Clinical and laboratoristic remission after cryosupernatant plasma exchange in thrombotic thrombocytopenic purpura

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We describe a case of thrombotic thrombocytopenic purpura (TTP) resistant to conventional therapy with fresh-frozen plasma (FFP)-plasma exchange (PEX) as well as to steroids, immunoglobulins, vincristine, dipyridamole, dextran and iloprost, achieving complete remission with cryosupernatant-plasma exchange. Our case shows the effectiveness of cryosupernatant PEX, when FFP-PEX and alternative therapies have failed.

Thrombotic thrombocytopenic purpura (TTP) is a rare and multisystemic disorder characterized by platelet aggregation, formation of platelet-rich thrombi and impairment of blood flow in the microvasculature.[1] The clinical features include neurological signs and possible renal involvement, fever, microangiopathic hemolytic anemia and severe thrombocytopenia. Only 70-80% of acute TTP respond to conventional plasma exchange (PEX) using fresh-frozen plasma (FFP),[2] and the effectiveness of alternative agents remains elusive.[3] We have seen a case of TTP resistant to conventional therapy achieving complete remission after cryosupernatant plasma exchange.

A 28-year-old woman was admitted to the hospital because of jaundice and severe anemia. Hemoglobin was 74 g/L associated with a reticulocyte count of 20%. Indirect bilirubin level was 68.4 µmol/L with a Coomb's negative test. LDH level was 845 U/liter and platelet count was 3×10^9/L. The peripheral blood smear showed burr and helmet cells. This constellation of finding supported the diagnosis of TTP.

After three days of plasma exchange (fresh frozen plasma 1800-2000 mL per session/daily), drowsiness and left hemiparesis developed. Minimal recovery was seen after a second cycle of plasma exchange with fresh frozen plasma and additional treatment with high-dose immunoglobulins (600 mg/kg iv daily for 5 days), vincristine (1 mg iv weekly for 2 weeks), methylprednisolone (60 mg iv daily for about 10 weeks), dextran 40 (250-500 mL iv for about 3 weeks), dipyridamole (800 mg os daily for about 12 weeks), and prostacyclin (10 ng/kg/min iv for about 4 weeks). Figure 1 shows the correlation between the platelet count and the different treatments. Platelet count stabled above 15×10^9/L with no evidence of any hemorrhagic episode. Soon after, fluctuating neurological signs developed and FFP/PEX was discontinued. Cryosupernatant (plasma from which cryoprecipitate has been removed) PEX was then daily performed for two consecutive weeks and twice a week thereafter for about one month. LDH blood level normalized and platelet count ranged from $250\text{-}300\times10^9$/L. Nine months later, the patient had no neurological deficit and remained in complete clinical and laboratoristic remission.

On the basis of the possible involvement of high molecular weight, von Willebrand factor (HVWF) multimers in the pathogenesis of TTP,[4,5] the use of cryosupernatant (plasma depleted of cryoprecipitate) seems to be reasonable due to the fact that it is relatively deficient of VWF multimers.[6-9] Furthermore, considering the effectiveness of cryosupernatant, it is tempting to speculate that even the use of high affinity monoclonal antibodies against glycoprotein IIb/IIIa may represent a promising alternative in refractory TTP. In fact, these antibodies can block the well-known interaction[10] of HVWF multimers with the platelet membrane glycoprotein IIb/IIIa.[10]

In conclusion, our report confirms previous experiences and furthermore, strongly supports the need for a randomized trial to confirm the first-line use of cryosupernatant in the treatment of TTP.

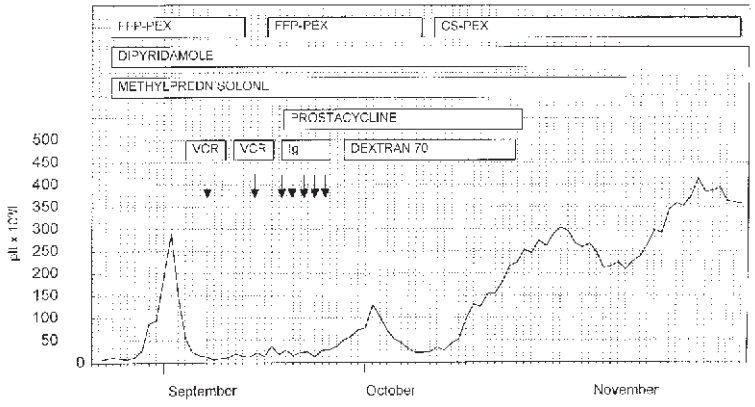

Figure 1. Correlation between platelet counts and different treatment attempts.

FFP-PEX: plasma exchange with fresh-frozen plasma.
CS-PEX: plasma exchange with cryosupernatant.
VCR: vincristine.

Key words

Plasma exchange, cryosupernatant plasma, thrombotic thrombocytopenic purpura

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Post-transplant cerebral toxoplasmosis diagnosed by magnetic resonance imaging

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Cerebral toxoplasmosis is a rare late complication in allogeneic bone marrow transplanted patients. Neuroradiological findings may suggest the correct diagnosis. We report a patient in whom cerebral magnetic resonance imaging (MRI) showed a lesion characteristic of toxoplasmosis. Anti-toxoplasma treatment led to clinical and radiological improvement. MRI seems to be a valid tool for detection and follow-up of cerebral toxoplasmosis.

Toxoplasmosis is the most common opportunistic infection affecting the central nervous system in patients with AIDS. Cerebral toxoplasmosis occurs in about 30% of toxoplasma-seropositive patients when $CD4^+$ cells fall below 100/mL.[1] It has also been reported in bone marrow transplanted (BMT) patients.[2,3] In principle, a definitive diagnosis of toxoplasmosis must be supported by positive serology and proven by histological evidence of tachyzoites in a brain lesion. However, in BMT recipients brain biopsy may not be performable because of thrombocytopenia, and serology may be uninformative because of concurrent immunosuppression. We report a case of cerebral toxoplasmosis in a patient with Hodgkin's disease (HD) who underwent allogeneic BMT followed by donor lymphocyte infusions (DLI) for a post-transplant relapse.

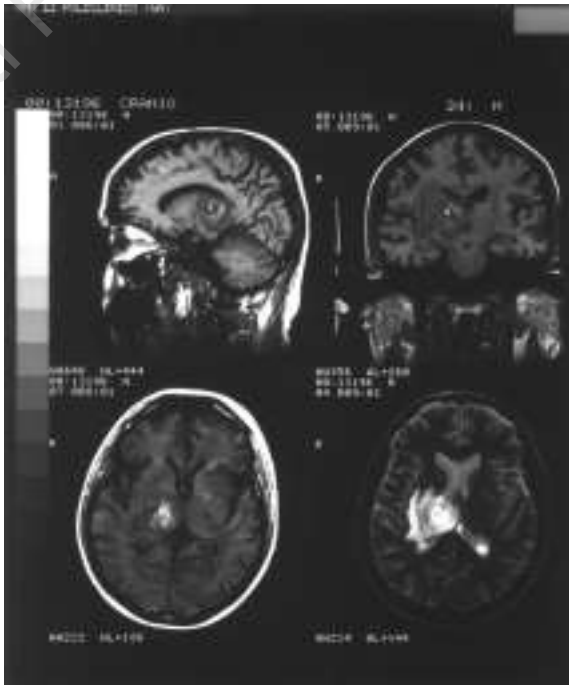

Figure 1. Cerebral MRI. Gradient recalled echo (GRE) sagittal and coronal T1-dependent pre-contrast images (top) demonstrate a round lesion in the right thalamus, with several concentric components (central: high intensity; intermediate: low intensity; peripheral: high intensity) surrounded by a low intensity edema. In the edematous region a post-contrast (Gadolinium-DTPA) GRE image (bottom, left) shows the presence of a thin ring-shaped enhancement. In the spin-echo (SE) T2-dependent image (bottom, right) the center of the lesion appears hypointense.

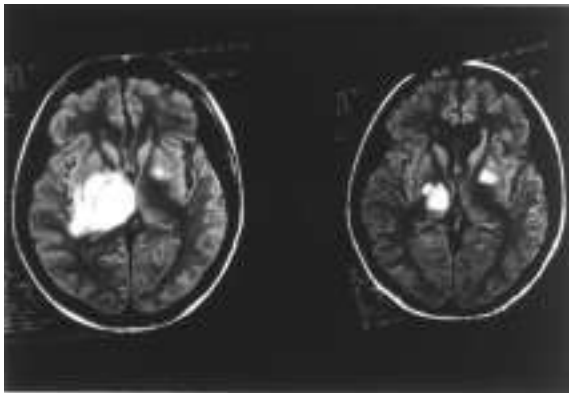

Figure 2. Pre-and post-therapy SE proton density-dependent images showing the presence of a second lesion in the left pallidus. In the post-therapy image (right) the dimension of the main lesion is markedly reduced, with disappearance of the edematous component. No change is observed in the smaller lesion.

HD, nodular sclerosis, stage IV B was diagnosed in a 22 year-old man in October 1995. After 16 doses of VEBED (vinblastine, epirubicin, bleomycin, etoposide, deflazacort), he achieved partial response. In June 1996 the patient underwent allogeneic BMT from his HLA-identical sister. Conditioning was busulfan-cyclophosphamide; graft versus host disease (GVHD) prophylaxis was carried out with cyclosporine; *Pneumocystis carinii* prophylaxis was performed by aerosolized pentamidine. Engraftment was documented on day +25. Six months later, with a bone marrow karyotype XX, mediastinal and liver HD relapse occurred; the patient was treated by a single C-MOPP course and two DLI (infused lymphocytes: 0.25 and 0.9×10^8/kg, respectively). Complete remission (CR) was achieved, but severe cutaneous and hepatic GVHD occurred, requiring intense immunosuppression by cyclosporine and methylprednisolone. A month later fever and neurological symptoms appeared. ESR and serum LDH were elevated. Brain CAT scan showed a hypodense right thalamic lesion of undefined nature; magnetic resonance imaging (MRI) showed a target-shaped lesion in the right thalamic area with a spontaneous hyperintense centre on T1-weighted images (Figure 1), likely due to hemorrhage, and a hypointense centre on T2-weighted images. A smaller hyperintense lesion was present in the left lenticula. Brain biopsy could not be performed because of thrombocytopenia. A low titer of serum anti-toxoplasma IgG was detected. Pyrimethamine-sulfadiazine treatment was started, leading to marked clinical and neuroradiological improvement within two months (Figure 2). At present, pyrimethamine-sulfadiazine treatment is continued at lower doses. HD is still in CR; the patient presents mild cutaneous and hepatic GVHD, and some neurological disturbances persist.

Toxoplasmosis has to be considered a possible cause of focal cerebral disease in allogeneic BMT patients.[4] Main risk factors are delayed immunological reconstitution and prolonged immunosuppression needed to control severe forms of GVHD. In absence of definite histological evidence, a presumptive diagnosis of toxoplasmosis can be based on MRI findings and is confirmed by response to specific anti-toxoplasma treatment. Peculiar findings at MRI are multiple round shaped lesions, with ringed or nodular gadolinium enhancement and mass effect.[5] The lesions are most often localized at the cerebral cortical or corticomedullary junction. A target-shaped lesion with hypointense centre on T2-weighted images is considered typical,[5] while other findings, such as the ringed gadolinium enhancement, may also be found in other diseases.[6] In our case, as in others,[7] aerosolized pentamidine was an insufficient prophylaxis against toxoplasmosis. The intense immunosuppression used to control the DLI-induced GVHD has certainly favored the infectious complication; on the other hand, DLI treatment may have contributed to achieve HD remission. To the best of our knowledge, no other case of relapsed HD has been treated by DLI.[8]

Key words

Cerebral toxoplasmosis, bone marrow transplantation, magnetic resonance imaging, donor lymphocyte infusion

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Thrombopoietin: a potential T-helper lymphocyte stimulator. Change in T-lymphocyte composition and blood cytokine levels in thrombopoietin cDNA transferred mice

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The aim of this study was to evaluate the effect of thrombopoietin (TPO) on T lymphocyte in Balb/c mice delivered hTPO cDNA with plasmid vector. Both mature and immature T lymphocytes in central organs increased, but only the CD4$^+$ subset was preferably proliferated in circulation. High serum IFN-γ was coinciding with the declination of platelet counts, but TNF-α was positively associated with the platelet count, while high IL-2 level was similar to the course of TPO expression. Our data suggested that TPO is a stimulator for T lymphocytes, especially the CD4$^+$ subset.

Accumulating materials have enlightened the participation of thrombopoietin (TPO) in immunological processes. TPO stimulates the proliferation of endothelial cells and enhanced their expression of cell adhesion molecules.[1] It also indirectly induces the production of interferon-α *in vitro*.[2] We previously observed macrophage proliferation and endothelial cell activation in the spleens of TPO gene therapy mice. Here we have investigated the T lymphocyte composition and cytokine production of mice with TPO cDNA delivery.

Female Balb/c mice were delivered with the pcDNA3/hTPO plasmid as previously described.[4] The dose was 60 μg plasmid DNA for each mouse. Null-treated mice as control. Mononuclear cells isolated from thymocytes, plenocytes, fumer marrow cells and blood cells were directly stained with FITC conjugated-anti-mouse CD4 (L3T4) and PE conjugated-anti-mouse CD8a (Ly-2)(Pharmingen). Samples were analyzed using a FacStar flow cytometer (Becton Dickinson). For each sample, 5,000 events were acquired. Sera collected from 5 mice at set times were analyzed in duplicate with IFN-γ, IL-2 ELISA kits (Genzyme) and TNF-α kits (Biotinge, China).

Both mature T lymphocytes (CD4$^+$ or CD8$^+$) and immature ones (CD4$^+$CD8$^+$) in central and peripheral immune tissues were affected. They assumed different characters in the 2nd week. As Figure 1 shows, in marrow and spleen, all the three T lymphocyte subsets increased, while only the immature cells increased in the thymus. However, CD4$^+$ subset predominantly and selectively increased in blood.

Serum IFN-γ, TNF-α and IL-2 concentrations began to change early within 24 hr. of gene delivery (Figure 2). IFN-γ peak was 9-12 folds that of the controls and maintained from the 2nd week. Striking peak TNF-α level was about 120 folds of the controls but only occurred in the first week. Meanwhile, IL-2 was high at the most times.

Our data indicated the stimulatory effect of TPO on T cell production. In this process, T helper subset was subject to be selectively enhanced, as implied by the increase in blood CD4$^+$ cells. Elevated immature but low mature T ratio in the thymus might suggest a speed-up development. Furthermore, high IFN-γ and IL-2 production implied activation of Th1 and possibly NK cells in the time.

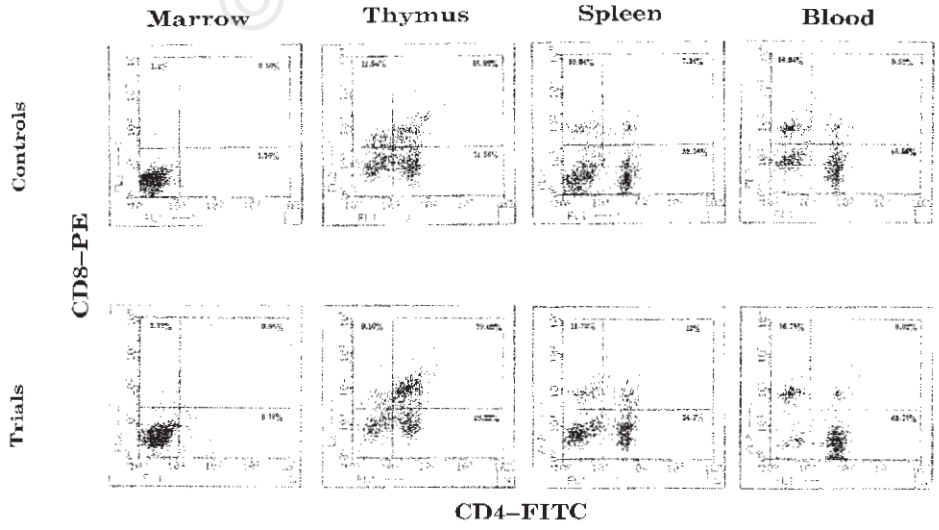

Figure 1. T-lymphocyte frequency alterations.

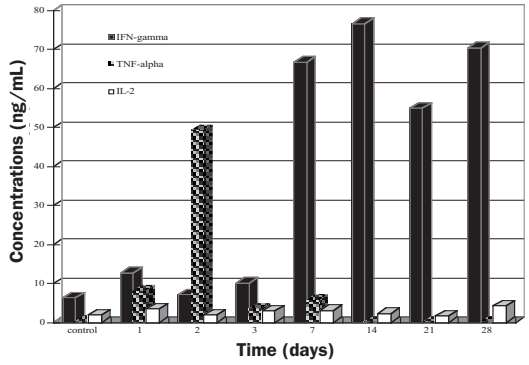

Figure 2. Serum cytokine kinetics of TPO gene delivery mice.

IFN-γ is inhibitory to CFU-Mk.[3] Although a contrary conclusion was recently gained,[4] its striking increases that coincide with the declination in platelet count and accompanying the fluctuation of platelet counts[5] inferred its down-regulatory role in thrombopoiesis. TNF-α can stimulate the proliferation of a human megakaryocytic cell line.[6] However, it showed little correspondence with platelet count in the later stages. The change of IL-2 was associated to TPO expression[7] that might support a direct stimulatory role of TPO on T lymphocytes. Although TPO indirectly induced IFN production *in vitro*[2] here, the proliferation of T lymphocytes might give a better explanation for the cytokine overproduction.

Several groups effectively promoted mice platelet production by TPO over-expression.[5,8,9] Recently, it was noticed that following platelet peak, the adenovector-mediated hTPO delivery had induced autoantibodies against TPO in Balb/c mice and resulted in pathological changes.[10] Was the activation of T lymphocyte part of such reactions? With the plasmid vector, first, we kept TPO expression for much longer than the platelet peak[7] without its being neutralized by the possible autoantibodies; second, our primary analysis of marrow megakaryocyte did not observed its reduction during this process. Furthermore, we recently observed that hTPO cDNA delivery thoroughly induced the turnover of tumor infiltration T lymphocyte phenotypes from $CD8^+$ to $CD4^+$ that accompanied significant retardation of the implanted tumor (unpublished data). The immunological responses did not seem auto-reactive.

Key words

Thrombopoietin, T lymphocyte, IFN-α, TNF-γ, IL-2

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Phenotypic changes in neutrophil granulocytes after G-CSF administration in patients with acute lymphoblastic leukemia under chemotherapy

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Phenotypic changes in neutrophil granulocytes (NG) after G-CSF have been scarcely studied. Using flow cytometry, we analyzed the changes of CD11b, CD14, CD33, CD71, HLA-DR, CD10, CD16 and CD15 on NG after G-CSF treatment in 6 patients with ALL receiving

intensification chemotherapy and in 10 control subjects. After G-CSF we found: expression of HLA-DR, a higher expression of CD11b, CD71 and CD14, a decrease in CD10 positivity, and fluorescence intensity in CD15 and CD16. After administration of G-CSF, the NG of patients with ALL express an immature phenotype as well as markers of proliferation.

In addition to the increase in the number of neutrophil granulocytes (NG), functional and phenotypic changes have occurred after G-CSF treatment. However, latter changes have been scarcely studied.[1,2] The aim of this study was to analyze these changes in ALL patients receiving G-CSF to enhance NG recovery after intensification chemotherapy.

Six adult patients with ALL receiving intensification chemotherapy (PETHEMA ALL-93 protocol[3]) were studied. The patients received 5 µg/kg G-CSF (Filgrastim®, Amgen-Roche) by subcutaneous injection once a day from the day after the end of chemotherapy to the day in which NG count was over 1×10^9/L (median 9 days after the last day of chemotherapy, range 5-13) in two consecutive determinations. Ten cycles of chemotherapy were evaluated. Neutrophil phenotype (CD11b, CD14, CD33, CD71, HLA-DR, CD10, CD16 and CD15) was analyzed within 24 hours following the last G-CSF administration by double immunofluorescence technique, and cell surface membrane antigens were detected by quantitative flow cytometry using a FACScan™ (Becton Dickinson, San José, CA, USA). The number of cells counted for this analysis was 15×10^3. Acquisition gates were set to exclude dead cells and aggregated material. Forward versus side scatter display was used to define granulocytes. For each MoAb the percentage of positive cells was established using the Lysys II software from Becton Dickinson. The mean intensity of fluorescence of CD15 and CD16 was also studied.

There were no differences in NG surface antigenic expression among the 10 control subjects and the 10 studies performed to the 6 ALL patients before G-CSF administration (Table 1). Table 1 also shows the antigenic changes observed before and after treatment with G-CSF. The most outstanding feature was

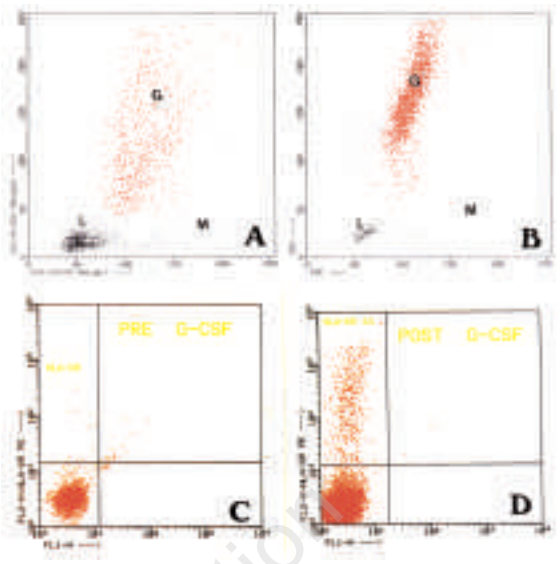

Figure 1. Forward versus side scatter display (FACScan Becton Dickinson) for granulocytes before (A) and after G-CSF (B). Flow cytometric analysis of NG stained with HLA-DR antigens before (C) and after (D) G-CSF administration.

the HLA-DR expression on NG (Figure 1) in ALL cases after G-CSF treatment compared to controls and to the same studies before G-CSF treatment. Other changes included a higher expression of CD71, CD14 and CD11b (although the latter was not statistically significant), a decrease in the CD10 expression and in CD15 and CD16 fluorescence intensity.

G-CSF has been employed in ALL[4] and has produced a reduction in the duration of the period of neutropenia, with no influence on either CR or relapse rates. The most outstanding finding in this study was the confirmation of the marked HLA-DR expression on NG after G-CSF administration. Such a finding was previously observed in a patient with chronic idiopathic neutropenia under G-CSF treatment.[5] The

Table 1. Expression of surface markers on neutrophils from control subjects and patients with ALL, before and after G-CSF administration.

	CD14	CD71	HLA-DR	CD10	CD11b		CD15		CD16	
	%	%	%	%	%	MI	%	MI	%	MI
Controls (n=10)	1±0.5	0.3±0.4	0.2±0.2	53.2±20.2	98±1.3	94±53	98±0.7	3437±445	94±4.5	178±55
ALL before G-CSF (n=6, 10 episodes)	1.2±0.9	0.4±0.7	0.3±0.4	39.6±15.3	96±1.9	102±48	98±0.5	2645±629	94±4.5	182±60
ALL after G-CSF (n=6, 10 episodes)	5.1±1.8	10.6±6.0	4.5±1.2	0.4±0.5	93±4.8	125±46	97±1.8	1679±985	79±19	86±55

*Results are expressed as mean±standard deviation. °p=0.001; *p=0.01, Student t-test; MI = mean intensity.*

marked expression of HLA-DR together with the decrease in CD10 and decrease in the mean intensity of fluorescence of CD15 and CD16 on neutrophils, suggest the presence of NG with immaturity features in patients receiving G-CSF for neutropenia secondary to intensification chemotherapy. An alternative significance for increased HLA-DR expression could be a higher proliferating activity of NG after treatment, also confirmed by the higher CD71 expression.

Several changes in the expression of neutrophil antigens following G-CSF administration (i.e. increased CD11b, CD66b, CD64, CD18, CD35, CD32, CD13, CD16 and CD45) have been observed, but this expression usually returns to normal after several hours.[1,2,6-8] Some of these changes indicate an enhanced adherence and phagocytic capacities. Our study confirms that NG after G-CSF administration also express the CD71 antigen, indicative of their active proliferation.[9] We also observed an increase in CD14 expression in NG of ALL patients after G-CSF administration, as previously mentioned.[7,10] and such a receptor may be important for achieving efficient response to infections caused by Gram-negative bacteria. Different from other studies in which normal human volunteers treated with G-CSF have been employed,[1,2] our control subjects did not receive G-CSF. However, the phenotypic profile of NG in control subjects was the same as that of ALL patients before chemotherapy, strongly suggesting that the phenotypic changes observed in the same ALL patients after G-CSF administration were due to the effect of this cytokine on NG. The results of our study suggest that after G-CSF administration there is not only an increase in NG number and an enhancement of the functional properties,[11,12] but also that these NG carry features of immature phenotype. The biologic significance of this feature remains to be ascertained.

Key words

Acute lymphoblastic leukemia, G-CSF, neutrophil granulocytes, surface antigens

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Treatment of chronic myeloid leukemia in relapse after umbilical cord blood transplantation

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Umbilical cord blood (UCB) is increasingly used as a source of hematopoietic progenitor cells for allotransplantation. Donor-derived buffy coat cells are considered optimal treatment for leukemia relapses after transplantation of allogeneic bone marrow. Experience with relapses after UCB transplants are sparse. Here we report a girl who received an UCB transplant for

chronic myeloid leukemia, relapsed after three years, failed to respond to donor buffy coat cells, but achieved a complete hematologic, cytogenetic, and molecular remission on interferon-α.

Since the first umbilical cord blood transplantation (UCBT) performed in 1988, over 230 patients received this form of hematopoietic cell transplant.[1,2] We reported the first UCBT for Philadelphia chromosome positive (Ph^{+}) chronic myeloid leukemia (CML).[3] Here we present the follow-up of this patient who relapsed 3 years post-transplant, failed to respond to donor buffy coat cells, but achieved a second complete remission on interferon. To our knowledge this kind of treatment in a recipient of UCBT has not been published previously.

In July 1991 we performed an UCBT for Ph^{+} CML in a 28-month-old girl. The donor was her HLA-identical sister. Conditioning was performed with cyclophosphamide and total body irradiation. She received 8.7×10^{7}/kg nucleated cells. GM-CSF was administered because of slow granulocyte recovery. Otherwise the transplant course was uneventful, and she became transfusion independent after 50 days. At that time cytogenetic analyses and blood group testing showed donor type hematopoiesis. The patient is a native of Bosnia and being caught in the turmoil of war, did not appear for control until end of 1993. At that time blood counts and cytogenetic analyses were normal. However, in July 1994 while hematologically still in remission a molecular and cytogenetic relapse occurred. *bcr2/abl2* transcript was detected using reverse transcription and PCR (RT-PCR). Besides t(9;22), t(12;18) was found. The percentage of Ph^{+} metaphases increased, so we decided to treat her with donor derived buffy coat cells. In January 1995 she received the following buffy coat cells from her original donor who was at that time four years old: 5.7×10^{8}/kg nucleated cells, 4.0×10^{8}/kg CD3, 2.5×10^{8}/kg CD4, and 1.2×10^{8}/kg $CD8^{+}$ cells. We observed no signs of GvHD. Blood counts and rate of Ph^{+} metaphases remained unchanged. In September 1995, eight months after buffy coat infusions, interferon-α, 3,000,000 U/day five days per week, was started. Molecular (RT-PCR) and cytogenetic analyses were done in regular intervals. In January 1996 a complete molecular and cytogenetic remission was obtained. Six years after UCBT and 20 months after starting interferon she is well, still in complete molecular and cytogenetic remission, and with normal blood counts.

The optimal treatment of leukemia in relapse after UCBT is unknown.[4] Donor buffy coat infusion is an effective treatment for CML in relapse after marrow transplantation.[5-7] Although interferon is often added, it is unknown whether it increases the response rate. Results are better if the buffy coat is used earlier, i.e. before hematological relapse.[8] The response to buffy coat can occur up to six months after infusion. Therefore it seems that our patient did not respond to buffy coat infusion, but to treatment with interferon-α. It is tempting to speculate that this is due to the immaturity of T-cells present in the blood of the four-year-old donor, and that additional extrinsic interferon was necessary to achieve a sufficient graft-versus-leukemia effect. Still, this remains a completely unproven hypothesis, perhaps meriting further studies.

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Key words

Chronic myeloid leukemia, umbilical cord blood transplantation, interferon-α.

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