Mx Gene Control of Interferon Action: Different Kinetics of the Antiviral State Against Influenza Virus and Vesicular Stomatitis Virus

HEINZ ARNHEITER* AND OTTO HALLER*

Institute for Immunology and Virology, University of Zurich, Zurich, Switzerland

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The allele Mx regulates the extent to which interferon alpha/beta inhibits the growth of influenza viruses in mouse cells such as peritoneal macrophages. The time course of induction of the antiviral state against an influenza A virus is comparable in macrophages with and without Mx and is similar to that found with vesicular stomatitis virus. In contrast, the decay of the antiviral state against influenza virus is markedly slower in Mx-positive cells and slower than that against vesicular stomatitis virus observed in either Mx-positive or Mx-negative cells. Thus, after removal of interferon alpha/beta, Mx-positive cells remain protected against influenza virus at times when they have lost protection against vesicular stomatitis virus. These results suggest that interferon alpha/beta treatment activates different antiviral mechanisms, each acting against distinct groups of viruses and each independently controlled by host genes.

Cells treated with interferon (IFN) require several hours of incubation to develop antiviral protection. When IFN is removed, protection decays until full susceptibility to viral infection is regained. Thus, the time course with which the antiviral state is induced and subsequently decays may influence the IFN sensitivity of a distinct cell type or virus.

We have previously reported that alleles at the Mx locus of a mouse selectively influence the potency of IFN alpha/beta to inhibit influenza virus replication (1, 4–7). The allele Mx enhances the protective effect of IFNs against influenza viruses without influencing the protection against other viruses (1, 7). We have now analyzed the effect of the Mx allele on the kinetics of the development and the decay of the antiviral state against an influenza A virus and vesicular stomatitis virus (VSV), using virus yield reduction in mouse peritoneal macrophages as a simple assay.

Peritoneal macrophages were harvested from A2G mice (homozygous for Mx0) and A/J mice (lacking Mx) as described previously (5, 7). Cells were cultured in 24-well tissue culture trays (10⁶ cells per well) for 3 weeks before use in an experiment. We found that this time was required for these primary cultures to express uniform susceptibility to viral infections (5, 7).

To assay the time course of the development of the antiviral state, A2G and A/J cells were exposed to various concentrations of mouse IFN alpha/beta, induced by Newcastle disease virus in C243 sarcoma cells and partially purified to 10⁷ U per mg of protein (15), or to control medium. After removal of IFN, cultures were washed with medium containing sheep antibodies to mouse IFN (AIFN) at a dilution sufficient to neutralize 10,000 U of IFN (3). The cells were then infected with the influenza A virus M-TUR (11) at a multiplicity of infection of 10 in the further presence of AIFN. Virus yields are shown in Fig. 1.

A2G cells responded to lower doses of IFN and appeared to acquire their antiviral state more rapidly that did A/J cells. It is likely that these kinetic differences are a direct reflection of the higher IFN sensitivity of A2G cells; in cases in which a similar maximal protection was achieved on A2G and A/J cells (using a high dose of IFN on A/J cells and a low dose on A2G cells), the time course of induction of the antiviral state was similar in the two cell types. In addition, the time course of induction obtained in these macrophage cultures was comparable to those obtained in established cell lines challenged with different viruses (2, 13). With VSV (1) as the challenge virus, no cell-dependent differences in the development of the antiviral state were seen (Fig. 1c).

To assay the kinetics of the decay of the antiviral state, macrophage cultures were ex-

† Present address: Laboratory of Molecular Genetics, National Institute of Neurological and Communicative Diseases and Stroke, Bethesda, MD 20205.
posed to various IFN concentrations for 18 h. IFN was then removed and replaced by AIFN, and at various time intervals thereafter, cultures were infected, and virus yields were assayed 18 h later.

A representative decay experiment is shown in Fig. 2. With increasing time of incubation after removal of IFN, viral titers increased. In A2G cells challenged with M-TUR virus, the antiviral protection was long lasting. For instance, after 40 U of IFN per ml, susceptibility was not regained over the test period of 10 days (Fig. 2a). In A/J cells, the antiviral state was short lived. Full susceptibility to M-TUR virus was found 1 to 4 days after removal of IFN, even when high doses were used (Fig. 2b). Thus, the decay of protection observed with M-TUR virus in A2G cells was unusually slow—in fact, slower than most decay rates so far described (14)—even when cells in a quiescent stage (similar to our nondividing macrophages) were used (9).

With VSV as challenge, no such differences in the rates of the decay of the antiviral state were found, and the time course of the decay was similar to that seen in A/J cells challenged with M-TUR virus and was also similar to that observed in other mouse (14) or human (2) systems (Fig. 2c). Thus, A2G cells remained protected against M-TUR virus at a time when they had regained full susceptibility to VSV, indicating that the antiviral states against M-TUR virus and VSV decayed independently. It has been reported previously (8) that decay rates of IFN-induced resistance vary with the test virus used. However, these studies dealt with one cell type, and it was therefore impossible to decide whether the different viruses exhibited different sensitivities to a common antiviral mechanism or whether different viruses were inhibited by distinct mechanisms, each of which would decay with its own kinetics, as discussed by Hallum (8). Because in our case the IFN sensitivity of influenza virus varied with the host cell genotype, whereas that of VSV did not, we can exclude the possibility of differences in the sensitivity of these two viruses to a common antiviral mechanism. This is in agreement with the fact that IFN treatment may inhibit replication of different viruses in different ways and at different steps of replication (10). These and our present observations support the hypothesis that IFN activates several defense mechanisms, each inhibiting certain groups of viruses (4).

To test whether the differences in the decay rates were relevant for the time course of virus growth after IFN treatment, A2G and A/J cells
were infected in the presence of AIFN after IFN treatment, and viral titers were determined at various time points after infection (Fig. 3). In A/J cells, growth of M-TUR virus was reduced for only a short period of time, and final viral titers were as high as in non-IFN-treated cultures. In A2G cells, however, viral titers never reached levels found in non-IFN-treated control cells, even when low doses of IFN (e.g., 4 U/ml) had been used to protect the cells. With VSV as challenge virus, no such difference between A2G and A/J cells was observed (Fig. 3c and d). This suggests that the rate of decay of the antiviral state is a relevant parameter for the time course of viral growth.

Cells from A2G and A/J animals differ at many loci besides $Mx$ (4). If the slow decay of the antiviral state in IFN-treated macrophages and resistance of animals in vivo were due to the same allele, the two properties should cosegregate in appropriate backcross animals. Earlier genetic studies have indicated that $Mx$ is a dominant allele (12) and that among backcross animals segregating for $Mx$, only those animals carrying $Mx$ have macrophages capable of expressing resistance to influenza virus in vitro (11). We therefore tested (A/J × [A2G × A/J] $F_1$) backcross mice for resistance to infection with M-TUR virus, and we tested the corresponding macrophages for decay of the antiviral state in culture.

Twenty-nine backcross animals were earmarked, and several individual cultures were established from the macrophages of each animal. The cultures were kept for 3 weeks to allow for uniform expression of susceptibility to in vitro infection. One culture from each animal was then treated with IFN, washed, and exposed to AIFN for 6 days. When now infected with M-TUR virus, macrophages from 13 animals showed no cytopathic effect after infection, and 18-h virus yields were $10^3$ to $10^4$ times lower than those from corresponding cultures not treated with IFN. Macrophages from the remaining 16 animals showed massive cytopathic effect, with titers as high as in control cultures. The mice were then infected with a lethal dose of M-TUR virus. Fifty percent of these animals were expected to carry the allele $Mx$ and to survive infection. In good approximation, 13 mice survived, and 16 died. In addition, survival or death of an individual mouse corresponded to virus resistance or susceptibility of its IFN-treated macrophages.

**FIG. 2.** Decay of the antiviral state. Macrophage cultures were treated with IFN alpha/beta at the doses indicated (units per milliliter) for 18 h, starting on different days. IFN was removed, and the cultures were kept in the presence of antibodies to IFN (3) for the time periods indicated. The cultures were then simultaneously infected with either M-TUR virus (a and b) or VSV (c) in the further presence of anti-IFN antibodies. Supernatant fluids were harvested 18 h thereafter. The mean titers from four independent cultures are given. (a) A2G cells; (b) A/J cells; (c) A2G (○) and A/J (●) cells. Asterisks indicate titers in control cultures not treated with IFN.
Thus, when infected 6 days after IFN treatment, macrophages prepared from mice surviving the infection were protected, and those from mice that succumbed to infection were not. Although based on a small number of backcross animals, these results suggest that resistance of animals in vivo and the slow decay of the antiviral state of cells in culture are genetically linked, and that both traits are due to the same allele, \( M_x \).

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**LITERATURE CITED**


**FIG. 3.** Virus growth in macrophages treated with IFN. Peritoneal macrophages were exposed to IFN alpha/beta at the doses indicated (numbers indicate units per milliliter) for 18 h and then were infected with either M-TUR virus (a and b) or VSV (c and d) in the presence of antibodies to IFN (3). After being washed, antibodies were kept present during the entire incubation period. Samples of the supernatant fluids were removed at the times indicated, titrated for infectivity, and replaced with fresh medium. The viral titers represent the mean value from four cultures. (a and c) A2G macrophages; (b and d) A/J macrophages.
