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Immune Response against Ovalbumin in Rats Colonized with an Ovalbumin-Producing *Escherichia coli* and the Influence of Feeding Ovalbumin

Key Words

Immune response
 Hypersensitivity
 Oral tolerance
Escherichia coli

Abstract

The influence of feeding ovalbumin (OA) on the development of IgE/IgG antibodies and delayed-type hypersensitivity (DTH) against OA was studied in rats colonized from birth with an *Escherichia coli* genetically manipulated to produce OA. At 21 days of age, colonized pups and pups with a normal intestinal flora were weaned onto either an OA-containing or a conventional diet without OA. At 2 months of age the colonized rats showed an increased DTH reaction to OA, but they did not have any anti-OA antibodies in serum. The rats were then immunized intracutaneously with OA in Freund's complete adjuvant. After immunization the colonized rats fed the conventional diet had a significantly higher DTH reaction to OA and significantly higher serum levels of IgE anti-OA antibodies than the uncolonized rats on the same diet. The colonized rats eating the OA-containing diet showed a 73% decrease in the DTH reaction to OA and also significantly lower levels of IgE and IgG antibodies against OA compared with the colonized rats fed conventional diet. The dams colonized as adults by the OA-producing *E. coli* developed IgE anti-lipopolysaccharide antibodies in serum while the pups colonized via the dams at birth did not. Neonatal colonization with an *E. coli* strain producing OA resulted in increased DTH reactivity against OA and priming for secondary IgE anti-OA response. Feeding the animals an OA-containing diet from weaning abrogated this intestinally induced hypersensitivity and rendered the animals orally tolerant to OA.

Introduction

The development of the immune response against antigens exposing the gut-associated lymphoid tissue depends on the way the antigen is administered. For instance, feeding soluble antigens to adult and neonatal animals often leads to a state of tolerance to subsequent immunization, both in

terms of in vivo T-cell reactivity and antibody response [1–5]. In addition, in a previous study we have shown that the gastrointestinal immune system discriminates between bacterial and dietary antigens, in that germ-free rats monocolonized with an *Escherichia coli* strain and simultaneously fed ovalbumin (OA) only responded with antibodies to the bacterial antigens, but not the dietary antigen [6]. On the

Received:
 March 16, 1994
 Accepted after revision:
 July 25, 1994

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 1018–2438/94/1054–0381
 \$8.00/0

other hand, when germ-free rats were monocolonized with an OA-producing *E. coli* strain they responded against OA with both IgG and IgA antibodies [7]. Recently [8] we also demonstrated that conventional rats colonized from birth with an OA-producing *E. coli*, developed an enhanced delayed-type hypersensitivity (DTH) reaction to OA after immunization, along with elevated IgE and IgG antibodies against OA, but no response to the bacterial lipopolysaccharide (LPS) and fimbriae antigens. Thus, when OA is presented to the intestinal immune system of young conventional rats by a bacterium, it induces a DTH reaction as well as an IgE response. This can be seen as a model of a natural way of inducing immunologically mediated hypersensitivity to dietary antigens via the intestine. It has previously been shown that parenterally induced immune responses can be down-regulated by subsequent feeding of the antigen [5, 9]. Therefore, we wanted to study if subsequent feeding of an antigen can reverse the hypersensitive state seen in our model.

Materials and Methods

Animals

Eight-week-old Sprague-Dawley rats were obtained from BK Universal, Stockholm, Sweden. Rats of the same strain, born by dams who had been colonized with the genetically manipulated *E. coli*, were also raised in our own animal house.

Rat Diet

The experimental diet consisted of a standard rat chow where half of the protein content was replaced with equal parts of egg protein and milk whey protein [6]. The standard rat diet is based on cereals, fish, soy and yeast with a dry weight protein content of 24% (R3; Ewos, Södertälje, Sweden).

Expression of OA in *E. coli*

The plasmid pOMP21 was obtained by fusing cDNA for OA to the lac-operon [10, 11]. In the protein synthesized, the first seven amino acids derive from β -galactosidase; the seventh residue is connected to the fifth residue of OA. The localization of the OA, when the plasmid is expressed in *E. coli* K-12 is periplasmic and cytoplasmic, but the protein is not secreted [12]. In the present study the pOMP21 plasmid was introduced into the *E. coli* strain O6K13H1 [7]. The plasmid also carries a gene which confers ampicillin resistance to the bacteria.

Bacterial Colonization

Eight-week-old female rats were given streptomycin (Evans Med. Ltd, Langhurst, Horsham, UK) in the drinking water (5 g/l) for 1 day. They were starved for 24 h and then given 10^{10} genetically manipulated *E. coli* in 1 ml bicarbonate buffer (0.2 M), through a stomach tube. The rats were given water containing ampicillin (0.5 g/l, Doktacillin; Astra, Södertälje, Sweden) ad libitum during the whole period of colonization. Fecal cultures were done on ampicillin agar at regular intervals. The rats were mated immediately after colonization.

Table 1. Experimental groups

	Group				
	1	2	3	4	5
Colonized	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	–	–
Diet	Std	Std	OA	OA	Std
Immunized	PBS	OA	OA	OA	OA
Challenged	OA	OA	OA	OA	OA

OA = Ovalbumin in the diet or in FCA for subcutaneous immunization; Std = standard diet; *E. coli* = colonized from birth with an *E. coli* strain producing OA; PBS = phosphate-buffered saline.

Experimental Procedure (table 1)

The experiment included five groups of pups. Groups 1–3 were delivered by conventional dams colonized with the OA-producing bacteria. Hence, the pups in these groups were naturally colonized at birth with the OA-producing bacteria. Weekly fecal cultures were done on ampicillin agar to assure that they retained the bacteria. The pups in groups 4 and 5 were born by conventional dams carrying a normal intestinal bacterial flora. Groups 1, 2 and 5 were weaned at 3 weeks of age onto a standard rat diet without OA and were used as controls in the DTH experiment. The pups in groups 3 and 4 were weaned at 21 days of age onto the experimental diet fed ad libitum for 5 weeks. The intake of OA from this diet was estimated to 0.8 g/rat/day.

When the rats in groups 2–5 were 8 weeks old they were immunized with a subcutaneous injection on the back with 50 μ l OA (20 mg/ml) (Sigma, St. Louis, Mo., USA) emulsified in Freund's complete adjuvant (Difco Laboratories, Detroit, Mich., USA). Two weeks later, all rats in groups 1–5 were given a challenge intradermally in one ear with 20 μ l OA (2.5 mg/ml) in phosphate-buffered saline (PBS) and 20 μ l PBS in the other ear. The increase in ear thickness was measured 24 h later with a micrometer (Oditest; Kröplin, Hessen, Germany).

Collection of Serum

When the pups were weaned onto a different diet, the dams were sacrificed and blood samples taken. In addition, blood samples were collected from the tail vein of all rats before immunization. After measurement of the increase in ear thickness, all rats were sacrificed and blood samples collected. The serum was also collected from the dams 3 weeks, postpartum. Serum was stored at -20°C until analyzed.

Antibody Determinations

Serum samples were analyzed with an enzyme-linked immunosorbent assay (ELISA) for IgG and IgE antibodies against OA and LPS as described previously [8]. The primary and secondary antibody for IgE measurements were monoclonal anti-rat IgE (MCA 193; Serotec, Oxford, UK), diluted 1,000 in PBS-Tween and goat anti-mouse IgG alkaline phosphatase-conjugated antibody (Tago, Inc., Burlingame, Calif., USA) diluted 1/4,000 in PBS-Tween. The IgG measurements were done simultaneously and we used rabbit anti-rat IgG (Zymed Laboratories, Inc., San Francisco, Calif., USA) diluted 1/10,000 in PBS-Tween and goat anti-rabbit IgG alkaline phosphatase-conjugated antibody, (Sigma Immuno Chemicals, St. Louis, Mo., USA) diluted 1/10,000 in PBS-Tween. The specificity of the antibody reagents was extensively tested against rat myeloma proteins (PharMingen, San

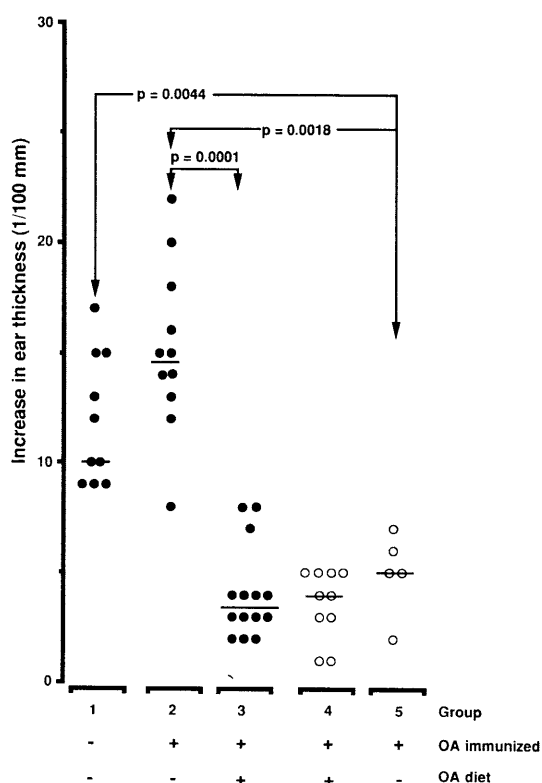


Fig. 1. DTH reactions against OA measured as increase in ear thickness in rats colonized with an *E. coli* strain producing OA (●, groups 1–3), and in control rats (conventional rats which had never been exposed to OA, ○, groups 4 and 5). The animals were immunized intracutaneously with OA in Freund's complete adjuvant (+) 2 weeks (groups 2–5) before challenge with OA in the ear (groups 1–5). The rats in groups 3 and 4 were fed with OA-containing food (+). Each symbol represents 1 rat and the bars denote the medians.

Diego, Calif., USA) and polyclonal rat IgG. All values were calculated and compared with a standard sample with the aid of a computer program and expressed as arbitrary ELISA units in the diagrams.

Statistics

The data were tested for statistical significance by the Mann-Whitney U test and Spearman rank correlation test.

Results

DTH Response to OA

The results of colonization with the genetically manipulated *E. coli* on the DTH reaction against OA is shown in figure 1. The colonized animals, whether immunized or not, showed a significantly greater increase in ear thickness than uncolonized rats immunized with OA (groups 1 [$p = 0.0044$]

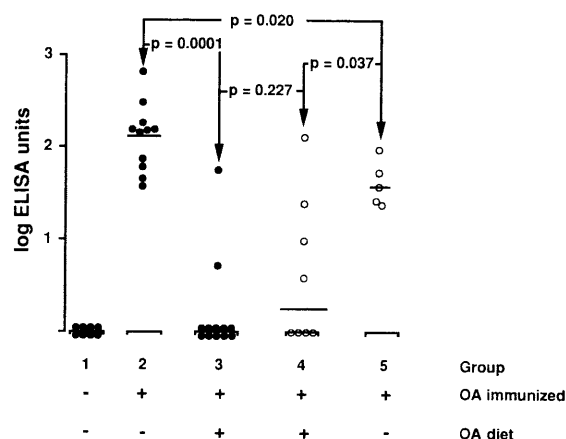


Fig. 2. IgE antibodies in serum against OA recorded by ELISA in 10-week-old rats, which were immunized intracutaneously with OA in Freund's complete adjuvant (+) 2 weeks earlier (groups 2–5). Filled circles (●) are rats colonized from birth with an *E. coli* strain carrying a plasmid, which codes for production of OA (groups 1–3). Empty circles (○) are control rats with a conventional intestinal bacterial flora. The rats in groups 3 and 4 were fed with OA-containing food (+). Each symbol represents 1 rat and the bars denote the medians.

and 2 [$p = 0.0018$] versus group 5). In contrast, rats colonized with the bacteria and fed OA (group 3) had a significantly lower DTH reaction ($p = 0.0001$) than the colonized animals fed normal diet (group 2) and were indistinguishable from the noncolonized OA-fed rats in group 4.

IgE and IgG Antibody Response to OA and LPS

The IgE and IgG antibody activity against OA are shown in figures 2 and 3. There were no detectable antibodies against OA before immunization in the colonized rats (group 1), which was in contrast to the DTH reaction. However, the colonized rats were primed by the colonization, since immunization of the rats with OA resulted in a significantly higher level ($p = 0.020$) of IgE anti-OA antibodies in the colonized rats than in the control rats (group 2 versus 5). Feeding the OA-containing diet significantly reduced both the IgE and the IgG antibody response against OA ($p = 0.0001$ for both isotypes) in the colonized and immunized rats (group 3). The uncolonized rats on the OA diet (group 4) also showed a significantly reduced IgE and IgG response to OA ($p = 0.037$ and 0.012 , respectively) as compared to the controls (group 5). Most of the animals in the two groups fed the experimental diet did not develop any IgE or IgG antibodies to OA at all. The animals colonized

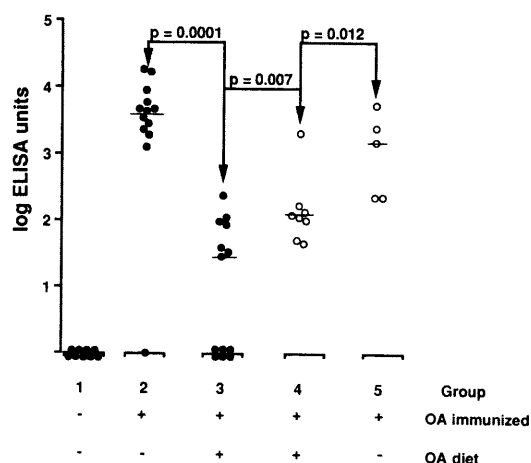


Fig. 3. IgG antibodies in serum against OA recorded by ELISA in 10-week-old rats, which were immunized intracutaneously with OA in Freund's complete adjuvant (+) 2 weeks earlier (groups 2–5). Filled circles (●) are colonized from birth with an *E. coli* strain carrying a plasmid, which codes for production of OA (groups 1–3). Empty circles (○) are control rats with a conventional intestinal bacterial flora. The rats in groups 3 and 4 were fed with OA-containing food (+). Each symbol represents 1 rat and the bars denote the medians.

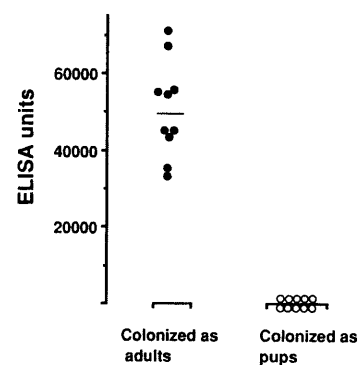


Fig. 4. IgE anti-LPS antibodies in serum recorded by ELISA in rats colonized with an *E. coli* strain at different ages. Filled circles (●) are dams colonized with the *E. coli* as adults and the empty circles (○) are their pups colonized at birth with the same *E. coli*.

from birth did not produce any IgE antibodies to the O antigen of the *E. coli* used (fig. 4). However, the dams which were colonized as adults developed IgE anti-LPS antibodies.

Discussion

In the present study we have demonstrated that feeding OA to the colonized rats suppresses the priming effect of the OA-producing bacteria on the immune system and renders the animals refractory to OA challenge in FCA. The feeding of OA abrogated both the DTH and the antibody response to OA.

The OA produced by the bacteria is retained in the periplasmic space and not secreted, so there will probably be a very limited amount of free OA in the intestine of the colonized animals [7]. It is likely that antigen in the diet is taken up over the whole surface of the intestine while in the colonized nonfed animals OA are predominantly taken up in a bacterial context in the Peyer's patches. The large amount of OA and OA peptides present in the small intestine of the rats fed OA has a potential of stimulating T-suppressor cells, which may result in down-regulation of the immune re-

sponse induced by the bacteria in the Peyer's patches. Occasionally we have been able to grow the colonizing bacteria from the Peyer's patches which confirms that bacteria can be taken up in the Peyer's patches.

In our previous study using this model [8] we observed both an IgE and an IgG antibody response against OA in rats that were colonized from birth via their mother. In the present study, however, there were no detectable IgE and IgG antibodies to OA in response to the colonization alone. This difference could reflect a lower degree of colonization of the rats and may only be quantitative, since the OA-producing bacteria were sufficient to produce a DTH response and to prime the animals for an IgE response. However, the weekly fecal cultures on ampicillin agar consistently showed the presence of the ampicillin-resistant modified *E. coli*.

The novel finding that feeding of OA completely abrogated intestinally induced hypersensitivity in the colonized animals suggests that the immune system is able to differentiate between the same antigen according to its physical appearance, i.e. whether soluble or particulate.

In contrast to OA, the bacterial antigen LPS, which was probably present in higher amounts than the OA in the gut of the colonized animals not fed OA, induced tolerance or at

least the rats did not respond to its presence in the intestine. The induction of tolerance against bacterial antigens and its importance for the protection against streptococcal cell wall-induced arthritis has been suggested in a study where germ-free rats became refractory to arthritis induction when they were colonized with a conventional bacterial flora as adults [13]. In our hands it seems as if the animal had to be new-born or at least neonatal to become unresponsive to the bacterial antigens, since adult animals colonized with the *E. coli* even responded with IgE antibodies against the LPS antigen.

We have previously shown that the feeding regimen used in this study induces an almost complete refractoriness to parenteral challenge [4]. The present findings show that we can also turn a sensitivity reaction induced via the gut into a

state of unresponsiveness by feeding the antigen in a soluble form. This could provide a potential model for the study of the possible role of the bacterial flora in the induction of hypersensitivity reactions to food antigens.

In conclusion, a high amount of fed antigen suppresses intestinally induced hypersensitivity caused by a common food antigen presented in a bacterium.

Acknowledgements

The skilful technical assistance of Ms. Helena Kahu and Mrs. Eva Ågren is greatly appreciated. The study was supported by grants from the Swedish Medical Research Council, Grant No. 215, and the Ellen, Walter and Lennart Hesselman Foundation for Medical Research.

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