Intracellular expression of P-170 glycoprotein in peripheral blood mononuclear cell subsets from healthy donors and HIV-infected patients

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ABSTRACT

Background and Objective. **P-glycoprotein (P-gp) is a transmembrane efflux pump that actively extrude a variety of unrelated drugs from cancer cells, leading to the so-called multidrug resistance (MDR) phenomenon. However, P-gp has also been found in normal bone marrow and peripheral blood mononuclear cells (PBMC). Recently, the presence of P-glycoprotein in PBMC from human immunodeficiency virus (HIV)-infected patients has also been investigated and a phenotype-associated P-gp expression has been detected.**

Design and Methods. **A total of thirty-eight HIV-1 positive patients with a mean age of 34 years (range, 24-41 years) were studied after an informed consent. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on a Ficoll/Hypaque and P-glycoprotein expression was investigated on lymphocyte population by single and double-color immunofluorescence techniques. We investigated: i) both surface and intracellular expression of the P-gp molecule in different PBMC subsets, ii) P-gp expression modifications occurring during HIV infection, and iii) the effect of HIV-gp120 on the expression of P-gp by T lymphocyte subsets from healthy donors.**

Results. **Our experimental findings indicate that: a) P-gp glycoprotein can be detected on an intracellular level in different PBMC subpopulations (mainly CD8+ T lymphocytes, CD16+ NK cells and CD14+ monocytes); b) this intracellular expression is decreased in specific PBMC subsets (i.e. T-CD8+ and NK-CD16+) from HIV-infected patients and c) a rearrangement was obtained when CD4+ and CD8+ lymphocytes from healthy donors were exposed *in vitro* to the HIV-binding glycoprotein gp120.**

Interpretation and Conclusions. **Our results indicate that P-gp glycoprotein can also be expressed intracellularly and can be rearranged in PBMC subsets from HIV-infected patients.**
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Keywords: AIDS, HIV, leukocyte, multidrug resistance, P-glycoprotein

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Multidrug resistance (MDR) is a phenomenon of serious clinical importance in which a cell exposed to a single drug becomes cross-resistant to a large number of structurally unrelated compounds.[1,2] A specific gene, the human *mdr1* gene and its encoded product identified as a 170 kDa transmembrane phosphoglycoprotein named P-glycoprotein (P-gp), seems to mediate, although not exclusively, this phenomenon.[1,3-5] It has also been reported that a variety of normal human tissues express the *mdr1* gene.[6] This finding, together with the sequence and structural homology that P-gp shares with some known transport proteins,[7] suggested the possibility of a role for P-gp as an active pump system not related to cancer cell resistance.[8]

P-glycoprotein has been found as expressed on the surface of normal human hematopoietic cells with excretion functions similar to those observed in other cell types.[9-11] In particular, P-gp seems to contribute to effector functions and/or cytokine secretion.[11-15] A variable expression of surface P-gp in other peripheral blood lymphocyte subsets than cytotoxic CD8+ T cells and natural killer (NK) cells, i.e. B cells and CD4+ T helper cells has also been reported.[9-11] Finally, recent studies suggested a role for P-gp in pathologic conditions diverse from cancer, e.g. during the course of acquired immunodeficiency syndrome (AIDS).[14,16,17]

Based on recent reports showing an intracellular P-gp expression in tumor cells[18,19] where it may represent a constitutive intracytoplasmic detoxification system able to influence both drug-dependent and independent cell homeostasis,[6,14,20] we investigated this molecule in healthy donors and HIV-infected patients. In particular, by using flow cytometry and immunocytochemistry for P-gp assessment, we evaluated: i) both intracellular and surface expression of P-gp in different PBMC subsets, and ii) the occurrence of intracellular P-gp expression changes during HIV infection. In addition, since it has been reported that the HIV-binding glycoprotein gp120 is able to activate several lymphocyte functions pos-

sibly via the up-regulated expression of immunoregulatory cytokines,[21,22] we also analyzed whether this protein induces *in vitro* alterations of surface and intracellular P-gp expression in mononuclear cells from healthy controls.

Materials and Methods

Study population

A total of thirty-eight HIV-1 positive patients with a mean age of 34 years (range, 24-41 years) were studied after an informed consent. HIV positivity was determined by enzyme-linked immunosorbent assay (ELISA) with confirmation by Western blot analysis. HIV-infected patients were divided into two subgroups based on the 1993 revised CDC classification system, which categorizes individuals on the basis of clinical conditions associated with HIV infection (A, B and C) and CD4+ T lymphocyte count (*Centers for Disease Control: 1993 revised classification for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults, 1992*). There were 19 asymptomatic (ASY)/persistent generalized lymphadenopathy (PGL) (11 ASY; 8 PGL) (A1/A2) (group 1) and 19 AIDS patients (C3) (group 2). Twenty-six anti-HIV seronegative healthy individuals with a mean age of 30 years (range, 25-33 years) were also studied as a control group. The absolute peripheral blood lymphocyte count (±SD) was: $1648\pm99/mm^3$ in control group, $1496\pm76/mm^3$ in A_1/A_2 patients and $646\pm96/mm^3$ in C3 patients. The mean CD4+ cell number in group 1 and group 2 was 393 (range, 230-800)/mm^3 and 52 (range, 4-106)/ mm^3, respectively. There was no history of prior chemotherapy or any malignancy in patients included in this study.

Isolation of mononuclear cells (PBMC)

Heparinized venous blood was collected from patients and controls. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on a Ficoll/Hypaque (LSM, Litton Bionetic, Kensington, MD, USA) density gradient. Cells were washed two times in phosphate buffered saline (PBS) and resuspended in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 2% heat-inactivated fetal calf serum (FCS). Cell viability was confirmed by trypan blue dye exclusion test. Isolated mononuclear cells were then processed at the same time for both flow cytometry and fluorescence microscopy analysis.

Monoclonal antibodies

P-glycoprotein expression was investigated on lymphocyte population by single and double-color immunofluorescence techniques. The following isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (MAbs) were used to characterize cell subsets: anti-CD16 (Leu11) or CD56 (Leu19) for NK cells, CD20 (Leu16) for B

cells, CD4 (Leu3a) and CD8 (Leu2a) for T subsets and CD14 (LeuM3) for monocytes (Becton Dickinson, Mountain View, CA, USA). Anti-CD45-FITC and anti-CD14-PE (Leucogate Simultest, Becton Dickinson) served as markers for validating lymphocyte scatter gating. All MAbs were used for saturating concentrations as recommended by the manufacturer.

P-glycoprotein reagents

Monoclonal antibodies MM4.17[23] and MRK-16 (Kamiya, Thousand Oaks, CA, USA), which recognize two distinct human-specific epitopes of extracellular domains of the *mdr1*-P-glycoprotein isoform were used in this study. MM4.17 is an IgG2a$_k$, monoclonal immunoglobulin reacting with a continuous linear epitope on the apical part of the 4th loop of P-gp. MRK-16 is an IgG2a$_k$ monoclonal immunoglobulin reacting with a conformational epitope distributed on the 1st and 4th loop of P-glycoprotein. These MAbs specifically recognize living human cells expressing P-gp. The optimal concentration for flow cytometry and immunocytochemistry studies was 10 µg/mL. FITC-labelled goat anti-mouse (GAM) IgG (Becton Dickinson) was used as second-step reagent.

Flow cytometry

Expression of surface P-gp on several resting lymphocyte populations was evaluated in 20 HIV-infected individuals (10 asymptomatic/PGL, 10 AIDS). Briefly, following isolation on Ficoll/Hypaque, PBMC were stained immediately with unconjugated anti-P-gp MAb (MM4.17 and MRK-16) for 30 min at 4°C. For dual-fluorescence, anti-P-gp/GAM-FITC-stained cells were incubated with 80 µL of normal mouse serum (1 mg/mL) for 30 min. at 4°C to block any free anti-mouse Ig binding sites prior to addition of a second PE-conjugated MAb. Samples were fixed with 1% paraformaldehyde and then analyzed by flow cytometry. Quotes of mononuclear cells were analyzed for surface P-gp expression after fixation in 2.5% paraformaldehyde for 30 min at 4°C. Controls with isotypic control antibodies (IgG2a and IgG1) (Becton Dickinson) were used in all experiments. Samples were analyzed using a FACScan flow cytometer (Becton Dickinson) equipped with a 15-mW, 488-nm, air-cooled argon ion laser. Forward and side light scatter was collected in linear mode and served to exclude unwanted events (i.e., debris, dead cells, and aggregates from counting). Fluorescence signal was collected in log mode. Five thousand suitable cells were analyzed using Lysys II software (Becton Dickinson).

Cell cultures and recombinant HIV-gp120 (rgp120)

The expression of surface P-glycoprotein was also studied, by flow cytometry, on recombinant HIV-gp120-stimulated CD4+ and CD8+ T lymphocyte sub-

sets. Briefly, PBMC (1×10^6 cells/mL) were incubated for 24-72 hr in RPMI-1640 supplemented with 10% FCS-50 µg/mL gentamycin in the presence of recombinant HIV-1-III B gp120 at 3 µg/mL. This preparation was purchased by Intracel (London, England) and was produced in a baculovirus expression system. Cross-linking with anti-CD3 MAb (Sigma Chemicals) was used as positive control for P-gp induction.[14] Cell viability in all experiments exceeded 90% when assessed by dye exclusion method. At the above indicated time points, PBMC were harvested, extensively washed with PBS and stained for double-fluorescence assay as above reported.

Fluorescence microscopy

Mononuclear cells were isolated from healthy donors and HIV-infected patients through the standard methods as described above.

The use of a Cell Sorter for separation of different cell subsets was avoided because of the resulting low cell viability and, mainly, because of the artifactual morphological features obtained. Double fluorescence analyses were then performed. For cell surface labeling, similar quotes of each sample were incubated for 40' at 4°C with a 1:10 dilution of the following monoclonal antibodies to a lineage specific marker: anti-CD4, CD8, anti-CD16 and/or CD56, CD20 and CD14, then fixed with 3.7% paraformaldehyde in PBS at 4°C. For P-gp intracellular labeling, after adhesion on polylysine-coated coverslips, each PBMC subset was made permeable with Triton X-100 0.5% for 5' at room temperature and then incubated for 30' at 37°C with P-gp-specific MAbs (MM4.17 and MRK-16). After several washes in PBS, samples were incubated for 30' with TRITC-conjugated IgG at a working dilution of 1:50, mounted with glycerol-PBS (1:1) and observed with a Nikon Microphot fluorescence microscope. Irrelevant mouse immunoglobulins were used in negative control samples.

Experiments were also performed by using a Sarastro 2000 Confocal laser microscope. This apparatus, made up of an argon laser beam and a specific image analyzer system (Sarastro) allowed us to detect antigenic sites by single focus planes. For each PBMC subset, the percentage of cells expressing P-gp on the surface and/or intracellularly was evaluated by fluorescence microscopy. At least 300 cells were evaluated for each sample at the same high magnification (×3000).

The pictures were taken by using the same objective diaphragm and the same exposure conditions. Prints were performed by using the same photographic paper having the same contrast features.

Statistical analysis

Mean value±SD were compared using Student's t-test for independent means. P values ≤ 0.05 were considered significant.

Results

P-glycoprotein surface expression on peripheral blood lymphocytes (PBL) from HIV-infected patients

Initial studies to investigate the degree of surface P-glycoprotein expression on PBL in healthy controls and HIV-infected patients revealed in both cases a percentage of positivity lower than 12%. It should be noted, however, that the staining of the cell population with MM4.17 was specific because control MAbs did not stain the population. A more detailed analysis of P-gp in subgroups of patients (asymptomatic/PGL and AIDS) showed that patients with AIDS had a decreased, although not statistically significant, percentage of peripheral lymphocytes bearing a detectable surface P-gp molecule compared to asymptomatic/PGL patients and controls. Basically the same results were obtained using another MAb (MRK-16) toward an extracellular epitope of the P-glycoprotein domain (controls: 10.2±0.6; HIV+ as a group: 5.3±1). In agreement with another report,[24] by studying the effect of fixation in detecting P-gp glycoprotein, we found that the proportion of PBL expressing surface P-gp could vary considerably. In fact, as observed in both control and HIV-infected patients (though much more significantly in the latter group), fixation of the cells prior to incubation with the MM4.17 monoclonal antibody increased the level of reactivity (P-gp+ lymphocytes: controls-unfixed 11.5±0.1; fixed 16±0.8; HIV+ patients-unfixed 6.6±0.2; fixed 10.3±1.2). However, in order to elude conflicting results due to different experimental conditions, only data from unfixed PBL were considered.

Two-color immunophenotyping of PBMC

The phenotypic analysis of the coexpression of P-gp by lymphocyte subpopulations and monocytes revealed that in both controls and in HIV-infected patients approximately 60% of CD20+ B lymphocytes and 80% of CD14+ monocytes expressed surface P-glycoprotein (Table 1). When the frequency of CD8+ cells and CD4+ T cells and CD56+ NK cells bearing P-gp was evaluated, a lower proportion of positive lymphocytes was detected, with no significant differences between controls and HIV-infected subjects (even when patients were subdivided according to the CDC classification). Comparable results were obtained using MM4.17 or MRK-16 MAbs.

Immunocytochemical analyses

Double fluorescence analyses considering different PBMC subsets, i.e. CD4+ and CD8+ T cell subsets, CD16+ NK cells, CD20+ B lymphocytes and CD14+ monocytes from healthy donors and HIV-infected patients, and parallel expression of P-gp were carried out (Figures 1-5). A permeation method was performed, as stated in *Materials and Methods* section, allowing us to detect this antigen

Table 1. Percentage of PBMC and specific subpopulations reacting with MM 4.17.

% Lymphocytes	Controls (n = 10)	ASY/PGL (A1/A2) (n = 10)	AIDS (C3) (n = 10)	HIV+ patients (as a group) (n = 20)
Whole PBL	11.5±0.1	8.9±0.4	6.6±0.32	7.7±0.2
CD4+/P-170+	2.6±0.3	2.2±0.4	1.8±0.2	2.0±0.1
CD8+/P-170+	1.8±0.4	1.5±0.2	1.0±0.3	1.2±0.2
CD56+/P-170+	1.3±0.2	1.1±0.5	1.0±0.2	1.0±0.2
CD20+/P-170+	5.8±1.5	4.1±0.9	2.8±0.5	3.4±0.5*
% Monocytes	**Controls (n = 10)**	**ASY/PGL (A1/A2) (n = 10)**	**AIDS (C3) (n = 10)**	**HIV+ patients (as a group) (n = 20)**
CD14+/P-170+	93±0.1	91±0.8	88±0.5	89±1

cases where there is a statistically significant difference between HIV+ patients and controls. Statistical analysis performed by Student's t test. Abbreviations: ASY, asymtomatic; PGL: persistent generalized lymphadenopathy.

Figures 1-5. Immunofluorescence micrographs illustrating the rearrangement of the P-gp in some cell subsets. In CD8+ T lymphocytes (Figure 1) from healthy donors (a), the expression of P-gp is clearly cytoplasmic while is essentially located at the plasma membrane level in the HIV-infected patients (b). Similar analyses carried out in CD16+ natural killer (NK) cells (Figure 2), showed that the intracellular expression of P-gp (a) was decreased in blood samples from HIV-infected patients (b). Figure 2c (below) shows intracellular expression of P-gp in CD16+ cells as visible by confocal microscopy. By contrast, CD14+ monocytes, in which the intracellular expression of P-gp was particularly well evident, appeared substantially unaltered in blood samples from HIV-infected subjects (Figure 3a-b). Figure 3c (below) shows intracellular distribution of P-gp in monocytes as viewed by confocal microscopy. Finally, CD4+ T lymphocytes (Figure 4a-b), as well as CD20+ B lymphocytes (Figure 5a-b), showed a P-gp positivity which seems to be localized only on their surface (in blood samples from healthy donors, Figures 4a and 5a, and HIV-infected patients, Figures 4b and 5b, respectively). Arrow in Figure 4b indicates a large monocytic cell. (All the micrographs are of the same magnification: ×3000; confocal microscopy: ×5000).

Percentage of cells with a prevalent intracellular expression of P-gp
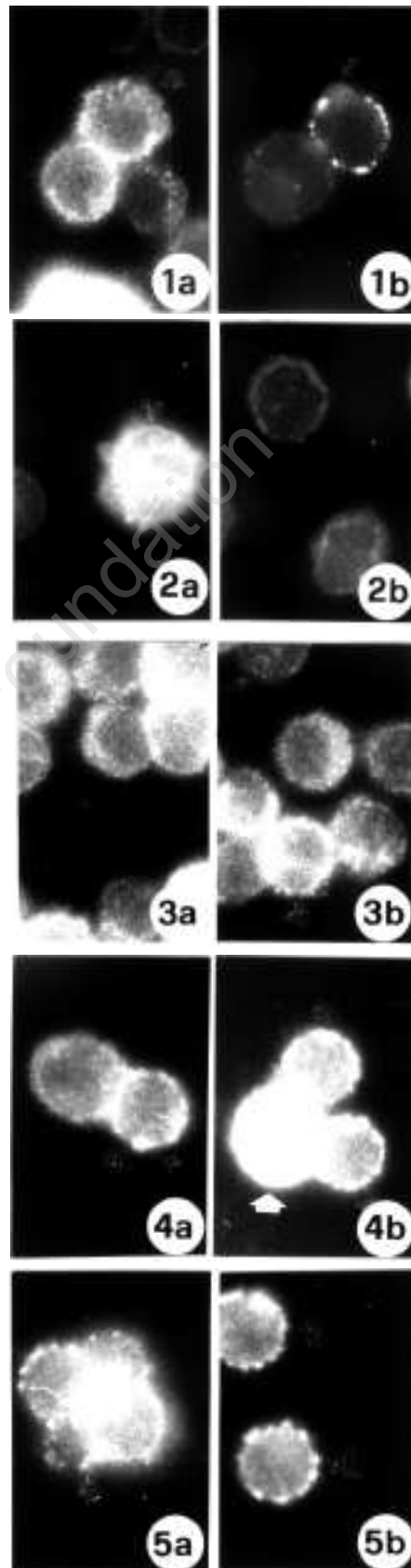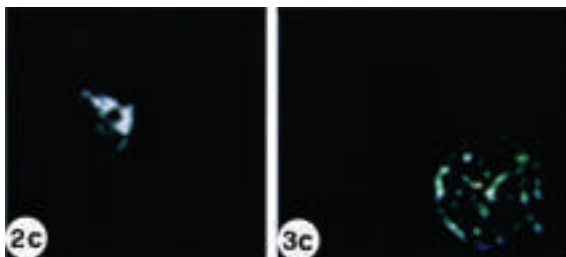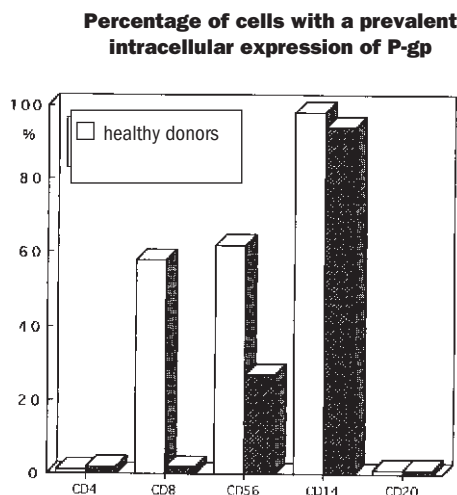
Figure 6. Histogram showing the percentage of PBMC expressing intracellular P-gp in healthy donors and HIV-infected patients.

in the cell cytoplasm by using the MM4.17 MAb. As a general rule, we have to note that the morphology of lymphocytes is characterized by a extremely high nucleus/cytoplasm ratio. Thus, because of the relatively low *amount* of cytoplasm in these cells, the detection of cytoplasmic positivity can be depicted as a *cortical* or *apical* presence of the antigen while the surface expression was detectable as a thin-layer cell surface positivity. In particular, the intracellular apical expression of the P-gp molecule was well evident in PBMC from healthy donors belonging to the CD8+ T subset (Figure 1a) while it appears to be expressed only on the cell surface of the same cells from HIV-infected patients (Figure 1b). Similar analyses were carried out in CD16+ NK cells (Figure 2a-c) and CD14+ monocytes (Figure 3a-c). The former shows a marked intracellular expression of P-gp (Figure 2a) which was significantly reduced in samples from HIV-infected individuals (Figure 2b). By contrast, the latter (CD14+) displayed an unaltered cortical expression of P-gp (Figure 3a-b). Confocal microscopy analyses confirmed the above findings. Figures 2c and 3c show intracellular distribution of P-gp in CD16+ cells (Figure 2c) and CD14+ cells (Figure 3c) as observed by using this technique. In the other cell subsets considered (i.e. CD4+ T cells (Figure 4a-b) and CD20+ B lymphocytes (Figure 5a-b), only a surface expression of P-gp was detected while their cytoplasm remained negative. In consideration of these qualitative results, a quantitative analysis was also carried out as specified in *Materials and Methods* (Figure 6). The results obtained indicated that: a) the intracellular expression of P-gp is mainly detectable in cells from healthy donors; b) the

intracellular expression of P-gp is detectable in about 60% of CD8+ T lymphocytes and CD16+ NK cells from healthy donors and c) P-gp was detected in the great majority of CD14+ monocytes (more then 90%) of both healthy donors and HIV-infected patients (Figure 6). Parallel experiments performed by using MRK 16 Mab confirmed above results (not shown).

Effect of rHIV-gp120 on P-glycoprotein expression

The expression of P-gp appears to be somewhat increased after the exposure to 3 µg/mL of HIV-gp120 glycoprotein. More specifically, P-gp surface expression on freshly isolated CD4+ and CD8+ T lymphocytes, appeared to be gradually and moderately increased with culture time as detected by flow cytometry analyses. Figure 7 shows an example of the expression of P-gp kinetic on CD4+ and CD8+ lymphocytes following treatment of normal PBMC with HIV-gp120. A time-dependent enhancement of P-gp surface expression was detected in both T lymphocyte subsets that peaked at 24-48 hrs (Figure 7). Immunofluorescence microscopy confirmed data obtained by flow cytometry and demonstrated that the exposure to HIV-gp120 glycoprotein exerted a marked alteration in the expression of P-gp in CD4+ cells (not shown) and CD8+ cell subsets (Figure 8a-b) in which a light rearrangement of P-gp expression is detectable (compare Figure 1a, b and Figure 8b). In fact, other subpopulations considered appeared to be less affected by the viral-binding gp120 antigen-induced activation (not shown).

Discussion

It has been previously demonstrated that P-glycoprotein (P-gp) is located in the plasma membrane of MDR tumor cells and in tissues which have excretory and detoxifying functions in common,[6,20] while few studies concerning the intracytoplasmic localization of the molecule have been carried out.[18,19] Therefore, given the growing importance of this system in patients with HIV infection,[14,16,17] we have analyzed both surface and cellular P-gp expression in peripheral blood mononuclear cells (PBMC) from HIV-infected subjects. Our results show that P-gp distribution in PBMC is not restricted to the plasma membrane but it is also intracellular and susceptible to be rearranged in certain lymphocyte subsets from HIV-infected subjects (mainly cytotoxic CD8+ T cells and CD56+/CD16+ natural killer cells). Interestingly enough, similar results were obtained with HIV-gp120-activated PBMCs from healthy controls, although it has to be established whether the *in vitro* P-gp 120 reflects the pattern of stimulation operating *in vivo*.

Regarding mononuclear blood cells, previous reports have indicated: i) P-gp is expressed on the
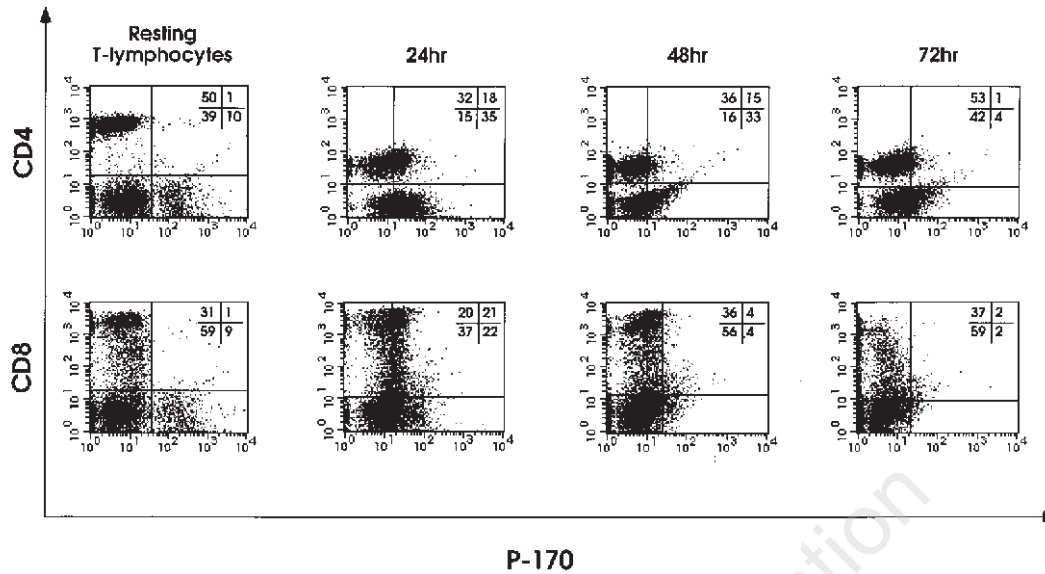
Figure 7. Kinetics of induction of P-gp expression on CD4+ and CD8+ T lymphocytes. PBMC of a healthy patient were stimulated with HIV-gp120 at 3 mg/mL. Cultures were harvested at the indicated time points, and the cells stained with GAM/FITC-conjugated anti-P-gp MAb (X-axis, log scale) and PE-conjugated anti-CD4 and anti CD8 MAbs (Y-axis, log scale). Numbers in the top right corner of each scattergram indicate the proportion of cells in the respective quadrants.

surface of normal human lymphocytes and monocytes;[9-11] ii) this surface expression can be modulated.[11,14,25] However, while surface P-gp can be easily detected by flow cytometry in all leukocytes, discrepancies exist on the degree of this expression and on its hierarchy among lymphocyte subsets and monocytes because of the diversity of the techniques and reagents employed. In agreement with a number of studies,[4,26-28] the expression of surface P-gp we detected among normal mononuclear cells
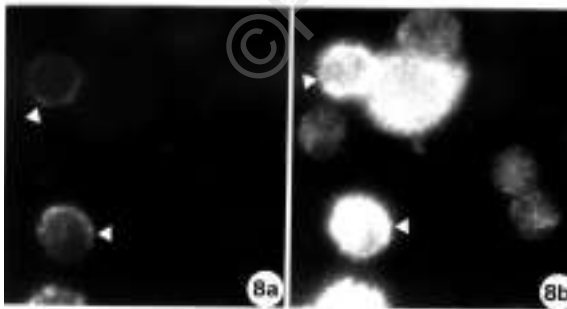
Figure 8. Immunofluorescence micrographs illustrating the arrangement of the P-gp in CD8+ T cells after exposure to HIV-gp120 glycoprotein. Double staining immunofluorescence micrographs illustrating the same microscopic fields for the detection of surface cluster designation antigens (arrows indicate CD8+ T cells, Figure 8a) and for intracellular P-gp antigens (Figure 8b, right panel; arrows indicate the same cells shown in Figure 8a after 48 hrs exposure to HIV-gp120 glycoprotein).
(Micrographs are of the same magnification: ×3000).

suggests that P-gp is constitutively expressed at levels that cannot significantly affect drug resistance. Moreover, the differential P-gp expression among mononuclear lineages we observed is in agreement with Gupta et al.,[11,17] who reported relatively high levels of surface P-gp in B lymphocytes and monocytes as well as an activation-induced amplification of membrane P-gp in T cells using the MAb MRK-16. Thus, the resulting apparent discrepancy between intracellular and surface P-gp expression changes in specific lymphocyte subsets during HIV infection, i.e. lack of detectable increase of surface P-gp despite a reduced intracellular molecule expression, may require a series of tentative explanations.

First, the absence of a detectable increase in the degree of P-gp surface expression in lymphocytes from HIV-infected patients may reflect the relatively inefficient function of the pump in resting conditions.[29] This is consistent with observations that exposure to a substrate up-regulates surface P-gp through, perhaps, a transient conformational shift to an active state. Following this activation P-gp may revert to its initial state, as already suggested for other surface molecules.[30,31] By analogy, there is evidence that P-gp can occur in more than one topological form with some of the protein membrane helices being outside the membrane bilayer. The different topologies may represent different functional states of P-gp.[32] Our finding that gp120-induced activation transiently increases surface P-gp in both CD4+ and CD8+ subsets from healthy

donors, while reducing intracellular P-gp may indirectly confirm these hypotheses, suggesting that P-gp may be involved in some metabolic functions that are modulated during T lymphocyte activation.

Second, a certain function that this protein exerts in different mononuclear cell subpopulations could be hypothesized (reviewed in #17). Monocytes, for example, express both surface and intracellular P-gp with no changes either during HIV infection or following cellular activation. Similarly, it has been shown that monocytes, despite surface P-gp expression, remain in an inactive state even after substrate exposure and retain rhodamine123 (a P-gp specific fluorescent dye) in an assay for P-gp function.[33,34] This seems to suggest a different functional role for P-gp other than that of a drug/dye extrusion. For this reason, given the homology that P-gp shows with HAM1 transport protein in macrophages,[35] a role for P-gp in antigen processing/presentation has been hypothesized.[17]

Finally, the intracellular P-gp function pathway may efficiently operate independently from the degree of surface P-gp expression.[36] According to this hypothesis, an emerging scenario suggests that P-gp acts prevalently at the intracellular level, accumulating specific substrates into a vesicular export system without assembling P-gp molecules at the plasma membrane level.[36,37] As a consequence of such process, intracellular P-gp may prevent the substrates from reaching their target macromolecules.[36,37] Taken together, these findings may have important clinical implications since P-gp recognizes and transports 3'-azido-3'-deoxythymidine (AZT) and other antiretroviral nucleoside analogs,[38] as well as several MDR-related cytotoxic agents, e.g. vinca alkaloids and anthracyclines,[3,39] employed in patients with HIV-associated neoplasms (mainly B-cell lymphomas and Kaposi's sarcoma). In fact, although we have reported that *in vivo* and *in vitro* treatment with AZT and related drugs do not modify lymphocyte surface P-gp expression and function,[14] no data are presently available concerning the level reached in cells expressing a P-gp system by those drugs that are repeatedly found in the HIV disease and are known to be recognized and pumped out of cells by P-glycoprotein.

In conclusion, the results here presented show an intracellular P-gp expression in normal leukocytes that has yet not been reported. Moreover, our findings also suggest a role for P-gp as a modulated, phenotype-associated molecule whose alteration during HIV infection could play a role in the long-term treatment of immunocompromized patients.

Contributions and Acknowledgments

WM, RC, GD and LO were responsible for the study design. MBL, GR and MC contributed to data analysis and paper writing.

Disclosures

Conflict of interest: none.
Redundant publications: no substantial overlapping with previous papers.

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