CHAPTER 12

Passive Immunization

OUTLINE

As described in Chapter 1, it was not long after Louis Pasteur's initial discoveries that the sources of immune protection were found in the blood and were called antibodies. It was demonstrated that blood serum containing antibodies to bacterial toxins such as those from tetanus or diphtheria could be transferred from an immune animal to a susceptible individual and so confer protection (Fig. 12.1). Thus the recipient was "immunized" without mounting an immune response—passive immunization. Horses were the major source of these "antitoxins" because of their size and ease of management. Passive immunization was widely employed in the 1920s and 1930s against human pathogens such as *Streptococcus pneumoniae, Neisseria meningitides,* and *Haemophilus influenzae,* in addition to tetanus and diphtheria. With the advent of cheaper and easier to use antimicrobials and antibiotics such as penicillin and streptomycin, it fell into disuse. Passive immunization only persisted for use in toxin-mediated diseases such as tetanus and botulism, virus diseases such as rabies, and in snake envenomation (Table 12.1). It is now staging a comeback. Polyclonal antibodies generated in immunized animals and monoclonal antibodies generated in the laboratory are increasingly employed in the treatment of diverse animal and human diseases.

Immunoglobulins

The major antibody in mammalian serum is a protein called immunoglobulin (Ig)G. This is a Y-shaped protein of about 160 kDa. In passive immunization, whole or semipurified serum, or IgG obtained from an immune animal, is injected into or fed to another animal. If it is injected into an animal of the donor species then the injected antibodies will simply be removed through normal catabolic processes. If IgG is injected into an animal of a different species it will act as a foreign antigen and trigger an immune response. Such a response will result in its prompt elimination. It is therefore highly desirable to minimize the antigenicity of IgG. The simplest way of doing this is to treat the IgG with a protease such as papain or pepsin. These split the IgG molecule into two or three fragments. The first fragment to be cleaved off is the "tail of the Y" (Fig. 12.2). This fragment can be crystalized and so is called the Fc fragment. It does not contribute to toxin or virus neutralization so it can be discarded. The rest of the IgG molecule consists of the two joined arms of the Y is called Fab'2. This fragment retains the antibody activity. Further proteolytic digestion separates this into two antigen-binding fragments each called Fab. These too are functional. Elimination of the Fc region greatly reduces the antigenicity of the preparation although the smaller fragments do have a shorter half-life than intact IgG.

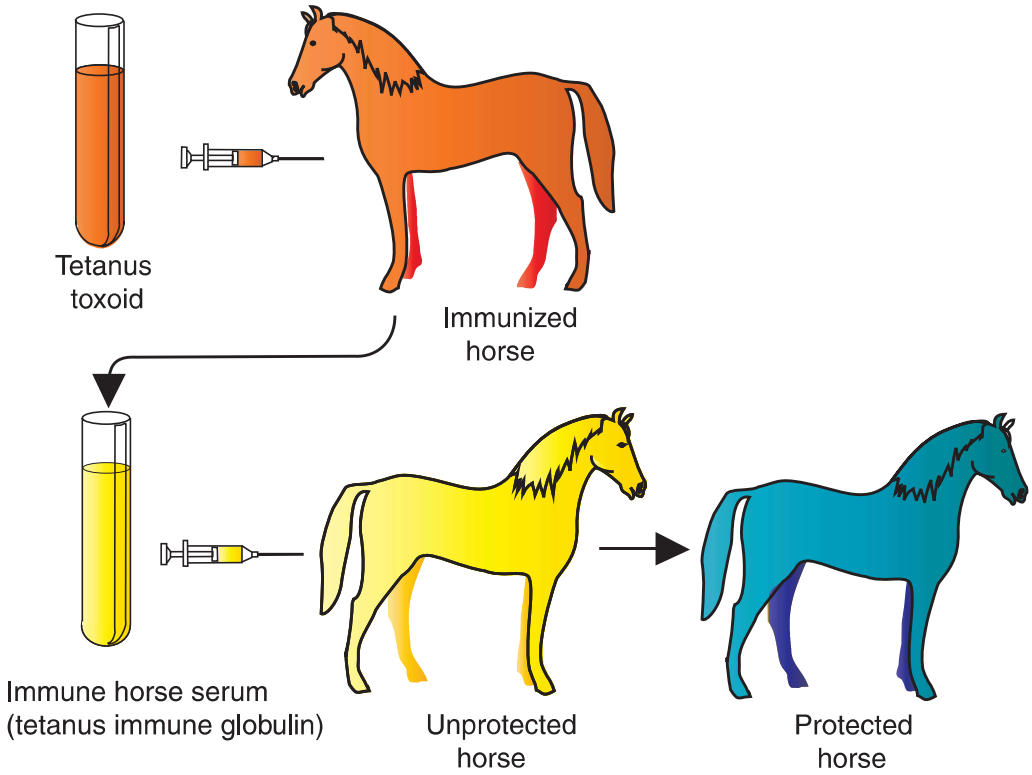

Fig. 12.1 The principle of passive immunization. Thus serum from an immunized animal contains antibodies. When injected into another animal these can confer immediate, but temporary, immunity.

TABLE 12.1 ■ **Licensed Polyclonal Antibody Products for Animal Use in the United States**

Function	Examples
Antibacterial	*Escherichia coli* (+K99)
	Rhodococcus equi
	Streptococcus equi
	Salmonella typhimurium
	Trueperella pyogenes
Antitoxins	*Clostridium botulinum* Type B
	Clostridium perfringens types C and D
	Clostridium tetani
	Crotalidae (rattlesnake) antivenin
Antiviral	Bovine rotavirus-coronavirus
	West Nile Virus

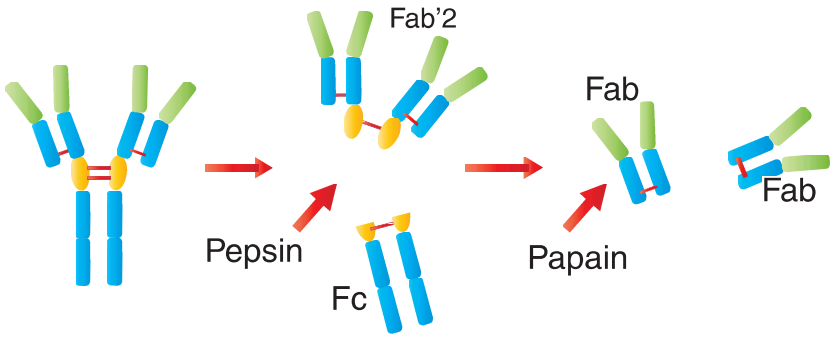

Fig. 12.2 If immunoglobulins are injected into another species they act as antigens and are removed rapidly. If fragmented by treatment with proteases and unnecessary components such as the Fc region removed, this immunogenicity is minimized.

As discussed later, modern molecular techniques also make it possible to alter the nonantigen-binding parts of immunoglobulins so that they too are identical to the recipient species and almost completely eliminating their antigenicity.

Polyclonal Antibodies

A natural immune response to a complex antigen such as a bacterium or virus activates large numbers of B cells that in turn generate a diverse mixture of antibodies, each with a different antigen-binding specificity. Most pathogens have a complex structure and present the immune system with many different epitopes. As a result, multiple B cell clones are stimulated to respond. These clones produce polyclonal antibodies. Polyclonal antibodies with their mixture of specificities can bind collectively to many different antigens. (This is in contrast to monoclonal antibodies that are derived from a single clone of B cells and bind only a single targeted epitope.)

IMMUNIZED DONOR ANIMALS

Passive immunization requires that antibodies be produced in donor animals by active immunization and that these antibodies then be given to susceptible animals to confer immediate protection. Serum containing these antibodies may be produced against a wide diversity of pathogens. For instance, they can be produced in cattle against anthrax, in dogs against distemper, or in cats against panleukopenia. They are most effective when protecting animals against toxigenic organisms such as *Clostridium tetani* or *Clostridium perfringens*, using antisera raised in horses. Antisera made in this way are called immune globulins and are commonly produced in young horses by a series of immunizing injections. The clostridial toxins are proteins that can be denatured and made nontoxic by treatment with formaldehyde. Formaldehyde-treated toxins are called toxoids. Donor horses are initially injected with toxoids, but once antibodies are produced, subsequent boosters may contain purified toxin. The responses of the horses are monitored, and once their antibody levels are sufficiently high, they are bled. Bleeding is undertaken at intervals until the antibody level drops, when the animals are again boosted with antigen. Plasma is separated from the horse blood, and the globulin fraction that contains the IgG antibodies is concentrated, titrated, and dispensed.

CHICKEN EGG YOLK

When an egg develops in the ovary of chickens, it contains a rich food source, the yolk. The yolk also contains a high concentration of chicken antibodies called IgY. IgY is the avian functional equivalent of mammalian IgG. Approximately 30% of the chicken's IgY (but only 1% of its IgM or IgA) will transfer from the plasma to the yolk. Thus a single egg yolk may contain up to 250 mg of IgY. If the hen is first vaccinated, then their eggs will contain high levels of antibodies against that antigen. If these yolk antibodies are simply fed to a mammal they will confer local immunity. Passive immunization by feeding egg yolk immunoglobulins is a relatively simple and economical method of protection against some enteric diseases. For example, chicken egg yolk antibodies can protect calves against diarrhea caused by group A rotaviruses. Seven days of IgY treatment significantly suppressed virus shedding, duration of diarrhea, and disease severity when compared with untreated calves. Similar benefits have been recorded in piglets and poultry.

Dried egg yolk powder from chickens has been administered to newborn puppies in milk replacer before closure of intestinal absorption (first eight hours after birth). Puppies supplemented in this way show significantly greater weight gain compared with controls. Weaned puppies receiving hyperimmune egg powder from chickens immunized against *Escherichia coli* and salmonella in the form of food supplementation had improved fecal quality and increased fecal

IgA. Likewise egg powder containing antibodies to canine parvovirus 2 (CPV2) protected puppies against CPV2 challenge.

BLOOD PLASMA

Spray-dried blood plasma is used as a feed additive for pigs. It contains high concentrations (20%) of immunoglobulins. It has been shown to improve weight gain and resistance to some pathogens. Thus it also protects against *E. coli* colonization. The beneficial effects appear to reside in the immunoglobulin fraction. However, it is possible that viruses such as porcine epidemic diarrhea virus may survive the spray-drying process. Pooled abattoir blood plasma is another possible source of purified IgG. Fed to piglets for seven days postweaning, it reduces the severity of postweaning diarrhea.

MILK WHEY

The major immunoglobulin in bovine milk is IgG. When casein is precipitated from milk during cheese manufacturing the liquid whey that remains contains small amounts of protein, 10% of which is IgG. However large volumes of whey are needed to obtain significant amounts of immunoglobulin for passive immunization.

Antitoxins

CLOSTRIDIUM TETANI

Antitetanus immunoglobulin (also called tetanus immune globulin or tetanus antitoxin) for veterinary use is produced in hyperimmunized healthy horses. Notwithstanding its equine origin, it can be used in cattle, sheep, pigs, dogs, and cats, as well as in horses. It is available in vials of 1500 and 15,000 units, and it contains thiomerosal and/or phenol to inhibit microbial growth. Deep, dirty wounds, especially when contaminated with soil or manure and tissues are devitalized sites where *Clostridium tetani* can grow and secrete its toxin. This toxin must be neutralized if clinical tetanus is to be avoided. Antitoxin should also be administered to nonimmune animals after castration, docking, and any surgical procedure conducted at sites where tetanus is known to be present. The half-life of equine IgG ranges from 27 to 39 days. Tetanus antitoxin given intramuscularly provides immediate immunity that lasts about 7 to 14 days in species other than horses.

To standardize the potency of different immune globulins, comparison is made to an international biological standard. In the case of tetanus immune globulin, this is done by comparing the dose necessary to protect guinea pigs against a fixed amount of tetanus toxin with the dose of the standard preparation of immune globulin required to do the same. The international standard immune globulin for tetanus toxin is a quantity held at the International Laboratory for Biological Standards in Copenhagen. An international unit (IU) of tetanus immune globulin is the specific neutralizing activity contained in 0.03384 mg of the international standard. Tetanus toxoid may also be measured in limes flocculation (Lf) units. These are determined by an in vitro flocculation test. They measure the quantity and antigenicity of a toxoid but not its potency. One Lf unit is the amount of toxoid neutralized by 1.4 IU of tetanus immune globulin.

Tetanus immune globulin is given to animals to confer immediate protection against tetanus. At least 1500 IU of immune globulin should be given subcutaneously or intramuscularly in the neck to horses and cattle; at least 500 IU to calves, sheep, goats, and swine; and at least 250 IU to dogs. The exact amount should vary with the amount of tissue damage, the degree of wound contamination, and the time elapsed since injury. Tetanus immune globulin is of little use once the toxin has bound to its target receptor and clinical disease appears. Notwithstanding this, some

veterinarians seek to improve its prognosis by administering high doses of antitoxin, 10,000 to 50,000 units to horses and cattle, and 3000 to 15,000 units to goats and sheep. Animals with slow-healing puncture wounds may be given a second dose in seven days.

Although immune globulins give immediate protection, some problems are associated with their use. For instance, when horse tetanus immune globulin is given to a cow or dog, as described earlier, the horse proteins will be perceived as foreign, elicit an immune response, and be rapidly eliminated.

If repeated doses of horse immune globulin are given to an animal of another species, this may provoke IgE production and allergic reactions. Additionally, the presence of high levels of circulating horse antibodies may interfere with active immunization against the same antigen. This is a phenomenon similar to that seen in newborn animals passively protected by maternal antibodies.

Mixtures of different monoclonal antibodies directed against multiple toxin epitopes are now being tested as possible replacements for polyclonal antiserum. These are more readily standardized than polyclonal antisera.

Serum Sickness

When tetanus began to kill large numbers of soldiers during the First World War, the use of tetanus antitoxin increased dramatically. Physicians gradually increased the amount of antitoxin administered to severely wounded soldiers. However, soldiers who had received a very large dose of equine antitetanus serum developed a characteristic illness about 10 days later (Fig. 12.3). This was called serum sickness and consisted of a generalized vasculitis with erythema, edema, urticaria of the skin, neutropenia, lymph node enlargement, joint swelling, and proteinuria. The reaction was usually of short duration and subsided within a few days. A similar reaction can be produced experimentally in rabbits by administration of a large intravenous dose of antigen. The development of sickness coincides with the formation of large amounts of immune-complexes in the circulation. The experimental disease may be acute if it is caused by a single, large injection of an antigen, or chronic if caused by multiple small injections. In either case, animals develop

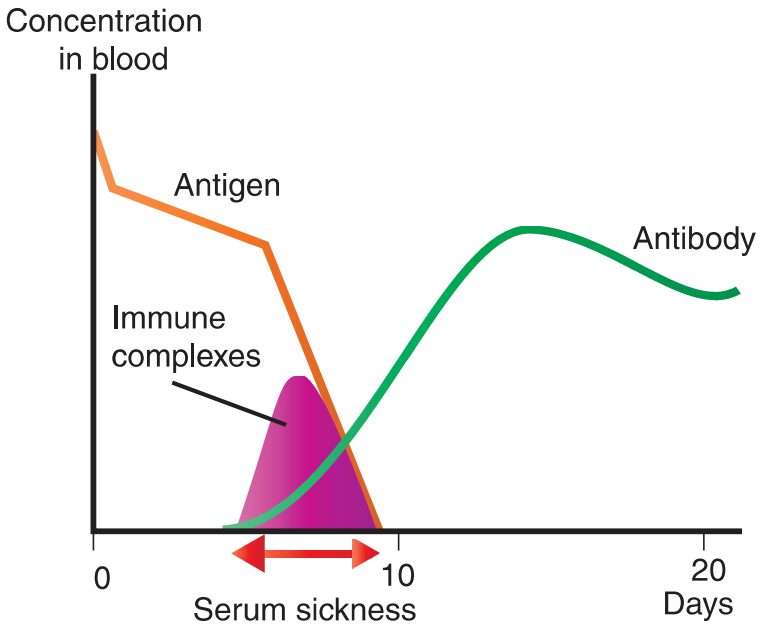

Fig. 12.3 If large amounts of foreign serum are injected into an animal, immune complexes form within a few days. These immune complexes are deposited in tissues such as the kidneys and joints to cause serum sickness.

glomerulonephritis and arteritis. For this reason, tetanus immune globulin of human origin is now preferred for the prevention of tetanus in people whenever possible.

CLOSTRIDIUM BOTULINUM

Clostridium botulinum antitoxins are commercially available. Antiserum to the type C toxin has been successfully used in ducks and mink. Type B toxin causes lethal toxicosis in foals (Chapter 15). Early administration of 30,000 IU of antitoxin intravenously has been reported to be effective in treating botulism in foals.

CLOSTRIDIUM PERFRINGENS, TYPES C AND D

This antitoxin of equine origin is given subcutaneously to prevent enterotoxemia (overeating disease) caused by *Cl. perfringens* toxinotypes C and D in calves, adult cattle, lambs, sheep, and goats, and by type C in piglets. (Type D is not known to cause disease in pigs). Protection lasts approximately 14 to 21 days. It is not standardized, and so for dosing refer to the product label. For disease treatment, the dose should be doubled.

Immune Sera

NEONATAL FOALS

Immune sera are especially useful in neonatal animals that may have failed to receive sufficient colostral antibodies. For example, commercially available horse serum, fresh frozen plasma, or horse IgG may be administered orally soon after birth or intravenously if gut closure has occurred.

Commercially available hyperimmune plasma is available to assist in the prevention of *Rhodococcus equi* pneumonia on infected farms. It should be administered as soon as possible after birth. At least 1L of licensed hyperimmune plasma should be administered to foals no older than 2 days of age. If the potential for further challenge is high, a second dose should be administered to foals between 14 and 32 days of age. This treatment reduces the severity of the disease in experimentally infected foals although it is not curative. Adult horses are susceptible to strangles caused by *S. equi* and equine antisera may be available to provide immediate protection to horses in contact. An antiserum is also available against West Nile virus.

NEONATAL CALVES

Neonatal calves that have received insufficient colostrum may be given normal bovine serum or purified IgG. They may also receive specific antisera against *E. coli, Trueperella pyogenes, Pasteurella multocida, Salmonella typhimurium,* and *Cl. perfringens* antitoxin either alone or in combination. They may also receive antibodies to bovine coronavirus and rotavirus. In general, antibodies against systemic infection are given subcutaneously, whereas antiserum against enteric disease is administered orally.

The use of commercial bovine serum is not without risks, especially if it comes from pooled random animals. Bovine virus diarrhea virus is an occasional but pervasive contaminant, whereas many other viruses may be detected by exquisitely sensitive metagenomic techniques. Ultraviolet or gamma radiation will inactivate viruses in serum, but specialized equipment is required.

NEONATAL PIGLETS

Equine antisera against *E. coli* and *Cl. perfringens* are available for use in piglets. They are given orally to treat neonatal diarrhea.

RABIES

Standard treatment of a human bitten by a rabid animal includes immediate postexposure prophylaxis. After thorough washing and flushing of the wound, local infiltration into and around the wound site or sites with rabies immune globulin (RIG) serves as a barrier to viral spread. The immune globulin will provide neutralizing antibodies at the site of exposure and prevent viral spread until the active immunization with a rabies vaccine takes effect. RIG is usually a polyvalent antiserum of human or equine origin. Human RIG is however expensive and not available in many less developed countries. Currently studies are ongoing on the use of carefully designed broad-spectrum monoclonal antibodies for this purpose.

SNAKE AND OTHER VENOMS

The primary causes of venomous snakebites in the United States are the pit vipers of the family Crotalidae—rattlesnakes, moccasins, and copperheads. Snake venom is a complex mixture and as many as 50 components contribute to its destructive properties. Collectively they cause soft tissue necrosis, vasculotoxicity, coagulopathy, cytotoxicity, and necrosis. This results in severe tissue damage, hypotension, and neurological impairment.

Administration of polyvalent crotalid antivenom is the preferred treatment for pit viper envenomation. The prompt use of this antivenom limits swelling and reverses the coagulopathy caused by the venom. Antibodies neutralize the toxin hemolysins, vasoactive, and myotoxic activities. However, these venoms contain toxins that cause immediate necrosis at the bite site so the antivenom may not prevent local tissue necrosis, especially if treatment is delayed. Early intravenous administration of the antivenom is essential when treating snake-bitten animals. Infusion should start slowly to determine if there are any immediate hypersensitivities. If all is in order then the flow rate can be increased. Ideally it should be administered within 4 hours of the bite, but it will still be beneficial if given within 24 hours. On the other hand, if the venom reaches the bloodstream, such as when an artery is pierced, then death may be inevitable despite use of antivenom.

At the present time there are two approved antivenoms available in the United States: Antivenin Crotalidae Polyvalent (ACP) (Wyeth-Ayerst) and CroFab Crotalidae Polyvalent Immune Fab (Ovine) (BTG International Inc.). ACP is minimally purified and still contains horse IgG and albumin. It is licensed for animal use. It has been used to treat humans for many years. ACP may not prevent the thrombocytopenia seen in some patients bitten by a timber rattlesnake (*Crotalus horridus*) or reverse the neurologic effects of Mojave rattlesnake (*Crotalus scutulatus*) venom. ACP generally requires the use of multiple vials depending on severity of the bite, size of the snake, and time elapsed, but cost may then be a factor. Additional doses may be given every 2 hours.

Crotalidae Polyvalent Immune Fab (Ovine) (CroFab, FabAV) is produced by immunizing sheep with 1 of 4 crotaline snake venoms: *Crotalus atrox* (Western diamondback rattlesnake), *Crotalus adamanteus* (Eastern diamond rattlesnake), *Crotalus scutulatus* (Mojave rattlesnake), and *Agkistrodon piscivorus* (Eastern cottonmouth). The IgG is then digested with papain to produce antibody fragments (Fab and Fc), and the more immunogenic Fc fragment removed. The four individual monospecific Fab preparations are then combined in equal amounts to form the final product. This product is about five times as potent as ACP. FabAV is efficacious against *Crotalus viridis* (Prairie rattlesnake), but ACP is not. As described earlier, Fab fragments are less immunogenic and less likely to induce adverse events. However, being much smaller molecules they are cleared more rapidly and thus have to be administered more frequently, perhaps as often as every six hours.

There are also two effective foreign antivenoms. One is a polyvalent product (Antivipmyn) manufactured in Mexico. The second is a polyspecific IgG product of equine origin (Polyvet-ICP) from Costa Rica. Antivipmyn, a Fab'2 antibody fragment antivenom, is cleared from the body

faster than IgG but slower than the Fab fragments. One or two vials appear to be effective in the dog. Smaller dogs may require more vials. Polyvet-ICP is not specifically directed against North American pit vipers. Both anaphylaxis and serum sickness are potential complications of antivenom treatment.

HUMAN PASSIVE IMMUNIZATION

Humans have a greater diversity of passive immunization products available than animals. These include antitoxins against tetanus, botulism, diphtheria, and anthrax; immune globulins against viruses such as rabies, varicella zoster, cytomegalovirus, measles, hepatitis A and B, respiratory syncytial virus, and vaccinia; antivenoms against numerous snake species, and also black widow spiders.

Monoclonal Antibodies

Monoclonal antibodies are another potential source of passive protection for animals (Box 12.1). There are several different ways to produce them. In the original method described in 1975, they were produced from mouse plasma cell tumors—myelomas (Fig. 12.4). This was done by fusing normal plasma cells making the antibody of interest with immortal myeloma cells grown in tissue culture. The resulting mixed cell is called a hybridoma. These hybridomas divide rapidly in tissue culture. Clones that produce the desired antibody are grown in mass culture and the supernatant fluid harvested. Unfortunately these mouse antibodies are immunogenic in other species and rapidly cleared from the body.

A strategy that is used to minimize this involves the genetic manipulation of hybridomas by replacing the mouse with human or other constant regions. For example, mouse myeloma variable regions can be attached to dog constant regions to make a "caninized" monoclonal antibody for use in that species. By subsequently modifying the sequence in the V region framework regions, the monoclonal antibody may be fully caninized. A caninized monoclonal antibody, directed against interleukin-31 (Il-31) is used to prevent itch in dogs with atopic dermatitis.

Several other methods have been used to produce human monoclonal antibodies and could be readily applied to veterinary species (see Fig. 12.4). For example, phage display techniques are commonly used. In this technique, the DNA encoding variable region genes, from heavy and light chains, are prepared from blood B cells. These genes are then paired to generate Fab regions. This generates a huge library of genes that are randomly inserted into filamentous bacteriophages. The phages are then exposed to a surface covered with the target antigen. Only phages displaying antigen-binding sites of the correct specificity will bind to the target antigen. After washing to remove any unbound phages, the bound phages are eluted and amplified by infecting *E. coli*. These steps can be repeated several times, thus effectively enriching the selected phages.

BOX 12.1 ■ Nomenclature

The nomenclature of monoclonal antibodies has been approved by the World Health Organization and other national bodies. All monoclonal antibody names end with the stem *mab,* whereas polyclonal antibodies end in *pab*. The previous letter *o* identifies its species of origin as mouse, and *u* denotes human origin. The letters before that refer to the antibody's target, such as *lim* for the immune system, *ci* for the circulatory system, *ne* for the nervous system and *tum* for cancer. The stem for humanized antibodies is *zu*, and that for veterinary use is *vet*. Finally, the prefix has no special meaning, but it should contribute to an easily pronounceable name. Adalimumab is a human monoclonal antibody that acts on the immune system (specifically antiTNF).

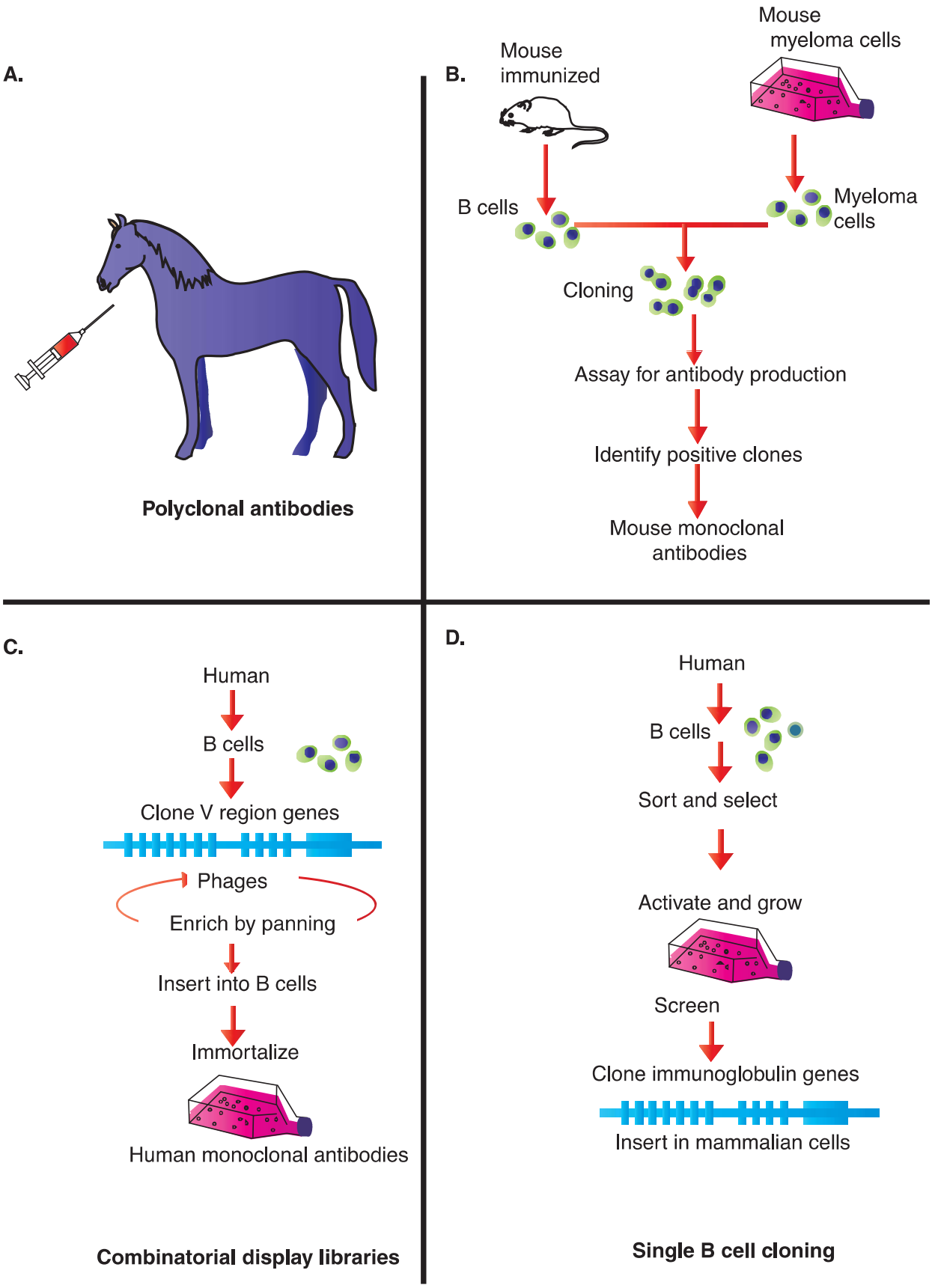

Fig. 12.4 (A) Polyclonal antibodies are generated simply by immunizing an animal and collecting the antibodies produced. (B) Originally monoclonal antibodies were produced using mouse myeloma cells fused with normal plasma cells to generate hybridomas. These hybridomas produce mouse monoclonal antibodies. Recently, other methodologies have been developed that enable monoclonal antibodies to be generated in species other than mice. (C) Immunoglobulin genes are cloned into a host such as a bacteriophage, yeast, or bacterium. These are then cloned and the clones making useful antibodies are selected and enriched. (D) The B cells from an individual making polyclonal antibodies can be activated and the individual cells analyzed to determine the antibodies they are producing. Selected cells can then be cloned and immortalized.

Once isolated, the relevant gene encoding the Fab of interest can be excised, sequenced, and cloned into immortal B cells.

An alternative method is to clone antibody-encoding genes from single B cells from an immune individual. (This can be either a naturally infected and recovered individual or an immunized one.) The desired clones are identified by a single-cell polymerase chain reaction or by panning on an antigen-covered surface in a manner similar to that used for phages. It is then possible to select those cells that make the most potent antibodies, especially those that make broadly neutralizing antibodies. Memory B cells are the major source of these antibodies and they can be immortalized by infecting them with Epstein-Barr virus, an oncogenic herpesvirus. These single B cell cloning techniques have been very effective in identifying key protective antigens.

Monoclonal antibodies, because of their purity, can be used for passive immunization. Many different "humanized" monoclonal antibodies are being employed to treat cancers and suppress inflammatory and autoimmune diseases. Similar species-modified monoclonal antibodies are being increasingly employed in veterinary medicine.

ANTIMICROBIAL MONOCLONAL ANTIBODIES

Although monoclonal antibodies have not been widely employed as therapeutic agents in infectious diseases, this is an obvious future development. They would be very useful in responding rapidly to emerging viral diseases. In humans multiple monoclonal antibodies have been prepared against different strains of Ebola virus. These have great potential for the emergency treatment of infected persons. Likewise monoclonal antibodies against Zika virus might protect human fetuses from congenital Zika syndrome. Another such example would be the use of these antibodies, directed against conserved epitopes, to protect against newly emerged pandemic strains of influenza. Only one antiviral monoclonal antibody (mAb) is commercially available. It is directed against respiratory syncytial virus—palivizumab. It should be pointed out that monoclonal antibodies are very expensive to produce, (about 100 times that of a polyclonal hyperimmune globulin) and a general assumption is that they are only effective for prophylaxis, something that active vaccination may do better.

ANTIINFLAMMATORY MONOCLONAL ANTIBODIES

Among the most successful applications of monoclonal antibodies is their use to decrease unwanted inflammatory reactions. In humans these antibodies may be directed against inflammatory cytokines such as tumor necrosis factor (TNF)-α or its receptor. They have proven very effective in the treatment of diseases such as rheumatoid arthritis or systemic lupus. Many of these are now being developed for animal use.

Atopic Dermatitis

IL-31 is the major cause of the severe itching in canine atopic dermatitis. The production of IL-31 in affected skin can be neutralized by administration of a caninized monoclonal antibody—Lokivetmab (Cytopoint, Zoetis), directed specifically at canine IL-31. The antibody is injected subcutaneously. It binds to circulating IL-31 and prevents it from binding to the IL-31 receptor. In double blind, placebo-controlled trials, a single dose has provided relief from itch and a reduction in disease severity in dogs with chronic atopic dermatitis.

ANTICANCER MONOCLONAL ANTIBODIES

Monoclonal antibodies are increasingly used for the treatment of cancers. They are usually directed against specific tumor cell antigens or against immunosuppressive signaling molecules. They are discussed in Chapter 23.

Equine Serum Hepatitis

On rare occasions, horses may develop lethal, acute hepatic necrosis 30 to 70 days after receiving an immune globulin of equine origin. This is called equine serum hepatitis or Theiler's disease. Theiler noted hundreds of cases of hepatitis in horses that had received equine antiserum. It has occurred in horses that have received antiserum against tetanus, botulism, *S. equi*, anthrax, influenza, and encephalitis, and also pregnant mare serum, and equine plasma. It has also occurred after active immunization against equine encephalitis and rhinopneumonitis when the vaccines were prepared using fetal equine cells. Certain serum mixtures or just a single vaccine batch may be associated with outbreaks of the disease. Occasional cases have been described in untreated horses living with affected animals, suggesting that the disease is transmissible.

Recently four novel viruses have been identified in horses with Theiler's disease. Three are flaviviruses (nonprimate hepacivirus, Theiler disease-associated virus, and equine pegivirus) and the fourth is equine parvovirus-hepatitis (EqPV-H). Theiler's virus was identified during an outbreak of acute hepatitis in horses that had received equine botulinum antitoxin. In another example 15 out of 17 (88%) horses injected with a specific lot of immune globulin were carrying the pegivirus. The disease was severe, with 53% to 88% mortality. Clinical signs included anorexia, icterus, excessive sweating, and neurological abnormalities. Clinical chemistry confirmed severe liver damage with high liver enzyme levels, ammonia, and bilirubin. However, the pegivirus is reported to be a common infection in horses in the United States and Europe and is not hepatotropic.

In a more recent situation, a horse that died 65 days after receiving tetanus antitoxin was shown to be infected by a parvovirus. It was present in the tetanus antitoxin administered to this horse nine weeks previously. This equine parvovirus-hepatitis (EqPV-H) virus belongs to the *Copiparvovirus* genus and is related to the bovine and porcine parvoviruses. Experimental inoculation of EqPV-H into two healthy adult horses resulted in the development of acute hepatitis 80–90 days later. Subsequent studies have demonstrated that EqPV-H was present in 18 consecutive cases of Theiler's disease whereas the other viruses were not consistently present. EqPV-H appears to be endemic in some horse populations because 13% of a random sample had circulating antibodies against it. In another study 9/10 horses with Theiler's disease and 20/37 in-contact horses were positive for EqPV-H by polymerase chain reaction (PCR).

Commercial tetanus antitoxin is usually heated at 60°C for one hour, but parvoviruses are relatively heat resistant. Some antitoxins have added phenol or thiomerosal as preservatives, but these may inactivate the flaviviruses while being insufficient to inactivate EqPV-H. Producers of equine blood products must ensure that their products are free of all these viruses (Box 12.2).

BOX 12.2 ■ Bovine Antibodies

Some cattle immunoglobulin molecules are unusually large because they use a long third hypervariable polypeptide loop (CDR3), which may contain up to 69 amino acids. This length is caused by a very long germline Dh2 gene segment. As a result, these CDR3s fold into a long beta-stranded stalk supporting a disulfide-bonded "knob" domain located far from the antibody surface. The benefits of this structure to cattle are unclear.

However, cattle immunized against human immunodeficiency virus (HIV) will produce strongly neutralizing antibodies. The knob and stalk structure can block the virus CD4-binding site much more effectively than can human antibodies. These neutralizing bovine antibodies may be used prophylactically or therapeutically against HIV and other major human pathogens.

(From Sok, D., Le, K.M., Vadnais, M., Saye-Francisco, K.L., et al. [2017]. Rapid elicitation of broadly neutralizing antibodies to HIV by immunization in cows. *Nature*, 548, 108–111.)

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Abstract: Immediate protection of an animal can be achieved by passive immunization—the administration of preformed antibodies to a susceptible animal. These antibodies have historically been produced in hyperimmunized horses. The most widely employed are antitoxins directed against clostridia, such as tetanus or botulism, and snake venoms. Polyclonal antibodies are also administered to neonatal animals to protect against infections such as *Rhodococcus equi* in foals. Monoclonal antibodies are more specific reagents that are currently being used for the treatment of atopic dermatitis, certain cancers, and selected infectious diseases. Intravenous immunoglobulins are being used to treat autoimmune diseases.

Keywords: immunoglobulins, polyclonal antibodies, antitoxins, immune sera, monoclonal antibodies, passive immunity, serum hepatitis, intravenous immunoglobulins.