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STUDIES ON VIRUS-INDUCED CELL FUSION

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Progress Report

We are studying the mechanism of cell fusion induced by fusion-causing mutants of Herpes Simplex Virus Type 1 (HSV-1). We have isolated a number of these mutants which, unlike the wild type strain KOS, cause extensive cell fusion during an otherwise normal infection. These mutants are designated syn because of their ability to cause the formation of syncytia, cells containing many nuclei, during infection. The mutation is not conditional-lethal, but rather should be thought of as a plaque morphology mutation. A year ago we proposed to develop fluorescent probes to study cell fusion, to compare the glycolipid composition of wild type and syn mutant infected cells, to determine the mechanism by which β -galactosidase inhibits cell fusion, to examine cell fusion using electron microscopy, and to determine the killing efficiencies for decays of adenosine-2-³H and guanosine(adenosine)-8-³H in mammalian cells. Our progress on this research is as follows.

Publications of previous work supported by this agency are given as Appendices 1-4. Three manuscripts are also in preparation for proposed research cited above, and these are summarized in Appendices 5-7 and abstracted below. The remaining items of research proposed a year ago are still under investigation.

We have now developed two simple quantitative assays which can be used to measure the kinetics of cell fusion under a variety of experimental conditions. In one assay a Coulter counter is used to measure the remaining number of small single cells as a function of time after infection (App. 3). In this assay infected cells forming a monolayer on a glass surface are harvested with a trypsin-EDTA with a trypsin-EDTA solution which separates cells. Cells were considered to be fused if they were not separated into single cells by the harvesting procedure.

A second fusion assay using two fluorescent probe molecules, derivatives of fluorescein (F18) and rhodamine (R18) that partition into cellular membranes, has also been developed and characterized (App. 5). The bases for assay is the detection of resonance energy transfer between F18 and R18 which occurs when they are in close proximity (less than 50 Å). Under these conditions R18 and F18 will both fluoresce if only F18 has been excited due

to non-radiative resonance transfer of energy from F18 to R18. The amount of R18 fluorescence is a measure of the degree to which the probes are mixed. Following fusion a rapid intermixing of the probes is expected. Experimentally, infected cells labeled with F18 or R18 are mixed, seeded into petri dishes and the R18 fluorescence measured as a function of time. When cell fusion occurs an increase in enhancement of R18 fluorescence is observed. The assay has been fully characterized and the results obtained agree in all respects with the Coulter counter and microscope assays. In addition, this assay offers several advantages over other methods. An entire fusion kinetics curve can be obtained from a single petri dish, allowing many curves to be obtained simultaneously. Also, fusion between non-identical cell populations can also be easily observed.

Neutral sphingoglycolipids (glycolipids) were studied in wild type and syn mutant infected cells (App. 6). Wild type and syn mutant infected cells were labeled with ^3H -galactose or ^{14}C -galactose, respectively, from 4-10 hr after infections. Cells were harvested and mixed, and the lipids extracted. The lipids were then co-chromatographed on silica gel G thin-layer plates and autoradiograms prepared. The glycolipids extracted from wild type infected cells contain shorter oligosaccharide chains relative to those from syn mutant infected cells. In other experiments it was also found that the sugar chains are shorter in the wild type infected relative to those for uninfected cells. Therefore, it would appear that the HSV-1 genome specifies genetic information for a gene product that is concerned with some aspect of oligosaccharide metabolism. This gene product could be a glycosyl transferase, a glycosidase, or a regulatory protein. The data are also consistent with the interpretation that cell fusion in syn mutants of HSV-1 are caused by an alteration in oligosaccharide metabolism. Fusion could be due to an altered oligosaccharide composition in glycolipids, glycoproteins, or both. Because of the interesting results obtained, further studies are anticipated (see Research Proposal).

Some time ago we discovered that preparations of β -galactosidase blocked fusion. Studies during the past year have revealed that the active factor in the preparations was not β -galactosidase but $(\text{NH}_4)_2\text{SO}_4$, which is present in high concentrations in many commercial preparations of β -galactosidase (App. 7).

It was found that NH_4Cl also blocks fusion, and that none of the series of alkali chlorides (LiCl , NaCl , KCl , RbCl , and CsCl) was inhibitory. In this series, the ionic radius of Rb^+ is identical to that of NH_4^+ . We conclude, therefore, that the inhibition is due to the presence of the NH_4^+ ion and that it is not a generalized effect of monovalent cations. We have not eliminated the possibility that NH_3 is the actual active agent, although at the pH of our medium, the concentration of NH_3 is only 1% that of NH_4^+ . The concentration of NH_4Cl required for complete inhibition of fusion is 20 mM. This concentration shows little or no effect on virus adsorption or growth. The protein and glycoprotein profiles, as examined by SDS-gel electrophoresis are not affected by NH_4^+ . At present we have no molecular explanation as to the nature of the NH_4^+ inhibition of cell fusion.

PUBLICATIONS

1. S. Person, W. Snipes and F. Krasin. Mutation production from tritium decay: a local effect for ^3H -2-adenosine and ^3H -6-thymidine decays. Mutation Res. 34, 327-332 (1976).
2. F. Krasin, S. Person, R. D. Ley and F. Hutchinson. DNA cross-links, single-strand breaks and effects on bacteriophage T4 survival from tritium decay of ^3H -2-adenine, ^3H -8-adenine and ^3H -8-guanine. J. Mol. Biol. 101, 197-209 (1976).
3. S. Person, R. W. Knowles, G. S. Read, S. C. Warner and V. C. Bond. Kinetics of cell fusion induced by a syncytia-producing mutant of herpes simplex virus type 1. J. Virol. 17, 183-190 (1976).
4. F. Krasin, S. Person, W. Snipes and B. Benson. Local effect for ^3H -5-cytosine decays: production of a chemical product with possible mutagenic consequences. Accepted for publication in J. Mol. Biol.
5. T. Holland and S. Person. Effect of NH_4Cl and other agents on cell fusion induced by herpes simplex virus. MS in preparation.
6. P. M. Keller and S. Person. Assay for virus induced cell fusion by a fluorescence enhancement technique. MS in preparation.
7. Merilee Ruhlig and S. Person. Glycosphingolipids synthesized in cells infected with syncytia-producing mutants of herpes simplex virus. MS in preparation.

Related Research:

1. R. W. Knowles and S. Person. Effects of 2-deoxyglucose, glucosamine, and mannose on cell fusion and the glycoproteins of herpes simplex virus. Accepted for publication in J. Virol.
2. E. C. Pollard, S. Person and Mildred Rader. Inuction of mutagenesis by error-prone repair with ionizing radiation and ultraviolet light in Escherichia coli. Submitted for publication in Radiation Res.

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