

# TECHNICAL LITERATURE

## IMMUNOGLOBULIN Y (IgY): Summary of Human Studies

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### INTRODUCTION

#### KEY CONCLUSIONS

- IgY antibodies from egg yolk are the functional equivalent to IgG found in human serum, placenta and colostrum.
- Only 2-10% of the IgY found in immunized hens is antigen-specific, therefore, gram quantities, unpurified or expensive IgY preparations have been used until now.
- New proprietary technology from IGY Immune Technologies & Life Sciences produces, cost effective, commercial quantities of IgY isolate at very high purities.
- Numerous human clinicals using antigen-specific IgY have shown promise in enhancing the immune system's response to infections.
- Clinical studies have been initiated by IGY Immune Technologies & Life Sciences to demonstrate the benefits of IgY isolate on the immune function and inflammatory processes of healthy individuals.

The vertebrate immune system's primary goal is to protect the individual against microorganisms: viruses, fungus, bacteria and parasites. It has been divided in two major parts, one being non-specific and innate and the other specific and acquired. The acquired immune system uses two different approaches: the humoral immune response and cellular immune response. The recognition factors of the humoral immune response are immunoglobulins (antibodies), soluble proteins synthesized in response to foreign substances (antigens), which are secreted by plasma cells derived from B lymphocytes[1]. In humans, there are five classes of immunoglobulins: IgG, IgA, IgM, IgD and IgE. Chickens produce three classes: IgY, IgA and IgM. IgG and IgY are the main antibodies found in mammal and avian's serum, respectively. Although structurally different, both immunoglobulins are functionally equivalent[3].

One major difference between the mammal and the avian immune systems is the mechanism by which these animals provide passive immunity to their offspring. In mammal, IgG reaches the fetus/newborn through the placenta or colostrum. While in avian, IgY antibodies are transferred, selectively, by receptors from the hen's blood to the yolk[5]. A single egg yolk contains approximately the same amount of IgY as 30 ml of blood[9]. The typical annual production of IgY from one hen can be approximately 20-40 g, with very low day-to-day variation reported from individual laying hens[9, 10]. This huge quantity of consistent IgY antibodies and their ability to bind antigens with high specificity and affinity have led to their broad use in scientific and medicine disciplines. Furthermore, advances on production and purification, the lack of cross-reactivity with human rheumatoid factors[13], and their inability to activate the mammalian complement system, have open new opportunities for antigen-specific IgY in the treatment of bacterial, fungi, protozoa and viral infections in humans[7].

To stimulate the production of antigen-specific IgY in a hen, the desired antigens are injected in the form of a vaccine containing an adjuvant. The latter are non-specific B-cell stimulating immune modulators which improve the immune response to an antigen[3]. The most common site of injection is intramuscular. Although, the amount of IgY in the egg can be affected by various factors, such as age, breed of the chicken, antigen, adjuvant and injection route, IgY yields from 60 to 150 mg per egg have been reported. Interestingly, only 2-10 % of the IgY in an immunized hen has been shown to be antigen-specific[5].

Numerous studies have been conducted with oral and topical applications of antigen-specific IgY in humans[18]. A summary of published clinicals will be briefly discussed below. Many of these studies required either grams (4.5-13.5 g) of egg-yolk powder or expensive IgY preparations. Through a patented technology, IGY Immune Technologies & Life Sciences, Inc., is now capable of producing commercial relevant (kilograms) quantities of egg-based products with up to 95% IgY purity.

## PUBLISHED HUMAN CLINICALS

### Respiratory Care

#### **Good effect of IgY against *Pseudomonas aeruginosa* infections in cystic fibrosis patients[2]**

Researchers in Sweden continued an open study on 17 Swedish patients with cystic fibrosis (CF). The patients were instructed to gargle with a 70 ml solution containing approximately 50 mg of IgY (equivalent to the normal IgY amount present in half an egg) against two *P. aeruginosa* strains for two minutes, every evening, for up to 12 years. Twenty-three Danish CF patients served as control.

**Results:** There was a statistical reduction ( $P = 0.028$ ) on positive cultures for *P. aeruginosa* on the treated versus the control groups. In the IgY treated group only two patients (12%) became chronically colonized with *P. aeruginosa* compared to seven patients (30%) in the control group. The IgY group had better pulmonary function than the control group. These results suggest that Anti-*Pseudomonas* IgY has great potential to prevent *P. aeruginosa* infections.

#### **Oral administration of specific yolk antibodies (IgY) may prevent *Pseudomonas aeruginosa* infections in patients with cystic fibrosis: a phase I feasibility study[4]**

Thirty-eight patients with cystic fibrosis (CF), who had the first colonization with *P. aeruginosa* eradicated with antibiotics, were divided into two groups. The treated group ( $n=17$ ) gargled daily for two minutes with an IgY-antibody preparation (50 mg/day, equivalent to the normal IgY amount present in half an egg), purified from eggs of hens immunized with *P. aeruginosa* bacteria. The control group ( $n=21$ ) did not gargle with the preparation.

**Results:** The study showed that the period between the first and second colonization with *P. aeruginosa* was significantly ( $P = 0.015$ ) prolonged for the treated vs. the control group. Once colonization took place, the treated group had only 2.5 sputum cultures positive for *P. aeruginosa* per 100 months of observation versus 13.7 in the control group. Furthermore, five (24%) patients became chronically colonized with *P. aeruginosa* in the control group while none of the treated patients were chronically colonized. No adverse events were reported.

#### **Immunoglobulin IgY pseudomonas A clinical trial for cystic fibrosis treatment (IMPACTT)[6]**

The results from Phase I and II clinical studies proved that daily gargles with Anti-*Pseudomonas* IgY solution significantly improved CF patients. Therefore, the European Commission has granted Swedish researchers (Dr. Anders Larsson and collaborators) with over 5 million Euros to conduct a Phase III Clinical Trials called IMPACTT. This study is in progress.

### Oral Care

#### **Retention of specific yolk IgY in the human oral cavity[7 8].**

Carlander (2002) studied the presence of IgY antibodies in the saliva of healthy subjects treated with *P. aeruginosa*-specific IgY. The individuals rinsed and gargled with 70 ml of the IgY solution (0.7 mg/ml concentration) for one or two minutes and then swallowed. The saliva was collected immediately, 8 hours and 24 hours after the treatment and analyzed using Enzyme-linked Immunosorbent assay (ELISA).

**Results:** The tests showed the presence of active antibodies in the saliva from almost all subjects the next morning. Active antibodies were detected in saliva from only a few of the subjects after 24 hours. Individuals who rinsed for two minutes had higher residual active levels. The results suggest that the antibodies may bind to the bacteria and probably prevent adhesion of bacteria to the oral cavity.

#### **Effects of egg yolk antibody against *Porphyromonas gingivalis* gingipains in periodontitis patients[11]**

Five patients with detectable levels of *P. gingivalis*, visible signs of gingivitis and bleeding on probing (BOP) were enrolled in the study. Five pairs of contralateral anterior single-root teeth were selected per individual and randomly treated with anti-*P. gingivalis* egg yolk antibody (IgY-GP) and subgingival scaling and root planing. The control teeth were only treated with root planing. Approximately 30-60 mg of 20% IgY-GP ointment was used for each periodontal pocket.

**Results:** In the IgY-GP group, there was a significant reduction ( $P<0.05\%$ ) in the mean probing depth four weeks after the treatment. The BOP was significantly decreased in the IgY-GP group compared to the control group at week 4. The number of *P. gingivalis* bacteria significantly increased ( $P<0.05\%$ ) in the control group. These results suggest that the use of antigen-specific IgY preparation may be effective in the management of periodontitis.

### **Anti-cell-associated glucosyltransferase immunoglobulin Y suppression of salivary mutans streptococci in healthy young adults (Abstract) [12]**

In this study, young healthy adults self-administered lozenges containing either anti-*Streptococcus mutans* cell-associated glucosyltransferase IgY (anti-CA-gtf IgY) or a placebo, each day, for five days.

**Results:** The levels of *S. mutans* in the treatment group decreased significantly ( $P < 0.001$ ) as compared to placebo, suggesting that daily intake of lozenges containing anti-CA-gtf IgY can suppress oral colonization by *S. mutans*. This is one of the few studies suggesting the benefit of antigen-specific IgY oral supplement for dental care.

### **Effects of topical application of immunoglobulin yolk on mutans streptococci in dental plaque (Abstract) [14]**

Twenty-four healthy adults were randomly assigned to either an anti-*Streptococcus mutans* IgY containing spray (treated group,  $n = 9$ ), a 0.15% chlorhexidine (antibiotic) spray (positive control,  $n = 9$ ) or phosphate buffered saline spray (negative control,  $n = 6$ ). All individuals used their treatments three times daily for three weeks. Pooled plaque samples from the smooth surfaces of teeth were collected before administration (baseline, day 0), during treatment (days 7, 14 and 21) and post treatment (days 28, 42 and 56) for microbial analysis. Plaque indices were also recorded.

**Results:** *S. mutans* numbers in the dental plaque was suppressed significantly following three weeks of treatment with IgY. Low counts of *S. mutans* persisted for at least 5 weeks post treatment with IgY. No changes in plaque index were observed. The results suggest that the immunization with specific immunoglobulin yolk against *S. mutans* could be helpful in reducing colonization in human dental plaque.

### **Oral treatment with yolk antibodies for the prevention of *C. albicans* infections in chemotherapy treated children. A feasibility study[15]**

In this open label study, eight patients diagnosed with acute lymphatic leukemia and treated with chemotherapy were assigned to prophylactic ( $n=4$ ) or control ( $n=4$ ) groups. The prophylactic group gargled with anti-*C. albicans* IgY (50 mg/day, equivalent to the normal IgY amount present in half an egg) and rinsed their mouth after tooth brushing every evening. The control group did not receive IgY. There was also an untreated historical group with nine patients.

**Results:** None of four patients treated with anti- *C. albicans* IgY got candidiasis. In the non-treated groups (control and historical), three out of four (3/4) control patients and four out of nine (4/9) historical patients had clinical signs of candidiasis and required antimycotic drugs. This feasibility study suggests the potential of antigen-specific IgY to prevent oral candidiasis in immune-compromised individuals.

## **Digestive Care**

### **Effect of dietary anti-*Helicobacter pylori*-urease immunoglobulin Y on *Helicobacter pylori* infection[16]**

Seventeen asymptomatic volunteers diagnosed as *H. pylori*-positive by the 13C-urea breath test (UBT) were enrolled in the study. An anti-*H. pylori* urease IgY (anti-HpU IgY) powder containing 900 mg of Ovalgen was taken three times per day, about 30 min after each meal, for four weeks.

**Results:** After the four weeks of treatment, 13C-urea breath test values were significantly decreased ( $P<0.01$ ) in 13 of the volunteers. However, none of the subjects showed *H. pylori* eradication. The results suggest that the use of anti-HpU IgY may provide a novel approach to the control of *H. pylori* infection.

### **Suppressive effect of functional drinking yogurt containing specific egg yolk immunoglobulin on *Helicobacter pylori* in humans[17]**

Forty-two volunteers who tested positive for *H. pylori* using a 13C-urea breath test were recruited. The subjects were divided randomly into a test group and a control group. The test group consumed one bottle (150 mL) of drinking yogurt containing 1.5 g of egg yolk IgY-urease, 3 times daily (45 mg of IgY-urease/d), for 4 weeks. The control group consumed IgY-urease-free yogurt of the same volume.

**Results:** The 13C-urea urea breath test values significantly decreased ( $P<0.001$ ) in the test group compared with the control group. Thus, indicating a suppression of *H. pylori* infection by the consumption of the drink fortified with IgY-urease. Furthermore, the researchers showed that it was possible to formulate a commercial drinking yogurt with IgY-urease, but reported a reduction of 15% in IgY-urease activity after three weeks of storage, which may be associated with a decrease in pH.

### **Randomized placebo-controlled clinical trial of immunoglobulin Y as adjunct to standard supportive therapy for rotavirus-associated diarrhea among pediatric patients (Abstract) [19]**

Fifty-four dehydrated and rotavirus-positive children were randomized into an Anti-Human Rotavirus IgY (Rotamix IgY) group and placebo IgY group. Only 52 children completed the study (placebo,  $n=22$ ; treated,  $n=22$ ). The patients were monitored for volume and duration of oral rehydration fluid (ORF), intravenous fluid (IVF) intake, daily stool frequency, overall duration of diarrhea, and duration of rotavirus shedding.

**Results:** Compared to the placebo group, the Rotamix IgY group had statistically significant reductions in mean ORF intake ( $p=0.004$ ), mean duration of intravenous fluid administration ( $p=0.03$ ), mean duration of diarrhea from day of admission ( $p<0.01$ ), and mean duration of rotavirus clearance from stool from day of admission ( $p=0.05$ ). The results from this study suggest that the use of antigen-specific IgY may be a safe and effective strategy to manage acute diarrhea in children.

### **Randomized, placebo-controlled, clinical trial of hyperimmunized chicken egg yolk immunoglobulin in children with rotavirus diarrhea (Abstract) [20]**

In this randomized, double-blind, placebo-controlled study, 79 children with known rotavirus diarrhea were assigned to receive either 10 g hyperimmune egg yolk (containing IgY immunized with human rotavirus strains, HEY group) or a similar preparation obtained from nonimmunized chicken (placebo group). Both groups took the preparations daily, in four equally divided doses, for four days. Daily stool frequency and amount, oral rehydration solution (ORS) intake, and presence of rotavirus in the stool were monitored for four days.

**Results:** In the HEY group, there were significant reductions in stool output ( $P = 0.03$ ) and ORS intake on day 1 ( $P = 0.008$ ) and greater virus clearance on day four ( $P = 0.02$ ) compared to placebo. However, there was no difference in diarrheal duration between the groups. These results suggest that's antigen-specific IgY may be effective in the treatment of rotavirus-induced diarrhea in children.

## **Sport Performance**

### **Increased muscular strength and enhanced muscle repair with hyperimmune egg protein supplementation[21]**

Twenty-four recreational active males were randomly assigned to either hyperimmune egg (HIE) or an egg protein placebo (PLA) group. The researches described the HIE as a powdered, pure egg derived from chicken immunized with more than 26 killed pathogens. The participants were supplemented with 4.5 g/d for 2 days, 9 g/day for 2 days and 13.5 g/day for 6 days of either HIE or PLA. On days 1, 8, and 10 participants performed one repetition maximum (1RM) testing using a flat bench press and parallel back squat, followed by maximal reps at 70% of the respective 1 RM for each exercise. Muscle soreness was assessed 24-hr after 1RM testing using 10 cm visual analog scale.

**Results:** The results showed significant ( $P<0.05$ ) increases in upper body muscular strength and endurance between HIE on Day 8 and Day 10 as compared to Day 1. No improvements were seen for PLA on Day 10 on the above endpoints. This study suggests that IgY may provide benefits on muscle performance and recovery in healthy individuals subjected to strenuous exercise.

### **Hyperimmune egg protein decreases submaximal heart rate and increases peak power[22]**

Twenty-four recreational active males were randomly assigned to either hyperimmune egg (HIE) or an egg protein placebo (PLA) group. The researches described the HIE as a powdered, pure egg derived from chicken immunized with more than 26 killed pathogens. The participants were supplemented with 4.5 g/d for 2 days, 9 g/day for 2 days and 13.5 g/day for 6 days of either HIE or PLA. On days 1, 8, and 10 participants performed five minutes submaximal exercise bouts on a treadmill at 0%, 3% and 6% grade at constant speed to measure heart rate (HR) changes. To determine peak anaerobic power, each subject performed a 30 sec Wingate test using 7.5% own body weight.

**Results:** Changes in HR from Day 1, at all submaximal intensities, was significantly lower ( $p<0.05$ ) between HIE and PLA on days 8 and 10. Peak power was significantly greater ( $p<0.05$ ) between HIE and PLA on day 8. The HIE group improved ( $P<0.05$ ) peak performance at day 10 compared to Day 1. This study suggests that IgY may provide benefits on muscle performance in healthy individuals subjected to strenuous exercise.

## **Cardiovascular Care and Inflammation**

### **Pilot study on the effect of hyperimmune egg protein on elevated cholesterol levels and cardiovascular risk factors[23]**

This double-blinded, placebo-controlled study was conducted with 44 volunteers from the US Army who exhibited elevated serum total cholesterol (180-290 mg/dl). The subjects were randomly assigned to three treatment groups: untreated (no drink), placebo (nutritional drink) and treated (nutritional drink with hyperimmune egg). All participants consumed their assigned products for 26 weeks and received dietary education.

**Results:** After 6 months of intervention, the placebo and untreated groups had increased total cholesterol levels in the blood, while the treated (drink-product containing IgY) showed no significant changes in total cholesterol. Furthermore, the ratio of total cholesterol to high-density lipoproteins and the apolipoprotein B level remained essentially unchanged in the treated group. Triglyceride and apolipoprotein A-I values did not change significantly in any of the groups. The authors suggest that IgY containing eggs could be beneficial in the regulation of serum lipoprotein levels and thereby on cardiovascular health.

## Administration to arthritis patients of a dietary supplement containing immune egg: an pen-label pilot study[24]

Thirteen arthritis patients; five with rheumatoid arthritis (RA), five with osteoarthritis (OA) and three with psoriatic arthritis (PA), were enrolled in this open-label study for 12 weeks. All patients consumed a drink fortified with 4.5 g powdered "immune egg" for 8 weeks and stopped taking the drink for the last 4 weeks. A physician global assessment was performed at the start of the study and then at 30, 60 and 90 days using a scoring system. The following joints were score for pain: fingers, wrist, elbows, shoulders, toes, fee, ankles, knees, hips, neck and lower back.

**Results:** Overall, the arthritis patients consuming the "immune eggs" had significant ( $P<0.05$ ) improvement in mean global assessment (MGA) scores at 30 and 60 days compared to baseline. However, not all the patients had similar degree of improvement. Patients with OA exhibited statistically ( $P<0.05$ ) lower MGA scores 30 and 60 days after treatment, while only a positive trend was seen in the RA patients. No significant effect was reported with PA patients. The MGA scores correlated well with personal evaluations provided by the patients. The results of this study suggest that the "immune egg" preparation may have anti-inflammatory properties.

## CONCLUSIONS

Immunoglobulins are soluble proteins synthesized by vertebrates for protection against foreign substances or antigens. IgG and IgY are the most abundant antibodies present in mammal and avian's serum, respectively. Both immunoglobulins are functionally equivalent and provide passive immunity to their offspring through the placenta and colostrum (human) or egg (hen).

Sixty to 150 mg of IgY per egg have been reported in immunized hens, but only 2-10% of the IgY is antigen-specific. Therefore, either gram quantities or expensive IgY preparations have been required until now. New proprietary technology from IgY Immune Technologies & Life Sciences, Inc., is capable of producing commercial relevant (kilograms) quantities of egg-based products with up to 95% IgY purity.

Numerous human clinicals using antigen-specific IgY have shown promise in enhancing the immune system's response to infections. Of great consequence is the use of anti-*P. aeruginosa* IgY as a prophylatic agent for cystic fibrosis patients. The positive results from several years of patient intervention have resulted in a multimillion Euro grant from the European Commission for a phase III clinical trial. Furthermore, in September 2008, the European Medicines Agency granted Anti-*Pseudomonas* IgY an Orphan Drug Designation.

Emerging science suggests further benefits of antigen-specific IgY antibodies for other human health conditions; such as cardiovascular, sport performance and inflammation. However, more studies are needed to substantiate early findings. Two clinical studies have been commissioned by IGY Immune Technologies & Life Sciences to study the benefits of purified IgY on the immune system and inflammatory processes of healthy individuals.

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