Immunomodification and the Expression of Virulence in Mice by Defined Strains of Semliki Forest Virus: the Effects of Myocrisin and l-Asparaginase

By C. J. BRADISH, K. ALLNER AND R. FITZGEORGE
The Microbiological Research Establishment, Porton Down, Salisbury, U.K.

(Accepted 14 April 1975)

SUMMARY

Studies in mice of the modes of immunomodification imposed by cyclophosphamide, have been extended to comparative studies with Myocrisin, l-asparaginase and interferon. It has been shown for mice infected i.p. or i.c. by an avirulent clone of SFV, that the potentiation of disease may be marked by many distinct changes in the type and rate of response.

For low i.p. doses of virus, enhancement (Myocrisin, l-asparaginase) or impairment (interferon) of the efficiency of infection may be associated with death (potentiation by Myocrisin) or protection (immunoenhancement by l-asparaginase).

For higher doses of virus the increased mortality after infection (primary potentiation) is determined within 2 or 3 days and appears to be due to inhibition of phagocytosis (Myocrisin and l-asparaginase) and of T cell functions (l-asparaginase and cyclophosphamide). The increased incidence of death after challenge (secondary potentiation) appears to be due to inhibition of B cell functions (cyclophosphamide) associated with suppression of antibody synthesis and persistence of viraemia.

These results are discussed in relation to the expression of virulence by a heterogeneous and replicating antigen. The critical cellular and humoral changes which occur within 2 or 3 days of infection are emphasized.

INTRODUCTION

It was shown in the previous paper (Bradish, Allner & Fitzgeorge, 1975) that for young mature mice infected by an avirulent clone of Semliki Forest virus, the potentiation of disease by cyclophosphamide may occur with or without suppression of antibody synthesis and modification of the course of viraemia. These distinct modes of immunomodification were determined by the time of drug administration in relation to virus infection and the consequent cellular responses. These events determined the expression of virulence within about 2 days of infection by virus and about 3 days before conventional humoral or cellular indicators were available.

In view of these inferences it was desirable to make supporting studies with drugs having possibly sharper and more specific effects either upon the functions of T or B cells, or upon the even earlier phagocytosis and processing of virus as a replicating antigen. The several effects of Myocrisin, l-asparaginase and interferon are considered in this paper and discussed in terms of the regulatory processes which combine to control the expression of virulence.
METHODS

Strains of Semliki Forest virus (SFV). These were as in the previous paper (Bradish et al. 1975) and earlier reports (Bradish, Allner & Maber, 1971). Most experiments were with the avirulent strain A7.C1 or its derived clone A7(74).C1 which efficiently immunize young mature mice (≈ 100 p.f.u./i.p. PD_{50}) against lethal challenge two days later by the virulent strain L10.C1 of SFV (≈ 100 p.f.u./i.p. L{D_{50}}). Basic dose and response relationships in the absence of drug treatment are given in the previous paper.

Assays of virus infectivity and interferon or antibody activity. These were based on the counting of plaques formed in agar suspension of primary chick embryo cells (CEC) or mouse L cells. The medium for all dilutions was Parker's 199 at pH 7.4 with 10% pre-tested calf serum or 0.3% BSA. The inoculated doses of virus to be related to animal responses were titrated simultaneously in mice and cell suspensions (Bradish et al. 1971). Antibody activities were determined through plaque reduction in the CEC-suspension system (Fitzgeorge & Bradish, 1973) and interferon activities through plaque reduction in a similar L cell suspension system (Bradish & Allner, 1970). Antibody activities are expressed logarithmically as the serum neutralization index (SNI).

Procedures in mice. Mice of strain PR, A2G or C3H from Allington Farm, Porton, were used in groups of 10 or more of defined age (±10%). Sex was also defined but only pooled results are quoted since males and females showed very similar responses. In general, mice received a confirmed dose of virus i.c. or i.p. in 0.025 ml on day zero.

Assessment of animal responses

The outcome of infection. The operational basis of these experiments is that of the previous paper (Bradish et al. 1975) in which the incidence of mice dying after primary virus infection (D %) or after subsequent lethal challenge (S %) is compared in control and drug-treated groups. In each group the mice surviving the challenge infection (P %) are regarded as immunized or protected by the primary infection.

The changed responses in infected animals due to immunomodification by the chosen drug (suffix i) thus define a primary potentiation of disease as the increased incidence of deaths before challenge (D_{i} - D_{0})%. Additionally, a secondary potentiation is indicated by the increased incidence of deaths following challenge (S_{i} \times 100/C) %; the latter term includes the correction for the extent of lethality (C %) in control mice receiving only the challenge virus infection.

The efficiency of infection. The present studies are also particularly concerned with the efficiency of infection, regardless of the outcome of that infection as death (D) or immunization (P). The efficiency of infection, defined as dose for 50% infection or p.f.u./ID_{50}, was obtained directly from paired titrations of infectivity as p.f.u./ml and as ID_{50}/ml in mice (Bradish et al. 1971). Any positive response in mice of death, protection or antibody synthesis indicated infection. Such titrations, using tenfold dilutions of virus, were made in groups of mice treated at various times with the required drug.

Drugs. These were prepared at appropriate concentrations in the medium for virus and were administered as 0.1 ml i.p. at 2 to 4 h before virus (day 0) or on the indicated days before (−) or after (+) virus inoculation.

Sodium auro-thio-malate (Myocrisin; May and Baker Ltd, Dagenham, Essex) was administered once as 7 mg i.p./mouse. Experimental conditions were generally as described by Allner et al. (1974), and only 10 (7%) of 150 mice at 30 to 35 days old showed toxic deaths within 8 weeks.
Virus, virulence and immunomodification

Fig. 1. Responses of groups of 10 to 20 mice to i.p. infection on day 0 by 10^6 p.f.u. of the avirulent strain A774.C1 of SFV. Mice also received single i.p. doses of 7 mg Myocrisin on the day shown. Curve A: 30 to 35 days old mice of strain A2G (○) or C3H (▲). Curve B: 30 to 35 days old PR mice (■) or 130 to 170 days old A2G (○) mice. Curve C: 130 to 170 days old PR mice (□). The mean day of death in primary potentiation for each group is shown at each point.

The l-asparaginase (Erwinia asparaginase NSC 106977) was kindly supplied and assayed by our colleague Dr H. E. Wade (Wade & Rutter, 1970; Ashworth & MacLennan, 1974). This was isolated from cultures of the soft-rot Erwinia carotovora and part of its activity may have been due to l-glutaminase. At 4000 International units per mouse i.p. there were fewer than 5% toxic deaths within 8 weeks.

The mouse interferon was produced in rolling-cultures of mouse L cells and assayed against standards as described by Bradish & Allner (1970). Preparations were purified (> 10^6 units/mg) and concentrated (> 10^6 units/ml) as summarized by Fantes (1973). There was no significant toxicity in mice at doses up to 10^6 units.

RESULTS

Immunomodification by Myocrisin

It has been shown (Allner et al. 1974) that the probable blockade of the phagocytic function of macrophages by Myocrisin at day 0 is associated in mice with modifications in both the efficiency and the outcome of infection by defined strains of SFV.

Features to be noted in the potentiation of an otherwise avirulent infection by Myocrisin at day 0 (7 mg/mouse/i.p.) are that there is no elevation or persistence of viraemia or inhibition of antibody synthesis, at least in terms of the gross humoral activity. The potentiation of the avirulent infection by Myocrisin is marked by an acceleration and elevation of virus replication in the brain and an approach to the polioencephalitis with neuronal destruction which characterizes primary infection by the virulent L10.Cl strain of SFV. Additionally, immunomodification by Myocrisin is marked by primary potentiation (enhanced lethality following primary avirulent infection) without significant secondary potentiation (impairment of protection in subsequent survivors). Thus, of all the mice which survived (372) the avirulent infection potentiated by Myocrisin, only 3.2% (12) were
Table 1. Responses of groups of 20 to 30 PR or A2G mice of 30 to 150 days old to benign infection by 10⁶ p.f.u. i.p. or i.c. of avirulent virus (strain A774.C1) and potentiation by 7 mg i.p. Myocrisin administered 2 to 4 h before

<table>
<thead>
<tr>
<th>Strain of mice and age</th>
<th>Extent of primary potentiation and mean survival time</th>
<th>Mean survival time*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myocrisin + A774.C1 i.c.</td>
<td>Myocrisin + A774.C1 i.p.</td>
</tr>
<tr>
<td>A2G 30-33 days</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>2:6 days</td>
<td>6:6 days</td>
</tr>
<tr>
<td>125-150 days</td>
<td>90%</td>
<td>56%</td>
</tr>
<tr>
<td></td>
<td>3:9 days</td>
<td>7:8 days</td>
</tr>
<tr>
<td>PR 30-37 days</td>
<td>90%</td>
<td>56%</td>
</tr>
<tr>
<td>150 days</td>
<td>26%</td>
<td>7:8 days</td>
</tr>
<tr>
<td></td>
<td>6:5 days</td>
<td>8:7 days</td>
</tr>
</tbody>
</table>

* See Table 2 and previous paper for other control series.

susceptible to later lethal challenge by the virulent strain of SFV. This effect of Myocrisin at day 0 resembles that for cyclophosphamide administered 3 or more days before virus (Bradish et al. 1975). Since no detectable change in antibody synthesis is involved, these effects may be due to an inhibition of T cell stimulation or proliferation with almost normal expression of B cell functions.

The influence of time of administration of a single dose of 7 mg i.p. of Myocrisin with respect to avirulent infection on day 0 by a massive i.p. dose of 10⁶ p.f.u. of SFV (strain A774.C1) is shown in Fig. 1. The overall controls showed 6-7% (I₀) lethality due to Myocrisin alone and a response (D: P: S) to the avirulent infection alone of 11% primary deaths (25): 87% protected (187): 2% challenge-susceptible (4).

The primary potentiation by Myocrisin is shown by two bell-shaped curves according to the age of the mouse. The lower curve (Fig. 1) for mice 130 to 170 days old receiving 7 mg Myocrisin may be raised to the position of the upper curve if 15 to 20 mg Myocrisin/mouse is administered. The potentiation of infection in younger mice of 30 to 35 days old is shown by the upper curve, except for young PR mice which showed a maximum primary potentiation of about 60% at the highest acceptable dose of 7 mg Myocrisin (Allner et al. 1974). In view of this sensitivity-toxicity limitation for PR mice, the limited-outbred strains A2G and C3H were used in most subsequent experiments.

These results demonstrate that primary potentiation of the avirulent infection by Myocrisin is maximal at almost 100%, if the drug is administered from about 2 days before to 2 days after virus infection. Under these conditions the mean survival times, shown as number of days above points in Fig. 1, range from 6:0 to 8:5 days and approach the mean survival time of 6:0 days (Table 1) following primary infection i.p. by the virulent strain. This rapidity of lethal response in primary potentiation is distinct from that of over 11 days observed following administration of cyclophosphamide at optimum times, or of Myocrisin (Fig. 1) at 4 or more days after avirulent virus. Thus, only Myocrisin promotes a fast primary response similar to that following i.p. infection by the virulent strain.

These features of primary potentiation of an otherwise avirulent infection, are extended in Table 1 to a comparison of the influences of the route of virus infection. The primary potentiation by Myocrisin (7 mg i.p.) of an i.c. infection is invariably about 30% higher than that observed for an i.p. infection, unless both are near maximal. Similarly the potentiation in the younger mice is about 50% higher than that in the older mice. In consequence of this the older mice of Porton random (PR) strain show significant potentiation of an i.c. infection, but not of an i.p. infection (Fig. 1), and are significantly less responsive to the influence of
Table 2. Responses of groups of 15 to 25 PR mice of 35 and 55 days old infected by 300 to 3000 p.f.u. i.p. of avirulent strain (A774.C1) of Semliki Forest virus and potentiated by 4000 U i.p. of l-asparaginase

<table>
<thead>
<tr>
<th>Day of administration</th>
<th>Response of mice as numbers and (%)</th>
<th>% primary potentiation*</th>
<th>Mean day of death of mice in D</th>
<th>Mean SNI† before lethal challenge in P and S mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p. of 4000 U l-asparaginase</td>
<td>Dead (D)</td>
<td>Protected (P)</td>
<td>Challenge susceptible (S)</td>
<td>D1 - D0</td>
</tr>
<tr>
<td>-14 55</td>
<td>1 (6)</td>
<td>13 (77)</td>
<td>3 (17)</td>
<td>Nil</td>
</tr>
<tr>
<td>-1 35</td>
<td>9 (60)</td>
<td>3 (40)</td>
<td>0 (0)</td>
<td>55</td>
</tr>
<tr>
<td>0 55</td>
<td>4 (19)</td>
<td>13 (62)</td>
<td>4 (19)</td>
<td>14</td>
</tr>
<tr>
<td>0 35</td>
<td>13 (54)</td>
<td>11 (46)</td>
<td>0 (0)</td>
<td>49</td>
</tr>
<tr>
<td>±3 35</td>
<td>8 (53)</td>
<td>7 (47)</td>
<td>0 (0)</td>
<td>48</td>
</tr>
<tr>
<td>±3 35</td>
<td>7 (35)</td>
<td>8 (47)</td>
<td>5 (25)</td>
<td>30</td>
</tr>
<tr>
<td>Avirulent virus only</td>
<td>35, 55</td>
<td>3 (5)</td>
<td>49 (74)</td>
<td>14 (21)</td>
</tr>
<tr>
<td>Virulent virus</td>
<td>35, 55</td>
<td>54 (83)</td>
<td>2 (3)</td>
<td>9 (14)</td>
</tr>
</tbody>
</table>

* Expressed throughout as the increased incidence of primary deaths over that shown following avirulent infection of control groups.
† SNI, serum neutralization index as log (antibody activity) estimated through plaque inhibition (see Methods).

Animals were bled for antibody assay at 14 and 21 days and before lethal challenge by 10^4 p.f.u. i.p. of virulent virus. The first entry shows the SNI for animals shown to be protected (P) against challenge, the second entry for animals susceptible (S) to challenge.

Myocrisin than are mice of the A2G or C3H strains. In all cases the median survival time in the potentiated i.c. infection is shorter than that in the potentiated i.p. infection and, as before, approaches the survival time in the primary i.p. infection by the virulent strain L10.C1. For the more sensitive A2G strain of mice the survival time in the potentiated i.c. infection approaches the survival time in the primary i.c. infection by the virulent strain. These survival times are significantly shorter than those observed under similar conditions for avirulent infections potentiated by the administration of cyclophosphamide (Bradish et al. 1975).

Immunomodifications by l-asparaginase

The distinctions between the modes of immunomodification by cyclophosphamide and Myocrisin prompted tests on the action of l-asparaginase in view of its low toxicity, short half life and activity in inhibition of proliferating T or B lymphocytes (Ashworth & MacLennan, 1974).

When l-asparaginase was administered intraperitoneally, effects were maximal for doses of about 4000 units and minimal below about 1000 units: of 110 control mice which received 4000 units i.p. of l-asparaginase, only 5 died during the experiment. The extent of primary potentiation due to this dose of l-asparaginase is shown in Table 2 for 35 days old PR mice which also received an infecting dose of 10^2.3 to 10^4.3 p.f.u. i.p. of avirulent virus: about 50 % (primary potentiation) of these infected mice died if l-asparaginase was administered from one or more days before to a day after the avirulent virus. Secondary potentiation was negligible. The mean survival time before primary death was 7.5 days whereas that for the control mice infected by the virulent strain was 5.8 days. Thus, as for cyclophosphamide but unlike Myocrisin, the primary potentiation of this avirulent infection by l-asparaginase failed to elevate its virulence to that shown by the virulent strain of virus. Primary potentiation by l-asparaginase follows a similar course to that for cyclophosphamide and suggests a major effect upon the stimulation or proliferation of T cells.

Table 2 also shows the humoral antibody activities as the median serum neutralization
Fig. 2. Influence of 4000 units $i.p.$ of l-asparaginase upon efficiency of infection in mice $i.p.$ of virulent (L10.C1) or avirulent (A7.C1) strains of SFV. ▲, avirulent strain (A7.C1) in 60 days old PR mice; ○, avirulent strain (A7.C1) in 36 days old C57 mice; ●, avirulent strain (A7.C1) in 36 days old PR mice; ■, virulent strain (L10.C1) in 60 days old PR mice.

index (SNI) for mice surviving to 14 or 21 days after primary infection: all younger mice showed high antibody activity and, as with cyclophosphamide, evident recovery of antibody synthesis to almost the levels shown by mice receiving only avirulent virus. Of these mice 89% (±) were protected against the subsequent challenge by virulent virus at 14 to 21 days after primary infection and thus showed minimal secondary potentiation. This result differs from that for cyclophosphamide (Bradish et al. 1975) but resembles that for Myocrisin.

**Efficiency of infection**

A second and distinct effect due to l-asparaginase is the enhancement of the efficiency of primary infection. This is unrelated to the outcome of infection as death or protection, since both virulent (L10.C1) and avirulent (A7.C1) strains of SFV showed an efficiency of $i.p.$ infection (Fig. 2) of about 250 p.f.u./ID$_{50}$ in normal mice and 10 or less p.f.u./ID$_{50}$ in mice primed by l-asparaginase 4 h before. This effect on the efficiency of infection by virus strains of any virulence has also been found for Myocrisin (Allner et al. 1974) but not for cyclophosphamide. This effect is not shown if the drugs are administered three or more days after virus infection.

**Adjuvant effect at low doses of virus**

A third and regular activity of l-asparaginase, which has not been shown for either cyclophosphamide or Myocrisin, is an enhanced protective and antibody response for critical low doses (see later Table 3) of avirulent virus. Thus (Fig. 3) control mice receiving only avirulent virus were protected only if infected by at least 2000 p.f.u. $i.p.$, as confirmed by their high humoral antibody activity at 14 days after infection and before virulent challenge. By contrast
Fig. 3. Illustration of three consequences of immunomodification: influence of dose of virus and immunomodification by l-asparaginase upon responses of 36 days old C57 mice. In (a) △, ● mice received 4000 units i.p. of l-asparaginase at 2 h before virus infection; in (b) ∆, ○ mice received only the indicated i.p. doses of avirulent virus of strain A7.C1. The adjuvant effect is well illustrated at 20 p.f.u., the primary potentiation of disease at 2000 p.f.u., and the increased efficiency of infection by the difference between these response-doses. ○, ●: primary lethality; △, ▲: SNI.

Table 3. Mean dose thresholds for responses of normal and Myocrisin or l-asparaginase-treated, 30 to 40 days old mice to i.p. administration of SFV (strain A774.C1) with about 20 virus particles per p.f.u.

<table>
<thead>
<tr>
<th>Response</th>
<th>Threshold doses as p.f.u. administered i.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: no primary infection or immunization</td>
<td>Normal mice: Below 400</td>
</tr>
<tr>
<td></td>
<td>Treated mice: Below 15</td>
</tr>
<tr>
<td>B: primary infection without primary immunization</td>
<td>400 to 10⁶</td>
</tr>
<tr>
<td></td>
<td>15 to 3 × 10⁶</td>
</tr>
<tr>
<td>C: primary infection and immunization</td>
<td>Over 10⁶</td>
</tr>
<tr>
<td></td>
<td>Over 3 × 10⁶</td>
</tr>
</tbody>
</table>

mice primed by 4000 units (U) of l-asparaginase at 4 h before virus infection by only 20 to 2000 p.f.u., showed high antibody activity and full protection against subsequent challenge by virulent virus. Infection by doses of avirulent virus above 2000 p.f.u. i.p. showed the primary potentiation already discussed. Thus the 25- to 100-fold enhancement of infection in mice by l-asparaginase (Fig. 2) was associated with the protection of 74% (7/10) infected by lower and formerly non-infecting doses of virus. For Myocrisin the similarly enhanced
efficiency of infection was associated with rapid primary death (81%: 80%) at such formerly non-infecting doses of virus; these differences are highly significant. The suppression by Myocrisin of the protective influence of the avirulent virus particles in a virus population has been discussed (Allner et al. 1974).

Relative infective and immunogenic efficiencies

An important feature in these experiments is the composition of the inoculated virus population in terms of infective virus particles and non-infective but potentially immunogenic virus particles and subunits (Neurath & Rubin, 1971). For SFV samples of the present type it has been shown (Cameron & Bradish, 1972) that one infective unit (p.f.u.) is associated with about 6 to 60 intact virus particles which may be identified by electron microscopy. It has also been shown that the 50% infective dose (ID50) is about 250 p.f.u. i.p. for the normal 30 to 40 days old mouse but less than 20 p.f.u. i.p. (Fig. 2, 3) after the presumed inhibition of primary phagocytosis by Myocrisin or l-asparaginase. In parallel with these observations it was found that if infective virus particles were inactivated by formalin (0.1% for 4 days at 4°C) then an i.p. dose of about 10^9 to 10^10 virus particles (or 10^9 p.f.u. before inactivation) was required in normal mice to produce 50% protection against lethal challenge or detectable recovery of specific neutralizing activity in serum. A similar threshold of 10^8 to 10^10 virus particles was observed for inactivated influenza A virus in mice by our colleagues Dr G. Appleyard and Dr J. Oram (personal communication).

These quantitative factors are summarized together in Table 3 in order to emphasize the distinct conditions and dose-range requirements for primary infection or primary immunization. In the intermediate range (B) immunization is delayed until primary infection and replication have amplified virus antigens to the levels required for primary immunization (C).

Most of the experiments reported in this paper have used i.p. inocula of 10^5 to 10^6 p.f.u. which correspond with case (B) in which responses must be related to the input of infective virus particles and the infective or non-infective products of the first replications in the host’s cells.

DISCUSSION

It has been shown in this and the previous paper that, according to dose of virus and time of drug administration, an otherwise benign and immunizing infection by avirulent virus may be modified to show one or more of several distinct changes in the pattern of the hosts’ responses: (1) The efficiency of infection (p.f.u./ID50) may be enhanced (Myocrisin and asparaginase), unchanged (cyclophosphamide), or impaired (interferon). (2) If the efficiency of infection is enhanced, then formerly non-infective doses of virus may kill (primary potentiation by Myocrisin) or immunize (enhancing or adjuvant effect of asparaginase). (3) Higher and normally immunizing doses of virus may kill early or late after primary infection (primary potentiation) or fail to protect against lethal challenge (secondary potentiation).

A summary of these time and dose effects and of their association or not with the suppression of antibody synthesis is given in Table 4. Results for interferon are included in order to emphasise the range and timing of these effects, and the continuity between specific and non-specific responses.

It is convenient to discuss the several virus-cell-antibody interactions upon which the above changes may depend in terms of three sequential but overlapping phases in infection and immunity: these are the phases of invasion and replication, of immune stimulation, and
Table 4. Summary of influences of immunomodifications upon the efficiency and outcome of an avirulent SFV infection in young mature mice

<table>
<thead>
<tr>
<th>Drug and dose per mouse i.p.</th>
<th>Day of drug with respect to virus</th>
<th>Change in efficiency of infection as ID₅₀/p.f.u. estimated at a range of low virus doses (Table 3)</th>
<th>Primary potentiation without major suppression of antibody synthesis</th>
<th>Secondary potentiation with major suppression of antibody synthesis</th>
<th>Mean survival* time in primary potentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocrisin, 7 mg</td>
<td>−2 to +2</td>
<td>Enhanced by × 30 to × 100 with potentiation of disease (ID₅₀ = LD₅₀)</td>
<td>50 to 100% according to age and strain of mice</td>
<td>Nil</td>
<td>7–9 days*</td>
</tr>
<tr>
<td>l-asparaginase, 4000 U</td>
<td>−2 to +2</td>
<td>Enhanced by × 30 to × 100 without potentiation of disease (ID₅₀ = PD₅₀); the adjuvant effect</td>
<td>About 50%</td>
<td>Minimal</td>
<td>7–9 days*</td>
</tr>
<tr>
<td>Cyclophosphamide, 4 mg (see previous paper)</td>
<td>{−1 to −3}</td>
<td>No change</td>
<td>About 50%</td>
<td>Minimal</td>
<td>11–14 days*</td>
</tr>
<tr>
<td></td>
<td>{+1 to +2}</td>
<td>No change</td>
<td>About 40%</td>
<td>About 40%</td>
<td></td>
</tr>
<tr>
<td>Mouse L cell interferon, 3000 U (to be published)</td>
<td>{−1 to −2}</td>
<td>Inhibited by 1/6 to 1/6</td>
<td>Up to 20%</td>
<td>Nil</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>{+1 to +2}</td>
<td>No change</td>
<td>Nil</td>
<td>Nil</td>
<td>—</td>
</tr>
</tbody>
</table>

* To be compared with survival for 5 to 6 days (Table 2) after direct virulent infection i.p.
finally of regulation of the hosts' responses (feed back control and the expression of virulence).

Since infective virus is a replicating antigen (Johnson & Mims, 1968; Zisman, Hirsch & Allison, 1970) the first phase of invasion, replication and amplification of virus antigens must precede the phase of immune stimulation (Table 3). This applies unless the inoculum contains an exceptionally high concentration of non-infective but immunogenic or interfering components: for arboviruses of the present kind this requires an input dose of virus probably in excess of $10^7$ p.f.u. or $10^9$ virus particles.

It is during the first phase of invasion and replication by low doses of virulent or avirulent virus that the efficiency of infection is enhanced by Myocrisin or l-asparaginase, but not by cyclophosphamide. This effect is presumably due to an inhibition of the elimination of virus particles by pinocytosis or phagocytosis (Nelson, 1969; Lashkin & Lechevalier, 1972). The balance of infective virus particles available to infect local susceptible cells increases by up to 100-fold compared with the situation in the normal mouse in which probably only 1% of input infective virus particles may achieve infection and replication. During this phase the efficiency of infection for treated adult mice approaches that for untreated suckling mice and emphasises the role of cellular maturation in determining the early changes in the efficiency of infection. These effects occur regardless of the outcome of infection as virulent or avirulent (Bradish et al. 1971; Walder & Bradish, 1975). Furthermore, virulent and avirulent infections cannot be readily distinguished in the majority of mice during the first 2 or 3 days following infection since both generate similar patterns and levels of viraemia, interferon activity and tissue infectivity (Murphy, Harrison & Collin, 1970; Bradish et al. 1971; Pusztai, Gould & Smith, 1971; Bradish & Allner, 1972).

The further phases of immune stimulation and response regulation depend upon the production by replication of secondary populations of infective virus particle and an array of non-infective components which may be immunogenic (Neurath & Rubin, 1971) or interfering (Baron, 1973; Huang, 1973; Darnell & Koprowski, 1974; Woodward & Smith, 1974). The proliferation of virus and production of terminal lesions at central target sites (CNS) may now be regulated or blocked by the activities of the non-infective components: these may act by direct interference (autointerference or interferon) or through their stimulation of informed cells, protective antibodies and soluble factors (Thind & Price, 1968; Davies & Carter, 1973; Greaves, Owen & Raff, 1973; Wheelock & Toy, 1973).

Here the mechanisms of the later immunomodifications by Myocrisin and l-asparaginase diverge (Table 4) since Myocrisin enhances infectivity to kill (ID$_{50}$ = LD$_{50}$) but l-asparaginase enhances infectivity to protect (ID$_{50}$ = PD$_{50}$). This adjuvant effect of l-asparaginase at low doses of virus (Makinodan et al. 1970) may indicate an enhanced immune stimulation following inhibition of the phagocytosis, but not of the transport and focusing, of the necessary antigens. At higher doses of virus the central proliferation of virus is more vigorous and may now be insufficiently delayed or limited by the regulatory cells or antibodies, particularly if l-asparaginase also inhibits the proliferation of T cells. A differentially greater sensitivity of T cells than B cells to the action of l-asparaginase has been reported by Berenbaum, Cope & Jeffrey (1973). If, as these authors suggest, B cells enjoy a partially protective micro-environment, then this is evidently less effective against the action of cyclophosphamide which (Table 4 and Bradish et al. 1975) influences antibody memory and the development of protection against challenge (secondary potentiation) to an extent not observed with l-asparaginase.

Myocrisin potentiates the infection by low or high doses of avirulent virus almost to the level of virulence associated with primary virulent infection. Thus, deaths following primary
infection (primary potentiation) are more numerous than those following l-asparaginase treatment and more rapid than those following cyclophosphamide treatment. This efficient potentiation is probably due to a general inhibition of phagocytosis and of the subsequent transport and focusing of antigens by macrophages. Such impairment of a later regulatory process (macrophage–T cell), in addition to the enhancement of infectivity, is consistent with the observation that at 4 to 6 days after avirulent infection the Myocrisin treated animals have elevated brain infectivities and marked polioencephalitis with neuronal destruction (Allner et al. 1974): this occurs without obvious impairment of antibody synthesis.

More direct tests within the first 4 days of the properties of ‘early’ serum in passive protection or of ‘early’ cells in adoptive immunity encounter the difficulties of detection already discussed (Bradish & Allner, 1972; Bradish et al. 1975).

In summary, and within the limitation of our indirect approach, the overall indication from this group of studies is that immediately after peripheral infection, the actual efficiency of infection (p.f.u./ID$_{50}$) is determined by the probability that potentially infective virus particles will not be lost by phagocytosis, pinocytosis, interference (interferon or auto-interference) or wasteful adsorption. This is followed by virus replication in local tissues leading to the almost exponential upsurge of viraemia and blood-interferon activity. But immune stimulation is also active and the decline of viraemia and interferon activity from about the 35th hour is a first indication of humoral antibody activity. So far these mechanisms are not determinants of virulence since virulent and avirulent infections follow the same course. But further immune stimulations have developed and, unless macrophage–T cell–B cell interactions are delayed or blocked within the first 2 days and certainly before the 3rd day, the proliferation of virus in target tissues will be regulated by informed cells and specific antibodies. The stimulation and proliferation of B cells within the first 48 h appear to be essential to the expression of avirulence through the development of immunity and protection against lethal challenge. Antigen transport and the functions of T cells within the first 48 h appear to control the expression of virulence through regulation of the severity of first clinical signs or the time and incidence of primary deaths. Thus the expression of virulence is regulated normally at many stages of invasion and stimulation and a normally avirulent infection may be potentiated in different ways by interference at one or more of these stages. The distinct responses to a standard infection of mice of different strains and ages also indicate regulations at these several stages (Walder & Bradish, 1975). This emphasizes the detail of the host response as an important and quantitative specification of virus virulence and heterogeneity.

The authors are grateful to their colleagues, Sara Albery, P. Blake, B. Gerdes, R. Ling and S. Leighton for their invaluable support.

REFERENCES


(Received 14 November 1974)