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Tritiated-Thymidine Incorporating Cells in the Peripheral Blood of Normal and Splenectomized Rats.* (32160)

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An intimate relationship exists between the spleen and the circulating blood cells of mammals. For example, following splenectomy, changes have been reported in the structure and numbers of circulating erythrocytes and there is a sustained increase in platelet count and peripheral leukocyte numbers(1,2). In rats, splenectomy induces a granulocytic and mononuclear leukocytosis(3,4), and elevation in platelet counts(5,6), but peripheral erythrocyte numbers remain unchanged(4,7).

In the present investigation, the proliferative potential of peripheral mononuclear leukocytes from normal and splenectomized rats was studied. It was found that tritiated-thymidine (H^3T)-incorporating cells were present in small numbers in the peripheral blood of normal rats and that splenectomy caused a significant increase in the numbers of these cells in the circulation.

Materials and methods. Seventy-five male rats (200-250 g) of a modified Long-Evans strain, free of *Bartonella muris,* were maintained on Purina chow and tap water *ad libitum* was used. These were divided into 3 groups. The first group (42 rats) was splenectomized as described by Farris and Griffith(8). The second group (21 rats) was sham-splenectomized by carefully exteriorizing and then returning the spleen to the peritoneal cavity and the third group (12 rats) comprised unoperated controls. On post-operative days 1-4, 7, 10 and 23, 6 splenectomized and 3 sham-operated rats were exsanguinated by aortic puncture and total white cell counts and blood smears were prepared from the blood of each rat. Tritiated thymidine (2 μc/ml whole blood; Schwarz BioResearch, Sp. Act. 15.0 c/mMole) was added to the heparinized blood (Heparin Sodium, Fisher, 100 units/ml whole blood), collected from each rat and the mixture incubated (with constant agitation) at 37°C for 1 hour. Each sample was then centrifuged 5 minutes at 1200 rpm. Most of the supernatant was removed without disturbing the buffy coat cells which were resuspended in residual supernatant with Pasteur pipettes to give a uniform cell suspension. Smears made from each suspension were air dried, fixed in methanol, processed autoradiographically and stained as described by Monette *et al*(9). Exposure time was 12 days at 5°C. Control blood samples were treated identically. Labeling incidences were determined by counting 5000 peripheral mononuclear cells for each animal. From this and the total white cell count, the absolute numbers of labeled cells per mm^3 of blood was calculated.

Results. No mortality or anemia developed in the splenectomized rats indicating the absence of *Bartonella muris* infection. The absolute numbers of H^3T-incorporating cells in the circulation of splenectomized, sham-splenectomized and normal intact rats are indicated in Fig. 1. It may be seen that small numbers of mononuclear cells in the peripheral blood of normal rats were capable of incorporating H^3T. One week after sham-splenectomy there was a 2-fold increase in

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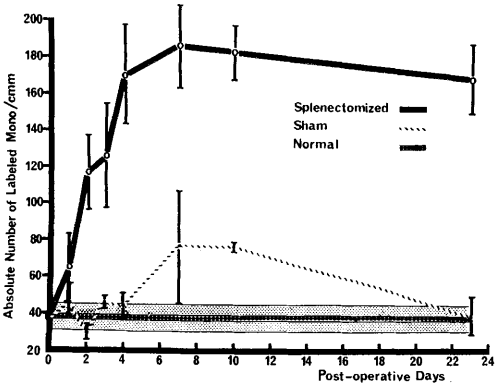

FIG. 1. Absolute number of labeled mononuclear cells per mm^3 of aortic blood from splenectomized, sham-splenectomized, and intact normal rats following one hour's incubation with tritiated thymidine. Each point in the splenectomized group represents the mean of 6 rats; in the sham-splenectomized group, 3 rats. The normal group shows the mean of 12 rats. Vertical bars represent ± 1 S.E.M.

the number of these cells in the blood but they returned to normal levels by post-operative day 23. Forty-eight hours after splenectomy the numbers of H^3T-incorporating cells in the circulation were markedly increased. The highest value was 4.5 times greater than normal on about day 7 and persisted for at least 23 days.

In heavily labeled cells, the exposed grains often obscured nuclear morphology making cytological identification impossible. However, the more lightly labeled cells that could be recognized cytologically included the following:

1. Histiocyte-like cells (greater than 20 μ in diameter) with eccentrically placed nuclei and abundant pale blue-staining, agranular cytoplasm (Fig. 2a).

2. Monocytic cells (15 or more μ in diameter) with large indented nuclei. Present in the moderately basophilic cytoplasm were clear regions adjacent to the nuclear indentations (Fig. 2b).

3. Large plasmocyte-like cells (about 20 μ in diameter) with large eccentrically placed nuclei and archoplasmic regions present in otherwise basophilic cytoplasm (Fig. 2c).

4. Large blast-like cells (over 20 μ in diameter) with large, round, centrally placed nuclei, well dispersed chromatin and homogeneous, deeply basophilic cytoplasm (Fig. 2d).

5. Large mononuclear cells (about 20 μ in diameter) with large, round nuclei and scanty cytoplasm. The chomatin material showed slight clumpings (Fig. 2e).

6. Lymphoblast-like cells (greater than 20 μ in diameter) with large eccentrically placed nuclei and intensely basophilic, homogeneous cytoplasm (Fig. 2f).

7. Medium sized lymphocyte-like cells (about 10-16 μ in diameter) with basophilic to extremely basophilic cytoplasm, (Fig. 2g) and small (8-10 μ diameter) lymphocytes (Fig. 2h).

The predominantly labeled cell types in the normal and sham-operated rats were the histiocyte-like and plasmocyte-like cells. In splenectomized rats labeled monocytic cells, blast-like cells and small lymphocytes were found most frequently as well as labeled granulocytic (Fig. 3a and b) and erythrocytic precursors. Labeled and unlabeled mitotic figures were observed in both normal and splenectomized rats, with higher incidence in the latter. This would indicate that the H^3T-incorporating cells were synthesizing DNA.

Discussion. It has been reported that a small percentage of normal peripheral blood cells in human(10) and in mouse(11) were able to incorporate H^3T. The present studies have demonstrated that H^3T-incorporating cells were also present in the peripheral blood of normal rats. They included small, medium, and large lymphocytes, monocytes, histiocyte-like plasmocytic-like and blast-like cells. Although the small lymphocyte is generally considered a post-mitotic cell(12,13), small numbers of them were capable of incorporating DNA precursor and after splenectomy their numbers were increased. The origin, fate, and significance of these peripheral DNA-synthesizing cells are unknown. They may represent part of the mobile stem cell pool suggested by Cronkite *et al*(14) and may be the cells responsible for giving protection to lethally irradiated mice(11).

The spleen is a major lymphoid organ and its removal may stimulate compensatory hypertrophy in other lymphoid tissues (15, 16). Thus, circulating cells of lymphoid origin may be induced after splenectomy to divide as a result of feedback stimuli for com-

pensatory hyperthrophy, or normally fixed, proliferative lymphoid tissue cells may be caused to migrate to other sites to regenerate splenic nodules, a phenomenon known to occur following splenectomy(17). On the other hand, splenectomy may remove some splenic humoral factor that inhibits leukocyte production and release(3,18,19). With the inhibitor removed, increased release of blood cells (including DNA-synthesizing mononuclear cells, granulocytic and erythroid precursors) into the peripheral blood could occur. It is also possible that the spleen may normally trap circulating immature and abnormal mononuclear cells which would constitute a splenic activity analogous to its erythrocyte "culling" and "pitting" function suggested by Crosby(20) and others(21,22). If this is so, splenectomy would allow immature cells capable of DNA-synthesis and mitosis to remain within the circulation. One or any combination of the above mentioned

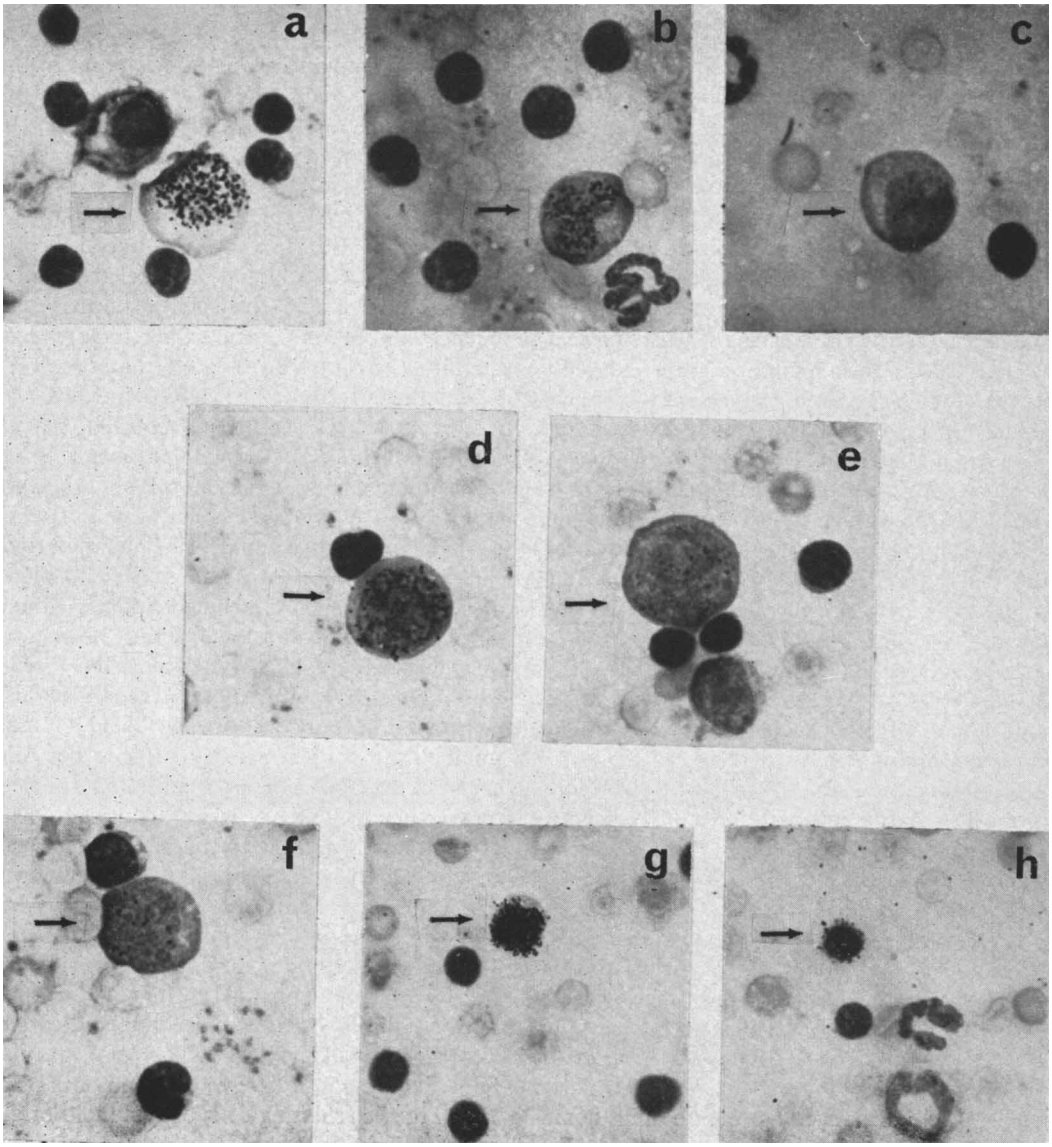

FIG. 2, a-h. H^3T-incorporating cells observed in the peripheral blood of rats (about 970×). (see text for detailed description).

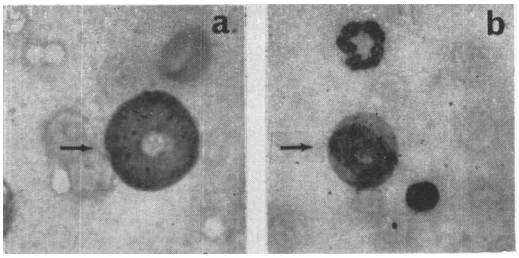

FIG. 3, a and b. H^3T-incorporating granulocytic precursors observed in the peripheral blood of splenectomized rats. (about 970×).

possibilities could account for the increase in H^3T-incorporating cells noted in the circulating blood of splenectomized rats.

The reason for the small, transient increase in H^3T-incorporating cells in the circulation of sham-operated rats observed in the present studies is obscure. It may represent a simple inflammatory condition in these rats caused by the operation. This is known to increase the numbers of DNA-synthesizing cells in the peritoneal cavity(23). On the other hand, handling and exteriorizing the spleens of these animals may have resulted in altered splenic function (*i.e.*, transient hyposplenism).

Summary. Aortic blood from normal, sham-splenectomized, and splenectomized rats was incubated with tritiated thymidine (2 μc/ml) for 1 hour at 37°C and the labeling incidences for the buffy coat leukocytes determined autoradiographically. It was found that a small percentage of mononuclear cells in the peripheral blood of normal rats were capable of incorporating tritiated-thymidine. Following splenectomy the numbers of these cells increased more than 4-fold for 23 days post-operation. The labeled cell types are described and possible causes for their increase in numbers after splenectomy are discussed.

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