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Review

Antibody production: Polyclonal-derived biotherapeutics☆

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Abstract

Antibody based therapies using monoclonal or polyclonal antibodies are emerging as an important therapeutic approach for the treatment of a number of diseases. With increasing emphasis on new technologies associated with monoclonal antibody expression and purification, the clinical need of polyclonal therapeutics for treatment of a variety of specific illnesses and infections is often overlooked. Despite being largely abandoned in the early twentieth century due to the development of antibiotics, polyclonal antibody therapeutics are today widely used in medicine for viral and toxin neutralization and for replacement therapy in patients with immunoglobulin deficiencies. Over the past 20 years, intravenous immunoglobulins have shown beneficial immunomodulatory and anti-inflammatory effects in many illnesses. Hyperimmune antibody preparations have been used over the past century for the treatment of a variety of infectious agents and medical emergencies, including digoxin toxicity, snake envenomation and spider bites. Here, we examine the contemporary techniques and applications, and assess the future therapeutic potential, for polyclonal-derived antibody therapeutics.

Keywords: Polyclonal; Antibody; Hyperimmune; Serum; Immunization; Therapeutic

Contents

1. Introduction

Antibodies play an important role in the defense against invading pathogens. They specifically recognize and bind to foreign antigens, resulting in the activation of a number of immune effector functions capable of selectively eliminating foreign micro-organisms, viruses and molecules. The earliest recorded attempts to induce immunity were performed by the Chinese in the fifteenth century [1]. Dried crusts derived from smallpox pustules were either inhaled or inserted into small cuts in the skin (variolation) to provide protective immunity. This

Abbreviations: AIDS, anti-immune deficiency syndrome; cGMP, current Good Manufacturing Practice; CHO, Chinese Hamster Ovary cell line; Fab, antigen binding fragment; $F(ab)^2$, IgG fragment comprised of two covalently coupled antigen binding regions; Fc, antibody crystalline fragment; HAC, human artificial chromosome; HIV, human immunodeficiency virus; IVIG, intravenous immunoglobulin; mAb, monoclonal antibody; pAb, polyclonal antibody; Tc, transchromosomic; TNFα, tumour necrosis factor alpha

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technique was improved by the English physician Edward Jenner in 1798 and was used to develop a smallpox vaccine by inoculating volunteers with the fluid from a cowpox pustule [2]. The experimental work of a number of scientists in the 1890s [3,4] demonstrated that administration of a serum 'antitoxin' could protect unimmunized animals against diphtheria, and soon after the administration of hyper-immunised animal serum was used for the treatment of a variety of diseases [1]. The administration of hyper-immunised serum for the treatment of infection in human patients was introduced in the 1890s for the treatment of diphtheria. Over the next 20 years animal-derived polyclonal antibodies were introduced for use as serum therapy to treat pneumonia, meningococcal meningitis, scarlet fever, diphtheria and measles [5]. By the 1930s serum therapy was used to treat a variety of bacterial infections including pneumonia (*Streptococcus pneumoniae*) [6], anthrax (*Bacillus anthracis*) [7] and botulism (*Clostridium botulinum*) [8], however the efficacy of the treatment varied depending on the type and severity of the infection and the accuracy of microbiological diagnosis.

Modern hyper-immune polyclonal antibody therapeutics used for the treatment of acute illness and medical emergencies are typically derived from human donors or animals with elevated serum levels of specific polyclonal antibodies. Examples of marketed (approved) polyclonal therapeutics are shown in Table 1. Increased serum titres of specific antibodies due to infectious agents or toxins may occur as a result of natural infection, or after immunization with the corresponding antigen (Fig. 1). As hyper-immunised serum contains a mixed population of antibodies against multiple epitopes, polyclonal antibody preparations are often considered more efficacious (especially for the treatment of acute illness and medical emergencies) as they can bind and neutralize multiple epitopes on the disease-causing agent. To minimize antigenicity against the species-specific Fc region, hyperimmune antibodies derived from animals are often digested with proteases such as papain [9] or pepsin [10] and the antigen binding fragments (Fabs) purified by chromatography [11]. The generation of monovalent Fab or divalent $F(ab)^2$ fragments significantly reduces hypersensitivity reactions and enhances product safety. Polyclonal-derived hyperimmune therapeutics have been marketed for many years and are considered the most effective treatment for potentially lethal snake and spider envenomation (Table 1). Although the administration of animal-derived (often ovine or equine) antibodies or fragments may have a potential clinical disadvantage due to risk associated with hypersensitivity, serum sickness and anaphylaxis [12], serum-derived polyclonal antibodies have been used successfully for many years to treat snake bites [13] and poisoning with digoxin [14], digitoxin (and a range of structurally similar cardiotoxins, such as toxins from the oleander plant (*Nerium oleander*) [15]. Manufacturers of polyclonal-derived antibody products have introduced a variety of steps to ensure pathogen inactivation and clearance. Animal-derived material used for polyclonal biopharmaceutical production is often sourced from countries with a long history of absence of relevant transmissible spongiform encephalopathies (TSEs) [16]. Countries such as Australia have always ensured strict animal import control, maintaining a disease free status for a number of diseases that are currently endemic in other parts of the world. Animals used for biotherapeutic production are usually contained within closed flocks and obtained from approved suppliers, records of all routine sheep husbandry activities are maintained and animals often require routine stock inspections and daily examination. Extensive flock health control and monitoring is usually undertaken, including routine viral screening of serum and other adventitious agents. As with the production of monoclonal antibodies and other recombinant biotherapeutics, downstream purification

Table 1
Examples of currently available polyclonal therapeutics[a]

Marketed polyclonal therapeutic	Product description	Therapeutic application
Animal derived		
CroFab™	Crotalidae (*Crotalus atrox, Crotalus adamanteus, Crotalus scutulatus, Agkistrodon piscivorus*) Polyvalent Immune Fab (Ovine)	Rattlesnake antivenom
DigiFab™	Digoxin Immune Fab (Ovine)	Digoxin toxicity/oleander poisoning
ViperaTAb™	Affinity Purified, European Viper Antivenom (Ovine) Fab	Common adder (*Vipera Ammodytes, Vipera Aspis, Vipera Berus*) antivenom
Atgam®	Lymphocyte immune globulin, anti-thymocyte globulin (Equine)	Immunosuppressive therapy
Antivenin (Micrurus fulvius)	Antivenin (*Micrurus fulvius*) Equine origin	North American Coral Snake antivenom
Antivenin (latrodectus mactans)	Antivenin (*latrodectus mactans*) Equine origin	Black widow spider antivenom
Diptheria Antitoxin	Diphtheria Antitoxin (Equine)	Treatment of diptheria
Lymphoglobuline®	Anti-thymocyte (Equine) immunoglobulin	Immunosuppressive therapy
Thymoglobulin	Anti-Thymocyte globulin (Rabbit)	Immunosuppressive therapy
Human derived		
Respigam®	Respiratory syncytial virus immune globulin intravenous (Human)	Respiratory tract infection
BayRab®	Rabies Immune Globulin (Human)	Suspected rabies infection
BayTet®	Tetanus Immune Globulin (Human)	Prophylaxis against tetanus
BayGam®	Immune Globulin (Human)	Passive protection against hepatitis A
Cytogam®	Cytomegalovirus immune globulin intravenous (Human)	Prophylaxis against cytomegalovirus disease
Nabi-HB®	Hepatitis B Immune Globulin (Human)	Passive protection to hepatitis B

[a] Data from US Food and Drug Administration, Centre for Biologics Evaluation and Research, licensed Establishments and Products (table may not represent a complete list).

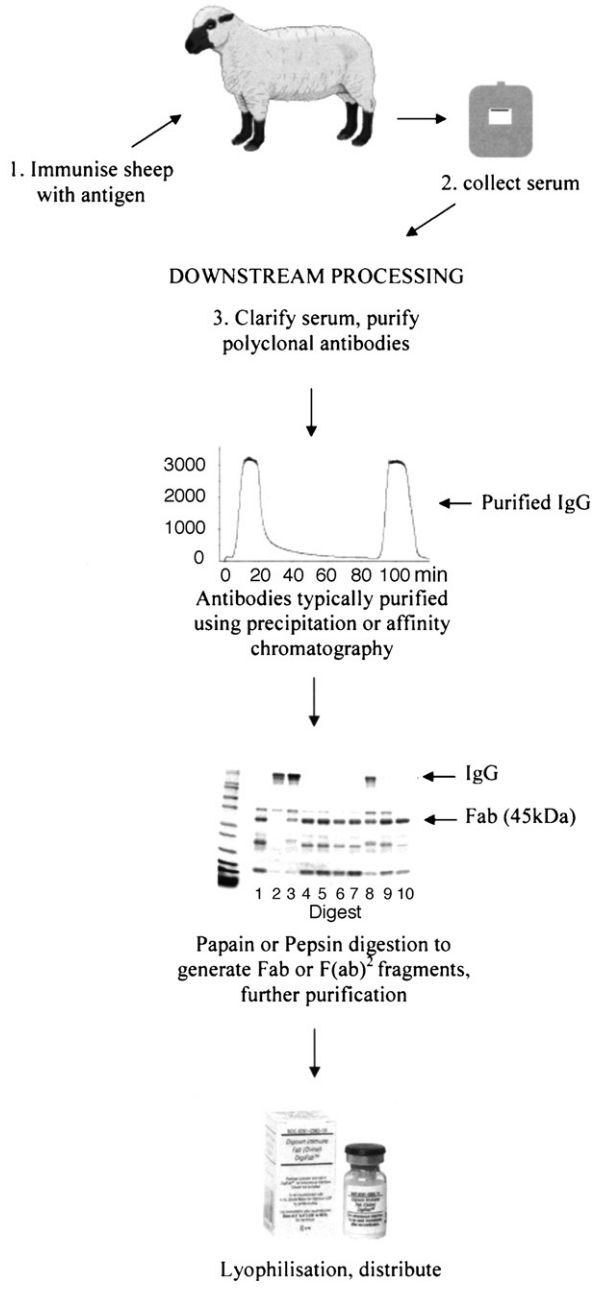

Fig. 1. Production of ovine polyclonal Fabs including DigiFab™ (Digoxin Immune Fab (Ovine)). (1) Animals are immunized with antigen, serum titres monitored to assess the immune response. (2) Serum containing a polyclonal mixture of specific and non-specific antibody. (3) Total IgG is purified using precipitation or affinity chromatography, cleaved with a proteolytic enzyme such as papain and the specific Fab fragment further purified to remove non-specific Fabs and minor impurities.

processes also typically includes two orthogonal process steps for antiviral clearance [17] (validated using appropriate model viruses) to ensure patient safety and fulfill the necessary regulatory requirements [18]. Venom- or toxin-specific Fabs are generally well tolerated and clinically effective for the treatment of potentially life threatening toxicity or envenomation [19].

Another advantage of polyclonal therapeutics is the ability of the polyclonal fragments to neutralize similar (yet chemically distinct) toxins or infectious agents—the polyclonal-derived biotherapeutic CroFab™ (Crotalidae Polyvalent Immune Fab (Ovine)) primarily used for Crotalidae (North American rattlesnake) bites has also been used for the treatment of hognosed viper (*Porthidium nasutum*) envenomation [20] and due to the polyvalent nature of the Fab isoforms has potential applications for the treatment of other venomous snake bites. At least one other ovine polyclonal Fab is in late stage clinical development. CytoFab™, initially developed by Protherics (London, UK) is designed to neutralize tumour necrosis factor alpha (TNF-α) and is currently under development by AstraZeneca as a treatment for severe sepsis. The body's response to severe infection involves the release of numerous cytokines (including TNF-α) which cause inflammatory syndrome. CytoFab™ effectively neutralised TNF-α in a Phase II clinical study and has the potential to become the next product to reach the market for the treatment of acute sepsis, illustrating the potential application of modern biopharmaceutical development to traditional therapeutic antibody technologies. Here, we review contemporary techniques and applications for the production and future therapeutic potential of polyclonal-derived antibody therapeutics.

2. Hyperimmune (pAb) polyclonal therapeutics

Serum-derived polyclonal antibodies from animals (Fig. 1) are currently the preferred and often the only therapeutic choice for selected acute medical emergencies to eliminate complex, poorly characterized mixtures of target antigens. Purified polyclonal antibodies are often digested using specific proteases such as papain [9] or pepsin [10], and the specific antigen binding fragments (Fabs) further purified to remove the Fc fragment and other impurities and contaminants. CroFab™, used for the treatment of crotalidae (rattlesnake) envenomation, the predominant venomous snakebite in the United States, has dramatically changed snakebite management since its release in December 2000 [21]. CroFab™ has been shown to be highly efficacious in treating both the local and systemic toxic effects of crotaline envenomation [22].

3. Pooled polyclonal antibodies

Many human polyclonal therapeutics are fractionated plasma products or plasma derivatives manufactured from human blood by the selective separation and purification of target proteins [23,24]. Polyclonal immunoglobulins for intravenous use are purified at production scale from plasma obtained from blood samples taken from several hundred to thousands of human donors. Polyclonal intravenous IgG (IVIG) has been used for many years for the treatment of patients with antibody deficiencies. This may be due to a genetic disorder, disease, or to treatment such as chemotherapy. Polyclonal IVIG was first demonstrated as an effective therapy for patients with primary immunodeficiency syndromes. Although IVIGs have become the major plasma product on the global blood product market [17], this review focuses on raised or expressed hyperimmune or recombinant polyclonal immunoglobulins. IVIG purifica-

tion has been discussed previously in a number of informative reviews [25–28].

4. Monoclonal (mAb) antibody cocktails

A seemingly simple approach to generate a polyclonal mixture of recombinant therapeutic antibodies would be to combine several monoclonal therapeutics with the desired specificities into a single drug product. Several antibody cocktails have been developed in the hope of providing a cost-effective and safe replacement to human polyclonal antibodies and resolve some of the potential limitations of monoclonal antibody therapies. A number of these antibody cocktails have been evaluated using *in vivo* models, including human rabies virus-neutralizing monoclonal antibody cocktails [29] and radiolabelled monoclonal antibody (mAb) mixtures for the treatment of carcinoma [30]. Although a mAb 'cocktail' approach may confer many of the advantages of polyclonal-derived antibody products, there are major commercial and technical obstacles to overcome as a result of the requirement for large-scale cGMP manufacture [31,32]. Due to an inherent heterogeneous cell culture with different growth rates, expression rates, and genetic stability between cell lines, process validation and product characterisation would be required for each constituent monoclonal antibody, with purified antibodies blended during final formulation of the biotherapeutic. Furthermore, due to potential variation in antibodies (isoelectric points, hydrophobicity, solubility and tendency to aggregate) the blended antibody cocktail may show distinct biochemical characteristics that differ from each of the individual mAb components. The commercial investment to develop several distinct mAb product streams and the regulatory requirements involved may therefore have a considerable impact on the commercial viability of the therapeutic and may be a limiting factor of this approach for many therapeutic indications.

5. Antigen-specific recombinant polyclonal antibodies

A number of new technologies are emerging for the potential large-scale production of 'next generation' polyclonal antibodies. Symphogen (Sympogen A/S, Denmark) has developed proprietary technology for the production of recombinant polyclonal antibody preparations, produced using mammalian cell culture [33]. Symphogen have developed a proprietary expression platform, the Sympress^{TM} technology, for manufacturing recombinant therapeutic polyclonal antibody therapeutics. Polyclonal mixtures of recombinant human antibodies are expressed using a modified mammalian expression system, which involves the site-specific integration of a single antibody expression plasmid into a constant genomic site within the host cell DNA. This technology differs from traditional mammalian transfection and expression systems which typically involve the random integration of the plasmid: the DNA may be re-arranged and inserted into different sites in the genome. The result is a variety of genomic integration sites, differing plasmid numbers per cell and compositional growth (variable expression levels between cells known as compositional growth bias) of

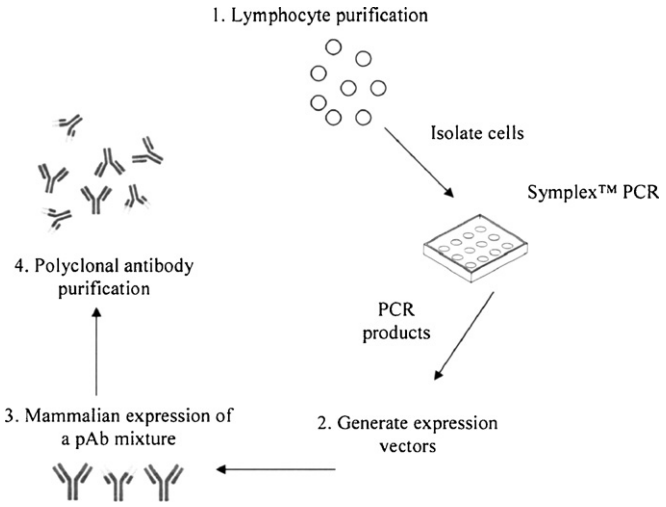

Fig. 2. Production of recombinant human polyclonal antibodies. (1) Blood donation and B lymphocyte isolation. (2) Site-specific integration of a single antibody expression plasmid at the same genomic site of each cell. All antibody constructs share identical constant regions. (3) Constructs expressing the desired antibodies are transferred to Chinese Hamster Ovary (CHO) cells and selected for stable integration. (4) A polyclonal mixture of antigen specific human antibodies may be expressed and purified.

the transformed cells during upstream processing. The Symphogen technology permits the site-specific integration of a single antibody expression plasmid into the same genomic site of each cell. This eliminates genomic integration effects and permits a consistent level of expression of antibody. A mixture of transformed cell lines (expressing multiple mAbs of desired specificities) may therefore produce consistent levels of a recombinant human polyclonal antibody mixture termed 'symphobodies' (Fig. 2). Although recombinant human polyclonals produced using Symphogen's technology have yet to be assessed in the clinic, several antibody therapeutics are in pre-clinical development.

6. Transgenic polyclonal therapeutics

Human antibodies produced using transgenic technologies have the potential for the large-scale production of human polyclonal antibodies. The biopharmaceutical company Hematech LCC (Connecticut, USA) has used human artificial chromosomes and emerging transgenic technologies to produce cattle that express functional human antibodies [34]. Researchers utilized a human artificial chromosome (HAC) containing the entire unarranged sequences of the human immunoglobulin heavy and light chain loci to transfer the human antibody genes into bovine primary foetal fibroblasts. The HAC is composed of chromosome 22 containing few genes other than those required for antibody expression. To clone bovine embryos, HACs were introduced into cow cells that were subsequently fused with bovine oocytes to produce transchromosomic (Tc) calves (Fig. 3). The results indicate that >80% of cow cells contained the HAC, with a number of immune cells containing correctly assembled antibody genes [35]. Cows carrying complete human antibody genes could be immunized using the desired target antigen and

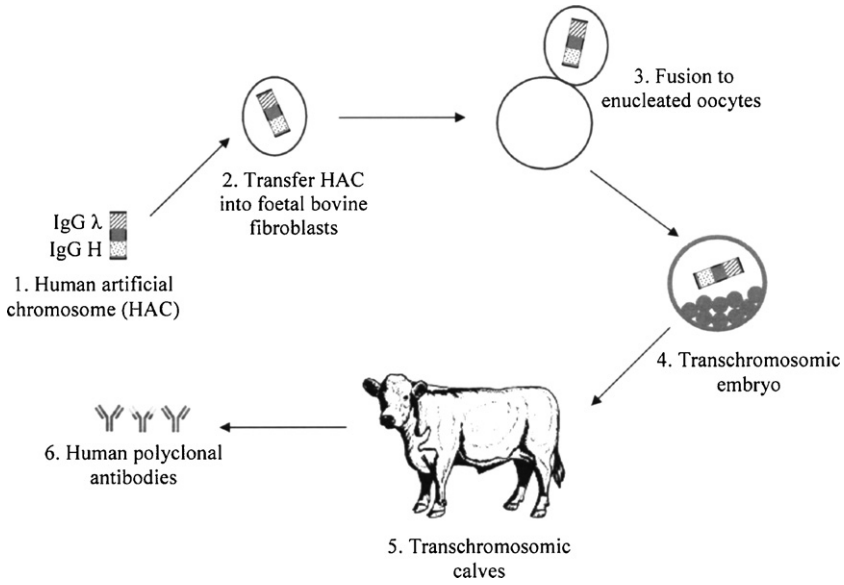

Fig. 3. Production of human polyclonal antibodies using transchromosomic (Tc) calves. (1) Human artificial chromosome vector (HAC) carrying the human Ig heavy and λ light chain sequences. (2) The HAC is transferred from a CHO clone into foetal bovine fibroblasts. (3) Enucleated oocyte couplets are fused and the fibroblast nucleus transferred, resulting in the formation of an embryo. (4) Tc embryos are cultured *in vitro*. (5) Embryos implanted into recipient cows, foetuses removed, re-established and evaluated. Regenerated Tc embryos transferred to receipents to produce Tc calves. (6) Human polyclonal antibodies expressed. Cows carrying human antibody genes may be immunized against target agents.

the human polyclonal antibodies collected and purified. Several challenges remain before transchromosomic animals may be used for large-scale production of human polyclonal antibodies. Because transchromosomic calves retain the ability to express bovine antibody genes, expression of bovine antibodies may dominate over expression of the human antibody genes. In addition, chimaeric antibodies containing a combination of human and bovine heavy and light chains may also be present. Therapeutic Human Polyclonals Inc. (California, USA) have developed a technology platform to produce the next-generation polyclonal antibody therapy with products focusing on infectious diseases and cancer. The PolyTarg^{TM} platform utilizes transgenic rabbits for the production of humanized antibodies [36]. Origen Therapeutics Inc. (California, USA) [37] have developed technologies for the isolation, culture and genetic modification of avian embryonic cells for the production of fully human polyclonal antibodies. Although still in development, the use of transchromosomic animals for the production of human polyclonal antibodies has the potential for the production of biotherapeutics for the treatment of a variety of illnesses, including infectious disease and cancer.

7. Polyclonal antibodies for biodefense applications

Polyclonal antibodies show enormous potential as treatments to combat anthrax, smallpox and other biological warfare agents, mimicking the passive immunization that occurs naturally thus offering protection against a variety of pathogens including diphtheria, tetanus, streptococci and mumps [38,39]. Polyclonal therapeutics may be advantageous by providing the initial protection upon exposure to a toxic agent in the absence of a vaccine, or when levels of an immune response are low. Although vaccines may provide the necessary protection against biological warfare agents, several factors must be considered when using such an approach. An active immune response against the desired pathogen does not always correlate to protective immunity and the role of memory B cells in long-term immunity often depends on the incubation period of the pathogen. In addition, serum antibodies typically peak 10–14 days after primary immunization [1] and efficient protection may require repeated immunization to maintain high levels of neutralizing antibodies. Passive immunization with specific polyclonal antibodies or fragments targeted against disease-causing agents have the advantage of offering clinical protection immediately after exposure to pathogenic bacteria or viruses. *B. anthracis*, the causative agent of anthrax is a potential target candidate for polyclonal antibody therapy [40].

8. The future of polyclonal antibody therapeutics

The use of polyclonal based antibody therapeutics is undergoing a revolution. Although monoclonal antibodies may be considered ‘sniper bullets’ targeting a single specific epitope on a disease-causing agent, the Achilles’ heel of monoclonal therapeutics for the treatment of a variety of diseases may be the monovalent nature of the interaction. Polyclonal antibody therapy may be described as a targeted ‘machine gun’ approach, providing polyvalent interactions that permit the application of therapeutic strategies against multiple epitopes and targets. Another major advantage of polyclonal antibodies raised against a selected target in hyper-immunised animals is that the most immunogenic epitopes are ‘naturally selected’ for by the host. This may also permit the development and manufacture of polyclonal products that may target numerous biochemical path-

ways. In addition to the purified polyclonal approaches described above, several biopharmaceutical companies are also evaluating active immunotherapy and vaccine strategies utilizing cytokine derivatives, viral toxins or cellular factors released by pathogenic cells to elicit an innate human polyclonal antibody response for the treatment of a number of diseases including AIDS, cancer and some allergies [41]. Neovacs SA (Paris, France) has recently signed a development and licensing agreement and has advanced clinical-stage partnerships with at least one of its promising anti-cytokine therapeutics, a leading candidate for treatment of HIV infections [42]. Cytokine targeted immunotherapy may be used as a single therapeutic, or potentially combined with other treatments including conventional immunotherapies (targeting the disease-causing agent) or chemotherapy. Several attempts to use polyclonal-derived antibody therapeutics for the treatment of cancer have been reported. Therapies using anti-tumour polyclonal antibodies derived from animal or human antisera for the treatment of cancer have been described as early as the 1980s [43,44], and recently the generation of recombinant polyclonal antibodies for colorectal cancer therapy using tumor-reactive phage display libraries has been reported [45]. Although polyclonal antibodies have the potential to become a new generation of multi-targeted cancer therapeutics, the clinical success of these biotherapeutics will depend on the technology to express (or induce through optimised immunisation procedures) and characterise high titre polyclonal mixtures and also purify sufficient quantities to meet market demands. With continuing advances in antibody production, the future looks bright for polyclonal-derived antibody therapeutics.

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References

[1] J. Kuby, Immunology, second ed., W.H. Freeman and Company, New York, 1994.
[2] A.J. Stewart, P.M. Devlin, J. Infect. (2005) (Epub ahead of print).
[3] F. Katscher, Nature 381 (1996) 468.
[4] R.C. Bonnabeau, Minn. Med. 66 (1983) 331.
[5] A. Casadevall, M.D. Scharff, Clin. Infect. Dis. 21 (1995) 150.
[6] A. Casadevall, M.D. Scharff, Antimicrob. Agents Chemother. 38 (1994) 1695.
[7] P.F. Lucchesi, N. Gildersleeve, JAMA 116 (1941) 1506.
[8] C.O. Tacket, W.X. Shandera, J.M. Mann, N.T. Hargrett, P.A. Blake, Am. J. Med. 76 (1984) 794.
[9] C. Cresswell, A.R. Newcombe, S. Davies, I. Macpherson, P. Nelson, K. O'donovan, R. Francis, Biotechnol. Appl. Biochem. 42 (Pt 2) (2005) 163.
[10] P. Kumpalume, R. Boushaba, R.G.A. Jones, N.K.H. Slater, Trans. IChemE Part C, 80, 88.
[11] M.B. Hansen, A. Lihme, M. Spitali, D. King, Bioseparation 8 (1999) 189.
[12] R.F. Clark, P.E. McKinney, P.B. Chase, F.G. Walter, Ann. Emerg. Med. 39 (2002) 671.
[13] R.C. Dart, S.A. Seifert, L. Carroll, R.F. Clark, E. Hall, L.V. Boyer-Hassen, S.C. Curry, C.S. Kitchens, R.A. Garcia, Ann. Emerg. Med. 30 (1997) 33.
[14] V. Bara, Emerg. Nurse 9 (2001) 16.
[15] R. Safadi, I. Levy, Y. Amitai, Y. Caraco, Arch. Intern. Med. 23 (155) (1995) 2121.
[16] M. Farshid, R.E. Taffs, D. Scott, D.M. Asher, K. Brorson, Curr. Opin. Biotechnol. 16 (2005) 561.
[17] A. Buchacher, G. Iberer, Biotechnol. J. 1 (2006) 148.
[18] International Conference on Harmonisation of Technical Requirements for Registration of Pharamceuticals for Human Use: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. (ICH) 5QA (R1), 1997.
[19] T.W. Smith, Review of clinical experience with digoxin immune Fab (ovine), Am. J. Emerg. Med. 9 (2 Suppl. 1) (1991) 1–6 [Discussion 33–34].
[20] J.G. Schier, S.W. Wiener, M. Touger, L.S. Nelson, R.S. Hoffman, Ann. Emerg. Med. 41 (2003) 391.
[21] A new snake antivenom. Med. Lett. Drugs Ther. 43 (1107) (2001) 55–56.
[22] G. Juckett, J.G. Hancox, Am. Fam. Physician 65 (2002) 1367.
[23] R. Lemieux, R. Bazin, S. Neron, Mol. Immunol. 42 (2005) 839.
[24] A. Durandy, V. Wahn, S. Petteway, E.W. Gelfand, Int. Arch. Allergy Immunol. 136 (2005) 217.
[25] T.D. Martin, Int. Immunopharmacol. 6 (2006) 517.
[26] R.J. Looney, J. Huggins, Best Pract. Res. Clin. Haematol. 19 (2006) 3.
[27] S. Jolles, W.A. Sewell, S.A. Misbah, Clin. Exp. Immunol. 142 (2005) 1.
[28] M. Ballow, Am. J. Health Syst. Pharm. 62 (2005) S12.
[29] M. Prosniak, M. Faber, C.A. Hanlon, C.E. Rupprecht, D.C. Hooper, B. Dietzschold, J. Infect. Dis. 188 (1) (2003) 53 (Epub 2003 Jun 23).
[30] Y.Q. Song, G.F. Wang, X.L. Dai, H. Xie, Cell Res. 8 (1998) 241.
[31] Medicines Control Agency, Rules and Guidance for Pharmaceutical Manufacturers and Distributors, TSO Press, Norwich, UK, 2002.
[32] FDA Code of Federal Regulations (CFR), Title 21 Food and Drugs, National Archives and Records Administration, Office of the Federal Register, USA, 2004.
[33] J.S. Haurum, S. Bregenholt, IDrugs 8 (2005) 404.
[34] J.M. Robl, P. Kasinathan, E. Sullivan, Y. Kuroiwa, K. Tomizuka, I. Ishida, Theriogenology 59 (2003) 107.
[35] Y. Kuroiwa, P. Kasinathan, Y.J. Choi, R. Naeem, K. Tomizuka, E.J. Sullivan, J.G. Knott, A. Duteau, R.A. Goldsby, B.A. Osborne, I. Ishida, J.M. Robl, Nat. Biotechnol. 9 (2002) 889.
[36] Therapeutic Human Polyclonals Inc. http://www.polyclonals.com.
[37] Origen Therapeutics Inc. http://www.origentherapeutics.com.
[38] A. Casadevall, L.A. Pirofski, Expert Opin. Biol. Ther. 5 (2005) 1359.
[39] S. Bregenholt, J. Haurum, Expert Opin. Biol. Ther. 3 (2004) 387.
[40] A. Casadevall, Emerg. Infect. Dis. 8 (2002) 833.
[41] D. Zagury, R.C. Gallo, Drug. Discov. Today 9 (2004) 72.
[42] Neovacs SA, http://www.neovacs.com..
[43] G.A. Currie, Br. J. Cancer 26 (1972) 141.
[44] S.A. Rosenberg, W.D. Terry, Adv. Cancer Res. 25 (1977) 323.
[45] J. Sharon, M.A. Liebman, B.R. Williams, J. Cell Biochem. 96 (2005) 305.