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Microbes and Infection 10 (2008) 396–404

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Original article

A rapid recombination assay of HIV-1 using murine CD52 as a novel biomarker

Jun-ichi Sakuragi*, Sayuri Sakuragi, Masahisa Ohishi, Tatsuo Shioda

Department of Viral Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Received 11 October 2007; accepted 30 December 2007
Available online 9 January 2008

Abstract

Biomarkers are commonly used for verification of infection in conjunction with the development of viral vectors or experiments involving virus infection. Leukocyte surface antigens (CDs) are a prime option for biomarkers since they can be easily visualized and analyzed by flow cytometry after indirect fluorescent staining. For analyses of human cells, murine CD24 (Heat Stable Antigen: HSA) and CD90.2 (Thy-1.2) are currently being used. In the study reported here, we attempted to develop a rapid system for measuring retroviral genome recombination efficiency. For this purpose, we looked for an alternative CD molecule which could be used as a marker on a viral vector concurrently with other markers. We found that murine CD52 is suitable for this purpose because of its small gene size, low inhibitory effect on virus production, and measurable level of surface expression. With this novel biomarker, we succeeded in developing a rapid viral recombination measuring system using a flow cytometer.

Keywords: Retrovector; Biomarker; CD52; Recombination; Flow cytometry

1. Introduction

The use of retroviral vectors for gene delivery has become very common in recent years. To determine the efficiency of gene induction and/or to monitor the fate of induced cells, a variety of selectable markers have been incorporated into retroviral vectors. The genes of products that confer resistance to toxic compounds are widely used from the start of vector development, since their stable expression enables positive selection of cells induced by prolonged treatment with antibiotics [1]. Fluorescent proteins such as eGFP and RFP are widely used as markers because of their high stability, minimal toxicity, and non-invasive detection [2].

Cell surface molecules, such as leukocyte surface antigens (CDs), constitute another type of commonly used selectable marker antigens. Staining cells with fluorescent dye-conjugated antibodies and analyzing them with a flow cytometer enables the rapid and quantitative detection of transferred gene expression in the desired target cells while they are still alive. However, most of the genes used as selectable markers are relatively large, leaving limited space in the retroviral vector for other genes of interest. The human hematopoietic cell surface antigen CD24 and its murine homologue, the heat stable antigen (HSA), are two of the few exceptions because of their relatively small gene size (about 0.24 kb) and potential for cell surface expression. Because of these properties, CD24 and HSA are widely used as biomarkers [3,4]. Another surface antigen, CD90.2 (Thy-1.2), is also in common use as a biomarker although its gene size (488 bp) is larger than that of HSA [5].

To monitor retroviral infection and viral genome recombination in cells, several good vector assay systems have been developed [6,7]. Rhodes et al. described an attractive method using two similar vectors with mutated eGFPs and surface markers to measure their infectivity and recombination rate [8]. These systems are based on the principle that only a single

* Corresponding author. Department of Viral Infections, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita City, Osaka 565-0871, Japan. Tel.: +81 6 6879 8348; fax: +81 6 6879 8347.
E-mail address: sakuragi@biken.osaka-u.ac.jp (J.-i. Sakuragi).

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doi:10.1016/j.micinf.2007.12.016

DNA is formed in an infectious retroviral particle [9]. Although this system promises reasonable and stable results, it requires multiple cell sorting and expansion, so that it takes considerable time and effort to perform. In addition, the vectors carry IRES sequences for marker gene expression, sequences which do not exist in the native retroviral genome. We therefore wanted to modify this recombination assay system since we intended to evaluate HIV-1 genome recombination in a situation more similar to viral physiological conditions and to simplify the experiments. We attempted to generate retrovectors which could express two or more biomarkers at the same time for the development of the recombination assay system. Since it was therefore necessary to find a new biomarker, we explored the database and found that murine CD52 (mCD52), a protein of the CD24/HSA family, might have the characteristics required for our purpose.

2. Materials and methods

2.1. DNA constructs

The replication competent HIV-1 proviral clone pNL4-3 [10] and pMSMBA [11], a derivative of pNL4-3, were used as progenitors for the mutant constructs described below. The HSA gene was amplified from the plasmid pNLrHSAS [12], also a derivative of pNL4-3, the vpr gene of which was replaced with the HSA gene, with a pair of primers (XbaH-SAonF: 5′-TCTAGAGCCGCCATGGGCAGAGCG-3′, and EcoRIHSAstpR: 5′-GAATTCTCTAACAGTAGAGATG-3′). pNLrHSAS was digested with NheI, blunt-ended with a Blunting-High kit (Toyobo, Osaka, Japan), and ligated to generate pNLrHSASNh. The amplified fragment was replaced with the XbaI-EcoRI region of pNLrHSASNh to add a Kozak sequence upstream of the ATG codon of the HSA gene to enhance its expression, and the resultant plasmid was named pNLrH. pNLrH was then digested with HpaI and XhoI, and the HpaI-XhoI fragment of pGEMHnGX [13] including the EGFP gene was inserted in the corresponding position to construct pNLrHnG.

The murine CD52 (mCD52) gene coding fragment was generated by synthesizing three oligonucleotide probes (CD52atg-95: 5′-ATGAAGAGCTTCCTCCTCTTCCTCAC TATCATTCTTCTGGTTGTGATTCAGATACAAACAGGAT CCTTGGGACAAGCCACTACGGCCGCTTCTGG-3′, CD52 cmpl170-116: 5′-GGCACCCGCATCGATGATGGATGAGG CCCCACTCTTTAAGGGGGTTTTTTTGGTGGAGGTGCTG TTTTTGTTAGTACCAGAAGCGGCCGTAGTGG-3′, and CD5 2fwd151-stp: 5′-CCATCATCGATGCGGGTGCCTGCAGTTT CCTCTTCTTTGCCAATACCTTAATGTGCCTCTTCTACC TCAGCTGA-3′) following sequential PCR amplification with two pairs of primers (XbaKozCD52F: 5′-TCTAGAGCCGC CATGAAGAGCTTCCTCCTCTTCC-3′, CD52RevCla: 5′-GG CACCCGCATCGATGATGGATG-3′, CD52FwdCla: 5′-CCAT CATCGATGCGGGTGCCTGC-3′, and CD52RevStp: 5′-TCA GCTGAGGTAGAAGAGGCAC-3′). The 0.23 kb amplified fragment was purified, ligated to the pGEMTeasy vector (Promega, Madison, WI) to construct pGEMmCD52, and verified for its sequence authenticity. pNLrHnG was digested with XbaI and EcoRI, and the XbaI and EcoRI fragment of pGEMmCD52, including the mCD52 gene, was inserted at the corresponding position to construct pNLrCnG. A base substitution mutation was then introduced into the start codon of the eGFP gene of pNLrHnG to eliminate eGFP expression (ATG to TAA) in order to allow for the construction of pNLrHnGΔN. A frame shift mutation of the eGFP gene at the 0.6 kb position from the start codon was introduced as reported elsewhere [8] (mutant pON-H6: one-base substitution and one-base insertion to introduce a stop codon and a frame shift) into pNLrCnG for the construction of pNLrCnGΔC. Furthermore, two-base substitution mutation at the hairpin loop of SL1 (GCGCGC to GTGCAC) was introduced into pNLrCnGΔC to construct SL1MrCnGΔC.

2.2. DNA transfection

293T cells [14] (approximately 3×10^6) were seeded on dishes (diameter 100 mm) the day before transfection with plasmid DNA (total 5 μg) using the calcium phosphate precipitation method [15]. The day after transfection, the supernatant was replaced with fresh medium.

2.3. Virus infection

At 48–72 h post-transfection, the media was centrifuged and the supernatant was used for infection into T-cell lines (MT-4 and M8166).

2.4. RT-PCR assay

Two days after transfection, 293T cells were harvested and total cellular RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA). RNAs were treated with RQ1-DNaseI (Promega) for removal of contaminated DNAs. Reverse transcription (RT) reaction using 5 μg of total RNA with Superscript III (Invitrogen) was performed according to the manufacturer's instructions, and one-twentieth of the RT products was used for the PCR template. Two sets of primer pairs were prepared to detect the mCD52 gene (Forward: 5′-TCTAGAGCCGC CATGAAGAGCTTCCTCCTCTTCC-3′; reverse: 5′-TCAGC TGAGGTAGAAGAGGCAC-3′) and the GAPDH gene (forward: 5′-CCACATCGCTCAGACACCAT-3′; reverse: 5′-GGC AACAATATCCACTTTACCAGAGT-3′). Amplified products were subjected to agarose gel electrophoresis and visualized by means of ethidium bromide staining.

2.5. Flow cytometric analysis

Mock-infected cells, an empty vector, and HSA/eGFP/mCD52/Thy-1.2-infected cell populations in growth medium were first centrifuged and washed twice in PBS(−) supplemented with 10% Blocking One solution (Nacalai Tesque Inc., Kyoto, Japan). Aliquots of the cells were then stained with an anti-mCD52 rat monoclonal antibody (MBL Co. Ltd., Nagano, Japan) for 30 min, washed twice, incubated with Allophycocyanin (APC)-labeled anti-rat Ig polyclonal

antibody (BD Biosciences, San Jose, CA) for an additional 30 min, and washed twice. The cells were then stained with directly conjugated anti-murine HSA-Phycoerythrin (PE) antibody and anti-murine Thy-1.2-biotin antibody (both from BD Biosciences) for an additional 30 min, washed twice, and stained with PerCP-Cy5.5-conjugated Streptavidin (BD Biosciences). After antibody labeling, two further washes in PBS(−) were performed, the last together with 1% formaldehyde to fix the cells. Finally, the cells were analyzed on a FACSCalibur (BD Biosciences).

3. Results

3.1. Generation of mCD52 expressing HIV-1 vectors

To construct multi-marker carrying retrovectors, we initially tried to use the existing biomarkers. Within the HIV-1 genome, the vpr, env, and nef coding regions were replaced with HSA, Thy-1.2, and eGFP genes in various combinations. While the HSA gene was well expressed under any conditions, the Thy-1.2 and eGFP genes performed well only when located within the nef coding region (data not shown). Any vector carrying the Thy-1.2 or eGFP gene in the vpr or env region produced little or no viral particles. Hence, we needed to find a novel marker with approximately the same potential as CD24/HSA and that could be used concurrently with them.

Human and murine CD52s (h/mCD52) belong to a group of very small GPI-anchored sialoglycoproteins which include CD24/HSA and with size and protein properties resembling those of CD24. Human CD52 is abundantly expressed on lymphocytes and monocytes, and is also expressed in non-lymphoid tissue in epithelial cells of the distal epididymal and deferent ducts from which it is transferred to the surface of sperm [16]. The gene size of mCD52 is only 222 bp and encodes 74 peptides (Fig. 1A). A computer search found no significant homology of mCD52 to any known molecules except hCD52 at either the DNA or amino acid sequence level [17]. Although it retains a certain homology to hCD52 within the N- and C-terminal signal region, the amino acid sequence of the mature peptide region of mCD52 is significantly different from that of its human homologue [16]. In addition, it has been suggested that the monoclonal antibody BTG-2G, which is the only commercially available anti-mCD52 monoclonal antibody (mAb) [17], can recognize peptides containing KKTPL [18]. This sequence is unique to mCD52 (Fig. 1A) and thus no cross-reaction of the antibody with hCD52 can be expected. These characteristics suggested to us that mCD52 could be a candidate for a novel selectable marker of gene transfer in human cells. We cloned the mCD52 gene by synthesizing and amplifying its DNA primers and inserting it in place of the vpr gene of HIV-1 to construct various retrovectors. Fig. 1B shows representative schematics of the vectors we constructed for the experiments described below. We confirmed expression of the mCD52 gene by means of an RT-PCR assay (Fig. 1C). A transcription of the mCD52 gene was clearly detected in cells transfected with the vectors carrying the mCD52 gene. The production of the viral antigen from cells transfected with vectors carrying the mCD52 insertion was only moderately reduced compared to that from cells transfected with the wild-type or the vectors carrying HSA (data not shown). This indicated that the effect of mCD52 insertion into the viral genome on virus production was nearly negligible.

3.2. Detection of cell surface expression of mCD52 by mAb

We first used a flow cytometer to verify surface expression of the mCD52 protein and its detection with an mAb. 293T cells were then transfected with pNLrCnG, which carries both the mCD52 and eGFP genes as biomarkers. Two plasmids, pNLNh and pNLrHnG, were used for transfection as parallel controls. Unlike pNLrCnG, pNLrHnG carries an HSA gene instead of an mCD52 gene. Forty-eight hours after transfection, the cells were stained with anti-mCD52 mAb, APC-anti-RatIg antibody, and phycoerythrin (PE)-conjugated anti-HSA mAb. The cells were fixed with 1% formaldehyde-containing PBS(−), and analyzed with a flow cytometer. The expressions of eGFP, HSA, and mCD52 were detected through channels FL1, FL2, and FL4, respectively. The results showed good expression and separation of the three marker genes (Fig. 1D), suggesting that these markers could be utilized concurrently within a cell for discrimination of their expression. Double marker positive cells accounted for about 11% (NLrCnG) and 14% (NLrHnG) of total cells.

3.3. Four-color analysis of transduced cells using three surface markers and eGFP

As expression of mCD52 was clearly distinguishable from the expressions of HSA and eGFP, we attempted to identify gene transductions mediated by multiple retrovectors by means of four biomarkers using all fluorescence channels (FL1–4) of a flow cytometer. For this purpose, the vectors pNLrCnGΔC (NLC) and pNLrHnGΔN (NLH) were prepared. NLC carries the mCD52 gene and NLH the HSA gene, and both carry inactivated eGFP genes. In addition, another surface marker gene, murine Thy-1.2, was employed for analysis. The vector pNLΔBgThy [5] (NLT) carries the Thy-1.2 gene in place of the nef gene, features deletion of the Env gene of pNL4-3 and does not carry the eGFP gene. Biotin-conjugated anti-Thy-1.2 mAb and Avidin-PerCP-Cy5.5 were used for staining and labeling Thy-1.2. The order of cell staining is shown in Fig. 2A. 293T cells were transfected with pCG-VSVG and various combinations of retrovectors. Two days post-transfection, the cells and supernatants were harvested, and the cells were stained with the mAbs and analyzed with a flow cytometer (data not shown). As expected, four biomarkers were detected independently and no cross-reaction was observed. The harvested supernatants were then used for the infection assay with MT-4 cells (Fig. 2B). Similar to the finding for 293T, infection of retrovectors resulted in a satisfactory expression and detection of marker genes with a flow cytometer. The bottom row of the panel in Fig. 2B shows

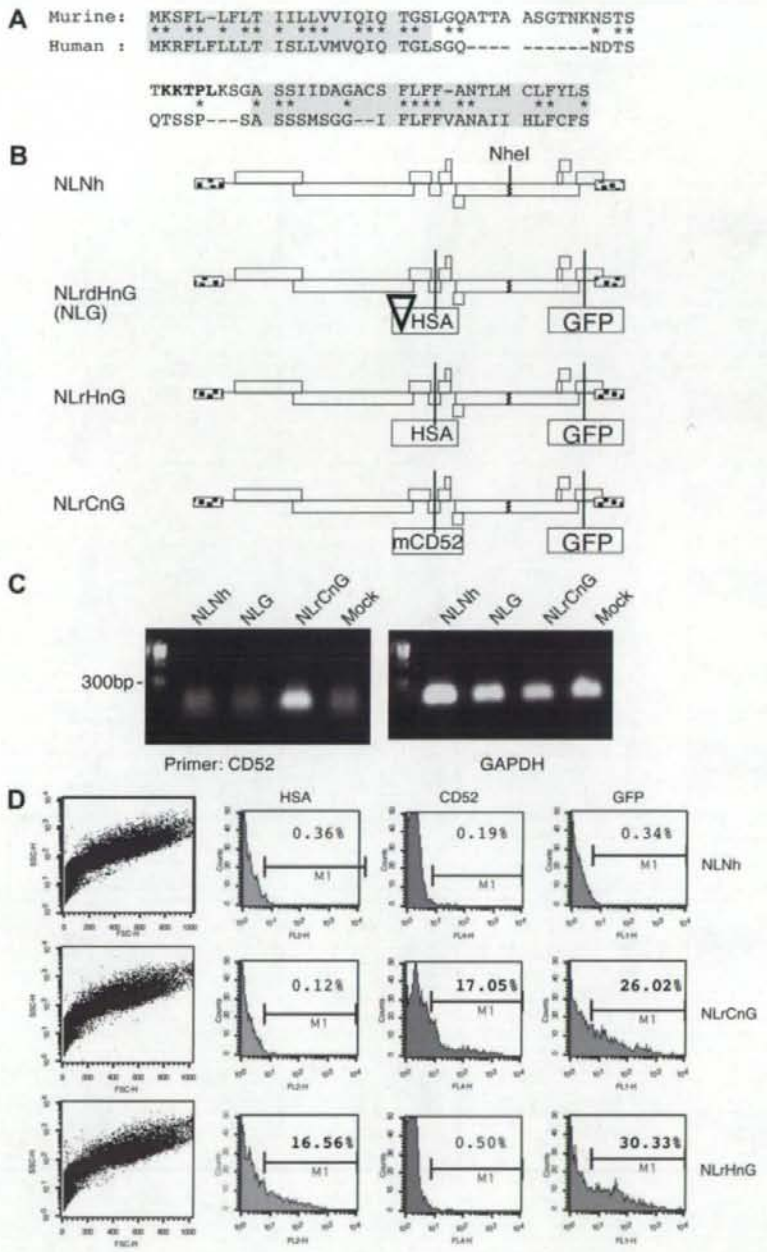

Fig. 1. Construction of murine CD52 expressing HIV-1 vector. A) Comparison of murine and human CD52 protein sequences. N-terminus signal sequences and C-terminus GPI-addition sequences [22] are shaded. The BTG-2G monoclonal antibody recognition sequence [18] are shown in bold. B) Schematic of constructed HIV-1 vectors. Plain boxes represent ORFs of HIV-1 genes and stippled boxes represent LTRs. The env gene in each vector was inactivated by frameshift mutation introduced at the NheI site. The boxes labeled HSA, GFP, and mCD52 represent marker genes inserted in the vectors. The triangle represents the location of mutations introduced to inactivate the gene. C) RT-PCR assays to confirm mCD52 expression. 293T cells were transfected with the plasmids shown in Fig. 1B. Cells were harvested 2 days post transfection and total cellular RNA was extracted. RT products of 5 μg of total cellular RNA were used for PCR amplification. Detection of the expression of the GAPDH gene was used for control. Faint signals for mCD52 in control lanes were non-specific since they also appeared in RT negative controls (data not shown). D) Surface expression of mCD52 molecules caused by transfection. 293T cells were transfected, harvested, and stained with anti-mCD52 Rat monoclonal antibody, APC-conjugated anti-Rat IgG antibody, and PE-conjugated anti-HSA monoclonal antibody. Four charts in one row represent one set of analyses of one sample. Plasmids used for transfection and molecules detected by cytometric analysis are indicated to the left of the rows and above the columns respectively. Percentages of positive cells are shown in each histogram.

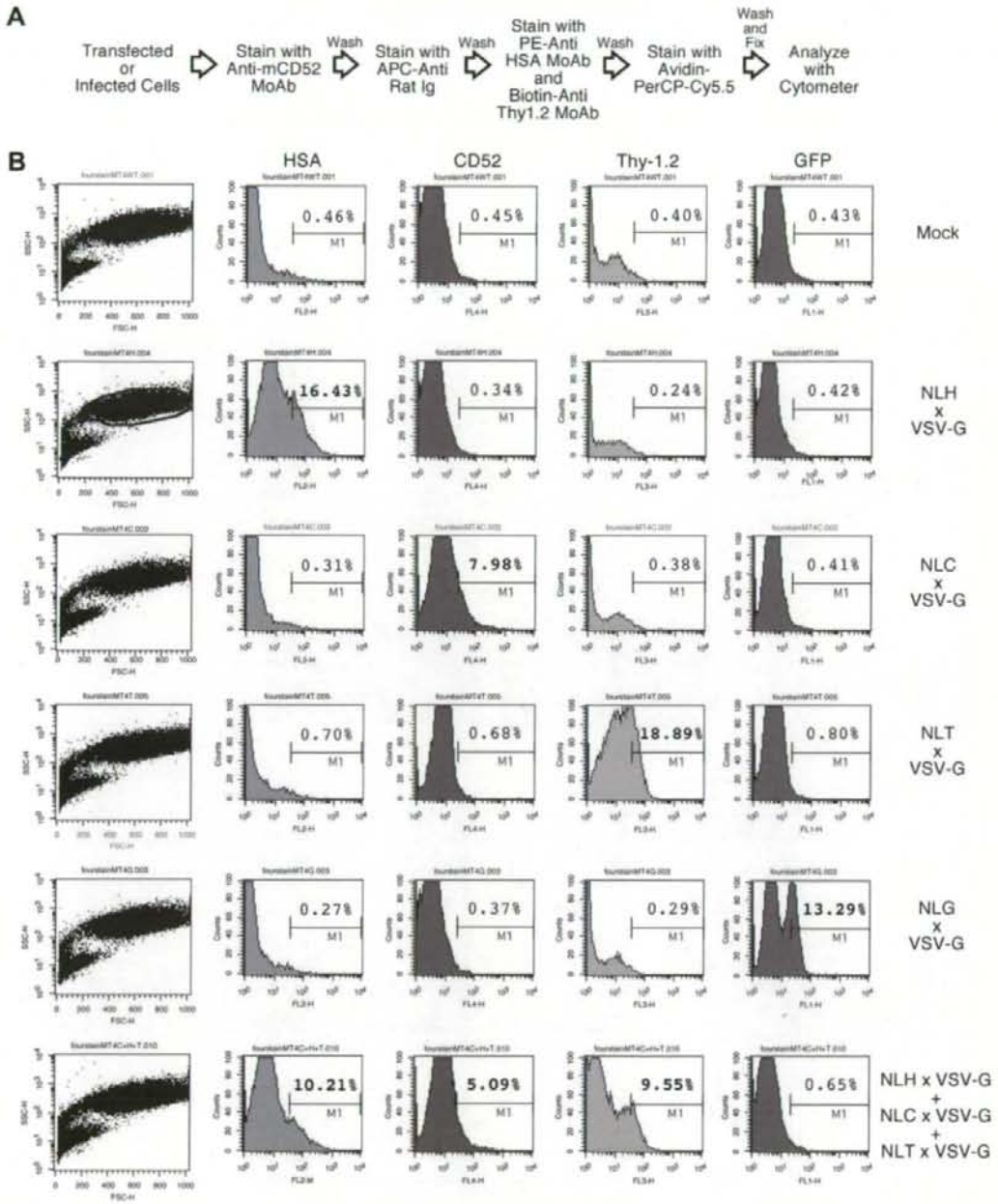

Fig. 2. Simultaneous detection of four biomarkers transduced by retrovectors. A) Flowchart of cell staining of the assay. B) MT-4 cell infection experiment. The supernatants of 293T cells transfected with pCG-VSVG and various retrovectors were collected and used for infection into MT-4. Six charts in one row represent one set of analyses of one sample. The notations are the same as those for Fig. 1D. The "x" between two or three plasmids indicates co-transfection of the plasmids.

that co-infection of the mixture of three vectors resulted in a lack of eGFP expression, thus indicating that no recombination between GFPΔN and GFPΔC from different virus particles had occurred. We therefore proved that these three surface markers could be conveniently leveraged to distinguish the gene induction of different vectors simultaneously, and that their utilization could be expected to have a wide range of applications.

3.4. Construction of HIV-1 genome recombination assay

We then attempted to develop a simple system to monitor retroviral infection and viral genome recombination in cells. The system we designed is shown in Fig. 3. Two similar vectors with the same or different dimerization signals (DLS) were constructed (Fig. 3A) and co-transfected together with or without the VSV-G expression vector (pCG-VSVG) (Fig. 3B). Vector A carries a surface biomarker (Mark-A) and an inactivated eGFP gene with amino-terminal mutation (GFPΔN), while vector B carries another biomarker (Mark-B) and an inactivated eGFP gene with carboxyl-terminal mutation (GFPΔC). After transfection, the released virions can be expected to co-package the homo- or hetero-dimerized vector genome, while the ratio of homo- to hetero-dimerization should be one-to-one if the genome expression efficiency of the two vectors is similar (Fig. 3C). Without VSV-G, the expression of Mark-A and -B indicates transfection efficiency of the vectors, and no eGFP expression should be observed. With VSV-G expression, pseudotyped virions have been observed to cause retro-transduction to the producer cells [13] and the number of marker genes expressing cells in the transfectant increases with an increase in the occurrence of retro-transduction. Recombination of the two vectors can be assumed to occur only in retro-transduced cells, and is monitored in terms of further restoration and expression of the eGFP gene (Fig. 3C). Transfection and retro-transduction efficiency are measured by expression of Mark-A and -B, while the transduction efficiency is estimated by subtracting marker gene expression ratios of the VSV-G negative sample (bA, bB%) from those of the positive sample (rA, rB%). Finally, the recombination efficiency is estimated by calculating the ratio of eGFP expressing cells (rG−bG%) in vector-transduced cells. If we assume that one of the recombination events always occurs between the ΔN and ΔC mutation of eGFPs during reverse transcription, 50% of the recombination events should result in reconstitution of the eGFP gene. In addition, 50% of the virions from doubly transfected cells possess a hetero-dimerized genome, and thus have the potential to reconstitute eGFP. This means that a ratio of eGFP positive cells of 25% in Mark-A or -B positive cells should be the maximum value for recombination. We therefore adopted this maximum ratio for easy indexing by quadrupling the numeric results (Fig. 3C).

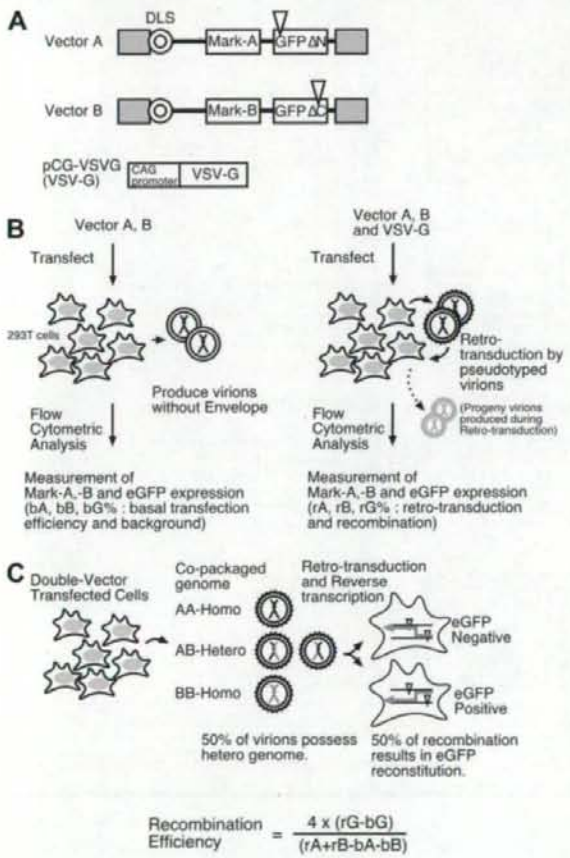

Fig. 3. The system for estimating HIV-1 recombination efficiency using retro-transduction realized by means of pseudotyping. A) Schematics of the vectors planned for the system. Symbols are the same as those for Fig. 1B. Concentric circles represent encapsidation/dimerization signals (E/DLS). B) Experimental design of the system. Without VSV-G, co-transfection of the vectors results in production of non-infectious virions and expression of marker genes (left). With VSV-G pseudotyping, the generated virions infect the cells within the transfected cell culture (retro-transduction), and marker gene expressions are enhanced. C) Estimation of recombination efficiency. Co-transfected cells produce 25% A-A and 25% B-B homo-dimerized genomes, as well as 50% A-B hetero-dimerized genomes containing virions. Fifty % of the opportunity for genome recombination results in reconstitution of the eGFP gene.

We assessed the efficacy of this system by constructing and testing several vectors derived from HIV-1. We found that the combination of vpr substitution with HSA or mCD52 as surface markers and nef substitution with mutated eGFP genes yielded satisfactory results in terms of virion production, infectivity, and marker expression. We also used this system to verify the recombination between the wild-type retrovectors and those derived from a dimerization initiation site (DIS) mutant of HIV-1 (Fig. 4). DIS is located in DLS and it has been suggested that it performs core functions in viral genome recombination [19]. The vectors NLC and NLH carry the same DIS, whereas pSL1MrCnGΔC (SL1MC) carries a two-base substitution on DIS (Fig. 4A), so that heterogenome dimer formation between NL- and SL1M-vectors can be assumed to be reduced. In this experiment (Fig. 4B), 3–5% of all cells were surface marker positive cells in non-pseudotyped samples (NLH × NLC, NLH × SL1MC), which constitutes evidence of the efficiency of transfection and marker expression. eGFP fluorescence was not detected in these samples, confirming that ΔN or ΔC mutation inactivated the functional eGFP expression. In contrast, HSA/mCD52 expression was dramatically enhanced by about 80% in pCG-VSVG co-transfected samples due to retro-transduction. Efficient reconstitution of eGFP was observed in the homo-DIS sample (20% of total cells) whereas only limited eGFP expression was detected in the hetero-DIS sample (4%), thus indicating diminished occurrence of recombination as also reported elsewhere [19]. Recombination efficiency was

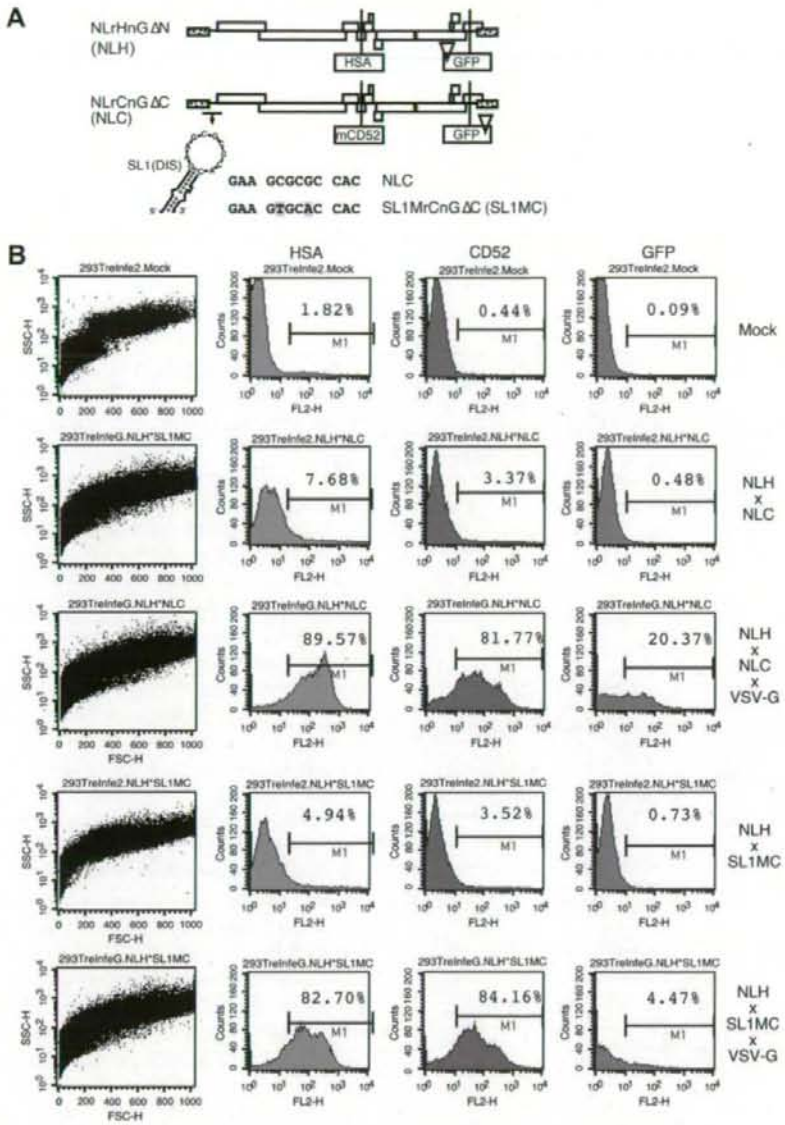

Fig. 4. Detection of HIV-1 recombination. A) Schematic of the vectors actually constructed for the recombination estimation system. Two nucleic acids on DIS of pNLrCnGΔC were substituted for each other (5′-GCGCGC-3′ for 5′-GTGCAC-3′) to construct pSL1MrCnGΔC. B) Genome recombination assay using 293T cells. The notations are the same as those for Fig. 2B.

estimated at 0.5 for NL-NL [4(20.37 − 0.48)/(89.57 + 81.77 − 7.68 − 3.37) = 0.496] and 0.09 for NL-SL1M [4(4.47 − 0.73)/(82.70 + 84.16 − 4.94 − 3.52) = 0.094]. The efficiency of heterogenome dimerization between NL and SL1M mutant estimated with the system devised by us [20] was reduced to about 30% of that of homo-dimerization (data not shown), which suggests there is a parallel interrelation between genome dimerization and recombination. These results clearly showed that our system utilizing retro-transduction is effective and quite practical for evaluation of recombination.

4. Discussion

In this report, we described the practical use of a new reporter gene, mCD52, and the development of a rapid recombination assay system for HIV-1. We focused on the mCD52, which is a protein of the HSA family, since its gene is as small as that of HSA and was assumed to possess similar properties. The results of our study demonstrated that mCD52 can be a useful biomarker since its insertion into the vpr region resulted in its expression from the HIV-1 genome similar to that of HSA.

The recombination assay system we developed is relatively simple, easy and fast. Infection to the transfected cells (=retro-transduction) is the key component of the system, and without it, this system cannot be operated. Retro-transduction is inevitable as far as using the VSV-G protein for pseudotyping, since the VSV-G pseudotyped vector can infect virtually any kind of cell [13]. On the other hand, it is not easy to attain high enough infectivity of the vectors to calculate the recombination rate without VSV-G. We therefore constructed this system with VSV-G by taking advantage of retro-transduction for a quick estimation. For reliability of the calculation, however, the efficiency of retro-transduction is a matter of concern. If the titer of the vectors is too high, multiple infections would occur in a single cell. As the estimate of the recombination rate is based on the assumption of a "single-hit" infection per cell, too high an efficiency of vector production might bias the estimate. In several reports, the recombination frequency of HIV-1 in cultured cells is estimated at about once per 0.9–1 kb of viral genome [6,8]. Our result for the normal recombination rate was 0.5 times per 0.6 kb, so that the estimated frequency is once per 1.2 kb. Since this value represents a lower efficiency than previously reported, it may be the result of multiple infections reflecting a high infection rate (most of the cells in the culture were marker positive as seen in Fig. 4). To prevent biased estimates, a reduction in vector infectivity is needed for this system. In fact, when we reduced the titer of the vector appropriately to minimize "multiple-hit" infection, the calculated recombination ratio was as one event per 0.8 kb, which was very close to the previously published data (data not shown).

There is a possibility that viral genome-derived cDNA and transfected plasmid DNA recombine in retro-transduced cells. We performed an additional experiment to verify this possibility. The 293T cells were cotransfected with the plasmid NLH, pCG-VSVG, and the plasmid carrying only GFPΔC gene. After three-day incubation, we observed no appearance of GFP positive cells in retrotransduced cell culture, whereas a certain number of HSA positive cells appeared (data not shown). This clearly showed that the recombination between the GFPΔN gene derived from viral cDNA and the GFPΔC gene derived from the transfected plasmid was undetectable during retro-transduction. Thus, we believe that plasmid-cDNA recombination is negligible in this system. Like in other established systems [6,8], the recombination rate estimated by our system only reflects that in the nef coding region. By changing the structure of the vectors, it may be possible to further study the recombination rate in regions other than the nef region.

We did not encounter any practical problems such as alterations of cell viability caused by the expression and staining of mCD52. Specific mAb (Campath-1H) treatment of human CD52 has been shown to lead to extensive eradication of CD52 positive cells by complement activation and is thus utilized for bone marrow transplantation therapy [21]. Although our system includes anti-mCD52 mAb treatment, the mAb we used was different from Campath-1H, and the treatment of cells in our study lasts only for a short time prior to cell fixation. In addition, complement components in cell culture media were inactivated by heat inactivation of serum. Although mCD52 seemed to cause no serious defects in the experiment, mCD52 expression may have some deleterious effect under certain conditions such as in *in vivo* experiments. The potential problems associated with wider application of our system thus need to be investigated.

In conclusion, mCD52 constitutes a novel option for a reporter gene which can be leveraged concurrently with other biomarkers. We could demonstrate that mCD52 is a useful marker for transduction by retrovectors, and the utility of this system may be extended to various viral and non-viral gene transfer systems. With this new marker, we developed an easy-to-use HIV-1 recombination assay system, which is expected to be useful for studying and determining the recombination ratios of many viral strains and/or mutants at one and the same time.

Acknowledgements

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labour, and Welfare, and the Health Science Foundation, Japan.

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