Studies on an Interferon-sensitive Mutant of Mengovirus: Effects on Host RNA and Protein Syntheses

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SUMMARY

Interferon induces an activity which strongly inhibits the growth of is-1, an interferon-response mutant of mengovirus. This activity is not expressed in protected cells infected with either is+ (the wild-type parent) or vaccinia virus, or in cells infected with is-1 in the presence of actinomycin D. A failure of is-1 to shut off host RNA and/or protein synthesis could explain these observations. The present paper, however, shows that is-1 and is+ are equally effective in suppressing host syntheses, and suggests that is+ actively inhibits the interferon-mediated activity directed against is-1.

Simon et al. (1976) isolated an interferon-response mutant of mengovirus (is-1), which has an enhanced sensitivity to interferon. They showed that the final yield of the wild-type virus, is+, was 10- to 30-fold greater than that of is-1 in L cells pretreated with interferon. Under our present conditions, pretreatment of cells with 10 units/ml interferon results in a 30- to 100-fold difference. The final yield of is-1 from untreated cells is typically 50 to 100% of wild-type virus. However, treatment of the cells at the time of the infection with the inhibitors of host RNA synthesis, actinomycin D (Simon et al., 1976) or DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; G. S. Fout & E. H. Simon, unpublished observations), or co-infection of is-1-infected cells with either is+ (Simon et al., 1976) or vaccinia virus (G. S. Fout & E. H. Simon, unpublished observations) reverses the is-1 phenotype. These data imply that an induced, short-lived cellular activity is needed for the expression of the mutant phenotype and that either the synthesis or action of this activity can be blocked by actinomycin D, DRB, is+ or vaccinia.

Mengovirus (Apriletti & Penhoet, 1978; Baltimore et al., 1963; Plagemann, 1968) and vaccinia (Salzman et al., 1964) can act like actinomycin D and DRB by blocking the production of the host activity at the level of RNA synthesis. These viruses also inhibit host protein synthesis and may act at this level as well (Bablanian et al., 1978; Baltimore et al., 1963; Egberts et al., 1976; Falcoff & Sanceau, 1979). If is-1 was deficient in either of these activities, a difference in the ability of is+ and is-1 to block host RNA or protein synthesis should be detectable. The data we present show that both viruses block RNA and protein syntheses equally well, and are consistent with the suggestion that is+ and vaccinia (but not is-1) produce substances that specifically inhibit the cellular activity (Simon et al., 1976). Alternatively, the host activity may be activated only during infection with is-1.

Sucrose gradient analysis of RNA extracted from uninfected L cells pulse-labelled with 3H-uridine for 30 min reveals three main classes of RNA: ribosomal RNA precursors (>28S), 18S ribosomal RNA and 4S RNA (Plagemann, 1968). The pattern is unchanged for the first 2 to 3 h following infection with mengovirus, but subsequently cellular RNA synthesis is rapidly inhibited and virus RNA appears (Apriletti & Penhoet, 1978; Baltimore et al., 1963; Plagemann, 1968). This experiment was repeated using mengovirus strains is+ and is-1 with similar results. However, it was difficult to quantify the inhibition of ribosomal RNA synthesis since the virus RNA co-sedimented with a major ribosomal RNA precursor. In addition, the effects of the viruses on messenger RNA synthesis could not be determined.
Fig. 1. RNA synthesis in uninfected and mengovirus-infected cells. Total RNA from mock-infected L cells (◊) or from cells infected (m.o.i. 4) for 3.5 h with is+ (●) or is-1 (▲) was separated into (a) poly(A)- and (b) poly(A)+ fractions by oligo(dT) chromatography, and sized on linear-log sucrose gradients. The positions of transfer RNA, 18S and 28S ribosomal RNAs are shown above (a). The 36S and 45S RNA positions were calculated as in Brakke & Van Pelt (1970). Fractions from each gradient were pooled and assayed for infectious RNA (c). [●], Poly(A)- RNA from is+-infected cells; [□], poly(A)+ RNA from is+-infected cells; [□], poly(A)- RNA from is-1-infected cells; [■], poly(A)+ RNA from is-1-infected cells.

Fig. 2. Polyacrylamide gel analysis of proteins synthesized in uninfected and mengovirus-infected cells. 35S-methionine labelled proteins from mock-infected L cells (lane 1) or from cells infected (m.o.i. 4) for 6 h with is+ (lane 2) or is-1 (lane 3) were analysed on 10 to 20% polyacrylamide gels and detected by fluorography. Protein standards were: β-galactosidase (130000 mol. wt.), bovine serum albumin (68000 mol. wt.), ovalbumin (43000 mol. wt.), DNase I (31000 mol. wt.) and RNase A (13700 mol. wt.). Mengovirus proteins are identified on the right of the gel.

To resolve these problems, RNA was pulse-labelled for 30 min and separated into poly(A)- and poly(A)+ fractions before linear-log sucrose gradient analysis. Linear-log sucrose gradients were prepared according to the procedure of Brakke & Van Pelt (1970) and centrifuged for 8 h at 239 000 g and 13 °C. Fig. 1 (a) shows the effect of is+ and is-1 infection...
on poly(A)- RNA at 3.5 h post-infection. It is clear that both viruses inhibit ribosomal and transfer RNA syntheses. Since 18S ribosomal RNA is rapidly processed from 45S ribosomal precursor and transported into the cytoplasm (Weinberg et al., 1967), its absence implies that little or no 45S ribosomal precursor is being made in virus-infected cells. An infectious RNA assay (McCutchan & Pagano, 1968) showed that greater than 90% of the mengovirus RNA went into the poly(A)+ fractions (Fig. 1c). Hence, the RNA peaks from virus-infected cells at approx. 34S probably represent heterogeneous and messenger RNA rather than virus material.

Fig. 1(b) shows the results of virus infection on poly(A)+ RNA. It is clear that both viruses inhibit messenger RNA synthesis to about the same extent. The amount of total is-1 RNA (Fractions 16 to 20) is typically less than is+. This probably reflects the small difference in final yield seen in untreated cells. In subsequent experiments performed before the start of virus RNA synthesis, both viruses showed a similar inhibition of cellular RNA across the entire gradient. Although the inhibition of cellular RNA synthesis is most striking at high multiplicities of infection, cells were infected with only 3 to 5 p.f.u./cell in these experiments to duplicate our standard conditions. The data in Fig. 1(b) do not eliminate the possibility that the is-1 virus may inhibit a different spectrum of messages than is+. However, Apriletti & Penhoet (1978) have been unable to detect a change in the specificity of RNA polymerase II following mengovirus infection of L cells. Their evidence suggests that inhibition is due primarily to a decrease in the number of active polymerase molecules.

Infection of L cells with mengovirus also leads to an inhibition of protein synthesis (Baltimore et al., 1963; Egberts et al., 1976; Falcoff & Sanceau, 1979). As with RNA synthesis, the inhibition appears 2 to 3 h post-infection, progressively increases throughout the rest of the infection cycle, and is related to the multiplicity of infection (Falcoff & Sanceau, 1979). Fig. 2 shows the effect of mengovirus infection on proteins synthesized in vivo at 6 h post-infection. Proteins were labelled with 35S-methionine for 1 h and then the cells were lysed with 0.2% Triton X-100 as described by Gupta (1979). The 600 g pellets, which contain all major virus proteins, were applied to a 10 to 20% polyacrylamide gradient gel prepared as described by Maizel (1971). Even at the low m.o.i. we employed, there is a striking decrease in host protein synthesis. Both viruses blocked synthesis of most proteins, while permitting, to varying degrees, the continued synthesis of a few specific species. It is important to note that this difference was a function of the particular host protein and not of the virus. This conclusion is supported by preliminary studies using two-dimensional gel electrophoresis (data not shown). Thus, while the results do not rule out the possibility that a particular protein(s) could be made in is-1-, but not is+-infected cells, they strongly argue against it.

The results show that the is phenotype is not likely to be due to a general inhibition of host-specific RNA or protein synthesis by the virus. This means that the phenotypic reversion of is-1 to is+ by agents such as actinomycin D, DRB, is+ and vaccinia must be due to some specific effect on the interferon system. Actinomycin D and DRB probably block the synthesis of the mRNA for an interferon-mediated antiviral activity which strongly inhibits is-1 replication. This antiviral activity is evidently not sensitive to mengovirus shut off. Thus, its phenotypic absence in is+-infected cells is likely to be due to either (i) a virus-specific inactivation of the activity or (ii) a failure of the activity to be activated.

Ito et al. (1978) have presented evidence favouring the first possibility. They showed that L cells persistently infected with a temperature-sensitive mutant of haemagglutinating virus of Japan produced antiviral and anticellular activities in response to interferon at the non-permissive temperature, but not at the permissive temperature. This may mean that the production of a virus-mediated 'anti-interferon action' blocks the development of the antiviral state. Vaccinia and is+ may also produce anti-interferon activities which are directed against
certain interferon-mediated functions, and which may explain the different sensitivities of viruses to interferon.

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REFERENCES


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