Acta virol. **29**: 334—337, 1985

EARLY PROTECTION TO JUNÍN VIRUS OF GUINEA PIG WITH AN ATTENUATED JUNÍN VIRUS STRAIN

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Received October 22, 1984

Summary. — Inoculation of guinea pigs with attenuated XJO Junín virus (JV) strain confers protection against challenge with pathogenic XJ-JV strain starting as early as 3 days post infection (p.i.). The protection increased continuously up to 100% by 30 days p.i. Neither stimulation of non-specific cell mediated mechanisms by previous BCG sensitization nor circulating interferon (IFN) seemed essential for such protection. The early detection of the virus in guinea pig organs considered the site of primary JV multiplication suggests that early resistance phenomenon was attributed mainly to direct viral interference.

Key words: early protection; Argentine haemorrhagic fever; guinea pig; viral interference; Junín virus

Several animals are susceptible to the experimental infection with Tacaribe and Junín viruses, members of the *Arenaviridae* family. Both viruses are lethal for newborn mice eliciting an immunopathologic encephalitis (Weissenbacher *et al.*, 1975). Besides, Junín virus (JV), aetiologic agent of human Argentine haemorrhagic fever (AHF), also kills the guinea pig and the marmoset *Callithrix jacchus* with haemorrhagic signs attributed to a direct virus action, while Tacaribe virus induces a subclinical infection only (Weissenbacher *et al.*, 1975).

Guinea pigs and *C. jacchus* can be protected against challenge with the pathogenic XJ-JV by previous inoculation of attenuated JV strains or of heterotypic Tacaribe virus (Weissenbacher *et al.*, 1975). Complete protection achieved from 30 days post vaccination had been attributed to the development of a humoral immune response evidenced by longlasting specific anti-Junín neutralizing antibodies (NA), detectable 20 or 40 days post infection (p. i.), when attenuated JV or Tacaribe virus were used, respectively. Furthermore, in either case, protection was reported as early as 3 days p. i. though the overall pattern depended on whether heterotypic or homologous strains were employed (Boxaca *et al.*, 1981; Damonte *et al.*, 1978). When XJO-JV attenuated strain was used, protection reached 37.5% at 3 days p. i., 75% at 9 days p. i. and increased steadily up to 100% within 30 days.

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Table 1. Effect of BCG stimulation on the early protection induced by XJO-JV in guinea pigs

Group of guinea pigs	3 days p.i.			9 days p.i.		
	D /T	% P*	DOD Avge	D /T	% P*	DOD Avge
BCG-XJO	6/9	33	17.7	1/8	87.5	17
XJO	3/4	25	19.7	1/4	75	14
BCG	4/4	0	15.2	4/4	0	14.3
C	4/4	0	15.0	4/4	0	14.8

D/T: No. of deaths/No. of total guinea pigs, P: protection (proportion of survivors), DOD Avge: Average day of death post challenge

* The authors are aware of the approximate value of percentage calculations because of low number of animals used. The data are significant as confirmation of previous results (Boxaca *et al.*, 1981).

Cellular immune mechanisms or viral interference phenomena have been advanced (Boxaca *et al.*, 1981; Damonte *et al.*, 1978) to explain the prompt protection, which cannot be ascribed to a humoral immune response. To explore the feasible mechanisms of this type of pretection, three lines of approach were followed: non-specific stimulation of cellular immune mechanisms, early virus presence in organs using a highly sensitive technique and determination of circulating interferon (IFN).

For stimulation of cell-mediated immunity, 25 guinea pigs were sensitized with 0.15 ml of a BCG (Pasteur 1173 strain, Batch 1378) stock suspension containing 3×10^6 viable bacilli. Then 28 days later Mantoux reaction was performed in 4 randomly selected guinea pigs to check sensitization. Seventeen animals (BCG-XJO group), together with 8 non-sensitized guinea pigs (XJO group) were inoculated with 10^3 PFU of XJO-JV by intramuscular (i. m.) route. Eight tuberculin-sensitized guinea pigs (BCG-group), and 8 non-infected and non-sensitized ones were used as controls. Three days p. i., 9 BCG--XJO guinea pigs, 4 XJO, 4 BCG and 4 normal ones were challenged with 10^2 PFU of the XJ-JV pathogenic strain by i. m. route while the remaining animals of all groups were likewise challenged 9 days post XJO. Animals were observed for 40 days and their mass and death were recorded.

As shown in Table 1 no significant differences were found between final survival rates of BCG-XJO and XJO guinea pigs when challenged at 3 or 9 days p. i. However, the day of death was somewhat delayed and haemorrhagic signs were not regularly present at autopsy, in contrast to the BCG and normal guinea pigs which died, as expected, between 12 and 17 days post XJ infection with typical AHF signs. Percentage of protection agreed with values obtained in previous experiments (Boxaca *et al.*, 1981).

Virus distribution was studied in a group of 10 guinea pigs inoculated i. m. with 10^3 PFU of XJO. On days 3, 6, 9 and 15 p.i. two animals were killed by bleeding. Cell suspensions of spleen, lymph nodes and blood were cocultivated with a suspension of 10^5 Vero cells (ATCC-CC18L)/ml, using MEM (GIBCO 410-1100) plus Hanks' solution and 5% lactoalbumin hydrolysate with 8% calf serum for growth and 3% for maintenance. Supernatants collected were tested for infectious virus in Vero cell cultures and in newborn

Table 2. Virus, circulating IFN and NA in early samples of guinea pigs infected with attenuated XJO-JV strain

GP No.	Sampling (days p.i.)	Virus isolation			(Circulating)	
		Spleen	Lymph nodes	Blood	IFN	NA
1	3	—	cont	—	—	<5
2		—	+	—	—	<5
3	6	+	+	+	—	<5
4		+	+	+	—	<5
5	9	+	+	+	—	<5
6		+	+	+	—	<5
7	15	+	+	+	—	5

Virus in organ suspensions or serum samples cocultured with Vero cells was confirmed by i.c. mouse or stationary Vero culture inoculation.
IF was measured as the protective serum effect on primary guinea pig kidney cultures, challenged with VSV. NA were assayed in Vero cell cultures by serum dilution — constant virus (100 $TCID_{50}$ XJO) technique.
cont = contaminated

mice as already described (Boxaca *et al.*, 1984). Virus was demonstrated as early as 3 days p.i. in lymph nodes of at least one of the two animals killed and in all samples taken there after (Table 2), while in agreement with previous data, specific NA were barely detectable in only one animal by 15 days p.i. (Boxaca *et al.*, 1981).

Levels of circulating IFN were studied in the above 3-, 6-, 9- and 15-day serum samples. Aliquots were acidified with 1 mol/l HCl up to pH 2 and kept overnight at 4 °C. After neutralizing with 2% $NaHCO_3$ and diluting 1 : 15, samples were stored at −70 °C. Protective effect was tested on monolayers of primary guinea pig kidney cultures treated with serial 1 : 2 serum dilutions. Treated and normal cell cultures were challenged with 10^2 $TCID_{50}$ of vesicular stomatitis virus (VSV) and observed up to 24 hr. No IFN was detected in three successive tirations for each sample.

As already mentioned, the pathologic changes caused by both attenuated and pathogenic JV strains in experimental hosts such as the newborn nouse, are known to be mediated by the host's cellular immune response (Weissenbacher *et al.*, 1975), whereas no evidence supporting this mechanism has been found in the guinea pig infected with pathogenic strains. Moreover, some findings demonstrate that these strains suppress cell mediated immunity (CMI) (Carballal *et al.*, 1981; Galassi *et al.*, 1982). In contrast, in attenuated XJO-JV infected guinea pigs no alterations in CMI have been detected. As CMI is promptly established after infection with LCM, an another arenavirus (Marker and Volkert, 1973), we suspected this mechanism in the early protection conferred by the attenuated JV strain. However, the lack of correlation between non-specific CMI increase and survival rate failed to support our original view, although the somewhat prolonged survival and the absence of haemorrhagic signs at autopsy suggest that partial protection takes place in these animals as previously described (Boxaca *et al.*, 1981).

Although circulating IFN was demonstrated in low titres between 9 and 96 hr p.i. in attenuated XJC13-JV guinea pigs (Dejean *et al.*, 1983), no IFN was between 3 and 15 days p.i. in our experimental conditions, employing the closely related attenuated XJO-JV strain but different doses and route of infection. Though IFN involvement cannot be entirely ruled out, it seems inessential for this prompt protection, which is in agreement with the poor IFN response to different JV strains observed in various hosts even when attenuated strains have been used (Holstein *et al.*, 1975; Weissenbacher *et al.*, 1975) and with negligible effect on JV multiplication detected when exogenous IFN or IFN inducers were employed "*in vitro*" or "*in vivo*" (Weissenbacher *et al.*, 1975).

In spite of low titres, our early detection of virus in organs considered the site of primary JV multiplication points to direct viral interference causing the prompt resistance described. Although in Tacaribe virus-infected guinea pigs early protection was found accompanied with easily detectable virus in organs, the further development differs from XJO-elicited protection. In both cases late resistance is attributed to specific anti-JV humoral response, but in the Tacaribe virus induced protection abrogation was observed around 13 days p.i. before NA become detectable. When XJO-JV was given early and late protections overlap and a continuously increasing resistance develops, due probably to the closer antigenic relationship between protecting and challenging strains. Briefly, these preliminary results suggest that attenuated XJO-induced early protection should be ascribed mainly to direct viral interference.

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