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MODIFICATION OF JUNIN VIRUS NEUTROPISM IN THE GUINEA PIG MODEL

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Summary. — Virulent and attenuated Junin virus (JV) strains have been employed to study the influence of virus passage history on the neurotropism for guinea pigs. Five i.p. successive passages (P1-P5) of the pathogenic JV-XJ strain and of the attenuated XJO variant were performed in guinea pig spleen. Viral titrations of organ suspensioms were made through P1-P5 passages. The XJ strain produced a widespread infection in P1 guinea pigs with viral dissemination to all organs except brain, in P5 animals the brain has been involved as well. XJO-infected P1 guinea pigs showed lower viral titres than XJ-infected P1 animals, and again, the virus reached the CNS in P5 only. The passaging by i.p. route was shown to enhance CNS invasivity of the XJ strain as well as to maintain the XJO neurotropism for guinea pigs. Neurotropism of both strains seemed somewhat affected by the passage history of the virus and the inoculation route appeared critical for its expression. In addition, the neurotropic potential of the attenuated strains has apparently remained unaltered.

Key words: Junin virus; neurotropism; CNS involvement

Introduction

It was shown (Guerrero *et al.*, 1969) that the XJC13 strain of Junin virus (JV) besides proving attenuated for guinea pigs and human beings, was able to induce an immunological response which protected the animals against wild type virus infection. These experiments opened the way for the development of an attenuated vaccine against Argengine haemorrhagic fever (AHF). In spite of the extensive body of information accumulated up to date, however, some questions remain unanswered. In order to clear some of these problems, we consider that further research is essential on the degree of JV neurotropism evidenced by the encephalitic forms of the human disease (Rugiero *et al.*, 1969; Biquard *et al.*, 1977) as well as on the pathomorphology of lesions

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observed in experimental infections of guinea pigs (Avila *et al.*, 1981; Boxaca *et al.*, 1982) and monkeys (Carballal *et al.*, 1980; Weissenbacher *et al.*, 1979).

It is well known that the properties of a viral population obtained under given experimental conditions depend not only on the genetic information of the parental virus but also on the host's features favouring to certain virus mutants. This has been repeatedly observed for LCM virus, a member of the *Arenaviridae* family like JV. Soon after its identification, Traub (Traub, 1937; Traub, 1938) showed that LCM pathogenicity for mice and quinea pigs depends on the infecting virus strain passage history. Other investigators confirmed these observations (Schwartzman, 1946; Hotchin *et al.*, 1962; Tosolini and Mims, 1971), although in some cases, findings were not entirely in agreement (Popescu and Lehmann-Grube, 1976). Lately, other authors (Suzuki and Hotchin, 1971) dealt with this problem anew showing that by successive passages through mouse brain an "aggressive" viral variant was obtained, which killed newborn mice and produced early death in adult mice. On the other hand, liver-passed "docile" virus induces persistent infection when inoculated into newborne mice and a late death or persistent infection in adult mice. Both variants are known to coexist in the blood of persistently infected animals.

All these facts led us to study the possibility of obtaining a viscerotropic variant of JV by successive passages through extraneural tissues in the experimental model. In this paper we present the results of passaging the JV-XJ strain and its XJO variant (Guerrero and Boxaca, 1980) through guinea pig spleen.

Materials and Methods

Virus. The virulent XJ strain and the attenuated XJO variant (Guerrero and Boxaca, 1980) of JV were used. XJ stock was a 10% suspension of guinea pig spleen plus lung harvested 12 days after intramuscular (i.m.) infection and XJO stock was a 10% mouse brain suspension harvested 7 days after intracerebral (i.c.) infection. Titers were $10^{5.2}$ and $10^{8.2}$ LD_{50}/ml, respectively.

Animals. Randomly bred guinea pigs, 300—350 g body weight, inoculated by intraperitoneal (i.p.) route and two-day-old Rockland mice infected by i.c. route.

Cell cultures. Stationary cultures of Vero cells, (ATCC-CCL81) were employed. Growth medium consisted of MEM (Gibco 410-1100) plus Hank's solution supplemented with 8% inactivated calf serum (ICS) and 0.5% lactalbumin hydrolysate. For maintenance MEM with 3% ICS was used.

Experimental design. Five succcessive passages, P1—P5, of infected guinea pig spleens were performed according to the following procedure. Four guinea pigs were inoculated with 10^3 PFU of JV-XJ. At 14 days p.i. or earlier, if a significant decrease in body weight took place 2 animals were killed and a 10% suspension of pooled spleens in Hank's solution was prepared, After centrifugation 1 hr at 10,000 rev/min the supernatant was titrated in Vero cells and in mouse brain; 10^3 PFU was used as inoculum for the next passage. JV-XJO was treated likewise, but as virus recovered from spleens was insufficient, only 2 guinea pigs were inoculated from P2 onwards. Sera and 10% suspensions of separately pooled homologous spleens, lymph nodes, livers, lungs, hearts, adrenals, kidneys, bone marrows and brains from P1 and P5 guinea pigs were prepared as above and titrated in newborn mice. Blind passages were performed when necessary. Titres were calculated by Reed and Muench's method and expressed as LD_{50} per ml or g of tissue. Remaining guinea pigs were kept under observation and necropsies were performed on all animals found dead. Animals surviving after 30 days p.i. were challenged with JV-XJ.

Results

In all cases, viral titers in the spleen of guinea pigs infected with the XJ strain ranged from 4.7 to 6.8 LD_{50} (Fig. 1). In P1 animals virus was widespread in all organs except brain, while in P5, it also appeared unexpectedly in brain, reaching 3.3 LD_{50}. Signs of CNS involvement were lacking and no significant differences in viral titres were found in given organs (Fig. 2). All the animals inoculated with XJ strain and kept under observation died between 12 and 16 days p.i. showing the typical haemorrhagic alterations described for experimental AHF. As already observed (Boxaca *et al.*, 1982) among the animals killed, 50% failed to exhibit these lesions. JV-XJO titres in spleen decreased from 3.6 LD_{50} in P1 guinea pigs to barely detectable levels starting in P3 (Fig. 1). In P1 animals the virus was present in lymph nodes, spleen, lungs, kidneys and adrenals although titers proved lower than those found in XJ-P1 guinea pigs. In P5 animals virus appeared in brain, as observed with XJ strain, and 1 log increase in virus titre was found in lymph nodes and adrenals, while lung titers decreased from 3.6 to almost undetectable levels (Fig. 2). Animals surviving P1-P3 passages with XJO resisted to JV-XJ challenge. As shown in Fig. 1, XJ spleen viral titers proved closely parallel either in mouse or in tissue culture through all 5 passages, while XJO virus was detected from P2 only by mouse titrations.

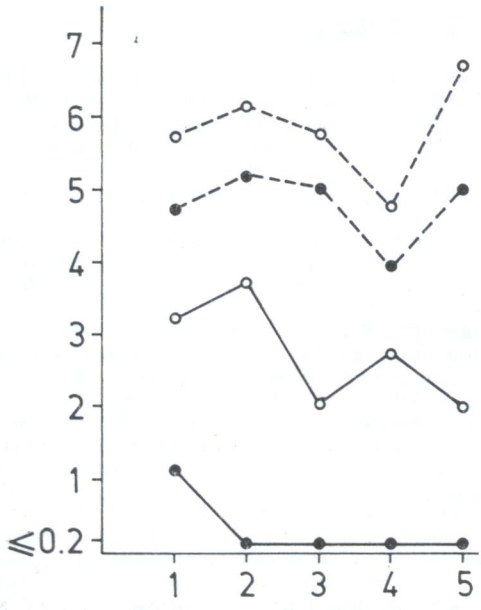

Fig. 1.
Viral titres in guinea pig spleens obtained through 5 passages by i.p. route of virulent JV-XJ strain and its XJO attenuated variant
Titrations were simultaneously performed in suckling mouse brain (XJ ○----○; XJO ○———○) and Vero cells (XJ ●----●; XJO ●———●). Values are expressed as LD_{50} or $TCID_{50}$/g, respectively.
Abscissa: passage number; ordinate: virus titre in log LD_{50} or in log $TCID_{50}$.

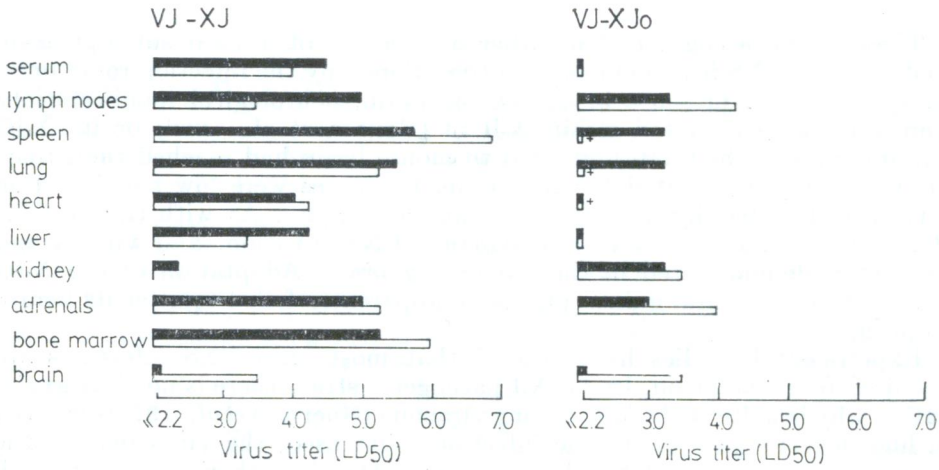

Fig. 2.

Viral spread in P_1 (black columns ■) and P_5 (empty columns □) guinea pigs infected with JV-XJ strain or JV-XJO variant

Titers were obtained by i.c. inoculation to suckling mice and expressed in LD_{50}/g of organ or ml of serum.

(+) means viral isolation after a blind passage.

Discussion

It is well known that for many viruses the host can exert a selective pressure in favour of a certain virus mutant, producing a viral population differing in some respects from the parental virus. Apparently, this is also valid for JV (Candurra and Coto, personal communication), but the expression of some properties, as the potential strain pathogenicity and tissue tropism in a given host, are affected not only by the history of virus passages but also by the infection route (Boxaca *et al.*, 1980). When inoculated by i.m. route, the virulent XJ strain regardless of its passage history has not yet been detected in guinea pig brain (Guerrero *et al.*, 1977; Avila *et al.*, 1981), while attenuated strains were often found to reach the CNS (Avila *et al.*, 1981). However, the i.p. route seemed to enhance CNS invasion (Boxaca *et al.*, 1982) for both guinea pig-adaped strains, as confirmed by the results presented here. In our experiments, no decrease in neurotropic potential of pathogenic or attenuated JV strains was achieved by the selection of viral particles adapted to guinea pig spleen. After 5 successive i.p. passages, both strains increased, or at least maintained, their original neurotropism.

Mouse brain or guinea pig spleen XJ stocks inoculated by i.m. route to the guinea pig proved lethal with extensive viral dissemination throughout all organs but brain (Guerrero *et al.*, 1977). However, when the guinea pig-adapted virus was administered by i. p. route, more than one i.p. passage was necessary for expression of the neurovirulence potency. Then JV was easily detected by the usual isolation technique.

These findings suggest that either a selection of a viral subpopulation took place or CNS invasion has been conditioned by the infection route only. In spite of viral presence in guinea pig brain, neurological manifestations were absent. Attenuated strain XJC13 (Boxaca *et al.*, 1982) or its XJO variant (unpublished data) adapted to mouse brain had reached the guinea pig CNS when inoculated by i.m. route, but titres were low and infection was only detected by sensitive isolation techniques. As with the virulent strain, the i.p. route seemed to enhance CNS invasion, and virus could be readily demonstrated in the guinea pig brain. Adaptation of the XJO variant to extraneural guinea pig tissue apparently failed to alter its neurotropism.

Experimental studies have proved that most viral CNS infections are acquired from the blood. In JV-XJ pathogenic strain there is no clear explanation why in spite of the long lasting viraemia (Guerrero *et al.*, 1977) observed in high levels regardless to the infection route used, the virus reached the brain only when inoculated by i.p. route. On the other hand, although viraemia proves barely detectable, attenuated strains reach the brain more easily, supporting the idea of higher potential neurotropism of these strains. It seems likely that i.p. inoculation, while favouring viral phagocytosis by macrophages, may promote a rapid dissemination of the virus into the brain.

Summing up, these observations suggest that attenuated strains apparently have a higher neurotropic potential than the pathogenic prototype strain and show that neurotropism is somewhat affected by the passage history of the virus. In addition, the inoculation route appeared to be critical for the expression of this viral marker.

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References

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

Biquard, C., Figini, H. A., Monteverde, D. A., Somoza, M. J., and Alvarez, F. (1977): Manifestaciones eurológicas de la fiebre hemorrágica argentina. *Medicina* (Bs. Aires) **32**, 193—199.

Boxaca, M. C., Guerrero, L. B. de, Frigerio, M. J., Rondinone, S. N., and Rabinovich, R. D. (1980): Algunos aspectos dela infeción experimental del cobayo con una variante atenuada del virus Junín. *Medicina* (Bs. Aires) **40**, 521—530.

Boxaca, M. C., Guerrero, L. B. de, Elsner, B., Avagnina, A., Gomez, M. de las M., and Lopez, S. (1982): Efecto de la inoculación intracerebral del virus Junín en el cobayo. *Medicina* (Bs. Aires) **42**, 284—294.

Carballal G., Cossio, P. M., Arana, M. R., Nagle, C., and Casanova, M. B. (1980): El Cebus sp como modelo experimental para la fiebre hemorrágica argentina. *Medicina* (Bs. Aires) **40**, 734.

Guerrero, L. B. de, and Boxaca, M. C. (1980): Estudio preliminar de una variante atenuada del virus Junin derivada de la cepa prototipo XJ. *Medicina* (Bs. Aires) **40**, 267—274.

Guerrero, L. B. de, Weissenbacher, M. C. and Parodi, A. S. (1969): Immunización contra la FHA con una cepa atenuada del virus Junín. Estudio de una cepa modificada del virus Junín. Inmunización de cobayos. *Medicina* (Bs. Aires) **29**, 1—5.

Guerrero, L. B. de, Boxaca, M. C., Weissenbacher, M., and Frigerio, M. J. (1977): Infección experimental del cobayo con virus Junín. Cuadro clínico, diseminación y eliminación de virus. *Medicina* (Bs. Aires) **37**, 271—278.

Popescu, M., and Lehmann-Grube, F. (1976): Diversity of lymphocytic choriomeningitis virus: variations due to replication of the virus in the mouse. *J. gen. Virol.* **30**, 113—122.

Rugiero, H. R., Parodi, A. S., Ruggiero, H., Cintora, F. A., Magnoni, C., and Milani, H. (1969): Sinthesis médica sobre fiebre hemorrágica argentina, Ministerio de Bienestar Social. Buenos Aires.

Schwartzman, G. (1946): Alterations in pathogenesis of experimental lymphocytic choriomeningitis caused by prepassage of the virus through heterologous host. *J. Immunol.* **54**, 293—304.

Suzuki, S., and Hotchin, J. (1971): Initiation of persistent lymphocytic choriomeningitis infection in adult mice. *J. infect. Dis.* **123**, 603—610.

Tosolini, F. A., and Mims, C. A. (1971): Effect of murine strain and viral strain on the pathogenesis of LCM infection and a study of footpad responses. J. *infect. Dis.* **123**, 134—144.

Traub. E. (1937): Immunization of guinea pigs with a modified strain of lymphocytic choriomeningitis virus. *J. exp. Med.* **66**, 317—324.

Traub, E. (1938): Factors influencing the persistence of choriomeningitis virus in the blood of mice after clinical recovery. *J. exp. Med.* **68**, 229—250.

Weissenbacher, M. C., Calello, M. A., Colollas, O. J., Rondinone, S., and Frigerio, M. J. (1979): Argentine hemorrhagic fever: a primate model. *Intervirology* **11**, 363—365.