EXPERIMENTAL STUDIES OF SEMLIKI FOREST VIRUS

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ABSTRACT

Semliki Forest virus (SFV) vectors have been developed to provide a convenient system to express protein-encoding sequences in virtually any animal cell. In mice, outcome of infection varies according to age of the mouse and strain of the virus and can include acute encephalitis, sub acute demyelinating meningoencephalomyelitis, and persistent subclinical central nervous system (CNS) infection. All strains of virus are virulent in mice infected <12days of age. The L10 strain is also virulent in mice >14 days age, whereas theA7 (74) strain is a virulent.

Key Words: Semliki Forest Virus, CNS, Heamaglutinin, Mice.

INTRODUCTION

Semliki Forest virus (SFV) is a mosquito-borne virus that naturally circulates in sub-Saharan Africa. The virus is an alpha virus of the family to gaviridae. Natural human and equine infections have been described (Mathiot et al., 1990). Different strains have been designated as virulent or avirulent according to their virulence inadult mice. The L10, V13, and Osterrieth strains and the strain designated prototype are virulent (Bradish*et al*, 1971; Glasgow *et al*, 1991). Semliki Forest virus (SFV) is a positive stranded RNA enveloped virus that belongs to the alpha virus genus along with Sindbis and Venezuelan equine encephalitis (VEE) virus.1 Vectors based on these viruses are now gaining increasing recognition for the expression of heterologous proteins *in vivo*.2Virulence in mice has been characterized for several natural isolates and their laboratory-passaged strains (Bradish et al., 1971). SFV particles carrying only recombinant RNA are formed and are used to infect cells for analysis of protein expression. The most characterized a virulent strain of SFV, A7 (74), is virulent in mice infected at the age of 11 days or less, but is avirulent in older mice; virus dissemination in the central nervous system (CNS) is increasingly restricted with age (Oliver et al., 1997).The virus is closely related to Chikungunya virus, responsible recently for an outbreak of severe arthralgia in the islands of the Indian Ocean (Schuffenecker et al., 2006). In 4–5-week-old mice, intraperitoneal inoculation of SFV A7(74) results in a high-titre plasma viraemia from which virus is seeded into perivascularfoci in the brain and spinal cord; there is little spread of virus from cell to cell, foci do not enlarge with time and the infection is restricted in mature neurons (Fazakerley et al.,1993). SFV A7(74) remains avirulent following direct intracerebral inoculation, but inoculation by this route results in a widespread infection of oligodendrocytes in the major white matter tracts (Fazakerley et al., 2006). The type I IFN system has also been demonstrated to be crucial for the protection of mice from nominally avirulent strains of the related alpha viruses Venezuelan equine encephalitis virus and Sindbis virus (Grieder &Vogel, 1999; Ryman et al., 2000). Strains of SFV and eastern equine encephalitis virus vary in their sensitivity to IFN (Aguilar et al., 2005; Deuber & Pavlovic, 2007). In

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a1987 survey in the Central African Republic, SFV was isolated from 22 patients with fever, severe persistent headaches, myalgia, and arthralgia (Mathiot *et al*, 1990).

MATERIAL AND METHOD

The sedimentation-enumeration method described for quantitative studies of haemagglutination by Semliki Forest virus (SFV) has been used for the quantitative assay of antibody through its activity for combination with virus haemagglutinin. SFV haemagglutinin was prepared by inoculating the brains of suckling mice with the vI3strain of SFV (Bradish, Allner & Maber, I97I) and treating the harvested brains with fluorocarbon. SFV infection of laboratory mice provides attractable model system for the study of virus pathogenesis and in particular virus encephalitis (Fazakerley, 2004). Infectious SFV4 virus was generated from a cDNA plasmid derived from the prototype strain of SFV (Liljestro¨m & Garoff, 1991).Studies by gel filtration and equilibrium density gradient centrifugation showed that the haemagglutinin was homogeneous and similar to the infective particle in size and density (Cameron, I969). SFV4 marker virus containing the gene for enhanced green fluorescent protein (eGFP) was constructed by inserting the coding sequence for eGFP followed by the foot-and-mouth disease virus 2Acleavage sequence between that for the capsid protein and p62 in the virus structural protein open reading frame. This strategy has been used previously to construct an eGFP-labelled Sindbis virus (Thomas et al., 2003).Dilutions of a rabbit hyper immune serum prepared against the specified strain (vI3) of SFV (R. B. Fitzgeorge & C. J. Bradish, to be punished) were reacted with different dilutions of SFV haemagglutinin in borate buffer (pH 9"o), containing0.2% (w/v) bovine serum albumin, at room temperature (23 °C for 30 min. An equal volume of a suspension of goose red blood cells (RBC) in phosphate buffered saline (PBS) was then added to each reaction mixture to give a final optimum pH 6.3 and an overall RBC concentration of 10^7/ml. Each mixture was then sampled and observed microscopically by the sedimentation-enumeration method (Cameron & Bradish, I972) for the formation under standard conditions of RBC aggregates of various sizes. From the observed concentration and distribution of size-specified aggregates, the total numbers of RBC-RBC bonds (B) and of red blood cells (R) were determined and from these the extent of agglutination was calculated (Cameron & Bradish, I972) as the average number of a hemagglutinin-specific RBC-RBC bonds/red blood cell. This is indicated by $(B/R)_{H}$ -- (B/R) o, where the second term expresses the correction for spontaneous agglutination in the absence of haemagglutinin. Residual haemagglutinating activities calculated and appear as a series of parallel lines displaced from the haemagglutinin-only control according to the antiserum dilution or antibody concentration used. Clearly, and as interpreted through the greater the initial concentration of antibody, the lesser the quantity of haemagglutinin remaining in suspension to be detected by the agglutination of the RBC finally added.

RESULT AND DISCUSSION

We have shown (Cameron & Bradish, I972) that the linear region of the haemagglutination characteristic represents by its position the virus-specific haemagglutinating activity (H) or concentration of available, haemagglutinin. Thus the horizontal or vertical displacement of the reaction lines due to the increasing concentration of antibody indicates the reduction of haemagglutinating activity or concentration of available haemagglutinin. In quantitative terms, the reduction of the logarithm of the haemagglutinating activity is proportional to the depression of the extent of agglutination,

$$\text{Log}\ (Ho/H_{Ab}) =, \alpha\ [(B/R)_{H}\text{--}(B/R)\ ab] = \alpha\delta$$

Here g is a reaction constant, and $(B/R)_{Ab}$ or $(B/R)_{H}$ the extents of agglutination in reactions with or without antibody, respectively. The dependence of this reduction of agglutination upon the concentration of antibody is a number of experiments under different conditions with dilutions of a single rabbit anti-SFV serum. This implies that the reduction of haemaggtutinating activity by antibody in excess is determined by the concentration of antibody but is independent of the initial concentration of haemagglutinin. Levels of IFN transcripts were assayed by QPCR. QPCR was used in preference to assaying functional IFN as RNA levels are less likely to be affected by levels of blood-derived material, particularly as SFV A7 (74) is known to disrupt the integrity of the blood–brain barrier (Parsons & Webb, 1982).Thus, by analogy with the mechanism and analysis of virus neutralization by antibody in excess (Bradish et al. 1962) we may write, Serum 'haemagglutination-inhibition' index - $\beta\delta+\log D_{Ab}$. Here D_{ab} is the overall dilution (denominator) of antiserum in the reaction system. The serum 'haemagglutination-inhibition' index, like the serum neutralization index (SNI), is the logarithm of constant times the concentration of antibody and is indicated for these experiments as 3.6 + 0.3 by the intersection of the ' best-fit' line with the ordinate. The slope, β, of the relationship in characterizes the mechanisms of agglutination and of the prior combination of antibody and haemagglntinin according to the percentage law. Quite apart from the merits of a quantitative analysis and a potentially absolute interpretation, information of the present type is not available through current pattern or photometric tests which depend upon unspecified distributions of aggregates and quantal observations of convenient but arbitrary reaction mixtures and end-points. The first description ofIFNAR-12/2 mice noted that SFV was rapidly fatal in these mice but the strain of SFV used was not stated (Mulleret al., 1994).It is of interest to note that the typical rabbit anti-SFV serum (Bradish et al. I970 quoted in this study showed a serum neutralization index (SNI) of about 4 log units in tests based on plaque reduction in agar suspensions of primary chick-embryo cells (Bradish et aL I97I). In culture, CNS cells including neurons and glial cells have been observed to activate the type I IFN system (McKimmie& Fazakerley, 2005; Prehaud et al., 2005). Intrathecal synthesis of IFNs and IFN-activated protein expression have also been demonstrated in patients with CNS virus infections (Dussaix et al., 1985; Ogata et al., 2004).

This near-identity of the neutralization and haemagglutination-inhibition indices suggests that the early mechanism through which antibody blocks virus infection of the chick cell is similar to that by which antibody blocks virus agglutination of the goose red blood cell. Since picorna- and arbo-virus particles generally combine rapidly with antibody molecules (Bradish & Crawford, 196o; Bradish et aL I962) to form stable complexes showing both antigen- and antibody-sites (amphoteric), it is probable that the complexes of antibody with virus haemagglutinin are formed equally rapidly. The type I IFN system has been shown to protect mice against the spread of other viruses in the CNS including Theiler's virus, Bunyamwera virus, Dugbe virus, Hantaan virus, influenza A virus, vesicular stomatitis virus, lymphocytic choriomeningitis virus, Sindbis virus and Venezuelan equine encephalitis virus (Boyd et al., 2006;Bridgen et al., 2001; Fiette et al., 1995; Garcia-Sastre et al.,1998; Grieder & Vogel, 1999; Koerner et al., 2007; Mulleret al., 1994; Ryman et al., 2000; Wichmann et al., 2002). The subsequent agglutination of RBC is then by stable, amphoteric complexes which, as anticipated by the percentage law, are not inhibited further by the excess of unabsorbed antibody. As documented previously, SFV A7(74)is efficiently neuro invasive, but in the adult mouse brain it is restricted in its ability to replicate in and spread between mature neurons (Fazakerley

et al., 1993, 2006; Oliver &Fazakerley, 1998; Pusztai et al., 1971).

Mouse neurons, both in culture and in the adult mouse brain, can respond to IFNs (Ousman et al., 2005; Wang & Campbell, 2005; Wang et al., 2002; Ward & Massa, 1995). Type I IFN responses have also been shown to protect ependymal cells from measles virus, meningeal cells from Sindbis virus and oligodendrocyte, ependymal and choroid plexus cells from Theiler's virus infections (Fiette et al., 1995; Mrkic et al., 1998; Rymanet al., 2000). Although the method of sedimentation-enumeration may be applied to the quantitation of haemagglutination or haemagglutination-inhibition in myxo- or other virus systems (Cameron, 1969), the mechanisms of inhibition by antibody in these systems may not follow the percentage law and the equations above. In such cases the serum' haemagglutination-inhibition' index would require to be replaced by an alternative constant appropriate to the reaction-kinetics of the system.

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REFERENCES

Aguilar, P. V., Paessler, S., Carrara, A. S., Baron, S., Poast, J., Wang, E., Moncayo, A. C., Anishchenko, M., Watts, D. & other authors (2005).Variation in interferon sensitivity and induction among strains of eastern equine encephalitis virus. J Virol 79, 11300–11310.

Boyd, A., Fazakerley, J. K. & Bridgen, A. (2006). Pathogenesis of Dugbe virus infection in wild-type and interferon-deficient mice.J Gen Virol 87, 2005–2009.

Bradish, C. J., Allner, K. & Maber, H. B. (1971). The virulence of original and derived strains of Semliki Forest virus for mice, guinea pig sand rabbits. J Gen Virol 12, 141–160.

Bridgen, A., Weber, F., Fazakerley, J. K. & Elliott, R. M. (2001).Bunyamwera bunya virus non-structural protein NSs is a nonessential gene product that contributes to viral pathogenesis. Proc Natl AcadSci U S A 98, 664–669.

Cameron, k. R. & bradish, c. J. (I972). The kinetics of haemagglutination by Semliki Forest virus: a new sedimentation-enumeration method. Journal of General Virology I6, I35-I52.

Cameron, k. R. (1969). Quantitative studies on haemagglutination. The mechanism and kinetics o f haemagglutination by Semliki Forest virus. Ph.D. Thesis, University of Edinburgh.

Deuber, S. A. & Pavlovic, J. (2007). Virulence of a mouse-adapted Semliki Forest virus strain is associated with reduced susceptibility to interferon. J Gen Virol 88, 1952–1959.

Dussaix, E., Lebon, P., Ponsot, G., Huault, G. & Tardieu, M. (1985).Intrathecal synthesis of different a-interferons in patients with various neurological diseases. Acta Neurol Scand 71, 504–509.

Fazakerley, J. K. (2004). Semliki Forest virus infection of laboratory mice: a model to study the pathogenesis of viral encephalitis. ArchVirol Suppl 18, 179–190.

Fazakerley, J. K., Cotterill, C. L., Lee, G. & Graham, A. (2006). Virus tropism, distribution, persistence and pathology in the corpus callosum of the Semliki Forest virus-infected mouse brain: a novel system to study virus–oligodendrocyte interactions. Neuropathol Appl.

Fazakerley, J. K., Pathak, S., Scallan, M., Amor, S. & Dyson, H. (1993). Replication of the A7 (74) strain of Semliki Forest virus is restricted in neurons. Virology 195, 627–637.

Fiette, l., aubert, c., muller, u., huang, s., aguet, m., brahic, m. &bureau, j. F. (1995). Theiler's virus infection of 129sv mice that lack the interferon alpha/beta or interferon gamma receptors. J Exp Med181, 2069–2076.

Garcia-Sastre, A., Durbin, R. K., Zheng, H., Palese, P., Gertner, R., Levy, D. E. & Durbin, J. E. (1998). The role of interferon in influenza virus tissue tropism. J Virol 72, 8550–8558.

Grieder, F. B. & Vogel, S. N. (1999). Role of interferon and interferon regulatory factors in early protection against Venezuelan equine encephalitis virus infection. Virology 257, 106–118.

Koerner, I., Kochs, G., Kalinke, U., Weiss, S. & Staeheli, P. (2007).Protective role of beta interferon in host defense against influenza Avirus. J Virol 81, 2025–2030.

Mathiot, C. C., Grimaud, G., Garry, P., Bouquety, J. C., Mada, A., Daguisy, A. M. & Georges, A. J. (1990). An outbreak of humanSemliki Forest virus infections in Central African Republic. Am J TropMed Hyg 42, 386–393.

McKimmie, C. S. & Fazakerley, J. K. (2005). In response to pathogens, glial cells dynamically and differentially regulate Toll-like receptor gene expression. J Neuroimmunol 169, 116–125.

MeBradish, C. J., Allner, K. & Maber, H. B. (1971). The virulence of original and derived strains of Semliki Forest virus for mice, guinea pigs and rabbits. J Gen Virol 12, 141–160.d Hyg 42, 386–393.

Mrkic, B., Pavlovic, J., Rulicke, T., Volpe, P., Buchholz, C. J.,Hourcade, D., Atkinson, J. P., Aguzzi, A. & Cattaneo, R. (1998).Measles virus spread and pathogenesis in genetically modified mice.J Virol 72, 7420–7427.Neurobiol 32, 397–409.

Ogata, S., Ogata, A., Schneider-Schaulies, S. & Schneider-Schaulies, J. (2004). Expression of the interferon-a/b-inducibleMxA protein in brain lesions of sub acute sclerosing panencephalitis.J Neurol Sci 223, 113–119.

Oliver, K. R. & Fazakerley, J. K. (1998). Transneuronal spread of Semliki Forest virus in the developing mouse olfactory system is determined by neuronal maturity. Neuroscience 82, 867–877.

Oliver, K. R., Scallan, M. F., Dyson, H. & Fazakerley, J. K. (1997).Susceptibility to a neurotropic virus and its changing distribution in the developing brain is a function of CNS maturity. J Neurovirol 3, 38–48.

Ousman, S. S., Wang, J. & Campbell, I. L. (2005). Differential regulation of interferon regulatory factor (IRF)-7 and IRF-9 gene expression in the central nervous system during viral infection. J Virol79, 7514–7527.

Parsons, L. M. & Webb, H. E. (1982). Blood brain barrier disturbance and immunoglobulin G levels in the cerebrospinal fluid of the mouse following peripheral infection with the demyelinating strain of Semliki Forest virus. J Neurol Sci 57, 307–318.

Prehaud, C., Megret, F., Lafage, M. & Lafon, M. (2005). Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon. J Virol 79, 12893–12904.

Ryman, K. D., Klimstra, W. B., Nguyen, K. B., Biron, C. A. & Johnston,R. E. (2000). Alpha/beta interferon protects adult mice from fatal Sindbis virus infection and is an important determinant of cell and tissue tropism. J Virol 74, 3366–3378.

Schuffenecker, I., Iteman, I., Michault, A., Murri, S., Frangeul, L., Vaney, M. C., Lavenir, R., Pardigon, N., Reynes, J. M. & other authors (2006). Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med 3, e263.

Thomas, J. M., Klimstra, W. B., Ryman, K. D. & Heidner, H. W. (2003).Sindbis virus vectors designed to express a foreign protein as acleavable component of the viral structural polyprotein. J Virol 77, 5598–5606.

Wang, J. & Campbell, I. L. (2005). Innate STAT1-dependent genomic response of neurons to the antiviral cytokine alpha interferon. J Virol79, 8295–8302.

Ward, L. A. & Massa, P. T. (1995). Neuron-specific regulation of majorhistocompatibility complex class I, interferon-b, and anti-viral state genes. J Neuroimmunol 58, 145–155.

Wichmann, D., Grone, H. J., Frese, M., Pavlovic, J., Anheier, B., Haller, O., Klenk, H. D. & Feldmann, H. (2002). Hantaan virus infection causesan acute neurological disease that is fatal in adult laboratory mice.J Virol 76, 8890–8899.