PROLONGED SURVIVAL OF RABBIT THORACIC DUCT LYMPHOCYTES IN A DIFFUSION CHAMBER

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In a recent review (1) Trowell has summarized much of the current information about the life span of the lymphocyte, and Yoffey and Courtice in their earlier book (2) have provided further data about this still unsettled question. In addition, a careful analysis of the information to be gained about the life span of the lymphocyte from isotope labeling studies has been made by Cronkite *et al.* (3). From the work presented in these reviews, there emerges the cautious hypothesis that there are two populations of lymphocytes, one long lived and the other short lived. This concept of Otteson (4) and of Hamilton (5) is supported by the experimental data of Cronkite *et al.* (3).

The evidence presented here shows that viable rabbit thoracic duct lymphocytes were present in a diffusion chamber that was left in a host animal for 200 days. Since mitoses were not observed after 14 days, the presence of a long lived component in this lymphocyte population was indicated.

MATERIALS AND METHODS

The diffusion chamber technique (described in detail elsewhere (6)) allows the investigator to grow cells of one animal species in the peritoneal cavity of another animal species. The peritoneal fluid of the host enters the chamber through fluid-permeable, but cell-impermeable, cellulose membrane filters and provides the metabolites needed for the growth or survival of cells contained in the chambers. In the present experiments, rabbit cells were grown in diffusion chambers placed in the peritoneal cavity of CAF_1 female mice by laparotomy. These were "double" chambers in which the cells were grown on a center Millipore filter that was enclosed on both sides by two other membrane filters.

Five milliliters of thoracic duct lymph were collected from a fistula in the neck of a healthy adult male white rabbit by Dr. E. Hardenbergh of the Naval Medical Research Institute, Bethesda, Maryland. Ten chambers containing 3.6×10^6 cells and 5 chambers containing 0.5×10^6 cells were prepared and inserted into mice. The chambers were removed at intervals of 3, 10, 14, 21, 33, 88, and 200

FIGURE 1

Group of living lymphocytes floating free in the chamber fluid. Each cell exhibits the anterior ruffled membrane and posterior tail characteristic of the moving lymphocyte. Phase contrast. × 900.

FIGURE 2

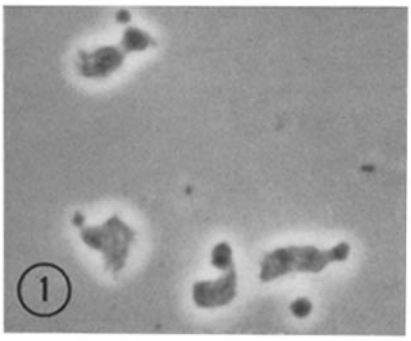
Higher magnification showing detail of a moving lymphocyte. Phase contrast. × 2000.

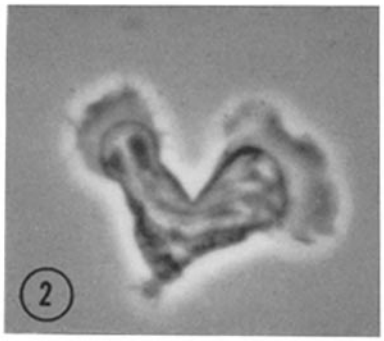
FIGURE 3

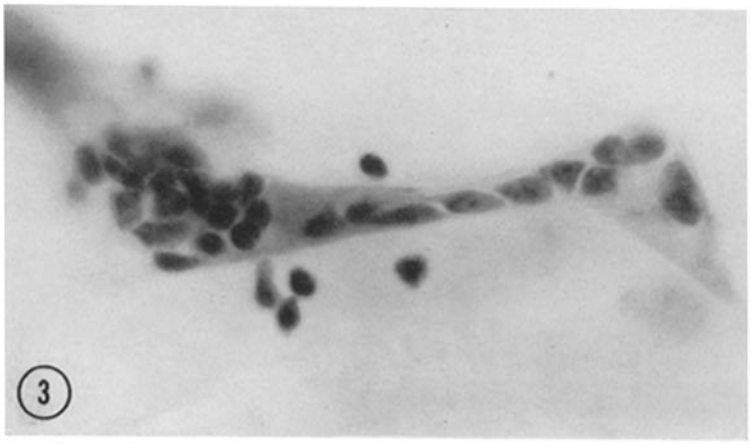
Lymphocytes clustered within and around a histiocyte. Eosin-azure stain. × 1000.

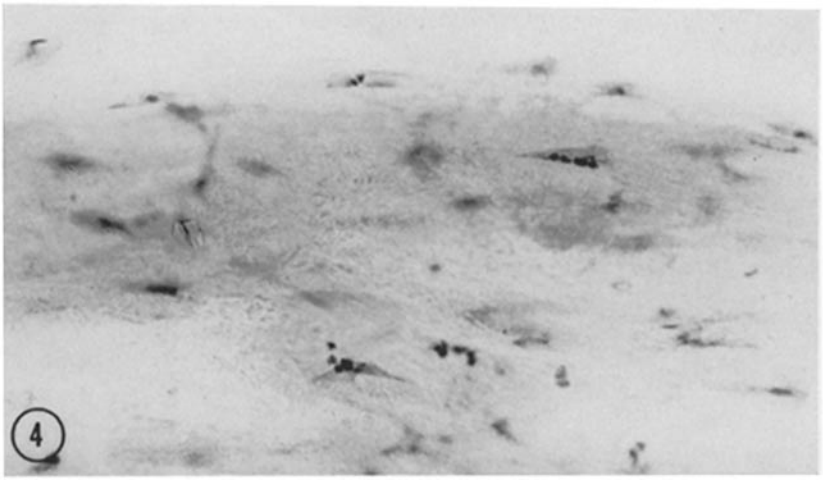
FIGURE 4

Low power view of clot within the chamber, showing the distribution of the histiocytes and the lymphocytes associated with them. Eosin-azure stain. × 300.









days. The fluid in the chambers was collected and examined for the presence of cells with the phase microscope, and the center Millipore filters, to which cells usually clung, were fixed, stained, and mounted permanently on slides.

RESULTS AND DISCUSSION

Of the 15 chambers that were put into mice, only 2 were allowed to stay in the animal for 200 days. The appearance of cells in the chambers opened at intervals from 3 to 88 days has already been reported (7). One of the chambers opened at 200 days had an initial inoculum of 0.5×10^6 cells. Only one small lymphocyte was seen in the fluid of the chamber, and no cells were attached to the center Millipore filter. In the second chamber (initial inoculum, 3.6×10^6 cells) there was a small, loose, fibrous clot, part of which was examined with the phase microscope and part of which was allowed to remain attached to the filter for fixation and staining.

With the phase microscope, many living lymphocytes were seen associated with the clot and many were observed floating free in the fluid. It was not possible to make an exact quantitation of the total number of cells in the fluid; however, 100 cells were counted in a small droplet of fluid and from this it could be estimated that the cell number was in the order of 5 to 10 thousand. Some of these cells are shown in Figs. 1 and 2. The cells exhibited the type of locomotion typical of the lymphocyte, and the amount of cytoplasm relative to nucleus placed these cells in the class of small lymphocytes.

In the fixed and stained clot, there were numerous small clusters of lymphocytes each associated with one or two larger cells of the histiocyte type. A low power picture of these clusters is shown in Fig. 4. Fig. 3 represents one of these clusters in more detail. Many of the lymphocytes appear to be surrounded by the cytoplasm of the larger cells.

The technical question whether or not the chamber remained intact and excluded host cells for such a long period can be dismissed for this reason. If host cells entered the chamber, polymorphonuclear leukocytes, mast cells, and especially histiocytes in large numbers would be present. These cells form a pattern of growth which is easily recognized and which is different from that seen in this chamber.

The two points of interest to be derived from the above observations relate to (*a*) the life span of the lymphocyte and (*b*) the nature of the association of the lymphocytes with histiocytes. It is apparent that lymphocytes can survive for 200 days in a diffusion chamber, but speculation as to the exact life span of these cells appears to be fruitless because of the possibility of intervening cell divisions. Mitoses among the cells grown in the chambers were quite numerous up to 14 days but were not observed thereafter (7). It may be said, then, that after two weeks in the chambers cell division was not an important factor in maintaining the cell number and that the cells present at 200 days truly represent a long lived population.

The association of lymphocytes with other cells is a well documented phenomenon that has been interpreted in a variety of ways. One concept is that the lymphocyte is a source of reutilizable DNA and protein for cells that ingest them (8, 9, and also literature cited in these references). Trowell (9, 10) suggests that reticulum cells that have "eaten up small lymphocytes" are capable of transforming into large lymphocytes and that the DNA contributed to the reticulum cell becomes part of the large lymphocyte. This "feedback" mechanism might govern the total lymphocyte population.

There is, on the other hand, good evidence that the lymphocyte needs the histiocyte or a related cell for its own survival. The work of Bichel (11) and deBruyn (12) shows that in some cases lymphocytes will not survive in the absence of another cell type.

The relationship between the lymphocytes and the histiocytes in the diffusion chamber is difficult to interpret. Intermediate cell types between the histiocyte and the large lymphocyte were observed as early as 7 days in the chamber (7) and the histiocytes were not seen in division. This indicates that the histiocyte, apparently derived from the large lymphocyte, is also a cell capable of long survival and that its presence has apparently provided the proper environment for the survival of recognizable small lymphocytes.

SUMMARY

Lymphocytes obtained from rabbit thoracic duct were present in a diffusion chamber that was left in the peritoneal cavity of a mouse for 200 days. Several thousand small lymphocytes were observed in the chamber fluid and many more were attached

to the Millipore filter of the chamber. These latter lymphocytes were found in close association with histiocytes that apparently were derived from large lymphocytes. Since mitoses were not observed after 14 days, the presence of a long lived component in this lymphocyte population is indicated.

Received for publication, August 27, 1961.

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