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JUNÍN VIRUS PERSISTENCE IN MICE

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Summary. — Newborn mice surviving intracerebral infection with Junín virus (JV) strain XJ showed viral persistence in brain up to 140 days post-infection (p.i.). Mild meningoencephalitis or encephalitis, but not the neutralizing antibody titres (NtAb) correlated with virus presence.

Key words: Junín virus; viral persistence; Arenavirus; mice

The majority of members of *Arenaviridae* family are known to establish persistent infections in their rodent hosts as a basic maintenance mechanism of the virus in nature. For Junín virus (JV), the aetiologic agent of Argentine haemorrhagic fever, persistent infection has been reported not only in its main reservoir *Calomys musculinus*, but also in experimental hosts such as rats (Weissenbacher *et al.*, 1986) or guinea pigs (Malumbres *et al.*, 1984). How wild and laboratory rodents are able to harbour JV chronically with or without evident late disease remains unknown, but it is quite likely that factors other than the immune response may be also involved.

The best studies experimental model for JV infection, the suckling mouse, was most useful in elucidation of the pathogenesis of acute disease. In this connection, the thymus dependent mechanism in the development of lethal encephalitis affecting about 90 % of immunocompetent mice has been amply demonstrated by means of congenitally athymic (Weissenbacher *et al.*, 1983) or neonatally thymectomized mice (Nota *et al.*, 1977).

As regards the mice surviving experimental JV infection, clinical signs are lacking but brain virus titres are quite high in thymus-impaired mice, whereas in immunocompetent animals no brain virus isolation has been reported, except for a single communication (Boxaca and Giovanniello, 1975).

Our preliminary finding that mortality could be diminished by infecting euthymic mice before 24 hr of age instead of the normally used 48—72 hr, encouraged us to study the feasibility of JV persistence in this model. Accordingly, 131 Rockland mice under 24 hr of age were inoculated intracerebrally with 10^4 PFU of XJ-JV strain: 101 developed characteristic fatal

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Table 1. JV isolation from brain and neutralizing antibody titres in mice surviving the acute disease

Mouse no.	Days p.i.	JV-isolation[a]		Nt Ab titre	Mouse no.	Days p.i.	JV-isolation		Nt Ab titre
		D.m.	Co−Ct				D.m.	Co−Ct	
1	45	4.2	+	17	16	100	ND	+	112
2	45	3.0	+	17	17	100	〃	+	80
3	45	3.2	+	ND	18	100	〃	+	640
4	45	2.7	+	40	19	100	〃	+	640
5	45	2.7	+	320	20	100	〃	+	540
6	45	1.7	+	220	21	100	〃	+	ND
7	45	2.2	+	56	22	100	〃	+	10
8	45	2.7	+	440	23	100	〃	−	10
9	45	3.2	+	640	24	100	〃	−	56
10	45	−	+	460	25	100	〃	−	ND
11	45	−	+	20	26	100	−	−	1 050
12	45	−	+	450	27	100	−	−	640
13	100	−	+	840	28	100	−	−	1 100
14	100	−	+	1 200	29	120	−	−	80
15	100	−	+	1 280	30	140	−	+	620

[a] JV isolation was attempted from 10% brain homogeneates by direct method (D.m.) or from fluids obtained by cocultivation of samples with Vero cells (Co−Ct). Titrations were performed by CPE on Vero cell monolayers, titres are expressed as log of $TCID_{50}$. Neutralizing antibodies (Nt Ab) were titrated by the constant virus (JV-$XJCl_3$, 100 PFU) varying serum dilution method on Vero cell monolayers by CPE. ND: not done.

Table 2. Correlation between JV isolation and histologic changes in surviving mice at 100 days p.i.

Mouse no.	JV isolation (brain)	Histologic changes[a] encephalitis	meningitis
16	+	+	+
17	+	+	+
18[b]	+	+	+
19[b]	+	+	+
20	+	+	+
21	+	+	−
22	+	+	−
23	−	−	±
24	−	−	−
25	−	−	−

Mouse numbers correspond to the same individual animals given in Table 1;
[a]: moderate (+), very mild (±) or absence of histologic changes (−)
[b]: JV antigen detected by PAP technique

encephalitis but the remainder (22.9 %), despite mild neurologic signs during the acute phase, survived showing neither sequelae nor late disease.

Between 45 and 140 days p.i., 30 animals were killed by bleeding under light anesthesia to harvest blood, brain, and in 15 cases also spleen and kidney. Virus isolation was attempted in kidney and spleen as well as in half of each brain and whole blood sample, which were processed by cocultivation with Vero cells. In 20 cases the other half of each brain sample was used for direct viral assay (Table 1). Throughout, virus presence was demonstrated on Vero cell monolayers by CPE. Isolates were identified as JV by 50 % plaque reduction neutralization test with hyperimmune guinea pig anti-JV serum (titre 1 : 1 250).

JV was found in the brain at least 140 days p.i. Direct assay was successful in 9 out of the 12 samples taken at 45 days: titres ranged from 1.7 to 4.5 log/g tissue, while all 8 samples harvested at 100—140 days proved negative. On the other hand, coculture proved positive in 23 out of all brain samples (Table 1). In samples negative by direct assay, titre was estimated as $\leqq 1.2$ log. There was a drop in infectious virus levels in brain at 100 days. The lack of virus rescue from all spleen, kidney and blood samples except for one spleen taken at 45 days, suggest that hardly any infectious virus was released from the CNS or more probably that effective peripheral clearance had taken place.

Humoral response to JV appeared unaffected as Nt Ab were usually present throughout, regardless of brain virus isolation. At 100 days p.i., histologic brain alterations were studied in 10 randomly chosen mice (Table 2) by processing the left hemisphere which was fixed, dehydrated and paraffin-embedded for immunolabelling and haematoxylin-eosin staining. In 8 brains slight meningoencephalitic signs appeared. Meningitis was characterized by lymphocyte and macrophage exudate. In nervous parenchyma there

were scattered cuffs arranged in 2 or 3 round cell layers, mostly consisting of lymphocytes plus a few perivascular macrophages. Neither focal necrosis nor polymorphonuclear exudate was observed. JV was regularly found in brains with meningoencephalitis or encephalitis alone, but proved undetectable when no alteration or meningitis appeared.

Interestingly, 2 JV positive brains (mice 18—19, Table 2) tested by PAP technique (Lascano and Bería, 1983) exhibited JV in brain cortex, basal nuclei and pons neurons. Although less neurons appeared affected than during the acute phase infection, antigen deposits were denser and Purkinje cells were more affected as previously observed in chronically JV-infected rats (Weissenbacher *et al.*, 1986).

Though isolates were regularly identified as JV, differences with the parental strain were detected. JV present in 1—3 mice brains at 45 days (Table 1) exhibited turbid instead of standard JV lytic plaques (Weber *et al.*, 1985) as well as homologous interference demonstrated by plaque reduction test was most probably due to interfering particles as already reported for LCM (Lehman-Grube *et al.*, 1983); ts mutants did not appear in culture at temperature of 39 °C and 37 °C (Ceriatti *et al.*, 1983; Weber *et al.*, 1985).

Virulence was somewhat lower in JV rescued at 100 days as shown by 3 biological markers: the 2.9 lethal efficiency ratio vs standard 1.5 in 14-day-old-mice (Contigiani and Sabattini, 1977), the 0 vs 90—100 % standard mortality or the 25 % vs 10 % standard mortality in 2-day-old Wistar rats infected by intracerebral or intraperitoneal routes, respectively (Avila *et al.*, 1981*a*), and the drop of guinea pig standard 100 % mortality to 75 %, with few if any haemorrhagic signs (Avila *et al.*, 1981*b*).

Although the fact that some mice survived the acute phase of XJ-JV infection cannot be explained at present, our results show that these animals acquired long lasting viral persistence in the CNS accompanied by mild encephalitis and generally by Nt Ab presence.

The failure of virus rescue from 37.5 % of brain samples taken at 100 days p.i. suggests that virus level dropped from 45 days p.i. on. Moreover, the fewer cortical neurons involved, the different pattern of JV antigen distribution, and the lower degree of histologic alteration observed at 100 days p.i., as compared with the JV meningoencephalitis described for the acute phase of disease (Nota *et al.*, 1977) point to age-dependent host restriction of virus replication.

The close correlation observed between virus isolation and encephalitic signs further supports this contention. The postulated restrictive mechanisms may explain the suspected progressive amelioration of encephalomyelitis, as well as the modification of virus characteristics observed.

Since no correlation could be found between the presence of Nt Ab and the brain virus isolation, the role of antibodies in JV replication requires further elucidation. Moreover, preliminary data on cyclophosphamide treatment appeared to lower antibody levels transiently, but brain virus titre remained unchanged (unpublished data). However, no antibodies were detected in persistently JV-infected athymic or thymectomized mice although brain

virus titre was higher than in our immunocompetent persistently infected animals, ascribing to immune response a regulatory role in viral replication.

Given the mass of data available on the acute phase of the disease in the mouse, together with the findings reported here, this animal may prove useful for research on the establishment and modulation of chronic JV infection. To our knowledge this is the first report on Junín virus causing a persistent non-lethal infection and histopathologic brain changes in mice inoculated soon after birth.

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References

Avila, M. M., Galassi, N. V., and Weissenbacher, M. C. (1981*a*): Argentine hemorrhagic fever: a biologic marker. *Intervirology* **15** 97—102.

Avila, M. M., Laguens, M., Laguens, R., and Weissenbacher, M. C. (1981*b*): Selectividad tisular e indicadores de virulencia de tres cepas del virus Junín. *Medicina (Bs. Aires)* **41**, 157—166.

Boxaca, M. C., and Giovanniello, O. A. (1975): Transmisíon vertical del virus Junín en una colonia cerrada de ratones. *Medicina (Bs. Aires)* **35**, 460—468.

Ceriatti, F. S., Damonte, E. B., and Coto, C. E. (1983): Induccíon de mutantes termosensibles del virus Junín por efecto del 5 fluorouracilo. *Rev. Arg. Microbiol.* **15**, 105—112.

Contigiani, M. S., and Sabattini, M. S. (1977): Virulencia diferencial de cepas del virus Junín por marcadores biológicos en ratones y cobayos. *Medicina (Bs. Aires)* **37**, Suppl. 3, 244—251.

Lascano, E. F., and Berría, M. I. (1983): Immunoperoxidase study of astrocytic reaction in Junín virus encephalomyelitis of mice. *Acta neuropath.* **59**, 183—190.

Lehman-Grube, F., Martinez Peralta, L., Bruns, M., and Lohler, J. (1983): Persistent infection of mice with Lymphocytic Choriomeningitis virus, pp. 43—103. In H. Fraenkel-Conrat and P. R. Wagner (Eds): *Comprehensive Virology* (Vol. 18), Plenum Press, New York, N. Y.

Malumbres, E., Boxaca, M. C., Guerrero, L. B. de, Berría, M. I., and Lascano, E. F. (1984): Persistence of attenuated Junín virus strain in guinea pigs infected by intramuscular or intracerebral routes. *J. infect. Dis.* **149**, 1022.

Nota, N. R., Nejamkis, M. R., and Giovanniello, O. A. (1977): Patogenia de la encefalitis del ratón infectado por virus Junín. *Medicina (Bs. Aires)* **37**, Supl. 3, 114—120.

Weber, E. L., Guerrero, L. B. de, and Boxaca, M. C. (1985): MRC5 cells, a model for Junín virus persistent infection. *J. gen. Virol.* **66**, 179—183.

Weissenbacher, M. C., Calello, M. A., Quintans, C. J., Panisse, H., Woyskowsky, N. M., and Zannoli, V. H. (1983): Junín virus infection in genetically athymic mice. *Intervirology* **19**, 1—5.

Weissenbacher, M. C., Lascano, E. F., Avila, M. M., and Berría, M. I. (1986): Chronic neurologic disease in Junín virus infected rats. *J. med. Virol.* (in press).