Plasma Interleukin-3 (IL-3) and IL-7 Concentrations in Children with Homozygous Beta-thalassemia

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Summary
Plasma levels of IL-3 and IL-7 were studied in 23 patients with homozygous beta-thalassemia in order to determine whether these cytokines are involved in abnormalities in erythropoiesis and immune responses as observed in thalassemic patients. No significant difference was found in plasma IL-7 concentrations between thalassemic patients and healthy controls and it was suggested that IL-7 is not a cytokine involved in cellular immunological alterations in beta-thalassemia. However, the number of thalassemic patients with detectable IL-3 concentrations was significantly higher. It was concluded that IL-3 production has to be studied in detail in order to learn more about the involvement of this cytokine in erythropoiesis of thalassemic patients.

Introduction
Cytokines play key roles in the control of haemopoiesis and immunity. These are biologically active molecules which are mainly produced by immune-competent cells and regulate immune response, inflammation and haemopoiesis. Haemopoiesis is controlled by at least 30 known cytokines. Some of these, such as erythropoietin, is present at all times, whereas others such as IL-3, IL-7 are produced in response to specific stimuli.

Recently, plasma levels of some cytokines have been studied in patients with different types of anaemia, such as sickle cell anaemia, auto-immune hemolytic anaemia and iron deficiency anaemia in order to determine whether selected cytokines have a role in abnormalities of erythropoiesis and immune response in these conditions. For example, highly elevated levels of plasma erythropoietin in beta-thalassemic patients has been known since 1987. However, Jelkman et al. have shown the inhibitory effect of IL-1 and TNF-alpha, and stimulatory effect of IL-3 on erythropoietin production in 1994.

Besides abnormalities in erythropoiesis, patients with thalassemia major are characterized by a partial functional immunodeficiency determined by increased activity of CD8+ suppressor/cytotoxic lymphocytes and reduced activity of the CD4+ helper/inducer subset. In addition, B lymphocytes have found to be highly activated in patients with thalassemia major. IL-7 is secreted by stromal cells of the bone marrow and acts predominantly on T and B lymphocyte progenitors. It functions as a growth and differentiation factor for both T and B cell precursors. IL-3 is a multi-colony stimulatory factor and is involved in proliferation of early progenitors of haemopoietic cells including erythrocytes.

Plasma IL-3 and IL-7 concentrations in beta-thalassemic patients were measured in order to determine whether they are useful for a better definition of immunological and erythropoietic alterations.

Materials and Methods
Twenty-three homozygous beta-thalassemic patients were included in the study. The age of the patients ranged from 2 to 18 years with a mean 9.4 ± 5.1. These patients had required blood transfusions since the ages of 4–15 months; at the time of the present study, transfusions of one unit of packed red blood cells were being given at 4-week intervals. Blood samples were obtained from the subjects just before a scheduled...
transfusion. Fifteen healthy normal children with an age range 2–17 years (mean 10.4 ± 7.4) were included as a control group.

IL-3 and IL-7 (Amersham, Aylesbury, UK) concentrations were measured in plasma using enzyme-linked immunosorbent assay (ELISA). The minimum detectable concentrations of plasma IL-3 and IL-7 by this method are 1.5 and 1.2 pg/ml, respectively. Statistical analyses were performed using Student's t-test and Chi square test.

**Results and Discussion**

All patients and healthy controls had detectable concentrations of IL-7. No significant difference (P > 0.05) was found in plasma IL-7 concentrations between thalassemic patients and healthy controls (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Thalassemic patients</th>
<th>Healthy controls</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>IL-3 (pg/ml)</td>
<td>6.9 ± 17.1</td>
<td>0.2 ± 0.5</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Range</td>
<td>(0–56.1)</td>
<td>(0–1.5)</td>
<td></td>
</tr>
<tr>
<td>Detectable IL-3</td>
<td>39%</td>
<td>13%</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>IL-7 (pg/ml)</td>
<td>37.8 ± 26.0</td>
<td>46.0 ± 32.5</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Range</td>
<td>(3.5–78.1)</td>
<td>(12.1–119.1)</td>
<td></td>
</tr>
<tr>
<td>Detectable IL-7</td>
<td>100%</td>
<td>100%</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

IL-3 concentrations were found detectable in 9/23 (39 per cent) thalassemic patients and 2/15 (13 per cent) healthy controls (P < 0.05) (Table 1). Quantitatively, there was no significant difference (P > 0.05) between these two groups, because of the range of IL-3 concentrations and high standard deviation in thalassemic group (Table 1).

A series of immunological abnormalities has been described in patients with homozygous beta-thalassaemia. Lombardi et al. have measured serum levels of IL-2, IL-6, TNF, soluble (s) CD4, sCD8, sCD23, sCD25 in transfusion-dependent patients with beta-thalassaemia major, and found increased TNF, sCD8, sCD23 and sCD25 and lower sCD4 values compared to normal controls. In Lombardi's study, IL-2 and IL-6 were found to be undetectable or within the normal range in all patients. Recently, similar to Lombardi's study, an abnormal cell-mediated immunity has often been reported in thalassemic patients, but to date the mechanism of immunological alteration remains unclear. IL-7 as a growth and differentiation factor for both T and B cells has not been found to be elevated or different than healthy controls in thalassemic patients in our study and it was suggested that IL-7 is not a cytokine involved in immunological alterations in thalassemia major.

In general, haemopoietic growth factor production is a cascade reaction regulated in such a way as to permit flexible and integrated responses to diverse haemopoietic stresses. Increasing levels of GM-CSF and IL-3 appear to be associated with increased haemopoiesis. In our study, although no significant difference in plasma IL-3 concentrations between thalassemic patients and healthy controls was found, the number of thalassemic patients with detectable IL-3 levels were significantly higher than control population. According to the data reported here, the involvement of IL-3 production in erythropoiesis of thalassemia major patients has been suggested.

**References**