

Antigen avoidance in a mountain environment: Influence on basophil releasability in children with allergic asthma

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Background: The influence of natural antigen avoidance in an environment free of relevant allergens (Istituto Pio XII, Misurina, Italian Alps, 1756 m) and of antigen exposure (sea level) on basophil releasability, as well as on bronchial hyperreactivity (BHR) and specific IgE serum level, were investigated in a group of children with asthma who were allergic to *Dermatophagoides pteronyssinus*.

Methods: Twenty allergic children with asthma participated in the study. Spontaneous and antigen-induced histamine release, BHR, and serum IgE were investigated at the time of admission, after 40 and 80 days of antigen avoidance, and after 15 days of exposure at sea level.

Results: Significant drops in antigen-induced basophil histamine release, BHR, and specific IgE serum level but not in spontaneous basophil histamine release were observed after 40 days of antigen avoidance and were confirmed at a further evaluation after 40 more days. After 15 days of antigen exposure at sea level, specific antigen-induced basophil histamine release, BHR, and serum IgE but not spontaneous basophil histamine release increased promptly, even if not significantly.

Conclusion: In children with allergic asthma, antigen-induced basophil releasability, BHR, and specific IgE serum levels appear to be modifiable by periods of antigen avoidance or exposure. (*J ALLERGY CLIN IMMUNOL* 1993;92:644-50.)

Key words: Asthma, altitude, antigen avoidance, basophil, releasability

Natural antigen avoidance, as achieved for patients allergic to *Dermatophagoides* species when they move from the sea level to a high altitude environment free of the offending allergen, is capable of inducing a reduction in bronchial hyperreactivity (BHR) to nonspecific stimuli.¹⁻⁵

Previous studies showed a good correlation between the degree of BHR and basophil histamine release, supporting the hypothesis that in patients with asthma BHR may be present not only in the bronchi but also in the mediator-releasing system.⁶

The purpose of the present study was to eval-

Abbreviations used

AIHR:	Antigen-induced histamine release
BHR:	Bronchial hyperreactivity
Dpt:	<i>Dermatophagoides pteronyssinus</i>
PC ₂₀ :	Provocative concentration causing a 20% fall in FEV ₁
SHR:	Spontaneous histamine release

uate the influence of a period of natural antigen avoidance in an environment free of relevant allergens on BHR, spontaneous histamine release (SHR), antigen-induced histamine release (AIHR), and specific serum IgE levels.

METHODS

Patients and experimental design

Twenty allergic children with asthma as defined by the American Thoracic Society,⁷ 15 boys and 5 girls, participated in the study. They were enrolled in the study at the time of their arrival at the residential house, Istituto Pio XII (Misurina, Italian Alps, m 1756),

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an environment that is free of *Dermatophagoides* organisms because of low humidity due to altitude. All of the children, aged 10.6 ± 1.7 years (mean $\pm$ SD), had a history positive for bronchial asthma and positive skin prick test results for *Dermatophagoides pteronyssinus* (*Dpt*). Fourteen members of the study group also had positive skin prick test results for seasonal allergens, such as grass pollen or *Parietaria* species.

The study was performed during the autumn and winter, which is out of season for all the seasonal allergens; therefore, it was possible to evaluate changes in the investigated parameters with the exclusion of any specific antigen stimulus other than *Dermatophagoides* species. Baseline evaluations for BHR, specific serum IgE to *Dpt*, SHR, and AIHR from basophil leukocytes were performed for each subject within 48 hours of arrival at the residential house (time A). The same parameters were evaluated again after 40 ± 6 days (mean $\pm$ SD) (time B) and after a further 40 ± 6 days (time C) of stay at high altitude. None of the children who participated in the study returned to sea level from time A to time C. A further evaluation was performed after a period of 15 days of natural antigen exposure, which occurred during Christmas vacation at home (time D).

None of the children had been receiving oral corticosteroids or antihistamines for at least 1 month before and during the study period. Inhaled steroids, cromolyn, and nedocromil sodium were suspended 1 or 2 days before admission to the hospital and throughout time A to C. The above named inhaled drugs were allowed from time C to D, but they were suspended at least 48 hours before time D. Inhaled β_2 -agonists had been allowed when needed throughout the study, but they were suspended at least 24 hours before the time when tests were performed. The study was approved by the Istituto Pio XII Ethical Committee.

Evaluation of respiratory function and bronchial reactivity

Respiratory function was evaluated by a Vitalograph Compact Spirometer (Vitalograph Ltd., Buckingham, England). Forced vital capacity, forced respiratory volume in 1 second (FEV₁), and forced midexpiratory flow were measured.

BHR was evaluated by methacholine bronchial provocation test.⁸ The test was performed with a Mefar dosimeter (Mefar, Brescia, Italy), which can nebulize 0.01 ml of solution in 1.2 seconds, generating particles that range from 0.5 to 3 μ m in diameter. All inhalations were performed by slow inspiration, starting from functional residual capacity without reaching total lung capacity, followed by a 5-second period of breath holding. Before the provocation with methacholine, five inhalations of buffer were performed. One minute after the last inhalation of buffer solution, FEV₁ was recorded again, and the challenge proceeded only if the change in FEV₁ was less than 10%. Provocation was performed on each patient with increasing doses of methacholine. Data were plotted so that concentrations

of inhaled methacholine appeared logarithmically on the abscissa, and percentage decrease of FEV₁, compared with FEV₁ measured after buffer control nebulization, appeared arithmetically on the ordinate. The concentration of methacholine required to produce a fall of 20% in FEV₁, defined as provocative concentration causing a 20% fall in FEV₁ (PC₂₀) (in milligrams per milliliter), was calculated as described by Rosenthal.⁹

Histamine release assay

Venous blood (8 ml) was drawn into a plastic syringe anticoagulated with 0.1 mol/L ethylenediaminetetraacetic acid (1.2 ml) and sedimentated for 90 minutes with 2.4 ml of dextran-dextrose sterile solution. Dextran-dextrose solution was previously prepared with 6 gm of dextran 75 6% (Pharmacia, Uppsala, Sweden) plus 3.12 gm of dextrose (Carlo Erba, Milano, Italy) in 100 ml of saline solution. The supernatant, which contained leukocytes, platelets, and plasma, was transferred to another tube and centrifuged at 250 g for 10 minutes at 4° C. The pelleted cells were resuspended in 10 ml of Tyrode's buffer and centrifuged at 250 g for 10 minutes at 4° C. After two more washes in Tyrode's solution, the cells were resuspended in Tyrode's ACM solution at a final concentration of 90 to 100×10^6 basophils per milliliter. Basophil leukocytes were calculated in duplicate with a Fuchs-Rosenthal chamber (Sarringia, Germany) and alcian blue staining.¹⁰

The histamine release assay was performed in three series of polystyrene tubes in duplicate. Either 0.5 ml of Tyrode's ACM solution, or 0.5 ml of Tyrode's ACM solution plus 11.15 IU of *Dpt* allergenic extract (Bayopharm Italiana s.r.l., Milano, Italy) or 0.5 ml of HClO₄ 6% were added while the tubes were kept in an ice bath. The optimum concentration of antigen for the histamine release assay was determined from a dose-response curve obtained at the beginning of the study,¹¹ and the dose was kept constant throughout the study period. A volume of 0.5 ml of cell suspension was added to each tube, and the racks were transferred to a 37° C shaking bath. After 60 minutes, the reaction was stopped and the tubes were centrifuged at 700 g for 15 minutes at 4° C. The supernatant was collected, added to a 1:1 volume of HClO₄, and centrifuged at 1000 g for 15 minutes to deproteinize the histamine-containing solution.

The solutions were assayed for histamine by a fluorometric automated method according to a modification of the technique described by Siraganian and Brodsky¹¹ in which a histamine Autoanalyzer II (Technicon Industrial System, Terrytown, N.Y.) was used. Histamine content per basophil was calculated quantitatively by comparison with standard samples.

Results for basophil histamine release were expressed as percent of basophil histamine total content. AIHR values were calculated as the difference of total histamine release of antigen-challenged basophils minus SHR. The sensitivity of this method in our laboratory is 0.6 ng/ml. The intraassay coefficient of variation

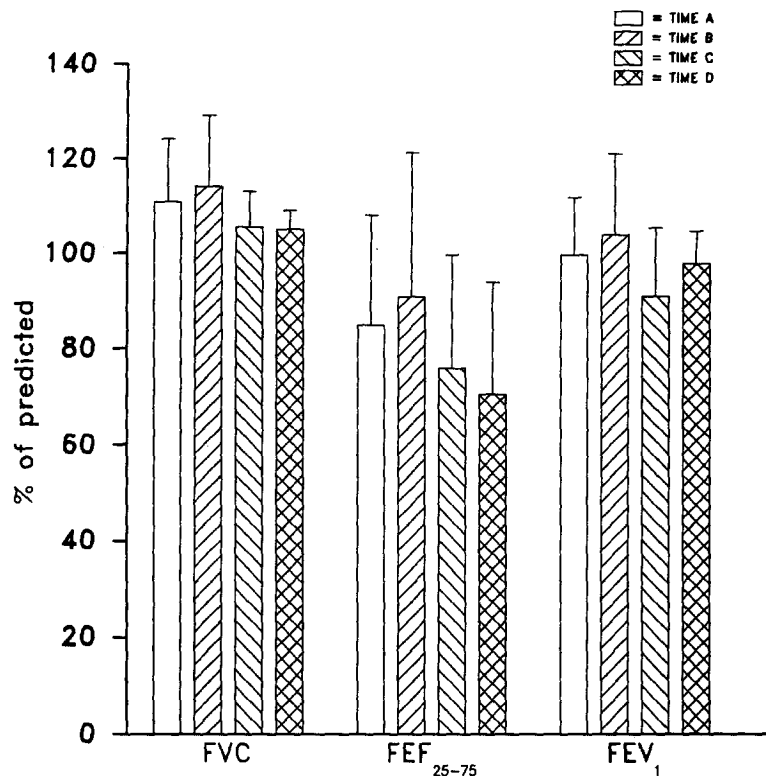


FIG. 1. Mean values for pulmonary function parameters at different times in the study.

is $\pm 2.2\%$ at 20 ng/ml, and the interassay coefficient of variation is $\pm 5.1\%$ at 20 ng/ml.

Specific IgE assay

Venous blood (3 ml) was collected from antecubital veins and assayed for *Dpt*-specific serum IgE with the use of the CAP System FEIA (Pharmacia). The intraassay coefficient of variation is $\pm 5\%$, and the interassay coefficient of variation is $\pm 8.6\%$.

Statistical analysis

Data were compared by means of the Wilcoxon test. Differences were considered significant when the p value was less than 0.05. Correlations between parameters were determined by means of Spearman's rank correlation test.

RESULTS

All of the children admitted to the study ($n = 20$) participated to time A and time B determinations. Four children were discharged from the residential house after time B, and nine more children were discharged after time C. All of these subjects were excluded from the study at the time of their discharge. Comparisons between different times of the study have been performed on the paired data, including values from the

subject actually participating in the study at both times of each comparison. Therefore A versus B comparison included 20 subjects; A versus C and B versus C included 16 subjects; A versus D, B versus C, and C versus D comparisons included seven subjects.

Data for respiratory function are plotted in Fig. 1. No significant modification in forced vital capacity, FEV₁, or forced midexpiratory flow was observed at the different times in the study.

Results for PC₂₀, specific serum IgE, SHR, and AIHR are summarized in Fig. 2.

PC₂₀ values (mean $\pm$ SEM) increased from a baseline value of 3.276 ± 0.946 mg/ml (time A) to a value of 6.766 ± 1.869 mg/ml after 40 days of antigen avoidance (time B) ($p < 0.005$) and further increased at time C, reaching a value of 7.387 ± 2.115 mg/ml ($p < 0.005$ vs time A, NS vs time B). After the short time of antigen exposure (time D), mean PC₂₀ value was 7.136 ± 3.322 mg/ml (NS vs time C). Serum-specific IgE concentrations (mean $\pm$ SEM) were 58.485 ± 7.059 kU/L time A, 45.067 ± 5.711 kU/L at time B ($p < 0.001$ vs A), 48.722 ± 8.093 kU/L at time C ($p < 0.02$ vs A, NS vs B), and 62.629 ± 8.055 kU/L at time D (NS vs A, B, C).

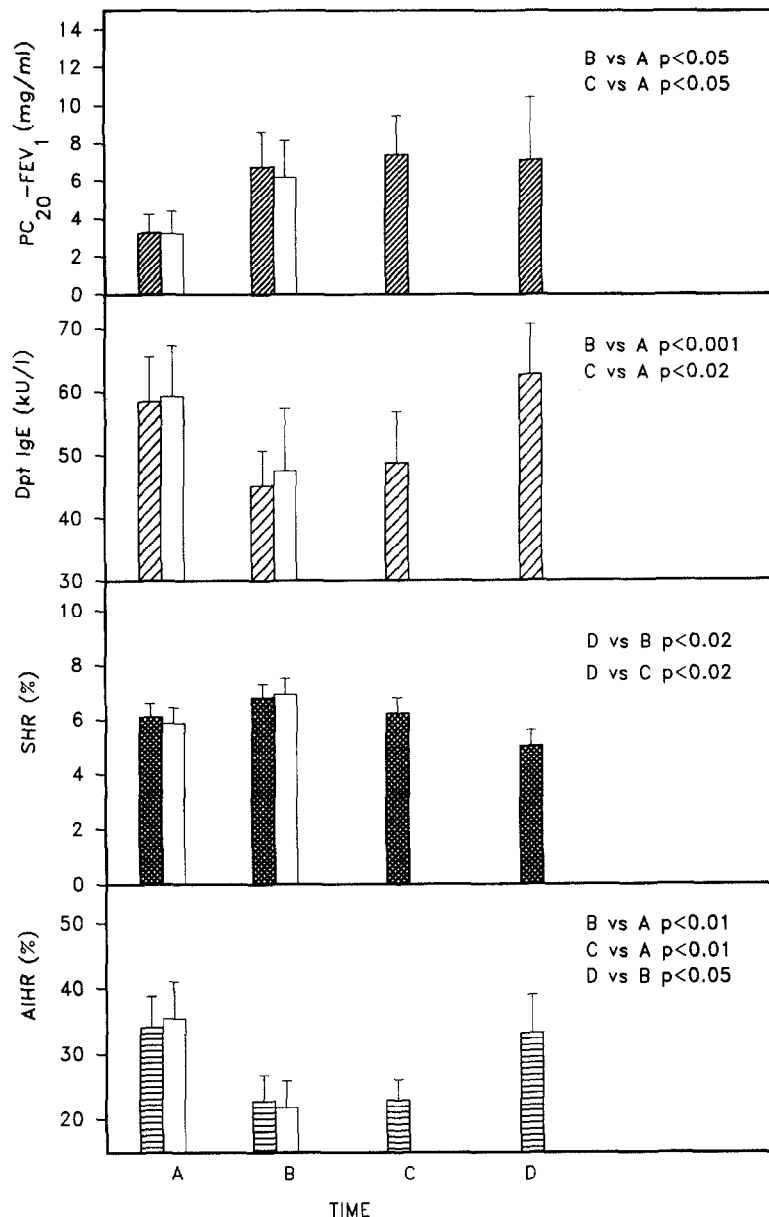


FIG. 2. Mean values (±SEM) of BHR, serum-specific IgE to Dpt (Dpt IgE), SHR, and AIHR at different times in the study. Significant changes are shown. Open bars at times A and B represent mean values including only patients who were still present at time C ($n = 16$).

SHR (mean ± SEM) was $6.08\% \pm 0.512\%$ at time A, $6.770\% \pm 0.543\%$ at time B (NS vs A), $6.200\% \pm 0.561\%$ at time C (NS vs A and B), and $5.029\% \pm 0.569\%$ at time D (NS vs A, $p < 0.02$ vs B, $p < 0.02$ vs C). AIHR (mean ± SEM) was $34.183\% \pm 4.714\%$ at time A, $22.76\% \pm 4.012\%$ at time B ($p < 0.01$ vs A), $22.90\% \pm 3.19\%$ at time C ($p < 0.01$ vs A, NS vs B), and $33.271\% \pm 5.776\%$ at time D (NS vs A and C, $p < 0.05$ vs B). Histamine quantity per basophil (mean ± SEM) was 0.368 ± 0.013 pg at time A,

0.340 ± 0.018 pg at time B, 0.344 ± 0.016 pg at time C, and 0.393 ± 0.043 pg at time D; no significant difference was observed among the different times in the study.

Possible correlations between the different parameters were investigated at time B because at this time in the study we had the highest number of subjects and none of them had been receiving any drug, other than inhaled β_2 -agonists, for at least 48 hours before the beginning of the study. The results of correlation tests are shown in Fig.

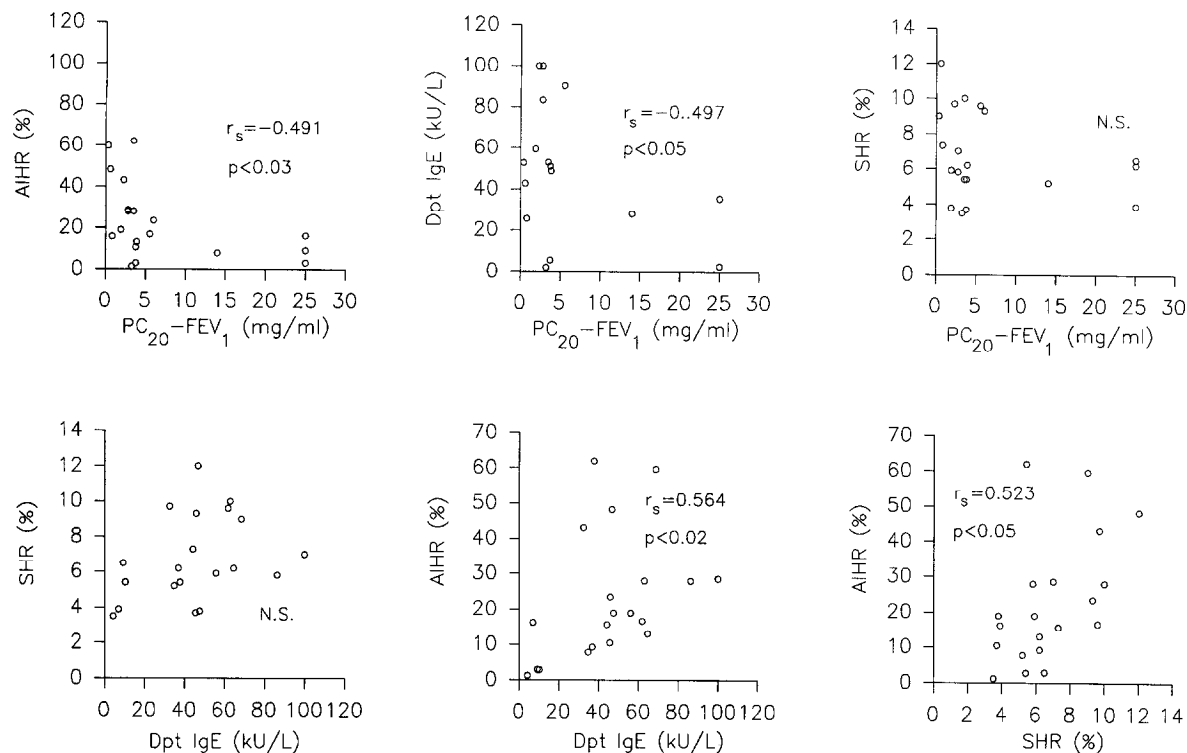


FIG. 3. Scatter diagrams showing correlations between parameters.

3. Significant correlations were found between specific IgE serum level and PC_{20} ($r_s = -0.4970$, $p < 0.05$), AIHR and PC_{20} ($r_s = -0.4912$, $p < 0.03$), specific serum IgE and AIHR ($r_s = 0.5643$, $p < 0.02$), and SHR and AIHR ($r_s = 0.5233$, $p < 0.05$). No significant correlation was found between specific IgE and SHR or between PC_{20} and SHR. No correlation was observed between histamine release and histamine content per basophil.

DISCUSSION

The routine around-the-year analysis of samples from mattresses in the residential house, Istituto Pio XII, located in an alpine environment at 1756 m, always failed to reveal the presence of mites. In contrast, evaluation of mite content in the dust from the houses in the area served by the residential house, performed by guanine test (ranging from 0 = no mites to 3 = high level of antigen), showed a level of positivity (mean $\pm$ SEM) of 1.72 ± 0.076 , independent of the season. This suggests a relevant presence of mites in the houses, as a result of the high humidity in this area of Italy. Therefore a prolonged stay in the residential house, free of mites because of the low humidity characteristic of the alpine environment

and because of an accurate control of the indoor temperature, which is constantly between 20° C and 22° C,¹² represents an ideal measure of antigen avoidance for children with asthma who are allergic to *Dermatophagoides* species. In addition, the migration of children from sea level to the alpine environment and back again, as during summer or Christmas vacations, offers a natural antigen avoidance/exposure challenge model. This model allows us to investigate whether different parameters may be influenced by a natural alternation of avoidance and exposure to mites.

The results of the present study confirm our previous reports showing that bronchial responsiveness³ and specific IgE serum levels¹³ are reduced by a prolonged stay in the alpine environment. The PC_{20} mean value significantly increased after the first 40 days of antigen avoidance, and the increasing trend was maintained during the time of the stay in Misurina; however, the short period of exposure at home during the Christmas holidays did not allow any significant increase in bronchial reactivity (Fig. 2). The lack of a significant increase in bronchial reactivity from time C to time D may be explained not only by the short period of antigen exposure but also by the possibility for use of inhaled steroids and/or cromolyn

or nedocromil sodium during this part of the study according to the specific needs of each patient. In a similar fashion, specific IgE antibodies to *Dpt* significantly decreased after the first part of the avoidance period. The maximum fall in IgE levels was observed within the first 40 days of exposure, because of the average half-life of 2.3 days.¹³ However, a level of IgE antibody persisted even during the period of antigen avoidance and increased after environmental exposure, which is typical for allergic subjects.¹⁴ The increase in mean specific IgE level observed at time C in comparison with that observed at time B is likely to be due to the significant number of subjects who dropped out of the study at time C. In fact, as shown in Fig. 2, if only data for patients present at both times are examined, there is no significant modification. The lack of statistical significance for the increase of IgE levels between times C and D in our study population is probably due to the very low number of subjects who were able to complete the last part of the study ($n = 7$). Effectively, as shown in Fig. 2, the mean specific IgE serum levels after the period of antigen exposure reached a value even higher than baseline.

SHR determinations show an almost flat-shaped curve during the time of the study. A slight but significant reduction of SHR, demonstrated after the 15 days of antigen exposure during the Christmas holidays, is difficult to explain even in consideration of biologic fluctuation. Furthermore, in the present study we have obtained low SHR levels, which were less than 10% in all but in two cases; therefore, these levels were within the normal range.¹⁵ The real significance of SHR in patients with asthma has been discussed extensively in the literature. Neijens et al.⁶ found a correlation among basophil SHR and bronchial reactivity and exercise-induced bronchial reactivity. The same authors, in a further study, suggested that similar microtubule-correlated mechanisms may be responsible for both bronchial and basophil hyperresponsiveness in patients with asthma.¹⁶ In contrast, in a recent study by Wahn et al.,¹⁷ an increased SHR in patients with asthma has been observed only in a subgroup and only in "washed" cell preparations but not in whole blood. The authors suggested that SHR in the absence of any stimulus is unlikely to be a relevant mechanism for the pathogenesis of asthma in vivo.¹⁷ Furthermore, on the basis of the present data, according to the report by Akagi and Townley,¹⁵ we were unable to find any significant correlation between serum IgE level and SHR. How-

ever, the correlation test performed in our group of patients, which showed a positive correlation between SHR and AIHR, suggests that basophils with higher spontaneous releasability are somewhat more prone to degranulate under antigen stimulus. Nevertheless, the interpretation of SHR remains very difficult, because it is only an in vitro phenomenon and results appear to depend critically on how the cells are handled. Thus results from different studies are difficult to evaluate and compare.

In contrast to SHR, AIHR in our experimental design appears to be highly influenced by the natural exposure/avoidance regimen and closely related to IgE serum levels and to BHR and IgE serum levels. These findings confirm and further extend a previous observation by Gamboa et al.¹⁸ who showed that patients allergic to *Dpt* release more histamine during those periods of the year when mite growth is facilitated by the environmental conditions and demonstrated a close relationship between AIHR and the patients' clinical situations.¹⁸ The level of antigen exposure in the patients' houses was not assessed specifically for the present study; however, the results about specific IgE to *Dpt* and AIHR suggest either that the level of antigen exposure is significantly greater in the patients' houses than in the residential house in the Alpine environment or, less likely, that changes of the investigated parameters and the level of antigen exposure are not related at all.

In conclusion, AIHR, but not SHR, appears to be directly and promptly modifiable by periods of antigen avoidance or exposure in subjects with asthma.

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