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Influence of thalidomide on immunophenotype of malignant plasma cells

Wpływ talidomidu na fenotyp nowotworowych plazmocytów

INTRODUCTION

Multiple myeloma (MM) is a B cell malignant disease affecting primarily the elderly population. Once the diagnose is made expected survival does not exceed four years. Despite all the efforts patients with recurrent or high risk refractory multiple myeloma still have a very poor prognosis. Therefore much attention is put recently to discover a novel, less toxic and more efficient anti- myeloma agents. So far the efforts have concentrated on high dose chemotherapy or PBSCT, which did not prove significant efficacy in elderly patients. In such circumstances thalidomide has emerged again.

It has been demonstrated that thalidomide is an effective drug in multiple myeloma patients (1]. It modulates T cell subsets function and cytokine production [3]. Thalidomide has an antiangiogenic activity and immunomodulatory effect on secretion of cytokines, but its anti-myeloma effect is not well understood [4, 5].

MATERIALS AND METHODS

30 patients with refractory or relapsed multiple myeloma, 25 with IgG and 5 with IgA MM, have been treated with thalidomide. Starting dose was 200 mg daily, and titrated by 100 mg increments at every week, until the maximum tolerated dose was achieved. Most of the patients were receiving doses of 400 mg, with maximum 600 mg. Patients were recommended to take daily dose as a single dose at bedtime. Cells' phenotype was checked before and after 4 and 8 weeks of therapy.

Lymphocytes were isolated from peripheral blood or bone marrow with Lymphoprep (Nycomed, Norway), rinsed twice with PBS, incubated with monoclonal antibodies and analysed by flow cytometry technique (Cytoron Absolute, Ortho Diagnostic System).

At the same time peripheral blood- and bone marrow sera were isolated and frozen. Cytokine concentrations were assessed by ELISA method, using standardised, specific kits (Endogen and BioSource International).

RESULTS

Perhaps the most important changes we have observed were those concerning the number of myeloma cells. Percentages of CD38+/CD138+ (BB4) cells are shown in Table 1/Figure 1.

Table 1. Percentages of CD 38+ CD 138+ cells in peripheral blood and bone marrow of multiple myeloma patients during thalidomide treatment

	Bone marrow			Peripheral blood		
	Before	4 weeks	8 weeks	Before	4 weeks	8 weeks
Total	31.2 ±12.9	16.3 ±6.9 b	13.2 ±5.8 b,c	18.0 ±7.7	10.3 ±4.3 b	8.6 ±3.3 b,d
Responders	32.0 ±14.2	6.4 ±2.1 b,**	3.2 ±1.1 b,c,**	19.7 ±8.3	4.4 ±1.8 b,**	3.0 ±0.7 b,**
Non-Responders	30.4 ±13.2	26.2 ±10.7	23.2 ±10.8	16.3 ±7.5	16.2 ±9.0	14.2 ±5.4

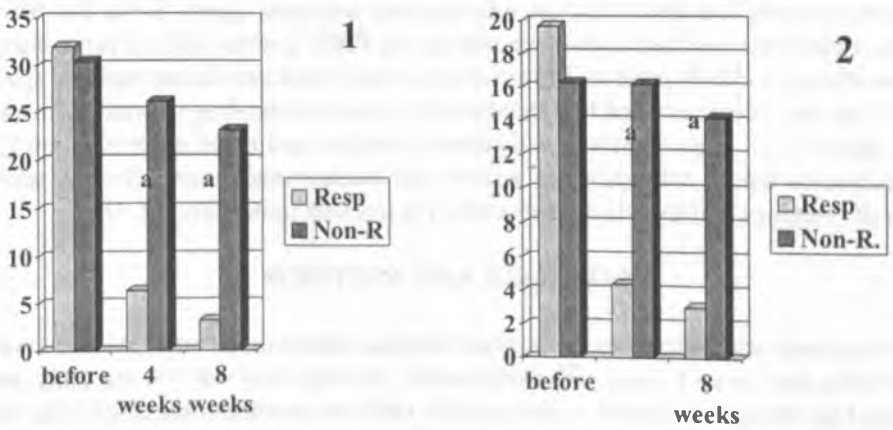

Figure 1. Percentages of CD38+CD138+ cells in peripheral blood (2) and bone marrow (1) in the course of thalidomide treatment

Table 2. Percentages of CD 20+ CD 138+ cells in peripheral blood and bone marrow of multiple myeloma patients during thalidomide treatment

	Bone marrow			Peripheral blood		
	Before	4 weeks	8 weeks	Before	4 weeks	8 weeks
Total	5.2 ±2.1	2.8 ±1.0 a	2.4 ±1.3 b	1.3 ±0.5	1.4 ±0.5	0.6 ±0.2
Responders	5.5 ±1.8	1.3 ±0.5 b,**	0.4 ±0.2 b,c,**	1.4 ±0.8	0.9 ±0.4	0.2 ±0.2 a,**
Non-Responders	4.9 ±2.0	4.3 ±1.5	4.0 ±1.9	1.2 ±0.8	1.9 ±0.6	1.0 ±0.3

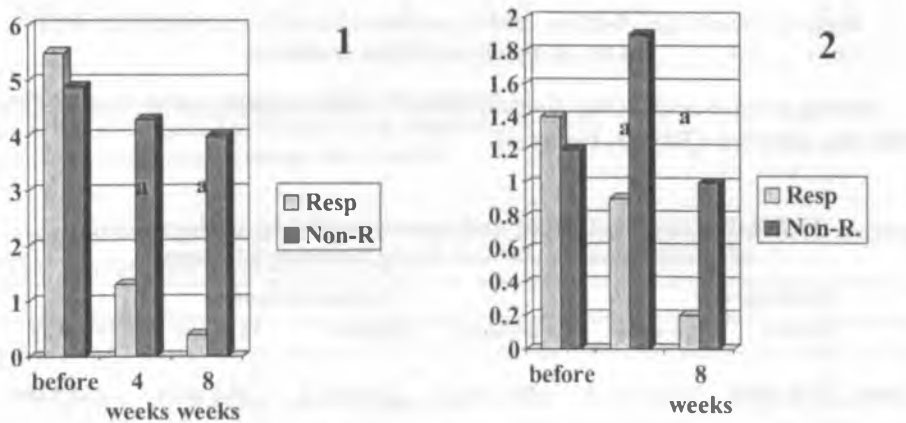

Figure 2. Percentages of CD20+CD138+ cells in peripheral blood (2) and bone marrow (1) in the course of thalidomide treatment

Percentages of CD20+ cells expressing CD138+ (BB4) antigen have also changed as described in Table 2/Figure 2.

Table 3. Percentages of CD 4+ cells in peripheral blood and bone marrow of multiple myeloma patients during thalidomide treatment

	Bone marrow			Peripheral blood		
	Before	4 weeks	8 weeks	Before	4 weeks	8 weeks
Total	19.5 ±8.9	19.6 ±9.1	20.1 ±6.0	22.9 ±11.7	24.5 ±13.6	27.1 ±15.6
Responders	19.4 ±9.2	25.6 ±6.2 a,**	26.8 ±12.4 a, **	22.7 ±8.2	29.5 ±14.2 b, **	34.9 ±17.5 b, **
Non-Responders	19.6 ±6.9	13.6 ±5.7 a	13.4 ±9.8 a	23.1 ±10.5	19.5 ±9.9	19.3 ±8.1 a

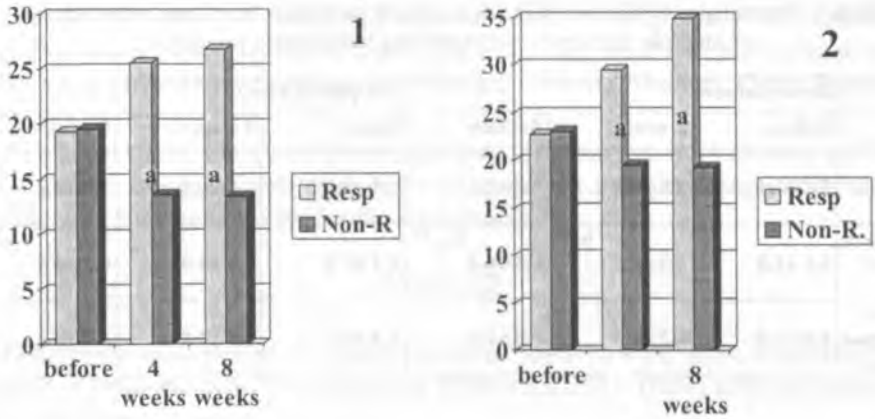

Figure 3. Percentages of CD4+ cells in peripheral blood (2) and bone marrow (1) in the course of thalidomide treatment

Among patients responding clinically to the treatment significant increase of CD4+ cells was observed (Table 3, Figure 3).

Table 4. Percentages of CD 8+ cells in peripheral blood and bone marrow of multiple myeloma patients during thalidomide treatment

	Bone marrow			Peripheral blood		
	Before	4 weeks	8 weeks	Before	4 weeks	8 weeks
Total	27.5 ±15.2	37.4 ±17.4 a	39.1 ±16.7 a	26.8 ±12.1	35.6 ±13.9 a	37.5 ±18.0 a
Responders	26.5 ±16.4	44.1 ±19.3 b, *	46.0 ±19.3 b, **	26.3 ±10.5	44.0 ±17.4 b, **	45.3 ±17.5 b, **
Non-Responders	28.5 ±13.8	30.7 ±14.8	32.2 ±12.9	27.3 ±11.6	27.2 ±12.6	29.7 ±12.8

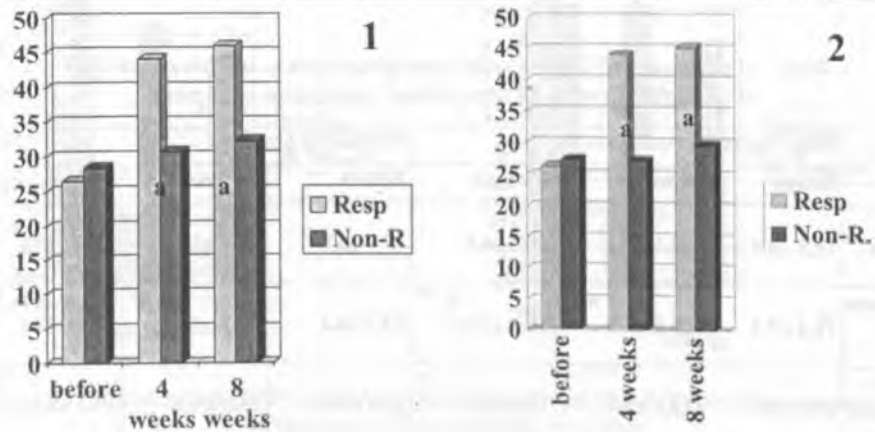

Figure 4. Percentages of CD8+ cells in peripheral blood (2) and bone marrow (1) in the course of thalidomide treatment

The same pattern of response was marked in number of CD8+ cells, as indicated in Table 4/Figure 4.

Table 5. Expression of activation markers on peripheral blood and bone marrow CD3+ T lymphocytes of MM patients during thalidomide treatment

	Bone marrow			Peripheral blood		
	CD69	CD25	DR	CD69	CD25	DR
Before treatment	4.3 +/-3.1	7.4 +/-4.5	9.9 +/-3.2	3.2 +/-1.8	7.6 +/-4.2	5.6 +/-3.7
After 4 weeks	5.1 +/-2.6	8.8 +/-4.9	5.8[a] +/-4.7	4.2 +/-2.9	8.6 +/-3.9	6.8 +/-2.9
After 8 weeks	5.3[a] +/-3.8	7.0 +/-5.2	3.4[c] [b] +/-1.9	4.8[a] +/-2.1	6.8 +/-4.1	5.2 +/-3.2

[a] $p < 0.05$ as compared to values before treatment
[b] $p < 0.001$ as compared to values before treatment
[c] $p < 0.05$ as compared to values after 4 weeks

Expression of different activation markers on peripheral blood and bone marrow CD3+ lymphocytes is shown in Table 5.

DISCUSSION

Thalidomide (N(alpha)-phthalimidoglutarimide) has a number of properties that can make it part of an effective regimen for treating multiple myeloma patients. As demonstrated by Hasslett et al. [2]. Thalidomide enhances cell mediated immunity by directly co-stimulating T cells, which results in IFNγ and IL-12 increased production. In our group of patients treated with thalidomide we have observed this particular stimulatory effect, which may contribute to final positive result.

After 4 and 8 weeks of treatment a significant increase in CD4+ and CD8+ cells both in peripheral blood and bone marrow samples was observed. It confirms the suggestion that thalidomide upregulates Th2-type immunity. What gives that observation even more significance is the fact, that there were statistically significant differences between patients who respond, or not, to the treatment (Table 3/Figure 3, Table 4/Figure 4). These results correlated with decreased number of CD38+/CD138+(BB4+) cells (Table 1/Figure 1).

The significant increase in expression of T cell early activating markers CD69 was observed. Other activating markers such as CD25 and HLA DR did not change significantly (Table 3). It is worth mentioning that there are some particular differences among activation markers. CD69 is a very early activation marker which appears on the cell surface within four hours after stimulus. HLA DR, on the other hand, is late activation marker. Finally CD 25 is an α subunit of IL-2 receptor.

Interestingly, the percentage of CD20+/CD138+(BB4+) cells was also decreased and correlated with decreased number of CD38+/CD138+(BB4+) cells (Table 2/ Figure 2).

It seems that thalidomide can provide activation signals to T lymphocytes, thus promoting T cell response. It affects mainly CD8+ subpopulation, that could be stimulated by CD3+ T cells

Different expression of T activating markers suggest different pathways of signal transduction (Il-2, Il-12, TNF) however further studies are required.

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STRESZCZENIE

U 30 chorych z opornym na chemioterapię lub nawrotowym szpiczakiem plazmocytowym w leczeniu zastosowano thalidomid w dawce 400–600 mg dziennie. Chorych podzielono na dwie grupy zależnie od odpowiedzi klinicznej: z dobrą odpowiedzią (*responders*) i słabą odpowiedzią (*nonresponders*). W obu grupach oceniono fenotyp immunologiczny limfocytów krwi i szpiku. Badano odsetek komórek limfoidalnych oraz markery aktywacji przed i w 4, 8 tygodniu leczenia. Ocenę wykonano przy użyciu technik cytometrii przepływowej. Stwierdzono istotne obniżenie odsetka nowotworowych plazmocytów (komórki CD38+/CD138+) oraz zwiększenie odsetka limfocytów pomocniczych CD4+ i cytotoksycznych CD8+ w grupie z dobrą odpowiedzią kliniczną. Potwierdzono, że thalidomid jest skutecznym lekiem w leczeniu opornych na chemioterapię postaci szpiczaka plazmocytowego. Obserwuje się zmienność odpowiedzi na leczenie thalidomidem, co znajduje odzwierciedlenie w odsetku poszczególnych subpopulacji limfocytów.