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# CAT SENSITIZATION ACCORDING TO CAT WINDOW OF EXPOSURE IN ADULT ASTHMATICS

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(2872 words)
Running head: Cat sensitization according to exposure

## 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 ($\chi^2$ 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 $p \le 0.05$.

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 $\ge$ 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 IgG4 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.

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**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)
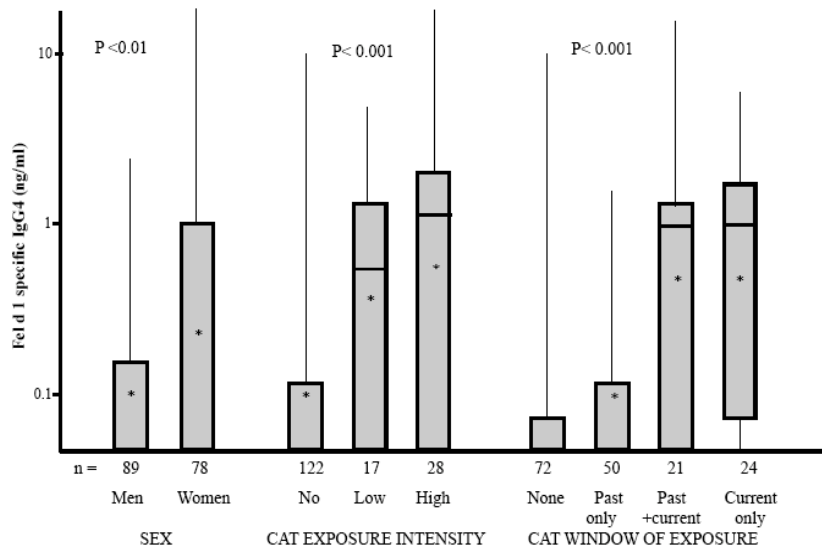High : cat allowed in the bedroom (current)



**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.