

High-dose exposure to cat is associated with clinical tolerance – a modified Th2 immune response?

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Summary

Background Allergen-specific IgG4 antibodies, it is suggested, may be protecting against allergy development by blocking responses. Levels are proposed as a marker of modified Th2 response.

Objectives To assess the levels of IgE, IgG1 and IgG4 antibodies to cat in relation to cat exposure, asthma and allergic diseases.

Methods We studied a population-based sample of 412 schoolchildren of 12–13 years of age. Parents of 402 children completed a questionnaire covering their child's medical history, the keeping of cats and other background data. Skin prick tests (SPTs) to common aeroallergens were performed in 371 of the children. Blood samples for the analyses of IgE, IgG1 and IgG4 antibodies were obtained from 309 of them.

Results All children had an immune response to cat, predominantly of the IgG1 subclass. The levels of cat-specific IgG1 and IgG4, but not IgE, were high in children currently keeping a cat. Children with asthma had increased levels of cat-specific IgE and IgG1, and children with a positive SPT to cat also had increased IgG4. The presence of IgG4 was not associated with asthma or sensitization, unless there was a simultaneous production of IgE. Twenty-five percent of the children had an immune response with only IgG4, and no IgE antibodies to cat. This group of children had the highest frequency of cat-keeping, but a similar prevalence of asthma and allergy as those with neither IgE nor IgG4 antibodies to cat.

Conclusion Cat-keeping was associated with a modified Th2 response, producing IgG4 but not IgE antibodies. This immune response was not associated with an increased risk of asthma or allergy. However, the IgG4 antibodies did not directly mediate any protective effect.

Keywords cat, IgE, IgG4 introduction, sensitization, tolerance

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Introduction

In the development of asthma and other allergies, the role of early animal exposure is still controversial. A dose–response relationship between cat allergen exposure and subsequent IgE antibody formation to cat dander has been shown, at least for a lower dose range [1]. Furthermore, the prevalence of asthma and sensitization to cat increases with increasing prevalence of cats in the area [2], which might be due to an increased indirect (or low dose) exposure to cats. In contrast, however, several recent studies have indicated that the keeping of cats and other animals from early childhood is associated with less manifest allergy [3–7]. This supposed tolerance development may be generally protective against, for instance, asthma, as well as preventing lasting sensitivity to the particular animal that the child was exposed to [2, 3, 8–10]. It is not known what mechanism(s) could lie behind this development of tolerance, although Platts-Mills et al. [11] recently suggested the modification of Th2 immune response

as one possible explanation. This interesting hypothesis implies that Th2 response is not necessarily associated with the production of IgE antibodies, as high-dose allergen exposure may instead induce IgG4 antibody production. Such a modified Th2 response would not be associated with an increased risk of asthma. Our aim was to test the hypothesis of modified Th2 response on differing populations, i.e. to assess the levels of IgE, IgG1 and IgG4 antibodies to cat in relation to cat exposure, asthma and allergic diseases.

Methods

Subjects

In 1991, we administered a cross-sectional population-based questionnaire study to schoolchildren in two Swedish regions ($n = 2481$). One was Kiruna, a mining town in the northernmost interior, and the other Göteborg, a city on the Southwest coast. All 7–9-year-old children were included in Kiruna, and in Göteborg we chose a sample of schools with

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7-year-olds covering known variations in socio-economic scale and in environmental pollution. A stratified subsample of 412 children was selected from the main sample for interviews and allergy testing (the selection procedure, the stratification of the subsample and the questions used, as previously described [12, 13]).

Questions about disease and symptoms were:

Has your child ever had asthma or asthmatic bronchitis?
Have there been any symptoms last year?

Has your child ever had allergic rhinitis/conjunctivitis (ARC)? Have there been any symptoms last year?

Prevalence refers to symptoms at any time during the last year.

Information on current cat-keeping was obtained from the interviews, asking if they currently have a cat. Information on previous cat-keeping was obtained from the questionnaire, asking: 'have you ever during the child's first year of life had a cat living inside your house?'. The term 'exposure' was used to describe those children who were exposed to high levels of cat antigen by having a cat living inside their house.

These 412 children were contacted once again in 1996 for a follow-up. The children in Göteborg were at that time 12 years old, and in Kiruna 12–13 years old. Follow-up included a questionnaire to be answered by the parents, and the children underwent interviews, skin prick testing to common aeroallergens (also as previously described [12]) and blood sampling for IgG and IgE antibody analyses. Retrospective questionnaire data were from the 1996 follow-up.

Antibody analyses

A conventional ELISA was used to determine IgG subclass antibodies as described by Jenmalm et al. [14]. In short, Costar 3590 micro-titre plates (Life Technologies AB, Täby, Sweden) were coated with 10 µg/mL of purified [15] cat dander extract (ALK, Hørsholm, Denmark) in phosphate-buffered saline. Free binding sites were blocked with human serum albumin (Chemicon, Stockholm, Sweden). Human serum samples or buffer without added serum were added to duplicate wells as blanks and then monoclonal antibodies to the human IgG subclasses (IgG (clone HP6064), IgG1 (clone HP6069) and IgG4 (clone HP6011), Bio Zac AB, Järfälla, Stockholm, Sweden). Alkaline phosphatase-conjugated rabbit anti-mouse IgG (Sigma-Aldrich, Stockholm, Sweden) was applied, with *p*-nitrophenyl phosphate (Sigma-Aldrich) as substrate. The optical density (OD) was read at 405 nm and the values were expressed as arbitrary units (AU) derived from the ODs of the reference serum curve after subtracting the blanks. The reference sera, calibrated according to a semi-quantitative method [14], were from patients undergoing cat immunotherapy. Titres in the reference sera were for antibodies to cat dander extract: IgG1 366 AU and IgG4 868 AU. IgG1 antibody levels below the detection limit were set to 3 AU.

Where IgG4 antibodies were not detectable with conventional ELISA, a more sensitive enzyme-amplified ELISA was employed. The coating, blocking and addition of human serum samples, monoclonal antibodies to IgG4 and alkaline phosphatase-conjugated rabbit anti-mouse IgG were as

described above. AMPAK kits (Dakopatts AB, Älvsjö, Sweden) were used for the substrate reaction. NADPD in diethanolamine buffer was allowed to react with the phosphatase for 20 min followed by 10 min incubation with an amplifier solution. OD was measured at 492 nm in AU deduced from the ODs of the reference serum curve after subtracting the blanks. Generally, inter-assay coefficients of variation were below 15% in the conventional and enzyme-amplified ELISAs. IgG4 antibody levels below the detection limit were set to 7 AU.

The levels of IgE antibodies to cat were analysed with the high-sensitivity protocol (incubation of serum samples overnight) of Magic Lite™, a commercial chemoluminometric method, according to the recommendations of the manufacturer (ALK, Hørsholm, Denmark). IgE levels below the detection limit were set to 0.05 kU/L.

Statistics

The sizes of the cross-sectional study population and the stratified subsample, were originally calculated to detect differences in the prevalence of asthma and allergy between Kiruna and Göteborg expected from those previously reported [16]. Statistical analyses were performed with Epi2000, version 1.1.2. The Kruskal–Wallis test for two groups was used to assess the difference between median values in continuous data, and the differences between proportions were calculated with χ^2 tests, Fisher's exact test and multiple logistic regression. The Ethics Committee of Sahlgrenska Academy, Göteborg University, approved the study.

Results

Of the 412 children who participated in the study, 402 (98%) returned the questionnaire, 371 (90%) accepted SPTs and 309 (75%) accepted blood tests.

All children had an immune response to cat with measurable cat-specific antibodies. All but one of them (99.9%) had cat-specific IgG1 antibodies, whereas 45% had IgG4 and 50% had IgE. The levels of IgG1 and IgG4, but not IgE, were higher in children currently having a cat (Table 1).

Children with current asthma had higher cat-specific IgE levels, slightly higher cat-specific IgG1 levels and similar levels of cat-specific IgG4 than those with no asthma (Table 2). In

Table 1. Levels of cat-specific antibodies in children with and without current cat-keeping; first and third quartiles within brackets

	No cat <i>n</i> = 276	Current cat-keeping <i>n</i> = 33	<i>P</i> -value
IgE (kU/L)	0.1 (0.05/2.65)	0.05 (0.05/0.1)	0.4
IgG1 (AU)	30 (17/62)	158 (55/1042)	<0.0001
IgG4 (AU)	7.0 (7.0/18)	37 (15/68)	<0.0001

kU/L, kilo units per litre; AU, arbitrary units.

Table 2. Median levels of cat-specific IgE, IgG1 and IgG4 antibodies in 12–13-year-old children in relation to current asthma and a positive SPT to cat (first and third quartiles within brackets)

Antibody	Current asthma			Positive SPT to cat		
	No <i>n</i> = 270	Yes <i>n</i> = 39	<i>P</i> -value	No <i>n</i> = 244	Yes <i>n</i> = 63	<i>P</i> -value
IgE (kU/L)	0.05 (0.05–1.3)	12 (0.05–210)	<0.001	0.05 (0.05–0.06)	23.7 (5.9–79)	<0.001
IgG1 (AU)	30 (17–73)	52 (23–108)	0.05	27.5 (16–68)	57.6 (3–150)	<0.001
IgG4 (AU)	7 (7–20)	7 (7–25)	0.41	7 (7–18)	14.4 (7–27)	0.003

Units as in Table 1. SPT, skin prick test.

Table 3. Subgroups of the children according to their antibody response to cat allergen (percentages within brackets)

	Group A (IgE ⁻ /IgG4 ⁻) <i>n</i> = 143	<i>P</i> -value (A vs. B)	Group B (IgE ⁻ /IgG4 ⁺) <i>n</i> = 76	<i>P</i> -value (B vs. C)	Group C (IgE ⁺ /IgG4 ⁻) <i>n</i> = 42	<i>P</i> -value (C vs. D)	Group D (IgE ⁺ /IgG4 ⁺) <i>n</i> = 42
Cat first year	8 (5.6)	0.18	8 (10.5)	0.28	2 (4.8)	0.65	3 (7.1)
Cat last year	7 (4.9)	<0.0001	21 (27.6)	0.003	2 (4.8)	0.65	3 (7.1)
Cat first or last year	14 (9.8)	<0.0001	24 (31.6)	0.003	3 (7.1)	0.29	6 (14.3)
Asthma ever	19 (13.3)	0.76	9 (11.8)	0.047	11 (26.2)	0.34	15 (35.7)
Asthma last year	12 (8.4)	0.40	4 (5.3)	0.007	9 (21.4)	0.32	13 (31.0)
ARC ever	37 (25.9)	0.43	16 (21.1)	0.006	19 (45.2)	0.03	29 (69.0)
ARC last year	27 (18.9)	0.41	11 (14.5)	<0.001	18 (42.9)	0.048	27 (64.3)
Eczema ever	60 (42.0)	0.98	32 (42.1)	0.28	22 (53.4)	0.66	20 (47.6)
Eczema last year	40 (28.0)	0.89	22 (28.9)	0.82	13 (31.0)	0.81	12 (28.6)
No parental history	54/139 (38.8)	0.59	26/74 (35.1)	0.92	14/41 (34.1)	0.64	12/41 (29.3)
Single parental history	62/139 (44.6)	0.85	34/74 (45.9)	0.77	20/41 (48.8)	0.66	18/41 (43.9)
Double parental history	23/139 (16.5)	0.66	14/74 (18.9)	0.81	7/41 (17.1)	0.29	11/41 (26.8)
Cat IgG4 (median, AU)	Undetectable		22.4		Undetectable		27.5
			<i>P</i> = 0.18				
Cat IgE (median, kU/L)	Undetectable		Undetectable		8.4	0.02	22.3

Units as in Table 1. ARC, allergic rhinoconjunctivitis.

children with a positive skin prick test (SPT) to cat, the levels of all three antibodies were higher than in SPT-negative children.

In Table 3, the children are divided into four groups according to their antibody response. 'Group A' comprises children with no detectable IgE or IgG4 antibodies to cat. 'Group B' has IgG4, but not IgE to cat. 'Group C' has IgE but not IgG4. The children in 'Group D' had both IgE and IgG4 antibodies to cat. Asthma and ARC were more common in the two IgE-positive groups (Groups C and D) as compared with the two IgE-negative groups (Groups A and B) (OR 4.5, 95% CI 2.11–9.65 for asthma last year and OR 5.5, 95% CI 3.05–9.94 for ARC last year), and this was independent of any accompanying IgG4 response.

Children with IgG4, but not IgE antibodies to cat (Group B) had, as a group, the highest exposure to cat (OR 6.0, 95% CI 1.69–21.4) and the difference remained statistically significant after adjustment for area of living, parental history

of allergy, gender and sensitization to grass pollen (OR 5.6, 95% CI 1.41–22.2), as compared with children with IgE but not IgG4 to cat (Group C).

To test if the presence of cat-specific IgG4 antibodies had any protective effect on asthma and SPT reactions to cat, we compared children with no cat-specific IgG4 antibodies (Groups A and C), with a number of children from groups B and D having an arbitrary chosen high level of IgG4 of at least 50 AU. The prevalence of asthma last year was 11% (21/185) in children with no detectable IgG4 and 15% (4/27) in those with high levels of at least 50 AU (*P* = 0.60). Fifteen percent (28/184) of those with no IgG4 response had a positive SPT to cat, as compared with 30% (8/27) in those with 50 AU or more (*P* = 0.06). A possible association between high IgG4 antibody levels and a low prevalence of SPT reactions to cat was further explored in the subgroup of children with cat-specific IgE antibodies. In these children, the median diameter of the SPT reaction to cat allergen was

similar, irrespective of whether they had cat-specific IgG4 antibodies or not (data not shown).

Discussion

All children had an immune response to cat, and this was particularly pronounced in children with current cat-keeping. Increased antibody levels were also seen in children with asthma or a positive SPT to cat. Children who responded with only IgG4, and not IgE, had as a group the highest exposure to cat. The presence of IgG4 antibodies was not associated with asthma or allergy, unless there was a simultaneous production of IgE, nor was IgG4 in itself associated with less allergy. It may be suggested that high antigen exposure induces tolerance by shifting the production of cat-specific antibodies from IgE to IgG4, which from an allergy point of view seems to be harmless.

IgG4 especially has been regarded as a Th2-derived antibody since, like IgE, it is regulated by IL-4 [17, 18]. Despite this, IgG4 does not trigger inflammatory reactions; instead it might have several different protective properties [19]. Recently, it was also suggested that tolerance might be due to a modified Th2 response characterized by the production of IgG4 antibodies [9]. We were able to identify a group of children with such a modified Th2 response. These children had, as a group, the highest exposure to cat and this remained statistically significant after adjusting for parental history and sensitization to grass pollen. They also had less asthma or ARC than the children responding with IgE but not IgG4. It is, however, unlikely that the reduced frequency of asthma and ARC associated with this particular immune response was directly mediated by IgG4, since we found no protective effect of IgG4 antibodies on either asthma, ARC, sensitization or the size of the SPT reaction. Instead, it seems more likely to assume that the shift, from IgE to IgG4, results in less asthma and ARC probably due to lower levels of IgE antibodies. High allergen exposure, or factors associated with animal-keeping, may enhance the switch from a Th2 response characterized by IgE production and allergy to a modified type, dominated by IgG4 antibodies not associated with allergy.

The relationship between animal exposure and allergy development is complex and different scenarios may result, depending on the circumstances when exposure occurs. Cumulated allergen dose, exposure during infancy or during other age periods, as well as microbial exposure from the animal, may all influence the outcome in different ways. It has been hypothesized that the protective effects associated with growing up on a farm or in a developing country are due to increased exposure to microbes or microbial components inducing maturation of the immune system, making a Th2 response less likely [20, 21]. Similar mechanisms may explain the protective effect associated with pet-keeping during early childhood, since the levels of microbial components are higher in houses with a dog [22], and it has recently been reported that two or more pets may be more protective than merely one [7]. However, a specific and dose-dependent relationship has also been observed between the immune response and the particular allergen in question. Thus, there is

a dose-response relationship between low and moderate exposure to cat and subsequent sensitization to cat, both in young children [1] and adults [10], whereas a negative association seems to follow high-dose exposure [2, 3, 8, 10]. In the latter cases, the association was specific in the sense that cat-exposed individuals were less often sensitized to cat allergen [3, 10], whereas horse-exposed subjects were less often sensitized to horse [8]. These findings indicate that tolerance may not only be generalized, due to immune deviation away from a Th2 response, and suggest that a partial and allergen-specific tolerance may also exist following prolonged exposure to high allergen doses. The latter type of tolerance would be characterized by a modified Th2 response, with a shift from allergy causing IgE antibody production to the production of the more harmless IgG4 subtype.

The present study was cross-sectional and we therefore focused on IgE, IgG1 and IgG4 antibody levels in relation to current cat-keeping, with the assumption that it is related to cumulative exposure. This approach reduces the risk of recall bias, but increases the risk of selection bias. We know from previous studies [3] that some families avoid having pets because of allergy in the family. The significance of these selection mechanisms is, however, difficult to interpret, since pet-keeping is also very common in families with asthma or allergy [23, 24]. In the present study, we have adjusted for such selection mechanisms by adjusting for parental history of allergy, and whether the child had developed other types of allergy, namely sensitivity to grass pollen.

In summary, our findings support that high-dose allergen exposure may be associated with a modified Th2 response, involving IgG4 but not IgE antibody formation. This immune response is not associated with clinical allergy or asthma, probably because of less IgE production and not because of any protective effects mediated by IgG4.

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References

- 1 Wahn U, Bergmann R, Kulig M, Forster J, Bauer CP. The natural course of sensitisation and atopic disease in infancy and childhood. *Pediatr Allergy Immunol* 1997; 8:16–20.
- 2 Roost HP, Kunzli N, Schindler C et al. Role of current and childhood exposure to cat and atopic sensitization. European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1999; 104:941–7.
- 3 Hesselmar B, Åberg N, Åberg B, Eriksson B, Björkstén B. Does early exposure to cat or dog protect against later allergy development? *Clin Exp Allergy* 1999; 29:611–7.

- 4 Svanes C, Jarvis D, Chinn S, Burney P. Childhood environment and adult atopy: results from the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1999; 103:415–20.
- 5 Nafstad P, Magnus P, Gaarder PI, Jaakkola JJ. Exposure to pets and atopy-related diseases in the first 4 years of life. *Allergy* 2001; 56:307–12.
- 6 Remes ST, Castro-Rodriguez JA, Holberg CJ, Martinez FD, Wright AL. Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not of atopy. *J Allergy Clin Immunol* 2001; 108:509–15.
- 7 Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002; 288:963–72.
- 8 Hesselmar B, Åberg B, Eriksson B, Åberg N. Allergic rhinoconjunctivitis, eczema, and sensitization in two areas with differing climates. *Pediatr Allergy Immunol* 2001; 12:208–15.
- 9 Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; 357:752–6.
- 10 Custovic A, Hallam CL, Simpson BM, Craven M, Simpson A, Woodcock A. Decreased prevalence of sensitization to cats with high exposure to cat allergen. *J Allergy Clin Immunol* 2001; 108:537–9.
- 11 Platts-Mills TA, Vaughan JW, Blumenthal K, Pollart Squillace S, Sporik RB. Serum IgG and IgG4 antibodies to Fel d 1 among children exposed to 20 microg Fel d 1 at home: relevance of a nonallergic modified Th2 response. *Int Arch Allergy Immunol* 2001; 124:126–9.
- 12 Åberg N, Hesselmar B, Åberg B, Eriksson B. Increase of asthma, allergic rhinitis and eczema in Swedish schoolchildren between 1979 and 1991. *Clin Exp Allergy* 1995; 25:815–9.
- 13 Åberg N, Sundell J, Eriksson B, Hesselmar B, Åberg B. Prevalence of allergic diseases in schoolchildren in relation to family history, upper respiratory infections, and residential characteristics. *Allergy* 1996; 51:232–7.
- 14 Jenmalm MC, Holt PG, Björkstén B. Maternal influence on IgG subclass antibodies to Bet v 1 during the first 18 months of life as detected with a sensitive ELISA. *Int Arch Allergy Immunol* 1997; 114:175–84.
- 15 Løwenstein H, Lind P, Weeke B. Identification and clinical significance of allergenic molecules of cat origin. Part of the DAS 76 Study. *Allergy* 1985; 40:430–41.
- 16 Åberg N, Engstrom I, Lindberg U. Allergic diseases in Swedish school children. *Acta Paediatr Scand* 1989; 78:246–52.
- 17 Jabara HH, Loh R, Ramesh N, Vercelli D, Geha RS. Sequential switching from mu to epsilon via gamma 4 in human B cells stimulated with IL-4 and hydrocortisone. *J Immunol* 1993; 151:4528–33.
- 18 Lundgren M, Persson U, Larsson P et al. Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. *Eur J Immunol* 1989; 19:1311–5.
- 19 Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* 2002; 105:9–19.
- 20 Björkstén B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999; 29:342–6.
- 21 von Mutius E. Environmental factors influencing the development and progression of pediatric asthma. *J Allergy Clin Immunol* 2002; 109:S525–32.
- 22 Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001; 163:322–8.
- 23 Arshad AH. Pets and atopic disorders in infancy. *Br J Clin Pract* 1991; 45:88–9.
- 24 Nafstad P, Magnus P, Gaarder PI, Jaakkola JJ. Exposure to pets and atopy-related diseases in the first 4 years of life. *Allergy* 2001; 56:307–12.