Elevated Serum Concentrations of Interleukin-10 in Nonneutropenic Patients with Invasive Aspergillosis

To the Editor—Prolonged neutropenia has been considered to be a key risk factor for development of invasive aspergillosis. However, invasive aspergillosis is increasingly recognized in nonneutropenic patients with phagocytic dysfunction [1–3].

Interleukin (IL)–10 exerts inhibitory effects on immune responses [4], including the functional suppression of phagocytic cells against *Aspergillus fumigatus* [5, 6]. Mice expressing high serum IL-10 levels show decreased resistance to aspergillosis and are protected by neutralization of this cytokine [7]. In this model, IL-10 was validated as a signature cytokine for Th2 response. These parallel in vitro and in vivo studies demonstrated that Th1 immunity confers a protective effect while Th2 increases susceptibility to aspergillosis.

We hypothesized that a similar Th1/Th2 dysregulation and a switch to a Th2 immune response may contribute to development and unfavorable outcome of invasive aspergillosis in humans. We therefore measured IL-10, IL-12, and tumor necrosis factor (TNF)–α in 38 sequential serum samples from 7 nonneutropenic immunocompromised patients with 8 episodes of documented invasive aspergillosis (figure 1). One patient had recurrent invasive aspergillosis after premature discontinuation of therapy for which he received a second course of antifungal therapy. For each patient, serum samples were drawn at baseline and at regular intervals after the initiation of therapy until discontinuation of study drug because of improvement or failure. After collection, the samples were stored at −70°C. Sixteen apparently healthy blood donors served as controls of cytokine measurements. Serum concentrations of IL-10, IL-12, and TNF-α were measured by ELISA (R&D Systems) in an automated reader (Flow Laboratories). We used Fisher’s exact test to evaluate differences in proportions.

The median patient age was 39 years. Baseline absolute neutrophil counts were 159–12,582 cells/μL. Serum IL-12 and TNF-α levels were <5 and <4.4 pg/mL, respectively (lower limits of detection) in all 54 serum samples from patients and controls. Serum IL-10 levels were undetected in all controls. By comparison, IL-10 was detected in the serum samples from 6 of 7 patients (all but patient 2) during 8 invasive aspergillosis episodes (*P* < .001).

Two patterns of correlation of serum IL-10 concentrations and outcome of invasive aspergillosis were observed. In 6 patients (1, 2, 3α, 3β, 4, and 5), favorable outcome (complete response in 2, partial response in 1, and stable disease in 3) was associated with either undetected or low levels during the entire course (1, 2, 3β, and 5) or with high baseline levels that decreased to undetectable levels during follow-up (3α and 4). In the second pattern, which was seen in 2 patients with poor outcome (6 and 7), IL-10 increased during the progression of invasive aspergillosis, until death despite therapy. Thus, 6 of 6 patients with favorable outcome had undetectable, low, or decreasing IL-10 levels versus 2 of 2 with poor outcome who had increasing IL-10 levels (*P* = .036). These findings are consistent with experimental observations [4, 5, 7] and suggest an association between elevated serum IL-10 levels and development of invasive aspergillosis and a possible correlation with unfavorable outcome in nonneutropenic immunocompromised patients.

Whether changes of IL-10 concentrations are the cause or the result of the progression of invasive aspergillosis is not well understood. We propose that IL-10 causes antifungal dysfunction of phagocytic cells, permitting *A. fumigatus* to evade host defenses. The growth of *A. fumigatus* in tissues may lead to higher IL-10 levels in the blood, as a reflection of immunoregulation in compromised hosts [10]. If this hypothesis is con-
Figure 1. Clinical course and serum interleukin (IL)-10 concentrations of patients with invasive aspergillosis. All patients were nonneutropenic and treated on protocol to receive liposomal amphotericin B intravenously (7.5–12.5 mg/kg/day) [8]. Lower limit of detection of IL-10 by ELISA was 2 pg/mL. Values of IL-10 concentrations are plotted as means of duplicate measurements. Invasive aspergillosis was diagnosed as proven or probable according to criteria of the European Organization for Research and Treatment of Cancer–National Institute of Allergy and Infectious Diseases Mycoses Study Group [9]. Response to therapy was defined as follows: complete, disappearance of all attributable symptoms and signs of invasive aspergillosis; partial, improvement of attributable clinical signs and symptoms; stable disease, minimal to no change of attributable signs and symptoms; failure, progressive disease. Favorable outcome consisted of complete response, partial response, or disease stabilization. Numeric scales indicate serum IL-10 levels (in pg/mL). Time course of infection in days (d) is shown on horizontal axis. AA, aplastic anemia; BMT, bone marrow transplantation; CGD, chronic granulomatous disease; CSA, cyclosporine A; F, female; HIV, human immunodeficiency virus infection; M, male; MDS, myelodysplastic syndrome; MM, multiple myeloma; Pt, patient; y, year.

To our knowledge, this is the first report to analyze IL-10 expression in humans with invasive aspergillosis and to demonstrate an association between Th2 response, reflected as increased serum IL-10 levels, and invasive aspergillosis in nonneutropenic patients. The results from this pilot study contribute to a rational foundation for a comprehensive multicenter investigation of cytokine expression in invasive aspergillosis.

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<table>
<thead>
<tr>
<th>Pt no</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Underlying disease</th>
<th>Immunosuppressive agents at onset</th>
<th>Site</th>
<th>Serum IL-10 levels (pg/mL)</th>
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<tbody>
<tr>
<td>1</td>
<td>31</td>
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<td>Pericardium</td>
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<td>Complete response</td>
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<tr>
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<td>41</td>
<td>M</td>
<td>MDS</td>
<td>CSA</td>
<td>Lungs</td>
<td>Stable disease</td>
</tr>
<tr>
<td>3β</td>
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<td>MDS</td>
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<td>4</td>
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<tr>
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<td>AA</td>
<td>CSA</td>
<td>Lungs</td>
<td>Failure (death due to progressive infection)</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>F</td>
<td>HIV</td>
<td>None</td>
<td></td>
<td>Failure (death due to progressive infection)</td>
</tr>
</tbody>
</table>

In a larger study, therapeutic inhibition of suppressive effects of IL-10 by anti-IL-10 antibodies or immunoenhancing cytokines (e.g., IFN-γ or granulocyte-macrophage colony-stimulating factor [GM-CSF]) and use of serum IL-10 levels as a marker of disease progression could be considered.

How aspergillus triggers a Th2 response reflected as elevated IL-10 levels in these patients is uncertain. Mononuclear cells from healthy donors respond to A. fumigatus by secreting IFN-γ, GM-CSF, TNF-α, and IL-2 but not IL-10 [10]. Immunocompromised patients might respond with a Th2 and not a Th1 profile and this may lead to increased susceptibility to invasive aspergillosis. High circulating IL-10 levels could suppress antifungal activity of phagocytes [5, 6] and down-regulate other immune responses, leading to progression of invasive aspergillosis.
References


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