Antiviral Research, 1 (1981) 263–267
Elsevier/North-Holland Biomedical Press

DEVELOPMENT OF AGE-DEPENDENT RESISTANCE TO SINDBIS VIRUS ENCEPHALITIS: CORRELATION WITH INACTIVATION OF VIRUS WITHIN THE BLOOD STREAM

ROBERT L. HIRSCH[1] and DIANE E. GRIFFIN[1,2]

Howard Hughes Medical Institute Laboratories in the Departments of [1]Neurology and [2]Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, U.S.A.

(Received 12 May 1981; accepted 7 July 1981)

The clearance of Sindbis virus from the blood stream and its localization in the reticuloendothelial system (RES) was studied in mice susceptible (2 weeks old) and resistant (6 weeks old) to fatal Sindbis virus encephalitis. No significant differences in the relative capacity of 2-week-old and 6-week-old mice to remove ^{125}I-labeled virus from the blood stream and to localize virus within the liver were observed. However, the decline of infectious virus was more rapid in the blood of adult mice. These studies suggest that, in addition to physical removal of virus by the RES, inactivation of virus in the blood stream plays an important role in limiting viremia during infections with Sindbis virus.

Sindbis virus non-specific resistance age-dependent resistance reticuloendothelial cell system

INTRODUCTION

In many viral infections the severity of disease decreases with increasing age of the host. The mechanisms responsible for the acquisition of resistance with aging vary with the host and virus under investigation [4,7,18,19]. However, for most viral infections the mechanism for development of age-dependent resistance is unknown. Sindbis virus, an alphavirus of the togavirus group, causes an acute encephalitis in mice in which mortality is age-dependent [16]. Previous studies have shown that virus-specific immune responses, including lymphoproliferative and antibody responses, are similar in the older resistant and younger susceptible animals [5]. Similarly, differences in interferon induction [20,21] and natural killer cell responses [13] do not appear to explain age-dependent susceptibility. Subcutaneous inoculation of Sindbis virus results in a viremia that is significantly greater in younger susceptible mice than in older resistant mice [5]. It has been suggested that the reticuloendothelial cell system (RES) of weanling mice is capable of clearing more virus than the RES of suckling mice [6]. The present studies were undertaken to determine whether the susceptibility of younger animals to fatal Sindbis virus infection could be correlated directly to a deficiency of RES function or to other factors operating independently of the RES.

0166–3542/81/0000–0000/$02.50

EXPERIMENTAL

Sindbis virus (AR339) grown on BHK-21 cells was purified by sucrose density gradient centrifugation [9] and labeled with ^{125}I by the lactoperoxidase method [17]. Unbound ^{125}I was removed by extensive dialysis of the labeled virus preparation. The labeling procedure did not significantly affect infectivity of the virus and the specific activity of the virus preparation was approximately 1 count per minute (c.p.m.) per plaque-forming units (p.f.u.) of virus. BALB/c mice were obtained from Charles River Animal Breeding Facilities (Wilmington, MA). In the study of virus clearance from the blood stream, animals were inoculated, via the intracardiac route, with 5×10^6 p.f.u. of Sindbis virus, diluted in phosphate-buffered saline (PBS). Two-week-old animals received this dose of virus in 0.1 ml and 6-week-old mice in 0.2 ml.

For study of the clearance of infectious virus, the amount of virus in blood was determined by plaque assay on monolayers of BHK-21 cells [5]. For study of the clearance of ^{125}I-labeled virus, whole organs and known volumes of blood (70 μl) were obtained and counted in a Nuclear Chicago Gamma Spectrometer. Individual mice were assessed at each time point.

To correct for the amount of label in organs due to the presence of blood, blood volumes of organs were determined following the injection of ^{51}Cr-labeled autologous red blood cells [3]. The following calculation was used to determine the amount of ^{125}I-labeled virus in organs, exclusive of label in the organs due to the presence of blood: net ^{125}I c.p.m./organ=Gross ^{125}I c.p.m./organ $-$ [^{125}I c.p.m./ml blood $\times$ organ blood volume (ml)].

Serum levels of C3 were determined by radial immunodiffusion in 1% agarose in PBS containing a 1 : 10 dilution of goat anti-mouse-C3 (Cappel Labs, Cochranville, PA). Results are expressed as precipitin ring diameters (mm) measured at 48 h.

Fig. 1 shows the results of an experiment in which RES function in susceptible and resistant mice was evaluated. ^{125}I-labeled Sindbis virus was injected by the intracardiac route, and samples of blood, liver, spleen, and lungs were obtained at frequent intervals. No significant differences in the rate of clearance of labeled virus from the blood of 2- and 6-week-old mice were observed. Furthermore, with the exception of one time point (2 h), there were no differences in the uptake of labeled virus into the livers of 2- and 6-week-old mice (data not shown). Only small amounts ($< 1.0\%$) of labeled virus were taken up by spleen and lungs in both groups of mice.

This study showed that there were no significant differences in the clearance and localization of labeled virus between susceptible and resistant mice. In order to evaluate the clearance of infectious virus, 2- and 6-week-old mice were injected with 5×10^6 p.f.u. virus and infectious virus was quantitated in the blood. Significantly more virus was present in the blood of 2-week-old mice at all times studied (Fig. 1). In addition, it appears that, for both groups of mice, infectious virus is inactivated at a faster rate than labeled virus is removed from the blood (Fig. 1).

Serum C3 levels in pooled serum from mice of both ages were identical (6.0 mm ring diameter).

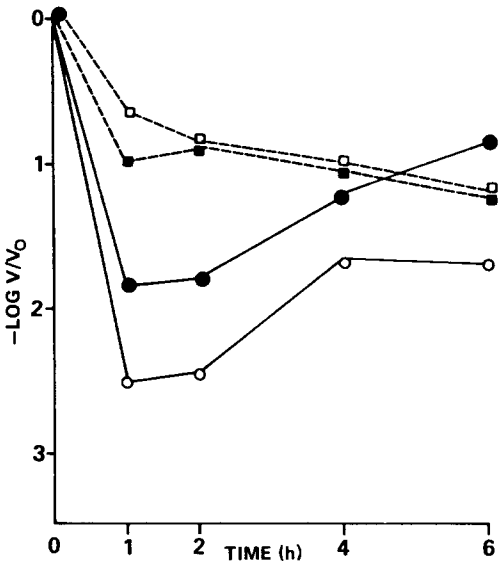

Fig. 1. Clearance of ^{125}I-labeled virus (-----, c.p.m.) and decline in infectivity (——, p.f.u.) from the blood of 2-week-old (•, ▪) and 6-week-old (○, □) mice. Data are expressed as the $-\log_{10}$ amount of virus (p.f.u. or label) present at any one time, divided by the amount present at time 1 min. Since the blood volume of 2-week-old mice was 2.6 times less than that of the 6-week-old mice, values for the 2-week-old animals were divided by 2.6 to correct for the initial higher concentration. Blood from six individual mice in each age group was assessed for each time point of the clearance curve of labeled virus; blood from five individual mice in each group was assessed for infectivity.

These studies demonstrate that, although the clearance of radiolabeled virus particles from the blood of young susceptible and older resistant mice is comparable, the clearance of infectious virus is more efficient in the older animals. These observations suggest that inactivation of virus within the bloodstream is an important aspect of the clearance of viremia and that a deficit in serum inactivation may contribute to the susceptibility of younger mice to fatal Sindbis virus infection.

Previous studies have suggested that the macrophage plays an important role in recovery of adult mice from other alphavirus infections [1,14]. However, there is no evidence that these functions are important in the development of age-dependent resistance, and, in fact, previous studies with Sindbis virus suggest that changes in RES function with aging do not contribute to its development [6]. The present experiments confirm these findings.

Two mechanisms seem to contribute to clearance of virus from the blood stream. In addition to physical clearance of virus by the RES, there is inactivation of viral infectivity. The latter appears less efficient in young mice in these short-term studies. Thus, the greater magnitude of viremia observed in young mice during natural infection [5] may be due to an inability to inactivate infectious virus in the blood stream. Since it has been previously shown that the length of viremia in Sindbis infection correlates with severity

of infection of the central nervous system [8], the inability of the blood to inactivate virus may result in a more severe form of encephalitis in younger mice. Since Sindbis virus activates the third component of complement (C3) [12] and complement depletion has been shown to prolong the viremia in Sindbis virus infections]8,11], maturational changes in levels of certain complement components may be of importance in this aspect of viral clearance. However, the inability of young mice to inactivate Singbis virus within the blood stream does not appear to be related to differences in serum C3 levels (as measured immunochemically), since these were identical in the two age groups. It remains possible that other complement components or perhaps the levels of functional C3 previously shown to be lower in younger animals [2] are of greater importance in limiting viremia.

Differences in viral clearance between younger susceptible and other resistant animals may contribute but cannot alone account for the development of age-dependent resistance. Direct intracerebral inoculation of virus which obviates the need for viremia to produce encephalitis still does not produce a fatal infection in the mature animal. This observation suggests that factors operative within local sites of replication as well as within the blood stream are also important in the development of age-dependent resistance.

ACKNOWLEDGEMENTS

This project was supported in part by U.S. Public Health Service Grant NS 15749. R.L.H. is a Research Associate of the Howard Hughes Medical Institute and D.E.G. is an Investigator of the Howard Hughes Medical Institute.

We wish to thank Dr. Richard Johnson for helpful discussions. We also thank Norman Karasanyi for excellent technical assistance and Linda Kelly for preparation of the manuscript.

REFERENCES

1 Allner, K., Bradish, C.J., Fitzgeorge, R. and Nathanson, N. (1974) Modifications by sodium aurothio-malate on the expression of virulence in mice by defined strains of Semliki Forest virus. J. Gen. Virol. 24, 221–228.

2 Ferreira, A. and Nussenzweig, V. (1975) Genetic linkage between serum levels of the third component of complement and the H-2 complex. J. Exp. Med. 141, 513–517.

3 Finbloom, D.S. and Plotz, P.H. (1979) Studies of reticuloendothelial functions in the mouse with model immune complexes. I. Serum clearance and tissue uptake in normal C3H mice. J. Immunol. 123, 1594–1599.

4 Gallily, R., Warwick, A. and Bang, F.B. (1967) Ontogeny of macrophage resistance to mouse hepatitis in vivo and in vitro. J. Exp. Med. 125, 537–548.

5 Griffin, D.E. (1976) Role of the immune response in age-dependent resistance of mice to encephalitis due to Sindbis virus. J. Infect. Dis. 133, 456–464.

6 Hackbarth, S.A., Reinarz, A.B.G. and Sagik, B.P. (1973) Age-dependent resistance of mice to Sindbis virus infection: reticuloendothelial role. J. Reticuloendothel. Soc. 14, 405–425.

7 Hirsch, M.S., Zisman, B. and Allison, A.C. (1970) Macrophages and age-dependent resistance to herpes simplex virus in mice. J. Immunol. 104, 1160–1165.

8 Hirsch, R.L., Griffin, D.E. and Winkelstein, J.A. (1978) The effect of complement depletion on the course of Sindbis virus infection in mice. J. Immunol. 121, 1276–1278.
9 Hirsch, R.L. and Griffin, D.E. (1979) The pathogenesis of Sindbis virus infection in athymic nude mice. J. Immunol. 123, 1215–1218.
10 Hirsch, R.L., Griffin, D.E. and Johnson, R.T. (1979) Interactions between immune cells and antibody in protection from fatal Sindbis virus encephalitis. Infect. Immun. 23, 320–324.
11 Hirsch, R.L., Griffin, D.E. and Winkelstein, J.A. (1980) The role of complement in viral infections. II. The clearance of Sindbis virus from the bloodstream and central nervous system of mice depleted of complement. J. Infect. Dis. 141, 212–217.
12 Hirsch, R.L., Winkelstein, J.A. and Griffin, D.E. (1980) The role of complement in viral infections. III. Activation of classical and alternative complement pathways of Sindbis virus. J. Immunol. 124, 2507–2510.
13 Hirsch R.L. (1981) Natural killer cells appear to play no role in the recovery of mice from Sindbis virus infection. Immunology 43, 81–89.
14 Jahrling, P.B. and Scherer, W.F. (1973) Growth curves and clearance rates of virulent and benign Venezuelan encephalitis virus in hamsters. Infect. Immun. 8, 456–462.
15 Johnson, R.T. (1964) The pathogenesis of herpes virus-encephalitis. II. A cellular basis for the development of resistance with age. J. Exp. Med. 120, 359–367.
16 Johnson, R.T., McFarland, H.F. and Levy, S.E. (1972) Age-dependent resistance to viral encephalitis: studies of infections due to Sindbis virus in mice. J. Infect. Dis. 125, 257–262.
17 Marchalonis, J.J. (1969) An enzyme method for the trace iodination of immunoglobulins and other proteins. Biochem. J. 113, 299–303.
18 Mokhtarian, F. and Shah, K.V. (1980) Role of antibody in recovery from K-papovavirus infection in mice. Infect. Immun. 29, 1169–1179.
19 Overman, J.R. (1954) Antibody response of suckling mice to mumps virus. II. Relation to onset of antibody production to susceptibility to mumps virus infections. J. Immunol. 73, 249–255.
20 Reinarz, A.B.G., Broome, M.G. and Sagik, B.P. (1971) Age-dependent resistance of mice to Sindbis virus infection: viral replication as a function of host age. Infect. Immun. 3, 268–273.
21 Vilcek, J. (1964) Production of interferon by newborn and adult mice infected with Sindbis virus. Virology 22, 651–653.