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Production of IgY Anti-Mouse IgG Antibodies from Chicken Eggs

Siriporn Kritratanasak[1], Sawitree Chiampanichayakul[2] and Watchara Kasinrerk[1,3]

SUMMARY IgY technology offers several advantages over antibody production in mammals. In this study, we applied IgY technology for the production of anti-mouse IgG polyclonal antibodies and developed a FITC conjugate reagent. Two hens were immunized 3 times with mouse IgG, one via the pectoralis and the other via the calf muscles. Specific antibodies could be detected in the sera two weeks after the immunization, and maximum levels were reached at week 10. The hen which was immunized via the pectoralis muscle produced a much higher antibody response than the hen immunized via the calf muscle. In egg yolk, specific antibodies appeared 2 weeks after the first immunization, reached a plateau after week 11 and remained high until week 20. IgY were extracted from egg yolk by sodium sulfate precipitation. Approximately 40 mg of IgY could be extracted from a single egg. The extracted IgY was labeled with FITC. The so-produced antibody-FITC conjugate reacted to all mouse IgG isotypes and could be used to determine leukocyte sub-populations in blood samples by flow cytometry.

As described more than 100 years ago, avian maternal antibodies are transferred from serum to egg yolk conferring passive immunity to embryos and neonates.[1] IgY antibodies were the evolutionary ancestors of mammalian IgG and IgE antibodies.[6] IgY technology is based on extracting the antibodies from the egg yolk.[2] IgY technology has a number of advantages to antibody production, the most apparent being the non-invasive collection of antibodies. Another advantage is the enhanced immune response against conserved mammalian proteins exhibited in birds due to their phylogenetic distance.[3] This makes the production of antibodies against conserved mammalian proteins usually more successful in chicken than in other mammals. The yield of egg yolk IgY antibodies is higher than that of IgG antibodies obtained by conventional immunization methods.[4] In chicken, approximately 1,500 mg of IgY can be harvested each month, and between 2 and 10% are specific IgY.[4] Compared to antibody production in other mammals, the IgY technology offers several advantages[5]: (i) no bleeding, only egg collection is required upon immunization; (ii) IgY isolation is simple; and (iii) very low quantities of antigen are required to obtain high and long-lasting IgY titers in the egg yolk from immunized hens.[3]

Despite the similarities between IgY and IgG antibodies, there are also some profound differences in their structure. The molecular mass of the IgY heavy chain is 67-70 kDa, whereas the molecular mass of the mammalian IgG heavy chain is approximately 50 kDa.[6] The greater molecular mass of IgY is due to a higher number of heavy-chain constant domains and carbohydrate chains. With regards to function, there are four important differences between IgY and IgG. Firstly, IgY does not bind to protein A or protein G. Secondly, IgY does not bind the rheumatoid factor (RF). IgG molecules often cause false

From the [1]Department of Clinical Immunology, [2]Department of Clinical Microscopy, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand, [3]Medical Biotechnology Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Chiang Mai 50200, Thailand.
Correspondence: Watchara Kasinrerk

positive results due to interaction with RF in immunoassays.[7] This phenomenon does not occur in the case of IgY. Thirdly, chicken egg-yolk IgY does not interfere with mammalian IgG, and finally, IgY does not activate mammalian complement.[8] These differences bring great advantages to the application of IgY technology in many medical areas such as diagnosis,[9-16] xenotransplantation,[17] and therapy.[8,18-20]

Many immunological techniques such as agglutination, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and immunofluorescence assay (IF) have been employed in diagnostic applications. The techniques using labeled reagents for detecting antigens and antibodies, e.g., ELISA and immunofluorescence, are exquisitely sensitive and specific. At the present, mouse monoclonal antibodies are widely used in labeled immunoassays. Polyclonal antibodies against mouse IgG are therefore used as secondary antibodies in those immunoassays. In this study, the IgY technology was established in our laboratory and employed to produce anti-mouse IgG antibodies for utilization in an immunoassay. For production of antibodies in chicken, the pectoralis and calf muscle immunization routes were compared. The kinetics of the antibody responses in both serum and egg yolk was also investigated. A large amount of polyclonal antibodies against mouse IgG were produced using the IgY technology. The obtained IgY anti-mouse IgG antibodies were conjugated with FITC. These conjugated antibodies could be used as secondary antibody in indirect immunofluorescence techniques.

MATERIALS AND METHODS

Purification of mouse IgG

Pooled normal mouse serum was purified by a protein G coated Sepharose column (Zymed Laboratory Inc., San Francisco, CA) according to the method described elsewhere.[21] The obtained IgG were concentrated by ultrafiltration and dialyzed against phosphate buffered saline (PBS) overnight. The concentration of antibody was determined by measuring the absorbance at a wavelength of 280 nm. The purity of the purified IgG was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The isotypes of the isolated IgG were determined using an isotyping enzyme-linked immunosorbent assay kit (Sigma, St. Louis, MO).

Chicken Immunization

Five-months-old egg laying Rosehorn hens were kept in individual cages with ample food and water throughout the course of the study.

At day 0, the first hen (SK-1) was immunized via the pectoral muscle with 500 μg of mouse IgG in complete Freund's adjuvant. The second hen (SK-2) was injected via the calf muscle with the same dose of antigen. Both hens were boosted twice with 500 μg of IgG in incomplete Freund's adjuvant at days 14 and 28. Eggs and blood were collected from both hens every one and two weeks, respectively. For blood collection, 2 ml of blood were collected from a wing vein. The sera were isolated from the collected blood and stored at -20°C.

Extraction of IgY from egg yolk by salt precipitation

The egg yolk was separated from the white of the egg and washed thoroughly in distilled water. Five milliliters of yolk were diluted 9 times with distilled water and kept at 4°C overnight. After incubation, the yolk mixture was separated into 2 layers. Thirty milliliters of the upper layer were collected and centrifuged at 9,000 x *g*, at 4°C for 30 minutes. Then 3.8 g Na_2SO_4 were slowly added to 20 ml of supernatant. After 90 minutes stirring at room temperature, the precipitate containing IgY was pelleted by centrifugation at 9,000 x *g*, at 4°C for 30 minutes. The precipitate was resuspended with 2 ml distilled water and dialyzed against PBS at 4°C overnight. The IgY concentration was determined by measuring at an O.D. of 280 nm. The isolated IgY was adjusted to 1 mg/ml with PBS and stored at -20°C.

Indirect ELISA for determination of chicken anti-mouse IgG antibodies

Each well of an ELISA plate (NUNC, Roskilde, Denmark) was incubated with 100 μl of 10 μg/ml mouse IgG at 4°C overnight. The plate was then washed 4 times with PBS containing 0.05% Tween 20 (PBS-Tween). Thereafter, the plate was blocked with PBS containing 2% BSA (W/V). One hundred microliters of various dilutions of sera or IgY extract were added into the wells and incubated at 37°C for 1 hour. The plate was then washed with PBS-Tween for 4 times. One hundred microliters of peroxidase conjugated rabbit anti-chicken immunoglobulin antibodies (Dako,

Glostrup, Denmark) were added and incubated at 37°C for 1 hour. After a washing step, OPD-H_2O_2 substrate was added to each well and incubated at room temperature in the dark. The reaction was stopped with 4N H_2SO_4 and the absorbance was determined at 490 nm using an ELISA reader.

Fluorescein isothiocyanate (FITC) labeling of IgY anti-mouse IgG antibodies

One milligram of IgY anti-mouse IgG was resuspended in 0.1 M $NaHCO_3$, pH 9.0. The FITC solution (10 mg/ml in DMSO) was added dropwise into the antibody solution. The antibody/FITC mixture was incubated at room temperature for 90 minutes with gentle stirring and protected from direct light. After that, the solution was dialyzed against PBS overnight. The FITC to protein (F/P) ratio was determined by measuring the absorbances at 495 and 280 nm.

Indirect immunofluorescence

Fifty microliters of EDTA-whole blood or U937 cells were added to 50 µl of 20 µg/ml monoclonal antibodies (mAbs) specific to various leukocyte surface molecules. The cells were then incubated on ice for 30 minutes. After incubation, the cells were washed twice with PBS containing 1% BSA and 0.02% NaN_3. Fifty microliters of FITC conjugated chicken anti-mouse immunoglobulin antibodies were added and incubated on ice for 30 minutes. For the lysis of red blood cells, one ml of FACS lysing solution (Becton Dickinson, San Jose, CA) was added into the cell suspension and incubated at room temperature for 10 minutes. The cells were washed twice with 1%BSA-PBS-NaN_3 and fixed with 1% paraformaldehyde in PBS and analyzed by a flow cytometer using Cell-Quest software (Becton Dickinson).

RESULTS

Preparation of mouse IgG

To prepare mouse IgG, one milliliter of normal mouse serum was added into a protein G-Sepharose column. A total amount of 2.6 mg of protein was obtained. To verify whether the obtained protein was IgG, the obtained eluate was subjected to a SDS-PAGE under both non-reducing and reducing conditions. Under non-reducing condition, a single high molecular weight band appeared on the top of the gel (Fig. 1, lane 2), whereas under reducing conditions 2 bands at a molecular weight of 51 and 26 kDa corresponding to the heavy and light chains of mouse IgG were obtained (Fig. 1, lane 3).

To identify the isotypes of the isolated IgG, the eluate was subjected to a sandwich ELISA using isotype specific antibody. The eluate contained all IgG isotypes, i.e., IgG_1, IgG_{2a}, IgG_{2b} and IgG_3 (Fig. 2).

Production of chicken anti-mouse IgG antibodies

In sera, anti-mouse IgG antibodies could be detected two weeks after the first immunization in both hens (Fig. 3). The antibody levels increased after the third immunization and reached the maximum level at week 10 after the initial antigen immunization. Three months after the last antigen immunization, the antibody levels decreased. Immunization via the pectoralis muscle (SK-1) induced much higher antibody responses than calf muscle immunization (SK-2) (Fig. 3).

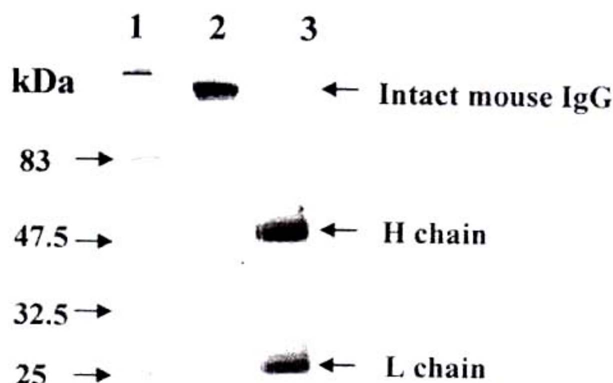

Fig. 1 SDS-PAGE analysis of mouse IgG isolated by protein-G Sepharose chromatography. The eluate obtained from the protein-G Sepharose column was subjected for SDS-PAGE under non-reducing (lane 2) and reducing (lane 3) conditions. Molecular weight markers (kDa) are shown in lane 1.

Eggs were collected from both hens every week and the IgY were extracted. The IgY extracts had a concentration of 1 mg/ml and were diluted and examined for their anti-mouse IgG titers. As was observed in the sera, anti-mouse IgG antibody titers could be detected two weeks after antigen immunization and increased after the third immunization (Fig. 4). The antibody concentration reached a plateau at week 11, and remained high for at least 20 weeks after antigen immunization. Similar to the sera, eggs collected from hens immunized by pectoralis immunization showed a better antibody response than those from calf muscle immunization (Fig. 4).

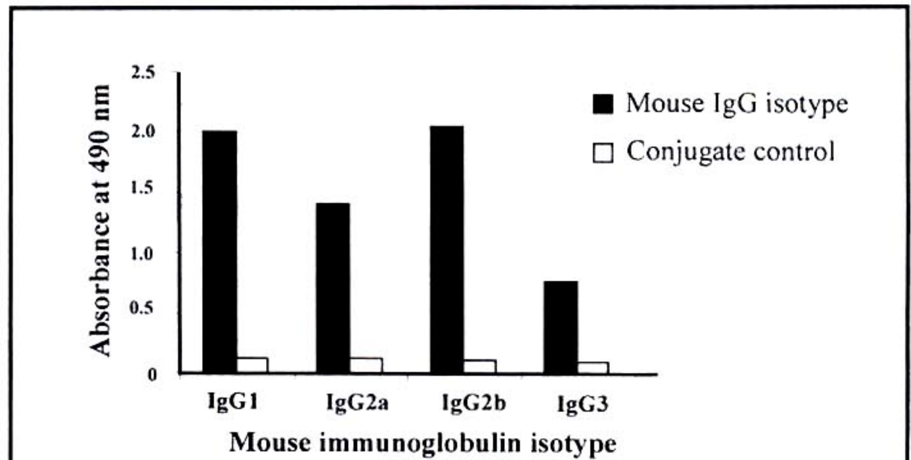

Fig. 2 The absorbance of the isolated mouse IgG determined by sandwich ELISA using a goat anti-mouse IgG isotype coated plate and rabbit anti-mouse immunoglobulins-HRP as conjugate. The O.D. of the conjugate control is the O.D. obtained from wells coated with anti-mouse IgG in the absence of tested materials and presence of conjugate.

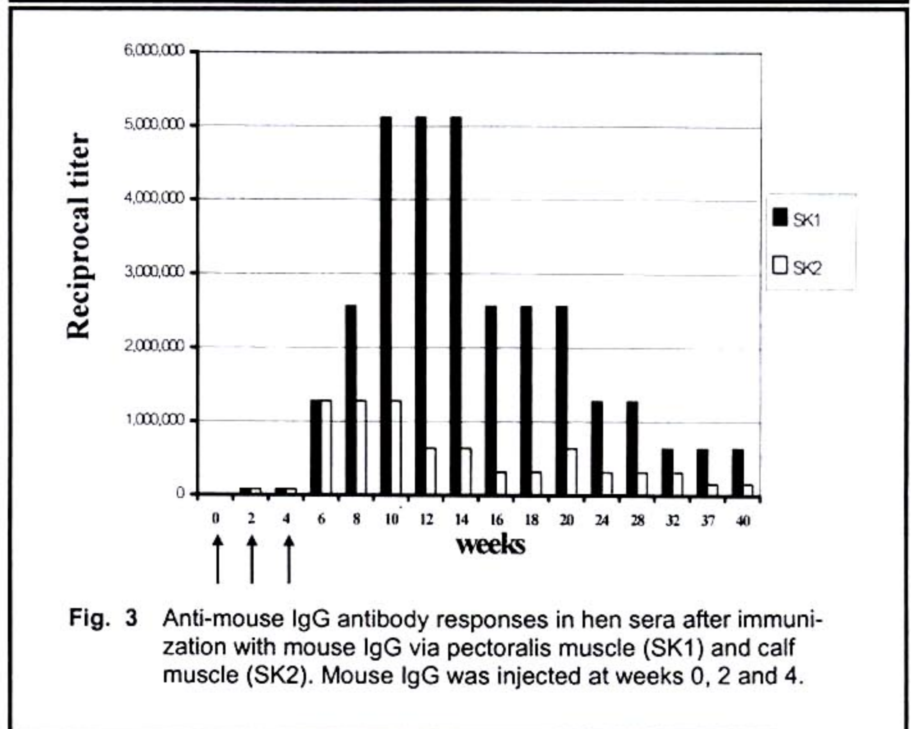

Fig. 3 Anti-mouse IgG antibody responses in hen sera after immunization with mouse IgG via pectoralis muscle (SK1) and calf muscle (SK2). Mouse IgG was injected at weeks 0, 2 and 4.

Preparation of FITC conjugated IgY anti-mouse IgG antibodies

Eggs were collected from SK1 at the time of the maximum antibody response and IgY was extracted from them. After the purification process, the purity of the IgY was assessed by SDS-PAGE. As shown in Fig. 5, under non-reducing conditions, a major high molecular weight band of intact IgY was observed on the top of the gel. However, a faint band with a molecular weight of approximately 33 kDa was also observed. Under reducing conditions, two protein bands, which are attributed to the heavy and light chains of IgY were observed (Fig. 5). Two faint bands at 39 and 45 kDa were also observed. The results indicated that the majority of extracted proteins were immunoglobulin. By the water dilution and salt precipitation method, approximately 30-50 mg of protein could be extracted from a single egg. The extracted IgY was then labeled with FITC. To verify the specificity of the produced conjugate, leukocytes and U937 cells were stained with mouse IgG and IgM mAbs. As shown in Fig. 6, the produced conjugate reacted to all IgG isotypes and IgM mAbs. These results indicated that the conjugate could be used as second antibody-FITC conjugate for immunofluorescence assays.

The produced conjugate was then employed in immunofluo-

rescence techniques for the determination of lymphocytes and monocytes. As shown in Fig. 7, leukocytes were stained with OKT3 (anti-CD3 mAb), MT14/2 (anti-CD14 mAb) and MEM-97 (anti-CD20 mAb), which were IgG1, IgG2b and IgG1 isotypes, respectively. The generated conjugate could be used to determine T cells, monocytes and B cells and showed similar FACS profiles as those obtained by using a commercial conjugate (Silenus, Boronia, Victoria, Australia) (Fig. 7).

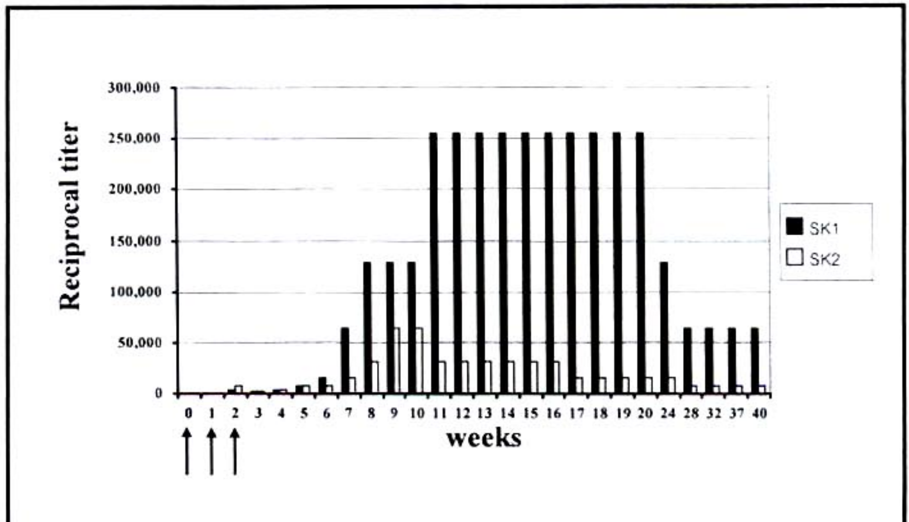

Fig. 4 Anti-mouse IgG antibody responses of IgY extracted from egg yolk after immunization with mouse IgG via pectoralis muscle (SK1) and calf muscle (SK2). Mouse IgG was injected at weeks 0, 2 and 4.

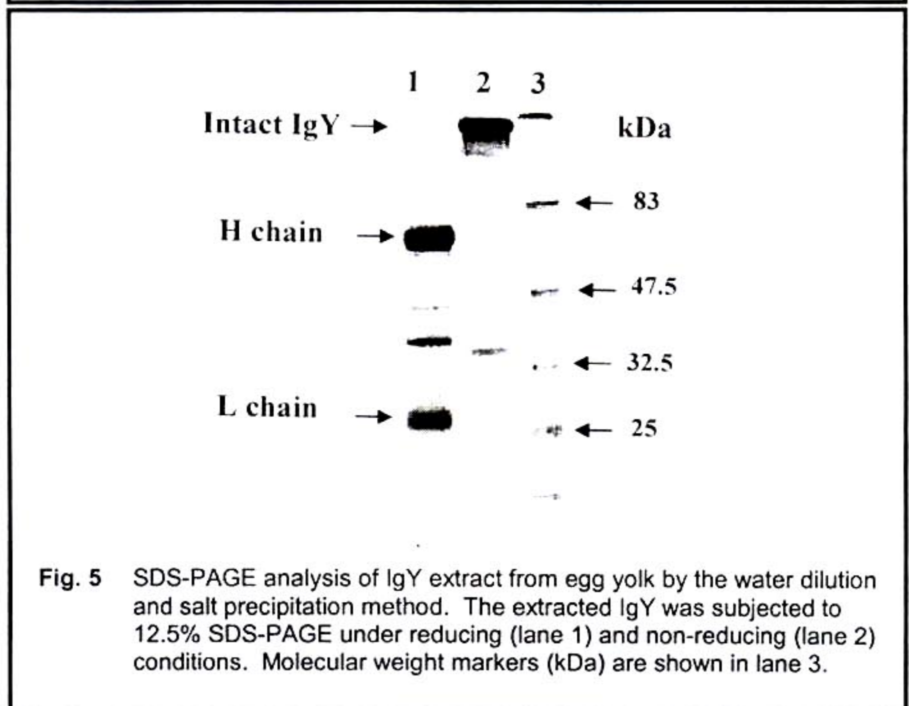

Fig. 5 SDS-PAGE analysis of IgY extract from egg yolk by the water dilution and salt precipitation method. The extracted IgY was subjected to 12.5% SDS-PAGE under reducing (lane 1) and non-reducing (lane 2) conditions. Molecular weight markers (kDa) are shown in lane 3.

DISCUSSION

In avian species, IgY is an immunoglobulin in the egg yolk that was transferred from the maternal circulation to confer passive immunity to embryos and neonates.[22] The amount of IgY transported is known to be proportional to the maternal serum IgY concentration.[23] In chicken, it is easy to induce humoral immune response, as only 20-30 μg of antigen are sufficient to induce the response. The specific antibodies appeared 20 days after antigen immunization, reached a plateau after 30 days, and remained high until at least day 81.[3] The induced antibodies were passed to the egg yolk in a high quantity. In a single egg, approximately 75-100 mg of IgY is present.[24] IgY of up to 1,500 mg can be harvested from eggs each month.[4] Compared to other animals, only larger mammals such as sheep can produce relatively similar amounts of antibodies. When compared to rabbits, production of antibodies in chicken eggs is much higher.[25] Isolation of IgY antibody from an egg is easy to perform and the collection of blood which is painful for the animals can be omitted. Because of these advantages, the production of IgY antibody from egg yolk, termed IgY technology, has been developed. Several investigators used IgY technology to produce polyclonal antibodies against a protein of interest.[26-32] The IgY specific antibodies can be applied in both immunodiagnostics and immunotherapy.[9-20]

In this study, we applied IgY technology to produce anti-mouse IgG antibodies. For this purpose, mouse IgG was isolated from serum by affinity chromatography using a Protein-G Sepharose

column.[33] The obtained IgG was verified by SDS-PAGE. Under reducing condition, 2 bands corresponding to the heavy and light chains of IgG were observed. While under non-reducing condition, a single high molecular weight band of an intact IgG molecule was observed on the top of the gel. No other protein band was observed indicating that the IgG obtained was rather pure.

Immunizations, were carried out by two different intramuscular routes, via the pectoralis and calf muscles. Both routes induced specific antibody after antigen immunization. However, different degrees of antibody responses were observed. Pectoralis immunization induced much higher antibody titers than calf muscle immunization. This result is in concurrence with previous reports.[3,29] In the present report, however, only one hen was used with each immunization route. The different antibody responses could be due to a difference in the individual immune response of each hen. It is, therefore, a speculation to conclude that the pectoralis muscle immunization is better than calf muscle immunization. A higher number of hens are required to confirm whether the pectoralis muscle immunization is the superior immunization route.

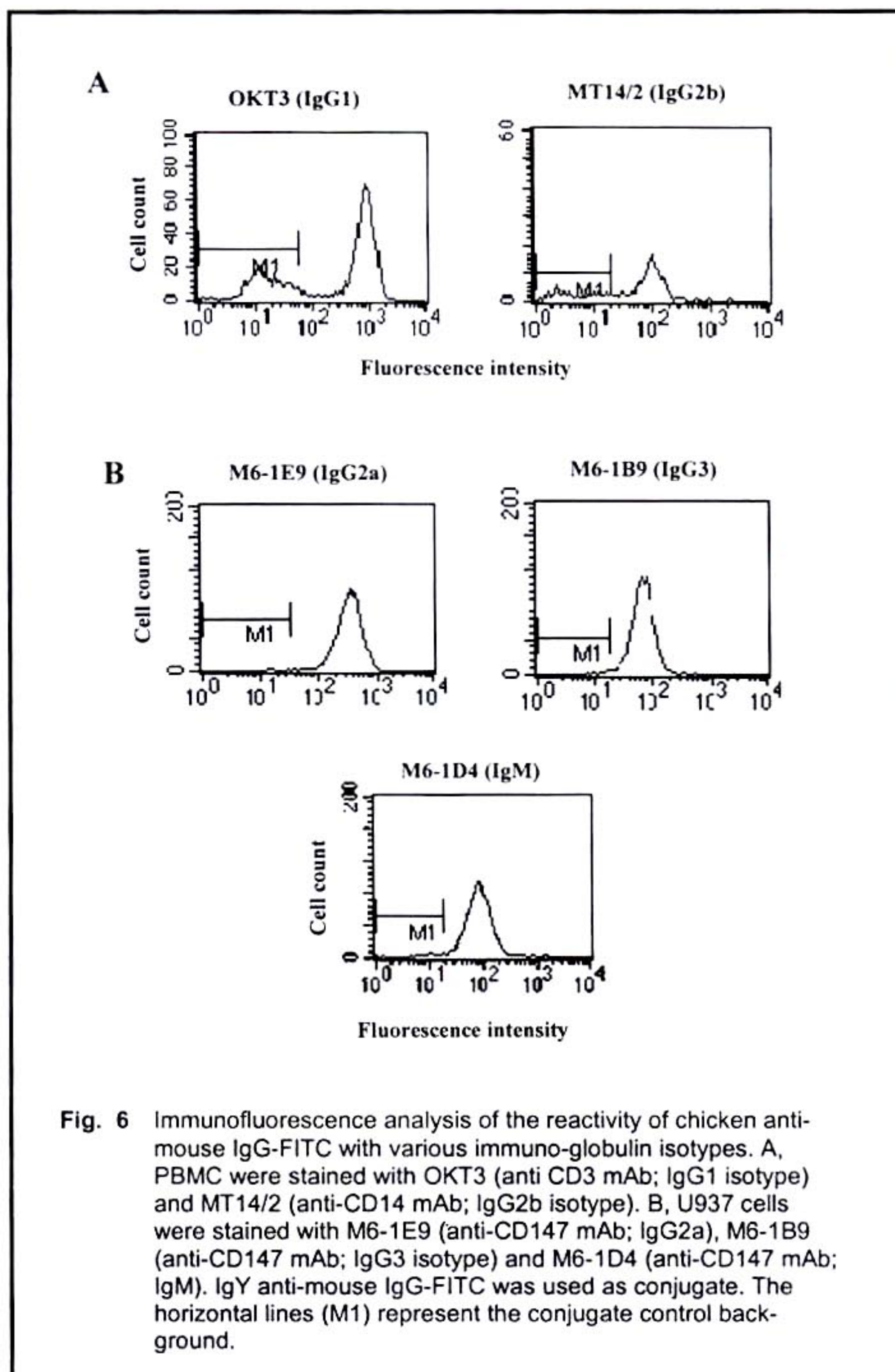

Fig. 6 Immunofluorescence analysis of the reactivity of chicken anti-mouse IgG-FITC with various immuno-globulin isotypes. A, PBMC were stained with OKT3 (anti CD3 mAb; IgG1 isotype) and MT14/2 (anti-CD14 mAb; IgG2b isotype). B, U937 cells were stained with M6-1E9 (anti-CD147 mAb; IgG2a), M6-1B9 (anti-CD147 mAb; IgG3 isotype) and M6-1D4 (anti-CD147 mAb; IgM). IgY anti-mouse IgG-FITC was used as conjugate. The horizontal lines (M1) represent the conjugate control background.

Several methods have been reported for the extraction of IgY from egg yolk.[34-37] The sodium sulfate precipitation proved to be a simple, reliable, environmentally compatible and economic method.[38] In this study, the water dilution and sodium sulfate precipitation method was therefore carried out. Besides extracting IgY, this method causes contamination with a small amount of other proteins (Fig. 5). Approximately 40 mg of IgY can be extracted from a single egg.

After antigen immunization, the antibody titers in the sera as well as the amounts of extracted IgY were determined by ELISA. In sera, by pectoralis muscle immunization, low anti-mouse IgG antibody titers were detected after the first immunization. The antibody continuously increased after the second and the third immunization. The kinetics of the antibody responses observed in our study are

corresponding to previous reports.[3] In the egg yolk, high IgY titers were obtained after pectoralis immunization. They reached a plateau seven days after appearing in the serum. Our results suggested that production of antibody in chicken is simple and a high quantity of antibody can be obtained from both serum and egg. The IgY technology is therefore an efficient alternative method for the production of polyclonal antibody.

For their use as a conjugate in an indirect immunofluorescence assay , the IgY anti mouse IgG antibodies extracted from the eggs were then labeled with FITC. The prepared FITC conjugate reacted to all isotypes of mouse IgG. However, it also showed reactivity with mouse IgM. This phenomenon is likely to be due to the reactivity of the light chains of the immunoglobulins as both IgG and IgM isotypes contain the same light chain isotype. The prepared conjugate was then employed in an indirect immunofluorescence assay for determining leukocyte sub-populations. In this study, white blood cells were stained with anti-CD3, anti-CD14 and anti-CD20 mAbs and analyzed by flow cytometry. In comparison to the commercial conjugate (Silenus), the FACS profiles obtained from the self-prepared and commercial conjugates were similar.

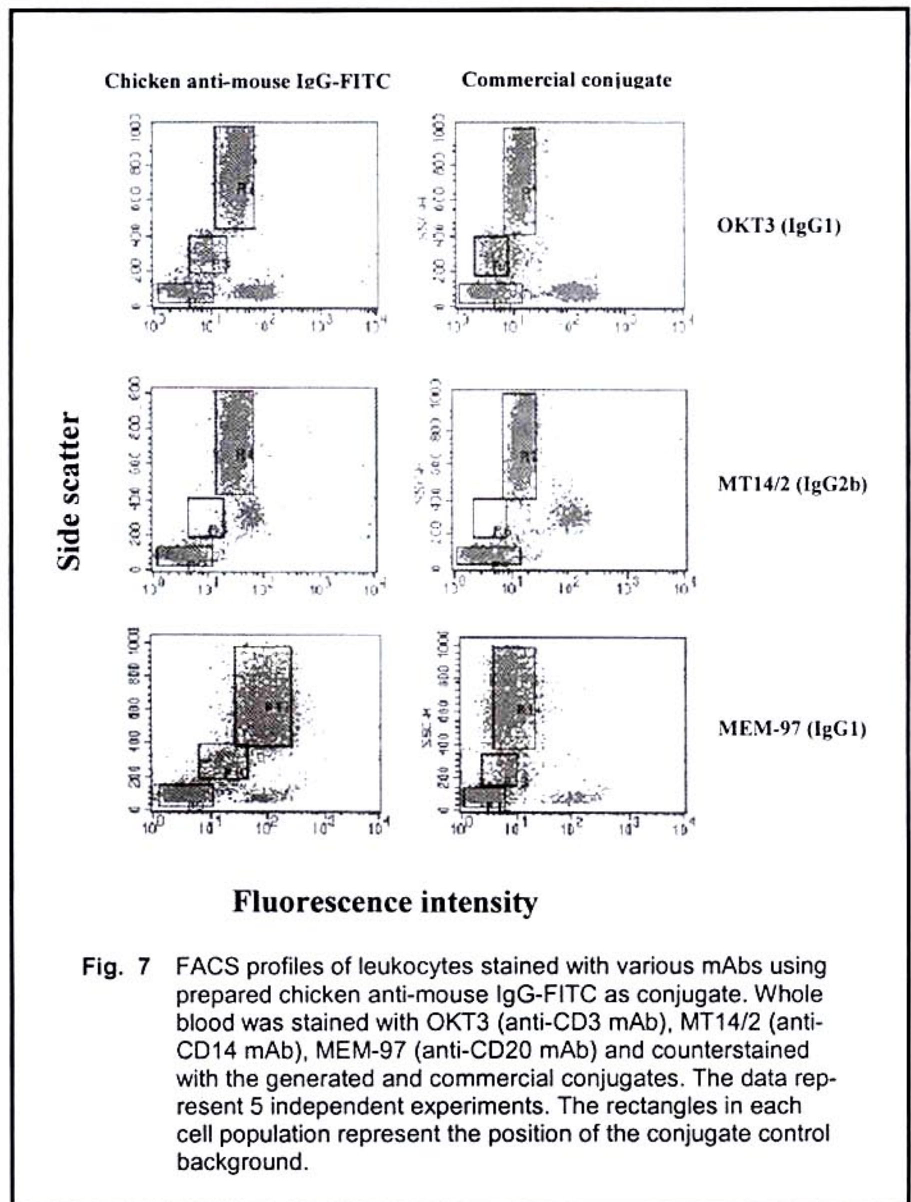

Fig. 7 FACS profiles of leukocytes stained with various mAbs using prepared chicken anti-mouse IgG-FITC as conjugate. Whole blood was stained with OKT3 (anti-CD3 mAb), MT14/2 (anti-CD14 mAb), MEM-97 (anti-CD20 mAb) and counterstained with the generated and commercial conjugates. The data represent 5 independent experiments. The rectangles in each cell population represent the position of the conjugate control background.

We, therefore, recommend the use of IgY technology to produce polyclonal antibody against mouse IgG. This technique is beneficial to the immunized animals, due to non-invasive antibody harvesting, with the added convenience of simple egg collection. Large amounts of antibody can be obtained from a single egg. It is expected that in the future, this technology will play an increasing role in research, diagnostics and immunotherapy.

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REFERENCES

1. Klemperer F. Ueber naturliche Immunitat und ihre Verwertung fur die Immunisirungstherapie. In: Naunyn, B., Schmiedeberg, O, eds., Archiv Fur Experimentelle Pathologie und Pharmakologie, Verlag von FCW. Vogel, Leipzig, Einunddreissigster Band.1893. XV.
2. Schade R, Henklein P, Hlinak A. Egg yolk antibody: State of the art and advantageous use in the life sciences. In: Zutphen LFM, Balls M, eds. Welfare and Ethics: Animal Alternatives. Amsterdam, Elsevier, 1977; pp. 973-81.

3. Gassmann M, Thommes P, Weiser T, Hubscher U. Efficient production of chicken egg yolk antibodies against a conserved mammalian protein. FASEB J 1990; 4: 2528-32.
4. Schade R, Burger W, Schoneberg T, *et al.* Avian egg yolk antibodies. The egg-laying capacity of hens following immunization with antigens of different kind and origin and the efficiency of egg yolk antibodies in comparison to mammalian antibodies. Altex Alternativen Zu Tierexp 1994; 11: 75-84.
5. Fischer M, Hlinak A, Montag T, Claros M, Schade R, Ebner D. Comparison of standard methods for the preparation of egg yolk antibodies. Tierarztliche Praxis 1996; 24: 411-8.
6. Warr GW, Magor KE, Higgins DA. IgY: clues to the origins of modern antibodies. Immunol Today 1995; 16: 392-8.
7. Davalos-Pantoja L, Ortega-Vinuesa, JL, Bastos-Gonzalez D, Hidalgo-Alvarez R. A comparative study between the adsorption of IgY and IgG on latex particles. Biomater Sci Polym Ed 2000; 11: 657-73.
8. Carlander D, Kollberg H, Wejaker PE, Larsson A. Peroral immunotherapy with yolk antibodies for the prevention and treatment of enteric infections. Immunol Res 2000; 21: 1-6.
9. de Almeida CM, Quintana-Flores VM, Medina-Acosta E, Schriefer A, Barral-Netto M, Dias da Silva W. Egg yolk anti-BfpA antibodies as a tool for recognizing and identifying enteropathogenic *Escherichia coli.* Scand J Immunol 2003; 57: 573-82.
10. Gutierrez Calzado E, Cruz Mario E, Samon Chavez T, Vazquez Luna E, Corona Ochoa Z, Schade R. Extraction of a monospecific Coombs-reagent from chicken eggs. ALTEX 2003; 20: 21-5.
11. Kollberg H, Carlander D, Olesen H, Wejaker PE, Johannesson, Larsson A. Oral administration of specific yolk antibodies (IgY) may prevent *Pseudomonas aeruginosa* infections in patients with cystic fibrosis: a phase I feasibility study. Pediatr Pulmonol 2003; 35: 433-40.
12. Cipolla A, Cordeviola J, Terzola H, *et al. Campylobacter fetus* diagnosis: direct immunofluorescence comparing chicken IgY and rat IgG conjugates. ALTEX 2001; 18: 165-70.
13. Erhard MH, Schmidt P, Zinsmeister P, *et al.* Adjuvant effects of various lipopeptides and interferon-gamma on the humoral immune response of chicken. Poul Sci 2000; 79: 1264-70.
14. Hommel U, Behn II, Hauschildt S. IgY antibodies as secondary reagent in FACS analysis. ALTEX 1996; 13: 40-4.
15. Hlinak A, Kruger M, Bartels T, Sasse M, Claros M, Schade R. Studies on diagnostic applications of egg yolk antibodies against *Bordetella bronchiseptica.* ALTEX 1996; 13: 70-2.
16. Staak C. Egg yolk antibodies (IgY) in routine diagnostic work. ALTEX 1996; 13: 73-75.
17. Fryer J, Firca J, Leventhal J, *et al.* IgY antiporcine endothelial cell antibodies effectively block human antiporcine xenoantibody binding. Xenotransplantation 1999; 6: 98-109.
18. Carlander D, Kollberg H, Larsson A. Retention of specific yolk IgY in the human oral cavity. BioDrugs 2002; 16: 433-7.
19. Mine Y, Kovacs-Nolan J. Chicken egg yolk antibodies as therapeutics in enteric infectious disease: a review. J Med Food 2002; 5: 159-69.
20. Almeida CM, Kanashiro MM, Rangel Filho FB, Mata MF, Kipnis TL, da Silva WD. Development of snake antivenom antibodies in chickens and their purification from yolk. Vet Rec 1998; 143: 579-84.
21. Harlow E, Lane D. Monoclonal antibodies. In: Harlow E, Lane D, eds. Antibodies: a laboratory. New York, Cold Spring Harbor Laboratory, 1988; pp. 285.
22. Rose ME, Orlans E, Buttres N. Immunoglobulin classes in the hen's egg: their segregation in yolk and white. Eur J Immunol 1974; 4: 521-3.
23. Loeken MR, Roth TF. Analysis of maternal IgG subpopulations which are transported into the chicken oocyte. Immunology 1983; 49: 21-8.
24. Maya Dc, Vasantha BM, Krishnan LK. Development of viper-venom antibodies in chicken egg yolk and assay of their antigen binding capacity. Toxicon 2000; 40: 857-61.
25. Svendsen BL, Hau J. Chicken eggs in polyclonal antibody production. Scand J Lab Anim Sci 1960; 23: 85-91.
26. Chuanjing W, Rong-Jiang Y, Ji Z, Shing B, *et al.* Production and characterization of a novel chicken IgY antibody raised against C-terminal peptide from human thymidine kinase 1. J Immunol Methods 2003; 277: 157-69.
27. Wu M, Chen Q, Wang YX. Preparation of egg yolk immunoglobulin (IgY) against p53 protein expressed in *E coli.* Di Yi Jun Da Xue Xue Bao 2003; 23: 491-3.
28. Di Lonardo AD, Marcante ML, Poggiali F, Hamsoikova E, Venuti A. Egg yolk antibodies against the E7 oncogenic protein human papillomavirus type 16. Arch Virol 2001; 146: 117-25.
29. De Ceuninck F, Pastoureau P, Agnellet S, Bonnet J, Vanhoutte PM. Development of an enzyme-linked immunoassay for the quantification of YKL-40 (cartilage gp-39) in guinea pig serum using hen egg yolk antibodies. J Immunol Methods 2001; 252: 153-61.
30. Sun S, Mo W, Ji Y, Liu S. Preparation and mass spectrometric study of egg yolk antibody (IgY) against rabies virus. Rapid Commun Mass Spectrom 2001; 15: 708-12.
31. Hlinak A, Schrodl W, Witt S, Schade R, Kruger M. Production of egg yolk antibodies against human cell associated antigens. ALTEX 1996; 13: 76-7.
32. Schade R, Henklein P, Hlinak A, de Vente J, Steinbusch H. Specificity of chicken (IgY) versus rabbit (IgG) antibodies raised against *Cholecystokinin octapeptide* (CCK-8). ALTEX 1996; 13: 80-5.
33. Frank MB. Antibody binding to protein A and protein G bead [online]. Available http://www.omrf.ouhsc.edu/~frank/ProteinA.html (15 April 2003).
34. Stalberg J, Larsson A. Extraction of IgY from egg yolk using a novel aqueous two-phase system and comparison with other extraction methods. Ups J Med Sci 2001; 106: 99-110.
35. Chang HM, Lu TC, Chen CC, Tu YY, Hwang JY. Isolation of immunoglobulin from egg yolk by anion polysaccharides. J Agri Food Chem 2000; 48: 995-9.
36. Hansen P, Scoble JA, Hanson B, Hoogenraad NJ. Isolation and purification of immunoglobulins from chicken eggs using thiophilic interaction chromatography. J Immunol Methods 1998; 215: 1-7.
37. Schwarzkopf C, Thiele B. Effectively of different methods for the extraction and purification of IgY. ALTEX 1996; 13: 35-9.

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