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Induction of IgE antibodies and T-cell reactivity to ovalbumin in rats colonized with *Escherichia coli* genetically manipulated to produce ovalbumin

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SUMMARY

The immune response to ovalbumin (OA) and the bacterial antigens, lipopolysaccharide (LPS) and fimbriae were studied in conventional rats colonized from birth with an *Escherichia coli* strain producing OA. The colonized rats had developed IgE antibodies against OA, but not against the fimbrial or the LPS antigens from the *E. coli* at 2 months of age. At this time all rats were primed with OA given intracutaneously in Freund's complete adjuvant. Two weeks later the colonized rats showed a 35% greater delayed-type hypersensitivity (DTH) reaction to OA, measured as ear swelling, than the controls. Thus bacteria carrying antigens resembling potential allergens might aggravate, or participate in the induction of allergic symptoms. In addition such bacteria could be efficient vaccine vectors in protection against parasites. The study illustrates the importance of the mode of antigen presentation for the subsequent immune response.

INTRODUCTION

One of the most important entries for antigenic material to the lymphoid system is through the intestinal epithelium. Food proteins and intact micro-organisms, are brought into contact with the gut-associated lymphoid tissue (GALT) by translocation,[1] or by invasion.[2] Depending on how and where the antigen is presented different responses can be achieved in the GALT.[2-4] Feeding soluble antigens to adult and neonatal animals often leads to a state of tolerance, both in terms of antibody response and T-cell reactivity.[5-8] On the other hand oral immunization regimes can induce secretory IgA (sIgA) antibodies on mucosal surfaces and in exocrine secretions and IgG antibodies in serum.[9-11] Previously we found that feeding rats an ovalbumin (OA)-containing diet for several weeks only induced IgG antibodies in serum and no sIgA antibodies,[12,13] which has also been shown in pigs[14] and humans.[15] However, recently, we have shown that germ-free adult rats monocolonized with a genetically manipulated *Escherichia coli* strain producing OA respond with a vigorous production of serum IgG and IgM antibodies against OA, as well as IgA, IgG and IgM antibodies to OA in secretions.[16]

With this in mind we wanted to study the immune response to OA and bacterial antigens in conventional rats, colonized from birth with the genetically manipulated *E. coli* producing OA.

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MATERIALS AND METHODS

Animals

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

Expression of ovalbumin in E. coli

The plasmid pOMP21 was obtained by fusing cDNA for ovalbumin to the lac-operon.[17,18] In the protein synthesized, the first seven amino acids derive from beta-galactosidase; the seventh residue is connected to the fifth residue of OA. When the plasmid is expressed in *E. coli* K-12 the localization of the OA is periplasmic and cytoplasmic, but the protein is not secreted.[19] In the present study the pOMP21 plasmid was introduced into the *E. coli* strain O6K13H1.[16] 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, U.K.) in the drinking water (5 g/l) for 1 day. They were starved for 24 hr and then given 10^{10} *E. coli* pOMP21 in 1 ml bicarbonate buffer (0·2 M), through a stomach tube. All rats were given water containing ampicillin (0·5 g/l, Doktacillin, Astra, Södertälje, Sweden) *ad libitum* during the whole period of colonization. Faecal cultures were done on ampicillin agar at regular intervals. The rats were mated immediately after colonization and the pups from these dams were used in the experiment.

Experimental procedure

All experiments were performed on conventional rats housed in a normal animal house and included three groups of animals. The first group was delivered by dams colonized with the OA producing bacteria. Weekly faecal cultures on ampicillin agar were done to ensure that these pups were colonized and retained the bacteria. The second group was born by conventional rat dams carrying a normal intestinal bacterial flora. These pups were weaned and kept on normal rat standard diet without OA. This group of rats with no access to OA was used as control in the delayed-type hypersensitivity (DTH) experiment. The third group included 10 pups born by conventional rat dams. These pups were weaned at 21 days of age onto an OA-containing diet[8] fed *ad libitum* for 5 weeks. The intake of OA from this diet was estimated to be 0·8 g per rat and day. This third group was used as a control group for the IgE antibody response against OA. At 8 weeks of age the two first groups of rats were given a subcutaneous (s.c.) injection with 50 μl OA (Sigma, St Louis, MO) [20 mg/ml in a mixture of phosphate-buffered saline (PBS) and an equal volume of Freund's complete adjuvant] on the back. Two weeks later, they were given a challenge intradermally in one ear with 20 μl OA (2·5 mg/ml) in PBS. Unimmunized conventional 10-week-old rats (group 2) served as control for non-specific inflammatory reactions. The increase in ear thickness was measured 24 hr later with an Oditest (Kröplin, Hessen, Germany).

Blood samples were taken from the tail vein of all rats before immunization, and stored at −20° until analysed.

Antibody determination

Serum samples were analysed with an enzyme-linked immunosorbent assay (ELISA). Polyvinyl microtitre plates (M-22, Dynatech, Alexandria, VA) were coated with 100 μl of OA (50 μg/ml), phenol-water extracted O6 lipopolysaccharide (LPS) (2 μg/ml)[20] or type 1 fimbriae (2 μg/ml) all dissolved in PBS and incubated overnight at room temperature. The fimbrial antigen was a generous gift from Dr C. Brinton (Pittsburgh, PA). The LPS was prepared by the phenol-water extraction method.[20] Next morning the plates were washed three times in PBS containing 0·05% Tween (PBS-Tween), with 2 min between each wash.

The samples were diluted in PBS-Tween at 1/10, 1/50 and 1/250 for IgE measurements and 1/100, 1/500 and 1/1000 for IgG measurement. One hundred microlitres of each dilution was added to the plate and incubated for 6 hr in a moist chamber at 25°. The plates were then washed three times in PBS-Tween. One hundred microlitres of biotinylated monoclonal anti-rat IgE (MCA 193 B, Serotec, Oxford, U.K.), or 100 μl of affinity-purified peroxidase-labelled anti-rat IgG (Cappel, West Chester, PA) was added at an optimal dilution and incubated overnight in a moist chamber. The next day the plates were washed as described above. Extravidin-alkaline phosphatase conjugate (Sigma) diluted 1/5000 in PBS-Tween, 100 μl, was then added to the IgE plate and incubated for 1 hr at room temperature. The plates were washed again with PBS-Tween. One hundred microlitres of paranitrophenyl-phosphate (1 mg/ml) in diethanolamine buffer (1 M, pH 9·8) was added to the wells. The absorbance was recorded at 405 nm (Titertec Multiscan, Flow Labs, MacLean, VA) and the antibody activity was expressed as the absorbance obtained at 1/50 (IgE) or 1/100 (IgG) sample dilution after 100 min of reaction time. The specificity of the reagents have been extensively tested against rat myeloma immunoglobulins (PharMingen, San Diego, CA) and polyclonal rat IgG.

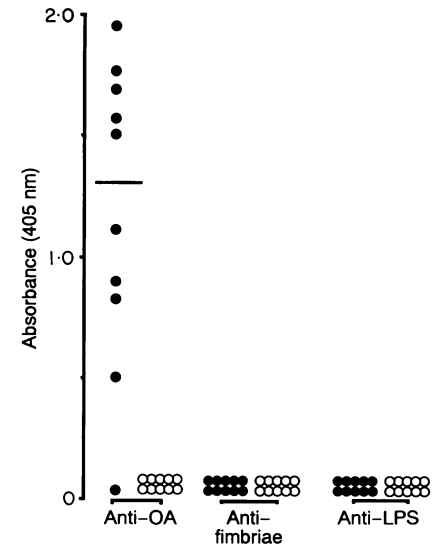

Figure 1. IgE antibodies (1/50 sample dilution) in serum against OA, fimbriae and LPS recorded by ELISA in 8-week-old rats colonized from birth with an *E. coli* strain carrying a plasmid, which codes for production of OA (●); control rats with a conventional intestinal bacterial flora fed an OA-containing diet since weaning (○). Each symbol represents one rat and the bars denote the medians.

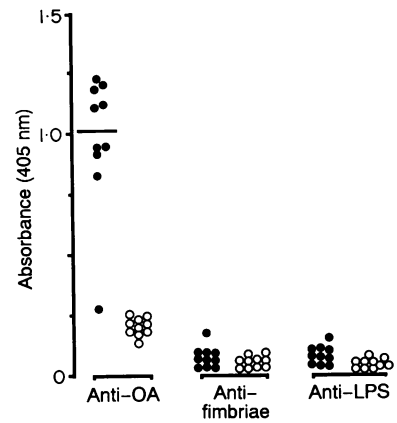

Figure 2. IgG antibodies (1/100 sample dilution) in serum against OA, fimbriae and LPS recorded by ELISA in 8-week-old rats colonized from birth with an *E. coli* strain carrying a plasmid, which codes for production of OA (●); control rats with a conventional intestinal bacterial flora fed an OA-containing diet since weaning (○). Each symbol represents one rat and the bars denote the medians.

Statistics

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

RESULTS

The IgE and IgG antibody responses

The IgE and IgG antibody activity against OA in the colonized rats compared to control rats (group 3 fed OA) is shown in Figs

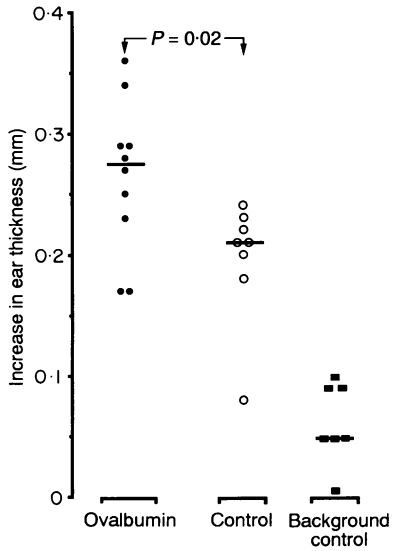

Figure 3. The DTH reaction against OA measured as increase in ear thickness in rats colonized with an *E. coli* strain producing OA (●), and in control rats (conventional rats which had never been exposed to OA, group 2, see Materials and Methods) (○). The animals were immunized intracutaneously with OA in Freund's complete adjuvant 2 weeks before challenge with OA in the ear. Background level control rats (■) are conventional rats only challenged with OA in the ear (from group 2). Each symbol represents one rat and the bars denote the medians.

1 and 2. The rats carrying the OA-producing *E. coli* bacteria had IgE and IgG antibodies against OA, but not against the bacterial antigens LPS and fimbriae. The control rats (group 3), who had been eating OA diet *ad libitum*, had no detectable IgE or IgG antibodies against either OA, LPS or fimbriae. There was a significant correlation between the IgG and IgE anti-OA antibody levels (Spearman rank correlation = 0·83, $P = 0·003$, $n = 10$).

DTH response against ovalbumin

The effect of colonization with the genetically manipulated *E. coli* on the DTH reaction against OA is shown in Fig. 3. The colonized rats and the control rats (group 2 without access to OA) were given a s.c. injection with OA in Freund's complete adjuvant and 2 weeks later challenged intradermally in the ear with OA. The rats which were colonized with the OA-producing bacteria had a 35% greater increase in ear thickness [mean increase = 0·27 mm, standard deviation (SD) = 0·06] than the control group harbouring a conventional intestinal bacterial flora (mean increase = 0·20 mm, SD = 0·05, $P = 0.02$). The increase in ear thickness ranged from 0·08 to 0·24 mm for the rats with the conventional flora and 0·17 to 0·36 mm for the rats carrying the OA-producing bacteria. The non-specific background increase in ear thickness achieved in the unprimed rats ranged from 0·00 to 0·10 mm (Fig. 3). There was no significant correlation between the IgE level and the DTH reaction (Spearman rank correlation = 0·29, $n = 10$).

DISCUSSION

This study shows that rats colonized from birth via their mother with a genetically manipulated *E. coli* producing OA, developed IgE and IgG antibodies against OA, but not against fimbriae or LPS. This differs partly from the results obtained when we monocolonized adult germ-free rats with this OA-producing *E. coli*. These rats responded with IgG and IgM antibodies against both LPS and type 1 fimbriae.[16] The difference in antibody responses between the animals in the present study and the monocolonized rats could be due to a lower number of the genetically manipulated bacteria in the intestine of the current animals due to competition from other bacteria and thus exposure to lower levels of antigen in these rats. Low levels of antigen have been shown to favour the IgE response, whereas higher antigen levels normally induce IgG antibodies and suppress the IgE antibody formation.[21] The present findings are probably not a result of antigen dose, since germ-free rats which were monocolonized with the genetically manipulated *E. coli* and presumably heavily exposed to bacterial antigens developed IgE antibodies against LPS and fimbriae, but only low levels of IgE antibodies against OA (A. Dahlman, S. Ahlstedt, Å. Hanson, E. Telemo, A. E. Wold and U. I. Dahlgren, in manuscript). In addition the animals in the current study developed both IgE and IgG antibodies to the OA antigen.

Therefore the observed IgE response against OA, but not against the bacterial antigens, is probably due to a specific unresponsiveness or down-regulation of the immune response against the fimbrial and the LPS antigens. This is partly in agreement with the result of earlier studies where LPS did not induce IgE antibodies in conventionally reared mice, even though proteins extracted from the bacteria did.[22] Thus it seems that adult germ-free animals which are colonized with a bacterial strain can respond against true bacterial antigens, i.e. LPS and fimbriae,[16] while conventional rats naturally colonized during the neonatal period do not.

On the basis of a previous study, we have no evidence that the ampicillin given to the colonized animals could affect the B- or T-cell responses (S. Ahlstedt and U. Dahlgren, unpublished observations).

The specific down-regulation in the conventionally reared animals could have been conveyed to them by their mother, carrying the bacteria during the pregnancy, by transport of immune cells, antigen-specific antibodies or anti-idiotypic antibodies via the placenta and/or the milk. Indeed the colonized mothers had IgG anti-LPS, but not anti-fimbrial antibodies in the milk (U. Dahlgren, manuscript in preparation). In addition, a direct effect of the bacteria on the immune system of the neonatal pups cannot be ruled out. If that is so this study suggests that it would be beneficial to be colonized with Gram negative bacteria as early in life as possible since it might be desirable to avoid the development of IgE antibodies against bacterial antigens.

The animals exposed to the bacteria producing OA exhibited an enhanced DTH reaction to the antigen. T-cell reactivity, reflected as DTH reaction, has been shown to be particularly sensitive to tolerance induction upon feeding antigen.[6,7,23] Since the OA produced by the bacteria is not released, but retained in the periplasmic space, a very limited amount of free OA is likely to be present in the intestine, which might be insufficient for tolerance induction.[16] If the bacteria are taken up primarily in the Peyer's patch (PP) they will be processed and presented to the immune system in a different way as compared to soluble food proteins. The net result could be that processing in the PP compartment mainly stimulates a CD4 T-helper response, while

processing by the epithelium and cells in the lamina propria mainly stimulates a CD8 T-suppressor response.[24,25]

From the results of the present study it seems as if certain bacterially delivered antigens have the capacity to stimulate different T-cell subtypes, bringing about both cell-mediated and humoral immune responses. Since both these branches of the immune system are activated, such bacteria might be ideal vectors for vaccines against parasites where IgE-, IgG- as well as T-cell-mediated immunity can be important.

In conclusion the current data indicate that bacteria carrying antigens resembling dietary proteins can cause both enhanced DTH and high levels of IgE antibodies to these antigens. Thus bacteria expressing such antigens might be responsible for induction or aggravation of allergic diseases. The outcome of the bacterial exposure may depend on (1) the effect of the bacteria on the immune system with the mode of presentation, or (2) factors transmitted from the mother to the foetus/neonate, or (3) a combination of these alternatives. This is presently being investigated in our laboratory.

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