Lymphocyte Production and Life-span in the Bone Marrow of the Guinea Pig

By Cornelius Rosse

Guinea pigs were given 14 daily injections of 3H-thymidine to label a proportion of cells with a slow rate of turnover in addition to rapidly proliferating cells. In the bone marrow the only unlabeled cells were some reticular, endothelial, and plasma cells, damaged cells, and 14.1% of small lymphocytes. Six weeks after discontinuation of 3H-thymidine 7% of the marrow lymphocytes remained labeled. In guinea pigs injected every 4 hr with 3H-thymidine for 4 days to label all cells entering DNA synthesis, 14.4% of small lymphocytes remained unlabeled along with some reticular, endothelial, phagocytic, monocytoid, damaged, and plasma cells. The pattern of appearance of labeled lymphocytes was consistent with the kinetics of transitional cells that function as their precursors. Thus, in the bone marrow of the guinea pig the majority of lymphocytes have a short life-span and a rapid turnover, whereas about 14% turn over more slowly and 7% have a life-span exceeding 4 wk. In this respect the kinetics of marrow lymphocyte production differs from that of the rat.

The conclusive evidence for the heterogeneity of lymphocytes in terms of life-span was obtained from studies of the kinetics of lymphocyte production in the rat. Similar studies have not yet been adequately performed in other species.

In the present work two types of experiments were performed to determine the life-span and the rate of production of small lymphocytes in the bone marrow of guinea pigs.

In the first group of experiments the life-span of lymphocytes was assessed. 3H-thymidine was administered over a prolonged period in order to label not only rapidly proliferating cells but also a proportion of those cells that have a slow rate of turnover. One daily injection was given to guinea pigs for 14 days. After discontinuation of 3H-thymidine, the concentration of the radioactive marker is progressively reduced in proliferating cells to a level that can no longer be detected by radioautography. At the time when isotope reutilization can be excluded, the cells found to contain label are cells that were formed during the period of availability of the label and survived to the time of sampling. In these experiments, 6 wk was the longest period of time tested.

In the second group of experiments the rate of lymphocyte production was measured. 3H-thymidine was injected repeatedly at 4-hr intervals into guinea pigs.

From the Department of Biological Structure, University of Washington, School of Medicine, Seattle, Wash.

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Cornelius Rosse, M.B., Ch.B.: Associate Professor, Department of Biological Structure, University of Washington School of Medicine, Seattle, Wash.
pigs for periods up to 4 days. Since the DNA synthetic period (S phase) in mammalian cells exceeds 4 hr, all cells that enter DNA synthesis, and their progeny, will be labeled on radioautographs. Only those cells will remain unlabeled that do not enter DNA synthesis for periods longer than 4 days and were formed prior to the commencement of $^3$H-thymidine injections. In a steady state the appearance of labeled cells should be a measure of their rate of formation.

**Materials and Methods**

Male guinea pigs of the Hartley strain with normal white cell count and hematocrit were used. In the first group of experiments injections of $^3$H-thymidine were commenced after weaning when the animals weighed around 300 g. In the second group of experiments the animals weighed approximately 400 g. 

$^3$H-thymidine (specific activity 6.7 Ci/mM) was injected intraperitoneally in doses of 1 μCi/g body weight after shaving and treating the site of injection with an antiseptic solution. The animals were killed 30 min after the last injection except for those killed at 1, 2, and 3 hr.

The white cell count was repeated at the termination of the experiment and the animals were killed by exsanguination. The absolute number of cells per cubic millimeter of bone marrow was determined using a quantitative hemocytometric technique. For radioautography, smears were prepared of the buffy coat of blood and of bone marrow suspended in serum. After coating with Kodak NTB2 nuclear emulsion, the radioautographs were exposed in batches for 1, 2, and 4 wk and were subsequently stained with MacNeal’s tetrachrome stain. One thousand cells were classified on blood and bone marrow smears. It was shown previously that such counts give a valid representation of cell distribution. In each animal, 1000 pachychromatic small lymphocytes were identified and the percentage of labeling was determined. The curve of silver grain distribution over lymphocytes and the grain count over erythrocytes indicated that labeling of cells with three or more grains was not due to background.

**Results**

The blood picture and myelograms of all animals fell within the range of normal.

*Life-span of Marrow Lymphocytes*

At the conclusion of 14 daily injections of $^3$H-thymidine 85.9% of small lymphocytes were labeled (Fig. 1A). Beside small lymphocytes, the only
other cell types in which 100% labeling was not achieved were reticular and endothelial cells, mature plasma cells, and occasional damaged cells.

Two weeks after discontinuation of \(^3\)H-thymidine, 17.6% of small lymphocytes remained labeled (Fig. 1E) while a high percentage of other cells showed uniform low grain density indicating considerable label reutilization. At 4 and 6 wk after discontinuation of \(^3\)H-thymidine, however, no label could be seen in erythroblasts or granulocytes. At 4 wk 6.9% and at 6 wk 7.2% of small lymphocytes remained labeled (Figs. 1F, 1G) and, therefore, had a life-span of 4–6 wk, or at least 4 wk, if the possibility of label reutilization is taken into account up to 2 wk following discontinuation of \(^3\)H-thymidine. Expressed in absolute numbers, they are 1000 times as numerous as the number of labeled lymphocytes of blood contained within the marrow of the same animals as calculated from the bone marrow blood volume.

In addition to small lymphocytes, the following cell types were found to be labeled at 6 wk: reticular cells, damaged cells, plasma cells, some monocytoid cells, and transitional cells (percentages were not estimated).

**Rate of Lymphocyte Production**

In agreement with numerous previous observations, 30 min after the injection of \(^3\)H-thymidine, bone marrow small lymphocytes were not labeled, indicating that these cells do not synthesize DNA. From 1–4 hr, labeling remained at 1% but thereafter a continuous increase was observed: 3.9–4.3% at 12 hr, 19.5–23.6% at 24 hr, 47.4–48.3% at 48 hr, 70.5% at 72 hr, and 85.6% at 96 hr (Fig. 2). Although 100% of erythroblasts and granulocytes were labeled at 4 days, 14.4% of small lymphocytes remained unlabeled. The only other unlabeled cells were stromal elements (reticular and endothelial cells), macrophages, monocytoid cells, plasma cells, and a few damaged cells. These findings closely correspond to those observed at the completion of 14 daily injections of \(^3\)H-thymidine.

The proportion of unlabeled small lymphocytes was consistent on radioautographs exposed for 1, 2, or 4 wk, although identification of labeled cells was difficult or often impossible due to the high grain density on autographs exposed for 2 and 4 wk. The latter, however, convincingly demonstrated the presence of unlabeled cells.

The rate of appearance of labeled small lymphocytes was not uniform over the observation period (Table 1). Labeled lymphocytes appeared at the fastest rate between 12 and 24 hr. Thereafter, a progressive decline followed, which suggests that 100% labeling could not be anticipated for some time and its attainment could not be predicted by extrapolation of the curve in Fig. 2, thus confirming in the marrow the presence of a group of lymphocytes with a slow rate of turnover.

**DISCUSSION**

The findings reported in these studies are in overall agreement with previous reports showing rapid turnover of the majority of bone marrow lymphocytes, but differ from these reports in demonstrating the presence of long-lived lymphocytes within the marrow. The 14% of small lymphocytes that
remained unlabeled at the completion of \( ^{3}\text{H}\)-thymidine administration in both experiments represent a population distinct from lymphocytes with a rapid turnover. Of the marrow lymphocytes, 7% are derived from precursors that can be labeled with 14 daily injections of \( ^{3}\text{H}\)-thymidine and that survive for at least 4 wk. In these respects, guinea pig bone marrow appears to be different from that of the rat\(^{11}\) when studied by similar methods. Lymphocytes that survive for a prolonged period of time have been demonstrated in the marrow of rats born to mothers continuously labeled with \( ^{3}\text{H}\)-thymidine throughout gestation.\(^{15}\) The present experiments indicate that guinea pig bone

\[\begin{array}{c|c|c}
\text{From} & \text{Hours of TTH} & \text{To} & \text{Percentage Increase per Hour} \\
\hline
0 & 12 & 0.34 \\
12 & 24 & 1.45 \\
24 & 36 & 1.25 \\
36 & 48 & 1.18 \\
48 & 60 & 0.90 \\
60 & 72 & 0.97 \\
72 & 96 & 0.63 \\
\end{array}\]
marrow contains lymphocytes with a slow rate of turnover in a greater proportion, many of which are formed postnatally in the growing animal. The importance of these differences in terms of function, however, remains to be investigated.

Since small lymphocytes themselves do not incorporate $^3$H-thymidine, the rate of their labeling is determined by the kinetics of their precursors, which are confined to the marrow in the guinea pig and also in the rat. Transitional cells have been identified as the precursors to bone marrow small lymphocytes. Their in vivo kinetic behavior has been determined previously and is consistent with the pattern of appearance of labeled small lymphocytes. The upswing in the percentage of labeled small lymphocytes commenced in accord with the "G2 + mitosis" interval for transitional cells. The rate of increase in labeled small lymphocytes was the greatest just before and after a 100% labeling was attained in basophilic transitional cells (24 hr). The increase in the per cent labeling per hour began to decline in the population of small lymphocytes after this point (Table 1), as would be expected because of the presence of long-lived cells in the marrow lymphocyte pool. Thus, the studies reported confirm the evidence that the great majority of small lymphocytes with rapid turnover are derived from transitional cells within the bone marrow. In addition, they demonstrate the presence of long-lived lymphocytes within the marrow, the origin of which is not yet known.

REFERENCES

LYMPHOCYTE PRODUCTION

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