Differential Expression of Human Immunodeficiency Virus Coreceptors, by CEM, CEM_VBL, 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 inoculation, HIV-1 protein and infectious virus production were decreased (70-fold) in CEM cells overexpressing P-glycoprotein (P-gp; VBL100 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 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 have examined the expression of HIV receptors in CEM, VBL100, and E1000 cells. The VBL100 cells were analogous to those used by Speck et al. [1] and have been selected for P-gp overexpression with vinblastine [7]. Prior to these studies, Western blot analysis had been used to show a similar expression of MRP-1 and MRP-2 in the parental CEM cell line (data not shown). Similarly, the E1000 cells have been selected for MRP-1 overexpression when epirubicin is used [8], and our experiments confirmed a similar expression of P-gp and MRP-2 in the parental CEM cell line (data not shown).

CD4 and CD8 were quantified in CEM, VBL100, and E1000 cells, by flow cytometry, as described elsewhere [9]. In addition, CXCR4 and CCR5 were quantified using specific antibodies 12G5 and CCR5-01, respectively (obtained from the National Institute of Biological Standards and Control, South Mimms, UK). Prior to incubation, cells were fixed with appropriate antibodies for 1 h at room temperature. After 3 washes in Hank’s balanced salt solution, cells were incubated with fluorochrome (fluorescein isothiocyanate, phosphatidylethanolamine)—conjugated secondary antibodies. Cells were then washed 3 more times, before being resuspended in 500 μl of CellFix. For flow-cytometric analysis, the forward and side scatters of the cells were measured simultaneously on an EPICS-XL flow cytometer (Coulter Electronics), and the lymphocyte population was electronically gated to exclude debris. The fluorescence of the cells was plotted against the number of events. The data were registered on a logarithmic scale, and the median fluorescence was calculated. An isotype control antibody was used for each of the antibodies, and the median fluorescence was deducted to control for nonspecific binding. Analysis of variance (ANOVA) was used to determine receptor-expression differences between the 3 cell lines.

Levels of CD8 (n = 6) and CCR5 (n = 4) were not statistically different in VBL100 and E1000 cells, compared with parental CEM cells (figure 1). However, a statistically lower expression of CD4 was observed in VBL100 (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 E1000 (4.19 ± 0.59; n = 6; P < .0005; 95% CI for the difference between the means, 7.01–10.86 [by ANOVA]) cells, compared with control CEM cells (13.13 ± 2.55; n = 6; figure 1). Furthermore, a differential expression of CXCR4 was observed in VBL100 (3.5 ± 0.32; n = 6; P < .01; 95% CI for the difference between the means, 0.45–2.52 [by ANOVA]) and E1000

M. Resti, C. Azzari, L. Galli, M. De Martino, and A. Vierucci
Department of Pediatrics, University of Florence, and Pediatric Hospital A. Meyer, Florence, Italy

References

Reprints or correspondence: Dr. Massimo Resti, Dept. of Pediatrics, Via Luca Giordano 13, 50132 Firenze, Italy (m.resti@meyer.it).

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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 variance. **$P<.01$; ***$P<.0005$. $(7.1 \pm 1.2; n = 6; P < .0005; 95\%$ CI for the difference between the means, $-3.19$ to $-1.13$ [by ANOVA]) cells, compared with control CEM cells $(4.9 \pm 0.75; n = 6$; 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 cellular entry. To allow an accurate correlation of transporter proteins with viral infectivity, infectivity studies must incorporate analysis of HIV-1 receptor expression.

Andrew Owen, Becky Chandler, Jenny Ford, Saye Kho, and David Back
Pharmacology Department, University of Liverpool, Liverpool, United Kingdom

References

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Reprints or correspondence: Dr. Andrew Owen, Pharmacology Research Laboratories, University of Liverpool, Block H, First Floor, 70 Pembroke Place, L69 3GF, Liverpool, United Kingdom (aowen@liv.ac.uk).

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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-VIM-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-VIM-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-