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Antibodies: An Alternative for Antibiotics?

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ABSTRACT In 1967, the success of vaccination programs, combined with the seemingly unstoppable triumph of antibiotics, prompted the US Surgeon General to declare that “it was time to close the books on infectious diseases.” We now know that the prediction was overly optimistic and that the fight against infectious diseases is here to stay. During the last 20 yr, infectious diseases have indeed made a staggering comeback for a variety of reasons, including resistance against existing antibiotics. As a consequence, several alternatives to antibiotics are currently being considered or reconsidered. Passive immunization (i.e., the administration of more or less pathogen-specific antibodies to the patient) prior to or after exposure to the disease-causing agent is one of those

alternative strategies that was almost entirely abandoned with the introduction of chemical antibiotics but that is now gaining interest again.

This review will discuss the early successes and limitations of passive immunization, formerly referred to as “serum therapy,” the current use of antibody administration for prophylaxis or treatment of infectious diseases in agriculture, and, finally, recent developments in the field of antibody engineering and “molecular farming” of antibodies in various expression systems. Especially the potential of producing therapeutic antibodies in crops that are routine dietary components of farm animals, such as corn and soy beans, seems to hold promise for future application in the fight against infectious diseases.

(Key words: recombinant antibody, plant, alternative, antibiotic, expression system)

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“SERUM THERAPY,” THE OLDEST ANTIMICROBIAL THERAPY

Early Medical Successes

By the dawn of the 20th century, the etiology of many infectious diseases had been discovered through the work of Pollender and Davaine, who identified *Bacillus anthracis* as the first specific bacterial agent to be the cause of a disease (Buchwald and Pirofski, 2003). A few years later, Robert Koch and Louis Pasteur provided final proof for the germ theory, and the introduction of pure culture and dye staining technology by Koch and Ehrlich established bacteriology as an exact science (Conn and Conn, 1929). This swift progress in the knowledge of host-microbe interactions led to the first fundamental discoveries in immunology, such as immune therapy and vaccination. These discoveries led to the development of immune serum as a new, pathogen-specific anti-infective therapy (Baldry, 1965).

Serum therapy is the administration of immune serum from immunized animals or convalescent humans for the

prevention or treatment of infectious diseases (Buchwald and Pirofski, 2003). Behring and Kitasato established the principle of serum therapy in 1890, when they discovered that immunity against diphtheria toxin and tetanus toxin results from the presence in blood of substances they dubbed antibodies. Most importantly, they demonstrated that immunity could be transferred to naïve animals by serum of animals that had previously been challenged with nonlethal doses of a crude toxin preparation (Behring and Kitasato, 1890). Serum therapy earned Behring the Nobel prize and led to the production of the first commercially available antimicrobial therapy for clinical use in 1893: a sheep serum against diphtheria toxin (Winau and Winau, 2002). Because all that is required for efficacy of antibody treatment in the case of toxin-mediated infectious diseases is the formation of a stable immune complex between the toxin and the antibody, preventing the toxin from entering and damaging any target cells, it is not surprising that these were the first success stories of serum therapy. For instance, serum therapy reduced mortality from diphtheria from 50 to 80% to 6 to 15% (Buchwald and Pirofski, 2003). Antisera against cholera vibrios could transfer immunity to control animals and also kill the bacteria in vitro. Other examples

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Abbreviation Key: scFv = single chain variable region fragment.

include the immunoneutralization of tetanus toxin (Grundbacher, 1992) and toxins responsible for scarlet fever (pyrogenic exotoxins) (Weisse, 2001; Buchwald and Pirofski, 2003).

When attempts were made to use serum therapy for other bacterial diseases such as pneumococcal and meningococcal infections, it soon became clear that the efficacy of antisera against these diseases was not dependent on toxin neutralization. Indeed, little was known about the characteristics of antibody molecules, and antitoxins were thought to be derived by modification of the toxin.

We now know that binding of antibodies to a pathogen sets off a broad variety of effector mechanisms that go far beyond the simple prevention of attachment and invasion. Although antibiotics kill microorganisms directly or interfere with their replication, antibodies use much more versatile mechanisms that include the promotion of phagocytosis at the site of infection, activation of the complement cascade followed by an inflammatory response and attraction of phagocytes, and initiation of antibody-dependent cellular toxicity executed by monocytes, neutrophils, and natural killer (NK) cells. Antibody administration thus has the capacity to enhance immune function in immunosuppressed patients. In addition, antibodies can cause agglutination of bacteria and viruses (thus lessening the number of infectious units), restrict mobility of the pathogen, and inhibit microbial metabolism and growth when antibodies bind to bacterial transporter proteins (Oral et al., 2002).

A major obstacle for these mechanisms to be activated proved to be the existence of microbes with multiple serotypes. Although early studies in rabbits established the principle that pneumococcal infection could indeed be treated with antibodies, the fact that *Streptococcus pneumoniae* has different serotypes was a major obstacle for clinical application of immune sera (Henrichsen, 1999). By 1920, effective immune sera were available for serotypes 1 and 2, but for some reason an antiserum against type 3 could not be developed. Fatality rates of type 1 pneumococcal pneumonia could be reduced to as low as 5% if serum therapy was begun within 24 h after the onset of the symptoms (Casadevall and Scharff, 1994). However, if the serotype was of the untreatable type (or misdiagnosed) antibody therapy obviously failed (Krause et al., 1997). Another success story is the treatment of an epidemic of meningitis due to *Neisseria meningitidis* (meningococcus) in 1904 in New York City, which caused a mortality rate of 60 to 80% if left untreated. Depending on the age of the patient and the delay between infection and treatment, serum treatment reduced the fatality rate to 20 to 30% (Buchwald and Pirofski, 2003).

The routine use of serum therapy was abandoned due to a number of drawbacks such as the occurrence of serum sickness, the risk of disease transmission, and lot-to-lot variations of different serum preparations, but the most important factor reducing the application of serum therapy was certainly the advent of antibiotic therapy in the mid 1930s (Casadevall, 1996).

Limitations of Passive Immunization

Although the above success stories of serum therapy dating back from before World War II may seem anecdotal now, they illustrate very well the potential and limitations of passive immunization. Even though excellent results were obtained with the neutralization of bacterial toxins, the variability and multiplicity of bacterial epitopes posed serious problems for the use of serum therapy against the majority of bacterial diseases. This is still true today; antibodies are a superior therapeutic choice for the neutralization of toxins (as illustrated by the use of antisera against snake venoms), but treatment of other bacterial diseases requires fast and accurate microbiological diagnosis followed by swift and, if necessary, repeated administration of an adequate dose of high quality antiserum via a route that will effectively reach the pathogen for neutralization. Indeed, there is unfortunately no such thing as an effective broad-spectrum antiserum. This combination of requirements in most cases disqualifies antibodies as a first choice antimicrobial therapy.

From the point of view of antibody production, careful target selection is of the utmost importance, and although it is important that the antibodies are pathogen specific, monospecific antibodies, such as monoclonal antibodies, may not be a good choice because they are too specific. Narrow specificity is advantageous because it prevents development of resistance among nontargeted microorganisms and avoids disturbance of the normal microflora. However, narrow specificity becomes a disadvantage in cases of mixed infections that cannot be treated with a single antibody preparation. It also decreases the potential market for the drug. Moreover, genetic variability and immune evasion capabilities characterize many pathogens. The capacity to generate novel specificities thus becomes crucial for any successful antibody therapy. Next to the challenge of antibody specificity, the production cost of sufficient quantities of high quality antibodies is considerable and certainly higher than the cost of routine chemotherapy. Unless intended for therapy of enteric pathogens, therapeutic antibodies need to be administered systemically. In addition, systemic administration ideally requires antibodies of the same species in order to avoid anti-isotype immune reaction against the therapeutic agent, which is an obvious obstacle in human medicine (Casadevall and Scharff, 1994).

Present Day Medical Use of Antibody Therapy Against Infectious Diseases

In spite of the obvious drawbacks of passive immunization, antibody therapy is still relevant in the modern arsenal of antimicrobial therapeutics. First, antibodies are the best option for therapy and prophylaxis of a small number of viral diseases, for which antibiotics obviously are not a treatment option. Applications include postexposure prophylaxis of hepatitis B and varicella as well as treatment of viral diseases for which no vaccines or specific

drugs are available, such as rabies and Ebola (Keller and Stiehm, 2000). However, also bacterial diseases are on the list of candidates for antibody therapy, especially those for which preventive or therapeutic approaches are imperfect, not available, or no longer available. Pathogens include vancomycin-resistant *Enterococcus*, *Bordetella pertussis*, drug-resistant *Mycobacterium tuberculosis*, methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (reviewed by Keller and Stiehm, 2000; Oral et al., 2002; Buchwald and Pirofski, 2003).

Finally, antibody therapy has an increasing number of applications in which it is possible to use inexpensive sources of animal antibodies, such as bovine colostrum antibodies and egg yolk antibodies. With the exception of the systemic administration of purified egg yolk antibodies against snake venoms (Thalley and Carroll, 1990; Carroll et al., 1992; Almeida et al., 1998; Devi et al., 2002; Maya Devi et al., 2002), all these applications involve peroral administration of antibodies for the treatment of enteric infections, such as enterotoxigenic *E. coli*, *Helicobacter pylori*, the dental caries causing *Streptococcus mutans*, and rotaviral infections. Basically, these are almost the identical types of diseases that are treated with antibodies in the context of animal agriculture, as discussed in the section below (Carlander et al., 2000).

DOES ANTIBODY THERAPY HAVE A PLACE IN AGRICULTURE?

Antibody Therapy and Prophylaxis: An Extension of Maternal Immunity

Antibody therapy and prophylaxis have a natural place in animal agriculture for the simple reason that mammalian species such as pigs, horses, sheep, and cows do not transmit maternal immunity prenatally (antibodies do not cross the barrier of the placenta, as is the case in humans) but postnatally through colostrum antibodies (Korhonen et al., 2000a,b; Barrington and Parish, 2001; Van de Perre, 2003). In poultry, maternal antibodies are transmitted to the offspring via the yolk of the eggs, (Carlander et al., 2000; Mine and Kovacs-Nolan, 2002). As a consequence, colostrum and egg yolk were the first (and to the best of our knowledge still the only) sources of antibodies that are routinely used for prophylaxis and therapy of infectious diseases in an agricultural setting, for which cost of antibody production, antibody production capacity, and simplicity of administration (i.e., orally, through the feed) are critical factors.

Bovine colostrum and chicken egg yolks have indeed evolved naturally as antibody carriers par excellence, and colostrum was recognized as a vehicle for immune factors by Paul Ehrlich as early as 1892 (Ehrlich, 1892). During colostrum formation, immunoglobulins are transported across the secretory epithelium of the mammary gland by specific, receptor-mediated transport at a rate of as much as 500 g/wk. First colostrum contains between 30 and 200 mg of immunoglobulin per milliliter, the majority (75%) of which is IgG1 (Korhonen et al., 2000b). In a

similar fashion, circulating antibodies in the plasma of a laying hen are transported by the granulosa cell layer and deposited into the yolk during the last days of oogenesis (Hatta et al., 1997a). An egg can contain as much as 25 mg of IgY per milliliter of yolk (Rose et al., 1974). Given that the volume of an egg yolk is approximately 15 mL and that laying hens produce close to 300 eggs per year, this represents a dazzling production capacity approaching 100 g of antibody per hen per year. For a house with 10,000 layer hens, this represents a capacity of 1,000 kg of antibody per year.

One of the reasons antibody transfer through colostrum and egg yolk works so remarkably well in a natural situation is the occurrence of a short window of opportunity immediately postnatally during which the gastrointestinal environment is relatively mild and uptake of intact antibodies occurs (McFadden et al., 1997). This is the case only for the first 2 to 3 d of life; beyond this point uptake of intact antibodies no longer occurs, and any beneficial effects of antibody treatment need to be within the limits of the gastrointestinal environment. Those include challenges such as the acidic pH of the stomach and the presence of proteolytic enzymes; thus, natural transfer of maternal immunity is limited to the first couple of days of life. Nevertheless, human intervention has proven that peroral administration of antibodies beyond the time of enteric antibody absorption can indeed be quite effective, as described in the next paragraph.

Successful Applications of Colostrum and Egg Yolk Antibodies

Although systemic administration of highly purified egg yolk or colostrum antibodies is a theoretical possibility and has been described in experimental models (Thalley and Carroll, 1990), the bulk of antibody treatment with colostrum- and egg-derived antibodies involves peroral treatment of enteric pathogens (Korhonen et al., 2000a; Carlander et al., 2002). Applications are very similar in humans and in agricultural species, and in principle, colostrum antibodies and egg yolk antibodies seem to be used interchangeably. The best known examples include the treatment of enterotoxigenic *E. coli* (ETEC) in humans (Tacket et al., 1988; Freedman et al., 1998), pigs (Marquardt et al., 1999; Owusu-Asiedu et al., 2003), and calves (Ikemori et al., 1992), the dental caries causing bacteria *Streptococcus mutans* in humans (Filler et al., 1991; Hatta et al., 1997b), fatal salmonellosis in calves (Yokoyama et al., 1998), and the treatment of gastric infections with *Helicobacter pylori* in humans (Shimamoto et al., 2002; Shin et al., 2002). Also enteric viral diseases are currently treated successfully with colostrum or egg yolk antibodies. Those include rotaviral infections in humans (Ebina et al., 1983; Ebina et al., 1985; Davidson et al., 1989), calves (Kuroki et al., 1994), and piglets (Hennig-Pauka et al., 2003) and coronaviral infections in calves (Ikemori et al., 1997).

Limitations of Antibody Therapy in an Agricultural Context

Although the benefits of feed supplementation with pathogen-specific antibodies from hyper-immune colostrum and egg yolk are undisputed, it would be an exaggeration to call oral antibody therapy common practice, especially in an agricultural context. This undoubtedly has to do with the fact that production of standardized immune colostrum and egg yolk therapeutics, although theoretically and technically relatively simple, is labor intensive and, thus, cost intensive. As a consequence, these therapeutics are not used on a general scale (e.g., like antibiotics or vitamin and mineral supplements), and treatment of individual animals on a case-by-case basis represents an additional obstacle. Finally, there is still a widespread misconception that orally administered antibodies simply get digested and inactivated in the gastrointestinal tract, just like any other protein. Consequently, orally administered antibodies are not viewed as very effective drugs, at least not compared with small, simple, and stable molecules such as antibiotics.

Although it is true that orally administered antibodies are subjected to potential denaturation by the acidic pH of the stomach and degradation by proteases, active antibodies can still be detected in stool samples in percentages varying from very low levels to as much as 50% of the orally administered dose (Carlander et al., 2000). Studies have shown that parts of the antibodies remain intact in pepsin and trypsin digests, but there is considerable cleavage of the antibodies into Fab and $F(ab')_2$ fragments. It is important to note that these fragments still have the capability to bind to the antigen and display neutralizing activity, but that they may largely escape detection methods that depend on the presence of the antibody's Fc fragment that carries most of the isotypic determinants recognized by secondary reagents (Kuby, 1992). In addition, it is possible to administer antibodies in a stomach-resistant formula in order to increase the ratio of intact antibodies in the intestine (Reilly et al., 1997).

CURRENT DEVELOPMENTS AND FUTURE PERSPECTIVES

The Advent of Antibody Engineering

In recent years, recombinant antibody technology has gained importance in the generation of diagnostic and therapeutic molecules for pharmaceutical and industrial applications (Hayden et al., 1997). By using this technology, immunoglobulin genes or their fragments can be cloned into bacteria, and large quantities of recombinant antibodies can be produced rapidly in bacterial cultures (Pluckthun, 1991). As shown in Figure 1, the single chain variable region fragment (scFv) is the smallest fragment of an immunoglobulin molecule that has the same specificity and affinity as the full size antibody (McCafferty et al., 1990; Winter et al., 1994; Pini and Bracci, 2000). The scFv is a recombinant antibody fragment constructed from im-

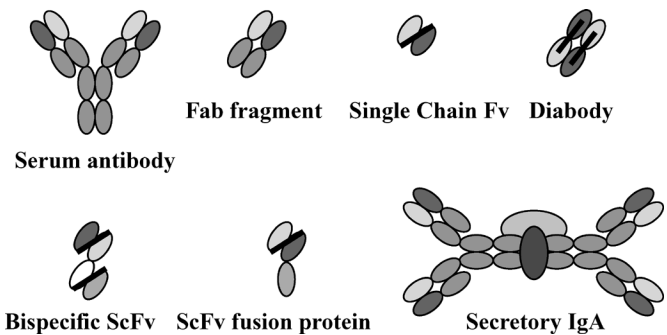


FIGURE 1. The various antibody formats that can be obtained by antibody engineering. All of the above antibody types have been expressed in various plant systems (Fischer et al., 2003). ScFv = single chain variable region fragment.

munoglobulin variable regions of heavy chain (V_H) and light chain (V_L) domains covalently held together by a flexible polypeptide linker (Bird et al., 1988; Huston et al., 1988) in such a way that the COOH-terminus of heavy chain links to the NH_2 -terminus of light chain. Recently, scFv fragments developed with recombinant antibody technology have proven to be effective in inhibiting the binding of their respective antigens to their receptors (Casalvilla et al., 1999; Cirino et al., 1999; Nagesha et al., 2001).

These developments are important for antibody therapy for two reasons: (1) antibodies can be engineered to fulfill very specific functions, and, more importantly, (2) totally new and previously unimagined expression systems can be used for the production of antibodies. Indeed, production systems now range from bacterial and yeast cell culture, over mammalian cell culture systems, to transgenic animals and transgenic plants (Dyck et al., 2003; Kipriyanov and Le Gall, 2004). Especially transgenic plants seem to offer immense possibilities with regard to future application in antibody therapy and prophylaxis in farm animals.

Plantibodies: The Future for Agricultural Antibody Prophylaxis and Therapy?

As mentioned above, in order for peroral antibody prophylaxis and therapy to become common practice and a valid alternative to antibiotics, a system needs to be designed that allows the production of gigantic amounts of antibodies of consistent quality (specificity, affinity, and concentration). In addition, the ideal system should provide such antibodies at a cost that is comparable with that of antibiotics and in a form that allows for straightforward administration, for instance by simply mixing the therapeutic agent in the diet. The only antibody expression system that combines these seemingly irreconcilable conditions is the transgenic plant system.

Initially, the strategy used for antibody production in plants was to transform tobacco plants separately with immunoglobulin light and heavy chain cDNA to express the light or the heavy chain protein. When these 2 plants were crossed, the resulting progeny produced a func-

tional antibody (Hiatt et al., 1989). The next advancement in this area was the demonstration of secretory IgA production in plants (Ma et al., 1995). This was accomplished by producing 4 different transformant types, each expressing a murine monoclonal antibody κ -chain, a murine monoclonal antibody α -chain, a murine J-chain, or a rabbit secretory component. A series of sexual crosses among these plants was performed to obtain progeny expressing all 4 components. In this final progeny, the 4 recombinant proteins were assembled into a functional, dimeric, and secretory immunoglobulin. Because scFv (see Figure 1) retain antigen-binding specificity in a single protein chain, they are much easier to produce. There are several reports demonstrating the use of plant systems to produce functional scFv (Fischer et al., 1999; De Jaeger et al., 1999; Vaquero et al., 1999; Torres et al., 1999; Stoger et al., 2000; Warzecha and Mason, 2003). Advances in these technologies have reached a point where several small biotechnology companies are operating currently to produce antibodies and other proteins of pharmaceutical interest in plants. The development of these companies is fueled by an expected annual expansion of 13% in the demand for protein pharmaceuticals with estimated revenue of \$25 billion per year.

The cost of purified recombinant antibodies from maize is currently estimated at no more than \$0.1/g, compared with \$300/g in animal cell culture, \$1 to 2/g from transgenic animals (milk or eggs), and \$1/g for microbial fermentation (Hood et al., 2002). In transgenic plants, the production cost is to a large extent dependent on the antibody expression level in the seeds, which is on average around 1% of the dry weight. Importantly, because antibodies can be expressed in a variety of crops that are normal components of animal diets, including not only maize but also soybean and wheat, it follows that no downstream processing and purification of the crude antibody preparation is needed, which represents 95% of the production cost of the pure protein. Additional benefits of addition of antibody-containing seeds to the diet include the obvious ease of storage and administration and the gradual and continuous infusion-like release of the antibody in the environment of the gut.

Even though this scenario is in theory perfect, several practical obstacles still need to be overcome. Probably the biggest stumbling block for now is the time and effort it takes to transfer the expression of an active antibody from a bacterial system to a plant system. This process takes anywhere between 1 and 2 yr, depending on the antibody format and the crop of choice (Hood et al., 2002). In addition, transgenic plants are currently used for the production of single molecules (e.g., the production of one monoclonal antibody per transgenic specimen). As mentioned above, monospecific antibody therapies are not optimal for fighting enteric pathogens, because the pathogen might mutate under the immune pressure of single antibody specificity and thus escape destruction. Therefore, ideally one would want to produce a transgenic seed containing an oligoclonal or polyclonal mix of pathogen-specific antibodies, which is currently not attainable tech-

nically, unless one would simply prepare a mix of monoclonal seeds. This approach requires extremely detailed knowledge of host-pathogen interactions to allow for the selection of the optimal target molecules, whether it is a pathogen molecule that is essential in invasion or persistence or an intestinal receptor molecule of the host. Finally, as with any genetically modified organism, containment (including separation from the human food chain), legal regulations, and acceptability by the public cannot be neglected.

In conclusion, there is well-founded confidence that recently developed antibody production technologies will be able to provide us with the massive amounts of highly specific antibodies at an affordable price. Only then will passive immunization become a realistic alternative strategy to chemotherapy for the large-scale prevention and treatment of infectious diseases in agriculturally important species. Proof of concept with regard to the use of maternal antibodies for the protection of neonatal farm animals against infectious diseases is as old as nature itself. However, it seems as if only our fairly recent capability to produce antibodies in transgenic crops will enable us to achieve similar results through human intervention in the not-too-distant future.

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