Acta virol. **35** : 144 - 151, 1991

RAPID VASCULAR CLEARANCE OF TWO STRAINS OF JUNIN VIRUS IN *CALOMYS MUSCULINUS:* SELECTIVE MACROPHAGE CLEARANCE

M. S. CONTIGIANI, S. I. MEDEOT* G. E. DIAZ, M. S. SABATTINI

Instituto de Virologia „Dr. J. M. Vanella", Facultad de Cs. Medicas, Universidad Nacional de Cordoba, Ciudad Universitaria, Estafeta 32, -5016- Cordoba, Argentina

Received June 5, 1990

Summary. - Clearance of Junin (JUN) virus strains with different virulence for *Calomys musculinus* (Cm) was followed using the Candid #1 virulent and CbaFHA 5069 attenuated strains. In addition, virulent virus albino mice (AM) were included as control host and Venezuelan equine encephalitis (VEE-VI) virus as control virus. The virus inoculum (Vo) and the blood samples (Vt) obtained at different times post-inoculation (p.i.) were titrated on Vero cells and the cleared plaque forming-units (PFU) were calculated as the log Vt/Vo. In Cm both JUN virus strains were cleared rapidly (within 5 min the Candid #1 strain and within 10 min the CbaFHA 5069 strain); meanwhile, VEE-VI virus could be recovered from blood until 30 min p. i. Furthermore, JUN and VEE-VI viruses showed the same behaviour in Am as in Cm. We conclude that the JUN virus strains of different virulence for Cm did not show differences in their clearance from the blood of these animals. Moreover, the rapid clearance observed was independent of the animal host and viral dose.

Key words: Junin virus; blood clearance; Calomys musculinus

Introduction

Junin virus (JUN), the aetiologic agent of the Argentine Haemorrhagic Fever (AHF), is a member of the *Arenaviridae* family (Fenner, 1976). Studies performed during the 1960's demonstrated that field mouse *Calomys musculinus* (Cm) was the main JUN virus reservoir (Sabattini *et al.*, 1977). Different progression of infection with wild and high level passaged strains has been demonstrated; one of the latter was proposed as live human vaccine. Wild

---

*Research fellow of CONICOR (Cordoba province Research Council, Argentina)

strains of JUN virus develop persistent infection in adult and newborn Cm, characterized by long lasting viraemia, virus presence in brain, saliva, lymphatic organs in the absence of antibody response (Sabattini *et al.*, 1977; Martinez Peralta *et al.*, 1979; Sabattini and Contigiani, 1982; Medeot *et al.*, 1987; Medeot *et al.*, 1988). On the other hand, with high level laboratory passaged strains viable virus could not be recovered from the blood. Only in newborn Cm the virus was detected in brain showing clearance between 30 to 60 days p. i. (Medeot *et al.*, 1987; Medeot *et al.*, 1988).

Venezuelan equine encephalitis virus (VEE) and other alphaviruses show inefficient clearance rates by the reticuloendothelial system (RES) resulting in high level viraemia; meanwhile benign strains were shown to be cleared rapidly by the RES (Jahrling *et al.*, 1973). Therefore, the clearance of virus from blood decreased viraemia below the hypothetical threshold levels necessary for the virus to invade the critical target tissues (Jahrling *et al.*, 1977). Thus, differences in viraemic level caused by the different strains could results in a different progression of infection.

Here we extend the abovementioned concept to JUN virus infection of Cm in order to explain the different behaviour of wild and passaged virus strains (Medeot *et al.*, 1987; Medeot *et al.*, 1988). Blood clearance curves of CbaFHA 5069 wild strain and of the Candid #1 strain, a JUN virus human vaccine candidate, and clearance of viraemia after blockade of macrophages with silica were determined in adult Cm.

Table 1. The virus strains used in this study

Virus	Strain	Passage history	Virulence for Cm*
Junin	Candid #1 (Human vaccine)	XJGp-2Am-44FRhL-19	Virulent
		XJGp-2Am-44FRhL-19+Am-1	Virulent
		XJGp-2Am-44FRhL-19+Am-2	UK
	CbaFHA 5069	Am-5	Attenuated
VEE-VI	Ag80-663	Am-2	UK

Gp: Guinea pig Am: Swiss albino mice FRhL: foetal rhesus lung cells certified
* Lethality via i. c. inoculation; UK: unknown

Materials and Methods

Virus strains. JUN and VEE virus strains, their passage histories and virulence for Cm and Am are listed in Table 1. Candid #1 strain (Barrera Oro, J., personal communication) was kindly provided by Dr. J. I. Maiztegui, National Institute of Viral Haemorrhagic Disease (INEVH), Pergamino, Argentina, as the culture supernatant harvested at 96 hr p. i. on certified foetal rhesus lung cells. Other virus stocks were suckling mouse brain suspensions harvested at 10 to 12 days p. i., centrifuged at 10, 000 rev/min. All virus stocks were stored at -80 ^{0}C until tested.

Animals. Both species used in this study were outbred. The Cm colony was iniciated in our laboratory with field animals from a nonendemic zone in Cordoba Province and proved to be free of lymphocyte choriomeningitis and JUN viruses (Sabattini *et al.*, 1977) and of Theiler's virus (unpublished data). Albino Swiss mice (Am) were specific pathogen free animals from INEVH. In accord with previous work (Medeot *et al.*, 1987; Medeot *et al.*, 1988) and due to the difficulties with bleeding of animals, adult Cm and Am were used.

Silicon dioxide, average particle size between 1 - 5 μm was obtained from Sigma (Catalogue No. S 5631). It was sterilized with hot air and suspended in isotonic saline 100 mg/ml. Immediately before use the silica particles were dispersed to brief exposure of ultrasonic vibration.

Virus assay. The cloned 76 Vero cell cultures were employed as test host for virus assay. They

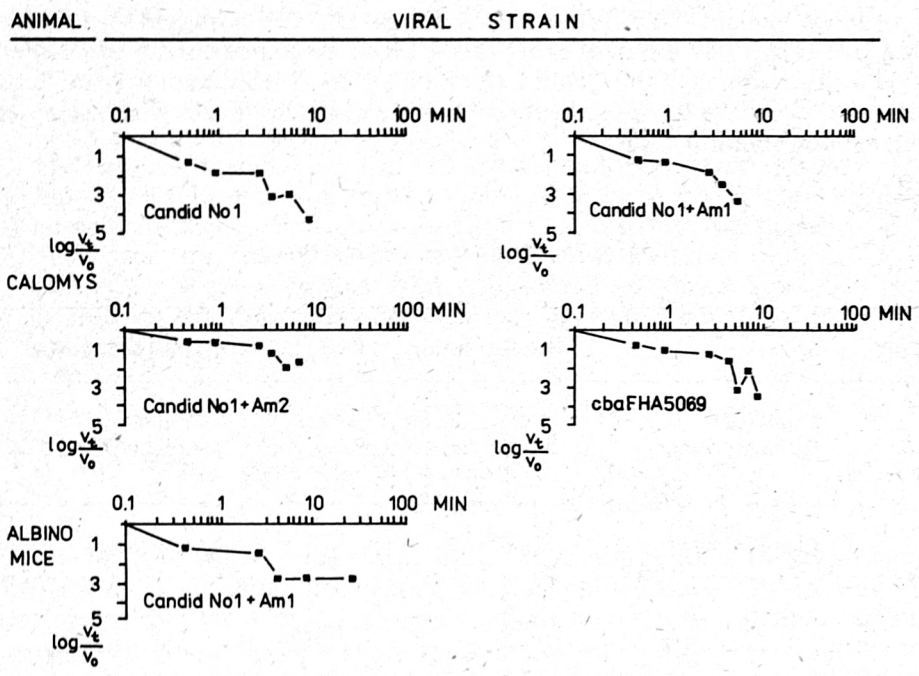

Fig. 1

Clearance rates of different strains of Junin virus from *Calomys* and Albino mice in the blood after intracardiac inoculation

Each point represents a geometric mean of results from 5 to 8 animals.
Abscissa: minutes after cardiac inoculation; ordinate: log V_t/V_o.

were grown in Minimal Essential Medium (MEM) with glutamine and supplemented with 10% of foetal calf serum (FCS) and 5 mg% gentamicine. When grown to confluency (within 24 to 48 hr) the monolayers were inoculated with tenfold dilutions of specimens and incubated under agarose overlay as previously described (Contigiani *et al.*, 1977).

Viral clearance rates. Animals were inoculated under either anesthesia by cardiac route with 3.2 to 3.9 log PFU of JUN virus or 3.1 to 5.0 log PFU of VEE virus in 1 ml bovine serum albumine (BSA) containig 1 mg of Evan's Blue (Jahrling *et al.*, 1973). At 30 seconds, 1, 3, 4, 6, 8, 10, and occasionally 30 min p. i. 0.1 ml of blood was taken from the orbital sinus mixed with heparin and diluted (1:10) with chilled sterile Hank's solution containing 10% FCS. After centrifugation at 10, 000 rev/min for 30 min the supernatant fluids were descarted, equal volumes of Hank's sol. with 10% FCS were added and the samples were stored at - 80^0C. The Evan's blue concentration was measured spectrophotometrically at 610 nm. Determination of the standard curve showed a linear relationship between 0 and 20 μg of Evan's blue per ml.

The specific clearance of virus from blood was calculated by comparing the observed concentration of virus in blood at each tipe p. i. (Vt) with the concentration expected if no clearance had occurred (Vo), by using the Evan's blue dilution method. The log of the fraction Vt/Vo is mathematically equivalent to the log PFU of virus cleared (Jahrling *et al.*, 1973). Differences in Vt/Vo between the different strains at 4 and 10 min were evaluated by the use of the Student's t test ($P < 0.05$).

Macrophage blockade with silica. Nine adult Cm were inoculated with silica, and JUN virus according to the procedure summarized in Table 2. Thereafter, the specific clearance of virus in blood of these animals was determined as described. Neither mortality nor morbidity was observed in the control group III.

Results

As is shown in Fig. 1 the two strains of JUN virus tested in Cm were cleared rapidly. To determine whether these results depend on the host or on the virus we followed the JUN virus clearance in Am. The Candid #1 strain was cleared more rapidly in Am as in Cm. As is shown in Table 3, nearly 100 % of the input virus was cleared within 4 min in Cm inoculated with Candid #1 and Candid

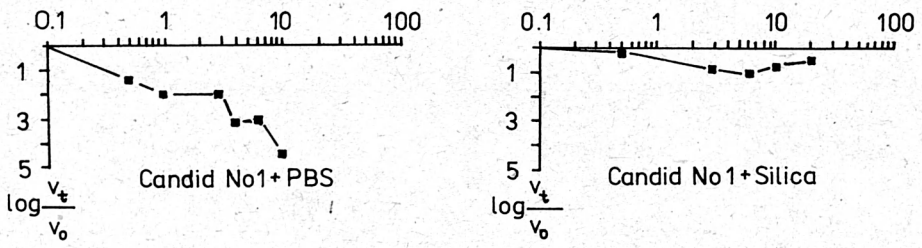

Fig. 2

Clearance rates of Candid #1 strain of Junin virus from *Calomys* after the intracardiac inoculation pretreated with PBS (I), and pretreated with silica (II)

Each point represents the geometric mean of results from 3 animals.

Abscissa: minutes after cardiac inoculation; ordinate: log V_t/V_o.

#1 + Am-1, whereas the CbaFHA 5069 and Candid #1 + Am-2 strains lasted 10 min to be cleared. The clearance differences observed between Candid #1 and Candid #1 + Am-1 on one hand and CbaFHA 5069 on the other were corroborated by Student's test ($P = 0.01$). No differences were found among Candid #1 + Am-2 and CbaFHA 5069 ($P > 0.05$) (Table 3). Furthermore, Candid #1 strain was cleared at slow rates from Cm blood when they were previously treated with silica. The virus was essentially not cleared within 20 min after inoculation (Fig. 2). Silica impaired the macrophages of the RES within 2 hr before virus inoculation. We also included the VEE-VI strain as another control (Fig. 3). The Ag80 663 strain of VEE-VI virus was cleared relatively slowly, virus was recovered from Cm and Am blood unitl 30 min p. i. No correlation with the amount of virus inoculated could be demonstrated.

Discussion

We showed that both strains of JUN virus that have different virulence for Cm were faster taken up from the blood (Fig. 1). The low differences observed could not explain the different biological behaviour. The high and prolonged

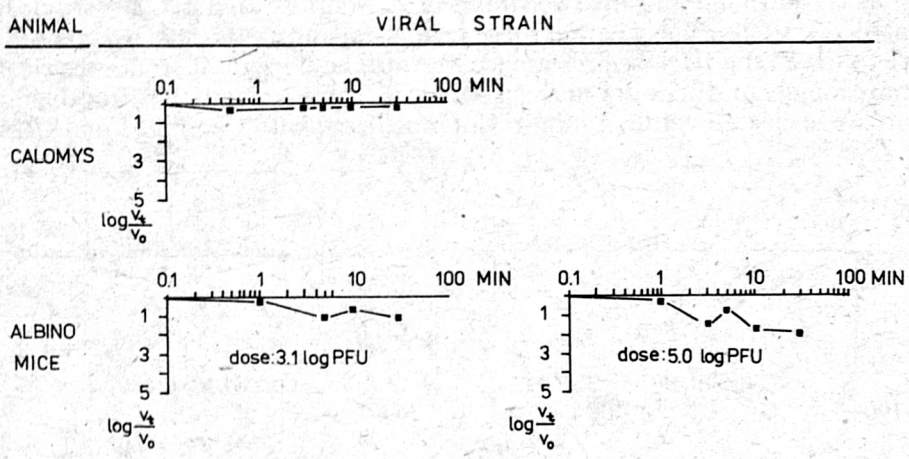

Fig. 3
Clearance rates of VEE-VI virus from *Calomys* and Albino mice bloods after intracardiac inoculation
Each point represents a geomteric mean of results from 5 animals.
Abscissa: minutes after cardiac inoculation; ordinate: log V_t/V_o.

Table 2. Design of the experiment with silica

Group	No. of animal	Treatment*	Time of inoculation
I	3	PBS	-2 hours
II	3	Silica (50 mg/i.p.)	-2 hours
III	3	Silica (50 mg/i.p.)	-2 hours

* All animals except those in group III were inoculated intracardiacally on time 0 with 3.8 log PFU of Candid #1 strain; i.p.: intraperitoneal route

viraemia with the CbaFHA 5069 strain (Medeot *et al.* 1987; Medeot *et al.*, 1988) was not related to low clearance. Moreover, the results obtained with Candid #1 strain in Cm after the macrophage blockade suggest that the rapid clearance observed is due to faster uptake of the virus by RES cells (Fig. 2).

As expected, the rapid clearance of JUN virus was independent of host species and of virus dose; with JUN virus we obtained the same results in Cm and Am (Fig. 1). On the other hand, the Ag80 663 strain of VEE-VI virus showed different clearance in both hosts used (Fig. 3).

The salient result of our work is that we did not observe a correlation between the high and prolonged viraemia of Cba FHA 5069 strain and its low

Table 3. Statistical evaluation of virus clearance between CbaFHA 5069 and Candid #1 strains of Junin virus

Sample Time (minutes)	Virus strain	Virus cleared %	P*
4	CbaFHA 5069	41.5	--
	Candid #1	77.2	0.01
	Candid #1 + Am-1	78.3	0.01
	Candid #1 + Am-2	36.9	>0.05
10	CbaFHA 5069	98.1	--
	Candid #1	100	>0.05
	Candid #1 + Am-1	100	>0.05
	Candid #1 + Am-2	98.3	>0.05

* The Student's test was used to compare the strains

clearance from blood as it was previously postulated for Mengo and VEE viruses (Campbell *et al.*, 1967; Jahrling *et al.*, 1973). However, a disassociation between the clearance rates and pathogenicity of virulent and benign strains of VEE virus for guinea pigs was also demonstrated (Jahrling *et al.*, 1977). Differences in virulence between strains were correlated with a restricted ability to replicate in and destroy the host tissue. Factors that determine the different replication and pathogenesis of the two strains of JUN virus should be studied in detail. More understanding of the persistence of wild strains of JUN virus in Cm and of the different pathogenesis of attenuated and wild strains is also needed. Recently, it has been reported that the XJC13 attenuated strain of JUN virus inoculated by i. p. route to newborn Cm caused lethal encephalitis (Lampuri *et al.*, 1982; Laguens *et al.*, 1982). Columbie *et al.* (1986) suggested a T lymphocyte mediated immune mechanism for this damage of CNS. Preliminary experiments that have been done in our laboratory showed the different ability of wild and derived strains to replicate in the thymus of Cm. CbaFHA 5069 wild strains that effectively replicates in thymus caused a lack of the immune response that might be responsible for virus persistence in Cm (Contigiani *et al.*, in preparation). On the contrary, virus could not be recovered from thymus with derived strains that did not persist in this host. Further studies are necessary to determine the critical differences between wild and attenuated strains in mice.

Acknowledgements. We thank Dr. Laura Gonzalez for statistical calculations, Faculty of Agronomic Sciences, Cordoba National University, and V. R. Vega for technical assistance. This investigation was supported by grants of CONICET, SECYT, and Fundacion Emilio Ocampo, Argentina.

References

Campbell, J. B., and Colter, J. S. (1967): Studies of three variants of Mengo encephalomyelitis virus. IV. Afinities for mouse tissues *in vitro* and *in vivo. Virology* **32**, 69 - 73.

Contigiani, M. S. and Sabattini, M. S. (1977): Virulencia diferencial de cepas de virus Junin por marcadores biologicos en ratones y cobayos. *Medicina (Bs. As)* **37** *(supl. 3), 244 - 251.*

Coulombie, F. C., Laguens, R. M., and Coto, C. E. (1986): Administration of antithymocyte serum modifies the response of *Calomys musculinus* to Junin virus infection. *Intervirology* **25**, 56 - 60.

Fenner, F. (1976): Classification and nomenclature of viruses. Second report of the International Committee on Taxonomy of Viruses. *Intervirology* **7**, 55.

Jahrling, P. B., and Scherer, W. F. (1973): Growth curves and clearance rates of virulent and benign Venezuelan Encephalitis viruses in hamsters. *Infect. Immun.* **8**, 456 - 462.

Jahrling, P. B., Heisey, G. B., and Hesse, R. A. (1977): Evaluation of vascular clearance as a marker for virulence of Alphaviruses: Disassociation of rapid clearance with low virulence of Venezuelan Encephalitis virus strains in Guinea pigs. *Infect. Immun.* **17**, 356 - 360.

Laguens, R. M., Lampuri, J. S., and Coto, C. E. (1982): Patologia de la infeccion persistente del *Calomys musculinus* con una cepa atenuada del virus Junin. *Medicina (Bs. As.)* **42**, 273 - 279.

Lampuri, J. S., Vidal, M. C., and Coto, C. E. (1982): Respuesta del *Calomys musculinus* a la infeccion experimental con virus Junin. *Medicina (Bs. As.)* **42**, 61 - 66.

Martinez Peralta, L., Cosio, P. M., Sabattini, M. S., Maiztegui, J. I., Arana, R. M., and Laguens, R. P. (1979): Ultrastructural, immunohistochemical, and virological studies in organs of *Calomys*

musculinus infected with Junin virus by natural routes. *Medicina (Bs. As.)* **39**, 213 - 217.
Medeot, S., Contigiani, M., Diaz, G., and Sabattini, M. (1987): Persistencia diferencial de diversas cepas de virus Junin en *Calomys musculinus. Comunicaciones Biologicas* **6**, 174.
Medeot, S., Contigiani, M., Sabattini, M. (1988): Capacidad neuroinvasiva del virus Junin en huespedes de laboratorio. V Congreso Argentino de Microbiologia, Mar del Plata.
Sabattini, M. S., Gonzale de Rios, L. E., Diaz, G., and Vega, V. R. (1977): Infeccion natural y experimental de roedores con virus Junin. *Medicina (Bs. As.)* **37**, (supl. 3) 149 - 161.
Sabattini, M. S., and Contigiani, M. S. (1982): Ecological and biological factors influencing the maintenance of Arenavirus in nature, with special reference to the agent of Argentine Haemorrhagic Fever. *International Symposium on Tropical Arbovirus and Haemorrhagic Fevers,* Academia Brasileira de ciencias, Rio de Janeiro, pp. 251 - 262.