# COMPARATIVE ANALYSIS OF INTENSITY OF THYMIDINE-$H^3$ LABELING OF SPLENIC LYMPHOCYTES IN INTACT AND IMMUNIZED MICE

G. F. Maksimova

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The content of thymidine-$H^3$ in splenic lymphocytes of immunized and intact mice was studied 1 and 10 h after radioactive labeling. The animals were immunized by intraperitoneal injection of $5 \times 10^8$ sheep's red cells. The number of silver grains calculated per 1000 cells in the immune spleen 10 h after injection of labeled nucleoside was twice as many as initially. This index was unchanged in the intact mice. It is postulated that injection of the antigen stimulates migration of lymphocytes synthesizing DNA into the spleen.

It was shown comparatively recently that towards the end of the inductive phase of the primary immunological response to sheep's red cells the number of cells in the mouse spleen is increased by one-third over its initial value [3, 4]. The suggestion has been made that this increase in the cell mass of the organ is completely due to proliferation of spleen cells through activation of division by the antigen. However, these investigations did not take into account such an important factor as the migration of lymphocytes into and from the spleen.

In the investigation described below the possibility of migration of cells into the spleen during the immune response in mice was investigated.

## EXPERIMENTAL METHOD

CBA mice were immunized by intraperitoneal injection of $5 \times 10^8$ sheep's red cells. The animals received a single intraperitoneal injection of thymidine-$H^3$ (specific activity 290 mCi/mmole), in a dose of 1 $\mu$Ci/g body weight, 38 h after antigenic stimulation. Intact mice (control) received the tritiated precusor only. The animals were sacrificed 1 or 10 h after radioactive labeling. Films made from a suspension of spleen cells and fixed in absolute methanol were coated with type M liquid nuclear emulsion and exposed in darkness at 4°C for 8 weeks. The films were then developed, fixed, and stained by the Romanowsky–Giemsa method. By analysis of the autoradiographs the total labeling index (LI) of the lymphoid population and the distribution of its labeled cells by classes (blast cells, large, medium and small lymphocytes) were determined, and their frequency expressed in promille of the total number of lymphocytes (labeled and unlabeled). To determine the labeling intensity the number of silver grains above interphase nuclei of 100 cells of each class or more was counted. The geometric mean was taken as the index of the mean number of grains (MNG) per cell. By multiplying this index by the frequency of labeled cells of the corresponding morphological class, the mean number of grains (MNG/1000) characterizing the contribution of the cells of this type to the total number of grains, calculated per 1000 cells of the total lymphoid population of the spleen (TNG/1000) was obtained. By dividing this last figure of the total labeling index, expressed in promille, the mean number of grains per cell, reflecting the intensity labeling of the lymphoid population as a whole (TMNG) was obtained.

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TABLE 1. Content of Thymidine-$H^3$ in Splenic Lymphocytes of Mice Immunized with Sheep's Red Cells.

| Time of sacrifice (in h) | Labeling index of cells | Blasts | Large lymphocytes | Medium lymphocytes | Small lymphocytes | All labeled cells |
|---|---|---|---|---|---|---|
| 1 | Frequency (in %) | 15,3 (8,3) | 15,7 (10,7) | 33,3 (16,3) | 7,3 (4,3) | 71,6 (39,6) |
| | MNG | 47,8 (55,0) | 39,8 (38,6) | 30,2 (37,8) | 26,6 (25,8) | 35,7 (40,4) |
| | MNG/1000 | 732,0 (456,6) | 624,8 (413,0) | 1005,6 (616,2) | 194,2 (111,0) | 2556,6 (1594,8) |
| 10 | Frequency (in %) | 18,2 (13,0) | 20,0 (14,3) | 70,2 (41,3) | 72,8 (21,7) | 181,2 (90,3) |
| | MNG | 34,6 (21,1) | 32,4 (20,0) | 32,6 (19,0) | 31,4 (17,4) | 32,4 (19,2) |
| | MNG/1000 | 629,8 (274,3) | 648,0 (286,0) | 2288,4 (784,8) | 2286,0 (377,2) | 5854,5 (1721,4) |

## EXPERIMENTAL RESULTS

The results are given in Table 1. In the spleens of the unimmunized mice, 1 h after injection of thymidine-$H^3$ labeled cells accounted for 4%, and 10 h after injection, for 9% of the total number of lymphocytes. Under the influence of antigen an increase in the incorporation of labeled nucleoside into the lymphocytes was found. For instance, 1 h after radioactive labeling the LI for the immunized animals was 7.2%, rising to 18.1% 10 h after labeling. Consequently, during the 9-h period of observation, in both experimental and control animals, LI approximately doubled in value. This increase in the number of labeled nuclei could be the result of division of cells which had incorporated thymidine-$H^3$ during its circulation in the animal's body. As Table 1 shows, TMNG for the lymphoid population stimulated by antigen was indistinguishable from its value in the control. However, 10 h after injection of thymidine-$H^3$ considerable differences were found in the behavior of the labeled cells in the immunized and intact animals. For example, in the control group TMNG was only about half its initial level, while TNG/1000 remained virtually the same. In other words, the twofold increase in LI for the unstimulated lymphocytes corresponded to the decrease of 50% in the mean intensity of labeling. Consequently, in the intact spleen the labeled lymphocytes divided during the first 10 h after a single injection of thymidine-$H^3$, in agreement with data in the literature [2]. Meanwhile, in the immunized mice, the twofold increase in LI was unaccompanied by dilution of the label, since TMNG remained almost unchanged for 9 h, while TNG/1000 was more than doubled. This increase in total radioactivity in the immunized animals can be regarded as evidence of migration of lymphocytes into the immune spleen.

Since the cell population arising in the organ cancels out the dilution of the label due to cell division in the spleen, it must evidently have a higher intensity of labeling than cells in situ. The reason for the high content of thymidine-$H^3$ 10h after radioactive labeling could be either the very high rate of DNA synthesis (and, consequently, the short synthetic period of the cell cycle) or, on the contrary, the longest duration of the mean generation time of the migrating population. However, there is evidence in the literature that the S-phase of mammalian cells is relatively constant [1]. It therefore seems more likely that lymphocytes in the spleen at the time of injection of the label evidently divide more rapidly than cells migrating into it. Futhermore, having regard to the very considerable effect of these migrating cells on the indices of labeling intensity in the immune spleen, it can be postulated that under the influence of antigen there is massive settlement of the spleen by lymphocytes synthesizing DNA. As the differential count of the intensity of labeling by classes (Table 1) showed, during 9 h of observation the value of MNG/1000 increased most of all in the class of small lymphocytes. Probably the relative number of small lymphocytes is greater in the migrating population than in the spleen. On the other hand, the possibility cannot by ruled out that the antigen accelerates differentiation in the series: blast → large lymphocyte → medium lymphocyte → small lymphocyte.

It can accordingly be concluded that at the beginning of the inductive phase of the primary immunological response of mice to sheep's red cells the antigen stimulates not only cell proliferation of the lymphoid tissue of the spleen, but also migration of lymphocytes capable of synthesizing DNA into the spleen.

## LITERATURE CITED


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