Defective interferon-gamma production in patients with hairy cell leukaemia

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ABSTRACT

Background: Patients with hairy cell leukaemia (HCL) have an increased susceptibility to intracellular pathogens, such as mycobacteria and Listeria. Although several abnormalities of T-cell populations have been described in HCL, the effector mechanism responsible for the increased susceptibility to infections is not known.

Methods: Blood was collected from 11 patients with HCL and 22 age- and gender-matched volunteers. Proinflammatory cytokine production by freshly isolated mononuclear cells was stimulated with either lipopolysaccharide or various heat-killed microorganisms. Cytokine concentrations were assessed by specific ELISAs.

Results: We demonstrate that mononuclear cells harvested from HCL patients have a specific defect of IFNγ production when stimulated with a broad panel of bacterial stimuli. In contrast, the production of other proinflammatory cytokines, such as TNF, IL-1β and IL-6, did not differ between HCL patients and controls.

Conclusion: The specific defect in IFNγ production may play a role in the susceptibility of patients with hairy cell leukaemia towards intracellular pathogens.

KEYWORDS

Cytokines, hairy cell leukaemia, interferon-gamma, mycobacteria

INTRODUCTION

Hairy cell leukaemia (HCL) is a chronic B-lymphocyte malignancy, in which mature neoplastic B lymphocytes show hair-like protrusions. A major characteristic of HCL is splenomegaly, due to accumulation of malignant cells. In HCL, opportunistic infections, especially with facultative intracellular pathogens such as mycobacteria or Listeria, are prominent. The occurrence of these infections suggests a serious defect in the cellular immune defence provided by T lymphocytes and macrophages.

Although HCL is a B-cell malignancy, a series of abnormalities in the function of T cells have been also reported. There is abnormal T-cell activation, proliferation, clonal expansion and distribution; there is a decrease in memory T helper cells, an increase in splenic CD3-positive T cells positive for gamma delta T-cell receptors, and a restricted and skewed repertoire of the T-cell receptor family. Although these abnormalities all point to T-lymphocyte dysfunction, the exact mechanism behind the increased susceptibility to facultative intracellular infection is not clear.

In recent years, an increased susceptibility to mycobacteria and Salmonella species has been described in patients with genetic defects in the interleukin-12/interferon-gamma (IL-12, IFNγ) macrophage activation route. In these patients, the defects result in impaired IFNγ signalling, required to activate the microbicidal capacity of macrophages.

In the present study we have assessed the production of IFNγ by peripheral blood cells of patients with HCL, to obtain a better insight into the effector arm of cellular immunity in this disease. Since cytokines other than IFNγ are also relevant for host defence against infection, and cytokine production is regulated within what is known as the cytokine network, we measured a series of other relevant proinflammatory cytokines.

PATIENTS AND METHODS

Patients

Eleven patients diagnosed with HCL were included in the study (see table 1 for clinical characteristics). Two of these patients had a mycobacterial infection at clinical...
presentation. These patients had completely recovered from this infectious episode at the time of the present study. Patients had been treated previously with either cladribine (2-CDA), IFN\(\alpha\) or a combination of both. Medication was stopped at least two weeks prior to the assessment of cytokine production. All samples were obtained between 9 am and 11 am, and for each patient, two gender-matched volunteers were concomitantly tested (n=22, 20 men and 2 women, age 26 ± 7 years).

**Ex-vivo stimulation of cytokine production**

After obtaining informed consent, venous blood was drawn from the cubital vein of patients and healthy volunteers into three 10 ml EDTA tubes (Monoject). Isolation of mononuclear cells (MNC) was performed as described, with minor modifications. The MNC fraction was obtained by density centrifugation of blood diluted 1:1 in pyrogen-free saline over Ficoll-Paque (Pharmacia Biotech). Cells were washed twice in saline and suspended in culture medium (RPMI 1640 DM) supplemented with gentamicin 10 \(\mu\)g/ml, L-glutamine 10 mM and pyruvate 10 mM. The cells were counted in a Coulter counter (Coulter Electronics) and the number was adjusted to 5 x 10⁶ cells/ml. The MNC population consisted of approximately 80% lymphocytes and 20% monocytes, and no differences between patients and controls were apparent.

5 x 10⁵ MNC in a 100 \(\mu\)l volume were added to round-bottom 96-well plates (Greiner) and incubated with either 100 \(\mu\)l of culture medium (negative control), or one of the various stimuli: 10 ng/ml LPS (S. typhimurium; Sigma Chemical), or 1 x 10⁶ microorganisms/ml heat-killed (30 min, 100°C) S. typhimurium, Staphylococcus aureus, Mycobacterium tuberculosis or Candida albicans. After 24 hours (TNF, IL-1\(\beta\) and IL-6) or 48 hours (IFN\(\gamma\)) incubation at 37°C, supernatants were collected and cytokine concentrations were measured using specific ELISA kits (Pelikine, Sanquin, Amsterdam).

**Statistical analysis**

The experiments were performed in duplicate with blood obtained from patients and volunteers, and the data are presented as cumulative results of all experiments performed. The differences between groups were analysed by the Mann-Whitney U test. The level of significance between groups was set at p<0.05. The data are given as means ± SD.

**RESULTS AND DISCUSSION**

When MNC of HCL patients were stimulated with LPS or a panel of microorganisms, the IFN\(\gamma\) production after 48 hours of stimulation was significantly lower compared with healthy volunteers (figure 1A). In contrast, the production of the proinflammatory cytokines TNF\(\alpha\), IL-1\(\beta\) and IL-6 did not differ between HCL patients and controls (figure 1B-D). The specific defect in IFN\(\gamma\) production, in contrast to the normal synthesis of monocyte products such as TNF\(\alpha\), IL-1\(\beta\) and IL-6, suggests a selective T/NK-cell defect in HCL patients, and is not part of a more general defect in cytokine production. It is tempting to assume that such defective production also occurs in vivo and underlies the remarkable susceptibility of HCL patients to facultative intracellular pathogens.

Our finding that TNF\(\alpha\) production is normal is remarkable, since elevated serum concentrations of TNF\(\alpha\) have been reported in patients with HCL.\(^{10,11}\) As far as we know, defective IFN\(\gamma\) production has not been reported previously, despite the fact that there is extensive literature on T-lymphocyte abnormalities in this disorder.\(^{15}\) Cytokine gene expression in patients with HCL has been reported

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**Table 1. Clinical characteristics of the 11 patients with hairy cell leukemia (HCL)**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Year of diagnosis</th>
<th>HCL therapy</th>
<th>Mycobacterial infections</th>
<th>Monocytes (x 10⁶/ml)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>1987</td>
<td>IFN(\alpha)</td>
<td>No</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>1983</td>
<td>IFN(\alpha)</td>
<td>No</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>1994</td>
<td>2-CDA</td>
<td>No</td>
<td>232</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>1992</td>
<td>IFN(\alpha)</td>
<td>No</td>
<td>106</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>1984</td>
<td>Splenectomy</td>
<td>IFN(\alpha)</td>
<td>803</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>1995</td>
<td>2-CDA</td>
<td>Mycobacterial lymphadenitis</td>
<td>324</td>
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<tr>
<td>7</td>
<td>M</td>
<td>1996</td>
<td>IFN(\alpha)</td>
<td>No</td>
<td>128</td>
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<tr>
<td>8</td>
<td>F</td>
<td>2000</td>
<td>IFN(\alpha)</td>
<td>Disseminated M. kansasii</td>
<td>117</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>1986</td>
<td>IFN(\alpha)</td>
<td>No</td>
<td>264</td>
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<tr>
<td>10</td>
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<td>No</td>
<td>406</td>
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<tr>
<td>11</td>
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<td>1983</td>
<td>Splenectomy</td>
<td>IFN(\alpha)</td>
<td>690</td>
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</table>
by Kluin-Nelemans et al. These authors investigated cytokine mRNA in T-cell fractions from spleens of HCL patients, and found spontaneous gene expression for IFN-γ, IL-2, IL-4 and granulocyte-macrophage colony stimulating factor (GM-CSF). Their finding of increased IFN-γ mRNA in spleen and our report of strongly decreased IFN-γ production may suggest that there is either a translational defect in these T cells, or a compartmentalised IFN-γ production. Further studies are needed to elucidate this issue.

Decreased IFN-γ production as a likely explanation for infections caused by facultative intracellular microorganisms, such as mycobacteria, has been reported for a number of conditions. First of all, there are hereditary disorders of the IL-12/IFN-γ axis. In CD4 lymphopenia, either as a consequence of HIV infection or idiopathic, IFN-γ production is low due to the loss of CD4 cells. In addition, in hyperIgE syndrome (HIES/Job's syndrome), deficient IFN-γ production has been found when blood cells were stimulated with relevant microbial stimuli (Staphylococcus and Candida spp.). It should be noted that in these patients, the susceptibility to infection pertains to the pathogens mentioned, rather than to facultative intracellular pathogens. A similar finding has been described in chronic mucocutaneous candidiasis. Finally, anti-TNFα treatment in patients with rheumatoid arthritis may lead to deficient IFN-γ production and infections by both facultative intracellular pathogens and pyogenic microorganisms. A consequence of our finding is that recombinant IFN-γ treatment may be considered for patients with HCL suffering from serious infection, with facultative intracellular microorganisms.

Acknowledgements

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References


Figure 1. Cytokine production capacity in hairy cell leukemia (HCL)

PBMC from healthy volunteers (n=22, open bars) and HCL patients (n=11, closed bars) were stimulated with LPS (10 ng/ml), or heat-killed S. typhimurium, S. aureus, M. tuberculosis, or C. albicans. The cytokine concentrations were measured in the supernatant after 48 hours (IFN-γ) or 24 hours (TNF, IL-10, and IL-6). The data are expressed as means ± SD, *p<0.05.

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