Herpes Simplex Virus 1 Precludes Replenishment of the Short-Lived Receptor of Tumor Necrosis Factor Alpha by Virion Host Shutoff-Dependent Degradation of Its mRNA

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Cell function is tightly regulated by surface receptors. Earlier reports showed that herpes simplex virus 1 regulates by diverse mechanisms the presentation of antigenic peptides, downregulates the signaling pathways associated with receptor tyrosine kinases, and posttranslationally modifies members of the Src family of protein kinases. Here we report that the receptor for tumor necrosis factor alpha (TNF-R1) rapidly disappears from both the cell surface and total cell lysates in cells infected with wild-type virus or a variety of mutants but not in cells infected with the mutant ΔU1Δ41, which lacks the U1Δ41 gene, the virion host shutoff gene. The half-life of TNF-R1 appears to be less than 30 min in both mock-infected and infected cells. The disappearance of TNF-R1 correlates with the disappearance of cytoplasmic TNF-R1 mRNA in wild-type-virus-infected cells. The results suggest that by degrading the TNFRI mRNA, the virus precludes the replenishment of naturally decaying TNF-R1.

The cell surface is a giant antenna that receives signals from and transmits signals to the environment. When cells become infected with a virus, some of the signals emanating from the cells inform the environment of the presence of an intruder. Receptors, responding to the emanating signals, attempt to curtail viral replication or induce the infected cells to commit suicide. It is not surprising that viruses have evolved mechanisms to curtail the outflow of signals, to degrade receptors of signals that could have a deleterious effect on viral replication, or at the very least to block the execution of the signals received by the receptors. In the case of herpes simplex virus 1 (HSV-1), clear examples of interference with signaling are evidence that the virus blocks the presentation of antigenic peptides on the cell surface by major histocompatibility complex class I and II proteins (12, 16, 24, 27, 30). More recent studies indicate that interference with signaling is more pervasive. HSV-1 blocks exogenous interferon from affecting viral replication through degradation of the promyelocytic leukemia protein, the organizer of ND10 nuclear structure, which appears to regulate interferon response genes (3, 5), the acceleration of turnover of Jak1 (33), and degradation of IRF3 (22). At least one HSV-1 product, infected-cell protein 0 (ICP0), has been shown to down regulate the receptor tyrosine kinases, and posttranslationally modifies members of the Src family of protein kinases. Here we report that the receptor for tumor necrosis factor alpha (TNF-R1) rapidly disappears from both the cell surface and total cell lysates in cells infected with wild-type virus or a variety of mutants but not in cells infected with the mutant ΔU1Δ41, which lacks the U1Δ41 gene, the virion host shutoff gene. The half-life of TNF-R1 appears to be less than 30 min in both mock-infected and infected cells. The disappearance of TNF-R1 correlates with the disappearance of cytoplasmic TNF-R1 mRNA in wild-type-virus-infected cells. The results suggest that by degrading the TNFRI mRNA, the virus precludes the replenishment of naturally decaying TNF-R1.

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The objective of the second series of experiments was to measure the time course of disappearance of TNF-R1 from wild-type-virus-infected HEp-2 cells. Replicate cultures of HEp-2 cells were mock infected or exposed to 5 PFU of HSV-1(F), ΔICP0, C116G/C156A (Δ-R-F), or ΔU41 per cell. HSV-1(F) is the prototype wild-type strain used in this laboratory (8). ΔICP0 lacks the gene encoding ICP0, and Δ-R-F carries amino acid substitutions (C116G/C156A) that disable the RING finger domain of ICP0 (18, 20). ΔU41 contains the coding sequences of β-galactosidase inserted into the U41 open reading frame (26). The cultures were harvested at 2, 4, or 8 h after infection, and total cell lysates were processed as above and reacted with TNF-R1 mouse monoclonal antibody. As shown in Fig. 1B, TNF-R1 protein significantly decreased or virtually disappeared from cells at 2 h after infection with all viruses except ΔU41. In ΔU41-infected cells, the amount of TNF-R1 protein decreased, but not nearly as rapidly as in cells infected with wild-type virus. Thus, at 8 h after infection the amount of TNF-R1 protein in lysates of ΔU41-infected cells was higher than that in wild-type-virus-infected cells harvested at 2 h after infection.

The objective of the third series of experiments was to determine whether the rapid disappearance of TNF-R1 was dependent on cell type. In this series of experiments, replicate cultures of HEp-2 cells, U2OS, or HeLa cells were mock infected or exposed to 10 PFU of HSV-1(F), ΔICP0, Δ-R-F, ΔU41 or d120 per cell. Mutant virus d120 lacks the gene encoding ICP4 (7). The cells were harvested at 6 h after infection, processed as above, and reacted with anti-TNF-R1 antibody. In each cell line tested, TNF-R1 decayed in cells exposed to wild-type or mutant viruses except those exposed to ΔU41 (Fig. 2). In cells infected with this mutant virus, the amount of TNF-R1 decreased but were significantly higher than in other infected cell cultures. We conclude that the decay in TNF-R1 was ΔU41 virus dependent but not cell type dependent.

The objective of the fourth series of experiments was to determine whether the life spans of TNF-R1 in mock-infected and infected cells were similar. In essence, the question posed was whether TNF-R1 was actively degraded in infected cells in contradistinction to its fate in uninfected cells. Replicate cultures of HEp-2 cells were mock infected or exposed to 10 PFU of HSV-1(F) or ΔU41 per cell. Cycloheximide (100 μg/ml) was added at the time of mock infection or exposure to virus and maintained until harvest at 0.5, 1.0, 1.4, 2.0 or 2.5 h after infection. The total cell lysates were processed as described above and reacted with monoclonal antibody to TNF-R1 or polyclonal antibody to actin. The salient feature of the results shown in Fig. 3 is that at 0.5 h after exposure to cycloheximide in either infected or mock-infected cells, the amount of TNF-R1 was less than half the amount present at the time of addition of the drug (0 h). The results indicate that the half-life of TNF-R1 is less than 0.5 h in both infected and mock-infected cells.
experiment did not elicit evidence that TNF-R1 protein was actively degraded in infected cells.

The fundamental property of the virion host shutoff protein encoded by UL41 is that it acts as an endoribonuclease that selectively degrades mRNA (9, 29). If the disappearance of TNF-R1 protein were related to the nucleolytic activity of the UL41 protein, it would be expected that the TNF-R1 mRNA would be degraded in wild-type-virus-infected cells but not in mutant-virus-infected cells. In this series of experiments, replicate cultures of HeLa cells were mock infected or exposed to 5 PFU of HSV-1(F) or ΔU1-41 per cell. Cycloheximide (100 μg/ml) was added at the time of exposure of cells to virus (0 h) and maintained during the infection. The cells were harvested at 0, 0.5, 1.0, 1.5, 2.0, or 2.5 h after infection. Total cell lysates were separated on a 10% denaturing polyacrylamide gel and immunoblotted with anti-TNF-R1 antibody or antiactin antibody.

FIG. 3. Half-life of TNF-R1 in infected HEp-2 cells. HEp-2 cells were mock infected or exposed to 10 PFU of HSV-1(F) or ΔU1-41 per cell. Cycloheximide (100 μg/ml) was added at the time of exposure of cells to virus (0 h) and maintained during the infection. The cells were harvested at 0, 0.5, 1.0, 1.5, 2.0, or 2.5 h after infection. Total cell lysates were separated on a 1% agarose gel and probed with 32P-labeled full-length TNF-R1 cDNA.

FIG. 4. Northern blot of TNF-R1 in infected HeLa cells. HeLa cells were mock infected or exposed to 5 PFU of virus per cell. Cells were harvested at 1, 3, or 7 h after infection, and cytoplasmic RNAs were extracted and separated on a 1% agarose gel followed by Northern blot analysis with 32P-labeled full-length TNF-R1 cDNA.

ΔU1-41 mutants are avirulent in experimental animal systems. Although the U1-41 protein affects many different host mRNAs, there arises the question of whether the inability to block the interaction of TNF-α with its receptor contributes to the virulence of this mutant.

HSV employs a large number of diverse strategies to inactivate host functions inimical to its replication and spread. Some proteins are degraded by the action of E3 ligase activity of ICP0 (4, 6, 11, 14, 19, 21, 25, 31). Others are inactivated by phosphorylation by the viral protein kinases (2, 23, 28), by translocation into inoperative compartments of the cell (10, 13, 17, 34), or by recruitment to perform functions different from those expressed in uninfected cells (1, 15). In the case of TNF-R1, the strategy of HSV-1 is to take advantage of the short natural longevity of the protein.

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