Persistence of encephalitogenic arboviruses in brain cell culture

Brief Report

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Summary

Virus recovery from brain cultures of mice infected with either Semliki Forest and/or Langat depended on the time interval between inoculation of either virus. Mixed infections may alter the course of a disease.

The wide variation of clinical symptoms following a viral infection may be due to the presence of another recent or latent infecting virus. Of particular interest is the central nervous system (CNS) where many viruses are known to persist. The presence of more than one virus in a host at the same time may not be uncommon and has been reported for both natural infections and experimental conditions in animals and tissue culture. Since many viruses are known to persist in the CNS [10, 14, 17] it is possible that some of these latent viruses could influence the outcome of a subsequent viral disease. Epstein et al. [5] suggest that some viruses may be reactivated during an AIDS infection and the latent JC papova virus was found to be more widespread in the brains of AIDS patients than is seen in other immunodeficiency disorders [15]. Thus although immunosuppression renders the host more susceptible to virus infections, a latent infection is more likely to become reactivated during a secondary infection. This may be important in diseases such as multiple sclerosis (MS) thought to be of viral aetiology.

Fenner [7] describes a process of complementation whereby one virus is suppressed to the advantage of the other. This has been described for
the Tick-borne encephalitis and Lipovnik viruses [9] where interference was dependent on the age of the tissue cultures. Oaten et al. [12] showed that it was the time interval between administration of the two encephalitogenic viruses that influenced the outcome of the disease in mice.

It is of interest, therefore, to study the replication of dual virus infections in CNS tissue having given the original viruses in vivo but to study the subsequent replication in the target organ, the CNS, in vitro free of an immune response.

Swiss A2G mice of the St. Thomas's Hospital strain, 2–3 days old, were used. A group of 20 mice were inoculated intraperitoneally (i.p.) with 0.05 ml of Semliki Forest (SF) virus A7(74)/C2 strain containing $10^4$ ICLD$_{50}$/ml. A further group of 20 mice were inoculated i.p. with 0.05 ml of Langat TP21 strain (9th mouse brain passage) containing $10^4$ ICLD$_{50}$/ml (both provided by Dr. C. J. Bradish of the Microbiological Research Establishment, Porton Down, England). These 2 groups acted as controls. The 3 test groups were inoculated i.p. with Langat virus and inoculated simultaneously or challenged at 30 h or 4 days with SF virus. All mice were sampled at 22 h after the SF virus and at the same time in the Langat virus only control group. Uninfected mouse brains were used for control cultures. The cells were prepared as described by Evans and Webb [6] and aliquotted into 5 culture flasks. At various time intervals 1 ml of culture fluid from all flasks were analysed for virus infectivity as described by Oaten et al. [12]. The ICLD$_{50}$ was calculated using the method of Reed and Meunch [13]. Students t test was used for statistical analysis.

Figure 1A shows that Langat virus persisted in the cultures for over 110 days whereas SF virus was only detected until post-culture days (PCD) 45. When SF virus was inoculated 30 h after Langat virus the SF virus titres did not differ significantly from the single infection until PCD 22 when the titres were significantly higher in the dual infected cells (p < 0.05) (Fig. 1B). In this group the SF virus titres remained significant (> 1.0 log). The Langat titres in this group were significantly suppressed (p < 0.01) as compared to the control group for Langat (Fig. 1A). In the group given the viruses simultaneously (Fig. 1D) the SF virus titres were identical to the single infection but the Langat titres were significantly suppressed (p < 0.001) and not detected between PCDs 50 and 93. The reverse was seen when SF virus was inoculated 4 days after Langat virus (Fig. 1C). Langat titres were identical to those after the single infection (Fig. 1A) but the SF virus titres were significantly reduced (p < 0.001) and not detected between PCDs 13 and 35.

These results show that two serologically distinct arboviruses can co-exist in mouse brain cultures although this may result in the reduced replication of one virus. In no condition was either virus totally excluded.