The number of nucleoli expressed by the nucleolar coefficient in the granulopoietic bone marrow compartment in non-leukemic persons and patients suffering from chronic myeloid leukemia

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Nucleolar coefficient determined for each stage of the granulopoietic proliferating compartment of patients suffering from chronic myeloid leukemia and non-leukemic persons was characterized by a remarkable stability which indicated that the development of myeloblasts to promyelocytes and myelocytes was not accompanied by a decreasing number or disappearance of nucleoli.

According to previous studies the nucleolar number and morphology reflect the transcription of the ribosomal RNA and are related to cell cycle, proliferation and maturation.[1,2] However, despite of numerous notes on nucleoli in immature granulocytes,[1,3-7] the present knowledge on their number expressed by the nucleolar coefficient in the granulopoietic proliferating compartment (GPC) in non-leukemic persons and particularly in patients suffering from chronic myeloid leukemia (CML) is limited.[1] Therefore, the present study was undertaken to provide such missing information which might contribute to the knowledge on bone marrow granulocytic precursors. The increased GPC in CML[8,9] represents a very convenient model for such study due to its similarity to that in non-leukemic persons.[7]

Nucleoli in GPC were investigated in 10 control persons with non-leukemic hematological disorders and 35 patients with accelerated and chronic phase of CML without cytostatic chemotherapy or treated with α-interpheron or hydroxyurea. Nucleoli were visualized in unfixed bone marrow smears stained for the demonstration of RNA using methylene or toluidine blue as described previously.[10] To express the number of nucleoli per cell, the nucleolar coefficient for each stage of GPC was determined by dividing the number of nucleoli by the number of cells in which they were counted. Main nucleolar types including the percentage of *large active nucleoli* and *inactive* micronucleoli[1] were evaluated in at least 30 cells for each stage of GPC in each investigated person.

The results are presented in Table 1. In non-leukemic GPC the nucleolar coefficient did not show significant differences. Similarly, the nucleolar coefficient did not differ significantly in leukemic myeloblasts, promyelocytes or myelocytes when its values were compared within each group of patients. On the other hand, in comparison with non-leukemic

Table 1. Nucleolar coefficient in cells of GPC.

Cells	*CML phase*	*Cytostatic therapy*	*Nucleolar coefficient*	*Active nucleoli°*	*Micro-nucleoli°°*	*Number of patients*
Mbl	Controls	0	2.80 (0.09)[a]	75.75 (2.66)[a,b]	19.87 (2,41)[a,b]	10
	Accelerated	0	2.56 (0.15)	72.60 (3.20)	20.05 (3.84)	5
		+	2.58 (0.09)	69.39 (4.26)	23.00 (3.34)	10
	Chronic	0	2.33 (0.26)*	71.70 (2,86)	18.67 (3.11)	4
		+	2.62 (0.07)	75.25 (1.78)	18.76 (1.91)	16
Prmc	Controls	0	2.92 (0.05)	55.17 (2.74)	37.83 (3.24)	10
	Accelerated	0	2.33 (0.05)*	64.22 (12.88)	28.28 (0.88)	5
		+	2.44 (0.09)*	57.81 (7.51)	37.16 (7.70)	10
	Chronic	0	2.80 (0.10)†	55.50 (7.00)	34.90 (3.80)	4
		+	2.56 (0.08)*	62.01 (4.22)	33.21(2.56)	16
Mc	Controls	0	2.65 (0.11)	2.90 (147)	93.42 (2.01)	10
	Accelerated	0	2.60 (0.31)	6.73 (4.56)	88.20 (4.47)	5
		+	2.40 (0.16)	13.48 (4.53)	81.75 (3.47)	10
	Chronic	0	2.90 (0.40)	1,95 (1.95)	94.75 (3.45)	4
		+	2.70 (0.17)	9.59 (2.41)	87.72 (2.28)	16

Mbl: myeloblasts; Prmc: promyelocytes; Mc: myelocytes.
[a]Mean and S.E. (standard error of mean); [b]Percentage-mean and SE (of main nucleolar types see ref. #1, the percentage of resting ring shaped nucleoli is not reported. The reported percentage represents only complementary data).
°Large nucleoli with a relatively uniform distribution of RNA; see ref. #1; °°Micronucleoli representing "inactive" small nucleoli; see ref. #1.
**Significant difference in comparison with control non-leukemic persons using the t- test ($p < 0.05$); †significant difference in comparison with patients in the accelerated phase untreated with cytostatics using the t-test ($p < 0.05$).*

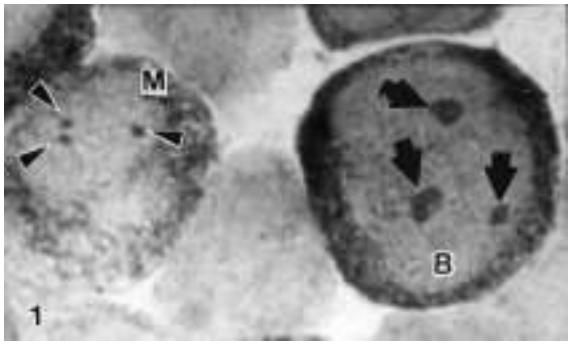

Figure 1. Micronucleoli (pointers) in a myelocyte (M). For comparison other types of larger nucleoli (arrows) are seen in a myeloblasts (B). Approximately ×1900.

persons, small but significantly lower nucleolar coefficient was noted in myeloblasts of untreated patients in the chronic phase of CML, promyelocytes in the accelerated phase of both untreated and treated patients as well as in the chronic phase of treated patients. Small differences of the nucleolar coefficient in the GPC between accelerated and chronic phase of CML were not significant except for promyelocytes of untreated patients in the chronic phase in which the nucleolar coefficient was slightly higher than in patients in the accelerated phase. However, all these differences were small; their interpretation is not possible and requires further studies.

The presented results demonstrated that the number of nucleoli expressed by the nucleolar coefficient in both non-leukemic and leukemic GPC was remarkably constant since it did not change substantially in the course of the myeloblastic development to promyelocytes and myelocytes. Thus, in contrast to some previous notes based on a different methodical approach,[3-6] this development was not characterized by a decreasing number of nucleoli or their disappearance, but by the transformation of large active nucleoli to inactive micronucleoli which were most frequent in myelocytes (see Table 1). In the present study, the visualization of micronucleoli invisible by panoptic procedures was facilitated by the procedure for the selective visualization of nucleoli due to their RNA content (Figure 1).[1,7]

Key words

Nucleolar coefficient in CML

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Autologous stem cell transplantation in multiple myeloma: a single center experience

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This study shows the feasibility and safety of autologous stem cell transplantation in 32 of 98 multiple myeloma patients referred to our Institution over a 3-year period. Complete response rate was 19% and partial response rate 58%. A significantly better outcome was shown among newly diagnosed patients in comparison with pretreated patients.

High dose treatment followed by autologous marrow or peripheral blood stem cell (PBSC) transplantation has been widely used in the treatment of multiple myeloma (MM), since it was shown to be able to induce long term responses with low morbidity and mortality in several trials.[1-4] The majority of published studies included patients selected according to eligibility criteria, but no mention has been reported on the feasibility of the study (the total number of MM from which patients for autologous stem cell transplantation [ASCT] were selected and the number of patients excluded). Moreover, which subgroup

Table 1. Response to transplantation in evaluable patients, related to disease status pretransplant.

Disease status pre-transplant	Total number of patients	Evaluable patients	Response to ASCT No. of patients (% of evaluable) CR	PR
CR	1	1	1 (100%)	//
PR	14	14	4 (29%)	10 (71%)
NR	11	10	0	5 (50%)
PD	6	6	1 (17%)	3 (50%)
Total	32	31	6 (19%)	18 (58%)

CR: disappearance of serum or urinary monoclonal component by immunofixation and plasma cells < 5% in bone marrow biopsy. PR: decrease greater than 50% in both measurable paraprotein and bone marrow infiltration. NR: all patients not satisfying PR criteria. PD: a 25% increase in serum paraprotein; a 90% increase of Bence-Jones proteinuria; new lytic lesions.

Table 2. Transplant-related data.

	Median (range)
Months from:	
Diagnosis to ABMT	17 (8-57)
End to therapy to harvest	4 (2-26)
Harvest to ABMT	4 (2-35)
ABMT to present	9 (5-37)
CD34×10^6/kg reinfused	3.2 (2.0-15.0)
Days to:	
PMN: > 0.5×10^9/L	11 (9-24)
PMN: > 1×10^9/L	13 (8-31)
PLT: > 20×10^9/L	12 (8-137)
PLT: > 50×10^9/L	21 (12-75)
No. of blood units transfused	4 (0-15)
No. of apheresis transfused	2.5 (1-23)
No. of G-CSF adminstration post-ABMT*	26
No. of febrile days/patients	4 (2-11)
No. of fever of unknown origin	20
No. of septicemia	6

*Abbreviations: PMN: neutrophils; PLT: platelets; *5 µg/kg/day.*

of patients should have the greatest benefit from ASCT and the timing of the procedure are still matters of debate.[5-7]

Herein we report our experience on the treatment of 32 MM patients who were autografted at our Institution between January 1994 and June 1997. These patients represent 32% of a total of 98 MM, which were referred to our center in the same period. Exclusion criteria from ASCT were: age older than 65 years (48%), first stage smoldering myeloma (5%), concomitant diseases (6%), previous extensive treatment (4%) and myeloma progression leading to complication or death during induction therapy (5%). At the start of induction treatment median age was 52 years (range 31-61); 9 patients were in stage I with a marrow plasmocytosis greater than 50%, 3 patients in stage II and 20 in stage III.

Twenty-three patients received only one line of induction chemotherapy, for a median of 4 monthly cycles (range 4-8). Four patients had a refractory and 5 a relapsing disease treated with at least two different chemotherapy regimens. Fifteen of 32 patients had a disease sensitive to conventional chemotherapy (reduction of the M protein greater than 50% in comparison with pretreatment values).

Bone marrow was harvested in 2 patients while in all others PBSC were collected after administration of 7 g/m^2 cyclophosphamide plus G-CSF at 5 µg/kg/daily. Conditioning regimen consisted of busulfan 16 mg/kg and melphalan 120 mg/m^2.

One patient, transplanted in progression, died of cerebral hemorrhage due to persistent thrombocytopenia 46 days after ASCT. Of the 31 patients acceptable for evaluation, 24 (77%) had a response, complete in 6 patients (19%) and partial in 18 patients (58%); 5 cases (17%) had no response and 2 (6%), both refractory before transplantation, progressed and died. Table 1 shows the response to ASCT related to status at transplant: for each category of pretransplant status, high dose treatment allowed the improvement of response rate in about 30 to 50% of the patients. Moreover, patients treated with only one induction regimen had a significantly better outcome than patients submitted to a second or third line treatment: in fact, in the former group 20/22 (91%) had a response (CR 23%) in comparison with 4/9 (44%) pretreated patients (CR 11%) (p=0.003). At a median follow-up of 9 months (range 5-57) from ASCT, 3 out of the 29 living patients, all belonging to the group of pretreated patients before ASCT, relapsed after a median of 18 months (range 17-36). Table 2 shows that hematological recovery was fast and adverse effects mild in all patients.

Our study shows feasibility and safety of ASCT in one third of the whole MM population. The best results were obtained in newly diagnosed MM, with a response rate of 91% and a CR of 23%, very close to those reported in larger series.[4,8] Outcome of our 9 pretreated patients was significantly poorer than that of newly diagnosed MM, suggesting that ASCT should be performed as part of the initial therapy, rather than delayed at the time of disease progression. Limited benefit from ASCT with a PR rate of 50% and no case of CR was also observed in resistant MM.

Although a longer follow-up of our patients' population will give further information, these results are strong enough to prompt us to program ASCT in the treatment of MM patients younger than 65 years as soon as possible. It also warrants the testing of new

strategies, such as positive stem cell selection and double autotransplant, to improve CR rate in this subgroup of patients.

Key words

Autologous stem cell transplantation, multiple myeloma

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Enzyme replacement therapy decreases hypergammaglobulinemia in Gaucher's disease

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We report the effects of enzyme replacement therapy in a patient with Gaucher's disease associated with a monoclonal gammopathy. Alglucerase induces a linear decline in immunoglobulin and β_2-microglobulin levels. This observation suggests that this treatment decreases the chronic antigenic stimulation commonly found in Gaucher's disease.

Gaucher's disease (GD) is characterized by genetic deficiency of lysosomal glucocerebrosidase. The subsequent accumulation of glycosylceramide in macrophages results in enlargement of the spleen and liver, bone marrow infiltration, and hematological disorders[1] and has been in part linked to the overload reticuloendothelial system.[2] A chronic B-cell stimulation, expressed by the development of hypergammaglobulinemia, has been documented in GD. We report the effects of enzyme replacement therapy on immunoglobulin abnormalities in a patient with type-I Gaucher's disease associated with hypergammaglobulinemia.

A 39-year-old man presented with a long history of GD with marked pancytopenia, hepatomegaly, splenomegaly, bilateral femoral head osteonecrosis and bone deformities. An M-component of IgGκ type (28 g/L). and three other smaller clones, respectively of IgG-κ, IgG-λ and IgA-λ type, were demonstrated by immunofixation electrophoresis. Serum IgA and IgM levels were respectively 1.45 g/L and 1.04 g/L. Low levels of Bence-Jones protein were found in urine (50 mg/L) and elevated β_2-microglobulin at 6.2 mg/L in serum (normal < 2.4 mg/L). No plasma cell proliferation was observed in bone marrow aspirate by immunofluorescence study. Extensive radiological investigations ruled out multiple myeloma or lymphoma.

Alglucerase (Ceredase®, Genzyme Co, Cambridge, MA, USA), administered at 60 IU/kg every two weeks, induced a linear decline in β_2-microglobulin level, which reached a quasi-normal value after 2 years (Figure 1). A parallel fall in IgA and IgM immunoglobulins, and a significant decline in the monoclonal IgG-κ paraprotein were observed (Figure 2). At immunofixation electrophoresis, the three smaller monoclonal gammopathies progressively disappeared. Moreover, Bence-Jones proteinuria vanished.

Polyclonal, oligoclonal or monoclonal hypergammaglobulinemia has been reported in GD.[2-4] In addition to marked splenomegaly or hepatomegaly, aseptic necrosis, chronic infections, liver diseases, decreased antigen clearance secondary to an overload reticuloendothelial system, excessive chronic antigenic stimulation secondary to distorted lipid metabolism, or defects in immunoregulation of B-cell function have been also implicated in the pathogenesis of the immune disorders.[3] Glucocerebroside has been found to activate immunoglobulin production by non-specifically stimulating macrophage interleukin-1 secretion.[5] As observed in our patient, the decline in β_2-microglobulin and gammaglobulins during alglucerase treatment suggests that enzyme replacement therapy may reduce the antigenic stimulation, either by restoring the macrophage ability to hydrolyze the glycosylceramide, leading to decrease macrophage stimulation, or by decreasing the amount of accumulated glycerylceramide. Reduction in hypergammaglobulinemia and in serum M-component level after splenectomy have been previously reported.[4, 6-8]

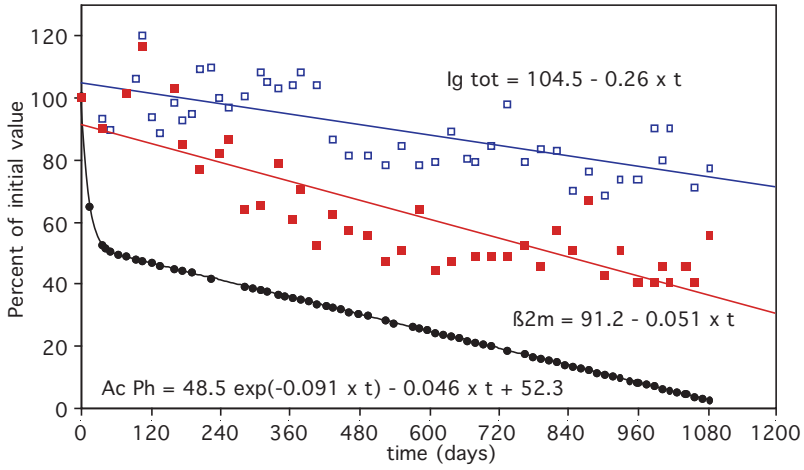

Figure 1. Comparison of the time-course of total immunoglobulin (Ig tot), β_2-microglobulin (β_2M) and acid phosphatase (Alc Ph) levels during enzyme replacement therapy, after normalizing of initial value to 100%.

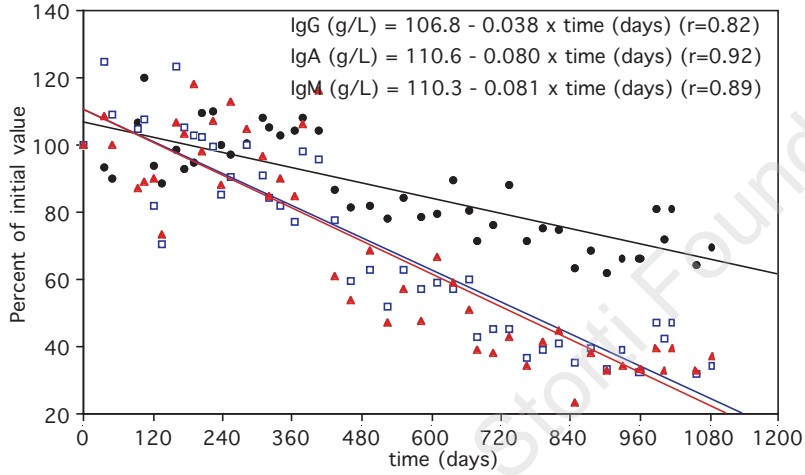

Figure 2. Time evolution of IgG (filled circles), IgA (open squares), and IgM (filled triangles) levels during alglucerase treatment, after normalizing of initial value to 100%.

Chronic immune disorders in GD might induce B-cell neoplasm.[9,10] One can therefore postulate that the treatment could contribute to decrease the risk of hematopoietic cancers. This hypothesis needs further investigations, but is worth evaluating when taking into account the cost of the treatment. Moreover, β_2-microglobulin level as a marker for the follow-up of patients with GD requires further studies.

Key-words

Gaucher's disease, hypergammaglobulinemia, β_2-microglobulin

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