Egg yolk antibodies (IgY) and its relevance in animal and human health-An updated review

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Abstract

Egg yolk represents an alternate source of antibody and provides some advantages over mammalian serum immunoglobulins. The principal immunoglobulin in avian blood is Immunoglobulin Y (IgY) and is transferred to egg yolk and then to their offspring and thus makes it possible for the non-invasive harvesting of antibodies in higher amount from eggs. Moreover, due to the structural differences and phylogenetic variations, IgY is more acceptable than mammalian antibodies for diagnostic purposes, as it does not interfere with mammalian antibodies and shows greater avidity for proteins retained in mammals. As a therapeutic and diagnostic tool, IgY has wide application in animal and human health. The emergence of drug resistance organisms causing respiratory tract infections is a critical problem for the health care system worldwide. Passive immunization is a potential alternative for this. Recent studies on the diagnosis and therapeutic effects of IgY against SARS-CoV-2 indicates the importance of this technique in the present context. This review refers to the use of IgY antibodies from chicken egg yolks against the infection caused by bacteria, viruses (including SARS-CoV-2) and parasites in human beings and in animals in addition to its use in food preservation, anti tumour and anti allergic activities.

Keywords: Egg yolk antibodies (IgY); human health; SARS-CoV-2; Egg yolk

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Introduction

IgY-technology (the production and extraction of specific IgY antibodies from egg yolk) is a novel method to produce antibodies for therapy and prophylaxis (Leiva et al., 2020). Protein molecules produced by the body in response to an antigen are called antibodies and are widely used in therapy, diagnosis and research due to their unique ability to bind with the precise targets. In the current scenario major chunk of available antibodies produced are of mammalian origin, especially from small rodents (Michael et al., 2010). One of the major disadvantages in using lab animals for the production of antibodies is the pain and suffering of animals during the procedures such as immunization, blood collection and sacrifice (Narat et al., 2003). Here comes the importance of noninvasive method like the usage of eggs, from which not only produce more amount of antibodies (IgY) with higher specificity compared to lab animals like rodents but also eliminates the ethical issues. (Narat et al., 2003, Schade et al., 1996).

In hens, IgY is transferred from blood and accumulates in egg (yolk) and then to their offspring. Hens are capable of producing 100 to 150 mg of IgY antibodies per yolk, in which 1 to 10% are specific IgYs in nature (Michael et al., 2010). In an year, a normal hen produces an average of 300 eggs which yield approximately 18 to 25 g of IgY antibody (Pauly et al., 2011). While coming to the cost of maintenance, a hen is less expensive to maintain than lab animals like rabbits and mice. From the point of animal and human health, IgY antibodies are used in immunotherapy (Rahman et al., 2013), immunodiagnostics (Cai et al., 2012), as a functional food (Horie et al., 2004), in neutralization of bacterial toxins (LeClaire et al., 2002) and venom of animals (Mendoza et al., 2012). Fc portion of IgY immunoglobulin is not capable of activating the complement system in humans (Larsson et al., 1992) by binding with protein G (Akerstrom et al., 1985) and to the rheumatoid factor (Larsson et al., 1991). IgY is more suitable for diagnosis as it displays greater avidity for mammalian conserved proteins and due to phylogenetic distance and structural differences when compared with mammalian antibodies (Gassmann et al., 1993).

Production of IgY immunoglobulin

Single antigens (Nucleic acids, polysaccharides, proteins) and complex type antigen such as bacterial,

virus, and parasite are being used to produce specific IgY in birds (Chalghoumi et al., 2009). In hens of seven to eight weeks of age, 10-100 µg (per ml) of antigen is injected in two to three sites (Michael et al., 2010) by intramuscular (I/M) route, usually in the breast muscle and leg muscle is usually avoided as it causes lameness (Schade et al., 1996). Oral administration of antigen can also be performed (Thibodeau et al., 2017). Different antigen concentrations can be combined with adjuvants as the induction of high antibody titer depends on the use of adjuvants and Freund's Complete Adjuvant (FCA) is commonly used due to its greater potential to induce antibodies in laboratory animals though it can cause severe inflammation at the site of injection (Chalghoumi et al., 2009). The titer of antibodies are influenced by a number of other factors also, like the type of antigen and dose, mode of application, frequency of inoculation, age and development stage of birds (Schade et al., 2005, Chalghoumi et al., 2009) and the higher titer can be maintained by booster inoculations for up to 150 days (Meenatchisundaram et al., 2011).

Inoculation

Minimum of two inoculations should be performed at an interval of four to six weeks before the period of egg laying. After 14 days of last immunization, IgY titer must be assessed. If the antibody titer is found to be decreasing more immunizations should be done during the egg laying period to increase titers (Schade et al., 1996). The number of injections required depends up on the dose and type of the antigen, and the adjuvant used. Highly purified IgY can be obtained by removal of lipid components from egg yolk, followed by precipitation of IgY with ammonium sulfate (NH₄)₂SO₄ (20%) (Araújo et al., 2010) supplemented by sodium chloride (NaCl) and addition or by ultra-filtration technique prior to ion exchange chromatography or gel filtration. Ethanol precipitation method can also be used at lower temperatures for purification of Immunoglobulin (Akita et al., 1992).

Applications of IgY in Therapeutics and prophylactics

The main advantage of IgY polyclonal antibody against infectious diseases is that it minimizes the risk of microbial resistance since it requires multiple

Figure 1: Production and application of IgY antibodies

genes for its synthesis (Rahman et al., 2013). IgY have shown antibacterial activity against *Salmonella typhimurium* (Li et al., 2016), *C. difficile* infection, (Pizarro-Guajardo et al., 2017), *H. pylori* (Malekshahi et al., 2011) and it has therapeutic effect against acne caused by *Propionibacterium acnes* (Revathy et al., 2017). IgY shows greater efficacy against bovine group A Rotavirus (RVA) (Oral administration) and against dengue fever, (Fink et al., 2017). Nasal, oral or spray applications of IgY are used against avian influenza A virus (H5N1) strain are found effective (Wallach et al., 2011).

IgY obtained from geese eggs were found to be effective against Andes virus (ANDV) (the etiological agent of the Hantavirus Pulmonary Syndrome (HPS)). They have prophylactic activity against *Candida albicans*, in elder peoples an oral gel preparation of IgY caused a reduction in the number of colony-forming units (CFU) on the oral cavity showing promise for prophylactic use against *C. albicans* oral infection (Tekeuchi et al., 2014). Sampaio et al., in 2014 found out that anti-*T. evansi* IgY when used in tandem with anti-hematozoa drugs increased the survival rates in animals. The antibodies are also shown anti tumor activity by inducing apoptosis in human breast cancer cells (Amirijavidv et al., 2016). IgY shows anti-obesity activity by inhibiting the dietary fat hydrolysis and thus reducing its intestinal absorption (Hirose et al., 2013).

Anti-allergic effect of specific IgY against the cytokines IL- β 1 and TNF- α in guinea pigs after inducing allergic rhinitis showed encouraging results (Wei-xu et al., 2016). Advantage of IgY as an antivenom over IgG is that it is easily and sufficiently purified and the absence of other serum proteins that would minimize the side effects due to nonspecific proteins. (Araujo et al., 2010, Sjostrom et al., 1994).

Applications of IgY in diagnosis

Fc portion of IgG react with the protein A of *Staphylococcus aureus* whereas the Fc portion of IgY does not react with protein A due to structural differences which makes IgY a relevant resource for more specific detection of different *S. aureus* strains and the toxins (Richman et al., 1982) and it was confirmed by high titer of IgY in ELISA and western blot technique (Walczak et al., 2016). Among the parasitic infections, the ability of IgY to detect the protozoan *Toxoplasma gondii* was checked by the development of IgY against the surface protein SAG1, which responded to the target antigen in ELISA and Western blot. (Cakir-Koc et al., 2015) Several authors have been investigating the potential of IgY in the detection of tumor markers. IgY against the antigenic peptide CA 15-3, is a widely used marker for breast cancer, and is used as a secondary antibody in sandwich ELISA for detecting CA 15-3 (Grzywa et al., 2014).

Against SARS- CoV-2

IgY immunoglobulin have excellent pathogen-neutralization in the respiratory tract and lungs, and has shown its efficacy to treat and prevent respiratory infections (Abbas et al., 2018). Overall significance for the use of monoclonal chicken egg yolk antibodies (IgY) using phage display method describes their potential passive immunotherapeutic application for the treatment and prevention of SARS CoV-2 infection, an approach that is easy, quick and safe to effectively treat patients. When compared to polyclonal antibodies, chicken monoclonal IgY antibodies proved to be more specific in the identification of a single unique epitope. Chicken scFv IgY antibodies produced against SARS CoV-2 spike protein (S) using phage display technology are expected to be a potential candidate for the development of antibody-based vaccines as an immunotherapy agent model for efficient mass production of high-affinity monoclonal IgY antibodies for the treatment of SARS CoV-2 spike protein (S) with standardized preparation for long-term effective use (Abbas et al., 2018).

As an agent for preservation of food

IgY produced against the *Listeria monocytogenes* showed an important inhibitory effect on the bacterial growth in the liquid medium and in the fish samples processed between 0°C and 6°C in a dose-dependent manner which showed that anti-L. IgY *monocytogenesis* is a possible antimicrobial agent and can be used in the food industry (Sui et al., 1946).

Protection against bioterrorism agent

IgY produced against the Staphylococcal enterotoxin is capable of saving people exposed to the substance B(SEB) which is a possible biological weapon. The findings with Rhesus monkeys showed the animals received anti-SEB IgY 30 min before or 4 h after lethal exposure to SEB aerosol survived (LeClair et al., 2002).

Conclusion

The benefits of IgY includes, lack of reaction to mammalian Fc receptors, low cost of development and ease of extraction. Compared to IgGs in mammals, they have higher specificity of the target and higher avidity of binding. To obtain IgY from egg yolk, several extraction methods are available and the choice of the

appropriate method depends on the purpose, which may require different degrees of purification, as well as the extraction size, cost and available technology. Besides, in view of its proven capacity to neutralize microorganisms, it is a valuable therapeutic resource which can be used in the case of antibiotic resistance and against the development of viral diseases for which there is very less number of anti-viral agents.

Authorship contribution statement

M.S. Sivaprasad: Conceptualization, Writing - Original draft, Writing - review & editing

V.K. Vinod: Writing - review & editing

K.S. Jisna: Figure designing, Writing - review & editing

Prasanth M. Nair: Writing - review & editing

Neha Parmar: Writing - review & editing

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Declaration of Competing Interest

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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