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Egg Yolk Antibody and Its Application

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Abstract A hen transfers her serum immunoglobulin G to the egg yolk (IgY) and gives immunity to her offspring Therefore, the hen egg can be an effective supplier of a large amount of antigen specific antibody that accumulates in the egg yolk. Antigen specific antibody has been widely used for immunological analysis in the field of diagnosis as well as pure scientific research The production and separation technology of IgY is demonstrated in the present study

Keywords: hen egg, yolk antibody, IgG, IgY, rotavirus, *Streptococcus mutans*, *Helicobacter pylori*

INTRODUCTION

A hen egg is the largest biological cell known that originates from one cell division [1] and is composed of various important chemical substances that form the basis of life. In other words, a hen egg is a storehouse of variable nutrients, protein, lipids, carbohydrates and other biologically active substances, including growth-promoting factors that are needed to produce a chick. In fact, when a fertilized egg is kept at 37°C in a humid environment for 21 days, the egg hatches However, this optimum environment for hatching also is the optimum environment for the growth of bacteria and viruses Therefore, the hen egg is facilitated with an effective protection system against infection from these microbes

Not all the phylaxis system has been revealed. We at least know that lysozyme, ovotransferrin, avidin, ovomucin and cystatin in egg white as well as phosbitin and yolk immunoglobulin in egg yolk contribute to anti-infection, and show anti-bacterial and anti-viral activities. Other important roles of these chemicals in the hen egg include immunopotentiation activity, metal ion trap and vitamin binding activity. In the field of immunology, egg yolk antibody became the object of attention. A recent study showed that the antibody in the egg yolk of immunized hens provides passive immunity to those who take the antibody orally. This oral passive immunization is a way of preventing intraoral and gastrointestinal infection of pathogens by taking antibody that combines pathogens to diminish infectiousiousness against the specific pathogens of the pathogens. This paper introduces egg yolk antibody (IgY, Yolk Immunoglobulin) and oral passive immunity by using IgY.

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SPECIFIC ANTIBODY AND ITS PREPARATION

Egg Antibody and Blood Antibody

Antibodies sometimes have been referred to immunoglobulins (Igs), and are found in humoral fluids (blood, saliva, milk, etc.) of the vertebrates. Five classes of immunoglobulins (IgG, IgA, IgM, IgD, and IgE) are known in humans, which are distinguishable in the structure and immunological function Animals produce Igs against almost all kinds of antigens (bacteria, virus, and foreign proteins, etc.) in their humor to defend against the invaded antigen. This humoral immunity is a biological defense system established in animals evolutionally; a specific immunoglobulin produced binds with its specific antigen to neutralize antigenicity.

In birds, three kinds of Immunoglobulins (IgG, IgA, and IgM) have been found in their circulating bloods. The hen transfers the immunoglobulins to her eggs. Hen's serum IgA and IgM are secreted together with other proteins to be the component of egg white at the oviduct, while serum IgG is specifically transferred through the yolk membrane into the yolk during its maturation. For instance, a receptor specific to IgG translocation is known to exist on the surface of the yolk membrane. It has been suggested that Igs in eggs are passive immunity, in a sense, because the antibodies in the egg originated from the mother hen are used to protect the new offspring from various infectious diseases In fact, IgG found in the egg yolk circulates in the blood and IgA and IgM, in the digestive tract of the hatched chick. The antibodies transferred from the hen to the chick via the latent stage of egg has thus an important immunological meaning for the newly developed chick in defusing various infectious diseases. This system of transfer of the antibodies to the offspring via biological latent form of eggs in birds is considered to be the same as the transfer of maternal immunity from a

mother to the neonate via her placenta in mammals

There have been several reports regarding the structural difference between mammalian IgG and yolk IgG as mentioned below:

1. The molecular weight of yolk IgG (1.8×10^4) is larger than that of mammalian IgG (1.5×10^4) [2].
2. The isoelectric point of yolk IgG is lower than that of mammalian IgG [2].
3. The binding of yolk IgG with Fc receptor on cell surface is lesser as compared to mammalian IgG [3].
4. Yolk IgG does not associate with mammalian compliments [3].
5. Yolk IgG does not bind to *Staphylococcus* protein A [4] or rheumatoid factor in blood, unlike mammalian IgG [5].
6. The whole structure of various sugar chains of yolk IgG indicates that there exists a unique structure in which glucose is conjugated at the non-reducing side of certain type of sugar chains, unlike mammalian IgG [6].

Based on these findings above, yolk IgG is specially called IgY.

Comparison of Preparation Methods of Specific Antibody

An antigen-specific IgG has been conventionally isolated from sera of animals such as rabbits, goats and sheep superimmunized with an aimed antigen. IgY can also be obtained from the egg yolk laid by a superimmunized hen. The preparation methods of IgG and IgY are comparatively shown in Fig. 1.

There are several advantages of the avian system for antibody production over that of mammalian.

IgY production from eggs is easier than that of IgG from serum. More than 100 mg of IgY can be isolated from one egg. An industrial scale production of IgY is possible because of the availability of a lager number of chicken farms and automation of egg breaking and processing. On the other hand, a complicated process maybe needed to obtain a huge amount of blood from immunized animals. Collecting eggs from laying hens does not require the bleeding of animals for antibody production, especially fitting the current regulation regarding animal use. Systematic immunization of hens has been done in farms as compared to other animals. Immunized hens can produce some of the antibodies that are not possible to be produced in mammals.

Several papers have been published on a comparative study of the productivity of antibodies through hen eggs and rabbit sera. Jensenius and his colleagues estimated that the total antibody activity of the eggs laid by a hen in a month is equivalent to that produced in a half liter of serum from an immunized rabbit [7]. Gottstein and Hemmeler compared the antibody production efficiency for *Echinococcus granulosus* as an antigen [8]. They reported that the quantity of IgY obtained from eggs laid by an immunized hen was 18 times greater than that of IgG isolated from the serum of an immunized rabbit. We also compared the productivity of IgY from the eggs laid by a hen over a year with that of IgG from the entire serum of a rabbit in which both animals were immunized with the same antigens. The results are summarized in Table 1.

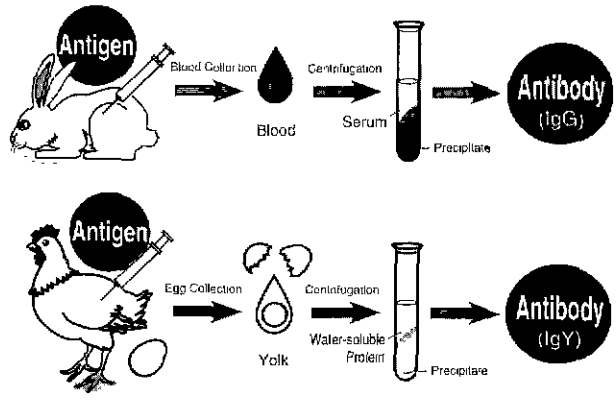

Figure 1 Preparation of specific antibodies

Table 1. Productivity of IgY and IgG

Immunized Animal	Rabbit	Hen
Source of antibody	Blood serum	Egg yolk
Kind of antibody	Polyclonal	Polyclonal
Quantity of antibody	*1.4 g/rabbit*	*40 g/hen*
(Quantity of specific antibody)		
Anti-HRV (MO) antibody	5.6×10^6 NT	600×10^6 NT
Anti-HRV (Wa) antibody	3.78×10^6 NT	520×10^6 NT
Anti-Mouse IgG antibody	0.7 g	11.2 g
Anti-Insulin antibody	0 g	2 g

NT: Nutralization titer HRV: Human rotavirus

EGG YOLK IMMUNOGLOBULIN (IgY) AND ORAL PASSIVE IMMUNITY

Prevention of Rotaviral Diarrhea

Rotavirus is a major pathogen of infectious gastroenteritis in infant and is now the leading cause of severe diarrhea in infants and young children [9,10]. It has been presumed that more than 2 million infant deaths annually are due to HRV diarrhea, mainly in the developing countries. Despite the continuous effort of the World Health Organization (WHO) in producing the vaccine against human rotavirus, no effective vaccine has been developed due to the facts that immunity, immunological response of infants is not fully established, and that human rotavirus (HRV) infection is characteristically localized to epithelial cells of the intestinal tract.

Prevention of HRV infectious disease by oral administration of the active antibody (passive immunization therapy) may be a promising application of anti-HRV antibody. Oral passive immunization has thus been investigated as an alternative to vaccination for prevention of HRV infectious disease. Several researchers demonstrated that oral administration of antirotavirus IgG or IgY is effective in controlling rotaviral diarrhea using several animal models [11-13]. Our experiment showed that immunizing hens with HRV (Wa and MO strains) results in bringing high levels of titer in the hen egg yolk. A high level of antibody titer is usually observed in any egg laid over a year after immunization Fig. 2 [14].

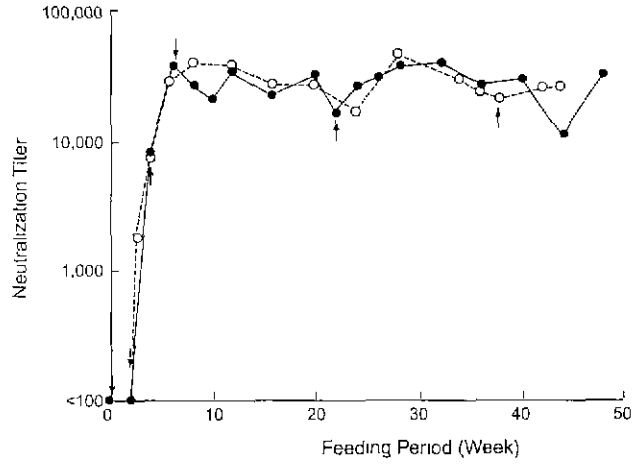

Figure 2. Change in neutralization titer against HRV Wa (●) and MO (○) strain of egg yolk from eggs laid after immunization

We immunized hens with inactivated HRV antigen, and anti-rotavirus IgY was purified from the eggs laid by the immunized hens The productivity of anti-HRV IgY from a hen, is 120 times greater than that from a rabbit as estimated per a head of animal. We also established an infection-test system to inoculate suckling mice with HRV by oral administration. Suckling mice were inoculated with HRV MO strain (3.5×10^7 FCFU/mouse) at 1, 3, 9, and 24 h after oral administration of anti-HRV (MO) IgY (225 mg IgY/mouse) No incidents of diarrhea were observed in all the mice that were administered IgY 1 h before HRV inoculation However, the mice administered IgY at 3, 9, and 24 h before HRV inoculation suffered from diarrhea at the incidence of 27.3, 41 7, and 93.3%, respectively. In the positive control group, the incidence of diarrhea was 83.3% (Fig 3) In our investigation, the preventive effect from HRV diarrhea in the suckling mice was found to decrease as the time gap between IgY administration and HRV inoculation was lengthened. This result indicates that the effectiveness of IgY for the prevention of HRV diarrhea depends greatly on the time of IgY administration before HRV infection [15]. The fact that a previous supply of IgY (22.5 μg/ mouse, 1 h before HRV infection) completely prevented HRV-induced diarrhea suggests that a sufficient amount of HRV-specific IgY for 5 to 6 children can be obtained from one immunized hen egg. This technology can be applied to prevent rotavirus infection of livestock (cattle and pigs), and the development of the feed and animal medicine containing the antibody is now in progress.

Prevention of Tooth Decay

Streptococcus mutans is thought to be the major causative bacterium of dental caries in humans [16] *Streptococcus mutans* has an enzyme called glucosyltransferase that produces cohesive glucans on the surface of the bacterium. These cohesive glucans make the bacteria adhere to the tooth surface, forming plaque. Tooth cavity is caused by lactic acid produced from *Lactobacilli* inside the plaque.

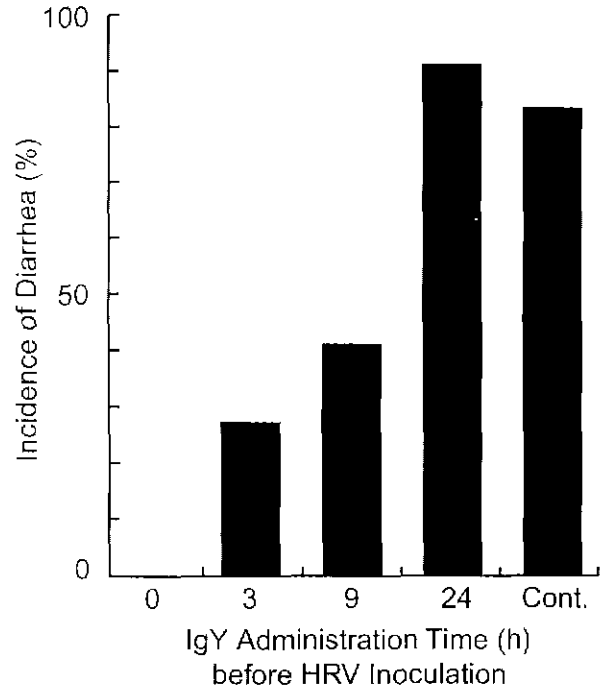

Figure 3. Passive immunization effect of anti-HRV IgY administered to suckling mice before inoculation of HRV

We prepared IgY against *Streptococcus mutans* serotype c, the main causative bacterium of dental caries in humans. An experimental rat caries model was used to test the cariogenic prevention effect of anti-*S. mutans* IgY Specific pathogen free rats infected with *S. mutans* which were fed a cariogenic diet containing immune yolk powder showed significantly lesser caries scores than those infected with the same strain and fed with a diet containing control (non-immune) yolk powder [17]

We also studied the effect of a mouthwash containing anti- *S. mutans* IgY against dental plaque formation in humans Saliva samples before mouth rinse and at 4 h after the first rinse were collected. Mouth rinse containing sucrose and immune IgY resulted in a significant decrease in the ratio of *S. mutans* per the total number of *Streptococci* in the saliva of all the volunteers (Fig. 4) [18] Therefore, the prevention of tooth decay is made possible by the administration of IgY in which IgY may have prevented *S. mutans* from adhering to the tooth surface.

Other Application of IgY

We have prepared a variety of antigen-specific IgY such as insulin, glutathione peroxidase and C reactive protein (CRP), which have poor antigenicity among mammals. Recently, we also demonstrated that IgY is an effective antibody as a functional food ingredient for gastroduodenal ulcer disease.

Helicobacter pylori was found in 1984 by Marshal and Warren [19] and is now known as a human pathogen associated with the development of gastric and

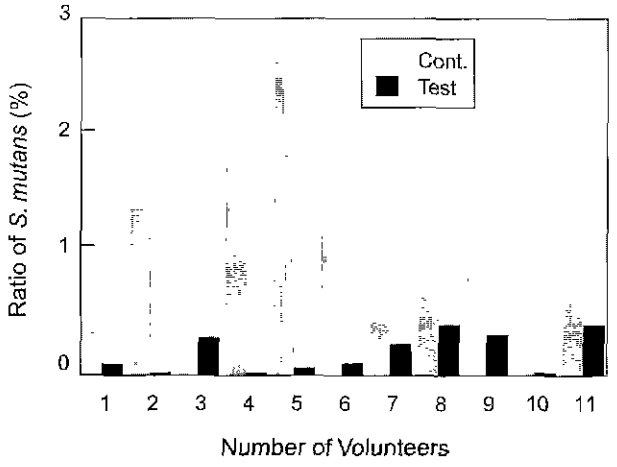

Figure 4. Changes in ratio of *S. mutans* (%) in saliva of 11 volunteers after mouth rinse test using sucrose solution containing IgY or control IgY

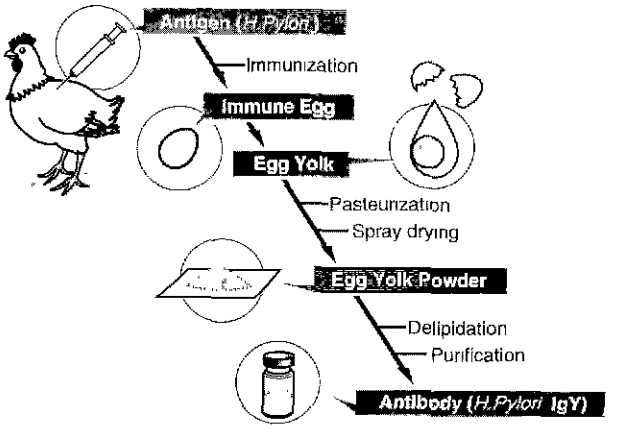

Figure 5. Purification of IgY against *H pylori*.

duodenal ulcers and gastric adenocarcimona Prevention of *H. pylori* infectious disease by oral administration of IgY may be a promising application of anti- *H. pylori* IgY In 1991, we demonstrated that IgY was effective against *H pylori* in that it inhibits *H pylori* from adhering to gastric mucasa *in vitro* [20]. One experiment showed that immunization of hens with *H pylori* results in bringing high levels of titer in the egg yolk (Fig 5). A high level of antibody titer is usually observed in an egg laid over a year after immunization. The anti- *H. pylori* IgY was purified, and clinical test was performed using 60 human volunteers who were proved to be positive to the *H. pylori* test with the Heath Examinator that indicated the effectiveness Massive evaluation system regarding this project has been undertaken.

In a sense, hens lay a 'golden egg' filled with specific antibodies almost every day. Focusing on this, the studies on IgY have been conducted extensively around the world. Among them, Japan is one of the most active countries in the research and development of IgY. The research groups aiming application of IgY are summarized in Table 2

Table 2. Major research groups of IgY

Country	Research Group	Application Field	Antigen
Japan	Osaka University	Food	*Streptococcus mutans*
	Ghen Corporation	Feed (cow)	Bovine rotavirus
		Feed (pig)	*Escherichia coli*
	Taiyo Kagaku	Food	*Streptococcus mutans*
	Kanebo Cosmetics	Cosmetics	*Propionibacterium acnes*
	Kyodo Nyugyo	Food	*Helicobacter pylori*
	Pharma Foods International	Food	*Helicobacter pylori*
	Kyoto Women's University	Diagnostic kit	Mouse monoclonal antibody
	Kao	Cosmetics	Split hair
USA	DCV	Food	Multi-bacteria
	MAC Associates	Feed (cow)	*Staphylococcus aureus*
Canada	University of Guelph	Feed (cow)	*Salmonella enteritidis*
	University of British Columbia	Food	Bovine milk IgG
	University of Manitoba	Feed (pig)	*Escherichia coli*
Sweden	Uppsala University	Medical	*Pseudomonas aeruginosa*
	Huddinge Hospital	Medical	Human rotavirus
Korea	Ildong Parmaceutical	Medical	*Helicobacter pylori*
	Doosan	Cosmetics	Propionibacterium acnes

CONCLUSION

Hens transfer blood serum immunoglobulin G which accumulate in the egg yolk during oogenesis. The antigen (pathogen)-specific antibody can be obtained in large quantities from eggs laid by hyper immunized hens. Considering the fact that a tremendous number of hens is immunized to protect them from several avian diseases and managed to lay eggs systematically, it is possible to apply IgY for passive immunization therapy. We have industrialized the manufacturing technology for the immunologically functional proteins of egg yolk. The higher productivity and mass production in the industrial scale strongly suggest the possibility of realizing the use of IgY to prevent infectious diseases through oral administration.

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