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Inhibition of Viral Replication by Interferons with Different Molecular Weights.* (31606)

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It has been reported that Newcastle disease virus (NDV), when inoculated into mice or cell cultures, stimulates the appearance of interferon with a molecular weight of about 30,000(1,2). In contrast, in mice injected with *E. coli* endotoxin(2) or statolon(3), an anionic polysaccharide from *Penicillium stoloniferum*, the interferon which appears in the circulation has a molecular weight of about 90,000. Except for their molecular weights, it has been impossible to distinguish the inhibitors produced in mice by nonviral stimuli from the interferon produced by virus (1,2,3). An attempt was made in our laboratory to determine the rate of adsorption of the different molecular weight mouse interferons to homologous cells. In agreement with the results of Buckler *et al*(4), we were unable to show the disappearance of interferon activity from fluid exposed to large numbers of mouse cells. In light of these indications that interferons can render cells resistant to virus in the absence of detectable uptake, it was decided to study the rates at which cells develop resistance to virus challenge after exposure to interferons with markedly different molecular weights.

Materials and methods. Cell cultures and media. L-cells (clone 929) were grown in 32 oz prescription bottles for 7 days with one change of Eagle's medium (MEM) supplemented with 10% calf serum. For interferon assays, all dilutions were made in Eagle's medium supplemented with 4% calf serum.

Interferon was produced in L-cells or in mice as follows. L-cell cultures were inoculated with the Herts strain of Newcastle disease virus (NDV) to give an input multiplicity of 5 to 10 infective particles per cell. After 1 hour of adsorption at 37°C, the inoculum was removed, the cell sheet was washed once, and fresh medium without serum was added to each bottle. After incubation at 37°C for 24 hours, the fluid was removed and clarified by low speed centrifugation. The fluid was brought to pH 2 with 0.1 N HCl, held for 5 days at 5°C to destroy infective

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virus, and then neutralized with 0.1 N NaOH. This pool contained 3,000 units of interferon per ml when assayed against vesicular stomatitis virus (VSV) by a plaque-inhibition technique which has been described elsewhere (5).

For *in vivo* production of interferon, mice were injected in the tail vein with 0.1 ml volume containing either 250 μg of *E. coli* (0113) endotoxin or 1000 μg of statolon. Blood was obtained by cardiac puncture using heparin-rinsed syringes, and plasmas were pooled prior to testing for interferon activity. Mice injected with endotoxin or statolon were bled at 2 or 8 hours, respectively, the time of maximum titer of interferon in the circulation. The plasmas contained 900 and 1,800 units of interferon per ml, respectively, for endotoxin- and statolon-stimulated interferon. The properties of the interferons produced by NDV, endotoxin, and statolon have been described(1,2,3).

Experimental. To study the rate at which cells developed resistance when exposed to the different interferons, cells were treated with a dose of interferon capable of producing complete inhibition of plaque formation. First, the smallest dose of inhibitor which completely prevented plaque formation when cells were exposed for 24 hours was determined. A multiple of this minimum 100% protective dose was then utilized in further experiments. For the NDV-, endotoxin-, and statolon-stimulated interferons, respectively, 10, 8, and 16 100% plaque inhibiting doses were employed.

L-cell monolayers in 60 mm petri dishes were treated with 3 ml of the different interferons, diluted to contain the desired inhibitor concentrations. Control cultures were treated with medium alone. After 1 hour of incubation at 37°C, half of the plates in the interferon-treated and control groups were washed 3 times with 5 ml of medium to remove the interferon. Six plates were immediately challenged with 40-60 PFU of VSV and overlaid with agar after 1 hour of adsorption. Medium (3 ml) was added to the other washed monolayer cultures and incubation at 37°C was continued. At intervals, the medium was removed from 6 cultures and a challenge dose of VSV added. Six control

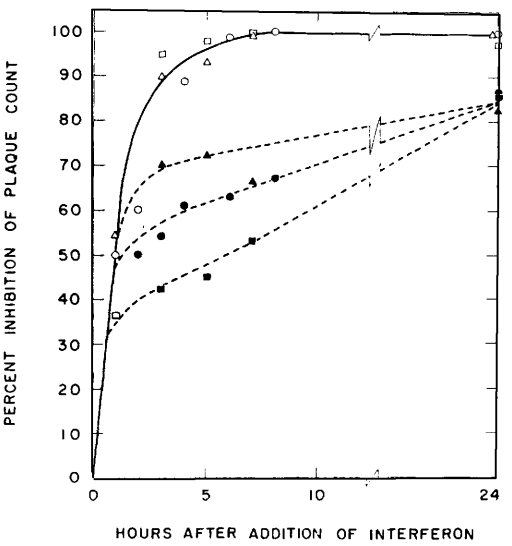

FIG. 1. Rate of development of resistance in L-cells exposed to interferons with different molecular weights. Interferons were prepared in L-cell cultures infected with NDV (circles), and in mice injected intravenously with *E. coli* endotoxin (triangles) or statolon (squares). In one set of cultures, the appropriate dose of interferon was left on cells for 1 hour, after which the cells were washed and tested for resistance after further incubation at 37°C (- - - -). In the other set of cultures the interferon was left on the cells throughout the entire experiment and resistance to infection with VSV was tested at the indicated times (——).

cultures, incubated with medium alone, were also challenged at each time interval. By this technique, we determined the rate of development of resistance to virus challenge after a one-hour exposure to the different interferons.

The rest of the cultures, which remained in contact with interferon, were washed and 6 cultures were challenged with VSV at each of the indicated times. Control cultures incubated with medium alone were washed and challenged at identical intervals. In this manner, the rate of development of resistance to virus challenge imparted by continuous exposure to the different interferons was tested.

The data presented in Fig. 1 show that after 1 hour of exposure to NDV- and endotoxin-stimulated interferons, the resistance of the cells was appreciable, the plaque count being reduced to about 50% of that obtained with untreated control cultures. With stato-

lon-stimulated interferon the inhibition obtained at 1 hour was 36%. When the interferons were left in contact with the cells, the inhibition rose to 90 to 95% at 3 hours, and to virtually 100% at 5 hours.

In the case of cultures exposed to the interferons for 1 hour and then washed, there was a gradual increase in resistance of the cultures to VSV. Even with only 1 hour of exposure, virus plaque inhibition had reached about 85% by 24 hours with all 3 interferons. A slower rate of development of cellular resistance was observed consistently with statolon. However, the differences from the other interferons were of minimum significance when compared to the error of the plaque inhibition method.

Discussion. Studies have been published by Lindenmann *et al*(6), Wagner(7), and Ho (8) which indicate that chick embryo cells develop an increasing resistance to virus infection when exposed to interferon for a short time, and then incubated at 37°C. When kept in contact with interferon, cells showed virtually complete resistance to virus after 3 to 4 hours of exposure(8).

The results presented here show that these observations not only can be extended to the mouse system, but also to interferons with markedly different molecular weights. No differences were seen when interferons produced by viral and nonviral stimuli were employed. The more rapid development of resistance in the cultures continuously exposed to interferon probably is a reflection of more rapid induction of new protein synthesis which is required before interferon-treated cells become resistant to virus infection(9-12).

Summary. No differences were found in the rates at which mouse cells develop resistance to virus challenge after exposure to interferons with markedly different molecular weights (30,000 or 90,000). These interferons were made in L-cell cultures in response to NDV or in intact mice stimulated with *E. coli* endotoxin or statolon. When kept in contact with interferon, cells showed virtually maximum resistance to virus after 3 to 4 hours of exposure. Exposure to interferon for 1 hour was followed by a gradual increase of resistance of cells to virus infection over the next 23 hours.

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