Antibodies have been used as therapeutics in various forms for over a century. Traditional immunoglobulin therapy has the advantage of reflecting the diversity of the natural immune response, but has limited clinical applications. During the past 10 years, many monoclonal antibodies have been successfully introduced to the market. The monoclonal antibody approach has the advantage of specificity, but often lacks efficacy in the treatment of diseases caused by complex antigens. Recombinant polyclonal antibodies are expected to have the advantage of specificity, but often lacks efficacy in the treatment of diseases caused by complex antigens. Recombinant polyclonal antibodies are expected to have the ability to tackle complex and highly mutagenic targets, and hold much promise for the future – but their large-scale industrial production has, until recently, remained elusive. However, new discovery and expression technology platforms – as discussed in the present review – have provided a means for the consistent and robust manufacturing of recombinant polyclonal antibody compositions, and thus constitutes a third generation of antibody therapeutics to enter clinical development.

Antibody Therapeutics

A key aspect of the body’s reaction to infection is the activation and clonal expansion of many different antigen-reactive B lymphocytes. Once these have matured into plasma cells (antibody-producing cells), each clone of cells will secrete its own unique specificity of antibody – thus, the invading pathogen will be met by a barrage of antibody molecules capable of binding at many different sites on its surface. The range of specificities and affinities of such a polyclonal response can shift with time making it ideal for combating infection. The importance of antibodies was recognised more than a century ago, when it was discovered that protection against certain toxins was conveyed by substances in the blood – referred to as antitoxins or antibodies. Since the first administration by von Behring (1) of antibodies in the form of animal-derived sera in the 1890s, antibody therapeutics have come a long way.

Traditional Immunoglobulin Therapy

Early antibody therapy involved purification of the immunoglobulin fraction of animal and later human donor plasma, and subsequent infusion in patients. Plasma-derived immunoglobulin from normal healthy donors offers the advantage of the polyclonal natural immune response, with both a diverse and specific repertoire, and remains a preferred choice in the treatment of selected conditions (2). Deriving immunoglobulin from human plasma that reflects the multitude of binding specificities in the natural immune response implies that only a small fraction of all the immunoglobulin injected is actually targeting the particular antigen of interest. One way of enhancing the amount of relevant antibodies is to use hyperimmune immunoglobulin. These are derived from individuals who have, for instance, recovered from an infection and have developed a high titre of antibodies against certain disease-related antigens. The products are therefore highly dependent on donor blood availability, both in terms of quantity and suitability, resulting in considerable variation between batches. In addition, screening technologies may fail to detect donor-derived pathogens, and thus immunoglobulin products carry a
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Monoclonal Antibodies

In the 1980s, Nobel Prize-winners Kohler and Milstein developed a method of producing highly specific antibodies: monoclonal antibodies (mAb). Hybridoma cells secreting antibodies of the appropriate characteristics could be obtained by fusing antibody-producing cells with immortalised cells, and then subsequent single cell cloning (4).

Monoclonal antibodies have revolutionised the use of pharmaceuticals of biological origin in all aspects of medicine (5). They provide the ability to have an unlimited supply of a single antibody that is clearly defined, and of reproducible affinity and specificity. In theory, mAb technologies allow the development of an antibody against any target of choice, whereas plasma-derived immunoglobulin products are highly dependent on sufficient and suitable blood donors.

The Development of Recombinant Antibodies

Adverse immune reactivity against non-human proteins after repeated use of hybridoma antibodies, which are mainly derived from rodents, are a concern in a clinical setting as these might lead to hyper-responsiveness and – in severe cases – anaphylaxis in immunocompetent individuals. These issues have been addressed by different approaches. One way has been to produce genetically engineered antibodies, for example, chimeric or humanised antibodies, where only the antigen-binding parts of the antibody are of animal origin, whereas the remaining regions of the antibody are replaced by human counterparts. Another way has been to completely avoid non-human antibodies through the development of new technologies, such as phage display and human antibody transgenic animals.

Phage display allows target-specific screening of large so-called combinatorial antibody libraries for the identification of potentially useful antibodies, which subsequently can be produced in large quantities. The technology uses bacteria and bacterial viruses (phages) to express and select recombinant antibodies that have the target recognition qualities of natural human antibodies (6). However, this method relies on random pairing of the antibody heavy and light chains, which disrupts the original pairing of the antibody chains, potentially leading to new reactivity as well as cross-reactivity patterns.

Transgenic animals, on the other hand, are engineered to replace their endogenous antibody genes with genes encoding human antibody sequences. Following immunisation with the appropriate target antigen, these transgenic animals develop target-specific immune responses and can thereby act as a source for antibodies homologous to human antibodies (3).

POLYCLONALS VERSUS MONOCLONALS

A natural antibody-mediated immune response involves a series of direct and indirect effector mechanisms. These are triggered by the synergistic action of antibodies, with a plurality of specificities binding several epitopes. The epitope-specific nature of monoclonal antibodies does not generate such a concerted action. Another inherent feature of monoclonal antibodies, which might have implications for their clinical use, is the fact that all the molecules compete for the same antigenic epitope and epitope density consequently becomes a limiting factor. The only way to improve efficacy is to increase the dose of drug; however, this also increases the risk of side effects resulting from the excess of unbound antibodies binding to tissues other than the target.

Nonetheless, mAb products have been successfully introduced into the clinical management of cancer, for example: Rituxan (Genentech/Biogen Idec), an anti-CD20 antibody approved for treatment of various B cell malignancies; and Herceptin (Genentech/Roche), an anti-Her2 antibody used in certain breast cancer indications. Both antibodies have improved the clinical response rates of anti-cancer treatment, but problems with significant relapse rates and drug resistance remain. Thus, it is believed by many that a mixture of antibodies may provide improved...
Figure 1: The Symplex™ technology. Antibody-producing cells are isolated from the blood of immune individuals by single cell sorting using flow cytometry.

Figure 2: The Symplex™ technology comprising establishment of a polyclonal master cell bank and production of a polyclonal antibody composition.

Antibody-heavy and light chains mRNA are reverse-transcribed, amplified and linked by the Symplex PCR, preserving the parental heavy and light chain pairing. Following high-throughput screening for antigen specificity, antibodies can be selected and expanded – ensuring the desired immune repertoire. Adapted from (14).

Three different recombinant polyclonal antibody preparations were produced to a minimal scale, Sym001:5/03, Sym001:5/21 and Sym001:5/23 & 24, and Sym001:5/03 and one for a clinical scale. Subsequently, these sera were characterised using flow cytometry and western blotting to evaluate the expression, functional and immunological activities.

Figure 3: Analysis of Sym001 purified from bioreactor cultures.

were used to visualise compositional reproducibility (13).

potency due to greater antigen coating, as well as reduced susceptibility to immune escape of the cancer (7). Likewise, the ability of pathogens to evade a polyclonal response is less probable, and recombinant polyclonal antibodies might very well offer a better alternative for the treatment of infectious diseases, where monoclonal antibodies often lose their effectiveness because of the antigenic drift presented by highly mutagenic targets as, for example, viruses (8).

Finally, the immunogenicity of therapeutic antibodies is a concern, not only as mentioned earlier because of adverse reactions leading to hypersensitivity and in severe cases anaphylaxis, but also because anti-drug antibodies may impact negatively on the pharmacodynamics of even fully human antibody drugs. Interestingly, an animal study performed in our laboratory has shown that the use of polyclonal antibody compositions generally evoked fewer blocking antibodies (anti-idiotypic antibodies) in the recipient than the respective monoclonal antibodies administered alone at the same dose range (9).

RECOMBINANT POLYCLONAL ANTIBODIES

The third generation of antibody therapeutics, recombinant polyclonal antibodies, aims to tackle the shortfalls of the first two generations of antibody therapeutics by mimicking nature’s way. Recombinant polyclonal antibodies binding in close proximity to multiple target epitopes will accordingly provide neutralisation by steric hindrance (antigens coated with antibodies are prevented from attaching to host cells or mucosal surfaces), and agglutination or precipitation (antibodies binding several soluble antigens cause aggregation and subsequent clearance). They are also believed to be capable of eliciting a range of secondary effector functions including activation of complement, opsonisation and antibody-dependent cellular cytotoxicity.

The first description of methods using phage display to isolate and generate antibody-encoding libraries – which can be utilised as a template for the development of recombinant polyclonal antibodies – was in 1994 by Sarantopoulos et al (10). These concepts have subsequently been used to develop libraries for the identification of polyclonal antibodies against, for example, colorectal cancer (7), Cryptosporidium parvum (11) and Rhesus D-expressing erythrocytes (12).

Mirroring Nature Using Symplex™ Technology

A new discovery platform, Symplex™ (see Figure 1), is a novel, high-throughput technology that allows direct cloning, screening and identification of antibody drug leads from human individuals generating disease-specific antibodies (through vaccination or natural immunity). Antibody-producing cells are isolated from the blood of immune individuals by single cell sorting. Antibody heavy and light chain mRNA are then reverse-transcribed, amplified and linked by Symplex™ PCR. Unlike phage display-based approaches, the key feature of this technology is the ability to capture immune antibody repertoires while preserving the original pairing of the antibody heavy and light chain (‘cognate pairing’). Using model antigens, we have been able to isolate a wide spectrum of unique antibodies which represent the genetic and functional diversity of the natural antibody response in the immune individuals (13). Furthermore, the technology preserves the diversity, affinity and specificity of the natural repertoire, and can thereby provide detailed insight into the nature and diversity of the natural human antibody response.

Manufacturing Polyclonal Recombinant Antibodies

 Constructs expressing selected antibodies, for example identified by Symplex™ technology, are sub-cloned into the mammalian expression platform, Sympress™ (see Figure 2) for manufacturing antigen-specific recombinant human polyclonal antibodies (14). Different from conventional expression technologies, Sympress™ employs a site-specific recombinase recognition system, ensures that only one copy of a plasmid is integrated into any one cell at the same genomic site in the producer cells. Thus, genomic position effects are minimised and manufacturing consistency and robustness are further
supported by selection of stably transfected producer cells. A polyclonal cell bank is subsequently generated by mixing the individual antibody-producing cell lines. Cells from the polyclonal cell banks are then used for seeding the bioreactors at the production facility. As exemplified by the manufacture of Sym001, production can be scaled up in a reproducible manner without altering the representation in the final product of the clones in the master bank (see Figure 3). We have analysed several production runs of Sym001 anti-RhD antibodies, and they all show batch-to-batch consistency in respect of their protein chemistry characteristic, as well as their antigen-binding properties and ability to elicit effector function in vitro (14).

CONCLUSION
Antibodies have been around as therapeutics for more than a century in various forms. First they were administered as immunoglobulins after extraction from suitable donor plasma. This first generation of antibody therapeutics which reflects the full diversity of the natural immune response, but also presents several disadvantages such as safety issues, lack of specificity and limited clinical applications. Nevertheless, plasma-derived immunoglobulin products are still the best treatment available for many clinical conditions.

The second generation of antibody therapeutics constitutes monoclonal antibodies and fragments thereof. These antibodies offer numerous advantages over plasma-derived immunoglobulins including better safety, reproducibility and defined specificity. Monoclonal antibodies constitute a substantial commercial success, especially in the last 10 years, and they will continue to do so with a promising pipeline of more than 100 molecules in development. However, their monospecific nature limits their clinical potential in the treatment of diseases caused by complex antigens.

Recombinant polyclonal antibodies – the most recent antibody therapy innovation – provide an answer to most of the challenges faced by the previous two generations of antibody therapeutics (15). Until now, the large-scale industrial production of polyclonal compositions has remained elusive. However, the development of an expression platform (Sympress™) has made it feasible to consistently manufacture recombinant polyclonal antibodies originating from immune human individuals, thereby opening the way for third-generation antibody therapeutics to progress to clinical development.

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References