Enrichment of Natural Suppressor Activity in a Wheat Germ Agglutinin Positive Hematopoietic Progenitor-Enriched Fraction of Monkey Bone Marrow

By Kikuya Sugiura, Susumu Ikehara, Nazareth Gengozian, Muneo Inaba, Evelio E. Sardiña, Hajime Ogata, Sang M. Seong, and Robert A. Good

Natural suppressor (NS) activity, the capacity of unprimed cells to suppress immunologic responses, is present in mouse, rabbit, and human bone marrow (BM). In this study we characterize NS activity in bone marrow cells of the rhesus monkey. Greatest NS activity was found in low-density cells (1.0600 to 1.0655 g/mL) obtained by density centrifugation on a discontinuous Percoll gradient. NS activity was further enriched when cells were separated by affinity for wheat germ agglutinin (WGA). Cells with high affinity to WGA demonstrated potent NS activity, whereas cells with low affinity to WGA had no NS activity. A significant relationship between NS activity and hematopoietic activity was demonstrated using in vitro assays of colony formation (CFU-GM and CFU-MIX). NS activity was not affected by treatment with monoclonal antibodies (MoAbs) to human Fcγ receptors (Leu, 11a,b,c) or treatment with MoAbs to monkey natural killer cells. These findings extend our prior observations by showing that cells with NS activity, which apparently have WGA receptors, are present not only in murine BM but also in monkey bone marrow, and suggest that such cells may be involved in immunoregulation by primitive cells of BM.

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affinity to wheat germ agglutinin coupled Sephadex 6B (WGA-
Sepharose 6B) beads (Sigma, St Louis, MO). To prepare a column,
0.8 mL of WGA-Sepharose 6MB beads were packed into a plastic,
disposable 1-mL syringe. A suspension containing 5 x 10^6 cells in 0.3
mL of Hank's balanced salt solution (HBSS) was loaded onto the
column and allowed to incubate at 4°C for 45 minutes. After the
incubation, cells with low affinity to WGA (WGA- cells) were
collected by washing with HBSS containing 2% FCS. To collect cells
with high affinity to WGA (WGA+ cells), HBSS containing 0.2
mol/L N-acetyl-D-glucosamine (GlcNAc; Sigma) was loaded onto
the column after the collection of WGA- cells, and the column was
then incubated at 37°C. Twenty minutes later, WGA+ cells were
euated from the column by washing with HBSS containing 0.2
mol/L GlcNAc and 2% FCS. Cells in each WGA+, WGA- fraction
or unseparated fraction were used in a suppressor assay or in an in
vitro CFU assay, or were stained with fluorescein isothiocyanate-
conjugated WGA (FITC-WGA) for analysis on a cytofluorometric
system (Epics C; Coulter Corp., Hialeah, FL).

Assay of suppressor activity. Suppressor activity of BM cells
was estimated according to ability to inhibit the mitogen responses
(RMs) of PBLs. MRs were determined by measuring incorporation of
[^1]H] thymidine into DNA. In brief, triplicate cultures were set up
in wells of 96-well flat-bottom microtiter plates (Costar, Cambridge,
MA). Each well contained 2 x 10^5 PBLs in 0.2 mL RPMI 1640
medium with 5% FCS and 5 x 10^-5 mol/L 2-mercaptopethanol
(2-ME; Sigma). The PBLs were cultured with various concentra-
tions of BM cells in the presence of concanavalin A (Con A; Sigma)
at 66 g/mL, or pokeweed mitogen (PWM; GIBCO) at 1%. The
cultures were incubated at 37°C for 48 hours in humidified atmo-
sphere of 5% CO_2 in air. [^1]H] thymidine was introduced during the
last 6 to 8 hours of culture. Percent suppression was calculated using
the following formula:

\[
\% \text{ Suppression} = 1 - \frac{\text{cpm (with BM cells)}}{\text{cpm (without BM cells)}}
\]

Assay of natural killer (NK) activity. NK activity of BM cells
was measured by the ability of effector cells to induce release of ^51Cr
from the erythroblast leukemia cell line K562. In brief, triplicate cultures were set up in wells of 96-well round-bottom microtiter plates (Costar, Cambridge, MA). Each well contained labeled K562 cells (1 x 10^6) and BM cells (1 x 10^4 or 5 x 10^4, as effector cells) in a total volume of 0.2
mL of RPMI 1640 medium with 10% FCS and 5 x 10^-5 mol/L
2-ME. Cells were incubated at 37°C for 4 hours in an atmosphere of
10% CO_2 in air. Percent specific lysis was calculated as
((cpm of experimental release - cpm of spontaneous release)/(cpm of
maximal release - cpm of spontaneous release)) x 100. Spontane-
ous ^51Cr release was determined with supernatant from wells
containing only target cells. To determine maximal release, labeled
target cells were incubated with detergent NP-40 at 37°C for 10
minutes.

In vitro colony forming assay. A culture to assay for granulocyte-
macrophage colony forming cells (CFU-GM) was set up in 35-mm
culture dishes (Corning Glass Works, Corning, NY). One millilitter of culture in each dish contained 1 x 10^4 BM cells, 0.36% agar
(Difco, Detroit, MI), 20% of GG-free horse serum (GIBCO), 12% GCT-conditioned medium (GIBCO), 5 x 10^-5 mol/L 2-ME, in
RPMI 1640 medium supplemented with sodium bicarbonate (0.06%),
sodium pyruvate (1.4 mmol/L), l-serine (12 ng/mL), I-asparagine
(22 ng/mL), dextran (molecular weight 500,000: 1 mg/mL),
penicillin (28 U/mL), and streptomycin (28 pg/mL). Dishes were
incubated at 37°C for 7 days in a humidified atmosphere with 5%
CO_2 in air. For mixed hematopoietic colony (CFU-MIX; CFU-
GEMM) assay, 1 mL of culture in each 35-mm dish contained 5 x
10^5 BM cells, 0.8% methylcellulose (4,000 cps; Aldrich Chem,
Milwaukee, WI), 30% GG-free horse serum, 5 x 10^-5 mol/L 2-ME,
2 U erythropoietin (Connaught Lab, Ltd, Willowdale, Ontario,
Canada), and 15% phytohemagglutinin-stimulated human leukocyte-
conditioned medium in Iscove's modified Dulbecco's medium
(IMDM). Dishes were incubated at 37°C for 14 days in a humidified
atmosphere with 5% CO_2 in air. After 14 days in culture, CFU-MIX
were identified using the criteria described by Powell et al. 13 Briefly,
counted colonies contained a minimum of 50 cells composed of both
hemoglobinized and nonhemoglobinized cells. Representative mixed-
cell colonies were separately picked up and stained with Wright-
Giemsas for morphologic analysis. It was confirmed that the colonies
contained erythroblast bursts, myelocytes, macrophages, and mega-
ephytes.

Treatment of bone marrow cells with MoAbs. Monoclonal and
anti-Leu 11 (anti-CD16) a, b, and c antibodies were purchased from
Becton Dickinson. MoAb to monkey NK cells (N4-2) used in this
study was developed by Gongozian et al. 19 who demonstrated its
phenotypic and functional specificity to this lymphocyte subset. Bone
marrow cells (10^9 cells/50 mL) were incubated with anti-Leu-1 (0.1
µg), anti-Leu-1b (0.1 µg), anti-Leu-1c (0.1 µg), or N4-2 MoAb for 45
minutes at 4°C. The bone marrow cells were then washed and
incubated with rabbit complement (1:4 dilution) at 37°C for 1 hour.
After the incubation the cells were extensively washed before being
used in the suppressor activity assay. The concentrations of
anti-Leu-1a, anti-Leu-1b, and anti-Leu-1c used were as recommended
for completely purge the corresponding cell populations. Complete
purging was confirmed by cytofluorometric analysis. The treatment
of N4-2 plus complement eliminated 100% of Leu-1a and Leu-1b
positive cells or eliminated 23% of Leu-1c positive cells.

Fig 1. Monkey BM cells were purged of T cells, B cells, and
and adherent cells as described in Materials and Methods (Unfrac.
BM). After the fractionation by equilibrium density centrifugation
on a discontinuous Percoll gradient, cells were collected in
fractions of various densities: Fr. 1, < 1.0600 g/mL; Fr. 2, 1.0600
to 1.0685 g/mL; Fr. 3, 1.0685 to 1.0700 g/mL; Fr. 4, 1.0700 to
1.0800 g/mL. Various numbers of fractionated or unfractied
BM cells were added to the culture of monkey PBL cells (2 x 10^6)
along with Con A (8 µg/mL). As a control, irradiated autologous
PBL cells were added to the culture. The striped areas indicates the
region on the graph where suppressor activity was not observed.
Five separate experiments were performed using BM obtained
from three monkeys.
RESULTS

Enrichment of suppressor activity was observed in the low-density fraction of BM cells purged of T cells, B cells, and adherent macrophages. Since natural suppressor cells have been defined as “null cells,” monkey BM cells were purged of mature T cells, B cells, plus G10 adherent macrophages. Suppressor activity in monkey BM was augmented after purging of all these cells (Fig 1) as previously reported with natural suppressor activity of the mouse. To further characterize cells exerting suppressor activity, treated BM cells were fractionated by equilibrium density centrifugation on a Percoll gradient. Since densities of various kinds of cells in human or monkey bone marrow have been reported by Olofsson et al20 or Moore et al,21 Percoll solutions of various densities (1.0600, 1.0655, 1.0700, and 1.0800 g/mL) were used to obtain the hematopoietic stem cell or precursor cell enriched fraction (Fr 2:1.0600 to 1.0655 g/mL), the monocytes or lymphocyte-enriched fraction (Fr 3:1.0655 to 1.0700 g/mL), and the myeloid cell enriched fraction (Fr 4:1.0700 to 1.0800 g/mL). As shown in Fig 1, cells in the low-density fraction (Fr. 2) exerted the strongest suppressor activity in Con A responses: 3 x 10^6 cells inhibited by 41% the proliferation of 2 x 10^5 responder cells. Cells in Fr. 2 also showed the strongest suppressor activity in the pokeweed mitogen (PWM) response (data not shown). Morphologically, the cells in Fr. 2 were round or oval and were 7 to 15 μm in diameter. Some of these cells had a large round nucleus and granules or lipid droplets in the cytoplasm (see Fig 3C). Almost all cells in Fr. 4 were myelocytes of the myeloid lineage (see Fig 3D). Cells in Fr. 3 were a mixture of cells found in Fr. 2 and Fr. 4.

Nonspecific suppressor activity of BM Fr. 2 cells. To determine whether the suppressor activity of BM Fr. 2 cells is effective in the context of major histocompatibility complex (MHC), BM Fr. 2 cells of monkey A were cultured with PBLs of MHC-mismatched monkey B as well as with the autologous PBLs. As shown in Table 1, BM Fr. 2 cells inhibited the proliferation of PBLs stimulated with Con A even if BM suppressor cells and PBL responder cells were MHC-mismatched. However, the suppressive effect of BM cells was stronger when BM cells and PBLs were identical at MHC. Because the proliferation of human PBLs stimulated with Con A was not suppressed but rather stimulated by the addition of monkey BM Fr. 2 cells (Table 2), the suppressor activity of monkey BM did not overcome stimulation.

Distinction of the NS cells from NK cells. Since Gengozian et al22 and Timonen et al23 have independently reported that NK activity in monkey or human BM cells is enriched in the low-density cell fraction, it was of interest to determine whether the suppressor activity we observed was being exerted by NK cells. As shown in Table 3, NK activity of rhesus monkey BM was enriched in the low-density cell

### Table 1. Nonspecific Suppressor Activity of Monkey BM Fr. 2 Cells

<table>
<thead>
<tr>
<th>Responder</th>
<th>Cells Added</th>
<th>No. of Cells Added</th>
<th>cpm ± SD (% suppression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey A PBL</td>
<td>0</td>
<td>326,706 ± 26,808</td>
<td>318,952 ± 5,813</td>
</tr>
<tr>
<td>Monkey A BM*</td>
<td>(2)</td>
<td>319,966 ± 16,381</td>
<td>231,196 ± 16,381</td>
</tr>
<tr>
<td>Monkey A PBL†</td>
<td>(1)</td>
<td>322,943 ± 6,812</td>
<td>315,377 ± 5,398</td>
</tr>
<tr>
<td>Monkey B PBL</td>
<td>319,926 ± 7,352</td>
<td>296,466 ± 37,014</td>
<td>260,039 ± 9,908</td>
</tr>
<tr>
<td>Monkey A BM*</td>
<td>(0)</td>
<td>319,926 ± 14,431</td>
<td>297,777 ± 4,097</td>
</tr>
<tr>
<td>Monkey B PBL†</td>
<td>(0)</td>
<td>319,926 ± 14,431</td>
<td>297,777 ± 4,097</td>
</tr>
</tbody>
</table>

### Table 2. Suppressor Activity of Monkey BM Cells Is Species Specific

<table>
<thead>
<tr>
<th>Responder</th>
<th>Cells Added</th>
<th>No. of Cells Added</th>
<th>cpm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human PBL</td>
<td>19,080 ± 608</td>
<td>18,630 ± 1,554</td>
<td>28,388 ± 3,067</td>
</tr>
<tr>
<td>Monkey BM*</td>
<td>19,554 ± 4,037</td>
<td>19,124 ± 2,357</td>
<td></td>
</tr>
<tr>
<td>Human PBL†</td>
<td>19,080 ± 608</td>
<td>18,026 ± 44,849</td>
<td></td>
</tr>
</tbody>
</table>

Three separate experiments were done each using BM cells and PBL cells from one monkey and PBL cells from a different monkey. A total of three monkeys were used in these experiments.

*Various numbers of BM Fr. 2 cells from monkey A were added to the culture of autologous (monkey A) or allogeneic (monkey B) PBL cells (2 x 10^6) with Con A (8 μg/mL).
†Irradiated PBL cells of monkey A or monkey B were added to the culture of autologous PBL cells (2 x 10^6) along with Con A (8 μg/mL).
Fractionated or unfractionated monkey BM cells were incubated with $^{51}$Cr-labeled K562 cells at $37^\circ$C for 4 hours. Three separate experiments were done using a total of three monkeys.

The suppressor activity was not affected by treatment with MoAbs to either human Fcγ receptors (anti-Leu 1 a, b, and c) that purge NK activity from human or monkey cells or to monkey NK cells that are N4-2 positive (Table 4). Cytomflowmetric analysis showed that 9% of cells in Fr. 2 were Leulla+ and 6% were Leulc+. Treatment with N4-2 or anti-Leulb antibody plus complement decreased NK activity of BM Fr. 2 cells by 93% or 75%, respectively. Thirty-three percent of NK activity was purged by anti-Leulla antibody plus complement treatment. Treatment of Fr. 2 with anti-Leullc antibody plus complement resulted in a less than 5% depletion of NK activity (data not shown).

Separation of BM Fr. 2 cells into WGA-positive and WGA-negative cells. Since potent NS activity in mouse BM had been found in the fraction of cells with high affinity to WGA,17 Fr. 2 cells of monkey BM were also separated into WGA-positive cells and WGA-negative cells to further characterize the cells exerting suppressor activity. As shown in Fig 2, two kinds of cell populations were demonstrated in the scatter of fluorescence intensity when BM Fr. 2 cells were stained with FITC-conjugated WGA (5 μg for 10⁷ cells in 1 mL). Forty-eight percent of cells in BM Fr. 2 were included in the population of the cells with increased number of surface receptor to WGA (WGA-positive cells: the right peak in Fig 2). However, if cells were passed through the

### Table 3. NK Activity of Monkey BM Cells

<table>
<thead>
<tr>
<th>Effector/Target</th>
<th>Specific Lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effector</td>
<td>100</td>
</tr>
<tr>
<td>Unfract. BM</td>
<td>3</td>
</tr>
<tr>
<td>Fr. 2</td>
<td>64</td>
</tr>
<tr>
<td>Fr. 3</td>
<td>26</td>
</tr>
<tr>
<td>Fr. 4</td>
<td>1</td>
</tr>
</tbody>
</table>

Fractionated or unfractionated monkey BM cells were incubated with $^{51}$Cr-labeled K562 cells at $37^\circ$C for 4 hours. Three separate experiments were done using a total of three monkeys.

**Fig 2.** Monkey BM Fr. 2 cells were separated into WGA+ cells and WGA− cells by passing the cells through a WGA-coupled Sepharose 6MB column as described in Materials and Methods. Cells separated or unseparated were stained with FITC-conjugated WGA (1 μg/mL) for 45 minutes on ice. After washing twice, the fluorescence intensity of cells was estimated in a cytofluorometric system.

### Table 4. Monoclonal Anti-Leu1 or Anti-Monkey NK Antibody Have No Effect on Suppressor Activity of Monkey BM Fr. 2 Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antibody</th>
<th>cpm ± SD (% suppression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>83,170 ± 3,431 (70)</td>
</tr>
<tr>
<td>BM Fr. 2</td>
<td>None</td>
<td>24,608 ± 1,624 (70)</td>
</tr>
<tr>
<td>BM Fr. 2</td>
<td>Anti-Leu1a</td>
<td>19,586 ± 4,797 (76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22,329 ± 4,256 (73)</td>
</tr>
<tr>
<td>BM Fr. 2</td>
<td>Anti-Leu1b</td>
<td>22,928 ± 4,212 (72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24,831 ± 2,200 (73)</td>
</tr>
<tr>
<td>BM Fr. 2</td>
<td>Anti-Leu1c</td>
<td>23,136 ± 3,815 (72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26,924 ± 4,346 (68)</td>
</tr>
<tr>
<td>BM Fr. 2</td>
<td>N4-2 (anti-monkey NK)</td>
<td>22,044 ± 3,875 (73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26,614 ± 5,198 (68)</td>
</tr>
</tbody>
</table>

Monkey BM Fr. 2 cells (1 x 10⁷) treated with or without MoAbs were added to the culture of monkey (autologous) PBL cells (2 x 10⁵) with Con A (8 μg/mL). Three separate experiments were done using a total of three monkeys.

* Treatment with rabbit complement at $37^\circ$C for 45 minutes.
† Treatment with MoAbs at 4°C for 45 minutes.
‡ Treatment with MoAbs at 4°C for 45 minutes followed by treatment with rabbit complement at $37^\circ$C for 45 minutes.
column of WGA-conjugated Sepharose 6MB beads, cells included in the WGA-positive cell population were reduced to only 4% (WGA-negative cell population). Almost all cells eluted by 0.2 mol/L GlcNAc solution were included in the WGA-positive cell population. Morphologically, the WGA-positive cell population contained round cells of medium size (8 to 10 μm in diameter). These cells had a large round nucleus and a small amount of cytoplasm. Myeloblastic cells were also included in the WGA-positive population (Fig 3A). On the other hand, promyelocytes and monocytic cells were enriched in the WGA-negative cell population (Fig 3B). Twenty percent of cells in WGA-negative fraction had the antigen recognized by anti-human Leu11a antibody, whereas only 2% of cells in the WGA-positive fraction and 9% of cells in unseparated fraction reacted with this antibody.

Enrichment of both NS activity and hematopoietic activity in WGA-positive cell population of the low-density fraction. As shown in Table 5, WGA-positive cells in BM Fr. 2 exerted potent suppressor activity: proliferation was inhibited 14% when 2 × 10⁵ responder cells were incubated with 3,000 WGA-positive cells, whereas WGA-negative cells exhibited little suppressor activity. In addition to suppressor activity, the hematopoietic activity of monkey BM was also enriched in the WGA-positive cells from Fr. 2 when an in vitro assay for both granulocyte-macrophage progenitors and multipotent hematopoietic progenitor cells was used (Table 6). Unseparated Fr. 2 cells demonstrated greater potency than the unfractionated BM cells or cells in fractions of other densities. This was true for hematopoietic activity as well as suppressor activity. Therefore, we conclude that the process of enrichment for the suppressor activity also enriches the hematopoietic activity of monkey BM (Fig 1, Tables 5 and 6).

DISCUSSION

In this study we characterize NS cells in monkey BM. These cells are CD2⁻ (non-T cells), surface Ig⁻ (non-B cells), Fcγ receptor⁻ and nonadherent. Cells with these characteristics have been found in hematopoietic organs, such as BM and neonatal spleen. Thus, NS cells are present in environments of considerable hematopoiesis, which strongly suggests that they not only suppress immune responses but may also play a crucial role in regulation of hematopoiesis. Indirect evidence for the latter speculation has been provided by Bennett and Marsh who showed that natural suppressor cells, which inhibit cytotoxic responses, are identical to the cells responsible for colony-forming units in culture (CFU-GM). We previously demonstrated that NS cells from mouse BM are highly enriched in a WGA-positive nonlymphoid cell fraction with densities between

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![Image](image-url)
1.063 and 1.075 g/mL. It is known that mouse hematopoietic stem cells are also enriched in this fraction, as reported by Visser et al., Spooncer et al., and Lord and Spooncer. It has also been reported that in monkey or human BM hematopoietic activity is enriched in a cell fraction of low density. Herein we have confirmed this finding and reported also that NS cell activity is enriched in the low-density cell fraction. Furthermore, we found that both NS and hematopoietic activity was enriched in a WGA-positive cell fraction, but not in a WGA-negative cell fraction nor in fractions with other densities.

Recently, Lemischka et al. investigated the dynamic behavior of hematopoietic stem cells in their differentiation using a technique for transferring foreign genes to hematopoietic stem cells. These investigators proposed that normal hematopoiesis results from sequential activation of different stem cell clones rather than from an average contribution of the entire stem cell pool. If this is true, then there should be a mechanism exercised by stem cells that suppresses activation of other stem cells but has no effect on those clones that have already started to differentiate. Our hypothesis that the differentiating stem cells themselves have suppressor activity that can down-regulate the activation of other stem cells seems to be a reasonable explanation of this phenomenon.

On the other hand, some investigators found that cells bearing Fcγ receptors exert NS or suppressor activity in rabbit BM or human BM, and that lymphokine-activated killer cells have NS activity. However, in our present study, NS activity was not affected by treatment with MoAbs to human Fcγ receptors (Leu 1a, b, c) or MoAb to monkey NK cells (N4-2). Therefore, we can conclude that natural suppression may define an activity rather than a specific cell lineage, that this activity does not reside in the NK population and does not require Fcγ receptors or cells bearing Fcγ receptors.

Some reports suggest that NS cells in BM or in fetal organs play a role in induction of tolerance to self-antigens or to alloantigens. In concert with our findings, these reports suggest that the biologic significance of NS cells may be to suppress an "overreaction" to self-antigens as well as to suppress responses to alloantigens. In this way such cells could participate in the induction of tolerance to self-antigens in the normal developmental process.

To obtain more crucial information that may ultimately be applicable to prevention of GVHD in allogeneic BM transplantation, experiments are currently underway to characterize, isolate, and culture NS cells from human BM.

ACKNOWLEDGMENT

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REFERENCES


Table 5. Monkey BM Fr. 2 WGA + Cells Have Potent Suppressor Activity

<table>
<thead>
<tr>
<th>Cells Added</th>
<th>No. of Cells Added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>cpm ± SD (% suppression)</td>
</tr>
<tr>
<td>None</td>
<td>163,230 ± 1,593</td>
</tr>
<tr>
<td>Fr. 2 WGA +</td>
<td>149,331 ± 2,664</td>
</tr>
<tr>
<td>(14)</td>
<td>(1)</td>
</tr>
<tr>
<td>Fr. 2 WGA -</td>
<td>171,960 ± 4,898</td>
</tr>
<tr>
<td>(1)</td>
<td>(6)</td>
</tr>
<tr>
<td>Fr. 2 WGA -</td>
<td>163,473 ± 9,414</td>
</tr>
<tr>
<td>(6)</td>
<td>(5)</td>
</tr>
<tr>
<td>Unfract. BM</td>
<td>168,293 ± 1,166</td>
</tr>
<tr>
<td>(3)</td>
<td>(18)</td>
</tr>
<tr>
<td>Irradiated PBL</td>
<td>174,921 ± 1,268</td>
</tr>
</tbody>
</table>

Table 6. Enrichment of Hematopoietic Activity in Monkey BM Fr. 2 WGA + Cells

<table>
<thead>
<tr>
<th>BM Cells</th>
<th>No. of CFU/105 Cells</th>
<th>Cell Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU-GM</td>
<td>CFU-MIX</td>
</tr>
<tr>
<td>Fr. 2 WGA +</td>
<td>506 ± 59</td>
<td>407 ± 14</td>
</tr>
<tr>
<td>Fr. 2 WGA -</td>
<td>15 ± 10</td>
<td>29 ± 9</td>
</tr>
<tr>
<td>Fr. 2</td>
<td>137 ± 18</td>
<td>237 ± 49</td>
</tr>
<tr>
<td>Fr. 3</td>
<td>19 ± 2</td>
<td>36 ± 18</td>
</tr>
<tr>
<td>Fr. 4</td>
<td>8 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>Unfract. BM</td>
<td>27 ± 4</td>
<td>48 ± 7</td>
</tr>
</tbody>
</table>

Various numbers of fractionated or unfractonated monkey BM cells were added to the culture of monkey PBL cells (2 x 10⁶) with Con A (8 μg/mL). As a control, irradiated autologous PBL cells were added to the culture. Three separate experiments were done using a total of three monkeys.

Abbreviation: ND, not done.