Cat sensitization according to cat window of exposure in adult asthmatics.

Marie-Pierre Oryszczyn, Ronald van Ree, Jean Maccario, Rachel Nadif, Francine Kauffmann, On Behalf of The Egea Cooperative Group

Clin Experim Allergy 2009 (in press)

CAT SENSITIZATION ACCORDING TO CAT WINDOW OF EXPOSURE IN ADULT ASTHMATICS

Marie-Pierre Oryszczyn[*,†], Ronald Van Ree[§], Jean Maccario[*,¶], Rachel Nadif[*,†], Francine Kauffmann[*,†] on behalf of the EGEA cooperative group.
[*] INSERM, U780, Epidemiology and Biostatistics, Villejuif, France
[†] Univ Paris-Sud, IFR69, Villejuif, France
[§] Academic Medical Center, Departments of Experimental Immunology and Otorhinolaryngology, Amsterdam, The Netherlands
[¶] Université Paris Descartes, Faculté des Sciences Pharmaceutiques, Paris, France

Correspondence : F Kauffmann, INSERM U780, 16 avenue Paul Vaillant Couturier, 94807 Villejuif Cedex, France Tel (33)1 45 59 50 72 Fax (33)1 45 59 51 69
E-mail : francine.kauffmann@inserm.fr
(2872 words)
Running head: Cat sensitization according to exposure
Supported by AFSSET-2005, ANR 05-SEST-020-02/05-9-97, ANR-06-CEBS and EU Framework programme for research, contract n° FOOD-CT-2004-506378, the GA2LEN project, Global Allergy and Asthma European Network »

ABSTRACT

Background In adults, there is limited information on tolerance to cat, which may be reflected by high IgG_4 without IgE sensitization. Early exposure to cat may play a critical role.
Objective The aim was to assess among adult the association of Fel d 1 IgG_4, Fel d 1 IgE, skin prick test response to cat and pet-related symptoms in relation to exposure to cat considering the period of exposure.

Methods Skin prick test response to cat, specific IgE and IgG_4 to Fel d 1 were assessed in 167 asthmatics recruited in chest clinics (40 years of age in average) from the French Epidemiological study on the Genetics and Environment of Asthma (EGEA). Childhood and/or current exposure to cat were studied retrospectively.

Results IgG_4 was higher in relation to current cat exposure (0.53 vs. 0.09 ng/ml ; $p < 0.001$) and higher in women than in men. The period of cat exposure was significantly related to Fel d 1 IgE, the IgE/IgG_4 pattern and cat wheal size. The lowest values of Fel d 1 IgE, cat wheal size, pet-related nasal or respiratory symptoms were observed in those with both childhood and current exposure as well as the highest proportion of the IgE-/IgG_4+ pattern observed in 1.4%, 4.0%, 38.1% and 12.5 % of those with -/-, +/-, +/+, -/+ childhood/current exposure respectively.

Conclusions Adult asthmatics exposed to cats since childhood present an immunologic pattern with high IgG_4 and low IgE. Continuous exposure may maintain a state of immunological tolerance to cat.
(249 words)
Key words: Adult, specific IgE positivity, cat, tolerance, asthma

Introduction

Most studies have reported that having a cat in childhood was protective towards IgE sensitization and atopic diseases [1-3] although a few reports have not reported such findings [4,5]. In childhood, both the period during which children are exposed (window of exposure) and the intensity of exposure have shown complex associations towards the development of sensitization [6]. Increasing evidence supports a protective role of cat in chidlhood towards atopic manifestations in adulthood [2,7], although the mechanisms are not clear. Whereas the hypothesis of endotoxin exposure associated to early contact with cats has been proposed in the context of the TH1/TH2 imbalance [8], recent hypotheses have proposed that the phenomenon depends on a TH2 modified reaction [3] in which increased IgG_4 played a key role and that the mammalian characteristic of cat allergen may explain that exposed subjects may be not sensitized, a phenomenon called tolerance [9]. There is no study on cat allergy with IgG_4 in adult asthmatics with an analysis on cat exposure over the lifespan.

The aim of the present study was to assess the characteristics of cat exposure (in particular the window of exposure) in relation to specific IgG_4, specific IgE to Fel d 1 and the IgG_4/IgE pattern, skin prick test response to cat and pet-related symptoms among asthmatic adults from the Epidemiological Study on the Genetics and Environment of asthma, bronchial hyperresponsiveness and atopy (EGEA) [10-12].

METHODS

Population

The EGEA study combines a case-control and a family study, and the protocol has been described elsewhere [2,10-14]. Briefly, asthmatic patients were recruited from six chest clinics in five French cities. Inclusion criteria used to define asthma in probands were based on self-reported answers to the four questions “Have you ever had attacks of breathlessness at rest with wheezing?”, “Have you ever had asthma attacks?”, “Was this diagnosis confirmed by a physician?”, and “Have you had an asthma attack in the last 12 months?”, or a positive response to at least two questions and a positive review of their medical record [13]. Previously, we have shown that exposure to cat in childhood was negatively related to allergy in adulthood among asthmatic cases and in a lesser extent in non asthmatic controls [2]. Out of the 213 adult proband cases, 167 with sufficient serum, were assessed for level of specific IgE, IgG and IgG_4 to cat.

Questionnaire and skin prick test response

Subjects answered a detailed questionnaire on the indoor characteristics of their households including questions relating to current and childhood exposure to domestic animals (cat, dog, rodents, birds, other). As a measure of high exposure to cat, it was further assessed whether the pet was allowed to enter the bedroom, as done in the ECRHS [7]. Among these adults, past exposure to cat was defined as exposure before 16 years of age, the limit chosen in EGEA for pediatric age. Current exposure was defined as owning a cat at the time of the survey. Triggers of asthma attacks, in particular related to pets and, more generally, questions regarding symptoms (in the last year) occurring in the presence of animals (cough, sneezes, runny nose, wheeze, shortness of breath) were recorded. Subjects were considered symptomatics if they reported cat-related respiratory or nasal symptoms, using a self-completed

questionnaire, which was shown to be valid versus an interview-based questionnaire [15] .

The day of examination, blood samples were drawn for subsequent serological testing and skin prick tests (SPT) to cat, *Dermatophagoïdes pteronyssinus, Cladosporium herbarum, Alternaria tenuis,* timothy grass, olive, birch**,** *Parietaria judaica***,** ragweed, *Aspergillus* and *Blattella germanica* were performed. Negative (diluent) and positive (histamine) controls were used. Cat weal size was measured as the mean of both diameters. Atopy to cat was defined by a positive response (weal diameter at least 3mm greater than the negative control) to cat allergen.

Measurement of total IgE, specific IgE, IgG and IgG_4 to Fel d 1

Total IgE was measured by RIA [16,17]. Specific IgE was measured by RAST [16] using Sepharose-coupled rFel d 1 using banked serum. The units were calculated using a standard curve of a chimeric monoclonal IgE antibody directed to Der p 2 and Sepharose-coupled *Der p 2* (1 IU=2,4 ng) [18]. Specific IgG was measured using Protein G Sepharose to bind IgG (or Sepharose-coupled mAb a-IgG_4 in case of IgG_4) in combination with radiolabeled purified Fel d 1.[19] A rabbit antiserum against cat dander extract, or a human serum pool of immunotherapy-treated patients in case of IgG_4, in combination with the same radiolabeled Fel d 1 was used as standard curve. Both standard curves were used from 5 to 5000 ng/ml Fel d 1-specific IgG. The limit of detection of the assay for Fel d 1-specific IgE, IgG and IgG_4 was 0.1 ng/ml and undetectable samples were assigned a value of 0.05 ng/ml. Unless otherwise specified, IgE refers to Fel d 1 IgE.

Statistical analysis

In a first step, the association of cat exposure was studied in relation to IgG_4. Then, the association of cat exposure was studied in relation to Fel d 1 IgE, cat wheal size and pet-related symptoms. In relation to previous findings of sex-related differences in asthma and allergy [11], all analyses were first done in each sex separately. Standard statistical tests (χ^2 or Fischer exact test when appropriate, and logistic regression for qualitative outcomes, analyses of variance and multiple linear regression analysis for quantitative outcomes) were performed. Tests were considered as significant where $p \leq 0.05$.

As numerous subjects had undetectable values for specific IgE and IgG_4, specific attention was given to the statistical aspects on these issues. Four strategies have been considered : 1) to perform analyses with dichotomous variables using cut-off derived from the literature (such as IgE $\geq$ 0.35 IU/ml) or based on the limit of detection (for IgG and IgG_4). This conservative strategy was performed for the main results. 2) to perform analyses on continuous variables after log transformation, but with non parametric tests 3) to perform analyses on continuous variables with standard tests. Analyses have been performed on log transformed data (total IgE, specific IgE, IgG and IgG_4 to Fel d 1) and results expressed in geometric means. Undetectable values were 44% for Fel d 1 specific IgE and 63% for IgG_4. All analyses performed led to similar p values as with method 2. 4) Finally, to address the imprecision of the offset and the underestimation of variance that occur in regressions based on censored data, maximum likelihood estimations were used. To accommodate the presence of covariables (age,

sex, and smoking), the method presented by Lyles et al 2001 [20] was adapted. Computations were performed with R [21]. All the analyses have been run using method 4. In the paper, results are presented for method 3 (usual test on continuous variables) when they were confirmed by method 4. It allows taking the benefit of using the whole information available while checking potential misinterpretation due to censorship.

RESULTS

Characteristics of the study sample

The population is described in table 1 and allergic markers, in particular related to cat, are presented in table 2. Fel d 1-specific IgE was correlated with Fel d 1 IgG-specific and IgG_4 (r=0.72 and r=0.61 respectively), and the association between Fel d 1-specific IgE and IgG_4 remained statistically significant when restricting the analysis to those with detectable values (r=0.28). IgG and IgG_4 were highly correlated (r=0.92). Subjects who were sensitized to cat (i.e. had a positive SPT) always had higher levels of Fel d 1 specific IgE (GM were 3.26 IU/ml vs .013 IU/ml for those with positive and negative skin prick test to cat respectively, $p < 0.001$) and 90% of them were polysensitised.

Factors associated with Fel d 1-specific IgG_4

Intensity of exposure and sex were independently associated with IgG_4 level (Figure 1 and Table 2), more precisely significantly higher levels of IgG and IgG_4 were observed in those subjects allowing cats in the bedroom (intensity of exposure) and in women compared to men (sex). Moreover, levels of Fel d 1-specific IgG_4 were higher ($p < 0.001$) in subjects with current exposure (geometric mean = 0.53 ng/ml) than in those with past exposure only (0.09 ng/ml). Smoking was unrelated to IgG_4 (data not shown). Taking into account age, sex or smoking did not change the associations of IgG_4 to cat exposure.

Cat sensitization

Cat IgE sensitization was then studied according to the window of cat exposure, i.e. past exposure only, both past and current exposure, or current exposure only. Three phenotypes for cat sensitization were considered: first Fel d 1-specific IgE titers in serum, second Fel d 1-specific IgE/IgG_4 pattern as dichotomous variables (positive/negative), and finally the cat wheal size. Current exposure to cat was significantly associated with higher Fel d 1-specific IgE (Figure 2), and those currently exposed tended to be more often IgE+ (44.4 vs. 30.3%; p=0.09), a trend which became significant after adjustment for age, gender and smoking, (OR=2.66 [1.21-5.82]). Asthmatics who had a cat in childhood had a lower level of Fel d 1-specific IgE in adulthood than those without (GM : 0.18 vs. 0.40 IU/ml; $p<0.05$), and the effect of cat in childhood remained significant after adjustment for age, sex and smoking. No interaction with sex was observed, but the relation was significant in women (GM: 0.17 vs 0.60 IU/ml; $p<0.05$) and not in men (GM: 0.20 vs 0.29 IU/ml). Considering windows of exposure, asthmatics with both current and childhood cat exposure were those with

the lowest Fel d 1-specific IgE level, whereas those exposed only currently had the highest level (Figure 2). This pattern was evidenced both in men and in women.

Considering the IgE/IgG_4 pattern, asthmatics exposed both in childhood and currently exhibited the highest percentage of IgE-/IgG_4+ (Figure 2). The proportion of the IgE-/IgG_4+ pattern was observed in 1.4 % for those with no exposure throughout life, 4% in those with only childhood exposure, 38.1% in those with both childhood and current exposure and in 12.5% in those with current exposure only. In those with both childhood and current exposure, nobody exhibited an IgE+/IgG_4- pattern.

Considering cat skin prick test response either dichotomously or by analysing cat wheal size showed a very consistent pattern with results on Fel d 1-specific IgE. Considering windows of exposure, the smallest cat wheal size were observed in those with both childhood and current exposures and the largest in those currently exposed only (Figure 2). The pattern on wheal size held when excluding those with wheal size equal zero, but was then not statistically significant.

Pet-related symptoms

Symptoms in relation to cat were studied by considering pet-related symptoms according to the window of cat exposure. The prevalences of nasal symptoms were 27.5%, 22.0%, 19.0% and 25.0% among those without cat, with exposure only in childhood, exposure in childhood and currently, and in those with only current exposure. For respiratory symptoms, the pattern was slightly more marked with prevalences of 27.5 %, 29.8 %, 14.3 %, and 33.3 %. Although not statistically significant, the combination of both types of pet-related symptoms, showed that the lowest percentage was observed in those with both childhood and current exposure.

DISCUSSION

In the EGEA study conducted in well characterized adult asthmatics, tolerance to cat among those continuously exposed since childhood was suggested, with a pattern combining high Fel d 1-specific IgG_4 and low IgE, supporting the hypothesis of a modified TH2 pattern, mostly described in children until now. Results were consistent when considering various markersFel d 1specific IgE, , cat wheal size and pet-related symptoms. Consideration of the combination of windows of exposure over the lifespan was critical. Detailed analysis confirms that early exposure to cat is essential for a pattern consistent with a protective effect but not enough : asthmatics who experienced early exposure exhibit an immunological pattern with high IgG_4 and low IgE only in the presence of current exposure. In the context of further epidemiological studies, Fel d 1-specific IgG_4 could be used as a quantitative marker of current exposure to cat. In adulthood, Fel d 1-specific IgE increased significantly less with Fel d1-specific IgG_4 (indicator of exposure) in asthmatics who were exposed to cat in childhood than in those who were not.

Strengths of the study are the good characterization of the asthmatics and the availability of exposure data over the lifespan. Limitations of the study were, the cross-sectional nature of the data and it should be emphasized that longitudinal data over the lifespan are necessary to disentangle issues of causality and selection [22]. Further, the

asthmatics were not taken at random from the general population and further studies are needed to assess the generalisability of the findings. All measures of Fel d 1-specific IgG_4 and IgE have been performed blinded towards exposure, phenotypic data. Exposures were collected retrospectively but measurement error in the report of exposure to pets in childhood has a poor reliability [23], appears marginal and unrelated to atopy [24]. Selective avoidance cannot easily be distinguished from protective effect in cross-sectional studies, but studies conducted both in children and adults suggest that selective avoidance can only explain part of the effects observed [24,25]. Because of the proportion of undetectable IgE and IgG_4, non parametric tests and likelihood ratios (data not shown) as well as categorical analyses were performed (Figure 1 panel 2) and led to the same conclusions.

Increasing evidence supports the hypothesis of a tolerance mechanism, with a modified TH2 pattern in children with early exposure to cat [3,26]. Observations in children further suggest that it is an allergen-specific phenomenon, which is not explained by exposure to endotoxin [26]. Observations in adults have been so far scanty [6, 27, 28]. Our results confirm and extend previous observations in adults and overall support the hypothesis of a tolerance mechanism, with a modified TH2 pattern, perpetuating the childhood phenomenon. In the EGEA study, we previously reported that early exposure to cat was associated with less cat atopy [2] among adult asthmatics. It is confirmed here that this is associated with lower Fel d 1-specific IgE. In the general population of ECRHS, current exposure assessed by measures of Fel d 1 in mattress was associated with Fel d 1-specific IgG_4, independent of Fel d 1-specific IgE positivity [9], which supports the hypothesis of Fel d 1-specific IgG_4 as an indicator of current exposure. The relevance of studying factors associated with IgG_4 goes beyond its role in successful immunotherapy or parasite immunology. B cells can switch directly from μ to ε or sequentially through γ_4 [29]. The latter most likely occurs at higher exposure, the former at lower exposure. As a consequence, in case of sequential switching epitope specificity of IgE and IgG_4 is identical. When IgE resulting from direct switching (low exposure) is later in life complemented by IgG_4 (high exposure) epitope-specificity will not necessarily overlap. Since IgE and IgG_4 antibodies have antagonistic functions, it is of great interest to understand how this process is controlled [30]. Beyond immunological research, there is therefore a need of more epidemiological studies with both parameters.

We performed what we called previously a longitudinal analysis of cross-sectional data [31] in two steps. The first step concerned the association of exposure with IgG_4 assuming that effect would be time invariant (i.e. the same in adulthood and in childhood). The second step concerned the association of exposure, considering then windows of exposure over the lifespan with cat IgE sensitization. We considered simultaneously current and past exposure. Overall, results show that it is not simply high IgG_4 in itself which initiates a potential protective effect, but that it is most likely dependent on the timing when the first IgE and/or IgG_4 antibody responses were induced. Similar results regarding the negative association of IgG_4+/IgE- towards symptoms have been observed for other mammalian allergens in occupational settings [32,33].

Our results, observed in asthmatics cannot be extrapolated to the general population, but understanding the effects of cat exposure among asthmatics is in itself

of great clinical interest. Few studies have addressed past and current exposure and it is difficult to compare our results in adults with those in children, such as in the study by Brunekreef et al [22]. In their study as well as in ours, the lowest prevalence of symptoms was in those with both current and past exposure, an observation compatible with both protection and selection. More sensitive measures of IgG_4 and larger samples are necessary to address the question in non asthmatics. It would be of great interest to take the opportunity of cohorts of children followed till the beginning of adulthood to test them regarding IgG_4 and IgE at several occasions to formally test such hypothesis. During childhood, it is worth noting that IgG to Fel d 1 correlated at age 5, 7 and 10 years (r=0.6) in the MAS cohort [34]. Whether subjects prone to increase markedly their Fel d 1-specific IgG_4 in presence of a cat remained similar over the life span should also be evaluated in longitudinal studies.

In conclusion, tolerance to cat was evidenced in adult asthmatics among those exposed to cats since childhood with an immunologic pattern with high IgG_4 and low IgE. More research is needed to understand the determinants of the occurrence and persistence of cat tolerance over the life span.

EGEA cooperative group
Coordination: F Kauffmann; F Demenais (genetics); I Pin (clinical aspects).
Respiratory epidemiology: Inserm U 700, Paris M Korobaeff (EGEA1), F Neukirch (EGEA1); Inserm U 707, Paris: I Annesi-Maesano; Inserm U 780, Villejuif: F Kauffmann, N Le Moual, R Nadif, MP Oryszczyn; Inserm U 823, Grenoble: V Siroux.
Genetics: Inserm U 393, Paris: J Feingold; Inserm U 535, Villejuif: MH Dizier; Inserm U 946, Paris: E Bouzigon, F Demenais; CNG, Evry: I Gut, M Lathrop.
Clinical centers: Grenoble: I Pin, C Pison; Lyon: D Ecochard (EGEA1), F Gormand, Y Pacheco; Marseille: D Charpin (EGEA1), D Vervloet; Montpellier: J Bousquet; Paris Cochin: A Lockhart (EGEA1), R Matran (now in Lille); Paris Necker: E Paty, P Scheinmann; Paris-Trousseau: A Grimfeld, J Just.
Data and quality management: Inserm ex-U155 (EGEA1): J Hochez; Inserm U 780, Villejuif: N Le Moual, C Ravault; Inserm U 946: N Chateigner; Grenoble: J Ferran

REFERENCES

1. Hesselmar B, Aberg N, Aberg B, Eriksson B, Bjorksten B. Does early exposure to cat or dog protect against later allergy development ? *Clin Exp Allergy* 1999; **29**:611-7.
2. Oryszczyn MP, Annesi-Maesano I, Charpin D, Kauffmann F. Allergy markers in adults in relation to the timing of pet exposure : the EGEA study. *Allergy* 2003;**58**:1136-43.
3. 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.
4. Melen E, Wickman M, Nordvall SL, van Hage-Hamsten M, Lindfors A. Influence of early and current environmental exposure factors on sensitization and outcome of asthma in pre-school children. *Allergy* 2001;**56**:646-52.
5. Wahn U, Lau S, Bergmann R, Kulig M, Forster J, Bergmann K, Bauer CP, Guggenmoos-Holzmann I. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol* 1997; **99** (6 Pt1): 763-9

6. Torrent M, Sunyer J, Garcia R, Harris J, Iturriaga MV, Puig C, Vall O, Anto JM, Newman Taylor AJ, Cullinan P. Early-life allergen exposure and atopy, asthma, and wheeze up to 6 years of age. *Am J Respir Crit Care Med* 2007 ;**176**:446-53.
7. Jarvis D, Zock JP, Heinrich J, Svanes C, Verlato G, Olivieri M, Villani S, Ponzio M, Leynaert B, Sunyer J, Dahlman-Hoglund A, Chinn S, Luczynska C, Norbäck D, Burney P. Cat and dust mite allergen levels, specific IgG and IgG_4, and respiratory symptoms in adults. *J Allergy Clin Immunol* 2007;**119**:697-704.
8. Liu AH. Something old, something new : indoor endotoxin, allergens and asthma. *Paediatr Respir Rev* 2004;**5** (Suppl A):S65-71.
9. Platts-Mills TA. The role of indoor allergens in chronic allergic disease. *J Allergy Clin Immunol* 2007;**119**:297-302.
10. Kauffmann F, Dizier MH, Annesi-Maesano I et al. EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy) - Descriptive characteristics. *Clin Exp Allergy* 1999 ; **29** (suppl 4):17-21.
11. Oryszczyn MP, Bouzigon E, Maccario J, Siroux V, Nadif R, Wright A, Kauffmann F. Interrelationships of quantitative asthma-related phenotypes in the Epidemiological Study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness, and atopy. *J Allergy Clin Immunol* 2007;**119**:57-63.
12. Smit LA, Siroux V, Bouzigon E, Oryszczyn MP, Lathrop M, Demenais F, Kauffmann F on behalf of the EGEA cooperative group. CD14 and Toll-like receptor gene polymorphisms, country living, and asthma in adults. *Am J Respir Crit Care Med*. 2009 ; 179 : 363-368
13. Kauffmann F, Dizier MH, Pin I, *et al*. Epidemiological study of the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy: phenotype issues. *Am J Respir Crit Care Med* 1997 ;**156**(4 Pt 2):S123-9
14. Oryszczyn MP, Annesi-Maesano I, Charpin D, Paty E, Maccario J, Kauffmann F. Relationships of active and passive smoking to total IgE in adults of the Epidemiological Study of the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy (EGEA). *Am J Respir Crit Care Med* 2000;**161**(4 Pt 1):1241-6.
15. Kauffmann F, Annesi-Maesano I, Liard R, Paty E, Faraldo B, Neukirch F, Dizier M. [Construction and validation of a questionnaire in respiratory epidemiology. Example of the questionnaire of the Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy (EGEA)]. *Rev Mal Respir* 2002;**19**:323-333
16. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. *J Allergy Clin Immunol* 1981; **68**:356-64.
17. Stallman PJ, Aalberse RC. Estimation of basophil-bound IgE by quantitative immunofluorescence microscopy. *Int Arch Allergy Appl Immunol* 1977; **54**:9-18.
18. Schuurman J, Perdok GJ, Lourens TE, Parren PW, Chapman MD, Aalberse RC. Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. *J Allergy Clin Immunol* 1997;**99**:545-50.
19. van Ree R, van Leeuwen WA, van den Berg M, Weller HH, Aalberse RC. IgE and IgG cross-reactivity among Lol p I and Lol p II/III. Identification of the C-termini of Lol p I, II, and III as cross-reactive structures. *Allergy* 1994 ;**49**:254-61

20. Lyles RH, Williams JK, Chuachoowong R. Correlating two viral load assays with known detection limits. *Biometrics* 2001; **57** : 1238-1244
21. R Development Core Team (2008). R : A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria ISB?N 3-9000051-07-0, URL http : //www.R-project.org [accessed January 10, 2009]
22. Brunekreef B, Groot B, Hoek G. Pets, allergy and respiratory symptoms in children. *Int J Epidemiol* 1992; **21** : 338-342
23. Svanes C, Dharmage S, Sunyer J, *et al*. Long-term reliability in reporting of childhood pets by adults interviewed twice, 9 years apart. Results from the European Community Respiratory Health Survey I and II. *Indoor Air* 2008 ;**18**:84-92.
24. Svanes C, Zock JP, Antó J, Dhamarge S, Norbäck D, Wjst M Heinrich J, Jarvis D, de Marco R, Plana E, Raherison C, Sunyer J; Early Life Working Group Of The European Community Respiratory Health Survey. Do asthma and allergy influence subsequence pet keeping ? An analysis of childhood and adulthood. *J Allergy Clin Immunol* 2006; **118** : 691-8.
25. Erwin EA, Wickens K, Custis NJ, Siebers R, Woodfolk J, Barry D, Crane J, Platts-Mills TA. Cat and dust mite sensitivity and tolerance in relation to wheezing among children raised with high exposure to both allergens. *J Allergy Clin Immunol* 2005;**115**:74-9.
26. Perzanowski MS, Ronmark E, Platts-Mills TA, Lundback B. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med* 2002 ;**166** :696-702.
27. Roost HP, Künzli N, Schindler C, Jarvis D, Chinn S, Perruchoud AP, Ackermann-Liebrich U, Burney P, Wüthrich B. Role of current and childhood exposure to cat and atopic sensitization. *J Allergy Clin Imunol* 1999; **104** : 941-947.
28. De Meer G, Toelle BG, Ng K, ovey E, Marks GB. Presence and timing of cat ownership by age 18 and the effect on atopy and asthma at age 28. *J Allergy Clin Immunol* 2004 ;**113**:433-8
29. Aalberse RC, Platts-Mills TA. How do we avoid developing allergy: Modifications of the TH2 response from a B-cell perspective. *J Allergy Clin Immunol* 2004 ; **113**: 983-986
30. Vercelli D. One cytokine, two isotypes. A Trojan Horse, Pandora's Box, and an evolving paradigm. *Am J Respir Crit Care Med* 2000 ; **162** : 586-590.
31. Kauffmann F, Maccario J. Cross-sectional analyses of longitudinal data and longitudinal analyses of cross-sectional data. *Eur Respir Rev* 2000; **10** (75):380-382
32. Krop EJM, Stapfel SO, De Vrieze H,van der Zee JS. Immunoglobulin E and G_4 antibody responses in ocupational airway exposure to bovine and porcine plasma proteins. *Int Arch Allergy Immunol* 2006; **39** : 237-244
33. Matsui EC, Diette GB, Krop EJM, Aalberse RC, Smith AL, Curtin-Brosan J**,** Eggleston PA. Mouse allergen-specific immunoglobulin G and immunoglobulin G4 and allergic symptoms in immunoglobulin E –sensitized laboratory animal workers. *Clin Exp Allergy* 2005; **35** : 1347-1353.
34. Lau S, Illi S, Platts-Mills TAE, Riposo D, Nickel R, Grüber C, Niggemann B, Wahn U; Multicentre Allergy Study Group. Longitudinal study on the relationship between cat aallergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood – report of the German Multicentre Allergy Study (MAS 90). *Allergy* 2005 ; **60** : 766-773.

TABLE 1. CHARACTERISTICS OF THE POPULATION

	Value
n	167
Age, m ± SD, years	40.2 ± 13.5
Males, %	58.3
Current cat exposure, %	27.0
Among the exposed, cat allowed in the bedroom, %	62.2
Childhood cat exposure, %	42.5
Lifespan cat exposure	
None, %	43.1
Childhood only, %	29.9
Current + childhood, %	12.6
Current only, %	14.4
Cat related symptoms, previous 12 months	
Nasal, %	24.8
Pulmonary, %	27.3
Nasal or pulmonary, %	36.6

TABLE 2. IMMUNOLOGIC MARKERS ACCORDING TO SEX.

	Men (n=89)	Women (n=78)
SPT positivity, any (11 allergens), %	75.6	60.5*
SPT positivity, cat, %	20.9	28.9
Cat weal size, m ± SD, mm	1.42 ± 2.93	1.72 ± 2.44
Total IgE, GM [95%CI], IU/ml	219 [19-2575]	141 [5-3849]*
Fel d 1 specific IgE, GM [95%CI], IU/ml	0.25 [0.005-13.9]	0.34 [0.004-31.3]
Fel d 1specific IgE ≥ 0.35 IU/ml, %	30.3	38.5
Fel d 1specific IgG, GM [95%CI], ng/ml	0.17 [0.003-9.25]	0.40 [0.003-50.48] *
Fel d 1specific IgG ≥ 0.1 ng/ml, %	31.5	48.7*
Fel d 1specific IgG_4, GM [95%CI], ng/ml	0.11[0.008-1.32]	0.22 [0.006-7.71] **
Fel d 1specific IgG_4 ≥ 0.1 ng/ml, %	29.2	46.2**

*p≤0,05 ; **p≤0,01

After adjustement for age and smoking, differences in total IgE (higher in men) and in Fel d 1 specific IgG_4 (higher in women) remained statistically significant.

Figure 1. Relationships between Fel d 1 specific IgG_4 with sex, cat exposure intensity and cat windows of exposure among adult asthmatics
Figures are box plots (with means represented by a star, and median by an horizontal line)
Low : cat not allowed in the bedroom (current)
High : cat allowed in the bedroom (current)

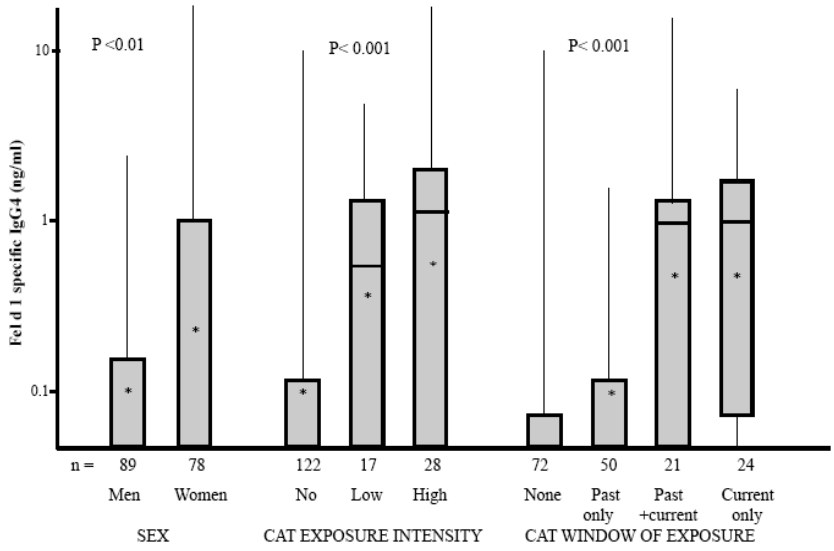

Figure 2. Fel d 1 specific IgE, IgE/IgG_4 pattern and cat weal size according to cat window of exposure among adult asthmatics
In box plots, means are represented by a star, and medians by an horizontal line. When median or mean are not figured, it is because there are extremely low.