates essential properties such as binding affinity and target specificity. The origin of the Fv can be diverse, e.g., hybridomas, human antibody libraries or rodents with a human antibody repertoire. Affinity maturation allows the binding affinity of the Fv to be improved or target selectivity to be modulated. The constant domain (Fc) of an antibody is responsible for interactions with immune cells; the associated properties of the Fc can also be modulated by engineering at several levels, e.g., altering the glycosylation status to modulate anti- and pro-inflammatory properties; modulation of antibody-dependent cellular cytotoxicity by site-directed mutagenesis that alters binding to Fc receptors; increasing the serum half-life by Fc engineering to increase binding to the neonatal Fc receptor (FcRn), which prevents IgG degradation; increasing complement activation by isotype chimerism.

For most diseases, multiple mediators contribute to overall pathogenesis by distinct or redundant mechanisms. The simultaneous blockade of multiple targets might therefore yield better therapeutic efficacy than inhibition of a single target. Building on the work of numerous research groups, in 2009 Fresenius Biotech received the first marketing approval for a bispecific (bivalent) antibody invented by Trion Pharma. Catumaxomab (Removab®), which targets the tumor-associated antigen EpCAM as well as CD3 on T cells, was approved in Europe for the treatment of malignant ascites. Other promising bispecific antibodies are undergoing evaluation in clincal studies, including blinatumomab (specific for CD19 and CD3), which is being investigated in Phase 2 studies of patients with minimal residual disease of B-precursor acute lymphoblastic leukemia.

The use of bispecific antibodies directed against two different, disease-relevant targets is another strategy that has been investigated, but with only limited success due at least partly to the highly heterogeneous mixtures that result from the multiple possibilities of immunoglobulin chain association, as well as scale-up and purification issues. These difficulties have been recently overcome by the dual variable domain (DVD)-Ig technology. This novel immunoglobulin was obtained by combining the variable domains of two characterized monoclonal antibodies (two VLs on the light chain and two VHs on the heavy chain), as demonstrated with IL-12- and IL-18-specific antibodies or with IL-1α and IL-1β-specific antibodies. This technology enables the distinct specificities of two antibodies to be engineered into a single, functional, dual-specific, tetravalent IgG-like molecule, with good production yields in a scalable CHO cell line. Another elegant approach consists of engineering an additional paratope in the variable domain of an existing antibody, allowing, for example, simultaneous binding to HER2 and VEGFA. Using either approach, the designed proteins can be produced as a homogeneous single, functional species with productivities similar to conventional IgGs, which was not the case for the previous bispecific antibody formats.

Structure-function relationship studies of chimeric, humanized and human IgGs with similar constant domains that aim to identify antibody micro-variants and investigate the affect of these variants on antigen binding, stability, pharmacokinetics and pharmacodynamics have been recently published. For example, high-resolution mass spectrometry methods in combination with ultra-performance separation techniques are routinely used at all stages of antibody discovery and development to assess antibody structure. New analytical tools such as these have resulted in the identification of minor antibody components, e.g., charge variants, glycoforms, disulfide bridge isoforms and other low level molecular species and aggregates. Knowledge derived from analytical studies is now being used during lead optimization to increase homogeneity and mitigate the chemistry, manufacture and control liabilities of preclinical antibody candidates through genetic engineering. The removal by mutation of instability or aggregation hot spots in the antibody complementarity-determining regions and the use of hinge-stabilized or aglycosylated IgG4 are just a few examples of methods that yield antibodies with improved pharmaceutical properties.

As editors of *mAbs*, a multi-disciplinary journal dedicated to the art and science of antibody research and

*Correspondence to: Janice M. Reichert; Email: janice.reichert@landesbioscience.com
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development, we look forward to publishing cutting-edge studies in these research areas. The amazing, multipurpose features of antibodies are exemplified in the 27 therapeutic mAbs now approved in the European Union or United States (see www.landesbioscience.com/journals/mabs/about for a complete list), including belimumab (Benlysta®) and ipilimumab (Yervoy®) approved in March 2011. Considering that there are three additional antibody-based molecules (raxibacumab, brentuximab vedotin, aflibercept) in regulatory review, over 300 novel candidates in clinical studies and a vast number undergoing discovery, we are confident that antibody research and development has a bright future.