expression unit in a reverse orientation were not affected by the anti-HIV transgene (Fig. 3c).

The ability of our lentiviral vectors to transduce the VPS4B dominant negative mutant for inhibition of HIV-1 release was then tested. GeneSwitch-293 cells were infected with vR-VPS4B-KQ and vR-Luc and sorted for hrGFP positive cells. When transduced cells were cultured with mifepristone, induction of transgene expression was seen (Fig. 3d). The inhibitory effect of transgenes on HIV-1 release from transduced cells was examined by transiently transfecting a plasmid DNA producing infectious HIV-1 virions (pNL4-3). In the presence of mifepristone, levels of HIV-1 production from vR-VPS4B-KQ-transduced cells dropped to 53.9 ± 8.5%, approximately half that seen in the absence of mifepristone treatment. However, no significant inhibitory effects on HIV-1 release were observed in vR-Luc or mock-transduced cells (112.3 ± 9.0% and 89.7 ± 3.1%, respectively). This indicated that induced expression of dominant negative mutant VPS4B proteins in transduced cells accounted for the observed inhibition of HIV-1 release. These results demonstrate the utility of our reverse vector to transduce an anti-HIV gene that functionally suppresses HIV-1 release in target cells.

Discussion

The basic principle of current gene therapy is to deliver genetic material to a population of cells in the body, thereby preventing a disease or improving the clinical status of a patient. Although, a key factor in successfully implementing gene therapy is the development of effective vector systems, a number of issues need to be addressed to apply them in a clinical setting. In terms of viral vector systems, one of the major problems is that insertion of cytotoxic or antiviral transgenes adversely affects viral titers during vector production. In this study, we incorporated a mifepristone-inducible gene expression unit into HIV-1-based lentiviral vectors to solve the problem of vector self-inhibition.

Previous studies have reported the delivery of various anti-HIV genes by HIV-1-based vectors in vitro and in vivo [31–36]. Some of the transgenes used in these studies target HIV-1 RNA sequences either directly or indirectly, aiming to inhibit transcription, nuclear translocation, or translation of viral RNA [32–36]. In these types of approach, the problem of self-inhibition can be solved by modifying the nucleotide sequence of a lentiviral vector such that the function of the vector RNA does not interfere with the anti-HIV transgene in producer cells. However, if the transgene targets a fundamental process of the HIV-1 life cycle, such as virion formation, another strategy to avoid self-inhibition is to express the transgene in a regulated manner such that its expression is blocked in producer cells and induced in target cells. This kind of approach would be of value in the transduction of a harmful gene into target cells. The data presented here demonstrate that a lentiviral vector bearing a regulatable gene expression unit is indeed capable of transducing cytotoxic (VSV M) and anti-HIV (VPS4B K180Q) genes into target cells without significant decrease in vector titer (Figs. 2, 3). In addition, induction of anti-HIV genes in transduced cells resulted in approximately 50% inhibition of HIV-1 release (Fig. 3e).

Expression of VPS4B-KQ mutant by transfection has been reported to inhibit HIV-1 release >100-fold [26]; although, the VPS4B-KQ expression induced by our mifepristone-regulatable system produced about 2-fold reduction in HIV-1 production (Fig. 3e). When we looked at the IRES-controlled hrGFP expression in mifepristone-induced cells that had been transduced by vR-VPS4B-KQ or vR-Luc and sorted, hrGFP expressions were only observed in 10.5% (vR-VPS4B-KQ) or 12.2% (vR-Luc) of the cells (data not shown). We speculate that uninducible population of cells was still permissive to HIV-1 production and thus lead to the observed 50% inhibition in the vR-VPS4B-KQ-transduced cells. During expansion of these transduced cells after cell sorting it is possible that some (e.g., gene shut-off) lost their ability to be induced by mifopristone. Besides, the cell sorting step might lead to this issue of lost inducibility. Therefore improving the way to enrich transduced cells should help to alleviate this problem.

To achieve tight regulation of transgene expression, enabling production of infectious vectors, it was necessary to place the mifepristone-inducible gene expression unit in the reverse orientation in the context of the lentiviral vector. Sirin and Park [12] tested the forward and the reverse orientations of a mifepristone-inducible gene expression unit in HIV-1-based lentiviral vectors and reported basal levels of transgene expression that were higher in lentiviral vectors bearing the expression cassette in the reverse orientation than those containing it in the forward orientation. This was in contrast to the findings presented here, where basal expression of the CD14 transgene in reverse vector-infected cells appeared to be lower than that in forward vector-infected cells (Fig. 1d). Similarly to the Sirin and Park [12] study, we used an HIV-1-based SIN vector in which the woodchuck post-regulatory element (WPRE) was inserted into the 3′-untranslated region of the viral genome (Fig. 1a). WPRE has been reported to increase the stability of RNA transcripts, thereby enhancing transgene expression from retroviral and lentiviral vectors [37]. Interestingly, WPRE functions only when placed in the sense orientation of a transgene and antisense WPRE actually shows an inhibitory effect on transgene expression [37]. In our reverse vector, WPRE

was positioned in the opposite orientation to the inducible gene expression unit (Fig. 1a), while the vector designed by Sirin and Park [12] contained WPRE in same orientation as the expression unit. Orientation-dependent elements such as WPRE can thus enhance basal expression of a transgene in both producer and transduced cells. In addition to WPRE, the SIN vector used in our study contained a hybrid 5′-LTR in which the U3 region was replaced with the CMV promoter [27]. We speculate that, in the context of our forward vector, these *cis*-acting sequences should increase background activity of the mifepristone-regulatable gene expression unit without induction, leading to leaky expression of cytotoxic/anti-HIV genes in producer cells and significant loss of vector titers (Figs. 2d, 3c).

One general drawback of regulatable gene expression systems, including the Tet and mifepristone systems, is that they necessitate delivery of two expression units into a target cell; one to express the transactivator and the other to express the transgene in response to the activator. To exclude differences in experimental conditions due to differing levels of transactivator expression, a cell line stably expressing the GeneSwitch transactivator was used as a target cell in this study. While Sirin and Park [12] also described a two-lentiviral vector system in which GeneSwitch and inducible gene expression units were cloned into separate vectors, this type of binary approach would produce populations of singly transduced cells with either transactivator or transgene, resulting in low inducibility. Single-lentiviral vectors bearing the entire regulatable unit have been developed in Tet systems [11, 38, 39]. This single-vector approach would be an attractive option for the mifepristone-regulatable system, bypassing the need for co-transduction of target cells with high amounts of virus. However, RNA virus-based vectors, such as lentiviral vectors, are limited in their cloning capacity for larger genes. Theoretically, lentiviral vectors can accommodate 7–7.5 kb of foreign DNA [1], yet this packaging capacity will be decreased by the insertion of additional regulatory sequences. Improvements to the mifepristone system that would allow incorporation of both transactivator and inducible units into a single-lentiviral vector would be necessary to design a more versatile vector.

The mifepristone-regulatable gene expression system reported here has a number of potential advantages that suit it to gene therapy applications in humans. First, the majority of the system consists of modified human proteins with no impact on cell viability. Second, the induction response is specific and rapid. Third, mifepristone is orally effective and the dose required for induction is within the range acceptable for clinical use [10]. Importantly, mifepristone has been approved by the Food and Drug Administration (FDA) for use in humans. Although, no gene regulatory system has yet been approved by the FDA for clinical use, lentiviral vectors in conjunction with a mifepristone-regulatable gene expression system are a promising step toward achieving successful gene therapy.

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