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Lab Test Results of HIV inactivation by electric current from patent 5,139,684 (of Kaali & Schwolsky 8-18-92)

EXPERIMENTAL RESULTS

Overview: A non-flow vessel or cell included a pair of platinum electrodes 1 mm apart inserted into a well 1.56 mm in length and 8.32 mm in depth. The non-flow vessel was connected to a direct current source capable of creating an electric field at a constant voltage and constant amperage. Into this well was laced a suspension of the human immunodeficiency virus type 1 (HIV-1) at a concentration of 1,000,000 infectious particles per ml. An aliquot of approximately 10 ul of the virus suspension was placed into the well. Thereafter, the viral suspension was exposed to direct currents ranging from 0 microamps (uA) for up to 12 minutes, to 100 microamps for up to 6 minutes. Intermediate currents of 25, 50 and 75 microamps were used to expose similar viral aliquots. After exposure of the viral suspension to electric currents, the contents of the non-flow vessel were removed and placed into sterile microtubes. 5 ul of each sample were removed and diluted with 95 ul tissue culture medium supplemented with 10% fetal calf serum (FCS. unborn calf blood)

In Experiment 1, the resuspended and treated viral stocks were incubated with a human T lymphoblastoid cell line named CEM-SS. This cell line, upon exposure to HIV-1, forms syncytia (giant cells). It is well documented that the viral titer (amount) used is directly correlated with the number of syncytia formed. Therefore, evaluation of infectivity of HIV-1 can be used with this assay. In contrast, Experiment No. 2 used a differnet human T lymphoblastoid cell line named H9. This cell line, in contrast to CEM-SS cells, produces, upon exposure to HIV-1, many viral particles. The amount of virus produced is proportional to the amount of virus to which the cells are exposed. Therefore, quantitation of viral particles, or more commonly associated viral protein (in this case reverse transcriptase), can be used as an index of viral infection. In both assays, the CEM syncytia forming assay and the H9 viral protein assay, similar type results were obtained. That is, with the CEM cells, although syncytium formation and quantitation is preferrable, one can quantitate the HIV-1 associated protein (reverse transcriptase) activity and conversely with the H9 cells, although reverse transcriptase quantitation is preferred, one can quantitate giant cell (syncytia) formation. Both of these assays are widely used as reproducible measures of viral infection and can be used to determine if alterations in viral infectivity as a product of this electrical treatment can be detected.

Experiment #1
Approximately 100,000 CEM-SS cells per sample were incubated with a treated or untreated (control) viral aliquot for up to 4 days. The cells were placed into microtiter plate wells and monitored for formation of syncytia every 24 hours by microscopic observation. In a standardized fashion, as it has been reported in the literature and is currently being conducted in many laboratories, the number of syncytia at 3 and 4 days was determined. Table 2 summarizes the results from a representative experiment using this assay. As can be noted, the number of syncytia formed was inversely proportional to the amount of electric current. That is, additionally, with increased current (100 vs 50 uA) there was a reduction in the number of syncytia formed. These results and those of additional experiments using the CEM-SS cell line indicate a consistent finding that electrical treatment of the RF strain of HIV-1 attentuates the virus potential for inducing syncytium formation in this cell line.

Experiment #2
A separate and independent assay to determine the ability of electric current to alter HIV-1 infectivity using H9 cells was employed. The basic strategy was similar to that used for the CEM cells with the exception that the initial suspension of treated and controlled (non-treated) viral stock was incubated with 100,000 H9 cells for 2 hours at 37 degees Celsius. Thereafter, the cell virus suspensions were further diluted to 5 ml in standard tissue culture medium. The cell-viral suspensions were then incubated for up to 14 days at 37 degrees Celsius with 5% carbon dioxide. At 3 day intervals (beginning at day 2), aliquots of cell suspension were removed from each sample. The aliquots were centrifuged at 1,000 rpm for 5 minutes in order to pellet the cells. After centrifugation, the supernatant and cell pellets were seperated. The supernatant was cyropreserved for subsequent reverse transcriptase assay and the cell pellets were resuspended in fixatives and maintained in a tissue bank for additional studies employing in situ hybridization and immunocytochemistry to detect qualitatively and semi-qualitatively viral infection by HIV-1. At the end of each experiment, the supernatant samples from each of the tests and time points were examined using standard reverse transcriptase assay. The results of the representative experiment are shown in Table 3. The results of this experiment indicate the ability of HIV-1 to infect H9 cells is attenuated by the magnitude of the electrical currents to which the virus is exposed. Additionally, at lower current magnitude, but with prolonged exposure time, attenuation of viral infectivity is achieved. That is, analogous to the results observed using syncytium formation and the CEM-SS cell line, either increased current or increased duration of

exposure time was inversely proportional to the amount of reverse transcriptase produced by the cell line.

In conclusion, these experiments which have been repeated several times, and those using the CEM-SS cell line, indicate at a statistically significant level that direct electrical current at biocompatible amperages for discrete exposure time intervals can attenuate the ability of HIV-1 to infect normally healthy cells which are susceptible to the HIV-1 AIDS virus.

TABLE 2
Syncytium Formation

Dilution of virus	(Number of Syncytia)				
1:20	TNTC	TNTC	28	66	15
1:40	TNTC	175	22	42	7
1:80	TNTC	90	20	25	4
1:160	180	44	9	9	2
1:320	115	28	4	6	0
1:640	70	10	0	2	0
1:1280	40	7	0	0	0
1:2560	28	4	0	0	0
1:5120	15	2	0	0	0
1:10,240	10	1	0	0	0
1:20,480	4	0	0	0	0
	0uA	25uA	50uA	75uA	100uA

(TNTC=too numerous to count)

TABLE 3
Reverse Transcriptase Activity
(count per million x .001)

uAmps/Time(min.)	Days of Incubation	
	2 days	4 days
0/6	0	13.8
0/12	0	11.7
50/3	0	9.1
50/6	0	9.1
50/12	0	4.8
100/3	0	5.7
100/6	0	3.6