

Importance of Early Allergen Contact for the Development of a Sustained Immunoglobulin E Response in a Dog Model

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Key Words

Allergy · Atopy · Sensitization · Immunoglobulin E · Dog

Abstract

Background: Immunoglobulin E (IgE)-mediated allergies are postulated to require early allergen contact and sensitization for the full development of sustained IgE levels.

Methods: Thirty-two Beagle dogs from seven litters selectively bred for their high IgE response were sensitized by subcutaneous injection of chicken ovalbumin (OVA), peanut extract and recombinant birch pollen allergen (Bet v 1). In half of the dogs from each litter, sensitization injections were started on the first day of life; the other half of the same litter was first sensitized at the age of 4 months. To evaluate whether early sensitization also predisposes the animals to IgE responses to other allergens later in life, we injected a recombinant timothy grass pollen allergen (Phl p 5) later on, at the age of 10–12 months. Allergen-specific serum IgE and IgG levels were evaluated with enzyme-linked immunosorbent assays. In addition, 21 dogs were challenged with aerosolized OVA to measure bronchoconstrictive changes in lung function. **Results:** Early sensitized dogs developed significantly higher OVA-specific serum IgE levels than late

sensitized dogs, in contrast to the IgG levels, which were lower in these dogs ($p < 0.001$). The increase in specific serum IgE and IgG following boosting remained different between the two groups for over a year. Titers of specific serum IgE and IgG were also different after sensitization with a new allergen injected later in life for the first time. Dynamic pulmonary compliance and resistance, both parameters for bronchoconstriction induced by OVA aerosol challenge, were also significantly higher in early sensitized dogs (for both parameters, $p < 0.01$). **Conclusions:** Contact with an allergen early in life is decisive for the development of sustained IgE levels and the development of IgE responses to additional allergens encountered later in life. Allergen avoidance during early life may have some preventive effect on IgE-mediated allergy in dogs.

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Introduction

The increasing incidence of allergic diseases in human beings [1, 2] as well as in companion animals has created an urgent need for new preventive or therapeutic strategies. Immunoglobulin E (IgE)-related atopic diseases oc-

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cur in dogs as well as in humans. For both species, the expression of the disease can be atopic dermatitis, and a genetic predisposition to atopy has been demonstrated [3–8]. By using Beagle dogs selected for a high IgE response by breeding, we established a dog model for human atopy [6, 9].

There is increasing evidence in humans that sensitization leading to IgE responses occurs through early allergen contact, possibly already in utero [10–15]. We have reported previously [6, 9] that sensitization of high IgE responder dogs at birth leads to sustained IgE responses, while delaying first contact with allergen prevents the formation of IgE. This, however, was observed after immunizing dogs from different parents at different times. Hence, the possible influence of subtle genetic and/or environmental differences could not be excluded. In order to prove that early sensitization, in addition to genetic predisposition, is an important prerequisite for a sustained IgE response, we split litters of high IgE responder dogs into two equal groups. One group received the first injection of allergens within the first 24 h of life, whilst the other group was first sensitized at the age of 4 months. In a follow-up experiment (at the age of 1 year), we boosted the animals again to see whether the IgE and/or IgG responses remained different. An additional allergen was also used for primary sensitization later in life in order to investigate whether development of an IgE response early in life opens the gate for multiple IgE responses later in life [9]. Changes in airway functions after inhalation of aerosolized allergens are described in the literature for IgE-sensitive children [16], mice [17], rabbits [18–20] and dogs [21]. We therefore also included airway provocation studies after the two early or late sensitization schedules in order to explore whether the potential differences in the development of IgE responses are also relevant for a clinical allergic response.

Materials and Methods

Animals

Beginning in 1992, Beagle dogs from a commercial dog breeder were mated in our Institute. We selected the breeding dogs on the basis of their ability to produce high allergen-specific IgE levels after early subcutaneous allergen injections [6, 9]. A total number of 32 newborn dogs from 7 litters (18 males and 16 females), offspring of so-called 'high IgE responder' parents, were included in this study and followed for up to 2 years. All dogs were nursed by their mothers and weaned at the age of 10 weeks. During all experiments, the guidelines of the Austrian law for the care and use of experimental animals were strictly followed.

Allergens and Allergen Sensitization

We used birch pollen allergen 1 (Bet v 1) and the timothy grass pollen allergen 5 (Phl p 5, *Phleum pratense* 5, PP5), both recombinant allergens from Biomay (Vienna, Austria), ovalbumin (OVA; 98% purity, Sigma, St. Louis, Mo., USA) and a purified protein extract from peanuts (PN; Ara B; Laboratoire de chimie des protéines, Université de Nancy I, Vandoeuvre, France). The allergens (for Bet v 1 and Phl p 5: 1 µg per injection; for OVA and PN: 10 µg per injection) were diluted in aluminum hydroxide (Serva Electrophoresis GmbH, Heidelberg, Germany) suspended in sterile water, mixed with 0.9% phosphate-buffered saline (PBS; 1:1) and administered subcutaneously in a total volume of 1.0 ml for puppies up to 4 months and 2.0 ml for adult dogs.

Initial Experiment in Young Dogs

A total number of 18 puppies from four different litters, all born between May 19 and August 12, 1997, was used for this first experiment. All four litters were split into equal groups, but two of the late sensitized dogs died within a few weeks after birth, leaving unequal numbers. The early sensitized group (n = 10) received one primary subcutaneous injection of OVA, recombinant birch pollen allergen (Bet v 1a) and PN within the first 24 h of life, followed by three subcutaneous booster injections at 2-week intervals, two more booster injections at 4-week intervals (weeks 10 and 14) and then one boost every other month (weeks 22, 30, 38 and 47). For the late sensitized group (n = 8), the sensitization schedule was the same, except for the fact that they received their primary allergen injection at 4 months of age (fig. 1). All animals were kept in the same kennels under the same conditions, and received the same food and the same vaccinations (Vanguard 7 at the ages of 9 and 13 weeks).

Follow-Up Experiment in Adult Dogs

Ten dogs from the initial experiment (8 early and 2 late sensitized) and 9 additional dogs from two other split litters (4 early and 5 late sensitized) for a total of 10 male and 9 female animals were included in a follow-up study which was carried out 1 year later. The animals were boosted at the age of 14–23 months with the same allergens to which they had been sensitized before (OVA, PN, Bet v 1). They were boosted twice at 4-week intervals and blood samples were taken weekly until 2 months after the second boost.

Late Initial Sensitization with a New Allergen

The same 18 dogs as in the initial experiment were used. At the age of 10–12 months, they received for the first time an injection of recombinant timothy grass pollen allergen (Phl p 5, alias PP5), followed by three booster injections at 2-week intervals and one 4 weeks later (fig. 2).

Blood Sampling

In all experiments, blood samples were taken on the day of the allergen injection as well as 7 and/or 14 days thereafter. In the early sensitized group, blood sampling was only started at the age of 6–8 weeks. Serum was separated and frozen in aliquots at –20°C until allergen-specific IgE and IgG levels were determined.

Antibody Determinations

An enzyme-linked immunosorbent assay (ELISA) method based on a previous report [22] was established for determination of allergen-specific IgE and IgG. Briefly, flat-bottomed 96-well Nunc Immunoplates (Maxisorp™) were coated with 100 µl/well of the respective

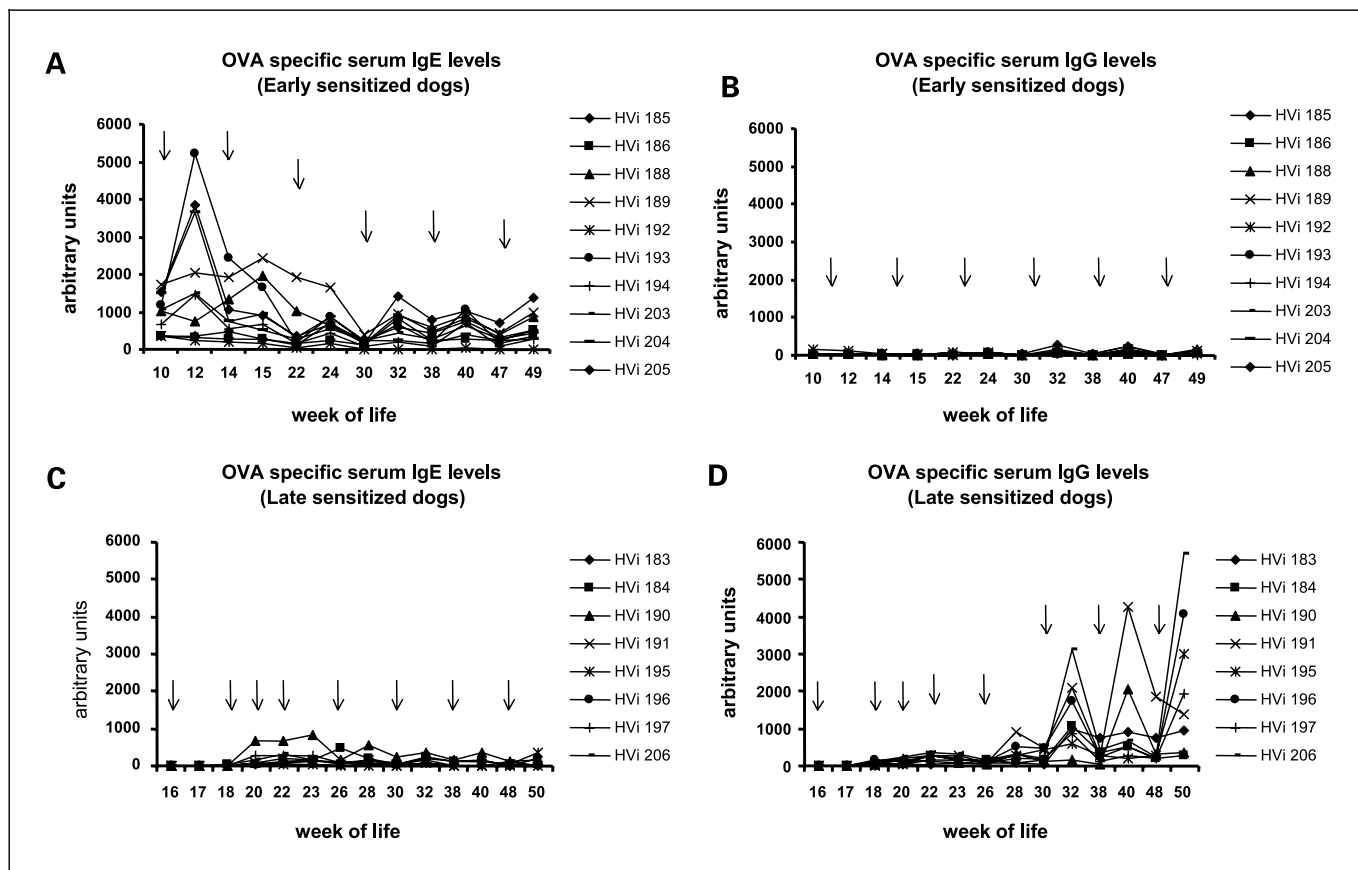


Fig. 3. OVA-specific serum IgE and IgG levels in early and late sensitized dogs during their first year of life. The immunizations are depicted by arrows (x-axis). The y-axis shows the OVA-specific serum IgE (and IgG) levels, which are expressed in RU for each single animal. The differences between early (A, B) and late (C, D) OVA-sensitized groups were significant ($p < 0.01$ for both).

Xact version 3.1, Cytel Software Corporation). Other data derived from early and late sensitized dogs were compared using multiple t tests after logarithmic transformation of the data to achieve normal distribution (Systat version 10, SPSS Inc.).

Lung function parameters were also expressed by the AUC of the response versus time curve, using the baseline determined after inhalation of aerosolized PBS before allergen challenge. Since the periods of measurement of the pulmonary functions differed among the animals due to respiratory arrest after challenge in some animals, data were grouped in two different measuring periods (<15 min and 10–20 min). The AUC of the pulmonary parameters in early and late sensitized dogs were compared using two-way analysis of variance, taking the measuring period as a second factor (Systat version 10). Resulting p values were adjusted for the multiple comparison situation using the method of Hochberg-Bonferroni.

Results

High IgE titers were produced only in early sensitized dogs, while high IgG titers were only seen in later sensitized animals.

Initial Experiment in Young Dogs

Figure 3 shows the differences in OVA-specific IgE and IgG levels between early (fig. 3A, B) and late sensitized dogs (fig. 3C, D; in the initial experiment). In the early sensitized group, the mean OVA-specific IgE levels were approximately 10 times higher than in the late sensitized dogs ($p < 0.001$; fig. 3A). Early sensitized dogs developed mean OVA-specific serum IgE levels of 1,932 RU (range 230–5,198 RU), falling to not lower than 180 RU (range 7–389 RU) 2 months after the 6th boost. These IgE levels could be maintained even at boosting intervals of

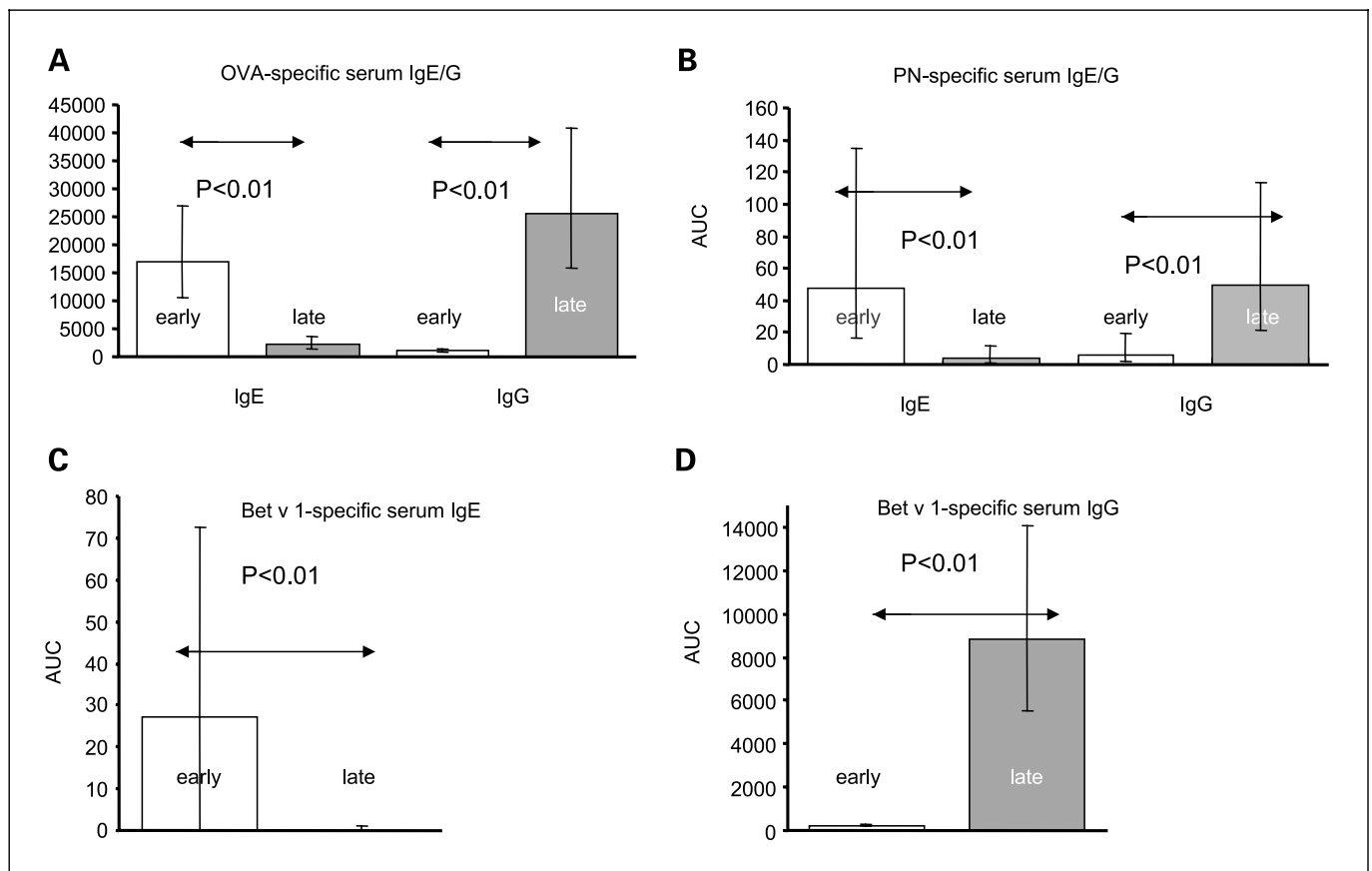


Fig. 4. Geometric means of OVA- (A), PN- (B) and Bet v 1 (C, D)-specific serum IgE and IgG levels in early (white bars) compared to late (gray bars) sensitized dogs. All differences between early and late sensitized groups were statistically significant.

about 2 months. The IgE levels rose in all of these dogs after each boost. The IgE levels remained boostable for at least 1 year in dogs that had their first sensitization early in life.

In the late sensitized dogs, relatively low specific serum IgE levels were found, the highest level being 828 RU at week 23 (mean 223 RU, range 33–828 RU), which dropped down to less than 10 RU in 4 dogs ($p < 0.001$ for the difference between early and late sensitization). In those animals, repeated injections of allergens had no boosting effect.

The OVA-specific serum IgG levels (fig. 3B, D, fig. 4A) correlated inversely with the levels of IgE. OVA-specific IgG ranged between 276 and 5,669 RU (mean 1,720 RU) in the late sensitized group (fig. 4D), while in the early sensitized group, the highest IgG values were much lower at a range of 43–239 RU (fig. 3B, 4A; $p < 0.001$ for the

difference between early and late sensitization). Similar differences could be observed for the other allergens, i.e. PN (fig. 4B; $p < 0.01$ for IgE, $p < 0.02$ for IgG) and Bet v 1 (fig. 4C, D; $p < 0.001$ for both IgE and IgG).

Follow-Up Study in Adult Dogs

A follow-up study showed that even at the age of 1 year or older, early sensitized dogs still produced higher allergen-specific IgE levels than late sensitized animals when boosted with allergens (fig. 5). The statistical analysis confirmed the significance of differences in levels of IgE ($p < 0.001$ for OVA, PN and Bet v 1) and IgG ($p < 0.01$ for PN and Bet v 1, but $p = 0.53$ for OVA).

Response to the Additional Antigen Phl p 5

When immunized for the first time at 10–12 months with Phl p 5, the difference in IgE level between early and

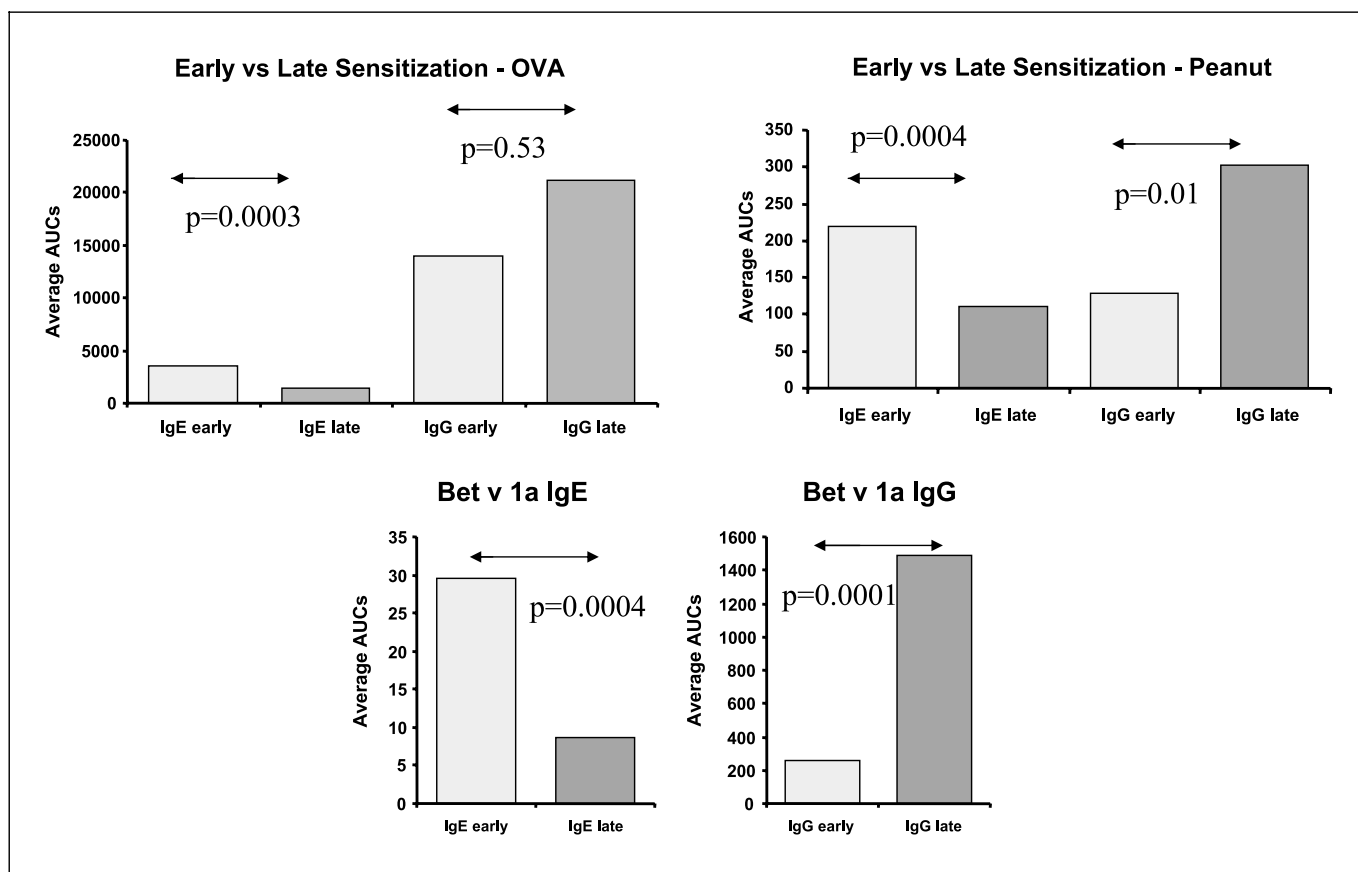


Fig. 5. Follow-up study with dogs older than 1 year after boosting with the various allergens. Results are expressed as mean AUC for OVA-, PN- and Bet v 1-specific serum IgE and IgG levels in early versus late sensitized dogs. The differences between the groups were highly significant except for OVA-specific IgG.

late sensitized dogs did not quite reach significance as was the case for the other allergens used earlier, but a trend towards high Phl p 5-specific IgE ($p = 0.07$) was still evident in early immunized dogs (fig. 6). In contrast, the Phl p 5-specific IgG levels were significantly higher in late immunized dogs as for all the other allergens ($p < 0.01$).

Late Sensitized Animals React Less Severely to Challenge with Aerosolized OVA than Early Sensitized Animals

Inhaled OVA significantly altered pulmonary function parameters in early sensitized dogs. In evaluation of pulmonary function following aerosol allergen challenge, the results for frequency must be looked upon with caution, since some of the dogs which showed severe bronchoconstriction after OVA challenge stopped breathing for 3–5 min and had to be artificially ventilated with oxygen in

order to save their lives. Furthermore, during the wake up phase of anesthesia, breathing frequency increases rapidly. Still, all early sensitized dogs except one (11/12) showed an increase in frequency compared to the baseline value (AUC from 6.99 to 1,091.40). All early sensitized animals (12/12) had to be artificially ventilated with oxygen. In contrast, only 4 out of 9 late sensitized animals displayed changes in respiratory frequency (AUC 169.5–803.41).

The two other parameters, dynamic compliance and resistance (fig. 7), were considered to be more reliable. All early sensitized dogs exhibited a sustained increase in resistance (AUC from 507.66 to 3,296.58) and decrease in compliance (AUC from –259.51 to –876.37), whilst only 4 out of 9 late sensitized dogs responded to the OVA challenge.

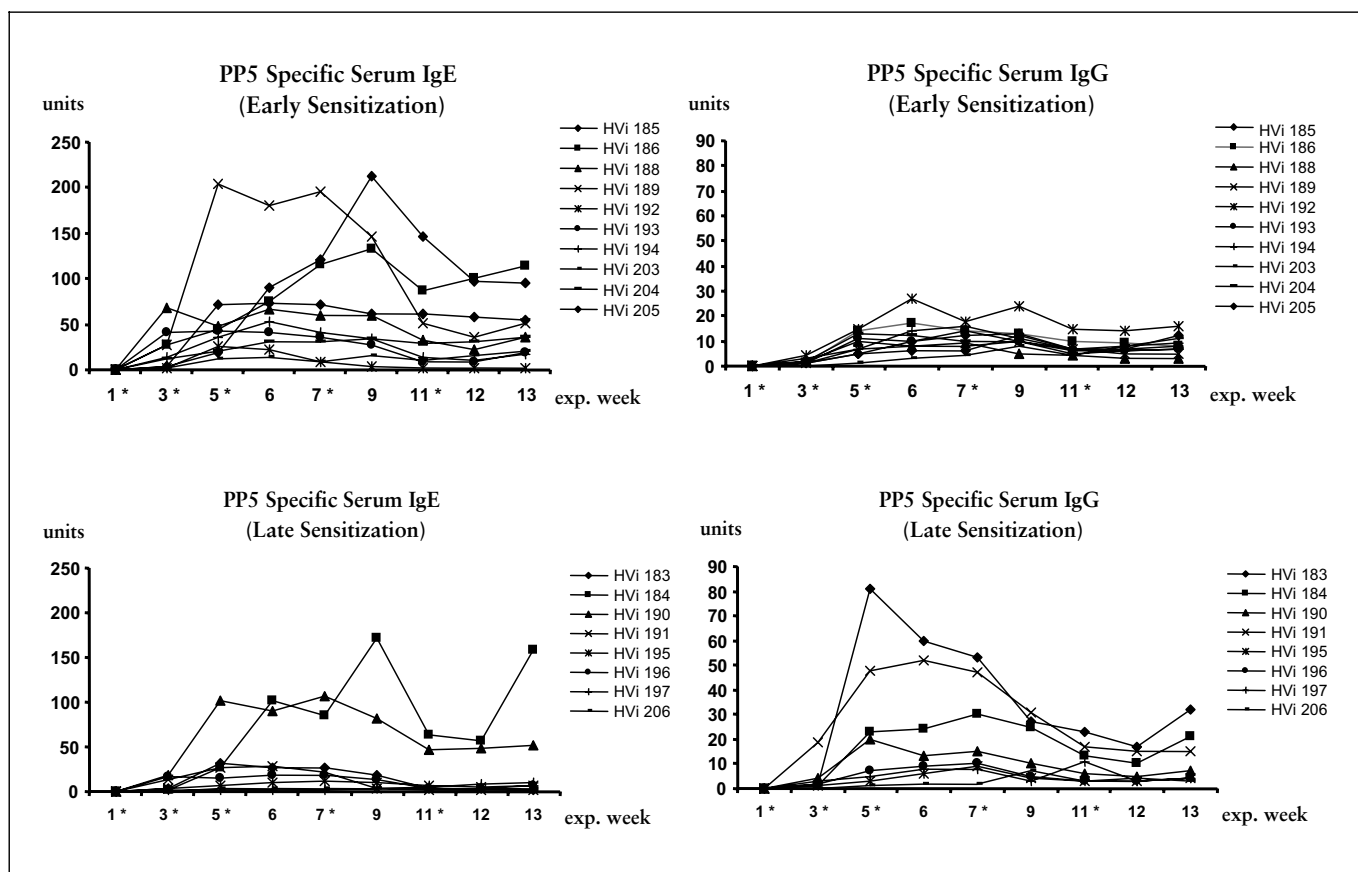


Fig. 6. Phl p5 (PP5)-specific IgE and IgG levels in early compared to late sensitized dogs over a period of 13 weeks after first immunization at the age of 10–12 months. The asterisks indicate the time points of the 5 allergen injections. A trend that early sensitized dogs still develop higher IgE and lower IgG in response to new allergen injections later in life can be seen. For IgG, this difference between the groups is significant ($p = 0.008$). For IgE, the p value is 0.069.

Taken together, changes in compliance and resistance were statistically significantly different between the two groups ($p < 0.01$ for both). Figure 7 includes OVA-specific serum IgE levels on the day of challenge. No strong correlation was found between airway responsiveness and serum IgE levels.

Discussion

The results of this study show that even dogs with a genetic predisposition to a high IgE response must be sensitized early in life to develop high and long-lasting serum IgE levels after allergen contact. In the early sensitized group, the mean OVA-specific IgE levels were 10 times higher than those in the late sensitized animals. The IgE response remained boostable for at least 1 year in dogs

that had their first sensitization early in life, while in the dogs sensitized later, IgE levels dropped to almost zero and were not boostable. For all allergens used, the differences between early and late sensitized dogs were highly significant. On the other hand, the late sensitized dogs developed allergen-specific IgG immediately after the first subcutaneous injection of allergen, while the IgG production was very poor and hardly boostable in the early sensitized dogs. The better ability of neonatal and young animals to produce IgE has already been described in the literature for rabbits [23], mice [24] and guinea pigs [25]. It has also been briefly mentioned that sensitization early in life fosters IgE production in dogs, but this point has not been systematically investigated before now [26, 27].

Dogs that had their first allergen contact early in life reacted to aerosolized OVA with severe bronchoconstriction. Some of the dogs did not start breathing until more

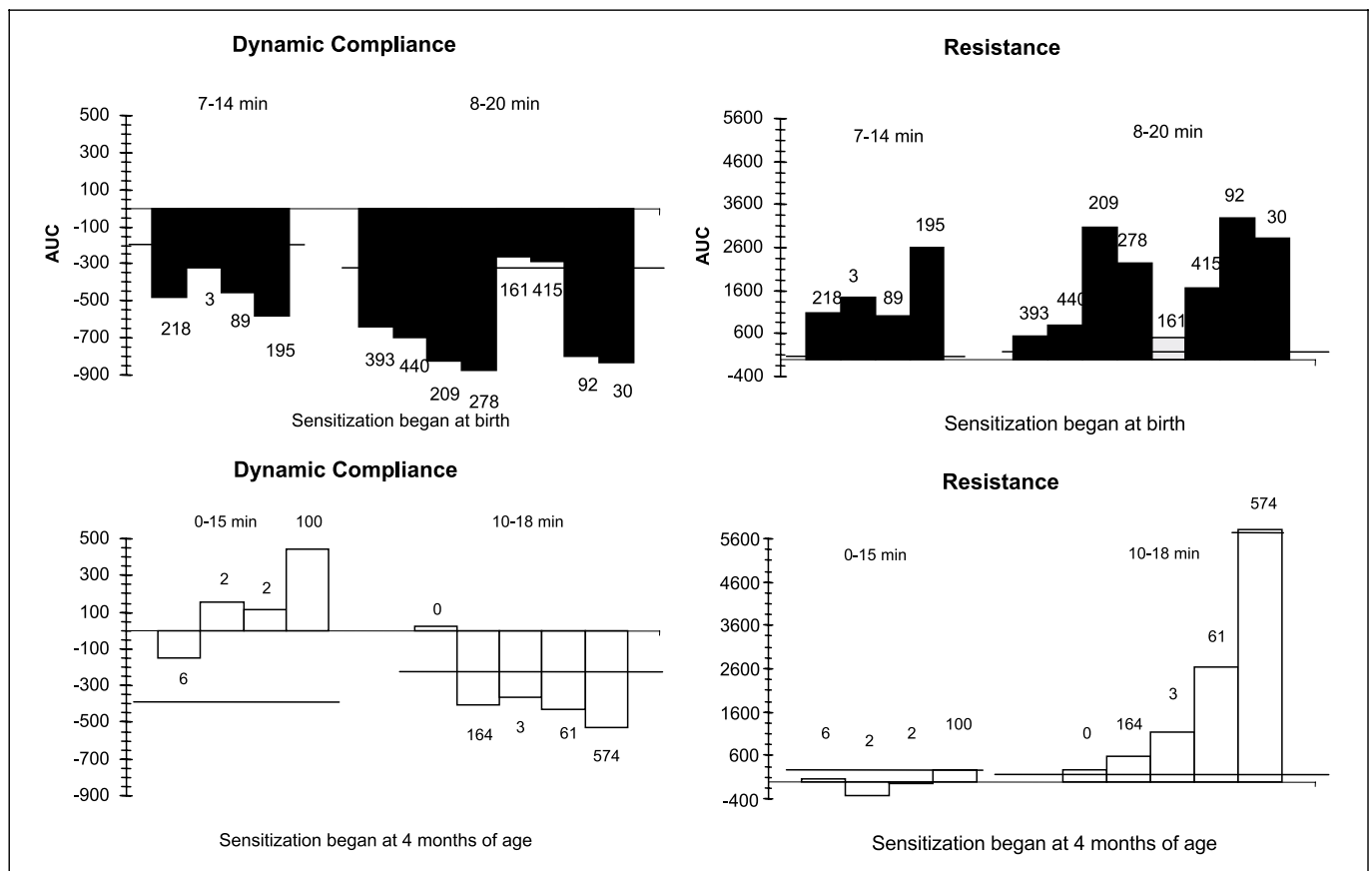


Fig. 7. Twelve early (black bars) and 9 late (white bars) sensitized dogs, each dog represented by one bar, were challenged with 15 breaths of aerosolized OVA (0.05% in PBS). Before challenge, a baseline for resistance, dynamic compliance and breathing frequency was set after 15 breaths of PBS alone. AUCs for the baseline deviation curves were calculated for dynamic compliance and resistance during

a certain period of observation (indicated in minutes above), depending on the severity of allergic response (time at which measurement of the pulmonary functions became possible following breathing assistance with oxygen). The numbers above or below the bars indicate OVA-specific serum IgE levels in arbitrary units (RU) on the day of challenge.

than 3–5 min after inhalation of aerosolized OVA. On the other hand, dogs that were first sensitized at the age of 4 months reacted moderately or poorly to the respiratory challenge with OVA. We conclude that airway hyperresponsiveness to OVA is clearly correlated with early sensitization.

These results lead us to speculate that the immature immune system (early allergen contact) of animals genetically predisposed to a high IgE response develops an IgE response after allergen contact which cannot easily be downregulated, whereas a more mature immune system (late allergen contact) develops both IgE and IgG, but IgE in that case becomes downregulated and therefore only transient. This fits with the T helper 1 (Th1)/T helper 2 (Th2) cell concept, in which an excess of one cell type will

suppress the other cell type by the production of a specific cytokine pattern. Alternatively, IgE-downregulating T regulator cells may develop in a mature immune system [28]. Several authors [1, 10, 12, 29–33] have reported the fact that in humans, the newborn's immune system is skewed in favor of Th2-driven responses and may remain skewed in adult atopic patients. An imbalance of interleukin (IL)-2 and interferon gamma, both prototype Th1 cytokines, versus IL-4 and IL-5, typical Th2 cytokines, in the newborn dog remains to be investigated.

The different response to Phl p 5 (used in the present study for sensitization later in life) after early or late sensitization to other allergens supports the hypothesis that early sensitization and sustained IgE response to at least one allergen is a high risk factor for the development of

multiple IgE-mediated allergies later in life. As a working hypothesis, we assume that the allergen which has sensitized the animal early after birth has an influence on the microenvironment by stimulating the release of IL-4 (and other mediators), which directs T cell differentiation after contact with a new allergen into the Th2 pathway.

Since the experiments described here with dogs from the same litters which were kept in the same surroundings and which received the same food show such clear differences in IgE responses depending on their sensitization protocol, it is clear that the timing of allergen exposure may be decisive for the future outcome of an individual with a high genetic risk of developing IgE-mediated allergy. The so-called 'hygiene hypothesis' assumes that the increase in IgE-mediated allergies is essentially due to the natural Th2-skewed response of newborns in the absence of environmental Th1-driving stimuli, e.g. bacterial infections [34–40]. In our case, however, the environmental and hygienic conditions of early and late immunized animals of the same litters were the same and the only difference was the time of first immunization. Therefore, the explanation for the failure of the late immunized animals to develop a sustained IgE response is probably that during maturation and in the absence of allergen contact, the immune system develops spontaneously from Th2-driven

to Th1 cell-driven responses. When an immature system is sensitized by allergen exposure, the balance of Th2/Th1 cells is permanently set in favor of Th2 cells, which will continuously inhibit Th1 cell differentiation even later in life, at least in early immunized genetically high IgE responder dogs. Whether the initiated IgE response in dogs, despite early immunization, can be turned off by external Th1-fostering stimuli later on will have to be further investigated.

In summary, we conclude that early allergen contact after birth leads to long-lasting IgE formation and allergen-specific airway hyperresponsiveness and is a high risk for expansion to additional IgE-mediated allergies later in life in dogs. Since these results are very similar to the experience of natural disease in infants, we believe we have established an animal model which imitates human disease and therefore allows the study of new intervention strategies for the treatment of allergic diseases.

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