

The Responses of Nude-Athymic Mice to Nominally Avirulent Togavirus Infections

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SUMMARY

Following intraperitoneal infection by an avirulent strain of Semliki Forest virus, athymic nude mice showed almost normal clearance of viraemia and a transitory peak of antibody activity at 5 to 9 days which fell to less than about 0.1% of the normal antibody activity from the 14th day. When nude mice received a transfer of normal spleen cells from sex-matched litter mates at 1 day before infection, a pattern of high and continuous antibody synthesis was established for at least the following 7 weeks.

This clear T-cell dependence of the regulation of serum antibody synthesis was unrelated to the development of regulatory (pre-challenge) or protective (post-challenge) immunity since, particularly for female nude mice, up to 60% were benignly and protectively infected in the absence of detectable antibody activity. The brains of such nude mice showed persistence of infectivity for at least 7 weeks at 10 to 10^4 p.f.u./brain after avirulent infection and at about 10^3 to 10^4 p.f.u./brain after virulent challenge. The prior transfer of normal spleen cells to nude mice enabled them to clear brain infectivity as efficiently as normal mice.

These results are discussed in terms of the evident interplay of both T-lymphocyte dependent and T-lymphocyte independent functions in the control of brain infectivity, in the expression of virulence and in the stimulation of regulatory and protective immunity.

INTRODUCTION

A number of recent studies with strains of Semliki Forest virus (SFV), Venezuelan equine encephalomyelitis virus and Yellow Fever virus have demonstrated that in the infection of the mouse, serologically indistinguishable strains of virus may differ in efficiency of infection, in expression of virulence, in immunogenicity and in the stimulation of protection against subsequent virulent challenge (Bradish *et al.* 1975*a, b*; Walder & Bradish, 1975; Bradish & Fitzgeorge, 1979). These distinguishable phases of the virus-host interaction reflect the selective stimulation and interplay of the several dynamic mechanisms by which the host may respond to invasion by replicating immunogens (Valdimarsson, 1976; Mims, 1977).

Reviews of the factors affecting virulence and immunogenicity must attempt to define and resolve these interacting phases of host response in increasingly quantitative terms. One experimental approach involves the comparisons of these phases of response in hosts modified by radiation or drug treatment (Nathanson & Cole, 1971; Bradish *et al.* 1975*a, b*). The alternative use of the nude-athymic mouse offers considerable advantages in view of this host's known and stable deficiency of thymus processed cells which cannot recover

unless restored by controlled transfers of T-lymphocytes (Pantelouris, 1968; Rygaard & Povlsen, 1974, 1977; Nomura *et al.* 1978).

The present study compares the courses of avirulent SFV infections in nude mice, with and without transfers of normal spleen cells, with those in their normal litter mates. Emphasis is given to the role of cellular and humoral factors in the modulation of the above phases of the virus-host interaction.

METHODS

Strains of virus. The strains of Semliki Forest virus (SFV) and of Venezuelan equine encephalomyelitis virus (VEEV) used in this study were as described previously (Bradish *et al.* 1971; Walder & Bradish, 1975). Briefly, the A774.C3 strain of SFV was obtained by clonal selections followed by three production passages in primary chick embryo cells (CEC). An intracerebral (i.c.) or intraperitoneal (i.p.) dose of 10 p.f.u. or more of the A774.C3 strain of SFV benignly protects at least 90% of adult mice against homologous lethal challenge. By contrast, the virulent L10.C1 strain of SFV (Bradish *et al.* 1971) is lethal within 7 days at a dose of less than 100 p.f.u. i.p. or i.c. in adult mice. Similar properties attach to the avirulent TC83 and virulent P2023 strains of VEEV (Walder & Bradish, 1975).

Assays of virus infectivities. Virus samples to be used for inoculation, or the suspensions of mouse tissues, were assayed for virus infectivity by counting plaques formed in 9 ml agar suspensions of primary chick embryo cells by 1 ml samples of the appropriate sample dilutions in Parker's 199 medium at pH 7.4. The procedure was essentially that of Bradish *et al.* (1971).

Preparation of mouse tissues. At appropriate times following primary or challenge infection (see results), mice were killed by fracture of the neck and their brains or other organs removed and homogenized individually in Griffiths tubes with 2 ml of Parker's 199 medium with 10% pre-tested calf serum. Blood samples were obtained sequentially and individually from the retro-orbital plexus of anaesthetized mice. After mixing with an equal volume of Heparin BP in saline (10 units/ml, The Boots Co. Ltd, Nottingham), plasma samples were held at -20°C until required for infectivity assay as above.

Tissues for histology were removed and fixed in 10% buffered-neutral formalin. Usually one half of a brain was used for infectivity assay as above and the other half fixed in formalin, processed by standard methods, embedded in paraffin wax and cut in $5\ \mu\text{m}$ sections before staining by haematoxylin and eosin.

Neutralizing antibody activity. The ability of undiluted or serially diluted mouse sera to neutralize virus infectivity was estimated by the use of a ring-inhibition test or a direct plaque neutralization test in agar suspensions of primary CEC (as above). These methods are strictly equivalent and in all cases standard rabbit anti-virus sera were included for comparison and uniformity (Fitzgeorge & Bradish, 1973). Results are expressed as the serum neutralization index (SNI) which is the logarithm of the standardized antibody activity or concentration.

Immunoglobulin class differentiation. Serum samples were treated with 2-mercaptoethanol and residual antibody activity, assayed as above, was taken to be due to the stable IgG classes of immunoglobulins (Svehag & Mandel, 1964).

Procedures in mice. Athymic-nude mice and their normal siblings were obtained from a colony maintained at Allington Farm, Porton. The breeding stock was derived from the Laboratory Animal Centre and the Institute of Animal Genetics, Edinburgh and represents a spontaneous mutation maintained on an outbred genetic background (Flanagan, 1966). Tests with T-cell mitogens confirmed that the athymic-nude mice showed no detect-

able T-lymphocyte responses. The stock was maintained in an environment compatible with the lowered immune competence of the athymic mouse (Rygaard & Povlsen, 1974). Mice from the same litter were segregated by sex at approx. 30 days old and used in these tests at 45 to 65 days old. Unless otherwise stated, all mouse inoculations were by the i.p. route.

Spleen cell transfer. Mice at 45 to 65 days old were killed at the times noted in the text by fracture of the neck and their spleens removed aseptically. Each spleen was passed through a stainless steel gauze (36 mesh) which was then washed through with 1 ml of Parker's medium 199 containing 10% calf serum, pH 7.4. The cell macerate was then passed three times through a 27 gauge syringe needle and the suspension allowed to settle for approx. 30 s. The debris-free supernatant suspension was then injected i.p. so that the appropriate sex-matched litter mate recipient received about one spleen equivalent of from 1 to 4×10^8 cells. Cell viability was 70 to 80% when examined in the presence of 0.4% eosin Y (BDH Chemicals Ltd, Poole, Dorset) in 15% heat-inactivated calf serum. Cells excluding eosin in the standard counting time of 5 to 30 min at room temperature were considered to be viable.

Plan of experiments for observations of mouse responses to infection. Groups of nude-athymic mice and their sex-matched siblings were injected i.p. (0.1 ml) with a standard dose of about 3000 p.f.u. of the avirulent strain of SFV (A774.C3) or VEEV(TC83). The results with the avirulent TC83 strain of VEEV generally confirm those for SFV but will not be quoted in detail.

Mice were either killed at various times following infection for assessment of tissue infectivities and lesions, or were bled sequentially while their general clinical responses were being observed. There was no distinction in responses between bled and unbled mice.

At about the 21st day following primary avirulent infection, the mice were bled and then challenge infected i.p. with 10^4 p.f.u. of the virulent strain of SFV (L10.C1) or VEEV (P2023). Challenged mice were observed for clinical signs for 14 to 21 days when they were killed to provide blood and tissues for assays of antibody and infectivity as above.

Observations on the distinct phases of host-response. Following primary encounter with virus and later challenge infection as above, the pattern of host-responses shows several distinct phases or components (Bradish *et al.* 1975*a, b*; Walder & Bradish, 1975) which must be resolved if humoral and cellular mechanisms are to be considered and placed in their temporal context.

(i) *The efficiency of infection* as expressed by ID_{50} per administered p.f.u. and judged by any indicator of positive infection.

(ii) *The outcome of primary infection* or expression of virulence as judged by the severity of clinical signs, their rate of development and the rate and quality of host recovery. For the present tests in mice virulence this is indicated by clinical signs and the time and incidence of death.

(iii) *Regulatory stimulations*: this refers to the mechanisms regulating antibody synthesis, viraemia and tissue infectivities. These accompany positive infection (i) and the expression of avirulence or virulence (ii). No secondary or challenge infection is involved.

(iv) *Protective stimulations and the outcome of secondary challenge infection.* This refers to the pattern of host-responses and less evident boost stimulations that accompany challenge infection by virulent virus. This embodies (i), (ii) and (iii) above but now modified as they apply to the status of the primed host. For the present tests in mice this is indicated by the incidence of survival and the levels of antibody activity, viraemia or brain infectivity following virulent challenge.

RESULTS

The synthesis of antibody following SFV infection

The results in Fig. 1 necessarily refer to combined results for several small groups of mice. A clear and consistent pattern emerged in which nude or sibling mice infected by the A774.C3 strain of SFV indicated first detectable antibody activity in blood at about the 4th day following infection. The antibody activity rose to a plateau of SNI of about 4 by the 11th day for siblings. In contrast, nude mice showed only a transitory synthesis of antibody with a peak SNI of about 3 by the 7th day which declined to negligible levels of about 0.1% of the sibling level by the 11th to 14th days.

Antibody levels in nude mice and their normal litter mates showed considerable individual variation at 5 to 7 days after infection while IgM was synthesized maximally as the dominant immunoglobulin class (see Methods).

In tests on nude mice after the 12th day of primary infection, only 2 of 28 showed significant antibody activity and this incidence was not enhanced by challenge with virulent virus (Fig. 1). These nude mice clearly failed to sustain normal antibody synthesis after primary and challenge infection, although the early transitory synthesis of IgM was relatively unimpaired and evidently not T-cell dependent.

Essentially similar results to those in Fig. 1 were obtained when nude mice and their normal littermates were infected by the avirulent TC83 strain of Venezuelan equine encephalomyelitis virus (unpublished data).

Restoration of capacity to synthesis antibody following spleen cell transfer from siblings

When 36 nude mice received spleen-cell transfer from sex-matched donor siblings at 1 day before infection by the A774.C3 strain of SFV, their capacity to synthesize antibody was restored uniformly to the level already demonstrated for normal siblings (Fig. 1).

These tests with an avirulent strain of SFV were again confirmed with the TC83 strain of VEEV and demonstrate that the normally sustained synthesis of serum antibody activity is critically T-cell dependent. By contrast, as shown below, the capacity to resist challenge by virulent virus is not clearly either T-cell dependent or antibody related.

The responses of nude and normal mice to primary and challenge infections

The results in Fig. 2 refer to groups of about 25 mice infected i.p. with 3000 p.f.u. of the A774.C3 strain of SFV. The % incidence of death following avirulent infection, death following virulent challenge, or protection is shown for nude mice, for their normal litter mates and for nude mice that received a transfer of sibling spleen cells at 1 day before primary infection. Details are given in Methods and in the Fig. 2 legend. Transfers of spleen cells at up to 21 days before infection gave essentially similar results.

The distinction between the incidences of the above responses for male and female nude mice is shown by the separate figures. Regardless of this sex difference, which precludes a coarse overall grouping of results, there is an evident impairment of the capability of the nude-athymic mouse to mount the sustained regulatory responses that would ensure an avirulent outcome to the primary infection. Similarly, the nude mouse fails to sustain the stimulations necessary to protect against challenge infection. Furthermore, only 50% to 70% of nude mice mount an effective protective response after the transfers of sibling spleen cells that totally restore their ability to maintain normal antibody synthesis (as shown in Fig. 1). This result extends the distinctions between the stimulations that provoke either antibody synthesis or protection against challenge.

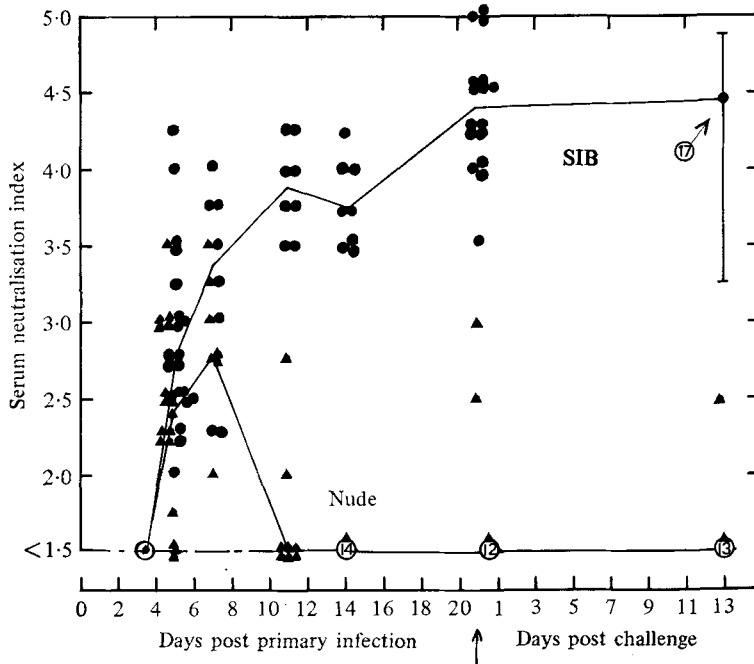


Fig. 1. The levels of virus neutralizing activity in blood for individual athymic-nude mice (\blacktriangle) and their normal litter mates (SIB; \bullet) at various times following primary i.p. infection by 3000 p.f.u. of the avirulent A774.C3 strain of SFV. The median levels of serum neutralization index (logarithm of antibody activity) are shown by the two curves. The numbers of mice at some points are shown circled.

The time of death after primary or challenge infection

The time of death of nude mice after primary infection by the A774.C3 strain of SFV was not the same as that for their normal siblings. Normal mice (Porton, A2G, Balb/c) and the normal siblings of the nude mice considered here showed few ($\sim 5\%$) deaths within 12 days after i.p. infection by the A774.C3 strain of SFV, and 100% lethality within 7 days when infected by the virulent L10.C1 strain. In contrast, 48 nude mice after primary avirulent infection or secondary virulent challenge showed 12 deaths by 12 days and 14 further deaths at 12 to 30 days. A similar pattern was apparent in the fewer deaths of nude recipients of sibling spleen cells in which 11 of 58 died within 12 days and 11 more between the 12th and 30th days.

Thus the pattern for normal siblings of efficient protection against virulent challenge and rare but early deaths was not restored to nude mice by the transfers of spleen cells that nevertheless completely restored their capacity to synthesize specific antibody or to clear brain infectivity (see below).

These results indicate a role of T-cells in the amplification of resistance to primary infection and of protection against challenge, although T-cells are not essential to these responses in about half the nude mice.

Viraemia in nude mice

Groups of nude mice and their sex-matched normal litter mates (as those of Fig. 3) were bled at various times after infection i.p. with 3000 p.f.u. of the A774.C3 strain of SFV. Mice were killed at various times to provide terminal samples of blood and brain tissue for individual infectivity assay and histology of brain and spleen.

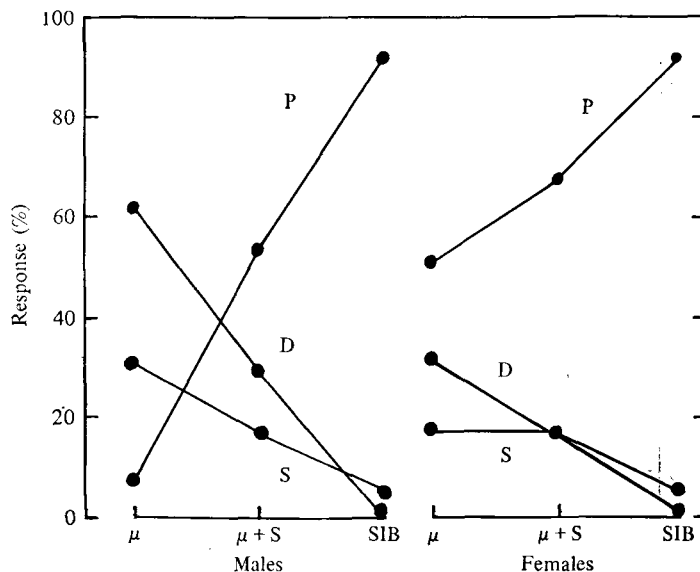


Fig. 2. The aggregated responses as % D, P or S (as below) for groups of about 25 nude (μ) mice and their normal litter mates (SIB) to primary avirulent infection by 3000 p.f.u. i.p. of the avirulent A774.C3 strain of SFV followed by challenge by 10^4 p.f.u. i.p. of the virulent L10.C1. strain of SFV at 21 to 30 days later. Also shown as $\mu + S$ are the responses to these virus infections of nude recipients of normal spleen cells from normal donors of the same sex (see Methods); D: incidence of death following primary A774.C3 infection; S: incidence of death following secondary L10.C1 challenge infection, due to inadequacy of protective stimulations; P: incidence of survival following virulent challenge. The specificity of these deaths is indicated generally by the noted signs of encephalitis and the recovery of SFV in the brains of moribund animals. Fewer than 20% of deaths were noted in nude or normal mice infected in parallel by avirulent strains of VEEV or SFV (unpublished data).

At the critical 2nd day after infection when normal mice of either sex show a maximum of viraemia (Bradish *et al.* 1975a) at 10^6 to 10^7 p.f.u./ml of blood, the nude mice of either sex also showed a maximum but lower viraemia of 10^3 to 10^5 p.f.u./ml of blood. This viraemia (lines A and B in Fig. 3a) was cleared almost completely (< 10 p.f.u./ml) by the fourth day in female nude mice or siblings, although the male nudes and siblings still retained a viraemia of 10^2 to 10^4 p.f.u./ml. The male nudes and siblings did not clear their viraemia until the 5th to 6th day following infection. The clearance of viraemia was most protracted in some male nude mice, the group showing the most severe responses (Fig. 2). The permanent elimination of viraemia by the 6th to 7th day in all mice was accompanied by a vigorous synthesis of antibody which was maximally established in the nudes of either sex by the 7th day at a mean level (SNI ~ 3) of about 3% of that shown in siblings (as in Fig. 1).

Early brain involvement in nude mice

The infectivities associated with sampled brain tissues were found to range widely and to be generally indistinguishable (Fig. 3) for nudes and siblings at up to the 5th day following infection. From the fifth day of infection, which is also critical in the brains of normal mice for the differentiation of virulent and avirulent infections (Bradish *et al.* 1975a), the brain infectivities fell for siblings (E in Fig. 3) but remained high for nudes and for nude-recipients of sibling spleen cells (C and D in Fig. 3). By the 9th day post-infection, both sibling brains and the brains of spleen-reconstituted nudes showed negligible infectivity (below 10 p.f.u./

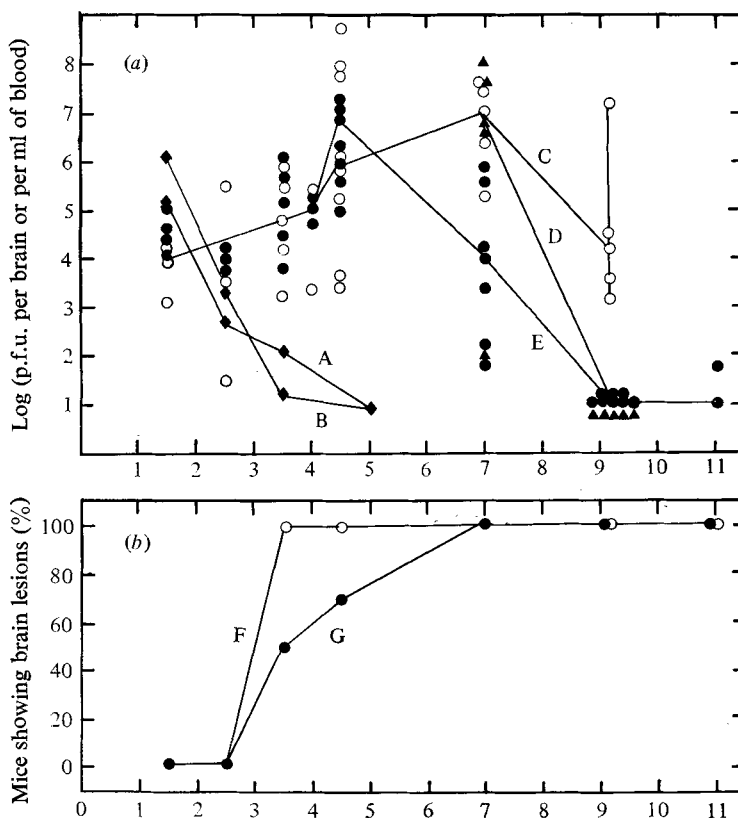


Fig. 3. (a) The infectivity of individual brains at various times following infection by the avirulent A774.C3 strain of SFV. ● and median line E: normal litter mates; ○ and median line C: nude mice; ▲ and median line D: nude mice recipients at 1 day before infection of spleen cells from normal sex-matched litter mates; ◆: median viraemia for male and female normal (A) and nude (B) mice as log (p.f.u./ml blood). See text for details. (b) Incidence of brain lesions at various times following infection in the above normal (G) and nude mice (F). Half brains were examined for infectivity (a) and for lesions.

brain), whereas the nude brain infectivities remained high at 10^8 to 10^7 p.f.u./brain (C and bar, ○—○, in Fig. 3).

In histological studies, all samples from nude mice showed deficiency of lymphoid tissue in spleen and mesenteric lymph nodes but there were no specific lesions in these tissues. In general, there was no detectable difference between the severity and extent of the encephalitis seen in siblings and nudes, or in the A2G mice also used in parallel studies. The only notable difference was the earlier incidence (Fig. 3) of positive lesions in nude mice. By the 6th or 7th day post-infection, when brain infectivities were already falling in siblings, all mice showed similar brain lesions of the patterns previously described (Zlotnik & Harris, 1970; Zlotnik *et al.* 1972).

Persistence of brain infectivity after primary avirulent infection

In order to clarify the later pattern of clearance of brain infectivity, nude mice and their normal litter mates were infected i.p. with 3000 p.f.u. of the avirulent A774.C3 strain of SFV. At 43 and 50 days after infection and without any previous bleedings, these mice were

Table 1. *Brain infectivity and serum antibody activity for 17 individual athymic-nude mice at 43 to 50 days following i.p. infection by 3000 p.f.u. of the avirulent A774.C3 strain of Semliki Forest virus*

Incidence in nude mice	Brain infectivity as log (p.f.u./brain)	Serum neutralization index
9/17	5.4, 4.2, 4.1 3.9, 3.8, 2.1 1.1, 0.8, 0.6	All < 1
2/17	Both < 0.3	Both < 1
6/17	All < 0.3	All 4 to 5

sacrificed and their brains sampled for infectivity assay. At the same time, blood was taken for assay of viraemia and antibody activity.

The results in Table 1 show that of 17 nude mice, all clinically healthy and non-viraemic, nine had significant brain infectivity of $10^{0.5}$ to $10^{5.5}$ p.f.u./brain and no detectable serum neutralizing activity. In contrast, the remaining eight of 17 nude mice showed no detectable brain infectivity (below 2 p.f.u./brain) and six of these had high serum neutralizing activity in blood of SNI 4 to 5.

This strong individual correlation of the persistence of brain infectivity and the absence of detectable serum neutralizing activity may indicate that nude mice respond steadily in one of two distinct ways. Alternatively, the nude mice showing high brain infectivity at the time of sampling may have only recently experienced a decline of circulating antibody activity and a rapid proliferation of virus in brain. The latter possibility suggests fluctuation in view of the earlier patterns (Fig. 3) of negligible antibody activity and high brain infectivity. Thus 3 of 8 nude mice sampled at 30 days post infection showed positive brain infectivities of 12, 30 and 120 p.f.u./brain.

Clearly, individual levels of brain infectivity may fluctuate widely with time if these depend upon subtle immunological balances and feed-backs in a host unable to sustain antibody synthesis.

Persistence of virus in brain following challenge infection

An essential feature of protective stimulation is that the now primed host should either not become infected or not show severe responses when an otherwise virulent challenging virus is encountered.

When 14 nude-athymic mice were infected i.p. by the A774.C3 strain of SFV, two died at 11 and 14 days (as for Fig. 2). The 12 survivors were challenged i.p. on the 23rd day by the virulent L10.C1 strain of SFV and only one of these mice died 11 days later. On the 37th day (14 days post challenge) none of these nude mice was viraemic and only two of the 11 showed serum antibody activity near the normal level (as for Fig. 1). All showed high brain infectivity at from $10^{2.9}$ to $10^{4.3}$ p.f.u./brain (as for Fig. 3 and Table 1). As anticipated, all primed and challenged normal litter mates showed no detectable brain infectivity or viraemia.

This failure of the T-deficient nude mouse to eliminate brain infectivity after immunization and virulent challenge is similar to, but much more regular than, its fluctuating failure to clear the persisting brain infectivity due to the avirulent priming virus. The fullest virulence of the challenge virus, which was uniformly lethal within 7 days in normal sibling controls, was nevertheless not expressed in the primed nude mice due to an evident T-independent protective stimulation in the absence of detectable serum neutralizing activity. The differential persistence or clearance of virulent and avirulent virus is under investigation.

DISCUSSION

The present paper is not strictly histological or immunological but dwells on the regulation of the progressive responses of individual mice from the initiation of infection, through the expression of virulence, to the development of protection against virulent challenge. The use of the nude-athymic mouse permits the assumption that the observed changes in host-responses are due to the degree of availability of thymus-processed lymphocytes (T-cells). The requirement in most cell transfer studies for the separation or *in vitro* confirmation of T-cell populations is obviated here by the demonstration *in situ* that nude mice, following a transitory synthesis of IgM antibody, show negligible continued synthesis of serum neutralizing antibody. This incapacity of nude mice was totally and permanently restored to 'normal sibling' levels by the transfer before infection of normal sibling spleen cells. This *in vivo* functional restoration was observed in 44 of 46 nude mice following infection by avirulent strains of Semliki Forest virus or Venezuelan equine encephalomyelitis virus (our unpublished data).

This clear pattern of the T-cell dependence of continued antibody synthesis (Pritchard & Micklem, 1974; Burns *et al.* 1975) was not simply related to the stimulation of immunity, either regulatory (pre-challenge) or protective (post-challenge). Thus, particularly in females, up to 60% of athymic-nude mice showed benign responses to the avirulent priming SFV and were fully protected against virulent challenge. The range of individual responses, the differences between the reaction of male and female nude mice and the differences between the responses to different strains of arbovirus (our unpublished data), emphasise that these virus-host interactions are a dynamic interplay of T-cell dependent and T-cell independent functions. Thus IgM synthesis and the clearance of viraemia may be closely associated and largely T-cell independent, but continued IgG synthesis and the clearance of virus-infectivity from brain are evidently T-cell amplified but not entirely T-cell dependent. It is premature to draw close conclusions from the striking inverse relationship between serum immunoglobulin activity and the long persistence of virus infectivity in brain until the penetration or synthesis of specific immunoglobulins in brain has been assessed (Fleming, 1977).

That the normal clearance of brain infectivity and the expression of virulence involve the interplay of both T-cell dependent and T-cell independent functions is shown further by the levels of brain infectivity detected in mice under different conditions. Nude mice show a fluctuating persistence of brain infectivity for at least 7 weeks after avirulent SFV infection unless they have received splenic T-cells from normal donors. Nevertheless, nude mice do not support the proliferation of virus to the lethally high levels in brain that characterize virulent infections in normal mice or 'avirulent' infections in other impaired mice (Bradish *et al.* 1975*b*). Thus, in athymic-nude mice, the proliferation of 'avirulent' SFV in brain is limited by T-independent mechanisms to levels of tolerated 'persistence' that are not cleared unless T-cell functions can be transferred or antibody synthesis stimulated through T-independent routes.

It has been observed that a presumed macrophage blockade in mice by treatment with Myocrisin (Allner *et al.* 1974; Bradish *et al.* 1975*b*) causes no detectable check in patterns of antibody synthesis but allows 'avirulent' SFV to replicate in brain to lethally 'virulent' levels. This suggests that the normal regulation and clearance of brain infectivity, and thence the expression of virulence, may be mediated by a combination of macrophage (phagocytosis and transport) and later T-cell functions. Such early sequential interactions between virus, macrophage and lymphocyte (Valdimarsson, 1976) contribute to the phagocytic limitation of the efficiency of infection as above, to the activation and modulation of

macrophage populations in key tissues (Silverstein, 1970; Rager-Zisman *et al.* 1976; Macfarlan *et al.* 1977) and to the regulation of information flow from proliferating virus to responsive lymphocyte populations responsible for antibody synthesis or specific-cellular activities. The latter aspects of immunogenicity (regulatory and protective) have been considered in this paper.

Recipient mice can be protected from death due to 'virulent' SFV or VEEV infections by adoptive transfer of spleen cells or passive transfer of antisera only if the donor mice are immunized at least 5 days before (Rabinowitz & Alder, 1973; Rodda & White, 1976). Such transfer observations are clearly difficult to relate to the mechanisms of initial immunological priming by viruses and to the macrophage lymphocyte interactions that initiate and control the patterns of regulatory and protective responses noted in this paper. Rodda & White (1976) have described a rapid, non-specific response of mice to togavirus infections (SFV and Kunjin virus) in which peritoneal cells sampled only two days later are highly cytotoxic to mastocytoma target cells, particularly if these are virus infected. These cytotoxic cells disappear between the 4th and 6th days as specific antibody and sensitized T-cells are first detected and become effective in passive transfer. The cells responsible for this early non-specific and antibody-independent cytotoxicity were first called macrophages but later shown to lack the characteristics of macrophages, T- and B-cells (Macfarlan *et al.* 1977) and to be distinct from normal peritoneal macrophages active in the virus specific and antibody dependent toxicity directed against virus-infected cells.

Doherty (1973) has studied the acute fatal meningoencephalitis in CBA/J normal and athymic mice that resulted from the intravenous infection by 10^8 p.f.u. of a highly virulent strain of Semliki Forest virus. The cellularity of cerebrospinal fluid (CSF) indicated that the onset of neurological symptoms and death within 7 days were not dependent upon invasion by inflammatory cells. Inflammation and levels of circulating antibody were unrelated. Similar results and lesions of encephalitis from day 5 were found for athymic-nude mice and their normal litter mates. Inflammation and CNS-damage were probably induced directly by virus. There was no general breakdown of the blood-CSF barrier and T-cells probably acted relatively late as an amplifier of the infiltration of brain and CSF by other mononuclear elements. This situation for SFV is quite distinct from that associated with infection by ectromelia virus or lymphocytic choriomeningitis virus in which T-lymphocytes are an essential trigger for the inflammatory process (Blanden, 1971; Cole *et al.* 1972).

In studies parallel and complementary to those of the present paper, S. Jagelman, A. J. Suckling, H. E. Webb & E. T. Bowen (personal communications), used the same A774 avirulent strain of SFV in a different CBA/Balb/c strain of normal and nude mice. There was now no inflammatory histology and no deaths in these nude mice and characteristic lesions of encephalitis were rare (30%). The levels of neutralizing antibody in the group-pooled-sera of nude mice remained high for 3 weeks at a level (SNI 4) little below that for normal mice. Brain infectivities persisted for up to 4 weeks in nude mice at levels similar to those in our study, apart from the suggestion of a 1000-fold rise at about the 21st to 28th day coincident with a 1000-fold decline in the group-pool serum neutralizing activity. This may reflect the sampling-fluctuations noted in our studies of individual brain-infectivities and serum antibody levels.

The various studies with virulent and avirulent strains of SFV in different strains of nude mice support the general conclusion noted in the discussion above that the phase of the virus-host interaction associated with the expression of virulence [see (ii) in Methods] involves an interplay between virus and several cellular components that is modulated by, but not strictly dependent upon, either T-lymphocytes or circulating antibody.

Further timed studies of cellular and immunoglobulin classes and activities in particular tissues, notably brain, will be required to clarify these regulations of this dynamic virus-host interaction and their expression through the several phases of host-response.

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