Expression of breast cancer resistance protein in peripheral T cell subsets from HIV-1-infected patients with antiretroviral therapy

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Abstract. The aim of the present study was to investigate the expression of breast cancer resistance protein (BCRP) in peripheral T cell subsets of human immunodeficiency virus 1 (HIV-1)-infected patients, and to analyze the association between the levels of BCRP expression and disease progression in HIV-1 infection. Peripheral blood mononuclear cells (PBMCs) were obtained from HIV-1-infected patients (n=118), including 92 patients with antiretroviral therapy (ART) and 26 patients without a history of ART. Control samples from 30 healthy donors were also analyzed. The expression levels of BCRP in T cells were evaluated by flow cytometry. A high inter-individual variability was observed in $CD4^+$ and $CD8^+$ T cells in the HIV-1-infected patients and healthy donors; however, the analyzed expression levels of BCRP were significantly higher in the HIV-1-infected group with ART than those in the group with no history of ART ($P<0.01$). Furthermore, the frequency of BCRP-expressing T cells was inversely correlated with $CD4^+$ and $CD8^+$ T cell counts in HIV-1-infected patients with ART. The results suggested that BCRP expression varied among HIV-1-infected patients and healthy donors but was significantly higher in HIV-1 patients undergoing ART. In conclusion, the present study suggested that overexpression of BCRP may be involved in disease progression of the HIV-1 infection and may participate in drug resistance to ART, thus contributing to the failure of highly active ART in HIV-1 therapeutics.

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Introduction

The adenosine triphosphate (ATP)-binding cassette (ABC) superfamily is a large family of highly conserved membrane proteins that transport a wide variety of substrates across the cell membrane. Overexpression of proteins which belong to the ABC family of transporters appears to cause cellular drug resistance (1). In this context, the most extensively studied members of these transporters are P-glycoprotein (P-gp) and multidrug resistance-related proteins (MRPs/ABCCs). Aside from P-gp and MRPs, which have been well analyzed and characterized, several studies have demonstrated that other drug transporters may also be important for cellular drug resistance (2). The breast cancer resistance protein (BCRP), which was isolated initially from the multidrug-resistant (MDR) human breast cancer cell line MCF-7/AdrVp is a protein that consists of 655 amino acids with a molecular weight of 72.6 kDa, which is encoded by the BCRP gene located on chromosome 4q22 (3). The BCRP protein exhibiting ATP-dependent drug efflux in the absence of P-gp or MRP-1 expression was revealed to be identical to the mitoxantrone resistance protein (MXR) and the placental ABC protein (ABCP), and was demonstrated to be associated with drug transport and multidrug resistance. BCRP is a half transporter that functions as a dimer and confers multidrug resistance to topotecan, mitoxantrone, doxorubicin and other associated compounds by ATP-dependent drug extrusion (4). BCRP expression is not confined to breast cancer cells; it is also detected in other tumor types and in several normal tissues, including in the apical membrane of placental syncytiotrophoblasts, the luminal membrane of the small and large intestines, the liver canalicular membrane, the ducts and lobules of the breast, the brain microvessel endothelium, lymphocytes, haematopoietic stem cells and at the blood-brain barrier (5-9). The overlapping localization of BCRP with P-gp suggested that BCRP may also be involved in pharmacokinetic drug-drug interactions. This hypothesis was verified in several earlier studies of antiretroviral drug treatment of human immunodeficiency virus 1 (HIV-1) infection (10-12).

Table I. Characteristics of participants.

Characteristic	HIV-1-infected antiretroviral-treated (n=92)	HIV-1-infected untreated (n=26)	HIV-1-uninfected (n=30)
Age, years	35 (22-55)	31 (24-46)	32 (21-42)
Female, no. (%)	18 (19.6)	6 (23)	12 (40)
$CD4^+$ T cell count, cells/mm^3	217 (3-615)	91 (6-231)	846 (611-1,215)
$CD8^+$ T cell count, cells/mm^3	409 (28-1,030)	218 (107-380)	1061 (372-3,494)
Plasma HIV RNA levels, log10 copies/ml	2.2 (1.6-4.2)	4.0 (3.3-5.5)	-

Data are median (interquartile range) values. HIV, human immunodeficiency virus.

In the treatment of HIV-1 infection, highly active antiretroviral therapy (HAART) consists of a combination of several antiretroviral drugs of different classes. The complexity of antiretroviral drug regimens and the need for additional drugs to treat co-morbidities increases the risk of drug-drug interactions in HIV-1 patients. The role of BCRP in the interactions between antiretroviral drugs remains elusive. Inhibition of BCRP by antiretrovirals may increase the toxicity of drugs *in vivo* and, as a result, decrease the effectiveness of HAART. Although preliminary investigations revealed that BCRP is also expressed in peripheral blood mononuclear cells, there are currently no studies assessing BCRP expression levels in $CD4^+$ and $CD8^+$ cells of HIV-1 patients undergoing antiretroviral treatment with drugs including nucleoside reverse transcriptase inhibitors (NRTIs). It is possible that the interaction of NRTIs with BCRP may reduce intracellular drug concentrations *in vivo*, resulting in an insufficient suppression of HIV-1 replication and making NRTIs less effective and bioavailable. Therefore, the present study was designed to measure the prevalence of BCRP expression in peripheral T cell subsets in HIV-1-infected patients, and to analyze the associations among the levels of BCRP expression, the efficacy of ART and the disease progression of HIV-1 infection.

Materials and methods

Patients. Patients that fulfilled the standard criteria to commence antiretroviral therapy (ART) were recruited into the study. The present study was approved by the ethics committees of Lanzhou General Hospital (Lanzhou, China) and Tangdu Hospital (Xi'an, China). Written informed consent was obtained from all the patients and the subjects consented to the study following full explanation of what was involved. Participants included 92 HIV-1-infected patients undergoing ART and 26 HIV-1-infected patients without a history of ART, who were consecutively treated at our hospital. Of these patients, 74 were male and 18 were female, with a mean age of 35 years (range, 22-55 years). All patients were hospitalized or followed up in our unit. Patients with ART commenced treatment with oral triple ARTs, which included zidovudine (AZT; 300 mg twice daily), lamivudine (3TC; 300 mg daily) and efavirenz (EFV; 600 mg daily). Following informed consent, peripheral blood was collected from the patients. On the basis of their treatment histories, patients were divided into two groups: HIV-1-infected patients with ART and HIV-1-infected patients without ART. Samples from 30 healthy blood donors were also included in the study as the controls (Table I).

Measurement of T lymphocyte counts. The T lymphocyte subsets of all three groups were measured by flow cytometry (FACScan; Becton-Dickinson, Cowley, Oxford, UK). Absolute $CD4^+$ and $CD8^+$ T lymphocyte counts were calculated from the white blood cell count. All measurements were conducted using the same flow cytometer and sample preparation over the duration of the study.

Virological assessment. Viral load measurements were batch tested at the end of the study on plasma samples stored at -80˚C. For the detection and quantitation of HIV-1 RNA in the plasma, the COBAS® TaqMan 48 analyzer (Roche Diagnostics, Mannheim, Germany) was used in a blinded fashion, according to the manufacturer's instructions. RNA was extracted from 500 μl plasma through a generic manual specimen preparation based on nucleic acid binding to glass fibers, and then the absorbed RNA molecules were eluted with an aqueous solution. The COBAS TaqMan 48 analyzer calculated the HIV-1 RNA titer in the test specimen based on the HIV-1 signal and provided the HIV-1 quantitation standard signal and lot-specific calibration constant. Reported values for the upper limit of quantitation (ULQ) and the lower limit of quantitation (LLQ) were 10,000,000 (7 log10) copies/ml and 40 (1.6 log10) copies/ml, respectively.

Isolation of PBMCs. PBMCs were isolated from venous blood samples and separated on Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO, USA) by density gradient centrifugation. Following isolation, the PBMCs were immediately cryopreserved in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and 10% dimethyl sulfoxide (Merck, Amsterdam, The Netherlands) and stored at -196˚C. The PBMCs were thawed with a step-by-step gradual dilution method as described previously (13). Cell viability was >90% as assessed by trypan blue exclusion.

Determination of BCRP mRNA by quantitative polymerase chain reaction (qPCR). Total RNA from $3x10^6$ PBMCs was extracted and purified using an RNeasy Mini kit (Qiagen,

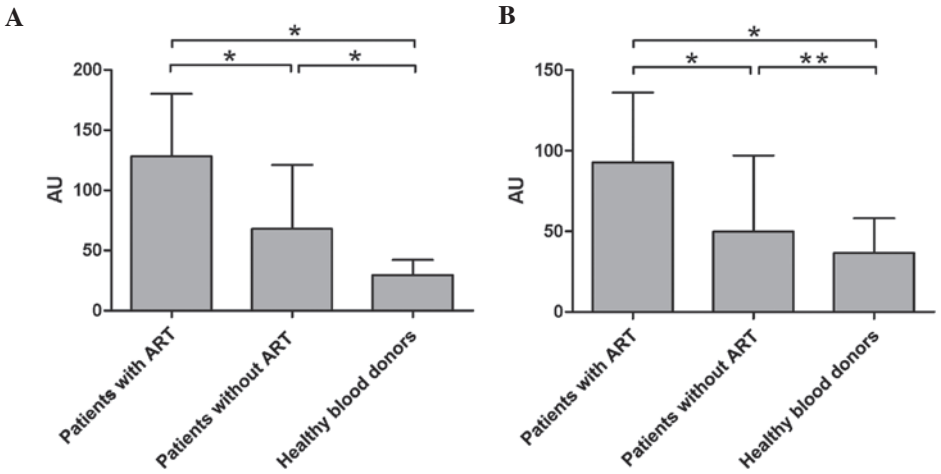

Figure 1. Transcript levels of BCRP determined by quantitative polymerase chain reaction in HIV-1-infected patients with ART, HIV-1-infected patients without ART and healthy donors. (A) Expression of BCRP mRNA on circulating CD4 T cells. (B) Expression of BCRP mRNA on circulating CD8 T cells. The expression of BCRP mRNA within $CD4^+$ and $CD8^+$ T cells increased in HIV-1-infected patients with ART and without ART. Values (AU) are expressed as the amount of transporter mRNA/amount of GAPDH mRNA ($x10^{-3}$). Values are presented as the mean ± standard deviation. The Mann-Whitney U test was used to compare differences among groups. Statistically significant differences between the groups are present as indicated; $^*P<0.01$ and $^{**}P=0.028$. BCRP, breast cancer resistance protein; HIV-1, human immunodeficiency virus 1; ART, antiretroviral therapy; AU, arbitrary units.

Hilden, Germany) according to the manufacturer's instructions. For first strand cDNA synthesis, 0.5 μg total RNA with 1 μl oligo (dT) 18 primer was incubated with 2 μg 10 nM dNTP mix, 20 U RNase inhibitor, 4 μg 5X reaction buffer and 200 U M-MuLV reverse transcriptase (RT; Revert Aid First Strand cDNA Synthesis kit; Fermentas, Hanover, MD, USA) in a total volume of 20 ml for 1 h at 42˚C. Negative controls were performed by replacing the enzyme with water.

Expression of mRNA of the BCRP was performed by qPCR in an Applied Biosystems 7500 qPCR system (Foster City, CA, USA) using SYBR-Green detection kit (SYBR Premix Ex Taq II ; Takara Bio Inc., Otsu, Shiga, Japan). GAPDH was quantified as an internal control. The PCR was performed in 50 μl solution, consisting of 4.0 μl cDNA sample, 25.0 μl 2X SYBR Premix Ex Taq II (including Taq DNA polymerase, reaction buffer and deoxynucleotide triphosphate mixture), 1.0 μl ROX reference dye, 16.0 μl ddH_2O and 2.0 μl of each primer (10 μM). The BCRP primers for qPCR were as described previously (14). Cycling parameters were as follows: 30 sec at 95˚C, followed by 40 cycles of 5 sec each at 95˚C and 1 min at 60˚C, and then by 15 sec at 95°C, 1 min at 60˚C and 15 sec at 95˚C. Relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method (15), which represents the fold change in gene expression, normalized to a housekeeping gene (GAPDH) and relative to the samples with the lowest mRNA expression. All samples were amplified in triplicate.

Expression of BCRP by flow cytometry. To detect BCRP expression, flow cytometric analysis was performed on the stored PBMC samples using fluorochrome-conjugated antibodies specific for the surface markers CD3, CD4, CD8 and BCRP. PBMCs (~$1x10^6$) diluted with 2 ml of phosphate-buffered saline (PBS) with 1% FBS were transferred to 5 ml sterile tubes. The cells were harvested at 300 g for 10 min at 4˚C. The following antibodies were used for staining: anti-CD3-peridinin chlorophyll protein (Percp) (BD Biosciences, San Jose, CA, USA), anti-CD4-phycoerythrin (PE) (BD Biosciences), anti-CD8-allophycocyanin (APC) (BD Biosciences) and mouse anti-BCRP fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (no. MAB4155F; Millipore, Billerica, MA, USA). The cells were incubated and stained at 4˚C in the dark for 30 min and were then analyzed with a four-color FACSCalibur analyzer (BD Immunocytometry Systems, San Jose, CA, USA). Acquisitions were performed with CellQuest Pro software (BD Immunocytometry Systems) and analysis was performed with FlowJo software version 8.6 (Tree Star Inc., Ashland, OR, USA). Isotype control antibodies were used to separate positive and negative cells in the FITC, Percp, PE and APC fluorescence channels.

Statistical analysis. Data are expressed as the mean ± standard deviation (SD) or number (%). Mann-Whitney U and χ^2 tests were used to compare differences among the study groups. Spearman correlation was conducted to assess the association between the frequency of BCRP-expressing T cells and the other indicated parameters. A value of $P<0.05$ was considered to indicate a statistically significant difference between values. All statistical analysis was performed with SPSS 16.0 for Windows software (SPSS, Inc., Chicago, IL, USA).

Results

Expression of transcripts for BCRP. Relative mRNA levels of the BCRP gene in the HIV-1-infected patients with ART, HIV-1-infected patients without ART and healthy donors are illustrated in Fig. 1. The BCRP gene was detected in all groups analyzed. The expression levels of BCRP mRNA in HIV-1-infected patients without ART and healthy donors were low and significantly reduced compared with those in the group of HIV-1-infected patients with ART. Analysis of the clinical and molecular characteristics of the patients

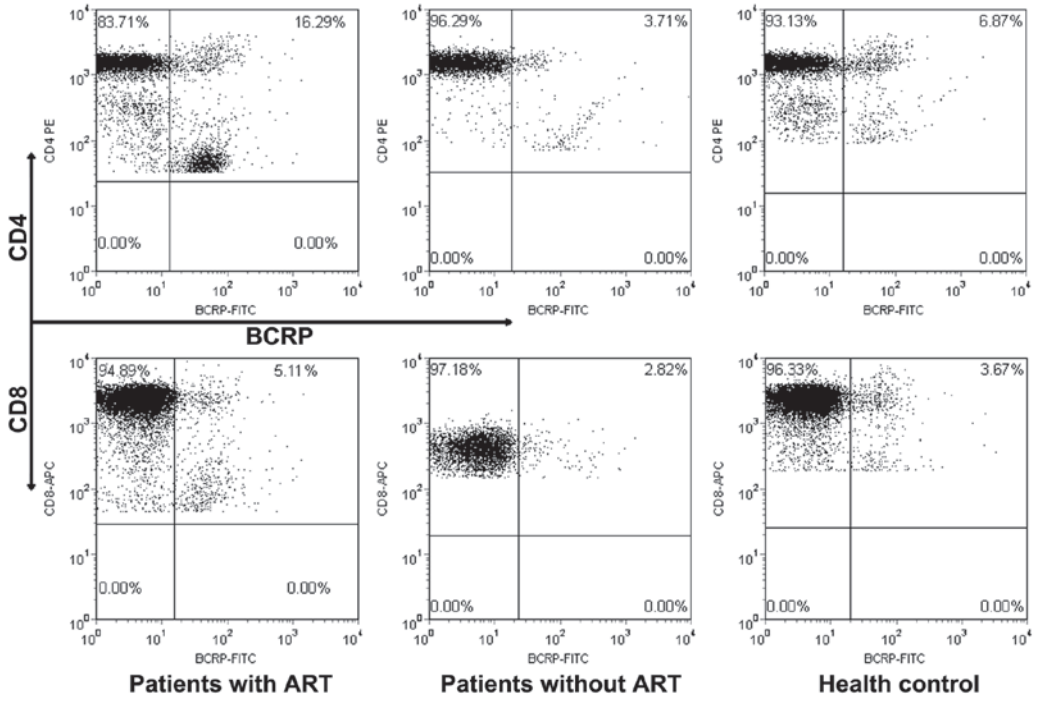

Figure 2. BCRP expression on peripheral T cell subsets obtained from control subjects and patients with HIV-1 infection. PBMCs from HIV-1-infected patients with ART, HIV-1-infected patients without ART and healthy blood donors were stained with directly conjugated antibodies against CD3, CD4, CD8 and BCRP. An isotype-matched antibody was used as a negative control. For each sample, 10,000 events were collected. BCRP, breast cancer resistance protein; HIV-1, human immunodeficiency virus 1; PBMCs, peripheral blood mononuclear cells; ART, antiretroviral therapy.

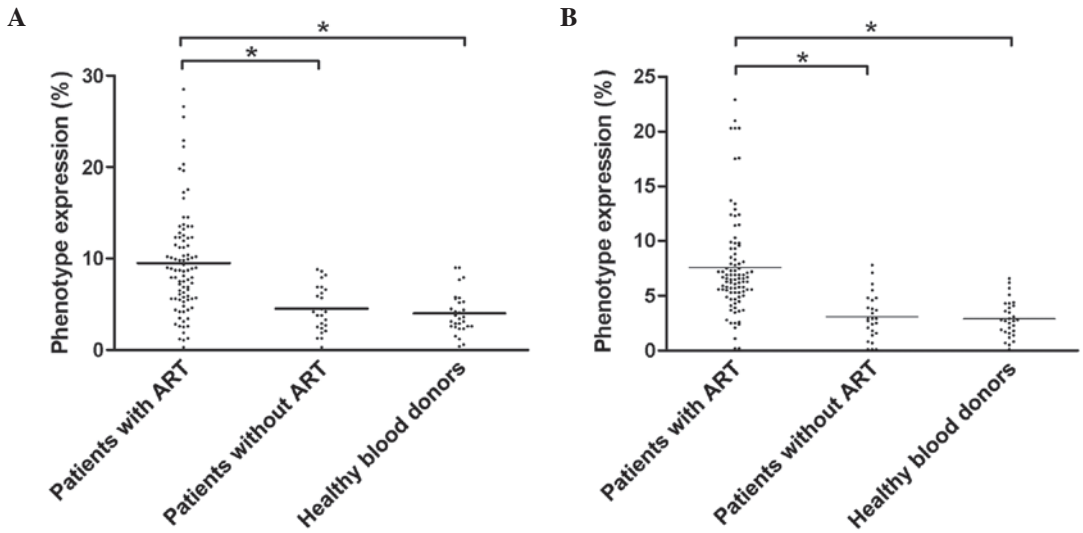

Figure 3. Elevated BCRP expression levels in circulating CD4$^+$ and CD8$^+$ T cells in HIV-1-infected patients. (A) Phenotype expression of BCRP on circulating CD4$^+$ T cells. PBMCs from normal controls, HIV-1-infected patients with ART and HIV-1-infected patients without ART were stained with antibodies against CD3, CD4 and BCRP. An isotype-matched antibody was used as a negative control. (B) Phenotype expression of BCRP on circulating CD8$^+$ T cells. PBMCs from the above patients were stained with antibodies against CD3, CD8 and BCRP. The percentages of BCRP cells within CD4$^+$ and CD8$^+$ T cell populations were increased in HIV-1-infected patients with ART. Each dot represents an individual data point and the horizontal lines represent the mean. The Mann-Whitney U test was used to compare the differences among groups (*P<0.01). BCRP, breast cancer resistance protein; HIV-1, human immunodeficiency virus 1; PBMCs, peripheral blood mononuclear cells; ART, antiretroviral therapy.

showed that BCRP expression was not correlated to age, gender or initial white blood cell count.

Expression of BCRP in CD4$^+$ T cells of HIV-1-infected patients. The expression of BCRP was assessed in 92 HIV-1-infected patients with ART, 26 HIV-1-infected patients without a history of ART and all 30 samples obtained from healthy donors (Fig. 2). As demonstrated in Fig. 3A, a high inter-individual variability was observed in the samples from all three groups, with all coefficients of variation (CV) being >50%. However, the extent of the variability was greatest in HIV-1-infected patients with ART (60.13%).

Despite the evident wide variability in all three groups, the analyzed expression levels of BCRP were significantly higher in HIV-1-infected patients with ART than those in HIV-1-infected patients without a history of ART (P<0.01) and

in the healthy donor group (P<0.01). The mean values (±SD) of the expression levels of BCRP were 9.52±5.73 (median, 8.8; range, 0.3-28.5) in the HIV-1-infected patients with ART; 4.52±2.45 (median, 4.2; range, 0.3-8.8) in the HIV-1-infected patients without a history of ART and 3.99±2.66 (median, 3.5; range, 0.4-9) in the healthy donor group.

Expression of BCRP in CD8⁺ T cells of HIV-1-infected patients. The expression of BCRP was measured in all of the collected samples (Fig. 2). As demonstrated in Fig. 3B, a high inter-individual variability was observed in samples from all three groups, with all coefficients of variation (CV) being >50%. The extent of the variability was also the greatest in HIV-1-infected patients without ART (67.59%). For HIV-1-infected patients with ART and for healthy donors, the variability detected for BCRP was 59.75 and 57.55%, respectively.

With a wide variability in all three groups, the expression levels of BCRP analyzed were significantly higher in HIV-1-infected patients with ART than those in HIV-1-infected patients without a history of ART (P<0.01) and in the healthy donor group (P<0.01). The mean values (±SD) of expression of BCRP were 7.58±4.53 (median, 6.6; range, 0.2-22.9) in HIV-1-infected patients with ART; 31±2.09 (median, 2.9; range, 0.1-7.8) in the HIV-1-infected patients without history of ART and 2.91±1.68 (median, 2.75; range, 0.1-6.6) in the healthy donor group.

Correlation between BCRP⁺CD4⁺T cells and CD4⁺ T cell count. To investigate whether the increase in the expression levels of BCRP correlated with CD4⁺ T cell counts, the CD4⁺ T cell counts from the recruited individuals were assessed. Due to the limited availability of material, it was not possible to evaluate the T cell counts for all patients in three groups. However, CD4⁺ T cell counts were detected in 39 samples of HIV-1-infected patients with ART, 18 samples of HIV-1-infected patients without a history of ART and 25 samples obtained from healthy donors. Spearman analysis revealed that there was a significant inverse correlation between the expression levels of BCRP and CD4⁺ T cell counts in HIV-1-infected patients with ART (Fig. 4; r=-0.4506, P=0.004). However, there was no correlation between BCRP⁺CD4⁺T cells and CD4⁺ T cell counts in HIV-1-infected patients without ART (Fig. 5) and healthy blood donors (Fig. 6).

Correlation between BCRP⁺CD8⁺T cells and CD8⁺ T cell count. To investigate whether the increase in the expression levels of BCRP correlated with CD8⁺ T cell counts, the CD8⁺ T cell counts in the recruited individuals were assessed. The CD8⁺ T lymphocyte count was assessed in 44 samples of HIV-1-infected patients with ART, 16 samples of HIV-1-infected patients without a history of ART and 21 samples obtained from healthy donors. Spearman analysis demonstrated that there was a significant inverse correlation between the expression levels of BCRP and the CD8⁺ T cell count in HIV-1-infected patients with ART (Fig. 7, r=-0.3801, P=0.0109). However, there was no correlation between BCRP⁺CD8⁺T cells and the CD8⁺ T cell count in HIV-1-infected patients without ART (Fig. 8) and healthy blood donors (Fig. 9). These results suggested that an increased

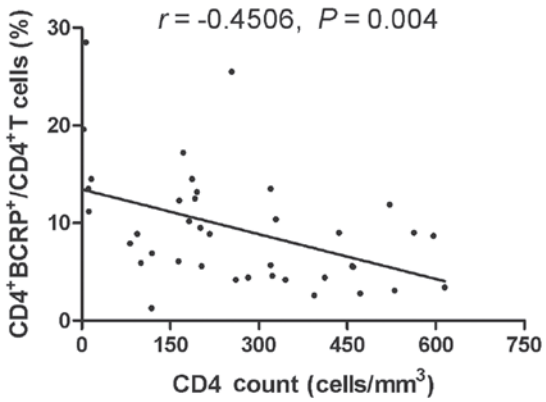

Figure 4. Association between CD4⁺ T cell count and BCRP⁺CD4⁺ T cell frequency in HIV-1-infected patients with ART. BCRP expression was measured with BCRP monoclonal antibodies. Spearman correlation analysis indicated a significant inverse correlation between BCRP⁺CD4⁺ T cell frequency and CD4⁺ T cell count in HIV-1-infected patients with ART (r=-0.4506, P=0.004). BCRP, breast cancer resistance protein; HIV-1, human immunodeficiency virus 1; ART, antiretroviral therapy.

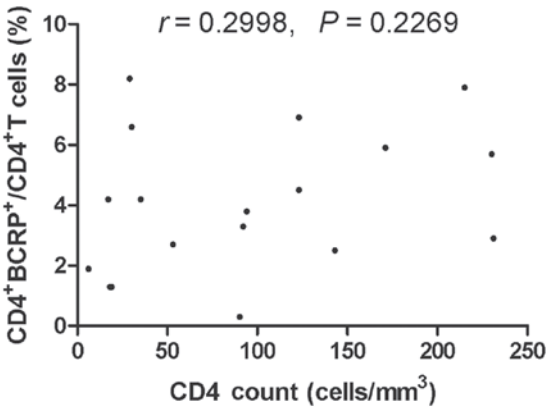

Figure 5. Association between CD4⁺ T cell count and BCRP⁺CD4⁺ T cell frequency in HIV-1-infected patients without ART. BCRP expression was measured with the BCRP monoclonal antibody. Spearman correlation analysis indicates there is no correlation between BCRP⁺CD4⁺ T cell frequency and CD4⁺ T cell counts in HIV-1-infected patients without antiretroviral therapy. BCRP, breast cancer resistance protein; HIV-1, human immunodeficiency virus 1; ART, antiretroviral therapy.

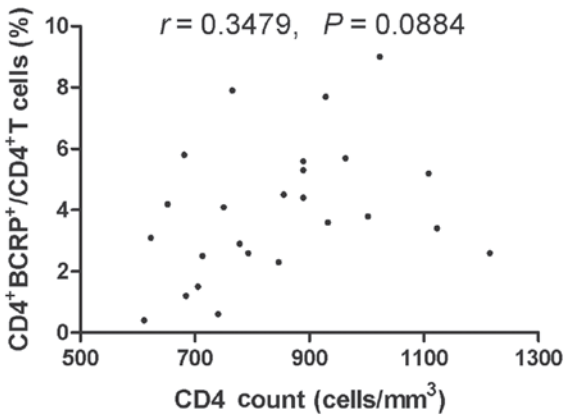

Figure 6. Association between CD4⁺ T cell count and BCRP⁺CD4⁺ T cell frequency in healthy blood donors. BCRP expression was measured with BCRP monoclonal antibodies. Spearman correlation analysis indicated there is no correlation between BCRP⁺CD4⁺ T cell frequency and CD4⁺ T cell count in this group. BCRP, breast cancer resistance protein.

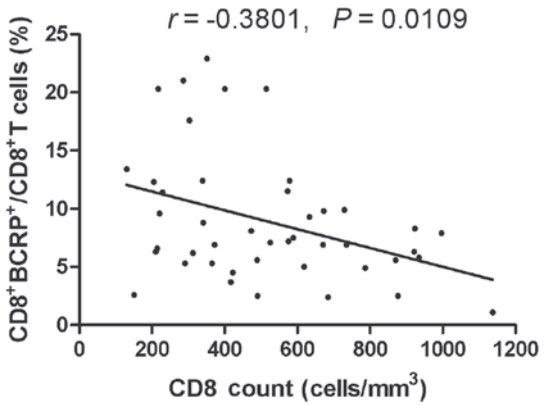

Figure 7. Correlation between $CD8^+$ T cell counts and $BCRP^+CD8^+$ T cell frequency in HIV-1-infected patients with ART. BCRP expression was measured with BCRP monoclonal antibodies. Spearman correlation analysis indicated a significant inverse correlation between $BCRP^+CD8^+$ T cell frequency and $CD8^+$ T cell count in HIV-1-infected patients with ART (r=-0.3801, P=0.0109). BCRP, breast cancer resistance protein; HIV-1, human immunodeficiency virus 1; ART, antiretroviral therapy.

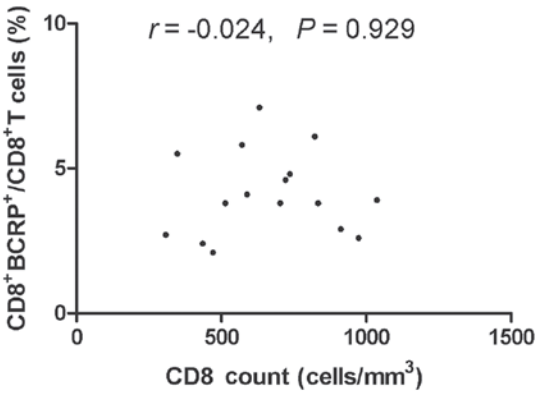

Figure 8. Association between $CD8^+$ T cell count and $BCRP^+CD8^+$ T cell frequency in HIV-1-infected patients without ART. BCRP expression was measured with BCRP monoclonal antibodies. Spearman correlation analysis indicated there is no correlation between $BCRP^+CD8^+$ T cell frequency and $CD8^+$ T cell count in HIV-1-infected patients without ART. BCRP, breast cancer resistance protein; HIV-1, human immunodeficiency virus 1; ART, antiretroviral therapy.

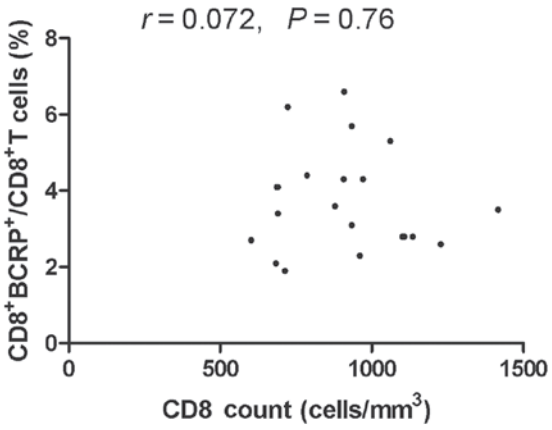

Figure 9. Association between $CD8^+$ T count and $BCRP^+CD8^+$ T cell frequency in healthy blood donors. BCRP expression was measured with BCRP monoclonal antibodies. Spearman correlation analysis indicated there is no correlation between $BCRP^+CD8^+$ T cell frequency and $CD8^+$ T cell count in this group. BCRP, breast cancer resistance protein.

BCRP expression and a corresponding low $CD4^+/CD8^+$ T cell count may be associated with a weaker immune response, leading to BCRP-associated drug resistance due to the administration of antiretroviral drugs in HIV-1-infected patients.

Correlation between the expression of BCRP and virological parameters. Spearman's correlation analysis was used to evaluate whether the expression levels of BCRP were associated with the plasma levels of HIV-1 RNA. The results revealed no evidence for the prognostic importance of BCRP expression in HIV-1 infection and no correlation was observed between BCRP expression and the plasma levels of HIV-1-RNA in HIV-1-infected patients with ART (P>0.05; data not presented).

Discussion

BCRP is a member of the ABC super family of transport proteins, which was first identified by Doyle *et al* (16) in a breast cancer cell line selected for its unique drug resistance in the presence of a P-gp inhibitor. Although not highly expressed in breast cancer, the protein was termed BCRP because it was first isolated from a breast cancer cell line. Following the identification of BCRP drug-resistant cells, its presence in cancer cells was scrutinized to determine its role in acquired drug resistance and the effect of its expression in normal tissues on therapeutic response.

It is well established that the ABC-transporter proteins may significantly modify the pharmacokinetic properties of several drugs, and it has been suggested that they may be critical in drug-drug interactions and the development of drug resistance. Several studies on tumor types and acute myeloid leukemia (AML) have been analyzed for BCRP expression and its potential value in clinical drug resistance, where a positive correlation between BCRP expression and drug resistance in AML has been identified. Drug interactions between and with anti-HIV-1 drugs, as well as viral resistance to antiretrovirals, represent a considerable limitation for the safety and efficacy of HAART. Of note, several studies have reported that certain members of the ABC-transporter protein family, including P-gp, MRP1, MRP4 and MRP5, are able to transport antiretroviral drugs (17-24). While evidence defining the roles of other transporters in the pharmacokinetics and the interaction of antiretroviral drugs is accumulating, the effect of BCRP on the efficacy of HIV-1 therapeutics is far from being elucidated. However, previous studies have identified that high levels of BCRP expression in $CD4^+$ T cells conferred cellular resistance to HIV-1 NRTIs (20,25-28).

The present study represents the first analysis, to the best of our knowledge, of BCRP expression in a large cohort of clinical samples of patients with HIV-1 infection. The data obtained revealed a large inter-individual variability in the protein expression of BCRP in HIV-1-infected patients (with ART or without ART) and in healthy donors. In HIV-1-infected patients, the extent of variability in $CD4^+$ and $CD8^+$ T lymphocytes was greater in the antiretroviral drug treated patients than in healthy volunteers, which suggests that the therapy may contribute to variable expression of the transporter. Indeed, antiretroviral treatment appeared to affect the expression of BCRP, since the expression of the transporters was significantly higher in the HIV-1-infected, treated group than in the

untreated group and in the healthy volunteers. These results appeared to be in agreement with *in vitro* studies that have identified that the expression of ABC-transporters in PBMC is increased in HIV-1 infection, as a result of antiretroviral treatment (29-32). However, in the HIV-1-infected untreated group, the extent of the variability in $CD8^+$ T lymphocytes was even greater than in the HIV-1-infected treated group, and there was a significant difference in BCRP expression at the mRNA but not the protein level. To address this, it should be considered that in HIV-1 infection, the expression of transporter genes may be differentially modulated by the HIV-1 infection status, at a transcriptional and post-translational level, and multiple factors, including HIV-1 replication and viral products, may reduce or increase the expression and the function of these transporters (31,32-35). In the present study, the BCRP expression did not correlate with the viral load value, although the transporter expression in HIV-1-infected patients was higher than that in the healthy donors. These data support the hypothesis that several factors may be involved in this process. Furthermore, the role of antiretroviral drugs in modulating the expression of ABC transporters had not been clear to date. The possibility that both therapeutic strategies and HIV-1 infection may modulate BCRP mRNA expression and its subsequent translation cannot be excluded.

The results of the present study demonstrated a significant inverse correlation between BCRP expression and $CD4^+$ cell count and a weak but significant inverse correlation between BCRP expression values and the $CD8^+$ cell count in HIV-1-infected treated patients. Although these data are difficult to interpret, they suggested that these membrane proteins may not only be expressed on $CD4^+$, but also present on $CD8^+$ cells, which has provided new evidence for its expression in another group of T cells. It has previously been demonstrated that ABC transporters are expressed on the surface of the two major HIV-1 target cells, $CD4^+$ T lymphocytes and macrophages (8,31,36-39); however, whether BCRP is expressed in CD8 or in other blood cells had not been documented until now. The present study has provided the first evidence, to the best of our knowledge, for a possible role of BCRP in cellular resistance towards NRTIs in the treatment of HIV-1 infection *in vivo*. Several groups have hypothesized that the expression of such proteins may decrease the efficacy of treatment (2,19-20,24,31,40). Therefore, any attempt to study the expression of these proteins during the treatment of HIV-1-infected patients may provide further insight into the effect of BCRPs on the efficacy of HIV-1 therapeutics.

However, the present study had a number of limitations. It was retrospective and was performed only on samples from patients receiving NRTI treatment, which limited the possibility of fully interpreting the results obtained. Inclusion of a group of patients subject to antiretroviral treatment and other antiretroviral regimens would have improved the clinical applicability of these results. Furthermore, the lack of time-dependent data on the expression of the transporter in the patients and healthy blood donors meant it was not possible to investigate the effect of such parameters on the expression of ABC-transporters. Such an analysis may have reinforced the significance of the data, allowing to define the importance of drug-drug interactions in the expression of the transporters examined.

The present study demonstrated that the expression of BCRP varied among HIV-1-infected patients and healthy donors, but is significantly higher in the HIV-1-infected group with ART. Furthermore, a significant inverse correlation was observed between the expression levels of BCRP and $CD4^+$ or $CD8^+$ T cell counts in HIV-1-infected patients with ART. These data appeared to indicate an unfavorable role of BCRP in anti-HIV-1 treatment. However, due to the variety of effects that the HIV-1 infection status appeared to have on the expression of the transporter, further studies, particularly prospective controlled studies, are required to exclude the possibility that ABC-proteins may contribute to the effect of ART, and to discover how HIV-1 infection and treatment may interact with these important cellular transporters.

In conclusion, the present study provided the first evidence, to the best of our knowledge, for a possible role of BCRP in the cellular resistance towards NRTIs in the treatment of HIV-1 infection *in vivo* by demonstrating that the BCRP protein is expressed in PBMCs from HIV-1-infected patients with and without ART. Furthermore, an inverse correlation exists between the expression levels of BCRP and the T cell count. BCRP expression in PBMCs, although at a low level, appears to be capable of reducing the efficacy of HIV-1 treatment by actively transporting substrates in antiretroviral drugs. Future studies should investigate BCRP expression and it associations with virological and immunological characteristics in a larger cohort of HIV-1-infected patients, to more reliably determine the effect of BCRP expression on clinical outcome.

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