The Responses of Normal and Athymic Mice to Infections by Togaviruses: Strain Differentiation in Active and Adoptive Immunization

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SUMMARY

Strains of yellow fever virus (YFV), Venezuelan equine encephalomyelitis virus (VEEV) and Semliki Forest virus (SFV) have been used to compare the stimulations of regulatory immunity (pre-challenge), antibody synthesis and protective immunity (post-challenge) in athymic-nude and normal mice. Similarly, direct assessments have been extended to athymic recipients of normal spleen cells and to adoptively immunized mice. The results indicate that the responses of mice to different togaviruses or strains of togaviruses may be differentially T-lymphocyte dependent at any one or more of the above three stages of host response. T-cell reconstitution or adoptive immunization may be effective only for the virus strains of highest immunogenicity. These results suggest a resolution of T-lymphocyte dependence at three levels of host response to virus infections. This approach may be of value in the similarly direct in vivo differentiation of other virus strains and as a practical framework for the consideration of the in vivo significance of the variety of in vitro lymphocyte markers.

INTRODUCTION

The nude-athymic mouse differs from its 'normal' heterozygous litter-mate in its lack of thymus-processed lymphocytes (T-cells; Pantelouris, 1968; Rygaard & Povlsen, 1974, 1977; Nomura et al. 1978). This enables the several phases of response to virus infections (Bradish et al. 1979) to be followed comparatively and in sequence in hosts that differ in a major cellular compartment. Furthermore the frequent uncertainty in correlation between cellular specifications in vitro (Katz, 1977) and the expression of in vivo functions does not arise.

This use of athymic mice and their litter-mates has been applied to the estimation of the T-cell dependence of regulatory immunity (expression of virulence), antibody stimulation and protective immunity following primary infection by distinct strains of Venezuelan equine encephalomyelitis virus (VEEV) and yellow fever virus (YFV). The virus strains have been specified in terms of their differing demands upon the T-cell status of athymic, reconstituted or adoptively-immunized mice. These results suggest one quantitative in vivo framework within which immunological and virological criteria of host-responsiveness may be brought together for deeper correlation.

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METHODS

Strains of viruses. The strains of virus used in this study are those defined in previous papers. The codes C1 and m1 in the strain designations below refer to one (or more) passages in suspensions of primary chick embryo cells or in the brains of suckling mice; these passage codes will not be used after this section of Methods. For Semliki Forest virus (SFV), the clonally defined avirulent strain A774.C3 (Bradish et al. 1971, 1972) was used for the primary intraperitoneal (i.p.) infection of mice by a dose of 3000 p.f.u. At 14 to 28 days later mice were challenged i.p. with 10^4 p.f.u. of the L10.C1 lethally virulent strain of SFV. For VEEV, the vaccine strain TC83.C1 and a strain of intermediate virulence, MUCAMBO.C1 (Walder & Bradish, 1975, 1979) were used as above for primary i.p. infection by 10^6 and 10^5 p.f.u., respectively. This was followed 14 to 28 days later by i.p. challenge with 3000 p.f.u. of the strain P2023.C1 which is virulent to man, horse and mouse. As will be discussed, the range of primary doses reflects strain differences in efficiency of infection and immunization.

For YFV, the human vaccine strain 17D.C1 (Arilvax; Burroughs Wellcome Ltd) and the strains virulent for man, Y1.m1 (Asibi, Ghana, 1927), Y27.m1 (Suarez, Columbia, 1936), D2.m1 (French Neurotropic from Martin, 1969) were used for primary i.p. infection at doses of 10^4 to 10^6 infective units [p.f.u. or suckling mouse intracerebral (i.c.) LD_{50} units]. The detailed passage histories and in vivo characteristics of these virus strains have been described (Fitzgeorge & Bradish, 1979). Following primary i.p. infection as above, mice were challenged 21 to 35 days later by i.c. administration of 10^4 infective units of the vaccine strain of YFV, 17D.C1, which was uniformly lethal in mice infected by this route.

Assays of virus infectivity. Virus samples to be used for primary infection or challenge of mice, or suspensions of mouse tissues, were assayed for infectivity by direct plaque counting. For this purpose, 1 ml samples of appropriate dilutions in Parker's medium 199 at pH 7.4 were incorporated with 9 ml of an agar suspension of primary chick embryo cells; this procedure has been described by Bradish et al. (1971).

For the strains of YFV which failed to produce clear plaques (Y1, Y27 and D2), virus infectivity assays were made by the inoculation of serial dilutions (0.025 ml) into the brains of 3 to 5 day-old suckling mice held in litter groups of about 10.

Assays of Neutralizing activity of mouse sera. Blood samples were taken sequentially and individually from the retro-orbital plexus of anaesthetized mice. After mixing in equal volume with 10 units/ml of heparin BP in saline, plasma samples were held at -20 °C until required for assay. Undiluted or serially diluted samples of mouse sera were assayed for virus neutralizing activity by plaque reduction tests in agar suspensions of primary chick embryo cells (Fitzgeorge & Bradish, 1973). Alternatively, the virus neutralizing activity was estimated by an equivalent zone-inhibition test also in agar suspensions of primary chick embryo cells. In all cases standard mouse or rabbit antisera were included for comparison and uniformity.

The challenge virus strains in these neutralization tests were A774 for SFV, TC83 for VEEV and 17D for YFV. These were adopted by reason of avirulence and the results were little influenced by the use of other strains.

Results are expressed as the serum neutralization index (SNI) which is the logarithm of the standardized antibody activity or concentration (Fitzgeorge & Bradish, 1973).

Spleen cell transfers. These procedures followed those recently described by Bradish et al. (1979). Mice at 40 to 70 days old were killed by fracture of the neck and their spleens removed aseptically. Each spleen was passed through 36 mesh, stainless steel gauze and washed through with 1 ml of Parker's medium 199 containing 10% calf serum at pH 7.4.
After passing three times through a 27 gauge needle, the spleen suspensions were allowed to settle for 1 min before testing for count, viability and, where appropriate, infectivity. Generally, recipient mice received one donor spleen equivalent of $1 \times 10^8$ to $4 \times 10^8$ cells from a sex-matched, litter-mate.

**Procedures in mice.** Athymic-nude mice and their ‘normal’ heterozygous litter-mates, all with an out-bred genetic background, were obtained from a colony maintained at Allington Farm, Porton. The maintenance and use of these mice at 40 to 70 days old was as described by Bradish *et al.* (1979). The parallel control tests made throughout this study in in-bred Balb/c or A2G mice will not be quoted in detail in view of their general confirmation of the findings for the ‘normal’ heterozygous mice.

As discussed in the previous paper (Bradish *et al.* 1979), the comparison of the responses to infection of nude-athymic mice and their normal litter-mates permits the assumption that observed changes are due to the degree of availability of competent, thymus-processed lymphocytes (T-cells).

**Plan of experiments for observations of T-lymphocyte control of host responses to infection.** As described under *Strains of virus*, groups of athymic mice and their sex-matched, normal litter-mates were infected i.p. by nominally avirulent strains of SFV, VEEV or YFV. The incidence of mice dying characteristically within specified times due to this primary infection (called D) indicated the proportion in which regulatory immunity (non-specific, cellular or humoral) had failed to check progressive invasion by virus (Bradish *et al.* 1979; Fitzgeorge & Bradish, 1979).

Survivors to 14 to 28 days were bled for survey of viraemia or serum antibody activity before being challenged by the i.p. (SFV or VEEV) or i.c. (YFV) administration of virus (see *Strains of virus*) which was uniformly lethal in control groups. The incidence of mice surviving to 28 to 35 days after lethal challenge (called P) indicated the proportion in which (i) early regulatory immunity had saved against the primary infection and (ii) later protective immunity had saved against the challenge infection. The incidence of mice dying characteristically after the challenge infection indicated the proportion (called S) in which early regulatory immunity saved against the primary infection despite the later failure of protective immunity to save against the challenge infection. Clearly, $D + P + S = 1$ or 100%.

**Criteria of response.** Since mice protected against (P) or susceptible to (S) the lethal challenge infection may have been either antibody converted (+AB) or not (−AB) prior to challenge, it follows that mice may now be divided into four responding groups (+P, −P, +S, −S) indicating their individual status in stages of regulatory and protective immunity. As noted here and in a previous paper (Fitzgeorge & Bradish, 1979), relatively few mice showed intermediate levels of neutralizing antibody activity so that normal antibody conversions to high activity levels (+AB) were usually sharply distinct from depressed or undetectable antibody conversions (−AB). This will be discussed later.

The direct application of these criteria (D, +P, −P, +S, −S) for regulatory and protective immunity to the assessment of the effectiveness of T-lymphocyte functions was compared in athymic or normal mice after various treatments. Thus the primary avirulent infections and later virulent challenges (see *Strains of virus*) were applied to normal mice, athymic mice or to athymic mice at 1 to 4 days after transfer of T-lymphocyte-rich spleen cell extracts from sex-matched normal litter-mates.

**Adoptive immunity.** In addition to the above transfers of normal spleen cells, primed spleen cells were transferred to athymic or normal mice from donor mice immunized by primary avirulent infection 2 to 4 weeks previously. This attempted adoptive immunization was tested by bleeding the recipients 2 to 3 weeks later for assay of antibody activity; also
Fig. 1. The development of neutralizing antibody activity in serum and protection against challenge following primary i.p. infection by $10^8$ p.f.u. of the TC83 Cl strain of VEEV. See Fig. 4 for the immune responses of these mice. A and ●, Nine of nine normal litter-mates of athymic mice shown to be protected against challenge on the 22nd day; B and □, five of ten athymic mice shown to be protected; C and □, five of ten athymic mice shown not to be protected; D and ▲, seven of nine athymic mice reconstituted with normal spleen cells at 1 day before primary infection and shown to be protected 22 days later; E and △, two of nine athymic mice reconstituted as above and shown not to be protected against challenge; F, the shaded area shows for comparison the profile of IgM neutralizing antibody synthesis already reported (Bradish et al. 1979) for athymic mice infected by the A774 C3 strain of Semliki Forest virus.

in the 3rd to 4th week the recipients were lethally challenged to complete the assessment of their status in protective immunity.

The immune or antibody-converted status of the donor mice was confirmed in each case. Spleen cell suspensions were also confirmed for non-infectivity and the recipient mice for non-infection.

RESULTS

Responses to VEEV infections

Groups of nine or ten athymic mice, normal mice and athymic recipients of normal spleen cells, were tested 1 day later by infection i.p. with $4 \times 10^8$ p.f.u. of the TC83 strain of VEEV. These mice were bled for antibody survey on the 8th and 20th days and then challenged i.p. with 3000 p.f.u. of the lethally virulent P2023 strain of VEEV. As summarized in Fig. 1, all athymic mice showed a declining level of neutralizing antibody activity, even after virulent challenge. The highest level of antibody activity was at day 8, a time previously shown to give a transitory peak of IgM synthesis in athymic mice after infection by the avirulent A774 strain of SFV (Bradish et al. 1979); these data are shown for comparison by the shaded areas in Fig. 1 and 2. No athymic mice (0/10) died following primary infection but only 50% (5/10) were protected against virulent challenge. Thus all athymic mice showed regulatory immunity but only 50% showed protective immunity and none showed persisting or rising levels of antibody activity. When nine athymic recipients of normal spleen cells were tested (Fig. 1), seven showed complete restoration of normal antibody synthesis and protection against virulent challenge. Only 22% (2/9) showed no restoration of antibody synthesis and were not protected against virulent challenge.

This pattern of responses in athymic mice, as for the avirulent strain of SFV (Bradish
Fig. 2. The development of serum neutralizing antibody activity following primary i.p. infection by \(10^{6.8}\) to \(10^{8.8}\) suckling mice i.c. LD_{50} units of the Y1.m1 and Y27.m1 strains of YFV. Responses to challenge are shown in Fig. 3. A, Mean and range for groups of ten A2G mice of 30 to 60 days old infected by strain Y1.m1 (A) or Y27.m1 (△) and shown to be protected against challenge on day 32. B, Mean and individual values for normal litter-mates of athymic mice infected by strain Y1.m1 (■) or Y27.m1 (□) and shown to be protected against challenge on day 24 to 30. C, Mean and individual values for athymic mice infected by strains Y1.m1 (●) or Y27.m1 (○) and shown not to be protected against challenge on day 24 to 30. 7, As C but for athymic mice that received transfers of spleen cells from sex-matched normal litter-mates at 4 days before the primary infection. The shaded area shows for comparison (see Fig. 1) the profile of IgM neutralizing antibody synthesis for athymic mice infected by the A774.C3 strain of SFV.

et al. 1979), indicates that for these representative alphaviruses, protective immunity and continued antibody synthesis are predominantly T-cell dependent and can be restored by the donation of normal T-cells before immunization. In contrast, regulatory immunity is not strictly T-cell dependent.

**Antibody synthesis following YFV infections**

The responses of the present athymic mice and their normal litter-mates to primary 'avirulent' i.p. infections by the strain Y1 and Y27 of YFV differed in many ways from the responses shown above to the alphaviruses VEEV and SFV. As shown in Fig. 2, the normal mice showed no detectable antibody synthesis at 7 to 8 days after primary infection and thereafter a slow rise to levels at 4 to 5 weeks which were as those for A2G mice. Antibody activity levels were at least tenfold lower in the athymic mice.

At least 12/18 YFV-infected athymic mice showed significant, but severely depressed, antibody levels at 2 to 5 weeks after infection: at this time SFV- or VEEV-infected athymic mice showed falling or negligible levels of antibody activity. Only the VEEV- and SFV-infected athymic mice showed the peak of antibody activity at about 7 to 8 days (Fig. 1 and 2). These features demonstrate that the course of detectable antibody stimulation and the components of virus immunogenicity are very different in mice infected by alphaviruses or flaviviruses.
Fig. 3. The incidence (%) of the responses of athymic-nude mice and their normal, heterozygous litter-mates to infections i.p. by $10^6$ to $10^9$ infective units of the indicated strains of YFV. The shaded columns in the histogram for athymic mice show the major changes in response due to T-lymphocyte deficiency. D, Deaths (%) following primary infection (minimal if regulatory immunity is effective). P, Protection (%) following lethal challenge at 3 to 5 weeks after primary infection (maximal if protective immunity is effective). S, Susceptibility (%) to challenge as above (minimal if protective immunity is effective).

**Responses to challenge following YFV infections**

In continuation of the above tests, groups of 9 to 24 athymic and normal mice were infected i.p. by the ‘avirulent’ strains defined. After bleeding 3 to 5 weeks later, these mice were challenged i.c. by the 17D strain of virus. A majority of the ‘normal’ heterozygous mice were protected against lethal challenge whereas the athymic mice were predominantly susceptible.

These results are shown as histograms in Fig. 3. The enhanced susceptibility to challenge in athymic mice is shown shaded in each case. The three YFV strains 17D, Y1 and Y27 all induce an effective regulatory immunity (no change in D) in athymic mice that is followed by an evident failure in protective immunity (major change from P to S). By contrast, the YFV strain D2 fails to induce an effective regulatory immunity in athymic mice, or is responsible for an exceptionally rapid or overwhelming CNS involvement (major change from P to D).
T-cells in active and adoptive immunization

The response histograms for athymic mice, normal mice and the athymic recipients of normal spleen cells are shown in Fig. 4. In all cases the indicated strains of virus were injected i.p. 1 day after the cell transfer and 3 to 5 weeks before lethal challenge under the conditions of the previous sections (see Strains of viruses).

The response histograms for the TC83 strain of VEEV (as for Fig. 1) demonstrate effectiveness of the spleen cell transfers in enhancing the protective immunity of athymic mice and in restoring their ability to synthesize antibody continuously; the median levels of serum neutralization index are shown in the P columns of the histograms. By contrast (Fig. 4), the MUCAMBO strain of VEEV was primarily lethal for athymic mice and neither this failure of regulatory immunity (major change from P to D) nor the failure of antibody synthesis was restored by the prior administration of normal spleen cells. This pattern was also shown by the D2 strain of YFV (Fig. 3). A further distinct pattern of response was induced by the Y1 strain of YFV (see Fig. 3) which, despite an effective regulatory immunity, failed to induce protective immunity or normal antibody synthesis in athymic mice even after spleen cell reconstitution (Fig. 4).

In all of these cases an effective stimulation of protective immunity and antibody synthesis was shown for the normal heterozygous mice. Furthermore, the spleen cell transfer operations that failed to restore T-cell functions in tests with strain Y1 (or Y27) of YFV or strain MUCAMBO of VEEV were nevertheless successful with strain TC83 of VEEV or strain A774 of SFV (Bradish et al. 1979). These features suggest, as will be dis-
Fig. 5. The incidence (%) of the responses of athymic, normal heterozygous and Balb/c mice to lethal challenge (see Methods) at 2 to 4 weeks following the adoptive transfer of spleen cells from immunized donors. The immunized donors are defined by the results shown in previous figures. The responses are shown as: AB, antibody conversion (%) at 2 to 4 weeks following spleen cell transfer; P, protection (%) against challenge in the week following the above bleeding. The numbers at the bases of the AB % columns show the mean neutralization indices (SNI) and this antibody activity level as a % of that in the donor.

Disussed, that the T-lymphocyte reconstitutions of the athymic mice were successful in cellular terms but that virus strains differ in their dependence upon T-cell level or competence for the stimulation of both regulatory and protective immunity.

Transfer of informed T-cells: adoptive immunity

In further tests on the transfer and competence of T-lymphocytes, spleen cell suspensions from donors in a state of established immunity were transferred to athymic or normal recipients. These recipients were then tested for antibody conversion and challenged 2 or 3 weeks later under the conditions already defined. The immune state of the donor mice in terms of antibody conversion or protection at 2 to 4 weeks after their immunization by infection with the various avirulent strains of virus is shown by the control information in previous figures. Additional tests on spleen cell extracts before transfer confirmed that these were not infective. Recipient mice showed no viraemia (strains of SFV or VEEV) and were therefore not directly infected by the donated suspensions.

The response histograms of Fig. 5 summarize the observed incidences of antibody conversion and protection against challenge for groups of at least six to ten mice. The numbers in the antibody columns show the mean neutralization indices (SNI) for the recipient mice and its level as a percentage of the mean antibody activity found in the donor mice. In general, and as found in other studies in normal mice in active or passive immunity, serum
neutralization indices above about 2.0 are associated with a positive incidence of protection against lethal i.p. challenge but, at the individual level, there is no correlation between protective immunity and the pre-challenge level of neutralizing antibody in serum. This lack of correlation is emphasized by the results (Fig. 5) for athymic mice as donors or recipients.

The results of Fig. 5 suggest that adoptive protective immunity and adoptive antibody conversion are generally effective in normal mice when the avirulent alphaviruses (A774 or TC83) are used for donor immunization. This is not so when the 17D strain of the flavivirus YFV is used for the immunization of normal donors or when athymic donors are used after alphavirus immunizations.

**DISCUSSION**

Table I summarizes the results of this study in terms of the direct indicators of host-response following primary avirulent infection and secondary challenge infection. The three sequential indicators of (1) survival or death (D) following primary infection, (2) antibody conversion or not (+AB, -AB) after primary infection or adoptive immunization, or (3) protection against (P) or susceptibility to (S) later virulent challenge, assess the three phases of host response (marked 1, 2, 3 in Table I) in which a T-lymphocyte dependence may be expressed. In all cases in this study, the normal mice showed the primary infections to be avirulent, antibody converting and non-lethal and so demonstrated an effective primary regulatory immunity (Bradish et al. 1979; Fitzgeorge & Bradish, 1979; Walder & Bradish, 1975, 1979). Similarly, these normal mice showed an effective protective immunity when further infected or boosted by challenge.

In comparison with these control observations in normal mice, the athymic mice maintained regulatory immunity against all virus strains except D2 (YFV) and MUCAMBO (VEEV); this deficiency at 1 in Table I, which was not restored by prior transfers of normal spleen cells, suggests that the efficient activation of T-lymphocytes in situ plays an important role in the regulation of the expression of virulence by these virus strains. Some aspects of the primary regulatory mechanisms that may be involved have been discussed by Doherty (1973), Jagelman et al. (1978) and Bradish et al. (1979). All other virus strains tested here stimulated an effective regulatory immunity in the athymic mouse (Table I) but failed to stimulate at the level of antibody conversion (2 in Table I) and again at the level of protective immunity (3 in Table I) against challenge. In the tests with strains of YFV these separate impairments in T-lymphocyte deficient mice were not restored by normal spleen cell donation or adoptive immunization. Despite this, successful restoration was observed under both of these conditions in the parallel tests with strains of VEEV (TC83) and SFV (A774).

These direct tests on the role of thymus-processed lymphocytes at three levels of host response to togavirus infections suggest that a gradient of virus strain dependence may be matched against a gradient of T-cell competence. This is indicated by the layout of the results summarized in Table I. Thus the TC83 strain of VEEV is generally the least aggressive in its influence on deficient mice and responds most sensitively to active or adoptive reconstitutions that fail in other cases. At the other end of the scale, the strains D2 (YFV) and MUCAMBO (VEEV) are avirulent and immunogenic only in the normal mouse. The further strains of SFV (A774) and YFV (17D, Y1 and Y27) show intermediate features. This gradation of T-cell dependence through three stages of host response (1, 2, 3 in Table I) must be related to the efficiency or rate of virus-information transfer through T-lymphocytes: lower numbers of normal spleen cells in reconstitution, or of primed cells in adoptive immunization, may be sufficient for the restoration of immune functions only if
### Table 1. Responses of normal and athymic mice in active and adoptive immunization*

<table>
<thead>
<tr>
<th>Host and prior treatment</th>
<th>VEEV strain TC83·C1</th>
<th>SFV strain A774·C3</th>
<th>YFV strain 17·C1, Y17·C1</th>
<th>YFV strain D2·m1</th>
<th>VEEV strain MUCAMBO·C1 and YFV strain D2·m1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice (heterozygous litter-mates of athymic-nude mice)</td>
<td>+P</td>
<td>+P</td>
<td>+P</td>
<td>+P</td>
<td></td>
</tr>
<tr>
<td>Athymic mice after transfer of spleen cells from normal mice</td>
<td>+P</td>
<td>+P</td>
<td>3/3</td>
<td>±S</td>
<td>D</td>
</tr>
<tr>
<td>Normal mice after transfer of spleen cells from immunized mice (adoptive immunity)</td>
<td>+P</td>
<td>+P</td>
<td>3</td>
<td>-S</td>
<td></td>
</tr>
<tr>
<td>Normal mice after transfer of spleen cells from immunized athymic mice (donor restricted adoptive immunity)</td>
<td>3</td>
<td>3/3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Athymic mice after transfer of spleen cells from immunized normal mice (recipient restricted adoptive immunity)</td>
<td>+S</td>
<td>-P/S</td>
<td>-S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Athymic-nude mice</td>
<td>-P/S</td>
<td>-S/D</td>
<td>-S</td>
<td>D</td>
<td></td>
</tr>
</tbody>
</table>

* Symbols used are as follows: +, neutralizing antibody activity at near-normal levels in serum when tested before virulent challenge at 2 to 4 weeks after active or adoptive immunization as shown; -, neutralizing antibody activity detected at markedly depressed or negligible levels; D, response of death after primary infection i.p. by the indicated strains of virus; P, response of protection against challenge at 2 to 4 weeks following active or adoptive immunization as indicated; S, response of death following challenge after active or adoptive immunization as above; P/S and S/D, mixed or partial responses as above; 1, deletion of regulatory immunity; 2, deletion of competence in serum antibody synthesis; 3, deletion of protective immunity.

The virus strains concerned are maximally immunogenic following infection and replication. Certainly the Y1 and Y27 strains of YFV show a significantly higher efficiency of infection and immunization than the 17D vaccine strain (Fitzgeorge & Bradish, 1979) yet all of these strains are more sensitively T-cell dependent than the most efficiently infective and immunizing A774 strain of SFV (Bradish et al. 1971, 1972) and the TC83 strain of VEEV of low efficiency of infection (Walder & Bradish, 1975, 1979).

A difference between these tests with alpha- and flaviviruses is that protective immunity has been assessed by i.p. challenge with VEEV or SFV, but by i.c. challenge with YFV. This reflects the fundamental distinction that virulent or avirulent strains of VEEV or SFV (alphaviruses) retain their character regardless of i.p. or i.c. route of infection in normal mice, whereas all the tested strains of YFV (flaviviruses) are essentially 'avirulent' by the i.p. route but 'virulent' by the i.c. route. It is thus probable that protective immunity against i.c. challenge is more critically dependent upon the competence and activation of T-lymphocytes than is that against i.p. challenge. Despite this, the graduated layout of Table 1 would be maintained on the evidence only of primary i.p. infections and pre-challenge levels of neutralizing antibody activity. Even for the A774 strain of SFV in athymic mice, established persistent infections in brain are tolerated but cannot be cleared unless competent T-lymphocytes are available (Bradish et al. 1979).

Regardless of the effects of strain and dose of immunizing virus (Fitzgeorge & Bradish,
and route of challenge infection, the assessment of protective immunity by lethal challenge must depend upon the dose and strain of ‘virulent’ virus employed and the time of its administration after the primary immunizing infection by the ‘avirulent’ strain of virus. Challenges in the present study have been made with the least ‘virulent’ strain of YFV and the most ‘virulent’ strains of VEEV and SFV. Furthermore, in control tests with the latter viruses, protection in normal mice at 7 days or more after immunization was effective against i.p. or i.c. challenge and against higher doses of challenge virus; this has not been tested in athymic mice.

Despite these quantitative cautions related to the doses and rates of replication of the immunizing and challenging viruses, it has been possible to identify at several levels (Table I) the balances between the intrinsic immunogenicity and pathogenicity of the primary virus in relation to the responsiveness of the tissues or cellular compartments involved. Closer definitions of these regulations of the dynamic virus-host interaction appear to require further histological and immunological correlations across the response-change levels noted in Table I (1, 2, 3).

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