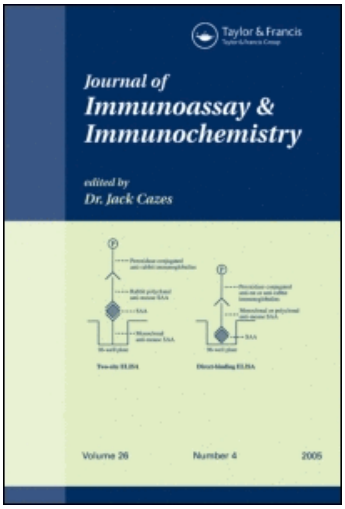

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

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To cite this Article Vieira, José Gilberto H. , Oliveira, Marli A. D. , Russo, Ewaldo M. K. , Maciel, Rui M. B. and Pereira, Aparecido B.(1984) 'Egg Yolk as a Source of Antibodies for Human Parathyroid Hormone (hPTH) Radioimmunoassay', Journal of Immunoassay and Immunochemistry, 5: 1, 121 — 129

To link to this Article: DOI: 10.1080/01971528408063002

URL: http://dx.doi.org/10.1080/01971528408063002

JOURNAL OF IMMUNOASSAY, 5(1&2), 121-129 (1984)

EGG YOLK AS A SOURCE OF ANTIBODIES FOR HUMAN PARATHYROID HORMONE (hPTH) RADIOIMMUNOASSAY

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ABSTRACT

Chickens were immunized with synthetic hPTH peptides 1-34 and 53-84. Serum from the best responder of each group was compared with IgG obtained from yolk of eggs layed by the same chicken, showing similar properties. A simple method for purification of IgG from yolk is described, allowing the obtaining of substantial amounts of anti-hPTH IgG without the need for bleeding the animals. We conclude that: 1) egg from chickens immunized with synthetic hPTH peptides are a convenient source of antibodies against these peptides; 2) this principle should apply to any other antigen to which chickens are good

 0197-1522/84/0501-0121$3.50/0

responders, (KEY WORDS: egg yolk IgG, hPTH radioimmunoassay, yolk IgG purification).

INTRODUCTION

The use of chickens as a source of antibodies for use in radioimmunoassay is increasing, specially where the phylogenetic distance between birds and mammals provides additional possibilities of obtaining antibodies reacting against mammalian antigens. Specifically in the field of radioimmunoassay (RIA) for human parathyroid hormone (hPTH) several investigators have already used chickens for immunization (1-3). However, the difficulty of bleeding and the small amount of blood obtained are important limitations to this procedure.

The transfer of specific antibodies, especially IgG, from hen serum to yolk is a well known process (4), and the use of eggs as a source of antibodies for various applications has recently been described (5). A hen laying several eggs per week could provide IgG equivalent to repeated bleedings, without any difficulty or harm to the animal.

Methods of separation of proteins (levitins) from lipoproteins (lipovitellins) and remaining yolk lipids have been already reported, and the processing of yolk on a routine basis can be rendered a simple procedure. We report herein a simple method for separating yolk IgG, and the results obtained with the immunization of chickens with synthetic hPTH peptides.

MATERIALS AND METHODS

Synthetic hPTH (1-34) used for immunization and iodination, and synthetic hPTH (53-84) used for immunization were obtained from Península Laboratories Inc., Belmont, California, U.S.A.. Synthetic hPTH (Tyr 52-84) used for iodination was obtained from Bachem Inc., Torrance, California, U.S.A..

Immunization

Six chickens were immunized by multiple intradermal injections, three with the 1-34 peptide and three with the 53-84 peptide. Each received 1,0 ml of an emulsion of equal parts of complete Freund's adjuvant (Difco) and 50 µg of the peptide in 100 mmol/ℓ acetic acid. Injections were repeated at monthly intervals for three months, and blood samples (from a wing vein) were obtained monthly after the third injection.

Egg Processing

A method based on that described by Burstein et al (6) was used. The collected eggs were kept at 4°C until processed. The separate yolk was diluted ten times with Tris-buffered saline (TBS: NaCl 140 mmol/ℓ; Tris/HCl 10 mmol/ℓ; pH 7,4; NaN_3 1 g/ℓ); the precipitate formed after dilution was separated by centrifugation. Precipitating solution, containing 1 part by volume of 3,0 mol/ℓ magnesium chloride and 5 parts by volume of phosphotungstic acid (48 g/ℓ) was added to the diluted yolk solution in a proportion of 1:10 parts by volume. After mixing,

the solution was kept at room temperature for 10 min and then centrifuged for 30 min at 4000 rpm. The clear supernatant was separated and a saturated solution of ammonium sulphate was slowly added under continuous stirring until a proportion of 33% was achieved. The procedure was carried out at 20°C, and after 15 min of stirring the solution was centrifuged for 15 min at 4000 rpm. The precipitate was solubilized in 10 ml of phosphate buffered saline (PBS: phosphate buffer 50 mmol/ℓ, NaCl 140 mmol/ℓ, pH 7,4), and dialysed against 2ℓ of PBS for 48 h at 4°C. The purified yolk IgG solution was finally diluted in an equal volume of glycerol (Merck) and kept at - 20°C. Recoveries were estimated by adding ^{125}I-labelled chicken IgG at the beginning of the process.

Iodination Procedure

The peptides were labelled by the chloramine T method (7) used as follows: 50 µℓ of phosphate buffer, 500 mmol/ℓ, pH 7,4 and Na ^{125}I (0,5 mCi, New England Nuclear, Boston, Mass., U.S.A.) were added to 10 µℓ of 100 mmol/ℓ acetic acid solution containning 0,6 µg of the peptide (Tyr 52-84 or 1-34). Chloramine T (50 µg) in 10 µℓ phosphate buffer 50 mmol/ℓ, was then added followed by an oxidation time of 30 seconds at room temperature. 150 µg of sodium metabisulphite in 50 µℓ of phosphate buffer 50 mmol/ℓ were used to stop the reaction (reagents from Merck Ind. Quîmicas, Rio de Janeiro, Brasil). The final reaction product was applied to a 0,9 X 20 cm column of Sephadex G-15 (Pharmacia

Fine Chemicals, Uppsala, Sweden) equilibrated with a ammonium acetate buffer 100 mmol/ℓ, pH 3,0, and eluted with the same buffer. The fractions containing the labelled immunoreactive peptide were pooled, divided in aliquots of 200 µℓ and stored at - 20°C. The specific activity obtained averaged 300 µCi/µg for the Tyr 52-84 peptide and 120 µCi/µg for the 1-34 peptide. Chicken IgG, purified by DEAE-cellulose chromatography, was also labelled by the chloramine T procedure using 500 µCi of Na ^{125}I and 100 µg of IgG.

Radioimmunoassay procedures

The buffer used throughout (RIA buffer) was phosphate 50 mmol/ℓ, pH 7,4, with 1 g/ℓ BSA (Sigma Fraction V) and 1 g/ℓ NaN_3. Standard curves were obtained by incubating 0,1 ml of the appropriate dilutions of the peptide, in assay buffer, with 0,1 ml of the antiserum diluted to produce 30-35 percent binding when no unlabelled peptide was present. Following an incubation at 4°C for 2 days, 0,1 ml of the respective labelled peptide (3.000-4.000 cpm for the 1-34 and 15.000 cpm for the 53-84 assay) was added and the incubation continued for 2 additional days at 4°C. Separation of bound and free ^{125}I - peptides was achieved by the addition of 0,1 ml PTH-free serum (charcoal treated outdated serum) followed immediatly by addition of 1,0 ml of a 175 g/ℓ solution of PEG 4.000, mixing and centrifugation at 2.500 rpm for 30 min at 4°C. After the removal of the supernatants, the pellets were counted in a scintillation spectrophotometer.

Results were expressed as percentage of the counts bound in the zero standard tubes, with correction for non-specificity.

RESULTS

Egg processing

Recovery of the yolk IgG during the purification procedure was studied using the ^{125}I-labelled chicken IgG. In five simultaneosly treated yolks, a mean recovery of 63% was obtained at the end of the procedure. The sequential study showed a mean recovery of 85% during the first step (precipitating of yolk lipids). Immunoelectrophoresis using a rabbit antiserum anti-total chicken serum showed that the protein obtained (after the yolk processing) consisted mainly of IgG (data not show).

Antibody production

Antibodies were produced by all chickens although only one of each group were of sufficient titer and affinity to be used in a radioimmunoassay procedure. Figure 1 shows the evolution of the titer (serum and yolk) in these two animals. Note that chicken # 6 (hPTH 1-34 group) mantained a stable titer during the whole study, whereas chicken # 5 (hPTH 53-84 group) responded to one booster injection with the titer declining thereafter.

Antibody Characteristics

Figure 2 shows the comparison between the yolk-derived IgG and serum in the hPTH (53-84) system. The standard curves and

the coefficients of affinity obtained were similar, suggesting that the same population of IgG anti-hPTH (53-84) were present in both assay systems. The results of the same study using the hPTH (1-34) system are shown in Figure 3. The slight differences observed in the coefficients of affinity and the 50% displacement may be related to higher B/T of curve B (yolk IgG).

DISCUSSION

In order to produce specific antibodies against synthetic hPTH peptides, we immunized different animal species, including chickens. The work of Jensenius et al (5) oppened the possibility of obtaining specific anti-hPTH Ig from eggs of chickens that were good responders. This helped to circumvent two intrisinc problems related to the production of antibodies in such small animals: the difficulties of bleeding and the small amounts of sera that can be obtained in each sampling.

The method of egg processing here described is simple and reproducible, yielding good recovery. Considering that IgG is the main Ig present in egg yolk (4) and the standard curves obtained with serum and yolk Ig, one can conclude that be desired specific IgG anti-hPTH were indeed extracted from the eggs. The parallel increase in serum titer and the appearence of specific antibodies in the yolk (Figure 1) corroborates these conclusions.

These findings demonstrate that: 1) antibodies against synthetic hPTH peptides can be raised in the chicken; 2) these antibodies are transfered to egg yolk and; 3) they can be easily extracted, yelding a substancial amount of antibodies without harm to the animal. We believe that this principle can be applied to any other antigen to which chickens are good responders, enhancing the importance of this animal species as an antibody producer.

ACKNOWLEDGEMENTS

We thank Prof. Dr. R.-D. Hesch, Medizinische Hochschule Hannover, West Germany, for his encouraging support and for reviewing the manuscript. We also thank the Laboratorio Fleury, São Paulo, Brasil, for the techinical support, Dr. Carlos Henrique de Mesquita and Dr. Claudio E. Kater for their help in preparing this manuscript.

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