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Efficient Foreign Gene Expression in Epstein–Barr Virus-Transformed Human B-Cells

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Epstein–Barr virus (EBV) is a herpesvirus that transforms B-cells (B-LCL) and has undergone intense scrutiny owing to its association with Burkitt's lymphoma, nasopharyngeal carcinoma, and immunoblastic lymphomas. B-LCL have also proven useful in the study of human immunology. We describe a novel system for inducing efficient foreign gene expression in B-LCL using biotinylated adenovirus as an endosome-disrupting agent. Plasmid DNA is coupled to the exterior of viral particles by streptavidin–polylysine chimeric proteins. Up to 67% of B-LCL may be induced to express foreign genes *in vitro* in transient expression systems, and gene expression lasts for at least 17 days. We have expressed firefly luciferase, β-galactosidase (β-gal), chloramphenicol acetyltransferase, HIV *gag*, and *env* genes, as well as infectious HIV, and the EBV-specific *BZLF* gene in B-LCL with this system. *In vivo* delivery of a β-gal reporter gene to B-LCL was documented in a SCID mouse model. Potential applications include study of genetic regulation of EBV infection and transformation events, study of potential gene therapies for EBV-related B-cell tumors, and production of antigen-presenting cells for use in immunologic assays. Because of the high percentage of cells transformed and the length of foreign gene expression, the possibility of examining foreign gene expression in transient assays, without selection for clonal populations, exists.

INTRODUCTION

Epstein–Barr virus (EBV) is a member of the herpesvirus group that transforms infected B-cells (B-LCL) (Schooley *et al.*, 1990). These B-LCL have proven useful for several human immunologic applications. They make target cells expressing appropriate HLA types for study of human cytotoxic T-cells (CTL). A common technique used to induce foreign gene expression in B-LCL is to infect with recombinant vaccinia viruses (Plata *et al.*, 1987; Walker *et al.*, 1987, 1988; Curiel *et al.*, 1993; McFarland *et al.*, 1993). Although vaccinia virus is efficient in inducing foreign gene expression in B-LCL, it is a lytic virus, and fresh target cells must be made for each assay. The vaccinia-based system has been exploited extensively for study of the CTL response to HIV (Plata *et al.*, 1987; Walker *et al.*, 1987, 1988; Curiel *et al.*, 1993; McFarland *et al.*, 1993). However, some of the promising candidate HIV vaccines are delivered by a vaccinia vector (Hu *et al.*, 1991; Fultz *et al.*, 1992). Thus, the use of the recombinant vaccinia virus system to study CTL responses to such vaccines is difficult or impossible due to high background vaccinia-specific target cell lysis. In addition to their use as target cells, B-LCL serve as efficient antigen-presenting cells (Lanzavecchia, 1985; Curiel *et al.*, submitted for publication). Thus, B-LCL expressing foreign genes may be used for the stimulation and propagation of antigen-specific human CTL.

Induction of stable foreign gene expression in B-LCL has been achieved experimentally, but has proven difficult. Electroporation of B-LCL has been used successfully in the study of potential toxin gene therapy for B-cell malignancies (Maxwell *et al.*, 1991) and in the study of oncogene expression (Lombardi *et al.*, 1987). However, electroporation suffers from the necessity of using large numbers (up to 10^7) of cells and its lack of applicability as a gene transfer system *in vivo*. Lipofectin has been used successfully to study the role of mucin expression in B-cell tumors, including B-LCL (Jerome *et al.*, 1992). A murine model for the development of human EBV-related B-cell lymphomas was recently described (Mosier *et al.*, 1989; Mosier, 1990; Purtillo *et al.*, 1991). Attention has thus turned to animal models for gene therapy of these tumors. A means of delivering genes of potential therapeutic benefit would be a valuable research tool.

We recently described a novel, high-efficiency gene transfer system based on receptor-mediated endocytosis using adenovirus as the DNA carrier (Curiel *et al.*, 1992; Wagner *et al.*, 1992). With this approach, we now demonstrate the efficient expression of reporter

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as well as HIV- and EBV-specific genes in B-LCL. By contrast, DEAE–dextran, electroporation, and lipofection were not shown to induce efficient expression of these genes in these cells. *In vivo* expression of a β-gal reporter gene in SCID mice reconstituted with B-LCL was documented. Potential applications of this system to the study of EBV gene regulation, human immunology, and gene therapy are discussed.

MATERIALS AND METHODS

Conjugate synthesis

Human transferrin–poly-L-lysine conjugates with average chain lengths of 190 lysines (TfpL190) or 290 lysines (TfpL290) (Wagner *et al.*, 1992), biotinylated adenovirus dl312, and streptavidin–polylysine conjugates were all prepared as described (Wagner *et al.*, 1991). Biotinylation of adenovirus does not significantly affect viral titer (Wagner *et al.*, unpublished observations). Human Ig–polylysine conjugates and anti-human Ig conjugates were prepared by conjugation through disulfide bridges after modification with succinimidyl 3-(2-pyridyldithio)propionate (SPDP; Pharmacia, Uppsala, Sweden) as described previously (Wagner *et al.*, 1990). Human Ig conjugates were constructed using human Ig (Sigma, St. Louis, MO) and poly-L-lysine (with an average chain length of 300 monomers) at a molar ratio of 1:1.6. Anti-human Ig conjugates were constructed using goat anti-human Ig (Southern Biotechnology Associates, Inc., Birmingham, AL) and poly-L-lysine (with an average chain length of 300 monomers) at a molar ratio of 1:2. Before use, antibodies were subjected to gel filtration (Sephadex G25; 150 m*M* NaCl, 20 m*M* HEPES buffer, pH 7.3). The procedure is as follows:

To a solution of 19.5 mg (122 nmol) of human Ig in 1 ml HBS, 39 μl of a 10 m*M* ethanolic solution of SPDP (390 nmol) was added with vigorous mixing. After 2.5 hr at room temperature, purification was performed by gel filtration (Sephadex G25; HBS) to give 1.5 ml of a solution of 118 nmol antibody modified with 252 nmol dithiopyridine linker. This solution was mixed under an argon atmosphere with a 0.9-ml solution of 120 nmol poly-L-lysine (average chain length 300 lysine monomers) in HBS, modified with 280 nmol 3-mercaptopropionate groups as described (Wagner *et al.*, 1992). After 18 hr at ambient temperature 0.2 ml of 5 *M* NaCl was added to the reaction mixture. Conjugates were isolated by cation-exchange chromatography (Mono S, HR 5/5, Pharmacia; gradient elution 22–100% buffer (50 n*M* HEPES, pH 7.9, 3 *M* NaCl)). The product was eluted at a NaCl concentration of approximately 1.8 to 2.1 *M*. After dialysis against HBS, 7 ml of conjugates containing 9.1 mg (57 nmol) human Ig modified with 90 nmol polylysine was obtained.

Anti-human Ig conjugates were prepared in similar fashion. Ig conjugates prepared in this fashion were uncontaminated by free Ig or free components (our unpublished observations).

Complex formation

Binary DNA complexes were prepared as follows: Biotinylated adenovirus d1312 (30 μl; 10^{12} particles/ml) in 50 μl HBS (20 m*M* HEPES, pH 7.3, and 150 m*M* NaCl) was mixed with 800 ng streptavidin–polylysine in 100 μl HBS. After a 30-min incubation at room temperature, a solution of 9 μg pCMVL (Plank *et al.*, 1992) in 200 μl HBS was added, and after an additional 30-min incubation at room temperature, a solution of 5.1 μg poly-L-lysine (average chain length 450 amino acids) in 150 μl HBS was added. Binary complexes containing other plasmids were prepared in analogous fashion using 3 to 9 μg plasmid DNA. Ternary DNA complexes containing adenovirus and transferrin, human-Ig, or anti-human Ig as a further ligand were prepared in analogous fashion, using 10.2 μg TfpL290, 12 μg human Ig–polylysine conjugate, or 10 μg anti-human Ig–polylysine conjugate, respectively, instead of polylysine in the final incubation step. Chloroquine incubations were performed by preincubating cells with 50 μ*M* chloroquine (Sigma, St. Louis, MO) from 15 min prior to 4 hr after transfection, after which time the drug was eluted.

Transfections

DNA complex solutions were added to 3×10^5 to 1×10^6 B-LCL in 24-well plates in 1 ml of RPMI 1640 plus 2% FCS for most experiments. Plasmids pYU-2 and pSG3.1 were prepared in serum-free medium. After 2 hr, 1 ml of RPMI 1640 plus 20% FCS was added. Further cell culture procedures were performed as described (Wagner *et al.*, 1991).

Plasmids

pYU-2 (encoding an HIV isolate derived from brain) and pSG3.1 (encoding a highly cytopathic strain of HIV) have been previously described (Li *et al.*, 1991; Ghosh *et al.*, 1993). pDOLHIVenv, which encodes HIV *env* (Freed *et al.*, 1989), and pDOLHIVenvR, which encodes the same *env* gene in the reverse orientation (Freed *et al.*, 1989) were obtained from the AIDS Reagent Program. pRSVβgal (MacGregor *et al.*, 1987), pCMVL (Plank *et al.*, 1992), pRSVL (deWet *et al.*, 1987), pCMV–CAT (Cann *et al.*, 1988), and pCMV–LTR–CAT (Cann *et al.*, 1988) have also been described. pCMVZ encodes the EBV replication activator gene *BZLF1* (Marschall *et al.*, 1991). pIBIRSVpA was constructed by incorporating the *luc* gene and polyadenylation signal of pRSVL into pIBI20 (International Biotechnologies, Inc., New Haven, CT). To produce p220gag, the

HIV *gag* insert of pDK1 (NIH, 1992) was prepared by sequential digestion with *Bam*HI and *Eco*RI. The resulting 1.7-kb fragment was blunted using Klenow and ligated into the *Sma*I site of pIBIRSVpA. Digestion of the resultant plasmid with *Bam*HI yielded a 3.0-kb insert which combined the HIV *gag* insert with the flanking regulatory regions derived from pIBIRSVpA. This fragment was cloned into the *Bam*HI site of p220.2 (Sugden *et al.*, 1985; Yates *et al.*, 1985). In the resulting plasmid, p220.2gag, transcriptional orientation of HIV *gag* was in the same direction as *EBNA-1*.

Cell lines

B-LCL were made as described (Walls *et al.*, 1989). Each cell line was labeled with its donor's initials as "EM, WO, RU, GU, or EC." The T-cell line C8166 was originally obtained from the American Type Culture Collection. The T-cell line H9 was obtained from Dr. Bruce Walker. All cells were maintained in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10–15% heat-inactivated fetal calf serum (Fisher, Pittsburgh, PA), 2 m*M* glutamine, 10 m*M* HEPES buffer, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Electroporation, DEAE–dextran transfection, and lipofection

Electroporations were performed as described (Cann *et al.*, 1988) using pulses of 250 or 300 V with a Bio-Rad gene pulser linked to a capacitance extender unit. 5×10^6 cells were pulsed in medium RPMI 1640 plus 10% FCS. DEAE–dextran (McCutchan and Pagano, 1968) and lipofectin (Life Technologies, Grand Island, NY) were used to transfect cells as described (Jerome *et al.*, 1992).

Assays for reporter gene expression

Assays for detection of luciferase (luc) (Plank *et al.*, 1992), HIV-induced syncytia (Chao *et al.*, 1989), β-galactosidase (β-gal) (Lim and Chae, 1989), and chloramphenicol acetyltransferase (CAT) (Gorman *et al.*, 1982) were performed as described. HIV p24 concentration was determined from culture supernatants and cell lysates using a commercial kit (Coulter, Hialeah, FL) according to the manufacturer's directions.

Vaccinia virus infection

Recombinant vaccinia virus vDK1 encodes HIV-1 *gag* (NIH, 1992). Infection was performed as described (Curiel *et al.*, 1993). Cell lysates were prepared by incubating 5×10^5 cells in 100 μl of 0.5% Triton X-100 at room temperature for 30 min followed by centrifugation at 600 *g* to remove cellular debris. Supernatants were stored at −20° until assay.

SCID mouse experiments

SCID mice of either sex, 6 to 10 weeks old, were purchased from Taconic. Animals were inoculated with 3×10^7 B-LCL by intraperitoneal injection. Fourteen days later 12 μg pCMVβgal conjugated to adenovirus/pL was injected in 0.5 ml HBS, and the animals were sacrificed by CO_2 inhalation after an additional 3 days. Recovered peritoneal cells were subjected to development with fluorescein β-di-D-galactopyranoside according to the manufacturer's directions (Molecular Probes, Inc., Eugene, OR) followed by staining with CD19-phycoerythrin (B-cells; Coulter) or $H2^d$-phycoerythrin (murine cells; Pharmingen, San Diego, CA) and subjected to two-channel FACS analysis using an EPICS Profile II cell sorter. This method allows discrimination of transfected human cells (fluorescein$^+$/CD19$^+$) from transfected mouse cells (fluorescein$^+$/$H2^d$+). Preliminary studies also indicated that elicited murine peritoneal cells in this model had detectable baseline β-gal activity (our unpublished observations).

RESULTS

Reporter gene expression in B-LCL

Data shown in all cases are from single experiments, but are representative of the values noted for repeated assays. Plasmid DNAs encoding β-gal, CAT, luc, EBV-specific *BZLF*, and the HIV *env* and *gag* genes were all efficiently expressed in this system. Optimal transfection conditions were determined using the CAT reporter plasmids. Initial experiments indicated that pCMV–LTR–CAT was more efficient in inducing CAT expression than was pCMV–CAT (Fig. 1C and data not shown). Using 3 μg of pCMV–LTR–CAT DNA, CAT expression was detected up to Day 6 following transfection and was maximal around Day 3 (Fig. 1A). High-level CAT expression was detected using as little as 10 μl of adenovirus in the complex (Fig. 1B). Therefore, 10 to 30 μl of adenovirus was used for subsequent experiments. Neither electroporation nor DEAE–dextran transfection of pCMV–LTR–CAT was efficient in inducing CAT expression in B-LCL, despite the use of varied electroporation and DEAE–dextran conditions and larger (but nontoxic) amounts of plasmid DNA (Fig. 1C and data not shown). Lipofection of 3 μg of pRSVL induced less than 300 light units of luciferase expression in B-LCL (not shown), which was not significantly different from background.

Initial experiments indicated that *luc* expression in B-LCL was equally efficient using either pCMVL or pRSVL (not shown). In assays using pCMVL, *luc* expression was maximal on Day 1 or 2 depending on the B-LCL line used (Fig. 2a). Substitution of polylysine for transferrin–polylysine in the formation of ternary complexes still allowed for significant luciferase reporter

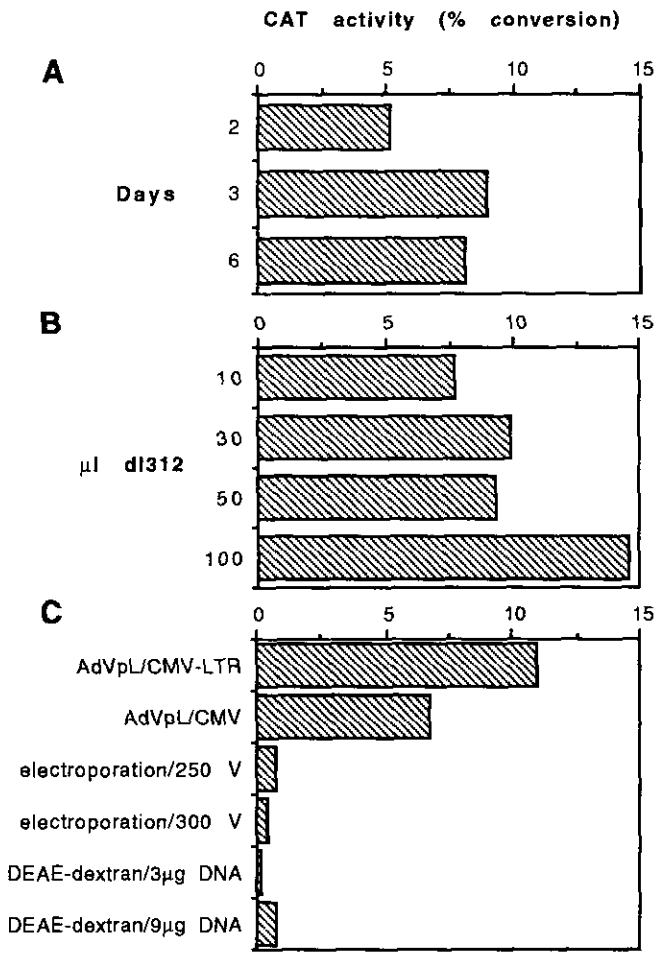

FIG. 1. Time course, viral dosage effect, and comparison of transfection methods using pCMV–CAT or pCMV–LTR–CAT gene constructs. Binary DNA/adenovirus complexes were used. (A) Time course of CAT expression using 3 μg of the pCMV–LTR–CAT construct and 30 μl adenovirus. (B) pCMV–LTR–CAT complexes containing 3 μg of plasmid DNA and 10 to 100 μl adenovirus dl312 (i.e., 10^{10} to 10^{11} viral particles) assayed at 3 days after transfection. (C) Comparison of gene delivery by adenovirus complexes, electroporation, and DEAE–dextran, assayed 3 days after transfection. Three micrograms of plasmid DNA was used except as noted. Untransfected cells had no detectable CAT activity.

gene expression (Figs. 2a and 2b). Under all experimental conditions tested, we noted variation in the absolute value for reporter gene expression from assay to assay. These variations were not large (usually less than a factor of 3). In all cases, however, the relative degrees of reporter gene expression by the different methods were the same.

Immunoglobulin-binding or Fc receptor-binding ligands

In this system, DNA complexed to chimeric polycationic proteins enters cells via receptor-mediated endocytosis after a binding event that physically attaches the complex to the target cell (Abrahamson and Rodewald, 1981; Nio *et al.*, 1989; Curiel *et al.*, 1992; Wagner *et al.*, 1992). We investigated whether ligands that bound surface Ig or Fc receptors, rather than transferrin receptors, would allow entry of DNA complexes into B-LCL. Both Ig–polylysine (which is predicted to bind to Fc receptors) and anti-Ig–polylysine (which is predicted to bind to surface Ig) used in the formation of ternary complexes induced up to three times more reporter gene expression in B-LCL than that induced when transferrin–polylysine or polylysine was used in the formation of DNA complexes (Figs. 3A and 3B). Preincubation of B-LCL with a threefold excess of free anti-Ig reduced reporter gene expression using the anti-Ig–polylysine complexes by approximately 40% (Fig. 3C), demonstrating that anti-Ig was allowing DNA entry by binding to surface-bound Ig.

Expression of HIV genes in B-LCL

B-LCL were induced to produce infectious HIV by transfection with pYU-2 or pSG3.1. p24 antigen detected in cell culture supernatants confirmed the presence of HIV gag proteins (Table 1), and production of infectious HIV was demonstrated by the ability of supernatants from these transfections to infect the susceptible cell line H9 (not shown). p24 levels detected in H9 cell supernatants following transduction with HIV-expressing plasmids were comparable to those detected using a cell-free live viral HIV dose of approximately 5000 $TCID_{50}/10^6$ cells (our unpublished observations), a large viral challenge. In comparison, gag production induced by recombinant vaccinia virus vDK1 was less than 18% as efficient. Envelope expression was confirmed by demonstration of syncytium formation up to 17 days after transfection when B-LCL transfected with pDOLHIVenv, but not pDOLHIVenvR, were cocultured with the indicator cell line C8166 (data not shown). HIV *gag* was also produced when B-LCL were transfected with p220.2gag, but at levels substantially lower than that induced by vDK1 infection or by transfection with proviral plasmid DNA (Table 1).

Expression of EBV-specific genes in B-LCL

pCMVZ was used to induce expression of ZEBRA, the *BZLF* gene product, in B-LCL (Fig. 4) using anti-Ig–polylysine in the formation of ternary complexes. ZEBRA is ordinarily expressed only in B-LCL undergoing lytic EBV infection (Countryman *et al.*, 1987). The functional consequences of ZEBRA expression in these cells are now under investigation in our laboratory.

As a basis for comparison, TPA stimulation variably induced ZEBRA expression in B-LCL, depending on the line tested. In some lines TPA failed to induce any detectable expression. In those B-LCL in which TPA did induce ZEBRA, expression was similar to that transduced by the adenovirus conjugates, as judged

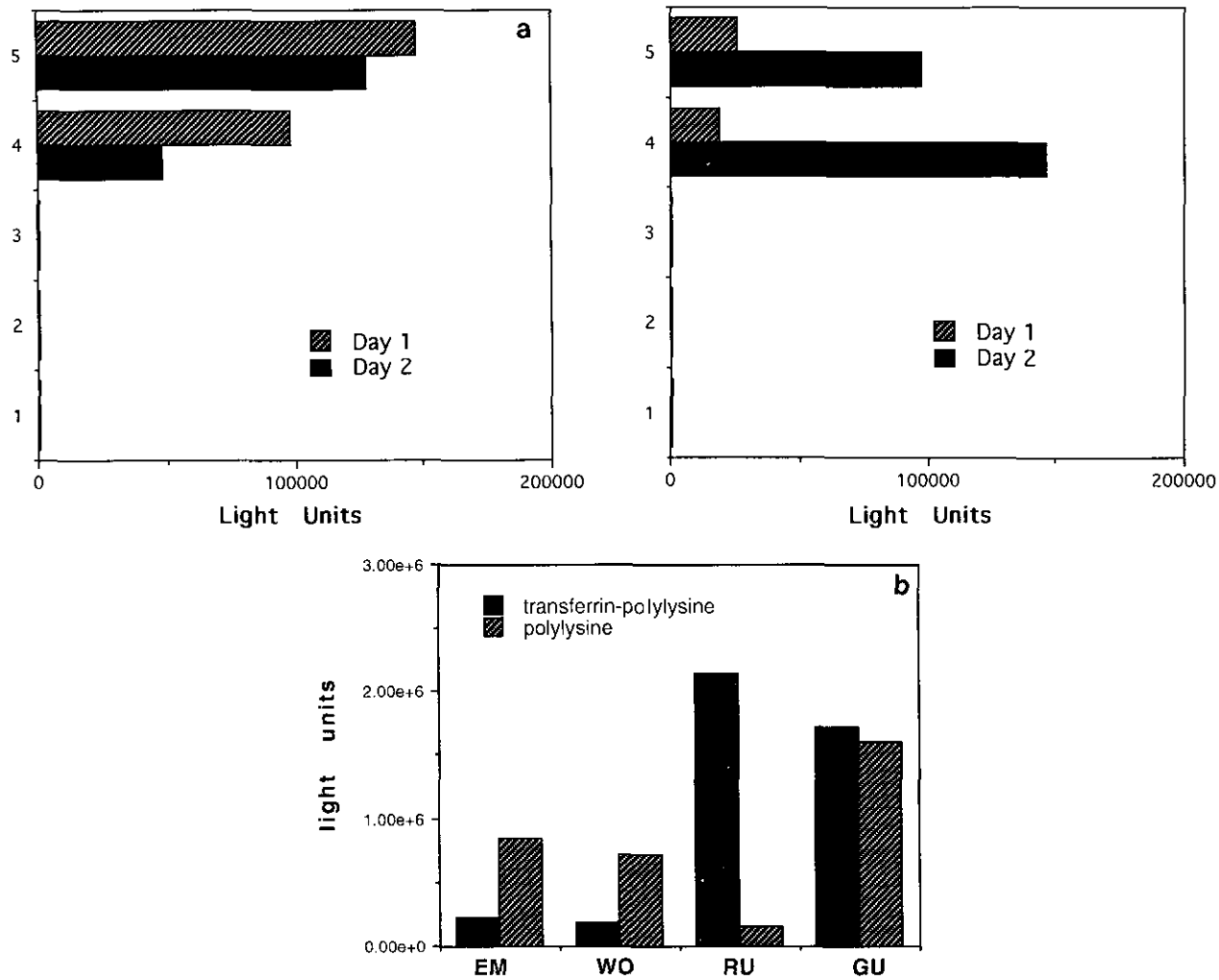

FIG. 2. Comparison of luciferase gene transfer to B-LCL using binary or ternary complexes. Binary complexes, containing pCMVL–DNA and adenovirus d1312, and ternary complexes, containing pCMVL–DNA, adenovirus d1312, and transferrin, were prepared as described under Materials and Methods and used for transfection experiments in the B-LCL lines EM, WO, RU, and GU. (a) Luciferase activity in EM (left) and WO (right) using the combinations of ligands and endosomolytic agents shown. Total luciferase activity in 1×10^6 cells is shown. 1, TfpL, no chloroquine; 2, TfpL + chloroquine; 3, TfpL + adenovirus; 4, pL + adeno-biotin/StpL; and 5, TfpL + adeno-biotin/StpL. (b) Comparison of luciferase activity induced in the B-LCL lines EM, WO, RU, and GU at 48 hr using ternary complexes prepared either with polylysine or with TfpL190. *Cells were harvested 48 hr after transfection and cell extracts were assayed for luciferase activity. Results shown are total luciferase* activity of the sample. TfpL190, transferrin–polylysine with an average chain length of 190 amino acids.

by band intensity on Western blots in which equal cell equivalents were loaded into each lane (data not shown).

Efficiency of transfection and time course of foreign gene expression

B-LCL were transfected with pRSVβgal using transferrin–polylysine, and the percentage of cells expressing β-gal over time was assessed by light microscopic examination. β-Gal was first detected on Day 3 following transfection and was present through Day 12, the latest time point tested. Sixty to 67% of cells were β-galactosidase positive at each time point tested, including Day 12 (data not shown). In addition, HIV-induced syncytia were observed up to 17 days following transfection with proviral DNA as described above.

Effects of gene promoters and EBNA-1/OriP on transient foreign gene expression

RSV, CMV, and MMLV LTR promoters all efficiently induced foreign gene expression in B-LCL in this system (Figs. 1–3, and data not shown). *luc* and HIV *gag* expression were similar when induced by plasmids that either encoded or lacked *EBNA-1* and *OriP* (data not shown).

In vivo gene transfer

The primary aim of these studies was to detail the use of this novel adenovirus system for expression of foreign genes in B-LCL. However, a pilot *in vivo* experiment was performed to assess the feasibility of using this system for *in vivo* gene transfer. Intraperitoneal

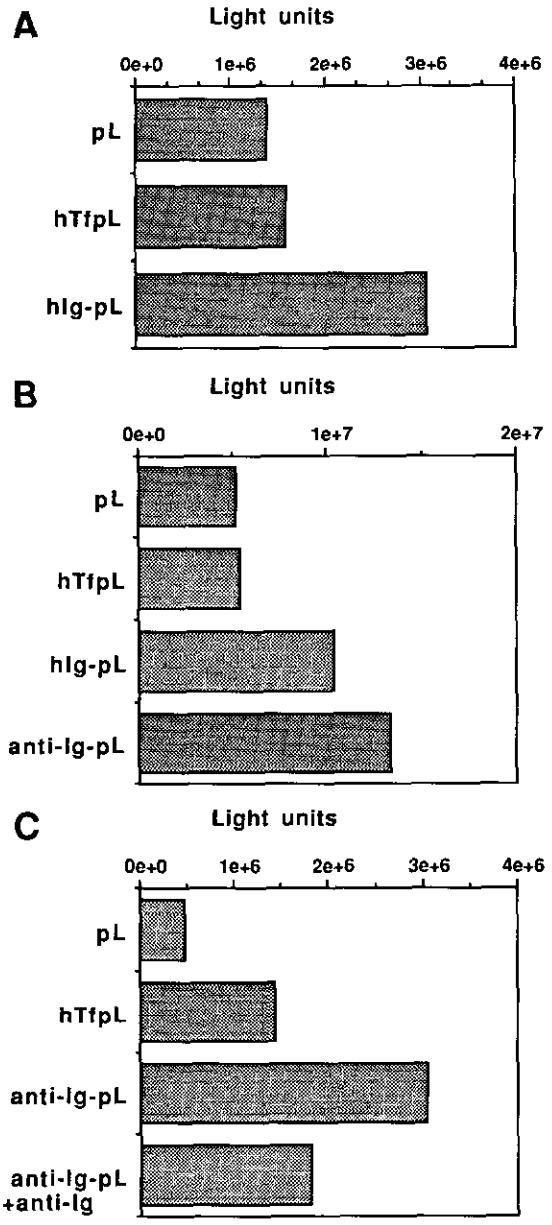

FIG. 3. Luciferase gene transfer using ternary complexes containing IgG or anti-human Ig as ligands for the FcγR or for surface Ig, respectively. Complex formation and transfection were performed as described under Materials and Methods, using 1×10^6 cells and hTfpL (TfpL290). Total luciferase gene expression of the sample 48 hr after transfection is shown. (A and B) The B-LCL line GU, assayed on two separate occasions. (C) The B-LCL line GU in the presence or absence of excess free anti-human Ig. pL, polylysine; hTfpL (TfpL290), transferrin–polylysine with an average chain length of 290 amino acids; hIg-pL, human Ig–polylysine chimeric protein; anti-hIg-pL, anti-human Ig–polylysine chimeric protein.

injection of B-LCL into SCID mice was chosen because the adenovirus conjugates in their current form are relatively unstable in blood (D. Curiel and E. Wagner, unpublished data).

A single SCID mouse challenged with intraperitoneal B-LCL was inoculated with 12 μg pCMVβgal using an adenovirus/pL conjugate that did not specifically target

TABLE 1

COMPARISON OF HIV *gag* EXPRESSION BY RECOMBINANT VACCINIA VIRUS VERSUS ADENOVIRUS-FACILITATED TERNARY COMPLEXES

Expression system	Supernatant	Cells
vDK1[a]	50.2	153.8
p220.2gag[b]	<5	9.7
pYU-2[b]	ND[c]	>880.0
pSG3.1[b]	ND	>880.0

[a] Vaccinia infection of 5×10^6 B-LCL was performed as described under Materials and Methods. Results expressed as pg/ml of p24 antigen at 24 hr after infection (optimal *gag* expression time for vDK1 in this system; not shown).

[b] Plasmids were transfected into 5×10^6 B-LCL using adenovirus-derived DNA complexes that employed transferrin–polylysine in the final incubation. Results expressed as pg/ml of p24 antigen at 48 hr following transfection for p220.2gag, and at 5 days following transfection for pYU-2 and pSG3.1 (optimal gene expression times for these plasmids in this system; not shown).

[c] ND, not determined.

B-cells, and the mouse was sacrificed 3 days later. Forty-two percent of recovered peritoneal cells were $CD19^+$ (B-cells), and of these, 22% were also $\beta\text{-gal}^+$ by dual FACS analysis (data not shown). Further *in vivo* work is in progress.

Technical notes provided to assist researchers in optimizing experimental conditions

Our experience suggests that suspension cells in general, and B-LCL in particular, are less susceptible than adherent cells to transduction using this system. Some of these differences are no doubt attributable to inherent properties of the cell lines tested and have not

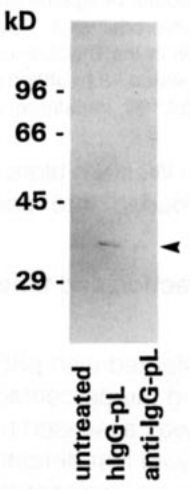

FIG. 4. Expression of *BZLF* in B-LCL. Western blot analysis of 5×10^5 cells from the B-LCL line WU transfected with pCMVZ. Detection of ZEBRA (arrow), the *BZLF* gene product, at 3 days post-transfection detected using antibody AZ 125, supplied by Dr. Alain Sergeant. Expression of *BZLF* appeared to be higher with anti-Ig-pL than with Ig-pL in the formation of ternary DNA complexes.

been systematically studied. Suspending cells in wells of small surface area (such as 24-well plates) appears to give gene expression levels superior to those observed when larger surface area receptacles are used, although this has not been systematically examined. High serum concentrations are clearly detrimental to efficient gene transfer (our unpublished observations). B-LCL may be transfected in serum-free medium with no apparent ill effects on the cells. Performance of the conjugates drops at ≥2% serum. Live adenovirus used in the formation of the conjugates killed all cell types tested by Day 6 of culture. Inactivated virus, on the other hand, has no apparent effect on the growth of B-LCL at up to 8 μg delivered DNA (data not shown). Above this level, cell growth will be inhibited in proportion to the DNA and adenovirus content of the conjugates. It is currently unknown if this inhibitory effect is related to the toxicity of the additional DNA, to the adenoviral proteins, or to some other component of the conjugates.

DISCUSSION

We have demonstrated that efficient, high-level foreign gene expression is induced in B-LCL by a novel gene transfer system (Curiel *et al.*, 1992; Wagner *et al.*, 1992) that uses adenovirus as an endosome disrupting agent and polylysine-linked ligands to allow DNA uptake by receptor-mediated endocytosis. Plasmid DNAs encoding CAT, β-gal, and *luc* reporter genes were all efficiently expressed in B-LCL using complexes made with biotinylated adenovirus plus streptavidin–polylysine and transferrin–polylysine in transient expression assays. Foreign gene expression was efficient in that up to 67% of cells in a transient expression system produced the foreign gene product. Long-lasting transient gene expression (up to 12 days for β-gal and up to 17 days for HIV envelope) was achieved despite the lack of *EBNA-1* and *OriP* in the expression plasmids used.

Adenovirus facilitates the expression of foreign genes by its ability to disrupt endosomes (Wagner *et al.*, 1990, 1991, 1992; Curiel *et al.*, 1992; Plank *et al.*, 1992). However, not all agents with endosome-disrupting ability are efficient in this regard, as we were unable to demonstrate a similar augmentation of foreign gene expression using chloroquine alone or in combination with adenovirus-containing DNA complexes (Fig. 2a, and Curiel *et al.*, unpublished observations).

As DNA complexes made with transferrin may bind to cells via transferrin receptors, this system may be successfully applied to cells which naturally lack adenovirus receptors. To enhance further the ability of complexed DNA to bind to B-cells, ligands were made to target B-cell Ig (anti-Ig–polylysine) or Fc receptors (Ig–polylysine). These ligands were found to enhance foreign gene expression in B-LCL by up to threefold compared to complexes made with polylysine or transferrin–polylysine, presumably by favorable interactions with these cell surface receptors. By contrast, other methods of inducing foreign gene expression in B-LCL such as electroporation, DEAE–dextran, and lipofection were not successful despite optimization of conditions. Whether decreased expression by these alternative methods was due to decreased DNA uptake or expression cannot be determined from this work.

One difficulty in the study of human antigen-specific CTL is that restimulation of these effector cells often requires the use of autologous antigen-presenting cells. Thus, the original donor must make repeated blood donations as a source of antigen-presenting cells. We (Curiel *et al.*, submitted for publication) and others (Lanzavecchia, 1985) have demonstrated that autologous B-LCL may serve as antigen-presenting cells suitable for stimulation of human immune cells. Thus, a B-LCL line induced to express the antigen of interest would serve as a useful and convenient source of autologous antigen-presenting cells in some instances.

Likewise, CTL target cells may be produced with this system. Currently, genes of interest are usually inserted into a vaccinia virus expression vector for efficient production of CTL target cells. With our system, the antigen-encoding plasmid may be used directly to produce a target cell, without the need to produce a recombinant vector. A further advantage in this regard is that immunity to recipients of vaccinia-based vaccines, such as those being tested for HIV, may be studied without interference from high background levels of vaccinia-specific killing. We have already demonstrated the expression of infectious whole HIV and individual HIV *env* and HIV *gag* with this system. The low level of HIV *gag* expressed by p220.2gag likely is due to the absence of HIV *rev* on the plasmid, a regulatory element known to be required for high-level *gag* expression (Malim *et al.*, 1990; Malim and Cullen, 1991). Tests of CTL target cells made with this system are currently being performed in our laboratory.

The system we describe here will also be useful for the study of gene therapy for EBV-related tumors. To that end we have constructed specific B-cell targeting ligands and expression vectors with B-cell-specific promoters. *In vivo* human B-cell-specific β-gal reporter gene expression has been demonstrated in SCID mice inoculated with B-LCL and treated with adenovirus/DNA/anti-Ig–polylysine conjugates. The study of regulation of EBV lytic and latent cycles in B-cells may be achieved as well. Transient foreign gene expression in B-LCL is sufficiently durable (up to 17 days) and effi-

cient (up to 67% of cells transformed) that study in some instances may be accomplished without the need to produce stably transformed cell lines.

In summary, efficient, high-level foreign gene expression in B-LCL may be induced with the novel gene transfer system we report here. Potential applications include production of novel antigen-presenting cells and CTL target cells for study of human cellular immunity, gene therapy for B-cell malignancies, and as an aid in the study of EBV genetic regulation.

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