APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1987, p. 708–709
0099-2240/87/040708-02$02.00/0

Vol. 53, No. 4

Inactivation of Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus by Formaldehyde-Based Reagents

LINDA S. MARTIN,* SHERRY L. LOSKOSKI, AND J. STEVEN McDOUGAL

Division of Host Factors, Immunology Branch, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Received 28 April 1986/Accepted 8 January 1987

Neutral buffered Formalin, a fixative used in most pathology laboratories, was found to inactivate human T-lymphotropic virus type III/lymphadenopathy-associated virus. Preparations containing this virus with infectivity titers of $>10^5$ were treated with 1% or greater neutral buffered Formalin; after treatment, virus was undetectable (titer, $<10^1$). In addition, when infected phytohemagglutinin-stimulated lymphocytres were treated with paraformaldehyde, transmission of the virus to other such lymphocytes was eliminated.

Although the human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) can be inactivated by many chemical agents, those working in pathology laboratories have been concerned about the survival of HTLV-III/LAV in tissues fixed with 10% neutral buffered Formalin (NBF) (1–4). Spire et al. reported that the virus was not inactivated, as determined by reverse transcriptase activity, by 0.1% Formalin (4). The purpose of our study was to determine whether greater concentrations of Formalin inactivate HTLV-III/LAV. In some experiments, infected cells were treated with paraformaldehyde (PF), a polymer of formaldehyde, to assess intracellular inactivation. PF is frequently used for fixation of cells for fluorescent cell sorter analysis. In addition, attempts were made to determine whether live virus exists in PF-treated infected cells, by using ultrasonic energy to release virus.

MATERIALS AND METHODS

HTLV-III/LAV was propagated in phytohemagglutinin-stimulated normal human lymphocytes (PHA blasts) as previously described (1, 2). Stock virus (50 μl) with an infectivity titer (50% infectious dose; ID_{50}) of $>10^5$ was treated at room temperature for 5 min with 50 μl of various concentrations of NBF diluted in saline (10% NBF, pH 7 = 3.7 to 4% formaldehyde, 6.5 g of Na_2HPO_4 per liter, and 4.0 g of NaH_2PO_4 per liter) or medium B (RPMI 1640 containing 100 U of penicillin per ml, 50 μg of streptomycin per ml, 2 mM L-glutamine, 10% [vol/vol] fetal calf serum, and 10% [vol/vol] interleukin-2 [Cellular Products, Buffalo, N.Y.]). Treated virus was then serially diluted in medium B and inoculated into indicator cells (PHA blasts); the ID_{50} was determined using a microculture infectivity assay as previously described (1, 2). Appropriate toxicity controls were performed in parallel as described (1, 2).

In sonication experiments, duplicate tubes containing 3×10^6 PHA blasts, infected with HTLV-III/LAV 4 to 6 days earlier, were treated with saline or various concentrations of PF in saline for 2 h at room temperature. After incubation, the cells were washed one time in saline and suspended in 1 ml of medium B. One tube from each duplicate set was sonicated on ice for 10 min in a closed container at maximum intensity, using a Fisher Sonic Dismembrator 300 equipped with a cup sample holder (Fisher Scientific, Pittsburgh, Pa.). Serial dilutions of each set of test samples were prepared, inoculated onto indicator cells (3-day PHA blasts), and assayed in the microculture infectivity assay. Stock virus suspended in medium B was also sonicated to assess the effect of sonication on suspended virus. To control for any toxic cellular substances that might be released by sonication, uninfected PHA blasts that had or had not been treated with PF were sonicated; then stock virus was added, and ID_{50} was determined.

RESULTS

HTLV-III/LAV was inactivated by treatment for 5 min at room temperature with NBF at concentrations of 1% or greater (Table 1). The results shown in Table 1 are from one experiment and are representative of those obtained in three separate experiments in which concentrations of 1% NBF or greater were necessary for virus inactivation. There was little or no reduction in ID_{50} when the virus was treated with concentrations ranging from 0.1 to 0.005% (data not shown).

We treated infected PHA blasts with various concentrations of PF and assayed for infectious virus. Some samples were also treated with ultrasonic energy to release any infectious virus that might be in the cells. One percent PF was used in these latter experiments since this is the concentration routinely used in our laboratory to fix cells from patients with acquired immune deficiency syndrome and the related disease complex for flow cytometry analysis. We had previously determined that virus suspended in medium B was inactivated by treatment with 0.5% PF (1). Representa-

TABLE 1. Inactivation of HTLV-III/LAV by NBF

Formalin concn (%)	Posttreatment ID_{50}[a]	Toxicity control ID_{50}
5.00	$<10^{1.0}$	$10^{5.67}$
2.50	$<10^{1.0}$	$10^{5.33}$
1.00	$<10^{1.0}$	$10^{5.12}$
0.50	$10^{2.20}$	ND[b]
0.25	$10^{3.79}$	$10^{5.09}$
Untreated	$10^{5.27}$	

[a] ID_{50} is the reciprocal of the dilution of virus inoculum that, when inoculated onto microcultures containing PHA blasts, results in detectable virus replication in 50% of the cultures (1).
[b] ND, Not done.

* Corresponding author.

TABLE 2. Sonication of HTLV-III/LAV-infected PHA blasts

Virus inoculum	ID_{50} of inoculum[a] after treatment with PF concn of:						
	0%	0.01%	0.05%	0.10%	0.25%	0.50%	1.00%
1. HTLV-III/LAV-infected PHA blasts	$10^{6.0}$	$10^{5.2}$	$10^{2.5}$	$<10^{1.0}$	$<10^{1.0}$	$<10^{1.0}$	$<10^{1.0}$
2. Sonicated HTLV-III/LAV-infected PHA blasts	$10^{5.5}$	$10^{4.5}$	$10^{2.0}$	$10^{1.3}$	$<10^{1.0}$	$<10^{1.0}$	$<10^{1.0}$
3. HTLV-III/LAV	$10^{3.16}$						
4. Sonicated HTLV-III/LAV	$10^{3.56}$						
5. HTLV-III/LAV added to PHA blasts	$10^{4.5}$						$10^{4.3}$
6. HTLV-III/LAV added to sonicated PHA blasts	$10^{4.3}$						$10^{4.4}$

[a] All inocula were titrated on PHA blasts. Uninfected PHA blasts were washed after treatment with PF and before addition of virus to the inoculum.

tive results obtained from one of three experiments are given in Table 2. Treatment of infected cells with PF at concentrations of 0.10% or greater rendered the cells noninfectious (Table 2, line 1). Furthermore, infectious virus was not released from sonicated cells (line 2). Virus was not inactivated by the sonication procedure itself (lines 3 and 4). To insure that the virus was not inactivated by toxic substances released by sonication of the PHA blasts, uninfected PHA blasts were treated as previously described and sonicated, and stock virus was then added (lines 5 and 6).

DISCUSSION

The data reported here indicate that the acquired immune deficiency syndrome virus is inactivated by 1% or greater concentrations of NBF (1% NBF = 0.37 to 0.4% formaldehyde). This result is similar to results we have reported for PF, a polymer of formaldehyde; in our previous study, concentrations of 0.5% or greater inactivated the virus (1). Since the virus was susceptible to 1% NBF, the 10% solution used in pathology laboratories should provide an adequate margin of safety. In an earlier study, Spire reported that 0.1% Formalin (0.037 to 0.04% formaldehyde) did not inactivate HTLV-III/LAV (4). We also found that this concentration of Formalin does not inactivate the virus.

Although retroviruses are thought to become infectious during the budding process in which the virus acquires its envelope, there may be some infectious virus within the cell, or the cellular environment may afford some protection for the virus from the effects of PF. This question was approached by treating virus-infected cells with PF, sonicating the cells to release potentially infectious virus, and testing for infectivity (Table 2). Although these experiments may not distinguish the precise mechanism of inactivation (i.e., penetration and inactivation of infectious virus versus an effect on the cell that halts replication of infection-competent virus), the data support the conclusion that, whatever the mechanism, infected cells treated with appropriate concentrations of PF can no longer transmit the virus to susceptible cells.

LITERATURE CITED

1. **Martin, L. S., J. S. McDougal, and S. L. Loskoski.** 1985. Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy associated virus. J. Infect. Dis. **152**:400–403.
2. **McDougal, J. S., S. P. Cort, M. S. Kennedy, C. D. Cabridilla, P. M. Feorino, D. P. Francis, D. Hicks, V. S. Kalyanaraman, and M. S. Martin.** 1985. Immunoassay for the detection and quantitation of infectious human retrovirus, lymphadenopathy-associated virus (LAV). J. Immunol. Methods **76**:171–183.
3. **Prince, A. M., B. Horowitz, H. Dichtelmueller, et al.** 1985. Quantitative assays for evaluation of HTLV-III inactivation procedures: tri(*N*-butyl)phosphate:sodium cholate and β-propiolactone. Cancer Res. **45**(Suppl.):4592s–4594s.
4. **Spire, B., F. Barre-Sinoussi, L. Montagnier, J. C. Chermann, W. Stephan, and R. C. Gallo.** 1984. Inactivation of lymphadenopathy associated virus by chemical disinfectants. Lancet **ii**:899–901.