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ACTION OF ANTITHYMOCYTE SERUM ON JUNIN VIRUS INFECTION IN RATS

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Summary. — Two-day-old rats resistant to intracerebral (i.c.) infection with XJ strain of Junin virus (JV), were rendered sensitive to JV by treatment with antithymocyte serum (ATS). The mortality reached 80%, the virus titres in brain were higher and the serum neutralizing antibodies dropped but brain lesions were absent throughout. The same host was susceptible to XJCl3 strain infection, which induced lethal encephalitis manifested by severe necrotic foci in cerebellum. ATS treatment conferred significant protection against this strain; the mortality was 63%, viral titres in brain remained unchanged but the lesions were mild as compared with non-treated animals. It seems likely that the XJ strain allowed the 2-day-old rat to develop serum antibodies against JV, while the XJCl3 strain unleashed an immunopathologic response.

Key words: Junin virus; rat; antithymocyte serum

Introduction

Intracerebral (i.c.) inoculation of JV in newborn mouse leads to encephalitis, which has been shown to occur due to a cell-mediated immunopathologic reaction (Taratuto *et al.*, 1973; Nota *et al.*, 1976; Giovanniello *et al.*, 1978). Besides, the mouse exhibits an age-dependent resistance to the lethal effect of the virus (Giovanniello *et al.*, 1980).

Wistar rats are known to behave in different ways according to their age and the JV strain employed (Avila *et al.*, 1981). Animals from 8 to 12 days of age inoculated i.c. with the XJ prototype strain showed maximal mortality (95%); in contrast 2-day-old ones infected by i.c. route developed subacute encephalitis with barely 5—10% mortality (Galassi *et al.*, 1981). In the latter case there was a spontaneous remission, although infectious virus persisted up to 6 months post inoculation (p.i.).

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Intracerebral inoculation with the attenuated XJCl3 strain of JV from birth up to 16 days of age, led to 95% mortality (Avila *et al.*, 1981). Typically, 2-day-old rats developed severe encephalitis manifested by widespread necrotic foci (Lascano *et al.*, 1981).

These findings support the use of the 2-day-old rats as a biological marker since the susceptibility of rats to JV infection is strain-dependent.

The present experiments were aimed at determining the immune mechanisms responsible for the development of the disease. The action of ATS was investigated since immunodepression is known to alter the outcome of many virus infections (Rook and Webb, 1970; Nathanson and Cole, 1971).

Materials and Methods

Animals. Pregnant outbred Wistar rats, raised in our faculty bioterium, were observed to establish day of litter birth. Two-day-old rats were infected i.c. with 10^3 LD_{50} of JV.

Virus. Prototype XJ and XJCl3 JV strains pathogenic or attenuated for guinea pigs, respectively, were used. The stocks were harvested from newborn mouse brain, infected i.c. 7 days previously with 10^3 LD_{50} of either strain. Their titres determined by Reed and Muench's method in newborn mice were $10^{7.3}$ LD_{50}/0.1 g for XJ strain and $10^{8.4}$ LD_{50}/0.1 g for the XJCl3 strain.

Virus titration. Ten-fold virus dilutions were prepared in Hanks' medium with 10% heat--inactivated calf serum and inoculated onto Vero cells. The cells were checked daily and virus titres were calculated.

Neutralizing antibody (Nt Ab) titration. The variable serum-constant virus method was used in Vero cells. The first day was registered, when infected cells in the absence of immune serum exhibited cytopathic effect. The serum titre was determined as the dilution capable to neutralize 50% of CPA.

Preparation of ATS. Four rabbits were inoculated subcutaneously in one flank (10^8 cells/rabbit) with thymus cells from 3 to 4-week-old rats, emulsified in Freund's Complete Adjuvant (FCA) (Difco). Three weeks later the animals received daily for 3 days 10^8 thymus cells/rabbit (no FCA) by intravenous route. Rabbits were bled one week later and their sera separately heated to 56 °C for 30 min, absorbed twice with rat red blood cells and stored at —20 °C.

ATS from each of the 4 rabbits was inoculated intraperitoneally into a pair of adult Wistar rats, while an additional control pair received normal rabbit serum (NRS). ATS activity was measured by the drop of leukocyte count performed 0, 4, 24 and 48 hr after injection 0.2 ml of ATS. A 50% or greater leukocyte decrease lasting for up to 48 hr was considered as a proof of ATS efficacy. As expected, the NRS-control rats recovered normal count by 24 hr.

Histology. Brains were fixed in 10% formaldehyde and embedded into paraffin. Paraffin sections were stained with haematoxylin and eosin and with the periodic acid Schiff reagent.

Experimental design is outlined in Table 1. During ATS treatment, rats received pooled sera

Table 1. Experimental design

Group	Number of animals	Treatment	Virus strain
A	103	ATS	XJ
B	85	ATS	XJC13
C	22	NRS	XJ
D	17	NRS	XJC13
E	62	—	XJ
F	61	—	XJC13

daily in the dose of 0.01 ml/g body mass for 9 days, starting one day before infection, then 3 times weekly for 2 weeks, with a flat dose of 0.1 ml in each case, altogether 15 doses. NRS treatment has followed an identical schedule. All rats were observed daily for 50 days; signs of acute infection and mortality were registered. Three rats from the groups A and E were killed on days 3, 6, 9, 16, 20, 23, 27, 33, 38 and 44 p.i.; three rats from groups B and F were killed on days 3, 6, 9, 13, 17, 21, 25 and 35 p.i. to determine brain viral titres and serum Nt Ab. In addition, for histologic studies, 10 rats from groups B and F, as well as 10 rats from groups A and E, were killed on days 9 and 29 p.i., respectively.

Results

Mortality curves of dead animals are given in Figs. 1-I and 1-II. Noteworthy, ATS treatment altered the course of i.c. infection with both virus strains. In the case of XJ-infected ATS-treated group (A) there was a sharp increase in mortality from day 25 p.i. up to day 50 p.i., when the accumulated percentage reached 80%. The XJ-infected, non-treated control group (E), exhibited 5% mortality (Fig. 1-I). On the other hand, the XJC13-infected ATS-treated group (B) exhibited 63% mortality with a striking delay in the average day of death; 50% mortality occurred on day 32 vs. day 12 for the XJC13-infected control group (F), which had no survivors (Fig. 1-II). NRS had no effect at all on infection with either strain. Fig. 2-I illustrates

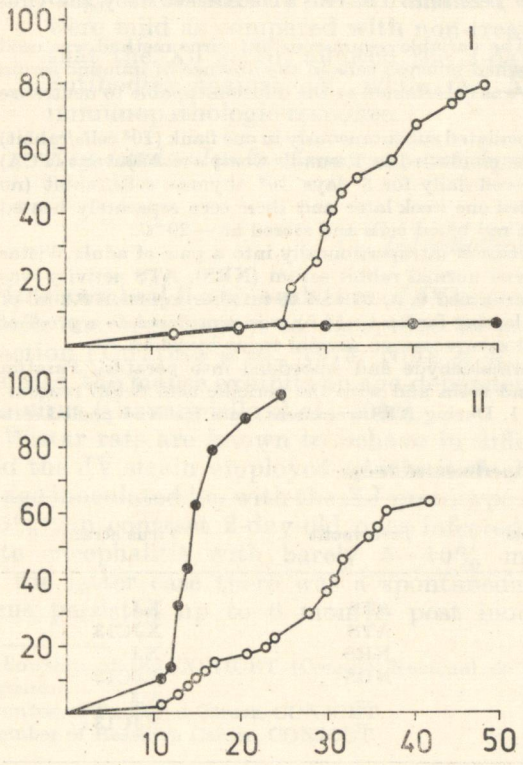

Fig. 1.
Total cumulative mortality in 2-day-old rats inoculated by i.c. route
I — XJ strain (groups A, E),
II — XJC13 strain (groups B, F).
Black circles: untreated rats (groups E, F).
Empty circles: ATS-treated rats (groups A, B).
Abscissa: days p.i.; ordinate: cumulative mortality (%).

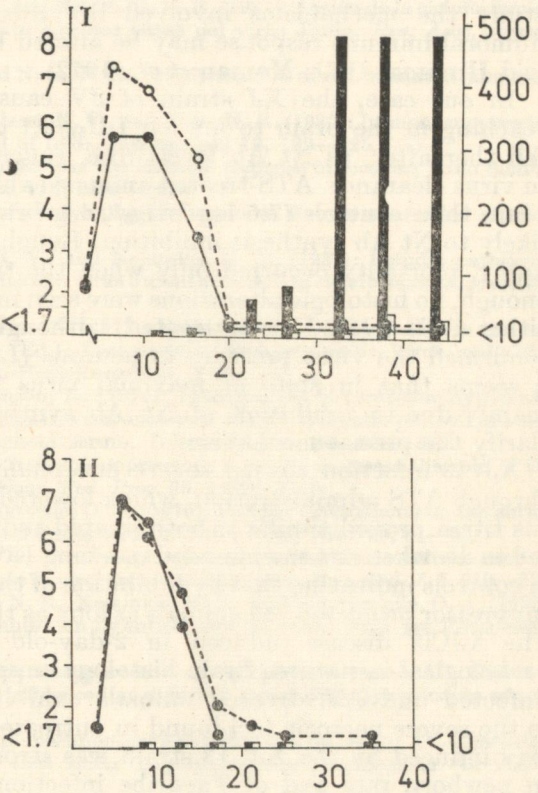

Fig. 2.

Virus in the brain and serum neutralizing antibody titres in 2-day-old rats inoculated by i.c. route
I — XJ strain (groups, A, E).
II — XJC13 strain (groups B, F).
Solid lines (———) untreated rats.
Interrupted lines (— — —): ATS-treated rats.
Empty columns: Nt Ab titres in ATS-treated rats (groups A, B). Black columns: Nt Ab titres in untreated rats (groups E, F).
Abscissa: days p.i. Left ordinates: virus titres in the brain; right ordinate: serum neutralizing antibody titres.

the amount of virus in brain and Nt Ab titres for XJ groups A and E. Brain virus titres in the former group proved higher, approaching a 100-fold ratio. Nt Ab values in the group E were patent as early as day 23 p.i. increasing markedly thereafter, while in the group A the remained below 1/20 throughout. Fig. 2-II illustrates brain virus and Nt Ab titres for XJC13 virus groups B and F. Brain virus titres were roughly parallel in both groups up to day 18, then disappearing for group F, but remaining detectable up to day 25 for group B. Nt Ab were absent throughout.

However, no brain lesions were found in XJ-infected rats, whether untreated or ATS-treated (groups A and E). In contrast, the XJC13 infected rats, exhibited severe necrotic foci when untreated (group F), whereas only mild histopathologic alterations were found in the ATS-treated group (B).

Discussion

It has been shown that immunosuppression may convert subclinical experimental infection into lethal disease (Ida and Hinuma, 1971; Griffin and Johnson, 1977). Immunosuppressors, such as ATS, have been used to

study the mechanisms involved in virus infection since cellular and/or humoral immune response may be altered by means of ATS treatment (Ida and Hinuma, 1971; Monjan *et al.*, 1972).

In our case, the XJ strain of JV caused subclinical infection in rats, reaching in the brain to 5.9 log LD_{50}/0.1 g tissue at day 6 p.i. Viral titres fell thereafter, as Nt Ab levels rose, suggesting the leading role of Nt Ab in virus clearance. ATS-treated animals exhibited higher titres of virus in the brain than controls (7.5 log LD_{50}/0.1 g brain tissue at day 6 p.i.) due most likely to Nt Ab synthesis inhibition. Roughly 80% animals died, but remarkably, mortality occurred only when the tratment had ended. Surprisingly enough, no histologic alterations were seen in the brain of XJ-infected animals either ATS-treated or untreated, although peroxidase-labelled antibodies confirmed the virus presence in neurons (Lascano, unpublished). Therefore, it seems that in spite of maximal virus replication in brain, death was mainly due to inhibition of Nt Ab synthesis. Studies are in progress to clarify the precise mechanism.

XJC13 infection caused severe encephalitis, capable of partial reversion through ATS administration, which lowered the mortality to 63%. Brain virus titres proved similar in both treated and untreated animals. Animals started to die when treatment ended reaching 15% mortality at day 20 p.i. vs. 85% in controls indicating that by inhibition of the immune response, the immunosuppressor protected the animals as long as the treatment had been continued. The XJC13 disease induced in 2-day-old rats appeared to be immunopathological in nature. Brain histology supported this conclusion as XJC13--infected and ATS-treated animals exhibited minimal lesions in contrast to the severe necrotic foci found in untreated infected rats. Thus, the pathology induced by the XJC13 strain was strongly reminiscent of LCM disease in newborn rats and of Tacaribe infection in neonatal mice (Borden and Nathanson, 1974; Monjan *et al.*, 1974).

In both — XJ and XJC13 — rat models, protracted ATS immunosuppression apparently inhibited T cell subsets including helpers, cytotoxic cells and effectors, impairing antibody formation and/or cell-mediated immune mechanisms (Zimmerman and Tsui, 1979; Zimmerman and Tsui, 1980). To sum up, it seems that the XJ strain allowed rats to develop antibodies and resist viral disease, whereas the XJC13 strain unleashed an immunopathologic response leading to fatal encephalitis. These results support our previous findings on cyclophosphamide suppression of the immune response in the JV infected rats (Galassi *et al.*, 1981).

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