3

Preventive vaccination against allergy: maternal allergen immunization protects offspring

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Introduction

There is evidence that mother starts the 'immunological education' of her child well before birth, and also that environmental influences *in utero* affect health and disease later in life (Terry and Susser 2001; Leon 2001). Together with early childhood, the gestational period is probably the most important period in relation to the formation of the immune system and the development of allergy (Von Mutius 2001; Peden 2000). Maternal imprinting of immune responsiveness in the offspring has been described, and may phenotypically appear as non-genetic inheritance (Lemke, Hansen and Lange 2003; Lemke and Lange 1999; Lange et al. 1999; Lundin et al. 1999). Maternal vaccination against tetanus has been claimed to induce active *in utero* immunization of the foetus (Vanderbeeken et al. 1985), and maternal helminth infection has been reported to sensitize *in utero* for parasite-specific cellular responsiveness in the offspring (Pit et al. 2000). Increasing focus has the last years been put on the *in utero* environment and materno-foetal interactions also in relation to the development of allergy as well as asthma (Warner et al. 1996; 1998; Hanson et al. 1997; Björkstén 1999; Christensen 2000; Cogswell 2000; Gillman and Rich-Edwards 2000).

Hypothetically, the *in utero* influence of factors reaching the unborn child via the mother could go in two directions. One direction could be increased allergy, caused by *in utero* sensitization (Herz et al. 2000a; 2000b; Platts-Mills and Woodfolk 2000; Szepfalusi et al. 2000b; Prescott et al. 1998) or other mechanisms, like those activated by maternal smoking. The other effect could be allergen-specific or more general non-responsiveness – *in utero* life is a time for development of immunological tolerance. For both directions mechanisms could be direct allergen exposure (Vance and Holloway 2002; Holloway et al. 2000; Szepfalusi et al. 2000a; Dahl et al. 1984), for example to allergen transported over placenta bound to IgG antibodies that are actively transported from the mother to the child (De Moraes-Pinto and Hart 1997; Saji et al. 1999), although the transport of immune complexes from mother to foetus remains controversial (Wood 1994). Other mechanisms could be immunoregulatory effects of maternal antibodies (Lange et al. 2002), cell-mediated effects (Seelig Jr. and Head 1987), or cytokines (Fusaro et al. 2002) or other soluble mediators. It is important to note, however, that the effects observed may be mediated via the mother's milk rather than by transplacental mechanisms (Okamoto, Freihorst and

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Ogra 1989; Szepfalusi et al. 2000a; Szepfalusi et al. 2000b; Jarrett and Hall 1979; 1983).

A number of investigators working with rodent models have reported suppression of the specific IgE response in offspring of both actively and passively immunized mothers, but also transmission of asthma susceptibility (Jarrett and Hall 1979; 1983; 1984; Roberts and Turner 1983; Seeger et al. 1998; Lange et al. 2002; Fusaro et al. 2002; Victor Jr. et al. 2003; Hamada et al. 2003)). The possibility to prevent allergy in the child by prophylactic immunization of the mother with common allergens should therefore be explored. Consequently, in a mouse model, we performed experiments to investigate what effects maternal immunization with allergen during pregnancy has on the IgE, IgG1 and IgG2a antibody responses to the same allergen in young adult offspring (Melkild et al. 2002).

Materials and methods

Mice

Mice of the inbred strain NIH/OlaHsd were used. Pregnant plug females were obtained after mating with syngenic males from Harlan UK Ltd. The pregnant mice were housed individually on BeeKay Bedding in Makrolon Type 3 cages in Scantainer filter cabinets in the animal facilities of the Norwegian Institute of Public Health, Oslo. The animals were marked by ear punching. Room temperature, humidity, air changes and light cycle were regulated. Pregnant mice were kept on ovalbumin-free mouse-breeding diet and tap water *ad libitum.* Offspring were similarly kept on ovalbumin-free mouse maintenance diet. After weaning, litter mates were separated according to sex and kept in separate cages. The experiments were approved by the local authorized competent person under the surveillance of the Norwegian Research Animal Authority, and were registered by the Authority.

Allergen and adjuvant

For all allergen immunizations ovalbumin (OVA, grade VII, Sigma) was used. $Al(OH)_3$ was used as an adjuvant. For immunization, 10 μg of OVA was given intraperitoneally (i.p.) or subcutaneously (s.c.) as appropriate with 2 mg of $Al(OH)_3$. For boosting we used 1 μg of OVA with 0.2 mg of $Al(OH)_3$ s.c. or 10 μg of OVA i.p. unless otherwise indicated.

Experimental design

Two types of experiments were performed. With the first protocol, mice were immunized i.p. 3 days into pregnancy, with boosting s.c. at mid-pregnancy (10 days into pregnancy), about 4 days before giving birth (17 days into pregnancy), and 5-6 days post partum. Control mothers were kept unimmunized. Offspring were weaned at about 3½ weeks of age, and were immunized when about 7½ weeks old. One offspring group was immunized i.p. with OVA and adjuvant, and boosted 15 days later. The other offspring group was immunized with OVA alone given as 2-μg doses i.p. four times at 5- to 8-day intervals. Some offspring were kept unimmunized. The offspring were bled before the first immunization and 1 week after the last booster. With the second protocol, pregnant mice were immunized s.c. once either 3, 10 or 17 days into pregnancy. Pregnant control mothers remained unimmunized. Offspring were immunized i.p. with adjuvant and boosted i.p. without adjuvant at 15 days.

Collection of blood samples

Blood was collected from the lateral femoral vein into heparinized capillary tubes after puncture of the vessel with a 21-G needle (Aaberge et al. 1992; Hem, Smith and Solberg 1998). At the end of each experiment, the animals were exsanguinated under CO_2 anaesthesia by heart puncture. Serum and heparin-plasma samples were stored at -20^oC.

Determination of mouse IgE, IgG1 and IgG2a antibodies to OVA

For all three antibody isotypes, microtitre plates were coated with rat anti-mouse IgE, rat anti-mouse IgG1, or rat anti-mouse IgG2a, and incubated with diluted mouse serum to capture IgE, IgG1 and IgG2a antibodies, respectively. This was followed by, in succession, incubation with biotinylated OVA, complexes of streptavidin and biotinylated alkaline phosphatase, and finally with colour-reagent substrate. Optical density was measured and compared to results with dilutions of standard sera included in every plate for standard curve generation. Results were expressed as arbitrary units.

Statistics

Data were analysed with SigmaStat® statistical software on a PC. The non-parametric Kruskal-Wallis one-way analysis of variance was used for comparison of several groups, and the Mann-Whitney rank sum test was used for pair-wise comparisons. Bonferroni correction for multiple comparisons was used.

Results

A. Proof of principle – immunization of the mother with allergen during pregnancy and the early post-partum period strongly reduces the specific IgE response in offspring

With the first protocol, mice were immunized three times during pregnancy and once one week post partum to cover most of the pregnancy and early lactation period. Mothers were shown to have produced anti-OVA IgE before the last booster dose, and to have high levels of OVA-specific IgE and IgG1 three weeks after the last booster dose. A comparatively smaller IgG2a response was observed in the mothers, and the mothers showed a predominantly Th2-dependent antibody response (IgE and IgG1 isotypes). Control mothers did not have detectable levels of specific IgE or IgG1 antibodies, and only marginally detectable anti-OVA IgG2a levels. The offspring one week before immunization showed low levels of anti-OVA IgG1 and IgG2a antibodies, presumably transferred from the mother.

After allergen immunization, the offspring of immunized mothers showed a markedly reduced OVA-specific IgE response compared to offspring from non-immunized mothers ($P<0.001$). This was seen both with and without $Al(OH)_3$ adjuvant for offspring immunization (Figure 1). The two ways of offspring immunization both revealed clear differences between offspring of immunized and non-immunized mothers. However, responses were stronger and showed a larger difference between group medians with adjuvant. The adjuvant method, therefore, seemed preferable for use in future experiments, at least with regard to IgE.

The OVA-specific IgG1 response showed the same pattern as the IgE response in the offspring immunized without adjuvant, with a markedly lower specific anti-OVA IgG1 response in offspring of allergen-immunized mothers. However, with adjuvant, no difference between offspring of immunized and non-immunized mothers was seen in the IgG1 response.

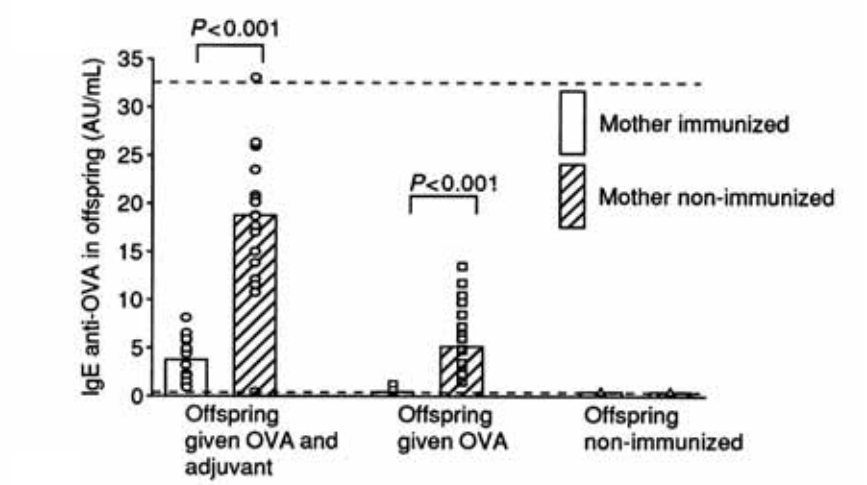

Figure 1. Specific anti-OVA IgE response in young adult offspring of mothers immunized (open bars) or not immunized (hatched bars) with OVA during pregnancy. Offspring was immunized either with OVA in $Al(OH)_3$ adjuvant (left panel, n = 16 and 18), or with OVA without adjuvant (middle panel, n = 25 and 18). Right panel (n = 9 and 9) shows IgE levels in non-immunized offspring. Lower dotted line denotes the detection limit for specific IgE, upper dotted line denotes the upper limit for quantitative measurement. Bars denote medians, symbols denote individual mice. (Reproduced with permission from Melkild et al. 2002)

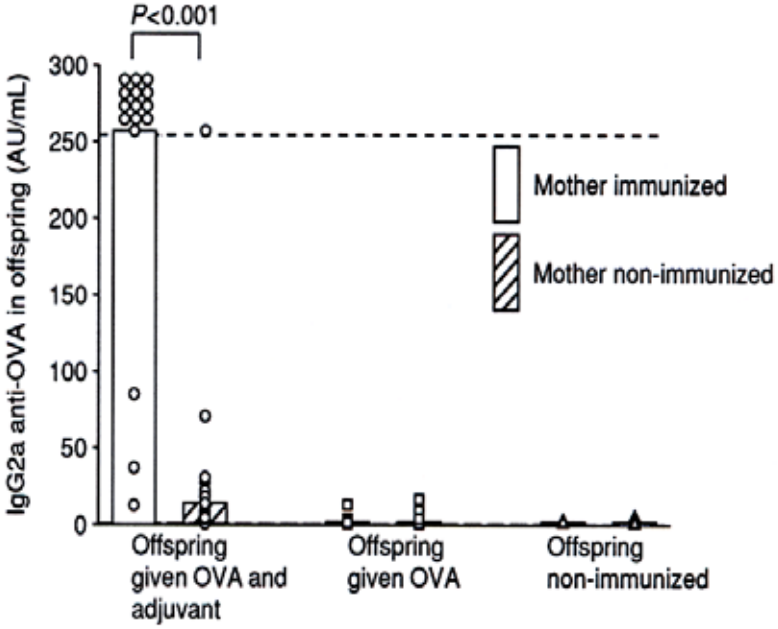

Figure 2. Specific anti-OVA IgG2a response in young adult offspring of mothers immunized (open bars) or not immunized (hatched bars) with OVA during pregnancy. Offspring was immunized either with OVA in $Al(OH)_3$ adjuvant (left panel, n = 16 and 18), or with OVA without adjuvant (middle panel, n = 25 and 18). Right panel (n = 9 and 9) shows IgG2a levels in non-immunized offspring. Lower dotted line denotes the detection limit for specific IgG2a, upper dotted line denotes the upper limit for quantitative measurement. Bars denote medians, symbols denote individual mice. (Reproduced with permission from Melkild et al. 2002)

In contrast, the allergen-specific IgG2a levels were greatly elevated in offspring of allergen-immunized mothers as compared to offspring of non-immunized mothers. However, this difference ($P<0.001$) was only observed when offspring were immunized with adjuvant. Without adjuvant, the OVA-specific IgG2a response was

modest and there was no difference between responses in offspring of immunized and non-immunized mothers (Figure 2).

Non-immunized control offspring of both immunized and non-immunized mothers showed no (IgE) or marginally detectable (IgG1, IgG2a) levels of specific antibodies.

B. Early-pregnancy allergen immunization of mother shows more reduced specific IgE levels in immunized adult offspring than late pregnancy immunization.

With the second protocol, mice were immunized at different times in pregnancy: about 3 days into pregnancy, at 10 days (mid-pregnancy) and about 4 days before giving birth. As with the first protocol, all the immunized mothers developed anti-OVA IgE and IgG1 antibodies, and also low levels of IgG2a antibodies. Similarly, low levels of anti-OVA IgG1 and IgG2a antibodies were detected in pooled plasma samples taken from offspring of immunized mothers one week before offspring immunization with OVA and $Al(OH)_3$.

Offspring of mothers immunized 3 days into pregnancy showed a significantly reduced allergen-specific IgE response compared to offspring of mothers immunized 17 days into pregnancy and offspring of non-immunized mothers ($P<0.006$ for both comparisons) (Figure 3). There were no statistically significant differences in the OVA-specific IgE response between other groups of immunized offspring.

The IgG1 response in offspring of mothers immunized in early pregnancy was significantly suppressed compared to the response in offspring of mothers immunized in mid- and late pregnancy, the difference between early and late immunization being statistically significant ($P<0.006$). However, the responses in offspring of mothers immunized in mid- or late pregnancy, which showed little difference between them,

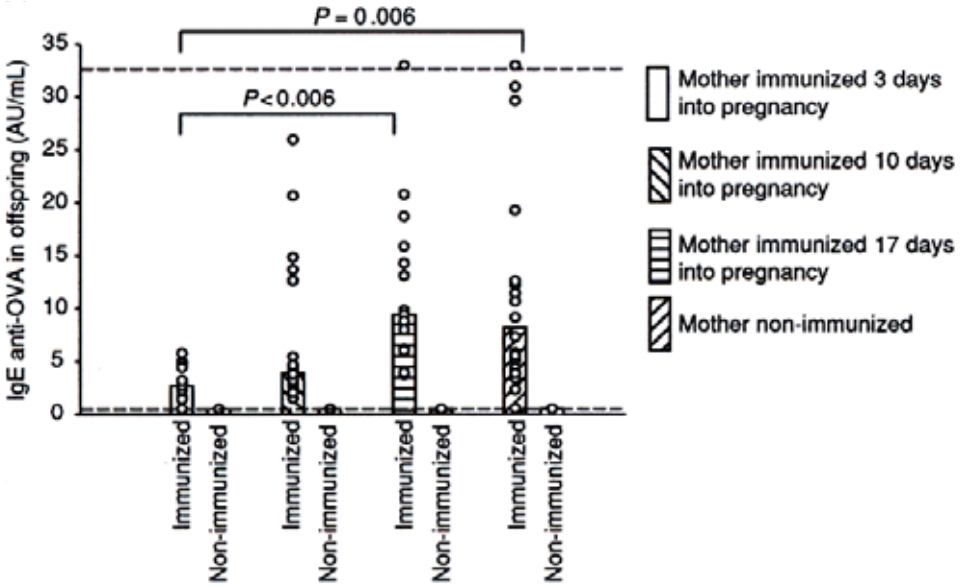

Figure 3. Specific anti-OVA IgE response in young adult offspring of mothers immunized with OVA 3, 10 or 17 days into pregnancy, or mothers not immunized. Left column in a pair shows non-immunized offspring, right column in pair shows offspring immunized with OVA in $Al(OH)_3$ adjuvant. For 3 days n = 13 and 12, for 10 days n = 19 and 18, for 17 days n = 15 and 15, respectively. Right pair shows IgE levels in offspring of non-immunized mothers (n = 18 and 13). Lower dotted line denotes the detection limit for specific IgE, upper dotted line denotes upper limit for quantitative measurement. Bars denote medians, symbols denote individual mice. (Reproduced with permission from Melkild et al. 2002)

were increased compared to the response in offspring of non-immunized mothers. Offspring of non-immunized mothers and offspring of mothers immunized in early pregnancy showed similar responses for IgG1 (Data not shown).

The OVA-specific IgG2a response showed a marginally non-significant difference between the immunized offspring groups (P=0.053), with the IgG2a response in offspring of mothers immunized in early and mid-pregnancy tending to be higher than in offspring of mothers immunized in late pregnancy (data not shown).

Discussion

Preventive vaccination against allergy has to be done before birth, because there is evidence that sensitization may take place *in utero*. Two approaches are possible. One is to immunize the foetus directly, for example by injecting modified or unmodified allergen into the amniotic fluid that the unborn child swallows. This approach has worked for experimental immunization *in utero* in sheep (Gerdts et al. 2000). However, it would appear less risky and more attractive if the mother could be immunized with a conventional vaccination procedure to prevent allergy in the child. Our experiments, together with those of others cited in the Introduction, indicate that vaccination of the mother to reduce allergy in the offspring may, indeed, be possible.

One question is the duration of the specific IgE suppression in the offspring. Fusaro (2002) reported suppressed specific IgE as well as IgG1, IgG2a and IgG2b production to dust mite allergen in offspring of immunized mothers if immunized at age 25 days and bled at 42 days, but not if immunized at age 45 days and bled at age 62 days. In our experiments, however, offspring have been immunized at age 40 – 55 days and still showed suppression. Seeger (1998) showed that reduced offspring IgE response to bee-venom phospholipase A_2 could be induced by maternal immunization with the allergen, but also by monoclonal antibodies to the allergen when given to the mother. Repeated allergen immunizations of the offspring up to age 6 months did not induce an IgE response, indicating long-lasting down-regulation of IgE responsiveness. Lange (2002) found that maternally transferred allergen-specific monoclonal antibodies induced a temporary suppression of the allergen-specific IgE response in the offspring during the first 4 months of life, but not thereafter, and not in the mothers. However, if the initial offspring immunization at 3 or 4 months was followed by further application of allergen, IgE suppression lasted up to an age of more than one year. Thus, after down-regulation of IgE responsiveness was first induced, allergen contact was necessary to maintain the low IgE responsiveness. This suggests that if a child is born with the proper immunological ‘imprinting’, exposure to allergen is beneficial because it will help maintain the allergen-specific IgE unresponsiveness, and allergen avoidance may be harmful. Also, the findings suggest that if vaccination of mother is implemented, allergen avoidance should probably not be practiced.

In man, IgG from the mother is actively transferred to the foetus. Also, the milk is rich in antibodies. It has been reported that cord-blood-specific IgG antibodies are associated with reduced allergy in later life (Jenmalm and Björkstén 2000). In rodents maternal IgG is transferred across the yolk sack during pregnancy and from the milk via the intestinal epithelium postnatally (Appleby and Catty 1983). Further, in man, it has recently been reported that amniotic fluid also contains IgE, evidently from the mother (Thornton et al. 2003). Thus, if the mother–child interaction with regard to down-regulation of specific IgE is mediated by maternal antibodies, it may take place both via trans-placental transport and via the mother’s milk. If the mother’s milk turns

out to be important in the rodent models, that does not necessarily represent a serious drawback with regard to using allergen vaccination in man, other than in those instances where the baby cannot be fed at least partially on the mother's milk.

Reduction of IgE synthesis does not necessarily mean reduced incidence of allergic disease, although there would be reasons to hope so, because specific IgE is such an important component of the mechanism in allergic disorders. However, it may turn out that vaccination will be most effective, for example, for allergic rhinoconjunctivitis, that it will have some effect for allergic asthma, and will have little effect on atopic dermatitis. Only clinical studies can give us the answer. Also, the effect on food allergy is an interesting question. The findings by Victor (2003) that maternal immunization with the house-dust mite *Dermatophagoides pteronyssinus* not only reduced IgE responses to mite allergen (but not to heterologous allergen) but also induced regulatory $CD4^{+}CD25^{+}$ T cells, suggest that the clinical expression of allergy may perhaps also be reduced.

Human data are scarce, but allergen-hyposensitization therapy given to pregnant mothers has been reported to reduce homologous allergy in the children (Glovsky, Ghekiere and Rejzek 1991). There is evidence that antibodies given orally in the neonatal period in rodents can affect the immune response for two generations (Lundin et al. 1999), so that foetal and neonatal influences on the immune system can have long-lasting effects. Maternal allergen immunization in man may therefore, if we are lucky, have an effect not only on the first but also on the second generation, and thus start a long-term trend of reduction of allergy in the population.

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