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Interferon Treatment Inhibits Pinocytosis

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Treating mouse L cells with crude or purified mouse interferon inhibited fluid-phase pinocytosis. Inhibition was maximum at 24 h after treatment with 1,000 U of interferon per ml and was dose dependent and reversible with time. Pinocytosis was inhibited when human and chicken embryo cells were treated with homologous, but not heterologous, interferons.

In addition to inhibiting viral replication, interferon treatment of cells produces a variety of effects on the plasma membrane (2, 3, 7, 9, 12, 15) and on associated cytoskeletal elements (23; I. Tamm, E. Wang, F. R. Landberger, and L. M. Pfeffer, *in* T. C. Merigan, R. M. Friedman, and C. F. Fox [ed.], Chemistry and biology of interferons, in press). Recently, Tamm et al. (in press) and Wang et al. (J. Cell Biol. **91**:238a, 1981) reported that interferon treatment of thioglycollate-elicited mouse peritoneal macrophages caused a marked change in the distribution of microtubules and 10-nm filaments in a major fraction of the cells; this alteration was correlated with an inhibition of pinocytosis. The results reported here describe some details of the inhibition of pinocytosis in cells treated with interferon.

Mouse L cells were pretreated with 1,000 U of crude mouse interferon per ml for 24 h before the uptake of horseradish peroxidase (HRP) was measured. Untreated control cells and interferon-treated cells both internalized HRP at a rate which was linear with respect to time and to the HRP concentration in the medium (Fig. 1A and B). However, the rate of HRP uptake in the interferon-treated cells was only about 20% of the control rate (Fig. 1A). The uptake of fluorescein-dextran was also inhibited, and examination by fluorescence microscopy (8) suggested that all the cells in the population were inhibited to a similar extent (data not shown).

To determine whether interferon treatment inhibits pinocytosis by altering the interaction between pinocytic vesicles and lysosomes, the subcellular distribution of internalized HRP was analyzed. Confluent monolayers of L cells were treated with crude mouse interferon (1,000 U/ml) for 24 h before HRP was added. The cells were then homogenized, and the postnuclear supernatants were centrifuged in isopycnic sucrose density gradients. The distribution of the HRP internalized by interferon-treated and untreated cells paralleled the distribution of the lysosomal marker *N*-acetyl-β-glucosaminidase (Fig. 2).

When L cells were treated for 24 h with increasing concentrations of crude mouse interferon, slight inhibition of HRP uptake was apparent at concentrations of 1 and 10 U/ml, and 50% inhibition was observed at 100 U/ml. At the highest dose tested (1,000 U/ml), HRP uptake was inhibited by 75% (Fig. 3). Similar dose-response data were obtained when L cells were treated with a purified mouse interferon preparation (Fig. 3).

HRP uptake was analyzed as a function of time after the addition of 1,000 U of interferon per ml to L cells. There was no detectable effect on HRP uptake at 4 h; however, by 8 h after the addition of the inhibitor, a 25% reduction was seen. By 14 h the rate of uptake had decreased to 1.3 ng/mg per min, a 50% reduction. The greatest inhibition (a decrease to 28% of control levels) was observed at 24 h (Fig. 4).

Cells which had been treated with 1,000 U of interferon per ml for 24 h were then incubated in interferon-free medium. After 24 h the uptake of HRP increased from 29 to 54% of control levels; by 72 h the HRP uptake was 97% of control levels (Fig. 5).

The effect of homologous and heterologous interferons was tested in mouse, human, and chicken embryo cells. Marked inhibition of HRP uptake was observed only in cells treated with homologous interferon (Table 1). A slight inhibitory effect occurred in heterologous cells, and this observation is consistent with reports of minor cross-species interferon activity (19).

The rate of pinocytosis and its response to interferon treatment varied in different lines of L cells. When HRP uptake was tested in a line of L

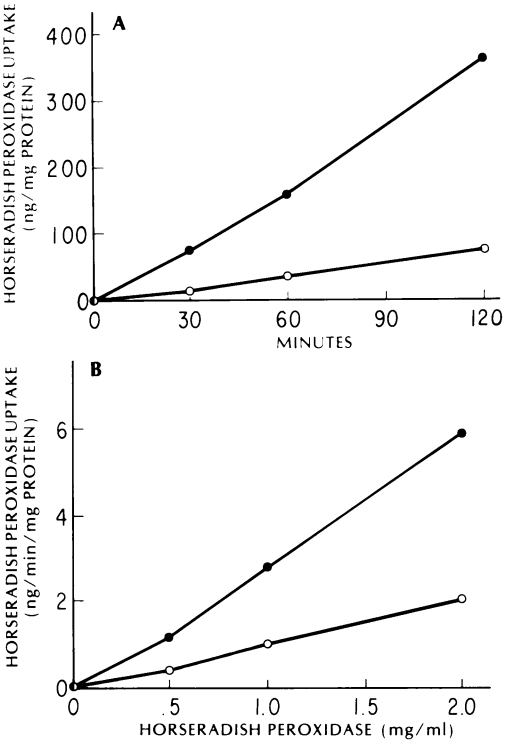

FIG. 1. Effect of interferon treatment on HRP uptake by L cells. L cells were cultured at 37°C in an atmosphere containing 5% CO_2 in Eagle minimal essential medium with 4% calf serum, penicillin, and streptomycin (26); confluent monolayers in 35-mm dishes were preincubated with complete medium (●) or complete medium supplemented with 1,000 U of crude mouse interferon per ml (○), which was prepared in L cells infected with Newcastle disease virus (27). HRP uptake by the cells was analyzed as described elsewhere (18). Each point represents the average of triplicate determinations. (A) L cells incubated for the times indicated in complete medium containing 1 mg of HRP per ml. (B) L cells incubated for 60 min in complete medium containing the indicated concentrations of HRP.

cells obtained from J. A. Lewis, the rate of pinocytosis was less than 30% of that seen in the L cells used here, and treatment with 1000 U of interferon per ml for 24 h caused a minimal (20%) inhibition of HRP uptake.

We showed that pinocytosis is inhibited in interferon-treated mouse, human, and chicken embryo fibroblasts treated with homologous, but not heterologous, interferon preparations. In mouse L cells, similar inhibition was obtained with both crude and purified interferon preparations, and crude mouse interferon caused the same slight inhibition in human cells as did purified interferon. It is thus unlikely that the effect was due to a contaminant in the preparation, although further studies will be required to establish this unequivocally.

A variety of agents have been shown to inhibit pinocytosis. Inhibition in cells treated with chloroquine, methylamine, or monensin (1, 11, 20, 25) is apparently the consequence of an effect on the intracellular events involved in the recycling of plasma membrane, and all three compounds cause extensive formation of vacuoles. In contrast, the inhibition of pinocytosis by interferon does not result in vacuole formation within the cell (unpublished data). The finding that HRP taken up by interferon-treated cells is transferred to lysosomes (Fig. 2) suggests that the interaction between pinocytic vesicles and lysosomes is not affected by interferon treatment. The most probable site of action for the interferon effect, then, is the cell surface; the inhibition of pinocytosis could be the consequence of a reduced rate of formation or stabilization of pinocytic vesicles. Alterations of the plasma membrane or of associated cytoskeletal elements (23) might be responsible for these changes.

It is well established that interferon treatment alters the distribution of structural proteins of

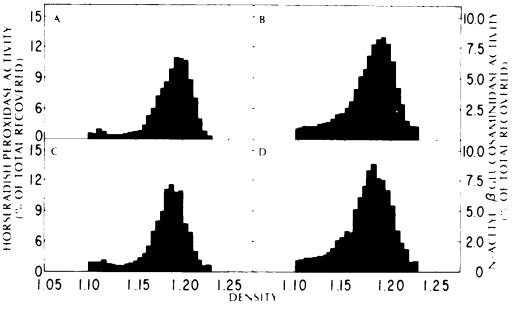

FIG. 2. Effect of interferon treatment on the subcellular distribution of HRP taken up by L cells. Confluent monolayers of L cells in 100-mm dishes were preincubated for 24 h in complete medium (A and B) or complete medium containing 1,000 U of crude interferon per ml (C and D). The cells were then incubated in complete medium containing 2 mg of HRP per ml for 2 h; the uptake of HRP by control and interferon-treated cells was 7.4 and 2.1 ng/mg per min, respectively (an inhibition of 71%). Postnuclear supernatants prepared after homogenization of the cells were subjected to isopycnic density gradient centrifugation and assayed for HRP (A and C) and for *N*-acetyl-β-glucosaminidase (B and D) as described elsewhere (10, 21). The recovery of *N*-acetyl-β-glucosaminidase from the gradients was 86 and 82% for control and interferon-treated cells, respectively, and recovery of HRP was 101 and 105%, respectively. The distribution of markers for other organelles (succinate dehydrogenase for mitochondria, externally iodinated proteins for plasma membrane) in the gradients was essentially as described by Tulkens et al. (21) for rat fibroblasts (not shown).

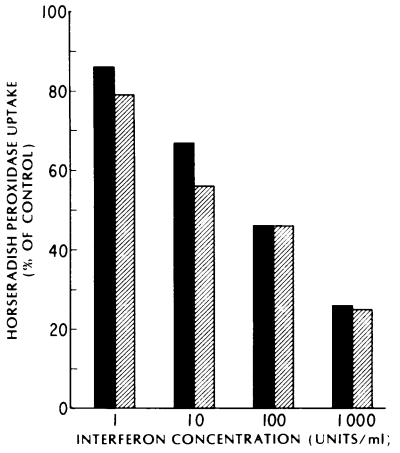

FIG. 3. Effect of interferon concentration on HRP uptake by L cells. L cells were preincubated for 24 h with either crude mouse interferon (solid bars) or a purified mixture of α and β mouse interferons (hatched bars) before incubation with 1 mg of HRP per ml for 60 min. The purified interferon (2.7×10^5 U/mg of protein) was obtained from Lee Biomolecular Laboratories, Inc. Uptake of HRP in control cells was 3.2 ng/min per mg of cell protein (average of two experiments) in the experiments measuring effect of the purified mouse interferon and 3.1 ng/min per mg (average of two experiments) for those measuring the effect of crude interferon. Other details are described in the legend to Fig. 1.

the cytoplasm (23; Tamm et al., in press). Although it is likely that the effects on cytoskeletal elements could explain the inhibition of pinocytosis (16), further work is required to establish a

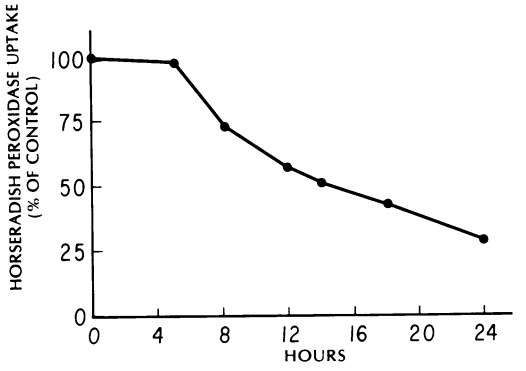

FIG. 4. Time course of inhibition of HRP uptake by L cells treated with interferon. L cells were incubated for the indicated times in the presence of 1,000 U of crude mouse interferon per ml. The uptake of HRP was determined after incubating the cells with 1 mg of HRP per ml for 60 min. Uptake of HRP by control cells was 2.5 ng/min per mg (average of two experiments), which was constant for the duration of the experiment. Other details are described in the legend to Fig. 1.

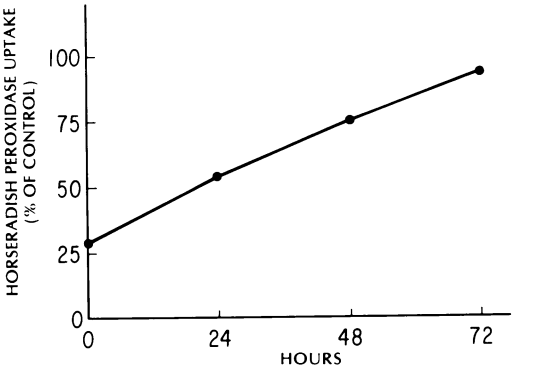

FIG. 5. Effects of incubation in interferon-free medium on HRP uptake by L cells pretreated with interferon. L cells were pretreated for 24 h with 1,000 U of crude mouse interferon per ml and then incubated for the times indicated in interferon-free medium. Uptake of HRP was measured after incubation with 1 mg of HRP per ml for 60 min at 37°C in complete medium. Uptake by control cells during the experiment was 3.7 ± 0.2 ng/min per mg; this remained constant during the experiment. Other details are described in the legend to Fig. 1.

cause and effect relationship between the two phenomena. The availability of lines of L cells which react differently to interferon should be useful for this purpose.

The concentration of interferon (100 U/ml) required to produce 50% inhibition of pinocytosis was significantly higher than the concentration (1 U/ml) needed to restrict virus replication. However, the higher concentrations of interferon required to inhibit pinocytosis are similar to the levels needed to protect cells against protozoa (22), bacteria (4), and chlamydia (6) or to inhibit cell growth (14).

Although the biological significance of the interferon-mediated inhibition of pinocytosis remains to be established, it is evident that the inhibition of uptake of intracellular pathogens could explain, at least in part, the protective action of interferon against such agents. In this connection, we showed that interferon treatment can inhibit the uptake of a virus (vesicular stomatitis virus) which enters the cell by pinocytosis (24). The inhibition of pinocytosis could also contribute to the effect of interferon on cell growth. The function of pinocytosis is not yet established (17), although uptake of nutrients and monitoring of the integrity of the cell surface (13) are among the more likely possibilities. Inhibition of either of these processes could influence the growth rate of cells. Interferon may thus represent a useful inhibitor for elucidating the biological significance of pinocytosis.

TABLE 1. Species specificity of interferon-mediated inhibition of HRP uptake

Interferon[a]	Inhibition of HRP uptake (%)[b] in:		
	Mouse L cells	Human diploid fibroblasts[c]	Chicken embryo fibroblasts[d]
Mouse			
Crude	67	12	21
Purified	65	20	ND[e]
Human	24	67	ND
Chicken (crude)	15	ND	64

[a] Cells were treated with 1,000 U of interferon per ml for 24 h. Mouse interferon was prepared as described in the legends to Fig. 1 and 3. Chicken interferon (2.4×10^3 U/ml) was prepared as described elsewhere (5). Purified human α interferon (2.3×10^5 U/mg) was obtained from Lee Biomolecular Laboratories.

[b] The uptake of HRP (determined with 1 mg of HRP per ml of medium) in mock-treated control cells was 3.1, 3.8, and 2.0 ng/min per mg of protein for mouse, human, and chicken embryo cells, respectively.

[c] Human diploid fibroblasts were obtained from John Armstrong, University of Pittsburgh. They were cultured as described in the legend to Fig. 1, except that calf serum was replaced with fetal calf serum.

[d] Chicken embryo fibroblasts were cultured as described elsewhere (26).

[e] ND, Not determined.

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