A CELL SURFACE ALTERATION IN MOUSE L CELLS INDUCED BY INTERFERON

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Summary: Mouse L cells grown in suspension culture when treated with L cell interferon have a greater electrophoretic mobility toward the anode than control cells. This change in electrophoretic mobility depends on the concentration of interferon in the medium and the duration of interferon interaction with the cells. It is concluded that the interferon-treated cells have a greater net negative charge on the cell surface than control cells and it is suggested that the cell surface is altered because of the interaction with interferon.

Interferon preparations cause biological effects in cells in addition to the induction of the antiviral state (1-8). Recent studies with interferon covalently attached to Sepharose beads (9,10) suggest that the antiviral state is initiated from the cell surface with no requisite entry of interferon into the cell. It has been suggested that the enhanced expression of surface antigens on murine leukemia L1210 cells after exposure of the cells to interferon preparations is the result of an interferon-induced modification of the cell surface (11). To test this proposal we have performed zonal electrophoresis (14) and have compared the electrophoretic mobilities of interferon-treated mouse L cells and untreated cells.

MATERIAL AND METHODS: Cells: Mouse L cells were grown in suspension cultures in Eagle's suspension culture medium (Joklik modified, Grand Island Biological Co.) supplemented with 7% fetal calf serum.

Interferon: Interferon was assayed on L cells by a microassay technique (13) using MM virus as the challenge virus. L cell interferon was purified to a specific activity of 1 x 10^8 units/ml as previously described (12). All units are expressed as international reference units. Preparations of "mock interferon" were of the same level of "purity" as the interferon but contained no interferon activity. Interferon-Sepharose was prepared as previously described (10).

Labeling of Cells with Radioactive Amino Acids: Either a [^{14}C] or [^{3}H] amino acid mixture was added to a culture of L cells at 2-4 x 10^5 cells/ml and the cells were allowed to grow in the radioactive medium for 24 hr. Cells were collected by centrifugation and resuspended in non-radioactive medium. Interferon was added to the [^{14}C]-labeled culture and both [^{3}H] and [^{14}C] cultures allowed to grow an additional 16 hr unless otherwise indicated in the figure legends.

Electrophoresis of L Cells: Electrophoresis of L cells was performed in glass tubes (20 cm x 0.6 cm ID) in a device previously described in detail (14). The modification required for analysis of intact cells substituted Ficoll

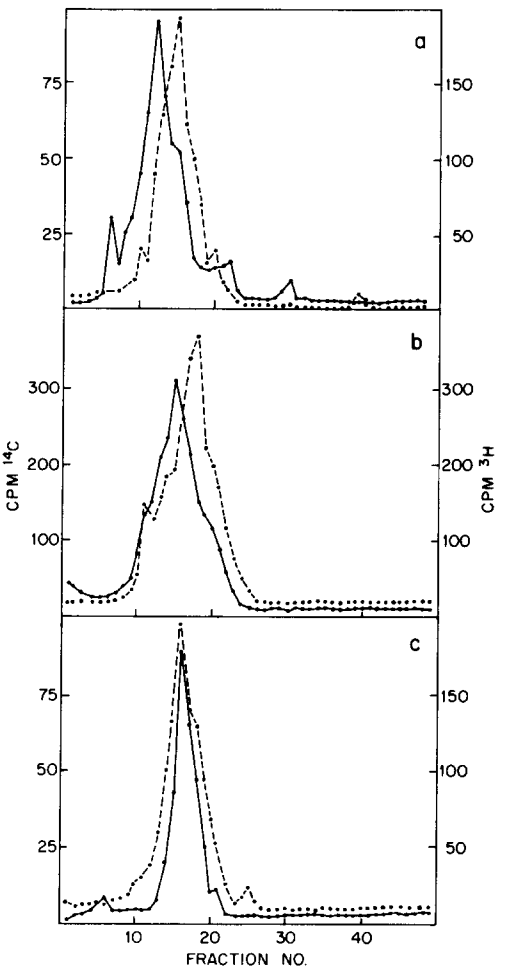

Figure 1. Electrophoretic mobility of L cells after treatment with soluble interferon (a) interferon-Sepharose (b) and "mock" interferon (c). L cells, labelled with [^{14}C] amino acids (0.1 μc/ml) were exposed to 150 units/ml soluble interferon, 100 units/ml interferon-Sepharose, or "mock" interferon for 18 hr. [^{14}C] labelled cells were mixed with [^{3}H] labelled (1 μc/ml) control cells and subjected to electrophoresis. Cell mobility is from right to left toward the anode. —— [^{14}C] interferon, interferon-Sepharose, or "mock" interferon treated cells. --- [^{3}H] control cells.

(ca. 600,000 m.w.) for sucrose in the density gradient (4%-10% w/v). The Ficoll gradient was prepared in 0.02 M sodium phosphate, pH 7.2, containing suspension culture medium at 10% the normal concentration (without serum) and 4% sucrose (electrophoresis buffer). The lower reservoir buffer was 0.2 M sodium phosphate, pH 7.2, containing 40% sucrose (w/v). L cells (1-2 x 10^4) were applied in 0.2 ml of a buffer of 0.001 M sodium phosphate, pH 7.2, containing 4% sucrose and 3% Ficoll. The electrophoresis buffer containing 2% Ficoll was layered over the cell sample, filling the tube. The upper reservoir was filled with 0.2 M sodium phosphate, pH 7.2, and the electrophoresis was performed at 25° for 7 hr at 4 ma per gradient. Gradients were fractionated and radioactivity determined as previously described (14).

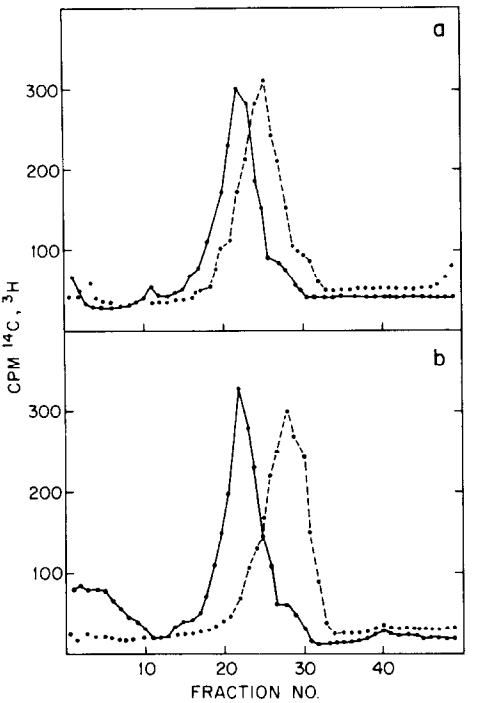

Figure 2. Electrophoretic mobility of L cells after treatment with trypsin (a) and neuraminidase (b).

[^{14}C] labelled L cells were treated with either 1.75 units/ml of trypsin or .01 units/ml of Cl. Perfringens neuraminidase for 40 minutes at 37°; with intermittent stirring. After treatment, the [^{14}C] labelled cells were mixed with [^{3}H] labelled control cells, diluted 1:10 into sample buffer, and analyzed as in Figure 1. — [^{3}H] cells. --- [^{14}C] cells.

RESULTS: The data in figure 1a show that L cells exposed to 150 units/ml of interferon for 20 hr have a greater electrophoretic mobility toward the anode than control cells. When cells are exposed to a similar concentration of antiviral units in the form of interferon-Sepharose an increase in the electrophoretic mobility is also observed (Figure 1b). It should be noted that the interferon-Sepharose was removed from the culture medium prior to mixing of the [^{14}C] and [^{3}H] labeled cells. Soluble interferon was not detected at any time while the interferon-Sepharose was in contact with the cells. Preparations of "mock" interferon caused no change in the cell electrophoretic mobility (Figure 1c) and moreover, L cell interferon did not cause a change in the mobility of human (HeLa) cells. That changes in the electrophoretic mobilities of cells can be observed by this method is supported by the data in Figure 2. Cells incubated with either trypsin (E.C. 3.4.4.4; Figure 2a) or neuraminidase (E.C. 3.2.1.18; Figure 2b) have

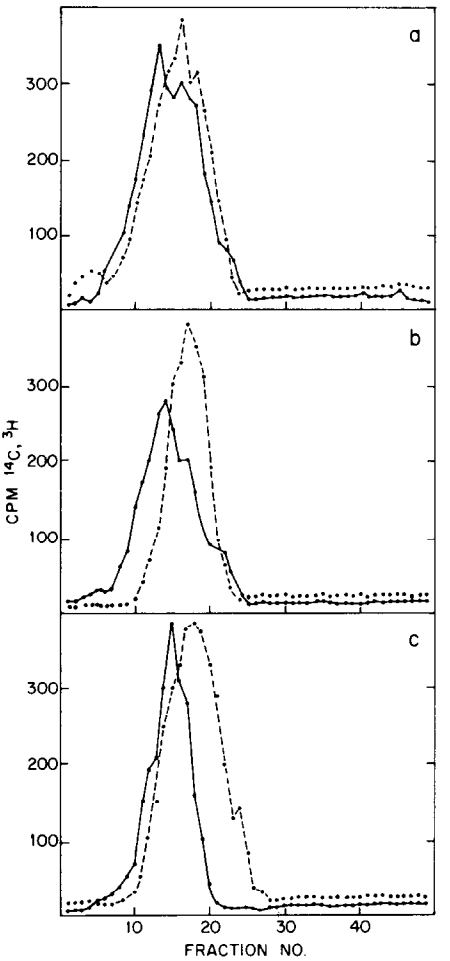

Figure 3. Effect of the concentration of soluble interferon on the electrophoretic mobility of L cells. L cells labelled with [^{14}C] amino acid were exposed to 10 (a) 50 (b), and 500 (c) units/ml of soluble interferon for 18 hr. Mixing of cells and electrophoresis performed as in figure 1. — [^{14}C]. --- [^{3}H].

mobilities that are less than control cells. Similar results have previously been reported (15).

The extent to which the antiviral state develops in cells is dependent on the concentration of interferon in the culture medium. The data in Figure 3 sugges that the change in mobility is also dependent on the concentration of the interfero in the medium, although the separation between species is not large enough to permit a detailed analysis.

A time-course study was done to determine the earliest time at which the increase in mobility could be observed after the addition of soluble interferon to

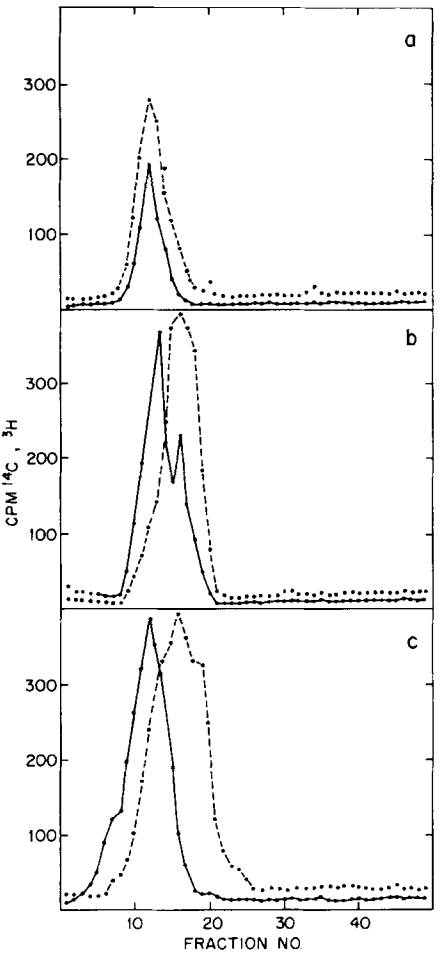

Figure 4. The increase in electrophoretic mobility of L cells as a function of time exposed to soluble interferon. L cells labelled with [^{14}C] amino acid were exposed to 150 units/ml of soluble interferon for 6 hr (a), 10 hr (b), and 20 hr (c). Mixing of cells and electrophoresis performed as in figure 1. — [^{14}C]. --- [^{3}H].

the medium. The data in Figure 4 show that the increase in mobility occurs between 6 and 10 hours after the addition of interferon. The time lag of 6 to 10 hr suggests that the interferon induces the cell to change its surface, i.e., a greater net negative charge, and is not a direct result of the attachment of interferon to the cell surface. The cells, after electrophoresis, have the same appearance in the light microscope as before electrophoresis. Greater than 95% of both control and interferon treated cells exclude the viable stain trypan blue either before or after electrophoresis.

DISCUSSION: The electrophoretic mobility of mouse L cells increases after interaction of the cells with interferon. This increase in mobility suggests that the interferon causes the L cells to increase the net negative charge on the cell surface. The time course for the development of the change in cell mobility parallels that for the induction of the antiviral state. Although there are no data which directly relate the change in electrophoretic mobility to the antiviral state we suggest that an interferon-induced alteration of the L cell surface as reflected in a change in electrophoretic mobility could be a prerequisite for the subsequent expression of the antiviral state. Moreover, cells treated concurrently with cycloheximide (100 μg/ml) and interferon did not show a change in electrophoretic mobility.

One explanation for the change in electrophoretic mobility might simply be the binding of the interferon to the cell surface with the bound interferon contributing the negative charge increase. This explanation seems unlikely for two reasons: (a) binding of interferon to the surface of the cell occurs in much less than 6 hrs (16) and (b) a mobility increase is observed after the removal of interferon-Sepharose from the cells. We therefore conclude that the change in electrophoretic mobility of L cells in induced by the interferon rather than resulting solely from the binding of interferon to the L cell surface.

Although the exact mechanism of interferon's antiviral action is still unclear, cells chronically infected with murine leukemia virus then treated with interferon produced virus but with reduced yield (17,18,19). It was suggested that the reduction in virus yield might be due to an inhibition of a late step in the maturation of the virus (17,18) or to an interferon-induced alteration in the cell surface (19). Furthermore, in a recent report (20) describing the inhibition by interferon of the binding of cholera toxin to mouse L cells it was suggested that the inhibition of cholera toxin binding may be caused by an alteration in the cell surface induced by interferon.

REFERENCES:

1. Paucker, K., Cantell, K., and Henle, W. (1962) Virology, 17, 324-338.
2. Gresser, I., Brouty-Boye, D., Thomas, M. T., and Macieira-Coelho, A. (1970) Proc. Nat. Acad. Sci. USA, 66, 1052-1058.
3. O'Shaughnessy, M. V., Lee, S. H. S., and Rozee, K. R. (1972) Can. J. Microbiol. 18, 145-151.
4. Stewart, W. E. II, DeClercq, E., Billiau, A., Desmyter, J., and De Somer, P. (1972) Proc. Nat. Acad. Sci. USA, 69, 1851-1854.
5. Gresser, I., Thomas, M. T., and Brouty-Boye, D. (1971) Nature New Biol., 231, 2
6. Huang, K. Y., Donahoe, R. M., Gordon, F. B., and Dressler, A. R. (1971) Infec. Immunity, 4, 581-588.
7. Lindahl, P., Leary, P., and Gresser, I. (1972) Proc. Nat. Acad. Sci. USA, 69, 721-725.
8. Stewart, W. E. II, Gosser, L. B., and Lockart, R. Z., Jr. (1971) J. Virol., 7, 792-801.
9. Ankel, H., Chany, C., Galliot, B., Chevalier, M. J., and Robert, M. (1973) Proc Nat. Acad. Sci. USA, 70, 2360-2363.

10. Knight, E., Jr. (1974) Biochem. Biophys. Res. Commun. 56, 860-864.
11. Lindahl, P., Leary, P., and Gresser, I. (1973) Proc. Nat. Acad. Sci. USA, 70, 2785-2788.
12. Knight, E., Jr. (1975) J. Biol. Chem., 250, 4139-4144.
13. Armstrong, J. A. (1971) Appl. Microbiol., 21, 723-725.
14. Korant, B. D., and Lonberg-Holm, K. (1974) Anal. Biochem., 59, 75-82.
15. Seaman, G. V. F. (1965) Cell Electrophoresis, p. 4, Churchill, London.
16. Friedman, R. M. (1967) Science, 156, 1760-1761.
17. Billiau, A., Edy, V. G., Sobis, H., and De Somer, P. (1974) Int. J. Cancer, 14, 335-340.
18. Friedman, R. M., and Ramseur, J. M. (1974) Proc. Nat. Acad. Sci. USA, 71, 3542-3544
19. Pitha, P. A., Rowe, W. P., and Oxman, M. N. (1976) Virology, 70, 324-338.
20. Friedman, R. M., and Kohn, L. D. (1976) Biochem. Biophys. Res. Commun., 70, 1078-1084.