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NOTES

In Vitro Infection of Murine Macrophages with Junin Virus

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Mouse peritoneal macrophages were successfully infected with two strains of Junin virus producing high titers with no apparent cell damage. Infected cultures survived longer than noninfected cultures. The pattern of virus released suggested a persistent infection. Virus replication was delayed in macrophages from mice previously immunized with Junin virus. These results support the opinion that macrophages are targets for virus replication in in vivo infections.

The role of macrophages in the pathogenesis of or defense against infection with highly pathogenic arenaviruses, such as Junin, Lassa, or Machupo, has not been well established. Immunofluorescence (IF) studies performed in humans and guinea pigs infected with Junin virus (JV) have shown specific viral antigens in cells of lymphatic and hemopoietic tissues with morphological characteristics of mononuclear phagocytic elements (1, 5), suggesting that macrophages could be targets for viral replication.

Concerning other arenaviruses, it was reported that lymphocytic choriomeningitis virus replicates in cultivated mouse peritoneal macrophages with no cytophatic effect (7) and, under special combinations of mouse and viral strains, it also replicates in splenic macrophages and mononuclear cells of infected mice (6).

In the present investigation, we studied the interaction of JV with mouse peritoneal macrophages from normal and JV-immunized animals to determine whether mononuclear phagocytic cells are targets for JV replication and whether any differences exist between cells from immunized and nonimmunized animals. The XJ (pathogenic) and XJ.Cl_3 (attenuated) strains of JV were used. Viral stocks were prepared in baby mouse brain as 10% homogenates as previously described (2).

Activated macrophages were obtained from adult Swiss mice injected intraperitoneally 3 days before with 3% starch in phosphate-buffered saline. Approximately 10^7 cells/ml were seeded in Leighton tubes and incubated for 2 h at 37°C in Eagle medium. After vigorous washings, 3×10^6 cells remained adhered to the glass. One day later, cells were infected with either the XJ or XJ.Cl_3 strain at a multiplicity of infection of approximately 0.1 50% lethal dose (LD_{50}) per cell. Virus was removed after 1 h of adsorption at 37°C, and the cultures were washed twice with phosphate-buffered saline and covered with minimal essential medium containing 3% fetal calf serum. Control cultures were mock infected with a suspension of noninfected mouse brain.

Up to 20 days after infection, the cover slips of the Leighton tubes were removed daily and processed for light and electron microscopy and IF studies. Phagocytic activity was assessed in parallel cultures by incubating viable cells with opsonized sheep erythrocytes (8). Virus infectivity was assayed in the supernatants by intracerebral inoculation of suckling mice. The same procedure was performed with macrophages obtained from mice immunized with 3 doses of 5×10^6 LD_{50} of JV strain XJ.Cl_3 administered intraperitoneally at 15-day intervals. The sera of immunized mice contained specific fluorescent anti-JV antibodies.

Control cultures remained viable up to day 9, but the number of adherent cells decreased gradually from 3.5×10^6 to 3×10^5. Strikingly, cultures of infected macrophages survived up to 20 days, when the experiment was interrupted. At that time, the cells appeared undamaged.

Both virus strains multiplied in cultivated peritoneal macrophages (Fig. 1). Viral titers attained by the XJ.Cl_3 strain were higher than those attained by the XJ strain, although both strains followed a similar pattern of infection. Virus was released to the supernatants in several growth cycles and was maintained throughout the observation period, suggesting that a persistent infection was established.

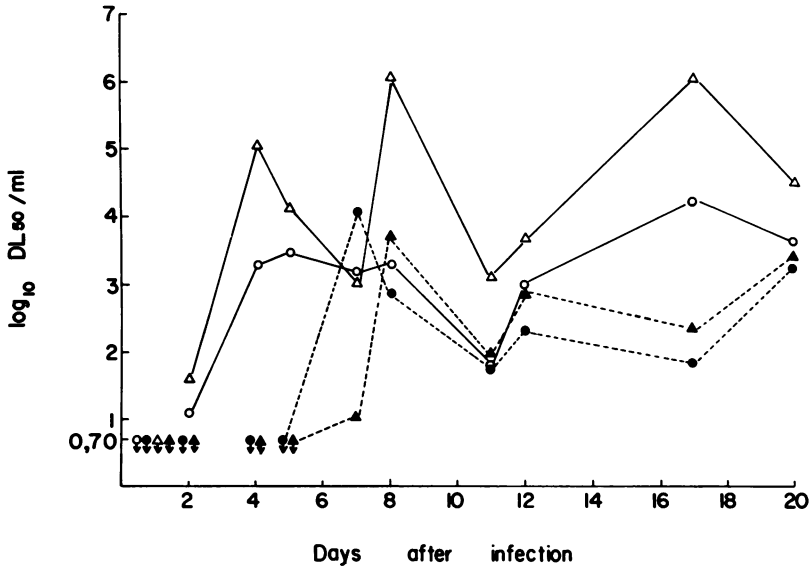

FIG. 1. Comparative growth curves of JV in mouse peritoneal macrophages from immunized (I) or nonimmunized (NI) donor mice. Leighton tubes containing 3×10^6 adherent cells were infected at a multiplicity of 0.1 LD_{50} per cell. Supernatant fluids were removed daily until day 20 after infection, and some of them were assayed for virus infectivity. Symbols: △, XJ.Cl_3 strain in NI macrophages; ○, XJ strain in NI macrophages; ▲, XJ.Cl_3 strain in I macrophages; ●, XJ strain in I macrophages.

From day 3 after infection, antigenic determinants of JV could be detected in the cytoplasm of infected macrophages (Fig. 2). The number of IF-positive cells increased from 10 to 30% (day 5), remaining constant until the end of the experiment. Occasionally, typical viral particles could be seen near infected macrophages.

In spite of the high virus titers produced by

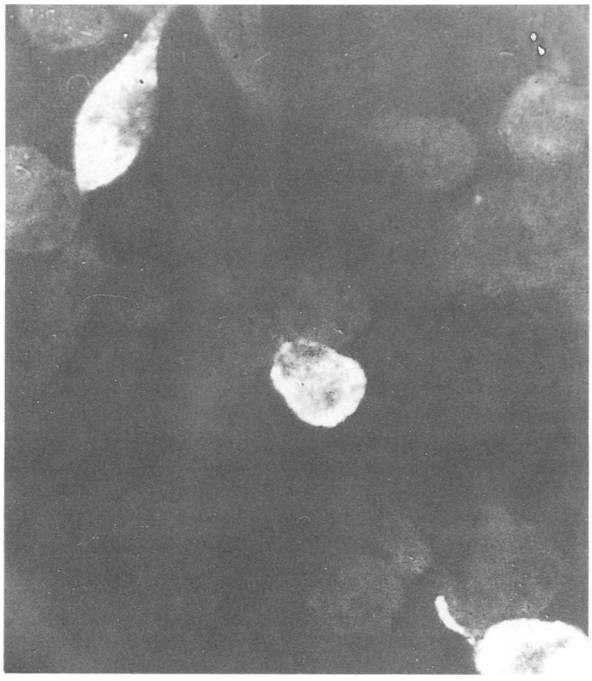

FIG. 2. Antigenic determinants of JV in infected macrophages detected by IF staining (×450).

infected macrophages, the phagocytic activity was not altered, and over 60% of the cells showed intracytoplasmic erythrocytes when incubated with opsonized erythrocytes. Control noninfected cultures behaved similarly. The only morphological alteration observed by light and electron microscopy of infected and noninfected cultures consisted of a marked cytoplasmic vacuolization, increasing with time. No other evidence of cell damage was found, and 80% of cells in the infected cultures remained viable and adherent to the glass until the end of the experiment.

Noninfected cultures obtained from immunized mice did not show IF-positive cells and did not release JV to the supernatants. The replication of both viral strains in macrophages from immunized donors was significantly delayed (4 to 5 days) compared with virus production in macrophages from nonimmunized mice, but once replication started, virus was released continuously to the supernatants, with titers of about 10^3 LD_{50}/ml (Fig. 1). The IF studies showed that antigenic determinants of JV were detectable only after day 5, with a pattern similar to that observed in cultures from nonimmunized mice.

Our results show that JV replicated efficiently in mouse peritoneal macrophages with no apparent damage. The cyclical pattern of virus growth curves resembled that obtained in mouse embryo cells persistently infected with JV (9). No explanation can be forwarded to account for this phenomenon. Another intriguing finding was the longer survival of infected macrophages than of noninfected ones. The lack of cell damage was similar to that reported for lymphocytic choriomeningitis virus (7), although in this case infection was followed only up to 72 h after infection.

Several mechanisms could be responsible for the delay observed in virus multiplication in macrophages from immunized mice. Although cytophilic antibodies could neutralize or prevent the adherence of virus particles to the macrophage membrane, the fact that cultures were established 1 day before infection makes this possibility unlikely. Since it is known that cell cultures persistently infected with JV are refractory to superinfection with homologous virus (9), it could be assumed that macrophages from immunized mice are persistently infected. This explanation seems to be improbable, since neither infectious virus nor antigenic determinants could be found in noninfected cultures. In consequence, it can be speculated that the different behavior observed is due to an immunological mechanism.

Whether macrophages are originally received from the host, producing immune interferon, or whether the cultures contain memory cells that are activated by infection, cannot be determined from our experiments.

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LITERATURE CITED

1. **Carballal, G., P. M. Cossio, R. P. Laguens, C. Ponzinibbio, J. R. Oubiña, P. C. Meckert, A. Rabinovich, and R. M. Arana.** 1979. Junin virus infection of guinea pigs: immunohistochemical and ultrastructural studies of hemopoietic tissue. J. Infect. Dis. **143**:7–14.
2. **Coto, C. E., E. B. Damonte, M. A. Calello, and M. C. Weissenbacher.** 1980. Protection of guinea pigs inoculated with Tacaribe virus against lethal doses of Junin virus. J. Infect. Dis. **141**:389–393.
3. **Damonte, E. B., and C. E. Coto.** 1979. Temperature sensitivity of the arenavirus Junin isolated from persistently infected Vero cells. Intervirology **11**:282–287.
4. **Doyle, M. V., and M. B. A. Oldstone.** 1978. Interactions between viruses and lymphocytes. I. In vivo replication of lymphocytic choriomeningitis virus in mononuclear cells during both chronic and acute viral infections. J. Immunol. **121**:1262–1274.
5. **González, P. H., P. M. Cossio, R. Arana, J. I. Maiztegui, and R. P. Laguens.** 1980. Lymphatic tissue in Argentine hemorrhagic fever. Pathologic features. Arch. Pathol. Lab. Med. **104**:250–254.
6. **Popescu, M., J. Lohler, and F. Lehmann-Grube.** 1979. Infectious lymphocytes in lymphocytic choriomeningitis virus carrier mice. J. Gen. Virol. **42**:481–492.
7. **Schwartz, R., J. Lohler, and F. Lehmann-Grube.** 1978. Infection of cultivated mouse peritoneal macrophages with lymphocytic choriomeningitis virus. J. Gen. Virol. **39**:565–570.
8. **Stuart, A. E.** 1967. Techniques for the study of phagocytes, p. 1034–1053. *In* D. M. Weir (ed.), Handbook of experimental immunology. Blackwall Scientific Publications, Oxford.
9. **Vidal, M. C., and C. E. Coto.** 1980. Persistent infection of mouse embryo cells with arenavirus Junin. Medicina (Buenos Aires) **40**:537–542.