

IgY-technology: application and trends

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The IgY-technology stand for the immunisation of chickens and the extraction of specific IgY antibodies (Ab) from egg yolk. Due to the non-invasive Ab isolation IgY-technology is internationally accepted as so-called alternative method. Despite of the aspect of animal welfare Ab production in chicken offers also outstanding economical advantages. i) purchase of chickens is much more cheap than purchase of rabbits. ii) One hen produces 17-35 g of total IgY/year. That is much more than the amount of Ab obtained from a rabbit in the same period (provided that regulations of animal welfare are observed). iii) That enormous Ab amount offers new fields of IgY application, particularly for therapeutic and/or prophylactic use in human and veterinary medicine. For example, there exists worldwide experience in successful treatment of diarrhoea in piglets, calves and also in children. Every year many children suffer or die from intestinal infections, particularly in non-developed countries. The administration of electrolyte solutions to infected children decrease the incidence of mortality but not the morbidity. Electrolyte solutions fortified with spec. Abs could decrease also the morbidity. Specific IgY Abs are used for treatment of infections with *Helicobacter pylori* a widespread infection normally eradicated by antibiotic therapy. In the last time *H. pylori* species are observed with resistance against antibiotics. Therefore, it is important to have effective alternatives. IgY Abs directed against *H. pylori* derived urease have been proved as effective. Recently, a drinking yoghurt fortified with anti-*H. pylori* urease Abs was tested successfully. The infection was not completely eradicated but significantly decreased. It could be proved that specific IgY prevent interactions between microorganisms and her target structures. There are a lot of other IgY application fields like prophylaxis in dental caries, treatment of celiac disease, application in aquafarming and anti-snake venom production. During the last few years the IgY-technology attracts also the interest of institutions working in the field of bioterrorism. There are several toxins and viruses suited for bio attacks, e.g. ricin, botulinum toxins, staphylococcal enterotoxins etc. Furthermore, today there is a big demand for specific Ab useful for detection and neutralisation of toxins in foodstuffs and toxins being candidates for bioterroristic attacks. IgY-technology is a fast developing field and we are convinced that the use of IgY will offer new alternatives and solutions to science, to medicine and to the society as a whole.

Keywords: IgY-technology; egg yolk antibodies; prophylaxis; therapy

Introduction

In 1893, Klemperer first described an experiment in which he demonstrated that the immunisation of a hen resulted in the transfer of specific antibodies (Abs) into the egg yolk. For a long time there was no scientific application for this knowledge, but when animal welfare became a matter of serious ethical concern for the scientific community, the results of Klemperer gained interest. In particular, this development was initiated by the work of Russell & Burch and the publication in 1959 of *The Principles of Humane Experimental Technique*. In the subsequent 20 years, more and more researchers recognised the importance of Klemperer's results. Since the 1980s, egg yolk antibodies (IgY Abs) have found a broader application, possibly due to the availability of commercial secondary

reagents such as IgY-purification kits, IgY-standards, and of labelled Abs (such as alkaline phosphatase, fluorescein isothiocyanate and peroxidase) specifically against IgY. Since 1996, IgY-technology (introduced by Dr Claus Staak in 1995) has become the internationally accepted term for describing the production and use of IgY Ab. In 1996 (Schade et al.), an European Centre for the Validation of Alternative Methods (ECVAM) workshop recommended the use of IgY instead of mammalian IgG, in order to minimise pain caused by invasive Ab sampling. This workshop also provided information about practical aspects of the rearing of laying hens, the immunisation of chickens, the use of adjuvants, and IgY extraction methods. In 1999 (Anonymous), the IgY-technology was approved as an alternative method for supporting animal welfare by the Veterinary Office of the Swiss Government (Office Vétérinaire Fédéral).

In the following 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 immunisation procedures, Ab-titre 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 bio-medical diagnostic is well documented by an increasing quantity of literature and is therefore not included in this review.

Advantages of IgY-technology

The most important aim of animal welfare is the reduction of painful manipulations. IgY technology fulfils this requirement, since chicken Abs can be easily sampled by a non-invasive method based on the simple action of egg collection, instead of the stressful bleeding of animals to obtain serum. IgY technology also offers outstanding economical advantages because the costs for hen keeping are lower than those for rabbits. Furthermore, the Ab production of a hen roughly corresponds to that of a large mammal, such as a sheep or a goat. Thus, an extraordinary amount of Ab can be produced from only one hen, approximately 17–35g 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 applied to 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, 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 immunised with the same mammalian antigen, very often the chickens respond with an Ab-specificity that can rarely be achieved in rabbits, as for instance, PIINP (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 recognised by many authors (for example Lösch et al. 1986, Carlander 2002, Narat 2003, Karlsson et al. 2004).

Applications of IgY in biomedical research and in human and veterinary medicine

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 new context of the so-called “functional food”.

IgY for therapeutical or prophylactic use in veterinary medicine

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), Löscher (Wiedemann et al. 1991), Erhard (Erhard et al. 1996), and Kuroki (Kuroki et al. 1997) have mostly performed the studies on the practical use of IgY (but see also Terzolo et al. 2003). These studies confirmed that the treatment of diarrhoea 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 binding of specific IgY to bacterial surface antigens, which caused significant changes in the bacterial surface structure. Another effect of IgY binding 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, minimising the risk of bacteria developing antibiotic resistance.

IgY for therapeutical or prophylactic use in human medicine

1 Treatment of intestinal infections in children

The adherence ability of many viral and bacterial pathogens is a major prerequisite for the successful colonisation 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.

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 a prophylactic reagent 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 minimises the use of antibiotics. Horie et al. (2004) carried out a study with 42 volunteers to test the protective effect of a drinking yogurt fortified with anti *H. pylori* urease IgY, obtaining a significant decrease in urea breath values of the treated group (fed with IgY-yogurt).

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 tumour 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, Pennsylvania, USA). Sunwoo and Sim (2004) reported on the use of IgY Ab against dietary gluten proteins which play a role in the autoimmune disorder of the celiac disease. The authors immunised 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.

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 (70ml, 0.7mg/ml IgY), given as a mouth rinse in the evening. A high level of the specific chicken Abs could be demonstrated in the saliva via an ELISA, for approximately 8 hours after the treatment. Later, the IgY concentration 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).

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 immunisation 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-immunised 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.

6 Use of IgY as tool in context of bioterrorism

To test the therapeutic use of IgY Abs LeClaire and colleagues (2002) produced IgY Abs against the highly toxic staphylococcal enterotoxin B (SEB). SEB is considered to be a potential biological warfare agent. Therefore, it exist an increasing necessity 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.

7 IgY as a tool in proteomics

A new and an interesting field of the use of the IgY-technology is the proteomic analysis. A problem in separation of complex protein mixtures by 2D-electrophoresis is the predominance of high-abundant proteins like albumin which disturb the monitoring of low-abundant proteins. Low-abundant proteins can be of high importance for identification and monitoring of several human (and animal) diseases. Recently, it has been shown that IgY Abs directed against these high-abundant proteins are in fact useful tools for their removal. In addition, these Abs work more specific than matrices with affinity to albumin like for example blue sepharose (Hinerfeld et al. 2004, Ahmed and Rice 2005, Huang et al. 2005).

Conclusions and future Prospects

Today, there is no doubt that chicken Abs can be produced and used, with minor modifications, in similar ways 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 (but 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 immunisation of chickens or dugs 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 future. In particular, due to the increasing resistance of microorganisms to antibiotics, research on all aspects related to the development of specific IgY against pathogenic microorganisms will have to be intensified. In 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 to science, to medicine and to the society as a whole.

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