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Recombinant polyclonal antibodies: the next generation of antibody therapeutics?

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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 very limited clinical applications. However, over the past ten years more than 30 monoclonal antibodies have been successfully introduced on to the drug market. The monoclonal approach provides the advantage of specificity, but lacks efficacy in the treatment of diseases caused by complex antigens. Recombinant polyclonal antibodies, the third generation of antibody therapeutics, have the ability to tackle complex and highly mutagenic targets, and will undoubtedly offer a promising commercial future.

For more than a century, antibodies have been used in various forms as pharmaceuticals. Since their first administration in the form of sera in the 1890s, they have come a long way with the development of monoclonal antibodies (mAbs), antibody fragments, domain antibodies and polyclonal antibodies today [1,2]. The underlying objective behind all the research in the field has been to improve the therapeutic potential. The original infusion of immunoglobulins extracted from human plasma had the advantage of reflecting the natural immune response, relating to the breadth of its repertoire and its diversity [3]. Several limitations including rarity of suitable plasma, batch-to-batch variation, cost and, not the least important, safety issues have, however, prevented the widespread use of immunoglobulin therapy in its original form.

The development of the hybridoma technique in 1975 revolutionized the antibody field. This technique allows the virtually unlimited production of pure, highly specific mAbs *in vitro* [4]. This crucial milestone, as well as the series of technical advances that followed, has led to great commercial success. Almost 200 mAbs are now in various stages of clinical development, and the ones that have already reached the market were expected to generate a revenue of US$12 billion in 2005 [5]. Among these, the disease area of cancer is advanced in terms of numbers of approved mAb therapeutics, as well as those in development. Rituxan® and Herceptin®, as cancer mAb therapeutics, collectively generated over US$3 billion in sales in 2003. Success stories also include Remicade®, a mAb approved for Crohn's disease and rheumatoid arthritis, which recorded the highest sales in 2003 – to the tune of US$2.3 billion [5].

Nonetheless mAbs have several disadvantages, some of which relate to their monospecific nature. Their effects – associated with blocking or activating downstream signalling pathways – do not cover the full spectrum of effector mechanisms of a natural immune response and mAbs are, therefore, less effective in the treatment of diseases that have complex target antigens. In cases of antigen mutation, or when facing a disease caused by a virus with multiple strains, mAbs can also become ineffective [6,7].

More recently, a third generation of antibody therapeutics has emerged in response to complex diseases, including infectious diseases and hard-to-target cancers. Recombinant polyclonal antibodies are pure and highly specific, providing an alternative to mAbs with additional characteristics to improve therapeutic potential. In particular, they have the ability to maintain activity against mutating antigens.

Contrasting polyclonals and monoclonals

Historical milestones

The importance of antibodies was first recognized in the 1890s when Pierre-Paul-Emile Roux and Alexandre Yersin identified an

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antitoxin reaction – against the enterotoxin produced by *Diphtheria bacillus*. Emil von Behring then identified a similar toxin–antitoxin reaction in tetanus, and discovered that small doses of *Diphtheria* or tetanus toxin could produce an immunity that was transferable from one animal to another via serum. Behring concluded that the immunity was conferred by substances in the blood that he called antitoxins or antibodies.

Although their immense potential was not fully realized at the time, antibodies rapidly found clinical applications, for example in the treatment of tetanus or botulinum intoxication [2,8]. However, there was limited understanding of the structural nature of antibodies and of their mechanisms of action. It was Paul Ehrlich (1908 Nobel Prize winner in medicine) who first envisioned the theory of immunity with his 'side-chain antibodies' theory. His key insight was to think of the specific molecular structure of a substance as the cause of specific biological effects. He believed that antigens and antibodies combined chemically in specific ways, like keys fitting into locks, and referred to antibodies as 'magic bullets' that could seek out disease-causing organisms and destroy them, at the same time avoiding any harmful effects on the body of the patient.

Advances in immunochemistry and immunobiology shed much light on antibodies in the first half of the twentieth century: their production by plasma cells, their protein nature, and their molecular structure. However, researchers still believed antibodies were formed on the antigen template. It was Niels K Jerne and Frank M Burnet in the 1950s [9] who developed the theory of antibody repertoire and clonal selection – the antibody repertoire is genetically predetermined and the encounter with antigen triggers selection and clonal expansion of the appropriate antibody-producing plasma cells.

The physiology of natural human antibody responses is now well understood and Erhlich's magic bullets have been used in various forms of passive immune therapy – from immunoglobulin infusions to recombinant mAb injections.

Traditional immunoglobulin therapy

Early beginnings of antibody therapy involved the purification of the immunoglobulin fraction of human donor plasma and its infusion in to patients. Plasma-derived immunoglobulin from normal healthy donors offers the advantage of mimicking the polyclonal natural immune response with a diverse and specific repertoire, and remains a preferred choice in the treatment of selected conditions. Plasma-derived immunoglobulins reflect the breadth of the human antibody repertoire and, yet, the specificity of the antibody response, with the presence of several antibodies against the pathogen's multiple epitopes increasing the chance of triggering effector mechanisms [3].

Deriving immunoglobulin from whole human plasma, reflecting the multitude of binding specificities in the natural antibody, implies that only a small fraction of all the immunoglobulin injected is targeting the antigen of interest. This can be partially overcome by the injection of hyperimmune immunoglobulin – derived from individuals who have developed a high titre of antibodies against certain disease-related antigens following (for instance) recovery from infection. Today, hyperimmune immunoglobulin is used for prophylaxis or therapy against infections with hepatitis B virus, respiratory syncytial virus (RSV), cytomegalovirus (CMV) and rabies virus, as well as tetanus, botulinum intoxication [2,8,3,10] and Rhesus D (RhD) alloimmunization [11,12].

A more widespread use of immunoglobulin products has been prevented by the fact that source plasma is only available for a limited range of infectious diseases. Moreover, the products are highly dependent on donor blood availability, both in terms of quantity and suitability, resulting in considerable variation between batches. In addition, screening technologies fail to keep up with constantly evolving viruses, thus, immunoglobulin products carry a potential risk of infectious disease transmission. Finally, the long process of blood collection, screening and immunoglobulin purification means plasma-derived immunoglobulins are expensive to produce.

Animal-derived immunoglobulins essentially overcome the manufacturing limitations of the human plasma-derived products. Thymoglobulin® (Genzyme), a hyperimmune immunoglobulin purified from the blood of rabbits immunized with human T lymphocytes, is successfully used in the treatment or prevention of solid organ transplant rejection. Thymoglobulin® suppresses immune cells (in particular T cells) responsible for acute organ rejection by binding to surface antigens of target cells. Other examples include equine immunoglobulin against rabies virus or botulinum toxin [13]. However, the animal origin of these products and all the inherent risks associated to their use in humans (hypersensitivity, anaphylaxis, anti-animal response and transmission of infectious pathogens including prions) considerably limit their clinical applications [14].

Monoclonal antibodies

In the 1980s, Nobel prize winners Kohler and Milstein developed a method of producing highly specific antibodies – mAbs. The technique involves fusing an antibody producing cell with an immortalized (multiple myeloma) cell line and subsequently isolating and cloning a hybridoma – a single immortalized B-lymphocyte clone that secretes antibodies with the desired characteristics [4].

It is true that mAbs have revolutionized the use of biologic pharmaceuticals in all aspects of medicine [15]. They provide the ability to have an unlimited supply of a single antibody that is clearly defined and of reproducible affinity and specificity, a major attribute in many experimental and clinical situations.

In theory – and this is their major advantage – mAb technologies allow the development of an antibody against any target of choice. As well as providing the added advantage of safety, mAbs are easier and cheaper to produce than their plasma-derived counterparts. Plasma-derived immunoglobulin products are also highly dependent on sufficient and suitable blood donors.

Development of recombinant antibodies

Adverse immune reactivity against non-human proteins after repeated use of hybridoma antibodies (mainly derived from rodents) has been addressed by different approaches. One way has been to produce genetically engineered antibodies (e.g. chimeric and humanized antibodies) where genes encoding the variable domains or only the actual binding region are animal in origin, whereas the genes encoding the remaining parts of the antibody are replaced by their human counterparts. Through the

development of new technologies to produce human mAbs, such as phage display and human-antibody transgenic animals, it has been possible to avoid non-human antibodies. Phage display allows screening of huge antibody libraries for potentially useful therapeutics that can subsequently be produced in large quantities. The technology uses bacteria and bacterial viruses (phages) to express and select recombinant antibodies that have all the target recognition qualities of natural antibodies. The phages are genetically engineered so that a particular antibody is fused to a protein on the phage's envelope, and the gene encoding the displayed antibody is contained inside the phage particle. Collections of these antibody-covered phages (or phage libraries) typically contain a billion different antibodies, a number comparable with that of the human immune system. The screening process is based on 'panning' the library against the target molecule, which is immobilized onto a solid surface. Phages with antibodies that recognize the target molecule bind tightly, whereas others are washed away. Bacterial cells are then transfected with the DNA contained within the bound phage, and used to produce more of the selected antibody for use in research of an antibody drug development candidate [16,17].

By contrast, transgenic animals are engineered so their endogenous antibody genes have been replaced with genes encoding human antibody sequences. Following immunization with the appropriate target antigen, these transgenic animals develop target-specific immune responses with human-like antibodies, where the molecular sequences of individual protein chains are homologous to those of human antibodies [14].

A natural antibody-mediated response involves a series of direct and indirect effector mechanisms that include direct neutralization, phagocytosis, complement-mediated destruction and antibody-dependent cellular cytotoxicity. These are elicited by the concerted action of antibodies with multiple specificities, binding several epitopes and acting in synergy. The monospecific nature of mAbs does not generate such a concerted action. Additionally, in the case of mAbs, all the molecules present in the composition compete for the same antigenic epitope and, hence, the epitope density is a limiting factor. To improve efficacy, there is a need for higher doses of drug, increasing the risk of side-effects, resulting from the excess unbound antibodies binding tissues other than the target (tissue cross-reactivity). Therefore, mAbs have a commercial potential in the treatment of diseases with a simple antigen [e.g. neutralization of tumour necrosis factor-α (TNF-α) in rheumatoid arthritis] but are probably less effective in neutralizing, killing or eliminating complex target antigens, such as infectious disease agents, cancer cells or allergens [18,19].

Certainly, mAb products have been successfully introduced into the clinical management of cancer (e.g. Rituxan® and Herceptin®). Rituxan® binds to the B-cell marker CD20, and mediates inhibition of proliferation, apoptosis and killing of lymphomas, whereas Herceptin® binds to HER2, a receptor on epithelial breast cancer cells. Both antibodies have improved the clinical response rates of anticancer treatment, but problems with significant relapse rates and drug resistance remain. Thus, it is believed by many that a mixture of antibodies could provide improved potency because of greater antigen coating and reduced susceptibility to immune escape of cancers [20].

Recombinant polyclonal antibodies

One aspect of the body's reaction to invasion by a microorganism is the activation and clonal expansion of 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 – the invading pathogen will be met by a barrage of antibody molecules capable of binding at many different sites on its surface. Such a polyclonal response, where the range of specificities and affinities can shift with time, is ideal for combating infection.

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. Polyclonal antibodies are capable of binding to several different epitopes on any given antigen. Thus, recombinant polyclonal antibodies binding in close proximity to multiple target epitopes are believed to be capable of triggering a range of effector functions including opsonization (enhancing phagocytosis of antigens), steric hindrance (antigens coated with antibodies are prevented from attaching to host cells or mucosal surfaces), toxin neutralization, agglutination or precipitation (antibodies binding several soluble antigens cause aggregation and subsequent clearance), activation of complement and antibody-dependent cellular cytotoxicity (antibodies enable the killing of target cells by natural killer cells and neutrophils).

mAbs also lose their effectiveness against highly mutagenic targets, including viruses, that can undergo random mutations and thereby evade the immune system (immune escape or antigenic drift). The ability of pathogens to evade a polyclonal response is less probable, and therefore recombinant polyclonal antibodies offer a better alternative for the treatment of infectious disease. In addition, the recombinant polyclonal antibody technology provides a better chance of retaining a therapeutic activity in the event of strain variation [21–23].

Recombinant polyclonal antibodies also offer many advantages over classical plasma-derived immunoglobulins. In traditional immunoglobulin therapy antibodies specific for the target only represent a small fraction of all the antibodies injected, resulting in a large quantity of immunoglobulin being wasted and increasing the tissue cross-reactivity and side-effects. The dilution of specific antibodies among non-specific ones also means a reduced effectiveness or the need for higher doses, frequently requiring intravenous (i.v.) infusion. Recombinant polyclonal antibodies overcome these shortfalls because, unlike plasma-derived immunoglobulins, these antibodies are pure and specific.

In addition, recombinant polyclonal antibodies can theoretically be raised against every antigenic target and do not suffer from lack of supply or plasma suitability problems, as is the case for serum-derived products. Finally, the new generation of antibodies does not carry the risk of infectious agent transmission (as the traditional method does) and, hence, should have an improved safety profile.

The first description by Sarantopoulos *et al.* [24] of methods to isolate and generate recombinant polyclonal antibodies was in 1994. It has been followed by reports describing development of recombinant polyclonal antibodies against breast cancer and colorectal cancer [25–27] and *Cryptosporidium parvum* [28].

Technologies in the commercial setting

Animal and human immunoglobulin companies

The market is divided between companies developing animal or humanized immunoglobulins and those developing human immunoglobulin products.

The first category includes Protherics (UK), a company developing therapies using sheep polyclonal antibodies to treat snake bites and digoxin overdose. Also in this category, THP (CA, USA) and Hematech (CT, USA) produce specific, humanized polyclonal antibodies from transgenic rabbits and cows, respectively, whereas Genzyme (MA, USA) produces rabbit polyclonal antibodies against human T cells (e.g. Thymoglobulin®) for the prevention of organ transplant rejection [10].

In the second category, the leaders of the i.v. human immunoglobulin market include Cangene (Canada), Baxter International (IL, USA) and Bayer (Germany). Cangene markets WinRho® SDF for the prevention of haemolytic disease of the newborn and for treatment of idiopathic thrombocytopenic purpura.

Monoclonal antibody companies

The advances in the production of humanized or human mAbs have led to an explosion of the antibody therapeutic market with >15 mAbs approved for market in the past decade, and >100 in development. This success rate has boosted biotech companies, and has renewed the confidence of pharmaceutical companies in this area of investment. Roche–Genentech has three major products in the antibody therapeutic market: Herceptin® targeting HER2 in breast cancer; Avastin® for colorectal cancer; and Rituxan®, the market leader, for the treatment of non-Hodgkin's lymphoma. Johnson and Johnson and Centocor are commercializing Remicade® for rheumatoid arthritis treatment, Muromab (anti-CD3) against transplant rejection and REOPRO® to prevent blood clots during certain heart procedures. Other remarkable commercial successes include MedImmune's Synagis® for lower respiratory tract disease caused by RSV, Imclone System's Erbitux® for metastatic colorectal cancer, and Humira® (Abbott–Cambridge Antibody Technology) for rheumatoid arthritis.

Key players in the mAb development field have taken different approaches: Dyax (MA, USA), Morphosys (Germany), BioInvent International (Sweden) and Cambridge Antibody Group (UK) use combinatorial phage library technologies; Abgenix (CA, USA), Medarex (CA, USA) and Kirin (Japan) use transgenic animals to produce humanized antibodies; and Protein Design Laboratories (CA, USA) humanizes murine antibodies [29,30].

Recombinant polyclonal antibody platform companies

Until the development of a new discovery and expression platform technology by Symphogen (Denmark), the large-scale industrial production of recombinant human polyclonal antibodies remained elusive.

The new discovery platform Symplex™ (see Figure 1) allows the cloning, screening and identification of truly human, antibody

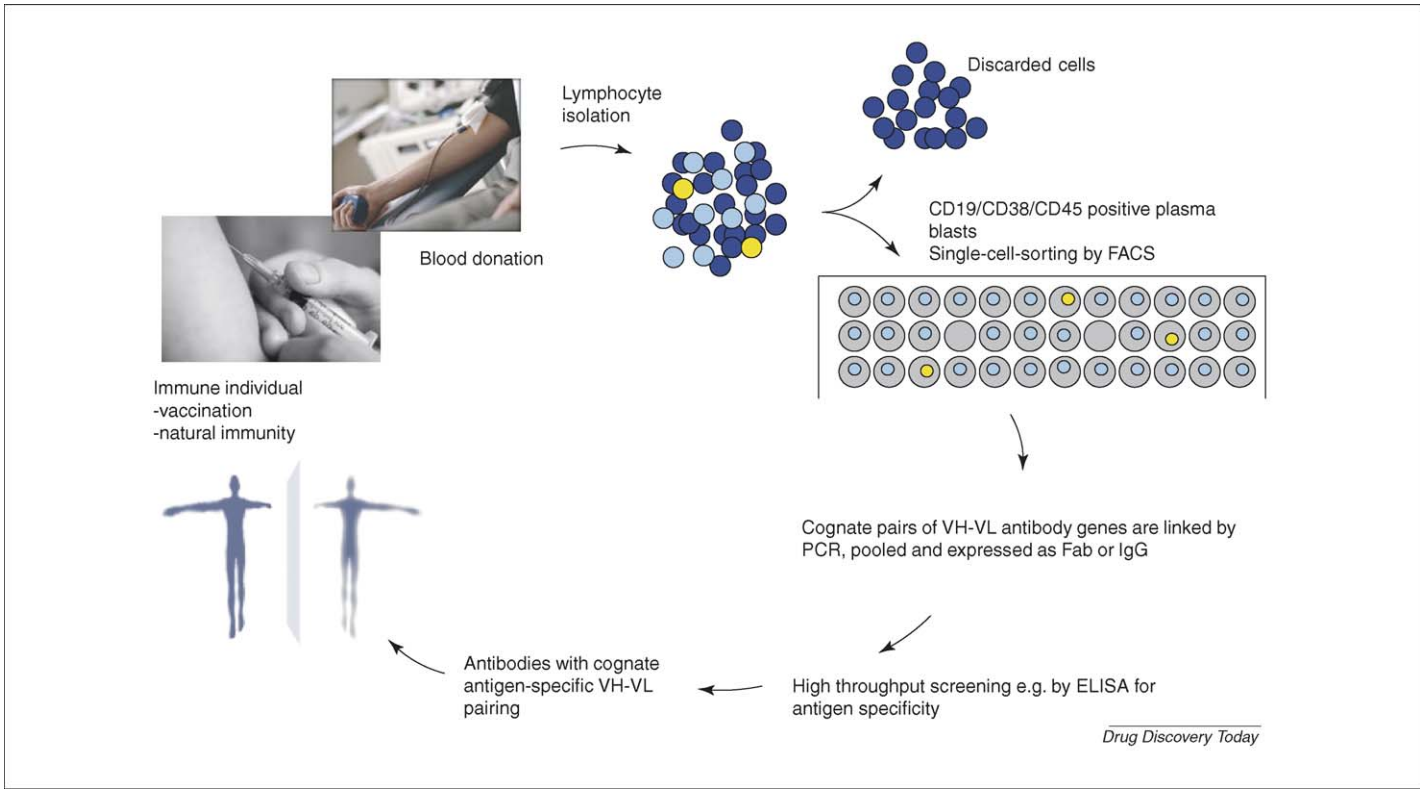

FIGURE 1

The Symplex™ technology. Antibody-producing cells are isolated from the blood of immune individuals by single cell sorting using flow cytometry. Antibody heavy- and light-chain mRNA is reverse-transcribed, amplified and linked by the Symplex™ PCR, preserving the natural heavy- and light-chain pairing. Following HTS for antigen specificity, antibodies can be selected and expressed, mirroring the natural immune response.

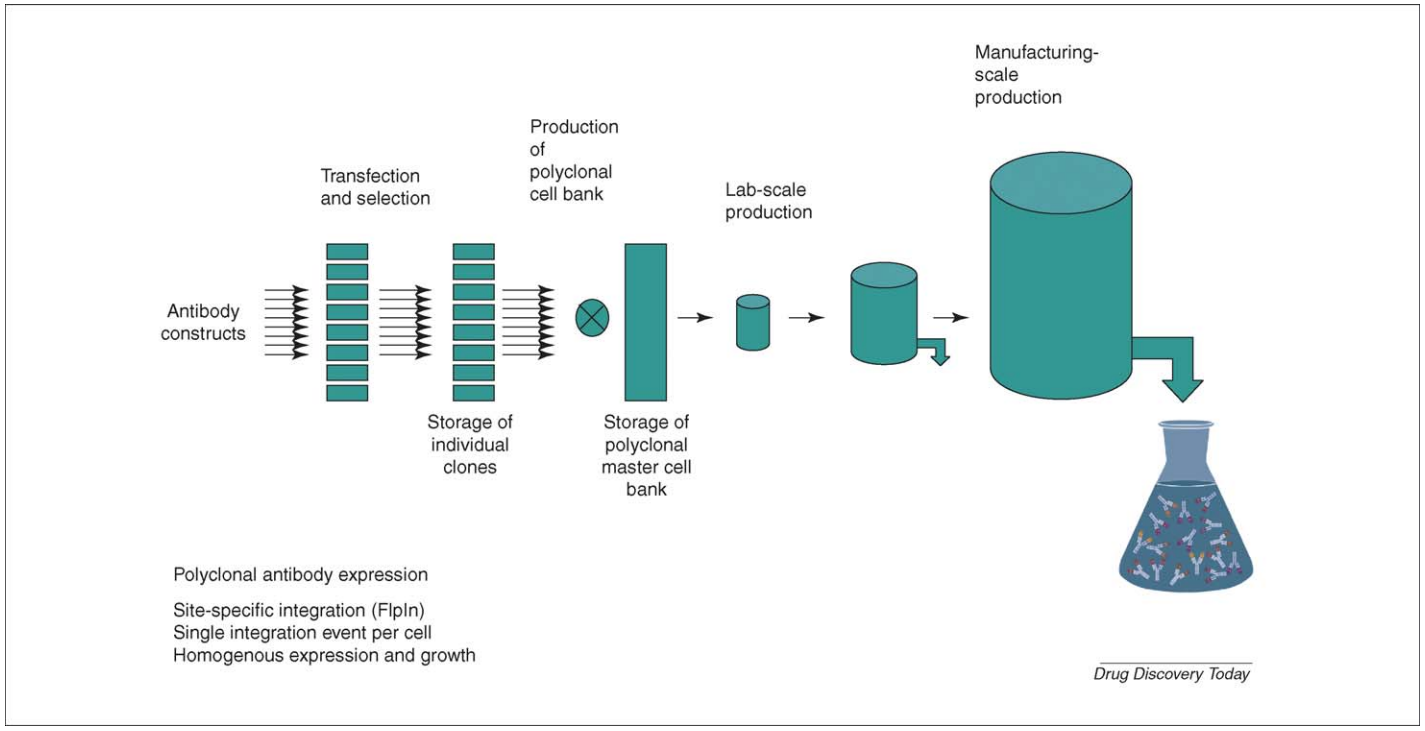

FIGURE 2

The Sympress™ technology. Constructs expressing each of the selected antibodies are transfected into mammalian cells, selected for stable integration and stored. Vials of each clone (producing the selected antibodies) are mixed together to constitute a polyclonal master cell bank, which is subsequently used as inoculation material for production. Production can be scaled up in a reproducible manner without altering the representation (in the final product) of the clones in the master bank.

drug-leads from individuals exhibiting disease-specific antibodies (through vaccination or natural immunity) [31]. 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 the Symplex PCR. Unlike phage-display-based approaches, the original pairing of the antibody heavy- and light-chain (cognate pairing) is preserved, saving a long process of screening for clones with heavy–light chain pairings of high affinity. In addition, the technology exactly preserves the diversity, affinity and specificity of the natural repertoire.

These antibody-encoding genes are then subcloned into the mammalian expression platform Sympress™ (see Figure 2) for the manufacturing of full-length, antigen-specific recombinant human polyclonal antibodies [32]. Unlike conventional expression technologies, Sympress™ employs site-specific integration, ensuring that only one copy of a plasmid is integrated into any one cell. Thus, genomic position effects are eliminated and manufacturing consistency is guaranteed. Constructs expressing each of the selected antibodies are then transfected into Chinese hamster ovary cells, selected for stable integration and banked. Vials of each clone (producing the selected antibodies) are then mixed together to constitute a polyclonal cell bank. Production can be scaled up in a reproducible manner without altering the representation in the final product of the clones in the master bank.

Validated by the production of truly human recombinant polyclonal antibodies that were specific for the tetanus toxin and the influenza virus, the potential clinical applications (both therapeutic and prophylactic) of the Symphogen technology are immense. Sym002, a hyperimmune vaccinia immunoglobulin (VIG), has a potential market of US$300 million. Sym001, Symphogen's lead product, is a recombinant human polyclonal anti-RhD composition consisting of 25 different anti-RhD antibodies. This product is intended for use in treatment of idiopathic thrombocytopenic purpura and haemolytic disease of the newborn, and is expected to enter the clinic in 2006, replacing a specific immunoglobulin anti-RhD that has already been proven safe and effective. The company's pipeline also includes anti-RSV antibodies (e.g. Sym003) and anticancer antibodies (e.g. Sym004).

Three generations of antibody therapeutics

Antibodies have been used 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 ideally reflects the full diversity of the natural immune response, but presents several disadvantages including safety issues, lack of specificity and limited clinical applications. Nevertheless, plasma-derived immunoglobulin products remain the only treatment available for many clinical applications.

The second generation, mAbs (including domain and fragment antibodies), offers numerous advantages including safety, reduced production costs, reproducibility and defined specificity. These therapeutics benefit from immense commercial success (especially in the past ten years) and will most certainly carry on doing so with

a promising pipeline of >100 molecules in development. Their monospecific nature, however, limits their clinical use for the treatment of diseases caused by complex antigens.

Recombinant polyclonal antibodies, the most recent development in antibody therapeutics, provide an answer to most of the challenges met by the two previous generations of antibody therapeutics. Fine-tuning of the technology will soon widen their clinical potential even further and the third generation of antibody therapeutics should become prominent in the antibody therapeutics market.

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