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Davis TA, Monroy RL, Donahue RE, MacVittie TJ

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**GM-CSF: A REGULATORY MOLECULE FOR NK ACTIVITY IN THE BONE
MARROW.**

Thomas A. Davis, Rodney L. Monroy, Robert E.
Donahue, and Thomas J. MacVittie.

Immunobiology and Transplantation Department
(T.A.D., R.L.M) Naval Medical Research Institute,
Bethesda, MD. 20814. Genetic's Institute (R.E.D),
Cambridge, MA. Experimental Hematology (T.J.M),
Armed Forces Radiobiology Research Institute,
Bethesda, MD. 20814

INTRODUCTION

The regulation of myeloid proliferation, differentia-
tion and activation by granulocyte-macrophage colony
stimulating factor (GM-CSF) has been recognized, (Monroy et
al., 1990). However, the role of GM-CSF in NK proliferation
and activation is unclear even though NK cells have been
shown to synthesize and secrete GM-CSF (Cuturi et al.,
1989). The activation of peripheral blood NK cells (Dempsey
et al., 1982) and their development from precursors in the
bone marrow (BM) (Lotzova and Savary, 1987) are suggested to
be regulated by multiple factors. We (Davis et al., 1990) have
shown that in vivo administration of GM-CSF to normal
monkeys resulted in a latent enhancement of peripheral blood
NK activity, with no measurable change during the adminis-
tration period. These suggested that GM-CSF treatment had
an effect on NK cell development in the BM. To evaluate
this possibility, we characterized the NK cell populations
in the BM of normal primates during and after GM-CSF
treatment. Our findings show a period of suppressed BM NK
activity followed by the transitional appearance of a unique
population of large lymphoid cells with an NK phenotype
(CD2⁺ CD4⁺ CD8⁻ CD16⁺) and lytic activity.



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MATERIALS AND METHODS

Adult (male, 9.8 ± 1.6 kg) rhesus monkeys (*Macaca mulatta*) were used. Monkeys were injected (s.c., bid, 5 days) with GM-CSF (5×10^5 U/kg/day, Genetic's Institute, specific activity of 1×10^4 units/mg). Heparanized peripheral blood (3 ml) and iliac crest bone marrow (20 ml) were aseptically obtained from anesthetized monkeys (ketamine hydrochloride, 10mg/kg, i.m.). All research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animal Resources", prepared by the Institute of Laboratory Animal Resources, National Research Council.

BM cells were separated by counterflow centrifugation-elutriation (CCE) as described elsewhere (Monroy et al., 1986). Briefly, low density bone marrow cells (LDBMC, $78-450 \times 10^6$) isolated by isopycnic separation over Ficoll-Hypaque were injected into a Beckman JE-6B elutriation rotor system at a loading flow rate of 6ml/min and a rotor speed of 2000 rpm. Cell fractions were collected by stepwise increases in flow rate: Fraction I, 8.5 ml/min, Fraction II, 10.0 ml/min, and Fraction III (rotor-off fraction).

Natural killer cell activity was measured in a ^51Cr release assay using a constant number (2.5×10^3) of K562 target cells and a variable number of mononuclear effector cells. Results expressed as percent specific release at an effector to target cell ratio (E:T) of 12:1.

RESULTS

Three distinct cell fractions were separated by CCE and morphologically characterized as (I) small lymphocytes, (II) intermediate-large lymphocytes, and (III) myeloid elements, containing 24%, 14% and 62% of the normal LDBMCs cells, respectively. The cellular composition and the number of cells recovered in fractions I and II did not change significantly over the longitudinal study. In contrast, the fraction III population was myeloid before and immediately following GM-CSF treatment but on days 21 and 35 a progressive increase in the number of lymphoid cells with a LGL morphology was detected.

The phenotype of each fractionated cell population is shown in Table 1. At day 7, a significant ($P \leq .01$) decrease (from 22% to 10%) in the percentage of CD2 positive cells was measured in LDBMCs due to an increase in myeloid

elements as a result of proliferation in response to GM-CSF. By day 35, 65% of the LDBMCs expressed a CD2⁺ phenotype. In comparison to changes in the LDBMC population, no significant changes in the relative distribution of CD4⁺, CD8⁺ or CD16⁺ were measured in fractions I and II, however an increase in CD2⁺ cells as detected in both fractions on day 35. On days 21 and 35, the increase in frequency of lymphoid cells in fraction III with a large granular lymphoid (LGL) morphology was paralleled by a significant increase in the percentage of cells expressing a NK cell marker (CD16) and a T-cell marker (CD2). However, these cells did not express either CD4 or CD8 surface markers.

Table 1. Expression of cell surface antigens on LDBMCs and CCE separated bone marrow cells from monkeys treated with GM-CSF.

Fraction	Day	Percent Positive Cells ^a			
		CD2	CD4	CD8	CD16
LDBMCs	0	22 ± 3	6 ± 1	16 ± 2	3 ± 2
	7	10 ± 2	3 ± 1	6 ± 1	7 ± 6
	21	32 ± 6	9 ± 3	13 ± 4	4 ± 2
	35	65 ± 18	13 ± 1	29 ± 11	7 ± 2
Fraction I	0	58 ± 3	17 ± 2	44 ± 4	10 ± 4
	7	80 ± 4	22 ± 3	55 ± 3	3 ± 3
	21	57 ± 10	15 ± 1	45 ± 5	7 ± 4
	35	78 ± 13	22 ± 4	56 ± 9	7 ± 3
Fraction II	0	62 ± 12	21 ± 2	38 ± 7	12 ± 7
	7	67 ± 18	23 ± 8	41 ± 11	11 ± 9
	21	84 ± 6	25 ± 4	53 ± 7	10 ± 7
	35	79 ± 8	28 ± 2	49 ± 4	6 ± 4
Fraction III	0	1.2 ± 1	0.4 ± 1	0.3 ± 1	1.5 ± 1
	7	7 ± 5	1.6 ± 2	2.1 ± 2	2.2 ± 1
	21	8 ± 2	1.7 ± 1	2.0 ± 1	8.7 ± 3
	35	53 ± 9	3.9 ± 2	2.5 ± 1	15 ± 5

a. Separated cells were stained with FITC conjugated monoclonal antibodies, and percent positive cells determined by flow cytometry. Results expressed as the mean percent positive cells ± 1SD from 3 monkeys.

Table 2. Characterization of NK activity in LDBMCs and CCE separated bone marrow following GM-CSF administration.

<u>Fraction</u>	<u>Day</u>	<u>NK activity^a</u>	
		<u>Prior Culture</u>	<u>Post Culture^b</u>
LDBMCs	0	53 ± 12	NT ^c
	7	27 ± 15	NT
	14	11 ± 8	NT
	21	29 ± 16	NT
	35	34 ± 7	NT
Fraction I	0	48 ± 12	68 ± 11
	7	20 ± 16	49 ± 7
	14	11 ± 4	NT
	21	27 ± 12	68 ± 10
	35	26 ± 12	69 ± 4
Fraction II	0	61 ± 7.5	82 ± 6
	7	39 ± 14	68 ± 13
	14	25 ± 5	NT
	21	47 ± 14	77 ± 3
	35	36 ± 5	72 ± 4
Fraction III	0	8 ± 8	12 ± 5
	7	10 ± 5	21 ± 11
	14	5 ± 7	NT
	21	20 ± 4	50 ± 16
	35	31 ± 3	32 ± 7

a. NK activity against K562 target cells in a 4-hr ⁵¹Cr release assay. Results expressed as mean percent specific release + 1SD (n=3) at an E:T ratio of 12:1.

b. Bone marrow cells were cultured for 5 days with 20 units/ml of rIL-2.

c. Not tested (NT).

NK cell activities of the isolated BM cell populations are presented in Table 2. LDBMCs from monkeys prior to treatment contained significant NK activity, with almost all of the activity recovered in fraction I (~55%) and II (~45%) cells. NK activity in LDBMCs, fraction I and fraction II was reduced to 20-40% of normal on days 7 and 14, with no change in fraction III NK activity. Normal NK activity in these cell populations did not return until after 35 days. Significant NK activity was detected in fraction III cells

on days 21 and 35, and this increase of activity occurred when there was a high frequency of LGL and CD2⁺ cells.

The suppressed NK activity in fractions I and II was abrogated by culture with rIL-2 (20 U/ml) for 5 days. The cytolytic capacity of fraction III cells was also significantly enhanced at day 21 by culturing in the presence of IL-2.

Unlike the bone marrow, no significant changes in peripheral blood activity was measured thru day 21, and in two monkeys peripheral blood NK activity was slightly elevated on days 28-35, returning to normal by day 42.

SUMMARY

We have demonstrated that GM-CSF administration to normal monkeys resulted in significant changes in NK cell activity within various bone marrow cell populations over a 5 week period. NK cell activity in the lymphoid fractions containing both small and large bone marrow NK cells was decreased for 2 weeks following GM-CSF treatment, returning to normal levels over the next 5 weeks. In addition, the recovery of the NK activity was accompanied by the appearance of large granular lymphocytes with a unique phenotype (CD2⁺ CD4⁻ CD8⁻ CD16⁺). Although the mechanism of action of GM-CSF on NK cell activity in the marrow is unclear, the results from these studies suggest that functional NK cells within the BM are decreased with GM-CSF administration and that this effect may be at the progenitor/precursor stage. Thus, we have identified that GM-CSF in vivo can affect not only NK cells in the peripheral blood but also the generation of functional NK cells in the BM. Furthermore, that this effect was detected over a prolonged period which reflected NK cell regeneration in the bone marrow.

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