Goats' milk of defective α_{s1}-casein genotype decreases intestinal and systemic sensitization to β-lactoglobulin in guinea pigs

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Summary. Contradictory results have been reported on the use of goats' milk in cows' milk allergy. In this study the hypothesis was tested, using a guinea pig model of cows' milk allergy, that these discrepancies could be due to the high genetic polymorphism of goats' milk proteins. Forty guinea pigs were fed over a 20 d period with pelleted diets containing one of the following: soyabean proteins (group S), cows' milk proteins (group CM), goats' milk proteins with high (group GM1) or low (group GM2) α_{s1}-casein content. Parenteral sensitization to GM1 and GM2 proteins was also assessed. The sensitization was measured (1) by systemic IgG1 antibodies directed against bovine or caprine β-lactoglobulin (β-lg), α-lactalbumin (α-la) and whole caseins, and (2) by intestinal anaphylaxis measured *in vitro* in Ussing chambers, by the rise in short-circuit current (ΔIsc) in response to milk proteins. Guinea pigs fed on CM and GM1 developed high titres (> 1500) of anti-β-lg IgG1, with an important cross reactivity between goat and cow β-lg. However, in guinea pigs fed on GM2, anti-goat β-lg IgG1 antibodies were significantly decreased compared with GM1 guinea pigs (mean IgG1 titres were 546 and 2046 respectively), and the intestinal anaphylaxis was significantly decreased ($3{\cdot}5 \pm 4{\cdot}5\ \mu A/cm^2$) compared with that observed in GM1 guinea pigs ($8{\cdot}3 \pm 7{\cdot}6\ \mu A/cm^2$). Animals receiving GM1 or GM2 proteins via the parenteral route developed a marked sensitization. These results suggest that the discrepancies observed in the use of goats' milk in cows' milk allergy could be due, at least in part, to the high genetic polymorphism of goats' milk proteins.

Keywords: Food allergy, milk, intestinal anaphylaxis, α_{s1}-casein.

Cows' milk allergy (CMA) is a frequent disease in infants under 2 years of age (Host & Halken, 1998). It is usually transitory, since the symptoms (intestinal or

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cutaneous) gradually disappear between 12 and 24 months of age, due to the development of a clinical tolerance to cows' milk (CM) proteins. The treatment is essentially nutritional and consists in the total elimination of CM proteins and their replacement by extensively hydrolysed milk formulas, and sometimes by other substitutes such as soyabean-based formulas or milks from different animal species including goats' milk (GM).

On the one hand, biochemical and immunochemical studies have shown that GM proteins are immunogenic in animals (Saperstein, 1960; Crawford & Grogan, 1961; McLaughlan *et al.* 1981*a*) and that important immune cross reactivity between goats' and cows' milk proteins is observed due to the presence of numerous common peptidic epitopes (Dean *et al.* 1993; Sabbah *et al.* 1997; Restani *et al.* 1999). However, Sabbah *et al.* (1997) reported that this immune cross reactivity was not completely correlated with the clinical symptoms.

On the other hand, there are clinical studies testing GM in the treatment of CMA patients, although the controlled clinical studies are few. The studies reported indicate that 50–80 % of CMA patients did not tolerate GM (Maree, 1978; Juntunen & Ali-Yrkkö, 1983; Birkbeck, 1984; Taitz & Armitage, 1994; MacDonald, 1995; Bellioni-Businco *et al.* 1999) suggesting that 20–50 % did display a clinical improvement when receiving GM. In a recent controlled clinical study, Bellioni-Businco *et al.* (1999) reported that 24 out of 26 CMA children had a positive double-blind, placebo-controlled, oral food challenge to GM, although the amount of GM needed to trigger the allergic reaction was five times that of CM.

In the present work we have hypothesized that discrepancies between clinical studies could be related to the high heterogeneity of GM used during oral provocation. In fact, goats have a high genetic polymorphism at the α_{s1}-casein locus, which is tightly connected to the amount of this protein in milk. Eleven alleles have been described corresponding to four different groups of casein expression ranging from 0 to 3·5 g α_{s1}-casein/l per allele (Grosclaude *et al.* 1994). Thus, the milk from homozygous goats at the α_{s1}-casein locus (α_{s1}-*CasA/A*) contains a high amount of α_{s1} casein (7 g/l) whereas the milk from homozygous *O/O* goats is almost devoid of α_{s1}-casein. In order to test whether the casein content of GM has an effect on its allergenicity, the IgE-dependent guinea pig model of CMA (Cuthbert *et al.* 1983) was used to compare CM and two genotypically different GM samples (α_{s1}-*cas A/A v.* α_{s1}-*cas O/O*) in their capacity to induce milk protein sensitization.

materials and methods

Preparation of milk protein-based diets

Goats' milk samples were taken from an experimental flock (Domaine Expérimental de Bourges, INRA, 18520 Arvord, France). We used two different goats' milks: GM1, a milk containing a maximal amount of α_{s1}-casein (7 g/l) and its counterpart, GM2, a milk containing only traces of α_{s1}-casein (0·7 g/l), i.e. one-tenth of that in the GM1 milk.

After thermization at 42 °C, the milk samples were microfiltered to remove fat globules and bacterial flora, ultrafiltered in order to concentrate proteins (caseins and whey proteins) and freeze-dried after diafiltration. A quantitative determination of α_{s1}-casein and β-lactoglobulin (β-lg) content of the concentrates was carried out using reversed-phase (RP)-HPLC (Jaubert & Martin, 1992). As expected, the

α_{s1}-casein concentration was very small (0·70 g/l) in GM2 milk, and ten times higher (7 g/l) in GM1 milk. Conversely, β-lg concentration was 60 % higher in GM2 milk than in GM1 milk (2·37 *v.* 1·50 g/l respectively).

The pellets used to feed the guinea pigs were prepared at INRA/APAE (Jouy-en-Josas, France). Milk proteins were introduced at a final concentration of 300 g/kg in the pelleted diet, the composition of which has been published previously (Darmon *et al.* 1998). During the preparation, all the ingredients were mixed at a 5 % final hydration, heated at 40–50 °C and granulated by air-drying.

Purification of milk proteins

The measurement of intestinal anaphylaxis in Ussing chambers and the enzyme-linked immunosorbent assay of IgG1 were carried out using milk proteins purified by the method of Andrews *et al.* (1985) using a preparative liquid chromatography system (Biopilot, Amersham Pharmacia Biotech, Europe, GmbH, Orsay, France). After isoelectric precipitation of caseins at pH 4·6, anion exchange chromatography (Source 15Q, Amersham Pharmacia Biotech) was used to separate the whey proteins β-lg and α-lactalbumin (α-la) in the supernatant. Caseins were purified using cation exchange chromatography (S-Sepharose fast flow, Amersham Pharmacia Biotech). Purification was confirmed using RP-HPLC (Waters 600 E) and a C4 column (Jaubert & Martin, 1992).

Animals and experimental protocol

Fifty-six male Dunkin-Hartley guinea pigs (200–250 g) at weaning (Charles River, Saint-Aubin les Elboeuf, France) were used in this study.

Oral sensitization. Animals were fed over a 3-week period with one of the four pelleted diets containing 300 g/kg of either soyabean proteins (group S, $n = 10$), or cows' milk proteins (group CM, $n = 12$), or goats' milk proteins containing a high amount (7 g/l, group GM1, $n = 9$) or low amount (0·7 g/l, group GM2, $n = 12$) of α_{s1}-casein. At 3 d before they were killed (after 21 d of dietary protocol), the animals fed on milk protein pellets were shifted to the soyabean pellets to keep their intestine free of allergic reactions until the anaphylactic responses were tested *in vitro*. Animals were fasted overnight before the experiment.

Parenteral sensitization. Animals were given injections of the freeze-dried goats' milk proteins (GM1, $n = 5$ or GM2, $n = 6$) reconstituted in water (30 mg/ml). Protein solution (1 ml) was injected intraperitoneally on day 1, and a second booster injection (0·3 ml) was given on day 15. The animals were killed on day 25, after an overnight fast, to study systemic sensitization (IgG1 plasma level) and local intestinal anaphylaxis.

Experimental protocol

On day 25, guinea pigs were anaesthetized with 90 mg sodium pentobarbital/kg given i.p. and weighed. The blood was obtained by cardiac puncture and the plasma samples were stored at −20 °C. A section of the small intestine (30 cm) was removed, starting 15 cm from the pylorus, and was carefully rinsed with cold Ringer's solution containing 140 mM-Na^+, 5·2 mM-K^+, 120 mM-Cl^-, 25 mM-HCO_3^-, 1·2 mM-Ca^{2+}, 2·4 mM-HPO_4^{2-}, 0·4 mM-$H_2PO_4^-$, 1·2 mM-Mg^{2+} and 2 mM-glutamine. Jejunal fragments were mounted in Ussing chambers to study anaphylactic response induced by purified milk proteins.

Morphological analysis of the intestinal mucosa

Jejunal segments (1 cm) from each animal were fixed in Bouin's solution (Sigma diagnostics), dehydrated, embedded in paraffin, cut into four sections and stained with hematoxylin, eosin and safranin for morphological analysis. A micrometer was used to measure total mucosal height, villus height and crypt depth. The number of intraepithelial lymphocytes/100 enterocytes was also evaluated.

Immune sensitization to milk proteins

Systemic IgG1 antibodies. In guinea pigs, anaphylactic antibodies essentially belong to the IgG1 subclass. Milk sensitization was therefore assessed at the systemic level by measuring plasma anti-β-lg, anti-α-la and anti-total casein IgG1 antibodies. We used a modified enzyme linked immunosorbent assay (ELISA) originally described by Kawabata *et al.* (1995). Briefly, the plates (Immulon 2, Dynex Technologies Inc, Polylabo, France) were coated overnight at 4 °C with each of the cows' or goats' milk proteins tested (100 μl of 0·1 mg/ml of each protein in 0·01 M-PBS, pH 7·2). The plates were then saturated with gelatin (10 g/l in 0·01 M-PBS) at 37 °C for 90 min. Plasma samples (100 μl) were serially diluted (from 1:4 to 1:16384) in PBS–Tween (0·5 ml Tween 20/litre in 0·01 M-PBS) and kept for 90 min at room temperature in the microtitre plates.

Finally, IgG1 antibodies were detected with a goat anti-guinea pig IgG1 peroxidase conjugate (1:7500; Nordic Immunology, Tebu, France) revealed by H_2O_2 and orthophenylene diamine. Plates were washed four times with PBS–Tween between each step. Positive titres were denoted as the last dilution giving an optical density at least two times higher than that of the background.

Local intestinal anaphylaxis to milk proteins

The intestinal anaphylaxis was used as a biological criterion of allergic sensitization. Eight adjacent jejunal segments from each animal were mounted in Ussing chambers as flat sheets with an exposed area of 0·5 cm². They were bathed on both sides with 12 ml Ringer's solution, which was continuously thermostated (37 °C), circulated, oxygenated and maintained at pH 7·4 with CO_2–O_2 (5:95, v/v).

The mucosal and serosal bathing solutions were connected via agar bridges to calomel electrodes for measurement of the transepithelial potential difference and to Ag–AgCl electrodes for current application. The tissue was kept under short-circuit conditions by an automatic clamping device (World Precision Instruments, Aston, UK) that cancelled out fluid resistance. The short-circuit current (I*sc*) was constantly recorded and the tissue was pulsed at 1 mV every 30 s. The I*sc* deflection was used to calculate electrical resistance (R) according to Ohm's law. I*sc*, PD and R, measured 30 min after tissue mounting, were taken as the basal electrical parameters.

The local intestinal immune response was then assessed by recording the maximal rise in I*sc* (ΔI*sc*) induced by serosal addition of 100 μg/ml of one of the cows' or goats' milk proteins. Intestinal tissues from milk-sensitized animals generally respond to antigen challenge by a type I hypersensitivity reaction named intestinal anaphylaxis. The latter is associated with fluid secretion and is characterized by a rapid rise in I*sc* owing to the net chloride secretion in the intestine (Cuthbert *et al.* 1983). Data from various experimental models of intestinal anaphylaxis indicate that such an electrogenic activity (rise in I*sc*) is induced by the degranulation of mucosal mast cells leading to the subsequent release of inflammatory mediators capable of stimulating chloride secretion.

Statistical analysis

Data were analysed by variance analysis or non-parametric tests. Multiple comparisons followed by group-to-group comparisons were performed using the general linear model procedure or the Kruskal–Wallis one-way analysis of variance, included in the NPAR1WAY procedure of the SAS package, version 6.12 (SAS Institute, Cary, NC, USA). Statistical analyses of the antibody titres were performed on logarithmic transformations. Results are presented as means ± standard deviation (SD), with n as the number of animals.

RESULTS

Nutritional status of guinea pigs

The nutritional status of guinea pigs from the four experimental groups of orally sensitized animals was satisfactory. No significant difference in body weight was observed among the different dietary groups at the end of the experimental protocol (body weight = 346 ± 17, 341 ± 32, 331 ± 35 and 367 ± 41 g in CM, GM1, GM2 and S groups respectively).

Among the guinea pigs that underwent parenteral sensitization, two had a fatal systemic anaphylactic reaction after the booster injection (one with GM1 and one with GM2) and could not be included in the analysis.

Morphological analysis of intestinal mucosa

As shown in Table 1, the morphological parameters of the proximal small intestine, including total mucosal height, villus height, crypt depth and the number of intraepithelial lymphocytes/100 enterocytes, were not modified by the dietary protocol, indicating the absence of mucosal lesions.

Oral sensitization to milk proteins

IgG1 antibodies. As shown in Fig. 1, IgG1 antibodies were essentially directed towards β-lg and antibody titres to α-la and casein were very low. The animals that received soyabean proteins did not develop any systemic response to milk proteins (mean titres = 1). In contrast, the animals having received CM, GM1 or GM2 developed anti-β-lg titres which were significantly higher than those observed in the control soyabean group ($P < 0{\cdot}0001$). No IgG1 responses directed to the other milk proteins, α-la and casein, were observed. There was an important cross reactivity between the cow and goat anti-β-lg IgG1 antibodies. Animals having received CM proteins in their diet developed high IgG1 titres to cow β-lg (mean titre = 1309) but it was noted that these antibodies reacted poorly with goat β-lg (mean titre = 406, $P < 0{\cdot}07$). Animals fed with the goats' milk proteins GM1 developed high IgG1 titres to goat β-lg (mean titre = 2056) and these antibodies also reacted with cow β-lg (mean titre = 1717). However, in animals that received GM2 proteins in their diet the antibody response to goat β-lg was low (mean titre = 546) and significantly lower than that observed with the GM1 diet (mean titre = 2056, $P < 0{\cdot}04$), but was not significantly lower than the antibody response to cow β-lg observed in CM guinea pigs (mean titre = 1309, $P = 0{\cdot}5$).

Interestingly, IgG1 antibodies from GM2 animals showed less specificity for cow β-lg than IgG1 antibodies from GM1 animals ($P < 0{\cdot}02$).

Intestinal anaphylaxis. The intestinal response was measured in Ussing chambers by assessing the milk protein-induced rise in short-circuit current (ΔIsc) in response

Table 1. *Histological analysis of the proximal small intestine of guinea pigs fed on different diets*

Groups…	Soyabean (S)	CM	GM1	GM2
Total mucosal height (μm)	601±66	581±56	582±70	543±101
Villus height (μm)	483±50	462±51	466±68	433±94
Crypt depth (μm)	117±22	118±15	110±31	113±13
C:V ratio	0·24±0·04	0·26±0·05	0·24±0·08	0·27±0·05
IEL	13·5±6·1	14·5±3·5	14·6±2·7	12·5±1·6

C:V ratio, crypt depth: villus height ratio; IEL, number of intraepithelial lymphocytes per 100 epithelial cells.

There were no statistical differences among the dietary groups.

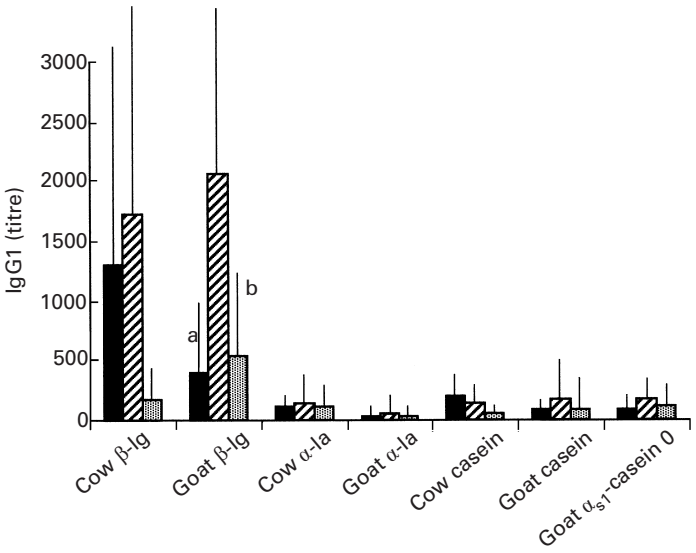

Fig. 1. Development of plasmatic anaphylactic antibodies (IgG1) in guinea pigs orally sensitized with cows' milk proteins (■), goats' milk proteins (▨) or goats' milk proteins defective in α_{s1}-casein (▩). In order to test cross reactivity, the specificity of IgG1 antibodies has been determined using the different cows' or goats' milk proteins. a, Different from cow β-lactoglobulin (β-lg) IgG1 titres in the CM group ($P < 0{\cdot}07$); b, different from goat β-lg IgG1 titres in the GM1 group ($P < 0{\cdot}04$). Results are means±SD, $n = 9$–12 animals. α-la, α-lactalbumin.

to the administration of bovine or caprine β-lg, α-la and total caseins. The basal electrical parameters (PD, I*sc* and R) showed no significant difference among all the dietary groups. In the S, CM, GM1 and GM2 groups, the potential difference was respectively 2·5, 2·7, 2·5, 2·4 mV and the electrical resistance was of 78, 87, 70, and 75 ohms.cm².

In the same way that IgG1 antibodies were directed towards β-lg only, intestinal anaphylaxis was triggered only by β-lg, but not by the other milk proteins like α-la or caseins. As shown in Fig. 2, the intestinal anaphylaxis in response to goat β-lg was lower in GM2 guinea pigs ($3{\cdot}5\pm4{\cdot}5\,\mu A/cm^2$) than in GM1 group ($8{\cdot}3\pm7{\cdot}6\,\mu A/cm^2$, $P < 0{\cdot}06$).

Considering individual biological responses (intestinal anaphylaxis) in each group of orally sensitized animals, we observed that 8 %, 30 % and 50 % of the CM, GM1, and GM2 animals respectively did not respond at all to β-lg ($\Delta Isc = 0\,\mu A/cm^2$).

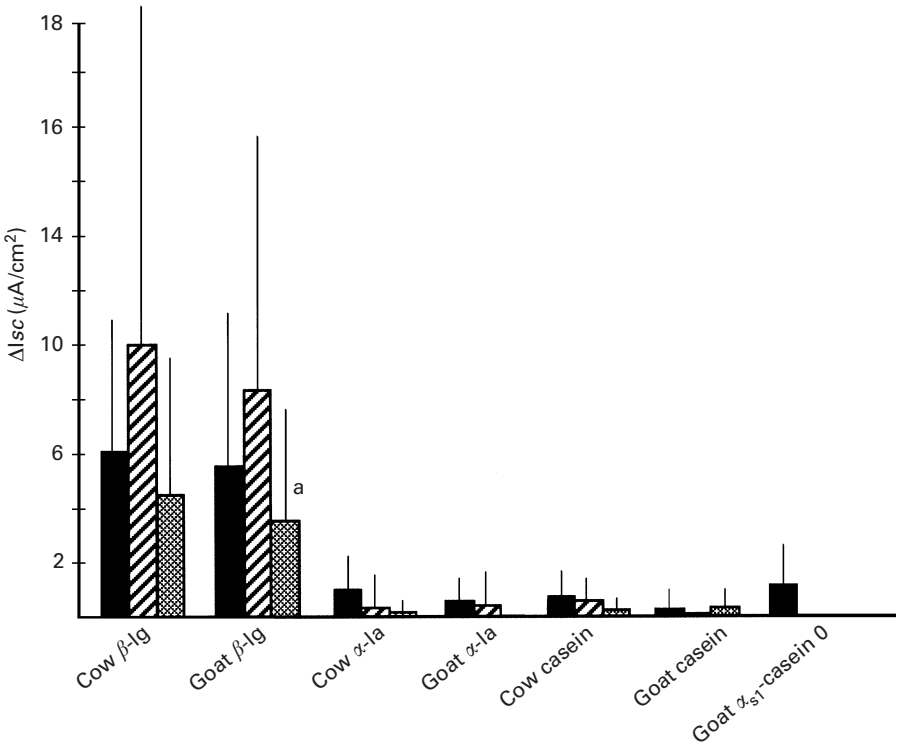

Fig. 2. Intestinal anaphylaxis, as measured by the rise in short-circuit current (Δ*Isc*) induced by the different cows' or goats' milk proteins, in guinea pigs having received orally cows' milk proteins (■), goats' milk proteins (▨) or goats' milk proteins defective in α_{s1}-casein (▩). a, Significantly different from the response to goat β-lactoglobulin (β-lg) observed in GM1 animals ($P < 0{\cdot}06$). Results are means ± SD, $n = 10$–12 animals. α-la, α-lactalbumin.

Parenteral sensitization to milk proteins

IgG1 antibodies. Guinea pigs sensitized intraperitoneally with GM1 or GM2 proteins developed IgG1 antibodies directed not only towards β-lg but also, to a lower extent, towards α-la and caseins (Fig. 3). Although there was a tendency to the lower IgG1 titres in animals sensitized with GM2 proteins compared with those fed on GM1 proteins, the difference between the titres of goat β-lg IgG1 in GM1 (mean titre = 5248) and GM2 (mean titre = 2252) animals did not reach statistical significance ($P = 0{\cdot}34$).

In general, IgG1 titres were much higher after parenteral than after oral sensitization. Particularly in the GM2 group, the anti-goat β-lg IgG1 titres were much lower in orally sensitized animals (mean titre = 546) than in parenterally sensitized animals (mean titre = 1888, $P < 0{\cdot}01$).

Intestinal anaphylaxis. In contrast to the results obtained with orally sensitized animals, in parenterally sensitized guinea pigs the intestinal anaphylactic response to β-lg was identical in animals having received intraperitoneal injections of GM1 or GM2 (Fig. 4).

As shown in Fig. 5, the GM1 proteins led to the same level of intestinal anaphylaxis after oral and parenteral sensitization ($8{\cdot}3 \pm 7{\cdot}6$ *v.* $6{\cdot}6 \pm 4{\cdot}4$ μA/cm²). In contrast, when the GM2 proteins were used the oral sensitization led to a lower

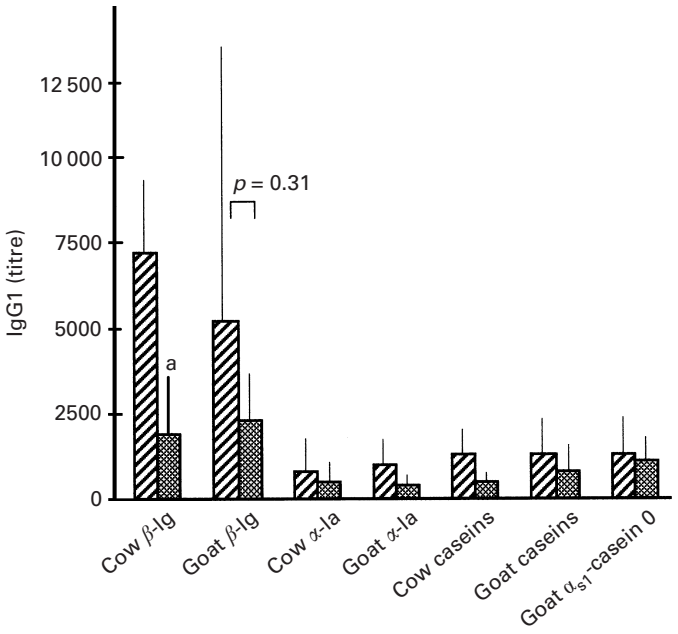

Fig. 3. Development of plasmatic anaphylactic antibodies (IgG1) in guinea pigs intraperitoneally sensitized with goats' milk proteins (▨, GM1) or goats' milk proteins defective in α_{s1}-casein (▩, GM2). Titres are higher than those obtained by oral sensitization. Although a tendency to a lower sensitizing capacity of GM2 compared with GM1 is observed, the difference did not reach statistical significance, except for titres against cow β-lactoglobulin (β-lg) which were lower in GM2 than in GM1 animals. a, Different from GM1 group ($P < 0{\cdot}02$). Results are means $\pm$ SD, $n = 5$ animals. α-la, α-lactalbumin.

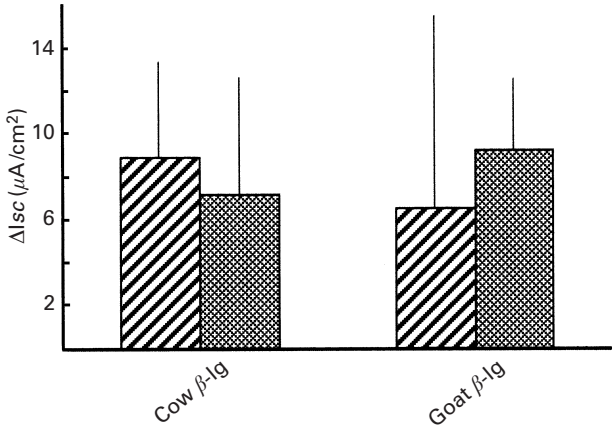

Fig. 4. Intestinal anaphylaxis, as measured by the rise in short-circuit current (ΔIsc) induced by cows' and goats' milk proteins, in guinea pigs having received intraperitoneally, goats' milk proteins (▨) or goats' milk proteins defective in α_{s1}-casein (▩). No differences in anaphylactic responses were observed among the different groups of guinea pigs. $n = 5$–6 animals. β-lg, β-lactoglobulin.

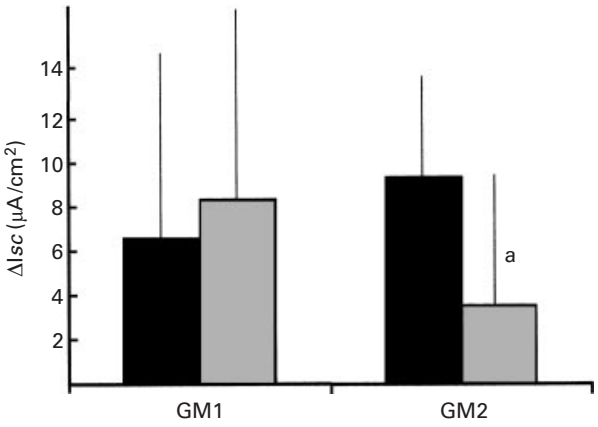

Fig. 5. Comparison of intestinal anaphylactic response in guinea pigs sensitized parenterally (■) or orally (▨) with goats' milk proteins GM1 (containing α_{s1}-casein) or GM2 (defective in α_{s1}-casein). Goat β-lactoglobulin was used to stimulate the tissues. a, Statistically different from parenterally sensitized GM2 guinea pigs ($P < 0{\cdot}06$).

intestinal anaphylaxis ($3{\cdot}5 \pm 4{\cdot}5\ \mu A/cm^2$) than the parenteral sensitization ($9{\cdot}3 \pm 3{\cdot}0\ \mu A/cm^2$, $P < 0{\cdot}06$).

DISCUSSION

The present results confirm an important immune cross reactivity, previously reported, between goats' and cows' milk proteins. They further indicate that, according to the goat genotype, milk protein composition can vary significantly, playing an important role in allergenicity.

Indeed, in goat species, a high genetic polymorphism has been reported at the α_{s1}-casein locus which is closely connected to the amount of this protein in milk (Grosclaude *et al.* 1987). In order to test whether the casein content of GM has an effect on its allergenicity, we have tested, using an animal model of milk sensitization, the capacity of GM of different genotypes to induce oral sensitization.

The guinea pig model of cows' milk allergy, initially described by Parish and co-workers (Parish *et al.* 1964) has been widely used to test the allergenicity of infant food formulas (McLaughlan *et al.* 1981*b*). Different induction and challenge routes have been used since sensitization can be obtained either by oral administration of milk antigens or by parenteral injections. In the present study we used this guinea pig model to compare the sensitizing capacities of cows' milk and goats' milk of different genotypes. The first goats' milk (GM1) was obtained from animals homozygous for the A allele at the α_{s1}-casein locus (α_{s1}-*CasA*), corresponding to the maximal amount of α_{s1}-casein in milk (7 g/l). In contrast, a genotype which expresses only traces of α_{s1}-casein from the defective allele (α_{s1}-*CasO*) at the relevant locus was used. This milk (GM2) contained 0·7 g α_{s1}-casein/l, i.e. one-tenth that in GM1 milk. As a consequence, the total amount of proteins in GM1 ($\sim$ 30 g/l) was higher than that observed in GM2 ($\sim$ 24 g/l). Since total milk proteins were extracted from GM1 and GM2 milk and freeze-dried before being incorporated into the pelleted diets at the concentration of 300 g/kg, the relative proportion of β-lg in GM2 diet was higher than that in GM1 diet (2·37 g/l *v.* 1·5 g/l).

In this animal model, oral sensitization with milk proteins led to a sensitization mainly towards β-lg but not towards the other milk proteins. This could be related,

as previously reported in guinea pigs and rats (Koritz *et al.* 1987), to the higher resistance of β-lg as compared with caseins or α-la, to the gastrointestinal enzymic hydrolysis. The reaginic IgG1 antibodies directed against cow β-lg were high in animals having received CM, whereas animals fed on GM1 developed high titres against goat β-lg. It must be pointed out that a high immune cross reactivity was found between cow and goat proteins. Animals sensitized with goats' milk proteins developed IgG1 antibodies that recognized goat or cow β-lg to the same extent. The reverse was not totally true, since animals sensitized with cows' milk proteins developed IgG1 reacting weakly with goat β-lg. The cross reactivity observed between cow and goats' milk proteins was also found when intestinal anaphylaxis was measured as an index of clinical reactivity.

The most striking result in the present work was the lower sensitizing capacity of the goats' milk GM2, devoid of α_{s1}-casein. Animals fed on GM2 developed significantly lower IgG1 antibody titres to β-lg and lower intestinal anaphylaxis than those fed on GM1.

These results suggest that the absence of α_{s1}-casein in GM2 milk could be responsible for the lower sensitizing capacity of β-lg. As intraperitoneal injection (parenteral sensitization) yields almost the same sensitization to goat β-lg with GM1 or GM2 milk proteins, it can be deduced that some modifications of the β-lg (conformation, binding to casein micelles) occurring in the milk may lead to differential enzymic degradation in the gastrointestinal lumen, and, in consequence, to the differences in intestinal absorption. Indeed, after oral administration, most of the proteins are hydrolysed by gastric and pancreatic enzymes, thus diminishing the amount of antigenic material that could cross the intestinal mucosa and possibly induce an allergic immune response. By contrast, after parenteral administration the proteins are directly delivered to the antigen presenting cells and therefore induce a more potent immune response. These observations may explain why in CMA infants, prick tests to goats' (or cows') milk proteins are often positive without correlation with the clinical symptoms observed after oral provocation with goats' (or cows') milk and suggest that immune cross reactivity does not mean constantly cross-allergenicity. The mechanisms by which a goats' milk with a very low α_{s1}-casein content (GM2) can be less allergenic than its counterpart containing high level of α_{s1}-casein (GM1) is unknown but could be related to biochemical interactions between caseins and β-lg. The digestion of β-lg, a protein known to be resistant to gastric pepsin hydrolysis, might be facilitated in the absence of α_{s1}-casein, as it is known that caseins and β-lg are tightly linked into the casein micelles. Further studies on such biochemical interactions are needed to understand better the decrease in immunogenicity and allergenicity of GM2 via the oral route.

Taken together, these results indicate that the allergenicity of milks may vary according to the proportion of constitutive proteins, and suggest than the efficiency of luminal hydrolysis of milk allergens such as β-lg could play a role in the development of allergic sensitization, the latter hypothesis remaining to be explored. The results also suggest that the discrepancies observed in the efficacy of goats' milk in the treatment of CMA children could be partly due to the type of goats' milk used in clinical studies (Juntunen & Ali-Yrkk, 1983; Birkbeck, 1984; Taitz & Armitage, 1994; Bellioni-Businco *et al.* 1999). It seems unlikely that goats' milk can substitute for extensively hydrolysed formulas successfully used to feed CMA children. The alternative possibility of using a defective goats' milk, however, could help in children at risk of food allergy or in developing countries where hypoallergenic formulas are not available.

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