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The Lang Strain of Reovirus Serotype 1 and the Dearing Strain of Reovirus Serotype 3 Differ in Their Sensitivities to Beta Interferon

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Replication of the Dearing strain of reovirus serotype 3 in mouse L cells was decreased 17- to 100-fold when a saturating dose of beta interferon (1,000 IU/ml) was used. Replication of the Lang strain of reovirus serotype 1 was inhibited only two- to threefold under similar conditions. It therefore appears that closely related strains of reovirus differ in their sensitivities to beta interferon treatment of mouse L cells.

Treatment of cells in culture with interferon (IFN) inhibits the replication of many families of viruses (see references 10 and 12 for recent reviews). Saturating doses of IFN can decrease replication of sensitive viruses more than 1,000-fold (12). In the case of the Dearing strain of reovirus serotype 3 (T3 Dearing), treatment of mouse L cells with type I IFN has been reported to decrease virus replication nearly 100-fold (20). Translation of reovirus mRNA to make viral proteins seems to be the step of virus replication most affected by IFN treatment (20). Interference in the translation of viral mRNA into protein may be the result of activation of the double-stranded-RNA-dependent protein kinase in virus-infected IFN-treated cells.

We have recently shown that mouse L cells infected with the Lang strain of reovirus serotype 1 (T1 Lang) contain an inhibitor of the IFN-induced protein kinase (2). The double-stranded-RNA-binding σ3 polypeptide (1) appears to be both necessary and sufficient for the kinase-inhibitory activity detected in extracts prepared from T1 Lang-infected cells (2). The presence of activity inhibitory for the IFN-induced protein kinase in reovirus-infected cells is a conundrum, since at least T3 Dearing has been reported to be sensitive to the antiviral effects of IFN (20) and to lead to the activation of the IFN-induced protein kinase in IFN-treated virus-infected L cells (13). Since T1 Lang and T3 Dearing appear to differ in the amount of kinase-inhibitory activity induced during viral infection (3), we decided to compare the sensitivities of these two strains to treatment of mouse L cells with IFN-β.

In order to compare the sensitivities of reoviruses T1 Lang and T3 Dearing to IFN, we treated confluent monolayer cultures of mouse L cells in minimal essential medium supplemented with 5% fetal calf serum with partially purified mouse IFN-β (1.3×10^8 IU/mg; Lee Biomolecular) or with a mock preparation of IFN (<32 IU/mg; Lee Biomolecular). After 24 h, the IFN-containing medium was removed and the cells were infected with T1 Lang or T3 Dearing at an equivalent multiplicity of infection (MOI) of 5 to 20 PFU per cell in minimal essential medium containing 1% fetal calf serum. Progeny virus was harvested after a single cycle of virus replication (24 to 32 h postinfection) and assayed by plaque formation on L cells. The composite results of three such experiments are shown in Fig. 1. As previously reported by Wiebe and Joklik (20), replication of T3 Dearing was inhibited approximately 100-fold by treatment of L cells with a saturating dose of IFN (100 to 1,000 IU/ml). In parallel experiments, IFN treatment only marginally affected replication of T1 Lang, leading to a two- to threefold reduction in production of progeny virus. Figure 2 shows the results of [^{35}S]methionine labeling performed on one of the sets of cultures for which composite results are shown in Fig. 1. Treatment of cells with 1,000 IU of IFN-β per ml decreased T3 Dearing protein synthesis 80 to 90%, while such treatment did not detectably decrease T1 Lang protein synthesis.

We have also analyzed replication of T1 Lang and T3 Dearing in cells treated with a saturating dose of IFN-β (1,000 IU/ml) as a function of time postinfection compared with replication in untreated cells. As shown in Fig. 3A, infectious progeny could be detected first at 12 h postinfection in T3 Dearing-infected cells not treated with IFN. IFN treatment slowed replication of T3 Dearing by at least 4 h. Production of infectious virus continued to slowly increase through 32 h postinfection. At all time points, IFN treatment decreased the production of infectious virus at least 10-fold.

Replication of T1 Lang (Fig. 3B) was approximately 4 h slower than replication of T3 Dearing. IFN treatment further slowed replication of T1 Lang by approximately 4 h. However, by late times postinfection (28 to 32 h postinfection), there was little decrease in replication of T1 Lang in IFN-treated cells compared with that in untreated cells. Similar results were seen when replication of reovirus was monitored by assaying for the incorporation of [^{35}S]methionine into viral proteins (data not shown).

On the basis of the rather long replication cycle of reovirus, and especially the somewhat longer replication cycle of T1 Lang than that of T3 Dearing, we thought that the strain differences that we have detected in IFN sensitivity might be due to differences in the sensitivities of the two strains to decay in the IFN-induced antiviral state. We therefore analyzed the sensitivities of the two viruses to continuous treatment with a high dose of IFN (10,000 IU/ml) rather than to pretreatment. Continuous treatment with 10,000 IU of IFN-β per ml decreased replication of T3 Dearing 50-fold and replication of T1 Lang 5-fold.

We detected an increased sensitivity of reovirus T3 Dearing to IFN treatment compared with that of T1 Lang by using partially purified virus (pelleted through a sucrose pad) and partially purified virus treated with antiserum to mouse IFN-α/β (data not shown). Differences in sensitivity to IFN for these two strains of reovirus were detected in subconfluent as well as confluent cultures of mouse L cells (data not shown). Addition of antiserum to mouse IFN-α/β to the medium during the virus replication cycle did not affect the

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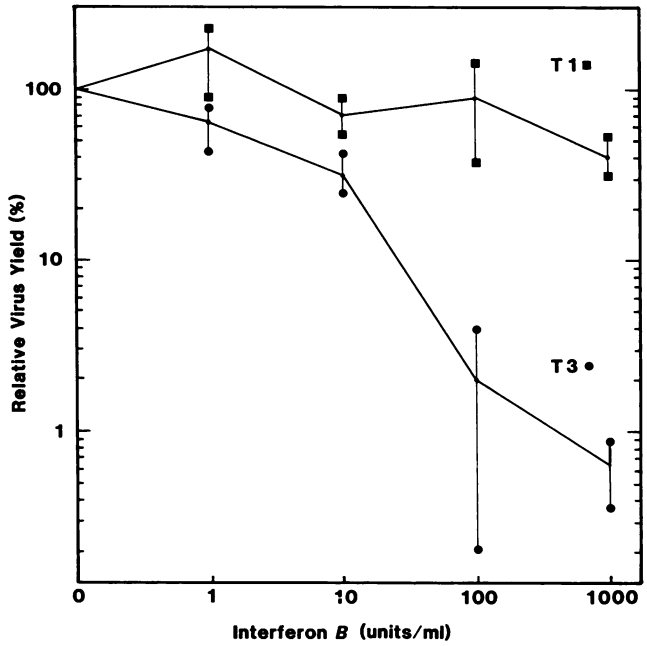

FIG. 1. Dose dependence of inhibition of reovirus replication after treatment of mouse L cells with IFN-β by using a virus yield reduction assay. Mouse L cells were treated and lysed and progeny virus was harvested as described in the text. The graph shows the average of the results of three independent experiments. In each of the three experiments, the MOIs for T1 Lang (■) and T3 Dearing (●) were the same.

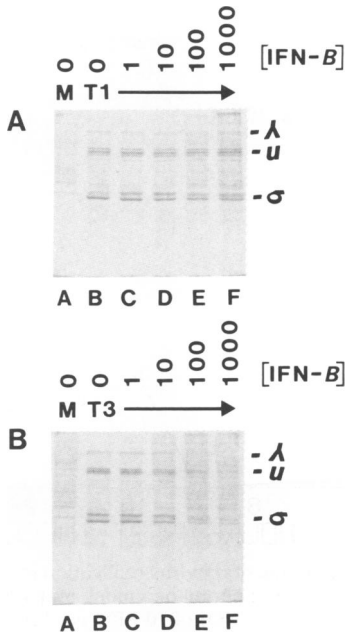

FIG. 2. Dose dependence of inhibition of reovirus protein synthesis after treatment of mouse L cells with IFN-β. Results of infection with T1 Lang (A) and T3 Dearing (B), both at an MOI of 10 PFU per cell, are shown. For one of the cultures for which the results are shown in Fig. 1, cells were labeled with [^{35}S]methionine for 30 min at 18 h postinfection and detergent lysates were prepared. Cells were pretreated with a mock preparation of IFN (lanes A and B) or with 1 (lanes C), 10 (lanes D), 100 (lanes E), or 1,000 (lanes F) IU of IFN-β per ml. For lanes A, cells were mock infected. Markers show the positions of the reovirus λ, μ, and σ proteins.

sensitivities of these viruses to pretreatment with IFN (data not shown).

All of the experiments described above were performed by using cells infected at an MOI high enough to guarantee simultaneous infection of more than 90% of the cells in the culture. To assay for IFN sensitivity, using a multicycle assay, we infected monolayers of IFN-treated or untreated L cells with approximately 100 or 1,000 PFU per plate. After virus adsorption, the cells were overlaid with agarose-containing medium supplemented with IFN or unsupplemented. Plaques were counted after 4 to 6 days. IFN treatment completely suppressed plaque formation by T3 Dearing, even when cultures were infected with approximately 1,000 PFU per plate. IFN treatment decreased plaque formation by T1 Lang 5- to 10-fold, although plaques were clearly visible, even on plates infected with approximately 100 PFU per plate.

These results suggest that reovirus T1 Lang and T3 Dearing differ markedly in the sensitivity of virus replication to treatment of mouse L cells with IFN-β. T3 Dearing was consistently more sensitive to the antiviral effects of IFN-β than was T1 Lang, with all of the protocols that we have tested. Analogous differences in sensitivity of reovirus replication to treatment with mouse IFN-β have been observed for independent isolates of T1 Lang and T3 Dearing and for an independent isolate of mouse L cells (4).

Previously, other workers have noted that T3 Dearing is a better IFN inducer than T1 Lang (15). We do not believe that the differences in sensitivity that we have detected are due to differences in induction of IFN during the virus replication cycle. Addition of antiserum to mouse IFN-α/β during the virus replication cycle did not rescue T3 Dearing from the effects of pretreating cells with IFN-β, suggesting that the presence of IFN during the virus replication cycle was not necessary for sensitivity to IFN. Addition of a large dose of IFN-β (10,000 IU/ml) during the virus replication cycle, after pretreatment, did not increase the sensitivity of T1 Lang compared with that of T3 Dearing. These results suggest that the IFN resistance of T1 Lang is not due to a lack of IFN during the virus replication cycle.

IFN sensitivity may vary, depending on the cell type used. Replication of reovirus T3 Dearing in human U cells (14), 293 cells (our unpublished observations), and, in some cases, HeLa cells (6, 7) is resistant to the antiviral effects of type I IFN, while replication of reovirus T3 Dearing in mouse L cells is sensitive to the antiviral effects of type I IFN (20). The results presented in this paper suggest that closely related strains of reovirus may also differ in their sensitivities to IFN treatment in the same cell line. Closely related strains of mengovirus (16) and herpes simplex virus (17) have previously been reported to differ in their sensitivities to IFN.

Resistance of adenovirus and vaccinia virus to IFN has been correlated with the presence of inhibitors of several of the IFN-induced enzymes. Both adenovirus and vaccinia virus appear to code for inhibitors of the IFN-induced protein kinase (5, 8, 9, 11, 18, 19). We have recently reported that reoviruses T1 Lang and T3 Dearing encode an inhibitor of the IFN-induced protein kinase (2, 3). The structural σ3 protein appears to be both necessary and sufficient for this kinase-inhibitory activity (2). The strains of reovirus used in the studies we report in this paper differ in the amount of σ3

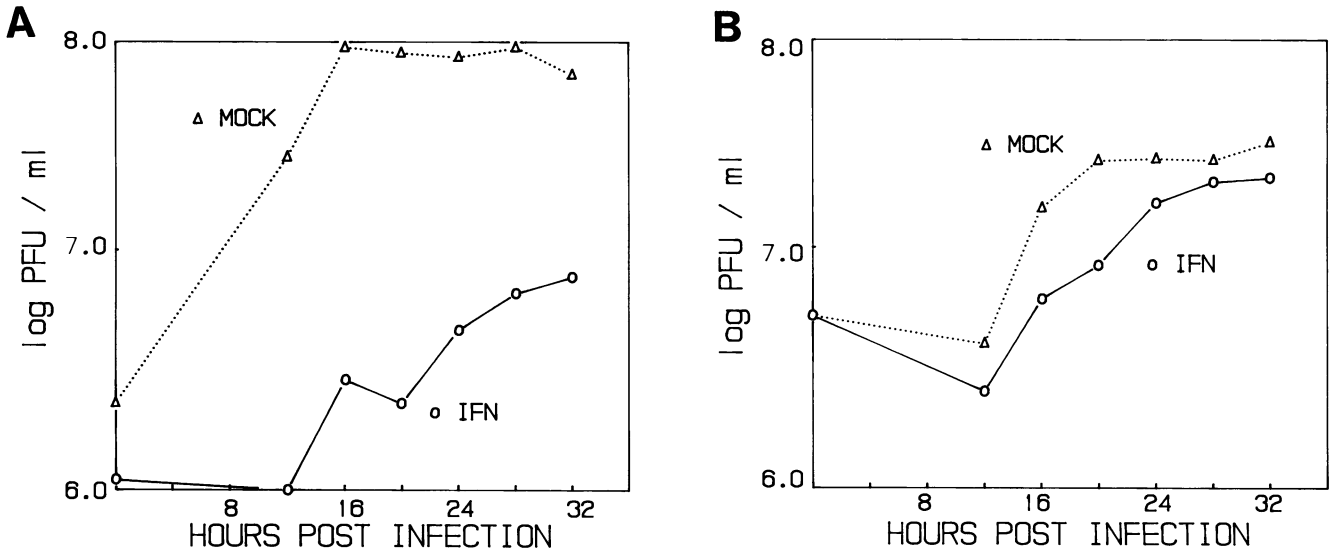

FIG. 3. Time course of reovirus replication in mouse L cells treated with IFN-β. Confluent monolayers of mouse L cells were treated with either 1,000 IU of IFN-β per ml or a mock preparation of IFN, as described in the text. After 24 h, cells were infected with either T3 Dearing (A) or T1 Lang (B) at an MOI of 10 PFU per cell. At the indicated times, cultures were harvested and the amount of infectious progeny was determined by plaque assay on mouse L cells.

and kinase-inhibitory activity induced in infected cells (3). While it is tempting to speculate that the differences in IFN sensitivity that we have described in this paper might be due to the differences in kinase-inhibitory activity induced by the two viruses, a genetic analysis of reassortants between the two reovirus serotypes is needed to determine whether these respective differences map to the same genes.

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