

Non-genetic inheritable potential of maternal antibodies

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Abstract

Maternal antibodies (IgG and IgA) not only provide passive protection against microbial infections, but also exert a variety of equally important active, idiotypically-mediated immunoregulatory functions. Since the generation of maternal antibodies depends entirely on the stimulation of the mother's immune system by external mainly thymus-dependent antigens, with long-lived antigen independent plasma cells in the bone marrow, maternal antibodies represent the mother's collective ontogenetic immunological experience. Although their stimulatory potential in mice is restricted to the neonatal imprinting period, maternal antibodies exert a life-long determinative influence which is even dominant over seemingly genetic predispositions. Therefore, the functional impact of maternal IgG antibodies appears phenotypically as a non-genetic inheritance.

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1. Introduction

It is already over 100 years ago that Paul Ehrlich demonstrated the protective function of maternally transferred antibodies against microbial infections in offspring [1]. His experiments seemed to demonstrate that maternal antibodies (matAb) act solely as passive protective mediators while having no influence on the ontogenetic development of the immune system, a view still widely accepted [1,2]. However, numerous investigations during the past few decades clearly demonstrated that matAb exert various immunomodulatory effects which might even be visible at an age when they themselves were no longer detectable in the serum [3–5], thus calling in question whether their function is restricted to the provision of passive protection. This conclusion is corroborated by several studies demonstrating that matAb may even enhance immune responses in murine offspring [5,6].

Since the immune system develops no tolerance to the idiotypic determinants of the antigen receptors of B and T cells, the pre-immune repertoire as well as antigen-activated B and T cells clones are subjected to internal idiotypic regulation [7–10]. In the present context, it is particularly important that the regulatory effects of such idiotype–anti-idiotype interactions in animal models have been reported (a) to be long-lasting or even permanent when induced during the

neonatal period [11–13] and (b) cannot only be induced by neonatally injected, but also by maternally transferred antibodies [11,14].

However, the determinative influence of idiotype–anti-idiotypic interactions is restricted to the neonatal period which in the mouse lasts for about the first 3 weeks of life [15–17]. This led to the conclusion that the “idiotype-directed selection of the *adult* B-cell repertoire appears to be limited to fetal–neonatal stages of development” [18]. Hence, “developmental windows” exist during early ontogeny [19] which are important for “immunological imprinting” [20]. These given facts beg the question whether the idiotypic interactions which are induced by matAb have any significance with regard to the protective function of the immune system against harmful influences from the environment?

2. Methods

The principle question arose as to whether an immune response in offspring was altered in any way when the mother had been immunized with that particular antigen. If matAb were merely functioning passively, the characteristic parameters of an immune response would not be altered when they had disappeared from the circulation and such mice would have to be regarded as normal mice. We tried to answer this question in three model systems with the following common experimental design (Fig. 1).

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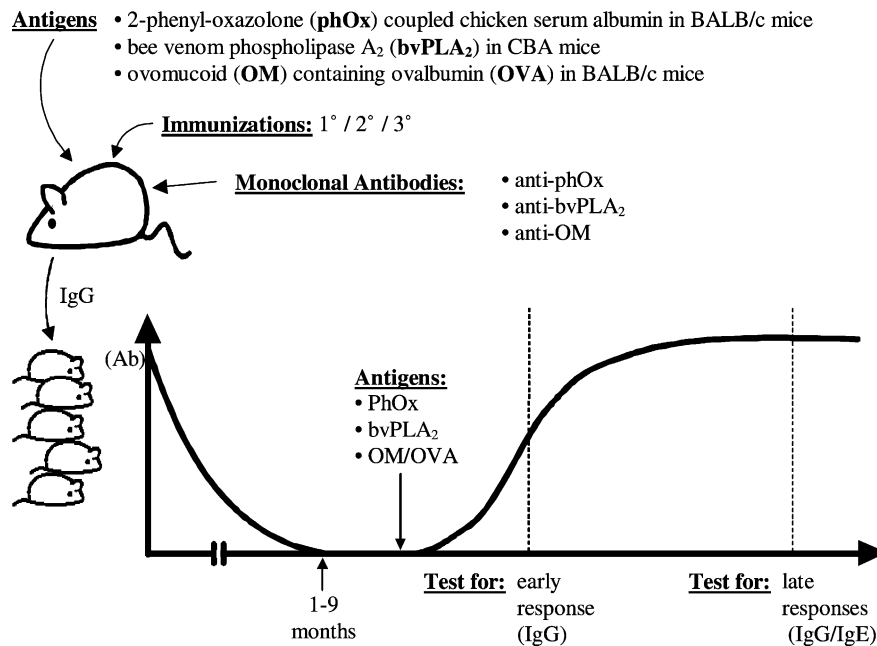


Fig. 1. Experimental design.

1. Female BALB/c mice were either immunized with the hapten carrier complex 2-phenyl-oxazolone (phOx) coupled to chicken serum albumin (CSA) or were injected with anti-phOx monoclonal antibodies (mAb), and the primary anti-phOx and anti-CSA immune responses in the offspring were analyzed.
2. CBA females were immunized with bee venom phospholipase A₂ (bvPLA₂) or were injected with anti-bvPLA₂ mAb and the offspring were tested for a suppressed IgE response (see below).
3. Female BALB/c mice were injected with mAb directed at ovomucoid (OM) which is a contaminant in certain preparations of ovalbumin (OVA). The IgG and IgE responses to OVA and OM were analyzed in the offspring after an immunization with nebulized OM-containing OVA via the airways.

3. Results

3.1. Maternal highly immune-maturated tertiary/quaternary anti-hapten IgG antibodies induce a complete dysregulation of a primary anti-hapten response in the offspring

The immune response of BALB/c mice to the hapten phOx has extensively been analyzed at the molecular level [21]. The primary day 7 response is dominated to 75–80% by IgG antibodies belonging to the Ox1 idiotypic (Id_{Ox1}) which is lost during the next 2 weeks by somatic mutations. Id_{Ox1} antibodies are of high affinity while the affinity of non-Id_{Ox1} is 10–100 times lower. Immune maturation during the primary and later responses is mainly based on the accumulation of

somatic mutations occurring in Id_{Ox1} antibodies. During secondary and tertiary immune responses, non-Id_{Ox1} antibodies are observed which are encoded by alternative V_H/V_L genes, and the propensity of non-Id_{Ox1} antibodies for somatic mutations is also known. Hence, we expected that the maternal pre-immunization model would tell us whether matAb act solely in a passive way or might be able to alter the parameters of the normal response. The results inevitably demonstrated that the offspring born to immunized mothers at an age when maternal IgG were no longer detectable in their sera cannot be regarded as ‘normal’ mice. (1) A secondary anti-phOx immune response in the mothers per se induced an increase of IgM anti-phOx antibodies in non-immunized F1 mice [5], a finding which has been confirmed in another system [22]. (2) A tertiary anti-phOx immune response or the transfer of highly mutated and immune-maturated quaternary anti-phOx antigen-free mAb induced a complete dysregulation of the clonal composition of the primary anti-phOx response in offspring at an age when the transferred matAb were no longer detectable [23]. First, in these experiments the Id_{Ox1} became exceedingly variable and was expressed by 10–90% of antibodies in individual animals. This is a fascinating result since the transferred tertiary and quaternary anti-phOx mAb exhibited extremely low idiotypic cross-reactivity which was tested with a whole set of syngeneic anti-Id_{Ox1} mAb [24,25]. Moreover, the observed variability of Id_{Ox1} expression at day 7 was even greater than the seemingly *genetically* based differences between various mouse strains [26]. Second, among primary day 7 antibodies of F1 mice born to tertiary immunized dams (3°moF1), half of the non-Id_{Ox1}, in comparison to non-Id_{Ox1} of control mice [23], showed a 5–100-fold enhanced affinity, and 60% exhibited an affinity identical to that of Id_{Ox1}, while the

remaining 40% had affinities of up to 7–25 times higher than Id_{Ox1} antibodies. Third, the genetic analysis of those primary day 7 antibodies of 3°moF1 mice revealed that the non-Id_{Ox1} were encoded by V_H/V_L genes and/or combinations thereof which were either new, not previously observed in the anti-phOx response or typical of memory responses in normal mice. Strikingly even those serologically Id_{Ox1}-negative antibodies which were encoded by the V_KOx1 gene and/or the V_HOx1 gene exhibited no somatic mutations which were identical to known affinity-increasing substitutions [21], making it highly unlikely that pre-immunization by transferred antigen phOx-CSA had occurred. Therefore, these results unequivocally demonstrated that maternal antibodies clearly do not only function as passive protective mediators, but exert an active idiotypically mediated, immunoregulatory influence on the nascent immune system of the neonate. The obvious benefits in the system studied are the enhanced IgM concentrations and the improved quality of the very first primary antibodies.

3.2. Maternally derived IgG antibodies suppress IgE responsiveness in offspring

In the two other models, we tested the IgE-suppressive effects of maternally derived IgG antibodies. This ability was demonstrated over 20 years ago by Jarrett and Hall who observed that immunization of female rats with OVA suppressed IgE formation in the offspring, even in an IgE high-responder strain and under experimental conditions which favor IgE responses by the use of minute amounts of the allergen and a Th2 cell-activating adjuvant [3]. We repeated these experiments in mice using bvPLA₂ as an IgE-inducing allergen. By use of preformed monoclonal anti-bvPLA₂ IgG, it could formally been proven that this IgE suppression functions independently of antigen [27]. In addition, recent experiments have demonstrated that transgenerational IgE suppression could not only be induced for parenteral, but also for those IgE responses which are stimulated by inhalation of nebulized allergen via the airways [28]. For both IgE responses to bvPLA₂ and ovomucoid, respectively, compelling evidence was presented that the maternally injected mAb were not the direct, IgE-suppressive effector molecules. First, IgE suppression was observed at an age of 3–4 months when the maternally injected mAb had long since disappeared from the circulation. Second, it might be argued that the maternally injected mAb were still present in an Fc receptor-bound form. Since IgG subclasses bind to FcR with different avidities [29], we prepared IgG2b and IgG2a switch variants from our IgE-suppressive anti-bvPLA₂ IgG1 clone. When tested for transgenerational IgE-suppressive function, all three IgG subclass mAb were equally effective although their binding avidity to FcR was quite different. Identical results were obtained in the third model system in which the IgE response was induced by inhalation of nebulized OM-containing OVA [28]. Third, although each of the maternally applied anti-OM mAb reacted

with a different epitope and could consequently not cover the entire allergen, the observed suppression was allergen, but not determinant-specific. Hence, masking of the whole molecules could not be responsible for the induction of IgE unresponsiveness. Finally, only the IgE response was suppressed, while IgG formation developed as in control mice.

Interestingly, in both systems, IgE suppression was not observed in the dams which had been injected with the anti-bvPLA₂ or the anti-OM mAb, and also not observed in the offspring when they were first immunized at the age of 6 months [28]. However, an encounter with both allergens before that age and further immunizations via their respective routes revealed a persistent IgE suppression up to an age of more than 1 year. Administration of OM via the airways induced an IgE response after i.p. presensitization which by itself did not provoke IgE, but did provoke high titers of IgG antibodies. Despite these high concentrations of systemically available IgG, inhalation of nebulized allergen induced a substantial IgE response. Hence, whereas neonatal IgG had a strong IgE-suppressive effect, much higher concentrations during adulthood were insufficient in this respect. These findings underscore the current opinion that early ontogeny is decisive for the development of human allergic diseases during adulthood [30]. Furthermore, the results demonstrated that murine maternal IgG indeed induce their immunomodulatory effects by themselves, but in addition they act in concert with environmental antigens when encountered during early ontogeny. This view is also corroborated by epidemiological studies in man which demonstrated that exposure to the environmental conditions on a farm during the first 5 years is most protective against asthma and allergy [31]. It is striking that an exposure to farming for only the first year of life induces an even stronger reduction of asthma, hay fever and atopic sensitization in later life than the period between 1 and 5 years of age [32].

4. Conclusion: maternal antibodies have a non-genetic inheritable potential

Since maternal IgG are the products of multiple immune maturation processes induced by thymus-dependent antigens, they have to be regarded as *acquired immunological phenotypic characters* which represent a great deal of the mother's entire immunological experience. This acquired maternal knowledge of the external world of antigens not only provides passive immunity as long as the newborn's immune system is immature, but also has a non-genetic inheritable potential for the offspring through an immunological imprinting effect during the neonatal period. This second function of matAb (a) enables anti-microbial protection even by non-antigen reactive, anti-idiotypic antibodies [33], (b) may be superior to seemingly genetically-based parameters such as the expression of dominant idiotypes or the IgE high-responder state of certain mouse strains and

(c) may thus have a therapeutic potential for the treatment of allergic diseases.

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