Absorption of antigens after oral immunisation and the simultaneous induction of specific systemic tolerance

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SUMMARY Antigenic proteins may be absorbed intact. We report here results from *in vivo* experiments in mice showing that the prior feeding of protein antigen may reduce the subsequent absorption of that antigen without altering its elimination from the circulation. This may be a function of local immunity. We have also shown that the same feeding regime can paradoxically induce a state of systemic tolerance and suggest that the two phenomena contribute to the safe handling of these antigens.

Large molecular weight proteins are absorbed intact by the intestine in quantities which are insignificant nutritionally but potentially important immunologically (Walker and Isselbacher, 1974). There is some evidence from *in vitro* experiments in rats to suggest that animals which have previously eaten an antigen absorb less of it subsequently (Walker *et al.*, 1972) and it has been suggested that this immune exclusion is a function of local antibody (Bazin *et al.*, 1973).

In 1946 Chase showed that the prior ingestion of dinitrochlorobenzene (DNCB) by rabbits reduces the subsequent sensitisation by that substance (Chase, 1946). Recent work has shown reduced antibody production to parenterally administered bovine serum albumin or sheep red blood cells when preceded by a period of antigen feeding (Thomas and Parrott, 1974; André *et al.*, 1975). We have performed experiments to investigate the paradox that both local immunity, causing exclusion of antigen, and also specific systemic tolerance can result from the same antigenic experience—feeding.

Methods

ANIMALS AND EXPERIMENTAL TECHNIQUES

Groups of 8 C₃H mice aged 2 to 3 months were used in all experiments and maintained on Oxoid diets

which contain no egg protein. Twenty-five milligrams of ovalbumin (grade III, Sigma) dissolved in 0.2 ml saline was administered daily for 14 days into the stomach of experimental animals through a Portex tube 1 mm in diameter. Control mice were given saline. The animals were then maintained on normal diet only for 14 days before study in one of three ways: (1) the absorption of ovalbumin; (2) the elimination of ovalbumin from the circulation; (3) the antibody response to parenteral immunisation with ovalbumin.

RADIOLABELLING

¹²⁵I-ovalbumin was prepared by using a modification of the chloramine-T method (Hunter and Greenwood, 1962). Ten micrograms of ovalbumin (grade V, Sigma) was iodinated with 1 mCi Na ¹²⁵I (Radiochemical Centre, Amersham) using 100 µg chloramine T in 45 µl phosphate buffer pH 7.4 at room temperature. The reaction was stopped immediately after mixing by the addition of 240 µg sodium metabisulphite in 100 µl of buffer. Excess iodide (20 µl 1% sodium iodide) and 5 drops of 5% human serum albumin in buffer were added and the labelled, monomeric ovalbumin separated from free iodide and aggregated protein by gel filtration on a Sephadex G150 column 30 cm by 1 cm.

RADIOIMMUNOASSAY (RIA)

Duplicate assay tubes (LP3 Luckham) were set up containing 50 µl test serum, 50 µl normal mouse serum, and 100 µl ¹²⁵I-ovalbumin adjusted to give approximately 60 000 cpm 100 µl rabbit anti-

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immune elimination—the disappearance of ^{125}I -ovalbumin from the blood after an intravenous injection was measured.

Similar groups were injected intravenously via the tail vein with 150 ng monomeric ^{125}I -ovalbumin in 0.05 ml phosphate buffer and bled sequentially from the retro-orbital plexus hourly for four hours using 20 μl capillary tubes. The radioactivity in the blood during the four hours after intravenous injection was no different in mice which had previously been fed ovalbumin than in those previously fed saline (Fig. 2).

Fig. 2 The elimination of ^{125}I -ovalbumin from the circulation after intravenous injection in mice previously fed ovalbumin (—) and in control, saline fed mice (---). Each point is the mean from eight mice $\pm$ 1 SEM.

The differences in serum concentration between experimental and control animals after ingestion of the antigen are therefore not due to differences of immune elimination and might be due to differences in absorption.

IMMUNE RESPONSES TO PARENTERAL IMMUNISATION WITH OVALBUMIN

Identical groups of mice were immunised with 1 mg ovalbumin in complete Freund's adjuvant by intraperitoneal injection 14 days after their last gavage. The animals were bled three weeks later and the antibody titre to ovalbumin measured by passive haemagglutination.

The mice not previously exposed to ovalbumin showed high titres of antibody to ovalbumin after parenteral immunisation (Fig. 3). The mice which had been fed ovalbumin showed significantly less antibody response ($P < 0.01$ Wilcoxon rank test). The titres of antibody in both groups fell by only one dilution when the assay was performed in the presence of 0.1 M 2-ME and the differences were maintained.

Fig. 3 Haemagglutinating anti-ovalbumin antibody response 21 days after parenteral immunisation with ovalbumin in complete Freund's adjuvant after the previous ingestion of ovalbumin or saline.

To test the specificity of this response three further groups of mice were fed saline, HSA, or ovalbumin in the same way for two weeks. After two weeks' rest all animals were immunised with 1 mg HSA in CFA intraperitoneally and the anti-HSA antibodies assayed using a similar passive haemagglutination of HSA-coated SRBC. Mice fed saline or ovalbumin showed similar high anti-HSA antibody responses, while animals fed HSA showed reduced responses (Fig. 4) ($P < 0.05$ Wilcoxon rank test).

Discussion

We have shown that feeding ovalbumin to mice reduced its subsequent absorption while simultaneously inducing a state of systemic tolerance; previously, these phenomena have been reported independently. This suggests a form of split tolerance as does the work of Kagnoff (1977), who showed that

Fig. 4 Haemagglutinating anti-HSA antibody response to parenteral immunisation with HSA in complete Freund's adjuvant after the previous ingestion of HSA ovalbumin or saline alone

previous ingestion of sheep red blood cells increased the IgA plaque forming cells in the spleen but reduced the IgM response after subsequent parenteral immunisation.

Following the original observation in humans that bovine antigens were detectable in the blood of infants shortly after weaning but that subsequently they disappear as antibodies appear (Lippard *et al.*, 1936), *in vitro* studies have shown immune exclusion after oral and parenteral immunisation (Walker *et al.*, 1972, 1973). *In vivo* studies have also been reported (André *et al.*, 1974; Walker and Bloch, 1977), though the amounts of antigen detected by André were surprisingly high and Walker's *in vivo* effects could have resulted from either immune exclusion or elimination. Our demonstration that oral feeding of antigen does not lead to its subsequent accelerated clearance suggests that the conventional mechanisms of immune elimination do not contribute to the altered handling of ingested antigen.

Immune exclusion may be a function of immunoglobulin, particularly secretory IgA (sIgA) (Bazin *et al.*, 1973). Serum IgA antibodies can perform this function (Stokes *et al.*, 1975) and it is likely that sIgA, with its intimate mucosal association (Tomasi and Bienenstock, 1968) and its innate resistance to digestion (Brown *et al.*, 1970; Steward, 1971), functions more efficiently. Walker's *in vitro* experiments suggest that IgG can do it too (Walker *et al.*, 1972).

IgA deficiency is associated with an increased incidence of antibodies to food proteins (Buckley and Dees, 1969), and with allergy, including food allergy (Ammann and Hong, 1971; Buckley, 1975). Transient IgA deficiency in the infants of atopic

parents precedes the development of allergic symptoms (Taylor *et al.*, 1973). Furthermore, allergen avoidance during the vulnerable period on these genetically predisposed children prevents the development of eczema (Matthew *et al.*, 1977). It is possible, therefore, that lack of IgA mediated antigen exclusion may result in a damaging allergic response to such antigens absorbed across the mucosa.

There is a significant, though unusual, association of IgA deficiency with coeliac disease (Hobbs, 1971; Booth, 1977) which might suggest that ineffective immune exclusion of gluten or one of its constituents induces damaging local immune reactions and produces the typical lesion in genetically vulnerable individuals. Defective antigen exclusion might also account for the immune phenomena associated with inflammatory bowel disease (Jewell and Hodgson, 1976) and the presence of circulating antifood antibodies in children with malnutrition (Chandra, 1975).

The mechanisms of orally induced tolerance are obscure. There are a number possible for tolerance to parenterally administered antigens (for review see the *British Medical Bulletin*, 1976) but there is evidence that at least some orally induced tolerance is different. The minute amounts of intact antigen absorbed make it likely that orally induced tolerance is of 'low zone' type and Thomas and Parrott (1974) showed that such tolerance to soluble protein antigen is T-cell dependent. However, the passive transfer to normal mice of contact sensitivity to picrylchloride and oxalazone with immune cells may involve both T or B cells (Asherson *et al.*, 1977).

The passive transfer with serum of orally induced tolerance to SRBC has also been reported (André *et al.*, 1975; Kagnoff, 1977) and André produced indirect evidence to suggest that it might depend upon IgA-antigen complexes. This led to the attractive hypothesis that IgA antibody-ingested antigen complexes might persist for a long time, perhaps because of lack of complement mediated opsonisation, thereby inducing tolerance. However, we have demonstrated in passive transfer experiments that IgA antibodies promote rapid elimination of antigen and increase rather than reduce the antibody response to subsequent immunisation (Swarbrick *et al.*, 1976).

Orally induced tolerance seems to be an appropriate response to common but otherwise harmless antigens. A breakdown of this process might lead to a variety of disease states; it provides an alternative explanation to immune exclusion for the pathogenesis of allergy (Okumura and Tada, 1971) or the immune phenomena associated with coeliac disease and inflammatory bowel disease. Bazin, using an

immunising regime similar to our own, has shown that antigen feeding can suppress subsequent IgE responses to injected antigens in rats (Bazin and Platteau, 1977) and as early as 1911 it was shown that young guinea-pigs fed maize (Wells and Osborne, 1911) oats, or egg-albumin (Wells, 1911) were resistant to anaphylactic sensitisation to these particular proteins.

Our observations suggest that the two phenomena—immune exclusion and tolerance induction—are not mutually exclusive. It is possible that they are interdependent and together would lead to the safe handling of ingested antigens.

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