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CULTURE OF NORMAL HUMAN BLOOD CELLS IN A DIFFUSION CHAMBER SYSTEM II. LYMPHOCYTE AND PLASMA CELL KINETICS

G. Chikkappa[1], A. L. Carsten[2], A. D. Chanana[2] and E. P. Cronkite[2]

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Address reprint requests to: G. Chikkappa, M.D.
V. A. Medical Center
Hematology Section (111)
113 Holland Avenue
Albany, New York 12208 U.S.A.

ABSTRACT:

Normal human blood leukocytes were cultured in Millipore diffusion chambers implanted into the peritoneal cavities of irradiated mice. The evaluation of survival and proliferation kinetics of cells in lymphocytic series suggested that the lymphoid cells are formed from transition of small and/or large lymphocytes, and the lymphoblasts from the lymphoid cells. There was also evidence indicating that some of the cells in these two compartments are formed by proliferation. The evaluation of plasmacytic series suggested that the plasma cells are formed from plasmacytoid-lymphocytes by transition, and the latter from the transition of lymphocytes. In addition, relatively a small fraction of cells in these two compartments are formed by proliferation. Mature plasma cells do not and immature plasma cells do proliferate. Estimation of magnitude of plasma cells formed in the cultures at day 18 indicated that at least one plasma cell is formed for every 6 normal human blood lymphocytes introduced into the culture.

INTRODUCTION:

The notion of formation of plasma cells from lymphocytes was proposed by Krompecher in 1898 (11) and restated by Maximow in 1923 (12) . Good described the formation of plasma cells from lymphocytes in brains of sensitized rabbits after repeated local injections of an antigen (8). Formation of plasma cells in diffusion chambers from rabbit thoracic duct, spleen and lymph node lymphocytes implanted into rabbits (10,17) and mouse spleen cells and mouse yolk sac cells implanted into mice (2, 18) has been reported. The formation of "plasmacytoid" cells from mouse marrow cells in a suspension culture system (5) has been observed. Development of plasma cells in semi-solid agar cultures from mouse marrow cells has also been documented (13). Formation of plasma cells from normal human blood lymphocytes was demonstrated by Douglas and Fudenberg in a liquid culture system (6), and from blood leukocyte suspensions by Cronkite et al in diffusion chambers implanted into mice (4). The nature of plasma cells formed was confirmed by their capacity to produce an immunoglobulin (10,13) and by the presence of large quantities of rough endoplasmic reticulum in their cytoplasma (6, 13). The purpose of this investigation was to demonstrate the light microscopic sequential events in the formation of plasma cells from normal human blood lymphocytes.

METHODS AND MATERIALS:

Selection of Normal Individuals:*

The blood from four normal individuals was obtained for the culture studies. They were apparently well and were not receiving any medications. Their total and differential leukocyte counts and other blood studies were normal.

* Informed written consent was obtained from each individual as required by the Human Study Committee.

Culture Studies:

Procedures of the culture were as described (3). In brief, the leukocytes were isolated from the blood samples using methyl cellulose solution to sediment the red cells. The leukocytes were then inoculated, 472 x 10^3/diffusion chamber (27.6% lymphocytes), into diffusion chambers made with Millipore filters. The chambers were implanted into the peritoneal cavities of 700 rad total body irradiated mice. At intervals, beginning at 2 hours after implantation, groups of 4-8 chambers were removed. An hour before harvesting the chambers, the host animals were injected with tritiated thymidine (^{3}HTdR) intraperitoneally in order to determine the fraction of cells in DNA synthesis. The chambers removed from the hosts were shaken in Pronase (0.5%) solution for 60-75 minutes and the total nucleated cell counts were made from the cell suspension of each chamber. The smears made from the chamber contents were autoradiographed and scored for the fraction of labelled cells (labelling index = LI) in each category and differential cell counts were performed.

Morphological Classification of Cells:

The light microscopic recognition of small and large lymphocytes, lymphoblasts, plasma cells and immature plasma cells was made according to Elves (7). Further, the cells with features of both lymhocytes and plasma cells were called plasmacytoid-lymphocytes (20). The cells with sizes in between the small lymphocyte and lymphoblast, light to deep blue cytoplasm, usually with a rather less prominent perinuclear hof, nuclear chromatin less clumpier than in the small lymphocyte but not as fine as in the lymphoblast and without or with a nuceolus (occasionally > 1) are classified as lymphoid cells.

RESULTS:

Numbers of various types of lymphocytes recovered from the chambers are shown in Figure 1. From the initial point of analysis, 2 hours after implantation (Figure 1, section A), the number of small lymphocytes increased to a maximum at day 1, and after having stayed at that level for another day, decreased to a low point on day 7. The lymphocyte count rose again to a second maximum at day 14 with a subsequent drop to the initial level on day 18. The large lymphocytes (Figure 1, section B) decreased to a low point by day 7 with subsequent fluctuations at a low level until termination of the study. The number of lymphoid cells (Figure 1, section C) was very low at the initial point of analysis. The count increased relatively abruptly at day 2, with subsequent gradual increase to a maximum by day 14. Thereafter, the cell count remained at that level until termination of the study. There were no lymphoblasts at initial point of analysis (Figure 1, section D). A very small number was found in some cultures at days 1, 2 and 4. The cell count then increased rapidly reaching a maximum on day 7, and remained at that level at all subsequent points of analysis.

The numbers and patterns of recovery of total lymphocytes (small and large lymphocytes, lymphoid cells and lymphoblasts), plasmacytoid-lymphocytes and plasma cells are shown in Figure 2. From the first point of analysis (Figure 2, section A), the lymphocyte count increased to an initial maximum on day 2 followed by a slight decrease on day 4. The cell count increased again to reach a second maximum by day 14 followed later by a slight decrease. The plasmacytoid-lymphocytes (Figure 2, section B) were first observed on day 7, and they increased exponentially up to day 14 and somewhat slowly thereafter until day 18.

The plasma cells (including immature plasma cells) were first detected on day 7. The cell count increased to a maximum by day 14 and remained at that level on day 18.

The ^{3}HTdR LI of cells in each category is shown in Figures 1 and 2. A small fraction of the lymphoid cells (Figure 1, section C) was labelled as opposed to a very large fraction in the lymphoblast class (Figure 1, section D). A small fraction of the plasma cells, immature in appearance (Figure 2, section C), were labelled on days 10, 14 and 18. A considerable fraction of the plasmacytoid-lymphocytes (Figure 2, section B) was labelled commencing from day 7, and the labelled cells persisted at subsequent points of analysis.

DISCUSSION:

The increase in number of lymphocytes during the first 24 hours in culture is probably not due to proliferation since ^{3}HTdR labelled cells were not seen at 1 hour. However, a small part of the increase could be due to division of cells that were in late S or G_2 when introduced into the DC. The increase in the lymphocyte count during the first 24 hours is most likely due to the poor recovery at 2 hours and an improved recovery at 24 hours (3). The reasons for the improved recovery at 24 hours are not clear.

During the first 7 days the lymphoid cells increased in the culture as the small and large lymphocytes decreased. This temporal relationship suggests, but does not prove, that the lymphoid cells are formed from the small and/or large lymphocytes. The exact relationship between the small and large lymphocytes is not clear from these studies.

The wave of lymphoblasts follows that of the lymphoid cells, suggesting that the lymphoblasts are formed, at least initially, by transition

from the lymphoid cells. Subsequently, however, there is evidence for proliferation in both compartments. The magnitude of transition of cells from lymphoid stage to the lymphoblast stage and proliferation within each of those compartments contributing to the total cellularity is difficult to assess.

The transition of small lymphocytes from many organs of several species into lymphoblasts has been demonstrated by many investigators in in vitro cultures, mainly in the presence of a mitogen, and in vivo, following an injection of an antigen, and in diffusion chamber culture systems similar to that used in the current study (4, 10, 14, 15, 16, 19). Further, the peak of lymphocyte transformation to lymphoblasts is at days 3 to 5 in in vitro cultures in the presence of a mitogen (15, 19). The maximum percent blasts (percent of total lymphocytes) found in our culture system was on day 7. This difference may be related to the lack of exposure of the cells to any conventional mitogen in our study. Diffusion of stimulatory factors from the host was probably responsible for the transformation and proliferation of lymphocytes in this diffusion chamber study.

The temporal relationship of formation of plasmacytoid-lymphocytes suggests that they are derived from lymphocytes and the plasma cells from the plasmacytoid-lymphocytes. There is evidence for proliferation in both the plasmacytoid-lymphocyte and plasma cell compartments, more in the former than latter, and all of the labelled cells in the plasma cell compartment were immature forms. The magnitude of cells formed in these two compartments by proliferation within and transition from one into the next cannot be ascertained from this study. Although the temporal

relationship of formation of lymphocytoid cells, lymphoblasts, plasmacytoid-lymphocytes and plasma cells suggests that they are formed by transition as well as proliferation, an alternate possibility is that all the lymphocytes introduced into the chambers died and the cells formed were the progeny of the stem cells committed to form lymphocyte-plasma cell series.

The total number of lymphocytes introduced into the culture was 1.3×10^5/chamber and the maximum number of plasma cells recovered at day 18 was 0.21×10^5/chamber. In other words, one plasma cell appears for every six normal human blood lymphocytes introduced into chambers during the culture period. This calculation does not take into account the probable constant turnover of the cells or the potential for later development of more plasma cells. When mouse yolk sac cells were cultured in a diffusion chamber system, 1 plasma cell for every 3 lymphocytes was observed (18). The difference in the magnitude of plasma cell formation from the mouse yolk sac cells and human blood lymphocytes could be due to several obvious variations in the methods, e.g. source of cells and/or culture conditions.

The B-lymphocyte is considered to be the precursor of plasma cell (1). The B-lymphocyte concentration in the blood of normal man is about 25% of the total lymphocyte population (9). Thus in the diffusion chamber culture system, one plasma cell is formed for every one and one-half normal human blood B-lymphocytes. This formation of plasma cell from the B-lymphocyte may be an important mode of disposal of the B-lymphocyte from normal man. The process may be defective in patients with chronic lymphocytic leukemia

and resulting in the accumulation of the lymphocytes in them. Studies are in progress to test this notion.

Although no direct proof is provided for formation of plasma cells from lymphocytes, there are other evidences in support of the notion. A compelling evidence for the formation of plasma cells from lymphocytes came from the work of Douglas and Fudenberg (6) who cultured the normal human blood lymphocytes in vitro in the presence of pokeweed mitogen. In their study formation of immature plasma cells at 72 hours and plasma cells at 7-10 days were documented. The plasma cells formed in their cultures and in cultures of other investigators had the well recognized light and electron microscopic and functional features (6, 13, 18).

The results of our studies demonstrate more clearly the temporal relationship of formation of various lymphoid cells and plasma cells from the normal human blood lymphocytes in a diffusion chamber culture system.

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LEGENDS FOR FIGURES

Figure 1

The left vertical axis (— o——) represents absolute number of cells recovered from the diffusion chambers (DC). The right vertical axis (vertical bars) represents the tritiated thymidine (^{3}HTdR) labelling indices. Absence of vertical bars at some points reflects that there were no labelled cells. The horizontal axis represents the time after the first implantation of the chambers into irradiated mice. The first point of analysis was at 2 hours after implantation of the chambers. At each point of analysis 4-8 chambers per study were evaluated and the results presented as the mean and standard error of 4 studies.

Figure 2

As in Figure 1

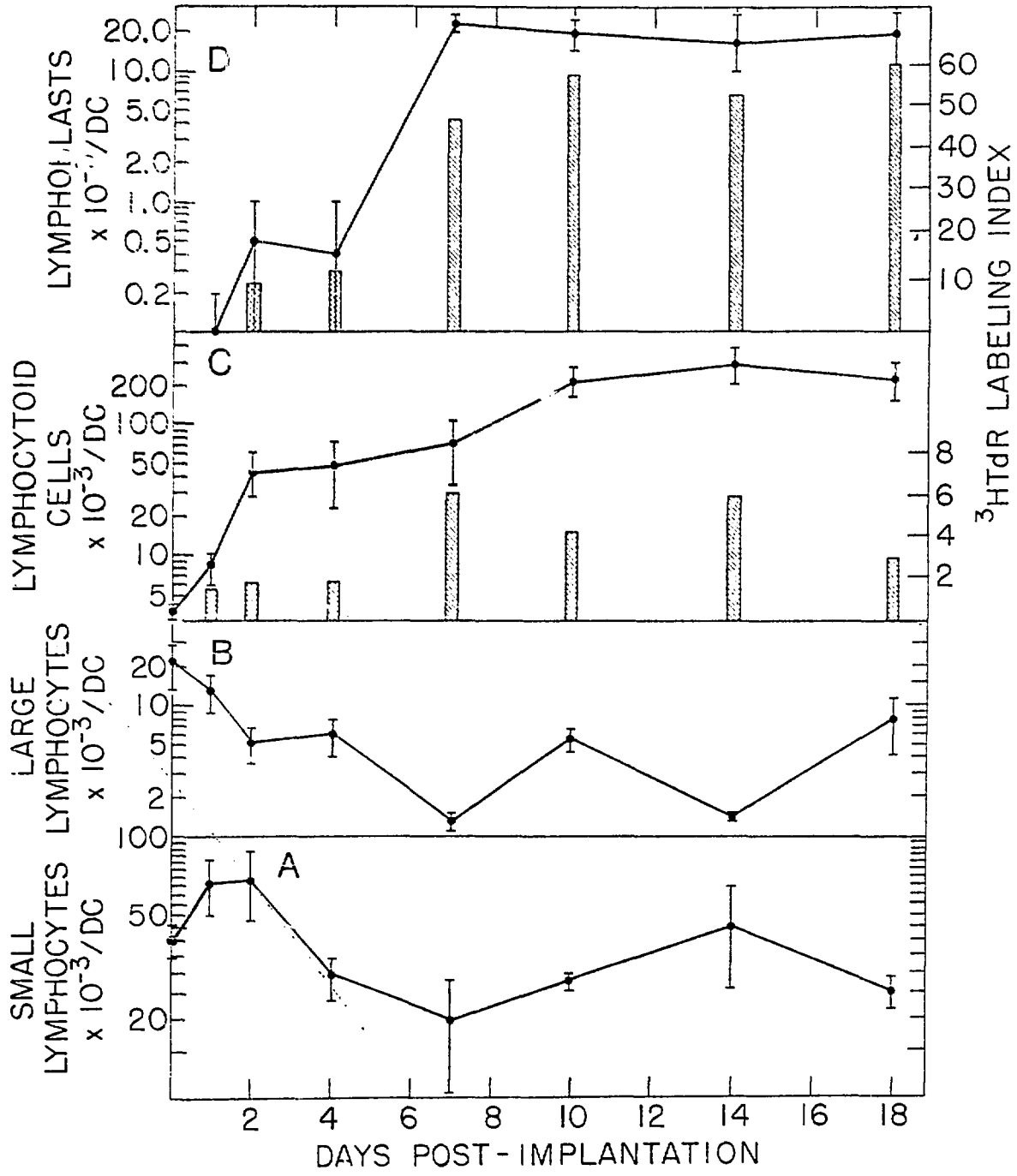

Figure 1 Neg. 10-833-76

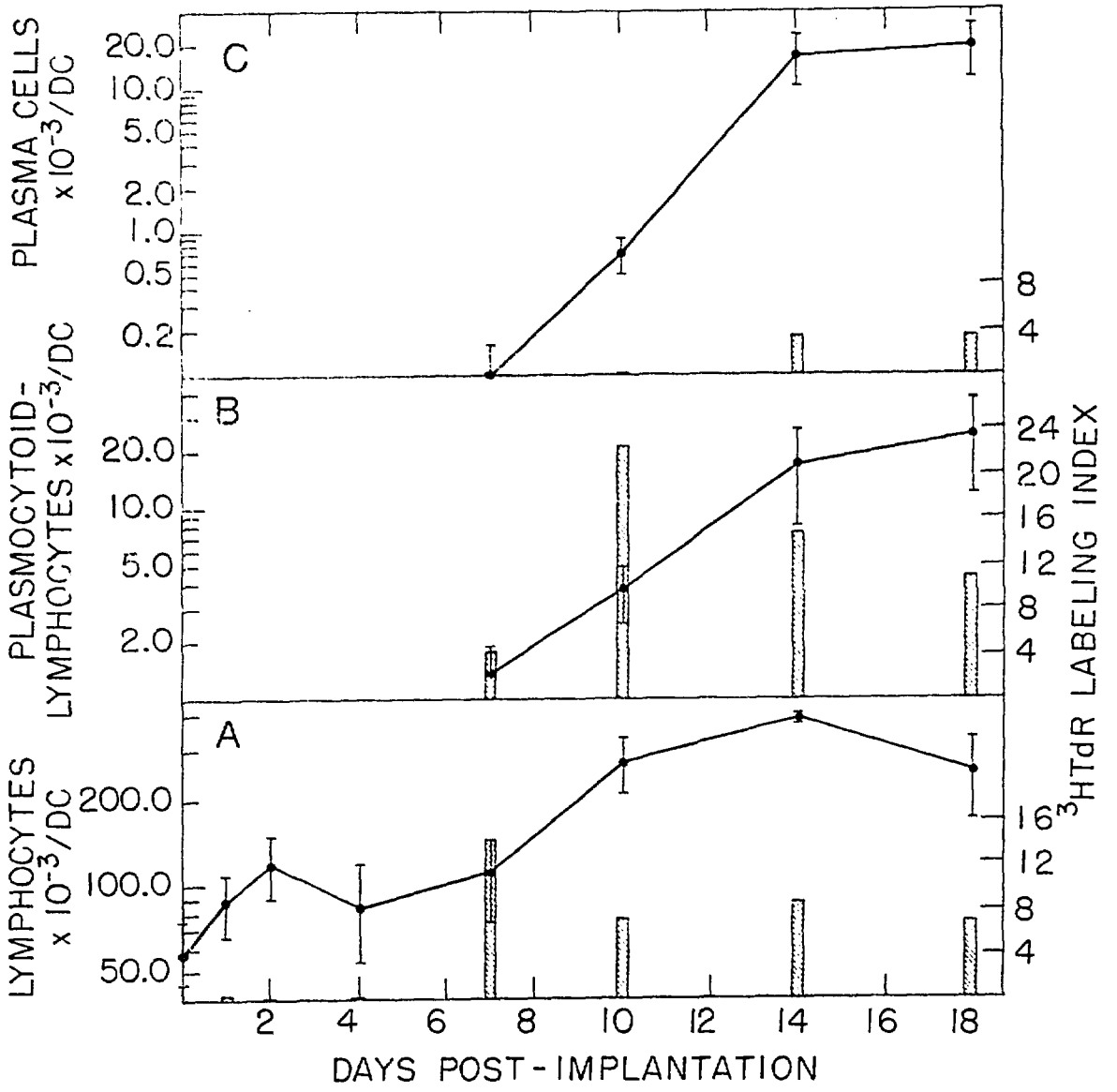

Figure 2 Neg. 10-834-76