Brief Definitive Report

DISTRIBUTION OF BLOOD MONOCYTES BETWEEN A MARGINATING AND A CIRCULATNG POOL

By R. van FURTH and W. SLUITER

From the Department of Infectious Diseases, University Hospital, 2300 RC Leiden, The Netherlands

Cell kinetic studies on mononuclear phagocytes have been done mainly in mice, because in these animals it is easy to obtain quantitative data on the numbers and kinetic parameters of monoblasts, promonocytes, and monocytes in the bone marrow, monocytes in the circulation, and macrophages in various organs and body cavities (1–7). Earlier studies (2, 6) on monocyte production in the bone marrow and the turnover of circulating monocytes did not suggest the existence of a marginating pool of blood monocytes. This report concerns a study on the blood volume of mice, which led to calculations that revealed the existence of a marginating as well as a circulating pool of monocytes.

Materials and Methods

Animals. Specific pathogen–free Swiss mice (Cpb/SE) of both sexes were obtained from The Central Institute for the Breeding of Laboratory Animals, TNO, Zeist, The Netherlands. Just before use, each mouse was weighed on a mechanical toploader (type 2254; Sartorius, Göttingen, Federal Republic of Germany).

Determination of Labelled Erythrocytes. A stock solution of labelled erythrocytes was prepared by addition of hexavalent sodium chromate of high sp act (200–900 Ci/g chromium, New England Nuclear, Boston, MA) in pyrogen-free saline containing 100 μCi to ~4 ml blood obtained by cardiac puncture under chloroform anesthesia. After incubation for 20 min at room temperature, the cells were washed once with an excess of saline (100 ml) to remove unbound chromium, and were then resuspended in 4 ml pyrogen-free saline. 100 μl of this suspension was injected intravenously into unanesthetized mice. After 20 min, ~0.3 ml blood was taken from the orbital sinus with a heparinized hematocrit capillary, and the radioactivity in 200 μl of each whole-blood sample and 100 μl of the stock suspension was measured with an autogamma spectrometer. After correction for the background level, the blood volume of each animal was calculated from the radioactivity (cpm) of the injected stock suspension and the blood sample taken 20 min after the injection (8).

Injection of Epinephrine and Leukocyte Counting. Specific pathogen–free Swiss mice (25 g body wt) received an i.v. injection of 0.5 μg epinephrine in 0.1 ml saline, or 0.1 ml saline alone (control mice). The saline was made pyrogen-free in the hospital pharmacy for use in humans; all other materials (tubes, syringes, etc.) were plastic and freed of pyrogen by gamma irradiation. For each mouse, ~20 μl blood was collected from the orbital venous sinus at the indicated time points. The total number of leucocytes was then determined, differential counts were performed, and the total numbers of monocytes, granulocytes, and lymphocytes per mouse were calculated and expressed as the mean and ±SE (9). The changes in the number of leukocytes and the differences between treated and control mice were evaluated for significance by analysis of variance.

This work was supported in part by the Netherlands Asthma Fonds.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/86/2/0474/09 $1.00
Volume 163 February 1986 474–479

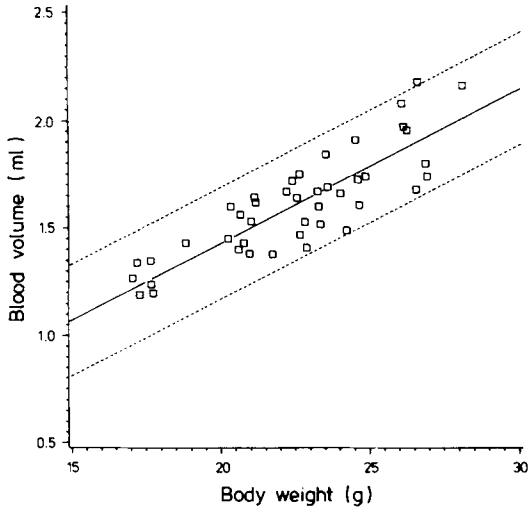

FIGURE 1. Blood volume in relation to body wt for Swiss mice. The 95% confidence limits (dashed line) were calculated with the data of individual mice.

Results

Blood Volume of Swiss Mice. Distinct correlation was found between the blood volume of Swiss mice and the weight of the animals (Fig. 1). Analysis of these data showed a linear correlation: $y = 0.0715x$ ($r^2 = 0.994$), in which y is the blood volume (in milliliters), and x is the body weight (in grams). Earlier, it had been assumed that the blood volume of Swiss mice weighing 25 g amounted to 3 ml, as determined in Akm mice (10). The smaller blood volume now found experimentally has, of course, consequences for calculations concerning the number of monocytes in the circulation.

Number of Circulating Monocytes. The mean number of leukocytes in the tail blood of Swiss mice with a mean body weight of 25 g amounts to 10^7 cells/ml (9, 11), and according to differential counts in blood smears, the tail blood contains 3.45% monocytes (11). This gives a value of 3.45×10^5 cells/ml for the mean number of monocytes, as consistently found for Swiss mice in the steady state (9, 11, 12). Since the blood volume of Swiss mice with a body weight of 25 g is 1.79 ml, the total number of circulating monocytes amounts to 6.18×10^5 cells/mouse.

Production of Monocytes in Bone Marrow. In a specific pathogen-free Swiss mouse weighing ~25 g, one femur contains 1.2×10^7 nucleated cells, 0.25% of which are promonocytes (13). Since the bone marrow of one femur represents 5.9% of the total bone marrow mass, as determined by the distribution of ^{59}Fe (14), the total number of promonocytes per mouse amounts to 5×10^5 cells. Promonocytes derive from dividing monoblasts (4, 15), and have a cell cycle time of 16.2 h (2). In each division, one promonocyte gives rise to two monocytes (16). The monocyte production in the bone marrow thus amounts to $2 \times (5 \times 10^5)/16.2 = 0.62 \times 10^5$ cells/h.

Comparison of Rate of Monocyte Production and Rate of Monocyte Efflux. In the steady state, the rate of influx of monocytes from the bone marrow into the

TABLE I

Leukocytes Number after Intravenous Injection of Epinephrine

Type of leukocyte	Substance injected*	Number of cells ($\times 10^3$/ml) 24 h before injection	Number of cells ($\times 10^3$/ml) at various times (min) after injection		
			5	10	60
Monocytes	Epinephrine	129 ± 11	148 ± 35	261 ± 40	97 ± 23
	Saline	121 ± 9	184 ± 79	276 ± 41	161 ± 43
Lymphocytes	Epinephrine	6,738 ± 291	8,214 ± 725	5,558 ± 685	2,770 ± 565
	Saline	7,006 ± 390	6,954 ± 669	6,106 ± 582	3,030 ± 237
Granulocytes	Epinephrine	1,079 ± 94	2,230 ± 284	1,697 ± 267	4,242 ± 576
	Saline	1,065 ± 64	1,735 ± 99	1,600 ± 76	4,478 ± 316

Values are means ± SE. $n \geq 9$ mice.

* Either 0.5 μg epinephrine in 0.1 ml saline or 0.1 ml saline alone given i.v.

circulation must equal the rate of efflux of monocytes from the circulation. Since it may be assumed that each monocyte formed in the bone marrow migrates to the peripheral blood, the monocyte influx into the peripheral blood must be 0.62×10^5 monocytes per hour. It has been found that monocytes leave the circulation randomly with a $t_{1/2}$ of 17.4 h (2). If the circulating monocytes (6.18×10^5 cells) comprise all of the monocytes in the circulation, the efflux of monocytes from the circulation would amount to $\ln 2/t_{1/2} \times 6.18 \times 10^5 = 0.25 \times 10^5$ monocytes per hour, which is much lower than the rate of monocyte influx (0.62×10^5 cells/h) into the circulation. This is not compatible with the principle that equilibrium between the rates of cell influx into and efflux from the circulation is maintained under steady-state conditions. Calculation shows that equilibrium will only occur if the total pool of blood monocytes amounts to $0.62/0.25 \times 6.18 \times 10^5 = 15.33 \times 10^5$ cells.

The only conclusion that can be drawn from these calculations is that the total pool of blood monocytes consists of not only a circulating pool (i.e., 6.18×10^5 cells) but also a marginating pool of monocytes (i.e., 9.15×10^5 cells), the latter accounting for about 60% of the total pool of blood monocytes under steady-state conditions.

Effect of Epinephrine on Number of Circulating Leukocytes in Mice. Since, in man, marginating leukocytes can be mobilized very rapidly after intravenous administration of epinephrine (17), this stimulus was applied to mice. The results show no differences between the course of the numbers of monocytes ($p = 0.75$), lymphocytes ($p = 0.93$), and granulocytes ($p = 0.95$) in mice given epinephrine and those given saline (Table I). A significant increase in the number of circulating monocytes was found 10 min, but not 5 min, after the injection of either epinephrine ($p = 0.002$) or saline ($p = 0.017$). The number of circulating granulocytes was significantly higher 5 and 10 min after the injection of either epinephrine ($p = 0.004$ and $p = 0.049$, respectively) or saline ($p = 0.003$ and $p < 0.001$, respectively).

Since mobilization of marginating leucocytes would have to occur within 10 min after administration of epinephrine (17–19), the increase in the number of circulating monocytes and granulocytes might reflect the existence of a marginating pool of monocytes and of granulocytes. However, this increase cannot be

ascribed to a specific reaction to epinephrine, because the course of the number of circulating leukocytes was the same when saline was injected.

Discussion

Establishment of the blood volume of mice in the steady state provided values for new kinetic calculations, and on the basis of these results, it must be concluded that the total pool of blood monocytes comprises not only a circulating pool but also a marginating pool. In earlier kinetic studies (1–7), we used the blood volume determined by others (10) in Akm mice (12 ml per 100 g body wt) for these calculations, and found no indications for a marginating pool, but it is now certain that the volume of the blood of Swiss mice is actually smaller (7.15 ml per 100 g body wt). If the number of monocytes in the circulating pool equaled the total number of blood monocytes, the total number of blood monocytes calculated with this value would be much too small to allow equilibrium between the influx and efflux of monocytes into and from the circulation, which would be in conflict with the steady-state principle. Furthermore, the efflux of monocytes would then be much too small to account for the renewal of macrophages in the peritoneal cavity (1, 20), the liver (3), lung (5, 6), spleen (7), and other tissues, under steady-state conditions.

Unfortunately, it is not possible to obtain direct experimental evidence for the existence of a marginating pool of monocytes in the mouse. The experiment on the mobilization of monocytes into the circulating pool by the injection of epinephrine was not convincing, since saline gave the same effect. This suggests that the stress caused by intravenous injection is sufficient to mobilize monocytes and granulocytes into the circulation; moreover, the source of these cells, i.e., the marginating pool or the bone marrow, is uncertain as well. Another approach, the transfusion of labeled monocytes, will also give unreliable results, because the collection of donor monocytes is in itself sufficient to change the surface of these cells such that they will immediately adhere to the endothelial surface of recipient mice. The existence of a marginating pool has not yet been demonstrated morphologically either.

For the present calculations, we used the mean number of monocytes in tail blood. Although earlier studies (9) have shown that blood collected from the orbital venous sinus and heart contains fewer circulating monocytes, most of the kinetic studies performed in mice have been done with leukocytes from tail blood, and therefore use of these values for our calculations of the monocyte pools is justified. The actual size of the marginating pool may be larger or smaller depending on the diameter of the vasculature and other conditions, such as a preference of monocytes to adhere to endothelium of a particular vascular bed (21).

In man, some evidence indicating the existence of a marginating pool of monocytes has been obtained. In a study on the marginating pool of granulocytes, the number of circulating monocytes increased significantly ($p < 0.001$ as calculated with the data of that study) within 15 min after the injection of epinephrine (17). From a kinetic study (22) done with autotransfused [^{3}H]-diisopropylfluorophosphate-labelled monocytes it was concluded that ~75% of the monocytes belong to the marginating pool, which is close to the size of the

marginating pool of granulocytes (~55% of the total blood granulocyte pool) reported for man (18, 19). Indications have been obtained that there is a ready exchange of granulocytes between the circulating and marginating pools (18, 19), and it may be assumed that this holds for monocytes as well.

Summary

Studies on the blood volume of SPF Swiss mice in the steady state led to reevaluation of the distribution of monocytes in the blood compartment. It was concluded from the results of our calculations that monocytes entering the circulation are distributed over a circulating and a marginating pool. The circulating pool accounts for ~40%, and the marginating pool for ~60% of the population of peripheral blood monocytes.

Received for publication 13 November 1985.

References

1. van Furth, R., and Z. A. Cohn. 1968. The origin and kinetics of mononuclear phagocytes. *J. Exp. Med.* 128:703.
2. van Furth, R., M. M. C. Dieselhoff-den Dulk, and H. Mattie. 1973. Quantitative study on the production and kinetics of mononuclear phagocytes during an acute inflammatory reaction. *J. Exp. Med.* 138:1314.
3. Crofton, R. W., M. M. C. Diesselhoff-den Dulk, and R. van Furth. 1978. The origin, kinetics and characteristics of the Kupffer cells in the normal steady state. *J. Exp. Med.* 148:1.
4. Goud, Th. J. L. M., and R. van Furth. 1975. Proliferative characteristics of monoblasts grown in vitro. *J. Exp. Med.* 142:1217.
5. Blussé van Oud Alblas, A., and R. van Furth. 1979. Origin, kinetics, and characteristics of pulmonary macrophages in the normal steady state. *J. Exp. Med.* 149:1504.
6. Blussé van Oud Alblas, A., H. Mattie, and R. van Furth 1983. A quantitative evaluation of pulmonary macrophage kinetics. *Cell Tissue Kinet.* 16:211.
7. van Furth, R., M. M. C. Diesselhoff-den Dulk. 1984. Dual origin of mouse spleen macrophages. *J. Exp. Med.* 160:1273.
8. Sluiter, W., L. W. M. Oomens, A. Brand and R. van Furth. 1984. Determination of blood volume in the mouse with ^{51}Chromium-labelled erythrocytes. *J. Immunol. Methods.* 73:221.
9. Sluiter, W., E. Hulsing-Hesselink, I. Elzenga-Claassen and R. van Furth. 1985. Method to select mice in the steady state for biological studies. *J. Immunol. Methods.* 76:135.
10. Kaliss, N., and D. Pressman. 1950. Plasma and blood volumes of mouse organs as determined with radioactive iodoproteins. *Proc. Soc. Exp. Biol. Med.* 75:16.
11. Thompson, J., and R. van Furth. 1970. The effect of glucocorticosteroids on the kinetics of mononuclear phagocytes. *J. Exp. Med.* 131:429.
12. van Waarde, D., S. Bakker, J. van Vliet, A. F. Angulo, and R. van Furth. 1978. The number of monocytes in mice as a reflection of their contribution and capacity to react to an inflammatory stimulus. *J. Reticuloendothel. Soc.* 24:197.
13. van Furth, R., J. G. Hirsch, and M. E. Fedorko. 1970. Morphology and peroxidase cytochemistry of mouse promonocytes, monocytes, and macrophages. *J. Exp. Med.* 132:794.
14. Chervenick, P. A., D. R. Boggs, J. C. Marsch, G. E. Cartwright, and H. M. Wintrobe.

1968. Quantitative studies of blood and bone marrow neutrophils in normal mice. *Am. J. Physiol.* 215:353.

15. Goud, Th. J. L. M., C. Schotte, and R. van Furth. 1975. Identification and characterization of the monoblast in mononuclear phagocyte colonies grown in vitro. *J. Exp. Med.* 142:1180.
16. van Furth, R., M. M. C. Diesselhoff-den Dulk. 1970. The kinetics of promonocytes and monocytes in the bone marrow. *J. Exp. Med.* 132:813.
17. Joyce, R. A., D. R. Boggs, U. Hasiba, and C. H. Srodes. 1976. Marginating neutrophil pool size in normal subjects and neutropenic patients as measured by epinephrine infusion. *J. Lab. Clin. Med.* 88:614.
18. Athens, J. W., S. O. Raab, O. P. Haab, A. M. Mauer, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe. 1961. Leukokinetic studies. III. The distribution of granulocytes in the blood of normal subjects. *J. Clin. Invest.* 40:159.
19. Cartwright, G. E., J. W. Athens, and M. M. Wintrobe. 1964. The kinetics of granulopoiesis in normal man. *Blood.* 24:780.
20. van Furth, R. 1985. New perspectives on the kinetics of mononuclear phagocytes. *In* Mononuclear phagocytes. Characteristics, physiology, and function. R. van Furth, editor. Martinus Nijhoff Publishers. Boston, MA. 201–208.
21. Harlan, J. M. 1985. Leukocyte-endothelial interactions. *Blood.* 65:513.
22. Meuret, G., G. Hoffmann. 1973. Monocyte kinetic studies in normal and disease states. *Br. J. Haematol.* 24:275.