

Allergic pulmonary and ocular tissue responses in the absence of serum IgE antibodies (IgE) in an allergic dog model

B. Zemann^{a,*}, M. Griot-Wenk^b, E. Marti^c, P. Mayer^a, C. Eder^a, M. Nefzger^a,
H. Schneider^b, A. de Weck^d, E. Lihl^a

^aNovartis Forschungsinstitut, Brunnerstrasse 59, 1235 Vienna, Austria

^bHealthEcon Ltd., Steinentorstrasse 19, 4051 Basle, Switzerland

^cInstitute of Animal Breeding, Bremgartenstrasse 109a, University of Berne, 3012 Berne, Switzerland

^d18 Rte de Beaumont, 1700 Fribourg, Switzerland

Abstract

Allergen-specific serum IgE may be insensitive as a marker for IgE-mediated reactions at the mucosal level. Five of six atopic beagle dogs developed high ovalbumin (OVA)-specific serum IgE levels after sensitization. This study aimed to show that these dogs still express allergen-specific IgE at the pulmonary and ocular mucosal levels and in the skin even when corresponding serum IgE was below the detection limit. When serum IgE levels were negative, all dogs exhibited allergic reactions at the tissue level. Specifically, they displayed positive ocular reactions after an ocular OVA challenge. After airway challenge with aerosolized OVA, five out of six animals reacted with decreased compliance and increased resistance of the lungs. Furthermore, an eosinophilia in the bronchoalveolar lavage fluid (BALF) was observed. Four weeks after the last exposure to OVA, IgE-positive BALF cells were seen in all animals. Six weeks on, all dogs still displayed positive skin reactions to OVA. This indicates that not only skin testing but also detection of ocular and pulmonary allergic tissue reactions including cell-bound IgE in BALF can serve as more sensitive and lasting surrogate markers of hypersensitivity in the allergic dog model than detection of allergen-specific serum IgE levels. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Allergies are chronic inflammatory conditions caused by aberrant immune reactions towards a variety of allergens. The most common clinical expression of allergies in humans includes allergic rhinitis, asthma, allergic conjunctivitis, atopic dermatitis and the less common life-threatening anaphylaxis.

Human therapeutic trials have often been based on findings derived from the commonly used mouse model. In contrast to rodents, dogs suffer from naturally occurring allergies that share features with the human diseases, in particular atopic dermatitis (Scott et al., 1995). Our colony of allergic dogs, whose high IgE response has been determined genetically and express features of atopic-like dermatitis, represent therefore a promising animal model to elucidate the pathophysiology as well as preventive and therapeutic strategies for allergies (de Weck, 1995). The objective of the study was to show that allergic dogs still express ovalbumin (OVA)-specific IgE at the pulmonary and

* Corresponding author. Tel.: +43-1-866-34317;
fax: +43-1-866-34582.
E-mail address: barbara.zemann@pharma.novartis.com
(B. Zemann).

ocular mucosal levels and in the skin when the corresponding serum IgE is below the detection limit.

2. Materials and methods

2.1. Sensitization of the animals

Six newborn genetically IgE high-responder beagle dogs from four litters were included in the experiment. Four times every 2 weeks the animals were sensitized by s.c. injection (1 µg/allergen/dog) to r-major birch pollen allergen (*Betula verrucosa*, Bet v1, Biomay, Linz, Austria) and r-timothy grass pollen (*Phleum pratense*, PP5; Biomay), starting on the day they were born (Fig. 1). At the age of 13 weeks, the sensitization schedule was repeated and immunization with OVA (10 µg/injection, Sigma Chemical) was included.

2.2. Parameters for evaluating allergic responses

The following parameters were applied to assess the allergic immune responses: differential bronchoalveolar lavage fluid (BALF) cell counts (week 27), humoral OVA-specific immune response in serum (week 42), pulmonary provocation tests (week 42), ocular challenge (week 42), IgE-specific immunostaining of BALF cells (week 50) and skin testing (week 56) (Fig. 1).

2.2.1. BALF cell counts

A pre-challenge BAL was carried out 1 week prior to allergen exposure to prevent nonspecific neutrophilia. Using a flexible fiber-optic endoscope (Olympus, Hamburg, Germany), 4 ml of 0.9% kg⁻¹ saline solution was administered and aspirated immediately.

The procedure was repeated 24 h after exposure to aerosolized OVA (15 breaths of 0.05% OVA/dog). Cytospin preparations of BALF were stained with May–Grünwald–Giemsa for differential cell counts.

2.2.2. Humoral immune response

Serum was collected prior to and 7 days after the airway challenge. The serum samples were subjected to capture ELISA for OVA-specific IgE and IgG levels. Results were expressed in relative ELISA units (REU).

2.2.3. Pulmonary provocation tests

Three parameters were used to confirm airway hyper-responsiveness: severe bronchoconstriction (defined as respiratory paralysis, arbitrarily assigned a percentage deviation from baseline of –100%), increase in lung resistance (change in pressure over change in flow) and decrease in dynamic compliance (change in volume over change in pressure). An endotracheal tube was inserted and connected to a Mark 7 Respirator (Bird Products, Palm Springs, CA, USA). After 15 breaths of aerosolized PBS, the baseline was set to compare the parameters before and after allergen challenge (15 breaths of aerosolized 0.05% OVA dissolved in PBS). Changes in lung function were recorded via software BioSystem XA, Version 1.5.7 f1 (Buxco Electronics, Troy, NY, USA).

2.2.4. Ocular challenge

The conjunctival provocation test was performed by dropping 100 µl OVA (1.0%) dissolved in Oxysept[®], a buffered 0.9% saline solution, into the left eye. The right eye served as a control and was only treated with diluent. Clinical symptoms (conjunctivitis, scleral

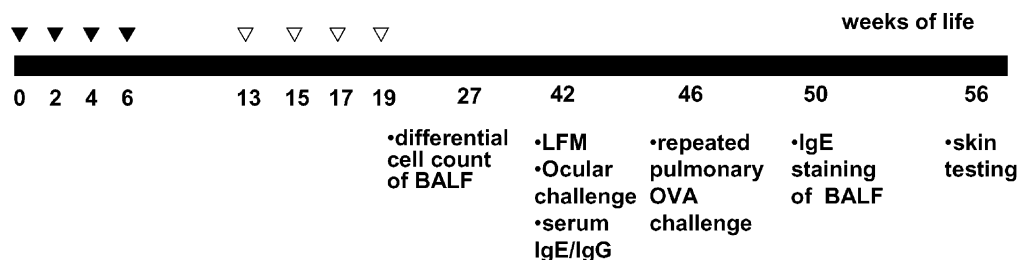


Fig. 1. Experimental set up. Newborn beagle dogs were sensitized to Bet v1 and PP5 (weeks 0, 2, 4, 6; closed arrows) and thereafter at the age of 13 weeks with the addition of OVA (weeks 13, 15, 17, 19, open arrows; BALF = bronchoalveolar lavage fluid).

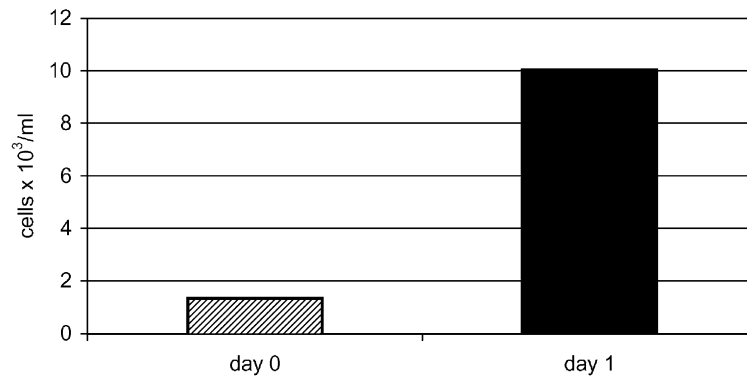
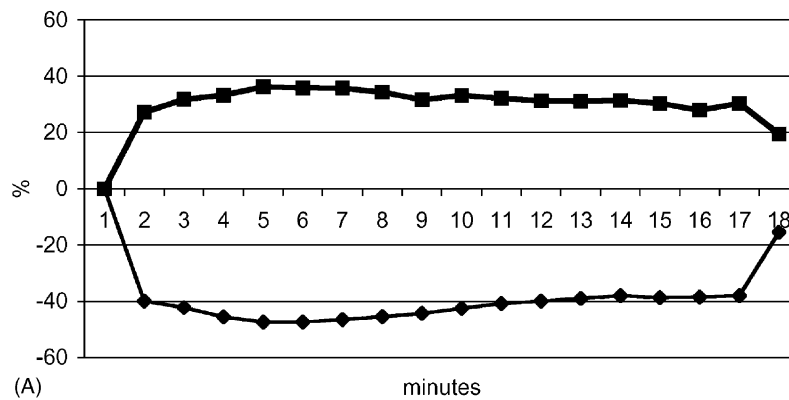
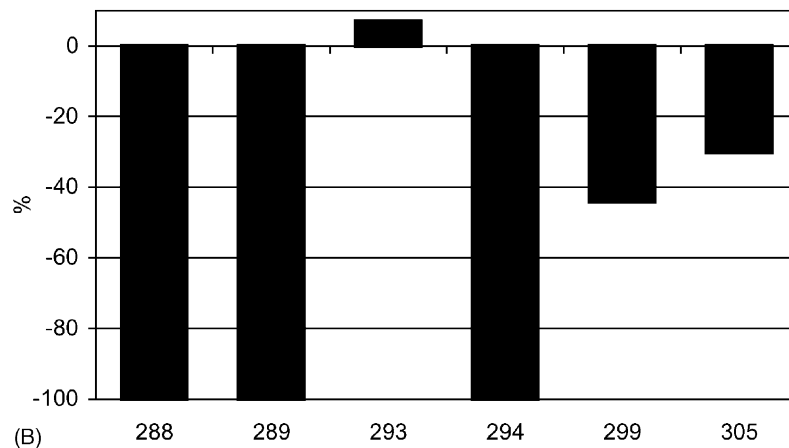


Fig. 2. Median number of eosinophils ($\times 10^3/\text{ml}$) in the BALF is shown before (hatched bar) and 24 h (black bar) after OVA challenge.



(A)



(B)

Fig. 3. Airway responsiveness in terms of pulmonary resistance and dynamic compliance is illustrated after OVA-challenge. (A) Representative example of one dog's (299) percentage change in airway reactivity over the observation time of 18 min (squares indicate airway resistance and diamonds indicate lung dynamic compliance). (B) The median percentage deviation in lung dynamic compliance compared to baseline is illustrated for all dogs. The numbers at the *x*-axis represent the dog's identification number. Severe bronchoconstriction occurred in three dogs (288, 289, 294) that was arbitrarily assigned a value of -100% .

injection, lid edema, and tearing) were evaluated 20 min after the challenge and scored from 0 to 4.

2.2.5. IgE staining of BALF cells

Ethanol (100%)-fixed cytospin preparations from BALF cells were incubated with dog IgE-specific mAb (CMG-Heska, Fribourg, Switzerland) followed by alkaline phosphatase-conjugated goat anti-mouse IgG (H + L) Ab (Jackson Immuno Research Lab., West Grove, PA, USA). Binding of Abs was detected by applying Naphtol AS-Mx Phosphat (1 mg/ml, Sigma Chemical) and Fast Red TR salt (6 mg/ml, Chroma-GesmbH, Münster, Germany).

2.2.6. Skin testing

Skin testing was carried out via i.d. injection of OVA at a concentration of 100 µg OVA/ml (dissolved in 0.9% saline solution). Histamine dihydrochloride (8 µg/50 ml, Allergopharma J. Ganzer KG, Reinbek b. Hamburg, Germany) served as positive control and the diluent as negative control. For better visibility of

the skin reactions, the dogs were pretreated with Coomassie[®] Brilliant Blue R 250 pure (Boehringer Ingelheim, Heidelberg, Germany), applied i.v. (1.0% in 0.5 ml kg⁻¹ saline solution). All in vivo testing was performed under general anesthesia (i.v. medetomidine (40 µg kg⁻¹) and ketamine hydrochloride (5 mg kg⁻¹)).

3. Results

All six dogs developed increased levels of eosinophils (Fig. 2) and neutrophils in the BALF 24 h after OVA exposure. Despite non-detectable OVA-specific serum IgE levels, 15 weeks after the last allergen contact, five out of six dogs showed a severe increase in airway resistance and a decrease in the lung dynamic compliance after the OVA challenge (Fig. 3A and B). Three out of six dogs even displayed a severe bronchoconstriction, resulting in a respiratory paralysis. Elevated OVA-specific serum IgE levels,

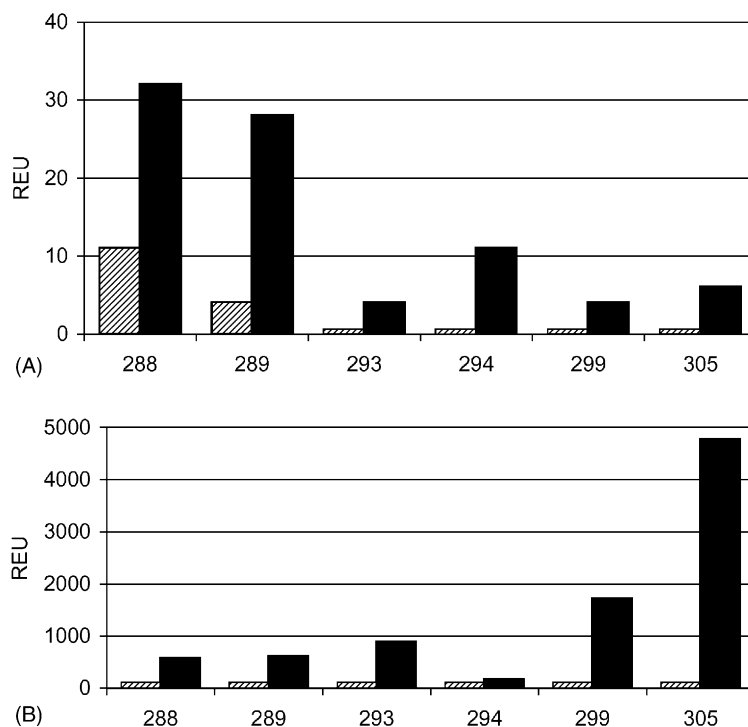


Fig. 4. OVA-specific serum IgE (A) and IgG (B) levels are illustrated for six dogs before (hatched bars) and after (black bars) pulmonary challenge with aerosolized OVA. The results are expressed in relative ELISA units (REU). The numbers at the x-axis represent the dog's identification number.

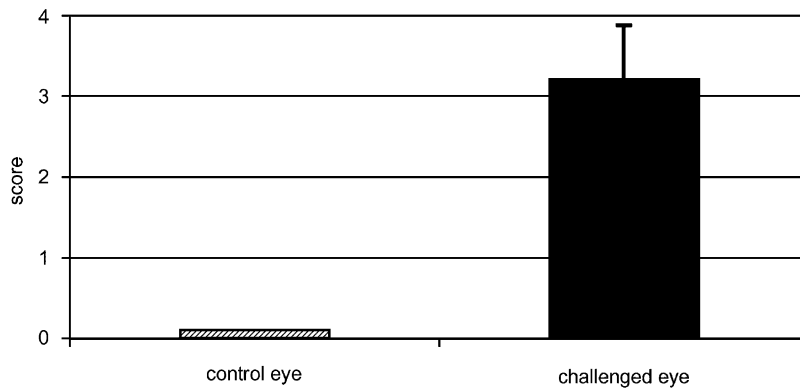


Fig. 5. Ocular reaction scores are illustrated after local OVA challenge in one eye in comparison to the control eye of each dog. The bars indicate mean scores of all six dogs plus 1 S.D. The ocular reaction was scored from no (0) to severe reaction (4).

measured 7 days after a pulmonary challenge with aerosolized OVA, demonstrated a systemic OVA-specific allergic response (Fig. 4A). In addition, these dogs exhibited increased OVA-specific serum IgG levels (Fig. 4B). The results obtained from the conjunctival provocation highlighted a significant clinical reaction of the eye (Fig. 5). IgE-positive BALF cells were present in all dogs. Also skin testing proved to be more sensitive than measurement of OVA-specific serum IgE. Ten weeks after the last allergen exposure, all dogs still showed a positive skin reaction; serum allergen-specific IgE levels decrease within 4–6 weeks after a challenge.

4. Discussion and conclusion

Skin testing is today the most common form of allergen provocation used in companion animals. Correlation between skin test thresholds and serum IgE levels has already been demonstrated in work carried out by Mueller et al. (1999). The results obtained from our experimental allergy studies within a defined allergic dog colony with limited natural exposure to allergens highlight that serum IgE may be negative, despite clearly positive allergic test results at the tissue levels, i.e., skin testing or ocular and pulmonary mucosae. It also reflects a longer half-life of tissue-bound IgE compared to serum IgE. Hence, allergen provocation at various sites beside the skin may be used for experimental studies, but in

turn could also be applied as a diagnostic tool in clinical patients.

Our former experiments have shown that OVA-specific serum IgE levels reach their maximum 2–3 weeks after the allergen challenge and usually decline below the detection limit within 3–6 weeks. Our experiments indicate that even months after the last treatment, the detection of mucosal allergic reactions, such as that at the pulmonary and ocular levels including cell-bound IgE in BALF, can serve as more sensitive and lasting surrogate markers of hypersensitivity than allergen-specific serum IgE levels. These findings differ from field studies with natural atopic patients with presumably continuous natural allergen(s) exposure where the serum levels of IgE and i.d. testing mostly correlate (Mueller et al., 1999). In contrast, a correlation can only be expected in our dog model shortly after allergen exposure.

Airway allergen provocation with subsequent measurements of airway reactivity could be life threatening for allergic patients. In contrast, specific conjunctival allergen challenges (including the evaluation of inflammatory cells in tear fluid) is a rather safe and easy test with a good precision (Moller et al., 1984). It has therefore become increasingly popular as a diagnostic tool in human medicine. This is the first description of this application in dogs. The ease of the procedure and safety issues makes this ocular allergy test also a potential candidate for more widespread diagnostic use in clinical canine patients.

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