

Chapter 25 Use of IgY Antibodies in Human and Veterinary Medicine

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1 Introduction

IgY technology, including the production and use of polyclonal IgY antibody (Ab), is a highly innovative and an expanding branch of biotechnology. In this chapter, we will discuss the advantages in the use of IgY Ab and the application of IgY in human and veterinary medicine (see also Kovacs-Nolan and Mine 2004). Other aspects such as immunization procedures, Ab titer development, IgY heat and acid stability and preservation of IgY solutions are reviewed in detail in Chacana et al. (2004), Hau and Hendriksen (2005), Leenars et al. (1999), Schade et al. (2000), and Schade et al. (2005). The usefulness of IgY Abs in biomedical diagnostic is well documented by an increasing quantity of literature and is therefore not included in this review.

2 Advantages of IgY Technology

The most important aim of animal welfare is to reduce painful manipulations. IgY technology fulfils this requirement in that chicken Abs can be easily sampled by a non-invasive method based on the simple act of collecting eggs. IgY technology also offers outstanding economical advantages because hens cost less to keep than rabbits. Furthermore, the Ab production of a hen roughly corresponds to that of a large mammal, such as a sheep or goat. Indeed, an extraordinary amount of Ab can be produced from only one hen—approximately 17–35 g of total IgY/chicken/year—of which 1–10% can be expected to be antigen-specific. This huge quantity of available Abs opens the door for new fields of IgY applications, such as immunotherapy and immunoprophylaxis for several viral and bacterial infections in veterinary and human medicine. In addition, IgY Abs have no cross reactivity with rheumatoid factors (Larsson et al. 1991) or human anti-mouse Ab (HAMA;

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Carlander et al. 1999). IgY Abs are unable to activate the mammalian complement system (Larsson et al. 1992) and have no heteroagglutinins (Calzado et al. 2003). Furthermore, several authors have reported that chickens often produce Abs against phylogenetical highly conserved mammalian proteins or peptides more efficiently than do rabbits (Karlsson et al. 2004). As a consequence, a conserved antigen can remain “masked” to the rabbit immune system, and thus cause only a weak or a “silent” response. Furthermore, if chickens and rabbits are immunized with the same mammalian antigen, very often the chickens respond with an Ab specificity that can rarely be achieved in rabbits, as for instance, with the N-terminal collagen propeptide PIIINP (Gerl et al. 1996), parathyroid-hormone-related protein (Rosol et al. 1993), transforming growth factor β 3 (TGF- β 3; Danielpour and Roberts 1995), and YKL-40 glycoprotein (De Ceuninck et al. 2001). The advantages of using chicken Abs have been recognized by many authors (for example Löscher et al. 1986; Carlander 2002; Narat 2003; Karlsson et al. 2004).

3 Applications of IgY in Biomedical Research and in Human and Veterinary Medicine

3.1 General Applications

Several publications have described the successful use of IgY Abs in a variety of research fields (for a review, see Narat 2003). IgY-based immunoassays are being used to measure the concentration of proteins or peptides via ELISAs, RIAs or other assays in clinical chemistry and basic research. IgY Abs are successfully used in immunohistochemistry for detection of antigens of viral, bacterial, plant, and animal origin, to assess the incidence of intestinal parasites in domestic animals (Schniering et al. 1996) and the contamination of foods with toxins or drugs (Pichler et al. 1998). During the past decade, IgY Abs have increasingly been used in therapy or prophylaxis of disease as well as in the new context of the so-called functional foods.

3.2 IgY for Therapeutic or Prophylactic use in Veterinary Medicine

3.2.1 Treatment of Intestinal Infections

Powdered whole eggs or yolks have been used in veterinary medicine as an inexpensive IgY source for the treatment of enteric diseases. The most famous example of a successful therapeutic/prophylactic use of IgY is the treatment of calves and piglets with specific Abs against *Escherichia coli* (K88, K99, 987P), rotaviruses, and coronavirus (for review see Mine and Kovacs-Nolan 2002 and Kovacs-Nolan and Mine 2004). Studies using both animal models and trials in field herds have been carried out. The groups of Yolken

(Yolken et al. 1988), Lösch (Wiedemann et al. 1991), Erhard (Erhard et al. 1996), and Kuroki (Kuroki et al. 1997) have performed the studies mostly on the practical use of IgY (see also Bilbao et al. 2006 and Terzolo et al. 2003). These studies confirmed that the treatment of diarrhea in calves and piglets with specific egg yolk Abs has achieved significant prophylactic and therapeutic benefits. Pokorova et al. (2000) administered IgY to protect dogs against canine parvovirus, and supposed that the protection was due to interactions between IgY and viral surface components. Sunwoo et al. (2002) demonstrated in vitro a marked growth inhibiting effect of specific IgY on *E. coli* 0157:H7, showing that growth inhibition was actually caused by the binding of specific IgY to bacterial surface antigens, which caused significant changes in the bacterial surface structure. Another effect of the binding of IgY to bacterial surface antigens is a marked impairment of bacterial attachment to the intestinal mucosa (Marquardt et al. 1999; Lee et al. 2002). Therefore, therapeutic IgY administration might reduce the clinical use of antibiotics, and thus minimize the risk of bacteria developing antibiotic resistance.

3.2.2 IgY Application in Aquafarming

IgY Abs have been successfully applied in treatments of fish farming diseases, for example in salmon and trout. Researchers from Japan have extensive experience in this field. For example, Hatta and colleagues demonstrated a successful treatment of *Edwardsiella tarda*-infected Japanese eels with specific anti-*E. tarda* IgY and showed protective effects when the IgY extracts were administered directly into the aquarium water (Hatta et al. 1994). Other authors achieved protective effects of specific IgY Ab against infection with *Yersinia ruckeri* (Lee et al. 2000) or against *Vibrio anguillarum* (Arasteh et al. 2004) in rainbow trout. Mostly, the Ab was incorporated in commercial pellet food. Furthermore, protection of shrimp (*Penaeus chinensis*) from infection with white spot syndrome virus (WSSV) has been described. Kim et al. (2004) produced IgY Abs against four different dominant proteins of WSSV and studied the neutralizing activity of these Abs. The authors demonstrated that shrimps survived an injection of WSSV preincubated with anti-WSSV Ab.

3.3 IgY for Therapeutical or Prophylactic use in Human Medicine

3.3.1 Treatment of Intestinal Infections in Children

The adherence ability of many viral and bacterial pathogens is a major prerequisite for the successful colonization of a higher organism, especially with respect to the host's respiratory and intestinal mucosae. It has been shown that specific IgY Abs against *Salmonella* antigens are able to inhibit in vitro the adhesion of this bacterium to epithelial cells (Lee et al. 2002). Casswall (1999), Carlander et al. (2000), and Sarker et al. (2001) investigated the action

of hyperimmune bovine colostrum (HBC) and IgY against human rotavirus isolated from infected children. The oral administration of IgY Abs resulted in a significant protective effect (Sarker et al. 2001). An anti-human rotavirus (strains Wa, RV5, RV3, ST3) IgY Ab was also effective, although to a lower extent than with HBC.

3.3.2 Treatment of *Helicobacter Pylori*

Therapeutic protection through IgY anti-*Helicobacter pylori* Abs has also been investigated in animals (Nomura et al. 2005) and humans (Shimamoto et al. 2002; Suzuki et al. 2004). Shin et al. (2003) were able to identify the immunodominant proteins of *H. pylori*. Antibodies with specificity against these proteins were more effective as prophylactic reagents as compared to Abs directed against the whole bacterial lysate. Altogether, all studies demonstrated a curative effect of the anti-*H. pylori* Ab. In most cases no complete *H. pylori* eradication could be achieved. But in view of the increasing bacterial resistance, the use of specific IgY Ab minimizes the need for antibiotics. Horie et al. (2004) carried out a study with 42 volunteers to test the protective effect of drinking yogurt fortified with anti *H. pylori* urease IgY, obtaining a significant decrease in urea breath values of the treated group.

3.3.3 Use of IgY for Treatment of Colitis and Celiac Disease

Worledge et al. (2000) demonstrated significant protective effects after oral application of specific IgY against tumor necrosis factor (TNF) in an experimental rat model for colitis. TNF is implicated in the pathogenesis of inflammatory bowel disease. The oral use of such Abs is considered to have fewer systemic side-effects than the intravenous infusion of a humanised murine anti-TNF monoclonal Ab (Infliximab, Centocor, Malvern, PA, USA). Sunwoo and Sim (2004) reported on the use of IgY Ab against dietary gluten proteins that play a role in the autoimmune disorder of the celiac disease. The authors immunized chickens with gliadins and low- and high-molecular glutenin. The resulting Ab can be used in different forms, such as table eggs, liquid and powdered eggs, and encapsulated nutraceuticals for treatment of celiac disease.

3.3.4 Treatment of Cystic Fibrosis

Carlander et al. (2002) studied the benefits of IgY as a prophylactic tool against infectious diseases in patients with cystic fibrosis (CF), the most common fatal genetic disease of the Caucasian population in Europe and the USA. CF is caused by a mutation of the gene for a chloride channel protein, which results in the secretion of an abnormally thick mucus. This leads to secondary infections in the respiratory tract, caused by several bacterial species, one of which, *Pseudomonas aeruginosa*, infects virtually all CF patients. The

researchers treated CF patients orally with an aqueous IgY anti-*P. aeruginosa* solution (70 ml, 0.7 mg/ml IgY), given as a mouth rinse in the evening. A high level of the specific chicken Abs could be demonstrated in the saliva by an ELISA for approximately 8 hours after the treatment. The IgY concentration then gradually declined, and was completely undetectable in the saliva 16 hours after the treatment. These oral IgY treatments were successful in reducing chronic *P. aeruginosa* infections in CF patients, and thus resulted in a decrease in antibiotic prescriptions (Kollberg et al. 2003).

3.3.5 Prophylactic use of IgY in Dental Caries

An effective local protection against plaque formation related to dental caries was achieved with anti-*Streptococcus mutans* IgY (Otake et al. 1991; Hamada and Kodoma 1996; Hatta et al. 1997; Chang et al. 1999; Smith et al. 2001). This passive protection was clearly shown with both SPF rats and human volunteers, following the use of either purified IgY or whole-egg powder. Active immunization against *S. mutans* glucan-binding protein B (GBP-B), under experimental conditions, induces good protection against experimental dental caries. This protection results from the continuous secretion of salivary Abs against GBP-B, which prevents the accumulation of *S. mutans* on the dental biofilm. The passive protection achieved by IgY is based on the same principle. In fact, the administration of IgY anti-*S. mutans* GBP-B via the diet and drinking water of experimentally infected rats caused a significant decrease in *S. mutans* aggregation on dental biofilms. In all these trials, a direct correlation was found between a given IgY dose and a reduction in the incidence of dental caries (Smith et al. 2001). Furthermore, the decrease in the *S. mutans* infection rate did not require continuous IgY administration (Smith et al. 2001). Hatta et al. (1997) evaluated the efficacy of oral IgY anti-*S. mutans* rinses in human volunteers. This IgY inhibited *S. mutans* adherence to saliva-coated hydroxyapatite discs by 59%, while the control IgY from non-immunized hens only gave an 8% inhibition. All these results strongly support the efficacy of oral treatments with anti-*S. mutans* IgY as a new alternative for reducing dental plaque in humans. Zhou et al. (2003) investigated the protective effect of an anti *S. mutans* IgY spray in adult volunteers. There was no difference in dental plaque indexes between controls and IgY-spray group although a significant decrease in *S. mutans* colonies could be demonstrated in the test group after three weeks of IgY application.

3.3.6 Use of IgY for Treatment of Poisonings

The protective effects of anti-venom IgY against rattlesnake toxins and scorpion toxins has been shown in a mouse model (Thalley and Carroll 1990). Almeida et al. (1998) showed similar results, producing IgY against venom from Brazilian snakes of the *Bothrops* and *Crotalus* genera. Related investigations are being performed in Bangalore (Vittal Mallya Research Institute of

Bangalore, India [<http://www.spiegel.de/spiegel/0,1518,231565,00.html>]). A Chinese group (Yu et al. 2004) produced anti king cobra venom based on IgY Ab with neutralizing activity. The use of anti-venom IgY is advantageous, since fewer immunological side-effects may be expected (see Sect. 3.1 this chapter). Gomez and colleagues (2006) produced specific IgY Abs against botulinum toxin type A that have been successfully used for immunoneutralization in a mouse model. Further, Lemley et al. (1995) produced an avian anti-ricin Ab and proved a protective effect in a mouse bioassay.

3.3.7 Use of IgY as a Tool in the Context of Bioterrorism

To test the therapeutic use of IgY Abs, LeClaire and colleagues (2002; see Table 1) produced IgY Abs against the highly toxic staphylococcal enterotoxin B (SEB). SEB is considered to be a potential biological warfare agent. Therefore, an increasing necessity exists to develop vaccines and therapeutic approaches for intoxication with SEB. The authors demonstrated the prophylactic and therapeutic application of anti-SEB IgY. Complete protection of mice and rhesus monkeys against a lethal SEB aerosol challenge has been observed when applied twenty minutes before or four hours after challenge.

Table 1. Toxin neutralizing activity of specific IgY-Ab. Two studies from 1893 and 2002

Klemperer F (1893) Ueber natürliche Immunität und ihre Verwerthung für die Immunisierungstherapie. *Archiv für Exp Pathol Pharmakol* 31:356–382

Repeated immunization (5 immunizations at intervals of 5–15 days each) of chickens with a tetanus culture (i.p. injection of increasing concentrations of virulent tetanus bouillon culture, from 5–30 ccm in steps of 5 ccm). After 4 weeks preparation of an egg yolk extract (11 ccm egg yolk mixed with 10 ccm saline). Treatment of mice with this extract (i.p.).

4 mice	2 mice	2 mice	2 mice
1 ml extract	0.5 ml extract	0.25 ml extract	0.0 ml extract (saline)
Next day administration of a lethal dose of tetanus bouillon culture (lethal dose 0.001 ccm, administered 0.0015 ccm s.c.).			
live	live	dead	dead

LeClaire et al (2002) Protection against bacterial staphylococcal enterotoxin B by passive vaccination. *Infect Immun* 70:2278–2281

Repeated immunization (4 immunizations at intervals of 2 weeks each) of chickens with bacterial staphylococcal enterotoxin B (250–500 µg SEB i.m.). After 8 weeks preparation of pure IgY (immunoaffinity chromatography). Administration of IgY 10 mg/kg or buffer (i.m. injection in monkeys), administration of toxin 5 × LD50 as aerosol (during the experiment the monkeys were anesthetized).

4 monkeys	4 monkeys	1 monkey	1 monkey
IgY 20 min before toxin challenge	IgY 4 h after toxin challenge	buffer 20 min before toxin challenge	buffer 4 h after toxin challenge
live	live	dead	dead

3.3.8 IgY as a Tool in Proteomics

A new and an interesting field in the use of IgY technology is proteomic analysis. A problem in separation of complex protein mixtures by 2D-electrophoresis is the predominance of highly abundant proteins such as albumin, which disturbs the monitoring of low-incidence proteins. Low-incidence proteins can be of great importance for identification and monitoring of several human (and animal) diseases. Recently, it has been shown that IgY Abs directed against these high-incidence proteins are in fact useful tools for their removal. In addition, these Abs work more specifically than do matrices with affinity to albumin, as for example Blue Sepharose (GE Healthcare, Amersham, Bucks., UK; Hinerfeld et al. 2004; Ahmed and Rice 2005; Huang et al. 2005).

4 Conclusions and Future Prospects

Today, there is no doubt that chicken Abs can be produced and used, with minor modifications, in ways similar to the use of mammalian Abs. It can also be said that, depending on the circumstances, the use of IgY Abs often has significant advantages over the use of mammalian Abs. Chickens have the potential to be used to complete the spectrum of animals used for Ab production. The production of chicken monoclonal Abs (and also recombinant IgY or genetically engineered IgY; Nakamura et al. 2004; Tsurushita et al. 2004; Park et al. 2005; Finlay et al. 2005) would combine the advantages of monoclonal Abs with the advantages of chicken Abs. In addition, a further interesting aspect is the immunization of chickens or ducks using DNA constructs (Cova 2005). It is to be expected that studies on the therapeutic or prophylactic use of IgY Abs will be intensified in the future. In particular, due to the increasing resistance of microorganisms to antibiotics, research on all aspects related to the development of specific IgY Abs against pathogenic microorganisms will have to be stepped up. In the future, IgYs will be universally used in science, including both veterinary and human medicine. IgY technology is a fast developing field and in this concise review we have only described some of its uses. We are convinced that, once accepted and widely used, IgY technology will offer new alternatives and solutions for science, medicine, and society as a whole.

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