Modifications by Sodium auro-thio-malate of the expression of Virulence in Mice by defined strains of Semliki Forest Virus

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SUMMARY

The responses of mice to infection by defined virulent or avirulent strains of Semliki Forest virus have been modified by the prior administration i.p. of sodium auro-thio-malate. This resulted in enhancement of the efficiency of infection and later modifications (potentiation) of the outcome of that infection. These distinct changes were associated with an enhanced virus invasion of the brain but not with any detectable depression of the humoral antibody response. These events are considered in relation to the multiple effects of alternative 'immunosuppressive' drugs.

Recent studies (Bradish, Allner & Maber, 1971, 1972; Bradish & Allner, 1972) on the expression of virulence in mice by defined strains of Semliki Forest virus (SFV) have been extended by consideration of the course of infection following immunosuppression (Nathanson & Cole, 1970, 1971) by cyclophosphamide and L-asparaginase (K. Allner, C. J. Bradish & R. Fitzgeorge, unpublished observations). These studies indicated the need to clarify the earliest stages of the transport and processing of virus since the potentiation of an otherwise avirulent or benign infection was not associated regularly with the suppression of the normal antibody response. Conversely, some animals without detectable circulating antibody were nevertheless solidly protected against repeated challenge by a lethal strain of SFV. Thus the response to and recovery from virus infection depended upon a series of component mechanisms which were modified in different ways by the drugs employed (Makinodan, Santos & Quinn, 1970).

As an approach to this problem we have observed the course of infection in mice previously treated with preparations associated with macrophage blockade (Zisman, Hirsch & Allison, 1970) or the control of autoimmune diseases (Robinson, 1971). Some properties of sodium auro-thio-malate (Myocrisin, May & Baker) are described in this paper since this compound proved to be particularly effective in the modification of the course of infection in mice by virulent or avirulent strains of SFV.

The procedures for the assay of virus infectivity by plaque counting in agar suspensions of primary chick embryo cells (CEC) have been described (Bradish et al. 1971). The defined virulent and avirulent strains of SFV and their distinct patterns of infection and interaction in mice have also been described (Bradish & Allner, 1972; Bradish et al. 1972). The strains of mice employed were obtained from Allington Farm, Porton, and were random-outbred (PR) or inbred (A2G, C3H, C57).

Preliminary tests were made in mice to determine the basic dose-toxicity relationships for a number of preparations. Groups of 10 A2G mice of 32 to 45 or 100 to 140-days old received intraperitoneal (i.p.) inoculations of 0.025 or 0.10 ml of graded doses of the appropriate preparations diluted in physiological saline. The toxicity of each preparation is shown in Fig. 1 as the % mortality within 12 days of inoculation. Tests for potentiation of
Fig. 1. The toxicity, as % mortality within 12 days, for 32 to 40 days old A2G mice, of a number of agents associated with macrophage blockade or the modification of autoimmune conditions. Each agent in physiological saline was inoculated as 0.025 or 0.10 ml i.p./mouse into groups of 10 or more mice. O-----O, soluble gold as sodium chloroaurate (NaAuCl₂H₂O; B.D.H., Poole, England); △-----△, colloidal gold (E. R. Squibb and Sons, New Brunswick, New Jersey, U.S.A.); □-----□, soluble gold as sodium auro-thio-malate (COONa.CH₃(CH₃)₂COONa; Myocrisin, May and Baker Ltd, Dagenham, England); ●-----●, colloidal carbon as Pelican ink (G. H. Smith and Partners, Colchester, England); ■-----■, colloidal carbon of < 5 μm diam. separated from activated charcoal (B. D. H. Poole, England; Fitzgeorge & Bradish, 1973); ▲-----▲, colloidal silica of < 5 μm diam. (Dörentrup Quartz No. 12, prepared by Dr K. Robuck, Institute of Occupational Medicine, Clinical Department, Essen University, Germany, and obtained through the courtesy of Dr A. C. Allison and Dr B. Zisman, M.R.C. Clinical Research Centre, Northwick Park, England).

disease were then made with each preparation at doses up to that indicating the maximum acceptable toxicity (10 to 15 % mortality).

The potentiation of infection by each preparation, administered 2 to 4 h before virus, was determined by the proportion of mice which died in consequence of drug intervention and within 12 to 14 days of infection (0.025 ml i.p.) by 10⁶ p.f.u. of the avirulent A774.C1 strain of SFV. It is known (Bradish et al. 1971, 1972) that the i.p. or intracerebral (i.c.) inoculation of this strain of SFV in mice of over 20 days old produces a benign and immunizing infection at doses of from 3 to > 10⁸ p.f.u.

Of the preparations shown in Fig. 1, only Myocrisin showed decisive potentiation of this avirulent i.p. infection and an acceptable separation of the drug doses for 50 % toxicity and 50 % potentiation. Myocrisin was therefore adopted for the following more detailed studies.

Results for potentiation by Myocrisin are shown in Fig. 2 for several strains of mice of 35 to 45 days old. The PR mice indicated their heterogeneity by a shallow response to the potentiation of virus infection by Myocrisin. The C57 inbred mice were considerably more sensitive to potentiation by Myocrisin and showed 50 % potentiation at 0.7 mg per mouse or ½ of the 50 % toxic dose. For the A2G mice, the dose for 50 % potentiation was 4 mg/mouse and about ¼ of the 50 % toxic dose. These results (Fig. 1, 2) indicate that the dose of Myocrisin must be determined with care for each strain of mice if maximal potentiation is to be associated with minimal toxicity. Thus for 35 to 45 days old A2G mice, potentiation exceeded toxicity by at least 60 % for Myocrisin doses of 7 to 10 mg/mouse or 0.25 to 0.50 mg/body weight. For A2G mice of 100 to 140 days old, two- to threefold higher doses of Myocrisin were required to produce responses similar to those shown above for the 30 to 45 days old mice.
Fig. 2. Dose-response curves for potentiation (——) and toxicity (-----) in 35 to 45 days old mice of different strains, • •, C57 inbred; • •, A2G inbred; ▲ ▲, Porton random bred (PR). Potentiation is defined as the % mortality within 12 or 14 days due to i.p. administration of Myocrisin, and infection i.p. at 2 to 4 h later by 10⁶ p.f.u. of the avirulent A774.C1 strain of SFV.

In order to relate the potentiation of disease by Myocrisin to changes in the levels of viraemia, brain infectivity or serum antibody, groups of PR mice of 31 to 34 days old were infected intraperitoneally with 10⁶ p.f.u. of the avirulent A774.C1 strain of SFV: half of these mice received 7 mg of Myocrisin i.p. at 2 h before the virus. Groups of five or ten of these control and treated mice were then killed every day for analysis on individual samples of brain infectivity, blood infectivity and neutralizing antibody activity. The procedures for analysis and interpretation of these results were as described by Bradish & Allner (1972).

The mean results shown in Fig. 3(a) indicate that the course of viraemia in infected mice was not influenced significantly (P = 46 %) by Myocrisin but followed closely the common pattern previously published for virulent and avirulent infections (Bradish & Allner, 1972) with a peak of about 10⁶ p.f.u./ml at 1 to 2 days and ≤ 10⁵ p.f.u./ml at 3 to 4 days. In contrast, the virus content of the brains of Myocrisin treated mice was very significantly (P < 0.01 %) 10- to 100-fold higher than that in mice receiving virus only. Although there was a wide range of individual variation of brain infectivity (Bradish & Allner, 1972) within each group (bars on Fig. 3a), the ranges for the Myocrisin treated groups did not overlap the means for the control groups. Results at the 7th day are biased in the Myocrisin group to the few survivors of least brain involvement. It is of interest to note that the level of brain infectivity (~ 10⁶ p.f.u./brain) associated with paralysis and death (Bradish & Allner, 1972) was exceeded by the Myocrisin treated mice, of which 97 % (44/45) died, but not by the control mice receiving virus only, of which only 15 % (6/40) died.

Photomicrographs of the brains of such mice (Fig. 5 to 10) indicated typical polioencephalitis with neuronal destruction only when virus infection was potentiated by Myocrisin: this is amplified in the figure legends.

When the individual sera (Fig. 3b) of the same mice were assessed for neutralizing activity there was no significant difference (P ~ 49 %) between the results for control and Myocrisin treated mice. Thus the potentiation of disease noted above was not associated with evident
Fig. 3. Development of viraemia, serum antibody and brain infectivity in 32 to 47 days old PR mice infected i.p. by $10^6$ p.f.u. of the avirulent strain (A774.C1) of SFV. ■—■, control mice receiving virus only; •—•, mice treated 4 h previously with 7 mg Myocrisin i.p.
suppression of antibody synthesis, at least in terms of the major humoral components detected in these assays (Bradish et al. 1971).

It should be noted that a neutralizing antibody activity (Fig. 3b) of about 10 neutralization units or SN1 (Fitzgeorge & Bradish, 1973) was established before the 3rd day. This threshold of detection of activity is estimated to be equivalent to about 10⁶ immunoglobulin mol/ml (Fitzgeorge & Bradish, 1973) and consistent with an equivalence between virus and antibody at or before the 2nd day of infection when levels of viraemia began to fall sharply.

A further effect of Myocrisin, which is distinct from the potentiation of disease discussed above, is the modification of the efficiency of infection, regardless of the outcome of that infection as death or benign protection. This is illustrated in Fig. 4 which shows results for the i.p. titration of graded doses of avirulent (A774.C1) or virulent (L10.C1) strains of virus in groups of 35 days old PR mice primed 2 to 4 h previously with i.p. doses of Myocrisin of from 0 to 7 mg/mouse. The p.f.u./LD₉₀ ratio (Bradish et al. 1972) was then estimated for each strain of virus and dose of Myocrisin.

For the avirulent (A774.C1) strain of SFV the p.f.u./LD₉₀ was over 10⁶ until at 5 mg Myocrisin/mouse it fell sharply to about 10 p.f.u./LD₉₀ at 7 mg Myocrisin/mouse. For the virulent (L10.C1) strain of SFV the p.f.u./LD₉₀ was from 300 to 1000 in untreated 35 days old PR mice and fell steadily as the dose of Myocrisin was increased again to about 10 p.f.u./LD₉₀ at 7 mg of Myocrisin/mouse. Thus the efficiency of infection for both virulent and
avirulent strains of virus was increased at least 30- to 100-fold by treatment with Myocrisin. In fact, the efficiency of infection was increased significantly by doses of Myocrisin (< 4 mg/mouse) which failed to show potentiation of disease.

In order to probe this effect more sensitively, mixtures of known proportions of virulent and avirulent strains of SFV (Bradish et al. 1972) were titrated similarly in mice primed by Myocrisin (Fig. 4). The p.f.u./LD₉₀ expressed in terms of the virulent component was again depressed significantly by doses of Myocrisin (1 to 4 mg/mouse) which failed to
potentiate disease. This indicated that the competitive protective potential of $10^6$ p.f.u. of
the avirulent component in the inoculum was abrogated by the action of these minimal doses
of Myocrisin. This enhanced sensitivity to the effects of small doses of Myocrisin, and pre-
sumably or other relevant drugs, is a particularly interesting application of the critical
virulent-avirulent interactions previously discussed (Bradish et al. 1972) as a component in
the expression of virulence.

These results suggest that the efficiency of infection and the expression of virulence are
determined by a series of cellular events which may be variously and separately modified by
Myocrisin or 'immunosuppressive' drugs. These distinct effects are not necessarily associ-
ated with detectable suppression of antibody synthesis. Our own observations of the effects
of Myocrisin upon an otherwise avirulent SFV infection may be summarised by the follow-
ing series of changes which culminate in lethality. (1) Enhancement of efficiency of infection
(Fig. 4). (2) Inhibition of development of active protection (Fig. 4) against challenge by
virulent virus. (3) No gross modification of humoral antibody response when detected at
3rd day or later (Fig. 3). (4) Enhanced recovery of virus from brain (Fig. 3) and greater
potentiation of i.c. than i.p. infection. (5) Flaccid paralysis prior to death associated with
typical polioencephalitis with neuronal destruction particularly marked in the cerebellum,
brain stem and spinal cord (Fig. 5 to 10).

Although the pharmacological action of Myocrisin is uncertain (Todd, 1967; Robinson,
1971), our observations on the enhancement of the efficiency of infection suggest that the
drug depresses phagocytosis and then modifies antigen transport in such a way that disease
is potentiated without gross modification of viraemia or antibody production. Studies by
Zisman et al. (1970, 1971) have demonstrated that silica and anti-macrophage serum
effectively potentiate the infection of mice by herpes or Yellow Fever viruses. In parallel to
our own results with SFV and Myocrisin, potentiated animals showed no significant depres-
sion of antibody response, although in any such studies important changes may have occur-
red in minor immunoglobulin classes or at the cellular level. Macrophages play an important
role in determining the initial course of virus infections (Mims, 1964; Silverstein, 1970;
Allison, 1972a, b) since they may first degrade virus or support its replication and then
contribute to antigen transport and to the induction of the immune response and to the

We are attempting to define more closely the modifications by ‘immunosuppressive’
drugs of these distinct cellular functions in host defence, in order to trace the earliest critical
events by which virulent and avirulent strains of virus, which may be serologically in-
distinguishable, are discriminated and then processed differentially by the host.

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