Serum Concentration of Soluble Interleukin 2 Receptors in Asthma

Correlation With Disease Activity

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We evaluated whether serum soluble interleukin 2 receptor (sIL-2R), a marker of T lymphocyte activation in vitro, could be useful to monitor disease activity in asthma. Venous blood was collected from 26 patients with acute severe asthma prior to the commencement of systemic corticosteroid therapy (day 1), 15 with stable disease, and 13 normal control subjects. Serum sIL-2R level was significantly higher in acute asthma (462.7 ± 36.1 U/ml; mean ± SEM) than stable disease (328.5 ± 30.4 U/ml; p = 0.013) which in turn, was significantly raised when compared with control subjects (239.0 ± 22.9 U/ml; p = 0.0003 vs acute; p = 0.036 vs stable). Nevertheless, sIL-2R concentrations in 11 patients with acute and 11 with stable disease did not exceed the upper limit of normal, i.e., mean ± 2 SD of the value in control subjects = 404.4 U/ml. Repeated measurements of sIL-2R in 24 acute asthmatics on day 3 revealed no significant fall (464.6 ± 37.2 U/ml, NS), although the reduction in sIL-2R was significantly correlated with the corre-

sponding improvement in peak expiratory flow (r = -0.52, p = 0.005). Following resolution of the acute attack, further measurements performed in 11 of these subjects on day 28 showed a significant fall in sIL-2R (p = 0.016). Our data showed that although serum sIL-2R was raised in asthma and, to a certain extent, might reflect disease activity, the considerable overlap of values between asthma of differing severity and normal control subjects precludes its clinical use as an index of asthma severity.

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CMI = cell-mediated immunity; GM-CSF = granulocyte-macrophage colony-stimulating factor; HLA = histocompatibility antigen; IFN-γ = interferon gamma; IL-2R = interleukin 2 receptor; PEF = peak expiratory flow; sIL-2R = soluble interleukin 2 receptor; PC20 = provocation concentration of a bronchoconstrictor agonist causing a 20 percent fall in FEV1 from baseline value

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Current evidence suggests that T lymphocytes may be involved in the regulation and expression of the inflammatory processes associated with asthma. Through the actions of its wide array of cytokines, these cells may regulate the synthesis of IgE antibody and facilitate the perpetuation of the intense eosinophilic response in the asthmatic airway. Furthermore, prominent lymphocytic infiltration has been described not only in patients who died of acute severe asthma, but also in those with milder disease. The demonstration of enhanced expression of three T-cell surface activation markers, interleukin 2 receptor (IL-2R), class II HLA and "very late activation" antigen in patients with acute severe asthma provides further support that cell-mediated immunity (CMI) may be an essential component in the pathogenesis of asthma. However, this notion is challenged by the finding that serum and urine levels of neopterin, a marker for activated CMI, were not elevated in patients with either acute or stable asthma.

Activation of T lymphocytes not only leads to the expression of IL-2R on the cell surface but also releases soluble interleukin 2 receptor (sIL-2R) molecules into the circulation. Various studies have confirmed the strong association between serum sIL-2R levels and the activation of T lymphocytes in vitro and have indicated that sIL-2R production is directly proportional to cellular IL-2R expression. Because of the small numbers of B cells and monocytes expressing cellular IL-2R, it is reasonable to assume the majority of sIL-2R in the serum is elaborated by activated T cells, and hence, its level can be used satisfactorily as a marker of T lymphocyte activation in vitro.

Markedly elevated levels of sIL-2R have been reported in patients with hematologic malignancy, autoimmune disorders such as systemic lupus erythematosus, and pulmonary disorders such as sarcoidosis and lung cancer. Recently, we have demonstrated that sIL-2R is useful as a marker of activity and extent of involvement in patients with active tuberculous lesions, and a rise in its level may herald a recurrence of nasopharyngeal carcinoma following radiotherapy. In this study, we have attempted to define whether circulating levels of sIL-2R could be used as an index of disease activity in asthma.

METHODS

Subjects

Three groups of nonsmoking subjects were studied (Table 1). Group A consisted of 26 patients with severe acute asthma admitted as emergency patients to the Prince of Wales Hospital with peak expiratory flow (PEF) <50 percent of the predicted value. None of them had received systemic corticosteroids before the study but 11...
were regularly taking inhaled steroids. Following hospital admission, they were all treated with nebulized β₂-agonists, oral or intravenous corticosteroids, and oxygen. Group B consisted of 15 patients with stable asthma who required no more than intermittent inhalation of β₂-agonists and regular inhaled steroids (9 patients). Their PEF was at least >60 percent of the predicted value. Group C was comprised of normal healthy volunteers who had normal lung function and were not receiving any medications. The atopic status of each subject in all three groups was determined by skin prick tests to the common allergens: Dermatophagoides pteronyssinus, Dermatophagoides farinae, mixed grass pollens, Aspergillus fumigatus, cat fur, and dog dander (Bencard, Brentford, UK). Atopy was defined as having a skin wheal response to two or more allergens with a diameter of ≥2 mm. Informed consent was obtained from all subjects, and the study was approved by the hospital ethical committee.

Histamine Bronchoprovocation

The procedure was performed on all subjects in groups B and C according to a protocol modified from the method described by Chai and coworkers. The aqueous solutions were administered as aerosols generated from a starting volume of 4 ml in a disposable nebulizer (Inspiron Mini-neb Nebuliser, C.R. Bard International, Sunderland, UK) connected to a dosimeter driven by compressed air at a pressure of 40 lb/sq in. Subjects wearing a noseclip were instructed to take ten consecutive breaths slowly from functional residual capacity to total lung capacity without holding between maneuvers. The dosimeter setting was adjusted so that this procedure generated 72 μl of aerosol of mass median particle diameter 4.7 μm. Subjects first inhaled ten breaths of 0.9 percent sodium chloride followed by measurements of FEV₁ after 1 and 3 min. If the FEV₁ did not fall by >10 percent of the starting baseline value, the histamine provocation was performed. Doubling concentrations of histamine acid phosphate (0.03 to 8 mg/ml) were inhaled at 1 and 3 min. The inhalations and airway caliber measurements were continued until FEV₁ had fallen by >20 percent from the lowest postsaline solution value. The percentage fall in FEV₁ from postsaline solution baseline was plotted against the concentration of histamine on a logarithmic scale and PC₂₀ derived by linear interpolation.

Blood Sampling and SL-2R Assay

Peripheral venous blood samples were taken from all 26 acute asthmatics at the time of hospital admission, before the commencement of systemic steroid therapy (day 1), on day 3 from 24 subjects and day 28 from 11 subjects. Venous samples were taken once only in stable asthmatics and healthy control subjects. Serum was separated from the clotted whole blood after centrifugation at 2,000 × g for 15 min and stored at −70°C before SL-2R assay. All serum samples were randomly assigned code numbers and assayed in a single batch without the knowledge of the clinical status of the patients.

The SL-2R was measured by a commercial assay kit (Cellfree; T Cell Science) using a sandwich technique with two different monoclonal antibodies that recognize different epitopes of the IL-2R. An aliquot of an IL-2R standard (T Cell Science, Cambridge, Mass) at a concentration of 1,000 SL-2R U/ml was used to generate a standard curve by measuring the absorbance values of serial dilutions of this standard.

Data Analysis

All figures are mean ± SEM unless otherwise stated, and the p<0.05 degree of significance was accepted. Unpaired data were compared by Mann-Whitney U test and paired data by Wilcoxon's signed rank test. Spearman's rank correlation was used to compare the relationships between change in SL-2R concentration and change in PEF in acute asthmatics, between SL-2R concentration and baseline PEF in all asthmatic subjects, and between SL-2R concentration and histamine PC₂₀ value in stable asthmatics.

Results

Patients with acute asthma had a mean PEF of 28.4 ± 2.2 percent predicted at the time of hospital admission. Their serum SL-2R levels on day 1 (462.7 ± 36.1 U/ml, n = 26) were significantly elevated when compared with those of stable asthmatics (325.5 ± 30.4 U/ml, n = 15, p = 0.013) and healthy control subjects (239.0 ± 22.9 U/ml, n = 13, p = 0.0003). Similarly, the levels in patients with stable

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Table 1—Characteristics of Subjects

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, yr</th>
<th>Sex, M/F</th>
<th>Atopy, %</th>
<th>PEF (%)</th>
<th>PC20 Histamine, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute asthma</td>
<td>26</td>
<td>37.0 ± 3.0</td>
<td>15/11</td>
<td>23/3</td>
<td>26.2 (10.4-48.0)</td>
<td>ND</td>
</tr>
<tr>
<td>Stable asthma</td>
<td>15</td>
<td>35.6 ± 3.7</td>
<td>4/11</td>
<td>12/3</td>
<td>88.6 (63.3-105.2)</td>
<td>0.38 (0.031-2.13)</td>
</tr>
<tr>
<td>Normal control subjects</td>
<td>13</td>
<td>19.2 ± 0.5</td>
<td>7/5</td>
<td>8/5</td>
<td>87.9 (73.0-111.0)</td>
<td>&gt;8</td>
</tr>
</tbody>
</table>

*PEF = peak expiratory flow; PC₂₀ histamine = provocation concentration of histamine causing a 20 percent fall in FEV₁, from baseline. ND = not done.
†Median values with range in parentheses.
FIGURE 2. Time course of serum sIL-2R in patients with acute severe asthma. Significant difference was seen between values on days 1 and 28 in those 11 subjects (549.8 ± 64.5 vs 464.6 ± 37.2 U/ml, p = 0.016) who had measurements done on all 3 days.

asthma were also higher than those in healthy subjects (p = 0.036, Fig 1). However, there was a considerable overlap of values between the two groups of asthmatics and the normal control subjects. Taking the mean + 2 SD of the concentration in normal control subjects as the upper limit of normal value, ie, 404.4 U/ml, only 15 (57.7 percent) subjects with acute asthma and 4 (26.7 percent) with stable asthma had elevated sIL-2R concentrations while the remaining subjects had values within the normal range (Fig 1).

Serum sIL-2R concentrations did not show any significant fall by day 3 in the 24 acute asthmatics who had assays performed on both days (483.0 ± 35.7 vs 464.6 ± 37.2 U/ml, day 1 vs day 3, NS, Fig 2). However, analysis on data in the 11 subjects who had assays performed on days 1, 3, and 28 demonstrated a significant fall in sIL-2R concentration by day 28 (p = 0.016, 549.8 ± 64.5 vs 386.7 ± 65.3 U/ml, day 1 vs day 28, Fig 2).

Following treatment with nebulized B2-agonist and systemic steroids, PEF increased significantly from 130.8 ± 9.5 L/min (28.5 ± 2.2 percent predicted) at the time of hospital admission to 246.9 ± 18.0 L/min (53.9 ± 4.3 percent predicted) by 8 AM on day 3 (p<0.0001). There was a significant correlation between this increase in PEF and the corresponding reduction in sIL-2R concentration in the 24 patients who had the assays performed on days 1 and 3 (r = -0.52, p = 0.005; Fig 3). A significant correlation was also observed between the sIL-2R concentration and PEF when day 1 data from acute asthmatics were analyzed together with those from subjects with stable asthma (r = -0.41, p = 0.004; Fig 4). However, no significant correlation was found between the serum concentration of sIL-2R and the degree of bronchial hyperresponsiveness in patients with stable asthma (r = 0.28, NS, Fig 5).

FIGURE 4. Correlation between serum sIL-2R and baseline PEF in 26 patients with acute severe asthma (day 1 data) and 15 with stable asthma.

FIGURE 3. Correlation between changes in serum sIL-2R and PEF from days 1 to 3 in 24 patients with acute severe asthma.

DISCUSSION

Although Corrigan and Kay20 have reported an elevated serum sIL-2R concentration in patients with acute severe asthma, they did not observe any increase in patients with stable disease. In contrast, Brown and coworkers21 have found increased sIL-2R concentrations in patients with stable asthma but this increase was of the same magnitude as that seen in acute asthma. In this study, we have not only confirmed that serum sIL-2R concentration was elevated in asthmatic patients, but also found that the increase was higher...
During acute exacerbation than when the disease was stable. The reason for these slightly conflicting results is unclear, although the use of plasma rather than serum for sIL-2R assay by Brown et al. might explain the differences between their results and ours. As far as one can gauge, the characteristics of our asthmatic subjects, assessed by the degree of airflow obstruction and atopic status, were similar to those studied by Corrigan and Kay and were therefore unlikely to account for the discrepancies in findings.

We have observed that following 2 days of high-dose systemic corticosteroid therapy, the fall of sIL-2R, though not different from baseline value, correlated significantly with the improvement in airflow obstruction (Fig 3). Serial measurements of sIL-2R in 11 patients with acute severe asthma showed significant reduction was achieved by day 28. Furthermore, a significant relationship was observed between the sIL-2R level and the degree of airflow obstruction when the two groups of asthmatics were analyzed together (Fig 4). Although our data suggest serum sIL-2R level may reflect the severity of asthma, significant overlaps were seen between values among the three groups of subjects. Thus >40 percent of patients with acute asthma and >70 percent of those with stable disease had levels within the normal range (Fig 1). Moreover, the lack of correlation between serum sIL-2R level and the degree of bronchial responsiveness to histamine further suggests that a single measurement of sIL-2R is neither useful for diagnosing asthma nor for assessing disease activity.

As mentioned earlier, the majority of serum sIL-2R is released by activated T lymphocytes. Our results therefore provide further evidence for T-cell activation in asthma. The CD4+ lymphocytes are likely to be a major source of the increased serum sIL-2R concentration as these cells recovered from patients with acute asthma have been shown to exhibit increased expression of IL-2R on their surface. Based on the patterns of their cytokines production, murine CD4+ cells are further classified into different subsets. Thus, TH1 cells synthesize IL-2 and IFN-γ, but not IL-4 or 5. In contrast, TH2 cells produce IL-4 and 5 but not IL-2 or IFN-γ. Both, however, are able to produce IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF). Recently similar subsets have also been found in humans. Current evidence suggests that in atopic asthma, these activated CD4+ lymphocytes are likely to be of the TH1 subset. With the release of IL-4 that stimulates the growth of mast cells and promotes B-cell synthesis of IgE, IL-5, and GM-CSF that enhance eosinophil adhesion to vascular endothelium and prolong the survival of this cell in culture, these cells are capable of orchestrating the recruitment and activation of mast cells and eosinophils in the asthmatic airways. The demonstration of a significant correlation between serum sIL-2R and blood eosinophilia in patients with nonatopic asthma has provided further support to this hypothesis. However, the recent finding of T cell induced by delayed type hypersensitivity may be responsible for the induction of airway hyperreactivity in mice suggests that TH1 cells may also be involved in asthma.

Serial measurements of sIL-2R in 11 patients with acute asthma showed a significant reduction by day 28 when their disease was in remission. This together with the significant correlation observed between the reduction in sIL-2R and the improvement in PEF from day 1 to day 3 suggest a possible mechanism for the efficacy of corticosteroids in the treatment of asthma may be related to inhibition of T lymphocyte function. Indeed, corticosteroids have been shown to down-regulate IL-2R and promptly reverse stimulated IL-2R expression on cultured T cells in vitro. Furthermore, proliferation of peripheral blood T lymphocyte from patients with steroid-resistant asthma is significantly less susceptible to the inhibition by glucocorticoids than that of steroid-sensitive asthmatics. Collectively, these findings further emphasize the role of T cells in asthma and suggest that regulation of their function may be important in the treatment of this disease.

Bronchial hyperreactiveness is a prominent feature in asthma. However, we have failed to observe any significant relationship between sIL-2R concentration and bronchial responsiveness measured as histamine PC20 in our subjects with stable asthma. This suggests that T lymphocytes may not be the only determinant for the development of bronchial hyper-reactiveness in asthma. In support of this, other pulmonary diseases such as sarcoidosis, although characterized by lymphocytic infiltration of the lungs, do not exhibit bronchial hyperreactivity. It is likely that other inflammatory cells, notably eosinophils and mast
cells, also participate in the inflammatory process leading to the development of bronchial hyperresponsiveness.\textsuperscript{30}

In conclusion, our data suggest that serum sIL-2R concentration may indicate the severity of asthma although the great overlap in values between acute and stable asthmatics suggests a single measurement may not be clinically useful as a marker of disease activity. However, the significant fall of sIL-2R concentration following resolution of an acute attack suggests that serial measurements of sIL-2R may be useful in monitoring disease activity. Further studies exploring such application are warranted. Finally, the present data provide further evidence for an important role for T lymphocytes in the pathogenesis of asthma.

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