QUANTITATIVE STUDY ON THE PRODUCTION AND KINETICS OF MONONUCLEAR PHAGOCYTES DURING AN ACUTE INFLAMMATORY REACTION

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During an acute inflammatory response in the peritoneal cavity both the peritoneal macrophages and the monocytes in the peripheral blood increase in number (1, 2). Labeling studies with [3H]thymidine have demonstrated that during an acute inflammation, just as during the normal steady state, the peritoneal macrophages derive from peripheral blood monocytes (1), which originate in the bone marrow from dividing promonocytes (3, 4).

Although the mitotic activity of the promonocytes (DNA-synthesis time and cell cycle time) and the kinetic characteristics of these cells have been studied quantitatively under steady-state conditions (4) and during treatment with glucocorticosteroids (5), similar data are not available for the acute inflammatory response.

The present quantitative study, performed during an acute inflammation, provided data on the production of monocytes in the bone marrow, the influx and efflux of these cells into and from the peripheral blood, and the migration of monocytes into inflammatory lesion of the peritoneal cavity. These results were compared with the findings during the normal steady state.

Materials and Methods

Animals.—Specific pathogen-free male Swiss mice weighing 25–30 g (Central Institute for the Breeding of Laboratory Animals, TNO, Bilthoven, The Netherlands) were used.

Cell Cultures.—The techniques for harvesting and culturing mouse bone marrow cells and peritoneal macrophages have been described in detail elsewhere (1, 3). Bone marrow cells were cultured for 6 h and the peritoneal cells for 2 h, both in Leighton tubes with a flying cover slip. The culture medium consisted of medium 199 (Microbiological Associates, Inc., Bethesda, Md.), to which 20% newborn calf serum (NBCS)1 (Grand Island Biological Co., Grand Island, N. Y.), 200 U/ml penicillin G, and 50 μg/ml streptomycin were added.

Cell Counts.—For the counting of bone marrow cells, the femora were flushed individually with 2 ml medium 199 and the cells collected in a plastic tube (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). After thorough mixing, the cell suspension was diluted 1:10 with Turk’s solution containing 6% acetic acid. The cells were counted in a Bürker hemocytometer and the number of nucleated cells per femur calculated. Differential counts were done in preparations made in a sedimentation chamber (6, 7). For each animal, the percentage of

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1 Abbreviations used in this paper: NBCS, new born calf serum.
monocytes was determined from the counts of at least 400 nucleated cells. The total number of monocytes per femur was derived from this percentage and the number of nucleated cells.

The number of promonocytes per femur was calculated from the promonocyte-to-monocyte ratio in 6-h cultures, and the number of monocytes calculated per femur (4). Since two femora represent 11.8% of the total bone marrow (8), the total number of promonocytes and bone marrow monocytes per mouse could be computed.

For the counting of the peritoneal macrophages, the peritoneal cavity was washed out with exactly 2 ml phosphate-buffered saline (pH 7.2) containing 50 U/ml heparin. The cells in undiluted suspensions were counted in a Bürker hemocytometer. After differential counting in preparations made in a sedimentation chamber, the total number of peritoneal macrophages per mouse was calculated.

Total peripheral blood leukocyte counts and differential counts were done in blood taken from the tail. In a leukocyte pipette the blood was diluted 1:20 with Turk's solution containing 6% acetic acid, and the nucleated cells were counted in a Bürker hemocytometer. All counts were made in duplicate. Differential counts were done in 200 leukocytes from at least two blood smears fixed in methanol for 30 min and stained with Giemsa's stain for 20 min. Since the blood volume of a mouse weighing 25 g is about 3 ml (9), the total number of monocytes per mouse could be calculated.

**Inflammation.**—An acute inflammation was provoked by injecting 1 ml sterile NBCS (GIBCO) into the peritoneal cavity.

**Labeling with [3H]Thymidine.**—For in vitro labeling, bone marrow cells and peritoneal macrophages were incubated for various periods in a Leighton tube in a medium containing 0.1 μCi/ml [3H]thymidine (sp act 6.7 Ci/mmol) (New England Nuclear, Boston, Mass.), washed 5 times with medium 199, quickly air dried, fixed in methanol for 30 min, and washed overnight in distilled water to remove all [3H]thymidine not incorporated into DNA. The cover slips were then prepared for autoradiography.

Peripheral blood monocytes were labeled in vitro by incubating a blood sample (obtained by puncture of the retro-orbital venous plexus and collected in a plastic tube containing 0.1 ml 5% [wt/vol] EDTA) with 0.1 μCi [3H]thymidine per ml blood for 2 h at 37°C. Smears were then prepared and fixed in methanol for 30 min, washed overnight in running tap water, given a last rinse in distilled water, and prepared for autoradiography.

It should be noted that the [3H]thymidine must be added immediately after harvesting of the cells and that when the cells must be handled before culturing, all solutions should contain the label to avoid underestimations due to decreased mitotic activity in vitro.

For in vivo labeling, the animals were given one intravenous injection of 1 μg [3H]thymidine per g body weight. At given time points, bone marrow samples were cultured for 6 h, peripheral blood smears were made, and peritoneal cells cultured for 2 h, after which the material was prepared for autoradiography.

The DNA-synthesis time of the promonocytes was determined in a group of mice given three serial intravenous injections of 1 μg [3H]thymidine per g body weight, exactly 1 h apart. 1 h after each injection, 6-h bone marrow cultures were prepared as described above.

**Autoradiography.**—Autoradiography was done as described in detail elsewhere (1). Cover slips and microscope slides were dipped in Ilford Nuclear Research Emulsion K5 in gel form (Ilford Ltd., Ilford, Essex, England). The slides were dried and stored in light-tight boxes at room temperature for 21 days. After development of the autoradiographs (Kodak Developer D-19 Eastman Kodak, Rochester, N. Y.) the preparations were stained with Giemsa's stain. The cultured peritoneal and bone marrow cells were stained for 20 min, the blood smears for 90 min.

Since background levels on preparations from nonlabeled animals showed less than three grains per nucleus, all cells with three or more silver grains over the nucleus were considered as labeled.
Identification of the Cells.—The morphological identification of promonocytes, bone marrow monocytes, peripheral blood monocytes, and peritoneal macrophages was based on the criteria described in a previous report (1, 3).

General Remarks.—Each time point represents the mean value of at least four animals.

RESULTS

Numbers of Peritoneal Macrophages and Monocytes in the Peripheral Blood and Bone Marrow during an Acute Inflammation.—During an inflammatory response, the numbers of mononuclear phagocytes change differently in the various compartments (Fig. 1). A normal mouse has $2.4 \times 10^6$ macrophages in the peritoneal cavity. After an intraperitoneal injection of 1 ml NBCS this number increases, reaching a maximum of $6.1 \times 10^6$ cells at 72 h.

The monocytes in the peripheral blood also increase during an acute inflammation but in a different pattern, i.e., from $1.0 \times 10^6$ cells in normal mice to a maximum of $3.0 \times 10^6$ at 48 h.

The monocytes in the bone marrow decrease gradually during an inflammation from $2.6 \times 10^6$ cells in normal mice to $1.7 \times 10^6$ at 96 h. For promonocytes, the $5.0 \times 10^5$ cells in normal mice increase during an inflammatory response to $6 \times 10^5$ at 12 h, and this number remains constant until 72 h, when it returns to the normal value.

From these data it is clear that during an acute inflammatory response in the peritoneal cavity, in the peripheral blood a monocytosis develops concomitantly with the increase in the number of peritoneal macrophages. The next question
to be answered was whether under these conditions the mitotic activity of the peritoneal macrophages and monocytes increases.

In Vitro and Pulse Labeling of Mononuclear Phagocytes during an Acute Inflammation.—After being incubated for 2 and 24 h in medium containing (³H)thymidine, the peritoneal macrophages of normal mice showed a labeling index of 3.6 and 4.1%, respectively, which agrees well with previous findings (1, 2). Initially, i.e., 12 h after an injection of NBCS, the peritoneal exudate macrophages had a lower labeling index, but thereafter this value lay within the range found in normal mice (Table I). Similar values for the labeling index of peritoneal macrophages were obtained in a pulse-labeling experiment in

| Time after NBCS* (h) | In vitro labeling± (%) | 2 h | 24 h | Pulse labeling§ (%) | In vitro labeled peripheral blood monocytes|%
|---------------------|-----------------------|-----|-----|---------------------|------------------------------------------|
| 0                   | 3.6                   | 4.1 | 2.4 | 2.3                 |
| 12                  | 0.2                   | 0.3 | 0.2 | 1.0                 |
| 24                  | 2.5                   | 3.9 | 1.7 | 1.3                 |
| 48                  | 3.0                   | 4.0 | 1.3 | 2.0                 |
| 72                  | 2.5                   | 1.6 | 1.2 | 2.8                 |
| 96                  | 1.7                   | 1.5 | 0.9 | 2.7                 |

* 1 ml NBCS intraperitoneally.
± Cultured in medium with 0.1 μCi/ml [³H]thymidine.
§ Harvested 1 h after 1 μCi [³H]thymidine/g body weight given intravenously, and incubated for 2 h in a nonradioactive medium.
∥ 0.1 μCi [³H]thymidine added to 1 ml EDTA blood and incubated for 2 h.

which peritoneal macrophages were harvested 1 h after an intravenous injection of [³H]thymidine and cultured for 2 h in a nonradioactive medium (Table I).

Peripheral blood monocytes of normal mice have a labeling index of 2.3% after incubation for 2 h in a medium with [³H]thymidine. During an acute inflammatory response the labeling index decreases slightly during the first 24 h, but thereafter shows about the same value as in normal animals (Table I).

In the smears, two categories of labeled monocytes could be distinguished: cells with a diameter of 11–14 μm, pale blue cytoplasm, and an indented, reniform, or horseshoe-shaped nucleus, and less mature monocytes with a diameter of 12–18 μm, dark blue cytoplasm, and an indented nucleus. In normal mice and after the NBCS injection, about two-thirds of the labeled monocytes are of this immature type.

It is known that promonocytes are actively dividing cells (4). In normal mice
these cells had an in vitro labeling index of 61.8% in 6-h bone marrow cultures (Table II); during an acute inflammatory response this index became higher. The in vitro labeling index of the bone marrow monocytes of normal mice was low (0.3%), equaling that of the mature monocytes in the peripheral blood, and also increased slightly during an acute inflammation.

The low labeling index of the peritoneal macrophages during an acute inflammation, just as under normal conditions, means that only a very small proportion of the peritoneal cells divide. Moreover, the absence of an increase in the labeling index during incubation with [3H]thymidine for 24 h or longer (1) indicates that the peritoneal macrophages lose their mitotic activity very soon. This may be an intrinsic characteristic of these cells, but may also be due to the in vitro conditions.

**TABLE II**

*In Vitro Labeling of Bone Marrow Monocytes and Promonocytes during the Normal Steady State and an Acute Inflammation*

| Time after NBCS* (h) | Labeled bone marrow monocytes† | Labeled promonocytes† |
|---------------------|-------------------------------|---------------------|
| 0                   | 0.3                           | 61.8                |
| 12                  | 0.2                           | 63.7                |
| 24                  | 1.2                           | 72.5                |
| 48                  | 1.4                           | 70.3                |

*1 ml NBCS intraperitoneally.
†6-h culture in medium containing 0.1 μCi/ml [3H]thymidine.

**Origin of In Vitro-Labeled Peritoneal Macrophages.**—Although the mitotic activity of the peritoneal macrophages is very low, an attempt was made to establish the origin of the cells labeled in vitro. For this purpose mice were treated with glucocorticosteroids because these drugs induce severe monocytopenia very rapidly, but do not grossly affect the number of peritoneal macrophages already present in the peritoneal cavity (2). Peritoneal macrophages obtained from mice given 15 mg hydrocortisone acetate subcutaneously and incubated for 24 h in a medium with 0.1 μCi [3H]thymidine, showed a decrease in the labeling index from 3.5% to nil 24 h after the injection (Fig. 2).

Since in normal mice glucocorticosteroids arrest the influx of mononuclear phagocytes from the peripheral blood into the peritoneal cavity (2) but do not affect the DNA synthesis of the mononuclear phagocytes either in vitro or in vivo (2, 5), these results demonstrate that the DNA-synthesizing peritoneal macrophages derive from the circulation. Indeed, DNA-synthesizing monocytes were found in the peripheral blood of normal mice (see Table I).

The rapid disappearance of labeled peritoneal macrophages during glucocorticosteroid treatment implies, furthermore, that once arrived in the peri-
toneal cavity, mononuclear phagocytes retain the capacity to synthesize DNA for only a short time. Supportive evidence for this assumption was obtained in an experiment in which peritoneal macrophages from normal mice were incubated first for various intervals in a medium without [3H]thymidine and then for 24 h in one containing [3H]thymidine. The results again show a rapid decrease of the labeling index, with values of 0-0.25% after 18 or more hours of preincubation in cold medium (Fig. 3). (These data derive from experiments performed by Dr. J. Thompson, University Hospital, Leiden, who kindly permitted us to use the results.)

Because glucocorticosteroids also suppress to a great extent the influx of monocytes into the peritoneal cavity during an acute inflammatory response (2), it was relevant to study the in vitro labeling of peritoneal macrophages in these mice. When the administration of hydrocortisone acetate was followed 48 h later by an intraperitoneal injection of NBCS, the labeling index rose gradually to 2.2% at 48 h after induction of the inflammatory lesion (Fig. 2).

Since the percentage of in vitro or pulse-labeled peritoneal macrophages does not increase during this kind of inflammation (see Table I), which indicates that there is no increase of local proliferation, the present results show that during this induced reaction in hydrocortisone-treated mice, DNA-synthesizing mononuclear phagocytes (conceivably of bone marrow origin) are still recruited from the circulation into the inflammatory lesion of the peritoneal cavity.

**In Vivo Labeling of Mononuclear Phagocytes during an Acute Inflammation.**

—Since the preceding experiments had indicated that the increase in the number of peritoneal macrophages during an acute inflammatory response is not
due to local proliferation, a quantitative study of the kinetics of the bone marrow and peripheral blood monocytes and the peritoneal macrophages was undertaken.

Mice were injected with 1 μCi [3H]thymidine per g body weight intravenously and 1 h later with 1 ml NBCS intraperitoneally. For purposes of comparison, earlier data (4) obtained in normal mice were used. The results, expressed as the total number of labeled monocytes and macrophages per mouse, were calculated from the labeling indices and total number of cells at each time point.

1 h after [3H]thymidine administration, 2.4% of the peritoneal macrophages are found to be labeled (i.e., 5.8 × 10⁴ cells). Since in all probability these cells are not labeled in the bone marrow compartment but are DNA-synthesizing macrophages residing in the peritoneal cavity (see above), the change in the number of the labeled peritoneal macrophages can be established more accurately if the observed number of labeled peritoneal macrophages is corrected by subtracting the number of labeled macrophages present at 1 h. This correction has been applied for both normal and inflammatory conditions.

In normal mice the number of labeled peritoneal macrophages is nil at 6 h, 2.7 × 10⁴ at 12 h, and reaches a maximum of 5.6 × 10⁴ cells at 48 h, after which a decrease sets in (Fig. 4). During an acute inflammation the number of labeled cells at 6 h is 1.8 × 10⁴, rising sharply to 63.0 × 10⁴ at 48 h and 70.5 × 10⁴ at 96 h.

The number of labeled monocytes in the peripheral blood of normal mice and of mice with an acute inflammation amounts at 2 h to 2.6 × 10⁶ and 3.7 × 10⁶ cells, respectively. In normal mice the number increases to maximally 61.6 × 10⁶ cells at 48 h; during the inflammatory response about twice this number, i.e., 119.3 × 10⁶ cells, was found (Fig. 4).
The total number of labeled bone marrow monocytes, blood monocytes, and peritoneal macrophages formed after a single injection of [3H]thymidine in normal mice and during an acute inflammation. Open symbols: normal mice; closed symbols: after NBCS i.p.

The number of labeled monocytes in the bone marrow showed a similar course under both conditions. 2 h after labeling the number was $4.8 \times 10^4$ labeled cells in normal mice and at 24 h there were $87.9 \times 10^4$ labeled cells; the other group of mice had $7.8 \times 10^4$ labeled monocytes at 2 h and $94.1 \times 10^4$ at 24 h. Only after 48 h was there a more rapid decrease in the number of labeled monocytes in mice with an acute inflammation, i.e. to $19.1 \times 10^4$ monocytes at 96 h; in the normal mice this value was $32.5 \times 10^4$ cells.

Determination of the DNA-Synthesis Time and Cell Cycle Time of the Promonocytes during an Acute Inflammation.—The DNA-synthesis time and cell cycle time were determined, both in normal mice and during an acute inflammatory response 12 and 24 h after the injection of NBCS (Table III). The criteria to be satisfied for the validity of the approach applied in this study have been discussed elsewhere (4). For normal mice the labeling indices of the promonocytes found initially ($i$) and 12 h after the injection of NBCS were almost the same, and at 24 h the index was a little higher. (A similar increase was also found in the in vitro labeling study; see Table II.) The hourly increase of the labeling index ($\Delta i$) was greater 12 h after a NBCS injection, and thus the DNA-synthesis time of the promonocytes ($t_s$), computed by dividing the initial labeling index by the increment of the labeling index per hour ($i/\Delta i$) (10), was shorter than in the normal steady state and 24 h after the induction of an acute inflammation ($P < 0.001$) (Table III).

The cell cycle time of the promonocytes ($t_c$), which is calculated by dividing the DNA-synthesis time by the initial labeling index ($t_s \times 100/i$) (11), amounts to 16.2 h in normal mice. About the same value (16.7 h) was computed 24 h
TABLE III

| DNA-Synthesis Time ($t_{12}$) and Cell Cycle Time ($t_c$) during the Normal Steady State and during an Acute Inflammation* |
|--------------------------------------------------|
| Normal                                           | Acute inflammation |
| 12 h‡                                            | 12 h‡              |
| i                                                | 72.9%              | 69.8% | 76.7% |
| $\Delta i$                                       | 6.2%               | 9.2%  | 6.0%  |
| $t_{12}$                                         | 11.8 h             | 7.6 h | 12.8 h |
| $t_c$                                            | 16.2 h             | 10.8 h| 16.7 h |

*Mice labeled with 1, 2, or 3 injections of 1 µCi $[^3]$H thymidine/g body weight given intravenously.

‡ Time after an intraperitoneal injection of 1 ml NBCS.

i = Initial labeling index of promonocytes 1 h after the first injection of $[^3]$H thymidine.

$\Delta i$ = Mean hourly increment of promonocyte labeling index after the second and third injection of $[^3]$H thymidine.

$t_{12} = i/\Delta i$.

$t_c = t_{12} \times 100/i$.

after the injection of NBCS, but a significantly shorter cell cycle time, i.e., 10.8 h ($P < 0.001$), was found 12 h after the injection.

These results demonstrate that the rate of division of the promonocytes increases during the first 12 h of an acute inflammatory response.

Production of Monocytes during an Acute Inflammation.—Monocytes originating in the bone marrow after the division of promonocytes are released into the peripheral blood, from which they disappear randomly (1). At any given time point the total production of monocytes can be computed from the total number of monocytes present in the bone marrow and in the peripheral blood and the number that has left the circulation. This last value can be calculated if the rate of disappearance from the peripheral blood can be determined. On the basis of the disappearance of the cohorts of the most heavily labeled peripheral blood monocytes (1), the exponential disappearance rate constant ($k$) for normal mice was found to be 0.03974 h$^{-1}$, which corresponds with a half time ($t_{1/2}$) of 0.6932/0.03974 = 17.4 h. During the first 24 h of the acute inflammatory response, $k$ proved to be 0.06946 h$^{-1}$ and the half time 10.0 h; thereafter, these values were the same as in normal mice.

Since 1 h after labeling 2.5% of the peripheral blood monocytes are labeled (i.e., $2.5 \times 10^4$ cells per mouse), in all likelihood representing the DNA synthesis of the monocytes present in the circulation (cf. in vitro labeling in Table I), the calculated number of labeled, newly formed monocytes is corrected by subtracting the number of peripheral blood monocytes labeled at 1 h. The total production of labeled monocytes thus amounts to:

$$P_{o-t} = (I_{bm} \times N_{bm})t + (I_{bl} \times N_{bl})t - (I_{bl} \times N_{bl})t-1 + k \int (I_{bl} \times N_{bl})dt,$$

in which $P_{o-t} =$ total number of labeled monocytes produced in time period
0 to \( t \), \( I \) = labeling index, \( N_{bm} \) = total number of bone marrow monocytes, \( N_{bl} \) = total number of blood monocytes, \( k \) = exponential disappearance rate constant of monocytes from the peripheral blood, \( t \) = time, and \( \int_0^t (I_{bl} \times N_{bl})dt \) = area under the curve of the numbers of labeled blood monocytes from \( t = 0 \) to \( t = t \).

According to this equation, in normal mice the total production of labeled monocytes amounts to \( 15.6 \times 10^5 \) cells during the first 24 h and to \( 20.9 \times 10^6 \) cells during the first 48 h, after which the production levels off (Fig. 5). During an acute inflammatory response induced by NBCS given intraperitoneally, the total production of labeled monocytes was greater, amounting to \( 22.0 \times 10^5 \) cells during the first 24 h and \( 34.3 \times 10^5 \) cells during the first 48 h (Fig. 5).

The rate of monocyte production can be calculated in two different ways: calculation A is performed with the values obtained for the total production of labeled monocytes and amounts to \( P_{0.5}/t \) monocytes per hour; calculation B is done with the number of promonocytes at each time point \( (N_{pro}) \) (Fig. 1) and

![Graph showing total production of labeled monocytes](image)

Fig. 5. Total production of labeled monocytes formed after a single injection of \(^{3}H\)thymidine in normal mice (open symbols) and during an acute inflammation (closed symbols). Production computed with the formula mentioned in Results.
the known cell cycle time \((t_c)\) of these cells at various time points (Table III),
the mean rate of monocyte production \((R)\) thus amounting to \(2 \times N_{pro}/t_c\) cells per hour.

The results of these calculations, which are given in Table IV, show excellent agreement in normal mice. During the inflammatory response the rate of production increased 1.5 times during the first 12 h, according to calculation A, and then leveled off. According to calculation B, at 12 h the rate of production lies between the values found during the first and second 12-h periods of the inflammatory response, and at 24 h was even lower, approaching the normal rate.

**TABLE IV**

| Rate of Monocyte Production during the Normal Steady State and during an Acute Inflammation |
|---------------------------------|------------------|------------------|------------------|------------------|
|                                 | Normal           | 0-12 h           | 12 h             | 12-24 h          | 24 h             |
|                                 | \(\times 10^5/h\) | \(\times 10^5/h\) | \(\times 10^5/h\) | \(\times 10^5/h\) | \(\times 10^5/h\) |
| Calculation A*                  | 0.65             | 1.06             | 0.78             |                  |
| Calculation B†                  | 0.62             | 0.96             | 0.70             |                  |

\(R = P_{0-t}/t,\)

\(R = 2 \times N_{pro}/t_c.\)

*R = production rate; \(P_{0-t}\) = total number of labeled monocytes produced in time period 0 to \(t; N_{pro} = \) total number of promonocytes; \(t = \text{time}; t_c = \text{cell cycle time.}\)

**DISCUSSION**

The main findings in this study concern quantitative data pertaining to the production and kinetics of mononuclear phagocytes during the normal steady state and the acute inflammatory response.

The inflammatory stimulus used, an intraperitoneal injection of NBCS, has its main effect on the mononuclear phagocytes, increasing the number of both the peritoneal macrophages and the peripheral blood monocytes by a factor of 2.5-3. In the peritoneal cavity this increase continues for more than 96 h, whereas the elevated number of blood monocytes decreases 48 h after the inflammatory stimulus. The granulocyte response is quite different: the number of these cells in the peripheral blood and peritoneal cavity shows a fourfold increase with the maximum at 12 h and then returns to normal levels 24 h after the injection (footnote 2, 11).

Two possibilities can be envisaged for the increase in the number of macrophages in the peritoneal exudate, one being local proliferation of these cells

\(^5\) Gassmann, A. E., and R. van Furth. The effect of azathioprine (Imuran) on the kinetics of monocytes and macrophages during normal steady state and the acute inflammatory reaction. Manuscript to be published.
and the other recruitment from the circulation. The former would require an increase of the in vitro and pulse-labeling indices of the peritoneal macrophages during the inflammatory response, but such an increase was not found. The small percentage of DNA-synthesizing macrophages always present in normal mice (12, 13) remained almost constant during the inflammatory response. It could even be demonstrated in an experiment with hydrocortisone-treated mice that in the normal steady state and during an acute inflammation the DNA-synthesizing macrophages are derived from the peripheral blood. It was also shown that after arriving in the peritoneal cavity the mononuclear phagocytes lose their capacity to synthesize DNA rather soon (within 24 h).

If DNA-synthesizing macrophages originate from the circulation, this would mean that DNA-synthesizing monocytes must be present in the peripheral blood. A small percentage of the monocytes are in fact labeled after incubation with [3H]thymidine in vitro. In our earlier study no in vitro-labeled monocytes were found (1), but the present technique appears to be more suitable for demonstrating DNA-synthesizing cells than the method we formerly used.

It has been reported that macrophages of the peritoneal cavity (12, 14-17) and at other sites (18, 19) will start to divide again during an acute inflammation associated with the state of cell-mediated immunity. In animals with cell-mediated immunity and treated with cortisone acetate labeling of Kupffer cells, albeit delayed, has also been found (20). The interpretation of these findings must be reconsidered, however, because the results of the present study indicate the recruitment of DNA-synthesizing mononuclear phagocytes from the circulation into the inflammatory exudate in hydrocortisone-treated animals. The resumption of mitotic activity of macrophages already present in the tissues during the state of cell-mediated immunity therefore remains to be confirmed. It is conceivable, namely, that DNA-synthesizing precursor cells of the bone marrow (e.g. promonocytes) move into the tissues under the influence of these specific stimuli.

The present study provides no evidence that the increase in the number of macrophages in the acute inflammatory exudate is due to local proliferation. The second possibility, i.e., that the peritoneal macrophages in the inflammatory exudate are of monocytic origin, has been amply demonstrated, and previous studies have also shown that these monocytes originate in the bone marrow from dividing promonocytes (1, 4, 13). The consequence of the migration of a large number of peripheral blood monocytes into the peritoneal cavity during an inflammatory response, concurrently with an increase in the number of monocytes in the circulation, is an augmented production of these cells. This increased production would require an increased number of promonocytes and/or an acceleration of the mitotic activity of the promonocytes. The number of promonocytes is indeed increased from the 12th to the 72nd h of the inflammatory response, but the cell cycle time of the promonocytes was found to be 5.4 h shorter 12 h after the inflammatory stimulus, and to have virtually
returned to the steady-state level at 24 h. The shorter cell cycle time appeared to be due mainly to a shorter DNA-synthesis time; the time of the $G_1 + G_2 + M$ phases (i.e., cell cycle time minus DNA-synthesis time) remained almost constant.

In this study the cell cycle time during the normal steady state was observed to be shorter than had been found previously (4), but we now consider it more accurate to allow only a 1-h interval between the repeated $[^{3}H]thymidine$ injections instead of the 2 h previously taken.

The rate of monocyte production was computed according to two different calculations based on completely independent experimental data (Table IV). For the normal steady state both methods gave a similar production rate. This excellent agreement confirms our earlier conclusion that promonocytes are the direct precursors of the monocytes and that the division of one promonocyte gives rise to two monocytes (4), because calculation B was based on this premise. These two kinds of calculation also supplement each other in another way, since the results clearly demonstrate that the increased production of monocytes during the first 12 h of the inflammatory response gradually levels off after that period.

To obtain more insight into the course of the mononuclear phagocytes during

![Diagram: Normal Steady State]

| Production | Influx 0–48 h | Efflux 0–48 h |
|------------|--------------|--------------|
| 0–48 h     | 13.6 x 10^5  | 7.4 x 10^5   |
| total: 20.9 x 10^5 monocytes | 19.2 x 10^5 monocytes | 12.8 x 10^5 monocytes |

**Tissues**

- Peritoneal cavity: *Present at 48 h* 6.3 x 10^5 macrophages
- Other tissues

![Diagram: Acute Inflammation in Peritoneal Cavity]

| Production | Influx 0–48 h | Efflux 0–48 h |
|------------|--------------|--------------|
| 0–48 h     | 27.2 x 10^5  | 15.3 x 10^5  |
| total: 34.3 x 10^5 monocytes | 30.5 x 10^5 monocytes | 16.5 x 10^5 monocytes |

**Tissues**

- Peritoneal cavity: *Present at 48 h* 6.3 x 10^5 macrophages
- Other tissues

**Fig. 6.** Comparison of the total production of labeled monocytes in the bone marrow, kinetics in the peripheral blood compartment, and migration into the peritoneal cavity, during the normal steady state and an acute inflammation in the peritoneal cavity.
an acute inflammation, the total production of monocytes, the kinetics of these cells in the peripheral blood compartment, and their migration into the peritoneal cavity were compared with the normal steady state (Fig. 6). The calculation was done for the first 48-h period, because after that there is almost no further production of labeled monocytes. During this period of the inflammatory response the total production of labeled monocytes is 64% greater than in normal mice. The total influx of labeled monocytes from the bone marrow into the peripheral blood (i.e., the number of labeled monocytes present in the peripheral blood at 48 h plus the number of labeled monocytes that have already left the circulation) is 2 times higher in mice with an acute inflammation than in normal animals. The total efflux of labeled monocytes from the circulation (calculated with the exponential elimination rate constant) is also twice the normal value during the inflammatory response. In normal mice at least 7.6% of the monocytes that have left the circulation arrive in the peritoneal cavity, whereas the inflammatory exudate in the peritoneal cavity contains about 11 times as much labeled macrophages, which accounts for 41.2% of the monocytes that have left the circulation. The remainder of the monocytes have conceivably migrated from the circulation to other tissues.

It is remarkable that 73% of the increment of labeled monocytes leaving the circulation form the increase in number of labeled peritoneal macrophages in the inflammatory lesion. It should be noted, however, that the number of labeled macrophages calculated to be in the peritoneal cavity at 48 h represents an underestimation of the influx into that compartment, since macrophages will also have left the peritoneal cavity during the first 48 h. However, data on the rate of elimination of peritoneal macrophages are not yet available.

Comparative quantitative studies on monocyte production during an acute inflammation on other species are not available. In high-turnover granulomata evoked in rats with *Bordetella pertussis* vaccine or complete Freund's adjuvant in 4-wk and 12-wk-old lesions, respectively, the influx amounted to $2 \times 10^5$ cells per 24 h (21, 22), which agrees quite well with the present findings. In carrageenan granulomata, which have a low turnover, the influx was about 0.1 of this value, or less, in 4-wk-old lesions (22).

The approach followed in the present study provides a way to study the mode of action of anti-inflammatory drugs, such as glucocorticosteroids (2) and azathioprine, on the production and kinetics of mononuclear phagocytes during an inflammatory response. The results of such a study will be reported separately.³

**SUMMARY**

The present communication concerns a quantitative study on the production and kinetics of mononuclear phagocytes during an acute inflammatory response as compared with the steady-state condition.

During an acute inflammation induced by an intraperitoneal injection of
NBCS, the peritoneal macrophages increase 2.5 times and there is a concomitant threefold increase of the monocytes in the peripheral blood. This increase of the peritoneal macrophages could be caused by a local proliferation of these cells or by the recruitment of monocytes from the circulation.

The results of the in vitro and pulse-labeling studies demonstrate that the mitotic activity of the peritoneal macrophages is not increased during the inflammatory response, which indicates that the increase in the number of these cells is not due to local proliferation. Evidence is also presented that the small proportion (maximally 4%) of peritoneal macrophages that synthesize DNA are very recently arrived from the circulation. In agreement with this is the finding that a small number (less than 3%) of the peripheral blood monocytes are capable of synthesizing DNA.

Since proof was obtained that the macrophages in the inflammatory peritoneal exudate originate from peripheral blood monocytes and the number of these cells in the circulation was also augmented, an increased formation of monocytes in the bone marrow was expected. Because increased monocyte production could be brought about by an increased number of promonocytes and/or an acceleration of the mitotic activity of the promonocytes, the various parameters of the cell cycle of these cells were determined. In normal mice the DNA-synthesis time of the promonocytes was 11.8 h, the cell cycle time 16.2 h, and the G1 + G2 + M phases 4.4 h. During the first 12 h of the inflammatory response a significantly shorter DNA-synthesis time (7.6 h) and cell cycle time (10.8 h) was found. At 24 h, these values approximated those found in normal mice.

Next, both the total production and the rate of production of the monocytes were calculated and compared for both conditions. This computation showed that the total production of labeled monocytes during the first 48 h of an acute inflammation was 64% greater than in normal mice. The rate of production, calculated in two ways (i.e., from the data of the total production and also from the data of the cell cycle time together with the total number of promonocytes) complemented each other very well. During the first 12 h of the inflammatory response the production rate was increased 1.5 times and then leveled off, reaching almost the normal rate after 24 h. Furthermore, the excellent agreement between the results of the two methods of calculation for the normal steady state confirmed once more that the promonocyte is the direct precursor cell of the monocyte, giving rise to the two monocytes after each division.

The kinetics of the monocytes in the peripheral blood was also altered during the inflammatory response. During the first 48 h, twice the normal number of labeled monocytes went from the bone marrow to the peripheral blood and twice the normal number also left the circulation. Furthermore, at least 70% of this increased number of labeled monocytes leaving the circulation migrated
into the inflammatory exudate of the peritoneal cavity, leading to a roughly 11-fold increase of labeled peritoneal macrophages.

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