Studies on the Multiplication of Lactic Dehydrogenase Elevating Agents

Recently, evidence was reported[1–3] to suggest that the plasma of mice infected with the lactic dehydrogenase (LDH) agent[4] contains at least two different virus particles. In this laboratory it was shown that the smaller virus (S particle) was not readily sedimentable by ultracentrifugation and not inactivated after incubation at 50°C for 60 min with *M* $MgCl_2$; the larger virus (L particle) was sedimentable and its heat inactivation enhanced by magnesium ions[3]. The present report will describe studies on the multiplication of these LDH elevating agents and present preliminary findings on the relationship between the two particles.

Materials and methods. Thirty-six C57BL/10J mice, approximately 3 months old, received an intraperitoneal injection (0.1 ml per mouse) of $10^{8.0}$ ID_{50}/ml of virus. At intervals of from 15 min to 12 weeks after infection, serum was collected from groups of 2-4 mice as described previously[5,6], pooled, and stored at −20°C. After thawing, aliquots (0.1 ml) of each serum sample were mixed with equal volumes of phosphate buffered saline (PBS), pH 7.2, of 2*M* $MgCl_2$, then incubated at 50°C for 60 min. Serial tenfold dilutions of the test materials were prepared in cold PBS and inoculated intraperitoneally (0.1 ml per mouse) into recipient mice. One week later, these test animals were bled and their serums assayed for LDH activity by the method described earlier[5,6]. Infective titers were calculated by the method of REED and MUENCH[7].

Results. The Figure shows the total virus titer (serum-PBS) at intervals after infection. Although virus was demonstrable in serum at 15 min, virus multiplication was not observed until 6 h. The titer increased rapidly, reaching a maximum, 10^{10} ID_{50}/ml, at 24 h, then fell approximately 3 logarithms to $10^{7.3}$ ID_{50}/ml by the end of the first week. Thereafter, a more gradual decline in serum titer was found with a decrease of approximately 3 logarithms over the next 9 weeks. Identical titers, $10^{4.5}$ ID_{50}/ml, were recorded at 11 and 12 weeks. A similar growth curve has been reported by NOTKINS and SHOCHAT[8], who used plasma rather than serum as their source of test material.

The titer of S particles (serum-$MgCl_2$) at intervals after infection is also shown in the Figure. Virus was recovered from serum at 15 min; virus multiplication was observed at 6 h. The maximum titer of the S particle, $10^{7.0}$ ID_{50}/ml, was reached at 24 to 36 h, then decreased 2 logarithms over the next 36 h. During the period 72 h to 12 weeks after inoculation, the titer remained essentially constant with extremes of $10^{4.0}$ ID_{50}/ml and $10^{5.3}$ ID_{50}/ml recorded.

Of particular interest is the finding that there was no significant difference between the total virus titer and the titer of S particles after the sixth week. This observation suggests that by 7 weeks after infection, all L particles have been removed from the serum. As such, it would appear that it is the S particle, rather than a mixture of both S and L particles, which accounts for the persistent viremia and elevated serum enzyme levels observed in infected animals.

At least two questions were raised by the above findings: (a) What is the relationship between S and L particles? If mice were inoculated with test materials presumed to contain only S particles (e.g. 10–12 week infected serum), there was no significant difference between the total virus titer and S particle titer in their serum at 24 h after infection. Further, the characteristic elevation in LDH activity was observed in 1 week sera from these animals. However, infective titers in 24 h serum from mice injected with test materials presumed to contain only L particles were similar to those recorded in mouse serum 24 h after an injection of plasma containing both L and S particles. Although several explanations are possible for the latter observation, all available evidence suggests that we have not yet obtained a 'pure' preparation of L particles. (b) By what mechanism is the L particle removed from host serum? Results obtained in preliminary experiments provide no evidence for the role of circulating antibodies. More definitive immunological studies are in progress[9].

Résumé. Observations sur la multiplication de deux particules différentes de virus dans le sérum de la souris après infection par l'agent de LDH. Le fait que la grande particule a disparu du sérum sept semaines après l'infection suggère que la petite particule est responsable de la virémie persistante et du taux élevé de l'enzyme dans le sang des animaux infectés.

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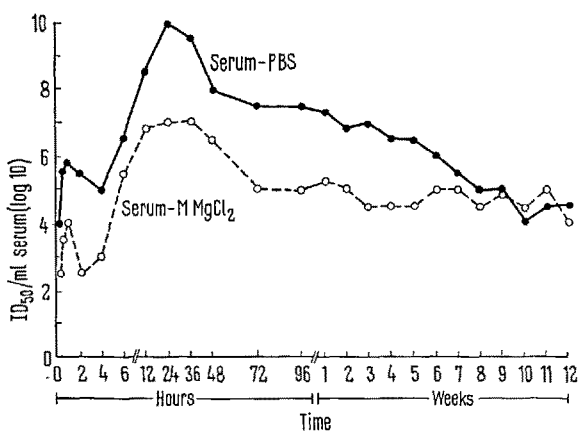

Total virus titer (serum-PBS) and S particle titer (serum-$MgCl_2$) at intervals after infection with 10^8 ID_{50}/ml. Each point represents the virus titration in pooled serum samples from 2 to 4 animals.

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