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Exclusion of polymeric immunoglobulins and selective immunoglobulin Y transport that recognizes its Fc region in avian ovarian follicles

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Abstract

In avian species, blood immunoglobulin (Ig) Y, the equivalent to mammalian IgG, is selectively incorporated into ovarian follicles, but other classes, IgA and IgM, are much less abundant in the follicles. Several mammalian Igs, including IgG and IgA, are also incorporated into ovarian follicles when administered to birds. To clarify the Ig structure required for incorporation into ovarian follicles, Ig uptakes were determined after the intravenous injection of chicken and human Igs. Three chicken Igs (cIgY, cIgA and cIgM) and two human IgAs (monomeric hIgA and polymeric hIgA) were labeled with digoxigenin, and their uptakes into quail (*Coturnix japonica*) egg yolks were determined by ELISA and SDS-PAGE. The uptake of cIgY was the highest among the three cIgs (22% of injected cIgY was recovered from egg yolks). Chicken IgA was efficiently incorporated into egg yolk when it formed a monomeric state. Pentameric IgM was untransportable into egg yolk. We also found that the uptake of monomeric hIgA was much more efficient than that of polymeric hIgA. These results suggest that the retention of the monomeric form contributes to the efficient transport of Igs into ovarian follicles. On the other hand, Ig uptakes among monomeric Igs nevertheless differed; for example, a time-course analysis showed that the rate of monomeric cIgY uptake was approximately eight times faster than that of monomeric hIgA. The injection of cIgY fragments Fc, Fab and $F(ab')_2$ resulted in the largest uptake of Fc fragment, with the same level as that of cIgY. These results suggest the presence of a selective IgY transport system that recognizes its Fc region in avian ovarian follicles.

Keywords: Fc fragment; IgY; Maternal antibodies; Monomeric immunoglobulin; Ovarian follicles; Quail

1. Introduction

In avian species, blood IgY, the avian counterpart of mammalian IgG, is incorporated into the egg yolk to confer passive immunity to the developing embryo before it can generate its own humoral immune response (Kowalczyk et al., 1985). In addition to IgY, heterologous Igs such as mammalian IgG and IgA are also incorporated into egg yolk when intravenously administered to laying hens (Mohammed et al., 1998; Morrison et al., 2001). In general, major yolk components are incorporated into ovarian follicles by receptor-mediated endocytosis at the oocyte membrane (Stifani et al., 1990; Barber et al., 1991; Mac Lachlan et al., 1994; Jacobsen et al., 1995; Vieira et al., 1995, 1996; Recheis et al., 2005). Igs are believed to be incorporated into ovarian follicles in a similar way, presumably by a specific receptor existing in ovarian

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follicles. However, the exact mechanism by which Igs are incorporated into ovarian follicles has not yet been fully elucidated; in addition, the Ig structure required for effective transport remains controversial.

Avian Igs consist of three classes, IgY, IgM and IgA, of which IgY is the most abundant in serum, ranging from 5 to 15 mg/ml in laying hens (Rose et al., 1974; Kowalczyk et al., 1985). IgM is the next most concentrated in serum (1–3 mg/ml), and IgA is present in the lowest concentration (0.3–0.5 mg/ml). Almost all of the Ig incorporated into egg yolk is IgY, while only trace amounts of IgM and IgA can be detected (Rose et al., 1974). It is not yet understood why avian IgM and IgA are excluded from egg yolk, but both Igs share certain structural characteristics: in serum, IgM and IgA are found in polymeric states. The majority of serum IgM exists as a pentameric state (Schranner and Lösch, 1986), while serum IgA exists in polymeric and monomeric states, and one-fifth of IgA exists in a monomeric state (Stevens, 1996). In contrast, serum IgY exists only in a monomeric state. These facts imply that the polymeric state of IgM and IgA restricts its deposition into egg yolk. Conversely, the incorporation of monomeric Ig may be blocked by its polymerization.

The Fc structure of Ig seems to be another limiting factor for Ig transport into egg yolk. The uptakes of monomeric Igs vary according to Ig classes or subclasses (Mohammed et al., 1998; Morrison et al., 2001). Both human IgG (hIgG) and hIgA are incorporated into chicken egg yolks. In contrast, there is no uptake of mouse IgG2b. In the case of hIgG, mutant hIgG with the CH2 domain deleted failed to be incorporated into egg yolk, implying that the Fc region is required for the incorporation of hIgG (Morrison et al., 2001). In addition to normal IgY, ducks also produce smaller Ig with a molecular mass of 120 kDa; this is referred to as IgY(ΔFc). IgY(ΔFc) lacks two constant regions of Fc, CH3 and CH4, and is present in serum. While both IgY and IgY(ΔFc) are incorporated into egg yolk, the full-length form is preferentially incorporated (Liu and Higgins, 1990). To the best of our knowledge, however, there has been no direct demonstration of the participation of the Fc region in the control of IgY transport into ovarian follicles.

To clarify the structural requirements for Ig transport into ovarian follicles, three classes of chicken Igs (cIgY, cIgA and cIgM) and two hIgAs (monomeric hIgA and polymeric hIgA) were injected into the blood of quail, and their uptakes into egg yolk and ovarian follicles were determined. Moreover, by injecting various IgY fragments into blood, we have identified structural region of IgY required for its efficient transport into ovarian follicles. A comparison of the Ig uptakes of the monomeric and polymeric states showed that the monomeric state of Ig contributes to its efficient transport into egg yolk. Importantly, cIgA in a monomeric state can be effectively transported into egg yolk. Moreover, by injecting various IgY fragments into blood, we have shown that the Fc structure is another limiting factor in the regulation of Ig transport into ovarian follicles.

2. Materials and methods

2.1. Animals

Female Japanese quail (*Coturnix japonica*) were purchased from a local hatchery (Cyubu-kagaku-shizai, Nagoya, Japan) and maintained individually with free access to water and a commercial diet (Power-Uzura; Toyohashi Feed Mills, Toyohashi, Japan) at a constant temperature of 23 ± 1 °C and with daily light period of 16 h. Egg production was recorded daily, and 11–17-weeks-old birds continuously laying were used for the present animal experiments. Animal care was in full compliance with the guidelines of the Nagoya University Policy on Animal Care and Use.

2.2. Authentic Igs and the preparation of DIG-labeled Igs

Chicken IgY and cIgA were purchased from Sigma–Aldrich (St. Louis, MO) and Inter-Cell Technologies (Jupiter, FL), respectively. Chicken IgM and cIgY fragments, Fc, Fab, and $F(ab')_2$, were obtained from Rockland (Gilbertsville, PA). Human IgA derived from blood and hIgA derived from colostrum were obtained from Athens Research & Technology (Athens, GA) and UK-Serotec (Oxford, UK), respectively. The Igs injected into birds were labeled with digoxigenin (DIG) by a DIG Protein Labeling Kit (Roche Diagnostics, Mannheim, Germany). An apparent molecular mass of DIG-labeled Ig was determined by 5% SDS-PAGE with reference to Precision Plus Protein Standards (Bio-Rad, Hercules, CA) under nonreducing conditions. The proteins were stained with a silver staining kit (Wako Pure Chemical Industries, Osaka, Japan) following the manufacturer's instruction.

After labeling, the concentration of DIG-labeled Ig was determined using an ELISA kit specific for each Ig (Chicken IgG, IgA or IgM ELISA Quantitation Kit and Human IgA ELISA Quantitation Kit; Bethyl Laboratories, Montgomery, TX). The concentration of cIgY Fc fragment was determined using a Chicken IgG ELISA