Differential Expression of Human Immunodeficiency Virus Coreceptors, by CEM, CEM_{VBL100}, and CEM_{E1000} Cells

To the Editor—We read with interest the findings by Speck et al. [1] that, after human immunodeficiency virus (HIV) type 1_{inm} infection, HIV-1 protein and infectious virus production were decreased (70-fold) in CEM cells overexpressing P-glycoprotein (P-gp; VBL_{100} cells) but were increased (50-fold) in CEM cells overexpressing multidrug-resistance protein (MRP)–1 (CEM-VM-1-5 cells), compared with control CEM cells. Extrapolating in vitro–culture findings to HIV-infected patients is difficult. However, we do know that there is a differential expression of P-gp during HIV infection [2] and that the presence of a common single-nucleotide polymorphism in ABCB1 has been related to clinical outcome [3]. The issue of the role of P-gp in viral infectivity [1, 4] is thus of vital importance as we seek to understand the many factors that regulate HIV-productive infection.

HIV-1_{inm} is a T-tropic strain that predominantly uses the CXCR4 coreceptor [5]. Recent reports suggest that HIV coreceptors and HIV-1 fusion are differentially regulated in CEM cells, compared with primary lymphocytes, suggesting that these cells may not be the optimal surrogate for infectivity assays [6]. To investigate the validity of using cell lines that have been selected for drug resistance, we examined the expression of HIV receptors in CEM cells, compared with parental CEM cells, after treatment with the CXCR4 antagonist SB225002. We have also used specific antibodies (2G5 and CCR5-01) to control for nonspecific binding.

Levels of CD8 (n = 6) and CCR5 (n = 4) were not statistically different in VBL_{100} and E_{1000} cells, compared with parental CEM cells (figure 1). However, a statistically lower expression of CD4 was observed in VBL_{100} (8.46 ± 0.71 [mean ± SD]; n = 6; P < .0005; 95% confidence interval [CI] for the difference between the means, 2.74–6.59 [by ANOVA]) and E_{1000} (4.19 ± 0.59; n = 6; P < .0005; 95% CI for the difference between the means, 4.19 ± 0.59; n = 6; P < .0005; 95% CI for the difference between the means, 2.74–6.59 [by ANOVA]) and E_{1000} (4.19 ± 0.59; n = 6; P < .0005; 95% CI for the difference between the means, 2.74–6.59 [by ANOVA]) and E_{1000} (4.19 ± 0.59; n = 6; P < .0005; 95% CI for the difference between the means, 2.74–6.59 [by ANOVA]).
and VBL100 cells. This result illustrates that CXCR4 and CD4 was similar in CEM Speck et al. [1], the surface density of with the parental line. In the study by 1–mediated drug resistance, compared CEM cells selected for P-gp and for MRP-1, expression of HIV receptors by transporter expression. [figure 1). These results clearly illustrate a differential expression of HIV receptors by CEM cells selected for P-gp and for MRP-1–mediated drug resistance, compared with the parental line. In the study by Speck et al. [1], the surface density of CXCR4 and CD4 was similar in CEM and VBL100 cells. This result illustrates that receptor-expression differences between cells selected for overexpression of a particular protein, but cultured under different conditions, may exist. Our findings, together with other reports [6], highlight the limitations of using cell lines as a model for assessing the role of transporters on HIV-1 infection. To allow an accurate correlation of transporter proteins with viral infectivity, infectivity studies must incorporate analysis of HIV-1 receptor expression.

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References

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Reply
To The Editor—The letter by Owen et al. [1] provides a possible explanation for the differences in productive human immunodeficiency virus (HIV)–1 infection that we reported in CEM cells selected to express differing amounts of the drug transporters P-glycoprotein (P-gp) and multidrug-resistance protein–1 (MRP-1) [2]. Their observation is potentially important and will enhance future studies to investigate the clinical relevance of drug-transporter expression in HIV-infected patients.

We agree with Owen et al. that HIV-infectivity studies must incorporate analysis of receptor and coreceptor expression. That is why we performed flow cytometry to quantify CD4 and CXCR4 expression in the 3 cell lines that we studied, using methodology nearly identical to theirs. However, in CCRF-CEM (control), CEM-VBL100 (P-gp), and CEM-VM-1–5 (MRP-1) cell lines, we found similar surface densities of CXCR4 and CD4 [2].

There are a number of possible explanations for the differences in CD4 and CXCR4 expression observed in the cell lines used in our experiments. First, as discussed by Owen et al., similar cells cultured under different circumstances in different laboratories may express proteins in different amounts. Many clones of the CEM cell line exist in different laboratories and may have evolved distinct traits. Second, the E1000 cell line is different from the CEM-VM-1–5 cell line that we used; E1000 was selected using epirubicin, whereas our cell line was selected using teniposide (VM-26) [3]. Using drugs or chemicals to select for transporter overexpression may affect targets other than the intended pro-

Figure 1. Expression of CD4, CD8, CXCR4, and CCR5, in CEM, VBL100, and E1000 cell lines, measured by flow cytometry. Data are presented as mean ± standard deviation of at least 4 experiments. Statistical analysis was performed using analysis of the variance. **P < .01; ***P < .0005.

(7.1 ± 1.2; n = 6; P < .0005; 95% CI for the difference between the means, −3.19 to −1.13 [by ANOVA]) cells, compared with control CEM cells (4.9 ± 0.75; n = 6; figure 1).