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25 February 1980

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## Abstract

Assessment of arenavirus sensitivities to an interferon (IF) of human cell culture origin was accomplished by a plaque reduction ($PR_{50}$) method in cells of monkey (BS-C-1) and human origin (Detroit 532). Arenavirus sensitivity to the human IF was compared in parallel titrations to the IF sensitivity of vesicular stomatitis virus (VSV).

Interferon bioassays in both cell types indicated that all arenavirus members were less sensitive to IF of human origin than was the standard challenge virus, VSV. Bioassay of human IF on BS-C-1 cells provided a wider spectrum of interferon sensitivity among the various arenavirus members than that shown for Detroit 532 cells. There was no apparent correlation between human IF sensitivity and human virulence with the arenaviruses tested.

No studies appear to have been devoted to the determination of the susceptibility of arenavirus (7) members to the antiviral activity of interferons, with the exception of lymphocytic choriomeningitis (LCM) (5). The universality of IF production by viral inducers in vitro is an important cellular response in relation to viral IF sensitivity, viral replication, defective interfering particle generation, and alterations in cellular nucleic acid and protein metabolism.

Arenaviruses are well known for their ability to persist in numerous cell types (3, 6). Measurement of their reactivity toward interferon(s) in cell cultures may serve a useful purpose to describe their behavior in certain infectious states, including persistent infection. Further, it was of interest to know whether human IF might be useful as an adjunct to immune therapy in natural and laboratory-acquired human infection with the virulent arenaviruses.

Although existing data pertaining to viral interference demonstrable within the arenavirus group suggest that viral interference is not mediated by IF (4, 8), and may be due to defective interfering particle formation (9, 11), the response of arenaviruses to a virus-induced interferon could aid in the further description of their persistence during infection. This study describes the determination of the sensitivity of several arenaviruses, including the human pathogens LCM and Machupo (MAC) viruses, to human IF.

Preliminary studies designed to determine a single cell type suitable for plaque assay in the IF sensitivity determinations revealed that Vero cells were suitable for plaque enumeration of all the viruses under study but were relatively insensitive to human fibroblast IF. BS-C-1 cells, although not the ideal system for plaque assay, were

sensitive to the IF preparation and were, therefore, utilized as the test menstruum for the major portion of the work. Later it was shown that several of the arenaviruses under study formed plaques satisfactorily on the human origin cell line, Detroit 532. A portion of the determinations were repeated on these cells for comparison purposes.

## MATERIALS AND METHOD

Cell Cultures. Cells of African green monkey kidney, BS-C-1, Vero and human foreskin cells, Detroit 532, were originally obtained from the American Type Culture Collection (ATCC). Cells were grown as recommended by ATCC procedures accompanying each cell culture; their propagation as monolayers for subculture was generally carried out in 800-$cm^2$ roller bottles. Cell cultures of diploid fetal rhesus monkey lung (FRhL-2) were originally obtained from Dr. J. C. Petricciani, Bureau of Biologics, NIH, Bethesda, Md, and were grown in Eagle's minimum essential medium (MEM) supplemented with L-glutamine, nonessential amino acids and 10% fetal bovine serum. Cell cultures were supplied for use principally in 25-$cm^2$ Falcon flasks. Vero cells were used after a 3-day growth period at 37°C ($1 \times 10^7$ cells/flask); BSC-1 and DBS-FRhL-2 cells were used 5-7 days after seeding ($6 \times 10^6$ to $1 \times 10^7$ cells/flask). Cultures of primary chick embryo fibroblasts supplied as monolayers were used 2-3 days after planting to provide stocks of VSV.

Viruses, propagation and assay. All arenaviruses except MAC virus, strain Malale, were obtained from the ATCC and maintained as viral stocks by Virology Division of this Institute. Amapari virus was propagated in DBS-FRhL-2 cells; the titer was $4 \times 10^4$ to $1.5 \times 10^5$ plaque-forming units (PFU)/ml in BS-C-1 cells after 7-11 days. Latino and LCM viruses were propagated in BS-C-1 cells and yielded $1 \times 10^7$ PFU/ml and $3 \times 10^6$ PFU/ml, respectively in BS-C-1 cells. Parana virus grown in suckling hamster brains yielded $4 \times 10^4$ PFU/ml on BS-C-1 cells in 7 to 8 days postinfection, and 1 to $2 \times 10^6$ PFU/ml on Detroit 532 cells 6 days postinfection. Pichinde virus was propagated in Vero cells and yielded 5 to $9 \times 10^4$ PFU/ml in BS-C-1 cells after 4 to 5 days and 1 to $2 \times 10^4$ PFU/ml in Detroit 532 cells in 3 to 6 days.

Tacaribe and Tamiami viruses were propagated in suckling mouse brains; the former yielded $3 \times 10^5$ PFU/ml in BS-C-1 cells in 3 to 7 days, whereas the latter yielded $5 \times 10^6$ PFU/ml in BS-C-1 cells in 3 to 5 days. Machupo virus was isolated from a human spleen and was plaque-purified and propagated in DBS-FRhL-2 monolayers. The titer was $3.6 \times 10^5$ PFU/ml in BS-C-1 cells at 9 days. Vesicular stomatitis virus was grown in primary chick embryo cell cultures and yielded $5 \times 10^7$ to $2 \times 10^8$ PFU/ml on BS-C-1 cells, and 1 to $3 \times 10^7$ PFU/ml on Detroit 532 cells in 2 to 3 days.

Interferon preparations. Interferon induction was carried out in 14-day old Detroit 532 cells contained in half-gallon roller bottles by the IF priming and superinduction procedure detailed by Billiau et al. (2). The priming IF was prepared in Detroit 532 cells by inoculation with the Herts strain (1) of Newcastle disease virus.

Titration of interferon sensitivity. Interferon preparations were diluted in bulk in a medium consisting of MEM supplemented with L-glutamine, nonessential amino acids, sodium pyruvate, lactalbumin hydrolysate, and 4% fetal bovine serum. Triplicate flasks of cells were inoculated with 3-ml quantities of each IF dilution and incubated at 36°C for 18 to 22 h prior to challenge with representative viruses. Each flask was inoculated with 0.5 ml of a predetermined dilution of challenge virus to yield 50 to 60 PFU/flask and incubated at 36°C for at least 1 h. Challenge with VSV was carried out in parallel with each arenavirus being tested. Each flask then received 4 to 5 ml of an agar overlay comprised of Eagle's basal medium supplemented with L-glutamine and 5% fetal calf serum. Plaques of all viruses in BS-C-1 cells were usually visible by oblique light prior to the day of optimal development and were generally counted after addition of a second agar overlay containing a concentration

of 1:26,000 neutral red. The median plaque-reduction ($PR_{50}$) titers of the IF preparations were obtained by plotting on probit paper the negative $\log_{10}$ of the IF dilution against percent plaque reduction (12).

One unit in the BS-C-1 bioassay for IF was equal to 0.71 international reference units (IRU), whereas one unit in the Detroit 532 cells was equal to 0.25 IRU using VSV as the challenge virus.

## RESULTS

Interferon sensitivity of arenavirus members in BS-C-1 cells. Plaques in these IF assays were enumerated after 2 to 3 days for VSV and from 4 to 9 days for the arenaviruses. As shown in Table 1, the arenaviruses displayed widely different sensitivities to human IF. None were as sensitive to IF as VSV. The variation in response, however, suggested that a presumptive grouping can be made based on their IF sensitivity relative to VSV. The first grouping consisted of Parana, Tacaribe, and Tamiami viruses with 7 to 10 times less sensitivity to IF compared to VSV. The second group, consisting of Amapari, MAC, and Pichinde viruses, was found to be 50 to 100 times less sensitive to IF. The least sensitive of the arenaviruses tested were LCM and Latino viruses which were > 100 to 600 times less sensitive than VSV. These comprised the third grouping.

Sensitivity of arenavirus members to human IF in human skin cells. Parana, LCM, and Pichinde viruses formed plaques reproducibly in Detroit 532 cells, thereby providing the basis for determinations of their sensitivity to human IF on human cells. The results shown in Table 2 demonstrate again that these arenaviruses were less sensitive to the human IF than VSV. As indicated, Detroit 532 cells required treatment with 4 to 10 times more IF to inhibit these arenaviruses than to inhibit VSV. Plaques in these IF assays were enumerated after 2 to 3 days VSV, and 5 to 8 days after arenavirus challenge.

## DISCUSSION

The spectrum of antiviral activity for human fibroblast IF was extended to include several arenaviruses in monkey and human cell lines. The BS-C-1 monkey cell line furnished a cell culture-IF test system which demonstrated a differential gradient in IF sensitivities among members of the arenavirus group relative to each other and to the IF sensitivity of VSV. Parana, Tamiami, and Tacaribe viruses were found to be examples of arenaviruses most sensitive to human IF, whereas LCM and Latino viruses were found to be the least sensitive to the human IF. The widely differing arenavirus sensitivities to human IF characterized in BS-C-1 cells were not demonstrably apparent when a limited number of arenaviruses were tested for their IF sensitivity in human skin cells. However, all arenaviruses examined were less sensitive to human IF than was VSV in either of the two cell lines utilized. Mims and Subrahmanyan (5) reported that LCM virus possessed mouse IF sensitivity about equal to the IF sensitivity of ectromelia virus in mouse macrophage cell cultures. While LCM virus was shown by those authors to be sensitive to an IF of mouse origin, the IF sensitivity of other arenavirus members relative to LCM virus for mouse IF remains to be determined. Since arenavirus members had been isolated originally from rodent species, their composite sensitivity to mouse IF might provide an additional parameter to describe the role of arenaviruses in this ecological habitat. The relative IF in vitro resistance of several nonpathogenic arenaviruses, notably Amapari, Latino and Pichinde viruses, would not support the hypothesis that interferon induced in vivo in response to infection plays a determinant role in the outcome of infection with these viruses. However, at the same time, these results do not rule out the possibility that administration of IF as an adjunct to

immunotherapy in the treatment of virulent arenavirus human infection, but might prove useful. A nonhuman primate model for MAC virus infection now exists (10) and could be utilized to test this hypothesis. If found effective, such treatments may be considered for use for "at risk" personnel in event of accidental exposure to a hazardous arenavirus.

An added criterion for the usefulness of in vitro testing for sensitivity to IF is the ability to characterize interactions between arenaviruses and BS-C-1 and Detroit 532 cell lines. Interferon sensitivity determination of a specific arenavirus in a specified cell line would aid in the descriptive molecular events, as suggested by Mims (6). In instances in which IF is not induced upon initial contact with arenavirus infection, the IF sensitivity of the virus would not seem to play an important role in the eventual outcome of the cellular infectious process. In arenavirus-cell interactions, where IF synthesis is induced either upon primary or perhaps secondary infection, consideration of the degree of IF sensitivity of the replicating arenavirus would be useful in attempts to more fully describe the infectious process.

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TABLE 1. Sensitivity of selected arenaviruses to human interferon as measured in cells of monkey origin (BS-C-1)

| Challenge virus | Day PFU read | Ratio of IF units required (Arenavirus/VSV[a]) |
|---|---|---|
| Amapari | 7 | 50 |
| | 9 | 100 |
| Latino | 8 | >630 |
| LCM | 6 | >100 |
| | 14 | >200 |
| Machupo | 9 | 53 |
| Parana | 5 | 10 |
| Pichinde | 4 | 65 |
| Tacaribe | 7 | 10 |
| Tamiami | 6 | 7 |

[a]Ratio of IF units required to inhibit the indicated arenavirus vs. VSV.

TABLE 2. Sensitivity of selected arenaviruses to human interferon as assayed in cells of human origin (Detroit 532)

| Challenge virus | Day PFU read | IF titer against Arenavirus ($\times 10^4$) | IF titer against VSV ($\times 10^5$) | IF units required (Arenavirus/VSA[a]) |
|---|---|---|---|---|
| LCM | 6 | 2.2 | 2.5 | 9 |
| | | 2.8 | 2.5 | 9 |
| Parana | 7, 8 | 6.3 | 2.2 | 4 |
| | | 5.6 | 4.0 | 7 |
| Pichinde | 5 | 4.0 | 4.0 | 10 |
| | | 1.0 | 1.0 | 10 |

[a]Ratio of IF units required to inhibit the indicated arenavirus vs. VSV.