

THE PATTERNS OF LABELING OF GERMINAL-CENTER CELLS
WITH TRITIATED DEOXYCYTIDINE

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INTRODUCTION

Autoradiographic studies of lymphoid tissue with tritiated thymidine ($[^3\text{H}]\text{TdR}$) have repeatedly demonstrated that thymus lymphocytes and germinal-center cells of lymph nodes and spleen are characterized by a weak labeling, whereas the majority of lymph node lymphocytes which occur outside of the germinal centers are intensely labeled (1-7).

An earlier study on mice by Osogoe and Ueki (8), on the other hand, has revealed that thymus lymphocytes are much more intensely labeled *in vivo* with tritiated deoxycytidine ($[^3\text{H}]\text{CdR}$) than with $[^3\text{H}]\text{TdR}$, and that the intensity of $[^3\text{H}]\text{TdR}$ labeling of these cells is significantly decreased by the simultaneous administration of unlabeled CdR. These findings indicate that, in thymus lymphocytes, CdR which is circulating in a considerable amount (9) is utilized for the formation of DNA-thymine and hence the incorporation of $[^3\text{H}]\text{TdR}$ into DNA-thymine in these cells is remarkably decreased.

As for the synthesis of DNA-thymine in the germinal-center cells of lymph node and other lymphoid tissues, any evidence in support of such a utilization of CdR has not yet been obtained. The present study was therefore undertaken to examine the patterns of labeling of the germinal-center cells with $[^3\text{H}]\text{CdR}$ in comparison with those labeled with $[^3\text{H}]\text{TdR}$, and to gain further information on the utilization of CdR in lymphocytes by autoradiographic techniques.

MATERIALS AND METHODS

Male rats of the Lewis strain which ranged in weight from 200 to 270 g were used. One group was injected

intraperitoneally with $[^3\text{H}]\text{CdR}$ (sp act 5.49 Ci/mM) in a single dose of 1 mCi per rat and a second group with $[^3\text{H}]\text{TdR}$ (sp act 6.7 Ci/mM), also in a single dose of 400 μCi per rat. A third group received a single intraperitoneal injection of 1 ml of 20% sheep red blood cells mixed with 1 ml of Freund's complete adjuvant in order to stimulate growth and new formation of germinal centers in the lymphoid tissues. After 10 days, the animals of the third group were then given intraperitoneally a single dose of either $[^3\text{H}]\text{CdR}$ (1 mCi per rat) or $[^3\text{H}]\text{TdR}$ (400 μCi per rat). Both $[^3\text{H}]\text{CdR}$ and $[^3\text{H}]\text{TdR}$ were obtained from New England Nuclear Corp., Boston, Mass. and Freund's complete adjuvant from Difco Laboratories, Inc., Detroit, Mich.

The animals of each group were sacrificed at intervals of 1, 24, 48 and 72 h after the injection of the labeled precursors. The thymus, mesenteric lymph nodes (MLN), spleen, and Peyer's patches of intestine were fixed in Carnoy's fluid, embedded in methacrylate, and 1 μm sections were prepared for autoradiography, as previously described by Rieke et al. (10). Smear preparations were also made from the thymus and MLN, fixed in Carnoy's fluid, and prepared for autoradiography. Autoradiographs were made using Eastman Kodak NTB-3 liquid emulsion, and the exposure time was 4 wk for sections and 7 days for smears. Both sections and smears were stained with pyronine-methyl green after photographic processing.

To confirm the incorporation of the labeled precursors into DNA, particularly that of $[^3\text{H}]\text{CdR}$, digestion of the sections was carried out with DNase and RNase as described by Amano (11), before dipping them in emulsion.

RESULTS

In the rats sacrificed 1 h after a single injection of $[^3\text{H}]\text{CdR}$, a high percentage of the germinal-center

cells of the MLN were intensely labeled, whereas the majority of lymphocytes, particularly lymphoblasts, which occur outside the germinal centers, were weakly labeled, except for a few scattered lymphocytes revealing fairly heavy labeling (Fig. 1). In the rats that received a single dose of [^3H]TdR 1 h previously, labeling of the germinal-center cells was very much weaker than that of the lymphocytes, particularly lymphoblasts, occurring in the diffuse lymphoid tissue outside of the germinal centers (Fig. 2).

Likewise, the germinal-center cells of spleen and Peyer's patches of intestine, as well as the lymphocytes of thymic cortex, were as heavily labeled with [^3H]CdR as were the germinal-center cells of lymph nodes (Figs. 5-7). In contrast, the intensity of [^3H]TdR labeling of the above-mentioned cells was much less than that of [^3H]CdR labeling of the same cells, as long as they were examined not later than 24 h after injection.

Examination of the cells which had been labeled either with [^3H]CdR or with [^3H]TdR revealed that silver grains were located over the nuclei. After DNase digestion of the sections before autoradiography, all the silver grains over the cells disappeared completely. On the other hand, RNase digestion of the sections caused no significant changes in the number of silver grains over the cells.

Most of the labeled cells of germinal centers, when examined 1 h after injection of [^3H]CdR, were large and medium lymphocytes (Fig. 1). After 24 h, however, smaller cells, i.e. medium and small lymphocytes, had become labeled and the labeling intensity of single cells was remarkably reduced (Fig. 3). After 72 h, the majority of the germinal-center cells revealed very weak labeling,

except for a few medium and small lymphocytes which were still fairly intensely labeled (Fig. 4).

Pretreatment of the rats with sheep red blood cells and Freund's complete adjuvant produced new formation and enlargement of germinal centers in MLN. However, with respect to the patterns of labeling of the germinal-center cells either with [^3H]CdR or with [^3H]TdR, no essential differences were recognized between the pretreated and untreated rats.

In the MLN of the pretreated rats, numerous large pyroninophilic blast cells were present in the paracortical area outside the germinal centers. The majority of such blast cells were heavily labeled with [^3H]TdR, while the labeling intensity of these cells with [^3H]CdR was much less than that with [^3H]TdR.

DISCUSSION

The present study has clearly demonstrated that the germinal-center cells and thymus lymphocytes label much more intensely with [^3H]CdR than with [^3H]TdR. Similar differences in labeling with [^3H]CdR and with [^3H]TdR were reported earlier for mouse thymus lymphocytes by Osogoe and Ueki (8).

Several factors which influence the incorporation rates of either TdR or CdR into DNA are to be considered: i.e. external concentration, DNA replication, endogenous synthetic pathway, degradation pathway, incorporation pathway, and pool size (9). The observed differences, however, between labeling with [^3H]CdR and with [^3H]TdR may be related mainly to the endogenous pathway of synthesis of DNA-thymine. Sugino et al. (12) have demonstrated by chemical methods that, in the thymus, CdR which is circulating in a con-

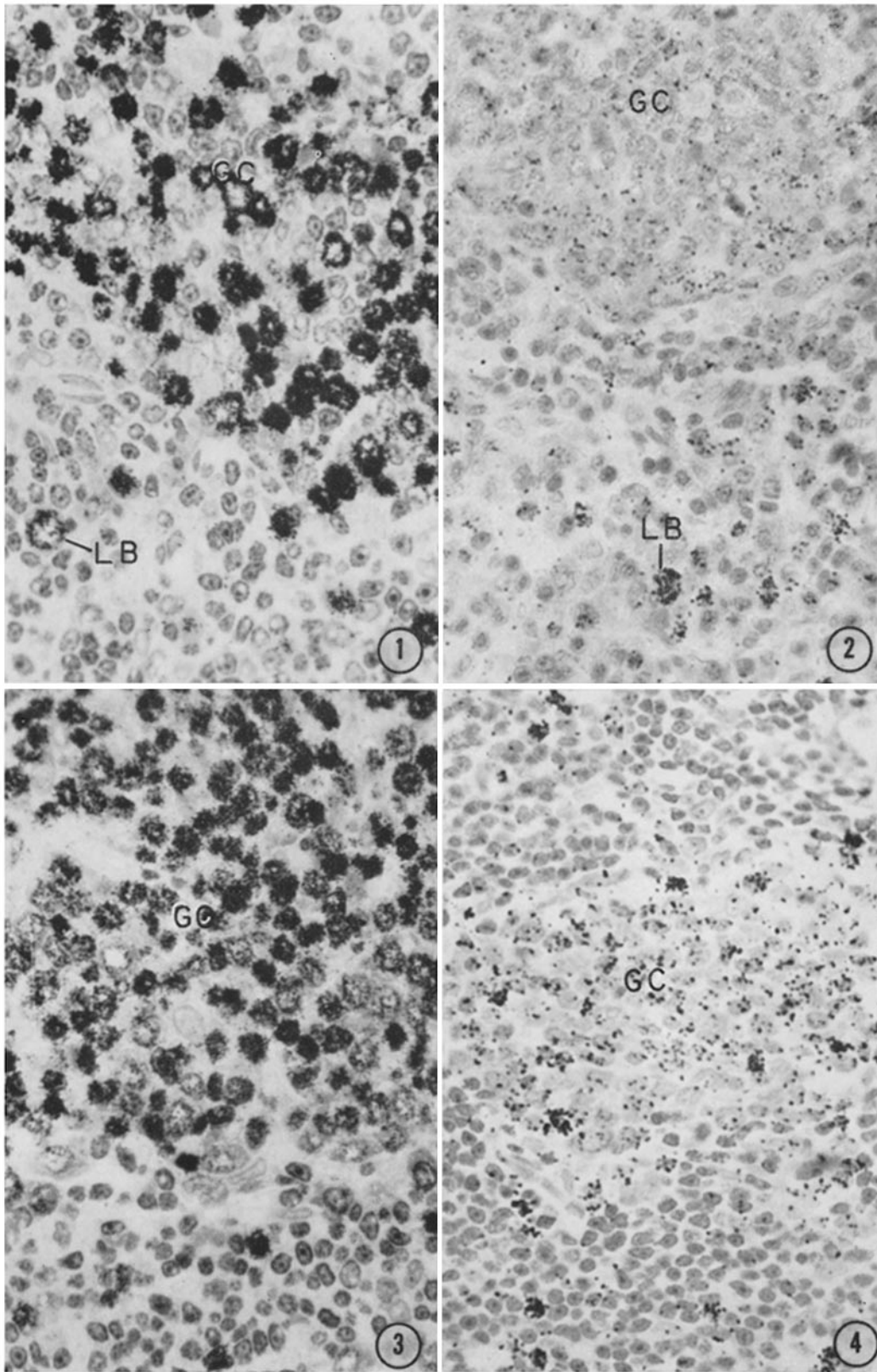
FIGURES 1-4 Autoradiographs of mesenteric lymph node in section showing the labeling patterns of the germinal-center cells. GC, germinal center; LB, lymphoblasts.

FIGURE 1 1 h after a single injection of [^3H]CdR. Notice the intensely labeled germinal-center cells as compared to the weakly labeled lymphoblasts in the surrounding corona. $\times 360$.

FIGURE 2 1 h after a single dose of [^3H]TdR. Notice weakly labeled germinal-center cells in contrast to the heavily labeled lymphoblasts in the surrounding corona. $\times 360$.

FIGURE 3 24 h after a single injection of [^3H]CdR. Notice that medium and small lymphocytes have become labeled. $\times 360$.

FIGURE 4 72 h after a single injection of [^3H]CdR. Notice that the germinal-center cells no longer show heavy labeling except for a few medium and small lymphocytes. $\times 360$.



siderable amount (9) is utilized for the formation of DNA-thymine. The present results provide evidence to indicate that not only the thymus lymphocytes but also the germinal-center cells are capable of utilizing CdR for the formation of DNA-thymine and hence the incorporation of [³H]TdR into DNA-thymine in these cells is strikingly decreased by the presence of CdR. This is further substantiated by the observation that the intensity of [³H]TdR labeling of either the thymus lymphocytes or the germinal-center cells was significantly reduced by the administration of unlabeled CdR (8, footnote 1).

In contrast, the lymph node lymphocytes, including large pyroninophilic blast cells, which occur outside the germinal centers, were found to be much more heavily labeled with [³H]TdR than with [³H]CdR. Moreover, it was revealed that the effect of unlabeled CdR, administered simultaneously with [³H]TdR, on the labeling intensity of these cells was not conspicuous (8, footnote 1). These findings indicate that in the majority of lymph node lymphocytes, other than the germinal-center cells, the capacity for synthesizing DNA-thymine endogenously from CdR is very limited and hence the incorporation of [³H]TdR into DNA-thymine in these cells is not so remarkably diminished by the presence of CdR as is the case for the germinal-center cells and thymus lymphocytes.

Finally, it should be noted that although the germinal-center cells were heavily labeled with [³H]CdR, the label of these cells had been diluted to a large extent within 72 h. Obviously, this is due to a rapid rate of division of these cells. However,

¹ Amano, M., and B. Osogoe. Unpublished observations.

the occurrence of a few medium and small lymphocytes which were fairly intensely labeled among the weakly labeled germinal-center cells, appears to indicate that some of the germinal-center cells might divide at a slow rate.

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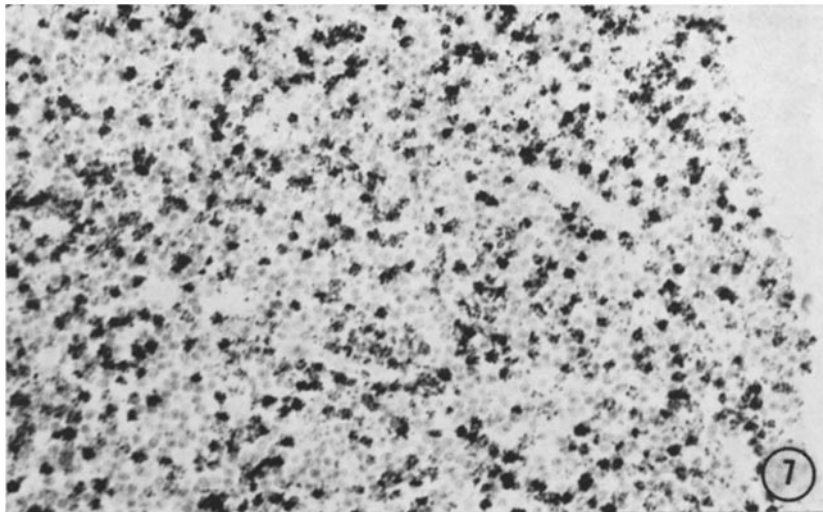
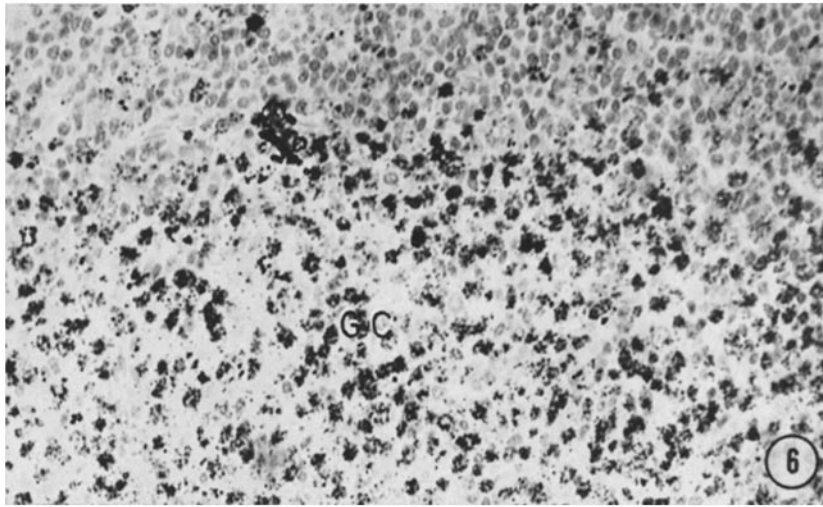
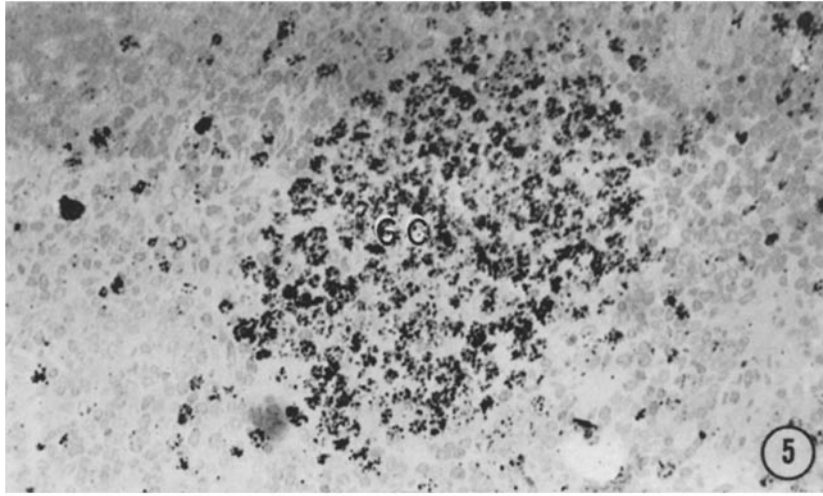
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FIGURES 5 and 6 Autoradiograph of spleen and Peyer's patch in sections showing the labeling patterns of germinal-center cells. GC, germinal center.

FIGURE 5 Spleen, 24 h after a single injection of [³H]CdR. Notice the heavily labeled germinal-center cells. × 180.

FIGURE 6 Peyer's patch, 24 h after a single dose of [³H]CdR. Notice heavily labeled germinal-center cells. × 180.

FIGURE 7 Autoradiograph of thymic cortex in section, 24 h after a single injection of [³H]CdR. Notice that the thymus lymphocytes are as intensely labeled as the germinal-center cells which are shown in Figs. 3, 5, and 6. × 180.



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