Original Article

Direct and prolonged exposure to dogs does not influence the degree of skin prick test positivity to dog allergen

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Abstract.
Background. The relationship between pet ownership and the risk of developing allergic sensitization to pet allergens is still controversial. We assessed the possible effect of direct exposure to dog allergen on skin reactivity in dog-sensitized patients. *Methods*. We studied, in a case-control trial, 116 adults sensitized to dog allergens (55 with a dog at home for at least 10 years and 61 without it). The degree of response was assessed by skin prick test, performed in quadruplicate with three concentrations of allergenic extract: A (1:20 w/v), B (1:200 w/v) and C (1:2000 w/v). The mean diameter of each wheal was assessed using a visilog image analysis software.
Results. No significant difference between the two groups in the wheal diameters induced by the three concentrations of dog allergen could be demonstrated.
Conclusion. The results of this study suggest that direct dog exposure in adults with respiratory allergy is not associated with greater cutaneous response to dog allergens, as compared to non exposed subjects.

Key words: Allergic sensitization, dog allergen, exposure, respiratory allergy, skin prick test.

Introduction

Several studies have consistently shown that a dose-response relationship exists between the degree of exposure to house dust mite allergens and the development of specific sensitization [1]. Other studies have also shown that inner city children are more likely to become allergic to indoor allergens (e.g. cockroach and mouse), when they are exposed to increased amounts of allergenic materials. On the contrary, studies conducted in rural communities have indicated a lack of association between an early and close contact of children with farm animals and development of respiratory allergy [2].

Also in the case of cat/dog allergens, the relationship between the degree of exposure in domestic environments and the risk of allergic sensitization is not clear. In fact, some trials have suggested a direct relationship between exposure and prevalence of sensitization to pets [3, 4]. In the last few years there was consistent evidence that the relationship between exposure to cat/dog allergens in domestic environments and the risk of allergic sensitization to cat/dog is not

linear [5, 6]. On the other hand, it was shown that in adults, the prevalence of sensitization to cat was lower when patients were exposed to very low or very high amounts of cat allergen [7]. Moreover, in adults, cat ownership can be associated with a lower prevalence of sensitization to both cat and dog [8].

The controversial aspects of the issue might be due, at least in part, to methodological problems, such as the differences in study designs, the modality of assessment of exposure, the considered outcomes, and the inclusion criteria [9-11]. Skin prick tests (SPT), due to their reliability and reproducibility, are widely used as markers of allergic sensitization in both clinical practice and epidemiological studies. However, in the large epidemiological studies the specific sensitization to pet allergens is usually assessed by standard skin prick tests, where a wheal diameter ≥ 3 mm is considered "positive" [12] and/or by specific IgE assay [13,14]. To our knowledge, there are presently no data about the degree of skin response to dog allergenic extract and its relationship with actual exposure. It can be reasonably expected that patients directly exposed to dog exhibit stronger skin responses to dog allergen. To test this hypothesis, we quantified the skin reactivity to dog allergens in two groups of dog-sensitized patients: one who owned and had owned the animal for at least 10 years (current and direct exposure) and the other who had never owned a dog. The measurement was carried out using an efficient and sensitive SPT method.

Materials and methods

Patients

Adult patients with immediate positive skin reaction to dog allergens were enrolled for this study. They were selected from a population of subjects living in the Naples area and consecutively evaluated for the first time at our Allergy Service between September 2001 and September 2002.

Inclusion criteria were: age between 15 and 55 years, clinical history of respiratory allergy in the last two years, skin reaction to positive control (histamine) and to the commercial dog extract > 4 mm. Those patients with a cat at home and those with severe skin diseases, malignancies or immunological disorders were excluded. Among patients with positive dog skin test we further selected: a) those who kept a dog at home for at least 10 years (i.e. direct and prolonged exposure), and b) those who had never owned a dog, had never worked with/near dogs and never stayed for working/personal reasons with dog owners (not directly exposed). Quantitative skin prick tests with dog extracts at various dilutions were performed in the selected patients. The classification of nasal and bronchial symptoms was performed according to International Guidelines. Thirty-five subjects sensitized to allergens other than dog and 20 non-atopic individuals served respectively as atopic and negative controls. All patients gave their informed consent before being submitted to quantitative skin prick tests (SPTs).

Skin prick tests

Routine SPTs for patients' screening were performed by the same operators and evaluated according to EAACI Position Statement [15]. Quantitative SPTs for assessing the degree of immediate hypersensitivity to dog allergen were carried out, in quadruplicate, using three concentrations of allergenic extract: A (1:20 m/v), B (1:200 m/v) and C (1:2000 m/v). A sterile plastic device (Stallerpoint, Stallergenes SA, Antony Cedex, France) was used for pricking and the results of the tests were read after 20 minutes. The contours of the wheals were outlined with a fine-tip rolling black pen and transferred by means of adhesive cellulose tape to record sheets. The diameters of the induced wheal were calculated through a Visilog image analysis software (Noesis, France). The geometric mean of the 4 wheals for each concentration was then calculated for each patient.

Allergen extracts

The commercial allergenic extracts used for screening SPTs were kindly provided by ALK Abellò Group (Lainate, Milan, Italy). The routine panel of allergens used at the screening included: *Dermatophagoides pteronyssinus, Alternaria alternata,* cat and dog hair, *Parietaria judaica*, Grass mix, *Artemisia vulgaris*, olive, birch, cypress and hazelnut. These allergens are the most common causative agents of respiratory allergy in our geographic area. The allergenic extracts for quantitative SPT were produced by Stallergenes SA (Antony Cedex, France). Briefly, dog dander (Greer, Lenoir, USA) were extracted at 1/20 (m/v) ratio in 4 g/L ammonium bicarbonate buffer for 24 hours at 4ºC with stirring. The extract was then centrifuged and the supernatant underwent clarifying filtration and ultra-filtration through a 0.22 µm membrane (Millipore). The extract obtained was stored as 1-ml aliquots and freeze-dried. The three solutions were prepared by reconstituting and properly diluting the freeze-dried extract with a 50% glycerol-saline diluent.

Prevalence of dog ownership in Naples area

The prevalence of dog ownership in the geographical area the patients belonged to was calculated by telephone interviews on a random sample of 2601 families, by simply asking whether they had or not a dog at home.

Statistical analysis

The geometric mean of the diameters of the wheal obtained with each of the three extracts was compared between the two populations using the Mann-Whitney's U test on SPSS 11.0 software.

Results

One hundred and sixteen subjects (50 male, mean age 27.8 years) were found to be sensitized to dog and other allergens: 55 out of them had owned a dog for at least 10 years (direct prolonged exposure), and 61 had never owned or had significant contacts with the animals (no direct exposure). None of them had previously received immunotherapy, since this was their first allergy diagnosis. The demographic and clinical characteristics of the patients are shown in Table 1, whereas Table 2 summarizes the results of the SPTs. The diameter of each wheal (expressed in mm) induced by solution A, B and C represents, for each patient, the mean value of the four wheal diameters.

There was no significant difference (Mann-Whitney test $p > 0.05$) in the wheal diameters induced by the three solutions between the two groups of patients. The three solutions gave invariably negative results in the groups of atopic patients who were not sensitized to dog allergens and in the negative controls. Interestingly, all dog-positive patients were also sensitized to other common aeroallergens (Table 2), and no subject displayed a single sensitization to dog.

Table 1. Characteristics of the patients

	Dog sensitized patients		**Atopic controls**	**Negative controls**
	Direct exposure	**No direct exposure**		
Nº patients	55	61	35	20
Age (years)	15-55	15-55	15-55	15-55
Mean age	27,82	26.7	22,6	35,7
Sex (M/F)	21/34	30/31	21/14	5/55
Dog ownership (yes/no)	55	61	16/19	2/18
Symptoms				
Rhinitis	55	61	35	0
Asthma	40	40	22	0
Conjunctivitis	25	25	18	0
SPTs positivity				
Dermatophagoides pt.	35	47	18	n.a
Parietaria judaica	36	44	21	n.a
Grasses	34	34	8	n.a
Artemisia v	14	9	7	n.a
Olea europaea	17	12	9	n.a
Alternaria a	4	10	2	n.a
Dog hair	55	61	–	n.a
Cat hair	35	47	–	n.a
Betula pendula	4	3	2	n.a
Corylus avellana	1	1	–	n.a
Cupressus sempervirens	1	1	–	n.a

n.a.: not applicable.

Table 2. Results of SPTs with three concentrations of dog allergenic extract

	NO DIRECT EXPOSURE TO DOG					DIRECT EXPOSURE TO DOG			
	Mean wheal diameter (mm)					Mean wheal diameter (mm)			
	H	A	B	C		H	A	B	C
Dog Sensitized Patients (n=61)	5.55 ±0.3	4.94 ±0.2	3.48 ±0.2	2.54 ±0.3	Dog Sensitized Patients (n=55)	5.22 ±0.2	4.63 ±0.4	3.29 ±0.2	2.47 ±0.2
Atopic Controls (n=19)	5.35 ±0.4	–	–	–	Atopic Controls (n=16)	5.39 ±0.3	–	–	–

H = Histamine HCL solution 10 mg/ml, A = Dilution 1: 20 m/v, B = Dilution 1:200 m/v, C = Dilution 1:2000 m/v.

Although no patient owned a cat, 80 (69%) out of the dog-sensitive subjects showed a SPT positivity also to cat. The prevalence of dog ownership in the Naples area was found to be 7.8%.

Discussion

The results of the studies that investigated the relationship between exposure to pet allergens and allergic sensitizations are conflicting. All epidemiological studies have evaluated the presence of sensitization to cat/dog allergens using only standard SPTs and/or RAST assay in large populations exposed or not to these animals. SPT positivity to natural allergen extracts represents the hallmark of immediate hypersensitivity and plays a key role in allergy diagnosis. However standard SPTs performed by a single conventional extract, in some circumstances may produce false positive or negative responses depending on different factors influencing skin test reactivity. The use of multiple concentrations of an allergenic extract, as performed in allergen standardization procedures, represents a more reliable method to assess the degree of immediate hypersensitivity.

The results of this study showed that a direct and continuous exposure to dog allergen is not associated with a higher degree of specific skin sensitivity to dog allergenic extracts. The use, in quadruplicate, of three different concentrations of allergenic material greatly reduced the risk of false positive or negative responses and represented a more reliable index of the cutaneous events associated with allergic sensitization. Our data provide therefore indirect evidence that the low amounts of dog allergen inhaled (probably as a consequence of occasional exposure) are sufficient to determine an immediate cutaneous hypersensitivity response.

It has been suggested that in communities with a high number of pets, passive exposure may be the primary cause of allergic diseases related to animals [16]. This is not the case in our study, since the prevalence of dog ownership was only 7.8% and it is likely that, in our community, the amount of allergen is relatively low in environments without dogs. This could be different with cats, since their allergens are ubiquitous, they can be present in environments where pets have never been kept [17, 18], and can be carried by clothing [19].

A gold standard measure of the exposure to pets has not been established [9]. In the available trials exposure was measured either by questionnaires or by measuring pet allergens in collected dust [14]. However, categorizing the degree of exposure by using questionnaires is difficult to quantify [9] and controversies still exist on the modality of collecting dust. For these reasons we have chosen to simply consider the presence of dogs at home for a long time as a reliable index of heavy direct exposure. Similarly, it is not possible to find a population of patients with "zero exposure" to dogs, since these animals are present in everyday life. Nevertheless, the clinical history (never had dogs at home, not staying at home with persons owning dogs, not working with dogs) reasonably confirmed for our "not directly exposed subjects", that the contact with dog allergens was similar to that of the general population. In addition, well-conducted studies have shown higher levels of allergens in houses containing domestic animals versus houses without pets [20]. Since all our dog-positive subjects showed also a SPTs positivity to other important aeroallergens, it was not possible to quantify the role of dog sensitization on patients' symptoms.

Our results suggest that sensitization to dog does not require a direct and prolonged exposure and can probably be induced also by low amounts of dog allergens. The degree of immediate hypersensitivity to dog allergen in not directly exposed patients is slightly higher than in subjects with a dog at home. A possible explanation of

this finding could be a "protective" effect of dog ownership determined by dog exposure, as previously hypothesized for cat allergens [6]. Recently Svanes et al [21] suggested that dog keeping in childhood could be protective in atopic subjects but not in non atopic ones.

From a clinical point of view, patients sensitized to dog allergens and not owning a dog should be alerted on the possibility of developing allergy (exactly as subjects with a dog at home), and should be warned to avoid massive inhalations of dog allergens (e.g. pet shops or dog shows) [22] to prevent possible exacerbations of respiratory symptoms.

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