and cis-platinum. Int J Radiat Oncol Biol Phys 1986; 12: 1415.
17. Giri PGS, Kimler BF, Giri UP, Cox GL, Reddy EK. Comparison of single, fractionated and hyperfractionated irradiation on the development of normal tissue damage in rat lung. Int J Radiat Oncol Biol Phys 1985; 11: 527.
18. Weissberg JB, Moulder JE, Fischer JJ, Parcells V. Effect of cycloheximide on skin and renal radiation tolerance in the rat. Radiat Res 1983; 96: 173.
19. Kimler BF, Henderson SD, Mansfield CM, Svoboda DJ, Cheng CC. Effect of dihydroxyanthroquinone (NSC 279836) and thoracic irradiation on long-term survival in rats. Cancer Res 1982; 42: 2656.
20. Covelli V, Metalli P, Briganti G, Bassani B, Silini G. Late somatic effects in syngeneic radiation chimaeras: II. mortality and rate of specific diseases. Int J Radiat Biol 1974; 26: 1.
21. Phemister D, Thomassen RW, Norrdin RW, Jaenke RS. Renal failure in perinatally irradiated beagles. Radiat Res 1973; 55: 399.
22. Guttman PH, Kohn HI. Age at exposure and the acceleration of intercapillary glomerulosclerosis in mice. Lab Invest 1977; 12: 250.
23. Bennett LR, Chastain SM, Flint JS, Hansen RA, Lewis AE. Late effects of roentgen irradiation: I. studies on rats irradiated under anoxic anoxia. Radiology 1953; 61: 411.
24. Hollcroft J, Lorenz E, Miller E, Congdon CC, Schweistnal R, Uphoff D. Delayed effects in mice following acute total-body x irradiation: modification by experimental treatment. J Natl Cancer Inst 1957; 18: 615.
25. Ginsberg SJ, Comis RL. The pulmonary toxicity of antineoplastic agents. Sem Oncol 1982; 9: 34.

Received 5 March 1986.
Accepted 13 June 1986.

705

GENOTYPIC ANALYSES OF CYCLOSPORINE-ASSOCIATED LYMPHOPROLIFERATIONS

We recently reported a series of 17 allograft recipients who developed lymphoproliferative disorders while on a cyclosporine-steroid-containing regimen (*1*). The results indicated good patient survival with conservative therapy, (i.e., control of local complications and reduction of immunosuppression), regardless of whether the lesions were monoclonal or polyclonal. Clonal designation in that report was based on immunoperoxidase staining of formalin-fixed, paraffin-embedded tissues. Appropriate caution was recommended regarding noncritical acceptance of clonal status, due to the known shortcomings of the immunoperoxidase technique (*1–3*).

Tissue from several cases has been analyzed for immunoglobulin gene rearrangements (J. S. and M. L. C.), using published techniques (*4*) (Table 1). Four of five cases so studied have shown evidence of monoclonality, supporting our original interpretation. Indeed, one case phenotypically categorized by us as polyclonal (No. 9) on the basis of a kappa:lambda ratio of 1.5:1 was shown to be monoclonal by gene analysis. Because the majority of cells were unstained by immunoperoxidase methods, the gene rearrangement results are consistent with a true monoclonal tumor or a monoclonal proliferation arising in a polyclonal background.

Three separate synchronous tumors from patient 12 showed different monoclonal patterns of rearrangements, suggesting either independent primary tumors or possible subclones derived from one original clone (*5*). Tissue from patient 16 exhibited clonal rearrangements only of kappa light chains. This verifies the monoclonal nature of the lesion and agrees with the original kappa designation of the tumor. Multiple bands in patient 17, originally designated as polyclonal, may instead indicate the presence of a small number of proliferating clones in the lesions.

TABLE 1. Immunoglobulin gene rearrangement studies in transplant recipients with lymphoproliferative disease[a]

Pt. No.	H	K	L	Interpretation
6	R	R	R	Monoclonal B cell
9	R	R	R	Monoclonal B cell
12 (F)	R	D	R	Monoclonal B cell
12 (G)	R	R	G	Monoclonal B cell
12 (I)	R	D	R	Monoclonal B cell
16	R	R	G	Monoclonal B cell
17	R	M	G	Multiclonal

[a] H: heavy chain region; K: kappa region; L: Lambda region; R: rearranged; D: deleted; G: germline; M: multiple; (F, G, I) = different gastrointestinal nodules.

Patients 6 and 12 are alive and well at 39 and 28 months, respectively, following tumor diagnosis. Both underwent surgical intervention and a reduction of immunosuppression. Patient 12 received no chemotherapy, whereas patient 6, diagnosed in 1982, did. Patient 17 died 17 months following tumor diagnosis. Death was consequent to a second heart-lung transplant for pulmonary difficulties. No tumor was found at autopsy. Patients 9 and 16 died a short time after diagnosis, as previously reported.

The correlation between the clinical results and gene rearrangement studies encourages us to employ a conservative approach based on operation followed by reduced immunmosuppression in the management of these tumors, even when monoclonality is demonstrated. However, at the same time, we recognize that significant differences of disease manifestation among different series may exist, as noted by Hanto et al. (*3*). These investigators pointed out the high frequency of gastrointestinal lymphomas in our series (*3*), This contrasts with the frequent central nervous system involvement seen in their cases (*6*). The reasons for these differences are not clear, but may reflect differences in the immunosuppressive regimens used. Only 1 of their 19 reported patients received cyclosporine (*6*), in contrast to all in our series (*1*). It thus appears prudent to apply our findings with this caveat in mind, until differences can be reconciled and generalizations established.

MICHAEL A. NALESNIK[1]
THOMAS E. STARZL,[2]
KENDRICK A. PORTER[3]
JEFFREY SKLAR[4]
MICHAEL L. CLEARY[4]
Departments of Surgery and Pathology
Presbyterian University Hospital
Pittsburgh, Pennsylvania
Department of Pathology
St. Mary's Hospital Medical School
London, England
Department of Pathology

[1] Department of Pathology, Presbyterian University Hospital.
[2] Department of Surgery, Presbyterian University Hospital.
[3] St. Mary's Hospital Medical School.
[4] Stanford University.

Laboratory of Experimental Oncology
Stanford University
Stanford, California

REFERENCES

1. Starzl TE, Nalesnik MA, Porter KA, et al. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporine-steroid therapy. Lancet 1984; i: 583.
2. Lymphoma in organ transplant recipients. (editorial) Lancet 1984; i: 601.
3. Hanto DW, Frizzera G, Gajl-Peczalska KJ, Simmons RL. Epstein-Barr virus, immunodeficiency, and B cell lymphoproliferation. Transplantation 1985; 39: 461.
4. Cleary ML, Chao J, Warnke R, Sklar J. Immunoglobulin gene rearrangement as a diagnostic criterion of B-cell lymphoma. Proc Natl Acad Sci USA 1984; 81: 593.
5. Cleary ML, Sklar J. Lymphoproliferative disorders in cardiac transplant recipients are multiclonal lymphomas. Lancet 1984; ii: 489.
6. Hanto DW, Gajl-Peczalska KJ, Frizzera G, et al. Epstein-Barr virus (EBV) induced polyclonal and monoclonal B-cell lymphoproliferative diseases occurring after renal transplantation. Ann Surg 1983; 198: 356.

Received 15 April 1986.
Accepted 13 June 1986.

EFFECT OF HYDERGINE ON CYCLOSPORINE-INDUCED NEPHROTOXICITY IN A RAT KIDNEY TRANSPLANTATION MODEL

Cyclosporine (CsA)* has now been established as a most effective immunosuppressive agent in man and various animal models (*1, 2*). Apart from one exception in living-related donor renal transplantation in diabetics (*3*), it has never been shown to be inferior, but is mostly superior, to conventional immunosuppression, when considering patient and graft survival. However several side effects have also been reported during CsA therapy—such as hepatotoxicity, nephrotoxicity, effects on the central nervous system (CNS), resulting in convulsion and tremor, anorexia, hirsutism, gum hypertrophy, and increased renin-angiotensin-aldosterone system (RAAS) activity (*4, 5*).

CsA associated nephrotoxicity, being reported ever since the first study of the use of CsA in clinical renal transplantation (*6*), is one of the most frequently occurring adverse effects of CsA therapy. This nephrotoxicity appears to be reversible and dose dependent. On the basis of renal scans, CsA nephrotoxicity has been attributed to acute tubular necrosis (*7*). Other studies in rats have proposed that the nephrotoxicity is caused by a glomerular rather than a tubular mechanism (*8*). However, absence of any histological change in patients manifesting nephrotoxicity has also been reported (*9*). The correlation of morphological changes with nephrotoxicity is still not clear.

Various attempts have been made to modify CsA-associated nephrotoxicity. Calne (*10*) suggested induction of forced diuresis with fluid and mannitol and delay of CsA administration until establishment of posttransplant diuresis. However, others suggest that this early posttransplantation complication was the result of acute rejection rather than CsA nephrotoxicity and that it reacted well to steroid therapy (*11*). Unsuccessful attempts in transplant patients have been made to correct nephrotoxicity by means of dopamine infusions (*12*). Attempts to accelerate CsA metabolism using hepatic Cyt-P450 enzyme-complex inducer, have been made in rats, resulting in reduced nephrotoxicity (*13*). Generally the strategy has been to reduce the CsA dose or convert to conventional therapy—however, these measures may be followed by rejection of the graft (*14*).

CsA has been reported to increase sympathetic nerve activity in spontaneous hypertensive rats (*5*). This was indicated as leading to stimulation of the RAAS-system, as well as release of angiotensin II, and inhibition of renal prostaglandin synthesis. Prevention of CsA nephrotoxicity in rats with prostaglandins has been described by Makowka et al. (*15*).

Hydergine is a partial α_1 and α_2 adrenoceptor agonist that also has a stimulating effect on dopamine and an antagonistic activity on the 5HT receptor. Considering these properties of Hydergine, it has been suggested that it might interfere with the proposed mechanism of CsA-induced nephrotoxicity (*5*). The present study, using a rat kidney allograft model, was undertaken to investigate whether Hydergine could prevent or offer partial protection from CsA-induced nephrotoxicity.

Male BN ($RT1^n$) and WAG ($RT1^u$) rats, weighing about 250 g were used as kidney graft donors and recipients, respectively. Kidney transplantations in the BN-to-WAG combination were performed using microsurgical techniques, as described earlier (*16*). Bilateral nephrectomy was done at the time of operation. CsA, diluted in olive oil, was administered i.m. in a volume of 0.2 ml. Hydergine (Sandoz) was administered i.p. in doses of 0.5 mg/kg/48 hr. Glomerular filtration Rates (GFR) were measured as clearances of ^{51}Cr-ethylenediaminotetraacetate using a method described in detail earlier (*17*). The relative glomerular filtration rate (RGFR; expressed as a percentage of normal GFR) was calculated by considering the GFR of WAG rats with both kidneys intact to possess an RGFR of 100% (*17*).

Serum creatinine (normal value 43±4 μmol/L), blood urea nitrogen (BUN, normal value 8.6±1.1 mmol/L), and serum Na and K values, were determined at regular intervals using standard analytical procedures.

The experimental design was as follows: Kidney transplantations in the BN-to-WAG combination were performed and the rats divided into two groups. One group was treated with CsA 15 mg/kg/48 hr from the day of transplantation for a period of 35 days (group 1, n=8). At the end of this period GFRs were determined. From day 42 onward these rats were treated with CsA 100 mg/kg/48 hr for a period of 21–28 days (group 1a). At the end of this period GFRs were determined again. A second group of kidney-allografted rats underwent the same treatment with CsA as the group above, except that they additionally received Hydergine, from the day of transplantation, 0.5 mg/kg/48 hr (groups 2 and 2a, n=8). All through this treatment period serum creatinine, BUN, Na, K, and body weight were determined regularly. The results were analyzed statistically by using the Student's *t* test.

In Table 1 the RGFRs of the groups treated with CsA and CsA + Hydergine are given. At the end of the first 35 days of treatment with low doses of CsA, the mean RGFRs of the CsA (group 1) and CsA + Hydergine groups (group 2) were 34.4± 8.2 and 28.2±16.7, respectively. This difference is not statistically significant. After completion of the treatment with a dose

* Abbreviations used: BUN, blood urea nitrogen; CNS, central nervous system; CsA, cyclosporine; GFR, glomerular filtration rate; RAAS, renin-angiotensin-aldosterone system; RGFR, relative GFR.