Immunogenicity in Mice of Temperature-sensitive Mutants of Vesicular Stomatitis Virus: Early Appearance in Bronchial Secretions of an Interferon-like Inhibitor

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SUMMARY

Temperature-sensitive mutants of vesicular stomatitis virus (Indiana serotype) rapidly induced resistance in mice to intranasal challenge infection with the highly virulent wild-type homotypic virus, and to a lesser extent with the heterotypic New Jersey serotype. Intranasal vaccination with mutant tsG44 (IV) resulted in early appearance (at 12 h) of a bronchial inhibitor which protected mouse L cells and chick embryo cells against infection with vesicular stomatitis virus. This bronchial inhibitor was inactivated by trypsin but did not exhibit the properties of immunoglobulins, defective-interfering virus or virus glycoprotein. It was active in both chick and mouse cells and was relatively labile to acid and heat, but the antiviral activity of this bronchial inhibitor was neutralized by a goat antiserum to type I mouse interferon.

An important approach to the development of virus vaccines is the use of live, attenuated viruses, capable of synthesizing enough virus proteins to provide a large antigenic stimulus but restricted in their replication so as not to produce disease manifestations. Although investigators are haunted by the spectre of long-term, deleterious effects caused by persistence of attenuated viruses, the search continues for suitable candidates for live virus vaccines (Chanock et al. 1975). Our laboratory (Wagner, 1974) has reported preliminary data on the vaccine potential in mice of the well-characterized temperature-sensitive (ts) mutants of vesicular stomatitis virus (VSV) which are restricted at 37 to 39 °C (Pringle, 1977). Compared to the highly virulent Indiana serotype of wild-type (ts~a) VSV, ts mutants in three complementation groups were found to be totally innocuous for mice when administered intranasally (i.n.); they also induced rapid and firm resistance to i.n. challenge infection with ts~a virus (Wagner, 1974). The surprisingly early development of resistance (at about 12 h) was tentatively attributed to bronchial secretion of an antiviral substance, originally assumed to be antibody. We report here that a VSV mutant induces a bronchial anti-VSV inhibitor which acts directly on host cells, and appears to be an interferon-like substance rather than antibody, defective-interfering (DI) particles, or VSV glycoprotein.

To determine the nature of early (and late) resistance to challenge infection with ts~a (San Juan strain) or with heterotypic VSV New Jersey wild-type (ts~a, Concan strain), mice were infected i.n. with ts~a mutants of all five complementation groups. Mutants tsG114 (I) and tsG44 (IV), isolated in Glasgow, U.K., were obtained from Craig R. Pringle; mutants tsO52 (II), tsO23 (III) and tsO45 (V), isolated in Orsay, France, were obtained from Pierre Printz. Stock preparations of the ts~a and the five corresponding ts~a mutants grown on BHK-21 cells had titres of \(2 \times 10^6\) to \(2 \times 10^{10}\) p.f.u./ml when plated on L cells at 31 °C (permissive temperature); the ts~a virus had a titre of \(2.3 \times 10^8\) p.f.u./ml at 31 °C (Hunt & Wagner, 1974; McAllister & Wagner, 1976). No plaques (< 10^3 p.f.u./ml) could be detected when any of the five ts~a mutants were plated at 39 °C (restrictive temperature), whereas ts~a and ts~a gave equal numbers of plaques at 39 and 31 °C.
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Fig. 1. Antiviral activity present in the bronchial secretions and sera of weanling mice sacrificed at intervals after intranasal infection with 10⁵ p.f.u. of tsG44 (IV). As previously described (Wagner, 1974), bronchial secretions were collected by tracheal aspiration of nutrient broth and blood was collected by cardiac puncture. Bronchial secretions and serum were tested for their capacity to inhibit plaque formation when mixed with 100 p.f.u. of ts⁺nd VSV plated on L cells. Results at each time represent the average from 6 mice.

Weanling male and female Swiss Webster mice weighing 10 to 12 g were lightly anaesthetized with Penthrane® and infected i.n. with 0.05 ml of virus, as previously described (Wagner, 1974). All mice survived infection with as much as 10⁶ p.f.u. of the five ts⁺nd mutants, but the 50% lethal dose (LD₅₀), calculated by the method of Reed & Muench (1938), was 4 x 10⁴ p.f.u. for ts⁺nd and 4 x 10⁵ p.f.u. for ts⁺+. Mice exposed to lethal doses of wild-type VSV showed cerebral symptoms on day 6 and were dead by day 11.

As an extension of earlier studies (Wagner, 1974), the immunizing capacity of all five ts⁺nd mutants was compared and quantified by i.n. infection of mice with graded doses of ts mutants, followed by i.n. challenge 6 days later with 100 LD₅₀ of VSV ts⁺nd or ts⁺+. The results were calculated as the dose of each ts mutant required to protect 50% of mice (PD₅₀) against challenge. We found that relatively low doses of each mutant (2 x 10⁸ to 1 x 10⁶ p.f.u.) afforded protection against homotypic ts⁺nd, but 20 to 1000 times more vaccine (2 x 10⁶ to 2 x 10⁷ p.f.u.) were required for mice to resist challenge with heterotypic ts⁺+. Mice previously injected i.n. with VSV ts mutants were not protected at all against i.n. challenge with the WS strain of influenza A virus (data not shown).

This heterotypic resistance to VSV ns, although limited, was somewhat surprising, because there is no cross-reactivity between the neutralizing antibodies directed against the major type-specific glycoprotein antigens of VSV⁺nd and VSV ns (Reichmann et al. 1978). The rapidity with which VSV ts mutants induce local resistance to i.n. challenge with wild-type VSV (by 12 h) had previously been attributed to rapid appearance of an antiviral substance in the bronchial secretions of the vaccinated mice; this bronchial inhibitor did not originally appear to be interferon and was assumed to be neutralizing antibody, possibly IgA (Wagner, 1974).

In an attempt to characterize this bronchial anti-VSV activity more fully, mice were infected i.n. with approx. 10⁷ p.f.u. of either tsG44 (IV) or ts045 (V); at intervals thereafter, bronchial washing and serum were collected as previously described (Wagner, 1974) and assayed for neutralization of VSV ts⁺nd plaques. Since bronchial washings routinely contained residual ts VSV for 72 h, the plaque neutralization assays were performed at the restrictive temperature of 39 °C. In confirmation of earlier experiments, tsG44 (IV) induced significant antiviral activity in bronchial secretions within 12 h (Fig. 1); the bronchial
antiviral activity declined by day 3 and disappeared thereafter. In contrast, no plaque-reducing activity could be detected in the bronchial secretions of mice at any time after i.n. infection with tsO45 (V) (data not shown). However, both mutants induced detectable plaque-reducing activity in serum, undoubtedly circulating antibody, by 3 days after i.n. infection, with peak titres by 4 to 5 days. We assume that failure to detect inhibitor in bronchial secretions of mice infected with tsO45 (V) is due to greater restriction of this mutant, which is clearly a less efficient inducer of interfering activity.

To detect the possible presence of neutralizing IgA, IgG and IgM antibody in bronchial secretions obtained from mice 12 to 72 h after i.n. infection with tsG44 (IV), a sensitive microtitre radio-immunoprecipitation assay was used. Varying concentrations of ts+~ virus, bronchial washings (or serum samples) from mice inoculated with tsG44 (IV) and 125I-labelled goat anti-mouse immunoglobulin were mixed. At no time was antibody of any type detected in bronchial washings, even though considerable amounts were present in serum of the same mice by day 3.

An alternative explanation for this early VSV resistance could be rapid induction in the bronchial mucosa of defective-interfering (DI) VSV particles (Huang, 1973) which are known to be generated specifically by ts mutants (Reichmann et al. 1971). Unlike neutralizing antibody, the interfering action of such putative DI particles should be detected by exposing cells to them before infection with plaque-forming VSV. To test this hypothesis, monolayer cultures of 2 x 10⁶ L cells were exposed for 60 min to 0.1 ml of bronchial washings from mice that had been inoculated with tsG44 (IV) or from control uninoculated mice; after three washings with warm medium, the monolayers were plated with 100 p.f.u. of tsG44. We found that bronchial washings of mice contained significant interfering activity (50% plaque reduction at 1:8 dilution) at 12 h and 24 h after i.n. infection with tsG44 (IV), but no significant activity (< 1:2) by 5 days p.i. (data not shown). However, this interfering activity in bronchial secretions was not due to VSV DI particles on the following criteria: (i) ultracentrifugation (> 100000 g) did not sediment the interfering activity; (ii) the interfering activity remained at the top of a 0 to 30% sucrose gradient after centrifugation at 30000 g for 1 h; (iii) the interfering activity was not excluded from a Sephadex G-100 column; (iv) no DI particles could be detected by negative-staining electron microscopy; and (v) the interfering activity was not reversed by VSV antibody.

Table I summarizes these and other preliminary data which further characterize the physical and biological properties of the VSV-interfering activity present in bronchial secretions of mice 12 to 48 h after i.n. inoculation of tsG44 (IV). Unlike type I mouse interferon (Lockart, 1973; DeMaeyer & DeMaeyer-Guignard, 1979), the bronchial inhibitor was relatively heat- and pH-labile and was not species-specific, as judged by comparable interference with VSV on chick embryo and mouse L cells; moreover, the inhibitor was partially virus-specific, with less activity against heterotypic VSV N, and none at all against influenza virus. The protein nature of the bronchial inhibitor was suggested by its inactivation by trypsin, but not by u.v. light at a wave length of 258 nm, and also by its nondialysability. The fact that it was not sedimented by ultracentrifugation and entered beads of Sephadex G-100 (but not of G-50) on chromatography suggest that the inhibitor has a mol. wt. between 50000 and 100000. The possibility that the bronchial inhibitor is the VSVβ glycoprotein generated by tsG44 (IV) appears unlikely because its antiviral activity was not significantly affected by antibody to the G protein of VSV.

Although the bronchial inhibitor of VSV induced by i.n. infection with tsG44 (IV) did not exhibit the characteristic properties of mouse type I interferon (DeMaeyer & DeMaeyer-Guignard, 1979), we tested antiserum directed against type I interferon for its capacity to neutralize the bronchial inhibitor. Dr Edward DeMaeyer of the Section de Biologie, Fondation Curie-Institut du Radium, Orsay, France, kindly provided us with goat anti-
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Table I. Properties of VSV inhibitor in bronchial secretions of mice 18 h after intranasal infection with 10^7 p.f.u. of tsG44 (IV)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control anti-VSV&lt;sub&gt;ind&lt;/sub&gt; activity on L cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56 °C, 1 h</td>
<td>98</td>
</tr>
<tr>
<td>80 °C, 1 h</td>
<td>0</td>
</tr>
<tr>
<td>pH 2−0, 1 h†</td>
<td>8</td>
</tr>
<tr>
<td>U.V., 85 ergs/mm²/s‡</td>
<td>93</td>
</tr>
<tr>
<td>Trypsin, 100 μg/ml/h§</td>
<td>15</td>
</tr>
<tr>
<td>Dialysis, 4 °C, 18 h</td>
<td>95</td>
</tr>
<tr>
<td>85000 g, 1 h</td>
<td>100</td>
</tr>
<tr>
<td>Sephadex G-50, void volume</td>
<td>100</td>
</tr>
<tr>
<td>Sephadex G-100, void volume</td>
<td>0</td>
</tr>
<tr>
<td>Plated on CE cells</td>
<td></td>
</tr>
<tr>
<td>G antibody, 1 h¶</td>
<td>89</td>
</tr>
</tbody>
</table>

* Bronchial secretions were collected by tracheal aspiration, pooled as previously described (Wagner, 1974) and diluted 1:4 in nutrient broth for treatment. They were added to L cell monolayers for 1 h; the monolayers were then washed three times with warm medium and challenged with 100 p.f.u. of VSV<sub>ind</sub>. Untreated washings produced a 50% reduction in plaques at 1:16 or 1:32. Results with treated samples are expressed as the percentage of the control antiviral activity remaining after treatment.

† Incubated with concentrated HCl for 1 h and then neutralized with concentrated NaOH.
‡ A=258 nm.
§ After 1 h, trypsin inactivated with 200 μg of soybean inhibitor.
¶ Bronchial secretions were added to chick embryo fibroblasts which were then washed and challenged with 100 p.f.u. of VSV<sub>ind</sub>.

We conclude from these studies that the antiviral activity present in bronchial secretions of mice 12 h after i.n. infection with tsG44 (IV) is related to type I mouse interferon, but differs from it in its acid lability and lack of cell species specificity. Differential sensitivity of VSV<sub>ind</sub>, VS<sub>N</sub>, and influenza viruses would not be unusual for an interferon. The minute amounts and crudeness of the material recovered in bronchial washings prevent further characterization of this interferon-like substance and determination of its relationship to the well-characterized pure type I mouse interferon (DeMaeyer-Guignard et al. 1978), one component of which (mol. wt. ~ 38,000) is acid-labile. Type II lymphocyte interferon (Wietzerbin et al. 1978) is relatively acid-labile, but it does not resemble the bronchial inhibitor because it is not neutralized by antiserum to type I interferon (DeMaeyer & DeMaeyer-Guignard, 1979). By 3 to 4 days after i.n. infection with tsG44 (IV), interfering activity disappeared from bronchial secretions; later resistance to VSV challenge coincided with appearance by day 3 to 5 of serum neutralizing antibody. This model system indicates that ts mutants can provide significant data on the possible use of attenuated live virus vaccines for promoting early resistance to acute virus infection.

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REFERENCES


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