

Evaluation of Allergenicity of Egg Yolk Immunoglobulin Y and Other Egg Proteins by Passive Cutaneous Anaphylaxis

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The allergenicity of egg yolk immunoglobulin (IgY) was studied using passive cutaneous anaphylaxis. It was observed that the intact yolk, water soluble fraction of the yolk, whole IgY molecule or its antigen binding (Fab') and crystallizable (pFc') fragments produced by pepsin digestion, elicited low IgE antibody response in mice when compared to egg white, a known allergen. The pFc' fragment of IgY was more antigenic than the Fab' fragment as determined by ELISA. Low cross-reactivity was also observed between the egg yolk proteins and egg white antibodies. The low IgE antibody response to the egg yolk proteins was associated with a higher suppressor T-lymphocytes or lower helper T-lymphocytes, or both, in comparison to egg white derived lymphocytes.

Keywords: *Allergenicity, IgY, egg proteins, passive cutaneous anaphylaxis*

INTRODUCTION

Utilization of immunoglobulin Y (IgY) from chicken eggs in immunotherapy and immunodiagnostics has been proposed by many researchers. Some of the potential uses of IgY in immunotherapy include the prevention or treatment of traveller's diarrhoea and prevention of dental carries. A potential problem which may be encountered with the utilization of IgY is allergenicity, since the structure of IgY is considerably different from that of mammalian IgG (Parvari *et al.*, 1988). Indirect evidence for the allergenicity of IgY was provided by Anet *et al.* (1985), who used radioallergosorbent test (RAST) and immunoblot analysis to compare the IgE binding of egg white and yolk. The main allergens were found in egg white, however, for a large proportion of the egg-sensitive patients, specific IgE binding to yolk constituents were also reported. The allergens were associated with each of the three yolk fractions; namely, the granules, livetins (fraction containing immunoglobulin) and low density lipoproteins. Bernhisel-Broadbent *et al.* (1991) reported that some egg-allergic patients exhibited specific IgE binding against one or more egg yolk derived anti-viral IgY.

Furthermore, cross-reactivity between bird dander and hen's egg has been reported, with livetins, especially, α -livetins being the major cross-reacting antigens (de Blay *et al.*, 1994; Szepefalusi *et al.*, 1994; Mandallaz *et al.*, 1988). This IgE-mediated allergy is designated as bird-egg syndrome. Consequently, for a small number of people who may be allergic to egg proteins, an attempt should be made to reduce or eliminate the allergenicity of IgY by chemical or physical modification.

It is known that the Fc fragment of the mammalian IgG molecule is the more antigenic portion of the molecule (Porter, 1959; Samesto *et al.*, 1983). We reasoned that removal of the Fc fragment could lower the number of possible allergenic sites on the IgY molecule without adversely affecting its function. Since IgY could be administered as the egg yolk (EY) or the water soluble fraction from egg yolk (WSF), the main objective of this experiment was to compare the relative allergenicity of EY, WSF, IgY and its Fab' and pFc' fragments to egg white (EW), a well known allergen. A secondary objective was to study the potential cross-reactivity between EW and the EY proteins.

MATERIALS AND METHODS

Animals

BALB/c male mice and Sprague Dawley rats were purchased through The University of British Columbia's Animal Care Centre. Mice were used at the age of 8–10 weeks while rats were used at the age of 10–12 weeks. Animals were fed a commercial diet (Laboratory rodent diet # 5001, PMI Feeds Inc., St Louis, MO, USA) in pellet form. This diet contained ground yellow corn, soy bean meal, dried beet pulp, fish meal, ground oats, brewers dried yeast, alfalfa meal, cane molasses, wheat germ meal, dried whey, meat meal, wheat middlings, animal fat preserved with butylated hydroxyanisole salts and essential minerals and vitamins. Mice were randomly selected and housed in groups of five or six in plastic cages and rats were housed in groups of two in metal cages. Animals were kept under conditions of controlled temperature (20–23°C) and lighting (alternating 12-h periods of light and dark).

Preparation of Antigens

Egg yolk and egg white. EY was separated from the EW, washed with distilled water to remove as much albumen as possible and rolled on a paper towel to remove adhering EW. The membrane was punctured and the yolk collected without the membrane. EY was diluted (10X) with phosphate-buffered saline (PBS, 0.01 M -sodium phosphate buffer, containing 0.14 M -NaCl, pH 7.0) and stored frozen until used. EW was diluted (20X) with PBS and stored frozen until used.

Water soluble fraction of egg yolk. EY was diluted with nine volumes of distilled water (acidified with 100 mM -HCl, predetermined to give a pH of 5.0 after dilution), and held for 6 h before filtration through Whatman No. 1 filter paper at 4°C. The filtrate or WSF was filter sterilized through a 0.45 μ m membrane and kept at 4°C until used. The WSF, so prepared, contained less than 1.5% of lipids in the original yolk.

IgY. The IgY was purified from WSF by successive precipitations with 18 and 14% sodium sulphate. The IgY (over 96% purity) was further purified by gel filtration using Sephacryl S200 (Pharmacia Biotech. Inc., Uppsala, Sweden) equilibrated with PBS (Akita & Nakai, 1992). Pure IgY was filter sterilized through a 0.45 μ m membrane and kept at 4°C until used.

Fab' and pFc' fragments. Fab' and pFc' fragments were prepared by digestion of IgY with pepsin in 50 mM -sodium acetate buffer at pH 4.2 for 3 h (Akita & Nakai, 1993). Digestion

was stopped by adjusting the pH to approximately 8. Gel filtration (Sephadex G-25, PD10 column; Pharmacia Biotech. Inc., Uppsala, Sweden) was used to exchange enzyme digest buffer into 10 mM -Tris-HCl buffer, pH 8.6. The enzyme digest was then applied to a DEAE-Sephacel column (2.0 × 4 cm) equilibrated with 10 mM -Tris-HCl buffer, pH 8.6, at a flow rate of 1.0 ml min⁻¹. The column was then washed with five bed-volumes of equilibration buffer. Pure Fab' was eluted with 100 mM -Tris-HCl, pH 8.0. Pure pFc' was eluted by 100 mM -Tris-HCl, pH 8.0, containing 200 mM -NaCl. Purified Fab' and pFc' were dialysed extensively against PBS for 48 h with several changes of the buffer, filter sterilized through 0.45 µm membrane and kept at 4°C until used.

Immunization

Groups of five BALB/c mice per treatment were immunized intraperitoneally with 10 µg of antigen adsorbed on 1 mg of aluminum hydroxide gel. Booster injections were given on days 30, 119 and 250 post primary challenge. Immunized mice were bled on days 43, 129 and 263 post primary challenge after a first, second and third booster injection, respectively. Antiserum was prepared from the collected blood as described by Akita & Nakai (1990). The antiserum thus prepared was stored at -20°C until used.

Passive Cutaneous Anaphylaxis

The IgE antibody specific for the antigens in mouse serum was assayed by heterologous passive cutaneous anaphylaxis (PCA) reaction in rat skin as described by Akita & Nakai (1990). However, in this study the amount of doubling dilutions of antiserum used for intradermal sensitization of rats was reduced from 200 µl to 100 µl. PCA titre was expressed as the reciprocal of the highest dilution of the antiserum with positive reaction, i.e. blueing of injection site. The absence of a reaction at five-fold dilution of the antiserum was regarded as no response. Variations of the titre for each sample was less than two-fold. Consequently, as reported by several researchers, variations of four-fold or more, were considered statistically significant (Tamura & Ishizaka, 1978; Pesce *et al.*, 1990; Hagen *et al.*, 1992). Heating the antisera at 56°C for 2 h eliminated the PCA reaction, thus confirming the PCA reaction to be IgE specific.

ELISA

ELISA was performed as described by Akita & Nakai (1990) with slight modifications. Immulon 2 microtiter plate (96 wells, Dynatech Laboratories Inc., Chantilly, VA, USA) was used as the solid support. Wells were coated with 100 µl of 0.01% of antigens (egg proteins) in PBS at 37°C for 1 h. Plates were washed three times with PBS-Tween (0.05% Tween 20), followed by a blocking step using 100 µl of 2% bovine serum albumin (BSA) for 30 min at 37°C. Plates were incubated with the appropriate egg proteins antisera (1000 × dilution) from mice, for 1 h at 37°C. The plates were then washed again three times with PBS-Tween and 100 µl of rabbit anti-mouse IgG coupled to alkaline phosphatase (Sigma Chemical Co., St. Louis, MO, USA, 1:1000 in PBS-Tween) were added to each well. After 1 h incubation at 37°C, the plates were washed again followed by addition of 50 µl of freshly prepared substrate solution (0.1% *p*-nitrophenyl phosphate disodium in diethanolamine buffer, pH 9.8). Absorbance was read at 405 nm using a ER-400 ELISA reader (SLT Labinstruments, Salzburg, Austria). For each plate, appropriate controls were included and corrections for non-specific binding of IgY antibodies and enzyme-labelled antibodies were done.

Isolation of Spleen Cells

Each animal was killed by cervical dislocation 13 days after the third booster injection was given. The spleen was removed, ruptured gently with a Ten Broek Homogenizer (Corning Inc. Corning, NY, USA) in 6 ml of minimum essential medium (MEM) containing 2% fetal

calf serum (FCS). Cells were transferred through filter mesh into 50 ml centrifuge tubes, brought up to 25 ml, centrifuged at $300 \times g$ for 5 min at 4°C and resuspended in 3 ml cold media. Cells from the same treatment were pooled.

Determination of Cell Viability

Cell viability was determined by the trypan blue exclusion method after lysing of red blood cells. Lysis was done by diluting the cell suspension 15 times with a lysing solution (8.99 g NH_4Cl , 1 g KHCO_3 and 37 mg tetrasodium EDTA in 1 l of distilled water) at room temperature. The cell-lysing mixture was allowed to stand for 4 min and centrifuged at $300 \times g$ for 5 min at 4°C. Cell pellets were washed twice with cold PBS and resuspended in the same volume of cold PBS + 2% FCS prior to lysis. Percent viable cells was determined by measuring the number of viable lymphocytes using trypan blue staining (25 μl of cell suspension diluted with 975 μl 0.1% trypan blue) and total number of lymphocytes using acidified methylene blue staining (25 μl of cell suspension diluted with 975 μl of 0.1% methylene blue + 2% acetic acid). All cell enumerations were made using a haemocytometer. Cell viability was found to be at least 70%.

Immunofluorescent Staining

Fluorescent staining was done in microtitre wells. Fifty μl of the 20 times diluted fluorescence labelled monoclonal antibodies (Becton Dickinson, Immunocytometry Systems, San Jose, CA, USA) generated against mouse Thy1.2 (total), Lyt 2 (suppressor or cytotoxic) and L3T4 (helper) T-lymphocytes were added to the microtitre wells. Cell suspension (50 μl , 2×10^7 cells ml^{-1}) was added to the wells and allowed to react for 30 min on ice. The cells were washed three times with PBS and cell samples were analysed with fluorescence activated cell sorter (FACS Star Plus, Becton Dickinson and Co., San Jose, CA, USA). 10 000 cells were scattered per sample. Results were expressed as percentage labelled cells of the total population.

Protein Determination

Protein content was determined by the micro Kjeldahl method of Concon & Soltess (1973). After digestion, the nitrogen content was determined on a Technicon Auto Analyzer II system. Protein content was calculated from nitrogen content using the conversion factor of 6.25.

RESULTS

Antigenicity of Egg Proteins

The antigenicity of egg proteins or their capacity to react with IgG antibodies raised against them was evaluated by ELISA. All of the antigens were found to be immunogenic (Figure 1). Similar observations were made after the first and second booster injections (results not shown). The results also show that the antigenicity of Fab' fragment is relatively lower than the pFc' fragment or the whole IgY molecule. However, it may be argued that the observed lower antigenicity of Fab' is a reflection of its lower binding capacity to the microtitre plate. Consequently, using IgY as the antigen, binding with antisera of IgY, pFc' and Fab' were assayed by ELISA (Figure 2). The higher absorbance values obtained for pFc', suggest either the presence of more antigenic sites or more antibodies raised against the pFc' portion of the molecule. It could therefore be inferred from these results that the pFc' fragment appeared to be more antigenic than the corresponding Fab' fragment.

Allergenicity of Egg Proteins

Heterologous PCA in rats was used to measure the relative efficacy of the egg proteins to generate IgE antibodies. PCA was performed with blood collected after first, second and third booster injections of mice. EW was found to be more allergenic than the yolk, showing a dramatic 16-fold increase in the PCA titre after the second boost (Table 1). Under the conditions

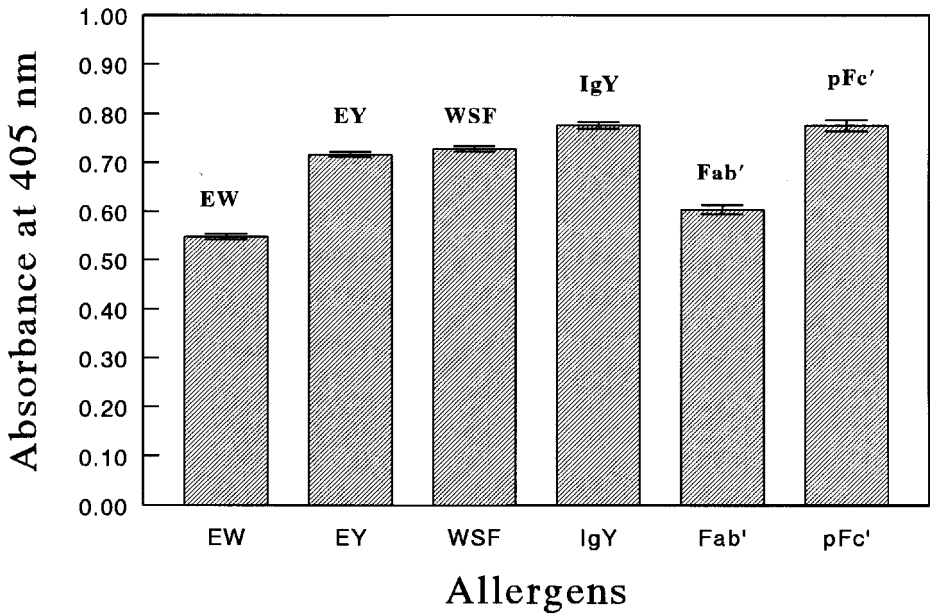


FIG. 1. Determination of capacity of egg proteins to produce specific IgG antibodies by indirect ELISA. Mice were bled 13 days after the final booster injection and pooled antisera ($1000 \times$ dilution) from each group was used for ELISA assay. Values are means of triplicate determinations $\pm$ standard deviations. The antisera are indicated on the top of bars.

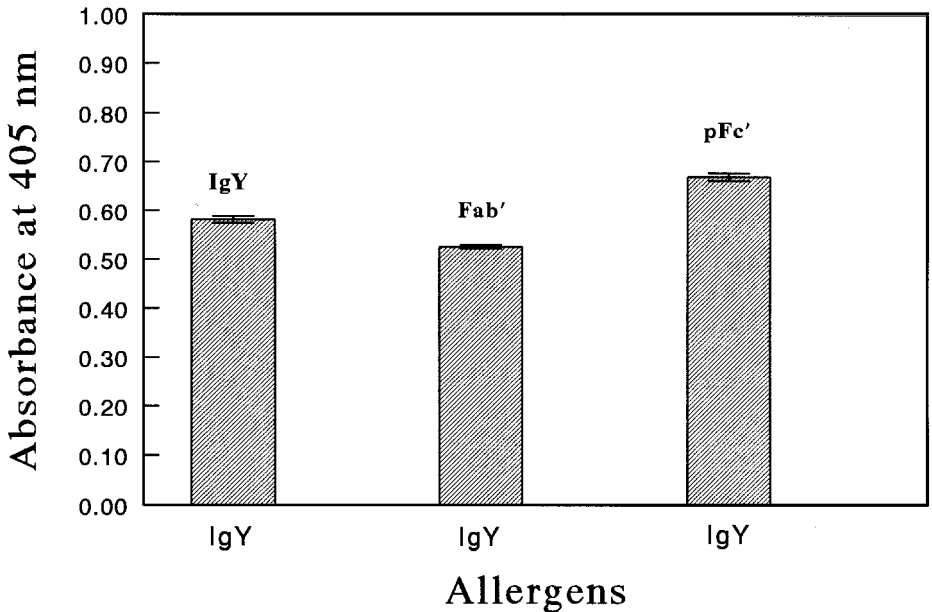


FIG. 2. Comparison of the antigenicity of IgY and its Fab' and pFc' fragments as determined by indirect ELISA. Mice were bled 13 days after the final booster injection and pooled antisera ($1000 \times$ dilution) from each group was used for ELISA assay. Values are means of triplicate determinations $\pm$ standard deviations. The antisera are indicated on the top of bars.

TABLE 1. Passive cutaneous anaphylaxis (PCA) titre in rats from mice immunized with different egg proteins and challenged by their corresponding proteins

Antigen ^b	Antisera	PCA titre values ^a		
		Days of bleeding		
		13 days after first boost	10 days after second boost	13 days after third boost
EW	Anti-EW	40	640	160
EY	Anti-EY	nr ^c	nr	nr
WSF	Anti-WSF	nr	40	20
IgY	Anti-IgY	nr	5	nr
Fab'	Anti-Fab'	10	20	10
pFc'	Anti-pFc'	20	40	20

^aPCA titres are reciprocals of the highest dilutions to give a positive response (> 4 mm blue lesion).

^bEW = egg white; EY = egg yolk; WSF = water soluble fraction of egg yolk.

^cnr = no response at the 1:5 dilution.

of the experiment, egg yolk failed to produce detectable IgE antibodies although it was found to be immunogenic (Figure 1). IgY also elicited no detectable IgE antibodies in mice after the first boost. A very low titre of 5 was obtained after the second boost. The WSF, Fab' and pFc' fragments showed low allergenicity compared to EW, but were more allergenic than IgY.

After the third boost, EW was still the most allergenic of the allergens studied, with EY and IgY showing no detectable IgE. However, the PCA titres were lower compared to the PCA values after the second boost. For example, a four-fold reduction for the EW and a two-fold reduction for WSF, Fab' and pFc' fragments were observed.

Cross-reactivity Between Egg Proteins

Cross-reaction has been demonstrated between EY and EW proteins (Anet *et al.*, 1985). Although IgY and its fragments showed low allergenicity, the potential cross-reactivity with other egg proteins was investigated. Using ELISA, the cross-reactivity between EW and IgG antibodies elicited against the yolk proteins were studied (Figure 3). Although some cross-reactivity was observed with WSF and pFc', essentially, no cross-reactivity was obtained between EW and the specific IgG antibodies produced against EY, IgY and the Fab' fragment.

When the cross-reactivities between the egg proteins were investigated using PCA, a number of interesting observations were made (Table 2). IgE antibodies against EW demonstrated very low cross-reactivity with EY proteins. No PCA response was observed when antisera raised against egg white were challenged with IgY or the Fab' fragment, or when antisera raised against IgY or the Fab' fragment were challenged with EW. The WSF and pFc' fragment both yielded very low PCA titres of 5 when used to challenge antisera from EW. When the antisera raised against the WSF was challenged with IgY, no PCA response was observed. This result suggest that the allergenicity demonstrated by WSF could be primarily due to other proteins present in the WSF. This observation and the failure of IgY antisera to give detectable PCA response when challenged with its fragments, is a confirmation of its limited capacity to elicit IgE antibodies in mice.

An interesting observation made from this experiment was the failure of pFc' antisera to react with the Fab' fragment, although the reaction between Fab' antisera and the pFc' fragment gave a PCA titre of 10. Although this may not be significant, a possible explanation for this finding is that there may be more allergenic sites, unique to the pFc' portion, on the IgY molecule.

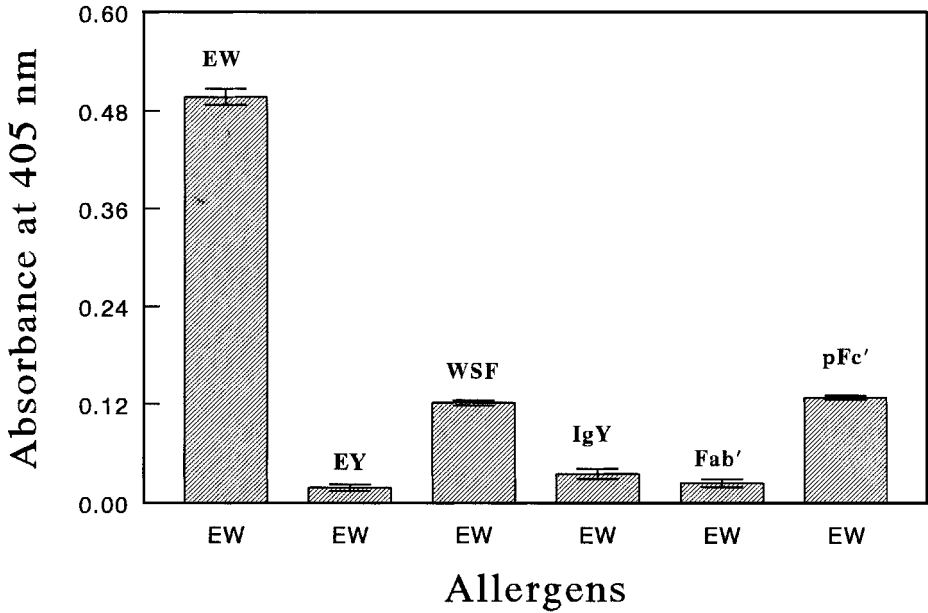


FIG. 3. Determination of cross-reactivity between egg white and egg yolk proteins antisera from mice by indirect ELISA. Mice were bled 13 days after the final booster injection and pooled antisera (1000 $\times$ dilution) from each group was used for ELISA assay. Values are means of triplicate determinations $\pm$ standard deviations. The antisera are indicated on the top of bars.

Determination of Murine T-Lymphocyte Subclasses

The phenotypes of the T-lymphocyte population produced by the various allergens are summarized in Table 3. This technique involved tagging T-lymphocyte surface markers labelled with fluorescence probes to lymphocytes isolated from mice spleen. The lymphocytes were then sorted according to the surface markers using a fluorescence antibody cell sorter. Mice immunized with EW showed lower levels of suppressor T-lymphocytes

TABLE 2. Passive cutaneous anaphylaxis (PCA) titre in rats from mice immunized with different egg proteins and challenged with other egg proteins for cross reactivity

Antisera ^c	PCA titre values ^a				
	Antigen ^b				
	EW	WSF	IgY	Fab'	pFc'
Anti-EW	640	5	nr ^d	nr	5
Anti-EY	nr	nr	nr	nr	nr
Anti-WSF	10	40	nr	nr	5
Anti-IgY	nr	nr	5	nr	nr
Anti-Fab'	nr	10	20	20	10
Anti-pFc'	nr	10	20	nr	40

^aPCA titres are reciprocals of the highest dilutions to give a positive response (> 4 mm blue lesion).

^bEW = egg white; WSF = water soluble fraction of egg yolk.

^cPooled antisera from mice in groups of five were prepared 10 days after the second booster injection and used for the PCA assay.

^dnr = no response at the 1:5 dilution.

TABLE 3. Determination of T-lymphocyte subclasses following immunization with some egg proteins in the spleen of mice

Antigen ^c	% Lymphocyte subclasses ^{a,b}		
	Thy 1.2	Lyt 2	L3T4
EW	34.4	6.6 (19.2)	28.4 (82.6)
EY	20.6	8.0 (38.8)	13.3 (64.6)
WSF	28.2	6.9 (24.5)	19.7 (70.0)
IgY	22.4	6.4 (28.6)	17.1 (76.3)
Fab'	39.8	10.1 (25.4)	24.5 (61.6)
pFc'	29.6	6.8 (23.0)	19.8 (66.9)
Control ^d	35.7	10.3 (28.7)	24.9 (69.8)

^aValues are means of duplicate determinations with deviations of less than 10%.

^bValues in parentheses correspond to % T-lymphocyte subclass of the total T-lymphocyte population.

^cEW = egg white, EY = egg yolk; WSF = water soluble fraction of egg yolk.

^dControl refer to the group immunized with aluminum hydroxide in PBS without test allergens.

(19.2%), compared to the control group (28.7%); and a higher level of helper T-lymphocytes (82.6%), compared to the control group (69.8%). On the other hand, unusually high levels of suppressor T-lymphocytes (38.8%) were obtained from the group immunized with EY.

The determination of the T-lymphocyte subclasses described herein, however, may be subject to some criticism. This method allowed the determination of the non-specific total T-lymphocyte and the subclasses. It may be argued, that in order to delineate the specific effect of the test allergens, the lymphocytes, specific against the test allergens should have been determined. However, this may present a technical problem since it is well known that T-cells recognize only processed antigens in the context of MHC antigens and do not normally recognize native antigens (Brandtzaeg *et al.*, 1991). Furthermore, the use of an enriched T-lymphocyte population may have improved the assay, since this may reduce the variations in the assay. In spite of these potential limitations, the data obtained by this assay may contribute to the possible explanations of some of the observations made in this study.

DISCUSSION

Egg proteins are well recognized as a major cause of food allergy in humans, especially in infants (Anet *et al.*, 1985). Since most of these allergens are associated with EW, many of the initial studies were focused on EW proteins. Consequently, ovalbumin (Elsayed *et al.*, 1986), ovomucoid (Kurisaki *et al.*, 1981; Matsuda *et al.*, 1985) and conalbumin (Langeland & Harbitz, 1983) have been studied in some detail. However, some of the EY proteins have since received increased attention. EY proteins such as phosvitin, apovitellenin I and VI (Walsh *et al.*, 1988) and IgY (Bernhisel-Broadbent *et al.*, 1991) have also been identified as potential allergens. These studies are based on the detection of allergen specific IgE by RAST in egg-sensitive patients. In this work, an animal model was used to study the capacity of some egg proteins to elicit IgE antibodies. Heterologous PCA, i.e. using mouse antibodies in the rat, was used to determine allergenicity since this test allows selective detection of functional mouse IgE (Ovary *et al.*, 1975).

The immune response of antigens is affected by a number of factors including nature and dose of antigen, use of adjuvants, the route of immunization, and the animal species and

strain (Hu *et al.*, 1990). Consequently, a variety of immunization schedules have been used to generate specific antibodies against various antigens. It is a rather difficult task, if not impossible, to select the best immunization schedule. Despite these uncertainties in the study of allergenicity of proteins in mice, the best results are obtained using high respondent mouse strains e.g. BALB/c (Chiorazzi *et al.*, 1977), aged between 8–10 weeks, with antigen doses between 0.1–10 µg per mouse and aluminum hydroxide as the adjuvant (Levine & Vaz, 1970; Holt *et al.*, 1981; Kawamura *et al.*, 1985). Consequently, these conditions were used in the immunization protocol.

The observation that EW was found to be allergenic is in agreement with several studies (Langeland, 1982; Hoffman, 1983). The high allergenicity of EW could be explained in part by the presence of low levels of suppressor and high levels of helper T-cell sub-populations in mice exposed to this protein. This combination may tend to facilitate the production of IgE antibodies.

In the present work, EY failed to elicit detectable IgE response, although it was found to be antigenic as determined by ELISA. Egg yolk contains about 30% lipids which are covalently or non-covalently bound to the proteins, especially in fresh eggs (Burley & Vadehra, 1989). It is known that attaching a hydrophobic ligand, e.g. fatty acid, suppresses the allergenicity of proteins (Segawa *et al.*, 1981; Akita & Nakai, 1990). Segawa *et al.* (1981) found that not only did such modified antigens have no immunogenicity by themselves, but they were also able to suppress both primary and secondary IgE antibody formation against the native antigen in mice upon injection both before and after the primary immunization with the modified antigen. These workers attributed the suppression of immunogenic effect to the priming of suppressor T-cells by the hydrophobically modified protein. In this work, a relatively high suppressor T-cell population was obtained for EY. Therefore, a possible explanation for the hypoallergenicity of EY could be the presence of lipids which may induce suppressor T-cell formation, consequently, suppressing the allergenicity of the EY proteins.

WSF, which consist of livetins and low density lipoproteins was found to elicit more IgE antibodies than IgY. This observation was attributed to the presence of other allergenic proteins in the WSF. However, because of the relative low capacity of WSF to elicit IgE antibodies compared to EW, administration of IgY as the WSF could provide an alternate and more economic way of administering IgY.

In this research, the reduction of allergenicity of IgY was based on the premise that the pFc' fragment may be more allergenic than the Fab' fragment. Consequently, removal of the pFc' fragment through enzymatic digestion is likely to reduce the allergenicity of the IgY. Our results showed that the pFc' fragment is indeed more antigenic than the Fab' fragment. However, removal of the pFc' portion failed to produce a molecule with reduced allergenicity. Although the Fab' was hypoallergenic, it was found to be relatively more allergenic than whole IgY molecule. A possible explanation is the smaller size (44 000 Da) of the Fab' molecule compared to IgY (180 000 Da; Akita & Nakai, 1993). It has been reported that the ideal size of food allergens range between 10 000 and 70 000 Da due to bridging requirement, capability of protein to initiate immune response and intestinal permeability (Taylor, 1992). It appears therefore, that the potential gain in reduced allergenicity by removal of some of the antigenic sites on the IgY molecule may have been offset by the reduction in molecular size.

Since most egg allergies are mainly attributed to the EW, the possibility of high cross-reactivity between EW and IgY or its Fab' fragment could increase the overall allergenicity of IgY and the Fab' fragment. The low cross-reactivity observed between the highly allergenic EW proteins and the IgY and its Fab' fragment may be an indication that these proteins do not share many common allergenic sites and therefore, the overall allergenicity of IgY and its Fab' fragment will not increase. Direct extrapolation should not be made from this rodent animal study to a human system. However, from the apparent lack of many shared allergenic sites between EW and IgY or the Fab' fragment, it is not unreasonable to expect similar observations in human subjects.

Edwards *et al.* (1983) reported that workers exposed to egg spray in a factory that produced dried EY, commonly had IgG precipitating antibodies against EY, but possessed no specific IgE antibodies, thereby suggesting that sensitization by EY may be uncommon. Even among atopic paediatric patients, Bernhisel-Broadbent *et al.* (1991) reported that 15 (54%) of 28 egg-hypersensitive patients, showed specific IgE binding against one or more of EY-derived antiviral chicken immunoglobulins, using immunodot blot analysis. It should be noted that the presence of allergen specific IgE as determined by immunodot blot assay in the above work may not be necessarily an indication of functional IgE. It is therefore conceivable that the actual number of patients with allergies directly attributed to IgY is smaller than expected.

In conclusion, EW was found to be more allergenic than the EY proteins studied, which included the whole yolk, the water soluble fraction of the yolk, IgY, and its Fab' and pFc' fragments. The low cross-reactivity observed between the EY proteins and EW may facilitate utilization of IgY whether administered as the yolk, the water soluble fraction, the purified whole IgY molecule or its Fab' fragment.

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