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6. Interferon Restriction of Target Organs for Lymphocytic Choriomeningitis Virus-Induced T Lymphocytes may be Lethal

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both 2′–5′-oligo(A) synthetase and protein kinase were markedly increased after HuIFN-α treatment but no significant increase of these enzyme activities was observed in RD114-C1 cells. On the other hand, in both A204 and RD114-C1 cells, a non-antiviral action of interferon, the inhibition of syncytium formations induced by u.v.-inactivated Sendai virus, was efficiently expressed by pre-treatment of cells with HuIFN-α at 100 units ml^{-1}. Furthermore, HuIFN-γ, kindly supplied by Dr K. Cantell, also showed these different effects on retrovirus production and EMCV replication in RD114-C1 cells. These results suggest that the mechanisms underlying the anti-retrovirus and the anti-cell fusion activities of interferon may be closely related, and that they are different from those of antiviral action against exogenous infection with EMCV.

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6. Interferon restriction of target organs for lymphocytic choriomeningitis virus-induced T lymphocytes may be lethal

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It is generally held that interferons play a role in recovery from acute primary viral infections. One of the most direct ways that this can be demonstrated is by use of anti-interferon serum. Rapid evolution of lethal disease in a number of murine infections (encephalomyocarditis, herpes simplex, Newcastle disease, Semliki Forest viruses) was observed when interferon liberated from virus-infected cells was neutralized by injection of anti-interferon serum. The neutralization suppressed the normal interferon effect, allowing unchecked dissemination of

virus (Gresser *et al.* 1976*b*). However, it appears to be precisely this antiviral property of interferon that *causes* death in adult mice infected intracerebrally (i.c.) with lymphocytic choriomeningitis (LCM) virus. We explain here how the rapid proliferation of LCM virus could spare these mice rather than kill them.

The unusual link between interferon and the pathogenesis of LCM has its origin in an observation made by Gresser *et al.* (1976*a*). They showed that mice injected daily with interferon for the first 6–8 days after birth died with a late-onset glomerulonephritis as early as 35 days after birth. They speculated and later demonstrated (Gresser *et al.* 1978) that the glomerulonephritis seen in LCM-infected mice was caused, at least in part, by virus-induced interferon during the neonatal period. This was shown by treating neonates with anti-interferon serum (Rivière *et al.* 1977). Furthermore, such treatment markedly lowered the early (within 14 days) high mortality rate observed after infection, and at the same time increased virus titres in the serum by 100-fold. This sparing effect of anti-interferon serum was also observed in adult mice infected i.c. with a strain of LCM that normally caused 100 % mortality within 6–12 days (Saron *et al.* 1981). Again, paradoxically, this sparing effect was accompanied by a 100–1000-fold increase in serum virus titres.

How could rapid dissemination of LCM virus help adult i.c.-infected mice to survive? A hypothesis to explain this evolved in our laboratory while we were studying the response of T lymphocytes to i.c. infection with two strains of LCM virus differing markedly in their pathogenicities. One of these strains, denoted 'aggressive', provoked a convulsive type of death in 100 % of the mice within 7–10 days; the other, denoted 'docile', usually killed less than 10 % of the mice. We wanted to determine if the 'docile' virus induced a T-cell response since it is well established that this response against the i.c.-inoculated LCM virus is critical for the induction of lethal disease of the central nervous system (c.n.s.). The target organ for these T cells has been assumed to be the brain because lymphocytes and macrophages infiltrate the virus-infected choroid plexus, ependyma and meninges. It was first necessary to assess T-cell function *in vitro* since a response *in vivo*, if made, was obviously masked. Using ^{51}Cr-labelled LCM-infected L cells, the cytotoxic T lymphocyte (CTL) activity was found to be about the same in mice infected with either type of virus (Pfau *et al.* 1982*a*). Since these CTLs are widely considered to be the equivalent of the T-cell population responsible for murine LCM pathogenesis, we began a search for the reason why CTLs induced by 'docile' virus were not lethal (Pfau *et al.* 1982*b*). A striking inverse correlation was found between the spread of the infection and lethality. With identical inoculum doses of 300 plaque-forming units (p.f.u.) the highly lethal 'aggressive' virus strain spread to visceral organs very slowly. On the other hand, 'docile' virus dissemination was rapid. Reinforcement of this correlation came from the observation that very high i.c. doses (10^5 p.f.u.) of 'aggressive' virus, which mimicked the rapid spread of 'docile' virus, were not lethal. Moreover, very low i.c. doses of 'docile' virus (0.3 p.f.u.), which mimicked the normally slow spread of 'aggressive' virus, were highly lethal. Adoptive transfer experiments (injection of lymphocytes from a donor mouse into a genetically identical recipient mouse) showed that a 300 p.f.u. inoculum of 'docile' virus induced a population of T cells in a donor mouse fully capable of causing c.n.s. disease in identically infected recipients. This disease-causing ability was lost if the donor cell transfer was delayed beyond 3 days after infection of the recipients, but could be preserved by lowering the size of the viral inoculum in the recipients. In contrast, immuno-suppressed recipients were fully susceptible to the lethal effects of adoptive transfer as late as 7 days after infection with 300 p.f.u. of 'aggressive' virus.

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The hypothesis that the outcome of the disease in mice is determined by the number of target organs 'seen' by the T-cell population responding to the LCM infection has received increasing attention over the last 10 years (Pfau *et al.* 1982*b*). It was first advanced by Gilden *et al.* (1972) to explain why adoptive transfers did not kill adult mice that had been infected with LCM virus as neonates. They suggested that in these 'carrier' mice, which had widespread tissue infection, lymphocytes were recruited to many target organs so that fewer reached the brain. This 'antigen sink' hypothesis explains our dose–response observations with 'aggressive' and 'docile' viruses and leads to the prediction that CTLs will be found in many organs during sparing infections but mostly in the brain during fatal infections. Preliminary evidence indicates that this is indeed so (Pfau *et al.*, unpublished).

Because the greater part of an intracerebral inoculum spills over immediately into the bloodstream, and because the two strains of LCM show no great difference in growth rate in tissue culture, it is not clear why 'aggressive' virus lags so dramatically behind 'docile' virus in its appearance and replication in the visceral organs. We have considered the role of interferon here. Its central importance in LCM infection in the adult can be shown by comparing the 'docile' and 'aggressive' virus infections. On the one hand, mice infected with 'docile' virus will develop c.n.s. disease with appropriately timed injections of interferon inducers (Jacobson *et al.* 1981). On the other hand, adult mice infected with 'aggressive' virus strains will be spared by treatment with anti-interferon serum (Saron *et al.* 1981; Pfau *et al.* unpublished). This sparing is accompanied by a 100–1000-fold rise in viraemia (Saron *et al.* 1981; Pfau *et al.*, unpublished), and we have found that the spread of the 'aggressive' virus to visceral organs under these conditions is at least as rapid as that of 'docile' virus. Again, we believe that the rapid dissemination of LCM virus ensured survival because multiple target organs were created, which diverted the focused T cell attack on the brain. If interferon slows the spread of the infection, where is the battle fought? Another current area of investigation is to determine if virus is cleared in the circulation or if its replication is blocked within the visceral organs.

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