Multi-factorial Specification of Virus–Host Interactions: Studies with Strains of Venezuelan Equine Encephalomyelitis Virus in Mice

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SUMMARY

Mice of different ages were infected i.p. or i.c. by 23 different strains of VEE virus. The course of the virus host interaction was specified in terms of the efficiency of infection, the outcome of infection as lethality or protection and the survival time. These separately quantifiable features all showed several host-maturation events that combine to provide a multifactorial specification of virus-strains and host-responses. This base-line for correlations with the responses of principal hosts (equidae and man) may be expanded to test correlations with the antigenic or in vitro characteristics of virus-strains.

INTRODUCTION

Strains or variants of a virus may differ in many molecular, serological or in vitro properties. Some genetically determined in vitro properties may serve as marker characteristics for the in vivo activities of virus strains if certain criteria can be satisfied. The most immediate of these criteria are that the marker correlation shall be established over a reasonable number of distinct virus strains, that the tested virus strains shall be essentially homogeneous and that the in vivo activity tested shall be defined and quantified with the precision applied to its in vitro correlate.

The expression of virulence, however, is only one of several parameters of the virus–host interaction and its putative correlation with any in vitro property requires that virulence should be clearly distinguished from such other in vivo characteristics as the efficiency of infection or the stimulation of immunity. These separate but interacting characteristics of the virus–host interaction depend in different ways upon the route of infection, the dose and heterogeneity of virus and the strain and status of the host (Walder & Bradish, 1975; Bradish & Fitzgeorge, 1979; Bradish et al. 1979).

An approach to the more detailed specification of the virus–host interaction has been made by the use of mice of different ages and increasing development of immunological or other competence. It was thus possible to 'titrate' the invasive and stimulatory potentials of different strains of VEEV against the potentials of the host to mount defensive responses against such invasion. On this basis some parameters of the virus–host interaction have been expanded against the scale of host-age or maturity. This provides a multi-component in vivo differentiation of virus-strains and offers a firm basis for the analysis of virus-strain heterogeneities and for the appraisal of tentative serological or molecular markers.
### Table 1  History and designation of strains of VEEV

<table>
<thead>
<tr>
<th>Letter Code</th>
<th>Designation of original strain</th>
<th>Source, origin and year of isolation</th>
<th>Further passage since isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A to K</td>
<td>As previously listed by Walder &amp; Bradish (1975).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>TC83/DETC/CC23817. Seed vaccine strain</td>
<td>Donkey (brain) No. 1. Debe Penal, Trinidad, 1934. 83 passes in foetal guinea-pig heart cells</td>
<td>TC83, SMB: one passage in suckling mouse brains (SMB)</td>
</tr>
<tr>
<td>M</td>
<td>TC83/DETC/CC23817. Seed vaccine strain</td>
<td>Donkey (brain) No. 1. Debe Penal, Trinidad, 1934. 83 passes in foetal guinea-pig heart cells</td>
<td>TC83, SMB G: large plaque variant from L above, as first clone selected from Vero cells</td>
</tr>
<tr>
<td>N</td>
<td>TC83/DETC/CC23817. Seed vaccine strain</td>
<td>Donkey (brain) No. 1. Debe Penal, Trinidad 1934. 83 passes in foetal guinea-pig heart cells</td>
<td>TC83, SMB P: small plaque variant from L above, as first clone selected from Vero cells</td>
</tr>
<tr>
<td>P</td>
<td>W-37947</td>
<td>Horse serum, Three Rivers, Texas, 1971</td>
<td>W-37947 (A24872) SMB (3): three passages in SMB</td>
</tr>
<tr>
<td>Q</td>
<td>IVIC PAn 23647</td>
<td>Sentinel hamster, Catatumbo, Venezuela, 1974</td>
<td>PAn 23647 VK (2): two passages in Vero cells</td>
</tr>
<tr>
<td>R</td>
<td>Mena-II</td>
<td>Human serum, Altamira Panama, 1962</td>
<td>Mena-II GPB SMB: one passage in guinea pig brain (GPB). One passage in SMB</td>
</tr>
<tr>
<td>S</td>
<td>Fe 3-7c</td>
<td>Culex melanconion spp. Florida, Everglades, U.S.A., 1963</td>
<td>Fe 3-7c SM(5)DE(1) SMB: five passes in SMB. One passage in DETC. One passage in SMB</td>
</tr>
<tr>
<td>T</td>
<td>388o</td>
<td>Human serum, Canito, Panama, 1961</td>
<td>388o SMB(3) VK(2) SMB: three passages in SMB. Two passages in Vero cells. One passage in SMB</td>
</tr>
<tr>
<td>U</td>
<td>Mucambo An 198586</td>
<td>Sentinel mice, Mucambo forest, Belem, Brazil, 1971</td>
<td>An 198586 (1) SMB: two passages in SMB</td>
</tr>
<tr>
<td>V</td>
<td>71 D 1252</td>
<td>From pool of 6 mosquitoes, Iquitos, Peru, 1971</td>
<td>71 D 1252 VK SMB: one passage in Vero cells. One passage in SMB</td>
</tr>
<tr>
<td>W</td>
<td>21t</td>
<td>Donkey brain, Trinidad 1934. 13 passes in embryonated eggs (virulent parent egg aced virus, PES) (Hearn, 1960)</td>
<td>21t SMB: twenty-one passes from PES in chronically infected L-cells. One passage in SMB</td>
</tr>
</tbody>
</table>
**METHODS**

*Virus strains.* The strains of VEEV studied are listed in Table 1. These were obtained from Dr C. H. Calisher of CDC, U.S.A. (GJ9-1BJ and W-37947), Dr V. Kubes, Universidad de San Carlos, Guatemala (Mena-11), Dr F. M. Wellings of the Florida Department of Health, Florida, U.S.A. (Fe-3-7c), Dr H. Hearn (1960; 214), and Dr G. Justines from MARU, Panama (3880 and 71-D 1252). Some properties of strains A to L of VEEV have been described previously (Walder & Bradish, 1975). Each virus strain received one additional passage in the brains of suckling mice of strain NMRI/IVIC or Porton (PR).

*Procedures in mice.* The infectivities of virus strain preparations used for subsequent tests were determined by intracerebral titration in suckling mice as described previously (Walder & Bradish, 1975). The doses of potentially infective units administered to mice of various ages is indicated throughout as suckling mouse i.c. LD<sub>50</sub> units: this is written as SM i.c. LD<sub>50</sub>. Groups of five to ten male random bred white mice of strain NMRI/IVIC or Porton (PR) were infected by the intracerebral (i.c.) or intraperitoneal (i.p.) route with graded tenfold doses of each test virus strain. Titrations were performed in mice of defined ages (± 2 days) as previously described (Walder & Bradish, 1975) using doses of 0.1 to 1000 SM i.c. LD<sub>50</sub> per animal.

Infected mice required to be tested for development of protection were challenged intraperitoneally with a single dose of 1000 SM i.c. LD<sub>50</sub> of a virulent strain of VEE virus [P2023 mc(5) G1 SMB] within 14 to 21 days of primary infection.

*Specification of host-responses.* Following primary infection with virus it is necessary to consider the efficiency of infection and the outcome of infection as distinct phases of host response (Walder & Bradish, 1975; Bradish & Fitzgeorge, 1979; Bradish *et al.* 1979). The efficiency of infection may be expressed as the reciprocal of the number of SM i.c. LD<sub>50</sub> units which need to be administered (i.p. or i.c.) to produce any positive indication of infection in 50% of the test group of mice. Thus in the present titrations in mice of a particular age, the incidence at any dose level of mice infected and dead or infected and protected indicated the total incidence of mice infected for the purposes of the ID<sub>50</sub> per ml calculation (Reed & Muench, 1938). The efficiency of infection was then calculated as the ratio:

\[
\frac{\text{ID}_{50} \text{ ml for mice of the specified age}}{\text{LD}_{50} \text{ ml i.c. for suckling mice}} \quad \text{or} \quad \frac{\text{ID}_{50}}{\text{SM i.c. LD}_{50}}
\]

*The outcome of infection.* Regardless of the above efficiency of infection, the outcome of primary infection by a particular strain of virus may be either death or protection against challenge. Virulence for mice of a particular age and input dose is indicated by death following primary infection for a majority of the test group. Similarly, avirulence is indicated by protection against virulent secondary challenge for a majority of the test group. If essentially all primarily infected mice die and none is protected, then the estimation of the 50% lethal dose (SM i.c. LD<sub>50</sub> per LD<sub>50</sub>) is admissible and will be equivalent to the reciprocal of the efficiency of infection. Similarly, if all infected mice are protected and none dies, then the 50% protective dose (SM i.c. LD<sub>50</sub> per PD<sub>50</sub>) may be calculated and will be equivalent to the reciprocal of the efficiency of infection.

In many cases groups of infected mice show the mixed responses of some killed by primary infection and some protected against subsequent virulent challenge. Thus, when LD<sub>50</sub> or PD<sub>50</sub> calculations are inadmissible, only ID<sub>50</sub> values are calculated or results are presented directly as the percentage of infected mice that die following confirmed primary infection by up to 10<sup>4</sup> SM i.c. LD<sub>50</sub> (Fig. 2b; Walder & Bradish, 1975).
Survival for over 14 days and protection against challenge

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**Survival time before death**

Since the time of death following primary infection is a putative marker for virulence, this was investigated in PR mice of different ages following i.p. infection by $10^4$ p.f.u. or SM i.c. LD$_{50}$ of distinct strains of VEE virus. In these tests all mice in each group of 10 died within the narrow ranges of times shown in Fig. 1, except for strain L(TC83) which switched to protection at 11 to 13 days. For the groups in which the mice survived primary infection for at least 14 days, all were protected against secondary challenge by virulent virus: this is shown by the shaded zone in Fig. 1.

The survival times before death increased rapidly for mice inoculated at around 6, 12, 22 and 36 days of age according to the strain of virus tested. Thus, such data provide four independent criteria for the differentiation of VEEV strains. The maturation events in PR mice at about 6 and 12 days old influence the responses to all VEEV strains tested other than strain P2023, whereas the maturation event at 36 to 40 days of age influences only the responses to strain Mucambo. In mice of about 22 days old the responses to strains 211 and P2023 are influenced but not that to strain Mucambo. These changes of response from death to protection may occur later for mice of strain PR than that shown below for mice of strain NMRI.

**The efficiency of infection**

The results in Fig. 2(a) summarize the inefficiencies of infection (ID$_{50}$/SM i.c. LD$_{50}$) for a number of strains of VEE virus in mice of the ages shown (± 2 days). For all the strains of VEE virus except N, the efficiencies of infection by i.c. and i.p. routes were indistinguishable and are shown by single lines in Fig. 2(a). As the ID$_{50}$/ml for a strain sample falls for older mice so the ratio ID$_{50}$/SM i.c. LD$_{50}$ falls to values as low as 0.001, or $-3$ on the log scale of Fig. 2(a).

These results fall into three distinct groups according to the impairment of the efficiency of infection and its variation with age of host. Major changes in efficiency of infection occur in mice of about 6, 12 or 22 days old and are expressed through infections by some strains of VEE virus but not others. Thus at least three separate criteria of age and efficiency of infection are available for the differentiation of strains of VEE virus and for the statement of response-maturity of the host. These representative results are expanded in Table 2.
Specification of VEEV-host interactions

Fig. 2. Responses of mice of defined age (±2 days) to infection by specified strains of VEEV. Responses are shown (a) by efficiency of infection as $ID_{50}$ for older mice per SM i.c. $LD_{50}$ and (b) by outcome of infection as % mortality after i.p. or i.c. administration of the strains shown. Strains are designated by the letter code given in Table I. Previous data for strain I are shown here schematically (Walder & Bradish, 1975).

In young adult mice of 30 to 40 days old the efficiency of infection, regardless of the outcome of infection, ranges from 1 to 0.001 $ID_{50}$ per input infective unit. These efficiencies of infection may be the same or very different for i.p. and i.c. routes.

The outcome of infection

As indicated previously (Walder & Bradish, 1975) and now expanded to more strains of VEE virus, mice infected i.p. or i.c. by different strains of virus show distinct ages for the response to change from death at younger ages to protection at older ages. This change may be specified by the age at infection at which 50% of a test group die and 50% show no primary responses but protection against virulent challenge.

As shown in Fig. 2(b), this age for change of response may occur at 6 to 8 days old, 11 to 14 days old, 18 to 24 days old, or not at all. Furthermore, i.p. and i.c. infections by one strain of virus may show the same or different ages for this change of response from death to protection. This clustering at four different levels of host-maturity corresponds with that already shown (Fig. 2a) for the independent characteristic of efficiency of infection.

The compounding of response characteristics

In Fig. 2(a and b), representative strains of VEE virus show definite ages for changes in the efficiency of infection and the outcome of infection. This information is expanded in Fig. 3 and Table 2 to include the 23 strains of VEE virus listed in Table I. The results in Table 2 refer mainly to mice of strain NMRI although similar results were obtained for key strains in PR mice. The results in Fig. 3 extend those of Fig. 2(a) and show the efficiencies of infection through i.p. and i.c. routes as grouped into eight distinct median curves that
Table 2. General differentiation in vivo of strains of VEEV in terms of critical ages of mice at infection for major changes in the efficiency of infection or outcome of infection

<table>
<thead>
<tr>
<th>Strain of virus (See Table 1 for letter code)</th>
<th>Intraperitoneal infection</th>
<th>Intracerebral infection</th>
<th>Efficiency of infection</th>
<th>Correlation with responses of principal hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6–7 days</td>
<td>10–12 days</td>
<td>18–22 days</td>
<td>6–7 days</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td></td>
<td>D→P;↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td></td>
<td></td>
<td>D→P;↓</td>
<td>0.001</td>
</tr>
<tr>
<td>V, W, J</td>
<td>↓</td>
<td>↓</td>
<td>D→P</td>
<td>0.001</td>
</tr>
<tr>
<td>H</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td>0.01</td>
</tr>
<tr>
<td>I</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>↓</td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>G</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>F</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>E</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>D, T, U</td>
<td>↓</td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>A, B, R, S</td>
<td>↓</td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>O, P, Q</td>
<td>No change at any age</td>
<td>No change at any age</td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

* D→P; outcome of infection changes from death in younger mice to immunization and protection against challenge in older mice.
↓↓ Major change of about −2 log units or more in efficiency of infection, as shown in Fig. 2 and 3.
↓ Change of about −1 log unit in efficiency of infection.
† −, No change of host response occurs at these ages.
Specification of VEEV-host interactions

Fig. 3. The efficiency of infection is shown as:

- $\text{ID}_{50}$ per ml for mice of age shown
- $\text{LD}_{50}$ per ml for suckling mice i.c.

For simplification of results for the 23 strains of virus shown in Table 1 and below, these efficiencies of infection are grouped together as single lines to within less than 0.5 log units of the values actually observed for infections at the age of mouse shown. Individual virus strains appear on two different lines if results following i.p. and i.c. infection differ at any age of mouse. The vertical broken lines at 6, 12, and 22 days of age summarize the major host-maturation events in terms of the survival time, efficiency of infection and outcome of infection already shown in Fig. 1 and 2. Lines a, virus strain L, i.p. route of infection only; b, strains F and H by i.p. route, L by i.c. route, I and K by both i.p. and i.c. routes; c, strain G by i.p. route only; d, strains E and N i.p., F i.e., D, J, T, U, V and W by i.p. and i.c. routes; e, strains E, H and L i.e., C and M by i.p. and i.c. routes; f, strain G i.e.; g, strains A, B, R & S by i.p. and i.c. routes; h, strain N i.e., O, P and Q by i.p. and i.c. routes.

summarize individual results to within ±0.5 log units. The precision of these curves and the changes at about 6, 12 and 22 days old are not determined only by individual points but by the whole continuum that distinguishes the developing pattern of efficiency of infection for that strain and route of infection.

Table 2 shows that these changes in efficiency of infection at about 6, 12 or 22 days old for a particular virus strain and route of infection do not correspond with the presence of a change in outcome of infection from death to protection. Changes in outcome of infection and efficiency of infection may occur separately or together at any of these three critical ages for host-maturation and so provide a multi-character differentiation of virus strains that allows their resolution into at least 14 distinct categories.

DISCUSSION

These studies extend those of a previous report (Walder & Bradish, 1975) and show that when mice of different ages are infected i.p. or i.c. with graded doses of various strains of VEE virus (as listed in Table 1), then there may be definite ages at infection for some strains at which the host shows one or more relatively abrupt changes in response. Thus for mice of about 6 to 7, 10 to 12, 18 to 22, or over 30 days old, any of the following independent changes may occur.
(i) The efficiency of infection (ID$_{50}$/input infective units), in terms of the reciprocal of the observed number of infective units required to infect half of the host-group, may remain the same or may decline significantly.

(ii) The outcome of infection, regardless of the efficiency of infection above, may remain the same or may change from death to protection.

(iii) For mice that die, the survival time before death may remain the same or may lengthen significantly until protection is established.

These characteristic changes and ages (Table 2) provide an array of distinct response characteristics for each virus strain that is more than quantitatively significant since it indicates probable cellular maturation events in host defence that separately control stages in the elimination of virus and the expression of virulence. These developing defences are effective only against some strains of VEE virus or routes of infection. This emphasizes the delicate dynamic balance between the potentials of a virus strain to involve critical sites and the potentials of the host to respond.

The lethality for adult mice (over 30 days old) following i.p. infection is shown in Table 2 as a marker for virulence for equidae and man. The several strains of VEE virus (L, M, N, K, V, W, J) that protect mice over 30 days old but are lethal for younger mice are primarily avirulent for horses but uncertain in the extent to which virulent sub-populations may emerge during natural passages. The present resolution of many strain differences offers a powerful tool for the study of such population selection mechanisms.

The comparison of any two lines in Table 2 indicates the tests in which these two strains or groups of strains may be most sensitively distinguished. Strains of highest or lowest efficiency of infection by any route may be of highest or lowest virulence. The sizes of plaques formed in monolayers or suspensions of Vero or primary chick embryo cells have not correlated with the above in vivo characteristics of the several virus strain samples (Walder & Bradish, 1975).

The continuity shown (Fig. 1) for the lengthening of survival time until protection is established suggests that survival time is more an indicator of the efficiency of immune stimulation by virus than of the expression of virulence. If the outcome of infection (expression of virulence) represents the balance between the destructive proliferation of virus and its regulation by immunological factors (Nathanson & Cole, 1970, 1971; Bradish et al. 1975a, b; Bradish & Fitzgeorge, 1979), then the lengthening of survival time is an indicator in mice of different ages of the efficiency of this immunological feed-back for different strains of virus. Thus the expression of virulence is only one indicator of the course of the virus-host interaction and it is equally important to quantify the distinct efficiency of infection and expression of immune stimulation. This must apply to both principal and model hosts and emphasizes the need for a multifactorial in vivo specification.

As defined in this paper, the efficiency of infection is assessed as the reciprocal of the number of infective units (SM i.c. LD$_{50}$) required to infect 50% of the host population. Some authors have called this ratio (or its logarithmic forms) a ‘virulence index’ (Halle & Zebovitz, 1977; MacKenzie, 1975) and have not distinguished the efficiency and the outcome of the virus-host interaction. Thus the parent and mutant strains of Japanese encephalitis virus (Halle & Zebovitz, 1977) show an equal efficiency of infection i.c. in suckling mice of about 0.05 or 1 in 20. In mice of 3 weeks old the efficiency of infection was still low for the parent strain but almost unity for the mutant strain. The outcome of infection i.c. was death in all cases so that both parent and mutant strains were virulent despite the lengthened survival before death and improved immunogenicity for the mutant strain. Similar comments apply to the study of strains of foot-and-mouth disease virus in mice by MacKenzie (1975). These differences illustrate again that wider criteria are required to resolve a critical scale of virulence-avirulence.
An important correlation must be considered between the efficiency of infection and the rate of elimination of infective virions following infection. The efficiency of infection defines the proportion of input virions that successfully infect. Clearance relates by difference to the proportion of virions \((V/V_0)\) that have not yet infected and can be detected in the bloodstream. A clearance rate \((\log (V/V_0) \div \text{time in min})\) of 0.3 log units in 10 min implies that 50% of infective virions following intravenous inoculation have been inactivated, eliminated or adsorbed. A rapid clearance rate of 3 log units in 10 min only doubles to 99.9% the dose of virus yet to be accounted for. These simultaneous mechanisms can only be resolved by detailed investigation. Tests in hamsters by Jahrling & Scherer (1973) suggest that, after intracardiac inoculation, three avirulent strains of VEE virus were cleared from plasma by about 2 log units in 10 min; six virulent strains were cleared by 0.1 to 1.0 units in 10 min. Similar results for monkeys have been presented by Jahrling et al. (1977). This suggestive inverse correlation of clearance with virulence raises the questions on interpretation outlined here.

Whatever the mechanisms in phagocytosis, transport, wasteful adsorption or physical inactivation that determine clearance, it is evident that infection is highly selective unless the efficiency of infection is nearly unity. For an efficiency of infection of 0.001, only one infective virion in 1000 may successfully infect, and this may represent a highly selected subpopulation of the strain sample having an acceptable receptor configuration or tissue tropism. Such finally infective virions evidently support no correlation between the expression of virulence and the several selective pressures they have survived. Clearly, older mice are infected only by a more highly selected residue of a virus-strain sample. It remains to be seen how the in vitro kinetics (Helenius et al. 1978) of receptor–virus reactions can be related to the actual efficiencies and outcomes of infections in vivo. These aspects of infection and heterogeneity are fundamental to the study of tropism, virulence and immunogenicity.

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REFERENCES


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