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Allergenic Properties of the Genetic Variants A and B of Bovine Beta-Lactoglobulin

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Abstract. The principal allergen in cow's milk is β -lactoglobulin (β -Lg) which is normally found as equal mixtures of the A and B genetic variants in pooled milk. The A and B variants were purified in large amounts and fed to guinea pigs. After 15 days, circulating immunoglobulins IgG1 and IgG2 to the milk proteins were detected, with a somewhat higher response to β -Lg A than B. Sensitised guinea pigs succumbed to anaphylactic shock when challenged intracardially with either variant. The higher response is likely to be due to amino acid residues found only in the A variant, lying in surface regions of the molecule.

Amongst the various protein components of cow's milk that can cause unpleasant allergic symptoms in young infants, β -lactoglobulin (β -Lg), which occurs only in ruminants, appears to be the most potent allergen [Freier et al., 1969]. Preliminary attempts to elucidate the molecular determinants of allergy have been performed on a mixture of genetic variants of β -Lg, as it is normally isolated [Haddad et al., 1979]. Six genetic variants of β -Lg have been so far identified, but of these, two (the A and B variants) are by far the most common [Aschaffenburg and Drewry, 1957]. Their homozygous forms occur with approximately equal frequencies, whereas the other variants are rather rare. The aim of the present study was to compare the allergenic properties of the A and B variants of β -Lg, using guinea pigs as a useful model for the human condition.

β -Lg A was prepared from low-fat whey protein concentrate from pooled milk by ion-exchange chromatography on Sepharose Q Fast Flow (Pharmacia, Milton Keynes, UK) equilibrated in 0.01 M sodium phosphate buffer (pH 6.2), and eluting with 0.2 M NaCl. β -Lg B was prepared from low-fat whey protein concentrate prepared from cow's milk homozygous for the B variant on Spherosil QMA (Rhône-Poulenc, Paris, France) using the same buffers. β -Lg

variants were concentrated and desalted on an Amicon DC10 unit. The preparations were found to give single bands on polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulphate (SDS), and the A and B variants were distinguished from each other by PAGE in the absence of SDS at pH 8.6 (fig. 1). Slower moving bands visible on this gel are likely to be due to polymerised forms of β -Lg [Townend et al., 1961]. In addition, the purified variants gave single peaks monitored at 280 nm upon high-performance liquid chromatography on Mono Q HR 5/5, eluting with a gradient to 0.5 M sodium acetate in 20 mM histidine acetate (pH 6) at 60 ml/h, or on TSK G-3000 SW, eluting at 35 ml/h with 0.05 M sodium phosphate (pH 6.2).

Groups of 3-week-old Dunkin-Hartley guinea pigs from Bantin & Kingman (Hull, UK) were sensitised by liquid diet of either whey protein concentrate from pooled milk (6.4 g/l protein), β -Lg A (5.2 g/l protein) or β -Lg B (5.2 g/l protein); each animal consumed between 100 and 150 ml/day. Solid food supplied was diet RGP, the protein content of which is fish meal. After 14 days, milk protein solutions were replaced by water. Serum samples from sensitised animals were taken at periods, and analysed for levels of IgG1, IgG2 and total IgG by ELISA using microtitre plates

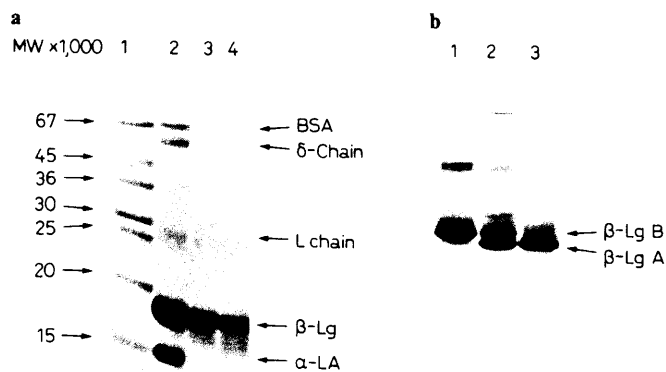


Fig. 1. **a** SDS-PAGE gel of the purified genetic variants of β -Lg. Shown are the molecular weight (MW) markers (lane 1), whey protein concentrate (lane 2), pure β -Lg A (lane 3) and pure β -Lg B (lane 4). BSA = Bovine serum albumin; α -LA = α lactalbumin. **b** PAGE gel in the absence of SDS. Shown are pure β -Lg B (lane 1), pure β -Lg A + B (lane 2) and pure β -Lg A (lane 3).

coated with the corresponding component of the liquid diet, either whey protein (16 μ g/ml), β -Lg A or β -Lg B (10 μ g/ml) as described by Heppell and Kilshaw [1982]. Antibodies bound were detected by peroxidase-conjugated goat anti-rabbit IgG, after incubation of the plates with either rabbit anti-guinea pig IgG1 or anti-guinea pig IgG2. Total IgG was measured by peroxidase-conjugated rabbit anti-guinea pig IgG. No antibodies to milk proteins were detected in serum samples taken at the commencement of feeding, nor in samples taken after 7 days. After 15, 21 and 28 days, significantly raised levels of IgG1, IgG2 and total IgG were detected (fig. 2). Titres of dilutions of sera that gave 50% of the maximum binding were calculated, and the mean and standard deviations calculated for each group of animals. Comparisons analysed by Student's *t* test showed that titres of IgG1, IgG2 and total IgG in response to β -Lg B were about 60% of the titres to β -Lg A ($p < 0.05$). There was no difference in the titres in response to β -Lg A and total whey protein.

Because the genetic variants of β -Lg differ in only a few residues in the polypeptide chains, it was of interest to see if all the sera resulting from sensitisation with β -Lg A contained antibodies that would bind β -Lg B, and vice versa. However, higher levels of antibodies were detected in animals sensitised with β -Lg

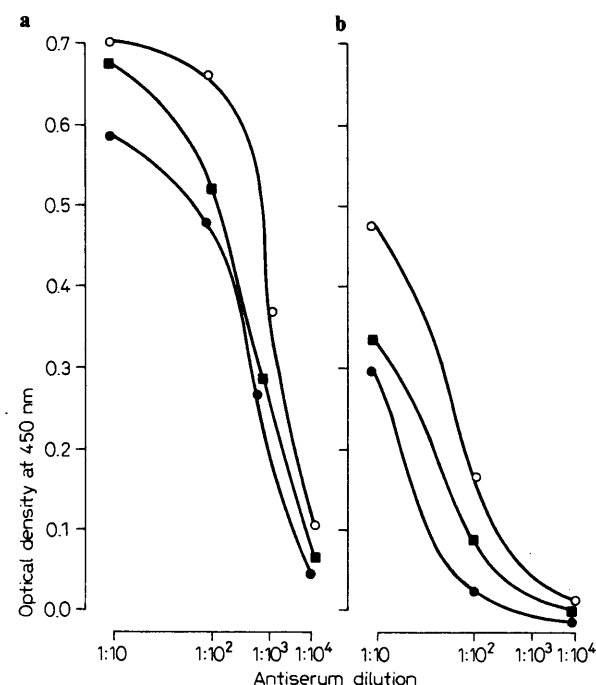


Fig. 2. Cross-over experiment. Antiserum from guinea pig sensitised with β -Lg A (**a**) and antiserum from guinea pig sensitised with β -Lg B (**b**) were tested for total IgG binding to whey protein concentrate (○), β -Lg A (●) and β -Lg B (■).

A irrespective of whether the A or B variants were used to coat the plates (fig. 2). In addition, it was shown that all antibodies to β -Lg A were removed from serum (0.1 ml) by incubation for 2 h at 37°C with a suspension of 0.05 ml of β -Lg B coupled to CH-Sepharose 6B (100 mg β -Lg B coupled to 2 g activated CH-Sepharose (Pharmacia) followed by centrifugation at 12,000 g. Thus, it is the immune response to β -Lg A that yields higher titres of antibodies rather than the presence in sera of antibodies that bind determinants unique to β -Lg A.

The effects of challenge by intracardiac injection of small quantities of the specific milk proteins with which each animal had been fed was observed. Five out of eight animals fed mixed whey proteins, 2 out of 4 animals fed β -Lg A and 3 out of 5 animals fed β -Lg B went into shock and died from anaphylaxis. The remaining animals in each group were observed to have difficulty in breathing due to bronchoconstriction over an 8-hour period, but did not die. It was not possible to detect any differences in these responses to the variant proteins. ELISA performed on serum samples withdrawn 30–60 min after challenge showed that

antibodies to the milk proteins were absent from the circulation, possibly by the formation of immune complexes.

Passive cutaneous anaphylaxis (PCA) [Watanabe and Ovary, 1977] performed over 4 h to detect IgG1 levels showed that, in agreement with ELISA measurements, skin reactions of between 1 and 2.5 cm occurred with sera at 1:5 dilutions withdrawn 15, 21 or 28 days after sensitisation. Sera taken at day 15 at 1:50 dilutions gave 5-mm responses, sera at day 21 at 1:100 or 1:200 dilutions gave 5-mm responses, and sera at day 28 at 1:200 dilution also gave 5-mm responses. Heating the sera at 56°C made no difference to the resulting skin reactions, indicating that they were due to IgG1 rather than IgE. PCA performed over 48 h was negative for all but one serum collected at day 15.

In keeping with the results of others [Devey et al., 1976; Heppell and Kilshaw, 1982], we found that guinea pigs fed a normal pellet diet but given cow's milk proteins in liquid form became sensitive to anaphylaxis, and were shocked by intravenous challenge. In addition, raised levels of IgG1 and IgG2 to the milk proteins were found in the circulation. Anaphylactic responses to β -Lg A and β -Lg B were similar, so that either variant alone could not be used as a safe hypoallergenic ingredient of food. Anaphylaxis is thought to result from cross-linking of antibodies on the surface of mast cells by allergens, followed by subsequent release of pharmacological mediators such as histamine or 5-hydroxytryptamine [Siraganian et al., 1975]. Thus, both β -Lg A and β -Lg B bear at least two determinants that are required for cross-linking. In agreement with the findings of Coombs et al. [1978], we found from PCA testing that in guinea pigs, in contrast to humans, the antibodies responsible for anaphylaxis are principally of the IgG1 type rather than IgE.

At pH values near its iso-electric point, each genetic variant exists as a stable dimer. Between pH 3.5 and 5.2, dimers of both variants associate to form octamers and the association is much stronger in β -Lg A [McKenzie, 1971]. The amino acid sequences of β -Lg A and β -Lg B differ in respect of residue 64, which in β -Lg A is Asp, and in the B variant is Gly, and also residue 118, which in β -Lg A is Val and in β -Lg B is Ala [McKenzie et al., 1972]. Disulphide bridges lie between residues 106 and 119 or 121, and also between residues 66 and 160. X-ray diffraction data have shown that the latter disulphide joins a central section of the β -strand structure with the C-terminal section

of the protein, which lies on the surface of the protein [Papiz et al., 1986], and thus comprises a probable antigenic determinant [Hopp and Woods, 1981]. Rather than yielding a unique epitope, the amino acid residues unique to β -Lg A appear to act in some way to yield greater stimulation of B lymphocyte clones. This greater response may be due, at least in part, to a more effective absorption of the intact protein across the epithelial layer of the gastro-intestinal tract [Taylor et al., 1973; Koritz et al., 1987], or to the more effective processing and presentation of the β -Lg A protein on the surface of T lymphocytes, or to its greater propensity for polymerisation. In either case, it is likely that the allergenic determinants are not identical to antigenic determinants [Stanworth, 1973].

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