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Maternal dietary antigens and the immune response in the offspring of the guinea-pig

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SUMMARY

Guinea-pig dams and their litters were raised on either a cow's milk protein-containing diet (MCD) or a milk-free diet (MFD). At 8 weeks of age all litters were challenged i.p. with 50 μg milk whey-protein concentrate (V67) and 100 mg $Al(OH)_3$ in saline. The immune response was estimated 2 weeks later as the serum IgG antibody titres against V67, β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) using enzyme-linked immunosorbent assay (ELISA) and the tracheal Schulze–Dale response to these antigens. Feeding milk protein antigen to dams from birth and during pregnancy induces antigen-specific hyporesponsiveness (tolerance) in their offspring, despite no direct contact between the offspring and the milk proteins. Tolerance seems to be induced by the antigen itself since withdrawal of the MCD 10 days before delivery reduced tolerance in the offspring. No tolerance was produced in the offspring of dams fed the antigen from 3 months of age (adult). β-LG appears to be a major antigen in milk whey while α-LA is a minor one since there was almost no antibody or tracheal response to α-LA in any of the animals tested. The results indicate that maternal antigen experience and antigens present during pregnancy are important for the subsequent immune response to these antigens in offspring.

INTRODUCTION

It is essential for the immune system to be able to discriminate between 'harmless' and potentially 'harmful' antigens. The gastrointestinal tract is exposed to numerous antigens to which an actively enhanced or suppressed immune response is maintained. Antigens presented orally have been shown to induce a state of antigen-specific immunological hyporesponsiveness (tolerance) in many different species. It has been shown in neonates (Filipp 1965; Hanson *et al.*, 1977; Heppel & Kilshaw, 1982) and in adults (Coombs, Devey & Anderson, 1978; Swarbrick, Stokes & Soothill, 1979; Strobel *et al.*, 1983; Kagnoff, 1978; Thomas & Parrot, 1974; Stokes, Swarbrick & Soothill, 1983). Oral tolerance is suggested as being a result of an active suppression maintained by antigen-specific T suppressor cells (Richman, Chiller & Brown, 1978; Ngan & Kind, 1978; Waters, 1979; Mowat *et al.*, 1982, Ferguson, Mowat & Strobel, 1983) and includes both cellular and humoral immune responses. A certain degree of maturation of the immune system seems to be necessary to achieve T-cell tolerance since it is not possible to tolerize mice before a few days of age (Hanson, 1981; Strobel & Ferguson, 1984).

Guinea-pigs are easily tolerized when fed an antigen early in life, but tolerance is more difficult to produce with increasing age (Coombs *et al.*, 1978; Heppel & Kilshaw, 1982), while in adult guinea-pigs sensitization by the oral route is a well-known phenomenon (Devey *et al.*, 1976, Coombs *et al.*, 1978).

There have been few reports on maternal antigen experience and the subsequent response in the offspring. Halsey & Benjamin (1976) showed that tolerance was induced in mice born to a mother injected with human gamma globulins. Pathriana *et al.* (1981) showed tolerance induction to soya protein in the offspring of rabbits fed soya, and Peri & Rothberg (1981) showed tolerance induction to bovine serum albumin.

In the present study, the immune response to cow's milk proteins (CMP) in the offspring of guinea-pig dams fed milk-containing diet from early in life, was studied.

Abbreviations: CMP, cow's milk proteins; α-LG, α-lactoalbumin; β-LG, β-lactoglobulin; MCS, cow's milk protein-containing diet; MFD, milk-free diet; OVA, ovalbumin.

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MATERIALS AND METHODS

Animals and experimental protocol

Eight female guinea-pigs of the Dunkin Hartley strain were delivered by caesarian section at 63 days of fetal age (2–3 days before normal delivery) with a mean weight of 76 ± 8 g. They

Table 1. Grouping of offspring

Experimental group	*n*	Litters fed	Dams fed
A	5	MFD	MCD (until delivery, then MFD)
B	2	MCD	MCD
C	6	MCD	MFD
D	4	MFD	MCD (until 10 days before delivery, then MFD)
E	4	MFD	MFD (until 3 months of age, then MCD until delivery)
Controls	10	MFD	MFD

were kept with four different lactating foster mothers and were raised to become the dams. Four of these were fed 4 ml/100 g body weight/day for 2 days of a cows milk whey-protein concentrate (V67; Semper, Stockholm), 50 mg/ml in saline, by stomach tube. From their first day of life they had ad lib access to a pelleted experimental milk-containing diet (MCD) made from normal guinea-pig diet (K1; Ewos, Södertälje) but with the fish protein replaced with V67 which made up 70% of the total protein content. Another four females were fed saline by stomach tube as above and had ad lib access to normal milk-free diet (MFD). One additional female (fed MFD from birth) was shifted from MFD to MCD at 3 months of age. At 6 months of age these nine females were mated and their offspring were divided into six different groups based on their own and their dams diet (A–E and controls) according to the protocol in Table 1. All groups had access to their diet until the end of the experiment.

At 3 months of age all animals in the second generation (group A–E and controls) were challenged by an i.p. injection of 50 μg V67 plus 100 mg $Al(OH)_3$ in 0·5 ml of saline, mixed 1 hr before injection. Two weeks after injection the animals were killed using CO_2, blood was taken by cardiac puncture and the trachea was dissected out. The blood was centrifuged at 3000 *g* and the serum was saved at $-20°$ until analysis.

Assay

Specific IgG, IgM and IgA titres to V67, bovine β-lactoglobulin (β-LG; no. L-2506, Sigma, St Louis, MO) and bovine α-lactalbumin (α-LA; no. L-4379, Sigma) were estimated using an enzyme-linked immunosorbent assay (ELISA) (Engvall, 1980). Chicken ovalbumin (OVA; no. A-5503, Sigma) was used as a negative control. Microtitre plates (Immunoplatte 1; Nunc, Roskilde, Denmark) were coated with antigen (1 μg/ml) in carbonate solution (pH 8·3) in a humid chamber over night at room temperature (RT). After washing with phosphate-buffered saline containing 0·05% Tween 20 (PBS+Tween) the samples were serially diluted to a final dilution of 1/390625 or 1/10935 and the plates were then incubated at $+4°$ for 16 hr. After washing either rabbit anti-IgG (diluted 1/20000 in PBS+Tween), IgM (1/2000) or IgA (1/2000) (nos 65–119, 64–322–1, 64–323–1, Miles, Slough, Berks, U.K.) were added and the plates were incubated at RT for 90 min. After washing peroxidase-conjugated swine anti-rabbit IgG (no. P217, Dako, Copenhagen, Denmark) diluted 1/1000 in PBS+Tween and containing 1% normal pig serum was added. After incubation for 2 hr at RT the plates were washed and substrate solution

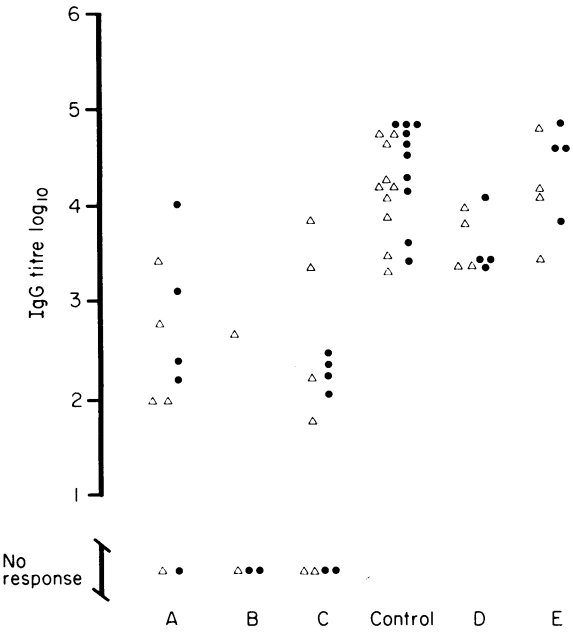

Figure 1. The specific serum IgG titre (log_{10}) against V67 (△) and β-LG (●) in guinea-pigs 2 weeks after i.p. challenge with 50 μg of V67 and 100 mg $Al(OH)_3$ in saline. Group A, MFD-fed born to dams fed MCD from birth; B, MCD-fed born to dams fed MCD from birth; C, MCD-fed born to dams fed MFD from birth; control, MFD-fed born to dams on MFD from birth; D, MFD-fed born to a dam fed MCD from birth until 10 days before delivery and then MFD; E, MFD-fed born to a dam fed MCD from 3 months of age.

(2.2′azinobis 3-ethylbenzthiazoline sulphonic acid; no. A-1888, Sigma), 1 mg/ml, was added. After 60 min the optical densities (OD) were recorded in an ELISA scanner (Titertek multiscan; Flow Lab., Solna, Sweden) at 405 nm. The titres were expressed as an end-point titre where the OD value differed 0·1 units from the background value of a normal guinea-pig serum.

The tracheal reponse to the antigens was recorded by an *in vitro* challenge using a slightly modified Schultz–Dale set-up (Schultz, 1910; Dale, 1913) and the response was related to that of histamine (no. H-7250, Sigma), 10^{-3} mg/ml, according to the following formula:

$$\frac{\frac{1}{\text{antigen deflection in mm}} \times \text{antigen conc. mg/ml}}{\frac{1}{\text{histamine deflection in mm}} \times 10^{-3}\ \text{mg/ml}}.$$

RESULTS

Guinea-pigs born to mothers fed the MCD (group A) showed a significantly reduced IgG titre (Fig. 1) and tracheal responsiveness (Fig. 2) to the whole skimmed milk powder V67, as well as to the individual protein β-LG ($P < 0·05$) compared to controls born to mothers on the MFD. Feeding both the mothers and the offspring the MCD (group B) or by feeding just the offspring MCD from birth (group C) gave the same result.

Changing the mothers diet from MCD to MFD 10 days before delivery caused an intermediate antibody response in the offspring (group D) and the titres to V67 and β-LG were

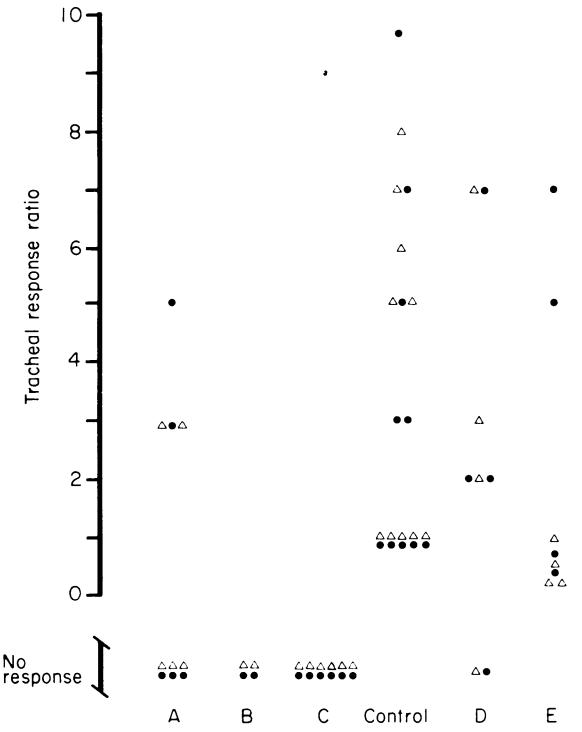

Figure 2. The *in vitro* tracheal response to V67 (△) and β-LG (●) as related to the response to 10^{-3} mg/ml of histamine in guinea-pigs, 2 weeks after i.p. challenge with 50 μg of V67 and 100 mg $Al(OH)_3$ in saline. Groups as in Fig. 1.

significantly ($P<0{\cdot}05$) lower than the controls (Fig. 1). Three out of four animals in this group showed tracheal response (Fig. 2).

Introducing the MCD to adult (3-month-old) mothers did not significantly alter the IgG response in the offspring (group E) compared with the controls, and all animals showed a tracheal response although slightly reduced compared to the controls (Fig. 2).

The response to β-LG alone was almost identical to that of the whole milk whey (V67) in all animals, while no response to α-LA was seen in the Schulz–Dale test of any of the animals. Only very low titres of IgG antibodies were found in four of the controls (data not shown).

Specific IgM antibodies were only detected in a few of the control animals and in two of the animals from group E, but in low titres not exceeding 1/4000 (data not shown). Serum IgA antibodies to milk proteins were not detected in any of the animals from the different groups.

DISCUSSION

The results of the present investigation clearly show that by including protein antigens in the mother's diet it is possible to alter the immune response to these antigens in the offspring. The offspring show a decreased serum IgG titre and tracheal response after challenge despite no direct contact earlier with the antigens. The results indicate that the tolerant state is produced by the antigen itself, since withdrawing the antigen 10 days before delivery markedly reduces the tolerance seen in the offspring (group D). The results in group C show that feeding the MCD to guinea-pigs from birth makes them tolerant to milk proteins, which implies that the dams fed MCD from birth also are tolerant to V67. This was, however, not tested. Since feeding the MCD to a dam from adulthood did not tolerize her offspring (group E) it seems to be crucial how the dam herself has been exposed to and handled the antigen. This observation was only made in the offspring of a single dam and should therefore be interpreted with some caution.

There has been no attempt to discriminate between the IgG and the IgE responses, but since the IgG levels and the Schulz–Dale response show the same pattern we assume that the tolerance seen also includes the suppression of a possible IgE response.

Despite α-LA comprising approximately 35% of the total whey protein (V67) fed, little or no serum antibody could be detected. This is consistent with the findings of other investigators (Ratner *et al.*, 1958; Devey *et al.*, 1976) and could be due to similarities with the homologous α-LA found in guinea-pig milk. β-LG, on the other hand, produced a vigorous response equal to that of the whole preparation (V67), indicating that β-LG is a major antigen in V67. β-LG was only found in ungulate milk and the lack of a homologous protein in guinea-pig milk might explain its immunogenicity in guinea-pigs. In clinical studies of cow's milk protein intolerance, β-LG has been shown to be a major allergen (Freier *et al.*, 1969; Kuitunen *et al.*, 1975).

Assuming that it is the presence of the antigen itself that produces the tolerance in the offspring, it is interesting to speculate on what route the antigen takes. Observations made in our laboratory show that by a single feed of CMP to pregnant guinea-pigs it is possible to detect small amounts of immunologically reactive milk proteins in the amniotic fluid (unpublished data). In the pregnant and lactating rat we have been able to detect CMP in the amniotic fluid (Dahl *et al.*, 1984) and in the milk (Telemo *et al.*, 1986) after feeding with CMP. We have also found that CMP deposited in the amniotic fluid are effectively absorbed by the fetuses and transmitted into their blood stream (Telemo, 1986).

Oral tolerance is believed to be an active state and apparently there are several different mechanisms involved in its induction and maintenance. The nature of the antigen and the dose administered are important factors (Tomasi, 1980). The biological age of the organism and hence the maturation of the immune system seems crucial for the ability to show tolerance (Hanson, 1981; Strobel & Ferguson, 1984) and it is necessary to consider the stage of maturation when comparing neonates from different species. The guinea-pig is relatively mature at birth and starts to eat solid food in addition to the mother's milk almost immediately. If the immune system is to be prepared for this immediate massive antigen exposure, the 'schooling' must take place *in utero*; this is supported by the present results.

Bruce & Ferguson (1986) found that serum from mice fed OVA produced a suppressed DTH response when injected into a recipient mouse, whilst injecting OVA directly failed to produce this response. The altered rsponse to 'biologically filtered' antigens must contribute to the effectiveness of the 'transfer' of tolerance from mother to offspring seen in this study.

With the growing evidences of an active priming of the offspring via maternal antibodies (idiotypes, anti-idiotypes or possibly immune complexes) (Rubenstein, Yeh & Bona, 1982; Stein & Söderström, 1984; Jarret & Hall, 1983), it is important to consider the immune response produced in the dams. It is

therefore likely that introducing the antigen to an adult guinea-pig dam produces a different response than feeding her from birth, as evaluated by the altered response in her offspring seen in this investigation (group E). The slightly reduced tracheal response seen in this group might be due to the influence of maternal antibodies. Maternal IgG has been shown to 'protect' the offspring from an IgE response (Jarret & Hall 1983).

In conclusion we propose that maternal antigen experience is of importance for the immune response to antigens in offspring. In general, we can speculate that a tolerant mother gives birth to a tolerized offspring.

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