INTERFERON AND CELL DIVISION

III. Effect of Interferon on the Division Cycle of L1210 Cells In Vitro

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We have previously described the inhibitory effect of interferon preparations on the multiplication of L1210 cells in vitro and presented the experimental results suggesting that interferon itself was the responsible factor (3, 4, 6). This inhibition could be due to a decrease in the number of cells capable of dividing, to a prolongation of the cell generation time, to a decrease in the doubling potential of each cell between subcultivation and the stationary phase, or to a combination of these effects. We present here the results of experiments designed to test each of these possibilities by analyzing the effect of interferon on the division cycle of L1210 cells.

MATERIAL AND METHODS

L1210 cells adapted to stationary suspension cultures were cultivated in nutrient medium R.P.M.I. 1640 (Gibco, Grand Island, N.Y.) supplemented with 20% heat-inactivated horse serum, L glutamine 1% (10 mM/ml), penicillin 200 units/ml, and streptomycin 40 μg/ml. Cells were subcultivated in Falcon plastic cell culture tubes (16 x 150 mm or in 15-ml glass

FIGURE 5 Part of an alveolar cell with its bordering band of microvilli (arrows). Microvilli are lacking over part of the free cell surface. At the center is a fat crater with the membrane torn away. The pitted surface within the crater represents the fat-cytoplasm interface; spherical particles, presumably of milk protein, have been deposited in the fat crater as well as elsewhere. × 4500.

FIGURE 6 A band of densely set microvilli marking a boundary between two cells runs horizontally across the center of this micrograph. Spherical particles of various sizes lying among microvilli probably are secreted protein particles. A dark hole in the cell surface at upper right may be the opening of a protein-containing vesicle fused with the cell membrane. × 18,000.

FIGURE 7 Stereomicrographs of microvilli along the boundary between two cells. × 22,000.

FIGURE 8 Cell surface with microvilli. The generally uniform contours of the microvilli are evident. The rough, finely pitted background may represent the actual cell surface, with its thin glycocalyx, or a thin precipitate of milk residues. × 43,000.

pharmacy bottles (2 ml/tube or bottle) placed in a Lwoff incubator in a 5% CO_2 atmosphere at 37°C. Every 24 hr, cells were counted in a hemacytometer and their viability was determined by the trypan blue dye exclusion test. In the figures, each point represents the average cell count of four cultures.

Purified mouse interferon (2) was obtained from the nutrient medium of monolayer cultures of MSV-Ia cells inoculated with Newcastle disease virus (NDV) (1). Control preparations consisted of medium from uninoculated cell cultures. In all experiments, purified interferon (320 interferon units/0.2 ml; specific activity 5 x 10^4 NIH International reference units/mg protein) was added to the nutrient medium at the time of cell subcultivation. Cells were then maintained without medium change until the stationary phase was attained.

For the measurement of deoxyribonucleic acid (DNA) synthesis, tritiated thymidine (TdR-^{3}H) was added (5 μCi/ml; specific activity 15 Ci/mmole) to duplicate cultures in each group, 5 hr after subcultivation and each day thereafter. 30 min after addition of TdR-^{3}H, the cells were washed and dissolved in 0.25 N sodium hydroxyde, and an equal volume of a 25% solution of perchloric acid was added (5). The incorporated radioactivity was measured in a liquid scintillator spectrometer after dissolving the acid-insoluble precipitate with soluene.

In other experiments, DNA synthesis was measured by radioautography.

PULSE LABELING: Cells were cultivated and labeled as described above but with 1 μCi/ml TdR-^{3}H. After the 30-min labeling, the cultures were washed twice with phosphate-buffered salt solution (PBS), and the cells were spread on duplicate slides. After air drying, the slides were dipped for 1 hr in acetic acid:methanol (1:3) and then in 2% perchloric acid (PCA) for 40 min at 4°C, and processed for radioautography (7). The percentage of labeled interphases was obtained from analysis of 500 cells/slide performed by two independent observers.

CONTINUOUS LABELING: At the time of subcultivation and on each day thereafter, 0.1 μCi/ml TdR-^{3}H was added to all cell cultures. Duplicate samples were fixed at different times as indicated in the respective experiments, and the cells were processed for radioautography.

The generation time was measured, adding TdR-^{3}H (0.5 μCi/ml) for 5 min to the cultures. After the labeling period (0 hr), the cells were washed and resuspended in medium with or without interferon supplemented with 10 μg/ml of nonlabeled thymidine (TdR) to dilute TdR-^{3}H. Cells were harvested and fixed at different times thereafter. The percentage of labeled mitoses was determined by two

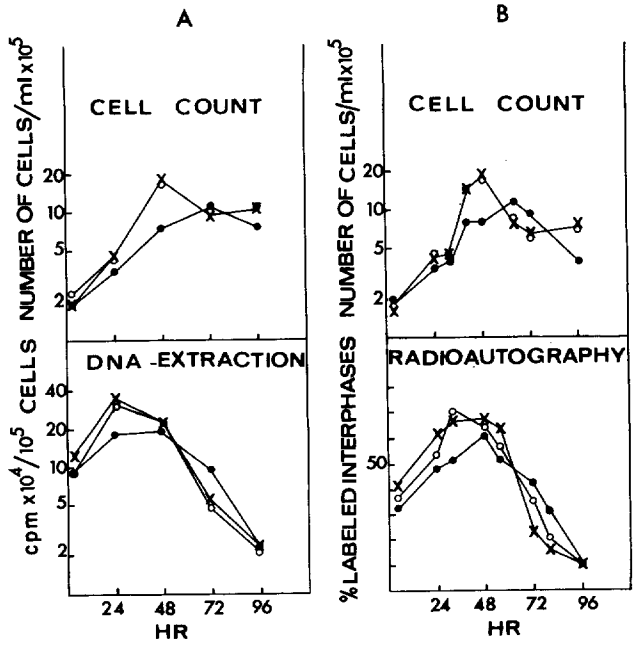

FIGURE 1 Growth curves and DNA synthesis after pulse labeling of control (×), mock interferon- (○), and interferon-treated (●) cultures measured by precipitation of acid-insoluble cell fractions (A) and by radioautography (B).

independent observers, each analyzing 50 mitoses/slide.

Cells cultivated in the presence of interferon during 24 hr and control cells were washed and subcultivated in interferon-free medium supplemented with Colcemid (Ciba Pharmaceutical Co., Summit, N. J.) (0.001 μg/ml). Every 2 hr, cells were spread on glass slides, fixed, and stained with toluidine blue, and the mitotic indices were determined from an analysis of 3,000 cells.

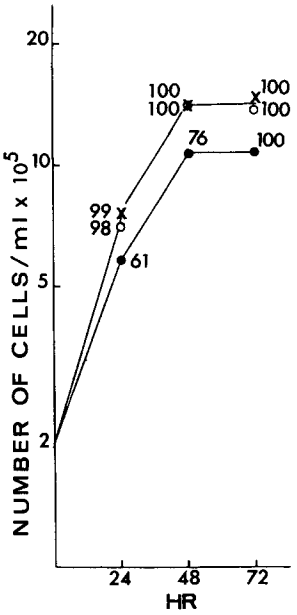

FIGURE 2 Growth curves of control (×), mock interferon (○), and interferon-treated (●) cultures labeled continuously with TdR^{3}H. Numbers represent the percentage of labeled interphases observed each day after subcultivation in the different cultures.

RESULTS

Division Cycle of L 1210 Cells Cultivated in the Presence of Interferon

Fig. 1 A illustrates the cell growth curve and the pulse labeling of control and interferon-treated L1210 cell cultures. The rate of cell multiplication in interferon-treated cultures was slower than in control cultures (comparison of the slopes of the growth curves), and the maximal cell concentration was attained at a lower cell density and 1 day later in interferon-treated cultures than in control cultures. The amount of DNA synthesized increased and reached a plateau, paralleling the cell growth curve, and then decreased to low values in both control and interferon-treated cell cultures (Fig. 1 A). Interferon-treated cells, however, synthesized less DNA than control cells during the 2nd day after subcultivation. Similar results were obtained with radioautographic techniques (Fig. 1 B).

Fig. 2 illustrates the growth curve and continuous labeling with TdR-^{3}H in control and interferon-treated cultures. 24 hr after subcultivation, 98–99% of cells in control cultures were labeled in contrast to 61% of labeled cells in interferon-treated cultures. 72 hr after cell subcultivation, 100% of the cells in interferon-treated cultures were labeled.

In the experiment illustrated in Fig. 3, cells were

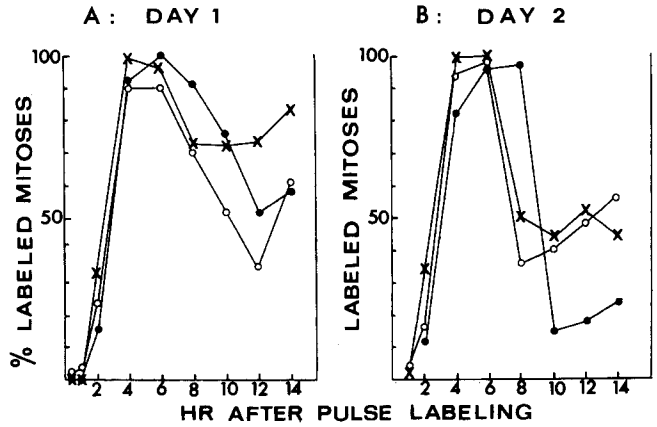

FIGURE 3 Percentage of labeled mitoses observed after pulse labeling of cultures during the 1st and the 2nd day after subcultivation of control (×), mock interferon- (○), and interferon-treated (●) cultures.

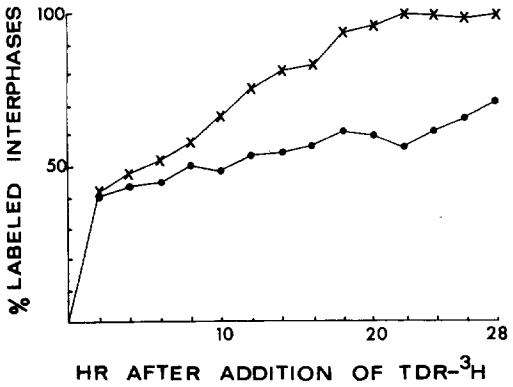

FIGURE 4 Percentage of labeled interphases observed at different times after subcultivation in the continuous presence of TdR-^{3}H of untreated cells (×) and cells previously cultivated (48 hr) in the presence of interferon (●).

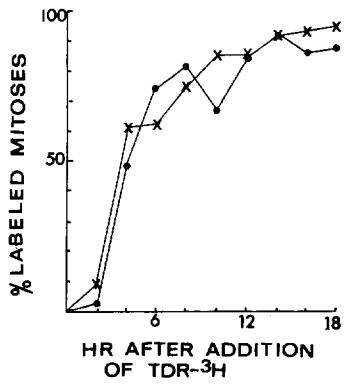

FIGURE 5 Percentage of labeled mitoses in the same experiment illustrated in Fig. 4.

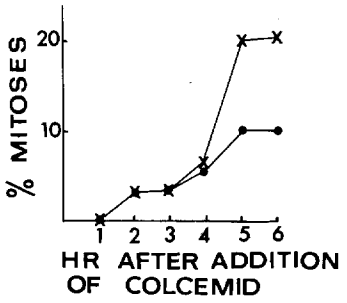

FIGURE 6 Mitotic indices at different times after subcultivation (in the presence of Colcemid) of untreated cells (×) and cells previously cultivated (24 hr) in the presence of interferon (●).

subcultivated and pulse labeled for the measurement of the generation time. Interferon or mock interferon was added immediately after labeling. The percentage of labeled mitoses was determined at 2-hr intervals (Fig. 3). The average generation times expressed as the interval between the addition of TdR-^{3}H and the lowest point in the curve before the second wave of labeled mitoses was similar in all groups (i.e., 12 hr). Fig. 3 B illustrates the generation time determined during the 2nd day of cultivation of control and interferon-treated cells (interferon had been added 24 hr previously at the time of subcultivation). The average generation time was similar in the three groups (8–10 hr).

Division Cycle of L 1210 Cells after Removal of Interferon

Cells cultivated for 48 hr in the presence of interferon were centrifuged, washed once with PBS, and subcultivated in the absence of interferon. TdR-^{3}H was added to the medium. 2 hr after subcultivation, 40% of cells in both control and interferon-treated cultures were labeled with TdR-^{3}H (Fig. 4). The percentage of labeled cells increased progressively but at a faster rate in control cultures than in interferon-treated cultures.

Analysis of the percentage of labeled mitoses in the same experiment, as illustrated in Fig. 4, showed that the kinetics of the appearance of labeled and unlabeled mitoses was similar in both cultures. Most of the mitoses appearing in the first 2 hr were unlabeled and represented cells that were in the G2 period at the time of subcultivation. The percentage of labeled mitoses increased at the same rate in both cultures (Fig. 5).

Fig. 6 illustrates the rate of entrance into mitosis (in the presence of Colcemid) of cells of control and interferon-treated cultures after removal of interferon from the medium. The rate was similar for both groups during the first 4 hr, and the plateau was attained at the same time although it was lower for cells previously cultivated with interferon. This indicates that more cells from control cultures entered division than from the interferon-treated cultures.

DISCUSSION

The data presented confirm the previous results (3–5) that mouse interferon decreases the rate of L1210 cell proliferation and the final cell density. Interferon does not appear to alter TdR-^{3}H incor-

poration since the amount of DNA synthesized per cell (Fig. 1 A) and the percentage of cells synthesizing DNA (Fig. 1 B) 5 hr after the cells were exposed to interferon were similar in control and interferon-treated cultures. The significant decrease in the number of interferon-treated cells synthesizing DNA during the 2nd day after subcultivation paralleled the inhibition of cell multiplication. Otherwise, the general pattern of the curves (Fig. 1 A and B) was the same for the different groups.

Inhibition of the multiplication of L1210 cells in the presence of mouse interferon may result from the following: (*a*) fewer cells in interferon-treated cultures capable of dividing, (*b*) a prolonged generation time, and (*c*) a decrease in the doubling potential of each cell. The continuous labeling of cells cultivated in the presence of interferon revealed that *all* interferon-treated cells divided at least once (Fig. 2). The finding that 100% of interferon-treated cells were labeled later than in control cultures suggested, however, a delay in the entry of some interferon-treated cells into the division cycle. Secondly, the cell generation time of cells cultivated in the presence of interferon was similar to that of control cultures. Thus, the third possibility was the most probable explanation for the interferon-mediated inhibition of cell division.

Analysis of the division cycle after withdrawal of interferon and subcultivation revealed that interferon-treated cells had been arrested in the same periods of the division cycle as control cells. Although the initial rate of entrance of interferon-treated cells into division was similar to that for control cells, the percentage of cells entering division was decreased. This could be caused by some residual interferon in the medium or to prolongation of the effect of interferon on cells.

Since the doubling potential of interferon-treated cells was decreased, the plateau was therefore attained at a lower cell concentration than in control cultures. One may postulate that interferon renders cells more sensitive to the various mechanisms that limit cell division as the cell concentration increases (8).

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