

## RAPID COMMUNICATION

Elevated Interleukin-8 Serum Concentrations in  $\beta$ -Thalassemia and Graft-Versus-Host Disease

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Neutrophil chemotactic and functional defects occur in  $\beta$ -thalassemia and in patients after bone marrow transplantation (BMT). Interleukin-8 (IL-8) is a novel chemotactic and activating peptide for neutrophils and can be detected in the circulation. IL-8 serum concentrations were evaluated in 30  $\beta$ -thalassemic patients before and after BMT. Serial samples from 16 patients were also studied. Fourteen sera from healthy children, 43 patients with chronic viral hepatitis, 16 patients on chronic transfusion treatment for various hematologic disorders, and 28 healthy adults were studied as controls. IL-8 was evaluated by an enzyme-linked immunosorbent assay. Patients with  $\beta$ -thalassemia had higher IL-8 concentrations than did normal controls, pa-

tients with liver disease, and patients on chronic transfusion.  $\beta$ -Thalassemic patients with severe liver siderosis and fibrosis had the highest IL-8 concentrations. After BMT in patients with successful engraftment, IL-8 concentrations decreased significantly. In contrast, in patients with acute graft-versus-host disease (GVHD), IL-8 concentrations were not statistically different from the concentrations found before BMT and were higher than in patients with no complications and patients with graft rejection. IL-8 may play a part in the immune dysregulation that occurs in  $\beta$ -thalassemia and may be involved in the immune mechanisms leading to GVHD.

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SEVERAL immunologic defects can be found in patients with  $\beta$ -thalassemia, among which the impairment of neutrophil phagocytic and killing functions is of the utmost importance.<sup>1</sup> As an alternative to blood transfusions and chelating therapy, bone marrow transplantation (BMT) has become an accepted treatment for  $\beta$ -thalassemia.<sup>2</sup> During graft-versus-host disease (GVHD), neutrophil chemotactic and phagocytic defects are well recognized<sup>3</sup> and  $\beta$ -thalassemic patients who undergo BMT may show profoundly depressed or dysregulated neutrophil functions.

The recently cloned interleukin-8 (IL-8), also known as neutrophil-activating peptide-1 (NAP-1), acts primarily and almost exclusively on neutrophils, stimulating chemotaxis and degranulation. This peptide can be produced by a variety of cell types, including large granular lymphocytes, macrophages, endothelial cells, fibroblasts, and synovial cells.<sup>4</sup> Activated monocytes, endothelial cells, and fibroblasts may produce IL-8 in response to exogenous and endogenous stimuli, such as lipopolysaccharide (LPS), tumor necrosis factor (TNF), and IL-1.<sup>5</sup>

It has been recently shown that IL-8 acts as a chemoattractant when intradermally injected, whereas it inhibits leukocyte endothelial cell adhesion when systemically administered,<sup>6</sup> suggesting that this cytokine may also play a complex

role in the regulation of inflammatory processes. Therefore, changes in IL-8 synthesis, site of production, or serum levels may be relevant in the pathophysiology of both  $\beta$ -thalassemia and GVHD. To assess this hypothesis we investigated IL-8 serum concentrations in patients with  $\beta$ -thalassemia before and after BMT.

## MATERIALS AND METHODS

*Patients.* From the series of 222 consecutive patients reported elsewhere<sup>2</sup> we randomly selected 30 (mean age, 9 years; range, 2 to 15 years; 17 males and 13 females), stratifying<sup>7</sup> them according to outcome of BMT. Ten had acute GVHD, 7 rejected the transplant, and 13 were disease-free survivors. Eleven had received up to 100 transfusions and 19 had received more than 100 transfusions before BMT.

Liver biopsy was performed in 27 patients as a part of liver disease assessment before transplantation. The presence of siderosis and fibrosis was evaluated by semiquantitative scoring (absent, mild, moderate, and severe). Seven patients presented with chronic active hepatitis and seven with chronic persistent hepatitis; none had cirrhosis.

Serum samples were obtained from all patients (mean  $\pm$  SEM: 22.1  $\pm$  3.6 days) before transplantation. In patients with acute GVHD or graft rejection, the sample taken after transplantation was obtained at the onset of transplant complications; in all cases samples were obtained at least 20 days after BMT. In patients with no GVHD or rejection, post-BMT samples were obtained at a comparable time (ie, after day 20). In addition, serial serum samples (3 to 10 samples) were obtained from 16 patients (8 with successful engraftment, 3 with graft rejection, and 5 with acute GVHD). Follow-up length varied from 50 to 360 days. Blood samples were obtained from each patient between 7:00 AM and 9:00 AM and left to clot at room temperature. Serum samples were stored at  $-70^{\circ}\text{C}$  until analysis.

The preparative regimen for BMT has been described previously.<sup>2</sup> Briefly, all patients received busulphan (3.5 mg/kg/d) for 4 consecutive days (day  $-9$  through  $-6$ ) followed by cyclophosphamide (4.0 mg/kg/d) for 4 consecutive days (day  $-5$  through  $-2$ ). Three patients were also treated with cyclosporin A (5 mg/kg/d). GVHD prophylaxis was performed using either cyclosporin A, methotrexate, or both in combination. Diagnostic criteria for infections, GVHD, and engraftment after BMT have been reported elsewhere.<sup>8</sup>

Control serum samples were obtained from 14 normal healthy children matched for sex and age (mean age, 8 years; range, 3 to 17 years; 6 males and 8 females) attending outpatient clinics at Pesaro Hospital (7 cases) and at King's College Hospital, London (7 cases) for routine checks.

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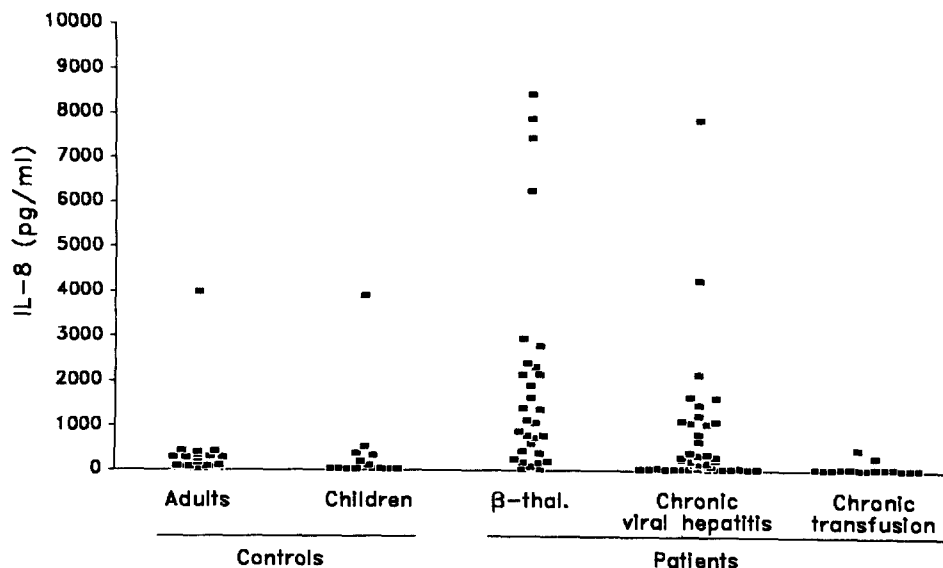
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**Fig 1.** Serum IL-8 concentrations in normal controls (adults and children) and in patients with  $\beta$ -thalassemia, chronic viral hepatitis, and on chronic transfusion treatment. ( $\beta$ -thalassemia v normal children, chronic viral hepatitis patients, and chronic transfusion treatment patients,  $P < .001$ .)



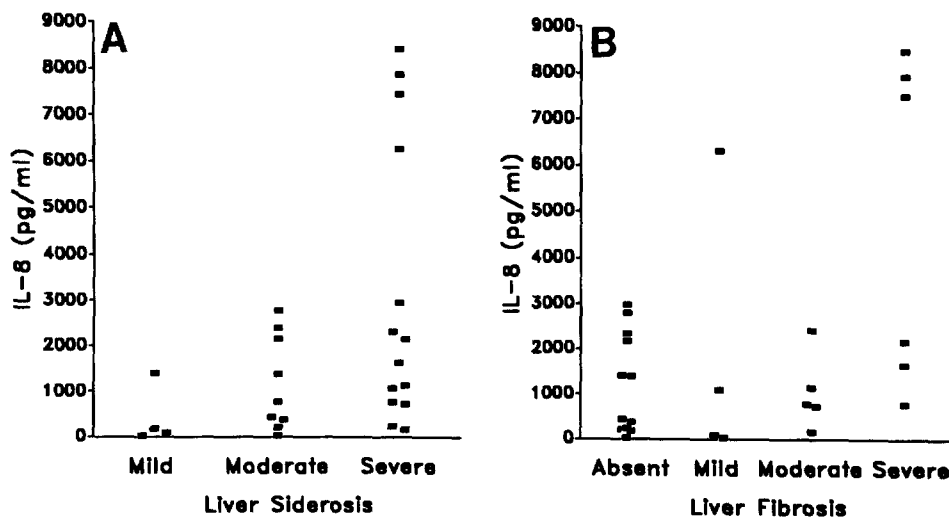
In addition, because  $\beta$ -thalassemic patients are on chronic transfusion treatment and often have chronic liver disease, we studied 43 patients with chronic viral hepatitis (mean age, 41 years; range, 15 to 74 years; 25 males and 18 females), 16 on chronic transfusion treatment for various hematologic disorders (mean age, 64 years; range, 24 to 90 years; 9 males and 7 females), and 28 healthy adults (mean age, 43 years; range, 24 to 58 years). Patients with chronic viral hepatitis had either hepatitis B virus infection (8 HBeAg<sup>+</sup>, 11 anti-HBe<sup>+</sup>, and 9 with hepatitis D virus superinfection) or hepatitis C virus infection (15 cases).

**IL-8 assay.** Serum IL-8 concentration was determined by a solid-phase double-ligand enzyme-linked immunosorbent assay (ELISA) as reported elsewhere.<sup>9</sup> The absorbance for each well at 405 nm was read with a BioRad 2550 EIA Reader (BioRad, Richmond, CA) interfaced with an IBM personal computer XT. Mean absorbance values from serum samples were plotted on a standard curve obtained from a reference IL-8 standard (nine dilutions, 0.03 to 10 ng/mL). The standard curve coefficient of correlation always exceeded 0.98. The optical density units were converted into nanograms per milliliter by the BioRad ELISA data analysis software.

Nonparametric tests were used to analyze data. Wilcoxon's test was used for paired observations and the Mann-Whitney U test was used for independent values. Kendall's coefficient of correlation was used to compare IL-8 concentrations and the results of liver histology (SPSS/PC+, Chicago, IL).

**RESULTS**

$\beta$ -Thalassemic patients had higher serum IL-8 concentrations compared with those of healthy children (mean  $\pm$  SEM patients, 1,938  $\pm$  439 pg/mL; healthy children, 379  $\pm$  274 pg/mL;  $P < .001$ ) (Fig 1).  $\beta$ -Thalassemic patients had higher serum IL-8 levels compared with those of chronic hepatitis patients (665  $\pm$  211 pg/mL,  $P < .001$ ) and patients on chronic transfusion (45  $\pm$  32 pg/mL,  $P < .001$ ) as well. Serum IL-8 concentrations in patients with chronic viral hepatitis and those on chronic transfusion were not significantly increased compared with those of healthy adults (252  $\pm$  140 pg/mL) (Fig 1).



**Fig 2.** Serum IL-8 concentrations in  $\beta$ -thalassemic patients with different degrees of (A) liver siderosis and (B) liver fibrosis.

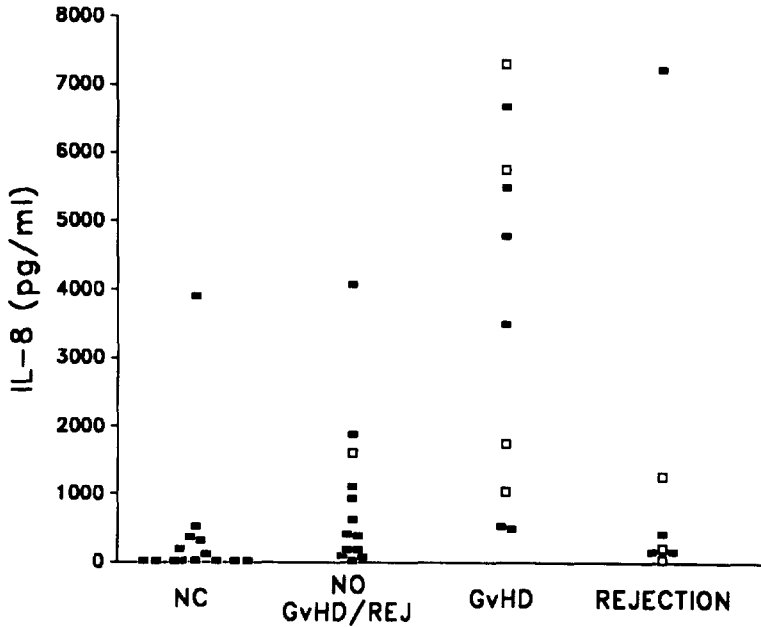


Fig 3. Serum IL-8 concentration in normal controls (NC) and  $\beta$ -thalassemic patients after BMT (GVHD v NO GVHD/REJ,  $P < .01$ ; GVHD v REJ,  $P < .05$ ). (■) No infection; (□) infection.

In  $\beta$ -thalassemic patients, IL-8 serum concentrations were not related to sex, the number of blood transfusions, chelation therapy, or ferritin concentration, whereas a significant direct correlation was found when IL-8 serum concentrations were compared with patients' age ( $P < .05$ ). There was no significant difference in IL-8 concentrations among the pretransplant samples from the three BMT outcome groups.

In  $\beta$ -thalassemic patients, we found a significant correlation between IL-8 serum concentrations and the degree of liver siderosis ( $P < .01$ ) (Fig 2A). Patients with severe liver fibrosis had significantly higher IL-8 serum concentrations than did those with no liver fibrosis ( $P < .05$ ) (Fig 2B). No relation was found between IL-8 concentration and the presence of chronic persistent hepatitis or chronic active hepatitis.

Acute GVHD patients had significantly higher IL-8 serum concentrations ( $3,720 \pm 830$  pg/mL) than did patients without complications ( $873 \pm 313$  pg/mL) and patients with rejection ( $1,336 \pm 992$  pg/mL) (Fig 3).

In patients who had a successful engraftment, IL-8 serum concentrations decreased significantly after BMT ( $P < .02$ ); in the seven patients we studied sequentially, IL-8 remained persistently low (Fig 4A).

In contrast, IL-8 serum concentrations did not decrease during acute GVHD and in the five patients we studied sequentially, IL-8 remained at high levels, with occasional peaks (Fig 5). In patients with graft rejection, there was no statistical difference between IL-8 serum concentrations before and after BMT. In three patients we studied sequentially after BMT,

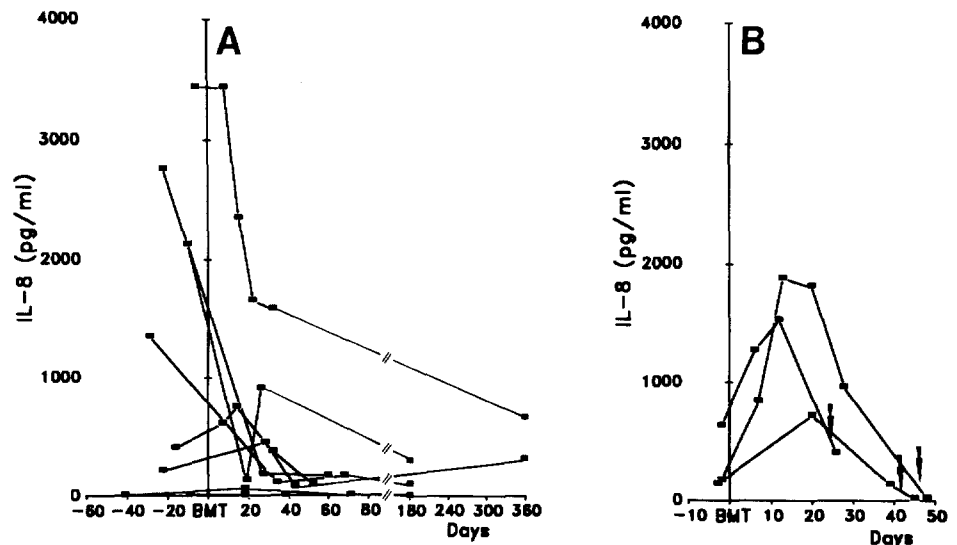


Fig 4. (A) Sequential serum IL-8 concentrations in seven  $\beta$ -thalassemic patients with successful engraftment. (B) Sequential serum IL-8 concentrations in three  $\beta$ -thalassemic patients with graft rejection. Arrows indicate the day rejection was diagnosed.

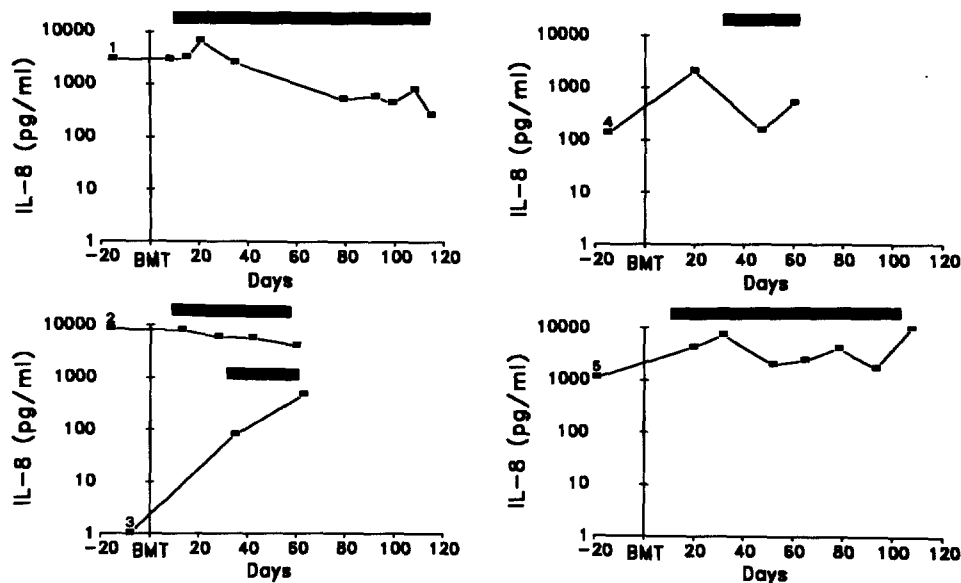


Fig 5. Sequential serum IL-8 concentration in five  $\beta$ -thalassemic patients with acute GVHD. Solid bars indicate the period of acute GVHD clinical manifestations.

IL-8 serum concentrations increased within 20 days and returned to normal levels at the time of rejection episodes (Fig 4B).

No relationship was found between IL-8 serum concentration and episodes of infection.

#### DISCUSSION

Our results show (1) increased IL-8 serum concentrations in  $\beta$ -thalassemia; (2) a persistent decrease after BMT in patients with successful engraftment; and (3) persistent high concentrations or increases during GVHD.

Circulating IL-8 can be detected in patients with septic endotoxaemia,<sup>10</sup> rheumatoid arthritis (RA),<sup>11</sup> and Felty's syndrome (R. Meliconi, personal communication, August 1992) and in liver patients after treatment with recombinant TNF $\alpha$  (rTNF $\alpha$ ).<sup>12</sup> Although IL-8 is primarily a neutrophil-activating peptide, these diseases differ widely with regard to neutrophil number and activation. Sepsis is characterized by a high number of activated neutrophils<sup>13</sup> and in RA large amounts of polymorphonuclear leukocytes (PMN) infiltrate joint synovial fluid. Felty's syndrome is associated with low numbers and lack of activation of neutrophils, whereas no significant changes in PMN have been recorded in liver patients or in patients receiving rTNF $\alpha$ .

We found high IL-8 serum concentrations in the majority of  $\beta$ -thalassemic patients. A likely cause of this IL-8 production could be ascribed to the transfusion-related continuous antigenic stimulation and iron overload with consequent macrophage activation. The relationship we found between IL-8 concentration and patients' age is in keeping with this hypothesis even though we did not find a correlation between IL-8 concentration and number of blood transfusions. Macrophages (and fibroblasts) can be responsible for IL-8 production either directly or indirectly via TNF $\alpha$  synthesis.<sup>5</sup> On the other hand, the low serum IL-8 concentrations found in the adult patients on chronic transfusion treatment suggest that in  $\beta$ -thalassemia there should be an additional, more

specific cause for IL-8 elevation. It should be noted that transfusion treatment begins at a very early age in  $\beta$ -thalassemic patients and the duration of this treatment is probably longer than in adults transfused for other hematologic disorders.

Chronic hepatitis is not per se a cause of IL-8 elevation, even if some patients with chronic viral hepatitis have high IL-8 concentrations. Nonetheless, the correlation between IL-8 concentration and the degree of liver siderosis and the finding of high IL-8 concentrations in  $\beta$ -thalassemic patients with severe liver fibrosis are intriguing, but are not clarified by this study.

Interestingly, an increase in superoxide anion production correlated with liver damage has been documented in  $\beta$ -thalassemia.<sup>14</sup>

After BMT, IL-8 concentration decreased significantly and persistently in patients with successful engraftment. This could be due to the end of the transfusion-related antigenic stimulation and to the immunosuppressive treatment. On the contrary, in patients with GVHD, IL-8 remained high and even presented late peaks.

Combined neutrophil defects have been documented after BMT, particularly in patients experiencing GVHD. Defects in PMN chemotaxis and bactericidal activity have also been shown in  $\beta$ -thalassemia. Therefore, high levels of circulating IL-8 are present in patients with  $\beta$ -thalassemia and GVHD both characterized by PMN defects in chemotaxis and activation.<sup>15</sup> In addition, few or absent neutrophils are present in the peripheral blood of patients after BMT.

It has been recently shown that intravascular IL-8 inhibits PMN migration at sites of inflammation by inhibiting the leukocyte-endothelial adhesion process.<sup>6</sup> Thus, high circulating IL-8 concentrations in  $\beta$ -thalassemia and GVHD may contribute to the chemotaxis defect shown by PMN. On the other hand, high IL-8 can activate PMN, which is in keeping with the finding of an increased superoxide anion production by neutrophils from  $\beta$ -thalassemic patients.<sup>14</sup>

Finally, we documented a transient increase in serum IL-8 before the clinical diagnosis of graft rejection in the three patients with follow-up samples available. Before we can ascribe a clinical value to IL-8 serum testing in graft rejection diagnosis, follow-up samples from a larger series of patients rejecting BMT should be evaluated.

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