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The Virulence of a Mouse-Adapted Semliki Forest Mechanism: a Model to Study the Pathogenesis

Abhimaan Tiwari[1] and Dr. N. Tiwari[2]
[1]Student, Department of Biology, Noorul Islam Centre for Higher Education, Thucklay, India
[2]Assistant Professor, Department of Biology, Noorul Islam Centre for Higher Education, Thucklay, India

[1]Corresponding Author: abhimaan.tiwari955@yahoo.com

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ABSTRACT
Vectors from the Semliki Forest virus (SFV) have been created to offer a simple method for expressing protein-encoding sequences in almost any animal cell. Acute encephalitis, subacute demyelinating meningoencephalomyelitis, and persistent subclinical central nervous system (CNS) infection are possible infection outcomes in mice, depending on age and viral strain. Infected mice under 12 days old exhibit severe symptoms of all viral types. While the A7 (74) strain is virulent, the L10 strain is equally pathogenic in mice older than 14 days.

Keywords: virulence, pathogenesis, semliki

I. INTRODUCTION

The Semliki Forest virus (SFV), which mosquitoes spread, naturally circulates in sub-Saharan Africa. The virus belongs to the Gaviridae family and is an alpha virus. Natural infections in humans and horses have been documented (Mathiot et al., 1990). Depending on their pathogenicity in adult mice, many strains have been labelled as virulent or avirulent. Virulent strains include the L10, V13, Osterrieth, and prototype strains (Bradishe et al., 1971; Glasgow et al., 1991). Along with Sindbis and the Venezuelan Equine Encephalitis (VEE) virus, the Semliki Forest virus (SFV) is a positive-stranded RNA-enclosed virus that belongs to the alphavirus genus. 1 Today, vectors based on these viruses are becoming more and more popular for the in vivo production of heterologous proteins. Several wild isolates and their laboratory-passaged strains have been studied for their virulence in mice (Bradish et al., 1971). To examine protein expression in cells, SFV particles are created that exclusively contain recombinant RNA. The most well-known and virulent strain of SFV, A7 (74), is virulent in mice infected when they are 11 days or younger but becomes avirulent in older animals because of age-related restrictions on virus propagation in the central nervous system (CNS) (Oliver et al., 1997). The virus shares a close relationship with the Chikungunya virus, which recently caused a serious arthralgia outbreak in the islands of the Indian Ocean (Schuffenecker et al., 2006).

Intraperitoneal inoculation of SFV A7 (74) in 4-5-week-old mice causes a high-titre plasma viremia from which virus is seeded into perivascular foci in the brain and spinal cord; there is minimal virus spread from cell to cell, foci do not enlarge over time, and the infection is limited to mature neurons (Fazakerley et al., 1993). Direct intracerebral inoculation leaves the SFV A7 (74) avirulent but results in extensive oligodendrocyte infection in the major white matter tracts (Fazakerley et al., 2006). Additionally, it has been shown that the type I IFN system is essential for defending mice against nominally avirulent forms of the related alpha viruses Sindbis virus and Venezuelan equine encephalitis virus (Grieder &Vogel, 1999; Ryman et al., 2000). IFN sensitivity varies across SFV and eastern equine encephalitis virus strains (Aguilar et al., 2005; Deuber & Pavlovic, 2007). In a 1987 study in the Central African Republic (Mathiot et al., 1990), 22 patients with fever, severe persistent headaches, myalgia, and arthralgia were found to have SFV.

II. METHOD AND MATERIALS

The quantitative assay of antibody activity for combination with virus haemagglutinin was carried out using the sedimentation-enumeration method established for quantitative investigations of haemagglutination by Semliki Forest virus (SFV). The vI3 strain of the SFV (Bradish, Allner, and Maber, 1997) was inoculated into the brains of suckling mice, and the harvested brains were then treated with fluorocarbon to produce SFV haemagglutinin. Lab mice infected with SFV serve as an appealing model system for the investigation of virus pathogenesis, particularly virus encephalitis (Fazakerley, 2004). A cDNA

plasmid produced from the SFV prototype strain was used to create the infectious SFV4 virus (Liljestrom & Garoff, 1991). Studies using equilibrium density gradient centrifugation and gel filtration revealed that the haemoglobin was homogeneous and similar in size and density to the infectious particle (Cameron, I969).

The foot-and-mouth disease virus 2A cleavage sequence was inserted between the capsid protein and p62 in the structural protein open reading frame to create the SFV4 marker virus, which has the gene for enhanced green fluorescent protein (eGFP). An eGFP-labelled Sindbis virus was previously created using this method (Thomas et al., 2003). Different concentrations of SFV haemagglutinin were reacted with dilutions of a rabbit hyperimmune serum made against the designated strain (vI3) of the virus by R. B. Fitzgeorge and C. J. Bradish at room temperature (23 °C for 30 minutes). The reaction took place in a borate buffer (pH 9"), containing 0.2% (w/v) bovine serum albumin. Each reaction mixture was then given an equivalent volume of a suspension of goose red blood cells (RBC) in phosphate buffered saline (PBS), bringing the ultimate optimal pH to 6.3 and the total RBC concentration to 107/ml. The production of RBC aggregates of various sizes under standard conditions was next investigated by sampling each combination and using the sedimentation-enumeration method (Cameron & Bradish, I972) to count the aggregates under a microscope.

The amount of agglutination was calculated (Cameron & Bradish, I972) as the average number of hemagglutinin-specific RBC-RBC bonds per red blood cell from the total numbers of RBC-RBC bonds (B) and red blood cells (R) based on the observed concentration and distribution of size-specified aggregates. (B/R) H—(B/R) o, where the second word expresses the correction for spontaneous agglutination in the absence of haemagglutinin, serves as a sign for this. Depending on the antiserum concentration or antiserum dilution employed, residual haemagglutinating activities are computed and displayed as a series of parallel lines offset from the haemagglutinin-only control. The amount of haemagglutinin remaining in suspension to be identified by the final addition of RBC agglutination is obviously reduced the higher the initial antibody concentration.

III. RESPONSE AND CONVERSATION

We have demonstrated that the linear section of the haemagglutination characteristic accurately reflects the concentration of accessible haemagglutinin or the virus-specific haemagglutinating activity (H) by its position (Cameron & Bradish, 1997). Therefore, the horizontal or vertical displacement of the reaction lines brought on by an increase in antibody concentration indicates a decrease in the haemagglutinating activity or concentration of accessible haemagglutinin. In terms of numbers, the decline in the amount of agglutination is proportional to the decline in the logarithm of the haemagglutinating activity.

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

Here, (B/R) Ab or (B/R) H, respectively, show the amount of agglutination in reactions containing or excluding antibodies. Several trials in various settings using dilutions of a single rabbit anti-SFV serum showed that this reduction in agglutination was dependent on antibody concentration. This suggests that the quantity of antibody, rather than the initial concentration of haemagglutinin, determines how much haemagglutinating activity is reduced by an excess of antibody. Using QPCR, IFN transcript levels were measured. Instead of measuring functional IFN, QPCR was utilised since RNA levels are less likely to be impacted by blood-derived material levels, especially since SFV A7 (74) is known to compromise the blood-brain barrier's integrity (Parsons & Webb, 1982). Thus, we can write serum "haemagglutination-inhibition" index - $\beta\delta+\log D_{Ab}$ by analogy with the mechanism and analysis of virus neutralisation by antibody in excess (Bradish et al. 1962). Here, Dab is the reaction system's overall antiserum dilution (denominator). The serum 'haemagglutination-inhibition' index, like the serum neutralisation index (SNI), is the logarithm of constant times the antibody concentration and is indicated for these tests as 3.6 + 0.3 by the intersection of the 'best-fit' line with the ordinate.

According to the percentage law, the slope β,of the connection characterises the mechanisms of agglutination and the preceding combination of antibody and haemoglobin. Information of the current type is not available through current pattern or photometric tests, which depend on unspecified distributions of aggregates and quantal observations of practical but arbitrary reaction mixtures and end-points. This is true even without considering the benefits of a quantitative analysis and a potentially absolute interpretation. Although the strain of SFV employed was not specified in the first report of IFNAR-12/2 mice (Mullere et al., 1994), it was recognised that these animals were immediately lethal to SFV. It is noteworthy that experiments based on plaque reduction in agar suspensions of primary chick embryo cells (Bradish et al., I970, referenced in this work) revealed that the average rabbit anti-SFV serum had a serum neutralisation index (SNI) of roughly 4 log units. The type I IFN system has been demonstrated to be activated in culture by CNS cells, including neurons and glial cells (McKimmie & Fazakerley, 2005; Prehaud et al., 2005). Patients with CNS viral infections have also been shown to produce intrathecal IFNs and express IFN-activated proteins (Dussaix et al., 1985; Ogata et al., 2004).

The fact that the neutralisation and haemagglutination-inhibition indices are so comparable shows that the early mechanisms by which antibodies prevent virus infection of chick cells and virus agglutination of goose red blood cells are related. It is likely that the complexes of antibodies with viral haemagglutinin are formed equally quickly because picorna- and arbo-virus particles frequently combine quickly with antibody molecules to form stable complexes showing both antigen- and antibody-sites (amphoteric) (Bradish & Crawford, 1960; Bradish et al., I962). It has been demonstrated that the type I IFN system can shield mice from other viruses that can infect the central nervous system (CNS), such as 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. The RBC is then subsequently agglutinated by stable, amphoteric complexes that, in accordance with the percentage law, are not further hindered by the excess of unabsorbed antibody. The ability of SFV A7(74) to replicate in and spread amongst mature neurons is constrained in the adult mouse brain, as has been previously shown (Fazakerley et al., 1993, 2006; Oliver &Fazakerley, 1998; Pusztai et al., 1971). SFV A7 (74) is an efficient neuroinvasive virus.

IV. CONCLUSION

According to Ousman et al. (2005), Wang & Campbell (2005), Wang et al. (2002), and Ward & Massa (1995), mouse neurons can react to IFNs both in culture and in the adult mouse brain. In addition, type I IFN responses have been demonstrated to protect oligodendrocyte, ependymal, and choroid plexus cells against Theiler's virus infections, meningeal cells from Sindbis virus, and ependymal cells from measles virus (Fiette et al., 1995; Mrkic et al., 1998; Ryman et al., 2000). Although the method of sedimentation-enumeration can be used to quantify the haemagglutination or the inhibition of the haemagglutination in myxovirus or other virus systems (Cameron, 1969), the mechanisms of antibody inhibition in these systems might not adhere to the percentage law and the equations above. In such circumstances, the serum's haemoglobin-inhibition index would need to be substituted with a different constant suitable for the system's reaction kinetics.

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