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Genetic differences in immune exclusion and partial tolerance to ingested antigens

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SUMMARY

The effect of feeding ovalbumin was studied in CBA, C_3H, SWR/J and A inbred strains of mice. In non-immune animals differences were observed in their abilities to absorb intact antigen and eliminate it from their circulation. Following oral immunization, C_3H mice were able to absorb significantly less ovalbumin than controls, however no evidence for immune exclusion was found in the other strains. Oral tolerance could be induced in all strains tested but in CBA and SWR/J mice oral immunization had to be continual, via the drinking rather than a single daily gastric intubation. The results suggest that genetic differences exist in the handling of antigen by the gut. If similar differences exist in man they may underly differences in susceptibility to food allergy.

INTRODUCTION

Protection from damaging hypersensitivity reactions at mucosal surfaces to fed antigens may be afforded by both immune exclusion and oral tolerance (Bazin & Platteau, 1977; Newby, Stokes & Bourne, 1980a), and we have previously shown that both events may be stimulated by the same feeding regime (Swarbrick, Stokes & Soothill, 1979). Genetically determined deficiency in the ability of mice to synthesize high affinity antibody (Soothill & Steward, 1971; Petty, Steward & Soothill, 1972) is associated with susceptibility to antigen–antibody complex nephritis (Devey & Steward, 1980). Susceptible strains being inefficient at immune elimination (Alpers, Steward & Soothill, 1972) and have poor macrophage function (Passwell, Steward & Soothill, 1974; Morgan & Soothill, 1975).

Immunopathological disease to extrinsic antigens, is dependant both upon antigen entry and a disordered immune response. Since the handling of antigen by the gut profoundly affects these responses in ways which may differ from the responses to injected antigen we have investigated the possibility that genetic variation exists, in the capacity of four inbred strains of mice to absorb antigen and develop immune exclusion and oral tolerance.

MATERIALS AND METHODS

Mice. Groups of sex matched, SWR/J, CBA, C_3H and A strain mice, aged 2–3 months, were maintained on oxoid diets which contain no egg protein. SWR/J and A strain mice were bred and

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maintained at the Animal House of the Institute of Child Health. CBA and C_3H mice were obtained from OLAC (1976) Ltd. (Oxford, UK).

Oral Immunization. This was attempted in two ways. (i) By gastric intubation. Twenty-five milligrams of ovalbumin (grade III, Sigma) dissolved in 0·2 ml saline was administered for 14 days into the stomach through a Portex tube 1 mm in diameter. Control animals received saline. (ii) In the drinking water. Half a per cent wt/vol. ovalbumin was added to the drinking water for 14 days and changed daily (equivalent to daily dose of 25 mg). Control animals received normal drinking water. In later experiments control animals received an equal dose of human serum albumin (HSA Fr.5, Sigma).

Absorption of ovalbumin. This was measured as described previously (Swarbrick *et al.*, 1979). Briefly, after an overnight fast mice received 50 or 40 mg ovalbumin in 0·2 ml saline by gastric intubation. Ovalbumin levels in blood collected from the mesenteric vein 45 min after feeding were measured by a liquid phase double antibody radioimmunoassay, as described. Gel filtration confirmed that the absorbed ovalbumin eluted in the same position as the native protein.

Elimination of ovalbumin. This was measured as described previously (Swarbrick *et al.*, 1979). Briefly mice were injected intravenously (i.v.) via the tail vein with 150 ng monomeric ^{125}I-ovalbumin and bled sequentially from the retro-orbital plexus over the next 6 h.

Immune responses to parenteral immunization with ovalbumin. These were measured as described previously (Swarbrick *et al.*, 1979). Mice were immunized by intraperitoneal injection of 1 mg ovalbumin in Freund's complete adjuvant (FCA) and antibody measured by haemagglutination after 21 days.

Statistics. Wilcoxon's rank sum test was used throughout.

RESULTS

Absorption of ovalbumin in four strains of unimmunized mice

The mesenteric vein serum concentration of ovalbumin measured 45 min after a single feed varied widely (Fig. 1); none was detected in three CBA and two SWR/J mice (less 0·5 ng/ml). C_3H mice had significantly higher levels than SWR mice ($P < 0·01$), but the other differences between strains were not significant.

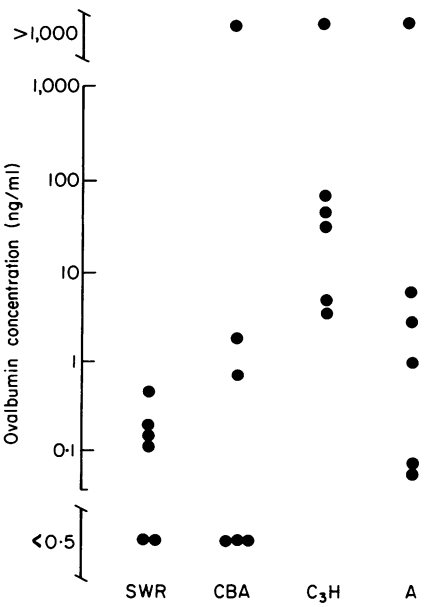

Fig. 1. Serum concentration of ovalbumin (ng/ml) in four strains of mice after an intragastric dose.

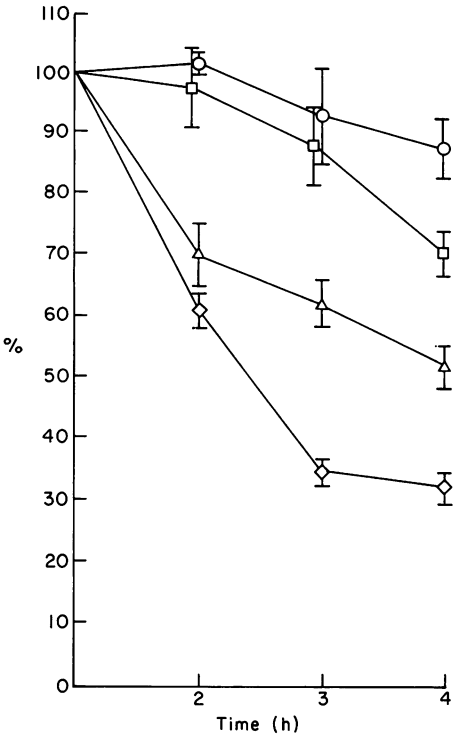

Fig. 2. The elimination of ^{125}I-ovalbumin from the circulation of four strains of unimmunized mice after intravenous injection. Each point is the mean from eight mice ± 1 s.e. (mean). Astrain (○), C_3H (□), CBA (△), SWR (◊).

The rate of non-immune elimination of antigen from the circulation was measured as radioactive counts in 20 μl of blood taken, up to 4 h after the injection (i.v.) of 150 ng ^{125}I-ovalbumin; the rate differed between strains, being greatest in the SWR/J mice and decreasing in the order CBA, C_3H & A (Fig. 2). This may account in part for the higher serum concentration of ovalbumin in C_3H mice than SWR mice after ingestion and could mask differences in antigen absorption.

Effect of oral immunization on subsequent antigen absorption in four strains of mice

The serum ovalbumin concentration 45 min after a feed of 40 mg of ovalbumin was measured 14 days after the completion of oral immunization (by gastric intubation) and compared with control (saline fed) mice. The results are shown in Fig. 3. Orally immunized C_3H mice had less circulating ovalbumin at 45 min than did controls ($P = 0{\cdot}05$; Fig. 3), but no similar immune exclusion was shown in the other strains.

To test the possibility that oral immunization might lead to a systemic immune response potentially capable of immune elimination, similar groups of the four strains were tested for their capacity to eliminate ^{125}I-ovalbumin injected intravenously. The results are shown in Fig. 4. There was no evidence in any strain for accelerated elimination of ^{125}I-ovalbumin in animals, who had previously received the antigen by gavage suggesting that strain observed differences result from variation of immune exclusion.

Effect of oral immunization on the subsequent immune response to injected antigens in four strains of mice

The systemic immune response to 1 mg ovalbumin in FCA was measured 14 days after completion of oral immunization (gastric intubation) and in controls and compared in four strains of mice.

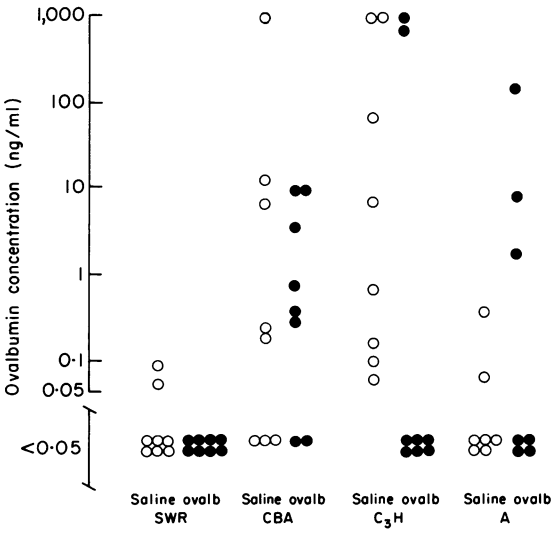

Fig. 3. Serum ovalbumin concentration in four strains of mice 45 min after intragastric administration. The experimental groups had been orally immunized with ovalbumin (●), the controls had received saline (○).

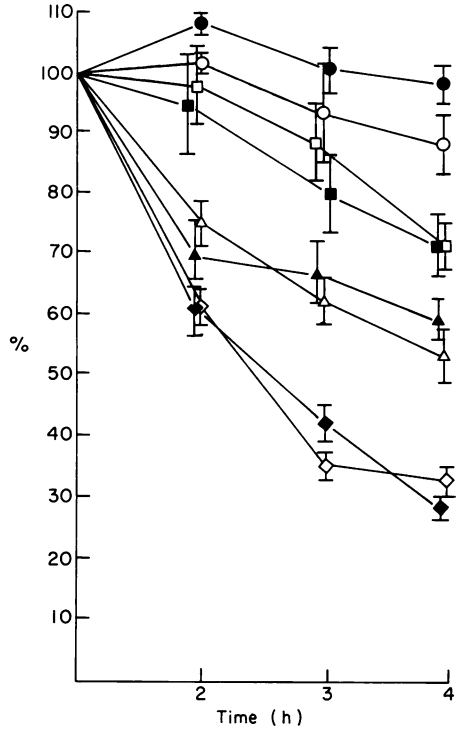

Fig. 4. The elimination of ^{125}I-ovalbumin from the circulation after intravenous injection of four strains of mice previously orally immunized with ovalbumin or fed saline. Each point is the mean from eight mice ± 1 s.e. (mean). Astrain (●, ○), C3H (■, □), CBA (▲, △), SWR (♦, ◊). Open symbols = saline fed, closed symbols = ovalbumin fed.

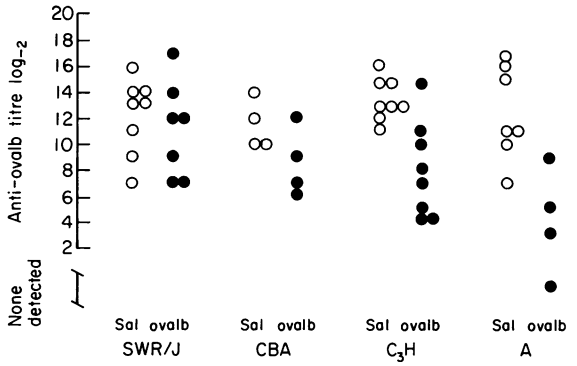

Fig. 5. Haemagglutinating anti-ovalbumin antibody response in four strains of mice, 21 days after parenteral immunization with ovalbumin in FCA, after the previous intragastric administration of saline (○) or ovalbumin (●).

There was no difference in serum antibody levels, 21 days after the injection between control and ovalbumin fed SWR/J mice (no tolerance was seen) and the trend for ovalbumin fed CBA mice to have a lower antibody titre was not significant. However, C_3H and A strain mice, previously fed ovalbumin, had reduced titres of anti-ovalbumin antibodies when compared to the saline fed controls ($P < 0{\cdot}01$) and $P < 0{\cdot}02$, respectively) (Fig. 5). 2-Mercaptoethanol (2-ME) (0·1M) reduced the median titres by only one dilution confirming that the major response was IgG, and the differences between experimental and control animals was maintained.

Effect of type of oral immunization of the subsequent immune response to injected antigens

Since the non-immune elimination of ^{125}I-ovalbumin was more rapid in strains in which tolerance was not obtained following administration of antigen by once daily gavage we also gave it in the drinking water over a 24 h period. As in the previous experiments anti-ovalbumin antibody levels in SWR/J mice fed ovalbumin by gavage were not significantly different from HSA fed controls, although they tended to be lower ($P < 0{\cdot}1$; Fig. 6). However, antibody titre in the groups fed the antigen in the drinking water were significantly lower ($P < 0{\cdot}01$) both than the controls and those receiving ovalbumin by gavage. Similar results were obtained when 2-ME was included in the assay. With CBA mice, ovalbumin feeding led to tolerance irrespective of the manner of oral immunization ($P < 0{\cdot}01$; Fig. 7), but the mice fed ovalbumin in the drinking water were significantly more tolerant than those receiving it by gavage ($P < 0{\cdot}01$). Again the differences were maintained when the assay was performed in the presence of 2-ME.

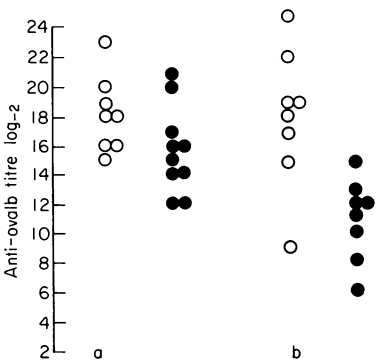

Fig. 6. Haemagglutinating anti-ovalbumin antibody response in SWR/J mice, 21 days after parenteral immunization with ovalbumin in FCA, following oral immunization with HSA (○) or ovalbumin (●) by either (a) gastric intubation or (b) antigen dissolved in the drinking water.

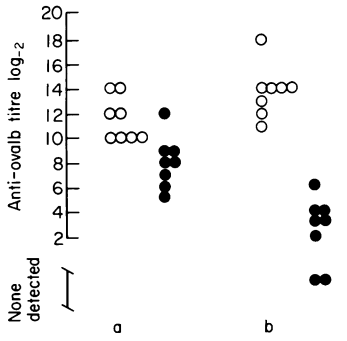

Fig. 7. Haemagglutinating anti-ovalbumin antibody response in CBA mice, 21 days after parenteral immunization with ovalbumin in FCA, following oral immunization with HSA (○) or ovalbumin (●) by either (a) gastric intubation or (b) antigen dissolved in the drinking water.

DISCUSSION

The results show that different inbred strains of mice differ in their handling if ingested antigens. C_3H mice demonstrate simultaneous immune exclusion and specific systemic tolerance after the same antigenic experience as we have reported previously (Swarbrick *et al.*, 1979). In contrast, CBA and A strain mice while showing tolerance after gavage with antigen do not develop immune exclusion; this suggests that these two functions are inherited independently.

Inter-strain differences in non-immune elimination may account for the same differences in macromolecular absorption and might mask the evidence for immune exclusion. The reason for these differences is unknown, but since the strains rank in reverse of that seen for ^{125}I-PVP clearance (Morgan & Soothill, 1975), it is unlikely that macrophage activity is significant factor in non-immune elimination of ovalbumin. Since however the molecular weight of ovalbumin is only 43,000 daltons and it will therefore pass through the glomerular basement membrane, strain differences of renal protein clearance may provide an explanation.

SWR mice could be made tolerant and CBA mice 'more tolerant' when antigen was administered in the drinking water rather than by gastric intubation. By presenting the antigen in this fashion it is possible that some remains in the circulation over a longer period and so induces tolerance. Since it is clearly established that a series of closely spaced injections are required to induce tolerance to rapidly eliminated antigens (Dresser & Mitchison, 1968), it is possible that the differences in the induction of oral tolerance may depend not only upon immune differences but also, non-specific handling of antigen.

Several other publications suggest that such differences in antigen handling occur, for example in the requirements for oral tolerance induction to ovalbumin in mice. For, whereas a single feed of 30 mg failed to make C_3H mice tolerant (Swarbrick, 1979), C57 Bl/6J × DBA/2J F1 hybrids and BALB/c mice could be tolerized by feeding 20 mg and 2 mg respectively (Hanson *et al.*, 1977; Mowat & Ferguson, 1981). Whilst, in view of reported effects of bacterial endotoxin in enhancing tolerance induction, (Newby, Stokes & Bourne, 1980a; Wannemuehler *et al.*, 1982) it is possible that these strain differences may reflect differences in gut flora associated with housing in different colonies it is unlikely since these studies reported that both germ free and conventional mice (whose intestinal tracts contain large numbers of Gram negative bacteria) could be affected by endotoxin. Similarly in studies with the parasite *Trichinella spiralis*, strain differences in IgE and homocytropic IgG, antibody production have been shown following intragastric delivery of the larvae (Perrudet-Badouz, Binaghi & Biozzi, 1975). Inter-strain differences have also been observed in rats, whilst Hooded lister rats make high levels of IgE antibody to ingested antigen (Jarrett *et al.*, 1976; Jarrett & Stewart, 1974), Wistars make low levels (Mota, 1964).

It is clear therefore that genetic differences exist in the immune response to ingested antigens, and that orally-induced tolerance and immune exclusion are inherited independently. Since

mucosal permeability and non-immune elimination may influence their induction, factors that regulate the induction of oral tolerance and exclusion must at least in part be antigen non-specific. Following the introduction of a new dietary protein, in addition to tolerance and exclusion non-specific effects are also generated and these include a transient increase macrophage function and an altered immune responsiveness to heterologous antigens (Newby *et al.*, 1980b). Oral tolerance to a second antigen, fed within 48 h of primary contact with non-related antigen, can also be prevented and these effects have been shown to be related to T cell activity and to be strain dependent (Stokes, Newby & Bourne, 1983). Since tolerance induction by the oral route can also be prevented by cyclophosphamide treatment (Mowat & Ferguson, 1981) it is likely that the inter-strain differences reported here may also reflect differences in T cell function. Clearly such diversity could contribute to different vulnerability to food allergy in such unphysiological circumstances as artificial infant feeding.

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