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Egg Antibody Farming and IgY Technology for Food and Biomedical Applications

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ABSTRACT : It has been recognized that the hen, like its mammalian counterparts, provides young chicks with antibodies as protection against hostile invaders. This system facilitates the transfer of specific antibodies from serum to egg yolk, and provides a supply of antibodies called immunoglobulin Y(IgY) to the developing embryo and the hatched chick. The protection against pathogens that the relatively immuno-incompetent newly hatched chick has, is through transmission of antibodies from the mother via the egg. Egg yolk, therefore, can be loaded with a large amount of IgY against pathogens which can immobilize the existing or invading pathogens during the embryo development or in day-old chicks. Thus, the immunization of laying hens to various pathogens results in production of different antigen-specific IgY in eggs. Egg yolk contains 8~20 mg of immunoglobulins (IgY) per ml or 136~340 mg per yolk, suggesting that more than 30 g of IgY can be obtained from one immunized hen in a year. By immunizing laying hens with antigens and collecting IgY from egg yolk, low cost antibodies at less than $10 per g compared to more than $20,000 per g of mammalian IgG can be obtained. This IgY technology opens new potential market applications in medicine, public health, veterinary medicine and food safety. A broader use of IgY technology could be applied as biological or diagnostic tool, nutraceutical or functional food development, oral-supplementation for prophylaxis, and as pathogen-specific antimicrobial agents for infectious disease control. This paper has emphasized that when IgY-loaded chicken eggs are produced and consumed, the specific antibody binds, immobilizes and consequently reduces or inhibits the growth or colony forming abilities of microbial pathogens. This concept could serve as an alternative agent to replace the use of antibiotics, since today, more and more antibiotics are less effective in the treatment of infections, due to the emergence of drug-resistant bacteria.

(Key words: immunoglobulin Y, pathogens, mammalian IgG, antibiotics, eggs)

INTRODUCTION

The avian egg is a reserved life form to the next generation which turns into a bird. An egg is a storehouse of all the substances necessary for a potential new life. Chickens produce immunoglobulins in blood against almost all kinds of antigens including bacteria, virus, and foreign substances in host defense. As described more than 100 years ago, avian maternal antibodies are transferred to egg yolk to protect embryos and neonates (Akita and Li, 1998).

Circulating immunoglobulin G(IgG) from the hen plasma is first sequestered in the yolk of maturing oocytes in the ovarian follicle via a receptor mediated process which recognizes intact Fc and hinge region of IgG (Akita and Nakai, 1992). As an egg is oviposited, as much as 200 mg of antibodies are present in the egg yolk, hence the term immunoglobulin of egg yolk (IgY) (Al-Haddad *et al.*, 1999; Benkirane *et al.*, 1998).

Using chicken as an antibody producer brings a number of advantages over conventional mammalian antibody and recombinant antibody production and serves as an alternative to antibody sources(Box 1). Combined with the egg industry's capacity to produce thousands of eggs per day and an existing technology for the efficient fractionation and purification of IgY, it is conceivable that kilogram quantities of antibodies could be produced on a daily basis.

Thus, IgY has been widely used as an important application of IgY for passive immunization therapy to treat enteric infections in humans and animals. Another application is the use of IgY as an immunological tool in the field of diagnostics as well as biomedical research. In this review, we summarize published data on properties and applications of IgY for prophylactic and diagnostic uses and suggest directions for its

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future use.

1. Structure of IgY

IgY is considered to be the evolutionary ancestor of mammalian IgG and IgE antibodies (Blais and Phillippe, 2000). Despite the similarities between IgY and mammalian IgG antibodies, there are somewhat differences in their structure. IgYconsists of two identical heavy (H) chains and two identical light(L) chains, which are linked by disulfide bridge(Fig. 1).

IgY has a molecular mass of ~180 kDa which is heavier than that(~150 kDa) of mammalian IgG. The greater molecular mass of IgY is due to an increased number of heavy-chain constant domains and carbohydrate chains. The H chain of IgY is 67 ~70 kDa and possess one variable domain(V), four constant domains(C) and no genetic hinge, unlike that of mammalian IgG (approximately 50 kDa) which has three C domains and a hinge region. Comparisons of C-region sequences in IgG and IgY show that the C2 and C3 domains of IgG are most closely related to the C3 and C4 domains of IgY, respectively, and that the equivalent of the C2 domain is absent in chains of IgG. The hinge region of IgY is much less flexible compared to that of mammalian IgG [5]. It has also suggested that IgY is a more hydrophobic molecule than IgG (Camenisch *et al.*, 1999).

2. Immunological Property of IgY

The structural characteristics of IgY is relevant to the immunological properties (Table 1). The differences of Fc regions between IgY and mammalian IgG, which include number and nature of carbohydrate chains, flexibility of switch region and the number of constant regions, lead to the different interaction of IgY with molecules as an antigen in comparison to that of mammalian IgG.

Most biological effector functions of Igs are activated by the Fc region. Such a role of Fc region of IgY is very poor in secondary effector capabilities in opsonization and complement fixation, although IgY is capable of binding to antigen strongly. IgY does not bind to protein A or G which are present on the surface membrane of *Staphylococci* and *Streptococci* other than mammalian IgG . The role of Fc region of IgY still remains unclear, but it is sure that chicken antibodies do not activate mammalian complement system and show no interaction with mammalian Fc receptors. Likewise, the reaction of antibody to cellular components may mediate inflammatory responses in the gastrointestinal tract. The vulnerable point makes IgY antibodies very attractive for peroral immunotherapy (Carlander *et al.*, 2000).

Another property in Fc region of IgY is no interaction with

BOX 1. Advantages of IgY Production

- Maintenance of a large flock of laying hens is inexpensive and practical, because large scale feeding of hens and the collection of eggs are less labor intensive and well integrated.
- Eggs as the source of IgY can be collected from laying hens by the non-invasive method, which is compatible with animal protection regulations, as compared to mammal's sera from which IgG is separated.
- Also, immunization of hens (vaccination) has long been applied to prevent hens from infectious diseases, indicating that immunization of hens is much more systematized to be effective than doing it for animals.
- A laying hen produces an average of 285 eggs in a year with a yolk of approximately 15 g whereas an immunized rabbit provides about 40 ml of sera. One gram of egg yolk contains about 10 mg of IgY whereas 1 ml of rabbit serum yields about 35 mg of IgG. An immunized hen produces about 43 g of antibodies per year.
- As egg yolk is known as a perfect food package, the isolation of IgY from the yolk is much easier than that of IgG from animal blood sera. For separation IgY, a large scale method is now applicable by automatic separation of the egg yolk with a machine.
- The immune response of chickens could be maintained for a long period of more than 20 weeks with two injections.
- On the contrary to the conventional method sacrificing animals to collect blood, using chicken is simple ggs laid by superimmunized hens.

Table 1. Comparison of immunological properties of IgY and mammalian IgG

Physico-chemical Properties	Avian IgY	Mammalian IgG
Molecular weight	180 kDa	150 kDa
Isoelectric point	>acidic	<acidic
Heat stability	>sensitive	<sensitive
pH stability	>sensitive	<sensitive
Immunological properties		
Protein A / protein G binding	no	yes
Interference with mammalian IgG	no	yes
Interference with rheumatoid factor	no	yes
Interference with human anti-mouse IgG antibody	no	yes
Activation of mammalian complement	no	yes
Fc receptor binding activity	Low	High

rheumatoid factor which causes disease associated with rheumatoid arthritis resulting from inflammatory responses by reacting with the Fc region of mammalian IgG. Due to the phylogenetic difference, IgY antibodies do not cross react with mammalian IgG and show no interference with human antimouse antibodies (Chang *et al.*, 1999). These differences bring great advantages to the application of IgY technology in many medical areas, such as xenotransplantation which is inhibition of xenograft rejection (Chang *et al.*, 2000), diagnostics, prophylaxis of pathogens and antibiotic-alternative therapy(Table 1).

3. Passive Immunization

Passive immunization differs from active immunization (vaccination) in that the former employs an antibody obtained from other animals. The oral administration of pathogen -specific antibodies is considered to be one of the most valuable applications of antibodies to result in prevention of infectious diseases. Such an important application requires antibodies to be made available in large quantities, at an acceptable cost and with high affinity for their targets. Thus, the laying hens may be alternative, as IgY from hen plasma is actively accumulated to egg yolk in daily basis and is present in high concentrations. The specific IgY preparations against enteric pathogens such as viruses, bacteria and parasites have been prepared on an industrial scale from eggs laid by hens immunized with selected pathogens and have previously shown to be effective as prophylaxis against infections.

Rotavirus are a major cause of diarrhea illness in human infants and young animals, including calves and piglets. Infections in adult humans and animals are also common. In a randomized, double-blind study, children with proven rotavirus diarrhea were treated with specific IgY for human rotavirus strains, indicating the effect of IgY in the treatment of rotavirus-induced diarrhea in children (Chang *et al.*, 2002).

Characteristics of pathogenic bacteria include the initiation of the infectious process and mechanisms such as transmissibility, adherence to host cells, invasion of host cells and tissues, ability to evade the host's immune system and symptoms of disease. Once pathogenic bacteria reside in the body, they must attach or adhere to host cells, usually epithelial cells. After the bacteria have established a primary site of infection, they multiply and spread directly through tissues or via the lymphatic system to the bloodstream.

The recent outbreaks of *E. coli* O157: H7 have been attributed to food-borne contamination in countries around the world. To cause disease, *E. coli* must first adhere to host intestinal epithelium, followed by bacterial colonization. Antibiotic therapy is not recommended early in the infectious process, because of disruption of the bacteria in the gut releasing Shigalike toxins. Antibodies can, in principal, bind the bacterial surface and then inhibit the bacterial adhesion to host intestinal epithelium. The complex of antibody and bacteria can be eliminated as a waste, so could antibodies replace antibiotics? The effectiveness of IgY in suppressing the activity of *E. coli* O157:H7 has been demonstrated by our study (Cippolla *et al.*, 2001). The specific binding activity of IgY leading to the inhibition of bacterial growth was explored by using immunoelectron microscopy by a negative staining and ultrathin sectioning method. These studies could visualize the interaction of bacteria with IgY in more detail than the ELISA technique and growth inhibition assay. The observation of immuno-gold particles labeling bacteria, furthermore, revealed the distribution of gold particles on and structural alterations of the bacterial surface. A similar result was also obtained from the growth

inhibition study of IgY against *Salmonella* (Cook *et al.*, 2001).

Salmonellosis is known to be a non-host restricted serotype causing diseases syndrome like gastroenteritis and systemic infections in human and animal species. The immune response of chickens against lipopolysaccharide (Davalos-Pantoja *et al.*, 2000), 14 kDa fimbriae (DeCeuninck *et al.*, 2001) and whole cell (Dera-Tomaszewska *et al.*, 2003) of salmonellae has been investigated on the possible control of salmonellosis. IgY specific against 14 kDa fimbriae of *S. enteritidis* was orally admini stered to mice infected with the corresponding bacteria. The result showed decrease of bacterial virulence. The passive immunization with IgY specific for *S. typhimurium* and *S. dublin* could prevent fatal salmonellosis in calves (Dera-Tomaszewska *et al.*, 2003). This indicates that IgY against the microbial pathogen may be used for the feed additives, which provide prophylactic and therapeutic function(Fig. 1)

Streptococcus mutans is a major etiologic agent of human dental caries. It has been shown in an experimental animal model that oral passive immunization using IgY specific to *S. mutans* was effective in protecting dental caries. The oral administration of IgY specific to *S. mutans* glucan binding protein B resulted in a statistically significant reduction in caries development in an experimental rat model (Di *et al.*, 2001). Furthermore, the effects of a mouth rinse containing IgY to *S. mutans* by the treatment of specific IgY powder prevented the establishment of this bacterium in dental plaque of humans *in vitro* and *in vivo*. The results supported the effectiveness of IgY with specificity to *S. mutans* grown in the presence of sucrose as an efficient method to control the colonization of *S. mutans* in the oral cavity of humans (Fortgens *et al.*, 1997).

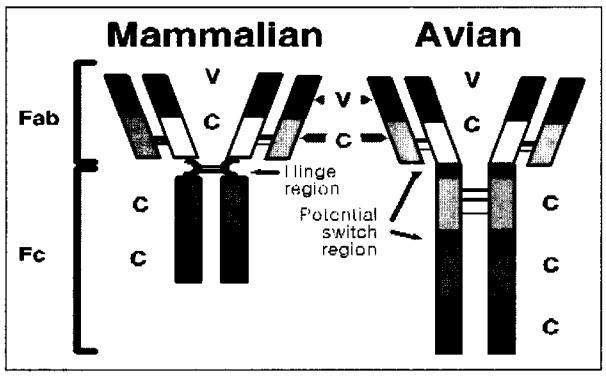

Fig. 1. Comparison of mammalian and avian immunoglobulin G.

Therefore, these studies provide evidence for the potential advantages of using IgY with specificity to *S. mutans* for controlling plaque levels and subsequent oral health problems associated with plaque accumulation.

Therefore, antibacterial properties of IgY demonstrated a protective role in foods or feeds, preventing contamination by pathogenic bacteria and consequently reducing the risk of pathogens-causing infection in humans or animals. To date there have been efforts to develop effective means for controlling or preventing food-borne diseases, which are mainly caused by pathogenic bacteria contaminating foods. IgY, as a food-based deterrent, may serve as a novel protective measure characterized by being economical, efficacious and safe.

4. Diagnosis

Antibodies have been used extensively as diagnostic tools in many different formats. Antibody-based immunoassays are the most commonly used type of diagnostic assay and still one of the fastest growing technologies for the analysis of biomolecules, toxins, and haptens. The first major milestone in antibody-based immunosaays was the development of the competitive binding assay, using radioisotope and later enzyme-labeled immunoassays. A frequently used approach for the detection of antigens involves an immobilized capture antibody, an antigen, and a labeled detection antibody. The antibodies in this assay are usually derived from mammals and the samples to be tested are often serum and plasma (Table 2). If anti-mammalian IgG antibodies or complement are present in the samples, they may block the antigen binding sites of the capture antibody and cause false positive reactions. In a bacterial or viral specimen test, mammalian capture antibody may also result in erroneous reactions due to the binding activity to protein A/G expressed by bacteria probably existing in samples. In contrast, IgY does not possess such immunological properties and thus can be used to avoid these interference problems. Many studies, therefore, have been conducted to show the feasibility of IgY application in diagnostic assays.

Moreover, IgY has been successfully used as immunological tools for other immunoassays such as western blotting (Fryer *et al.*, 1999; Halper *et al.*, 1999; Hatta *et al.*, 1997), dot blot (Holt *et al.*, 2000; Kim *et al.*, 1999), fluorescence (Cook *et al.*, 2001; Halper *et al.*, 1999; Klemperer, 1893; Larsson *et al.*, 1993; Lee

et al., 2002), immunoprecipitation (Lee *et al.*, 2002; Lemamy *et al.*, 1999; Li *et al.*, 1998), immunogold labelling (Cipolla *et al.*, 2001; Cook *et al.*, 2001; Li *et al.*, 1998; Morrison *et al.*, 2002), and immunohistochemistry (Fryer *et al.*, 1999; Noack *et al.*, 1999; Orsini *et al.*, 2001; Romito *et al.*, 2001). Immunoaffinity chromatography is a process for the isolation and purification of target molecules, using immobilized specific antibodies directed against the target molecule. This technique is considered a simple and mild process which can isolate materials with high purity, activity and stability. However, a more widespread use has been limited by high cost of the technique requiring large amounts of antibodies which should fall within parameters such as the efficiency of immobilization, antigenbinding capacity, useful life and reusability of immunoadsorbents. IgY, which can be simply produced in large quantities and high titers, may reduce such limitations and replace other sources of polyclonal antibodies or monoclonal antibodies conventionally used in immunoaffinity chromatography (Ruiz and Ruffner, 2002; Sarker, 2001; Shelver *et al.*, 1998). Therefore, immobilized IgY has been used successfully for the purpose of immunoaffinity isolation of lactoferrin (Sim *et al.*, 2000) and immunoglobulins from colostrums (Smith *et al.*, 2001). (Table 2)

5. Limitation of IgY

The immunization of chickens and the production of IgY have been well recognized to be simple, practical and economical as described in the above sections. Whole egg yolk and crude egg yolk can be used as an antibody source of prophylactics. However, the lipids in the yolk may interfere with the antibody activity in diagnostic assay. In this case, antibodies are usually purified from the yolk. Since IgY is mainly composed of ν-livetin, which is a larger molecule than any other α-, β-livetin in egg yolk, it is relatively easy to separate from other proteins in the water-soluble fraction of egg yolk. Various methods used for the purification of IgY have been explored in acidic condition [38], anionic polysaccharides (Sunwoo *et al.*, 1996), and affinity chromatography (Lee *et al.*, 2002; Sunwoo *et al.*, 1996; Tini *et al.*, 2002).

Table 2. Production of IgY specific to low immunogenic antigens against mammals

Antigen	Reference
Human insulin growth factor II receptor	Li *et al.*, 1998
Heat-shock protein (Hsp 70)	Warr *et al.*, 1995
Bovine interferon alpha	Romito *et al.*, 2001
Alpha-subunit of hypoxia-inducible factor-1	Lee *et al.*, 2002
Human melatonin mt1 receptor	Halper *et al.*, 1999
Naked DNA	Williams *et al.*, 2001
E7 oncogenic protein of human papillomavirus type 16	Lemamy *et al.*, 1999
Cartilage glycoprotein-39	Yokoyama *et al.*, 1998

A number of studies have also provided sufficient evidences of the suitability of IgY preparations for food supplementation: a safe and stable preparation of IgY by the water dilution method requiring no chemicals and resulting in no significant loss of IgY activity (60~90% of recovery); the stability of IgY to pasteurization at 60℃ for 3.5 min. However, the susceptibility of IgY to heat (> 75℃) and acid (< pH 3.0) may be a hindrance to the application of IgY as a food supplement. Some investigations have solved this problem by developing effective means, which is addition of sugars, glycerol, or glycine to IgY solution to improve the stability of IgY under processing conditions such as heat, acid, and high-pressure treatment (Tu *et al.*, 2001; Verdolvia *et al.*, 2000).

The preparation of IgY having appropriate storage properties is another essential consideration in its application. This may include storage of liquid products in the frozen state or at 2 ~ 4℃ with added preservatives to retard microbial growth, or storage of dried products. IgY preparations could be stored for 5 to 10 years at 4℃ without significant loss in antibody activity and also retain their activities after 6 months at room temperature or 1 month at 37℃. Freeze-drying for a purified IgY dried powder is a low temperature process, which is considered to minimize risk of bacterial growth and less destructive than spray- drying. To dry the egg yolk with IgY, spray-drying method should be used in economical ways, however, careful attention should be paid to this process which may lead to drying stresses at a high temperature more than 65℃.

6. Future Applications of IgY

Microbial food-borne diseases are responsible for serious

health problems in humans and animals due to pathogens such as *Escherichia coli* O157 : H7, *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., Enteropathogenic *E. coli*, viruses and parasites. IgY studies at our laboratory have demonstrated that specific IgY against *E. coli* O157:H7 and *Salmonella* is able to inhibit the growth of pathogens, eventually resulting in bacterial death. This research offers many advantages over traditional antibiotics and will probably provide the basis of a highly effective means of producing inexpensive antibodies in egg yolks as functional food and nutraceutical ingredients for the prophylactic treatment of humans and animals against enteric diseases.

It may also be the potential standard procedure to remove or reduce the health risk of pathogens contaminated in beef and food products. Most raw North American meat processed into ground beef patties may be tainted with the illness-causing *E. coli* O157:H7. It is also reported that over 60 % of beef cattle in North America are infected and shedding *E. coli* O157:H7 bacteria to the environment. The public health importance of *E. coli* O157 : H7 depends on the prevention of the bacterial contamination on the beef carcass. In this matter, a new approach to food safety is being investigated whether IgY can be sprayed onto carcass to help prevent bacterial contamination during processing or can be applied to a final packaging to inhibit bacterial growth and extend shelf life. At the industrial level, the IgY can be dissolved in water and sprayed onto meat carcasses to complement other processing methods, such as irradiation, or applied to final packaging. Such extra-protection methods would be welcome news for an industry that has been recently plagued with record-high meat recalls.

A new concept of IgY cocktail, pool of specific IgY against food-borne pathogens mentioned above, can be applied to the development of IgY powder, capsule and spice for the preventing bacterial infections from beef, salad, and other food products. The IgY cocktail may be most useful when traditional sanitation safeguards(i.e. rinsing, refrigeration, and thorough cooking) are unavailable or unreliable. There are possible uses of IgY cocktail for the prevention of food-borne illness caused by foods prepared outdoors or meals that are eaten away from home, especially at salad bars and food bars. The IgY cocktail could be helpful for travelers to foreign countries in which food-handling practices are suboptimal.

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