INTERFERON AND CELL DIVISION

A. MACIEIRA-COELHO, D. BROUTY-BOYÉ, M. T. THOMAS, and I. GRESSER. From the Institut de Cancérologie et d'Immunogénétique and Institut de Recherches Scientifiques sur le Cancer, 94-Vilbeuf, France

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...
pharmacy bottles (2 ml/tube or bottle) placed in a Lwoff incubator in a 5% CO₂ 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 10⁸ 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-³H) was added (5 µCi/ml; specific activity 15 Ci/m mole) to duplicate cultures in each group, 5 hr after subcultivation and each day thereafter. 30 min after addition of TdR-³H, the cells were washed and dissolved in 0.25 N sodium hydroxide, and an equal volume of a 25%/0 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-³H. 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-³H 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-³H (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-³H. Cells were harvested and fixed at different times thereafter. The percentage of labeled mitoses was determined by two independent observers.

![Figure 1](image-url)

**Figure 1** Growth curves and DNA synthesis after pulse labeling of control (X), mock interferon- (O), 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.

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 lower 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-3H 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 2](image-url)

**Figure 2** Growth curves of control (X), mock interferon (O), and interferon-treated (●) cultures labeled continuously with TdR-3H. Numbers represent the percentage of labeled interphases observed each day after subcultivation in the different cultures.

![Figure 3](image-url)

**Figure 3** Percentage of labeled mitoses observed after pulse labeling of cultures during the 1st and the 2nd day after subcultivation of control (X), mock interferon- (O), and interferon-treated (●) cultures.
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-3H 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-3H was added to the medium. 2 hr after subcultivation, 40% of cells in both control and interferon-treated cultures were labeled with TdR-3H (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-3H incor-
poration since the amount of DNA synthesized per
cell (Fig. 1 A) and the percentage of cells synthes-
izing DNA (Fig. 1 B) 5 hr after the cells were
exposed to interferon were similar in control and
interferon-treated cultures. The significant de-
crease in the number of interferon-treated cells
synthesizing DNA during the 2nd day after sub-
cultivation paralleled the inhibition of cell multi-
plication. 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 pro-
longed generation time, and (c) a decrease in the
doubling potential of each cell. The continuous
labeling of cells cultivated in the presence of inter-
feron 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 de-
lay in the entry of some interferon-treated cells
into the division cycle. Secondly, the cell genera-
tion time of cells cultivated in the presence of inter-
feron 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 inter-
feron-treated cells had been arrested in the same
periods of the division cycle as control cells. Al-
though the initial rate of entrance of interferon-
treated cells into division was similar to that for
control cells, the percentage of cells entering di-
vision was decreased. This could be caused by
some residual interferon in the medium or to pro-
longation of the effect of interferon on cells.

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

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