A GRAVIMETRIC SIMPLIFIED METHOD FOR NUCLEATED MARROW CELL COUNTING USING AN INJECTION NEEDLE

Toshiki SAITO1, Liu FANG2 and Kiyoshi MATSUMOTO2

1Nippon Institute for Biological Science, 9-2221-1 Shinmachi, Ome, Tokyo 198-0024, Japan
2Research Center for Human and Environmental Sciences, Division of Laboratory Animal Research, Shinshu University, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan

(Received December 18, 2004; Accepted March 23, 2005)

ABSTRACT — A simplified gravimetric marrow cell counting method for rats is proposed for a regular screening method. After fresh bone marrow was aspirated by an injection needle, the marrow cells were suspended in carbonate buffered saline. The nucleated marrow cell count (NMC) was measured by an automated multi-blood cell analyzer. When this gravimetric method was applied to rats, the NMC of the left and right femurs had essentially identical values due to careful handling. The NMC at 4 to 10 weeks of age in male and female Crj:CD(SD)IGS rats was 2.72 to 1.96 and 2.75 to 1.98 ($\times 10^6$ counts/mg), respectively. More useful information for evaluation could be obtained by using this gravimetric method in addition to myelogram examination. However, some difficulties with this method include low NMC due to blood contamination and variation of NMC due to handling. Therefore, the utility of this gravimetric method for screening will be clarified by the accumulation of the data on myelotoxicity studies with this method.

KEY WORDS: Gravimetric method, Marrow cell count, Bone marrow, Rats

INTRODUCTION

In myelotoxicity studies, the nucleated marrow cell count (NMC) is an important parameter. The NMC and myelogram impact on the evaluation of myelotoxicity of new drugs. Recently, the examination of immune function in repeated-dose toxicity studies has been required in the guidelines for toxicity testing. Bone marrow (BM) examination is a useful end point in evaluating immune function. Therefore, the importance of the BM examination will increase in toxicity studies. Several quantitative methods have been developed to determine the NMC in femurs. In many cases, the NMC in rats has been reported as values per volume (Yoneda et al., 1989) or per femur/humerus (Prion et al., 2001; Ulich and Castillo, 1991).

The NMC per mg of BM has been reported in detail by Chan et al. (1966). They have presented the quantitative method of NMC per mg of BM to evaluate cellular changes occurring in mouse BM. Briefly, BM was aspirated into a pre-weighted microcapillary tube. The NMC and total marrow cells were counted by using a hemocytometer and a Coulter electronic cell counter, respectively. They clarified the problem concerning the quantitative method for NMC per mg of BM, and showed important findings about the validity of the metrology and the measurement condition. Importantly, it was indicated that if three assumptions were valid, the NMC per mg of BM would reflect changes occurring in total marrow cellularity. The three assumptions were the following: 1) specific gravity of marrow was similar to that of plasma, 2) the volume of the marrow cavity remained constant, and 3) marrow cellularity varies indirectly with marrow tissue fluid content. Validating the three assumptions was necessary to obtain reliable data with this gravimetric method.

Since an injection needle is inexpensive, in the present study the BM aspiration was performed with an injection needle instead of a microcapillary tube. This is a key point of this gravimetric method, since the injection needle is easy to handle and unbreakable and
destruction of BM cells by dryness can be prevented by minimizing contact with air, which is possible with this type of needle. In addition, the measurement time was shortened by improvement of the performance of an electric balance and an automated multi-blood cell analyzer. In this paper, the outline of the procedure, differences of NMC between left and right femurs, and reference values of NMC in rats were investigated.

MATERIALS AND METHODS

Animals

Five male and five female Sprague-Dawley rats (Slc:SD, Japan SLC, Inc., Shizuoka, Japan) at 7 to 20 weeks of age, 14 male and 18 female Brown Norway rats (BN/SsN Slc, Japan SLC, Inc.) at 4 to 20 weeks of age, and 25 male and 26 female Matsumoto Eosinophilia Shinshu (MES) rats (Matsumoto et al., 1999) at 4 to 16 weeks of age were used to examine differences in NMC between right and left femurs. Ten male and 10 female Crj:CD(SD)IGS rats were purchased from Charles River Japan Inc. (Kanagawa, Japan) at 4, 5, 6, and 10 weeks of age and were used to determine the NMC reference values. This study was carried out in accordance with the Guidelines for Animal Experimentation of the Nippon Institute for Biological Science and of Shinshu University.

Measurement of NMC

In the gravimetric method, the animal was euthanized by bleeding under anesthesia with ether. A femur was removed and cut at the end. Either an injection needle (18−19 gauge) or a disposable stainless needle (1.25 mm I.D. × 70 mm), which was weighed before use, was inserted into the femur and then attached to a syringe. After a fresh BM was slightly aspirated, the needle was weighed again. The weight difference was assumed to be the amount of BM. The needle was then reattached to the syringe, and the BM cells were suspended in 2 ml of carbonate buffered saline. NMC was measured by an automated multi-blood cell analyzer (SF-3000, SYSMEX, Hyogo, Japan) with an analytical device for animals (SFVU-1, SYSMEX).

In the volumetric method, a femur was removed from a sacrificed rat. The femur was cut at the end and divided lengthwise. The BM was removed from the femur and placed on a watch glass. The bone marrow was aspirated using a blood cell diluting pipette and diluted 200-fold with Türk’s solution. The blood cell diluting pipette was mixed by a shaking apparatus for 15 min. The NMC was measured by a Bürger-Türk hemocytometer.

The difference of NMC between the left and right femurs was examined by the gravimetric method in Slc:SD, BN/SsN Slc, and MES rats. The reference values of NMC in Crj:CD(SD)IGS rats were measured by the gravimetric method using left femurs. The NMC of the left femur was compared with that of the right femur measured by the volumetric method using a blood cell diluting pipette.

Statistical analysis

The logarithmic transformed left NMC/right NMC ratios (log L/R) and the differences between the left NMC and right NMC (L-R) were calculated from the data of Slc:SD, BN/SsN Slc, and MES rats and then were analyzed by paired t-test (Snedecor and Cochran, 1967). The accepted level of significance was set at p<0.05.

RESULTS AND DISCUSSION

The difference in NMC between the left and right femurs in Slc:SD, BN/SsN Slc, and MES rats is shown in Fig. 1. The NMC was essentially identical from the left and right femurs, ranging from 4 to 20 weeks of age, regardless of the strain and sexes. No significant difference was observed in the log L/R and L-R. Malgor et al. (1988) reported that NMC from the left and right femurs of the same rats were similar. Our findings supported their result.

Fig. 1. Differences of NMC between left and right femurs. The NMC of left and right femurs in Slc:SD (■), BN/SsN Slc (▲), and MES (●) rats were measured by the gravimetric method using an injection needle.
The NMC in Crj:CD(SD)IGS rats is shown in Table 1. Using the gravimetric method, NMC at 4, 5, 6, and 10 weeks of age was 2.72, 2.39, 2.26, and 1.96 in males, and 2.75, 2.47, 2.38, and 1.98 ($\times 10^6$ counts/mg) in females, respectively. Although the NMC of males was similar to that of females in the gravimetric method, the NMC of females was slightly lower than that of males in the volumetric method. Chan et al. (1966) has suggested that the measurement error could be minimized by weighing a larger amount of bone marrow because marrow mass per bone is small. Since, in our gravimetric method, a large amount of BM (approximately 20 mg) was aspirated constantly by an injection needle, any measurement error could be minimized by careful handling. In contrast, the NMC was easily influenced by the total volume of the diluents in the volumetric method. These results were attributed to the handling procedures.

In both methods, the NMC of both sexes declined gradually from 4 to 10 weeks of age. In the wild-type control of osteopetrotic mice, the femoral BM cells increased postnatally, attaining adult levels by 6-8 weeks of age, and then remained stable to approximately 35 weeks of age (Begg et al., 1993). The total number of marrow cells per humerus slightly increased (no significant difference) with the body weight gain in mice (Chervenick et al., 1968). On the other hand, the volume of the marrow cavity was generally increased with the body weight gain. Therefore, it was considered that the age-related decrease in the NMC was attributed to reduction of marrow cell density. In the gravimetric method, it only requires a few minutes to measure NMC after aspirating BM, but some variability with this method includes low NMC by blood contamination and variation in NMC due to rough handling. However, those problems could be minimized by careful and swift handling of the BM.

In conclusion, this gravimetric method is a useful regular screening method to evaluate myelotoxicity in addition to myelogram examination. It is important to confirm the validity of the assumptions regarding the specific gravity of marrow, the volume of the marrow cavity, and marrow cellularity, which was suggested by Chan et al. (1966) in the method for quantitating cellularity per mg of bone marrow.

**ACKNOWLEDGMENT**

The authors wish to sincerely thank Mr. Tetsuo Fujii for his expert technical support in measuring the NMC.

**REFERENCES**


### Table 1. NMC in Crj:CD(SD)IGS rats.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Sex</th>
<th>Number of rats</th>
<th>Gravimetric method ($\times 10^6$ counts/mg)</th>
<th>Volumetric method ($\times 10^6$ counts/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Male</td>
<td>10</td>
<td>2.72 ± 0.26</td>
<td>2.34 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10</td>
<td>2.75 ± 0.16</td>
<td>1.99 ± 0.23</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>10</td>
<td>2.39 ± 0.17</td>
<td>2.04 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10</td>
<td>2.47 ± 0.12</td>
<td>1.92 ± 0.16</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>10</td>
<td>2.26 ± 0.21</td>
<td>2.15 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10</td>
<td>2.38 ± 0.15</td>
<td>2.12 ± 0.23</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>10</td>
<td>1.96 ± 0.21</td>
<td>1.72 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10</td>
<td>1.98 ± 0.15</td>
<td>1.69 ± 0.19</td>
</tr>
</tbody>
</table>

The NMC of left and right femurs was measured by the gravimetric method using an injection needle and the volumetric method using a blood cell diluting pipette, respectively. Figures represent the mean ± SD.