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), IFNα 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,9 with minor
modifications. The MNC fraction was obtained by density
centrifugation of blood diluted 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 10^6 cells/ml. The MNC population consisted of
approximately 80% lymphocytes and 20% monocytes, and no
differences between patients and controls were apparent.

5 x 10^5 MNC in a 100 µl volume were added to round-bottom
96-well plates (Greiner) and incubated with either 100 µl of
culture medium (negative control), or one of the various
stimuli: 10 ng/ml LPS (S. typhimurium; Sigma Chemical),
or 1 x 10^6 microorganisms/ml heat-killed (30 min, 100°C)
S. typhimurium, Staphylococcus aureus, Mycobacterium
tuberculosis or Candida albicans. After 24 hours (TNF, IL-1β and IL-6) or 48 hours (IFNγ) 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 IFNγ production after 48
hours of stimulation was significantly lower compared with
healthy volunteers (figure 1A). In contrast, the production of
the proinflammatory cytokines TNFα, IL-1β and IL-6 did not
differ between HCL patients and controls (figure 1B-D). The
specific defect in IFNγ production, in contrast to the normal
synthesis of monocyte products such as TNFα, IL-1β 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 TNFα production is normal is remarkable,
since elevated serum concentrations of TNFα have been
reported in patients with HCL.10-11 As far as we know,
defective IFNγ production has not been reported previously,
de spite the fact that there is extensive literature on
T-lymphocyte abnormalities in this disorder.12-15 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 10^5/ml)</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>1987</td>
<td>IFNα</td>
<td>No</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>1983</td>
<td>IFNα</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>IFNα</td>
<td>No</td>
<td>106</td>
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<tr>
<td>5</td>
<td>M</td>
<td>1984</td>
<td>Splenectomy</td>
<td>IFNα</td>
<td>803</td>
</tr>
<tr>
<td>6</td>
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<td>2-CDA</td>
<td>Mycobacterial lymphadenitis</td>
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<tr>
<td>7</td>
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<td>8</td>
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<td>Disseminated M. kansasii</td>
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</tr>
<tr>
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<td>IFNα</td>
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<tr>
<td>10</td>
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<td>2-CDA</td>
<td>No</td>
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<tr>
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<td>Splenectomy</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.

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REFERENCES


