High level transient gene expression in human lymphoid cells by SV40 large T antigen boost

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ABSTRACT

High level transient gene expression in lymphoid cells has always been challenging because of the difficulty to efficiently transfect such cells. This has precluded any attempt to clone cDNA encoding proteins by means of their specific biological function in lymphoid cells. We have developed a very efficient transient eukaryotic expression system analogous to the well-known expression system in COS cells. Firefly luciferase and human CD2 genes were used as reporter genes and cloned into the eukaryotic shuttle vector pCDM8 which contains the strong cytomegalovirus promoter and the SV40 origin of replication for autonomous plasmid replication in permissive host cells that express the large SV40 T Antigen. Co-transfection of the reporter plasmids together with an SV40 T Ag expressing plasmid resulted in the several fold amplification of either the Luc activity or the cell surface expression of the CD2 marker in a transient assay. The level of amplification was dependent on the strength of the promoter used to drive the SV40 T Ag expression and was correlated with the extent of autonomous replication of the reporter plasmid in transfected cells. This highly efficient transient gene expression by SV40 T Ag boost was suitable to several human cell lines, making this system of general interest for expression cloning strategies or other gene transfer application that need high level expression.

INTRODUCTION

The introduction of foreign DNA into eukaryotic cells allows the study of many aspects of molecular biology. Transient expression cloning systems have recently been developed which facilitated the isolation of cDNA encoding cell surface molecules like CD2 (1), CD28 (2), and ICAM-2 (3). The principle of this strategy is the transient transfection of a cDNA library into COS cells, followed by the isolation of cells expressing the gene of interest by means of specific monoclonal antibodies and the recovery of the plasmid DNA contained in the immunoselected cells. This technique relies on two critical points. First the shuttle vector utilized to construct the cDNA library (pCDM8) contains

the strong cytomegalovirus (CMV) promoter for high level transient gene expression in many mammalian cells and the SV40 origin of replication for autonomous plasmid replication in permissive host cells. The most widely used permissive cell line used in this system is the COS cell line. COS cells is a simian endothelial cell line transformed by a replication-defective mutant of SV40 (4). COS cells express the early viral genes, one of which encodes the Large T antigen. The SV40 Large T Ag has been shown to be the sole viral protein required for the autonomous replication of DNA containing the SV40 origin of replication (5); all other factors being provided by the host cell. More recently the same cloning strategy has been developed for use with Chinese hamster ovary cell line (CHO) (6). The availability of such a cloning system in a broad range of different cell types should theoretically enable the cloning of genes either by means of their tissue-specific biological function or by complementation in deficient cells. In addition to cloning purposes, the existence of a very efficient transient expression system could potentially supplant the need for stable transfected cell-lines in screening large panels of site directed mutations or in antisens experiments. The aim of our study was to appreciate whether high efficient transient gene expression in human lymphoid cells could be achieved by means of transient plasmid amplification using the SV40 T Ag boost strategy. To do so, we utilized the firefly luciferase (7) and the human cell surface molecule CD2 (1) as reporter genes which were co-transfected with another plasmid producing SV40 large T Ag.

MATERIAL AND METHODS

Plasmids constructions

Two plasmids with reporter genes were used (Fig.1a). pCD2 (1) contains the human CD2 cDNA in the πH3M eukaryotic expression vector (generous gift of A. Aruffo and B. Seed). pCDM-Luc comprises the 1.8 Kb firefly luciferase gene extracted from pCLuc (generous gift of U. Azan and O. Schwartz, 8) and inserted into the PstI-HindIII cloning sites of the pCDM8 eukaryotic expression vector (1). These two plasmids make use of the human cytomegalovirus (CMV) promoter for high level expression in mammalian cells. In addition, they both include the SV40 origin of replication for autonomous plasmid

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amplification in permissive host cells that express the large T antigen of SV40. pCDM-Luc also contains the polyoma virus (Py) origin of replication. These two plasmids were grown in MC1061/P3 bacteria under tetracycline and ampicilin selection.The tetracycline resistance of the pCDM8 shuttle vector is an important feature for plasmid rescue (see discussion).

Four plasmids expressing the large T antigen of SV40 were utilized (Fig.1b). In pLAS (generous gift of L. Daya-Grosjean, 9) the expression of the SV40 large T antigen is driven by its own promoter. In pLAS the SV40 origin of replication which is adjacent to the early promoter has been disrupted by a 13bp deletion so that this plasmid expresses high levels of T Ag. in mammalian cells but does not replicate in response to the T Ag. pCMV-Tag (6.1 Kb) was constructed by ligating the SV40 large T antigen gene as a 3.4 Kb BglII-EcoRI fragment from pKMT11 (generous gift of R.D. Gerard and Y. Gluzman,10) and the CMV-promoter containing 2.7 Kb HindIII-EcoRI fragment from pCMV-Luc. To construct the pHIV-Tag plasmid (6.8 Kb), the U3R region of the HIV-LTR (700bp) was first cloned into pGem4 resulting in the pHIV-LTR plasmid. Then a 3.4Kb BglII-EcoRI fragment from pKMT11, including a promoterless SV40 T Ag coding region was inserted in the EcoRI-HincII sites of pHIV-LTR. Finally, the pAcTag plasmid (12.7 Kb) in which the SV40 T antigen expression is driven by the human β actin promoter was made by replacing the mouse metallothionein (mMT) promoter of pKMT11 (0.6 Kb KpnI-BglII fragment) with the 5' flanking region of the human β actin gene from pHβApr1-neo (3.9 Kb EcoRI-HindIII fragment) (11). These four plasmids carry either ampicilin or kanamycin (pAcTag) resistance markers. None of them confer the tetracycline resistance (see discussion).

Cell culture

All DNA transfection experiments were performed by electroporation (12) using the Cellject apparatus (Eurogentec). A double pulse was applied to the cells. The first pulse was 1000 volts, 74Ω and 40μF which resulted in a time constant of 800−850μsec. The second pulse was 150V, 74Ω, 2100μF and lead to a time constant of 40msec. All cell lines were cultured in RPMI 1640, 10% FCS and were diluted two-fold with fresh culture medium 18h prior to transfection. Before electroporation the cells were sedimented and resuspended at 12.5 10^6 /ml in culture medium. 0.4 ml of the cell suspension was mixed with the plasmid DNA (1−5 μg), electroporated in cuvettes with a 0.4 cm gap between electrodes and immediately diluted to 1 10^6 cells/ml in culture medium at room temperature. Cultures were returned to 37°C, 5% CO2 for 20 to 72 h.

Luciferase assay

Luciferase assay was performed according to Nguyen et al. (13). Transfected cells were washed twice in ice-cold PBS and resuspended in lysis buffer (LB) containing 25mM Tris-PO4 (pH7.8), 8mM MgCl2, 1mM DTT, 1mM EDTA, 1% Triton X100, 1% BSA, and 15% Glycerol. Cell lysates were clarified from debris by a 5' centrifugation at 800g and stored at 4°C. Fifty μl of extract were mixed with 200μl of LB, 4μl of 20mM ATP and 25μl of 1mM Luciferin (Sigma) in a scintillation vial. After a 10 sec. incubation, light emission was monitored over 1 min. on the standard radioactivity channel of a scintillation counter (Tri-Carb 4000, United Technologies Packard) after disabling the coincidence circuit. Background activity was measured with LB, ATP and luciferin, and ranged

40,000−80,000 cpm. Background values were subtracted from sample values. Dose response study using purified luciferase enzyme (Sigma) revealed a linearity for luc activity up to 8 10^6 cpm (not shown). Any cell extract displaying an activity greater than 5 10^6 cpm was further diluted in LB prior to luciferase activity determination. Light emission determination were done in duplicate. To ensure homogeneity, total protein content was determined using the Biorad protein detection kit and the luciferase activity was finally expressed as cpm/μg.

Immunofluorescence

Immunofluorescence studies were completed on a Facscan (Becton Dickinson) according to standard procedures (14) using FITC-conjugated CD2 monoclonal antibody (Leu 5b), FIT-C-conjugated CD8 mab (Leu 2a) and FITC-conjugated CD19 mab (IOB4).

DNA replication assay

Transfected cells were washed twice in PBS. Dead cells were removed by Ficoll gradient centrifugation and low molecular

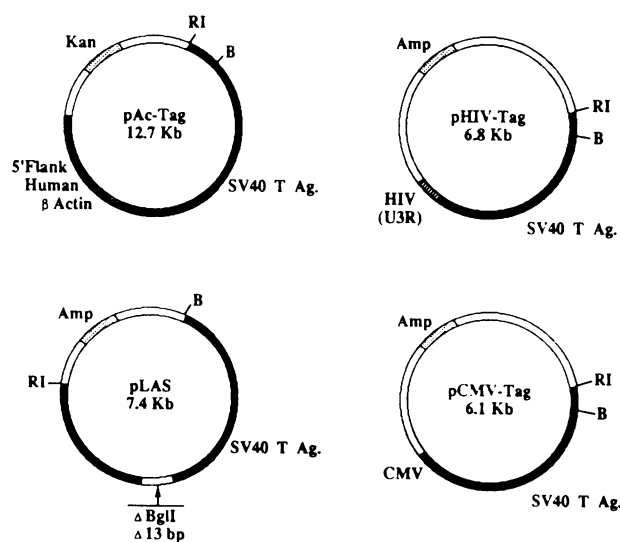## A Reporter Plasmids
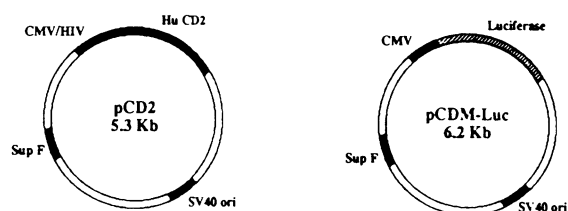
B SV40 T Ag, Expressing Plasmids

Figure 1. Plasmids. (A) Reporter plasmids encoding either the firefly luciferase gene (pCDM-Luc) or the human CD2 T cell marker (pCD2). (B) SV40 T Ag expressing plasmids in which the SV40 Large T Antigen expression is driven by the human β actin promoter (pAcTag), the U3R region of the HIV LTR (pHIV-Tag), the SV40 promoter (pLAS), or the cytomegalovirus (CMV) promoter (pCMV-Tag).

weight extrachromosomal plasmid DNA recovered by the method of Hirt (15). Plasmid DNA was then digested with HindIII alone, HindIII and DpnI, or HindIII and NdeII (MboI), electophoresed on 1% agarose gel, blotted onto nylon membrane and hybridized with ^{32}P labeled probe according to standard procedures (16). The probe was the CD2 encoding cDNA (1.5 Kb XbaI fragment) from pCD2.

RESULTS

Transfection conditions

The luciferase reporter system provides a very easy way to setup transfection conditions in order to get the optimal transient activity. However, the kinetics of maximal luc activity may vary depending on the cells used (17). To determine the best conditions of electroporation we utilized the B cell line Raji and the reporter plasmid pCDM-Luc (Fig 1) in which the luciferase gene expression is driven by the strong CMV promoter for high expression in a variety of eukaryotic cells. For the Raji cell line the peak Luc activity occurred at about 15 hours following the electroporation and dropped considerably thereafter (Fig 2). This early onset of Luc activity has been reported previously (17) and is in contrast with the usual 48 to 72 hours needed for maximum activity in CAT systems. Transfections were achieved by 'double-pulse' electroporation. The rationale behind this technique is that
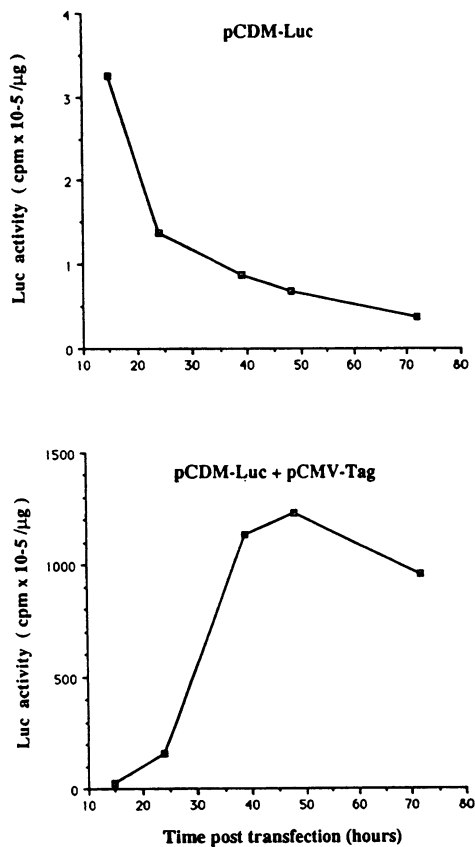

Figure 2. Time course of Luc activity following electroporation. 0.4ml of Raji B cells (12.5 10^6/ml) were electroporated with 1μg of pCDM-Luc alone (A) or together with 2.5μg of pCMV-Tag (B) and Luc activity was assayed at the time indicated. Electroporation conditions were 1000v, 74Ω, 40μF, 800μsec followed by 150v, 74Ω, 2100μF, 40msec.

the first pulse is meant to create holes in the cell membrane while the second pulse allows entry of the DNA into the cell. We found that the optimal voltage for the first pulse was 1000v with a time constant of 800μsec. Lower voltages considerably reduced the transfection efficiency while higher voltages resulted in complete cell mortality. The same was true for the second pulse for which the optimum was found around 150v with a time constant of 40msec.

pCDM-Luc reporter plasmid has been created by inserting the firefly luciferase gene (7) into the mammalian shuttle vector pCDM8. This plasmid includes both the SV40 and the polyoma (py) origin of replication for autonomous replication of the plasmid in cells expressing either the SV40 or the polyoma large T antigen (2). The capacity of the plasmid to replicate together with the very efficient transcription provided by the CMV promoter results in the high efficiency of transient expression in SV40 T Ag expressing cells like the COS cells which constitutively express large amount of SV40 T Ag. High efficiency transient transfection of lymphoid cells (both T and B cells) has always been difficult to achieve (18). We thought that the co-transfection of the reporter plasmid together with a plasmid producing SV40 large T Ag. could result in high level transient expression of the reporter gene. 1μg of pCDM-Luc was co-transfected with 2.5μg of pCMV-Tag and the luc activity was monitored over 72 hours after electroporation (Fig2). In the absence of T Ag. the maximum Luc activity was 3.2 10^{5}cpm/μg. When SV40 T Ag was present the Luc activity reached 1.2 10^8 cpm/μg which represents a 300-fold increase. The presence of T Ag. also modified the kinetic of Luc activity. Whereas Raji B cells transfected with pCDM-Luc alone showed a maximum Luc activity around 15 hours following transfection, the Luc activity was found maximum around 48h post transfection when pCMV-Tag was co-transfected, probably reflecting the time necessary for the pCDM-Luc plasmid replication to occur in response to the T Ag produced by pCMV-Tag.

Effect of different promoters on SV40 T Ag boost

We then tested this amplification model with plasmids that express the SV40 T Ag under different promoters. These plasmids are presented in Fig1. Raji B cells were co-transfected with 1μg of the pCDM-Luc plasmid together with 2.5μg of either one of the four T Ag producing vectors and the Luc activity was determined 72 hours after electroporation. The results are presented in table 1. As expected, the level of Luc activity obtained with pCDM-Luc alone (mean 2.5 10^{4}cpm/μg) was very weak after 72 hours since the optimal activity without amplification is reached 15 hours post transfection (Fig 2). The T Ag driven by the 5' flanking region of the human β actin gene lead to a very minor

Table 1. Effect of different promoter on the ability to induce an SV40 T Ag boost. Raji B cells were transfected with 1μg of pCDM-Luc and 2.5μg of the different SV40 T Ag expressing plasmids. The Luc activity was measured 72 hours after electroporation and normalized to the total protein content.

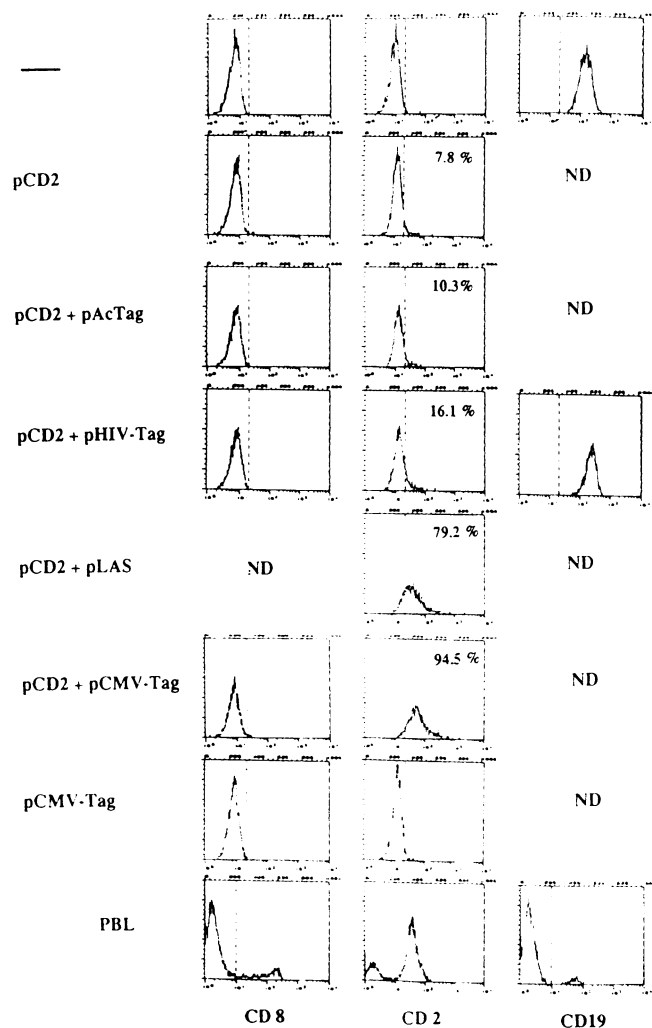| Plasmid | Promoter (Tag) | Luc activity (cpm/μg $\times 10^{-4}$) | | |
		Exp. 1	Exp. 2	Exp. 3
pCDM-Luc	–	2.3	3.7	1.5
pCDM-Luc + pCMV-Tag	CMV	3660	5600	4810
pCDM-Luc + pLAS	SV40	2400	2250	3430
pCDM-Luc + pHIV-Tag	HIV-LTR	650	820	478
pCDM-Luc + pAcTag	Hu. Actin	6.1	15.0	9.9
pCMV-Tag	SV40	0	0	0

Figure 3. Fluorescence analysis of human CD2 transient expression in B cell. Raji B cells were electroporated with 1μg of pCD2 alone or together with 2.5μg of different SV40 T Ag producing plasmids. Cells were harvested 72 hours after transfection and analyzed by flow cytometry on a Facscan for cell surface expression of the CD2 molecule using Leu-5b mab. CD8 (leu-2a) mab was used as negative control and CD19 (IOB4, B cell specific) as positive control. Fluorescence profiles using these three mab on normal human PBL is indicated.

Figure 4. Autonomous replication of pCD2 reporter plasmid. Raji B cells were transfected with 1μg of pCD2 and 2.5μg of SV40 T Ag expressing plasmid. Plasmid DNA was recovered from transfected cells and digested with HindIII alone (lane 1, 3, 5, 7, 10), HindIII and DpnI (lane 2, 4, 6, 8, 11), or HindIII and NdeII (lane 9, 12), the non transfected plasmid pCD2, digested with HindIII was used (lane 13) for size control.

amplification of the luc activity (mean 10^5 cpm /μg) . With the three plasmids utilizing viral promoters to drive the expression of the SV40 T Ag, the CMV promoter resulted in the strongest amplification of the signal (mean 4.7×10^7cpm/μg). The pLAS plasmid in which the SV40 T Ag is controled by its own promoter always gave a strong amplification (mean 2.7×10^7cpm/μg) albeit weaker than with the CMV promoter. Finally the pHIV-Tag plasmid lead to in an intermediate amplification (mean 6.5×10^6cpm/μg). This hierarchy in the amplification capacity of these different plasmids probably represents the level of transient expression of the SV40 T Ag in Raji B cells and is consistent with previous reports that used the CAT assay to show that the CMV promoter is by far the strongest amongst viral promoter both in COS cells and in primary human lymphocytes (19).

CD2 cell surface expression

The luciferase reporter system is a very powerful and rapid assay to test transfection efficiency but does not provide information
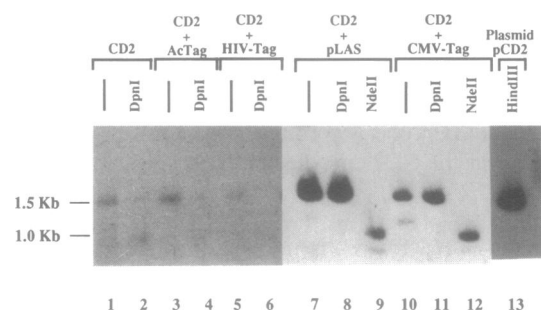
on a one cell basis. Since the amplification in the Luc activity we observed following the SV40 T Ag boost could be the result of either the higher expression of the Luc gene in a given cell or a greater number of cells expressing the transfected gene or both, we used a second reporter plasmid, pCD2 (Fig1), in which the human CD2 encoding cDNA expression is driven by the CMV promoter (1). CD2 is a T lymphocyte specific cell surface molecule present on about 80% of human peripheral blood lymphocytes (PBL) (fig3) but absent on human normal B lymphocytes and the Raji B cell line. Transfection of Raji B cells with pCD2 alone (Fig 3) resulted in only 7.8% of CD2-expressing cells after 72 hours. Moreover, the density of the CD2 molecule on the cell surface was less than the normal expression of this marker on human PBL. The co-transfection of SV40 T Ag expressing plasmids resulted in higher cell surface expression of the CD2 marker. This increase concerned both the number of CD2 expressing cells which reached up to 94.5% with pCMV-Tag as well as the cell surface density of CD2 which became similar to that of PBL. The same difference in the capacity of SV40 T Ag boost was noted among the different plasmids. pLAS (79.2% of CD2+ cells) was almost as competent as pCMV-Tag whereas with the HIV promoter the T Ag boost resulted in only 16.1% positive cells. The pAcTag plasmid (10.3%) did not lead to a significant amplification in this system. The transfection of Raji B cells with the pCMV-Tag plasmid alone did not resulted in any expression of the CD2 molecule. The CD8 T cell specific marker was used as a negative control for fluorescence. From this experiment it appears that the T Ag boost may result not only in the higher expression of the transfected gene within a single cell but may also increase the overall number of positive cells. Indeed, the rise in plasmid copy number due to autonomous replication provides a chance for daughter cells to inherit the transfected gene upon cell division, therefore optimizing the transfection efficiency.

Autonomous replication of the CD2 reporter plasmid

The ability of the different SV40 T Ag expressing plasmids to support the autonomous amplification of the pCD2 plasmid in transfected cells was then assessed by a transient replication assay (fig 4). This assay is based upon the fact that the restriction enzyme DpnI will only be active on DNA which have an adenine methylation pattern characteristic of growth in bacteria and does not cut DNA that replicated in eukaryotic cells. Conversely, the
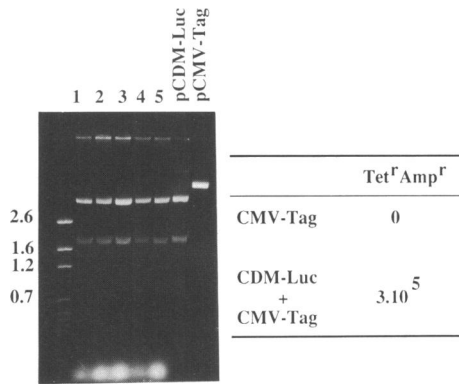
	Tet^{r}Amp^r
CMV-Tag	0
CDM-Luc + CMV-Tag	3.10^5

Figure 5. Rescue of pCDM-Luc plasmid following SV40 T Ag. boost. Raji B cells were transfected with pCMV-Tag or pCMV-Tag and pCDM-Luc, and hirt extract was prepared 48h following transfection and used to transform MC1061/P3 bacteria. A dilution of transformed bacteria were plated under Tet and Amp selection. The number of resistant colonies was determined and corrected to the different dilution factors. Five miniprep DNA from resistant bacteria were analyzed after Xba I digestion and compared to the purified pCDM-Luc and pCMV-Tag Xba I digested plasmids.

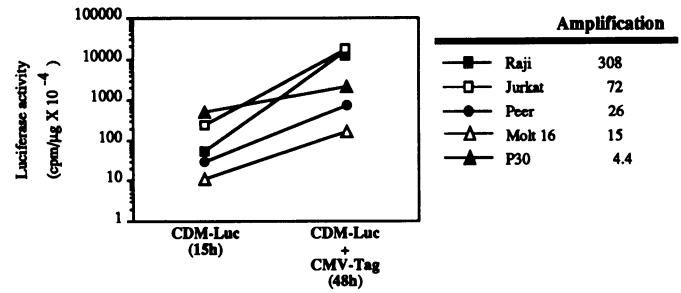| Amplification	
Raji	308
Jurkat	72
Peer	26
Molt 16	15
P30	4.4

Figure 6. Effect of SV40 T Ag boost in different cell lines.Jurkat, Peer, and Molt 16 are human mature T cell line and P30 is an immature T cell tumor. Conditions of elecroporation were identical as those described in figure 2 except that 210 volts were used in the second pulse for the T cells. The Luc activities at 48h following T Ag boost and at 15h without pCMV-Tag plasmid were used to determine the level of amplification.

NdeII enzyme which recognizes the same cleavage site is inhibited by methylation and will therefore only digest unmethylated plasmid DNA that replicated in the eukaryotic cells. The first indication of the autonomous replication of the pCD2 plasmid in transfected cells is given by the fact that in lanes 7 to 12 ten-times less hirt extract have been loaded on the gel compared with lanes 1 to 6 showing that the amount of pCD2 plasmid recovered from pLAS and pCMV-Tag transfected cells is much higher than with the other constructs. Moreover, the recovered pCD2 plasmid was totally refractory to DpnI digestion (lane 8 and 11) while completely cut with the NdeII enzyme (lane 9, 12). This pattern is specific of DNA that has replicated in the transfected cells. On the opposite, the amount of pCD2 plasmid recovered from Raji cells transfected with pCD2 alone was much lower (lane 1), was digested by DpnI (lane 2), and therefore represented input plasmid DNA from bacteria which did not go through autonomous replication in the transfected cells. The sensitivity of this assay did not allow the detection of an autonomous replication of the pCD2 plasmid in cells transfected with either pAcTag (lane 3 and 4) or pHIV-Tag (lane 5 and 6). This is in agreement with the poor amplification noted in both Luciferase activity (Table 1) and cell surface expression of the CD2 marker (fig 3) upon SV40 T Ag boost performed using these two constructs. The SV40 T Ag boost obtained with pLAS and pCMV-Tag lead to a similar extent of autonomous pCD2 replication as judged by the intensity of the bands in fig. 4 (lanes 7 to 12) which are not significantly different. This confirmed the results obtain with the Luc and CD2 assays.

We then tested if the transfected plasmid could be rescued. Raji B cells were transfected with pCMV-Tag or a combination of pCMV-Tag and pCDM-Luc. Hirt extracts were prepared 48h following transfection and used to transform MC1061/P3 bacteria. Transformants were analyzed under Tetracyclin and Ampicilin selection (Fig. 5). As expected the hirt extract derived from pCMV-Tag transfection did not give rise to any Tet^{r}Amp^r bacteria since this plasmid does not harbor the Tet. resistance gene (Fig. 1). In contrast, the hirt from 5.10^6 cells transfected with pCDM-Luc and pCMV-Tag gave rise to 3.10^5 Tet^{r}Amp^r

colonies. Miniprep DNA analysis from these bacteria confirmed the rescue of the pCDM-Luc plasmid only (Fig. 5).

Effect of SV40 T Ag boost in different cell types

We next attempted to extend the T Ag boost strategy to various human cell types, mostly T lymphoid cells which are known to be refractory to transfection (18). Jurkat, Peer and Molt16 are well-characterized mature human T cells whereas P30 is thought to be an immature T cell tumor. These cells are smaller than the Raji B cell line so that the best transfection efficiency was obtained with a 210volts second pulse instead of the 150v used with Raji cells (data not shown). One μg of pCDM-Luc was cotransfected with 2.5μg of pCMV-Tag and the Luc activity was measured 15h and 48h following the electroporation. The maximal luc activity was reached 15h following transfection in the absence of T Ag boost whereas 48h was necessary to get full amplification when SV40 T Ag was present in every cell line tested (data not shown). An amplification was obtained in the five cell lines but the degree of signal enhancement varied from cell to cell with a maximum of 300-fold in Raji and a minimum of 4.4-fold when using the P30 cell line (Fig 6).

DISCUSSION

The high efficient transient gene expression achieved by transfection into COS cells relies upon two critical features of this system. First, the shuttle vectors used contain the origin of replication of SV40 and a strong eukaryotic promoter (CMV for example).Second, the COS cells are transformed by a replicative-deficient mutant of SV40 and therefore constitutively produce the large T antigen of SV40, resulting in the autonomous replication of the shuttle vector and hence the higher level of transient gene expression of the transfected gene. In this report we have extended this method to potentially any human cell type. Studies on the biology of the SV40 virus revealed that the large T antigen is the only viral-encoded protein required for the virus replication to proceed, all the other factors being provided by the infected cells (5). These cellular factors include the DNA polymerase a and the DNA primase (20). Large T Ag. is a DNA binding protein whose function is the DNA unwinding upon specific interaction with its cognate DNA sequence the origin of replication (ori). This constitutes the first step in the DNA replication process. This minimal requirement lead us to test

wether the simple cotransfection of SV40-ori containing vectors together with SV40-Tag expressing plasmids could effectively result in elevated gene expression by SV40 T Ag boost in a transient assay. The utilization of the firefly luciferase gene as well as a cell surface molecule encoding gene as reporter markers established that the T Ag. supplied by cotransfection induced a considerable amplification in the expression of the luciferase reporter gene (300-fold increase with the pCMV-Tag plasmid). Fluorescence studies using CD2 monoclonal antibody revealed that this gain concerned not only the level of expression of the reporter gene within a given cell but also the overall number of expressing cells which could attain up to 95% compared with the 10% positive cells obtained in the absence of T Ag boost. The degree of signal amplification was correlated with the extent to which the reporter plasmid could autonomously replicate within the transfected cell. The ability of plasmid replication in our transient system was itself directly related to the strength of the promoter utilized to drive the expression of the SV40 T Ag., and confirmed previous studies describing the close relationship between SV40 T Ag synthesis and SV40 replication (10). Finally, this system was suitable to various different human cell lines making it of general interest. The SV40 T Ag boost strategy is not directly applicable to other species like the mouse because the murine DNA pol. α/DNA primase complex does not work on the SV40 Ori. (20). However, murine cells are permissive host cells for the polyoma virus (Py) both in vivo (21) and in vitro (20). Since the pCDM8 eukaryotic vector contains not only the SV40 Ori. but also the Py Ori. (1), one can presuppose that the cotransfection of this shuttle vector together with a plasmid expressing the polyoma large T antigen will result in a similar boost, therefore expanding the potential applications of this system.

One of the steps involved in expression cloning strategy calls for the recovery of the extrachromosomal plasmid DNA from the transfected cells, followed by its amplification in bacteria. None of the T Ag-expressing plasmids used in this study includes a tetracyclin resistance gene whereas the pCDM8 shuttle vector confers this drug resistance marker upon introduction into the MC1061/P3 bacterial strain. This last point is important for cloning applications because the T Ag-expressing plasmid rescued together with the reporter plasmid from transfected cells will not be subsequently amplified in bacteria under tetracyclin selection.

The availability of a universal eukaryotic expression cloning system should be a valuable tool for the cloning of new genes by means of their tissue specific biological functions. Among candidates are intracellular proteins which are difficult to purify and for which no sequence information and no antibodies are available but whose effects on the transfected cells can be detected by specific antibodies against the products of the protein's action. One example are the glycosyltransferases which need tissue specific acceptor substrates in order to synthesize the detectable product. Others are multimeric protein complexes in which one subunit can associate with a family of proteins like the integrins and for which antibodies to the whole complexes only are available. Other candidates are the tissue specific regulatory transcriptional factors. This method should also potentially allow the cloning of desease-related genes by complementation in deficient cells. Finally, apart from cloning purposes the high efficient transient gene expression technique presented here could reduce the need of establishing stable transfected cell lines in many situations where a high frequency of transfectant is required.

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