needed, we think that valve reconstruction, whenever possible, should be tried, even if later on valve deterioration should make valve replacement unavoidable.

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Interleukin-5 Levels of Pleural Fluid and Serum Samples in a Patient with P/E Syndrome*

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An increased production of IL-5 was detected in the pleural fluid (7.2 ng/ml) and in the serum samples (53 pg/ml) of a patient with P/E syndrome. Following steroid therapy, pleural fluid disappeared, eosinophilia improved and serum IL-5 concentration became undetectable. These results suggested that eosinophilia in the P/E syndrome is a consequence of increased production of IL-5, especially in the lung.

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Interleukin-5, a T-cell-replacing factor, shows eosinophil colony-stimulating activity in humans. We and others recently observed that transgenic mice expressing IL-5 have severe eosinophilia. These reports suggest that eosinophilia in some patients with allergy or parasite infection may be due to an enhanced production of IL-5.

Because the concentration of IL-5 can be determined with an ELISA method in mice and humans, we examined the concentration of IL-5 in the serum samples and pleural fluid of a patient with P/E syndrome.

Materials and Methods

Serum Samples and Pleural Fluids

Serum samples were obtained from a patient with P/E syndrome and from ten normal volunteers. Pleural fluid was obtained from a patient with P/E syndrome and from five other patients with carcinomatous pleurisy who showed no eosinophilia in their peripheral blood or pleural effusion. The pleural fluid samples were centrifuged to remove cells and debris. These test samples were stored at -20°C until use.

Reagents

Recombinant human IL-1, IL-2, IL-5, IL-6 and TGF-β were analyzed. Recombinant human IL-3, IL-4, G-CSF and M-CSF were obtained from Genzyme Corporation (Boston, Mass.). Furthermore, TB13, an anti-mouse IL-5 monoclonal antibody which can react with human IL-5, was purified as previously described.

ELISA Assay to Detect Human IL-5

For the assay of human IL-5, we slightly modified the ELISA method established to detect mouse IL-5. Briefly, polystylen plates were coated with TB13 (5 μg/ml) in PBS (10 mM phosphate, 140 mM NaCl, pH 7.4) overnight at 4°C. Nonspecific binding sites were blocked with PBS containing 2 percent bovine serum albumin for 2 h at room temperature. After washing the wells with PBS containing 0.05 percent Tween 20 (PBS-Tween), test samples were applied to the wells and incubated overnight at 4°C. Then, after washing again, polyclonal rabbit anti-human IL-5 IgG antibodies were added and incubated again overnight at 4°C.

Horseradish peroxidase-coupled goat anti-rabbit Ig (Bio-Rad Labs, Richmond, Cal) was added, followed by an hour's incubation at room temperature. After washing, 0.5 percent 3-(4-hydroxyphenyl)propionic acid in phosphate buffer (10 mM, pH 7.0) / 0.03 percent H2O2 was added and incubated 10 h at room temperature. The reactions were stopped by adding 0.25N NaOH containing 1.5 percent Na2CO3, and the fluorescence was measured at 405 nm (excitation 320 nm) on a fluorescence spectrometer (F-3000, Hitachi). Figure 1 shows a standard curve of this ELISA assay using rIL-5 (sensitivity: 2 pg/ml). The fluorescence of 2 ng/ml of recombinant IL-1, IL-2, IL-3, IL-4, IL-6, G-CSF, GM-CSF or TGF-β...
Recently, Samoszuk and Nansen detected IL-5 mRNA in the cytoplasm of Reed-Sternberg cells using an in situ hybridization technique and therefore suggested that eosinophilia in Hodgkin's disease was due to enhanced IL-5 production. Owen et al. reported a crucial role of IL-5 in eosinophilia of patients with IHES. Through an ELISA assay which can detect the picogram-order of IL-5 with ease, we could clarify a part of the in vivo role of IL-5.

In PIE syndrome, some antigens, for example fungi, drugs or parasites, elicit an immune response, which causes hyperproduction of eosinophils. Since the focus of this immune response is the lung, eosinophils accumulate there and migrating pneumonia develops. In our patient, eosinophilia was more marked in the pleural fluid than in the peripheral blood. That much more IL-5 was detected in the patient's pleural fluid than in the serum suggested that the lung probably was the center of the inflammation. The IL-5 production might have been limited to the lung and IL-5 leakage from the lung to the peripheral blood might have induced the peripheral eosinophilia. With steroid therapy, the pulmonary consolidation and the pleural effusion disappeared, eosinophilia was normalized and serum IL-5 concentration became undetectable. We conclude that IL-5 is important in the pathogenesis of PIE syndrome.

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Table 1—IL-5 Concentration in Serum Samples and Pleural Fluids

<table>
<thead>
<tr>
<th>Samples</th>
<th>IL-5 Concentration</th>
</tr>
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<tbody>
<tr>
<td>Normal serum (n = 10)</td>
<td>ND</td>
</tr>
<tr>
<td>Patient's serum (before steroid therapy)</td>
<td>53 pg/ml</td>
</tr>
<tr>
<td>Patient's serum (after steroid therapy)</td>
<td>ND</td>
</tr>
<tr>
<td>Patient's pleural fluid</td>
<td>7.2 ng/ml</td>
</tr>
<tr>
<td>Other patient's pleural fluids (n = 5)</td>
<td>ND</td>
</tr>
</tbody>
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*ND = not detected.