

ORIGINAL ARTICLES

Detection of four distinct groups of hen egg allergens binding IgE in the sera of children with egg allergy

B.J. Walsh^a, D.J. Hill^b, P. Macoun^a, D. Cairns^c and M.E.H. Howden^d

^aSchool of Chemistry, Macquarie University, North Ryde, N.S.W., Australia. ^bAllergy and Clinical Immunology Unit, Royal Childrens Hospital, Parkville, Vic., Australia. ^cSchool of Behavioural Sciences, Macquarie University, North Ryde, N.S.W., Australia. ^dDepartment of Biological Sciences, Deakin University, Geelong, Vic., Australia.

ABSTRACT

Background: There appears to be a lack of agreement in the literature on the allergenicity of hen egg proteins. This may be partly due to the use of impure proteins in some cases. Egg yolk proteins have also been largely ignored in such studies. We therefore set out to determine, using especially purified proteins, their relative allergenicity, and to observe whether there were any relationships between their potency and the sensitivity of patients to them.

Methods and results: The sera of 40 patients with clinically observed hen egg hypersensitivity were tested for specific IgE binding to purified egg white and egg yolk proteins using the radioallergosorbent test (RAST). Statistical treatment by correspondence analysis of the percent radioactive uptakes in the RAST to the 8 proteins demonstrated that there were four distinct groups of patients reacting in a similar way to four discrete sets of proteins.

Conclusions: The first three sets of allergens consisted of egg white proteins as follows: firstly, lysozyme and ovalbumin; secondly, ovomucoid; and thirdly, ovomucin. The fourth set contained the egg white protein ovotransferrin and the egg yolk proteins apovitellenins I and VI and phosvitin. The exis-

tence of patient groups may explain why various workers have reported different allergens to be important in egg hypersensitivity. A sufficiently large number of patients must be examined so as to give a representative distribution across each group, otherwise the results may be biased towards one allergen.

Key words: Hen egg. Food-allergy. Specific IgE. Allergens. Radioallergosorbent test. Statistical analysis.

RESUMEN

Antecedentes: Parece haber una falta de acuerdo en la literatura de la alergenicidad de las proteínas del huevo de gallina. En algunos casos esto puede deberse en parte al uso de proteínas impuras. Además, en tales estudios las proteínas de la yema del huevo han sido mayormente ignoradas. Por consiguiente nos hemos dispuesto determinar su relativa alergenicidad, usando proteínas especialmente purificadas y observar si había alguna relación entre su potencia y la susceptibilidad de los pacientes hacia ellas.

Métodos y resultados: Los sueros de 40 pacientes con clínicamente observada hipersensibilidad al huevo de gallina fueron examinados por fijación de IgE específica a proteínas purificadas de la clara y de la yema del huevo, utilizando para ello la prueba de radioalergenosorbente (RAST). El tratamiento estadístico por análisis de correspondencia del porcentaje radiactivo obtenido aplicando el RAST a las 8 proteínas demostró que habían cuatro grupos distintos de pacientes que reaccionan de manera similar a cuatro grupos separados de proteínas.

Conclusiones: Los tres primeros grupos de alérgenos consistían de proteínas de la clara del huevo,

Correspondence:

Dr. Merlin Howden
297 Halcrows Road
Glenorie, NSW 2157
Australia

contenían: primero, lisozima y ovoalbúmina; segundo, ovomucoide; y tercero, ovomucina. El cuarto grupo contenía la proteína de la clara ovotransferrina y las proteínas de la yema apovitellenins I y VI y fosfovitina. La existencia de grupos de pacientes puede explicar por qué diversos investigadores han indicado que los diferentes alérgenos son importantes en la hipersensibilidad del huevo. Un número suficientemente grande de pacientes deben ser examinados para poder obtener una distribución representativa en cada grupo, de lo contrario los resultados pueden denotar parcialidad a un alérgeno.

Palabras clave: Huevo de gallina. Hipersensibilidad a los alimentos. Alérgenos. IgE y específicos. Radioalergenosorbente. Análisis estadístico.

INTRODUCTION

As early as 1912 various proteins of egg white were implicated in egg allergy by both *in vivo* and *in vitro* investigations¹. The first report examined a child with acute egg hypersensitivity in which egg-white and ovomucoid, but not egg yolk, were reported to be active allergens by food challenge. Ovoglobulin and ovomucin were found to have only 25 % of the activity of ovomucoid¹. Miller and Campbell², on the other hand, reported that individuals sensitive to egg white reacted to ovomucoid, ovomucin, ovalbumin and lysozyme by skin test. There was no overall pattern of reaction and they suggested that sensitisation to egg white allergens is dependent upon individual variation rather than the nature of the antigen. Ovomucoid was reported to be the major skin-reactive protein in both raw and cooked egg white by Bleumink and Young³. They observed no reaction to ovalbumin, ovotransferrin and ovoglobulin. However, they did observe a reaction to lysozyme which they attributed to its irritant properties. Earlier investigations of raw and cooked egg are in conflict with this report. Ratner et al⁴ with the aid of passive transfer tests found that ovomucoid-sensitive individuals reacted to hard-boiled eggs while patients allergic to ovalbumin and ovoglobulin tolerated hard-boiled eggs. Conversely, Rhoden and Sutherland⁵ found that patients allergic to raw egg white reacted strongly to ovomucoid, while those sensitive to the heat labile portion of egg white also reacted with ovomucoid.

With the advent of improved immunological assays such as the radioallergosorbent test (RAST) the ability to define which proteins were involved in IgE-mediated egg allergy was greatly improved.

Hoffman⁶ reported that 25 out of 27 patients displaying symptoms on ingestion of egg gave positive RAST results. Three patients with strong anaphylactoid reactions to egg showed positive RAST tests to ovalbumin and ovomucoid. Both allergens may, however, have been slightly cross-contaminated. A later study by the same author⁷ using higher purity egg proteins indicated that ovalbumin, ovomucoid and ovotransferrin were important allergens, whereas lysozyme was only a weak allergen. Ovalbumin and ovomucoid and small amounts of ovotransferrin were detected in both hard- and soft-boiled eggs by radioimmuno-electrophoresis.

At this time Langeland⁸ and Langeland and Harbitz⁹ reported that for a group of egg-allergic children the most important allergens detected by crossed-radioimmuno-electrophoresis were ovomucoid, ovalbumin and ovotransferrin. Lysozyme was not detected as an allergen in this way. However, Holen and Elsayed¹⁰ found that lysozyme bound strongly to IgE in all of the sera of egg-allergic individuals that they studied, and concluded that lysozyme was one of the major allergens of egg white.

Egg yolk has largely been ignored by researchers, as early reports indicated that it was not allergenic¹¹. However Anet et al¹² recorded positive RAST scores for egg yolk in the sera of 36 egg-allergic patients. They also found that lysozyme bound specific IgE in the sera of four egg-sensitive patients. We have also recently reported IgE binding to several yolk proteins^{13, 14}. Some egg allergen fractions may be expected to give a high incidence of false positives *in vivo* due to their irritant property (e.g., lysozyme), especially for patients with atopic eczema. Often test procedures and materials were not well defined in the early literature. However these last two factors may not account for the wealth of conflicting reports of allergenicity. Therefore we have investigated binding of specific IgE in the sera of 40 egg-sensitive children to 8 purified egg white and yolk proteins by RAST in an effort to determine which of them are major allergens. The proteins were purified by high performance liquid chromatography (HPLC) where necessary to ensure purity. The results appear to explain the conflicting nature of reports on the allergenicity of egg proteins.

MATERIALS AND METHODS

Sera of egg-allergic patients

Sera were obtained from patients who were referred to the Allergy and Clinical Immunology Unit of the Royal Childrens Hospital, Melbourne, for evalua-

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