Circulating Interferon in Mice Infected with the Lactate Dehydrogenase-elevating Virus

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The lactate dehydrogenase-elevating virus (LDH-virus) (Riley et al. 1960; Riley, 1968) is unusual in a number of ways including its rapid rate of replication (its average replication cycle is approximately 30 min.), the production of high virus titres reaching peak concentrations of $10^9$ to $10^{11}$ ID $50/ml$. of blood plasma in 12 to 18 hr, and the rapid decline after 24 to 72 hr of 3 to 4 logarithms of virus infectivity to yield a stable lifelong viraemia of approximately $10^4$ to $10^6$ ID $50/ml$. Both the acute and chronic viraemias are clinically benign with no evidence of any tissue damage, loss of weight, decrease in longevity, or cytopathic effects in the host.

An understanding of the nature of the unknown inhibitory or virus-limiting conditions responsible for the abrupt decrease in virus concentration and for its continued suppression is obviously pertinent. The possible role of interferon in this process is the subject of this report.

Since the presence of interferon has been detected in the serum of mice infected with the LDH-virus (Baron et al. 1966; Du Buy & Johnson, 1965; Falke & Rowe, 1965; Burlingham & Riley, unpublished data, 1965), and since the striking decrease in virus titre was related in its timing more to typical interferon responses than to antibody production, studies were undertaken to establish the quantitative relationship between the virus titre and interferon concentration in the circulating blood during both the acute and chronic infective periods.

A uniform group of Swiss ICR/Ha female mice was inoculated intraperitoneally with the LDH-virus ($10^6$ ID $50/ml$.); at appropriate intervals, groups of five mice were bled from the brachial venous plexus and the blood was pooled. After clotting, the serum was removed, centrifuged at 2000 rev./min. for 15 min., acidified with 3 N-HCl to pH 2.0 and held at 4° for at least 6 days to destroy the LDH-virus infectivity. Shorter periods were less reliable. Samples were then centrifuged at 100,000 g for 2 hr to remove viral and other particulate material. The pH was adjusted to 7, and samples were either used immediately or stored at $-20°$. All samples were tested for residual LDH-virus infectivity before use by inoculating control mice and subsequently determining their plasma LDH levels. Control serum was obtained from normal mice injected with phosphate buffered saline and processed in the same way as the infected sera.

Serum samples to be assayed for interferon were tested for their protective activity in mouse embryo 1st-subcultures (Federoff, 1967) when the latter were challenged with vesicular stomatitis virus. After incubation with twofold dilutions of test serum samples in medium 512 (Fogh et al. 1964) the cells were washed twice with phosphate buffered saline and then challenged with $10^4$ TCD of vesicular stomatitis virus. This virus inoculum produced gross cytopathic effects in unprotected mouse embryo cultures within 24 hr. The cultures were considered protected if there
was less than 10% cytopathic effect 48 hr after challenge, while the controls were simultaneously destroyed. Interferon titres were expressed as the reciprocal of the highest dilution of the original material which protected at least 2 out of 3 of the cultures against vesicular stomatitis virus. Interferon samples were also tested against vaccinia virus challenge to confirm the accuracy of the technique using vesicular stomatitis virus.

The characteristics of the LDH-virus-induced interferon were as follows: It was resistant to pH 2.0 for long periods; non-sedimentable at 100,000 g for 2 hr; inactivated by trypsin; species specific (FL human amnion cells were not protected against vesicular stomatitis virus challenge, whereas primary mouse embryo cells were protected in vitro, as expressed by a lower LDH-virus titre in those cultures to which LDH-virus had been added); lacked virus specificity (vaccinia virus plaque formation was also inhibited by LDH-virus interferon); did not inactivate vesicular stomatitis extracellularly; and was stable at 4° and −20°.

![Graph](image)

Fig. 1. The relationship between the LDH-virus titre and production of interferon in serum of mice.

When the virus and interferon concentrations were followed after intraperitoneal injection of LDH-virus, a correlation was found between the increase of virus concentration and of interferon production for about 24 hr (Fig. 1). Serum interferon was first detected 12 hr after virus infection, and increased to its highest concentration at 20 hr, but decreased rapidly after 24 hr. Interferon was still detectable at 48 hr, but by 72 hr and for a continuing 8-week period it was no longer detectable in the blood plasma. The 3.5 logarithm decrease in virus concentration occurring after 24 hr was accompanied by the complete disappearance of interferon in the plasma.

The possibility of interference by residual infective LDH-virus was tested by injecting normal mice with each interferon-containing sample. All samples were found free of infective LDH-virus after 6 days at pH 2.0. However, in the event that virus concentrations less than 10^6.5 ID 50/ml. passed undetected and were responsible for the protective effect seen up to 48 hr, it was necessary to test the viral interference
potential of LDH-virus. Serum was harvested 24 hr and 72 hr after intentional LDH-virus infection and each pool was divided into 2 portions; one was treated with 3 N-HCl, the other was untreated. All were held at 4° for 7 days before examination for interference. Both the normal and acid-treated 24 hr serum samples gave similar interfering titres, while the preparations with lower virus titres harvested at 72 hr after infection showed no interference, even though at least 10^6 infective doses of LDH-virus were present in the untreated portion. These results illustrate the absence of significant interference by low titres of infective LDH-virus in vitro, and confirm the observations of others who failed to demonstrate LDH-virus interference or interferon production in vitro (Bendinelli, 1967; Evans, unpublished data; Du Buy & Johnson, 1966).

The increasing concentration of circulating interferon up to 24 hr, following LDH-virus infection, may explain the 3.5 logarithm decrease of the infectivity end point after the peak virus concentration was reached, and is consistent with observations that administration of interferon-suppressing actinomycin D following LDH-virus infection resulted in a higher virus titre during the first week (Crispens, 1966). However, the continued suppression of the initial high rate of virus replication in the absence of demonstrable serum interferon or antibody is puzzling. Antibody appears at a much later date and is therefore probably not concerned with this early virus suppression (Notkins et al. 1966; Rowson, Mahy & Bendinelli, 1966).

The absence of interferon in the serum after 48 hr does not necessarily rule out the possibility that interferon may still be present in the target tissues, and thus that its antiviral activity may persist. It is possible that small quantities of interferon may be produced continuously, and since the rate of interferon disappearance from the blood of normal mice is reported to be rapid (Baron et al. 1966) such levels might be difficult to measure. Since the clearance of colloidal carbon from the blood of LDH-virus-infected mice has been shown to be significantly impaired for the first few days of infection, and various enzymes have their clearance impaired for longer periods (Notkins, 1965; Riley, 1965), a correspondingly slower rate of interferon removal from the blood might facilitate or prolong its antiviral action.

The present data suggest that interferon plays some role in the suppression of LDH-virus replication. This is supported by other evidence demonstrating a temporary decline of LDH-virus titre when chronically infected mice were secondarily injected with Newcastle disease virus (Du Buy & Johnson, 1965), which is known to be an inducer of high interferon titres in vivo. This would suggest that the initial stimulation of interferon production by the LDH-virus had not induced a state of tolerance to secondary stimulation by Newcastle disease virus. Any suggestion of tolerance, however, would require reserved interpretation due to the lifelong persistence of infective LDH-virus in the plasma.

In conclusion, it seems likely that other factors such as the recovery of the reticuloendothelial system following its initial impairment, the presence of other LDH-virus inhibitors (Riley, 1968), and the possibility of the depletion of target cells may also play an integral role in the process of establishing the stable virus–host equilibrium.

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