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Efficient Electroporation of Primary Human Lymphocytes

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A number of molecular biological techniques require transfection of cells with recombinant DNA molecules. Our studies of human retroviruses have required us to develop a technique for foreign gene expression in primary human peripheral blood lymphocytes (PBL). Most clinical isolates of human immunodeficiency virus (HIV) do not grow in cultured cell lines, unlike tissue culture-adapted laboratory strains.[1] The human PBL is the primary target cell for HIV infection, and also for transformation by human T-cell leukemia viruses (HTLV). Therefore, we have defined conditions which permit electro-transfection of primary PBL and immortalized lymphoid and fibroblast cell lines, with efficiencies comparable to those achieved by calcium phosphate transfection of fibroblast cell lines.

PBL were prepared from fresh human blood by separation of mononuclear cells on discontinuous Ficoll-Hypaque gradients. Cells were cultured for 3 days in RPMI 1640 medium containing 20% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 1% PHA (Wellcome, HA15), and 30 U/ml recombinant IL-2. After 3 days, non-adherent cells increased in number by three- to five-fold due to stimulation by the T cell mitogen, PHA, and were subsequently maintained in the same medium without PHA (PBL growth medium). For electroporation, cells were taken directly from the PHA-stimulated cultures, washed once with ice-cold PBL growth medium, and resuspended at a density of 2×10^7 viable cells/ml. All subsequent steps were performed on ice. DNA (25 μg) was added to 0.25 ml of the cell suspension (containing 5×10^6 cells), mixed gently, then held on ice for 10 minutes. The mixture was then transferred to an ice-cold Gene Pulser cuvette (0.4 cm) and subjected to a single pulse of 960 μF, 250 V (625 V/cm) from a Gene Pulser apparatus equipped with a Capacitance Extender unit. After shocking, the cuvette was incubated on ice for a further 10 minutes, then the cells were transferred to 5 ml of PBL growth medium (room temperature), and incubated.

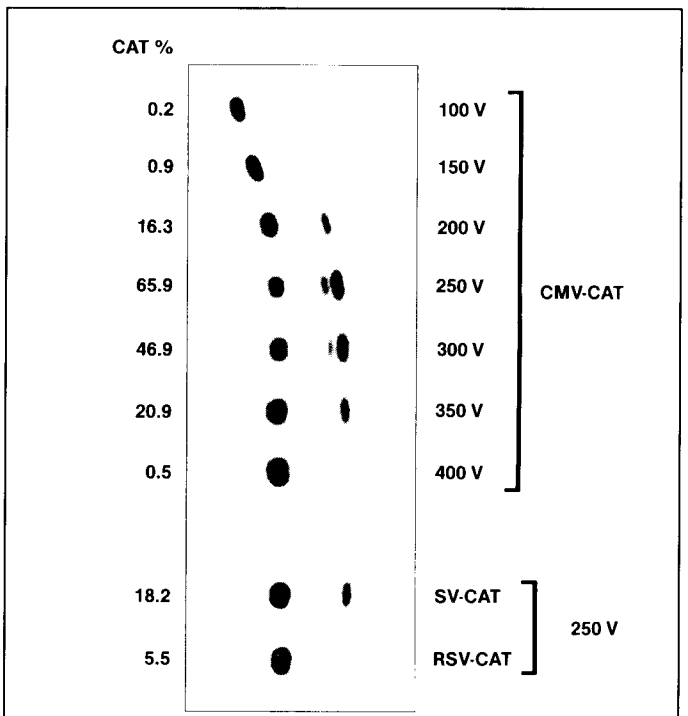

Fig. 1. Voltage optimum for electroporation of PBL and relative promoter strength. CMV-CAT DNA was introduced into PBL by electroporation at 960 μF. Maximum CAT activity in electroporated cells is achieved at an optimum of 250 V (625 V/cm) (left panel). The CMV-IE promoter functions three- to four-fold more efficiently than the SV-40 early promoter (SV-CAT), and twelve-fold more efficiently than the Rous Sarcoma Virus LTR (RSV-CAT) in these cells (right panel).

Initial experiments to determine the efficiency of DNA uptake and expression were performed by measuring chloramphenicol acetyltransferase (CAT) activity.[2] For efficient expression in lymphoid cells, we have utilized constructions based on the human cytomegalovirus immediate early promoter (CMV-IE), which functions very efficiently in all cell types[3] (Figure 1). We find that DNA uptake and expression is highest at the maximum value of the Capacitance Extender unit (960 μF). There is a sharp voltage optimum for CAT activity at 250 V, which corresponds to a pulse decay constant of approximately 45–50 msec under the conditions described above. We find that the medium used to suspend the cells for electroporation is critical to the results obtained. A comparison of different suspension media is shown in Table 1. Transient expression of CAT activity is highest at 24 hours after electroporation, and subsequently decreases.

Table 1. Comparison of Suspension Media

Buffer/Medium	Decay Constant (msec)	Relative CAT Activity (%)
PBS	45	8
HeBS	158	1
1 × Hepes	94	3
HBS	55	35
PBL growth medium (+ IL-2)	51	100
PBL growth medium (− IL-2)	51	92

Cells were suspended in the buffer indicated at a density of 2×10^7 cells/ml and electroporated at 960 μF, 250 V (625 V/cm).
PBS - 1 × phosphate buffer saline.
HeBS - 10% sucrose, 10 mM Hepes, pH 7.05.
1 × Hepes - 70 mM NaCl, 2.5 mM KCl, 350 μM Na_2HPO_4, 2.8 mM dextrose, and 21 mM Hepes, pH 7.05.
HBS - 1 × Hepes plus 10% sucrose.

Table 2. Trans-activation of HTLV and HIV LTRs in PBL

	CAT Activity (% conversion)		
	− *tax*	+ *tax*	Fold Activation
HTLV-I LTR-CAT	1.5%	60.6%	40 ×
	− *tat*	+ *tat*	
HIV LTR-CAT	3.9%	97.8%	25 ×

Recombinant DNA constructions consisting of HTLV and HIV LTRs linked to the CAT gene were introduced into PBL by electroporation. Vectors which express the HTLV *tax* gene or HIV *tat* gene were introduced by simultaneous electroporation with the LTR-CAT constructs (+ *tax*/ + *tat*). Basal activities of the LTRs in PBL were measured in the presence of the CMV expression vector without an insert (− *tax*/ − *tat*).

Using the conditions described above, we have been able to study *trans*-activation of the HTLV and HIV LTRs by their respective *trans*-acting factors. Data from these experiments are shown in Table 2. To our knowledge, this is the first experimental system described which permits the function of recombinant *tax* and *tat* protein expression constructs to be assayed in their normal target cells; i.e., primary human PBL. We are also routinely using this system to rescue infectious virus from molecular clones of HIV strains which do not grow in immortalized cells (Table 3). Since PBL cannot be transfected by other methods, this system permits us to perform *in vitro* genetic manipulations of primary HIV isolates and subsequently prepare virus stocks for phenotypic analysis.

Table 3. Rescue of HIV from Molecular Clones

Cell Type	gag p24 (pg/ml)			
	Day 3	Day 7	Day 10	Day 30
729-6	>2000	325	171	0
Jurkat	440	339	224	0
PBL	28	88	209	>2000

$HIV_{JR\text{-}CSF}$ is a primary clinical isolate which does not infect transformed cell lines.[1] DNA of an infectious molecular clone of $HIV_{JR\text{-}CSF}$ was introduced into the cells indicated, using the electroporation conditions described. Virus production was monitored by ELISA assay of *gag* p24 antigen in the culture supernatants. Although transient provirus production occurs in both EBV-transformed B-cell lines (729-6) and $CD4^+$ T-cell lines (Jurkat), ongoing infections are not established. In contrast, electroporated PBL which are susceptible to infection by $HIV_{JR\text{-}CSF}$ do become productively infected.

Although the major aim of our studies has been to achieve optimum efficiency of transient expression in primary PBL, we have also found that the same electroporation conditions result in a high frequency of stable transformants in immortalized lymphoid cell lines. Using vectors which express the *neo* gene via the CMV-IE promoter, we are able to isolate G418-resistant transformants of 729-6 (an EBV-transformed B-cell line) at a frequency of 1×10^{-3}, a value much higher than is normally achieved by other transfection methods. The electroporation conditions we have described therefore make available a number of experimental approaches in primary human lymphocytes which have previously been impossible, due to the difficulty of transfecting these cells. In addition, the same conditions also facilitate efficient expression of introduced genetic material in immortalized lymphoid and fibroblast cell lines.

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