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slgE and slgG to airborne atopic allergens: coupled rather than inversely-related responses

To the Editor,

Specific IgG (slgG) to allergens has been suggested to indicate a healthy immune response, as opposed to the pathological IgE response [1]. This is true for some anti-allergen responses, such as to insect stings and to many foods, as well as for allergen-specific immunotherapy (AIT). The situation in relation to low-dose airborne allergens, such as allergens from the prototypical atopic allergen sources pollen and mites, is still unresolved. Recently an analysis was published on the influence of geographical variability and route of exposure on allergen-specific IgG and IgE. The study was based on data obtained by an allergen microarray in sera from the French Epidemiological study of the Genetics and Environment of Asthma (EGEA, for details see [2] and the Online Repository). Based on an alternative way of analyzing the same data set we want to argue that the level of IgG to the clinically most relevant airborne allergens is strikingly low in subjects with slgE less than 0.3 ISAC standardized units (**IgE⁻**). This is important, because it may substantially change our views on the initial phase of allergic sensitization (as will be discussed below).

Methodological details on the selection of sera, allergens and data analysis procedure, as well as a discussion on the important but insufficient contribution of exposure, age and gender are presented in the Online Repository. The values shown in the figures are 1) the prevalence of high slgG, i.e. IgG higher than the median of slgG in subjects with slgE > 0.3 ISAC standardized units (ISU) to the same allergen (**IgE⁺**) and 2) the relative prevalence (RP), which is the prevalence of high slgG in the **IgE⁻** population compared to prevalence of high IgG in the **IgE⁺** population. In the present context, high slgG corresponds to a modest slgG level (in the µg/L range) compared to the IgG level following a full-blown antibody response originating in a mature GC (typically in the mg/L range). The scope of the presented analysis is exploratory: we want to show a phenomenon. For such an exploratory data analysis the use of 95% confidence intervals, as used in the figures, is appropriate.

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The evidence presented in favor of our main message (sIgG is low in **IgE⁻** subjects) is strong. For the two major allergens (mite group 1 and grass group 1) the analysis shown in figure 1 confirms preliminary results published almost 40 years ago[3,4]. Those results were not well noticed, possibly because of small sample sizes and the use of the currently unpopular radioisotope technology (a quantitative RadioImmunoAssay, RIA, see the Online Repository). Moreover, conflicting results were obtained by ELISA[5].

Our data show that the effect size for some, but not all, allergens is big, with a relative prevalence of high sIgG in the **IgE⁻** population that is often 10-100 fold below the statistical no-effect level (i.e. $RP=1$), with 95% confidence levels mostly well below 1, (figure 2, Table E1). For allergens from 8 families (Amb a 1, Art v 1, tree group 1, Ole e 1, grass group 2, mite group 2, Alt a 1, Can f 1) the prevalence of high sIgG was more than 10 times lower in **IgE⁻** than in **IgE⁺** subjects. For grass group 1 and mite group 1, the difference was substantially smaller when measured by the microarray than by RIA. The RP was close to 1 for 7 allergens (Pla a 2, mite group 1 and 5, Der p 4, 7, 21 and 23).

If we accept that lack of allergen exposure is an insufficient explanation for the low IgG response in **IgE⁻** subjects (see the Online Repository), another explanation is needed. One possible explanation is that atopic allergens are poorly immunogenic and fail to induce a B cell response in all but the most genetically predisposed subjects [6]. Even in these most reactive subjects the immune response is incomplete, germinal centers (GCs) fail to mature and the absolute amount of IgG produced is modest. However, some IgE-switched B cells become long-lived plasma cells. Upon subsequent exposure mucosal TH2 inflammation is induced, in which the IgE response diversifies and expands via indirect switching. Recently it has been established that a fully matured GC effectively prevents the development of long-lived IgE-switched B cells in several mouse models (reviewed in [7,8]).

In comparison to a non-atopic TH2 immune response (i.e. a “modified TH2 response” [9]), the atopic immune response (with pollen and mite as prototypical airborne allergen sources) results in subnormal B cell responses for both IgE and IgG. In the absence of mature GCs the IgG/IgE ratio remains low. In a modified TH2 response (a “healthy immune response” as mentioned in the introduction) the maturation of GCs results in the expansion of IgG-switched B cells and the deletion of IgE-switched B cells. The most obvious difference between these two routes is the IgG/IgE ratio. IgG-switched B cells are crucial precursors for the indirect route. Since only a small fraction of the IgG switched B cells will subsequently switch to IgE, the IgG/IgE ratio is predicted to be much higher than 1. In the modified TH2 response, for example following a bee sting, IgE is often undetectable. Our aim was to identify allergens that induce little or no detectable sIgG in the absence of sIgE, i.e. allergens that are associated with a low RP. In view of the published results we restricted our analysis to airborne allergens. Regrettably, we don’t have reliable information on the sIgG levels in mass units. Based on the approximately 10 times shorter half-life of circulating IgE compared to IgG, a 1:1 ratio of IgG/IgE producing plasma cells and assuming equal production rates, we would expect IgG levels in the 10-100 ng/ml range, whereas GC-derived IgG production levels are typically 10- to 100-fold higher (figure E2).

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Conflict of interest Statement

None of the authors have a conflict of interest in relation to this work.

Author Contributions

RCA, TP-M: substantial contributions to the analysis and interpretation of data; drafting the article; final approval of the version to be published.

CL, RV, VS, RN, JJ, JB: substantial contributions to the acquisition of data; revising the manuscript critically for important intellectual content; final approval of the version to be published.

List of abbreviations

AIT: allergen immunotherapy

EGEA: Epidemiological study of the Genetics and Environment of Asthma

GC: germinal center of a lymph node follicle

IgE⁻ : IgE < 0.3 ISU; IgE⁺ : IgE > 0.3 ISU

ISAC: Immuno Solid-phase Allergen Chip

ISU: ISAC standardized units

MeDALL: Mechanisms of the Development of Allergy

RIA: radioimmunoassay

RP: Relative Prevalence

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sIgE, sIgG: allergen-specific IgE, allergen-specific IgG

Lol p 1, Phl p 1, 2, 5, 6, 11; Amb a 1, Art v 1: grass and weed pollen allergens

Aln g 1, Bet v 1, Ole e 1, Pla l 1, Pla a 2: tree pollen allergens

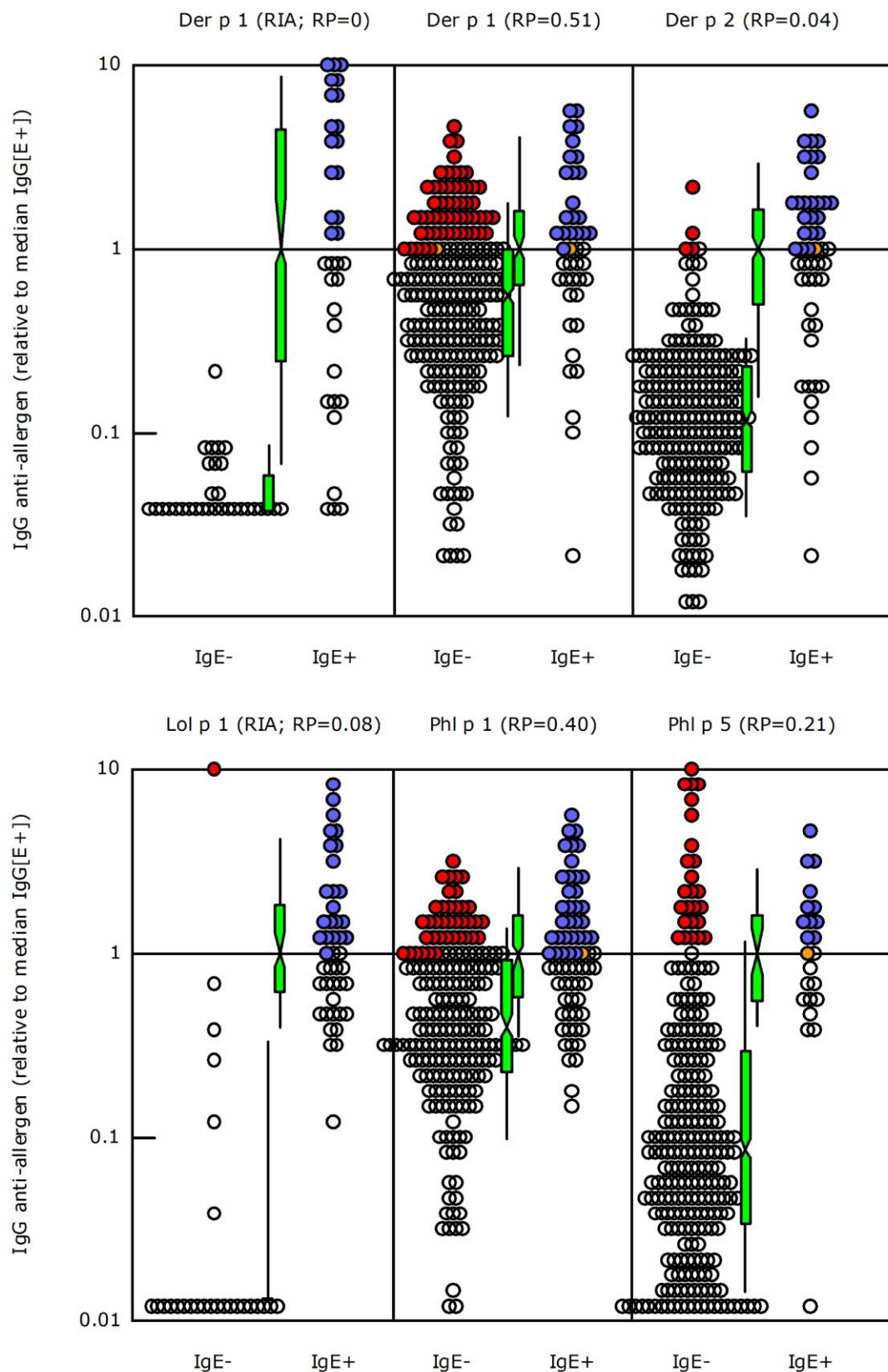
Fel d 1: cat allergen

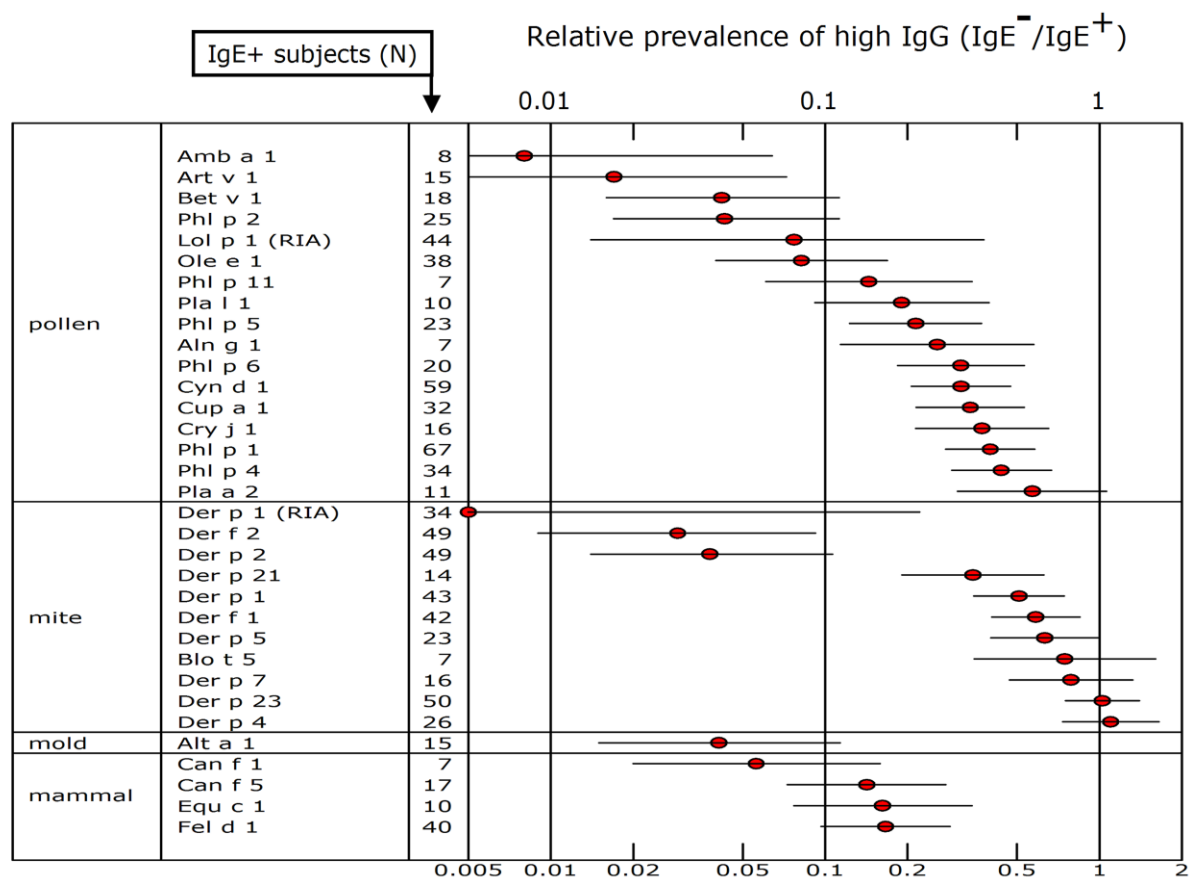
Der p 1, 2, 4, 5, 7, 21, 23; Der f 1, 2; Blo t 5: mite allergens

Legends to the figures

Figure 1. IgG to major allergens from mite (**1A**) and grass (**1B**). The results are expressed relative to the median of the IgG level of IgE^+ subjects. The red dots indicate $\text{IgG}^+ \text{IgE}^-$ sera, the blue dots indicate $\text{IgG}^+ \text{IgE}^+$ sera. Orange dots indicate sera with an IgG value equal to the median cut-off value. The subjects tested in the allergen-binding radioimmunoassay (RIA) are different from those tested in the microarray.

Figure 2. Relative prevalence (RP) of high allergen-specific IgG in IgE^- subjects compared to IgE^+ subjects. We only analyzed allergens for which >6 subjects (out of a total of 257) were IgE^+ . The numbers in the 3rd column indicate the total number of IgE^+ subjects. The error bars indicate the 95% confidence interval of the RPs.





Electronic Repository

slgE and slgG to airborne atopic allergens: coupled rather than inversely-related responses

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34 *Abbreviations:*

35 AIT: allergen immunotherapy

36 EGEA: Epidemiological study of the Genetics and Environment of Asthma

37 GC: germinal center of a lymph node follicle

38 IgE^- : $\text{IgE} < 0.3$ ISU; IgE^+ : $\text{IgE} > 0.3$ ISU

39 ISAC: Immuno Solid-phase Allergen Chip

40 ISU: ISAC standardized units

41 MeDALL: Mechanisms of the Development of Allergy

42 RIA: radioimmunoassay

43 RP: Relative Prevalence

44 sIgE, sIgG: allergen-specific IgE, allergen-specific IgG

45 Lol p 1, Phl p 1, Phl p 2, etc.: grass allergens

46 Fel d 1: cat allergen

47 Der p 1, Der p 2, etc.: mite allergens

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Electronic Online Repository

The Epidemiological study of the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy (EGEA)

In the present communication we discuss a subset of previously described serological results on the influence of geographical variability and route of exposure on allergen-specific IgG and IgE[E1]. The study was based on data obtained by an allergen microarray in sera from the French Epidemiological study of the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy (EGEA, <https://egeanet.vjf.inserm.fr>).

The EGEA cohort is composed of patients with asthma enrolled in chest clinics and their first-degree relatives, and a group of control subjects, all recruited in the early 1990s in 5 French cities. Protocol and characteristics have been previously published. Briefly, 2047 participants were enrolled at baseline (EGEA1) including 388 children (<16 years) and adult patients with asthma from chest clinics, their 1244 first-degree relatives, and 415 population-based control subjects [E2,E3]. Subjects were recruited through self-completed questionnaires and an overall matching by months at examination, age (decade), sex, and center. About 11 years later, this population was invited for a follow-up (EGEA2) and 1601 participants (77.1% of the original cohort + 58 new family members) were subjected to a complete examination [E4]. Written consent was obtained from all individuals. Ethical approval was obtained for both surveys (Cochin Royal Hospital, Paris, for EGEA1 and Necker-Enfants Malades Hospital, Paris, for EGEA2. As reported in more detail In the initial study [E1], 340 sera from different centers (Paris: n = 96; Lyon: n = 75; Marseille: n = 41; Montpellier: n = 22; Grenoble: n = 106) were analyzed. Grass pollen exposure is similar across France, whereas exposure to pollens of birch, cypress, and ragweed exhibits strong geographical variability. The population included 47% men, and the mean age was 43 years. Fifty-two percent of participants had allergic rhinitis ever and 38% had atopic dermatitis ever. Eighty-two participants reported having received some form of allergen immunotherapy (AIT). The core serological information consists of IgE and IgG antibody levels to 47 inhalant and food allergen components measured using the Mechanisms of the Development of Allergy (MeDALL) allergen microarray. Overall 49% of the population had allergen-specific IgE levels of 0.3 Immuno Solid-phase Allergen Chip (ISAC) standardized units (ISU) or more to at least 1 of the 47 allergens with no significant differences across the 5 geographical regions, except for allergens from region-selective pollen.

In the present communication we discuss a subset of those previously described serological results. The IgG data of one subject were not available, leaving 339 subjects. We were concerned regarding changes in antibody levels due to AIT. We did not have information on the details of the treatment, not even on the allergen(s) involved. Since IgG is expected to increase upon AIT, and the subjects are expected to be **IgE⁺**, the IgG level of the **IgE⁺** subjects is expected to increase, and thus the relative IgG level in the **IgE⁻** subjects to decrease. With the possible exception of Der p 23, this is indeed found. However, the RP of high sIgG is significantly less than 1 even if the subjects with an AIT history are excluded. This indicates that the AIT effect is insufficient to explain the observed low IgG in **IgE⁻** subjects. Nevertheless, we excluded these 82 AIT subjects from the main analysis, which is thus based on 257 subjects.

Our focus is on the major airborne allergens, so we excluded results on food and venom allergens and results for minor allergens, i.e. allergens with sensitization prevalence <2.5% (i.e. allergens with less than 7 IgE positive patients in these 257 subjects). This resulted in 31 allergens (16 from pollen, 10 from mites, 1 from a mold, 4 from mammals).

The frequencies of sensitization, gender and age is shown in table E1. Because the initial selection for EGEA was based on asthma rather than on sensitization and prevalence of non-allergic asthma increases with age, sensitization prevalence was low in the older age group. We therefore dichotomized on the median age of the sensitized subjects (32.4 yrs). The overall sensitization prevalence was 44% (young males: 79%, young females 51%, older males 35%, older females 30%).

Details on the Radio Immuno Assay (RIA)

In the paper that formed the basis of the present data analysis on the positive relation between IgE and IgG antibodies to allergens (in this case the grass group 1 allergen from *L. perenne*, Lol p 1, formerly called Rye 1), two groups were distinguished based on the clinical classification “hay fever” or “no hay fever”. IgE antibodies were measured using an antigen-binding procedure analogous to the IgG assay. Both sIgE and sIgG were measured using a “reversed RAST” procedure using purified iodinated allergen in combination with immune precipitation of allergen immune complexes by an anti-immunoglobulin reagent. This assay was less sensitive than the current assays for sIgE. All “no hay fever” patients were IgE-negative, but also one “hay fever” patient was IgE negative. Information on which of the IgG results were from the IgE hay fever patient without detectable IgE was not available. In the present analysis, the distinction is based on the presence or absence of sIgE. We used the worst-case scenario by selecting the highest IgG result among the hay fever patients. This data point is shown in Fig 1b of the main paper as the highest IgG value among the **IgE⁻** subjects.

Effect of the presence of a cat at home

We analyzed the effect of cat allergen exposure on the RP of high IgG to Fel d 1 in both **IgE⁻** and **IgE⁺** subjects. Figure E1 shows the absolute level of IgG to Fel d 1 in all 4 groups (with and without a cat at home, with or without sIgE to Fel d 1). As expected, IgG was higher in subjects with indoor cat exposure (right panel) than in those without indoor cat exposure (left). In both groups the Relative Prevalence (RP) of high sIgG is less than 1. The RP is lower (i.e. more different from 1) if no cat is present at home. This might reflect insufficient exposure in some of the non-allergic subjects [E4]. However, the more interesting finding is that even if a cat is present at home, sIgG is lower in **IgE⁻** subjects than in **IgE⁺** subjects. If allergen exposure was the only factor explaining sIgG levels, **IgE⁻** subjects would presumably be less likely to avoid exposure, and thus to have higher rather than lower sIgG to Fel d 1.

In figure E2 the 3 types of TH2 B cell responses are schematically contrasted.

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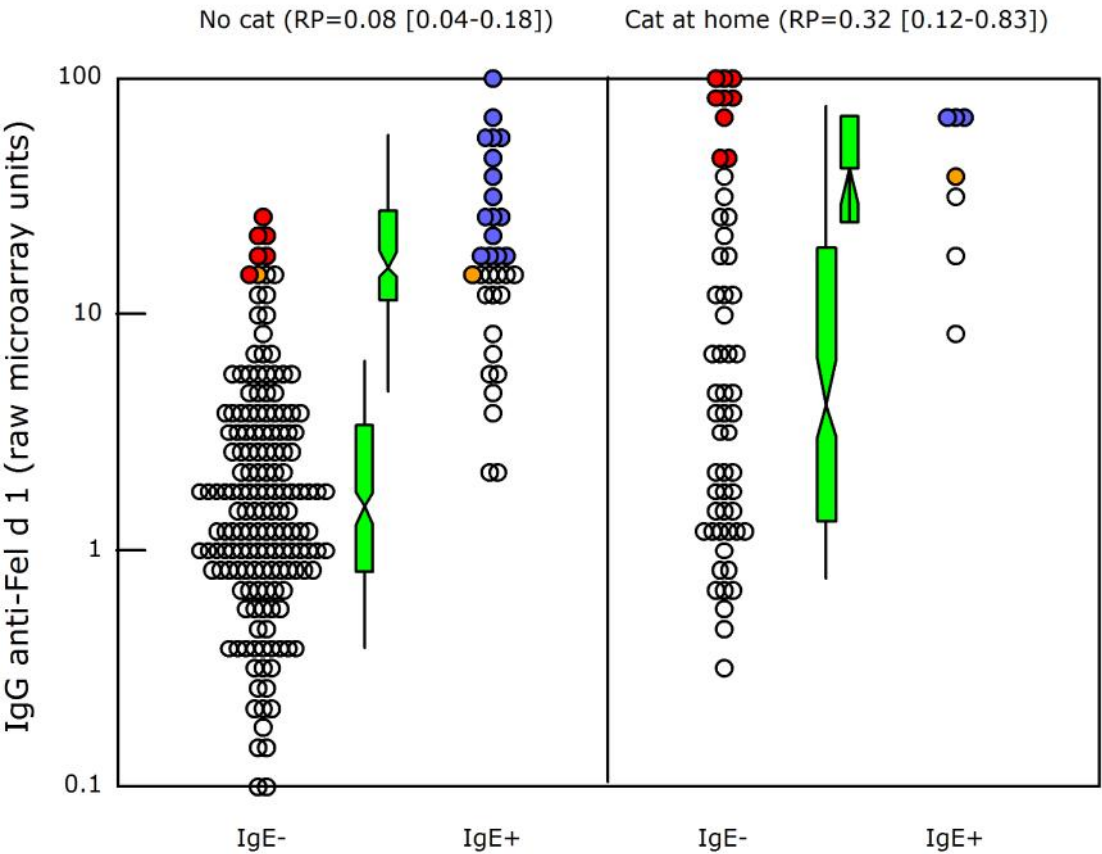
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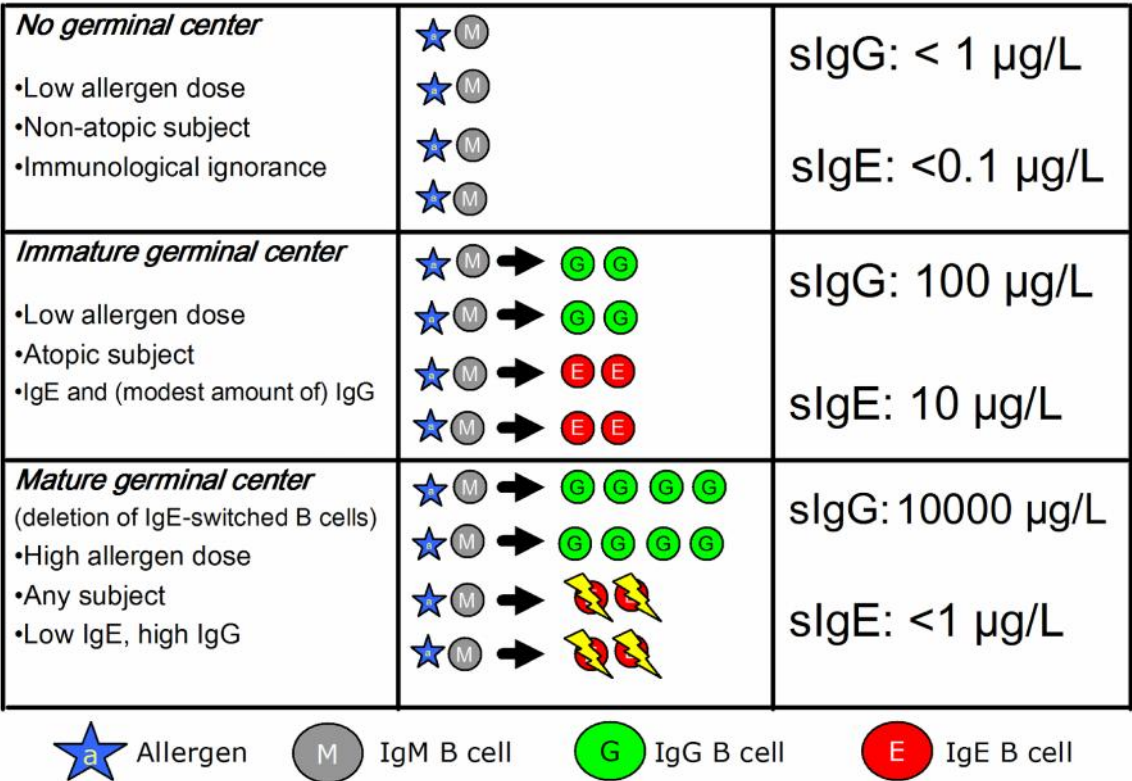
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Figure E1. Effect of indoor exposure to cat on the relative prevalence (RP) of high IgG to the cat allergen Fel d 1 in **IgE⁻** and **IgE⁺** subjects.

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Figure E2. Schematic overview of 3 types of TH2-type B cell responses: immunological ignorance (top), the atopic response (middle) and the modified TH2 response (bottom).

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		1=E+ (N)	2=median G[E+]	3=median G[E-]	4=median ratio	5=GM ratio	6=GM ratio (LL)	7=GM ratio (UL)	8=RP	9=RP (LL)	10=RP (UL)
pollen	Amb a 1	8	23.57	0.24	0.01	0.02	0.01	0.03	0.01	0.00	0.06
	Art v 1	15	4.50	0.10	0.02	0.04	0.02	0.06	0.02	0.00	0.07
	Bet v 1	18	16.92	0.75	0.04	0.07	0.04	0.13	0.04	0.02	0.11
	Phl p 2	25	8.87	0.19	0.02	0.03	0.02	0.05	0.04	0.02	0.11
	Ole e 1	38	7.59	0.68	0.09	0.12	0.08	0.19	0.08	0.04	0.17
	Phl p 11	7	2.14	0.30	0.14	0.37	0.16	0.82	0.14	0.06	0.34
	Pla l 1	10	3.82	0.94	0.25	0.23	0.11	0.47	0.19	0.09	0.39
	Phl p 5	23	8.33	0.71	0.08	0.12	0.06	0.24	0.21	0.12	0.37
	Aln g 1	7	2.17	0.66	0.30	0.22	0.10	0.48	0.26	0.11	0.57
	Phl p 6	20	3.19	0.75	0.24	0.27	0.15	0.48	0.31	0.18	0.53
	Phl p 1	67	8.46	3.37	0.40	0.41	0.31	0.54	0.40	0.28	0.58
	Phl p 4	34	7.08	2.11	0.30	0.27	0.16	0.46	0.44	0.29	0.67
	Pla a 2	11	7.81	3.33	0.43	0.40	0.18	0.91	0.57	0.31	1.06
Mite	Der f 2	49	8.40	1.21	0.14	0.16	0.12	0.22	0.03	0.01	0.09
	Der p 2	49	8.29	0.96	0.12	0.14	0.11	0.20	0.04	0.01	0.11
	Der p 21	14	7.23	3.40	0.47	0.67	0.42	1.09	0.35	0.19	0.62
	Der p 1	43	5.09	2.86	0.56	0.51	0.35	0.73	0.51	0.35	0.74
	Der f 1	42	10.85	7.09	0.65	0.77	0.59	1.01	0.59	0.41	0.85
	Der p 5	23	3.91	2.58	0.66	0.96	0.59	1.54	0.63	0.40	0.99
	Blo t 5	7	4.67	3.84	0.82	0.72	0.36	1.44	0.75	0.35	1.60
	Der p 7	16	3.20	2.27	0.71	0.56	0.29	1.08	0.79	0.47	1.32
	Der p 23	50	12.26	12.54	1.02	0.99	0.69	1.43	1.02	0.75	1.39
Mammal	Der p 4	26	0.27	0.32	1.19	0.80	0.50	1.27	1.10	0.74	1.64
	Alt a 1	15	7.22	0.66	0.09	0.11	0.07	0.17	0.04	0.02	0.11
	Can f 1	7	16.72	0.65	0.04	0.04	0.02	0.12	0.06	0.02	0.16
	Can f 5	17	16.07	1.14	0.07	0.11	0.05	0.22	0.14	0.07	0.27
	Equ c 1	10	7.31	0.98	0.13	0.16	0.07	0.35	0.16	0.08	0.34
	Fel d 1	40	17.70	1.77	0.10	0.12	0.08	0.19	0.17	0.10	0.28

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Legend to table E1

1=E+ (N)	Number of sIgE-positive cases
2=median G[E+]	Median of the sIgG of the IgE-positive cases
3=median G[E-]	Median of the sIgG of the IgE-negative cases
4=median ratio	Ratio of the IgG medians (IgE ⁻ /IgE ⁺)
5=GM ratio	Ratio of the IgG geometric means (IgE ⁻ /IgE ⁺)
6=GM ratio (LL)	Lower 95% confidence limit
7=GM ratio (UL)	Upper 95% confidence limit
8=RP	Relative prevalence of high sIgG (IgE ⁻ /IgE ⁺)
9=RP (LL)	Lower 95% confidence limit
10=RP (UL)	Upper 95% confidence limit