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Specific suppression of rat IgE responses with milk from immunized females and with feeds of serum antibody

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Summary. *Suckling by ovalbumin sensitized females suppressed the IgE anti-ovalbumin response of young rats. Suppression was also achieved after feeding rat pups with (i) immune adult rat serum and (ii) isolated serum IgG containing anti-ovalbumin antibody.*

Antigen-specific suppression of IgE responsiveness has been demonstrated in the offspring of immunized female Hooded Lister rats and was shown by cross-fostering to depend on the ingestion of milk from an immunized lactating female (Jarrett & Hall, 1979). Such suppression has not yet been shown in human infants and there is no evidence of a decreased incidence of allergic disease in the infants of atopic mothers (Hide & Guyer, 1981; Turner, Rosman & O'Mahoney, 1974).

Unlike breast fed human infants, the suckled newborn rat absorbs maternal immunoglobulin G from the gastrointestinal tract (Rodewald, 1973). It is possible that such antibody might influence the IgE response in suckled offspring by a feedback regulation (Tada & Okumura, 1970). We have investigated this possibility by studying the effect of ingested IgG anti-ovalbumin antibody on the IgE anti-ovalbumin response of the young rat and have shown that specific suppression does indeed occur.

Three main experiments were performed. In the first of these, adult female Hooded Lister rats (Animal Suppliers Ltd, London) were immunized within 7 days of mating with a single intraperitoneal injection of 10 mg ovalbumin (Sigma five times crystallized) and 10^{10} heat-killed *Bordatella pertussis* (Wellcome Research Laboratories). Fourteen days after injection the animals were lightly anaesthetized with ether and 2 ml of blood taken by cardiac puncture; there were no deaths. These sera were separated and shown to have high titres of agglutinating anti-ovalbumin antibody. The basic sequence of events in the immunization of weanling rats suckled by the immunized females is summarized schematically in Fig. 1. Weanlings were immunized at 22 days with an initial intraperitoneal injection of 1·0 μg ovalbumin and 10^{10} heat-killed *B. pertussis*, followed by a booster intraperitoneal injection of 1·0 μg ovalbumin on day 56. They were bled 4 days later by cardiac puncture.

Ten immunized adult female rats and ten unimmunized female rats were mated and each group produced six litters respectively. Litters from four immunized females were exchanged with litters from four unimmunized females within 12 hr of birth. These litters were then suckled by the fostering female until weaning and were then immunized as above. Two immunized females and two unimmunized females suckled their own litters. The rat pups were immunized as described and their IgE and IgG antibody responses determined (see below).

In the second experiment, rat pups born to unimmunized mothers were gavaged daily between day 5

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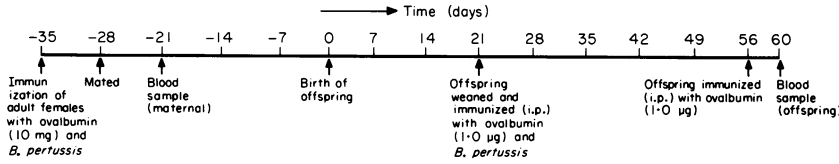

Figure 1. Schematic flow-diagram showing the basic sequence of events in the immunization of weanling rats suckled by immunized females. The cross-fostering and feeding experiments were superimposed on this basic design. Secondary IgE and IgG responses were measured in serum obtained on day 60.

and day 21 post partum with pooled immune rat serum obtained from adult male rats after three subcutaneous injections at 14 day intervals of ovalbumin (10 mg) in Freund's complete adjuvant. The serum was diluted in physiological saline to an IgG concentration similar to that in rat milk (McGhee, Michalek & Ghanta, 1975) and was given in volumes of 0·5 ml into the fasting stomach through a fine polythene tube 3 cm in length (Portex; internal diameter 0·28 mm).

In the third experiment, rat pups born to unimmunized mothers were gavaged with isolated rat IgG containing anti-ovalbumin antibodies. Serum (15 ml) from ovalbumin immunized male rats was applied to a column of DEAE-Sepharose CL 6B (30 × 3 cm) equilibrated in 0·1 M Tris-HCl buffer pH 8·0 (Karlsson, 1978). IgG antibodies to ovalbumin were eluted in the first peak and fractions corresponding to this region were pooled and concentrated by ultrafiltration to a final volume of 1·5 ml (protein concentration 7·3 mg/ml). Immunoelectrophoresis of this serum fraction against polyvalent anti-rat serum confirmed that it contained only IgG.

In all three experiments IgE antibody was measured by a paper radioallergosorbent test (Karlsson, Ellerson, Haig, Jarrett & Bennich, 1979) using ovalbumin-coated paper discs and ^{125}I-labelled rabbit anti-rat Fc_ε (kindly provided by Dr E. E. E. Jarrett). Positive control sera and newborn rat sera (containing no IgE antibody) were included in each assay as positive and negative controls. Results were expressed as counts bound relative to the binding obtained with newborn rat serum. Haemagglutinating antibody (presumably IgG antiovalbumin) was detected using chromic chloride treated, ovalbumin coated, erythrocytes after pretreatment of the serum with dithiothreitol to inactivate IgM antibodies (Kofler & Wick, 1977; Olsen, Weiblin, O'Leary, Moscowitz & McCullough, 1976).

The results of the first experiment are shown in Fig. 2. Secondary responses were significantly greater in rat pups from unimmunized females (group A) than in those from immunized females (group B)—$P < 0{\cdot}001$, Mann Whitney U test. The cross-fostering experiment confirmed that the milk of immunized females (groups D and F) is able to suppress the IgE response (Mann Whitney U test; Groups C and D, $P < 0{\cdot}01$ and Groups E and F, $P < 0{\cdot}05$).

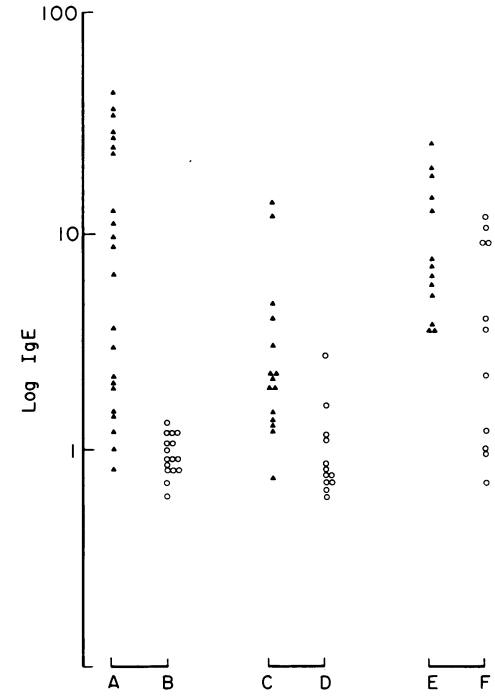

Figure 2. The influence of cross-fostering on the IgE anti-ovalbumin levels in the sera of six groups of rats from twelve litters. Groups were as follows: group A, offspring of unimmunized mother—suckled by unimmunized mother; group B, offspring of immunized mother—suckled by immunized mother; group C, offspring of immunized mother—cross-fostered by unimmunized mother; group D, offspring of unimmunized mother—cross-fostered by immunized mother; group E, offspring of immunized mother—cross-fostered by unimmunized mother; group F, offspring of unimmunized mother—cross-fostered by immunized mother.

In the second experiment pooled serum from rats immunized with ovalbumin was diluted 1:8 in 0·15 M NaCl, to achieve an IgG concentration of about 1·5 mg/ml, approximately that of rat milk (McGhee *et al.*, 1975). Eight rat pups from litters of two unimmunized females were gavaged with 0·5 ml of this preparation daily between day 5 and day 21. A control group of ten pups from the same litters were similarly treated with diluted serum from unimmunized adult rats. The weaned rats were immunized with ovalbumin on day 22 and specific IgE responses measured as before (see Fig. 3). The secondary IgE antiovalbumin response in rats treated with serum containing ovalbumin antibody was significantly less than that of controls ($P=0·005$, Mann Whitney U test). The degree of suppression was comparable with that seen in rats suckled by immunized females (Group B, Fig. 2).

In the third experiment six rat pups from one unimmunized female were gavaged each day between the ages of 5 and 15 days with 0·5 ml volumes of the IgG fraction of immune serum diluted 1:20 with 0·15 M NaCl to give a concentration of 0·36 mg IgG/ml. Nine untreated control rats from the same litter were suckled normally. At weaning, the six treated rats and six control rats were immunized with ovalbumin. IgE anti-ovalbumin was detected in both groups of immunized rats but not in the unimmunized controls (see Fig. 4). The median response was less in animals treated with the IgG preparation. Although this difference did not reach significance by the Mann Whitney U test ($P=0·06$), it was significant by t test on log transformed data ($t=2·75$, $P<0·05$), a more appropriate analysis with such small numbers.

The suppression of the IgE anti-ovalbumin response in rats fostered by ovalbumin-sensitized

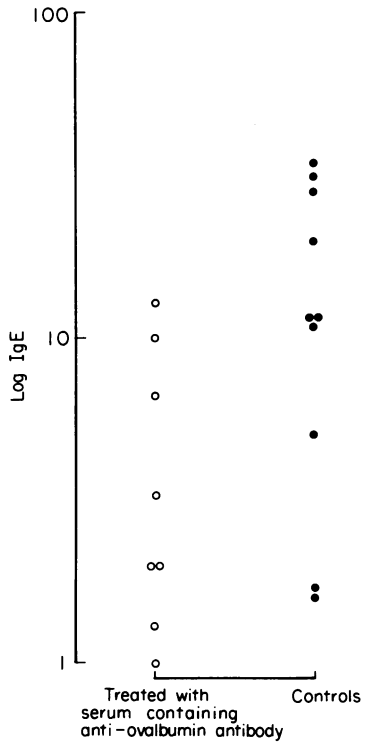

Figure 3. Secondary IgE anti-ovalbumin responses in rat pups born to unimmunized mothers and previously gavaged with adult rat serum containing anti-ovalbumin antibody (○). Litter mate control animals (●) received non-immune rat serum. IgE antibodies were measured by RAST and results expressed as counts bound relative to counts bound by newborn rat serum.

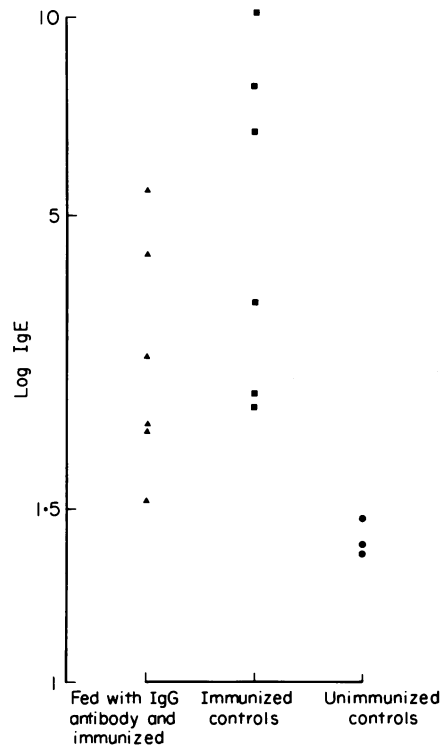

Figure 4. Secondary IgE anti-ovalbumin responses in rat pups born to unimmunized mothers and previously gavaged with isolated rat IgG containing anti-ovalbumin antibodies (▲). Litter mate controls were suckled normally and either immunized with ovalbumin (■) or remained unimmunized (●).

females, confirms the results of Jarrett & Hall (1979). A comparable degree of suppression was also shown in rats fed serum from ovalbumin-immunized animals suggesting that a component of serum, probably specific anti-ovalbumin antibody, ingested during the suckling period, prevents a subsequent IgE anti-ovalbumin response. Rats of weaning age have IgG concentrations in serum approaching those in adult rats (McGhee *et al.*, 1975) yet only a small amount of this immunoglobulin is acquired *in utero* and most of it is absorbed from milk ingested during suckling, probably through a mechanism involving specific Fc receptors on intestinal epithelial cells (Borthistle, Kubo, Brown & Grey, 1977). IgG from ingested serum is presumably absorbed in the same way. In our third experiment, limited by the amount of immunoglobulin available, there was a similar suppressive effect for IgG from immune serum. The dose of IgG was only 1·3% of the calculated amount normally acquired from milk and was therefore suboptimal. Though it is likely that IgG anti-ovalbumin suppresses the IgE response, it is possible that there are other suppressive factors too. The involvement of other mechanisms of suppression is also suggested by the observations of Jarrett *et al.*, (1979). These authors were only just able to detect circulating maternal anti-ovalbumin antibody in the offspring before immunization. Despite the suppression of IgE antibody, the total antibody and IgG antibody response of immunized rats from immunized females was greater than that of controls, suggesting that suppression was confined to the IgE response.

The suppression of the IgE response in rats of unimmunized mothers, cross-fostered to immunized lactating females was less complete than in non-crossed litters of immunized females (Fig. 2). This may be explained by inter litter variation in responsiveness or possibly by intrauterine suppression. The human foetus acquires IgG by the transplacental route (Brambell, 1966) and so an antigen-specific mechanism for prevention of sensitization may already exist at birth in the human neonate. The experiments with young rats suggest that the IgG mechanism would depend on the titre of specific maternal antibody in the neonate. The mechanism of suppression is not clear and it should be stressed that the experimental induction of IgE sensitivity described here is very different from the usual routes of sensitization via mucosal surfaces. Neonatal feeding practices might also be critical in the prevention of sensitization since an antigen non-specific enhancement of IgE responses by supplementary feeds in suckling rats has been observed (Roberts & Soothill, 1982) and may well operate in human infants. The antigen-specific suppressive mechanism may only protect during the first months of life when immunization, for example with Salmonella vaccine, is difficult to achieve (Smith & Eitzman, 1964) and atopic allergies have not yet become manifest. However, the effects of some of these protective mechanisms might persist throughout childhood, preventing later disease in the genetically predisposed individual. The therapeutic possibility of immunizing mothers in order to protect at-risk children awaits investigation.

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