Immune Functions of Immunoglobulin Y Isolated from Egg Yolk of Hens Immunized with Various Infectious Bacteria

Yoshiko Sugita-Konishi, Kazumi Shibata, Sung Seob Yun, Yukiko Hara-Kudo, Kunio Yamaguchi, and Susumu Kumagai

Department of Biomedical Food Research, National Institute of Health, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162, Japan
*The College of Agriculture and Veterinary Medicine, Nihon University, 3-34-1 Shimoannai, Setagaya-ku, Tokyo 154, Japan

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We studied the immune functions of IgY obtained from hens immunized with a mixture of formalin-treated pathogenic bacteria. The IgY inhibited the growth of Pseudomonas aeruginosa, the production of Staphylococcus aureus enterotoxin A, and adhesion of Salmonella enteritidis to cultured human intestinal cells (Caco 2). The results indicated that IgY specific for plural bacteria has effects useful toward prevention of bacterial diseases.

Key words: IgY; various infectious bacteria; enterotoxin A; immune functions; Caco-2

Many investigators have reported that immunoglobulin from egg yolk (called IgY) of hens immunized with an infectious pathogen is efficient in prevention of the disease caused by the pathogen. Specific IgY antibody raised against a single antigen such as rotavirus, E. coli, or 14-KDa fimbriae of Salmonella enteritidis, when given to experimental animals with the corresponding pathogen, was effective in protection of the animals from infection. These findings indicated that IgY, if obtained from hens immunized simultaneously with various pathogens, could have various functions against these pathogens. To examine this possibility, we isolated IgY (immune-IgY) from egg yolks of hens immunized with twenty-six strains of bacteria, and investigated its function against three infectious bacterial strains focused on in this study. These three bacteria, Pseudomonas aeruginosa, Salmonella enteritidis, and Staphylococcus aureus, were chosen to represent opportunistic, invasive, and toxin-producing bacteria, respectively.

Immunization to hens and isolation of IgY were done by the method of Hatta et al. As an immunogen, a mixture of twenty-six strains of formalin-treated bacteria was used, that is, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, A Type 1, Streptococcus pyogenes A Type 3, Streptococcus pyogenes A Type 5, Streptococcus pyogenes A Type 8, Streptococcus pyogenes A Type 12, Streptococcus pyogenes A Type 14, Streptococcus pyogenes A Type 18, Streptococcus pyogenes A Type 22, Aerobacter aerogenes, Escherichia coli, Salmonella enteritidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium, Haemophilus influenzae, Streptococcus mitis, Proteus vulgaris, Shigella dysenteriae, Diplococcus pneumoniae, Propionibacter acnes, Streptococcus sanguis, Streptococcus salivarius, Streptococcus mutans, and Streptococcus agalactiae.

Immunological function of immune-IgY against P. aeruginosa, S. enteritidis, and Enterotoxin A-producing S. aureus (gift from Dr. Igini) was studied, in comparison with control IgY, anti-S. mutans IgY obtained from hens immunized with S. mutans alone. All results are shown as mean ± standard error, with statistical significance (p < 0.05, p < 0.001) tested by Student’s t-test.

The reactivity of immune-IgY and control IgY with the three bacterial strains were measured by ELISA at the IgY concentration of 10 μg/ml. The activities of the immune-IgY with P. aeruginosa, S. enteritidis, and S. aureus, expressed at ELISA absorbance at 405 nm (mean ± SE of three measurements), were 0.828 ± 0.120, 1.339 ± 0.157, and 0.556 ± 0.012, respectively, and those of control IgY were 0.001 ± 0.0001, 0.042 ± 0.002, and 0.017 ± 0.001, respectively. These results confirmed that the immune-IgY reacted specifically with the three pathogens.

To assess the inhibitory effects of the immune-IgY on growth of P. aeruginosa, S. aureus, and S. enteritidis, the bacteria were cultured with the immune-IgY or control IgY at the IgY concentration of 1–10 mg/ml at 37°C for 2, 4, 6, 8, and 24 h in tryptase soy broth (TSB, Becton Dickinson, MD). The growth curve was drawn by the measurement of the turbidity (OD 550 nm) of viable bacteria in culture. Growth inhibition was noted in P. aeruginosa and S. aureus, but not in S. enteritidis (Fig. 1) even at 24 h (data not shown). P. aeruginosa has been known to induce opportunistic disease when it overgrows in the intestine. The observed inhibition of its growth indicates that the immune-IgY could be useful for prevention of opportunistic disease caused by overgrowth of this bacteria.

S. aureus used in this study produces enterotoxin A, which is a direct cause of S. aureus food poisoning, and therefore the effect of immune-IgY on production of enterotoxin A was examined. S. aureus (10^6 cfu/ml TSB) was cultured with immune-IgY at a concentration of 1 mg/ml or 10 mg/ml or with control IgY at 10 mg/ml. Supernatant of the culture were taken after 24 h and viable bacteria were counted on tryptase soy agar (TS agar, Becton Dickinson, MD) plates. The amount of enterotoxin A in the culture supernatant was measured by a latex particle agglutination assay (Denka Seiken, Japan). The toxin-producing activity expressed by the amount of the toxin produced by one colony forming unit (cfu) of bacteria was significantly suppressed at immune-IgY concentrations of 1 and 10 mg/ml (Fig. 2).

Generally anti-bacterial antibody is regarded to cause agglutination, blocking of attachment, opsonization of bacteria, or neutralization of bacterial toxin. The result demonstrates that immune-IgY had another function, suppression of the toxin-producing activity of bacteria.

S. enteritidis is an entero-invasive bacteria, for which infection adhesion to the intestinal epithelial cells is an essential step. The effect of immune-IgY on the adhesion of the bacteria to human intestinal cells was examined by using cultured intestinal cells, Caco-2 (AOAC, HTB37). The intestinal cells maintained by the method of Dharmathaphorn were seeded at the density of 10^3 cells per 500 µl of cell-culture medium in Lab-Tek Chamber Slides (Nunc, Inc. Naperville, IL). After 2 weeks, S. enteritidis

Abbreviations: IgY, Immunoglobulin Y; ELISA, enzyme-linked immunosorbent assay; TS agar, tryptase soy agar; TSB, tryptase soy broth; cfu, colony forming unit; PBS, phosphate-buffered saline.
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Fig. 1. The Effects of Immune-IgY on the Growth of Three Bacteria Strains.

Each kind of bacteria (10⁵ cfu/ml) in TSB was incubated with 1 mg/ml of immune-IgY ( ), 5 mg/ml of immune-IgY ( ), 10 mg/ml of immune-IgY ( ), or 10 mg/ml of control IgY ( ) at 37°C and the turbidity of the culture was measured at OD 550nm at 2, 4, 6, 8, and 24 h after culture. (a) Pseudomonas aeruginosa. (b) Staphylococcus aureus. (c) Salmonella enteritidis. They are expressed as mean ± SE values of triplicate measurements. Representative of three separate experiments. *p < 0.05.

Fig. 2. The Effects of Immune-IgY on the Production of Enterotoxin A of Staphylococcus aureus.

Staphylococcus aureus (10⁵ cfu/ml) in TSB was incubated with various amounts of immune-IgY or 10 mg/ml of control IgY for 24 h at 37°C in TSB. After culture, the amount of toxin in the culture supernatant was measured by a latex particle agglutination assay. The viable bacteria in the culture were counted by plating of TSA. The toxin-producing ability was expressed by the amount of enterotoxin in culture supernatant number of viable bacteria. They are expressed as mean ± SE values of triplicate measurements. Representative of three separate experiments. *p < 0.05; **p < 0.01.

Fig. 3. The Effects of Immune-IgY on the Adhesion of Salmonella enteritidis to Human Intestinal Epithelial Cells (Caco-2).

Salmonella enteritidis (10⁵ cfu/ml) was incubated with immune-IgY (10 mg/ml) or 10 mg/ml of control IgY for 15 min, and then was used for the adhesion assay. The adhesion assay was done as described in text. They are expressed as mean ± SE values of triplicate measurements. Representative of three separate experiments. *p < 0.05.

(2 x 10⁵ cfu/ml) incubated with immune-IgY (1–10 mg/ml) or control IgY for 30 min at room temperature were added to the apical side of Caco-2 cells, which were then left for 45 min at 37°C in CO₂ incubator, followed by rinsing the cells six times with PBS. Cell-associated bacteria were then released by incubating the infected cells with 0.2 ml of 1% Triton X-100 (Sigma Chemical Co., St. Louis, Mo) in saline for 10 min with vigorous agitation, and the bacteria were counted by plating on TS agar. The percent of adherence was expressed by (the number of bacteria associated with the cells/total number of bacteria added) x 100. The result showed that the immune-IgY significantly inhibited the adhesion of S. enteritidis to Caco-2 at concentration of 10 mg/ml (Fig. 3), suggesting that immune-IgY could inhibit the bacterial mobility and induce the agglutination of bacteria.

Thus these results demonstrate that the IgY from hens immunized with plural bacterial antigens have several effects toward prevention of bacterial diseases by inhibiting such processes involved as bacterial growth, toxin production, and adhesion to the intestinal cells. Such differences in the antibody effects among different bacterial species indicate that not only the agglutination of bacteria but also other functions of antibody, could be involved in the observed effects.

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References
3) H. Hatta, K. Tsuda, S. Akachi, M. Kim, T. Yamamoto, and T.