

Short communication

Effect on humoral tolerance (IgG and IgE) in dogs
by oral administration of ovalbumin and *Der p I*

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Abstract

The effect of oral administration of ovalbumin (OVA) or recombinant house dust mite allergen (*Der p I*) to dogs upon specific IgG and IgE reactions to subcutaneous immunization with these antigens was studied. Daily feeding of 10×10 g of OVA resulted in a non-responsiveness to subsequent parenteral immunization with OVA in two young dogs. The same two dogs were also immunized parenterally with *Der p I* and showed a pronounced IgG response against native *Der p I*, confirming that the non-responsiveness to OVA was antigen-specific. Thus, it has been demonstrated that it is possible to induce oral tolerance in dogs. Two other dogs of the same litter that received 2×10 mg of recombinant *Der p I* in a crude yeast lysate per os reacted to immunization with OVA with pronounced IgG and IgE production against OVA, further confirming the antigen-specificity of the OVA tolerance. However, tolerance to *Der p I* was not induced, as evidenced by a strong IgG response to immunization after per os application of the antigen, possibly because the oral dose was too small.

Abbreviations

ELISA, Enzyme-linked immunosorbent assay; OVA, ovalbumin.

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1. Introduction

Depressed immune response to a parenteral challenge with antigen after a period of oral administration with the same antigen has been demonstrated experimentally in laboratory animals (reviewed by Tomasi, 1980; Kern and Silbart, 1992). It has been proposed that the phenomenon of oral tolerance is the underlying mechanism involved in this discriminatory process (Mowat, 1987). Few data are available concerning how the systemic humoral response of dogs is affected by an initial intestinal contact with an antigen. The existence of oral tolerance mechanisms in dogs may provide effective and practical means of avoiding the development of sensitization to allergens, particularly to those derived from arthropods such as flea or house dust mite. However, the subversion of the tolerance mechanism by intestinal helminths or protozoa may be an explanation for the large number of dogs and foxes with no specific serum IgG response against intestinal infections with *Echinococcus* spp. (Jenkins et al., 1990; Gottstein et al., 1991).

The aim of this preliminary study was to compare the specific IgG and IgE response of dogs to parenteral immunization after prior feeding of an orally administered antigen, ovalbumin (OVA), and a major allergen of house dust mite *Dermatophagoides pteronyssinus* feces (*Der p I*) (Chua et al., 1988), respectively.

2. Materials and methods

2.1. Animals and experimental design

Four 12-week-old Australian cattle dogs from the same litter were vaccinated with Convac® (Commonwealth Serum Laboratories, Melbourne, Vic., Australia) and treated with Drontal® (Allwormer for Dogs, Bayer, Australia). The animals were fed exclusively with Meaty-Bites® and Chum® with lamb dog food supplied by Uncle Ben's of Australia, Ragland, N.S.W.

At the age of 13 weeks, dogs 109 (male) and 118 (female) were fed (Fig. 1(A)) daily for 10 days with 10 g egg albumin (Fluka, Buchs, Switzerland; diluted in water and mixed with dog food). Dogs 119 (male) and 178 (female) were fed (Fig. 1(B)) at the beginning and at the end of their 13th week approximately 10 mg recombinant *Der p I* in crude yeast lysate produced as described by Chua et al. (1992) (a gift from Dr. W.R. Thomas, Clinical Immunology Research Unit, Princess Margaret Hospital, Subiaco, W.A., Australia) in gelatin capsules (Parke-Davis Ltd., Caringbah, N.S.W., Australia). All four dogs were immunized subcutaneously at their 16th week (10 and 14 days after the end of OVA and *Der p I* feeding, respectively) with 1 mg ovalbumin (5×crystalline, Calbiochem®, San Diego, CA, USA) and with 400 µg purified native *Der p I* (purified as described by Greene et al. (1990); a gift from Dr. W. K. Thomas) mixed 1:1 with peanut oil as described by Haines and Penhale (1985). Blood samples were collected

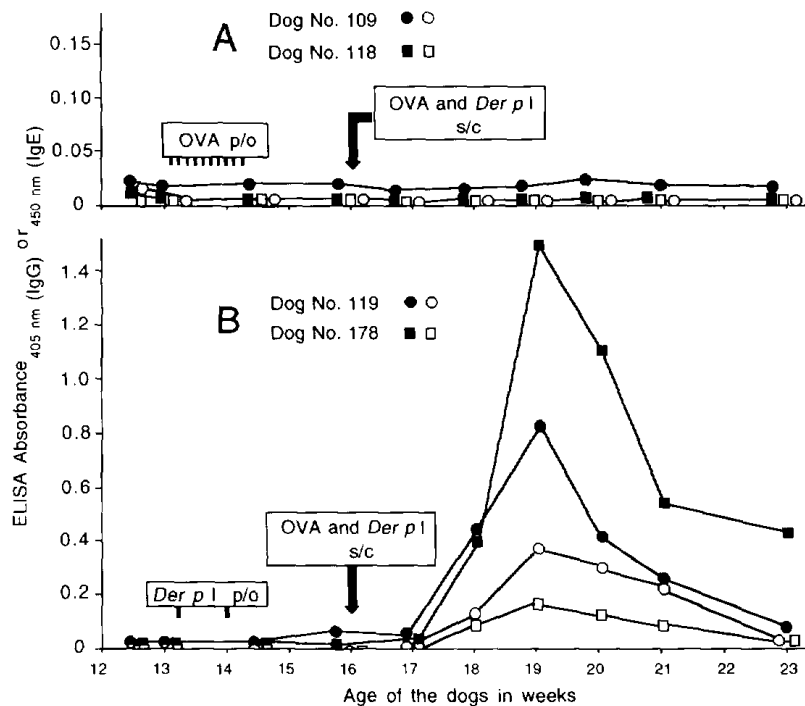


Fig. 1. Specific serum antibody responses of dogs to ovalbumin (OVA) (IgG, closed symbols; IgE, open symbols). Dogs were administered 10×10 g ovalbumin (A) or 2×10 mg recombinant *Der p I* in a yeast lysate (B) and subsequently immunized parenterally with 1 mg ovalbumin and $400 \mu\text{g}$ native purified *Der p I*, mixed 1:1 with peanut oil.

weekly for the whole experiment and frozen at -20°C until assayed for specific antibodies by enzyme linked immunosorbent assay (ELISA).

2.2. ELISA

Native purified *Der p I* ($4 \mu\text{g ml}^{-1}$) and ovalbumin ($50 \mu\text{g ml}^{-1}$) ($5 \times$ crystalline, Calbiochem) in 50 mM bicarbonate-carbonate buffer (pH 9.6) were used to coat 96-well microtiter plates (Nunc, Roskilde, Denmark) overnight at 4°C . Dog sera were diluted 1:200 for IgG and 1:40 for IgE detection in phosphate-buffered saline (pH 7.2) containing 0.2% v/v Tween 20 and 5% fetal calf serum (PBS-T-FCS). After addition of sera, the ELISA plates were incubated overnight at 4°C for IgG and for 4 h at room temperature for IgE detection. After these incubations, the plates were washed free of sera and goat anti-dog IgG(γ) conjugated to alkaline phosphatase (Kirkegaard and Perry Laboratories, Inc., MD, USA; diluted 1:200 in PBS-T-FCS) or rabbit anti-canine IgE conjugated to horseradish peroxidase (Calbiochem; diluted 1:50 in PBS-T-FCS) was added and incubated for 3 h at 37°C or 16 h at 4°C in a humidified chamber, respectively. The further steps of the ELISA were performed as previously described (Deplazes et al., 1994).

3. Results

3.1. Immune response to ovalbumin

Before parenteral immunization with ovalbumin and *Der p* I, including the period immediately after dogs were administered the antigens per os, only background reactions of OVA specific IgG and IgE antibody were found in all dogs (Fig. 1). After subcutaneous immunization of all four dogs with OVA and purified native *Der p* I, dogs which received OVA per os (Nos. 109 and 118) showed no increase in specific IgG or IgE levels against OVA (Fig. 1(A)). However, the two dogs which were administered *Der p* I per os but not OVA (Nos. 119 and 178) reacted with a pronounced specific IgG and IgE response against OVA in ELISA 2 weeks after the subcutaneous immunization with OVA and *Der p* I. Maximal absorbance values against OVA were found in these two dogs 3 weeks after parenteral immunization (Fig. 1(B)).

3.2. Immune response to *Der p* I

Before parenteral immunization with ovalbumin and *Der p* I, including the period immediately after dogs were administered the antigens per os, only background reactions of *Der p* I specific IgG and IgE antibody were found in all dogs (Fig. 2). Three weeks after immunization of dogs 109 and 118, increased levels

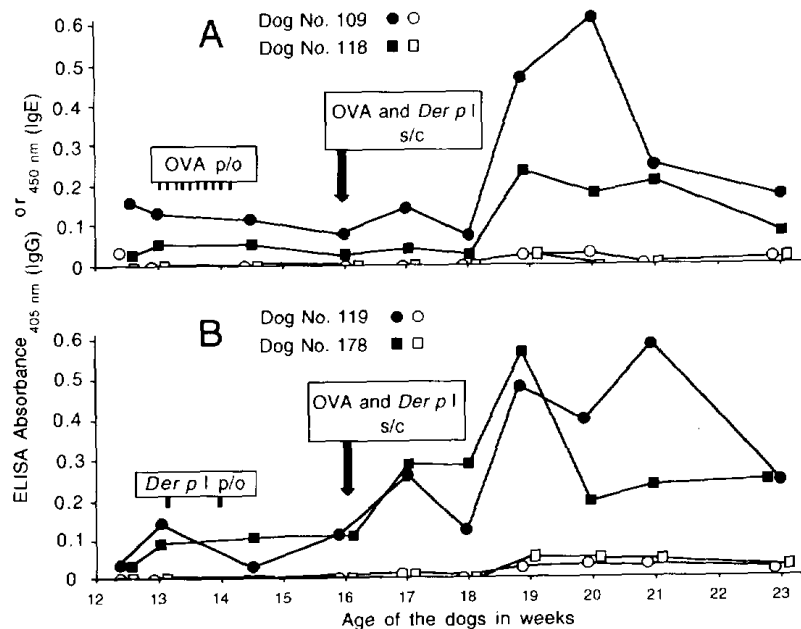


Fig. 2. Specific serum antibody responses of dogs to purified native *Der p* I (IgG, closed symbols; IgE, open symbols). Dogs were administered 10×10 g ovalbumin (A) or 2×10 mg recombinant *Der p* I in a yeast lysate (B) and subsequently immunized parenterally with 1 mg ovalbumin and 400 µg native purified *Der p* I, mixed 1:1 with peanut oil.

of specific serum IgG to *Der p* I were detected in both dogs (Fig. 2(A)). In contrast, the two dogs that received two oral *Der p* I administrations (Nos. 119 and 178) showed increased specific IgG levels against *Der p* I one week after parenteral immunization though the peak was 3 weeks after immunization (Fig. 2(B)). The IgE response of all four dogs was very low, as determined by ELISA against purified native *Der p* I antigen.

4. Discussion

The concept of oral tolerance has been previously demonstrated by Miyamoto et al. (1980) who induced a pronounced tolerance, as measured by specific IgE and IgG1 responses, against parenteral immunization with crude dust mite extracts by feeding mice every 2 days for 8 days with 10 mg of crude extract. At this stage it is not known to what degree these results can be extrapolated to non-laboratory animal models such as dogs. A major constraint on tolerance experiments with dogs is the availability of animals which have been kept under comparable feeding conditions for their entire lives. Therefore, for our experiment, we used four Australian cattle dogs from the same litter which were raised together. Because of the relatively small number of animals available for the experiment careful consideration had to be given to the experimental design. Thus, we opted to use very large amounts of antigen in attempts to induce tolerance in dogs to subsequent parenteral immunization 10–14 days after the final oral administration of antigen. We also tested two distinct antigens; ovalbumin (OVA) because of its known ability to induce oral tolerance in mice (Tomasi, 1980) and an allergen of house dust mite (*Dermatophagoides pteronyssinus*) feces (recombinant *Der p* I). Hence, two dogs were treated with OVA and two dogs were orally treated with *Der p* I, but all four dogs were subsequently parenterally immunized with both OVA and *Der p* I so that each experimental group represented the control for the other.

The results clearly demonstrate that suppression of specific IgG and IgE production is possible by pre-feeding large quantities of OVA as antigen. Thus, daily oral administration of 10×10 g of OVA appears to have produced a major suppressive effect on the subsequent response against OVA after parenteral immunization with OVA and *Der p* I in two young dogs (Nos. 109 and 118, Fig. 1(A)). The same two dogs, however, showed a pronounced IgG response against native *Der p* I (Fig. 2(A)), confirming that the non-responsiveness to OVA was antigen-specific. Furthermore, two other dogs of the same litter that received 2×10 mg of recombinant *Der p* I in a crude yeast extract per os reacted to immunization with OVA and *Der p* I with pronounced IgG and IgE production against OVA (Fig. 1(B)) also suggesting that the non-responsiveness to OVA by dogs 109 and 118 was antigen-specific.

Tolerance to *Der p* I was not induced in the two dogs that received 2×10 mg recombinant *Der p* I in a crude yeast lysate per os (Nos. 119 and 178). Both of these dogs reacted to immunization with OVA and native *Der p* I, showing in-

creased specific IgG levels against purified *Der p* I as early as 7 days after parenteral immunization (Fig. 2(B)). In contrast, the two dogs administered OVA per os displayed a specific IgG reaction to *Der p* I at the earliest 21 days after immunization. This suggests that oral administration of recombinant *Der p* I had a priming effect. The failure of oral administration of *Der p* I to induce tolerance to subsequent parenteral administration may be due to the doses used. Mowat (1987) reported that low per os doses of OVA enhanced the immune response to subsequent immunization whereas high doses induced tolerance. Similar observations were made by Miyamoto et al. (1980) using dust mite extracts. Thus, it is conceivable that the dose of *Der p* I used in our study may have been too low to induce a humoral IgG tolerance to *Der p* I in dogs. Unfortunately, the production of greater quantities of the antigen for in vivo experiments like those described here is quite difficult. Thus, a technically realistic maximal amount of recombinant *Der p* I (approximately 20 mg per dog) from the sediment of a 2500 ml culture was administered twice. Hence, experiments using different doses and administration regimes were not tested.

In conclusion, this preliminary study has established that oral tolerance can be induced in dogs. Larger scale investigations are now required to determine the influence of various parameters (for example, age of animals, mode of antigen administration, nature and concentration of the antigen and the duration of induced tolerance) on induced tolerance before the potential of feeding antigens as a prophylactic measure in the management of allergic diseases in dogs can be considered. Furthermore, studies using allergens with potential practical application such as flea allergens, the major triggers of allergic dermatitis in most dog populations (Halliwell et al., 1987), should be undertaken. Controllable induction of humoral tolerance, especially for IgE (Greene et al., 1993), could be very useful in this situation.

Acknowledgments

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