Cyclophilin B mediates cyclosporin A incorporation in human blood T-lymphocytes through the specific binding of complexed drug to the cell surface

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Cyclophilin B (CyPB) is a cyclosporin A (CsA)-binding protein located within intracellular vesicles and released in biological fluids. We recently reported the specific binding of this protein to T-cell surface receptor which is internalized even in the presence of CsA. These results suggest that CyPB might target the drug to lymphocytes and consequently modify its activity. To verify this hypothesis, we have first investigated the binding capacity and internalization of the CsA–CyPB complex in human peripheral blood T-lymphocytes and secondly compared the inhibitory effect of both free and CyPB-complexed CsA on the CD3-induced activation and proliferation of T-cells. Here, we present evidence that both the CsA–CyPB complex and free CyPB bind to the T-lymphocyte surface, with similar values of K_d and number of sites. At 37 °C, the complex is internalized but, in contrast to the protein, the drug is accumulated within the cell. Moreover, CyPB receptors are internalized together with the ligand and rapidly recycled to the cell surface. Finally, we demonstrate that CyPB-complexed CsA remains as efficient as uncomplexed CsA and that CyPB enhances the immunosuppressive activity of the drug. Taken together, our results support the hypothesis that surface CyPB receptors may be related to the selective and variable action of CsA, through specific binding and targeting of the CyPB–CsA complex to peripheral blood T-lymphocytes.

INTRODUCTION

Cyclophilins are represented in human tissues by at least five isoforms [1–5] and exhibit peptidyl prolyl *cis–trans* isomerase activity, leading to the *in vitro cis–trans* isomerization of Xaa-Pro peptide bonds [6,7]. However, the relevance of this activity to the biological functions of cyclophilins remains unclear. The immunosuppressant drug cyclosporin A (CsA) specifically binds to cyclophilins and abolishes the enzymic property of these proteins. Nevertheless, the inhibition of peptidyl prolyl isomerase activity is not directly involved in CsA-mediated immunosuppression. The complex formed by the drug with its specific receptor was found to bind to the phosphatase calcineurin, leading to the inhibition of enzymic activity [8]. The formation of a such ternary complex is thought to be a crucial step, which effectively blocks the early T-cell activation cascade and constitutes the basis of the immunosuppressive activity of CsA [9,10].

In organ transplantation, the sensitivity to CsA is a crucial factor for the acceptance of the graft. A high variability is observed among different patients and several *in vitro* studies have shown large individual variations of lymphocyte response to CsA treatment [11–13]. Furthermore, the selective action of the drug towards T-cells remains unclear. The understanding of the mechanisms involved in cell susceptibility to CsA is very important for the optimization of immunosuppressant therapy. An interesting field of investigation is to understand the involvement of cyclophilins in these phenomena. Indeed, variations of cell sensitivity to CsA might be related to the relative distribution of the intracellular cyclophilin isoforms. On the other hand, intrinsic differences of lymphocyte susceptibility might be related to variable incorporation of the drug into T-cells. The presence of a surface receptor for CsA remains controversial [14,15]. We have recently reported the characterization of T-cell surface binding sites for cyclophilin B (CyPB) [16]. This cyclophilin isoform was found to be located in reticulum vesicles [17,18] and released into biological fluids such as human milk and plasma [19,20]. In forming a complex with CsA before it enters the cells, extracellular CyPB might specifically target the drug to T-lymphocytes and consequently increase its cellular concentration.

To check this hypothesis, we have investigated the role of CyPB in specific CsA incorporation into T-cells. In our previous work, we have shown that CyPB specifically bound to T-cells but not to B-cells [16]. The surface-bound protein was partially internalized at 37 °C, even in the presence of CsA, and subsequently released into the medium in a degraded form. Here, we have first demonstrated specific binding and internalization of the CyPB–CsA complex, by using tritiated CsA as a radiolabelled probe. We have then investigated the inhibitory effect of the complex on CD3-induced activation and proliferation and shown that the immunosuppressive activity of CsA was enhanced after its CyPB-mediated incorporation. Taken together, our results suggest that CyPB may promote the incorporation of CsA into blood T-cells, without affecting its immunosuppressive activity. This raises the interesting possibility that the CyPB surface binding sites may be related to the selective and variable actions of CsA by targeting the drug to T-lymphocytes and thus increasing its intracellular concentration within these cells.

Abbreviations used: CsA, cyclosporin A; CyPA, cyclophilin A; CyPB, cyclophilin B; DPBS, Dulbecco's PBS; PBMCs, peripheral blood mononuclear cells.

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EXPERIMENTAL

Materials

[^{3}H]CsA (10.1 Ci/mmol) was a generous gift from Sandoz-France (Rueil-Malmaison, France). It was diluted in ethanol at a concentration of 250 μCi/ml. Recombinant CyPB was produced in collaboration with the Lycée Valentine Labbé (La Madeleine, France) and purified as previously described [19]. Citrated blood was obtained from the Centre Regional de Transfusion Sanguine (Lille, France). The lymphoblastic Jurkat T-cell line was routinely cultured in RPMI 1640 medium (Life Technologies, Paisley, Scotland, U.K.) containing 2 mM L-glutamine, 20 mM Hepes, 25 mM $NaHCO_3$, and gentamicin (5 μg/ml), pH 7.3, supplemented with 10 % (v/v) heat-inactivated fetal calf serum.

Preparation of radiolabelled ligands

[^{3}H]CsA–CyPB complex was obtained by incubating 100 μg of CyPB with a 10-fold molar excess of [^{3}H]CsA for 30 min at room temperature. Free CsA was removed by hydrophobic chromatography on a Sephadex LH-20 column (Sigma Chemicals Co., St. Louis, MO, U.S.A.) [19]. The specific radioactivity was estimated to be $(0.8–1) \times 10^6$ c.p.m./μg of CyPB. The stability of the complex was then verified at 4 °C and at 37 °C, and dissociated [^{3}H]CsA never exceeded 5 % of total radioactivity in our control. Moreover, the complex remained intact in citrate and phosphate buffers over the pH range from 4 to 9. ^{125}I-CyPB was obtained as previously described [16]. The specific radioactivity ranged over $(4–6) \times 10^6$ c.p.m./μg.

Surface binding assay

Peripheral blood mononuclear cells (PBMCs) were first isolated by density-gradient centrifugation on Lymphoprep separation medium (Nycomed, Oslo, Norway). T-lymphocytes were collected after depletion of monocytes and B-cells, as described in [21]. The purity of the population and the cell viability were estimated by flow cytofluorimetry analysis (FACScan Cytofluorimeter, Becton Dickinson). Cells were then resuspended in cold Dulbecco's PBS (DPBS) (Sigma) supplemented with 0.5 % BSA, to a final density of 4×10^6 cells/ml, and allowed to bind either [^{3}H]CsA–CyPB complex or ^{125}I-CyPB at various concentrations, for 1 h at 4 °C. Incubation (2×10^6 cells/sample) was performed in polyethylene tubes in order to limit non-specific adsorption of the ligand to the support. Non-specific binding was determined in the presence of a 200-fold molar excess of unlabelled ligand. After incubation, cells were centrifuged (400 *g*, 5 min), washed three times with 4 ml of cold DPBS and lysed by 100 μl of 0.1 M NaOH. In the [^{3}H]CsA–CyPB complex binding assay, cell lysates and aliquots of binding medium (50 μl) were resuspended in 3 ml of scintillation liquid (Lumac-LSV) and cell-associated (bound) and soluble (free) radioactivity was counted using a model LS 6000TA Beckman counter. In the case of ^{125}I-CyPB, cell pellets and incubation medium aliquots were directly counted using a model 1282 Compugamma LKB–Wallac counter.

Internalization kinetics

Cells (4×10^6/ml) were first allowed to bind either ^{125}I-CyPB or [^{3}H]CsA–CyPB for 1 h at 4 °C, until the binding site capacity reached a steady state. After washing off excess ligand, cells were resuspended in prewarmed binding medium at 37 °C. At various times, aliquots (1 ml) of cell suspension were removed and all subsequent steps were performed at 4 °C. Cell-free supernatants were collected and cells were submitted to a mild-acid treatment, by resuspending the pellet in 4 ml of ice-cold 50 mM glycine, 100 mM NaCl buffer, pH 4.5, for 10 min. The surface-bound, internalized and released radioactivity was counted in acid-sensitive eluate, cell extract and incubation medium, respectively. Non-specific binding was determined as described above.

Recycling of CyPB surface binding sites

Protein synthesis was inhibited by incubating cells with cycloheximide (20 μg/ml) for 30 min at 37 °C and during the remainder of the incubation. Treated cells were first incubated in the absence or presence of 50 nM unlabelled CyPB in culture medium at 37 °C. At various times, aliquots were collected and submitted to mild acid treatment to remove any remaining surface-bound ligand. After neutralizing by thoroughly washing with assay medium, specific ^{125}I-CyPB binding was determined.

Analysis of CsA immunosuppressive activity

PBMCs were collected as above and resuspended in RPMI 1640 medium (10^6 cells/ml), containing 2 mM L-glutamine, 20 mM Hepes, 25 mM $NaHCO_3$, and gentamicin (5 μg/ml), pH 7.3, and supplemented with 10 % (v/v) heat-inactivated fetal-calf serum. Cells were stimulated for 72 h in a 96-well plate in the presence of a monoclonal anti-CD3 antibody (Immunotech, Marseille, France), at 50 ng/ml. Lymphocyte activation was visualized by quantifying the expression of CD25 by flow cytofluorimetry. Briefly, cells were resuspended in cold DPBS–0.5 % BSA (10^6 cells/ml) and incubated in the presence of a monoclonal rat anti-CD25 antibody (Immunotech) for 1 h at 4 °C. After washing, a second fluorescein-conjugated anti-(rat IgG) antibody (Sigma) was added for 1 h. Cells were gated for forward- and side-angle scatters and data were expressed as the variation of fluorescence mean value with non-activated cells as a control. Cell proliferation was investigated by measuring the incorporation of [^{3}H]thymidine (18.5 kBq per well) for 5 h before harvesting. Results are expressed as an index of proliferation; 100 % represents the proliferation obtained in the absence of the drug. The half-inhibitory concentration (IC_{50}) was determined by incubating cells in the presence of increasing concentrations of free CsA and CyPB-complexed CsA from 12.5 to 1000 ng/ml. Analysis between the index of proliferation and CsA doses allowed us to estimate IC_{50} from the adjusted dose–response curve.

RESULTS

Binding of CyPB–CsA complex to human T-lymphocytes

CsA is known to enter blood cells in a non-specific manner and almost 60 % of the drug is recovered in the erythrocytes. The fact that CyPB could specifically bind to T-lymphocytes and mediates the incorporation of the drug into these cells suggests an interesting pathway for selective targeting of the drug to sensitive cells. To verify this hypothesis, the binding of both CyPB and CyPB–CsA complex to human blood T-lymphocytes was investigated.

The binding capacity of the CyPB–CsA complex to peripheral blood T-lymphocytes was investigated by using [^{3}H]CsA. Cells were allowed to bind various concentrations of [^{3}H]CsA–CyPB. The complex binding was specific since a 200-fold molar excess of unlabelled ligand inhibited [^{3}H]CsA–CyPB binding from 60 to 80 %. After subtraction of non-specific interactions from total counts, the binding was found to be concentration-dependent and saturable (Figure 1). Scatchard analysis resulted in a linear plot compatible with a single affinity site (Figure 1, inset) and

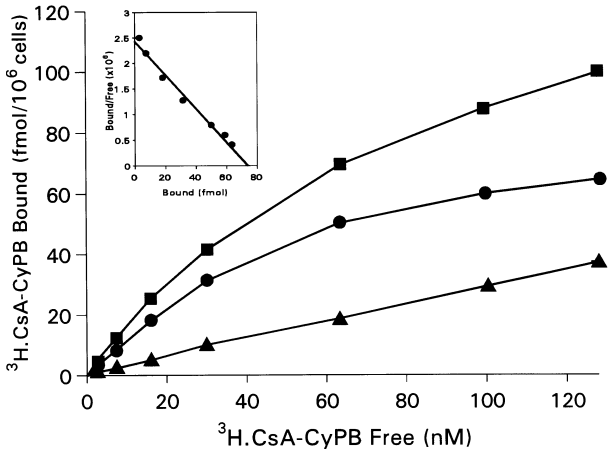

Figure 1 Surface binding of CsA–CyPB to human peripheral blood T-lymphocytes

Dose-dependence and saturation of CsA–CyPB binding were studied by incubating T-cells with the indicated concentrations of [^{3}H]CsA–CyPB complex for 1 h at 4 °C. The specific binding (●) was obtained after subtraction of non-specific (▲) from total counts (■). Points represent the mean values of triplicates at each ligand concentration. Inset is the Scatchard plot of the binding data.

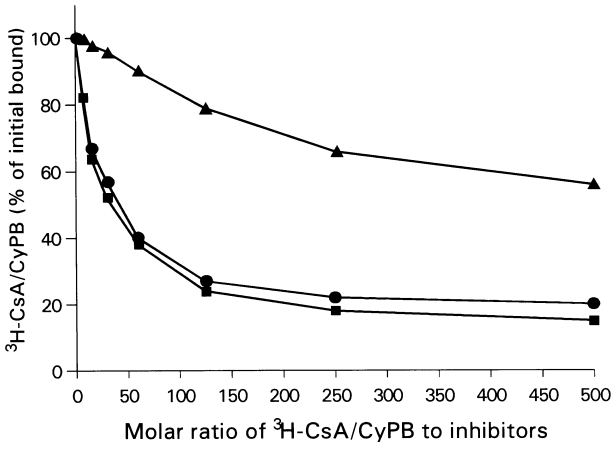

Figure 2 Competitive experiments for CsA–CyPB binding to human peripheral blood T-lymphocytes

Competitive binding experiments were performed by incubating T-cells in the presence of 50 nM of [^{3}H]CsA–CyPB and either unlabelled complex (●), uncomplexed CyPB (■) or free CsA (▲) at the indicated molar excesses. After 1 h at 4 °C, cells were washed and remaining cell-surface radioactivity was counted. Points represent the mean values of triplicates at each ligand concentration.

allowed us to determine the binding parameters, expressed as mean values ± S.E.M. from three separate experiments. The apparent dissociation constant (K_d) was 31 ± 9 nM and the number of binding sites was estimated to be 40600 ± 7600 per cell.

Radio-iodinated CyPB was previously shown to bind to Jurkat T-cells with an apparent dissociation constant of 12 nM and a number of binding sites of 35000/cell [16]. Similar experiments were reproduced with the radio-iodinated ligand, in order to compare the binding parameters of both free and CsA-complexed CyPB to peripheral blood T-lymphocytes and Jurkat T-cells. The parameters of ^{125}I-CyPB binding to Jurkat T-cells were very

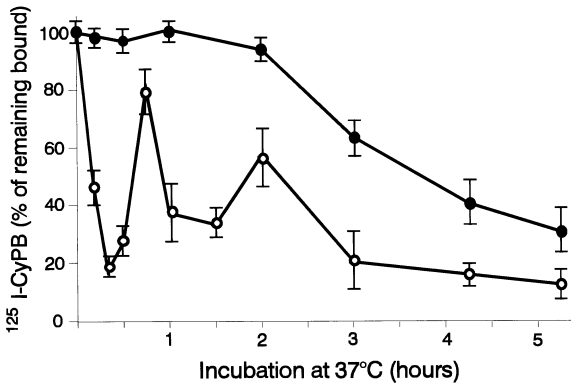

Figure 3 Ligand-dependent internalization and recycling of CyPB-binding sites in human peripheral blood T-lymphocytes

Cells were treated with cycloheximide and incubated at 37 °C in the absence (●) or presence of 50 nM CyPB (○). After being washed at the indicated times with acid buffer to remove surface-bound ligand, cells were allowed to bind ^{125}I-CyPB for 1 h at 4 °C. Data represent the percentages of remaining surface-bound ^{125}I-CyPB in comparison with control cells, and are expressed as mean values ± S.E.M. from three separate experiments.

similar to those obtained with peripheral blood T-lymphocytes (K_d = 18 ± 3 nM; n = 40300 ± 5800), suggesting that the CyPB surface binding sites were probably identical membrane proteins in both cell types. Since we have shown that the CyPB–CsA complex inhibited ^{125}I-CyPB binding to T-cells to the same extent as the uncomplexed protein, it may be postulated that CyPB and CsA–CyPB bind specifically to the same sites on the T-cell membrane. To verify this hypothesis, we also investigated the binding of the CyPB–CsA complex to the Jurkat T-cell surface. The parameters of complex binding were similar for both peripheral blood T-lymphocytes and Jurkat T-cells, and K_d and the number of sites were slightly higher than those obtained with ^{125}I-CyPB. These differences could, however, be due to the lower sensitivity in the binding assay using the tritiated complex instead of a radio-iodinated protein.

Finally, we verified the competitive properties of free CyPB and uncomplexed CsA for the inhibition of complex binding to the cell surface (Figure 2). As expected, free CyPB inhibited [^{3}H]CsA–CyPB binding to T-cells to the same extent as the unlabelled complex. In contrast, a large molar excess of CsA only weakly reduced surface-bound complex. This reduction could be explained by an isotopic dilution of tritiated drug rather than by specific competition for binding to the cell surface.

Ligand-dependent internalization and recycling of CyPB binding sites

In a previous study, we have demonstrated that surface-bound CyPB was internalized in Jurkat T-cells and subsequently released into the medium in a degraded form [16]. These experiments were reproduced here with peripheral blood T-lymphocytes and similar conclusions may be drawn (results not shown). In addition, we studied the ligand-dependent down-regulation of CyPB binding sites (Figure 3). Cycloheximide treatment gradually decreased the surface binding of ^{125}I-CyPB, suggesting that the binding sites are replaced on the cell surface with a half-life of 3 h 45 min. In contrast, the exposure of cycloheximide-treated T-cells to exogeneous CyPB produced an almost complete loss of cell surface binding in the first few minutes. Nevertheless, cells exhibited a gradual recovery of ^{125}I-CyPB binding. The surface binding recovered approx. 80 % of the original level in 45 min,

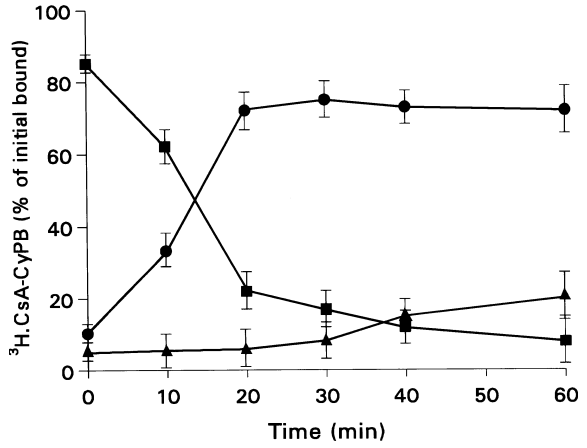

Figure 4 Time-course of the fate of CsA–CyPB surface-bound to human peripheral blood T-lymphocytes

Cells were first incubated with 50 nM [^{3}H]CsA–CyPB for 1 h at 4 °C. After washing off the unbound ligand, cells were allowed to internalize the complex at 37 °C. At the indicated times, aliquots were removed and incubation supernatant, eluate from mild-acid-treated cells and cell pellet radioactivities were counted. Ordinates represent the percentages of the total radioactivity initially bound to T-lymphocytes which was segregated within the cell (●), still surface-bound (■), and released in the incubation medium (▲). Data are mean values ± S.E.M. from three separate experiments.

before CyPB binding sites started a new cycle of endocytosis. These results demonstrate that CyPB binding sites are internalized in the presence of ligand and subsequently recycled to the cell surface. Similar experiments were performed in the presence of CsA without any alteration in CyPB endocytosis and binding site recycling, suggesting that the CyPB-complexed drug might be incorporated into T-lymphocytes through the internalization of the CsA–CyPB complex.

Internalization of CyPB–CsA complex within peripheral blood T-lymphocytes

To study whether the binding of the CsA–CyPB complex might promote drug incorporation, cells were allowed to bind [^{3}H]CsA–CyPB. After washing off the excess of unbound ligand, cells were incubated at 37 °C and the distribution of surface-bound, internalized and released radioactivity was analysed after a mild-acid wash (Figure 4). During the first few minutes, the acid-sensitive fraction decreased rapidly. At the same time, the acid-resistant fraction increased, until reaching a steady state after 20 min of incubation. No significant amount of radioactivity was found in the supernatant, demonstrating that the drug was no longer released from the cells. These results suggest that CyPB mediates CsA incorporation through the specific binding of the complex to the cell surface, but in contrast to the protein which is released into the supernatant in a degraded form, the drug accumulated within the cells. To confirm the involvement of CyPB binding sites in mediating CsA incorporation, similar experiments were performed in the presence of surface-trypsinated lymphocytes (results not shown). Neither specific binding of the [^{3}H]CsA–CyPB complex nor significant incorporation of the drug was observed, implying the existence of a membrane protein necessary for CyPB-mediated incorporation of CsA in T-lymphocytes.

Table 1 Effect of CyPB on CsA immunosuppressive activity

Lymphocytes were stimulated for 72 h by a monoclonal anti-CD3 antibody, in the presence of either CsA, CsA–CyPB complex or CyPB at 25 nM or 250 nM. T-cell activation and proliferation were visualized by variations of fluorescence mean value (FMV) associated with CD25 expression and by [^{3}H]thymidine incorporation experiments respectively, as described in the Experimental section. Data represent the percentages of remaining CD3-stimulation in comparison with control obtained in the absence of CsA and/or CyPB. They are expressed as mean values ± S.E.M. from three separate representative experiments.

	CD25 expression (% FMV)	[^{3}H]Thymidine incorporation (%)
Control	100 ± 9	100 ± 11
Free CsA		
25 nM	28 ± 5	45 ± 9
250 nM	12 ± 2	21 ± 4
CyPB–CsA complex		
25 nM	26 ± 2	31 ± 11
250 nM	12 ± 1	14 ± 7
Uncomplexed CyPB		
25 nM	80 ± 11	95 ± 16
250 nM	78 ± 8	98 ± 7

Immunosuppressive activity of CyPB-complexed CsA

In the following experiments, we verified the immunosuppressive activity of CyPB-complexed CsA. Indeed, the internalization of CyPB–CsA might lead to the sequestration of the drug in a subcellular compartment preventing the expression of its immunosuppressive activity. Two concentrations, 25 and 250 nM, were studied as they are respectively close to the K_d value and to saturation of CyPB-binding-site capacity. The effects of both CyPB-complexed CsA and free CsA on the anti-CD3 mixed-lymphocyte reaction were then compared. Thus, CyPB-complexed CsA inhibited CD25 expression to the same extent as free CsA without any significant variation. No significant effect was observed in the presence of uncomplexed CyPB, demonstrating that the protein had no inhibitory properties on the CD3-dependent activation of T-lymphocytes (Table 1). Cytogram analysis of the lymphocyte population shifting from the resting T-cell region to the activated window allowed us to determine an index of activation with similar conclusions (results not shown). Cell proliferation was then studied by [^{3}H]thymidine incorporation experiments. Results were very similar for both free and CyPB-complexed CsA, suggesting that the CyPB-complexed CsA exhibits anti-proliferative properties to the same extent as free CsA. The immunosuppressive effect of the CsA–CyPB complex is likely to be due to the internalization of complexed drug. Indeed a 200-fold excess of free CyPB dramatically reduced the inhibitory effect of CsA–CyPB, demonstrating binding-site occupancy on the T-lymphocytes blocked the incorporation and the consequent activity of CyPB-complexed CsA (results not shown). Finally, we investigated the influence of CyPB on the dose–response to CsA (Figure 5). The drug concentrations required for 50% inhibition of T-cell proliferation showed marked differences, since IC_{50} was estimated to be 40 ± 12 ng/ml in the presence of CyPB-complexed CsA while it was 175 ± 40 ng/ml in the presence of free drug. Taken together, these results demonstrate that CyPB-mediated incorporation of CsA enhances the activity of immunosuppressive drug, suggesting it might be related to differences in lymphocyte sensitivity.

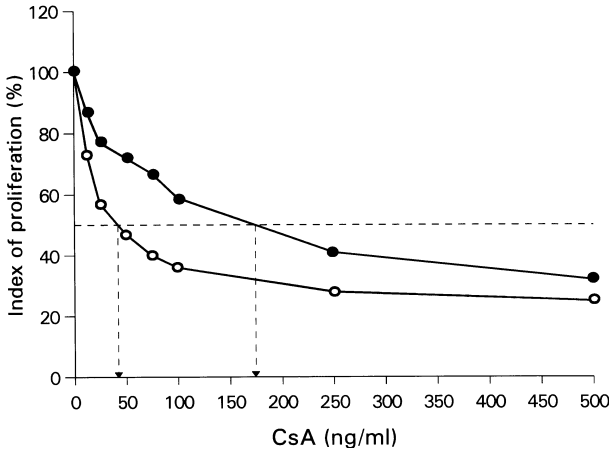

Figure 5 Half-inhibitory concentrations (IC_{50}) for lymphocyte CD3-induced proliferation in the presence of free and CyPB-complexed CsA

Representative dose–response curves were determined by [^{3}H]thymidine incorporation in CD3-stimulated lymphocytes exposed to increasing concentrations of either free CsA (●) or CyPB-complexed drug (○). The half-inhibitory concentrations (IC_{50}) were determined from the adjusted dose–response curves between index of proliferation and CsA concentrations. Data are mean values from three separate experiments.

DISCUSSION

Our previous work demonstrated that CyPB specifically bound to the surface of human lymphoblastic T-cells [16]. At 37 °C, the protein was internalized and subsequently degraded within the cell. Here we present new results concerning the properties of the CyPB–CsA complex towards human blood T-lymphocytes. As expected, the CyPB–CsA complex bound to blood T-cells in a specific manner, to the same extent as free CyPB. The complex and its binding site were internalized, but, in contrast to CyPB, the drug accumulated within the cell and the putative receptor was recycled to the cell surface. Moreover, the complexed drug retained its immunosuppressive properties, suggesting that CsA and its receptor dissociate and follow separate metabolic pathways within the cell. Finally, the analysis of drug concentration required for 50 % inhibition of T-cell proliferation showed that the CyPB-complexed CsA was more efficient than free CsA, suggesting CyPB and its surface binding site enhanced the drug activity.

Published data concerning CyPB are often divergent and poorly documented in contrast to the well-studied abundant cyclophilin A (CyPA). For instance, the subcellular localization of CyPB is the subject of debate. Arber et al. [18] suggest that the protein co-localizes with calreticulin in some intracellular vesicles which they called calciosomes. They have demonstrated that the protein is retained in these vesicles through its specific C-terminal extension. However, we have reported the characterization of CyPB in human biological fluids, using purification and ELISA procedures [19,20]. Price et al. [22] suggest that CyPB may act as a chaperone and escort the neosynthesized proteins along the sorting pathway. They also found this protein to be localized on the cell surface and released into the medium. Recently, the protein was even shown to be abundantly secreted together with HSP47 in escorting procollagen into the secretory pathway [23]. The possible role of CyPB in CsA-mediated immunosuppression has also been investigated. CsA is supposed to mediate its immunosuppressive action by forming a complex with intracellular cyclophilins and altering the activity of the Ca^{2+}-dependent phosphatase calcineurin [8–10]. The inhibition of the activity of this key enzyme in T-cell activation leads to the block of the cytosolic NF-AT (nuclear factor of activated T-cell) subunit dephosphorylation, preventing its translocation into the nucleus and the inducibility of interleukin-2 gene transcription. Due to its intravesicular localization, CyPB is unlikely to intervene in this process. An indirect role has been proposed, in relation with the relative concentrations of both CyPA and CyPB, in cells. Thus, Foxwell et al. [24] have suggested that the CsA-resistance of the fibroblastic cell line MRC5 may be due to the higher level of CyPB than CyPA. This hypothesis is in agreement with our selective ELISA measurements, which showed that lymphocytes exhibit very low concentrations of CyPB versus CyPA [20]. Other recent studies suggest a direct role of CyPB in CsA-mediated immunosuppression [25]. In fact, by using the two-hybrid system in yeast, Bram and Crabtree [25] have cloned and expressed a putative receptor for CyPB. This reticulum membrane protein, called CAML for 'calcium-signal modulating cyclophilin ligand', may be involved in the modulation of the Ca^{2+} transduction pathway. By inhibiting CyPB function, CsA might block the activity of CAML and consequently lead to a new mechanism of immunosuppression. Our results, however, suggest another alternative. Extracellular CyPB might indirectly interact in the immunosuppression mechanisms, through the specific targeting of the drug to sensitive cells.

According to the data of the literature and our own experiments, CyPB surface binding sites would be related to at least three functions. We showed that CyPB-binding sites were endocytosed in the presence of the ligand and recycled in T-cells as indicated by their re-expression on the cell surface. As Price et al. [22] suggested, CyPB could act as a chaperone and the binding sites might be involved in the return of CyPB to reticulum vesicles. This hypothesis is in agreement with the recent data of Baker et al. [26], who demonstrated that the cyclophilin homologue NinaA functions as a chaperone and recycles back and forth within the secretory pathway. The internalization of the immunosuppressive complex might be coupled to receptor recycling, leading to a long-term action of CsA through the continual accumulation of the drug within T-cells. This might explain the increase in CsA activity we obtained when T-lymphocytes were exposed to the CyPB-complexed drug. Furthermore, the inter-individual variations of lymphocyte sensitivity were shown *in vitro* to be related to the concentrations of CsA [13]. CyPB, by targeting the drug to T-cells, might promote its incorporation and consequently abolish the phenomenon of cellular resistance. We recently demonstrated that CyPB largely increased the drug susceptibility of T-cells from several healthy subjects. These data confirm the hypothesis that extracellular CyPB may be related to the variations in drug sensitivity (F. Allain, A. Denys and G. Spik, unpublished work). A second function might be as a scavenger receptor for released CyPB since the protein was found here to be partially degraded within T-cells. Besides targeting the drug to lymphocytes, the CyPB binding sites might be associated with the toxicity of CsA therapy. They may occur on liver and kidney cell surfaces, leading to a damaging accumulation of the drug in these tissues. The third function of CyPB-binding sites might be related to the chemotactic activity of cyclophilins [27,28]. The formation of the CyPB–CsA complex would lead to blocking of a chemokine-like activity and CsA might consequently act by inhibiting lymphocyte migration to the inflammation site. This last point is intriguing as it could in part explain the anti-inflammatory effect of CsA in psoriasis disease, where it was shown that the drug depletes both activated and non-activated T-cells from the epidermis and dermis of psoriatic patients [29].

In summary, we have shown that CyPB promotes the incorporation of CsA into blood T-lymphocytes through the specific binding of the complex to the cell membrane. Thus, the targeting of the drug and its consequent increase within cells might be related to the susceptibility of T-lymphocytes. Nevertheless, the function of CyPB receptors needs to be further investigated in an attempt to provide more explanations for the variability of CsA sensitivity and toxicity in CsA therapy.

This investigation was supported in part by the Centre National de la Recherche Scientifique (Unité Mixte de Recherche no. 111; Relations Structure–Fonction des Constituants Membranaires; Director: Professor A. Verbert), by the Université des Sciences et Technologies de Lille and by grants from the European Economic Community (Biomed Program Contract BMH1-CT93-1748). We are grateful to Dr. L. Vernillet for the generous gift of tritiated CsA derivative, to Dr. M. Zurini and Dr. N. R. Movva for the pCGF-72 plasmid, to Dr. D. Brevière for blood samples, to P. Michaux, J. P. Dumon and M. Binchet for their assistance in the production of recombinant CyPB and to Dr. B. Foxwell and Dr. R. Pierce for critical reading of the manuscript.

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Received 5 February 1996/18 March 1996; accepted 25 March 1996