PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 1268–1271 (1974)

Studies on Lymphocytes
XI. Differences in the Lymphocytopoietic Activity of Peripheral Lymphoid Organs[1,2] (37994)

G. L. GULLIANI, A. D. CHANANA, E. P. CRONKITE, AND D. D. JOEL

Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973

A number of studies of the mammalian lymphoid system have demonstrated that lymphocytes are produced at different rates in the bone marrow, thymus, lymph nodes, spleen, Peyer's patches, and possibly in other organized lymphoid structures throughout the body (1, 2). These lymphocytopoietic organs supply lymphocytes to the circulating blood lymphocyte pool (3–5) which is normally maintained at a relatively constant level. The regulation of the proliferative activity at different lymphocytopoietic sites is only partially understood. Studies in germ-free (6–8) and conventional (9–11) animals have shown that antigenic stimuli greatly influence the development, growth, and proliferative activity of peripheral lymphoid organs. These observations further suggest that the magnitude of proliferative activity in peripheral lymphoid organs may be determined largely by the degree of "normal" antigenic exposure. The present report supports this hypothesis.

Materials and Methods. Twenty field-bred, young adult goats (females and castrated males), weighing between 18 and 30 kg were studied. Sixteen of these animals comprised a control group for a separate experiment on the effects of extracorporeal irradiation of blood (ECIB) on the lymphoid system and received variable amounts of sham-ECIB. The technique used for sham-ECIB was similar to the one described for ECIB (12). Since the labeling indices of lymphocytes in the lymphoid organs of the sham-ECIB-treated animals were not significantly different from those in the corresponding lymphoid organs of nontreated animals, the values obtained from all 20 animals were pooled and the mean calculated for each of the organs tested.

In vivo flash labeling with tritiated thymidine (^{3}H-TdR, sp act 1.9 Ci/mmole, concn 1 mCi/ml, Schwarz Bioresearch Inc., Orangeburg, NY) was employed to assess the proliferative activity. Tritiated thymidine was injected intravenously at a dose of 0.2 μCi/g body weight. Twenty-two minutes after the ^{3}H-TdR injection, the goats were exsanguinated under general anesthesia (Fluothane, Ayerst Lab., New York). Samples of the spleen, prescapular lymph node, mesenteric lymph node, and Peyer's patches were harvested simultaneously 30 min after the ^{3}H-TdR injection.

Smears were prepared from cell suspensions of minced tissue, dipped in Kodak NTB_2 liquid emulsion (1:1 ratio of NTB_2 and sterile distilled water), exposed for 21 days at 4°C in light-tight boxes, and developed in Kodak D-19 developer. Developed slides were stained with Giemsa (Harleco, Philadelphia) 1:20 in phosphate citrate-buffered saline pH 5.75.

Autoradiograms were studied with an oil-

[1] Research supported by the U. S. Atomic Energy Commission.

[2] The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

immersion objective at 800 × magnification. Between 1000 and 1500 lymphocytes were scored per smear. Lymphocytes were classified into two categories, "small" and "non-small." Small lymphocytes were characterized by their relatively small size (as compared to other lymphocytes), dense nuclear chromatin, lack of visible nucleoli, and little or no visible cytoplasm. Occasionally a thin rim of the cytoplasm could be found around the nucleus of small lymphocytes. Lymphocytes with characteristics other than those described for "small" lymphocytes were included in the "non-small" category. The number of labeled and nonlabeled cells was recorded separately for each of the two categories of lymphocytes. Cells with 3 or more grains over the nucleus were considered labeled. The threshold of 3 grains was based upon the analysis of grain counts over a total of 10,000 lymphocytes from 10 smears prepared from blood samples harvested prior to the injection of ^{3}H-TdR but processed along with smears of samples harvested after ^{3}H-TdR injection. Grain counts in the background preparations did not exceed 2 grains per lymphocyte irrespective of cell category. Since small lymphocytes rarely if ever were labeled, the percentage labeling index was calculated for "total" and "non-small" populations of lymphocytes in each of the organs tested.

Results. Table I depicts the distribution of "small" and "non-small" lymphocytes in various lymphoid organs. The mean values for the percentage of "non-small" lymphocytes in these organs ranged between 21 and 33, with the largest fraction observed in the Peyer's patches and the smallest in the prescapular lymph node.

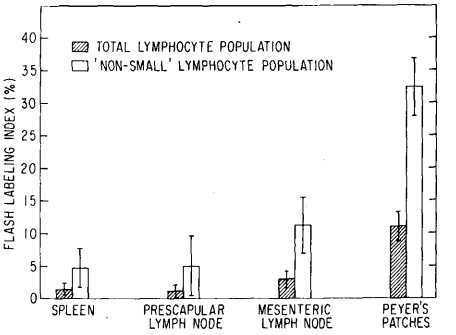

FIG. 1. Mean flash-labeling index with the standard deviation value obtained from 18–20 animals for total and "non-small" lymphocyte populations of various lymphoid organs.

The mean labeling indices and standard deviations obtained for each of the lymphoid organs investigated are shown in Fig. 1. Individual values are indicated in Table II. Proliferative activity indicated by the flash-labeling index was similar in the spleen and prescapular lymph node. The highest mean flash-labeling index among all of the organs investigated was found in the Peyer's patches. Statistical analysis (Student's *t* test) of the data revealed significant differences ($P < 0.001$) in the mean labeling indices between (i) the mesenteric lymph node and the spleen or prescapular lymph node and (ii) Peyer's patches and the mesenteric lymph node, prescapular lymph node, or spleen.

Discussion. Mitchell and associates (10) studied lymphocytopoiesis in various lymphoreticular organs in adult rats and observed that after *in vivo* flash labeling with ^{3}H-TdR the fraction of cells labeled was greater in the mesenteric lymph node and

TABLE I. Percentage of "Small" and "Non-small" Lymphocytes in Various Lymphoid Organs.

	Spleen (%)	Prescapular lymph node (%)	Mesenteric lymph node (%)	Peyer's patches (%)
"Small" (mean ± SD)	74.89 ±9.06	79.13 ±6.54	73.04 ±6.22	67.46 ±6.18
"Non-small" (mean ± SD)	25.11 ±9.06	20.87 ±6.54	26.94 ±6.22	32.54 ±6.18

TABLE II. Flash-Labeling Indices (%) of Total (A) and "Non-small" (B) Lymphocyte Populations in Peripheral Lymphoid Organs.

A				B			
Spleen	Prescap. lymph node	Mesenter. lymph node	Peyer's patches	Spleen	Prescap. lymph node	Mesenter. lymph node	Peyer's patches
0.8	1.5	2.9	11.3	4.2	—[a]	16.8	33.8
0.8	0.8	2.7	11.8	5.0	2.8	7.7	35.4
0.9	1.9	4.7	13.7	3.4	7.0	11.4	35.1
2.0	3.2	1.5	—[a]	8.0	4.9	6.5	—[a]
0.7	0.3	1.8	11.7	2.4	0.9	7.5	29.9
—[a]	0.8	2.1	10.4	—[a]	2.4	8.2	28.2
0.3	0.5	4.2	15.0	0.9	1.6	11.7	41.1
1.3	1.1	3.9	8.4	3.7	4.6	12.2	31.9
1.7	0.9	2.6	13.9	4.2	5.5	8.5	36.1
2.3	4.4	3.0	14.6	3.8	21.7	10.6	36.8
0.3	0.7	3.5	8.1	1.0	3.2	13.5	23.5
3.2	0.3	2.5	9.2	8.7	1.3	10.9	28.5
2.0	1.1	6.6	12.2	7.0	6.4	19.9	29.4
1.1	0.7	2.1	10.3	2.7	3.6	8.5	35.3
3.9	0.3	1.3	9.9	12.2	1.9	4.8	29.3
0.4	0.9	2.8	7.6	1.1	5.3	10.1	32.7
0.8	0.7	3.4	10.8	4.0	3.2	13.2	39.8
1.5	1.7	2.1	11.4	7.4	9.8	8.9	30.1
1.9	0.7	5.2	13.1	5.9	5.4	22.0	29.5
—[a]	0.3	1.9	7.4	—[a]	3.9	11.8	30.3
Mean 1.44	1.14	3.04	11.09	4.76	5.02	11.24	32.46
±SD ±0.96	±1.01	±1.31	±2.26	±2.99	±4.60	±4.32	±4.38

[a] Not investigated.

the spleen than in the popliteal lymph node. They did not find a significant difference between the labeling indices of cells in the mesenteric lymph node and spleen. These investigators did not limit their observation to lymphocytes only but determined the labeling index of the total nucleated cell population. The rat spleen, however, contains proliferating myeloid and erythroid precursors in addition to proliferating lymphocytes. Examination of autoradiographs of histologic sections of various lymphoid tissues harvested 1 hr after flash labeling with ^{3}H-TdR in guinea pigs (18) also revealed a greater number of labeled cells in Peyer's patches and mesenteric lymph node compared to those in the popliteal and axillary lymph nodes.

Koros *et al.* (13) examined the splenic content of plaque-forming cells (PFC) in mice after iv injection of varying doses of sheep red blood cells (SRBC). Compared to smaller doses of SRBC ($\sim 4 \times 10^4$), the injection of large doses of SRBC ($\geq 4 \times 10^7$) resulted in increased number of PFC in the spleen, suggesting a relationship between the degree of antigenic stimulation and the magnitude of proliferative activity of lymphocytes in the spleen.

In the present study, Peyer's patches, which by nature of their anatomical location apparently undergo a high degree of antigenic stimulation under normal circumstances, had the highest labeling indices. Evidence that Peyer's patches may be stimulated to proliferate by the transepithelial flow of intestinal antigens is provided for by the studies of Hess *et al.* (14) and Bockman and Cooper (15). These authors were able to demonstrate the uptake of particles (India ink and ferritin) from the intestinal lumen by the gut epithelium overlying the organized lymphoid structures.

The mean flash-labeling index of mesen-

teric lymph nodes, though lower than that of the Peyer's patches, was greater than those of the spleen and prescapular lymph nodes. The anatomical location of mesenteric lymph nodes like that of the Peyer's patches places them closer to continuous antigenic stimulation than other lymph nodes and spleen. Retention of charcoal particles in the mesenteric lymph nodes and Peyer's patches of coal miners or individuals treated with charcoal as a therapeutic measure, has been described (16).

The spleen and prescapular lymph nodes, presumably not normally subject to as great a degree of antigenic stimulation as the Peyer's patches and the mesenteric lymph nodes, had the lowest labeling indices among the organs tested.

Radiophosphorus (^{32}P) uptake studies performed by Andreasen and Ottesen (17) to determine the relative DNA turnover of various lymphoid organs in young mature albino rats also revealed that DNA turnover was faster in the Peyer's patches than in the lymph nodes and spleen. In these studies it was also observed that intestinal lymph nodes as compared to lymph nodes of skin had a greater DNA turnover rate. These observations are in accord with our results on the relative proliferative activity of peripheral organs tested by ^{3}H-TdR uptake.

In conclusion, these observations support the hypothesis that under normal circumstances, the magnitude of the proliferative activity of peripheral lymphoid organs (including Peyer's patches) may be determined by the degree of antigenic stimulation.

Summary. A comparative study of the proliferative activity of lymphocytes in the Peyer's patches, mesenteric lymph node, prescapular lymph node and spleen was performed. The proliferative activity was assessed by determining the flash labeling index after a single iv injection of ^{3}H-TdR. The mean flash-labeling index of the Peyer's patches was approximately 3× greater than that of the mesenteric lymph node which in turn had a mean flash-labeling index approximately twice that of the prescapular lymph node and the spleen. These results suggest that under normal circumstances the magnitude of proliferative activity of peripheral lymphoid organ is related to the degree of antigenic stimulation.

We thank Mr. C. Sipe, Mr. T. Weldon, Mr. F. Lawson, Mr. G. Borner, Mrs. K. Conkling, and Mrs. A. Alberico for their invaluable assistance.

1. Yoffey, J. M., "Quantitative Cellular Hematology," pp. 9–14. Thomas, Springfield, IL (1960).

2. Everett, N. B., and Tyler (Caffrey), R. W., Int. Rev. Cytol. **22**, 205 (1967).

3. Brahim, F., and Osmond, D. G., Anat. Rec. **168**, 138 (1970).

4. Williams, R. M., Chanana, A. D., Cronkite, E. P., and Waksman, B. H., J. Immunol. **106**, 1143 (1971).

5. Osmond, D. C., Anat. Rec. **165**, 109 (1969) (Review).

6. Gordon, H. A., Ann. N. Y. Acad. Sci. **78**, 208 (1959).

7. Miyakawa, M., Ann. N. Y. Acad. Sci. **78**, 221 (1959).

8. Thorbecke, G. J., Ann. N. Y. Acad. Sci. **78**, 237 (1959).

9. Hall, J. G., and Morris, B., Quart. J. Exp. Physiol. **48**, 235 (1963).

10. Mitchell, J., McDonald, W., and Nossal, G. J. V., Austral. J. Exp. Biol. Med. Sci. **41**, 411 (1963).

11. Rabin, B. S., and Rose, N. R., Immunology **19**, 239 (1970).

12. Sipe, C. R., Chanana, A. D., Cronkite, E. P., Joel, D. D., and Schnappauf, Hp., Radiat. Res. **25**, 684 (1965).

13. Koros, A. M. C., Henry, C., Nordin, A. A., and Jerne, N. K., Fed. Proc. **24**, 252, Abstr. 684 (1965).

14. Hess, M. W., Cottier, H., Sordat, B., Joel, D. D., and Chanana, A. D., Presented at the Conference on "A Re-examination of 'Non-Specific' Factors Influencing Host Resistance," Bern-Gurten (1972) (BNL 17004).

15. Bockman, D. E., and Cooper, M. D., Amer. J. Anat. **136**, 445 (1973).

16. Lubarsch, O., and Borschardt, H., "Handbuch der speziellen Pathologischen Anatomie und Histologie" (F. Henke und O. Lubarsch, eds.) Vol. IV/3, pp. 83–86. Springer, Berlin (1929).

17. Andreasen, E., and Ottesen, J., Acta Physiol. Scand. **10**, 258 (1945).

18. Olson, I. A., and Yoffey, J. M., *in* "The Lymphocyte in Immunology and Hemopoiesis" (J. M. Yoffey, ed.), pp. 358–361. Arnold, London (1967).

Received Oct. 1, 1973. P.S.E.B.M., 1974, Vol. 145.