

Lymphocyte transformation test with house dust mite (*Dermatophagoides pteronyssinus*) in normal children, asthmatic children and asthmatic children receiving hyposensitization

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Summary

In the first part of this study the proliferative response of lymphocytes (lymphocyte transformation test) to house dust mite (HDM) stimulation in cultures was studied in normal children ($n=16$), asthmatic children who never received hyposensitization (HS) ($n=50$) and asthmatic children receiving HS with HDM for at least 6 months ($n=20$). The results are expressed as disintegrations per minute (d.p.m.) and as stimulation index ($SI = \text{d.p.m. in the presence of the allergen} / \text{d.p.m. in the control culture}$). A positive SI (> 2) was found in 54% of the asthmatic children who never received HS, in 30% of the asthmatics receiving HS and in none of the normal children. Furthermore, between asthmatics with and without HS, the SI was not statistically different, although asthmatics without HS tended to have a higher SI (median value: 2.13 vs 1.38) ($P=0.10$). In a second series of experiments the effect of adding interleukin-2 (IL-2) to the lymphocyte cell culture was studied in asthmatic children with and without HS. Interleukin-2 induced an additional stimulatory effect on the lymphoproliferative response to HDM and to phytohaemagglutinin in patients who never received HS, but had no effect in patients receiving HS. We conclude that HS treatment seems to have an inhibiting effect upon this proliferative response, not only inhibiting the degree of the allergen-induced lymphocyte proliferation, but also inhibiting the sensitivity of proliferating lymphocytes for IL-2. These inhibiting effects upon lymphocytic activation could be responsible for the anti-inflammatory effects (i.e. suppression of the late asthmatic reaction) of HS.

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Introduction

Several studies have shown that allergic sensitization to house dust mite (HDM) (*Dermatophagoides pteronyssinus*) is the most common allergy in asthmatics in most parts of the world, affecting between 60 and 85% of patients as compared with 5–15% in control groups [1–5]. Moreover, there is evidence from avoidance studies and from bronchial provocation studies that HDM allergens are a major cause of bronchial hyperreactivity and asthma [6–8].

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Several studies have demonstrated a specific response of lymphocytes, reflecting T-cell sensitization, from HDM-sensitive subjects to allergens from HDM [9–17]. However, the specificity of this type of response is still a matter of debate [18–20]. Recently, a major development has been the isolation of mite-specific T-cell lines or clones by several groups [21]. Furthermore, the effect of hyposensitization (HS) with an allergen upon the *in vitro* lymphocytic transformation showed conflicting results [12,19]. Nevertheless, further study of this *in vitro* test could help the understanding of the underlying mechanisms of allergic asthma, as the regulatory function of T-lymphocytes in chronic asthma and in the late phase

found between atopic patients and normal persons [18,19]. Furthermore, recently O'Brein *et al.* [30], studying peripheral T-cell responses to the purified HDM allergens *Der p* I, *Der p* II and *Der f* II, showed that only HDM-sensitive patients responded to these mite allergens.

In the group of asthmatics who never received HS, no difference was found between the children with a positive SI and children with a negative SI for total IgE, specific IgG against HDM, severity scale of asthma and blood eosinophilia. This is in contrast with studies showing a relationship between the allergen-induced lymphoproliferative response and levels of specific IgE [12,14,30,31]. Also, the type of medical treatment seems to have no influence upon the LTT values as there was also no difference in SI value whether the children were treated with DSCG, inhaled corticosteroids or beta-agonists. However, this could be due to the withholding of anti-asthmatic treatment before blood sampling.

The SI of asthmatics without HS was higher than the SI of normal children. Between asthmatics without HS and asthmatics with HS, the SI was not statistically different, although asthmatics without HS tended to have a higher SI than asthmatics with HS ($P=0.10$). There was also no difference in the SI between normal children and asthmatics with HS. Furthermore, more asthmatics without HS tended to have a positive SI than asthmatics with HS (54% vs 30%; $P=0.12$). These data suggest, although it was not statistically confirmed, that only asthmatics who never received HS have a higher SI than normal children, providing evidence that the proliferative capacity of lymphocytes after exposure to the allergen, expressed as the SI, decreases following the administration of HS. Nevertheless, conflicting results have been published concerning the effects of HS upon the LTT. Rocklin *et al.* [32] reported that HS with ragweed extract suppressed lymphocyte proliferation in response to ragweed, apparently due to the generation of suppressor lymphocytes. In a study by Cavaillon *et al.* [19], studying HDM-sensitive atopics, no difference in the LTT was found between atopic patients receiving or not receiving HS, while in another study on HDM asthmatics it was found that the LTT with *Dermatophagoides farinae* was significantly lower in patients with long-term HS than in patients receiving no HS [12].

Additional proliferation of HDM-sensitized lymphocytes after subsequent incubation with IL-2 has been reported previously in asthmatic children [33,34]. In the present study we looked at the effect of subsequently adding IL-2 to the cell cultures incubated with HDM or with the non-specific mitogen PHA. In accordance with previous reports, an additional lymphoproliferative response was detected in children who never received HS, whether the cell culture was incubated with HDM or

PHA [33,34]. On the other hand, this effect could not be observed in asthmatic children receiving HS, suggesting that HS diminishes the sensitivity of lymphocytes to activation (i.e. cell division and proliferation) by IL-2 after allergen (specific) and mitogen (non-specific) stimulation. However, these results should be interpreted with caution as the study group of asthmatics receiving HS was small ($n=8$), which could result in a possible type II error (i.e. a negative result due to the small number of subjects).

Although the exact mechanisms of this decreased sensitivity of lymphocytes towards IL-2 caused by HS remain speculative, one possible explanation might be that HS induces the dominance of other sub-populations of lymphocytes (i.e. CD8-positive T-cells), unresponsive to IL-2, since Yoshizawa *et al.* [33] could demonstrate that the responder T-cells to IL-2 are very likely to be members of the CD4-positive subset [17,35,36]. In other studies, also in agreement with the present results, a decreased expression and release of IL-2 receptor by HDM-stimulated lymphocytes was shown in asthmatic children receiving HS [27,37,38]. Taken together, most studies, including the present study, show an inhibiting effect of HS on the allergen-induced proliferation of lymphocytes. Furthermore, the present results suggest a decreased sensitivity to IL-2 of HDM-specific T-cells induced by HS. As it has been shown that HS inhibits the occurrence of the late asthmatic reaction, it can be assumed that this *in vivo* inhibition of HS is due, at least in part, to an inhibiting effect of HS upon lymphocytic activation (i.e. proliferation and IL-2 sensitivity) induced by the allergen [22,23,25].

In conclusion, the present study demonstrates that about 50% of HDM-sensitive asthmatic children experience a specific HDM-induced proliferation of lymphocytes, suggesting the existence of circulating sensitized T-cells of which the exact role in the pathogenesis of allergic asthma remains to be established. Hyposensitization treatment seems to have an inhibiting effect upon this proliferative response, not only inhibiting the degree of the allergen-induced lymphocyte proliferation, but also inhibiting the sensitivity of proliferating lymphocytes for IL-2. These inhibiting effects upon lymphocytic activation could be responsible for the anti-inflammatory effects (i.e. suppression of the late asthmatic reaction) of HS.

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