

Peroral Immunotherapy with Yolk Antibodies for the Prevention and Treatment of Enteric Infections

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Abstract

Oral administration of specific antibodies is an attractive approach to establish protective immunity against gastrointestinal pathogens in humans and animals. The increasing number of antibiotic-resistant bacteria emphasize the need to find alternatives to antibiotics. Immunotherapy can also be used against pathogens that are difficult to treat with traditional antibiotics.

Laying hens are very good producers of specific antibodies. After immunization, the specific antibodies are transported to the egg yolk from which the antibodies then can be purified. A laying hen produces more than 20 g of yolk antibodies (IgY) per year. These antibodies also have biochemical properties that make them attractive for peroral immunotherapy: They neither activate mammalian complement nor interact with mammalian Fc receptors that could mediate inflammatory response in the gastrointestinal tract. Eggs are also normal dietary components and thus there is practically no risk of toxic side effects of IgY. Yolk antibodies have been shown in several studies to prevent bacterial and viral infections.

Key Words

Antibody
Chicken
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Immunoglobulin
Yolk
Review

Introduction

There is an increasing prevalence of antibiotic-resistant bacteria, which makes traditional antibiotics less effective. It is thus important to find alternatives to the use of antibiotics. Oral immunotherapy with specific antibodies is a treatment strategy that has been actively pursued in laboratory studies and

early-stage clinical studies for the last decade. Several sources of antibodies have been used in these studies, including human IgG, monoclonal antibodies, bovine colostrum, and chicken egg yolk antibodies.

Polyclonal antibodies recognize more epitopes on the bacterial surface, which makes it difficult for bacteria to avoid the antibodies by

mutations. They are also cheaper to produce than monoclonal antibodies.

Egg yolk is a very interesting source of specific antibodies for oral immunotherapy. Hens, which have been immunized with microbes, respond by producing specific antibodies against the microbe. These antibodies are actively transported to the egg yolk in large quantities from the blood of laying hens.

A laying hen produces more yolk antibodies than a rabbit can produce during the same time period, and the animal care costs are lower for the chicken compared to the rabbit. Egg yolk antibodies will thus be an inexpensive way of producing large amounts of specific antibodies. Chicken antibodies offer many advantages to the traditional mammalian antibodies when used for the detection of mammalian antigen. Owing to the evolutionary difference chicken IgY will react with more epitopes on a mammalian antigen, which will give an amplification of the signal (1). Chicken antibodies can also be used to avoid interference in immunological assays caused by the human complement system, rheumatoid factors, human anti-mouse IgG antibodies (HAMA), or human and bacterial Fc receptors (1). The antibodies can be purified in large amounts from egg yolk, making laying hens highly efficient producers of polyclonal antibodies. Several studies show that bacterial and viral infections can be prevented with egg yolk immunoglobulin (IgY) in a dose-dependent manner. Peroral administrations of specific antibodies from egg yolk have been used successfully to treat bacterial and viral infections in animals (2–5).

Chicken IgY

The low-molecular-weight serum antibody found in birds is IgY, whereas mammals have IgG. IgY is the main serum immunoglobulin in chicken. Chicken IgY (6), or chicken IgG as it was called earlier, is the functional equivalent of mammalian IgG in birds, but it differs

in many functional aspects to mammalian IgG (1). Chicken IgY consists of two light chains and two heavy chains and has a molecular weight of approx 180,000 Da. The heavy chain (upsilon, ν) has a weight of 66,000 Da and the light chain 22,000 Da. IgY is transported from the mother to the embryo via the egg yolk, and the egg yolk thus contains high concentrations of chicken IgY. Other immunoglobulin classes are present only in negligible amounts in the egg yolk. The IgY concentration, in egg yolk is higher than the serum concentration and a single egg yolk (approx 15 mL) contains more than 100 mg IgY.

The use of chicken egg yolk as a source for antibody production represents a reduction in animal use as chickens produce larger amounts of antibodies than laboratory rodents. It also makes it possible to eliminate the collection of blood, which is painful for the animal. The European Centre for the Validation of Alternative Methods (ECVAM) thus recommends that yolk antibodies should be used instead of mammalian antibodies for animal welfare reasons (7).

When an antibody reacts with the antigen, an immune complex is formed. Antigen–antibody complexes containing mammalian antibodies will activate the human complement system (1). Activated complement components are potent inflammatory mediators. Theoretically, immune complexes containing mammalian antibodies may interact with Fc and complement receptors in the mammalian gastrointestinal tract, causing cell activation. Immune complexes containing IgY do not activate the mammalian complement system and do not interact with mammalian Fc and complement receptors. In summary, yolk IgY seems to be well suited for peroral immunotherapy.

Kinetics of Orally Administered Immunoglobulins

The transit time for orally administered antibodies in the gastrointestinal tract is

between 12 and 36 h in infants and children (8,9). Orally administered antibodies are subjected to denaturation by the acidic pH of the stomach and degradation by proteases. In neonates, the gastric fluid has a pH close to neutral but the pH rapidly decreases to below 3.0 during the first days. Studies have shown that part of the antibodies remain intact in pepsin and trypsin digests but there is a considerable cleavage of the antibodies into Fab, Fab₂ and Fc fragments. However, Fab₂, and Fab fragments still have the capability to bind to the antigen and exhibit neutralizing activity.

Despite the degradation in the gastrointestinal tract, active antibodies can still be detected in stool samples. The percentage active antibody in the stool varies between very low levels to up to 50% of the orally given dose in different studies. These differences are probably due to differences in the pH of the stomach, enzyme activity, presence of antigen, and the gastrointestinal passage time. The methods used to detect the antibodies in stool may also influence the results. A large portion of the antibodies is digested to Fab₂ or Fab fragments. These fragments still bind to the antigen, but many immunological tests give different results with Fab fragments than with whole antibodies.

Antibodies are absorbed from the intestine in young piglets and calves. Low levels of IgY can be detected in the circulation, of calves or piglets that are treated orally with yolk antibodies during the first 24 to 48 h post natum (10). After this time period there will be no absorption of active antibodies from the gastrointestinal tract. No absorption of intact antibodies has been shown in humans. Blum et al. (11) and Eibl et al. (12) showed that there was no increase in serum immunoglobulin levels after oral administration of human immunoglobulins to infants. Also, Lososky et al. (9) could not detect any high-molecular-weight radioactivity in blood after oral administration of ¹²⁵I-labeled IgG. Thus, no systemic

effects can be expected after oral administration of yolk antibodies to humans.

Consumption of eggs is accepted in most cultures. Eggs are normal dietary components and there is practically no risk of toxic side effects of IgY. The mean egg consumption is 0.5–1 egg/d in Sweden. This amount of IgY is similar to or higher than the amounts used in several of the immunotherapy studies. However, allergic reactions to egg-derived immunoglobulins and to residual egg proteins may occur (13). It is important to consider egg allergy before starting immunotherapy with yolk antibodies in humans.

Prevention and Treatment of Bacterial Infections

Animals

Oral administration of spray dried yolk antibodies specific against *Salmonella typhimurium* or *S. dublin* has been shown to prevent *Salmonella* infections in calves. The antibodies were administered three times a day for 7–10 d after inoculation with *S. typhimurium* or *S. dublin*. All calves in the control group died, whereas only diarrhea and fever were observed in the group treated with the highest antibody titer. Thus, the antibodies could protect against fatal salmonellosis (14).

Yolk preparations from chickens immunized with purified outer membrane proteins, lipopolysaccharide, flagella, and fimbriae of *Salmonella* have also been tested in mouse models of experimental salmonellosis. The mice treated with specific antibodies had a significantly higher survival rate than mice fed normal egg yolk antibodies (14,15).

Yolk immunoglobulins have also been used to protect neonatal piglets against experimental enterotoxigenic *Escherichia coli* infections. Oral treatment with antibody preparations against the fimbrial adhesins K88, K99, and 987P considerably reduced the severity of diarrhea and reduced the mortality (16). Yolk

immunoglobulins have also been shown to prevent diarrhea in rabbits challenged with *E. coli* (17).

Humans

A mouth rinse containing egg yolk antibodies to *Streptococcus mutans* has been used to reduce the establishment of these bacteria in dental plaque of humans. The antibodies inhibited *S. mutans* adherence to saliva-coated hydroxyapatite discs in vitro and decreased the percentage of *S. mutans* per total streptococci in vivo (4).

We have also shown that gargling once a day with a yolk preparation specific to *Pseudomonas aeruginosa* prevents the colonization of these bacteria in the lungs of patients with cystic fibrosis (18). One of these patients has now been treated for more than 4 yr without any chronic colonization with *P. aeruginosa*.

Prevention of Viral Infections

Animals

Egg yolk and colostrum powders containing specific antibodies against bovine coronavirus (BCV) antigen were evaluated in a challenge model with a standardized dose of virulent BCV strain.

Daily treatment with these antibody preparations started 6 h until 7 d post-challenge. Control calves, which received no antibody, had severe diarrhea and all died within 6 d after infection. In contrast, calves fed milk containing egg yolk or colostrum with high antibody titers all survived and had positive weight gain. The study showed that specific antibody preparations protected against BCV-induced diarrhea in neonatal calves and that the egg yolk used provided a higher degree of protection compared to colostrum powder on a titer basis (19).

A field trial with oral administration of chicken IgY specific for bovine rotavirus (BRV) resulted in a significantly increased

mean body weight ($p < 0.05$) and a decrease in number of calves shedding high titer of BRV (G6) in stool compared to control calves ($p < 0.01$) (20). Oral administered yolk immunoglobulins have also been shown to protect suckling mice against experimental bovine rotavirus-induced diarrhea (21).

Humans

Oral treatment with bovine antirotavirus colostrum resulted in a reduction of rotavirus-associated diarrhea in infants (22). It also reduced the infection rate in children (23).

Prevention of Other Infections

Cryptosporidium is a zoonotic pathogen that infects the respiratory or gastrointestinal tracts of a large number of hosts including fish, birds, reptiles, and mammals. Cryptosporidium infections are usually associated with immunosuppressed hosts, but the infection may also occur in individuals with a normal immune system. The use of yolk preparations with high anti-Cryptosporidium activities caused a significant parasite reduction in a neonatal mouse model (24). Treatment with bovine anti-Cryptosporidium colostrum immunoglobulin has also been shown to induce a 100-fold reduction of oocyte excretion in healthy human volunteers (25).

Conclusions

Laying hens are a cost-efficient method for the production of large quantities of specific antibodies. Yolk antibodies do not activate the mammalian complement system nor interact with mammalian Fc receptors that could mediate inflammatory response in the gastrointestinal tract. Eggs are also normal dietary components and thus there is practically no risk of toxic side effects of IgY. Oral immunotherapy with yolk antibodies is thus an attrac-

tive approach for treatment of gastrointestinal infections in humans and animals that are difficult to treat with traditional antibiotics. The

increasing number of antibiotic resistant bacteria emphasize the need to find alternative to antibiotics.

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