

SHORT COMMUNICATION

SEASONAL VARIATION IN INTERLEUKIN 4 IN PATIENTS WITH HAYFEVER

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This study was performed to investigate the value of interleukin 4 as a marker of activity in mild atopic disease. We compared IL-4 levels to eosinophil cationic protein (ECP), a suggested inflammatory marker in allergic disease, in patients with hayfever. Patients with hayfever were assessed during January and then in late June at the height of the grass pollen season, and their levels of serum ECP and IL-4 compared. Serum ECP was determined by radio-immunoassay and serum IL-4 by a high-sensitivity enzyme-linked immunosorbent assay. ECP was found to increase significantly in patients with hayfever during the grass pollen season ($P<0.01$). Conversely, serum levels of IL-4 were found to decrease significantly over the same period when compared with winter values. ECP and IL-4 were not seen to correlate significantly with each other. The fall in serum IL-4 seen during the grass pollen season in the hayfever patients may reflect allergen driven upregulation of membrane IL-4 receptor expression or sequestration of cytokine producing cells to inflammatory sites. These findings suggest that serum IL-4 is a poor indicator of inflammatory status in allergic disease.

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There is considerable current interest in IL-4, largely related to its role in the induction of IgE synthesis by B cells^{1,2} and its function in the development of allergy. Others have demonstrated the importance of both eosinophils and T-lymphocytes in the local atopic inflammatory response.³ Eosinophils are suggested as an important source of IL-4.^{4,5} We have previously shown IL-4 to be raised in the serum of patients with mild atopy.⁶ In the light of these findings it would seem that serum levels of IL-4 might prove a useful indicator of inflammatory activity in allergic disease.

Eosinophil cationic protein (ECP) is a basic protein found in the granules of eosinophils and is widely regarded as a useful eosinophilic activation marker.⁷ The measurement of ECP in serum has been suggested as an indicator of treatment efficacy in seasonal asthma.^{7,8}

To assess the usefulness of IL-4 as an inflammatory marker, we have measured serum ECP and IL-4 in hayfever patients during the pollen season and in winter and compared them with normal subjects. Levels of ECP and IL-4 were correlated with each other and to eosinophils in both winter and summer.

RESULTS

The results for the normal subjects and hayfever group are summarised in Tables 1 and 2. In hayfever the mean eosinophil count was significantly raised in summer as compared with the winter ($P<0.01$). The summer and winter eosinophil counts in pollen hayfever were significantly higher than in normals ($P<0.01$).

TABLE 1. Eosinophil, IL-4 and ECP results for the normal participants

	Eosinophils ($\times 10^9/l$)	IL-4 (pg/ml)	ECP (ng/ml)
Summer	0.26 (0.1)	0.954 (0.465)	14.22 (8.84)
Winter	0.17 (0.09)	0.869 (0.600)	11.65 (6.32)

Results are quoted as group means, the values in parentheses are the standard deviations.

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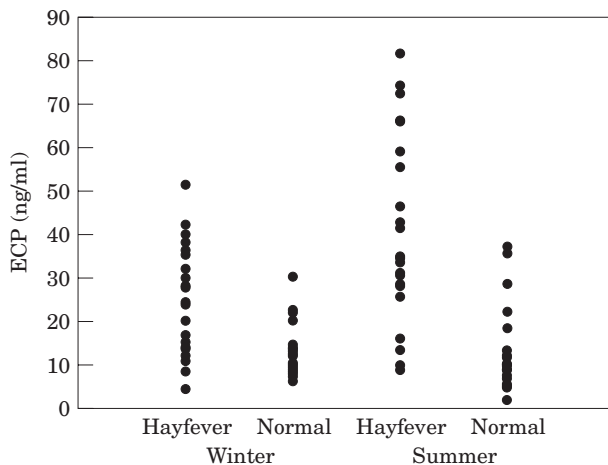
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KEY WORDS: eosinophils/ECP/IL-4/hayfever/seasonal variation/immunoassay

TABLE 2. Eosinophil, IL-4 and ECP results for the hayfever patients

	Eosinophils ($\times 10^9/l$)	IL-4 (pg/ml)	ECP (ng/ml)
Summer	0.54 (0.234)	1.608 (1.118)	40.77 (20.65)
Winter	0.38 (0.128)	2.825 (1.834)	20.22 (12.87)

Results are quoted as group means, the values in parentheses are the standard deviations.

**Figure 1. Seasonal variations in ECP levels.**

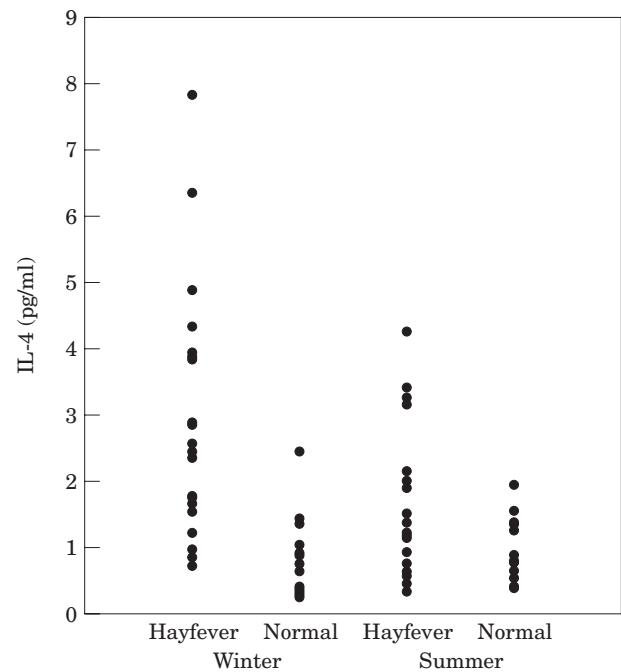
Serum ECP was higher in the hayfever group than the normals in both winter and summer. During the summer ECP was significantly raised above that seen in winter in hayfever ($P < 0.01$) (Fig. 1, Table 2), ECP was detectable in all patients.

Of the normals 50% (16/32) had no detectable IL-4 in summer and in winter 56% (18/32) had undetectable levels. In hayfever 38% (12/32) in summer and 31% (10/32) in winter had undetectable IL-4 (sensitivity = 100 fg/ml).

Seasonal variation in IL-4 can be seen in Figure 2. In those patients with hayfever IL-4 values were significantly higher than the normals in winter ($P < 0.01$) and summer ($P < 0.05$). However, IL-4 in winter was significantly higher compared with summer ($P < 0.01$) in the hayfever patients (Table 2).

IL-4 showed no significant daily variation in winter or summer and no significant reduction in recovery was seen in samples incubated for up to 8 h at 37°C.

There was no correlation between ECP and IL-4 in winter ($r = 0.394$, $P = 0.164$), or summer ($r = -0.284$, $P = 0.326$). There was no correlation between IL-4 and eosinophils in winter or summer, a positive correlation was seen between ECP and eosinophils, but only during the summer ($r = 0.661$, $P < 0.01$).

**Figure 2. Seasonal variation in IL-4 levels.**

DISCUSSION

Mean serum ECP was elevated in the hayfever group when compared to the control population and again these levels were shown to increase significantly during the grass pollen season. This finding was not unexpected giving the association of ECP to activated eosinophils.^{7,8}

Interestingly, IL-4 in hayfever, although raised compared to normals in both winter and summer ($P < 0.01$) fell significantly ($P < 0.01$) during the grass pollen season. This result would appear surprising in the light of the findings for ECP and total eosinophil counts. Others have demonstrated that eosinophils actively secrete IL-4.⁴ We have previously shown that IL-4 is increased over normal levels even in mild atopy.⁶

Other authors have suggested that factors such as epitope denaturation, enzymatic cleavage or binding to undefined ligands may in part be involved in the apparent reduction in cytokine levels, when these are quantified by immunoassay.^{9,10} In this study we were unable to demonstrate any substantive reduction in detectable IL-4 when sera from patients were incubated for up to 8 h with known amounts of IL-4. These sera were from subjects who had all demonstrated significant reductions between winter and summer IL-4 levels. This would suggest that such mechanisms are not responsible for the observed reduction in IL-4.

The reduction in IL-4 may be as a result of sequestration of T cells and/or eosinophils from the

peripheral circulation into inflammatory sites. IL-4 induces endothelium to express adhesion molecules that specifically attract eosinophils.^{11,12} It may result from antigen-driven upregulation of IL-4 receptors on responsive cells thus increasing cytokine consumption.

Given the significant summer fall in IL-4 shown in this study, it would be prudent to exercise caution if using serum IL-4 levels to assess inflammatory changes in atopy. This may be particularly so when there are marked temporal variations in allergen exposure.

METHODS

Patients

Thirty-two non-smoking patients, 16 males and 16 females with hayfever confirmed by a positive skin-prick test to grass with a minimum wheal size of 5 mm were selected. All used topical treatment only and discontinued medication for 48 h before sampling. Thirty-two normal, non-smoking non-atopic patients, 16 males and 16 females taking no medication, were selected as controls. The local ethics committee approved the study, participants gave informed consent in writing. Blood was collected by venepuncture.

Eight patients, four males and four females, had blood taken for 5 consecutive days during the winter and summer to determine daily variation in IL-4.

Blood counts

Full blood counts were performed using a Coulter MD18, differentials were carried out on Romanowsky stained blood films.

Serum ECP and IL4

ECP was assayed using a Pharmacia Radioimmunoassay System (Kabi Pharmacia Diagnostics, Uppsala, Sweden) and IL-4 was assayed using the Biotrak High Sensitivity ELISA System (Amersham International, Little Chalfont, Bucks, UK). The sera were stored at -70°C until required for analysis.

Determination of IL-4 consumption

Ten serum samples from the hayfever subjects obtained during the summer were incubated at 37°C with IL-4 at 5 pg/ml for 1, 2, 4 and 8 h and the samples assayed for IL-4 to determine consumption.

Statistics

Results were analysed using Minitab for Windows. Inter group variation was determined using the Wilcoxon matched-pairs test and correlations performed using the Spearman rank correlation coefficient. Significance was taken as $P < 0.05$.

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