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Immune Functions and Hematopoietic Progenitor Cell Activity in Plasmacytoma-Bearing Mice Cured by Melphalan

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A. Introduction

The incidence of secondary acute myeloid leukemia is higher in multiple myeloma (MM) than that predicted for normal persons. The etiology and pathogenesis of this leukemia is unclear, but it is commonly attributed to a leukemogenic effect of melphalan. The risk for secondary leukemia seems to be greater in MM than in other categories of cancer patients receiving similar drugs, and it is difficult to ascribe the high incidence of AML in MM to treatment alone. Host factors related to the primary malignancy might also be involved in the progression of a transformed but premalignant cell to clinical leukemia. Several immune effector mechanisms operating during the leukemia latency period were postulated to mediate surveillance of potentially malignant cells. We studied the immune profile of a mouse model bearing a transplanted plasmacytoma that was cured by melphalan for 1 year after tumor eradication and cessation of therapy. In melphalan-treated MM patients deficient immunity might be caused by the primary tumor per se or by the drug. We therefore assayed the same parameters in normal mice, without plasmacytoma, given melphalan in the same way. Marrow myeloid progenitor cell (CFU-C) growth was also studied. We were able to discriminate between long-term changes of immune and hematopoietic systems caused by melphalan from those related to the occurrence of the tumor in the past.

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B. Experimental Setup

On a day marked as zero, 1000 MOPC-315 plasmacytoma cells are inoculated intramuscularly to BALB/C mice. A growing tumor forms locally, causing the death of all untreated mice by day 50. Mouse groups studied were (a) *group T+M:* mice with plasmacytoma treated on day 14, when the tumor size was 0.5×0.9 cm, with 250 μg melphalan and again with 400 μg melphalan on day 24. The tumor disappeared and did not recur either locally or systemically during the entire study period; (b) *group M:* normal mice that were not inoculated with plasmacytoma cells but were treated with melphalan in the same way; (c) *untreated normal controls*. Survival rates on day 50 for mouse groups T+M and M were similar (86% and 92% respectively).

C. Results and Discussion

At monthly intervals for 1 year, nine to twelve mice from each group were assayed for a number of splenic immune parameters and marrow CFU-C growth (results at days 50 and 200 are depicted in Table 1). Life table analysis shows that the 1-year survival for group M was 33%, for group T+M 73%. In normal mice (group M), shortly

Table 1. Immune and hematopoietic functions of mice without (group M) or with (group T+M) plasmacytoma, treated with melphalan

Parameter	Day 50		Day 200	
	M	T+M	M	T+M
Allogeneic MLR	66	44	100	40
Thy1.2+ cells	76	46	95	60
IL-2 production	82	67	130	75
NK activity	31	108	90	60
Asialo GM1+ cells	0	0	111	104
sIg+ cells	50	0	108	132
CFU-C colonies	121	78	57	60

Results are expressed as percentage of the value of each parameter in normal untreated control mice. Day zero was the day of MOPC-315-cell inoculation.

after treatment with melphalan, the numbers of T (Thy1.2+) cells, NK (asialo GM1+) cells and B (sIg+) cells as well as T-cell function (allogeneic MLR), IL-2 production, and NK activity were all reduced. All these immune parameters recovered spontaneously by 3–4 months after treatment and remained whithin the normal range during the rest of the study. In plasmacytoma-bearing mice treated with melphalan (group T+M) similar reduced immunity was found shortly after treatment. However, mouse group T+M continued to manifest long-lasting cellular immune defects. They showed a significant reduction in T-cell number compared with group M and untreated controls, a capacity to proliferate in response to alloantigens, and IL-2 production up to 1 year after cessation of therapy. The number of NK cells was normal but their activity was slightly reduced. These data indicate that a short and intensive course of melphalan given to normal mice causes only transient immune deficiency shortly after treatment. However, plasmacytoma-bearing mice that receive melphalan develop long-term cellular immune deficiency, which is not due to melphalan alone. Interestingly, following a transient reduction, the splenic B-cell number in group T+M recovered and remained persistently higher than that in untreated controls and group M. Comparison with MOPC-315 cells ruled out the possibility that the B cells in group T+M were residual plasmacytoma cells. Contrary to immune parameters, the marrow CFU-C colony growth was persistently reduced in both the T+M and the M mouse group following treatment with melphalan.

Taken together, the data indicate that the presence of malignancy in the past played a role in the development of long-lasting immune deficiency, especially in T-cell number and function. On the other hand, melphalan per se causes long-lasting damage to a hematopoietic progenitor cell. We suggest that this mouse model may be useful for studying the role of immune aberrations related to the primary plasmacytoma in the development of overt leukemia from a hematopoietic stem cell, altered and maybe transformed by melphalan.