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Using egg IgY antibodies for health, diagnostic and other industrial applications
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Abstract: Immunoglobulin Y (IgY) is the major antibody in chickens, and is transferred in large quantities to the egg yolk to confer passive immunity to the developing embryo. IgY can easily be harvested from the yolk, and is an ideal alternative to using mammalian IgG antibodies. The following chapter discusses the production and purification of IgY, as well as its many advantages and applications, including the use of IgY as a diagnostic and analytical tool and for passive immunotherapy to treat numerous health conditions.

Key words: hen egg yolk antibodies (IgY), IgY purification, passive immunotherapy, diagnostics, immunoaffinity ligand.

17.1 Introduction

It has been over 100 years since the existence of immunoglobulin (Ig) Y was first described by Klemperer (1893). It was found to be the major serum antibody in hens, and termed IgY due to its enrichment in egg yolk (Leslie and Clem, 1969). Since then, the avian immune system, and IgY, has been studied extensively, revealing many important characteristics and applications of IgY. The laying hen is an ethical alternative for the production of large quantities of specific antibodies at a low cost (Schade and Hlinak, 1996). Owing to its favorable immunochemical characteristics when compared to mammalian IgG, IgY has found many applications in the medical and research fields, including areas such as diagnostics and biomarker discovery. IgY has perhaps received the most attention for its immunotherapeutic potential.

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Using egg IgY antibodies for health, diagnostic & other applications

Passive immunotherapy, the administration of pre-formed antibodies to prevent infectious diseases or treat existing conditions, may be one of the most valuable applications of antibodies, and is currently driven by the need to find alternatives to traditional antibiotic therapy (Reilly et al., 1997; Carlander et al., 2000). Antibody therapy is, however, often limited by the significant amount of antibody required for treatment (Hatta et al., 1997b). Since IgY is transferred in large quantities to the egg yolk, and can easily be extracted and isolated, eggs are an ideal source of specific antibodies for passive immunotherapy.

The following chapter describes structural, physical and immunochemical properties of IgY, as well as an overview of IgY production and purification, and its applications in diagnostics, analytical applications and immunotherapy.

17.2 Overview of the avian immune system and IgY biosynthesis

The avian immune system is made up of the bursa of Fabricius, bone marrow, spleen, thymus, the Harderian gland, lymph nodes, circulating lymphocytes and various lymphoid tissues. The thymus serves as the primary lymphoid organ for T-cell differentiation, while the antibody-synthesizing B-cells are produced in the bursa of Fabricius (Sharma, 1997; Carlander et al., 1999a). The spleen is the centre for plasma cell proliferation and memory B cells (Carlander et al., 1999a). The vertebrate immune system has the ability to produce an exceedingly high number of different antibody molecules, through the existence of multiple variable (V), diversity (D) and joining (J) elements in the genome (the germ line repertoire), as well as existence of somatic recombination processes and point mutations (Reynaud et al., 1985; Parvari et al., 1988). In the avian immune system, however, this combinatorial diversity is restricted, as the rearrangement of immunoglobulin genes is not an ongoing process, but rather takes place as a single wave during early embryogenesis. The total number of rearrangements from which the chicken B-cell repertoire may be generated is limited to the number of B-cell precursors in the bursa (Reynaud et al., 1989, 1991), estimated to be around 2–3 × 10⁴ cells (Pink et al., 1985). Despite the fact that chickens have an extremely limited number of immunoglobulin genes compared with mammals, they are capable of producing a wide range of immune responses and diverse antibody molecules (Sharma, 1997).

Three classes of antibody are found in the chicken, IgY, IgA and IgM, and are present in the serum at concentrations of 5.0, 1.25 and 0.61 mg/mL, respectively (Leslie and Martin, 1973). IgY, the functional equivalent of mammalian IgG, is the major antibody produced by chickens, and makes up approximately 75% of the total antibody population (Carlander et al., 2000). During egg formation, serum IgY is selectively transferred to the
yolk via a receptor on the surface of the yolk membrane specific for IgY translocation, in order to confer passive immunity to the developing embryo (Fig. 17.1) (Loeken and Roth, 1983; Tressler and Roth, 1987; Morrison et al., 2002; West et al., 2004; Tesar et al., 2008). Egg yolk can contain between 5 to 25 mg/mL of IgY, whereas IgA and IgM are found in the egg white, at concentrations of around 0.15 and 0.7 mg/mL respectively (Rose et al., 1974; Schade et al., 1991; Li et al., 1997).

17.3 Production and purification of IgY

17.3.1 Production

Several aspects of hen immunization, including the immunization route and vaccine adjuvant, have been studied in an effort to improve IgY production and yolk deposition (Levesque et al., 2007). Chang et al. (1999) demonstrated that intramuscular immunization resulted in higher levels of specific IgY when compared with immunization via the subcutaneous route. Oil-based adjuvants, such as Freund’s complete (FCA) and incomplete (FIA) adjuvant, continue to be the adjuvants of choice for IgY production (Trott et al., 2008); however, alternatives are continually being sought due to potentially severe injection site reactions (Wanke et al., 1996). The inclusion of bacterial components or immunostimulants in vaccine formulations has been shown to increase antigen-specific antibody levels in the yolk, and may require fewer injections and less antigen, and ultimately be more cost effective (Levesque et al., 2007; Trott et al., 2008; Herath et al., 2010). In fact, Levesque et al. (2007) found that adding synthetic oligonucleotides containing unmethylated CpG dinucleotides, characteristic of bacterial DNA (CpG ODNs), to FIA could
increase specific IgY production by 480%. The use of DNA vaccines has also been described for IgY production (Romito et al., 2001; Cova, 2005; Witkowski et al., 2009). This process involves immunizing birds with plasmid DNA encoding the antigen of interest, and not only avoids the potentially costly and tedious preparation of purified antigens, but can also overcome interference by maternal antibodies (Elazab et al., 2009).

17.3.2 Purification

Egg yolk is a liquid emulsion of water-soluble protein and a dispersed phase of lipoprotein particles. One of the major challenges in IgY purification is separating the water-soluble IgY from the yolk lipoproteins (Hatta et al., 2008). IgY purification typically involves isolation of the IgY-containing water-soluble fraction (WSF), followed by additional purification steps. Simple 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 (UF), has been reported for the isolation of WSF (Akita and Nakai, 1992; Kim and 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 (Jensenius and Koch, 1993), has also been employed, resulting in a purity of around 70% (Deignan et al., 2000). For these 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 separation by ultracentrifugation (McBee and Cotterill, 1979; Akita and Nakai, 1992; Akita and Li-Chan, 1998), organic solvent delipidation (Polson, 1990; Kwan et al., 1991; Horikoshi et al., 1993; McLaren et al., 1994), or use of lipoprotein coagulating agents such as polyethylene glycol (Akita and Nakai, 1993; Svendsen et al., 1995), dextran sodium sulfate (Jensenius et al., 1981), polyacrylic resin (Hamada et al., 1991) and dextran blue (Bizhanov and Vyshniauskis, 2000); however, application of these methods for large-scale IgY production is limited by problems related to food safety as well as cost constraints (Hatta et al., 2008). To address this problem, natural polysaccharides, which have been found to act as effective yolk lipoprotein coagulating agents (Hatta et al., 2008), have been employed, including sodium alginate (Hatta et al., 1988, 1990), xanthan gum (Akita and Nakai, 1993) and ι-carrageenan (Hatta et al., 1990). Chang et al. (2000) reported the precipitation of over 90% of lipoproteins from yolk using λ-carrageenan, sodium alginate, carboxymethyl cellulose and pectin.

A number of methods of purifying IgY following lipoprotein removal have been reported, and include ion exchange chromatography (McCannel and Nakai, 1990; Akita and Nakai, 1992; Fichtali et al., 1992, 1993; Ko and Ahn, 2007), ammonium sulfate precipitation (Ko and Ahn, 2007), hydrophobic
interaction chromatography (Hassl and Aspock, 1988), immobilized metal ion affinity chromatography (McCannel and Nakai, 1990; Greene and Holt, 1997), thiophilic interaction chromatography (Hansen et al., 1998), affinity chromatography using alkaline conditions (Kuronen et al., 1997) and synthetic peptide ligands, designed specifically for immobilizing IgY (Fassina et al., 1998; Verdoliva et al., 2000) which resulted in 92% purity and 78% recovery (Dong et al., 2008). Hernandez-Campos et al. (2010) recently demonstrated that UF purification of WSF using polyethersulphone (PES) or modified PES membranes increased purity and resulted in greater than 90% IgY recovery.

Recent proteomic analysis and identification of the proteins in WSF prepared by the water dilution method revealed that preparations obtained by this method are highly reproducible with low levels of cholesterols and triglycerides, and provides information that may be valuable for the prediction of potential allergic reactions to WSF preparations (Nilsson et al., 2008).

17.4 Advantages of eggs as an alternative antibody source

The use of chickens for antibody production provides several advantages over the conventional method of IgG production in mammals. Antibody production in chickens is much less invasive, requiring only the collection of eggs, rather than the collection of blood, and therefore is less stressful on the animal (Fig. 17.2) (Karlsson et al., 2004). Owing to the genetic distance between chickens and mammals, it is possible to produce antibodies

Fig. 17.2 Comparison of preparation of specific antibody from hen (Hatta et al., 1997a).
against highly conserved mammalian proteins that otherwise would not be possible in mammals. In contrast to mammalian systems, much less antigen is required to produce an efficient immune response, and sustained high antibody titers reduce the need for frequent injections (Larsson et al., 1998). In fact, it has been estimated that the productivity of antibodies in hens is nearly 18 times greater than that in rabbits, based on the weight of antibody produced per animal (Nakai et al., 1994), which translates to an overall reduction in animal use for the same level of antibody production. A laying hen typically lays around 240–280 eggs per year (Sim et al., 2000), and an egg yolk contains 100–150 mg of IgY. Therefore, around 28–42 g of IgY per year may be obtained from a single chicken (Karlsson et al., 2004).

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), as discussed in the previous section. Once purified, IgY has been found to be stable for years when stored at 4 °C (Larsson et al., 1993).

### 17.4.1 Structure and stability of IgY

Although IgG and IgY share a similar biological function, there exist some profound differences in their chemical structures and resulting immunoreactivity (Camenisch et al., 1999; Zhang, 2003). In contrast to IgG, which has a molecular mass of 150 kDa, the IgY molecule has a molecular mass of 180 kDa, owing to its increased number of heavy-chain constant domains and an extra pair of carbohydrate chains (Hatta et al., 1993a; Sun et al., 2001). In addition, the hinge region of IgY is shorter and less flexible (Warr et al., 1995), and the content of β-sheet structure in the constant domains of IgY has been reported to be lower (Shimizu et al., 1992), which may account for the differences in stability observed between IgY and IgG.

Both IgG and IgY contain Asn-linked oligosaccharides; however, the structure of oligosaccharides in IgY differ from those of any mammalian IgG, containing instead unusual monoglucosylated oligomannose-type oligosaccharides with Glc$_2$Man$_{7-9}$GlcNAc$_2$ structure (Ohta et al., 1991; Matsuura et al., 1993). The isoelectric point of IgY is lower than that of IgG (Polson et al., 1980), IgY does not associate with mammalian complement, and the binding of IgY with human and bacterial Fc-receptors on cell surfaces is less than that of IgG (Gardner and Kaye, 1982). Furthermore, it has also been suggested that IgY may be more hydrophobic than IgG, which matches the lipid-rich environment of the yolk (Davalos-Pantoja et al., 2000).

In general, when used as reagents for immunochemical or research purposes, where antibodies are stored and used under relatively mild conditions, stability does not warrant much attention. However, the stability of IgY under various processing and physiological conditions needs to be considered when they are to be used in industrial, food or medical applications (Shimizu et al., 1993b). IgY is stable in the pH range of 4–9, and up to temperatures of 65 °C.
Improving the safety and quality of eggs and egg products

(Shimizu et al., 1992; K. A. Lee et al., 2002), however this range may be extended by the addition of stabilizers such as sugars, complex carbohydrates, or polyols (Shimizu et al., 1994; Chang et al., 2000; K. A. Lee et al., 2002). IgY is relatively resistant to treatment with trypsin and chymotrypsin, but sensitive to pepsin digestion (Shimizu et al., 1988). Hatta et al. (1993b) found that almost all of the IgY activity was lost following digestion with pepsin; however, activity remained even after 8 hours incubation with trypsin or chymotrypsin.

Studies in humans have demonstrated that orally administered antibodies undergo a significant loss of activity upon passage through the stomach, and several reports have demonstrated around 4–20% survival of orally administered antibody, with similar results observed in both adults and infants (Blum et al., 1981; Hilpert et al., 1987; Roos et al., 1995). In order to circumvent the inactivation of IgY following oral administration, a number of encapsulation techniques have been examined. The microencapsulation of IgY using liposomes (Shimizu et al., 1993a) and multiple emulsions (Shimizu and Nakane, 1995) have been previously described; however, these have shown limited effectiveness, in some cases destroying antibody activity by the encapsulation procedure itself. The macroencapsulation of IgY using enteric-coated gelatin capsules has also been examined, and was found to significantly improve antibody stability (Akita and Nakai, 2000). The use of a pH-sensitive methacrylic acid copolymer and chitosan–alginate microcapsules (Kovacs-Nolan and Mine, 2005; Li et al., 2007, 2009a, b) have also been shown to increase IgY stability to gastric conditions both in vitro as well as in vivo.

17.5 Applications of IgY

17.5.1 Use of IgY as an immunoaffinity ligand

Immunoaffinity chromatography, involving the specific interaction between an antigen and its corresponding antibody, has been widely used for the purification of proteins from complex starting materials. The use of this process on a large scale is limited by the high cost of the technique, and problems relating to the production of antibody and the efficiency of immobilization (Akita and Li-Chan, 1998). Specific IgY, which can easily be produced on a large scale required for industrial applications, would provide an ideal replacement for other polyclonal or monoclonal antibodies currently used in immunoaffinity chromatography (Li-Chan, 2000). Although IgY is more sensitive to low pH than IgG, it was found that an IgY immunoaffinity column was capable of retaining stability when subjected to standard affinity chromatography conditions, and could be reused over 50 times without a significant decrease in binding capacity (Akita and Li-Chan, 1998).

More recently, the unique features of IgY have made it advantageous for use in proteomic applications (Huang et al., 2005). The depletion of abundant
proteins from complex biological samples containing a wide dynamic range of protein concentrations, such as plasma, serum and urine, prior to two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectrometry presents a significant challenge for proteomic analysis (Huang and Fang, 2008; Magagnotti et al., 2010). Immunoaffinity fractionation is one of the most effective methods used during sample preparation to improve the ability to detect low-abundance proteins (Huang and Fang, 2008). Owing to the ease of production of IgY and the ability to produce high affinity antibodies against conserved mammalian proteins, IgY is now widely used for immunoaffinity protein depletion in proteomic applications and biomarker discovery (Qian et al., 2008; Rajic et al., 2009; Polaskova et al., 2010).

17.5.2 Use of IgY in diagnostic and analytical applications
Antibodies serve as essential components in a variety of diagnostic assays used for the qualitative and quantitative determination of a wide range of substances (Schade and Hlinak, 1996). While the antigen-binding specificity of IgY is comparable to that of IgG (Hatta et al., 1997b), IgY presents several advantages that make it well suited for applications in medical diagnostics. A number of proteins exist whose amino acid sequences are well preserved among mammals, and when used as an antigen in mammals have limited or no antigenicity (Hatta et al., 2008). Evolutionary differences allow the production of high avidity IgY against conserved mammalian proteins, with a more diversified antibody repertoire than is possible in mammals (Olovsson and Larsson, 1993; Woolley and Landon, 1995; Carlander et al., 1999a). The use of IgY as a substitute for IgG in clinical testing can potentially eliminate false positives and often results in low background and less interference (Xiao and Gao, 2010). Human serum samples often contain an active complement system, which would be activated by mammalian antibodies. IgY, which does not activate the human complement system, eliminates the interference that would otherwise be caused by IgG (Larsson and Mellstedt, 1992). Human serum can also contain rheumatoid factor (RF) and human anti-mouse IgG antibodies (HAMA), which are well-known causes of false-positive reactions in immunological assays (Carlander et al., 1999a). RF reacts with the Fc portion of IgG, and HAMA, which can occur naturally in human serum, will bind to any mouse antibodies being used in an immunoassay (Carlander et al., 1999a). Since IgY does not react with RF (Larsson and Sjoquist, 1988; Larsson et al., 1991) or HAMA (Larsson and Mellstedt, 1992), it has been suggested as a replacement for IgG in immunological assays of human serum.

IgY has been used in numerous medical and veterinary diagnostic applications, including detection and quantitation of cancer biomarkers in breast and ovarian cancer and non-Hodgkin’s lymphoma patients (Grebenschikov et al., 1997; Al-Haddad et al., 1999; Lemamy et al., 1999; Pan et al., 2010;
Xiao and Gao, 2010), the diagnosis of gastric cancer (Noack et al., 1999), as well as for the detection of African horsesickness virus (Du Plessis et al., 1999), Mycobacterium avium subsp. paratuberculosis (Shin et al., 2009), Leptospira spp. in human blood (Vasconcellos et al., 2010), bovine leukemia virus (Juliarena et al., 2007), serotyping of foot-and-mouth disease (Veerasami et al., 2008), and detection of opiate drugs in urine (Gandhi et al., 2009).

IgY against the SARS-CoV nucleocapsid protein was incorporated into an immunoswab assay for point of care detection of SARS-infected individuals (Kammila et al., 2008).

Furthermore, IgY has been used in assays involving food safety concerns, including detection of Escherichia coli O157:H7 (Sunwoo et al., 2006), Listeria spp. (Kim et al., 2005), Campylobacter jejuni (Hochel et al., 2004), screening agricultural products for citrinin contamination (Duan et al., 2009), detection of ciguatoxin contamination in fish tissue (Empey Campora et al., 2008), the identification of allergens (Alessandro et al., 2009), and IgY against dichlorodiphenyltrichloroethane (DDT) was incorporated into a dipstick assay to test for pesticide contamination (Lisa et al., 2009).

## 17.6 Immunotherapeutic applications of IgY

Increases in antibiotic resistant bacteria and pathogens that do not respond to antibiotics, such as viral pathogens, along with an escalating number of immunocompromised individuals, has prompted much research into the administration of specific antibodies as an alternative to antibiotics and antimicrobial chemotherapy to treat infections (Casadevall and Scharff, 1995; Reilly et al., 1997). IgY is well suited to passive immunotherapy applications since it does not react with rheumatoid factors, human Fc-receptors, or HAMA, which are all well-known cell activators and mediators of inflammation (Larsson et al., 1993; Larsson and Carlander, 2003).

### 17.6.1 Oral administration of IgY

**Prevention of Pseudomonas aeruginosa infection in cystic fibrosis patients**

Respiratory infection caused by colonization of the airways by Pseudomonas aeruginosa (PA) is the major cause of morbidity and mortality in cystic fibrosis (CF) patients (Shale and Elborn, 1996), and once a chronic infection has been established it is very difficult to eliminate, even with the use of antibiotics (Kollberg et al., 2003). An ongoing study in CF patients has found that an oral rinse containing anti-PA IgY given on a continuous basis could reduce or prevent PA colonization, thereby reducing the need for antibiotic treatment (Carlander et al., 2000; Kollberg et al., 2003; Nilsson et al., 2007b, 2008).

*In vitro*, these antibodies were found to inhibit adhesion of the bacteria to epithelial cells, but did not inhibit bacterial growth, suggesting that the
IgY might be capable of interfering with the bacterial infection process and preventing colonization in CF patients (Carlander et al., 1999b). More recently, the immunoreactivity of these antibodies was examined in detail using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and PA flagellin was identified as the major antigen. The flagellin is important for establishing infections in hosts as well as being involved in PA chemotaxis, motility, adhesion and inflammation (Nilsson et al., 2007a). These results further support the ability of anti-PA IgY to prevent infection and host invasion.

The stability of the anti-PA IgY in saliva from healthy individuals was also examined (Carlander et al., 2002), and antibody activity remained after 8 hours. After 24 hours, the antibody activity had decreased significantly, but was still detectable in some subjects, indicating that oral treatment with specific IgY for various local infections, including the common cold and tonsillitis, might also be possible.

Prevention of Helicobacter pylori infection and gastric symptoms

Helicobacter pylori is a common cause of gastritis and gastric ulcers, and has been implicated in the development of gastric carcinomas (Dunn et al., 1997). The development of antibiotic resistance has prompted the investigation into alternative methods of treating and preventing H. pylori infection (DeLoney and Schiller, 2000). IgY produced against H. pylori reduced bacterial adhesion, growth and urease activity in vitro and decreased H. pylori-induced gastric mucosal injury and inflammation in an animal model (Shin et al., 2002). Since antibodies produced against whole-cell H. pylori might cross-react with other bacteria, including normal human flora (Shin et al., 2003), the production and efficacy of IgY against immunodominant H. pylori proteins or epitopes has also been described, including IgY against urease (Nomura et al., 2005), urease-derived peptides (Shin et al., 2004) and a 58 kD highly reactive H. pylori antigen (Hp58) (Attallah et al., 2009).

The effectiveness of anti-urease IgY has been demonstrated in humans. A functional drinking yogurt containing Lactobacillus acidophilus and Bifidobacterium spp. supplemented with 1% egg yolk IgY-urease was produced commercially, and given to volunteers testing positive for H. pylori (Horie et al., 2004). Volunteers consumed the IgY-containing yogurt three times daily for four weeks, following which urea breath test values and antigen detection in feces were significantly decreased, indicating suppression of H. pylori infection.

Prevention of dental caries and periodontitis

Porphyromonas gingivalis is one of the most important causative agents of periodontitis (Yokoyama et al., 2007b). In vitro, anti-P. gingivalis IgY dose-dependently decreased bacterial adhesion and hydrolytic activity (Yokoyama et al., 2007a), and reduced levels of P. gingivalis when applied as a gel to the teeth of periodontitis patients (Yokoyama et al., 2007b). The production of
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), have also been described and found to inhibit aggregation and hemagglutination *in vitro*.

*Streptococcus mutans* is believed to be the principal causative bacterium of dental caries in humans (Hamada and Slade, 1980), and IgY against *S. mutans* has also been shown to exert anti-cariogenic properties. IgY against *S. mutans* was examined for the passive prevention of dental caries (Hamada et al., 1991; Otake et al., 1991; Chang et al., 1999), and it was found that supplementation of 2% IgY-containing yolk powder to a cariogenic diet significantly lowered caries scores. Likewise, levels of *S. mutans* were decreased in volunteers who gargled with a mouth rinse containing anti-*S. mutans* IgY (Hatta et al., 1997c). IgY produced against the *S. mutans* glucan binding protein B (GBP-B), which is believed to be involved in *S. mutans* biofilm development, has also been examined. Using a rat model of dental caries, Smith *et al.* (2001) observed a decrease in *S. mutans* accumulation in rats treated with anti-GBP-B IgY, as well as a decrease in the overall amount of dental caries, as compared with control rats.

**Prevention of Escherichia coli infection**

Diarrheal diseases continue to be a health problem worldwide, especially in developing countries, where they are estimated to be responsible for 2.5 million infant deaths per year (Estrada-Garcia *et al.*, 2009), as well as causing economic losses in livestock, especially neonatal calves and piglets (Morris and Sojka, 1985). One of the most prominent causes of diarrheal diseases remains *Escherichia coli*, and a number of strategies to prevent *E. coli* infection using IgY have been examined.

The production of IgY against the fimbral antigens K88, K99 and 987P of porcine enterotoxigenic *E. coli* (ETEC), has been described. Anti-K88, -K99 and -987P IgY inhibited the binding of *E. coli* K88+, K99+ and 987P+ 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). Marquardt *et al.* (1999) also found that the oral administration of anti-K88 IgY resulted in 100% survival of ETEC-infected piglets, compared with a control group, and a reduction in the incidence and severity of diarrhea. The passive protective effect of anti-ETEC IgY, in neonatal calves has also been shown. Calves fed milk containing IgY had transient diarrhea, 100% survival and good body weight gain during the course of the study (Ikemori *et al.*, 1992).

More recently, IgY against recombinant adherence-associated proteins of *E. coli* O157:H7 was shown to reduce 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 produced against virulence factors of attaching and effacing \textit{E. coli} (AEEC) reduced bacterial adherence of porcine enteropathogenic \textit{E. coli}, as well as heterologous AECC strains, including human, bovine and canine enteropathogenic \textit{E. coli} and O157:H7, and found that oral administration of these antibodies reduced \textit{E. coli}-induced lesions in pigs challenged with the porcine enteropathogenic \textit{E. coli} strain ECL1001.

Furthermore, anti-\textit{E. coli} O78:K80 IgY was found to reduce \textit{E. coli} numbers and improve intestinal health in chickens challenged with \textit{E. coli} (Mahdavi et al., 2010), and IgY against mastitis-causing \textit{E. coli} (O111) dose-dependently inhibited \textit{E. coli} growth and enhanced phagocytic activity \textit{in vitro} (Zhen et al., 2008b). Amaral et al. (2008) demonstrated the \textit{in vitro} anti-\textit{E. coli} effects of IgY against EPEC O111, Shiga toxin-producing \textit{E. coli} (STEC) O111 and STEC O157, suggesting possible future therapeutic use in humans.

**Prevention of Salmonella infection**

\textit{Salmonella} Enteritidis (SE) and \textit{Salmonella} Typhimurium (ST) are the main causes of salmonellosis outbreaks in humans and infections in chickens (E. N. Lee et al., 2002). \textit{Salmonella} possesses several surface components which are virulence related, including outer membrane protein (OMP) (Isibasi et al., 1988), flagella (Fla) and, in some strains, fimbrial antigens (Thorns et al., 1990, 1992). The ability of IgY specific for OMP, LPS or flagella (Fla), to passively protect against experimental salmonellosis in mice has been demonstrated. \textit{In vitro} using a cultured human intestinal cell line (Caco-2), Chalghoumi et al. (2009) found that IgY against OMP of SE and ST reduced the \textit{Salmonella}-induced decrease in transepithelial electrical resistance (TER) of the infected Caco-2 cell monolayers and blocked \textit{Salmonella} sp. adhesion in a concentration-dependent manner, suggesting that passive immunization with \textit{Salmonella} OMP-specific IgY could be useful to prevent \textit{Salmonella} colonization in broiler chickens and the subsequent carcass contamination during processing. \textit{In vivo} in mice challenged with SE or ST, treatment with IgY against OMP, LPS or Fla resulted in a significantly higher survival rate when compared to control mice (Yokoyama et al., 1998b). \textit{Salmonella} infection in calves is also a significant problem, with ST and \textit{S. dublin} accounting for most cases of salmonellosis. Passive protection against ST and \textit{S. dublin} was investigated by challenging calves with SE or \textit{S. dublin}, and then orally administering anti-SE or anti-\textit{S. dublin} IgY. All control calves died within 7–10 days, whereas only fever and diarrhea were observed in calves given the high titer IgY (Yokoyama et al., 1998a).

**Prevention and treatment of rotavirus gastroenteritis**

Rotavirus is a major cause of acute gastroenteritis in infants in both developed and developing countries (Clark et al., 1999). \textit{In vivo}, IgY produced against murine, human and simian strains of rotavirus prevented rotavirus-induced

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diarrhea in mice (Yolken et al., 1988; Ebina, 1996). The anti-rotavirus effect was seen both when administered before and after viral challenge, suggesting its use for both the prevention and treatment of rotavirus gastroenteritis (Hatta et al., 1993a). The production of IgY against recombinant rotavirus coat proteins, VP8 (Kovacs-Nolan et al., 2001) and VP7 (Zhang et al., 2009) has also been described, and anti-VP8 IgY exhibited significant neutralizing activity against human rotavirus (HRV) in vitro, suggesting its potential use for the prevention and treatment of rotavirus in humans.

Neonatal calf diarrhea, caused by bovine rotavirus (BRV), is a significant cause of mortality in cattle (Lee et al., 1995). Using a mouse model of BRV infection, Kuroki et al. (1993) observed the protection against two strains of BRV using orally administered anti-BRV IgY. The passive protection of calves against BRV infection, using anti-BRV IgY, has also been demonstrated (Kuroki et al., 1994).

**Treatment of inflammatory bowel disease**

Inflammatory bowel disease (IBD), including Crohn’s disease and ulcerative colitis, is characterized by chronic inflammation of the gastrointestinal tract (Podolsky, 2002). Treatment typically involves corticosteroids and immunosuppressive agents; however, these have shown limited therapeutic efficacy and have been associated with severe side effects and long-term toxicity (Atreya and Neurath, 2008). 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 in patients receiving systemic anti-TNF therapy (Sandborn and Hanauer, 1999). Worledge et al. (2000) reported that orally administered anti-TNF-α IgY was capable of effectively treating acute and chronic phases of colitis in rats, as well as neutralizing human TNF-α in vitro, indicating its possible use for the treatment of IBD in humans.

**Use of IgY in aquaculture**

*Yersinia ruckeri* causes enteric redmouth disease, a systemic bacterial septicemia of salmonid fish (Stevenson et al., 1993), and the persistence of *Y. ruckeri* in carrier fish and shedding of bacteria in feces can present a continuing source of infection. Lower mortality rates and reduced infection rates were observed in rainbow trout fed anti-*Y. ruckeri* IgY when given before or after challenge with *Y. ruckeri* (Lee et al., 2000).

White spot syndrome virus (WSSV) causes high mortality and large economic losses in cultured shrimp (Lu et al., 2008). IgY produced against WSSV was shown to passively protect shrimp (Lu et al., 2008) and crayfish (Lu et al., 2009) from WSSV infection.

*Edwardsiella tarda* is a fish pathogen spread by infection through the intestinal mucosa, and Edwardsiellosis in Japanese eels is a serious problem for the eel farming industry, especially due to the appearance of antibiotic-
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resistant strains (Hatta et al., 1994). Eels challenged with *E. tarda* and then given anti-*E. tarda* IgY survived without any symptoms of infection, in contrast to the control eels which 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).

Prevention and treatment of other diseases
Specific IgY has been shown to be effective at preventing and treating several other diseases, including for the passive protection of chicks against infectious bursal disease virus (IBDV) (Eterradossi et al., 1997; Yousif et al., 2006) and avian coccidiosis caused by *Eimeria* spp. (Lee et al., 2009a, b), for the protection of piglets against porcine epidemic virus (PEDV) (Kweon et al., 2000), for the protection of calves against bovine coronavirus (BCV) (Ikemori et al., 1997), and for the protection of dogs against canine parvovirus-2 (CPV-2) (Van Nguyen et al., 2006). IgY has also been shown to be effective against *Candida albicans*, dose-dependently preventing *C. albicans* growth, adherence and biofilm formation *in vitro* (Wang et al., 2008; Fujibayashi et al., 2009) and preventing colonization in mice (Ibrahim el et al., 2008). Finally, the production and testing of IgY against influenza virus H5N1 and H1N1 has also been described. When administered before or after infection, the IgY prevented mice from infection and significantly reduced viral replication resulting in complete recovery from the disease, suggesting that IgY may be a safe and affordable alternative method for the control of influenza outbreaks (Nguyen et al., 2010).

17.6.2 Systemic administration of IgY
Neutralization of venom
Envenoming resulting from the bites of venomous snakes, scorpions or spiders is an important public health hazard in many regions, particularly in tropical and subtropical countries. The most widely used treatment is the direct injection of specific anti-venoms, often antibodies produced in horses or sheep, to neutralize the toxic and potentially lethal effects of the venom (Gutierrez et al., 2006). There have been several reports of the production and neutralization of venom by chicken anti-venom IgY (Paul et al., 2007; Meenatchisundaram et al., 2008a, b; Araujo et al., 2010), including the production of polyvalent anti-African snake venom IgY to be used against bites of several different snakes (de Almeida et al., 2008). IgY was found to have a higher bioactivity than anti-venoms traditionally raised in horses (Thalley and Carroll, 1990; de Almeida et al., 2008), and also has a lower likelihood of producing side effects such as serum sickness and anaphylactic shock, which can occur upon administration of mammalian serum proteins (Thalley and Carroll, 1990).
Prevention of Staphylococcus aureus infection and toxic shock syndrome

Staphylococcal enterotoxins are a family of bacterial superantigens produced by Staphylococcus aureus, and are associated with a number of serious diseases, including food poisoning and toxic shock syndrome (Fraser et al., 1976). Specific IgY has been shown to inhibit the production of S. aureus enterotoxin-A in vitro (Sugita-Konishi et al., 1996). It has also been reported that IgY against S. aureus enterotoxin-B (SEB) systemically administered both before and after challenge could protect rhesus monkeys from toxic shock syndrome (LeClaire et al., 2002), suggesting that it might be useful for both the prevention and treatment against lethal doses of S. aureus enterotoxins, and could be used to reduce or eliminate enterotoxin-mediated disorders.

Anti-S. aureus IgY has also been used in veterinary applications, where it was found to inhibit S. aureus growth in vitro (Zhen et al., 2008a; Guimaraes et al., 2009) and prevented bovine mastitis caused by S. aureus in cattle when administered by intramammary infusion (Zhen et al., 2009).

Prevention of rabies virus

Rabies remains a major public health problem in developing countries, claiming the lives of an estimated 55,000 people each year (Ertl, 2009). Infection with rabies virus causes encephalitis in humans that has a case fatality rate of almost 100% (Johnson et al., 2010). Motoi et al. (2005a, b) reported the production of anti-rabies IgY, raised against a part 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. Administration of anti-rabies IgY to mice infected with rabies virus 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 anti-rabies human or equine immunoglobulins (Motoi et al., 2005b).

IgY for the prevention of hyperacute rejection in xenotransplantation

Transgenic pigs are promising donor organisms for xenotransplantation as they share many anatomical and physiological characteristics with humans (Klymiuk et al., 2010); however one of the most important barriers with such xenografts is hyperacute rejection, mediated by natural antibodies in humans against pig antigens, complement fixation, and the rapid onset of intravascular coagulation (Sandrin and McKenzie, 1994). The major target of these natural antibodies is the carbohydrate epitope, Galα1-3Gal, which is expressed by all mammals except for humans, apes, and some monkeys. Besides humans and monkeys, chickens also lack Galα1-3Gal expression (Bouhours et al., 1998). Since IgY does not bind human complement or Fc-receptors, anti-α-Gal IgY may be a suitable candidate for use as blocking antibodies to inhibit the interactions that may contribute to xenograft rejection. Fryer et al. (1999) demonstrated that anti-α-Gal IgY blocked human xenoreactive antibody binding to both porcine and rat tissues in vitro, and inhibited lysis of porcine cells by human serum, suggesting that IgY could be of potential
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use in inhibiting pig-to-human xenograft rejection. Anti-α-Gal IgY was also found to significantly reduce the infectivity of porcine endogenous retrovirus (PERV), an α-Gal bearing virus which has emerged as a potential zoonotic agent, with possible pig-to-human transmission (Leventhal et al., 2001).

17.7 Conclusion and future trends

The production of IgY in chickens and the extraction of specific antibodies from egg yolk is increasingly attracting the interest of the scientific community, as evidenced by the significant growth in the amount of IgY-related literature (Schade et al., 2005). Indeed, the immunization of hens represents an ideal alternative to efficiently generate large quantities of polyclonal antibodies since housing is inexpensive, egg collection is non-stressful and non-invasive, and the isolation and purification of IgY is relatively simple and high-yielding (Zhang, 2003). Recently, it has also been suggested that the selective transport of IgY from the blood into the egg yolk may also provide a new strategy for delivering substances into the egg yolk in an attempt to produce designer eggs (Bae et al., 2010), and may further expand the application of IgY. Immunization protocols and adjuvant systems continue to be modified in order to increase specific antibody production, and the identification and production IgY against of new antigens will continue to lead to new applications of egg yolk antibodies for human health and research applications.

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