Granulocyte Transit from Bone Marrow to Blood

By Mary A. Maloney and Harvey M. Patt

The temporal course of granulocyte development has been rather well characterized. However, there is still a good deal of uncertainty about the behavior of the mature granulocytes in bone marrow. Interpretation of the chronology of appearance of mature cells after DNA labeling is open to question because of the unavoidable contamination of marrow samples with blood and possibly for other reasons as well. Thus, relatively little is known about the time spent by a mature granulocyte in the bone marrow and about the nature of its release to the circulating blood; for example, whether this follows first in-first out kinetics or is essentially a simple random process as in the case of granulocyte removal from blood. We have approached the problem indirectly by making a closely spaced study of the rise and fall of the progeny of presumably a single generation of tritiated thymidine labeled granulocytes in the blood of the dog. It was possible in this way to determine that granulocyte release from marrow was normally distributed with a mean time from labeling as a myelocyte to appearance in blood of 102 ± 13.8 hours.

Methods

The study was carried out in two young adult dogs, each of which received tritiated thymidine intravenously (0.3 mc./kg.; 360 mc./mM). Peripheral blood was sampled at hourly intervals after thymidine injection, in one dog from 48 to 103 hours and in the other from 89 to 143 hours. Total leukocyte and differential counts were made and autoradiograms of blood smears were prepared using AR 10 stripping film. The smears were stored in the dark at 4 C. for six weeks, then developed and stained with Ciemsa as described previously. A minimum of 2000 granulocytes was examined in each dog at each sampling time, and labeled cells were classified by grain count. A cell with four grains over the nucleus was taken to represent a definitely labeled cell (p < .01); background was below 0.4 grains per nuclear area. The hourly samples covered a broad distribution of times after labeling, that is from 48 to 143 hours; there was an overlap of 14 hours in the two dogs (from 89 to 103 hours), which facilitated comparison of the response. It was anticipated from earlier studies that labeled cells would not appear in the blood before 48 hours.

Results

The chronology of granulocyte appearance in blood is shown in Fig. 1. The curves were determined by calculation of the moving average of the per cent labeled cells for 10 adjacent points, i.e. by averaging the counts for hours 48

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Fig. 1.—Time course of labeled granulocytes (neutrophils) in the dog after a single injection of tritiated thymidine. (Based on studies in 2 dogs with overlap of hourly samples from 89 to 103 hours.)

Through 57, 49 through 58, etc. Since the counts were taken at hourly intervals, the moving averages in Fig. 1 refer to intervals of ±5 hours. It will be noted that labeled cells begin to appear at about 65 hours after H3 thymidine injection and that the rate of increase for cells with 4 grains or more (4+) is approximately linear at 1 per cent per hour from about 75 to 105 hours. The pattern of increase is qualitatively similar for the various grain classes (4+, 4−29, 4−14, 4−7); the rate of increase becomes progressively less from 4+ to 4−7 grains as a reflection of the decreasing number of labeled cells that are involved. The maximum values occur between 108 and 112 hours for all grain classes. It is of interest, and perhaps significant, that the peak seems to occur progressively later as the more heavily labeled cells are excluded from the population. Although the analysis was not of a sufficient duration to embrace the entire descending limb of the blood curve, there are obvious differences among the several populations of labeled cells with a clear indication of a pro-
found decrease when all of the labeled cells are considered and a slight decrease when only the lightly labeled cells are considered.

The data depicted in Fig. 1 reflect the balance between the entry and removal of labeled as well as of unlabeled cells. Although inferences can be made from comparison of the several blood curves, for example in regard to the appearance of successive generations of labeled cells and to the disappearance of the more heavily labeled cells, a more meaningful analysis would be possible if the blood curves could be corrected for concomitant cell loss. Several lines of evidence have suggested that granulocytes are removed randomly from the blood with a half time of approximately six hours.\textsuperscript{4,7} We have applied this rate of exponential loss to the blood curve for the most heavily labeled cells, those with 30 grains or more. Such cells would have been derived from myelocytes with 60+ grains because of the dilution of label at the first mitosis. From our knowledge of the initial myelocyte grain count distribution, blood granulocytes with 30+ grains can be taken to represent the progeny of a single myelocyte generation. Less than 0.4 per cent of initially labeled myelocytes have 60 grains or more and only rarely is a cell with over 80 grains encountered with the dosage, specific activity, and exposure conditions used.

A histogram depicting granulocyte release derived by correction of the blood curve for cell loss with a T_{\frac{1}{2}} of 6 hours is presented in Fig. 2. Five hour intervals were used for the analysis, the hourly counts being averaged over each interval and corrected for cell loss as follows:

\[ N(\Delta t) = N_{i+1} - N_{i} \exp(-k\Delta t) \]

where \( N \) = relative number of labeled cells

\( t_i, t_j = \) successive time intervals

\( \Delta t = t_j - t_i \)

\( k = \left( \frac{\ln 2}{T_{\frac{1}{2}}} \right) \)

It will be noted that this formula does not correct for loss in the emerging population during the five hour interval. While the calculation of the relative number of cells released per unit time is therefore an underestimate, the temporal distribution and mean time for the release of cells would remain essentially unchanged. The cumulative frequency distribution of release times along with the observed blood curve is shown in Fig. 3. The results are indicative of a normal distribution of release times from marrow to blood with mean and median times of 102 hours. When a shorter half time was used, e.g. 3 hours, the cumulative frequency distribution continued to rise; and when a longer half time was used, e.g. 12 hours, it began to decline. It is noteworthy that the time of attainment of the plateau in the cumulative frequency distribution corresponds to the onset of the final rapid decline of the 30+ granulocytes in blood which appears to be exponential with a T_{\frac{1}{2}} of 5 to 6 hours.

It is apparent from Figs. 2 and 3 that the release of a single generation of cells from marrow to blood covers a rather broad time span. The mean time from labeling of the DNA during the last myelocyte cell cycle to release of the mature granulocyte is 102 hours with a standard deviation of 13.8 hours. Since
the time from the mid-point of the DNA synthesis period to the appearance of label in the metamyelocyte is about 6 hours, the transit of the maturing non-proliferating granulocyte in bone marrow, i.e. from metamyelocyte to release of the segmented cell, is 96 hours. The mean metamyelocyte time is known to be about 20 hours, and the band time 26 hours which leaves 50 hours from the mean sojourn of the mature cell in marrow. The mechanisms governing the release of granulocytes from marrow to blood are not well understood. The mature cells must migrate and move into blood vessels where they may marginate or otherwise remain sequestered for a time. Hence, it is possible that most of the variance in the post-mitotic transit of the developing granulocytes may be related to the sojourn of the mature cell. In contrast to the exponential removal of granulocytes from blood, their release from bone marrow is clearly not a simple random process.

The finding that the transit time for the mature granulocytes is about twice that of the band cells is not consonant with the ratio of the two cell types in smears of bone marrow aspirates. In our experience, the ratio of segmented cells to band cells is usually 1 or less. We have no definite explanation of the apparent discrepancy other than to suggest that the proportion of segmented cells in the marrow aspirate is underdetermined perhaps, at least in part, for reasons analogous to the situation in peripheral blood. It is known in the latter case that there are two to three times as many granulocytes in blood as are revealed by the total granulocyte count. The normally unseen blood granulocytes are thought to represent marginated cells that are in equilibrium with cells in obvious circulation. That a large number of marrow granulocytes may be marginated or sequestered and in consequence not seen by conven-
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Fig. 3.—Cumulative frequency distribution of granulocyte release from bone marrow to blood for a single generation of labeled cells. (The time course of the corresponding labeled granulocytes in blood is shown in bottom curve.)

Fig. 3

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According to the studies by Raab et al.5 there are about $1.0 \times 10^9$ circulating and marginated granulocytes per kg. in the blood of dogs, and the mean transit time is 8 hours ($T\frac{1}{2} = 5.6$ hours). It follows therefore that there should be about $12 \times 10^9$ cells per kg. in the maturing non-dividing pool with a mean transit time of 96 hours. This is a minimum number since there is the possibility of other pathways for cell removal, e.g. by cell death. From the ratio of the number of proliferating to non-proliferating granulocytes, we can determine that there are approximately $16.8 \times 10^9$ granulocytes per kg. in the bone marrow of dogs, which is in excellent agreement with the estimate by Thomas.11

It will be noted in Fig. 1 that there is a slight separation between the curve for all labeled cells, i.e. 4+, and that for cells with 4 to 29 grains, which disappears after 125 hours. This time corresponds to the onset of the plateau in the cumulative frequency distribution of release times for cells with 30+ grains (Fig. 3). Cells with 30 grains or more represent only a small fraction of the total, and this is a reflection of the small number of initially labeled myelocytes with 60 grains or more. The progeny of a second division of such initially heavily labeled cells is included in the curve for granulocytes with 4 to 29 grains. However, because of the small numbers involved, blood granulocytes with 15 to 29 grains may also be taken to represent essentially the
progeny of a single generation. When the blood curve for this grain class is corrected for cell loss, the cumulative frequency distribution is similar to that for cells with 30+ grains. On the other hand, the more lightly labeled cells clearly represent multiple generations as indicated (a) by the progressive broadening of the top of the blood curves as the more heavily labeled cells are excluded from the analysis, and (b) by the eventual narrowing of the difference between the blood levels of granulocytes in the 4-7, 4-14, and 4-29 grain classes. Because of the large variance in the release of granulocytes to blood, it is difficult to demonstrate a clear separation of successive generations; the myelocyte generation time is of the order of 10–12 hours² and the release of the progeny of a single generation occurs over a period of some 70 hours. The recent work by Lark,¹² which suggests that chromosome segregation may not be entirely random, is also pertinent in this regard. If his findings should apply to proliferating granulocytes, grain count halving would occur for only two divisions after labeling with tritiated thymidine.

SUMMARY

The release of granulocytes from marrow to blood was studied in the dog by hourly sampling of the peripheral blood from 48 to 143 hours after injection of tritiated thymidine. Labeled granulocytes were classified by grain count and cells with 30 or more grains were considered to represent the progeny of a single generation. The blood curve for the most heavily labeled cells was corrected for exponential cell loss with a T½ of 6 hours. It was possible in this way to determine that granulocyte release from bone marrow was normally distributed with a mean time from labeling as a myelocyte to appearance in blood of 102 ± 13.8 hours. The mean transit time of segmented granulocytes in marrow is about 50 hours, which is indicative of a much larger pool of mature cells than formerly thought.

SUMMARIO IN INTERLINGUA

Le liberation de granulocytos ab le medulla ad in le sanguine esseva studiate in le porco per le obtention de specimens a intervallos de un hora ab 48 ad 143 horas post le injection de thymidina a tritium. Le marcate granulocytos esseva classificate secundo le numeration del granulos, e cellulas con 30 granulos o plus esseva reguardate como descendentes de un sol generation. Le curva de sanguine pro le plus fortemente marcate cellulas esseva corrigite pro le perdita exponential de cellulas con un T½ de 6 horas. In iste maniera il esseva possibile determinar que le liberation de granulocytos ab le medulla ossee esseva distribuite normalmente con un intervallo medie de 102 ± 13.8 horas ab le marcation como myelocyto usque ad le apparition del cellula in le sanguine. Le tempore medie de transito de segmentate granulocytos in le medulla es aproximativemente 50 horas. Isto suggestiona le existentia de un multo plus grande pool de cellulas matur quo lo que esseva supponite in le passato.

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