

Egg yolk antibodies for disease prevention

Abstract

The general structure of the egg yolk antibody, containing of two light and two heavy chains, is comparable to that of mammalian immunoglobulins. Oral administration of specific egg yolk antibody is an attractive approach against gastrointestinal infection in humans and animals. The use of IgY as antibiotic-alternative therapy have advantages: (1) The production of IgY is a non-invasive and inexpensive method; (2) Handling of animal is easy; (3) Don't need to bleeding of laboratory animals; (4) A laying hen is able to produce more than 20g of IgY per year; (5) Eggs are normal dietary components and there is no risk of toxic side effects of IgY. The increasing number of antibiotic resistant bacteria confirms the need to find alternative to antibiotics such as IgY. Yolk antibodies have been shown in several studies to prevent bacterial and viral infections. The example of a successful prophylactic use of IgY is the treatment of diseases with specific IgY antibodies against *Escherichia coli*, *Helicobacter pylori*, *Salmonella spp*, *Streptococcus mutans*, *Porphyromonas gingivalis*, *Pseudomonas aeruginosa*, *Candida albicans*, *rotavirus* and *coronavirus*.

Keywords: egg yolk antibody, prevention, prophylactic, pathogens bacteria, diarrheal disease, ovotransferrin avidin

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Introduction

Diarrheal disease caused by viral and bacterial pathogens remains the main cause of mortality in children in the developing world. The role of the gastrointestinal tract-associated lymphoid tissue in the prevention of diarrheal disease is important. The gastrointestinal tract-associated lymphoid tissue is the production of specific immunoglobulin A (IgA). This immunoglobulin assists in protection against pathogens bacteria by inhibiting binding, preventing colonization and neutralizing toxins.

A hen egg is a storeroom of variable nutrients; carbohydrates, lipids, protein and other biologically active substance that are needed to produce a chick. In point of fact, when a fertilized egg is kept at 37°C in a humid environment for 21 days, this optimum environment for the growth of bacteria. Yolk of egg is a rich source of immunoglobulin. Egg yolks from immunized hens can easily be protected in the prevention of bacterial diarrheal disease. The avian egg contains all of the necessary nutrients and growth factors required for the developing embryo, including antibodies that are transported from the blood of the hen into the egg yolk to provide immunity to the chick.

The hen egg is protected against infection by lysozyme, ovotransferrin avidin, cystatin and ovomucin in egg white and phosbitin and antibody in egg yolk. Chicken antibodies are low-level of serum immunoglobulin can be transported to the egg yolk, for protection of future generations. Egg yolk antibodies, now called immunoglobulin Y (IgY), Similar in function to the IgG but immunoglobulin Y with different physicochemical properties and biological activity as mammalian IgG. The structure of immunoglobulin Y differs considerably from mammalian IgG.¹

The use of chickens for antibody production has increased. Advantage of using birds is that the antibodies can be harvested from the egg yolk instead of serum. Therefore, the antibody productivity of an egg-laying hen is much greater than that of a similar sized mammal. The mass of antibody that can be yielded from one egg of an immunized hen is as much as that can be obtained from 300ml of rabbit blood. The use of chicken for the antibody production, display both a refinement and a reduction in animal use. The chicken is an excellent producer of antibodies. IgY is more highly localized in egg yolk than it is in serum of hen.²

Chicken egg yolk antibodies have been applied successfully for scientific, therapeutic, prophylactic and diagnostic purposes. The antibody in egg yolk of immunized hens provides passive immunity to those who take the antibody orally. This oral passive immunization is a way of preventing gastrointestinal infection. This paper introduce egg yolk antibody (IgY) and applications of egg yolk antibodies in passive immunity.³

Egg antibody

The general structure of the IgY molecule, consisting of two light and two heavy chains, is comparable to that of mammalian immunoglobulins. Their light (18,660kDa) and heavy (designated as ν , 65,105kDa) chains are also composed of V and C regions.⁴ The light chain contains one V and one C domain, and the heavy chain contains one V domain and four C domains (C ν 1, C ν 2, C ν 3 and C ν 4).⁵ The MW of IgY was found by mass spectrometry to be 167,250Da (that of IgG is about 160,000Da). Egg yolk immunoglobulins do not bind to bacterial Fc receptors and react with mammalian IgG or IgM, nor binding to the rheumatoid factor. The major differences observed between IgY and IgG are described in Table 1.

Table 1 Comparison of IgY and IgG antibodies

Comparison	Mammalian IgG	Avian IgY
Animal	Mammal	Birds, reptiles, amphibia
Main sources	Plasma	Egg yolk

Table Continued...

Comparison	Mammalian IgG	Avian IgY
Molecular Weight (SDS PAGE)	Whole: 150kDa L chains: 22kDa ×2 H chains: 50kDa ×2	Whole: 180kDa L chains: 21kDa ×2 H chains: 70kDa ×2
Basic structure differences	Flexible hinge region, shorter Fc stem with 2 pairs of carbohydrate groups	Less Flexible hinge, longer Fc region with 3 pairs of carbohydrate group
Stability	Stable at pH 3-10, up to 70°C	Stable at pH 4-9, up to 65°C
Antibody titer to mammalian antigens	Frequently low	Frequently high
Binding to Protein A/Protein G	+++	–
Binding to mammalian cell Fc receptor	+++	–
Immunocomplexes activate mammalian complement system	+++	–
Sustained antibody production	From 60 th day in rabbits	From 30 th day in chickens
Antibody production capacity	50–70ml sera/90 immunization day; 500–700mg of IgG	1 egg per day and 60 eggs/90 immunization days: 3-6g of IgY
Therapeutics	Well developed	To be further developed such as in antibiotic-alternative therapy
Applications	RIA, IRMA, ELISA, Western blot, FACS, etc.	RIA, IRMA, ELISA, Western blot, FACS, etc

IgY has been successfully used in conventional immunoassay, immunocytochemistry, immunoprecipitation, immunohistochemistry, cell sorting and cell staining. IgY-based immunoassays are being used to measure the concentration of proteins via RIAs, ELISAs or other assays in basic research. Although IgY antibodies have also been shown to have advantages in different applications, such as diagnostics and antibiotic-alternative therapy but One of the most valorous and hopeful areas of IgY research is its use for passive immunization to treat and prevent human and animal diseases. The adherence ability of many bacterial pathogens is a major essential for the successful colonisation of an organism, it has been shown that specific IgY antibodies are able to inhibit the sticking of bacteria to the epithelial cells.⁶

The use of specific IgY antibodies has been proven effective against a variety of intestinal pathogens, such as human and *bovine rotaviruses*, *bovine coronavirus*, enterotoxigenic *Escherichia coli*, *Edwardsiella*, *Salmonella spp.*, *Yersinia*, *Pseudomonas*, and *Staphylococcus*. Therefore, IgY antibodies are a promising alternative for the treatment and prevention of enteric infections, and passive immunotherapy application. However, its application is partly limited by its sensitivity to human gastrointestinal conditions.^{7,8} Effective protection against *Salmonella enteritidis*, *Salmonella typhimurium*, *Campylobacter jejuni*, *Escherichia coli* ETEC, *bovine rotavirus* and *corona virus* infections in mice, pig and calves has been obtained with the use of passively-administered egg yolk-derived antibodies.⁹

Orally administered Yolk immunoglobulins are subjected to denaturation by the acidic pH of the stomach and degradation by proteases. The authors observed that part of the antibodies remain intact in stomach proteases (pepsin & trypsin) digests. The fragments of cleavage by pepsin and trypsin still have the ability to bind to the antigen and display neutralizing activity.

Eggs are normal dietary components and there is actually no risk of toxic side effects of yolk immunoglobulin. Use of eggs is accepted

in most cultures. Powdered whole eggs or yolks have been used as an inexpensive alternative for the yolk immunoglobulin treatment of enteric diseases. The mean egg use is 0.5-1 egg in day. This amount of IgY is similar or higher than the amounts used in immunotherapy.

Immunization

The protocols of hen immunization, using different adjuvants, volume and dose of antigen, way of injection, vaccination frequency and interval were described. For the first injection the antigen was emulsified in the complete Freund's adjuvant and for two subsequent boosters in the incomplete adjuvant. Basically different immunizing protocols for each antigen and for each animal species have to be tested to find out which method induces the highest serum and egg yolk antibody titer. Usually 10-100µg of protein antigen in a final volume of 0.5-1ml is applied subcutaneously or intramuscularly in the breast muscle at two or three injection sites of 7-8week-old chicken (0.1mg/dose/animal).¹

Purification of IgY

Purification by saltwater

Akita and Nakai was modified the method for preparation of water-soluble fractions of yolk for the use of tap water as follows: Aliquots (10ml) of mixture yolks stored in 1:1 mixture with were further diluted 2-5 times with tap water, pH adjusted to 5.0 with 0.5 M HCl, and the mixture frozen in polypropylene centrifugation tubes at -20°C. After spontaneous thawing the aggregated egg yolk granules were sedimented by centrifugation at 13,500 g for 15min at 4°C and water-soluble fraction collected. Conditions for the IgY precipitation with NaCl were optimized. To the aliquots (30ml) of yolk water fraction, solid NaCl was added to prepare samples containing up to 2 M NaCl. Each sample was divided into two parts and pH was adjusted with 0.5 M HCl to 4.0 or 7.2 to check the behavior at different pH and concentration combinations. Mixtures were stirred for 2 hrs at

room temperature and then centrifuged at 3,700g for 20min at 4°C. Supernatants were discarded and the pellets dissolved in PBS (3ml). The purity and recovery of IgY in soluble fraction were monitored by the SDS-PAGE.^{3,6}

Purification by PEG

Polson introduced polyethylene glycol 6000 (PEG) precipitation method for IgY purification as a reference method. Briefly, an aliquot (100ml) of the mixture yolk was diluted (1:2) in PBS and precipitated with PEG to a final concentration of 3.5%. IgY were precipitated from the resulting supernatant using 12% PEG, then dissolved in PBS and re-precipitated with 12% PEG. Final IgY preparation was stored at 4°C.^{3,4}

Prevention and treatment of bacterial infections

The oral administration of yolk antibodies specific for any of the causative agents of diarrhea has proven successful for treatment of gastrointestinal infections. Oral administration of IgY specific against *Salmonella* has been shown to prevent salmonellosis in calves. The IgY could protect against *Salmonella* infections.¹⁰

Yolk preparations from chickens immunized with antigens of *Salmonella* have also been tested in mouse models of experimental salmonellosis. The group treated with specific IgY had a significantly higher survival rate than other groups that treated with normal egg yolk.¹¹ IgY antibodies have also been used to protect neonatal piglets against experimental enterotoxigenic *Escherichia coli* infections. The use of IgY specific against *Cryptosporidium* caused a significant

parasite reduction in mouse model. Oral treatment with IgY specific against *E. coli* have also been shown to prevent diarrhea in rabbits and reduced the mortality. Oral administered yolk immunoglobulin have also been shown to protect suckling mice against *bovine rotavirus* induced diarrhea and protected against bovine coronavirus induced diarrhea in neonatal calves.

The protection against dental plaque formation was achieved with anti-*Streptococcus mutans* IgY. A mouth rinse with IgY antibodies specific against *Streptococcus mutans* has been used to reduce the establishment of these bacteria in dental plaque.¹² The egg yolk antibodies inhibited *Streptococcus mutans* adherence to saliva-coated hydroxyapatite discs and reduced the percentage of *Streptococcus mutans* per total *streptococci*. Other researchers also shown that gargling with specific IgY antibodies against *Pseudomonas aeruginosa* (anti-P.a IgY) prevents the colonization of these bacteria in the lungs of patients with cystic fibrosis.¹³ Oral treatment with bovine anti rotavirus antibody resulted in a reduction of rotavirus associated diarrhea in newborn calves and reduced the infection rate in children.¹⁴ Oral treatment with anti-human rotavirus IgY Antibodies resulted in a significant protective effect and was also effective.¹⁵ The protective effects of anti-venom IgY against snake and scorpion toxins has been shown in animal.^{16,17} The authors have successfully produced specific IgY antibodies against botulinum and ricin toxin, which have been used for neutralization.¹⁸ The researchers shown that the prophylactic and therapeutic application of anti-SEB IgY completely protected rhesus monkeys against a lethal staphylococcal enterotoxin B (SEB) aerosol challenge. SEB is a potential biological warfare agent¹⁹ (Table 2).

Table 2 Uses of IgY for passive immunization

Agent	Effects of IgY	Reference
<i>Escherichia coli</i>	Protected against infection by <i>E. coli</i> and toxin of <i>E. coli</i> O157:H7	20,21
<i>Salmonella</i> spp.	Reduced rate of <i>Salmonella</i> -contaminated eggs and colonization	22
<i>Eimeria</i> spp.	Protected chicks against avian coccidiosis	23
<i>Pseudomonas aeruginosa</i>	Prevented or reduced colonization in lungs of cystic fibrosis patients	13,24
<i>Helicobacter pylori</i>	Reduced bacterial growth, gastric mucosal injury and Suppressed <i>H. pylori</i> infection	25
<i>Porphyromonas gingivalis</i>	Reduced levels of <i>P. gingivalis</i> of periodontitis patients	26
<i>Streptococcus mutans</i>	reduced the incidence and severity of dental caries	27
<i>Edwardsiella tarda</i>	Prevented and induced mortality in eels	28
<i>Yersinia ruckeri</i>	Reduced mortality and infection rates	29
<i>Vibrio anguillarum</i>	Protected against vibriosis	30
<i>Candida albicans</i>	Reduced <i>C. albicans</i> colonization	31
<i>Clostridium botulinum</i>	Blocked activity of neurotoxin	18,32
<i>Staphylococcus aureus</i>	Protected monkeys from a lethal dose of SEB	19
Venom	neutralize pharmacological effects of venoms	16,17

Conclusion

Use of Laying hens is a cost-efficient method for the production of large quantities of specific antibodies. Eggs are normal dietary components and thus there is practically no risk of toxic side effects of IgY. Oral immunotherapy with IgY antibodies is an attractive approach

for treatment of gastrointestinal infections in humans and animals. The increasing number of antibiotic resistant bacteria emphasize the need to find alternative to antibiotics. Therapeutic IgY administration could reduce the clinical use of antibiotics, and so could lower the risk that bacteria will develop antibiotic resistance.

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Conflict of interest

The author declares no conflict of interest.

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