

Reversal of the adult IgE high responder phenotype in mice by maternally transferred allergen-specific monoclonal IgG antibodies during a sensitive period in early ontogeny

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IgE is an important trigger in allergy and asthma, diseases whose development is suggested to depend on an initial sensitization in early life. While induction of murine IgE responses requires both a genetically based IgE high responder phenotype and defined experimental conditions, maternally transferred IgG can override these prerequisites and suppress IgE formation in an allergen-specific manner. Here, we show that maternally transferred monoclonal IgG, irrespective of their subclass and recognized epitopes, induce IgE unresponsiveness, which is effective for parenteral immunization with bee venom phospholipase A₂ as well as for airway-immunization with nebulized ovomucoid-containing ovalbumin. This IgE suppression is detectable in the offspring during the first 4 months of life, but not thereafter and not in the dams. However, when the initial immunization at an age of 3 or 4 months was followed by further application of both allergens via their respective routes, IgE suppression persisted up to an age of more than one year. If applicable to man, these findings may allow the development of a new strategy for the prevention of allergy and asthma by maternally transferred or neonatally injected allergen-specific mAb in combination with natural or prophylactic exposure to the respective allergens during early childhood.

Key words: Rodent / Allergy / Maternal IgG / IgE suppression

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1 Introduction

Allergy and asthma have assumed alarming proportions during the last decades [1, 2] and may soon reach pandemic dimensions. Although these diseases depend on a genetic predisposition, the observed increase seems to result from a change of the environment in industrialized and developing countries [1–3]. Allergy and allergic asthma are both associated with increased serum levels of allergen-specific IgE antibodies, the formation of which depends on the secretion of IL-4 from preferentially activated Th2 cells [4, 5]. Since allergy and allergic asthma can be treated effectively by an antibody-based anti-IgE therapy [6] in animal experimental systems [7, 8] as well as in patients [9–13], it can be concluded that allergen-specific IgE are causally involved in the genera-

tion of these diseases and that their symptoms largely depend on IgE antibodies. Therefore, one aim of specific immunotherapy [14] is the reduction of IgE serum levels by changing the balance from a Th2- to a more Th1-mediated response [15].

Experimental as well as clinical evidence suggests that early ontogeny is of prime importance for a switch towards the development of allergic diseases which is effective until adulthood [16]. It is assumed that the underlying mechanism for this determination is an initial sensitization to environmental allergens, which probably occurs during infancy and early childhood [17] or even *in utero* [18, 19]. Such an early sensitization might be expected to occur in a universal manner. However, not all individuals develop an IgE high responder phenotype [18, 20], so that other unknown conditional risk factors must be involved. Interestingly, it has been demonstrated that allergen-specific IgG antibodies either induced in female rats by immunization [21] or injected into pregnant mice as pre-formed monoclonal antibodies (mAb) [22] are transferred to the offspring and reverse a

[1 23239]

Abbreviations: BvPLA₂: Bee venom phospholipase A₂
OM: Ovomucoid phOx: 2-Phenylloxazalone

seemingly genetically-based IgE high responder phenotype in an allergen-specific manner. Therefore, it was of interest to study whether these IgE-suppressive effects of maternally transferred IgG antibodies could be related to and were dependent on a sensitive phase during early ontogeny. If so, application of IgG during this time period might prove effective in preventing the development of allergic and asthmatic diseases.

Here, we show that in two murine model systems monoclonal allergen-specific IgG, injected into the mothers, suppress IgE responsiveness in the adult offspring to the systemically-applied allergen bee venom phospholipase A₂ (bvPLA₂) as well as to inhaled ovomucoid (OM). IgE suppression in the offspring for both allergens was independent of IgG subclasses and epitopes recognized. Although this IgE suppression was only effective during the first 4 months of life, it could, by an encounter with the allergens during this period, be extended up to an age of more than 1 year.

2 Results

2.1 Subclass-independent suppression of the IgE immune response to bee venom phospholipase A₂ by maternally transferred allergen-specific IgG mAb

Since the IgE-suppressive function of maternal IgG is effective at an age when they could not any longer be detected in the serum [21–23], a direct suppressive action by IgG only seems possible if they were present in an Fc receptor (FcR)-bound form. However, in the absence of allergen, the formation of immune complexes cannot occur and, therefore, only the monomer-binding high-affinity FcγRI [19] may be involved. Since various IgG subclasses bind FcγRI with different affinities [24], we tested whether one of the subclasses might be a particularly potent IgE suppressor. CBA/J dams were injected with the anti-bvPLA₂ mAb MS613-γ1 or its γ2b or γ2a switch-variants or a bvPLA₂-negative control antibody. The offspring were initially immunized with IgE-inducing doses of bvPLA₂ at an age of 3 months. After 2–3 months, the control mice had developed specific IgG (data not shown) and IgE immune responses (Fig. 1a). In contrast, all three subclasses of maternally transferred anti-bvPLA₂ mAb MS613 (γ1, γ2b or γ2a) completely suppressed IgE formation to bvPLA₂ (Fig. 1a), thus demonstrating that IgE suppression by maternally transferred IgG mAb is independent of the IgG subclass. The IgG anti-bvPLA₂ responses of various experimental groups were found to be identical to those of control mice (data not shown).

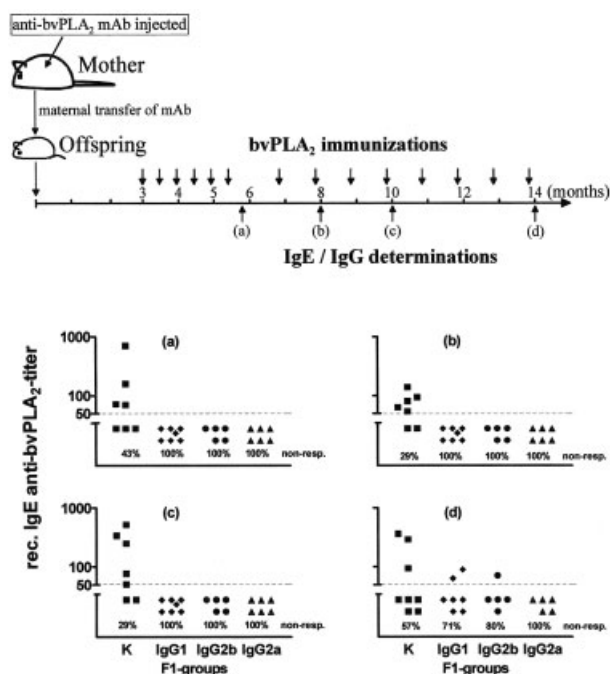


Fig. 1. IgE antibody response to bee venom phospholipase A₂ in CBA/J mice initially immunized at an age of 3 months. These mice had received mAb from their mothers which had been injected with either the control mAb NQ2/16.2 (γ1, κ; anti-2-phenyloxazalone, phOx) (F1 group K, ■), or anti-bvPLA₂ mAb MS613γ1 (F1 group IgG1, ◆), or two switch variants of this mAb, MS613γ2b (F1 group IgG2b, ●) or MS613γ2a (F1 group IgG2a, ▲). (a) The F1 mice were immunized every 2 weeks and the IgE response was measured 1 week after the sixth immunization when the mice were just under 6 months old. All mice were continued to be immunized by monthly injections of 0.1 μg bvPLA₂ and the IgE formation was analyzed in each case 1 week later. The IgE responses at ages of 8 (b), 10 (c) and 14 (d) months are shown. The antibody titers for individual animals are indicated. All symbols below the dashed line at a dilution of 1:50 represent sera that showed no reaction in the ELISA at this highest concentration tested. The percentage of these non-responders is indicated for each group.

2.2 Persistence of IgE suppression during further immunizations

The offspring were further immunized with IgE-inducing doses of bvPLA₂ at 1-month intervals and antibody responses were determined. Significantly, the IgE response to bvPLA₂ remained completely suppressed up to an age of 8 and 10 months (Fig. 1b, c). Even at 14 months, all mice born to MS613-γ2a-injected mothers still exhibited no IgE response, while among the offspring of MS613-γ1- or MS613-γ2b-injected dams there were 70–80% non-responders and only three mice showed marginal IgE-formation (Fig. 1d). Thus, either the

first encounter with the allergen had induced a persistent IgE suppression or the repeated administration of the allergen had extended this suppression until an age of more than 1 year. The overall amounts of bvPLA₂-specific IgG and its subclasses were also determined, but no significant differences between experimental and control mice were detected (data not shown).

2.3 Maternally induced immune modulation is no longer effective at an age of 6 months

The question arose as to how long this maternally-induced IgE-non-responsive state is maintained in the offspring before the first encounter with the nominal allergen. Therefore, another group of control and experimental F1 mice were initially immunized at an age of 6 months with IgE-inducing doses of bvPLA₂. During this response, both control and experimental mice developed bvPLA₂-reactive IgE antibodies, reaching plateau

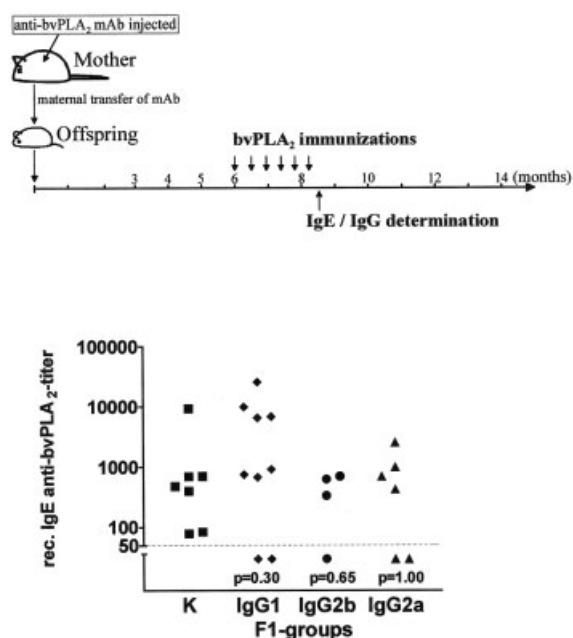


Fig. 2. IgE antibody response to bee venom phospholipase A₂ in CBA/J mice initially immunized at an age of 6 months. These mice had received mAb from their mothers, which had been either injected with the control mAb NQ2/16.2 (γ 1, κ ; phOx) (F1 group K, ■) or anti-bvPLA₂ mAb MS613 γ 1 (F1 group IgG1, ◆), or two switch variants of this mAb, MS613 γ 2b (F1 group IgG2b, ●) or MS613 γ 2a (F1 group IgG2a, ▲). The F1 mice were primed at an age of 6 months with following immunizations every 2 weeks and the IgE response after six immunizations at an age of about 8.5 months is shown. For further description see legend to Fig. 1.

levels at an age of about 8.5 months (Fig. 2). In relation to the previous experiment (Fig. 1), this demonstrates that IgE suppression by maternally transferred IgG ends between 3 and 6 months of age if exposure to the respective allergen does not occur beforehand.

2.4 IgE suppression by IgG mAb can not be induced in adult mice

Dams which had been injected with one of the three variants of bvPLA₂-specific mAb MS613 or a bvPLA₂-negative control mAb provided the opportunity to test whether allergen-specific IgG are also able to suppress an IgE response in adult mice. The IgE-inducing immunization of these dams was started when the offspring had reached an age of 3 months. Hence, the immune systems of both, dams and F1 progeny, had been influenced by the injected mAb for an identical period of time. In contrast to the offspring, the IgE antibody response of all dams was in no way suppressed (Fig. 3), thus, demonstrating that IgE-suppressive function of IgG is not operative in adults.

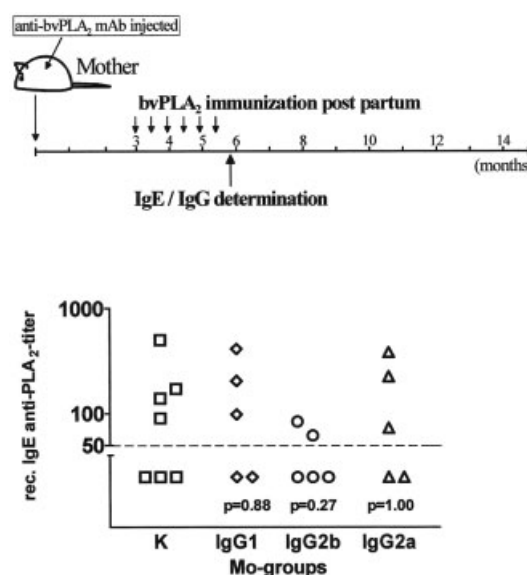


Fig. 3. IgE antibody response to bee venom phospholipase A₂ in CBA/J dams. The dams were either injected with the control mAb NQ2/16.2 (γ 1, κ ; phOx) (maternal control group K, □), or anti-bvPLA₂ mAb MS613 γ 1 (◇) or two switch variants of this mAb, namely MS613 γ 2b (○) or MS613 γ 2a (△). The dams were initially immunized when the progeny reached an age of 3 months, and the IgE response for individual animals at an age of a little less than 6 months is depicted. Further details are given in legend to Fig. 1.

2.5 Subclass-independent suppression of the airway-induced IgE immune response to ovomucoid by maternally transferred allergen-specific IgG mAb

IgE immune responses can also be induced by local immunization through inhalation of nebulized antigen preparations [25]. Since this local sensitization leads to the production of allergen-specific IgE, a cutaneous reactivity to OVA and an airway hyper-responsiveness, it is regarded as a murine model system of allergic bronchial asthma [26, 27]. This raises the question as to whether maternally transferred IgG mAb can also suppress IgE immune responses induced by inhalation of nebulized allergen. Since OVA preparations used for airway immunization contain small amounts of highly immunogenic ovomucoid (OM) as a contaminant [28], this allowed a specificity control by injecting anti-OM mAb into the dams and testing specific IgE responses to both OVA and OM in the offspring. Three independently raised OM-reactive mAb [OM30–15 (γ 1), OM7–3 (γ 2b) or OM16–8 (γ 2a)] reacting with different epitopes were employed or a negative control mAb. The offspring were initially immunized at an age of 4 months with a combination of i.p. injections and inhalation of nebulized OM-containing OVA.

During the pre-sensitizing i.p. immunization, all control and experimental mice developed solid IgG titers to OVA and OM, both in the range of 5×10^5 to 1×10^6 – 2×10^6 (data not shown), while, with the exception of two individuals, all mice remained negative for specific IgE antibodies. Two control mice showed marginal IgE-anti-OVA titers of 1:80 and 1:100, respectively, and one of them had an IgE-anti-OM titer of 1:60 (data not shown). After the first inhalative immunization, at an age of about 8 months, all control mice developed normal IgE responses to OVA as well as to OM (Fig. 4a and b), while the IgE-anti-OM response in experimental mice born to γ 1-, γ 2b- or γ 2a-anti-OM injected mothers were clearly suppressed (Fig. 4b). Compared to control mice, IgE antibody titers in the offspring of γ 1- and γ 2b-treated dams were significantly lower ($p=0.007$ and $p=0.035$, respectively). IgE-anti-OM formation was apparently suppressed in mice, which had received γ 2a anti-OM from the dams (Fig. 4b), though the difference relative to control mice was not statistically significant. Strikingly, the IgE response to OVA itself in the experimental groups was in the same range as in control mice (Fig. 4a), confirming that the IgE-suppressive action of maternally injected IgG is allergen-specific, but IgG isotype-independent as observed in the experiments with bvPLA₂ (see above).

Four months after the first inhalation, the mice were again immunized on 10 consecutive days with nebulized

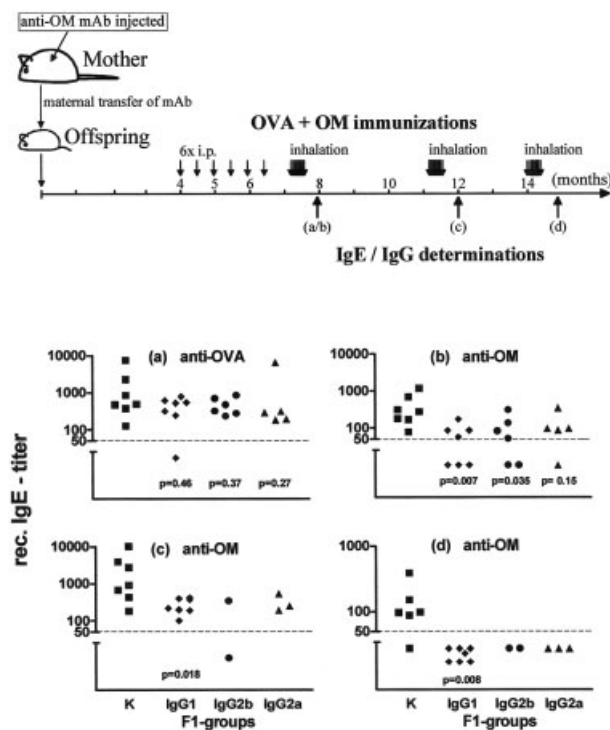


Fig. 4. Inhalation-induced IgE antibody responses to OVA and OM in 4-month-old BALB/c mice. They were immunized by an initial i.p. pre-sensitization with a preparation of OM-containing OVA (OVA grade V) at an age of 4 months followed by airway immunizations on 10 consecutive days with nebulized OVA/V. These mice had obtained from their mothers either the anti-phOx mAb NQ2/16.2 (γ 1, κ ; F1 group K, ■) or one of the independently raised anti-OM mAb OM30–15 (γ 1, κ ; F1 group IgG1, ◆), OM7–3 (γ 2b, κ ; F1 group IgG2b, ●) or OM16–8 (γ 2a, κ ; F1 group IgG2a, ▲). After the initial i.p. presensitization and the first inhalative immunization at an age of about 7.5 months, the IgE responses to OVA (a) and OM (b) were measured at an age of just under 8 months. (c) The mice were rested for 4 months and then challenged a second time with nebulized OM-containing nebulized OVA/V and the IgE responses to OVA (data not shown) and OM were measured 12 days later at an age of just under 12 months. (d) The mice were rested for another 3 months and, at an age of 14 months, they were again immunized via the airways on 10 consecutive days and the IgE-anti-OM response was determined 12 days later. The antibody titers of individual animals are indicated. p values indicate the statistical difference to the respective control group.

OM-containing OVA and the IgE response was measured 12 days later, at an age of about 12 months. In comparison to control mice, IgE antibody responses to OM in the offspring of anti-OM mAb-injected dams were still significantly suppressed (Fig. 4c), while the response to OVA equaled that of control mice (data not shown). When subjected to another round of airway immunization at an age of 14 months, control mice developed a

clear IgE response, while IgE-anti-OM titers of all animals in the experimental groups were below the detection level of 1:50 (Fig. 4d). IgE titers to OVA were essentially identical in control and experimental mice (data not shown).

2.6 IgE suppression by maternally transferred mAb is only effective during early ontogeny

To determine the duration of this maternally induced immunomodulation, another group of mice that had received one of the three OM-specific mAb or the OM-negative control mAb from their mothers, were allowed to rest until an age of 6 months before they were pre-sensitized by i.p. injections followed by airway immunization, both with of OM-containing OVA. The anti-OM immune response in control as well as experimental mice at an age of just under 10 months exhibited identical IgG

(data not shown) and IgE antibody titers (Fig. 5). Hence, as for the IgE response to bvPLA₂ (see above), the immunomodulatory IgE-suppressive effect of maternally transferred anti-OM IgG cannot be detected when the antigenic challenge occurs too late in life.

3 Discussion

These results show that maternally transferred monoclonal IgG of different subclasses suppress IgE immune responses to parenterally injected and inhaled allergen-preparations. Evidence, that a parenteral treatment is indeed effective throughout the body and is even able to influence airway-reactivity, is also derived from allergen-specific immunotherapy by subcutaneous injection of OVA [29]. This desensitization not only caused a decrease of IgE serum levels with a concomitant rise of IgG2a antibodies, but also induced an almost complete inhibition of eosinophil infiltration of the lungs and airway hyper-responsiveness, as detected by challenging the mice with inhaled OVA after immunotherapy. It is, therefore, conceivable that maternal IgG may also prevent IgE-mediated allergic asthma. Importantly, since all tested IgG subclasses of the anti-bvPLA₂ mAb MS613 directed at the same epitope and three different anti-OM IgG directed at different epitopes effectively suppressed IgE responses, it can be concluded that IgG-mediated IgE suppression does not depend on the recognition of specific epitopes.

The induction of an OVA-specific IgE response by airway immunization with nebulized OM-containing OVA was possible, despite the simultaneous presence of considerable amounts of humoral OVA-reactive IgG antibodies in these mice. This contrasts with the stringent IgE-suppression induced by maternally transferred monoclonal IgG-anti-OM, which were no longer detectable at the onset of immunization of F1 animals. Thus, while systemic application of IgG during early ontogeny via the mother induced long-term IgE unresponsiveness, humoral IgG present at the start of airway immunization in adult animals were ineffective. This emphasizes the importance of maternal IgG antibodies during early ontogeny.

Since the observed IgE suppression shows antigen specificity, it may be concluded that the antigen-binding sites of the employed IgG mAb are involved. However, the almost identical IgE suppression by all IgG subclasses tested argues against the possibility that functionally effective concentrations of our maternally injected IgG were still present in an FcR-bound form at the onset of immunization. Since up to this point both allergens were not involved, an immune complex-

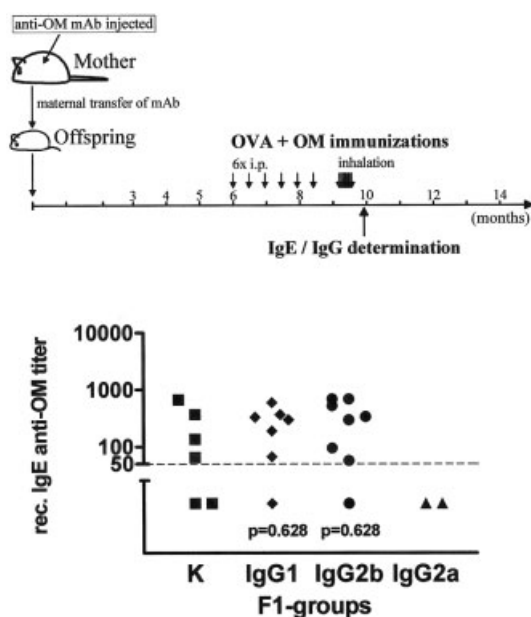


Fig. 5. Inhalation-induced IgE antibody responses to OM in 6-month-old BALB/c mice. The mice had obtained from their mothers either the anti-phOx mAb NQ2/16.2 (γ 1, κ ; F1 group K, ■) or one of the independently raised anti-OM mAb OM30–15 (γ 1, κ ; F1 group IgG1, ◆), OM7–3 (γ 2b, κ ; F1 group IgG2b, ●) or OM16–8 (γ 2a, κ ; F1 group IgG2a, ▲). The mice were initially i.p. pre-sensitized with a preparation of OM-containing OVA (OVA grade V) at an age of 6 months. After an inhalative immunization with OVA/V at an age of about 9.5 months, the IgE response to OM was measured at an age of just under 10 months. The antibody titers of individual animals are indicated. *p* values indicate the statistical difference to the respective control group.

formation with residual IgG mAb, which could be bound to low-affinity FcR expressed on a variety of immune cells [24], seems highly improbable. Due to the fact that high-affinity Fc γ RI binds IgG1 with much lower affinity than IgG2b and IgG2a [19], whereas all three maternally injected IgG subclasses induced an almost equal IgE unresponsiveness, the involvement of high-affinity Fc γ RI in mediating IgE suppression also seems impossible. Hence, it is not conceivable that residual FcR-bound IgG could induce the observed IgE suppression by directly binding and/or blocking the experimentally applied allergens. Furthermore, since one particular antibody is not able to block all determinants on either the bvPLA₂ or on the OM molecule, it is tempting to exclude the involvement of such a blockade as an effector mechanism.

The question, therefore, arises as to whether maternal IgG could induce IgE suppression in an indirect manner. Maternal antibodies are known to have a considerable influence on the neonatal immune system (reviewed in [30, 31]). For instance, they select idiotype-specific helper and suppressor T cells [32, 33] and thus are involved in the generation of the T cell repertoire [34, 35]. Since this influence is maintained until adulthood [36] and possibly throughout life [37], one could assume that our maternally injected bvPLA₂- or OM-reactive IgG mAb induced long-term T cell regulatory effects. However, this could only offer an explanation for IgG-mediated IgE unresponsiveness, if this isotype modulation could be connected with anti-idiotypic responses. Since this has not been described so far, an indirect mechanism for the exceptional ability of maternal IgG to induce IgE suppression is presently not at hand.

In the mouse, the time period during which maternally transferred IgG antibodies are able to exert a decisive influence on the further development of the immune system seems to be restricted to the first 3 weeks of life [32, 36, 38–40]. This ontogenetic period, thus, represents a 'developmental window' [41, 42], in which decisions governing the maturation of the immune system until adulthood are made. These conditions, in conjunction with the data presented here, allow the assumption of two developmental phases. The first, 'inductive' period permits an immunological imprinting [30] during the first 3 weeks of life. Although not tested so far, we assume that the IgE-suppressive, maternally transferred monoclonal IgG induce their immunomodulatory effect during this time. The second, 'effector' phase of this maternally induced modulation persists for about 4 months. It is not clear whether the primary encounter with the allergen during this period induced a permanent IgE unresponsiveness or whether secondary and tertiary experimental stimulations were necessary to prolong IgE suppression up to an age of more than 1 year. If the IgE-unresponsive

state is not exploited during the effector phase by an encounter with allergens, the maternally induced trans-generational isotype-regulatory effect seems to be permanently lost and, instead, the normal IgE high-responder phenotype is established. Hence, maternal experience in the form of IgG antibodies does not induce a persisting alteration in the nascent immune system, but has to be used in early ontogeny.

The relevance of these results, obtained in two different murine model systems, to the development of human allergy is not clear. A variety of epidemiological investigations have revealed a higher incidence of allergic and asthmatic diseases in children born to diseased mothers as compared to affected fathers [2, 43]. It has been argued that this approximately fourfold higher risk through the maternal line might be explained either by immune interactions between mother and child or by genomic imprinting [2]. The mechanism of this maternally induced immunomodulation is unknown, though maternal antibodies and cytokines may be involved. This higher maternally mediated risk seems to be in contrast to previous investigations in rats [21] and mice [22]. In these studies, even when the dams had been immunized in an IgE-inducing manner, the transfer of maternal IgG was sufficient to induce a strong suppression of IgE responsiveness in the offspring. Since the activatory or suppressive properties of maternal IgG can depend on their quantity [44], the preferential transmission of allergy and asthma by the mother may, in addition to other factors, be due to a qualitatively or quantitatively insufficient transfer of maternal IgG. In addition, a lack of exposure of the offspring to allergens during the postnatal period may also be responsible. In any case, both, the experimental situation in rats and mice as well as the maternally mediated enhanced risk in man emphasize the importance of an epigenetic influence of the mother for the development of allergic diseases.

The assumption of an effector phase during which an encounter with the allergen prolongs the IgG-induced IgE-unresponsiveness, might be helpful in understanding the significance of early ontogeny as the primary sensitizing period for the development of human allergic diseases in adults [1, 16–19]. Our results suggest that, under natural conditions, it would presumably be desirable for a mother to encounter allergens in such a way that a sufficient amount of specific IgG antibodies can be transferred to its offspring. These maternal IgG, which can be regarded as immunological information about the antigenic environment, induce an imprinting effect and are thereby decisive for an 'education' of the nascent immune system. However, a real long-term benefit could only be derived if the offspring themselves came into contact with the allergen during the effector phase

which, in the mouse, lasts for the first 4 months of life. In humans, an analogous time period of 5 years [1] may be indicated by the finding that exposure to the environmental conditions on a farm during the first 5 years is most protective against asthma and allergy [45]. It is striking that an exposure to farming for only the 1st year of life induces an even stronger reduction of asthma, hay fever and atopic sensitization in later life than the period between 1–5 years of age [46].

In summary, the data presented show that the imprinting effect by maternal IgG antibodies, once established, is able to reverse an IgE high responder phenotype in genetically identical individuals, *i.e.* it is dominant over a genetic predisposition and the experimental conditions necessary for the induction of IgE responses. Hence, maternal IgG antibodies interfere in an indirect way with the initial sensitization by environmental allergens and, thereby, determine whether or not an IgE high responder phenotype can develop during adulthood. This view provides the first explanation as to *why* early ontogeny can be decisive for a life-long allergic sensitization to environmental allergens [16, 17, 47]. Moreover, the findings raise hopes that the therapeutic application of allergen-specific IgG mAb may compensate for an insufficient transfer of maternal antibodies and may thus help to prevent the development of allergic diseases.

4 Materials and methods

4.1 Induction of IgE responses

CBA/J (H-2^k) and BALB/c (H-2^d) mice were obtained from Harlan/Winkelmann (Borken, Germany). IgE formation to bvPLA₂ was stimulated in CBA/J mice as described [22]. For induction of IgE immune responses of BALB/c mice, we used a combination of *i.p.* pre-sensitization, which by itself is not able to provoke the formation of IgE antibodies, and inhalations of nebulized antigen. BALB/c mice received six *i.p.* injections of 10 µg OM-containing OVA grade V (OVA/V, No. A 5503; Sigma, Deisenhofen, Germany) adsorbed to 2 mg Al(OH)₃ (Imject® Alum, Pierce; Rockford, IL) in bi-weekly intervals. At 26 days after the last injection, the mice were challenged on ten consecutive days each for 20 min by inhalation of nebulized OVA/V [1% solution of OVA/V in PBS, ultrasonically nebulized with a PARI/LC PLUS nebulizer (PARI GmbH, Starnberg, Germany)]. Control mice were challenged with nebulized PBS alone. Further immunizations via the airways were also performed on ten consecutive days in the same way. For the determination of humoral IgG and IgE antibody titers, the mice were bled from the tail vein, either 12 days after an *i.p.* injection or 12 days after the last inhalation.

4.2 mAb

After transfer from the dams, anti-bvPLA₂ mAb MS613 (γ1, κ) suppresses the IgE immune response to bvPLA₂ in CBA/J mice [22]. From this γ1 clone, γ2b and γ2a switch variants were isolated with the aid of isotype-specific antisera and magnetic cell sorting. The identity of V_L and V_H regions of mAb MS613 and its switch variants was checked by sequencing the respective PCR-amplified cDNA as described [48]. The variable region sequences of the mAb MS613γ1 and its γ2b and γ2a switch variants are available from GenBank under the accession nos. AF362833 to AF362838.

Three independent hybridoma cell lines secreting OM-specific mAb were selected after fusion of Ag8.653 myeloma cells and BALB/c spleen cells hyper-immunized with OM (T 2011, free of ovinhibitor; Sigma, Deisenhofen, Germany). The variable region sequences of these mAb (OM30–15; IgG1, κ), IgG2b (OM7–3; IgG2b, κ) or IgG2a (OM16–8; IgG2a, κ) were determined and are available from GenBank under the following accession nos.: mAb OM30–15–V_H=AF362839, V_L=AF362842; mAb OM7–3 – V_H=AF362840, V_L=AF362843; mAb OM16–8–V_H=AF362841, V_L=AF362844. Binding inhibition studies revealed that all three antibodies reacted with different OM epitopes (data not shown).

4.3 Transfer of mAb from dams to the offspring

Pregnant CBA/J and BALB/c mice were injected 10 days before and 2 days after delivery of their offspring with a total of 1.2 mg (each *i.p.* injection with 0.2 ml of a solution with 3 mg/ml) of purified anti-phOx control mAb NQ2/16.2 [49] or CBA/J females were injected with the IgG1-anti-bvPLA₂ mAb MS.613 or its γ2a or γ2a switch variants. BALB/c females were injected with the same amount of one of the three independent anti-OM mAb OM30–15 (γ1/κ), OM7–3 (γ2b, κ) or OM16–8 (γ2a, κ).

4.4 Determination of IgG and IgE antibody responses

At all indicated time points, not only IgE and total IgG, but also IgG-subclasses 1, 2b and 2a were measured. However, since IgG responses showed no clear and significant differences between the various groups, only IgE responses are shown. The determination of IgE and IgG immune responses to bvPLA₂ was performed as described [22]. The IgE antibody activities to bvPLA₂, OVA and OM were determined in 1:2 serially diluted sera and titers were calculated as that dilution which yielded three times the background values obtained without antibody. The immune response of BALB/c mice to OVA/V, which is contaminated with OM, was separately analyzed for IgE and IgG antibody activities to OM (T 2011) and OVA grade VII (A 7641; both from Sigma). Control experiments had shown that OM is essentially free of OVA. In contrast, OVA/V was found to contain considerable

amounts of OM, while the OM contamination of OVA grade VII (OVA/VII) was negligible (data not shown). Reactivity of our anti-OM antibodies with OVA preparations could be traced back to contamination with OM, but was not attributable to a true cross-reactivity with OVA itself.

4.5 Statistical analysis

Statistical significance was evaluated from individual values using the two-tailed Mann-Whitney two-sample rank test using the PRISM™ version 3.0 computer program (Graph-Pad, San Diego, CA). The level of significance between groups was set at $p < 0.05$.

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