Egg Yolk Antibodies for Passive Immunity

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Keywords

chicken antibodies, immunoglobulin Y (IgY), IgY production and purification, antibody therapy, antimicrobial, passive immunization

Abstract

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. Since the discovery of egg yolk antibodies, now called immunoglobulin Y (IgY), in the late 1800s, this process has been harnessed to produce antigen-specific yolk antibodies for numerous applications in the medical and research fields, including in areas such as diagnostics and proteomics. However, one of the most valuable and promising areas of IgY research is its use for passive immunization to treat and prevent human and animal diseases. The following review covers the key features and advantages of IgY and the production and purification of IgY from the egg yolk, as well as highlights some of the most promising applications of egg yolk antibodies in human and veterinary medicine.

INTRODUCTION

IgY: immunoglobulin Y

IgG: immunoglobulin G

Hens' eggs have long been recognized sources of nutrients, including large quantities of egg yolk antibodies. Immunoglobulin Y (IgY) is the functional equivalent of immunoglobulin G (IgG) in mammals and is transferred to the yolk to passively protect the developing chick. Three classes of antibody are found in the chicken: IgY, IgA, and IgM (Leslie & Martin 1973). During egg formation, IgY in the serum is selectively transferred to the yolk via a receptor on the surface of the yolk membrane specific for IgY translocation (Morrison et al. 2002, Tesar et al. 2008), whereas IgA and IgM are deposited into the egg white (Rose et al. 1974) (**Figure 1**).

Because of differences in the immunoreactivities of IgY and IgG, egg yolk antibodies have been used in many diagnostic and biomarker discovery applications. However, much research has focused on the use of IgY for passive immunization applications.

Passive immunization has recently become an even more attractive approach because of the emergence of new and drug-resistant microorganisms, diseases that are unresponsive to drug therapy, and individuals with impaired immune systems who are unable to respond to conventional vaccines. Also, passively administered antibodies have the ability to provide rapid and immediate protection; for example, against agents of bioterrorism (Casadevall et al. 2004). The reduction of antibiotic use in the livestock industry and increasing evidence that resistant organisms may pass from animals to humans, resulting in infections that are harder to treat (Yegani & Korver 2010), have led to numerous studies to examine the use of IgY in both human and veterinary medicine.

PASSIVE IMMUNITY VERSUS ACTIVE IMMUNITY

Active immunity refers to the process of exposing the individual to an antigen to generate an adaptive immune response. This response takes days or weeks to develop but may be long lasting. Passive immunity refers to the process of providing preformed antibodies to protect against infection (**Figure 2**), and it provides immediate but short-lived protection lasting several weeks

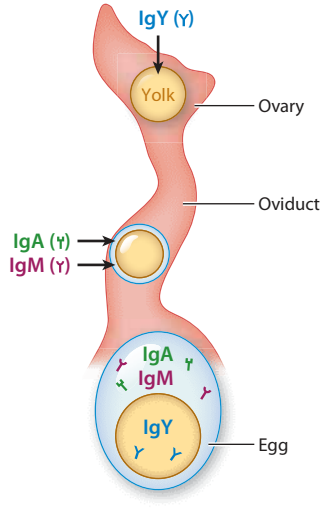

Figure 1

During egg formation, IgY (*blue*) is transferred from the blood to the egg yolk through receptors specific for IgY translocation. IgA (*green*) and IgM (*purple*) are later deposited into the egg white in the oviduct. Adapted from Hatta et al. (2008).

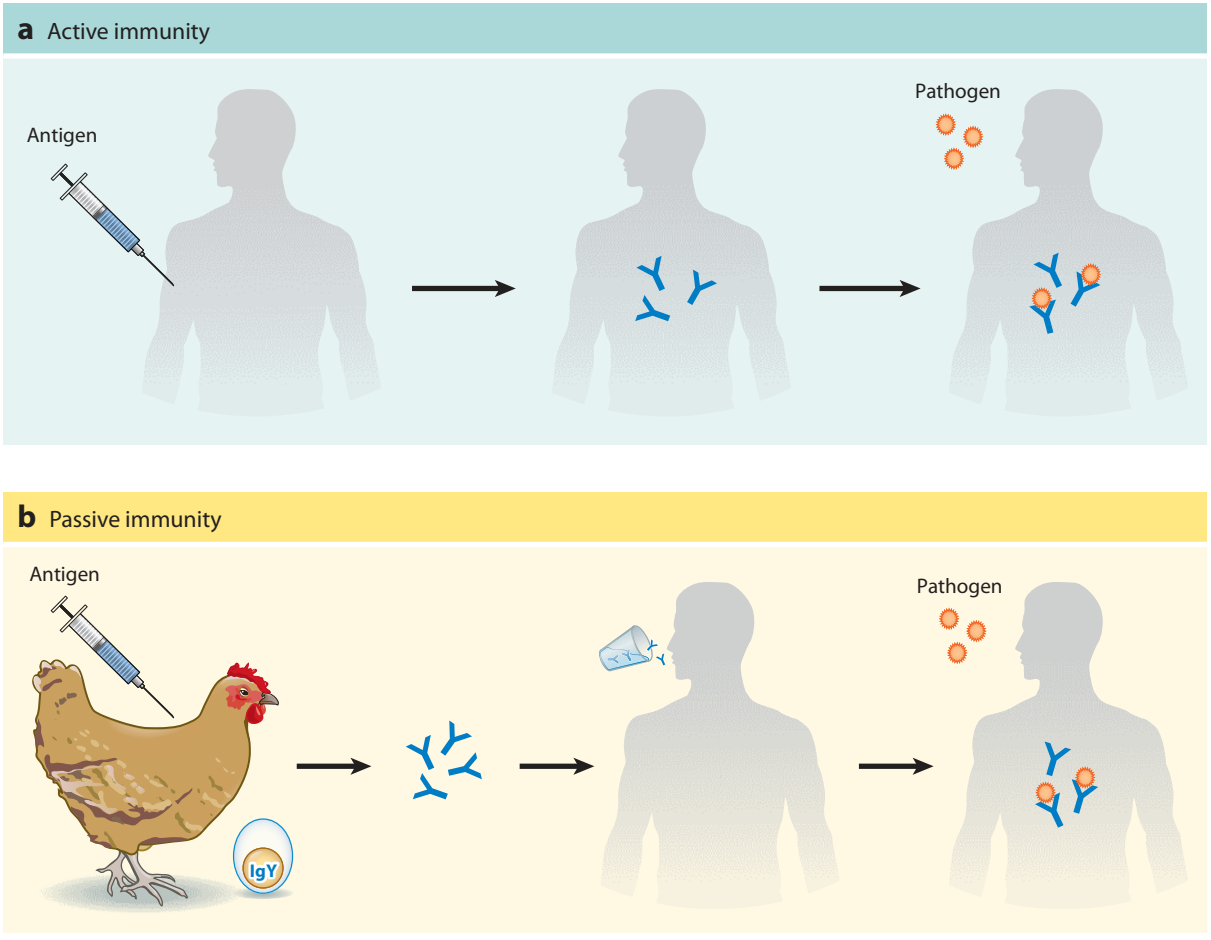

Figure 2

(*a*) Active immunity involves immunizing, or vaccinating, an individual with antigen to generate an adaptive immune response targeting the pathogen of interest. (*b*) In passive immunization, antibodies are isolated from another source (e.g., the yolks of immunized hens) and administered to susceptible individuals to provide pathogen-specific immunity.

to three or four months at most. Passive immunity can be classified as natural or acquired (Baxter 2007).

The immune system of the neonate is relatively immature, therefore maternal antibodies are passed to the offspring in order to confer natural passive immunity until the infant can develop its own immune response. In humans, maternal antibodies (IgG) are transferred from the mother to the fetus during pregnancy and to the neonate through IgA in the breast milk. Other mammals, such as cows, horses, pigs, sheep, and goats, obtain maternal antibodies via colostrum, which are then transported across the intestinal epithelium of the neonate into circulation (Chucri et al. 2010, Lilius & Marnila 2001, Van de Perre 2003).

In contrast, acquired passive immunity involves obtaining antigen-specific antibodies from another source (i.e., an immune individual or animal) and administering it to protect susceptible individuals (Baxter 2007). Because of the transient nature of the protection provided by passively transferred antibodies, repeated or continuous antibody administration is necessary, and large

amounts of preformed antigen-specific antibody are required, especially when used orally. Hens' eggs are an excellent source of large quantities of antibodies that can be used for passive immunization applications.

FCA: Freund's complete adjuvant

PLA_2: phospholipase A_2

FIA: Freund's incomplete adjuvant

ADVANTAGES OF EGGS AS AN ANTIBODY SOURCE

There are a number of advantages to using chickens for the production of antibodies. When considering antibody production for passive immunotherapy applications, chickens present a much more economical source of large quantities of specific antibodies (Schade et al. 2007). Nakai et al. (1994) estimated that the productivity of antibodies from yolk was nearly 18 times greater than that from rabbits, based on the weight of antibody produced per animal. IgY production is also less invasive, requiring only the daily collection of eggs compared to blood collection in mammals (Karlsson et al. 2004).

The genetic differences between chickens and mammals make it possible to produce antibodies against highly conserved mammalian proteins, which otherwise would not be possible in mammals, and much less antigen is required to produce an efficient immune response (Larsson et al. 1998). Similarly, chicken antibodies recognize different epitopes than mammalian antibodies, resulting in a different antibody repertoire (Carlander et al. 1999). In contrast to mammalian serum, egg yolk contains only a single class of antibody (IgY), which can easily be isolated from the yolk by precipitation techniques (Gassmann et al. 1990) and IgY does not activate mammalian complement or interact with mammalian Fc receptors that could mediate an inflammatory response (Carlander et al. 2000).

Finally, the vaccination of hens and automated collection and processing of eggs is already carried out on an industrial scale, making the large-scale production of IgY technically feasible (Cotterill & McBee 1995, Sharma 1999).

PRODUCTION OF IgY

The production of large amounts of IgY in a cost-effective manner is key to its successful use for passive immunization. To this end, several aspects of hen immunization have been studied in order to improve IgY production and yolk deposition, including immunization route, vaccine adjuvant, and type of antigen (Levesque et al. 2007).

The most common injection route is the intramuscular route, and Chang et al. (1999) demonstrated that intramuscular immunization resulted in higher levels of specific IgY when compared to antigen injected subcutaneously.

Oil-based adjuvants, such as Freund's complete adjuvant (FCA), remain the most effective adjuvants for antibody production; however, FCA has been associated with potentially severe injection site reactions (Wanke et al. 1996). Alternatively, the addition of immunostimulating components to vaccine formulations can markedly increase antibody levels in the yolk and utilize less antigen, thereby being more cost-effective (Herath et al. 2010, Levesque et al. 2007, Trott et al. 2008). Adding whole-killed *Streptococcus suis* or *Staphylococcus aureus* was found to increase IgY levels against phospholipase A_2 (PLA_2) by 51% or 62%, respectively (Trott et al. 2008). Moreover, Levesque et al. (2007) found that adding synthetic oligonucleotides containing unmethylated CpG dinucleotides, characteristic of bacterial DNA, to Freund's incomplete adjuvant (FIA) increased specific IgY production by up to 480%. The use of DNA vaccines has also been described for IgY production (Cova 2005). This process involves immunizing birds with plasmid DNA encoding the antigen of interest and eliminates the potentially tedious and costly process of purifying antigens.

Although the amount of IgY deposited into the yolk varies depending on several factors, including the age, breed of chicken, and antigen used, IgY yields have been reported to range from 60 to 150 mg IgY per egg (Cook & Trott 2010, Pauly et al. 2009). Given that a typical hen can lay approximately 325 eggs per year, this can result in a potential yield of around 20–40 g of IgY per year (Pauly et al. 2009), of which 2% to 10% is antigen-specific (Schade et al. 1991, Tini et al. 2002). Pauly et al. (2009) monitored IgY levels in chickens over a two-year period and found that although the laying capacity decreased in the second year, this was compensated for by a greater amount of total IgY per egg. Similar results were observed by Trott et al. (2009b), who found that older hens had higher IgY titers compared with younger hens.

One of the major challenges in IgY purification is separating the water soluble IgY from the yolk lipoproteins (Hatta et al. 2008), and a number of methods have been reported that result in different yields and purities. This typically involves isolating the IgY-containing water soluble fraction, followed by additional purification steps. Dilution of the yolk with water, which results in the aggregation of yolk lipoproteins at low ionic strength (Jensenius et al. 1981), followed by centrifugation or ultrafiltration, has been reported (Akita & Nakai 1992; Kim & Nakai 1996, 1998). Likewise, freezing and thawing of diluted yolk, producing lipid aggregates that are large enough to be removed by conventional low speed centrifugation, have also been used (Jensenius & Koch 1993), resulting in a purity of approximately 70% (Deignan et al. 2000). For dilution methods, pH and extent of dilution are very important for optimal IgY recovery, and Nakai et al. (1994) found that the best results were obtained using a six-fold water dilution, at pH 5.0.

Other methods of removing lipoproteins prior to IgY purification include organic solvent delipidation (Horikoshi et al. 1993, Kwan et al. 1991, Polson 1990) and use of lipoprotein coagulating agents, such as polyethylene glycol (Akita & Nakai 1993, Polson et al. 1980b, Svendsen et al. 1995) or dextran sulfate (Jensenius et al. 1981); however, application of these methods for large-scale production of IgY for passive immunization are limited by problems related to safety as well as cost constraints (Hatta et al. 2008). As an alternative, natural polysaccharides, including sodium alginate (Hatta et al. 1988), xanthan gum (Akita & Nakai 1993), λ-carrageenan (Hatta et al. 1990), and pectin have been found to be just as effective, precipitating more than 90% of lipoproteins from yolk (Chang et al. 2000).

V_L: antibody light chain, variable domain

C_L: antibody light chain, constant domain

V_H: antibody heavy chain, variable domain

C_H: antibody heavy chain, constant domain

MOLECULAR PROPERTIES OF IgY

Despite sharing a similar biological function, there are some striking differences in the structures and immunoreactivities of IgY and IgG. IgY is composed of two identical heavy (H) and two identical light (L) chains linked by a disulfide bridge and has a molecular mass of ~180 kDa, larger than mammalian IgG (~150 kDa). The light chain of both antibodies consists of one variable domain (V_L) and one constant (C_L) domain. The heavy chain of IgY consists of one variable domain (V_H) and four constant domains (C_H1, C_H2, C_H3, and C_H4), in contrast to IgG, which has only three constant domains, and IgY has a shorter and less flexible hinge region (Warr et al. 1995). Both antibodies contain Asn-linked oligosaccharides; however, IgY contains high-mannose-type oligosaccharides, which differ from those of IgG (Suzuki & Lee 2004, Taylor et al. 2009). In addition, the β-sheet content of IgY has been reported to be lower, suggesting that the conformation of IgY is more disordered and therefore less stable than IgG (Shimizu et al. 1992). The isoelectric point of IgY is in the range of 5.7 to 7.6 and is lower than that of IgG (Polson et al. 1980a), and it has been suggested that IgY may be more hydrophobic, which would correspond to the lipid-rich environment of the yolk (Davalos-Pantoja et al. 2000).

The stability of IgY under various processing and physiological conditions is an important consideration for passive immunotherapy applications. The activity of IgY has been shown to

decrease with increasing temperature and time. Minimal loss of activity was observed following heating between 60°C and 65°C but was decreased markedly by heating for 15 min at 70°C or higher (Hatta et al. 1993a; Shimizu et al. 1988, 1993b). However, addition of carbohydrates has been found to improve its heat resistance (Cook & Trott 2010, Shimizu et al. 1994). Likewise, it has been shown that the addition of disaccharides or complex carbohydrates to IgY prior to freeze-drying can improve stability during storage (Jaradat & Marquardt 2000, Nilsson & Larsson 2007).

IgY is relatively stable between pH 4 and pH 11 but displays a rapid reduction in activity above pH 12 (Hatta et al. 1993a, Lee et al. 2002b, Shimizu et al. 1988). At pH 3.5, IgY activity decreases and is almost completely lost at pH 3 because of rapid conformational changes (Shimizu et al. 1988, 1992, 1993b). However, pH stability may be improved by the addition of stabilizers, such as sugars, complex carbohydrates, and polyols (Chang et al. 2000, Lee et al. 2002b, Shimizu et al. 1994).

As with any protein, antibodies are susceptible to proteolytic digestion (Reilly et al. 1997). The sensitivity of IgY to pepsin digestion is highly dependent on the pH and enzyme to substrate ratio used (Hatta et al. 1993b). Shimizu et al. (1988) found that antibody activity was completely lost following pepsin digestion below pH 4.5 at an enzyme to substrate ratio of 1:20. Using similar conditions, Hatta et al. (1993b) found that 63% of IgY activity remained even after 4 h when pepsin was used at an enzyme to substrate ratio of 1:200; however, activity was rapidly lost using this ratio at pH 2. Following trypsin and chymotrypsin digestion, IgY retained 39% and 41% of its activity, respectively, after 8 h of digestion, despite the apparent production of peptides (Shimizu et al. 1988).

Gastric pH, enzyme levels, and gastric transit times can vary depending on a number of factors, including age and health status, thus inactivation of IgY during digestive processes may be a major concern for oral immunotherapy applications of IgY. Because gastric content and diet can also affect these parameters, it should be noted that the matrix in which IgY is administered (e.g., purified antibody versus whole egg yolk) may also affect antibody stability (Cook & Trott 2010, Jaradat & Marquardt 2000). In humans, oral IgA antibodies are transferred to the neonate via colostrum to confer passive immunity; however, it has been suggested that IgA in human milk may be protected from proteolysis by binding to a secretory component (Reilly et al. 1997). A number of studies have looked at the survival of orally administered antibodies from different species (i.e., bovine and avian sources) following passage through the gastrointestinal tract in both humans and animals, with varying results. Roos et al. (1995) found that 19% of orally ingested bovine IgG was still active following digestion in humans, whereas other studies have reported that up to 50% of orally administered antibodies could be recovered in adult or infant stool (reviewed in Reilly et al. 1997). On the other hand, Bogstedt et al. (1997) detected only minute amounts (<0.01%) of orally administered bovine IgG or chicken IgY in the stool of healthy adults.

In order to prevent inactivation of IgY following oral administration, a number of encapsulation techniques have been examined. Multiple emulsions (Shimizu & Nakane 1995) and microencapsulation of IgY using liposomes (Shimizu et al. 1993a) have previously been described; however, these have shown limited effectiveness. The macroencapsulation of IgY using enteric-coated gelatin capsules has also been examined and was found to significantly improve antibody stability (Akita & Nakai 2000). The use of a pH-sensitive methacrylic acid copolymer (Kovacs-Nolan & Mine 2005), chitosan-alginate microcapsules (Li et al. 2007, 2009a,b), and whey protein–based microcapsules (Cho et al. 2005) have also been shown to increase IgY stability to gastric conditions, both in vitro and in vivo. Recently, Xun et al. (2010) described the use of the cytoprotective agent sucralfate and found that it could improve IgY resistance to low pH and pepsin treatment, as well as enhance storage stability, in a dose-dependent manner. Finding an effective method of protecting IgY from degradation would not only ensure the delivery of a

consistent antibody dose but could also allow the effective dose to be lowered, thereby reducing cost (Bogstedt et al. 1997).

ETEC: enterotoxigenic *Escherichia coli*

SE: *Salmonella* Enteritidis

ST: *Salmonella* Typhimurium

PASSIVE IMMUNIZATION APPLICATIONS OF IgY

IgY Use in Veterinary Applications

Antimicrobials are used extensively in agriculture to both prevent disease and promote growth in livestock. However, the use of antibiotics, especially for growth enhancement, has come under scrutiny, as it has been shown to contribute to the increased prevalence of antibiotic-resistant bacteria (Mathew et al. 2007). As such, IgY has been examined as a feed additive for livestock to both target specific pathogens and improve growth and feed efficiency (Cook & Trott 2010) (**Table 1**).

IgY has been tested against a number of enteric pathogens. IgY produced against the porcine enterotoxigenic *Escherichia coli* (ETEC) fimbrial antigens K88, K99, and 987P was found to inhibit the binding of *E. coli* K88-, K99-, and 987P-positive strains to porcine epithelial cells (Yokoyama et al. 1992) and porcine intestinal mucus (Jin et al. 1998) in vitro. When given orally to piglets, these antibodies dose-dependently protected against *E. coli* infection (Yokoyama et al. 1992). More recently, Li et al. (2009b) found that when anti-K88+ ETEC IgY was encapsulated in chitosan-alginate microparticles, it exerted its antidiarrheal effects much faster (24 h versus 72 h postinfection in pigs given nonencapsulated IgY) and led to increased weight gain when compared to pigs fed nonencapsulated antibodies. The passive protective effect of anti–*E. coli* IgY in cattle has also been shown. Neonatal calves fed milk containing anti-ETEC IgY had transient diarrhea, 100% survival, and improved body weight gain (Ikemori et al. 1992). Likewise, the use of an IgY preparation against *E. coli* O157:H7 was found to reduce O157:H7 fecal shedding in feedlot steer (DiLorenzo et al. 2008a).

Bovine rotavirus (BRV) is an important cause of diarrhea in newborn calves, and local passive immunity is the most efficient protective strategy to control the disease (Vega et al. 2011). Kuroki et al. had previously found that orally administered anti-BRV IgY could protect against infection in mice (Kuroki et al. 1993) and in calves (Kuroki et al. 1994). More recently, it was shown that anti-BRV IgY-containing yolk provided up to 80% protection against BRV-induced diarrhea in neonatal calves when compared with calves given nonimmunized egg yolk (Vega et al. 2011), suggesting that supplementing newborn calves' diets for the first 14 days of life with BRV-specific IgY may be a promising strategy to prevent BRV-related mortality.

Salmonella Enteritidis (SE) and *Salmonella* Typhimurium (ST) are the main causes of outbreaks in humans and infections in chickens (Lee et al. 2002a). Chalghoumi et al. (2009) found that IgY against the outer membrane proteins of SE and ST reduced *Salmonella* sp. adhesion to intestinal epithelial cells in vitro, suggesting that passive immunization with *Salmonella*-specific IgY could be useful to prevent *Salmonella* colonization in broiler chickens. Moreover, feeding chickens egg powder containing SE-specific antibodies was found to reduce fecal shedding, cecal colonization, and the rate of *Salmonella*-contaminated eggs in experimentally infected chickens (Gurtler et al. 2004, Rahimi et al. 2007).

Other uses of IgY in cattle include the prevention of respiratory diseases and mastitis. Dahlen et al. (2008) found that an IgY preparation against several bovine pathogens administered intranasally reduced morbidity and mortality in calves. IgY against bovine mastitis–causing *E. coli* (O111) (Zhen et al. 2008) and *S. aureus* (Wang et al. 2011, Zhen et al. 2009) have both shown promise by inhibiting bacterial growth and internalization (Wang et al. 2011) and enhancing phagocytic activity of bovine macrophages (Zhen et al. 2008).

Table 1 Uses of IgY for passive immunization in humans, animals and aquaculture

Pathogen/antigen	Target species	Effects of IgY	Reference
Escherichia coli	Pigs	Protected against infection by K88+, K99+, and 987P+ *E. coli*	Yokoyama et al. 1992
	Pigs	Encapsulated anti-K88+ *E. coli* IgY enhanced protection and led to improved weight gain	Li et al. 2009a
	Cattle	Protected against K99+ *E. coli* infection in calves	Ikemori et al. 1992
	Cattle	Reduced O157:H7 fecal shedding in feedlot steer	DiLorenzo et al. 2008a
	Chickens	Improved intestinal health and immune responses in broilers challenged with O78:K80	Mahdavi et al. 2010
	Cattle	Inhibited growth and internalization of O111 and enhanced uptake by macrophages	Zhen et al. 2008
	Humans	Reduced binding of O157:H7 in vitro and protected mice from toxin challenge	Wang et al. 2010
Salmonella spp.	Chickens	Reduced rate of *Salmonella*-contaminated eggs in *Salmonella* Enteritidis (SE)-infected chickens	Gurtler et al. 2004
	Chickens	Reduced fecal shedding and cecal colonization in SE-infected broilers	Rahimi et al. 2007
Bovine rotavirus (BRV)	Cattle	Protected neonatal calves from BRV-induced diarrhea	Kuroki et al. 1994, Vega et al. 2011
Infectious bursal disease virus (IBDV)	Chickens	Protected chicks from IBDV infection	Yousif et al. 2006
Porcine epidemic diarrhea virus (PEDV)	Pigs	Protected piglets against PEDV infection	Kweon et al. 2000
Eimeria spp.	Chickens	Protected chicks against avian coccidosis	Lee et al. 2009a,b
Canine parvovirus (CPV)	Dogs	Protected dogs against CPV2-induced disease symptoms	Van Nguyen et al. 2006
Phospholipase A_2	Chickens	Improved growth and feed efficiency in chickens	Reviewed in Cook 2004, 2010
Others	Cattle	Reduced morbidity and mortality in calves due to respiratory pathogens	Dahlen et al. 2008
	Cattle	Reduced ruminal counts of *Fusobacterium necrophorum* and *Streptococcus bovis* and improved growth performance	DiLorenzo et al. 2006, 2008b
Pseudomonas aeruginosa	Humans	Prevented or reduced colonization in lungs of cystic fibrosis patients in long-term (>14 years) clinical studies	Carlander et al. 2000; Kollberg et al. 2003; Nilsson et al. 2007, 2008
Helicobacter pylori	Humans	Reduced bacterial growth, urease activity, and gastric mucosal injury in animal model	Shin et al. 2002
	Humans	Suppressed *H. pylori* infection in humans when incorporated into yogurt	Horie et al. 2004
Porphyromonas gingivalis and *Streptococcus mutans*	Humans	Reduced levels of *P. gingivalis* when applied to the teeth of periodontitis patients	Yokoyama et al. 2007b
	Humans	Reduced levels of *S. mutans* when used as mouth rinse	Hatta et al. 1997
	Humans	IgY against *S. mutans* glucosyltransferase reduced the incidence and severity of dental caries in rats	Kruger et al. 2004

(*Continued*)

Table 1 (*Continued*)

Pathogen/antigen	Target species	Effects of IgY	Reference
Tumor necrosis factor-α	Humans	Reduced inflammation in experimental colitis in rats	Worledge et al. 2000
Candida albicans	Humans	Reduced *C. albicans* colonization in mice	Ibrahim et al. 2008
Influenza	Chickens/ Humans	Protected mice from lethal H5N1, H5N2, and H1N1 challenge	Nguyen et al. 2010
Clostridium botulinum	Humans	Blocked activity of neurotoxins A and B in mice	Pauly et al. 2009, Trott et al. 2009a
Staphylococcus aureus	Humans	Protected monkeys from a lethal dose of *S. aureus* enterotoxin B	LeClaire et al. 2002
	Cattle	Reduced symptoms of clinical and experimental mastitis	Zhen et al. 2009
Venom	Humans	Shown to neutralize pharmacological effects of various venoms	Araujo et al. 2010; Liu et al. 2010; Meenatchisundaram et al. 2008a,b; Paul et al. 2007; de Almeida et al. 2008
Rabies virus	Humans	Reduced mortality in infected mice	Motoi et al. 2005b
Edwardsiella tarda	Fish	Prevented *E. tarda*–induced mortality in eels	Hatta et al. 1994
Yersinia ruckeri	Fish	Reduced mortality and infection rates in rainbow trout	Lee et al. 2000
Vibrio anguillarum	Fish	Protected rainbow trout against vibriosis	Arasteh et al. 2004
White spot syndrome virus (WSSV)	Shrimp	Protected shrimp and crayfish from WSSV infection	Lu et al. 2008, 2009; Kumaran et al. 2010

IgY has also been used for the passive protection of chicks against infectious bursal disease virus (Yousif et al. 2006) and avian coccidiosis caused by *Eimeria* spp. (Lee et al. 2009a,b), for the protection of piglets against porcine epidemic diarrhea virus (Kweon et al. 2000), and for the protection of dogs against canine parvovirus-2 (Van Nguyen et al. 2006).

Along with preventing disease, antibiotics can also improve growth performance and feed efficiency in livestock by reducing the number of immune-stimulating bacteria in the gut and minimizing the host antimicrobial inflammatory response (Cook & Trott 2010). To this end, the use of IgY targeting specific molecules involved in inflammation and animal growth has been described. Antibodies directed against the gut neuropeptides cholecystokinin and neuropeptide Y, believed to be involved in regulating appetite, as well as the enzyme PLA_2, which plays a role in the production of the inflammatory mediators prostaglandins and leukotrienes, have both been shown to improve growth and feed efficiency when fed to chickens (reviewed in Cook 2004, 2010). Similarly, Mahdavi et al. (2010) found that feeding yolk powder containing IgY against *E. coli* O78:K80 to chickens improved intestinal health and feed conversion ratio, and enhanced intestinal responses upon oral challenge. IgY feeding has also been found to have beneficial effects on growth in cattle. DiLorenzo et al. (2006, 2008b) found that feeding egg yolk preparations containing IgY against *Fusobacterium necrophorum* and *Streptococcus bovis* in place of antibiotics reduced ruminal counts of the target bacteria and improved feed efficiency and growth performance.

A recent proteomic analysis carried out on pigs infected with *E. coli* and *S.* Typhimurium and fed a diet supplemented with IgY showed that oral IgY administration could regulate the immune system and reduce the stress of microbial infections (Park et al. 2011).

IgY Use in Human Medicine

PA: *Pseudomonas aeruginosa*

CF: cystic fibrosis

IgY has been found to be effective against a number of human pathogens and diseases, both in vitro and in laboratory animal studies and clinical settings (**Table 1**).

One of the most successful clinical applications of IgY has been in the prevention of *Pseudomonas aeruginosa* (PA) colonization in the airways of cystic fibrosis (CF) patients. In 2008, orphan drug designation was granted for IgY antibody against PA for the treatment of CF in humans by the European Medicines Agency. PA is the major cause of morbidity and mortality in CF patients, and once a chronic infection has been established it is very difficult to eliminate, even with the use of antibiotics (Kollberg et al. 2003). Furthermore, there is the increasing risk of developing antibiotic-resistant strains (Nilsson et al. 2008). In ongoing trials in CF patients, a mouth rinse containing purified anti-PA IgY given on a continuous basis could significantly reduce or prevent PA colonization, thereby reducing the need for antibiotics (Carlander et al. 2000; Kollberg et al. 2003; Nilsson et al. 2007, 2008). These studies have shown that specific IgY is effective for immunotherapy for long treatment periods without negative side effects (Nilsson et al. 2007). The stability of the anti-PA IgY in the saliva of healthy individuals was also examined (Carlander et al. 2002), and antibody activity was shown to remain even after 8 h, supporting the potential application of IgY for other localized infections, such as the common cold and tonsillitis.

Another promising clinical application of IgY in humans is for the prevention of *Helicobacter pylori* infection. *H. pylori* is a common cause of gastritis and gastric ulcers, and the emergence of antibiotic-resistant strains has prompted the investigation into alternative treatment methods (DeLoney & Schiller 2000). In vitro, IgY against *H. pylori* reduced bacterial adhesion, growth and urease activity, and decreased *H. pylori*–induced gastric mucosal injury and inflammation in an animal model (Shin et al. 2002). Because antibodies produced against whole-cell *H. pylori* might also cross-react with normal human flora (Shin et al. 2003), the production and efficacy of IgY against immunodominant *H. pylori* proteins and peptides, including urease and urease-derived peptides (Nomura et al. 2005, Shin et al. 2004) and a 58-kD highly reactive *H. pylori* antigen (Hp58) (Attallah et al. 2009), have also been examined. A functional drinking yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium* spp. and supplemented with 1% antiurease IgY was produced commercially and given to volunteers testing positive for *H. pylori* (Horie et al. 2004). After four weeks, urea breath test values and antigen detection in feces were significantly reduced, indicating suppression of *H. pylori* infection.

Porphyromonas gingivalis is one of the most important causes of periodontitis. Anti–*P. gingivalis* IgY dose-dependently decreased bacterial adhesion and hydrolytic activity in vitro (Yokoyama et al. 2007a), and reduced levels of *P. gingivalis* when applied to the teeth of periodontitis patients (Yokoyama et al. 2007b). IgY against the *P. gingivalis* 40-kD outer membrane protein, which aggregates with other oral bacteria to form plaque (Hamajima et al. 2007), as well as *P. gingivalis* hemagglutinin (HagA), which allows the bacteria to adhere to gingival tissue cells (Tezuka et al. 2006), was also found to inhibit aggregation and hemagglutination in vitro. IgY against *Streptococcus mutans*, which causes tooth decay in humans, has been shown to exert anticariogenic properties. Levels of *S. mutans* were decreased in volunteers who gargled with a mouth rinse containing anti–*S. mutans* IgY (Hatta et al. 1997). IgY produced against the *S. mutans* glucan-binding protein B (GBP-B) (Smith et al. 2001), which is believed to be involved in *S. mutans* biofilm development, and the virulence factor glucosyltransferase (Kruger et al. 2004) also reduced the incidence and severity of dental caries in rats when compared with untreated control rats or rats given nonimmune egg powder, suggesting that IgY might be useful for high caries risk patients.

IgY has also been suggested for the treatment of *E. coli* infection in humans. IgY against adherence-associated proteins of *E. coli* O157:H7 reduced bacterial adherence in vitro in cultured

cells, suggesting they may have the potential to reduce *E. coli* O157:H7 colonization in hosts such as cattle and humans (Cook et al. 2007). Similarly, Girard et al. (2006) demonstrated that IgY directed against virulence factors of attaching and effacing *E. coli* reduced bacterial adherence of porcine enteropathogenic *E. coli* as well as heterologous human strains. Anti-Shiga-like toxin 1 (Stx1) IgY was also shown to effectively block the binding of *E. coli* O157:H7 Stx1 to cells in vitro and protect mice from toxin challenge (Wang et al. 2010), indicating IgY might be useful in cases of *E. coli* intoxication.

The role of specific IgY in treating inflammatory bowel disease (IBD) has also been examined. Tumor necrosis factor (TNF)-α is one of the key pro-inflammatory cytokines involved in the pathogenesis of IBD (Garside 1999), and immunotherapy using monoclonal mouse antibodies against TNF-α has been approved for use; however, it can be costly and adverse side effects have been reported (Sandborn & Hanauer 1999). Worledge et al. (2000) reported that orally administered anti-TNF-α IgY could effectively treat experimental colitis in rats, as well as neutralizing human TNF-α in vitro, indicating its potential for the treatment of IBD in humans.

IgY has been also used to prevent the growth, adherence, and biofilm formation of *Candida albicans*, an opportunistic fungal pathogen, in vitro (Fujibayashi et al. 2009, Wang et al. 2008), as well as to reduce *C. albicans* colonization in vivo in mice (Ibrahim el et al. 2008).

As mentioned above, immunization or exposure to an antigen or pathogen results in the development of an adaptive immune response and production of antigen-specific antibodies, a process which can take days to weeks. However, in some cases, for example in the case of novel or unknown pathogens such as pandemic influenza, immediate protection may be desired. Tsukamoto et al. (2011) reported the production of IgY against the pandemic influenza virus A/H1N1 2009, using a swine influenza virus vaccine strain. The resulting antibodies had strong cross reactivity to both the swine and human strains of the virus and were capable of neutralizing the A/H1N1 virus in vitro. Nguyen et al. (2010) purified IgY from commercially available eggs produced by chickens routinely vaccinated against the avian influenza virus H5N1 and found that when these antibodies were administered intranasally to mice before or after lethal infection with H5N1 or related H5N2 strains, they could prevent or significantly reduce viral replication, respectively, resulting in complete recovery. Moreover, the authors produced IgY against human H1N1 and found that it could similarly protect mice from a lethal influenza infection, indicating that IgY could be a potential alternative for the control of future influenza outbreaks.

The immediate protection afforded by antigen-specific IgY has also been suggested for protection against agents of bioterrorism. The production of IgY against the toxin ricin and the *Clostridium botulinum* neurotoxins type A (BoNT/A) and B (BoNT/B) has been described. Purified IgY was found to block the functional activity of the toxins both in vitro (Pauly et al. 2009) and in vivo in mice following preincubation of the toxin with toxin-specific IgY (Pauly et al. 2009, Trott et al. 2009a). IgY has also been shown to inhibit the production of *S. aureus* enterotoxin A in vitro (Sugita-Konishi et al. 1996), and IgY against *S. aureus* enterotoxin B administered 20 min before or 4 h after challenge protected rhesus monkeys from a lethal dose of aerosolized *S. aureus* enterotoxin (LeClaire et al. 2002).

IgY has also been used as an alternative to traditional antivenoms produced in horses, sheep, and goats to neutralize the toxic and potentially lethal effects of bites from venomous snakes, spiders, and scorpions. There have been reports of chicken IgY against a number of different venoms (Araujo et al. 2010; Liu et al. 2010; Meenatchisundaram et al. 2008a,b; Paul et al. 2007), including the production of polyvalent anti–African snake venom IgY for use against bites of several different snakes (de Almeida et al. 2008). IgY was shown to have a higher bioactivity than antivenoms traditionally raised in horses and also had a lower likelihood of producing side effects,

such as serum sickness and anaphylactic shock, that can occur upon administration of mammalian serum proteins (de Almeida et al. 2008, Thalley & Carroll 1990).

Motoi et al. (2005a,b) described the production of antirabies IgY against a portion of the G protein of rabies virus. In vitro, these antibodies bound virions and cells infected with the rabies virus and neutralized rabies virus infectivity. In vivo, administration of antirabies IgY to infected mice reduced mortality caused by the virus, suggesting that IgY directed against the rabies virus G protein could serve as a possible alternative to currently available antirabies antibody preparations made in humans or horses (Motoi et al. 2005b).

IgY Use in Aquaculture

Passive immunization has also been applied in the aquaculture industry, where infectious disease can result in significant economic loss (**Table 1**).

Edwardsiella tarda is a fish pathogen spread by infection through the intestinal mucosa, and Edwardsiellosis in Japanese eels constitutes a serious problem for the eel-farming industry, especially with the appearance of antibiotic-resistant strains (Hatta et al. 1994). Eels challenged with *E. tarda* followed by administration of anti–*E. tarda* IgY survived without any symptoms of infection, in contrast to control eels that died within 15 days (Gutierrez et al. 1993, Hatta et al. 1994). This antibody is now in use commercially, following a field test on nearly 2,400,000 tails of cultured eels with confirmed disease prevention with anti–*E. tarda* IgY (Hatta et al. 2008).

Enteric redmouth disease, caused by *Yersinia ruckeri*, is a systemic bacterial septicemia of salmonid fish, and the persistence of *Y. ruckeri* in carrier fish and shedding of bacteria in feces can present a continuing source of infection. Feeding anti–*Y. ruckeri* IgY before or after bacterial challenge resulted in lower mortality and reduced infection rates in rainbow trout (Lee et al. 2000). Similarly, IgY against *Vibrio anguillarum* protected rainbow trout against vibriosis for at least 14 days when given by intraperitoneal injection, oral intubation, or feeding (Arasteh et al. 2004).

White spot syndrome virus (WSSV) causes high mortality in cultured shrimp. IgY produced against WSSV was shown to passively protect shrimp (Lu et al. 2008) and crayfish (Lu et al. 2009) from WSSV infection when used as an immersion solution or incorporated into feed (Kumaran et al. 2010, Lu et al. 2009).

COMMERCIALLY AVAILABLE IgY PREPARATIONS

There are a number of IgY, or hyperimmune egg products for sale to treat specific diseases or for the promotion of overall health in humans, livestock, and companion animals. Although this list is by no means exhaustive, it highlights some of the IgY preparations that are currently commercially available. For example, Ovopron IgY from Pharma Foods International Co. Ltd, contains antibodies against *H. pylori* urease and has been incorporated into a number of finished products, including yogurt and tablets for the treatment and prevention of *H. pylori* infection in humans. i26®, developed by Arkion Life Sciences, is a product containing IgY and immune cofactors produced by hyperimmunizing hens with several antigens, including 26 human enteric pathogens, and in a number of clinical studies it has been reported to balance the immune system and maintain gut health, modulate autoimmune responses and improve joint and muscle health. A similar product, called IgY Recovery Proteins™, has been shown to lead to shorter recovery time, reduce muscle soreness, and improve overall performance in athletes. Likewise, i26® Companion has been developed to improve immune function in cats and dogs. Arkion has also developed a hyperimmune feed supplement containing IgY against bovine and porcine enteric pathogens (Protimax®), designed to improve performance of weanling pigs and calves. EW Nutrition, along

with Ghen Corporation in Japan, produces IgY supplements for both livestock and companion animals (Globigen®) as well as a full line of products for human health (Ovalgen®) containing IgY against a number of the pathogens described above targeting oral, stomach, intestinal, and skin and mucosa care. Similarly, AD Biotech Co. Ltd. and Dan Biotech Inc. produce several IgY-containing products (including Ig-Guard and Ig-Lock, respectively) that target gastrointestinal pathogens in livestock and companion animals as well as viral diseases in the aquaculture industry. Finally, Aova Technologies sells a line of products (BIG™) for pigs, cows, poultry, and the aquaculture industry that contains IgY against the enzyme PLA_2, and a number of animal trials have demonstrated significant improvement in feed efficiency, growth rate, carcass yield, and general health in livestock.

CONCLUDING REMARKS AND FUTURE RESEARCH

The production of specific antibodies in egg yolk continues to attract the attention of the scientific community, as evidenced by the significant body of IgY-related literature (Schade et al. 2005). With the push to reduce antibiotic use in livestock and the emergence of antibiotic-resistant pathogens, the role of passive immunotherapy is more important than ever.

Indeed, the immunization of hens is an excellent method to efficiently generate large quantities of antibodies because antibody production is nonstressful and noninvasive, and the isolation and purification of IgY are relatively simple and high yielding (Zhang 2003). However, the production cost of high-quality IgY for large-scale applications still remains higher than that of other drug therapies, such as routine antibiotics (Casadevall et al. 2004), suggesting that the cost of egg production may still be a significant barrier for the expanded use of IgY in some commercial applications (e.g., animal feed supplements) (Trott et al. 2009b). Trott et al. (2009b) found that aged or spent hens, although no longer valued for egg production for human consumption, were still a valuable source of high titer antigen-specific IgY and provided a more cost-effective source of eggs, thereby potentially reducing IgY production costs. At the other end of the cost spectrum, recombinant humanized IgY, in which the constant domain of the IgY antibody is replaced with corresponding human antibody domains, has been produced to provide antibodies that possess all the immunological advantages of IgY but may be more suitable for in vivo diagnostic or therapeutic applications in humans. Continuing research into IgY technology, improving chicken immunization protocols and adjuvants, as well as developments in extraction techniques and increasing antibody yield, will no doubt lead to new applications for IgY immunotherapy to improve human and animal health.

SUMMARY POINTS

1. Hens deposit large amounts of an antibody called IgY into the egg yolk to transfer passive immunity to the developing chick.
2. This process can be harnessed for large-scale antibody production by immunizing hens with the antigen(s) of interest, then collecting and isolating the resulting antigen-specific antibodies from the egg yolk.
3. IgY production using chickens is more cost effective, convenient, and less invasive than antibody production in mammals, making it a promising alternative for passive immunization applications.

4. Antigen-specific IgY has been shown to be effective at treating and preventing a number of different human and animal diseases, and commercially available IgY preparations have been shown to promote gut health and immunity in humans and improve feed efficiency and growth rates in livestock.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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