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# Assessment of immunosuppression by serum inhibition of alloreaction and measurement of cyclosporin A (CyA) serum levels in kidney graft recipients under CyA

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**Abstract.** The immunosuppressive effect of kidney graft recipient sera was studied on T-lymphocyte alloreactive line (4H) proliferation and compared to native cyclosporin A (CyA) and CyA metabolite concentrations determined by radioimmunoassay (RIA) using specific or nonspecific monoclonal antibodies. Three clinical groups were studied: (1) patients experiencing acute renal rejection episodes (CyA-R), (2) patients experiencing CyA-dependent nephrotoxicity episodes (CyA-TOX) and (3) patients in a clinically steady state (CyA-ST), according to their therapeutic regimen i.e., monotherapy (CyA alone) or polytherapy (CyA associated with prednisolone and/or azathioprine). Regardless of the clinical state, sera of patients in polytherapy displayed more inhibitory activity than those of monotherapy patients (24% and 40% inhibition of 4H proliferation, respectively, at sera dilution of 1:2), something which was no doubt due to the inhibitory activity of prednisolone on T-lymphocyte growth. In the two therapeutic regimens, CyA-ST patient sera exhibited the lowest inhibitory activity on the 4H line (45% and 65% inhibition of 4H proliferation in mono- and polytherapy, respectively, at sera dilution of 1:2). Sera from CyA-TOX patients were highly inhibitory (74% and 86% inhibition of 4H proliferation in mono- and polytherapy, respectively, at sera dilution of 1:2), in agreement with RIA assays showing increased native circulating CyA and CyA metabolites and daily CyA intake in this group as compared to CyA-ST. Surprisingly, CyA-R patient sera were no less inhibitory than those of CyA-ST patients on 4H-line, antigen-induced proliferation. This clinical group did not differ from others for CyA intake or level of circulating immunosuppressive molecules, suggesting that rejection could be associated with a state of interindividual variation in sensitivity to CyA. In addition, a polytherapeutic regimen seemed to modify CyA bioavailability in CyA-ST group patients, with a decreased CyA metabolite level as compared to their monotherapy counterparts (native CyA plus metabolite/native CyA ratio being 2.73 and 3.73, respectively). In contrast, in the CyA-R patient group, polytherapy appeared to be associated with an increase in CyA metabolite circulating levels (ratio 4.79). In view of the low inhibitory activity of CyA metabolites, this profile might lead to rejection.

**Key words:** Immunosuppression, cyclosporin A – Inhibition of alloreaction, cyclosporin A – Cyclosporin A serum levels, inhibition of alloreaction

Cyclosporin A (CyA) is an undecapeptide with immunosuppressive properties acting mainly on T-lymphocyte activation and widely used in organ transplantation. Routine monitoring of native compound levels are performed by high performance liquid chromatography [2] or, more commonly, of both native CyA and metabolite levels by a radioimmunoassay (RIA) using polyclonal rabbit antibodies [6]. Recently, the availability of monoclonal antibodies that react either with a specific epitope of the native CyA molecule or with an epitope shared by native CyA and some of its metabolites [12] has allowed for more sophisticated monitoring, suitable for large-scale routine practice. It is of interest to know which of these assays can provide accurate measurement of the actual immunosuppressive effect of the drug. In fact, the major CyA metabolite in humans – metabolite 17 – displays some inhibitory activity on antigen-specific T-lymphocyte allostimulation [3], although this molecule appears to be far less effective than the native one, suggesting that it is the native CyA that performs the major immunosuppressive function in vivo. In addition, although functional assays based on the capacity of sera to inhibit in vitro or selected T-lymphocyte alloreactive line growth would be obviously too long for routine clinical monitoring procedures, they may nevertheless contribute to a better assessment of the immunosuppressive activity of recipient sera resulting from the combined effects of native CyA and its metabolites, as

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**Table 1.** Renal function, cyclosporin A (CyA) doses and CyA trough levels in patients with rejection and CyA-induced nephrotoxicity[a]. Aza, Azathioprine; Pred, prednisolone

| Treatment | Number of cases | Blood creatinine levels (μmol) | | | CyA trough levels at onset (ng/ml) | | CyA daily intakes (mg/kg) |
|---|---|---|---|---|---|---|---|
| | | Before | At onset | After | Blood | Serum | |
| *CyA-R group* | | | | | | | |
| CyA alone | 7 | 155 (105–259) | 234 (168–355) | 145 (118–185) | 531 (320–765) | 130 (48–227) | 6.5 (4.9–4) |
| CyA + Aza | 3 | 175 (110–225) | 230 (216–250) | 170 (145–185) | 534 (437–604) | 130 (108–158) | 6 (3.1–8.6) |
| CyA + Aza + Pred | 5 | 155 (94–240) | 390 (178–830) | 174 (105–250) | 457 (200–771) | 122 (26–262) | 7 (2.2–9.4) |
| CyA + Pred | 1 | 145 | 180 | 130 | 581 | 154 | 6.8 |
| Total | 16 | 158 (94–259) | 278 (168–830) | 158 (105–250) | 511 (200–771) | 130 (26–262) | 6.6 (2.2–9.4) |
| *CyA-TOX group* | | | | | | | |
| CyA alone | 10 | | 146 (105–285) | 119 (75–163) | 943 [697][b] (613–1265) (400–112) | 313 (93–331) | 7.1 [5.2][b] (4–12) (3–11) |
| CyA polytherapy | 10 | | 278 (90–545) | 149 (70–225) | 895 [540][b] (251–1202) (175–780) | 278 (50–544) | 7.6 [6][b] (3–9.5) (1.5–7) |

[a] Main characteristics of patients with rejection (CyA-R) and with CyA-mediated toxicity (CyA-TOX). CyA blood levels after normalization of blood creatinine at the end of the episodes of the CyA-TOX group are given within brackets

[b] Levels given within brackets represent values after normalization of renal function

well as from other associated immunosuppressive drugs, such as prednisolone (Pred) or azathioprine (Aza).

We took advantage of the availability of kidney allograft recipients receiving either CyA as the sole immunosuppressive drug or CyA in combination with Aza and/or Pred to compare the capacity of their serum to inhibit an allogeneic stimulation in vitro on the basis of the serum CyA trough levels. In this study, we report for the first time on the relationships between these different monitorings of the immunosuppressive state of human kidney recipients in selected groups, including patients in a clinically steady state and those experiencing acute rejection or CyA-mediated nephrotoxicity episodes.

## Patients and methods

### *Patients*

Sera harvested 12 h after CyA intake from 165 kidney allograft recipients were studied. CyA was given twice a day and doses were adjusted throughout follow-up according to trough blood levels measured by polyclonal RIA (therapeutic range 300–600 ng/ml). Three groups of patients were studied. The first, a cyclosporin-rejecting group (CyA-R), was composed of 16 patients (10 males, 6 females; mean age 38 years, range 18–63 years) with reversible acute cellular rejection episodes occurring 8 months after transplantation (range 0.5–36). All rejection episodes were biopsy-proven. Sera were collected at the time of rejection diagnosis before antirejection treatment. CyA doses and concentration, as well as blood creatinine levels, are given in Table 1. The second group, a cyclosporin-related toxicity group (CyA-TOX), was composed of 20 patients (15 males, 5 females; mean age 42 years, range 16–63 years) who experienced acute nephrotoxic episodes 80 days after transplantation (range 13–100). Nephrotoxicity was defined as an increase in blood creatinine level, without clinical and/or histological symptoms of rejection and with normal graft ultrasound, and was confirmed by a diminution in blood creatinine level following CyA dose reduction (Table 1). Patients taking other drugs known to be nephrotoxic per se, in association with CyA or interfering with CyA availability, were not included. The third group was a cyclosporin steady state group (CyA-ST) and was composed of 129 patients (81 males, 48 females; mean age 40.5 years, range 12–64 years) with stable graft function for more than 6 months. Ninety-seven were under CyA monotherapy, whereas the others also received Aza and/or Pred. Patients in steady state renal function had blood creatinine levels of 146 (70–260) and 176 (60–360) μmol/l in mono- and polytherapy, respectively. Their respective blood CyA trough levels were 539 (129–1229) and 587 (135–1607) ng/ml and 100 (16–493) and 141 (23–859) ng/ml in serum. Doses of CyA were 5.3 (2.1–10.7) and 6.5 (3.5–12.5) mg/kg per day in mono- and polytherapy patients in steady state renal function, respectively. Since our original immunosuppressive protocol following first renal transplantation consisted of CyA alone after a 2-month postoperative period of triple therapy (CyA, Aza, and Pred), Aza and/or Pred were reintroduced following: (1) histologically documented chronic rejection, (2) recurrence of native nephropathy, (3) CyA-induced chronic nephrotoxicity (4) two or more acute rejection episodes, and (5) retransplantation.

### *Materials and methods*

*Sera.* Blood samples were collected 12 h after the last CyA dose intake in sterile vacuum tubes (Venoject) and kept at room temperature for 3 h to allow coagulation and distribution of CyA between blood cells and sera to stabilize. Samples were then centrifuged and sera were collected, aliquoted, and stored at −45 °C.

*Determination of blood and serum CyA trough levels.* Blood and serum CyA levels were routinely assessed by using polyclonal RIA kits (Sandoz, Basel, Switzerland) capable of detecting CyA and metabolites at concentrations ranging from 20 to 2000 ng/ml. Assays were performed according to the manufacturer's recommendations. In addition, CyA serum concentrations of samples used for in vitro studies on the 4H line were measured by RIA using both a CyA-specific monoclonal antibody (CyA-MS) and a nonspecific (CyA plus metabolites) monoclonal antibody (CyA-MUsp) contained in the Sandimmun Kit (Sandoz).

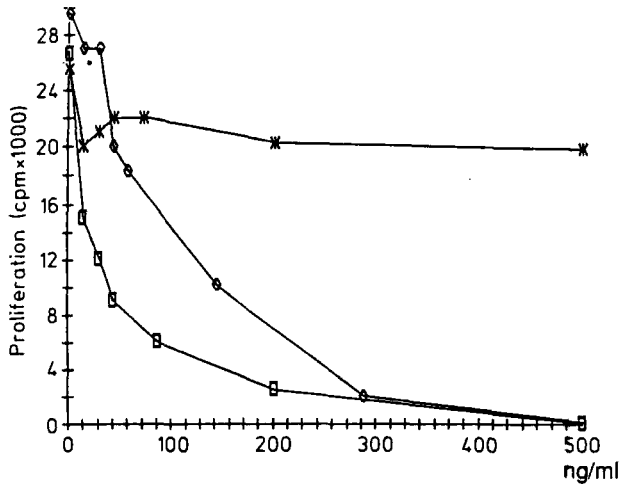


**Fig. 1.** Effect of cyclosporin A (CyA; O), azathioprine (Aza; *), and methylprednisone (MP; □) on 4H-line proliferation. 4H line (2.5 $10^4$ cells) were incubated with irradiated donor B-lymphoblastoid cells (5.$10^4$ cells) in the presence of various amounts of the different compounds tested. After 2 days of culture, proliferation was assessed on the incorporation of tritiated thymidine

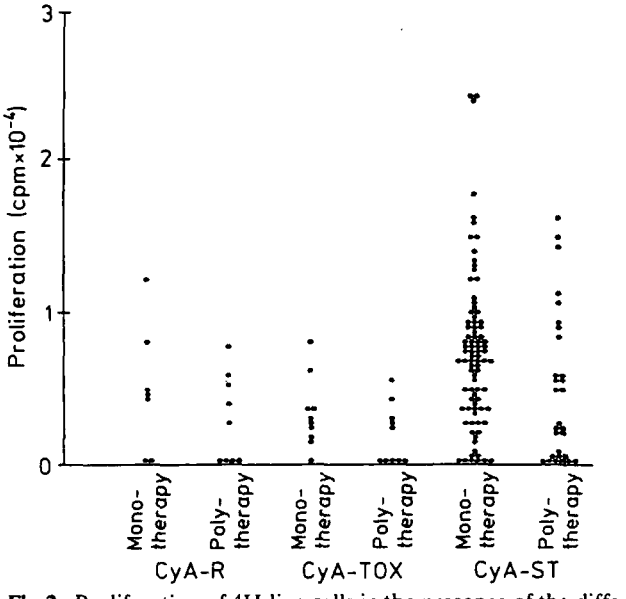


**Fig. 2.** Proliferation of 4H-line cells in the presence of the different sera groups used at the dilution 1:2. 4H-line cells (2.5 $10^4$) were incubated with irradiated donor B lymphoblastoid cells (5.$10^4$ cells) in the presence of the different sera at dilution 1:2. After a days of culture, proliferation was scored by tritiated thymidine incorporation as described in the text

*Serum inhibitory activity on 4H line growth. (a) 4H line:* This cell line was derived from cells invading a human rejected kidney allograft [1, 11]. Analysis of alpha, beta, and gamma T-cell receptor gene rearrangements [10] has shown that 4H is composed of two T-lymphocyte clones. 4H expresses a $CD2^+$, $CD3^+$, and/or $CD4^+$ $CD8^+$ phenotype and proliferates in response to specific antigens provided by a donor B-lymphoblastoid cell line obtained from kidney donor splenocytes immortalized with the Epstein-Barr virus [9]. *(b) Inhibition test:* Cultures were performed in U-bottomed, 96-well Greiner microplates in 0.16 ml final volume. Test sera were diluted (1:2; 1:10; 1:50; 1:125; and 1:625) in RPMI-1640 in a final volume of 80 µl 4H line cells (2.5.$10^4$), mixed with 5.$10^4$ irradiated cells from the donor B-lymphoblastoid cell line, to which were added 10% pooled human serum and 40 µg/ml gentamycin in the same volume (80 µl) in RPMI-1640. Test cultures were then incubated at 37 ÉC in 5% $CO_2$ for 2 days. Cells were pulsed with tritiated thymidine for the last 4 h of the incubation period, and the incorporation of thymidine was used as an index of cell proliferation (TRK61, Amersham, AS: 20–30 Ci/mmol, 0.5 µCi/well). Cells were then collected with an automatic cell harvester, and the incorporation of tritiated thymidine was measured in a beta scintillation counter.

*Results, expression, and statistical analysis.* Values obtained from sera of kidney recipients treated with CyA (alone or with other immunosuppressive drugs) were compared with those obtained from sera of 32 untreated, healthy donors. As these untreated control sera exhibited various levels of inhibition on cell proliferation at the informative dilutions tested (i.e., at 1:2 and 1:10 dilution, 49% and 12% inhibition of 4H proliferation, respectively), the values obtained with recipient sera were corrected according to this nonspecific sera effect. At each dilution considered, proliferation (counts per minute; CPM) in the presence of patient sera was expressed as a percentage of the response (CPM) obtained in the presence of control sera at the same dilution, according to the following formula: corrected proliferative response: (100/CPM of control sera) × CPM of patient sera. As distribution analyses indicated that some groups of values did not follow a normal distribution, we systematically used the nonparametric Mann-Whitney and Spearman tests for statistical comparisons.

## Results

### *4H line sensitivity to CyA, methylprednisolone, and azathioprine*

Figure 1 shows that 4H line sensitivity to the tested drug. 4H line exhibited sensitivity to CyA that compared to other alloreactive human T-cell clones we had previously studied (ID 50~50 ng/ml [3, 4]. This line also appeared to be extremely sensitive to corticosteroid (methylprednisolone) activity (ID 50~10 ng/ml). Azathioprine, on the other hand, exhibited a small effect on antigen-induced proliferation (ID 50 > 500 ng/ml). 4H line sensitivity was not sufficient to detect drug-circulating levels when sera were tested at higher dilutions than 1:10; therefore, only data obtained with recipient sera dilutions 1:2 and 1:10 have been considered.

### *Inhibition of tritiated thymidine incorporation of antigen-stimulated 4H line by sera of CyA-treated graft recipients*

Figure 2 shows the proliferation pattern of 4H line in the presence of the different groups classified according to clinical situations (rejection, CyA nephrotoxicity, and steady state) and to monotherapy and polytherapy regimens. Sera of the patient CyA-ST group (Fig. 2) in monotherapy exhibited the lowest average activity (45% and 23% of inhibition of 4H proliferation at dilution 1:2 and 1:10, respectively) compared to the other groups. 4H cells incubated with sera harvested at the time of rejection onset in CyA-R patients under CyA monotherapy had a 60% and 41% inhibition of 4H proliferation at dilution 1:2 and 1:10, respectively. Proliferation of 4H line was markedly decreased when CyA-TOX sera were used, with 74% inhibition of 4H line growth.

**Table 2.** Cyclosporin A (CyA) intakes and CyA serum levels (MUsp and MSp) in the different clinical groups tested. Statistical correlations. (No significant differences in all parameters studied were noted in polytherapy clinical groups between patients receiving CyA + Aza, CyA + Pred, or CyA + Aza + Pred. Aza, Azathioprine; Pred, prednisolone). * $P < 0.05$; ** $P < 0.01$

| Serum dilutions | CyA monotherapy | | | Polytherapy (CyA with Pred and/or Aza) | | |
|---|---|---|---|---|---|---|
| | Steady state ($n = 97$) | Rejection ($n = 7$) | Toxicity ($n = 10$) | Steady state ($n = 32$) | Rejection ($n = 9$) | Toxicity ($n = 10$) |
| CyA intake (mg/kg per day) | 5.3[a] | 6.3 | 6.8 | 6.5 | 6.1 | 7.3 |
| Serum native CyA MSp levels (ng/ml) | 30 | 36 | 60 | 48 | 29 | 98 |
| Serum native CyA and metabolites (CyA MUsp assay ng/ml) | 111 | 126 | 217 | 130 | 139 | 268 |
| CyA Usp/Sp ratios | 3.73 | 3.49 | 3.65 | 2.73 | 4.79 | 2.73 |

[a] Geometric means of corresponding values

**Table 3.** Correlation between 4H-line proliferation in the presence of recipient sera and cyclosporin A (CyA) serum levels as assessed by MSp and MUsp RIAs. Aza, Azathioprine; Pred, prednisolone

| CyA levels | 4H line proliferation | | | | | | |
|---|---|---|---|---|---|---|---|
| | CyA monotherapy | | | | Polytherapy (CyA with Pred and/or Aza) | | |
| | Serum dilutions | Steady state ($n = 97$) | Rejection ($n = 7$) | Toxicity ($n = 10$) | Steady state ($n = 32$) | Rejection ($n = 9$) | Toxicity ($n = 10$) |
| CyA MSp assay | D1:2 | $P < 0.01$[a] | NS | $P < 0.05$[a] | NS | NS | NS |
| | D1:10 | $P < 0.01$[a] | NS | NS | $P < 0.05$[a] | NS | NS |
| CyA MUsp assay | D1:2 | $P < 0.01$[a] | NS | NS | NS | NS | $P < 0.05$[a] |
| | D1:10 | $P < 0.01$[a] | NS | NS | $P < 0.05$[a] | NS | NS |
| Ratios CyA-MUsp /MSp | D1:2 | NS | NS | NS | NS | NS | NS |
| | D1:10 | NS | $P < 0.05$[a] | NS | NS | NS | NS |
| CyA doses mg/kg per day | D1:2 | NS | $P < 0.05$ | NS | NS | NS | NS |
| | D1:10 | NS | $P < 0.01$ | NS | NS | NS | NS |

[a] Negative correlation

Mean values of 4H line proliferation were always lower when the sera of patients under polytherapy were used as compared to monotherapy group values (Fig. 2). Again, the lowest proliferation was obtained with the CyA-TOX group: 86% and 71% inhibition of 4H proliferation at 1:2 and 1:10 serum dilutions, respectively. Further splitting of the polytherapy groups according to type of CyA-associated drug (i.e., corticosteroids or azathioprine) did not result in statistically significant differences in proliferative values. This was probably due to the small size of the samples. However, growth inhibition ranked as follows: CyA + Pred + Aza > CyA + Aza, according to what might be expected from results obtained by testing the effect of exogeneously added drugs on 4H-line cells (Fig. 1).

*CyA levels assessed by the specific and nonspecific CyA assays in the different groups of patients*

*Correlation between whole blood and serum CyA levels.* As addition of whole blood to the culture system was impossible, all experiments were performed with recipient sera. Trough CyA levels measured in whole blood and in sera, as routinely assessed for clinical monitoring using the rabbit polyclonal Sandimmun RIA kit (CyA-PUsp), showed a strong correlation ($r$: 0.8).

*Comparison of CyA serum levels in the different groups.* As inhibition studies on the 4H line could differ markedly according to the presence of various amounts of native CyA and CyA metabolies [3], we undertook their differential measurement by using the CyA-MSp and MUsp RIA. Table 2 summarizes the results and gives the CyA-MUsp/MSp ratios, together with the daily average CyA intakes for each recipient group.

Patients in steady state monotherapy had an average of 30 and 111 ng/ml (geometric mean) of CyA-MSp and CyA-MUsp, respectively, with an MUsp/MSp ratio of 3.73. Sera harvested during CyA nephrotoxicity episodes in monotherapy patients exhibited higher native CyA (Table 2, $P < 0.01$), as well as higher CyA-MUsp levels than other groups; their CyA-MUsp/MSp ratio was unchanged compared to that of the CyA-ST monotherapy group. Sera obtained from recipients at the onset of a rejection episode exhibited trough CyA-MSp, MUsp, and MUsp/MSp ratios roughly similar to those found in patients in steady state under monotherapy (Table 2).

Sera from CyA-ST patients under polytherapy had significantly higher native circulating CyA-MSp levels than those of their counterparts in monotherapy ($P < 0.01$). However, daily average intakes of CyA were also significantly higher than those in the monotherapy group (6.5 vs 5.3 mg/kg; $P < 0.01$). No statistical differences were observed between the two CyA-ST subgroups for the CyA-MUsp level; accordingly, ST patients in polytherapy had a lower CyA-MUsp/MSp ratio (2.73 vs 3.73; $P < 0.01$). As for their CyA-ST counterparts, sera of patients under polytherapy and experiencing CyA nephrotoxicity episodes also exhibited higher CyA-MSp and MUsp values than those in the other groups, but without modification of the CyA-MUsp/MSp ratio and drug intakes (Table 3). In contrast, sera harvested from patients under polytherapy who were tested at the onset of a rejection episode (CyA-R group) did not show modifications in MSp and MUsp CyA levels or in daily CyA intakes as compared to those obtained in the CyA-ST group; however, the CyA-MUsp/MSp ratio was found to be significantly higher (4.79; $P < 0.05$; Table 3) in this group than in the others.

*Correlations between 4H-line serum-mediated growth inhibition and CyA sera levels as assessed by specific and non-specific RIAs* (Table 3)

*Steady state patients.* Table 3 shows the distribution of CyA levels in sera of CyA-ST recipients on monotherapy according to the degree of 4H-line proliferation in the presence of recipient sera at dilutions 1:2 and 1:10. In this clinical group, 4H growth inhibition observed at both serum dilutions correlated significantly with the levels of CyA-MSp or MUsp ($P < 0.01$). There was, however, a large scatter in the values. Some sera with low CyA levels exhibited high growth inhibition, suggesting the role of a drug-unrelated serum effect, whereas low 4H-line growth inhibition ( > 65% proliferation) was only related to low CyA-Sp or Usp levels. As some patients, though clearly in a clinical steady state, nevertheless had some degree of renal insufficiency, with creatininemia above 150 $\mu M$, we checked for a possible correlation between blood creatinine levels and 4H-line growth inhibition. These two parameters were statistically linked ($P < 0.05$), suggesting an association between low-grade renal failure and 4H growth inhibition.

Like those of their monotherapy counterparts, CyA-ST polytherapy recipient sera induced 4H-line inhibition, which correlated with CyA-MSp and MUsp levels (Table 3; $P < 0.05$ for dilution 1:10).

*CyA-TOX and CyA-R patients.* In CyA-TOX group sera, 4H-line growth inhibition for the 1:2 dilution appeared to be correlated with CyA-MSp, but only with CyA-MUsp levels in polytherapy subgroups. The effects of CyA-R sera on 4H proliferation did not reach statistical correlation with CyA levels, which was probably due to the limited size of these recipient subgroups. Finally, no correlation was found between 4H-line growth inhibition, CyA-MUsp/MSp ratios, and daily CyA oral intakes, except for the CyA-R group in monotherapy (Table 3).

## Discussion

In this paper we have studied the relationship between clinical events and trough levels of native CyA alone or with CyA metabolites, as assessed by different RIAs. In addition, we have compared these CyA levels in the same recipient groups with the capacity of recipient sera to inhibit an allogeneic reaction in vitro. Culture conditions dictated the use of recipient sera instead of blood in functional tests. Although CyA levels (and Usp/Sp ratios) were not identical in sera and blood, something which was routinely tested clinically, there was a good correlation between these two measurements.

Although functional tests using in vitro allorecognition models are not adapted to clinical routine monitoring of CyA effects in transplanted patients, they may help to define recipient populations at risk and to assess the immunosuppressive status in polytherapy and monotherapy regimens. Interestingly, when we waited for high inhibition of proliferation in patients with CyA-related toxic episodes (with higher CyA-MSp or CyA-MUsp circulating levels), it was surprising to find that CyA-R recipient sera were also more inhibitory than those obtained from patients in a clinically steady state. Indeed, the inhibition rate of the 4H-line in CyA-R was comparable to that found in the CyA-TOX group. However, these results suggest that the rejection capacity of a given individual might not simply be related to a low circulating drug level or a low inhibition potential on allogeneic response. As rejecting patients had similar CyA intakes, CyA levels, and CyA-MUsp/MSp ratios to those in steady state (except in the CyA-R polytherapy subgroup), it is possible that renal failure-related accumulation of growth inhibition compounds [8, 18] might have interfered, since blood creatinine values were significantly higher in the CyA-R than in the CyA-ST or CyA-TOX groups (mean values 234 $\mu M$ compared to 146 or 146 $\mu M$ in monotherapy, and 267 $\mu M$ compared to 176 or 278 $\mu M$ in polytherapy, respectively). This possibility was, indeed, suggested by the observed significant correlation between 4H-line growth inhibition and creatinine levels.

As most of the patients studied were receiving CyA as the sole immunosuppressive drug, we were able to study this uncommon population and, thus, analyze the effect of the two other commonly used immunosuppressive drugs (i.e., steroids and Aza) on CyA levels and on the capacity of recipient sera to inhibit allogeneic reaction. Sera of recipients under polytherapy had consistently more inhibitory activity than those under CyA monotherapy, which was probably due to the well-documented effect of steroids [7, 16] or, to a lesser extent, Aza [5], on lymphocyte allogeneic proliferation, which synergizes with the CyA effect [5, 13, 14].

Differential assessments of native CyA and the pool of native CyA plus metabolites allowed a more detailed analysis of the pharmacological activity of the drug in the various clinical situations. CyA-TOX patients had a significantly higher CyA-Sp- as well as CyA-Usp level and higher drug intakes than others but did not show a significantly higher MUsp/MSp ratio, suggesting that the metabolites crossreacting with nonspecific antibody are not associated with the toxic event. As already emphasized, rejecting patients under monotherapy were not, however, "undertreated" on the basis of either their CyA daily oral intake or CyA blood or serum levels as compared to those in a clinically steady state. Another profile was found in recipients who rejected when under polytherapy, since they had shown a signifiantly lower serum native CyA (not detected in routine polyspecific RIA) compared to those found in CyA-ST patients and a statistically higher MUsp/MSp ratio. In view of the weak activity by the CyA metabolites studied thus far *in vitro* (mainly M17) compared to the native CyA molecule on allogeneic proliferative response [3], this profile could allow rejection to occur. Surprisingly, this high CyA-MUsp/MSp ratio was not a constant feature of all polytherapy subgroups but rather was restricted to the rejecting group. Whether this restriction was artifactual, owing to the small sample size analyzed, or related to specific metabolic pecularities of some patients at a risk of rejection, is impossible to determine from this study and requires further analysis of a larger group of rejecting recipients on CyA mono- and polytherapy. Taking into account the observations men-

tioned above, this study suggests that immunosuppression and nephrotoxicity could be restricted to the native form of CyA.

Taken together, our results offer a new insight into the characteristics of recipients at risk of rejection and provide an improved understanding of the mechanisms of polytherapy [15, 17] protocols as compared to those of CyA monotherapy. Indeed, the new possibility of native CyA monitoring, as well as the results relative to the capacity of recipient sera to interact in vitro on allorecognition in functional tests, do not suggest that rejecting recipients are at risk because of low circulating CyA levels but rather that they might have different CyA pharmacokinetics. It is also possible that these patients have an unimpaired cellular immune response, even though they have high CyA-MUsp/MSp ratios. Such hypothetical resistance of recipient lymphocytes to CyA could explain why some patients rejected while on polytherapy with sera exhibiting strong inhibition of the 4H-line growth.

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