Part VII
Approved Therapeutic Antibodies

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Oligoclonal and Polyclonal Antibody Preparations

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44.1
Introduction

Within the immunology world the definitions of monoclonal (one), oligoclonal (“few”), and polyclonal (many), either molecular or cellular, are understood. Although these, by definition, are clear, particularly in the antibody field, what is not clear is the pragmatic interpretation and implementation of what is necessary to use them. Any antibody preparation that is not monoclonal is then either oligoclonal or polyclonal and the difference is in degree. Oligoclonal means a few antibodies; so, at least two or more are combined in some meaningful way. At some point, the distinction between oligoclonal and polyclonal will blur and a relative number to help establish boundaries would be that preparations with more than 10 different specificities would be considered polyclonal and those with 2–10 different specificities would be considered oligoclonal. Such preparations as intravenous immunoglobulin (IVIg) can contain thousands of specificities, so would certainly be considered polyclonal. Recently, the technical and ethical limitations in generating antibodies have been discussed [1].

An important consideration in monoclonal and certainly oligoclonal antibody preparations is choosing the isotype of antibody to use. In most cases, this would be immunoglobulin G (IgG) related although individual isotypes, such as IgG1 and IgG3, may be useful. An oligoclonal antibody preparation can contain individual isotypes or a mixture of isotypes, depending upon the application. In addition, IgM oligoclonal preparations could be useful if the target antigen primarily elicited an immunoglobulin M (IgM) response.

44.2
Oligoclonal Antibodies

In response to an antigen, the natural immune response is an oligoclonal response and not a polyclonal response. This is primarily due to the targeting of a few elements of the immune response to the antigen, which is an oligoclonal response.

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As such, this is an important lesson to learn from nature in that since the natural immune response is oligoclonal, then perhaps this should be mimicked in developing a clinical treatment program in which an oligoclonal cocktail is used to treat patients, much the way the natural immune response works [2]. An *in vivo* oligoclonal immune response is the natural response to an antigen. Nature's oligoclonal ''preparations'' are antigen driven and result in the development of multiple germinal centers in immune organs such as lymph nodes, sites of isotype switching, and affinity maturation [3]. As a result of several germinal centers being formed from an antigenic challenge, an oligoclonal response is generated [3, 4]. The nature of the multiple germinal center antibody response generated could be any heavy chain isotype whether individual or a combination such as an IgM and an IgG.

Oligoclonal antibodies come in two varieties. One is synthetic and the other is natural. Synthetic oligoclonal antibodies are essentially manufactured and combined whereas natural oligoclonal antibodies are those that are harvested from donors and semi-purified to remove the bulk of the non-binding or other potentially interfering antibody molecules. An advantage of oligoclonal antibody preparations, synthetic or natural, is that they have the specificity of monoclonal antibodies (mAbs) combined with the sensitivity of polyclonal antibodies [5].

In addition to the specificity issues, another consideration in formulating oligoclonal antibody preparations is whether effector functions, such as Fc interactions, are important [5]. There are several types of Fc receptors and by formulating specific oligoclonal antibody preparations either certain subsets of Fc receptors can be targeted or perhaps all of them depending upon the application. Oligoclonal mAb preparations would have multiple modes of action that included not only traditional antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activity but also apoptosis and perhaps invoking an anti-idiotypic response [1, 2].

For oligoclonal mAb preparations some important considerations are whether the mAbs are to multiple epitopes on the same antigen (cocktail 1) or on different epitopes on different antigens (cocktail 2). A third possibility is mixing cocktail 1 and 2 to make a mix (cocktail 3; i.e., multiple antibodies to shared and unique epitopes on multiple targets). Each one of these combination cocktail formulations could have specific and unique applications both *in vitro* and *in vivo*.

The manufacturing of mAbs that will eventually be part of an oligoclonal preparation can be strictly controlled for Good Manufacturing Practice (GMP) regulations, unlike polyclonal preparations that are subject to batch-to-batch variations (see Section 44.7). Quality control issues would be minimized with compounded oligoclonal mAb preparations. For polyclonal antibody preparations immunized animals may be inconsistent, so manmade oligoclonal antibodies may remove this inconsistency. Moreover, mAbs can be selectively produced to specific epitopes and thereby controlled whereas this would be very difficult to precisely do with conventional polyclonal antibody preparations. Finally, the shelf-life of oligoclonal mAb preparations will be longer, thereby saving resources.

44.3
General Questions/Concerns

One particular question to ask is whether one should make one's own mAbs and custom combine them to make a custom oligoclonal mAb preparation or to commercially obtain already existing mAbs and combine them for your own specific use? The answer to this involves the amount necessary for the estimated work and whether a mAb to the target in question is commercially available. If small amounts are needed for benchtop-based experiments, then it may be more economical to commercially obtain the mAbs (assuming these mAbs are available). However, if no commercial mAbs are available to the target, then the investigator will have to make them. An important consideration here is whether more mAbs combined is a better approach than either a single or perhaps, two mAbs. Associated with this is the actual cost of manufacturing multiple mAbs, which may exceed any realistic return on commercialization.

The natural immune response is oligoclonal, so IgM antibodies do play a role. As such, will IgM oligoclonal antibody preparations or perhaps mixtures of IgG and IgM mAb oligoclonal preparations be useful? If only an IgM is available to the target in question, then this is what will be necessary to use. Furthermore, it is currently unclear what advantages oligoclonal preparations of immunoglobulin A (IgA) or immunoglobulin E (IgE) antibodies will have.

In addition to intact, whole antibody preparations another consideration is whether to use oligoclonal fragment combinations. A number of options are available with oligoclonal Fab, scFv oligos, diabody, tetrabody, and so on [6]. In these cases, caution is needed in the definition of what an antibody is. Fragments are not antibodies per se but rather binding molecules. Oligoclonal fragment combinations may be useful, but as they do not have an Fc region, certain elements of the immune response, such as effector functions, will not be available. Such oligoclonal antibody fragment preparations would be primarily useful in binding studies.

One interesting question is whether you can make an oligoclonal antibody preparation from polyclonal antibody sources such as IVIg? Significant depletion steps would then be necessary to reduce the heterogeneity seen in polyclonal preparations to get the number to 10 or less antibodies, work that may be difficult to justify.

44.4
Uses/Applications of Oligoclonal Antibodies

The first question is, why make an oligoclonal antibody preparation? In particular, what are the advantages over single mAbs and/or polyclonal antibodies? Oligoclonal antibody preparations will have two general uses, either as a therapeutic or a diagnostic. Current thinking with the FDA concerning therapeutic antibody preparations containing more than one antibody is that each antibody component

will be tested individually to establish safety and efficacy before they are combined [7, 8]. This could be a costly and time-consuming process if several antibodies are necessary for an oligoclonal preparation. Along with these FDA-related issues would be the necessary quality assurance/quality check (QA/QC) issues required for manufacturing multiple components of such a preparation.

A potential useful therapeutic application of an oligoclonal antibody preparation would be as a neutralization procedure such as would present with toxins of unknown origin, whether natural or manmade as in the case of chemical–biological weapons [9, 10]. Instead of trying individual anti-toxins, an oligoclonal preparation of certain key combinations could be more expedient. Another example is as a rabies therapy in which an oligoclonal preparation is composed of antibodies to individual components or epitopes of rabies antigens [11]. For diagnostic applications, it would be more expedient to screen for several antigens to establish what step or steps should be done next. The antigens could be related, such as in allergic pollens or unrelated physically although related chemically as in cross-reactive epitopes or with blood group or HLA-related specificities.

Depending upon how the oligoclonal preparation is composed the uses could have additive or synergistic effects in that binding of one antibody could influence the binding of other components for amplified effects, often seen in many immunoassays [12]. It is important that an oligoclonal mAb preparation should be based on specificity and functionality so that they compliment and synergize each other and not compete for the same or closely spaced epitopes. For antivirals, the target epitope may not be present on all strains, so in this case it would be necessary to have a combination of mAbs that recognize all strains [13].

For nontherapeutic applications such as immunoassays where the antibody requirements are not as strict as in clinical applications, oligoclonal antibody preparations would be easier to use [14]. In many of these immunoassays the assay reagents can be readily mixed and evaluated either individually or in combination. Titer and antibody affinity may play a role in how the immunoassays are performed.

For specific commercial applications, Speed Biosystems markets a mixture of ''10 carefully selected clones of monoclonal antibodies'' for use in immunohistochemistry studies [15]. They claim that such an oligoclonal preparation minimizes and lowers backgrounds in immunohistochemical analyses. Such multiple clones of mAbs are combined so that batch-to-batch consistency can be controlled. Since their oligoclonal preparation recognizes several epitopes on the target antigen, it has the same sensitivity as polyclonal antibodies.

Life Technologies markets a recombinant oligoclonal antibody preparation. They create a pool of mAbs against a specific antigen. These oligoclonal mAbs are produced by transfection of mammalian cells with heavy and light chain antibody cDNAs, thereby providing precise control of mAb properties.

Symphogen markets a two antibody mix to prions as well as recombinant polyclonal Abs for certain applications. Also, one product, Sym004, is a combination of anti-EGFR mAbs that synergistically inhibits cancer cell growth and survival [16], again suggesting that mAb combinations may have use in the clinic.

44.5 Infectious Disease

Davies *et al.* [17] showed that in a hamster model for *Clostridium difficile*, an oligoclonal mixture of three humanized IgG1 mAbs, which neutralize the TcdA and TcdB toxins, showed benefit at higher potency and neutralization, higher levels of protection, and higher valency of toxin binding compared to other agents. All are indicative of the benefits of an oligoclonal antibody preparation.

Meng *et al.* [14] showed that an antitoxin, XOMA3AB, consisting of three recombinant mAbs that potentially neutralize the known subtypes of type A botulinum neurotoxin (BoNT/A), may be beneficial over individual mAbs. XOMA3AB is an equimolar mixture of three human/humanized mAbs binding non-overlapping epitopes of BoNT/A with high affinity.

44.5.1 Virology

Both *et al.* [13] discussed the relevance of having a group of neutralizing mAbs and bispecific constructs useful in antiviral immunity. Oligoclonal combinations of mAbs that simultaneously target multiple viral epitopes is seen as broad coverage of different strains that could help reduce escape mutants. Virus targets are a particular issue due to the nature of viral strains and the mutants that can emerge, so an oligoclonal preparation is necessary to neutralize potentially heterogeneous virus populations. Examples of oligoclonal mAb combinations used as antivirals are against rabies [11], HIV [18], hepatitis B [19], and influenza [20].

44.5.2 Cancer

Jamnani *et al.* [21] showed that a combination of 12 nanobody clones derived from a camel library can be used to block several functional epitopes on the target HER2 antigen, thereby inhibiting the generation of escape mutants. It was also shown [12] that a mixture of two human mAbs showed a synergistic effect in decreasing tumor growth in a xenograft model that was greater than the individual mAbs.

44.6 FDA/Regulatory Considerations

The FDA has issued several guidelines [7, 22] on the requirements to characterize mAb combinations such as oligoclonal and polyclonal preparations. Since mAb combinations most likely have unique mechanisms of action that require the presence of all the antibodies, these combinations are clinically evaluated as a single mixture. In addition to these, such common questions as the product

stability, individual antibody characterizations, pharmacokinetic studies, and other analytical methods will need to be answered for the antibody combination.

Formulations of mAb preparations can take advantage of GMP manufacturing should the antibodies be used clinically. Combining mAbs in equimolar ratios would be the easiest for FDA approval. Should the antibody preparation be used *ex vivo*, as in most diagnostic tests, then different and simpler specifications will be necessary since no humans are directly involved.

44.7
Polyclonal Antibodies

Polyclonal antibodies are antibody preparations from immunized animals, normal or diseased individuals. They consist of complex mixtures of different antibodies produced by many different B-cell clones. Thus, by definition, mixtures of antibodies that are obtained from different B-cell resources or are isolated from the blood of target subjects are considered as polyclonal antibodies. They are a combination of immunoglobulin molecules directed against a specific antigen, each identifying a different epitope. Polyclonal antibodies are considered very effective because they ensure that a single antigen is recognized and attacked through its overlapping epitopes (antibody binding sites) by antibodies produced from multiple clones of B cells [23, 24].

Emil von Behring provided evidence that blood contained a substance that could counter diphtheria infections by transferring serum produced from animals immunized against diphtheria to animals suffering from it. Transferring the serum thus could cure the infected animals [25]. While the chemical nature of what exactly in the blood conferred this protection was not known, a few decades later it was shown that the protective serum could neutralize and precipitate toxins, and clump bacteria. These functions were the result of different substances that were later characterized as gamma globulins by Elvin A. Kabat in 1939 [26, 27]. The clonal selection theory was originally advanced by Paul Ehrlich to account for the wide range of antigens the immune system can recognize, which later was proved by the works of three scientists – Jerne, Talmage, and Burnet, who also documented that the specific receptors that could neutralize the agent were soluble molecules [28–30].

A single antibody can only bind to a small, specific area on the antigen. Consequently, an effective immune response often involves the production of many different antibodies by many different B cells against the same antigen; hence the term *polyclonal*. The antibodies thus produced in a polyclonal response are known as *polyclonal antibodies*, and are distinct from monoclonal antibody molecules, which are identical and react against a single epitope only, that is, are more specific. Although the polyclonal response confers advantages on the immune system, in particular, greater probability of reacting against pathogens, it also increases chances of developing certain autoimmune diseases resulting from the reaction of the immune system against native molecules produced within the host [31].

Overall immune responses in general are polyclonal in nature because each clone specializes in producing antibodies against a given epitope, and because each antigen contains multiple epitopes, each of which in turn can be recognized by more than one clone of B cells. To be able to react to innumerable antigens, as well as multiple constituent epitopes, the immune system requires the ability to recognize a very great number of epitopes in all; therefore, there exists a great diversity of B-cell clones [32].

When an antigen is recognized by more than one component of its structure, it is less likely to be ''missed'' by the immune system. Mutation of pathogenic organisms can result in modification of antigen and, therefore epitope [33, 34]. However, the immune system ''remembers'' what the other epitopes look like; the antigen, and the organism, will still be recognized and subjected to the body's immune response. Thus, the polyclonal response widens the range of pathogens that can be recognized [35].

44.8 Production of Polyclonal Antibodies

Antibody production is relatively a simple process, but requires consideration of several factors that range from the purpose and use (diagnostics, therapeutics, amount required, type of immunogen, antibody isotypes) [36, 37]. These factors complicate the process of not only production of polyclonal antibodies but also compatibility with the recipient. In addition, it is further complicated by the complex biological immune system of the immunizing host. It is well recognized that immunity of a living organism is not entirely predictable. Individual animals – even those that are genetically identical – respond differently to the same immunogen and immunization schedule that can result in generating different suites of specific antibodies against an injected antigen. Whole cells and large and complex molecules are more efficient than small compounds [37].

An antigen is any molecule that is identified as non-self by components of the immune system. An immunogen is an antigen that is able to evoke an immune response, including production of antibody via the humoral response. All immunogens are antigens, but not all antigens are immunogens [38]. It is important to distinguish between the terms *antigen* and *immunogen* because many compounds are not immunogenic, and successful production of antibodies against such antigens requires that they be made immunogenic by chemically attaching them to known immunogens before injection. For small peptides and small drug molecules it is frequently required to be chemically coupled to immunogenic carrier proteins, and be used in conjunction with adjuvants to increase the intensity of the immune response [39].

Antibody production involves preparation of antigen samples and their safe injection into laboratory or farm animals so as to evoke high expression levels of antigen-specific antibodies in the serum, which can then be recovered from the animal [40, 41]. Polyclonal antibodies are recovered directly from serum

(bleeds). Successful antibody production depends upon careful planning and implementation with respect to several important steps and considerations: (i) synthesis or purity of the target antigen (e.g., peptide or hapten), (ii) choice of an appropriate immunogenic carrier protein, (iii) conjugation of the antigen and carrier protein to create the immunogen, and (iv) immunization of animals using appropriate schedule and adjuvant formulation.

44.9 Immunogen Properties and Preparations

An immunogen is a substance that induces a specific immune response. Immunogenicity, which is the level of immune response it can elicit, largely depends on the chemical nature of the immunogen and the ability of the animal to respond to the immunogen. In addition, some substances are immunogenic in one species but not in another, and some substances are immunogenic in one individual (responders) but not in others (non-responders). Immunogenicity can be manipulated; therefore, the method of immunogen preparation is crucial [42]. Large size and significant complexity of chemical composition impart high immunogenicity. Proteins and polysaccharides are better immunogens than nucleic acids and lipids; however, lipids can function as haptens. As a rule, optimal immunogens have B-cell epitopes, T-cell epitopes, and MHC class II binding sites [43, 44]. Polysaccharides are generally T-independent immunogens because they are characterized by the repetition of the same antigenic determinant and are somewhat resistant to degradation [45].

The nature of antibodies produced in an immunized host is dependent on the preparation of the immunogen and the immunization protocol. Generally, purified molecules are used as immunogens. However, a major decision concerns how pure the antigen needs to be before starting the immunization schedule. Ideally, an immunogen should be purified to homogeneity for the production of monospecific antisera. Partially purified or crude immunogens could result in suppression due to ''antigenic competition'' [46]. This is because some proteins are more immunogenic than others. Haptens need to be coupled to a carrier, or larger protein, before immunization.

Immunization with non-denatured (i.e., native) proteins tends to produce antibodies against conformation epitopes, whereas immunization with denatured proteins tends to produce antibodies against linear determinants. Native proteins tend to be more immunogenic than denatured proteins [47]. A specific and high titer of polyclonal antibody could be produced using sonicated cellular preparations [48].

44.10 Carrier Proteins for Immunogen Preparation

A carrier molecule is any protein used to couple with peptides or other small molecules (haptens). The carrier protein, because of its large and complex nature,

confers immunogenicity to the conjugated hapten as well. There are a number of proteins that have been used as carriers, and have been selected on the basis of their immunogenic properties and availability of functional groups to which haptens can be coupled [49]. Bovine serum albumin (BSA) and ovalbumin are used carriers. These and other carriers, both modified and unmodified, are available commercially. In addition, reagents and protocols and reagents specific for coupling of haptens to particular functional groups (amino-, carboxyl-, and sulfhydryl-) on the carrier are available from various sources. Selection of carrier molecule and optimization of carrier to hapten ratios are critical factors to achieve the desired immune response to a hapten. In addition, a method should be in place to remove the carrier-epitope-directed antibodies from the end product. Among the various coupling reagents, the following are the most common approaches used to couple homo- or hetero-functional groups: (i) EDC (1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide) causes carboxyl and amine crosslinking, (ii) hetero bifunctional succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) is a non-cleavable crosslinking reagent. With this linker, amino group is coupled to sulfhydryl group, and (iii) glutaraldehyde conjugates amine-to-amine [49].

44.11 Choice of Animal

A wide range of vertebrate species have been used for the production of antibodies; however, mice and rabbits are the most widely used in research laboratories. Rabbits react to most immunogens, and relatively large quantities of antisera are obtained. The disadvantages of rabbits are that larger quantities of antigen are needed and rabbits often exhibit a high background because of heterologous antigens. Sheep and goats give very large amount of sera but they are difficult to maintain [36, 50]. Recently, molecular farming has opened up the opportunity of using plants for the production of antibodies and other proteins in plants. Plants are safe, extremely cost effective, and can carry out posttranslational modification [51, 52].

44.12 Adjuvants

Adjuvants are substances that enhance overall immune responses. Most adjuvants are comprised of two components. One component prevents rapid breakdown of the immunogen and promotes its slow release, causing a "depot effect." The second component non-specifically stimulates the immune system (mitogenic or polyclonal activation). These include lipopolysaccaride (LPS)-killed bacteria or muramyl dipeptide (MDP), which is a synthetic analog of the adjuvant-active component from the cell walls of mycobacteria [52–54].

44.13
Route of Injection

There are several possible routes to immunize animals; however, the route of injection has been known to affect the antibody response, both in terms of quantity of antibody produced and in the qualitative aspects of the antibody. Route of injection is dependent on the volume of immunogen to be injected. How quickly the immunogen should be released into the lymphatics or circulation is one of the determining factors of the route of injection. Rabbits are generally immunized subcutaneously at multiple sites to stimulate regional lymph nodes, whereas immunization of mice with large volumes can only be accomplished through intraperitoneal route. Intramuscular and intradermal injections are the best for slow release. Intraveneous immunization is only used for boosting with the immunogen containing no adjuvants [36, 55].

Initial contact with a new immunogen may evoke a primary response. The primary response is characterized by an initial rise in IgM followed by IgG [56]. Antibodies following the primary response are generally transient and will disappear from circulation. Subsequent exposure (e.g., booster injection) to the same antigen results in a much faster antibody response as well as in significantly higher levels of antibodies. Antibodies resulting from booster injection (secondary response) are predominantly of the IgG isotype. In addition, antibodies associated with the secondary response persist for a much longer time in the serum. This additional exposure to the antigen also results in a selection for plasma cells producing antibodies with a higher affinity for the antigen. This affinity maturation results in antibodies that react more strongly with the antigen [56, 57].

44.14
Collecting and Processing of Blood

Blood samples are typically collected 4–10 days after an immunization and tested for antibody titer. Since clotting factors interfere with many assays, serum is usually preferable over plasma. Serum is prepared by collecting the blood without anticoagulants and allowing the blood to clot for at least 30–60 min at 37 °C. Blood clotting can also be performed for 2–4 h at room temperature or overnight at 4 °C. The clotted blood is centrifuged to remove the clot and collect clear serum free of any blood cells. In some applications, it is also beneficial to inactivate the complement by heating the serum at 56 °C for 30 min. The following table gives blood collection parameters for various types of animals including chicken [47] (Table 44.1).

44.15
Antibody Purification

While whole sera can be used for most immunological assay applications, further purification of antibodies is generally not necessary. However, antibodies can

Table 44.1 Blood collection parameters for various species.

Species	Adult blood volume (ml)	Single sample volume (ml)	Exsanguinations volume (ml)	Adult body weight (kg)
Mouse	2.5	0.3	1.2	0.025–0.040
Hamster	9	0.5	3.0	0.085–0.150
Rat	30	2.5	12	0.3–0.5
Guinea pig	60	5.0	30	0.7–1.2
Rabbit	150–400	25–50	100–180	2–6
Goat (45 kg)	3000	400	1200	15–65
Sheep (60 kg)	4000	600	1500	20–70
Chicken	240	20	160	1.5–2.5

Taken from Ref. [47].

be partially purified and concentrated by precipitation with 40–50% saturated ammonium sulfate. This process removes a substantial portion of serum albumin, which is the major component of serum. Higher levels of purity can further be obtained after anion exchange chromatography on diethel-aminoethyl (DEAE) [58]. Affinity chromatography using protein A or G is also commonly used [59]. The various IgG subclasses from different animals exhibit a range of affinities for protein A and G, and can be differentially eluted from the affinity columns at different pH values.

Specific antibodies can also be purified from polyclonal sera using affinity purification methods where the antigen as ligand can be used to isolate the antibodies specific for that molecule. Furthermore, functionally monospecific antibodies, that is, antibody preparation reactive to an antigen but to different and dissimilar epitopes on the same molecule, are prepared by the high-resolution gel electrophoresis. It is also possible to pre-absorb serum to remove unwanted antibodies and to reduce background [58, 60].

44.16 Polyclonal Antibody Derives Therapeutics (Clinical Utility)

As reported by the Newcombe and Newcombe [61], antibody-based therapy using monoclonal or polyclonal antibodies is now well established as an important therapeutic approach for the treatment of a number of diseases. Owing to increasing emphasis on new technologies that are associated with monoclonal antibody expression and purification, the importance and need of polyclonal antibodies for the treatment of a number of autoimmune infections and infectious diseases has been neglected. Before the emergence of monoclonal therapeutics, polyclonal antibody therapeutics were widely used and are continued to be used in medicine for viral and toxin neutralization and for replacement of patients with immunoglobulin deficiencies. Intravenous administration of immunoglobulins has been shown

to exert beneficial effects through immunomodulatory and anti-inflammatory processes. In most cases, hyperimmune antibody preparations were used to overcome infectious diseases and medical emergencies such as digoxin toxicity and snake and spider bites [61]. In recent years, antibody-based therapies have returned as first-line therapy for a variety of diverse conditions that include viral infections, inflammatory disorders, and certain malignancies. Renewed interest in antibody-based therapies is a consequence of major advances in the technology of antibody production and the need for new therapeutic agents [62].

Examples of currently available polyclonal therapeutics include but are not limited to CroFab Crotalidae, a polyvalent immune Fab from Ovine for the treatment of rattle-snake bite [63]; DigiFab, a Digoxin Immune Fab from Ovine for the treatment of digoxin toxicity/oleander poisoning [64]; and ViperaTAb, an affinity purified Fab used as European Viper Antivenom (Table 44.2).

44.17 Recombinant Polyclonal Antibodies

Among the challenges to be faced in the next 10 years are the identification and validation of new targets, addressing the resistance to current drug treatments,

Table 44.2 Polyclonal antibodies in use for therapy [62, 65].

Marketed polyclonal therapeutic	Product description	Therapeutic application (Reference)
CroFab (animal origin)	Polyvalent immune Fab (ovine)	Rattlesnake antivenom [63]
ViperaTAb (animal origin)	Affinity purified, European viper antivenom (ovine) Fab	Common adder antivenom [66]
Antivenin (latrodectus mactans) (animal origin)	Antivenin (*Latrodectus mactans*) equine origin	Black widow spider antivenom
DigiFab	Digoxin Immune Fab (ovine)	Digoxin toxicity/oleander poisoning [64]
Thymoglobulin (animal origin)	Antithymocyte globulin (rabbit)	Immunosuppressive therapy [67]
Respigam (human origin)	Respiratory syncytial virus immunoglobulin intravenous (human)	Respiratory tract infection [68]
BayGam (human origin)	Immunoglobulin (human)	Passive protection against hepatitis A [65]
Nabi-HB (human origin)	Hepatitis B immune globulin (human)	Passive protection to hepatitis B [69]

and understanding target cross-talk and regulation. In the meantime, efforts are underway to decrease the costs of industrial production by increasing the productivity of the current cell lines, by developing alternative production systems and purification processes and by optimizing the design of more homogeneous and stable antibodies [70]. However, despite the fact that monoclonal antibodies and their cocktails have benefited human and animal health, there are several limitations and disadvantages that have been pointed out. To be able to circumvent the limitations and disadvantages associated with mAb technologies, antibody cocktails and human or animal serum, Symphogen has developed novel technologies, namely, Symplex and Sympress, to identify and manufacture antigen-specific recombinant human polyclonal antibodies. Symplex Technology is a process used to discover antibodies that are customized from plasma cells for a particular therapeutic application, and it involves direct isolation of the antibody genes from the immune system [71]. The Symplex technology captures a very broad representation of antibody species, reflecting the natural repertoire with affinities in the range of currently used monoclonal antibody drugs [72]. Sympress technology is the manufacturing platform used to produce recombinant polyclonal antibodies in high yields. This technology enables the production of consistent mixtures of polyclonal antibodies with high batch-to-batch consistency at an industrial scale [73]. In combination, the Symplex and Sympress technologies provide a means of screening, selecting, and manufacturing effective virus-specific recombinant human polyclonal antibody drugs. The topic of recombinant polyclonal antibodies to viruses has recently been reviewed by Bregenholt *et al.* [74].

44.18 Summary

Both oligoclonal and polyclonal antibody preparations have been used in the clinic, and as the technology matures, this use will only increase. The use of oligoclonal antibody preparations will eventually replace conventional polyclonal antibodies primarily because of advantages of more control and less variability. The same can be said of monoclonal antibodies in that for clinical applications oligoclonal combinations of antibodies will be used instead of monotherapy mAbs to better control and regulate the immune response. For most diagnostic assays mAbs will continue to be the reagent of choice. However, recombinant polyclonal antibodies manufactured through newer technologies such as Simplex and Sympless to identify and manufacture antigen-specific recombinant human polyclonal antibodies could make a significant impact in the arena of polyclonal antibodies as therapeutic drugs for infectious and autoimmune diseases, including cancer. While mono- and oligoclonal antibodies have proved to be of great significance, from cost point of view, polyclonal antibodies are expected to be economical therapeutics.

References

1. Glassy, M.C. and Gupta, R. (2014) Technical and ethical limitations in making human monoclonal antibodies (An Overview), in *Human Monoclonal Antibodies*, Humana Press, pp. 9–36.
2. Glassy, M. and McKnight, M. (2005) Requirements for human antibody cocktails for oncology. *Expert Opin. Biol. Ther.*, **5**, 1333–1338.
3. Victoria, G.D. and Nussenzweig, M.C. (2012) Germinal centers. *Annu. Rev. Immunol.*, **30**, 429–457.
4. Glassy, M.C. and McKnight, M.E. (1994) Pharming the human lymph node. *Expert Opin. Invest. Drugs*, **3**, 1057–1063.
5. Logtenberg, T. (2007) Antibody cocktails: next-generation biopharmaceuticals with improved potency. *Trends Biotechnol.*, **25**, 390–394.
6. Hudson, P.J. and Souriau, C. (2003) Engineered antibodies. *Nat. Med.*, **9**, 129–134.
7. U.S. Department of Health and Human Services (1997) Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, *www.fda.gov/downloads/BiologicsBlood Vaccines/GuidanceComplianceRegulatory Information/OtherRecommendationsfor Manufacturers/UCM153182.pdf* (accessed 06 December 2013).
8. Council, E. U. (2001) Directive 2001/83/EC of the European parliament and of the council of 6 November 2001 on the community code relating to medicinal products for human use. *Official Journal L*, **311** (28), 11.
9. Chen, Z., Moayeri, M., Crown, D., Emerson, S., Gorshkova, I., Schuck, P., Leppla, S.H., and Purcell, R.H. (2009) Novel chimpanzee/human monoclonal antibodies that neutralize anthrax lethal factor, and evidence for possible synergy with anti-protective antigen antibody. *Infect. Immun.*, **77**, 3902–3908.
10. Mazor, O., Ben-Arie, E., Mechaly, A., Rosenfeld, R., Marcus, H., and Ordentlich, A. (2010) in *The Challenge of Highly Pathogenic Microorganisms* (eds B. Velan, A. Shafferman, and A. Ordentlich), Springer, New York, pp. 275–285.
11. Bakker, A.B., Python, C., Kissling, C.J., Pandya, P., Marissen, W.E., Brink, M.F., Lagerwerf, F., Worst, S., van Corven, E., Kostense, S., Hartmann, K., Weverling, G.J., Uytdehaag, F., Herzog, C., Briggs, D.J., Rupprecht, C.E., Grimaldi, R., and Goudsmit, J. (2008) First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. *Vaccine*, **26L**, 5922–5927.
12. Glassy, M., McKnight, M., Kotlan, B., Glassy, E., and Koda, K. (2007) Cocktails of human anti-cancer antibodies show a synergistic effect in nude mouse tumor xenografts. *Hum. Antibodies*, **16**, 87–98.
13. Both, L., Banyard, A.C., van Dolleweerd, C., Wright, E., Ma, J.K.C., and Fooks, A.R. (2013) Monoclonal antibodies for prophylactic and therapeutic use against viral infections. *Vaccine*, **31**, 1553–1559.
14. Meng, Q., Li, M., Silberg, M.A., Conrad, F., Bettencourt, J., To, R., Huang, C., Ma, J., Meyer, K., Shimizu, R., Cao, L., Tomic, M.T., and Marks, J.D. (2012) Domain-based assays of individual antibody concentrations in an oligoclonal combination targeting a single protein. *Anal. Biochem.*, **421**, 351–361.
15. Speed BioSystems *www.speed biosystems.com/Antibodies/OligoClonal. aspx/* (accessed 06 December 2013).
16. Pedersen, M., Jacobsen, H.J., Koefoed, K., Hey, A., Pyke, C., Haurum, J.S., and Kragh, M. (2010) Sym004: a novel synergistic anti-epidermal growth factor receptor antibody mixture with superior anticancer efficacy. *Cancer Res.*, **70**, 588–597.
17. Davies, N.L., Compson, J.E., MacKenzie, B., O'Dowd, V.L., Oxbrow, A.K.F., Heads, J.T., Turner, A., Sarkar, K., Dugdale, S.L., Jairaj, M., Christodoulou, L., Knight, D.E.O., Cross, A.S., Herve, K.J.M., Tyson, K.L., Hailu, H., Doyle, C.B., Ellis, M., Kriek, M., Cox, M., Page, M.J.T., Moore, A.R., Lightwood, D.L., and Humphreys, D.P. (2013) A mixture of functionally oligoclonal humanized monoclonal antibodies that neutralize Clostridium difficile TcdA and TcdB with high levels of in vitro potency

shows in vivo protection in a hamster infection model. *Clin. Vaccine Immunol.*, **20**, 377–390.

18. Armbruster, C., Stiegler, G.M., Vcelar, B.A., Jäger, W., Köller, U., Jilch, R., Ammann, C.G., Pruenster, M., Stoiber, H., and Katinger, H.W.D. (2004) Passive immunization with the anti-HIV-1 human monoclonal antibody (hMAb) 4E10 and the hMAb combination 4E10/2F5/2G12. *J. Antimicrob. Chemother.*, **54**, 915–920.
19. Sawada, H., Iwasa, S., Nishimura, O., and Kitano, K. (1995) Efficient production of anti hepatitis B virus antibodies and their neutralizing activity in chimpanzees. *Appl. Microbiol. Biotechnol.*, **43**, 445–451.
20. Prabakaran, M., Prabhu, N., He, F., Hongliang, Q., Ho, H.T., Qiang, J., Meng, T., Goutama, M., and Kwang, J. (2009) Combination therapy using chimeric monoclonal antibodies protects mice from lethal H5N1 infection and prevents formation of escape mutants. *PLoS One*, **4**, e5672.
21. Jamnani, F.R., Rahbarizadeh, F., Shokrgozar, M.A., Ahmadvand, D., Mahboudi, F., and Sharifzadeh, Z. (2012) Targeting high affinity and epitope-distinct oligoclonal nanobodies to HER2 over-expressing tumor cells. *Exp. Cell. Res.*, **318**, 1112–1124.
22. Schenerman, M., Kozlowski, S., Webber, K., Gazzano-Santoro, H., and Mire-Sluis, A. (2004) CMC strategy forum report: analysis and structure characterization of monoclonal antibodies. *BioProcess Int.*, **2**, 42–49.
23. ElBakri, A., Nelson, P.N., and Abu-Odeh, R.O. (2010) The state of antibody therapy. *Hum. Immunol.*, **71**, 1243–1250.
24. Golden, J.W., Zaitseva, M., Kapnick, S., Fisher, R.W., Mikolajczyk, M.G., Ballantyne, J., Golding, H., and Hooper, J.W. (2011) Polyclonal antibody cocktails generated using DNA vaccine technology protect in murine models of orthopoxvirus disease. *Virol. J.*, **8**, 441.
25. Winau, F. and Winau, R. (2002) Emil von Behring and serum therapy. *Microbes Infect.*, **4**, 185–188.
26. Kabat, E.A. (1939) The molecular weigh of antibodies. *J. Exp. Med.*, **69**, 103–118.
27. Tiselius, A. and Kabat, E.A. (1939) An electrophoretic study of immune sera and purified antibody preparations. *J. Exp. Med.*, **69**, 119–131.
28. Silverstein, A.M. (2002) The clonal selection theory: what it really is and why modern challenges are misplaced. *Nat. Immunol.*, **3**, 793–796.
29. Schwartz, R.S. (2004) Paul Ehrlich's magic bullets. *N. Engl. J. Med.*, **350**, 11.
30. Forsdyke, D.R. (1995) The origins of the clonal selection theory of immunity: a case study for evaluation in science. *FASEB J.*, **9**, 164–166.
31. Nilsson, J. and Hansson, G.K. (2008) Autoimmunity in atherosclerosis: a protective response losing control? *J. Internal Med.*, **263**, 464–478.
32. Klinman, N.R. and Press, J.L. (1975) The B cell specificity repertoire: its relationship to definable subpopulations. *Transplant. Rev.*, **24**, 41–83.
33. Getzoff, E.D., Tainer, J.A., Lerner, R.A., and Geysen, H.M. (1988) The chemistry and mechanism of antibody binding to protein antigens. *Adv. Immunol.*, **43**, 1–98.
34. Varki, A. (1992) Diversity in the sialic acids. *Glycobiology*, **2**, 25–40.
35. Vivona, S., Gardy, J.L., Ramachandran, S., Brinkman, F.S.L., Raghava, G.P.S., Flower, D.R., and Filippini, F. (2008) Computer-aided biotechnology: from immuno-informatics to reverse. *Vaccinology*, **26**, 190–200.
36. Still, H.F. (2012) in *The Laboratory Rabbits, Guinea Pigs and Other Rodents* (eds M.A. Suckow, K.A. Stevens, and R.P. Wilson), Academic Press, Waltham, MA, pp. 259–274.
37. Marlies Leenaars, P.P.A., Hendriksen, C.F.M., de Leeuw, W.A., Carat, F., Delahaut, P., Fischer, R., Halder, M., Hanly, W.C., Hartinger, J., Jann Hau, J., Lindblad, E.B., Nicklas, W., Outschoorn, I.M., and Stewart-Tull, D.E.S. (1999) The production of polyclonal antibodies in laboratory animals: the report and recommendations of ECVAM workshop 35. *ATLA*, **27**, 79–102.
38. Sela, M. (1969) Antigens and antigenicity. *Naturwissenschaften*, **56**, 206–211.

39. Dey, A.K. and Srivastava, I.K. (2011) Novel adjuvants and delivery systems for enhancing immune responses induced by immunogens. *Expert Rev. Vaccines*, **10**, 227–251.
40. Zhao, X., Lin, X., Liu, G., and Wang, Z. (2012) Preparation of a polyclonal antibody against immunogenic fragment of bovine intestinal peptide transporter I (bPepTI). *Adv. Biosci. Biotechnol.*, **3**, 137–142.
41. Qi, Y.H., Shan, W.C., Liu, Y.Z., Zhang, Y.J., and Wang, J.P. (2012) Production of the polyclonal antibody against Sudan 3 and immunoassay of Sudan dyes in food samples. *J. Agric. Food Chem.*, **60**, 2116–2122.
42. Lee, N.H., Lee, J.A., Park, S.Y., Song, C.S., Choi, I.S., and Lee, J.B. (2012) A review of vaccine development and research for industry animals in Korea. *Clin. Exp. Vaccine Res.*, **1**, 18–34.
43. Dragovic, S.M., Conant, S.B., Gray, J.J., Zheng, M., Samir, P., Niu, X., Moutaftsi, M., Kaer, L.V., Sette, A., Link, A.J., and Joyce, S. (2013) Sculpting MHC class II–restricted self and non-self peptidome by the class I Ag-processing machinery and its impact on Th-cell responses. *Eur. J. Immunol.*, **43**, 1–11.
44. Blankenstein, T., Coulie, P.G., Gilboa, E., and Jaffee, E.M. (2012) The determinants of tumour immunogenicity. *Nat. Rev. Cancer*, **12**, 307–313.
45. Orson, F.M., Rossen, R.D., Shen, X., Lopez, A.Y., Wu, Y., and Kosten, T.R. (2013) Spontaneous development of IgM anti-cocaine antibodies in habitual cocaine users: effect on IgG antibody responses to a cocaine cholera toxin B conjugate vaccine. *Am. J. Addict.*, **22**, 169–174.
46. Bruder, J.T., Ango, E., Limbach, K.L., and Richie, T.L. (2010) Molecular vaccines for malaria. *Hum. Vaccin.*, **6**, 54–77.
47. Trier, N.H., Hansen, P.R., and Houen, G. (2012) Production and characterization of peptide antibodies. *Methods*, **56**, 136–144.
48. Sangdee, K., Thummabenjapone, P., and Sangdee, A. (2012) Evaluation of antigen preparation methods for polyclonal antibody production against Streptomyces spp. *Br. Microbiol. Res. J.*, **2**, 137–145.
49. Hermanson, G.T. (1996) *Bioconjugate Techniques*, Academic Press, San Diego, CA.
50. Hanly, W.C., Artwohl, J.E., and Bennett, B.T. (1995) Review of polyclonal antibody production procedures in mammals and poultry. *ILAR J.*, **37**, 93.
51. Abolade, A.S. and Ahmadu, R.O. (2012) Plants as animals alternatives in the production of antibodies and other therapeutic agents. *Asian J. Pharm. Biol. Res.*, **2**, 1–11.
52. Hua, J. and Hendriksen, C. (2010) Immunization for production of antibodies, in *Handbook of Laboratory Animal Science*, Essential Principals and Practices, 3rd edn, vol. **I**, Chapter 16 (eds J. Hau and S.J. Schapiro), CRC Press, Boca Raton, FL.
53. Cox, J.C. and Coulter, A.R. (1997) Adjuvants – a classification and review of their modes of action. *Vaccine*, **15**, 248–256.
54. Reed, S.G., Bertholet, S., Coler, R.N., and Friede, M. (2009) New horizons in adjuvants for vaccine development. *Trends Immunol.*, **30**, 23–32.
55. Swain, J.L., Sroller, V., Wong, C., Zhang, S., Halvorson, S.J., Herron, A.J., Kozinetz, C.A., and Butel, J.S. (2012) Effects of route of inoculation and viral genetic variation on antibody responses to polyomavirus SV40 in Syrian golden hamsters. *Comp. Med.*, **62**, 400–408.
56. Foote, J.B., Mahmoud, T.I., Vale, A.M., and Kearny, J.F. (2012) Cellular immunology and immune regulation: long-term maintenance of polysaccharide-specific antibodies by IgM-secreting cells. *J. Immunol.*, **188**, 57–67.
57. Codices, V., Martins, C., Novo, C., de Sousa, B., Lopes, A., Borrego, M., and Matos, O. (2013) Dynamics of cytokines and immunoglobulins serum profiles in primary and secondary Cryptosporidium parvum infection: usefulness of Luminex® xMAP technology. *Exp. Parasitol.*, **133**, 106–113.
58. Wongchuphana, R., Teyb, B.T., Tan, W.S., Subramanianc, S.K., Taipa, F.S.,

and Linga, T.C. (2011) Purification of rabbit polyclonal immunoglobulin G using anion exchangers. *Process Biochem.*, **46**, 101–107.

59. Grodzki, A.C. and Berenstein, E. (2010) Introduction to the purification of antibodies, in *Immunocytochemical Methods and Protocols*, Methods in Molecular Biology, vol. **588**, Chapter 2 (eds C. Oliver and M.C. Jamur), Humana Press, a part of Springer Science.

60. Agaton, C., Falk, R., Guthenberg, I.H., Göstring, L., Uhlén, M., and Hober, G. (2004) Selective enrichment of monospecific polyclonal antibodies for antibody-based proteomics efforts. *J. Chromatogr. A*, **1043**, 33–40.

61. Newcombe, C. and Newcombe, A.R. (2007) Antibody production: polyclonal-derived biotherapeutics. *J. Chromatogr. B*, **848**, 2–7.

62. Casadevall, A. (1999) Passive antibody therapies: progress and continuing challnge. *Clin. Immunol.*, **93**, 5–15.

63. Weant, K.A., Johnson, P.N., Bowers, R.C., and Armitstead, J.A. (2010) Evidence-based, multidisciplinary approach to the development of a crotalidae polyvalent antivenin (CroFab) protocol at a university hospital. *Ann. Pharmacother.*, **44**, 447–455.

64. Schaeffer, T.H., Mlynarchek, S.L., Stanford, C.F., Delgado, J., Holstege, C.P., Olsen, D., and Bogdan, G.M. (2010) Treatment of chronically digoxin-poisoned patients with a newer digoxin immune fab—a retrospective study. *J. Am. Osteopath Assoc.*, **110**, 587–592.

65. ElBakri, A., Nelson, P.N., and Odeh, R.O.A. (2010) The state of antibody therapy. *Hum. Immunol.*, **71**, 189–196.

66. Boels, D., Hamel, J.F., Deguigne, M.B., and Harry, P. (2012) European viper envenomings: assessment of Viperfav and other symptomatic treatments. *Clin. Toxicol.*, **50**, 189–196.

67. Gaber, A.O., Monaco, A.P., Russell, J.A., Lebranchu, Y., and Mohty, M. (2010) Rabbit antithymocyte globulin (Thymoglobulin): 25 years and new frontiers in solid organ transplantation and haematology. *Drugs*, **70**, 691–732.

68. Groothuis, J.R., Hoopes, J.M., and Jessie, V.G.H. (2011) Prevention of serious respiratory syncytial virus-related illness. I: disease pathogenesis and early attempts at prevention. *Adv. Ther.*, **28** (2), 91–109.

69. Stravitz, R.T., Shiffman, M.L., Kimmel, M., Puri, P., Luketic, V.A., Sterling, R.K., Sanyal, A.J., Cotterell, A.H., Posner, M.P., and Fisher, R.A. (2012) Substitution of tenofovir/emtricitabine for Hepatitis B immune globulin prevents recurrence of Hepatitis B after liver transplantation. *Liver Int.*, **32**, 1138–1145.

70. Beck, A., Wurch, T., Bailly, C., and Corvaia, N. (2010) Strategies and challenges for the next generation of therapeutic antibodies. *Nat. Rev. Immunol.*, **10**, 345–352.

71. Meijer, P.J., Andersen, P.S., Hansen, M.H., Steinaa, L., Jensen, J., Lantto, J., Oleksiewicz, M.B., Tengbjerg, K., Poulsen, T.R., Coljee, V.W., Bregenholt, S., Haurum, J.S., and Nielsen, L.S. (2006) Isolation of human antibody repertoires with preservation of the natural heavy and light chain pairing. *J. Mol. Biol.*, **358**, 764–772.

72. Meijer, P.J., Nielsen, L.S., Lantto, J., and Jensen, A. (2009) Human antibody repertoires. *Ther. Antibodies - Meth. Mol. Biol.*, **525**, 261–277.

73. Wiberg, F.C., Rasmussen, S.K., Frandsen, T.P., Rasmussen, L.K., Tengbjerg, K., Coljee, V.W., Sharon, J., Yang, C.-Y., Bregenholt, S., Nielson, L.S., Haurum, J.S., and Tolstrup, A.B. (2006) Production of target-specific recombinant human polyclonal antibodies in mammalian cells. *Biotechnol. Bioeng.*, **94**, 396–405.

74. Bregenholt, S., Jensen, A., Lantto, J., Hyldig, S., and Haurum, J.S. (2006) Recombinant human polyclonal antibodies: a new class of therapeutic antibodies against viral infections. *Curr. Pharm. Des.*, **12**, 2007–2015.