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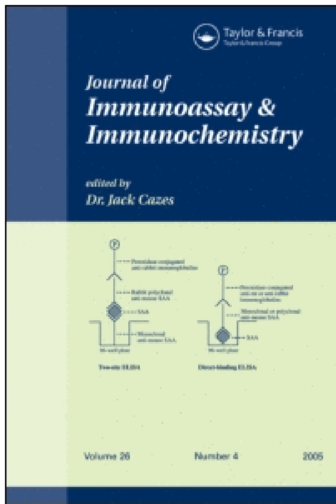

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IL-6 SECRETION BY EX VIVO WHOLE BLOOD CULTURES UPON ALLERGEN STIMULATION

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ABSTRACT

Allergen induced IL-6 synthesis by whole blood cultures was compared with skin prick allergen test results for the same group of individuals. Whole blood cultures from both allergic and non-allergic individuals secrete IL-6 at high allergen concentrations. When whole blood cultures from controls were incubated with serial dilutions of allergens it was found that IL-6 induction was abolished at lower allergen dilutions (allergen threshold concentration or ATC). When whole blood cultures from patients with allergic rhinitis were stimulated with ATC it was found that some allergens induced IL-6 secretion.

The allergens inducing IL-6 and the level of IL-6 secreted were dependent on the patient. The induction of IL-6 secretion by the cultures at ATC correlated very significantly with the patient's skin prick test results ($r = 0.711$; $p = 0.0003$).

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INTRODUCTION

Due to the central role of IL-6 in antibody production, several studies have been conducted to investigate the role of IL-6 in allergies. Studies done on allergic rhinitis patients have shown that their nasal secretions contain higher levels of IL-6 than those from control patients.(1) When allergic rhinitis patients were challenged with allergen, the level of IL-6 in their nasal secretions showed a further increase. Control patients did not show this increase in IL-6 activity.(1,2) *In vitro* studies using bronchial epithelial cells have shown that histamine is a potent stimulator of IL-6 secretion by these cells.(3) The process by which the IL-6 secretion is induced is histamine dose-dependent and occurs via the histamine H1 receptor.(3) The secretion of IL-6 is inhibitable by cycloheximide, indicating that histamine induces the secretion of newly synthesised IL-6. Other studies have also shown that the level of IL-6 secretion corresponds to the symptom score of these patients.(4) Patients with a single early phase response to allergen, exhibit a single early phase IL-6 peak, while dual responders i.e. patients with both an early and a late reaction to allergen show an early peak in IL-6 secretion followed by a late IL-6 peak.

Allergic contact dermatitis is also associated with increased IL-6 levels in plasma.(5) Experimental animal models of contact dermatitis have shown that there is an increase in IL-6 level in the draining lymph node cells upon sensitisation of the animal with chemicals.(6)

Studies have indicated that IL-6 secretion can be activated in monocyte cultures by the addition of specific allergens.(7,8,9) These authors have shown that the induction of IL-6 synthesis is mediated via an Fc receptor-mediated mechanism. Both allergen and IgE, specific for the allergen, must be present in the culture medium to induce IL-6 secretion.(7) These studies also showed that cells from non-allergic controls could be induced to secrete IL-6 upon allergen challenge by incubating these cells with IgE specific for the allergen prior to allergen addition.(7) This method is a very sensitive bio-assay for screening patients for specific allergies and avoids the danger of potential anaphylactic shock that is always a possibility when using *in vivo* skin prick tests for allergen screening.

This method has also been used to determine the efficacy of anti-allergic and anti-inflammatory drugs.(10) Successful drug treatment decreases the level of IL-6 secretion by cultured peripheral blood cells upon allergen challenge and by using this method of drug screening, several different drug preparations can be screened in parallel for effectiveness using patient blood from a single donation.

The aim of the present study is to investigate whether whole blood cultures (WBC) can be induced to secrete IL-6 upon stimulation with

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allergens and whether results obtained from the IL-6 induction correlated with data obtained using the skin prick test for the same allergens in the same individuals.

EXPERIMENTAL

Allergen Dilution

Allergen preparations in glycerol specifically manufactured for skin prick tests were used for all assays (Bayer, South Africa). Following are the allergens used and their respective Bayer catalogue numbers in brackets: grass mixture (2619), dog hair and dander (4084), Bermuda grass (1142), cat hair and dander (4810), tree mixture (2620), house dust mite (6692) and feather mixture (4350). Allergens were used as is for the skin prick test. For WBC the allergens were diluted with RPMI.

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Determination of Allergen Threshold Concentration (ATC) Using WBC from Non-allergic Patients

Assays were performed in 96-well culture plates (Nunc). Serial dilutions of allergens (skin prick inhalant allergens, Bayer, South Africa) in RPMI (Highveld Biologicals, South Africa) were added to wells (100 μL per well). One hundred μL of whole blood collected from a healthy donor was added to each well. The plate was then sealed with plastic wrapping and incubated at 37°C for 18 hours. At the end of the culture period the culture supernatants were assayed for IL-6 using an in-house ELISA assay as described previously.(11) The ATC was regarded as the highest concentration of allergen that did not induce IL-6 synthesis and this allergen concentration was used for subsequent assays using WBC from allergic individuals.

WBC to Monitor Allergen Induced IL-6 Production in Rhinitis Patients

Patients have been diagnosed as rhinitis sufferers by a general practitioner, upon looking at the patients' symptoms and clinical histories. Heparinised blood samples from consenting patients were submitted to the Microbiology Department, Tygerberg Hospital, by the general practitioner for allergen sensitivity screening. One hundred μL RPMI containing

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allergen at ATC was added to wells of 96 well culture plates. One hundred μL of heparinised blood from patients was added to each well. The plate was incubated at 37°C for 18 hours. At the end of the culture period the culture supernatants were assayed for IL-6 using an in-house ELISA assay as described previously.(11)

Skin Prick Test (SPT) for Allergies

SPT's were done using the same Bayer allergens (as used in the *in vitro* IL-6 release assays), according to the manufacturer's instructions. The tests were conducted by the general practitioner providing the blood samples. Testing was done on the volar surfaces of the arms from the axilla to 2.5 cm above the wrist. Hairy areas were avoided due to difficulty of interpreting results in these areas. The area to be tested was cleaned with alcohol, after which a drop of allergen was placed on the skin. Allergen drops were placed 2.5 cm apart. The area below the drop was then scarred using the scarifiers supplied with the allergens. Only the outer layers of the skin were disrupted. After ten minutes, excess allergen was wiped off the area and the size of the weals and erythmas were recorded.

The grade of SPT reactivity was classed according to the size of the erythma. Grade 0 indicated an erythma size less than 11 mm, grade 1 indicated an erythma size between 11 and 20 mm, grade 2 indicated an erythma size between 21 and 30 mm, grade 3 indicated an erythma size between 31 and 40 mm and grade 4 indicated an erythma size between 41 and 50 mm.

Statistical Methods

All values are representative of the mean of triplicate assays. Statistical analysis was performed on the variables using the Student's two-tailed *t*-test. Regression analysis and *p* values were calculated using the Microsoft Excel computer program.

RESULTS

The Effect of Allergen Concentration on IL-6 Secretion by WBC from Control Subjects

IL-6 induction occurs at high allergen concentrations for all the allergens tested (Figure 1A and B). High levels of IL-6 can be detected in the

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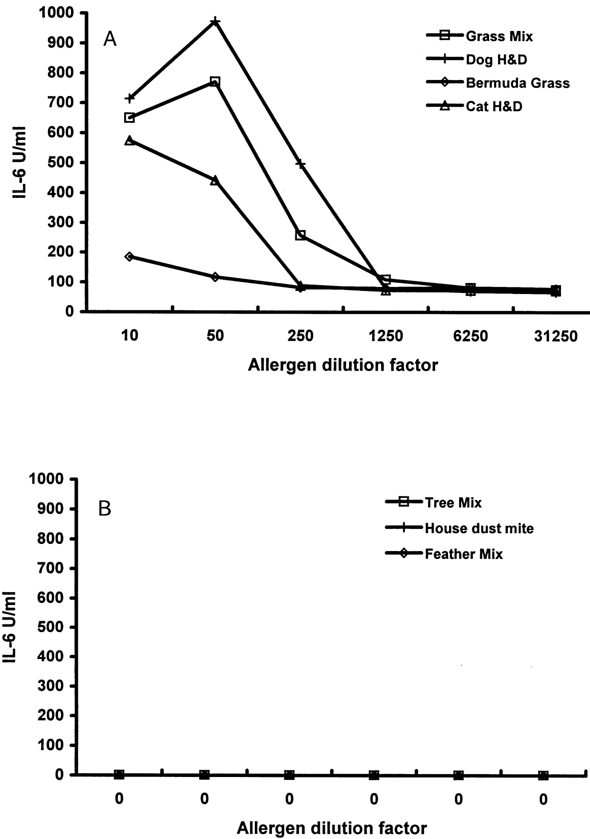

Figure 1. The effect of allergen concentration on IL-6 secretion by WBC from non-allergic subjects. WBC's from non-allergic subjects were incubated with different allergen concentrations. The amount of IL-6 secreted by the cultures were measured by ELISA.

supernatants from WBC at allergen dilutions less than 1/50. Serial dilution of the allergens resulted in decreased IL-6 secretion by the WBC. The allergen concentration giving no IL-6 induction was different for all the allergens tested and repeat assays with other non-allergic control patients gave similar results. The lowest dilution of allergen resulting in no IL-6 secretion (ATC) was selected for comparative assays with skin prick tests. The ATC were as follows: 1/1000 for grass mixture, 1/500 for dog hair and dander, 1/125 for Bermuda grass, 1/250 for cat hair and dander, 1/2000 for tree mixture, 1/125 for house dust mite, and 1/20000 for feather mixture.

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Allergen Induced IL-6 Secretion by WBC from Rhinitis Patients

WBC stimulated with some of the allergens at ATC resulted in IL-6 secretion. The allergens causing IL-6 secretion were patient specific. The level of IL-6 induced by a particular allergen was also dependent on the patient, i.e., the same allergen causing no IL-6 secretion in one patient could stimulate very high levels of IL-6 secretion in another. Repeat assays done three months after the initial WBC assay resulted in similar IL-6 induction patterns. Figure 2 (upper panel) is a typical diagrammatic representation of the IL-6 induction pattern for one of the rhinitis patients.

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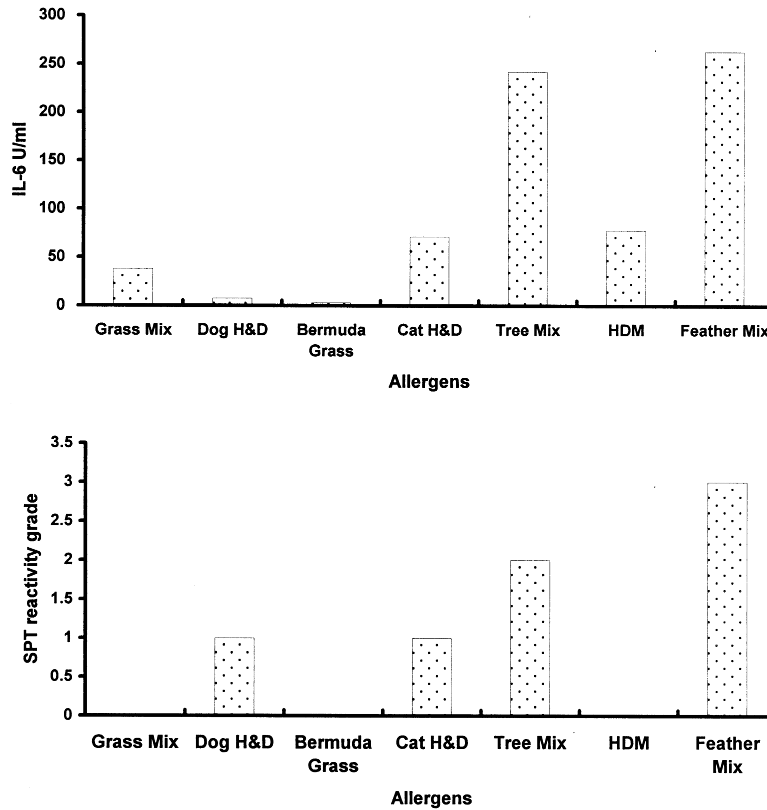

Figure 2. Comparison between SPT and IL-6 secretion results for allergic subjects. Upper panel: WBC's from an allergic subject were incubated with different allergens at ATC. The amount of IL-6 secreted by the cultures were measured by ELISA. Lower panel: SPT results for the same subject.

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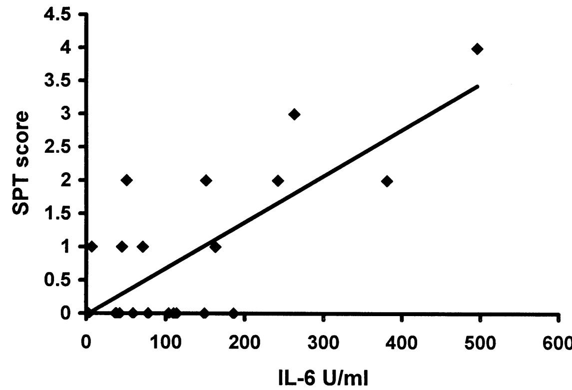

Figure 3. Comparison between SPT and WBC allergen screening results obtained for patients (number of patients = 3; number of points = 21) with allergic rhinitis.

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SPT on Rhinitis Patients

The three rhinitis patients tested by SPT all showed multiple allergen reactivities. Figure 2 (lower panel) is a diagrammatic representation of SPT results obtained for one of the patients tested. The results presented in Figure 2 (upper and lower panels) are from the same patient. By comparing these figures it can be seen that the allergens causing the highest grade of SPT reactivity are also the ones causing the higher levels of IL-6 induction by WBC from the same patient. At lower allergen reactivities the relationship between the two methods became less well defined when results obtained from a single patient are compared.

Comparison Between the WBC and SPT Test for Allergen Reactivity

Linear regression analysis was performed on the data (total data points = 21) using the Microsoft Excel computer program (Figure 3). The results shows that there is a highly significant relationship between the level of IL-6 induced using allergen stimulation of WBC and the SPT for allergens ($r = 0.711$ and $p = 0.0003$ two tailed).

DISCUSSION

The IL-6 induction assay that was used for the above experiments examined whether specific allergens induced inflammatory events in WBC

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from rhinitis patients. IL-6 secretion was used as a marker for this response due to its known central role, as pro-inflammatory cytokine, in the inflammatory response.

The study shows that allergen specifically stimulate WBC to synthesise IL-6 at ATC when the patient shows high SPT reactivity to that specific allergen. The very significant correlation between allergen induced IL-6 secretion by WBC and SPT shows that the WBC assay could become a useful alternative to the SPT whenever there is the possibility that a patient might go into anaphylactic shock upon SPT. Presently *in vitro* assays for allergen reactivities are all immunochemical in nature. These assays test for the presence of plasma IgE antibodies against the specific allergen and as such only test for IgE mediated or immediate allergic reactions. The WBC assay is an *in vitro* bio-assay for allergen reactivity. In this assay the *in vivo* response to allergen is mimicked by an *ex vivo* blood culture system.

The results presented in this paper confirm earlier reports that indicated an IgE-Fc dependent IL-6 induction mechanism when isolated monocytes from allergic patients were stimulated with specific allergen.(7) Studies with asthmatics have shown that allergen challenge caused an increase in CD14, the cell surface endotoxin receptor and LBP an endotoxin binding protein present in plasma of these subjects, while having no effect on the levels of these proteins in non-asthmatic subjects (12). The increase in CD14 and LBP increase endotoxin sensitivity and could induce these patients to mount an inflammatory response to levels of environmental endotoxins that would not normally induce inflammation. The WBC assay detects both IgE and endotoxin mediated inflammatory reactions and might thus be able to detect some of the non-IgE mediated allergic responses that are possibly due to allergen induced CD14 and LBP. The present study, in fact, also showed ($n = 7$, Figure 3) that, when an individual does not react on the skin prick assay, the whole blood cultures from that individual can still mount an inflammatory response to the specific allergen, indicative of a non-IgE mediated response.

Using WBC instead of isolated monocytes simplifies the allergen induced IL-6 procedure for testing allergen reactivity by eliminating cell separation methods. It also eliminates the possibility of contamination or activation of the monocytes that commonly occurs during monocyte isolation. This method, because of the presence of all the blood components, more closely resembles the *in vivo* situation than isolated monocytes. The amount of whole blood required to do a single assay was 100 μL and a single 5 mL donation of heparinised blood can be used to screen a maximum of 25 allergens in duplicate.

This study needs to be extended using more patients, since very few SPT and IL-6 results could be compared. Once properly validated, this assay

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could become a useful alternative to IgE assays for screening allergen reactivity. Studies are underway to screen more patients so that a larger number of points can be used for statistical analysis and validation of the WBC assay as an alternative test for allergen reactivity.

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