Isolation and Characterization of an IBT-dependent Mutant of Vaccinia Virus

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The antiviral activity of thiosemicarbazones against poxviruses was reported by Hamre, Bernstein & Donovick (1950). Thiosemicarbazone derivatives are clinically used in India and Africa. Appearance of drug-resistant and drug-dependent mutants in the course of viral therapy is a potential danger. Appleyard & Way (1966) and Ghendon & Chernos (1972) observed the selection of thiosemicarbazone-derivatives resistant mutants, during growth of the wild-type strain in the presence of these drugs.

We shall describe here the isolation and characterization of an isatin-β-thiosemicarbazone (IBT)-dependent mutant (IBT\(^D\)) of vaccinia virus, which needs IBT for its growth. The characteristics of this mutant are compared with those of the wild-type (wt) strain and an IBT-resistant mutant (IBTR), isolated in our laboratory.

The two vaccinia mutants IBTD and IBTR were isolated after treatment of cells infected with wt virus with the mutagenic agent iododeoxyuridine in the presence of IBT. Chick embryo cell monolayers were infected with vaccinia virus strain WR at an input multiplicity of 1 p.f.u./3 cells. After 45 min at 37 °C the culture was washed and Eagle's media (Eagle, 1959) containing 5 % calf serum, iododeoxyuridine (5 μg/ml) and 14 μM-IBT (Mann Research Laboratories, New York, N.Y.) was added. The culture was harvested 2 d later and was used for infection of new cultures in the presence of IBT. Virus from one of these cultures formed plaques in agar-overlaid cultures in the presence of IBT. Well-separated plaques were picked and grown into stocks in the presence of IBT. Tests on these stocks revealed that one of them formed plaques only in the presence of IBT (IBTD), while another formed plaques in both absence and presence of IBT (IBTR) (Fig. 1). Both mutants were plaque-purified and virus stocks were prepared in HeLa cell monolayers. Stocks of wt and IBTR were made in the absence of IBT while stocks of IBTD were prepared in the presence of 14 μM-IBT. The purity of the stocks of the mutant strains was further examined by picking isolated plaques from agar-overlaid cultures and plating them in the presence and absence of IBT. Sixteen plaques of IBTD, isolated from cultures containing IBT were all found to be of the IBTD character. All 19 plaques of IBTR, isolated from cultures without IBT, grew equally well in the absence and presence of IBT.

The growth curves of the IBTD mutant in HeLa cells in the presence, and in the absence of IBT are compared with those for the wt strain and the IBTR mutant in Fig. 2. The dependence on IBT of IBTD and the resistance to IBT of IBTR shown in these experiments indicates that these characteristics of the mutants are not restricted to a certain type of host-cell. Similar characteristics have also been observed in BSC-1 cells.

To find out whether the concentration of IBT which inhibits the growth of the wt strain is similar to that needed for supporting IBTD growth, the virus strains were grown in the presence of different concentrations of IBT (Fig. 3). The wt strain was inhibited more than 99 % at IBT concentrations larger than 20 μM, while concentrations of 10 μM and lower permitted partial growth. Full growth of the IBTD mutant was obtained with 5 μM-IBT; a further study revealed that 3 to 4 μM was the minimum concentration of IBT necessary. The IBTR mutant was able to grow equally well in the absence and presence of the IBT concentrations tested.
Fig. 1. Formation of plaques of vaccinia virus wr strain (WT) and of the two mutants IBT<sup>D</sup> and IBT<sup>R</sup> on chick embryo cells overlaid with Eagle's media containing 1 % Special Agar Noble (Difco Laboratories, Detroit, Mich.), 5 % inactivated calf serum, 0.0025 % neutral red and 14 µM-IBT. To duplicate Petri dishes IBT was not added. Photographs were taken after incubation for 5 d at 37 ºC.
Fig. 2. Growth curves of the wt, IBT<sup>D</sup> and IBT<sup>R</sup> strains in HeLa cells infected at a multiplicity of 1 p.f.u./cell with and without 14 µM-IBT. Virus was titrated by plaque assay on BSC-1 cells with the addition of IBT (14 µM) in the case of the IBT<sup>D</sup> strain. (A) Growth curves of IBT<sup>R</sup> and wt strains. (B) Growth curves of IBT<sup>D</sup> and wt strains. ▲, wt without IBT; △, wt with IBT; ■, IBT<sup>R</sup> without IBT; □, IBT<sup>R</sup> with IBT; ●, IBT<sup>D</sup> without IBT; ○, IBT<sup>D</sup> with IBT.

Fig. 3. The effect of different concentrations of IBT on the growth in HeLa cells of the wt strain and of the mutants IBT<sup>D</sup> and IBT<sup>R</sup>. Titrations were made in BSC-1 cell monolayers. Virus growth is plotted as a ratio of the yield at 22 h to the virus present after washing at the end of the 45 min adsorption period (O<sup>t</sup> sample). --- ● ---, wt; --- △ ---, IBT<sup>R</sup>; --- ○ ---, IBT<sup>D</sup>.
The reversion from dependence on IBT was examined following infection of HeLa cells with the IBT\textsuperscript{D} mutant in the absence of IBT. The culture was harvested at 22 h and the virus was titrated on BSC-1 cell monolayer. The revertants (2\% of the progeny virus) were found to be either sensitive or resistant to IBT, thus exhibiting the \textit{wt} and the IBT\textsuperscript{R} characters.

No significant differences in virus morphology among the three virus strains could be detected by electron microscopy of purified virus negatively stained with phosphotungstic acid. The ratio of infectivity to $E_{260}$ of purified virus suspensions was quite similar for the \textit{wt} and for the IBT\textsuperscript{R} mutant (9.3 $\times$ 10$^9$ and 1.7 $\times$ 10$^{10}$ p.f.u./$E_{260}$ unit, respectively), but over 100-fold lower for IBT\textsuperscript{D} (8.3 $\times$ 10$^7$ p.f.u./$E_{260}$ unit). This indicates that there are more non-infectious particles with IBT\textsuperscript{D} than with IBT\textsuperscript{R} and \textit{wt} strains.

The mutants IBT\textsuperscript{D} and IBT\textsuperscript{R} are immunologically similar to the \textit{wt} strain. Neutralization of all three strains was detected using human antivaccinial immunoglobulin.

The two mutants IBT\textsuperscript{D} and IBT\textsuperscript{R} described here differ from the wild-type strain, which is IBT-sensitive, by their IBT-resistance or dependence on the antiviral drug. Other characteristics of these mutants such as host-range, virus morphology, and immunological neutralization are apparently unaltered. Thus, these two mutants are of potential value as tools in fundamental studies concerning the functions of viral genes, as well as the genetic and non-genetic interaction between poxviruses. Studies in this direction and toward elucidating the mechanism of the antiviral activity of IBT are in progress in our laboratory.

To Dr Dov Karpas – in memoriam.

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REFERENCES


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