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PRODUCTION OF THE RAPEUTIC EGG YOLK ANTIBODIES IN GALLUS DOMESTICUS AND THEIR POTENTIAL

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Abstract:

Antibodies presently available for research, diagnostic and therapies are mostly mammalian monoclonal or polyclonal antibodies. Traditionally, bigger animals such as horses, sheep, pigs, rabbits and guinea pigs were used for the production of polyclonal antibodies, Chicken eggs present an ideal alternative antibody source to mammals, as the IgY in the chicken's blood is transported to the egg and accumulates in the egg yolk in large quantities. The yolks of eggs laid by immunized chicken have been recognized as an excellent source of polyclonal antibodies for over a decade. This simple non invasive approach presents an appealing alternative to conventional polyclonal antibody production methods. This review offers summarized information about production of edible antibodies used for passive immunization.

Key words: Immunoglobulin Y; Antibody; Chicken eggs; Immunization

Introduction:

Avian immunoglobulins transferred from the hens' blood into the egg yolk were named yolk immunoglobulins (IgY). They are an appealing alternative to mammal antibodies due to economical, ethical and animal welfare reasons. Nowadays, most frequently chosen mammals for polyclonal and monoclonal antibody production are rabbits and mice respectively. Both technologies have their advantages but also disadvantages. Major problem of monoclonal antibody production is that some antigens are weakly or not at all immunogenic for mice. In

polyclonal antibody production purification of antibodies from mammalian blood has been found to be low yielding and laborious in many cases. Both technologies also involve some steps each of which causes distress to the animals involved i) the immunization itself, ii) collecting of blood samples and iii) bleeding, which are a prerequisite for antibody preparation (Mojca Narat, 2003).

During the past 20 years, the use of chickens instead of mammals for antibody production has increased. A major advantage of using birds is that the antibodies can be harvested from the egg yolk instead of serum, thus making blood sampling obsolete. In addition, the antibody productivity of an egg-laying hen is much greater than that of a similar sized mammal (Hau & Hendriksen, 2005). Purification of immunoglobulin from mammalian blood is time-consuming and expensive. Today, hens are recognized as a convenient and inexpensive source of antibodies. It has been reported that the amount of immunoglobulin that can be yielded from one egg of an immunized hen is as much as that can be obtained from 300 ml of rabbit blood.

Antibodies from eggs:

Immunoglobulins (antibodies) can be readily produced in eggs by 174mmunized174 hens against specific antigens, serum antibodies of hyperimmunised hens are efficiently transferred and accumulated in the egg yolk. These Immunoglobulins can have broad applications from developing immunoassays to treating disease. Researchers have used egg antibodies in passive immunotherapy to treat a range of other diseases from bovine rotavirus in cattle to Mastitis in dairy cattle (Coleman, 1998). Antibody production in eggs is particularly advantageous because hens can be effectively 174mmunized, antibodies are readily deposited in the yolk, and eggs are a convenient and inexpensive food source.

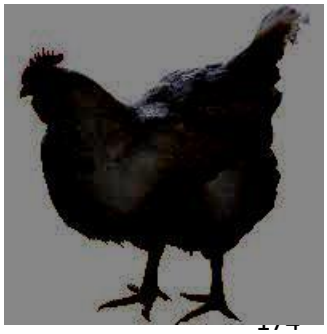
Antibodies are produced by the immune system of an animal in a specific response to a challenge by an immunogen. Immunogens (antigens) are molecules which can induce a specific immune response and are usually foreign proteins or carbohydrates or sometimes lipids and nucleic acids. Antibodies are secreted from plasma cells which have differentiated from B lymphocytes after appropriate stimulation by the foreign immunogen. Chicken egg yolk antibody (IgY) has received much attention in recent years because it can be easily prepared in high concentration and is both affordable and safe (Gassmann et al., 1990). IgY is successfully used in medical immune testing, diagnosis, heterografts and therapy. The use of chicken IgY in a double antibody sandwich ELISA for detecting African horse sickness virus by Du-Plessis et al. (1999).

New vaccine technology has led to vaccines containing highly purified antigens with improved tolerability and safety profiles, but the immune response they induce is suboptimal without the help of adjuvants. Gottstein and Hemmeler, 1985 reported Chickens store high contents of IgY in the yolk and are considered to be efficient antibody producers.

IgY production:

Immunization of the hens.

Specific IgY development and production can be achieved by immunizing laying hens with the target antigen. However, the resulting immune response of the immunized hens can not be very predictable. Mainly five factors influence this response: the antigen (dose and molecular weight),the type of adjuvant used, the route of application, the immunization frequency, and the interval between immunizations (Schade et al., 1996).



Antigen.

The immune response is triggered by contact of the organism with antigen, which is a structure that is recognized by the immune system as foreign ("nonself"). The dose of antigen influences significantly the immune response and the antibody titre that is evoked. Too much or too little antigen may induce suppression, sensitization, tolerance or other unwanted immunomodulation. found that the injection of antigen concentrations ranging between 10 μg and1 mg elicited good antibodies responses, and this was also reported by other researchers (Mahn, 1998).

Adjuvant.

The induction of high and sustainable eggyolk antibody titre reclaims the use of adjuvant. There are more than 100 known adjuvants, which differ in their chemical characteristics, their efficacy in stimulating the immune system, and their secondary side-effects. Freund's complete adjuvant (FCA) remains the most effective adjuvant for antibodies production in laboratory animals. In mammals, the use of this adjuvant leads systematically to severe inflammation at the injection site. In birds, the use of FCA does not seem to result in the same severe lesions as in mammals. The results of Gassmann et al., (1990) suggest that chickens show higher resistance to tissue damaging potency of FCA than rabbits. Svendsen et al.,(1996) also support this finding.

Route of application.

The most common route for antigen injection in hens for IgY production is the intramuscular route. Injection is usually performed in the breast muscle. Chicken can also be injected subcutaneously in the neck. With very young animals, it may be preferable to inject intramuscularly into the breast muscle, because subcutaneous injection is more difficult to perform and can therefore cause more distress (Schade et al., 1996).

Immunization frequency and interval between immunizations.

The total number of immunizations required depend on the type and dose of the antigen as well as the adjuvant employed. At least two immunizations have to be given. Yolk antibody titres should be checked 14 days after the last immunization. The success of an immunization protocol depends also on the interval between the first and second and subsequent immunizations. Often reported interval is two to four weeks (Tini et al., 2002).

Immunoglobulins:

The discovery and use of antibiotics and vaccination in animal agriculture have evolved from the management of small poultry flocks in the era prior to 1890s (Wehman, 1892) to the large consolidated units of today. The antibodies present in egg yolk have been termed IgY (Hatta et al., 1990). Thus, it is possible to obtain pathogen-specific IgY antibody from eggs laid by hens immunized against antigen (Shimizu et al., 1988). Since poultry farming is carried out on a large scale globally, eggs may be a suitable source of antibody for passive immunization, which requires large amounts of antibodies.

Over the past few years, we have successfully used the chicken egg yolk system to

produce polyclonal antibodies to enamel proteins and other calcified tissue matrix proteins (Nanci et al., 1996). Furthermore, the amount of antibodies produced from an egg is equivalent to that from 200 to 300 ml of mammalian blood, and the costs for animal care per unit production of antibodies are much lower in chicken than in mammals. However, the practical use of IgY in research and diagnostics is limited due to complex and time-consuming purification steps associated with the further purification of IgY (Akita and Nakai, 1992).

Isolation and purification methods for IgY:

Several methods were described in the 1950s for purifying IgY based on the strategy of separation of proteins (levitins) from lipoproteins (lipovitellins) and the rest of the yolk lipids using extraction with organic solvents with rather low yields of antibody. However, purification methods based on organic solvents like chloroform remain in use. Other methods are based on affinity chromatography or on dilution of the yolk followed by a freezing-thawing process after which the process consists of ion exchange chromatography and salt precipitations often combining a number of salts like for e.g. polyethylene glycol (PEG), dextran sulfate, dextran blue, sodium sulfate, ammonium sulfate, caprylic acid and sodium citrate. Hatta (1990) reported that the IgY remaining in this supernatant was isolated by DEAE-Sephacel column followed by salting-out with sodium sulfate resulting in almost pure IgY (98%) and the yield was 70-100 mg per egg. Water dilution method found to be superior in terms of ease of use and large scale production of IgY. This is simple rapid and efficient means of purifying IgY with high activity (Akita & Nakai, 1993).

Properties of IgY:

Laying hens transfer large amounts of immunoglobulin from serum to egg yolk of their eggs, where it serves as a means of passively protecting the developing chicks (Kariyawasam et al., 2004). An average egg may contain 100~150 mg of yolk immunoglobulins (IgY), and substantial amounts of specific antibodies may be collected and purified from the eggs of immunized hens (Akita and Nakai, 1992). The availability of large amounts of relatively inexpensive IgY from egg yolks makes it feasible to use these antibodies for passive immunization by oral administration or injection (Carlander et al., 2000). The efficacy of this approach has been shown in human and veterinary medicine for rotavirus diarrhea in humans. In aquatic species, IgY against Edwardsiella tarda was administered orally to passively immunize Japanese eels. These studies demonstrated that IgY could serve as an effective means against bacterial and viral infections (Van Nguyen et al., 2006).

Structure of rabbit IgG and chicken IgY.

Antibody stability:

IgY is fairly heat stable and most antibody activity remain after 15 min at 70°C. Incubation of IgY at pH above 4 is well tolerated, but at pH 2 and 37°C the activity is rapidly

decreased. The rapid activity loss is probably due to conformational changes, as the polypeptide is not broken down as observed by SDS-PAGE. The immunological activity of IgY is not affected by pasteurization at 60°C for 3.5 min. Addition of high concentrations of sucrose stabilizes IgY regarding heat denaturation, acid environment as well as high pressure. IgY fractions have been stored in 0.9% NaCl, 0.02% NaN_3 at +4°C for over 10 years without any significant loss of antibody titer. An egg can be stored in +4°C, with just a small loss of IgY activity for at least six months (Carlander, 2002).

Applications of IgY in Biomedical Research:

IgY Abs are used successfully in immunohistochemistry for detection of antigens of viral, bacterial, plant and animal origin, and also to assess the incidence of intestinal parasites in domestic animals Schniering, (1995) and the contamination of foods with toxins or drugs Pichler et al., 1998. During the past decade, IgY Abs have increasingly been used in therapy or prophylaxis of disease and also in the new context of socalled "functional food". Recent studies have compared the properties of specific IgY and IgG Abs originating from identically immunised animals. The results mostly confirm that IgY Abs can be used in the same way as IgG Abs, but additionally offer advantages in terms of specificity, cross-reactivity and/or sensitivity.

Powdered whole eggs or yolks have been used as an inexpensive alternative for the IgY treatment of enteric diseases in veterinary medicine. The most famous example of a successful therapeutic/prophylactic use of IgY is the treatment of calves and piglets with specific Abs against Escherichia coli , rotaviruses and coronavirus Ebina, (1996). Studies using both animal models and trials in field herds have been carried out. These studies confirmed that treatment of diarrhoea in calves and piglets with specific egg yolk Abs has achieved significant prophylactic and therapeutic benefits. Sunwoo et al. 2002 were able to demonstrate in vitro a marked growth inhibiting effect of specific IgY on E. coli 0157:H7, and showed that the growth inhibition was actually caused by the binding of specific IgY to the bacterial surface antigens, which caused significant changes in the bacterial surface structure. Another effect of IgY binding to bacterial surface antigens is a marked impairment of bacterial attachment to the intestinal mucosa. Lee et al., 2002. Thus, therapeutic IgY administration could reduce the clinical use of antibiotics, and so could lower the risk that bacteria will develop antibiotic resistance.

Conclusion:

IgY technology more popular and to convince the scientific community of its significant advantages. Chickens have the potential to be used to complete the spectrum of animals used for Ab production. The significant potential of avian antibodies for further use in immunodiagnostics and identification of disease markers, immunotherapy and the treatment and prevention of disease is expected. Since lot of benefits of IgY technology and its universal application in both research and medicine, it is expected that IgY will play an increasing role in research.

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