

Cyclosporin and renal graft histology

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SUMMARY The histology of renal allografts was compared in a series of 107 biopsies from patients receiving cyclosporin and 126 biopsies from patients receiving azathioprine and prednisolone. Patients receiving cyclosporin were converted to azathioprine and prednisolone 90 days after transplantation. Biopsies were taken routinely at 7, 21, 90, and 365 days, irrespective of clinical graft function and were examined "blind" by two independent observers. Interstitial haemorrhage was more common in patients treated with azathioprine and prednisolone corresponding with their poorer graft survival. Analysis of glomerular, tubular, vascular, and interstitial changes showed no other important differences between the two groups despite clinical evidence of reversible cyclosporin nephrotoxicity. Quantitation of interstitial fibrosis in 90 day biopsies showed it to be equal in prevalence after treatment with azathioprine and prednisolone and cyclosporin. It was preceded by diffuse interstitial cellular infiltration, a common finding in early biopsies. Diffuse cellular infiltrates were generally associated with higher serum creatinine concentrations and, if persistent, a poorer graft prognosis than focal infiltrates, but they were not always associated with renal dysfunction.

The nephrotoxic effects of the immunosuppressive agent cyclosporin have been well documented,¹ but the mechanism of toxicity has yet to be established. Many studies have indicated that the increased serum creatinine concentrations found in patients treated with cyclosporin may be corrected by withdrawal of the drug or diminution of its dose.²⁻⁵ More worrying are the reports which suggest that it can produce chronic and irreversible renal failure.⁶ Various renal histological abnormalities have been ascribed to cyclosporin, including vacuolation of proximal tubular epithelial cells and giant mitochondria,⁷ tubular epithelial cell microcalcification⁷; arteriolar hyalinosis and mucoid intimal thickening⁷; glomerular capillary and arteriolar thrombosis^{8,9}; and mononuclear cell infiltrates.¹⁰ It has been alleged that diffuse interstitial fibrosis is produced by chronic cyclosporin damage.^{11,12}

In the second Oxford cyclosporin trial, a prospective randomised trial of the use of short term (90 day) cyclosporin treatment in renal transplantation,¹³ patients in both treated and control groups had routinely undergone renal graft biopsies at 7, 21, 90, and 365 days after transplantation. These biopsies were

examined "blind" and tubular loss and interstitial expansion at 90 days were quantified. Results in the two groups were compared and are reported in this paper. The biopsy protocol not only enabled comparison of results at equivalent time intervals but also allowed analysis of sequential changes. In addition, it permitted the functional importance of different histological variables to be assessed, as biopsies were taken irrespective of graft function.

Material and methods

CLINICAL DATA

Biopsies from 100 recipients of cadaver renal transplant in the second Oxford cyclosporin trial were examined. Forty four of these patients received cyclosporin for the first 90 days after transplantation; the remainder received only azathioprine and prednisolone. Cyclosporin was given at 17.5 mg/kg/day for the first 30 days, reducing to 15 mg/kg/day until day 60, and then to 12.5 mg/kg/day until conversion at day 90 (these dosages were occasionally reduced in the presence of side effects or very high plasma concentrations of cyclosporin). At day 90 cyclosporin was stopped and azathioprine started at 2.5 mg/kg/day with 10 mg prednisolone twice daily. The 56 patients receiving azathioprine and

prednisolone alone were started at 2.5 mg/kg/day azathioprine and 15 mg prednisolone twice daily, reducing progressively to 10 mg twice daily by day 90. Acute rejection episodes were treated in both groups with 3–5 bolus doses of 0.5 g intravenous methylprednisolone. Biopsies were obtained after informed consent from all patients. They were taken routinely at 7, 21, 90, and 365 days after transplantation, as well as when clinically indicated. Biopsies that were considered to be inadequate were excluded from further analysis. This left 107 biopsies in the group treated with cyclosporin and 126 biopsies in the group treated with azathioprine and prednisolone for study (Table 1). Twenty eight patients underwent graft nephrectomy, 20 of whom had received azathioprine and prednisolone and 8, cyclosporin. Clinical rejection was assessed retrospectively on the basis that at least three of the following five criteria were fulfilled: graft tenderness or swelling; fever in the absence of identifiable infection; diminished urine output for two successive days (about 10% decrease/day); rise in creatinine (by about 10%) or dialysis for two successive days; response to steroid pulses.

BIOPSY ANALYSIS

All biopsies were fixed in formalin, and paraffin sections cut at 4 μ m were stained by routine haematoxylin and eosin, periodic acid-Schiff, Martius scarlet blue, and Goldner's trichrome methods for light microscopy. They were examined by two independent observers unaware of the treatment that each patient was receiving. The following changes were assessed and graded according to severity on a + to +++ scale: glomerular abnormalities including sclerosis, mesangial expansion, cellular infiltration and capillary thrombosis; abnormalities of tubules including epithelial necrosis and flattening, epithelial vacuolation, casts, cellular infiltration, and calcifications; interstitial changes—namely, cellular infiltration, oedema, fibrosis, and haemorrhage; vascular changes including intimal fibrosis, intimal oedema, cellular infiltration, and fibrinoid necrosis. Adequacy of the biopsy was judged by the number of glomeruli present. Size of vessels sampled was also noted.

QUANTITATION

Quantitative analysis of the tubulointerstitial ratio in biopsies taken at 90 days was performed by one of us (MSD) without knowledge of the treatment given. Only biopsies with renal cortex were used. Point counting¹⁴ was done on sections stained by Goldner's green trichrome method, using a Zeiss I integrating eyepiece and a x25 plane objective. Five hundred points were counted on each section, and the ratio of the number of points lying on tubules to the number lying on interstitium was calculated for each case.

Statistical analyses were performed using standard error of difference between percentages, Student's *t* test for differences between means, and χ^2 tests for comparative distribution of discrete variables.

Results

COMPARISON OF RENAL HISTOLOGY IN THOSE TREATED WITH CYCLOSPORIN AND CONTROL GROUPS

Glomeruli Glomerular abnormalities other than mild mesangial expansion were uncommon in both groups. Glomerular capillary thrombosis was noted in only two biopsies, both from patients receiving azathioprine and prednisolone. Glomerular scleroses were found with other features of chronic renal damage, notably arterial intimal fibrosis.

Interstitial cellular infiltration A striking phenomenon was the high percentage of early biopsies with a diffuse cellular infiltrate (Fig. 1). This was usually accompanied by interstitial oedema of varying severity. At day 90 most biopsies still had some degree of cellular infiltration, but focal infiltrates were more common than diffuse. At one year biopsies with absent or minimal cellular infiltration were predominant. No appreciable differences were found in either location or composition of the infiltrates in the two groups (Table 2). Mononuclear cells predominated in both, with a variable admixture of neutrophil and eosinophil polymorphs.

Interstitial haemorrhage Interstitial haemorrhage graded as moderate or severe occurred more commonly in patients treated with azathioprine and prednisolone than in those treated with cyclosporin: speci-

Table 1 *Material included in study*

Treatment	Patients	Needle core biopsies taken at				Graft nephrectomies
		7	21	90	365 (days)	
Cyclosporin	44	33	30	26	18	8
Azathioprine and prednisolone	56	44	35	33	14	20

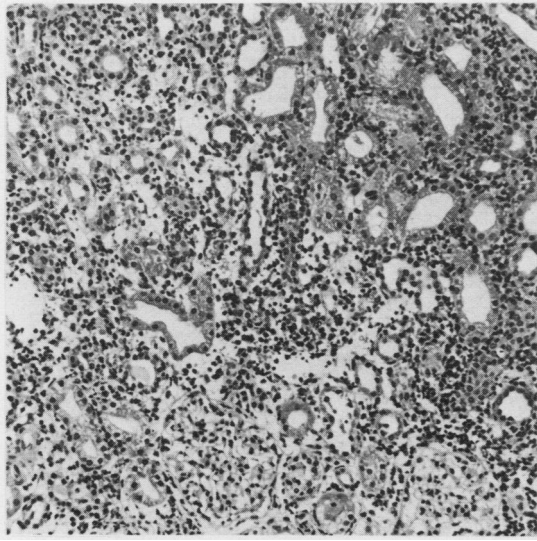


Fig. 1 Day 7 biopsy with diffuse interstitial cellular infiltration and interstitial oedema. (Haematoxylin and eosin.)

mens from 19 of 56 (34%) patients treated with azathioprine and prednisolone and five of 44 (11%) patients treated with cyclosporin showed this feature ($p < 0.01$).

Vessels Vascular changes specific for the group treated with cyclosporin were not identified. Intimal oedema was often found in biopsies with a diffuse cellular infiltrate in the interstitium and in cases in which the cellular infiltrate was severe it was often accompanied by intimal cellular infiltration. Intimal fibrosis was common in later biopsies. Nearly all patients with severe interstitial fibrosis had evidence of severe arterial intimal thickening whether treated with cyclosporin or azathioprine and prednisolone. It was impossible to determine the extent to which this affected small or large vessels, since large vessels were often not sampled. Fibrinoid necrosis was seen in only three biopsies, two from patients receiving aza-

thioprine and prednisolone and one from a patient receiving cyclosporin.

Tubules Tubular abnormalities were common in early biopsies and were usually associated with diffuse interstitial cellular infiltration and interstitial oedema. The most common change was tubular epithelial flattening accompanied by penetration of mononuclear inflammatory cells between epithelial cells into tubular lumina. Scattered casts were often present. No differences were observed in these features between the two groups, and no tubular abnormalities were identified that were specific for the patients receiving cyclosporin. Microcalcifications were fairly common in both groups and seemed to relate to preceding epithelial damage. Epithelial vacuolation was rarely seen. Variable tubular atrophy was present in later biopsies and this corresponded with the degree of interstitial fibrosis (Fig. 2).

Tubulointerstitial ratios (TIR) The extent of tubular atrophy and interstitial fibrosis in 90 day biopsies was assessed by calculating the ratio of the area occupied by tubules to that occupied by interstitium. Fig. 3 summarises the results. The mean tubulointerstitial ratios ($\times 100$) were: azathioprine and prednisolone 162.5 (SD (95.1)); cyclosporin 164.8 (87.7)—that is, there was no significant difference between the two groups. The qualitative nature of the interstitial fibrosis—namely, whether it was diffuse or focal—was also not found to differ. Comparison of TIR with previous subjective assessment of interstitial fibrosis showed substantial overlap between groups previously labelled 0 to + + +. Biopsies that had been labelled + + + fibrosis had TIRs ($\times 100$) ranging from 39–160; biopsies labelled + + fibrosis had TIRs ranging from 57–258; biopsies with + fibrosis ranged from 134–429, and “zero” fibrosis ranged from 338–489.

RELATION OF HISTOLOGY TO CLINICAL GRAFT FUNCTION

Forty two per cent of biopsies from patients receiving cyclosporin and 24% of those from patients receiving

Table 2 Interstitial cellular infiltration

Days after transplantation	Percentage of biopsies with:					
	Diffuse cellular infiltration		Focal cellular infiltration		Minimal cellular infiltration	
	Cyclosporin	Azathioprine and prednisolone	Cyclosporin	Azathioprine and prednisolone	Cyclosporin	Azathioprine and prednisolone
7	55	64	27	27	18	9
21	50	34	33	40	17	26
90	27	18	46	48	27	34
365	0	0	33	29	67	71

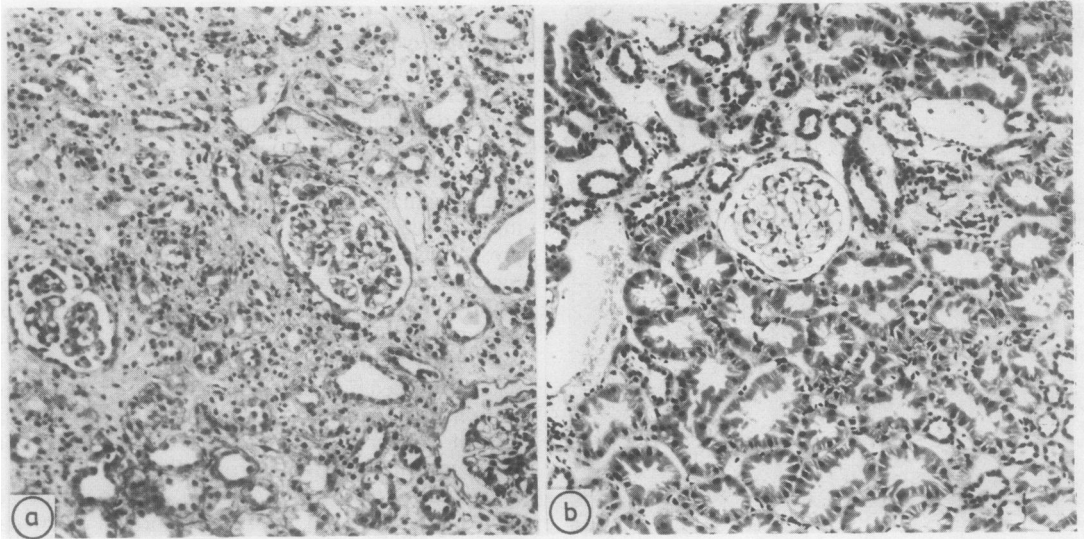


Fig. 2 Day 90 biopsies from patients on azathioprine and prednisolone showing; (a) severe interstitial fibrosis and tubular atrophy ($TIR \times 100 = 55$) and (b) minimal fibrosis ($TIR \times 100 = 219$). (Haematoxylin and eosin.)

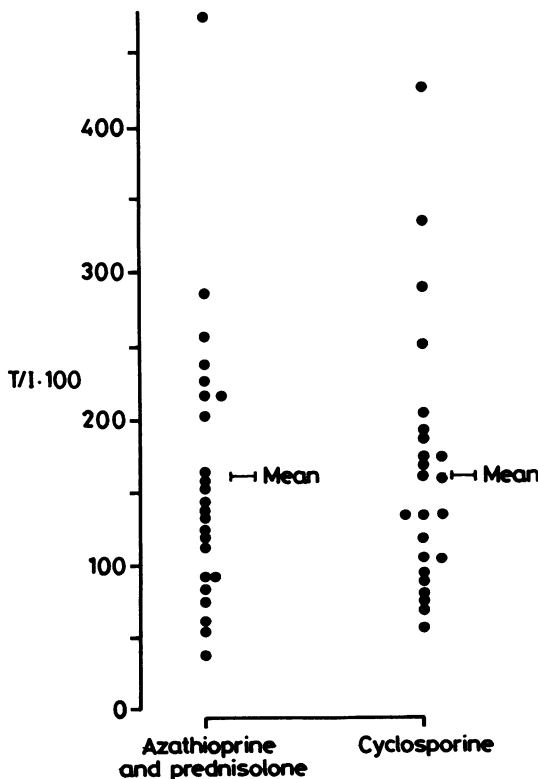


Fig. 3 Tubulointerstitial ratios $\times 100$ in biopsies taken at 90 days after transplantation from patients receiving azathioprine and prednisolone and cyclosporin.

azathioprine and prednisolone had diffuse cellular infiltration of the interstitium with no evidence of rejection on clinical criteria. About 80% of biopsies with focal infiltrates in both groups had stable renal function. The remainder had signs of clinical rejection (Table 3). Table 4 shows the mean serum creatinine concentrations of patients with grafts showing diffuse or focal cellular infiltration at 7, 21, and 90 days after transplantation. Patients with diffuse infiltrates had higher serum creatinine concentrations than patients with focal infiltrates, and patients receiving cyclosporin had higher serum creatinine concentrations than patients receiving azathioprine and prednisolone ($p < 0.02$), whatever the nature of the infiltrate. After conversion creatinine concentrations of all but one of the patients treated with cyclosporin fell, and this was irrespective of their graft histology at day 90. The magnitude of creatinine fall ranged from 11–129 $\mu\text{mol/l}$ (34–1458 mg/100 ml) but was not linked to any specific histological feature. The mean fall post conversion was 44 $\mu\text{mol/l}$ (497 mg/100 ml) for patients with good histology and 59 $\mu\text{mol/l}$ (667 mg/100 ml) for patients with fibrotic grafts.

RELATION OF HISTOLOGY TO SUBSEQUENT FATE OF GRAFT

Diffuse cellular infiltration Most patients in both groups with biopsies showing interstitial fibrosis of moderate or severe degree had had previous evidence of diffuse cellular infiltration of their grafts, with or without clinical signs of rejection. Prognosis was significantly poorer ($p < 0.01$) for grafts with a per-

Table 3 Relation of histology to clinical graft condition at time of biopsy

	Diffuse cellular infiltration		Focal or minimal cellular infiltration	
	Cyclosporin	Azathioprine and prednisolone	Cyclosporin	Azathioprine and prednisolone
Percentage of patients with clinical "rejection"	58	76	20	17
Percentage of patients with stable renal function	42	24	80	83

Table 4 Mean serum creatinine concentrations of patients with biopsies showing focal or diffuse cellular infiltration

	Days after transplantation					
	7		21		90	
	Diffuse	Focal	Diffuse	Focal	Diffuse	Focal
Mean serum creatinine ($\mu\text{mol/l}$) for patients receiving cyclosporin	606	456	439	197	314	177
Mean serum creatinine ($\mu\text{mol/l}$) for patients receiving azathioprine and prednisolone	522	255	378	166	255	105
Significance of creatinine differences between diffuse and focal infiltration for both groups	p < 0.02		p < 0.001		p < 0.001	

Conversion SI to traditional units: 1 $\mu\text{mol/l}$ sodium \approx 0.0113 $\mu\text{g/l}$

sistent diffuse infiltrate—that is, biopsies showing this feature on two or more successive occasions (Table 5). Ten of these cases progressed ultimately to nephrectomy, of which four were in the group treated with cyclosporin and six in the group treated with azathioprine and prednisolone. The nephrectomy specimens all showed features of severe chronic active rejection with diffuse cellular infiltrates in interstitium and vessels, and severe interstitial and vascular sclerosis and oedema. One case (cyclosporin) and four cases (azathioprine and prednisolone) had interstitial haemorrhage, infarctions, and vascular fibrinoid necrosis in addition.

Interstitial haemorrhage All but three patients (who were in the group treated with azathioprine and prednisolone) with moderate or severe interstitial haemorrhage required nephrectomy for acute rejection. Twelve of 20 nephrectomy specimens in the group treated with azathioprine and prednisolone and four of eight in the group treated with cyclosporin showed massive haemorrhage and infarction.

Glomerular capillary thrombosis and vascular fibrinoid necrosis One of two patients with biopsies showing glomerular capillary thrombosis and one of three with biopsies showing vascular fibrinoid necrosis subsequently required nephrectomy for acute rejection. These patients were both receiving azathioprine and prednisolone.

Discussion

This study failed to identify a specific histological marker of cyclosporin nephrotoxicity. No abnormality of renal graft histology was found in the group treated with cyclosporin, which did not also occur in the group treated with azathioprine and prednisolone. Clinical nephrotoxicity was manifest by raised serum creatinine concentrations which reverted to those of controls on conversion from cyclosporin to azathioprine and prednisolone at 90 days after transplantation.¹⁵ This was apparent in virtually all patients treated with cyclosporin, although to a varying degree. The magnitude of creatinine fall after conversion was not related to the nature of the cellular infiltrate, nor indeed to any other histological abnormality. Previous studies both on experimental animals¹⁶ and man⁷ have indicated that proximal tubular vacuolation may be produced by treatment with cyclosporin. This seems highly plausible as clinical studies indicate that cyclosporin may change tubular function, and epithelial vacuolation is a common marker of tubular damage produced by various agents. Nevertheless, the fact that epithelial vacuolation is found in renal graft biopsies both from patients treated with cyclosporin and those treated with azathioprine and prednisolone detracts from its usefulness as a marker of cyclosporin toxicity in renal

Table 5 Influence of persistent diffuse cellular infiltration on graft outcome

	Antecedent histology	
	Two or more biopsies with diffuse cellular infiltration	One or no biopsies with diffuse cellular infiltration
No of patients:		
Progressing to nephrectomy	10	3
Surviving graft with 90 day tubulointerstitial ratio \times 100		
< 100	7	3
100-200	6	10
> 200	0	10
Total	23	26

Only cases with a full complement of three sequential biopsies were included in this analysis.

transplantation. Epithelial vacuolation was rarely seen in this study, although the possibility that this was due to the method of tissue preparation cannot be excluded. Electron microscopy is a more sensitive method for detecting minor abnormalities of tubular epithelium.¹⁶ Epithelial microcalcification was a common finding in both groups, and accurate quantification of this feature was not undertaken.

The second type of abnormality that has been attributed to treatment with cyclosporin is vascular. Arterioles have been reported to show hyalinosis, necrosis, and mucoid intimal thickening of a type unlike that found in vascular rejection but similar to that seen in the haemolytic uraemic syndrome.⁷ Glomerular capillary thrombi have been described in bone marrow⁸ and renal⁹ transplant recipients treated with cyclosporin. Our study showed no differences between the vascular lesions of patients treated by either method and no evidence of cyclosporin induced glomerular capillary thrombi. Accurate quantitation of vascular abnormalities was not considered valid, as the size of vessels sampled varied substantially from one biopsy to another and changes were often focal in character. Observed vascular changes were all thought to be compatible with rejection, possibly superimposed on hypertensive changes in some cases.

Similarity of graft cellular infiltrates during treatment with azathioprine and prednisolone and cyclosporin agrees with one previous report¹¹ and suggests that these are all attributable to varying degrees and stages of "rejection" (where rejection is defined as the immunological reaction to allogeneic tissue rather than on clinical criteria). The conclusion that cellular infiltrates are not due to cyclosporin is supported by the quantitative observation that, if anything, cells are more numerous in grafts treated with azathioprine and prednisolone.¹⁷ Although infiltrates were rigidly classified as diffuse or focal, in practice there was a spectrum of changes from one to the other, diffuse infiltration with focal accentuation being a common finding. There was also a spectrum

of changes with time. The results suggest that diffuse infiltrates have greater clinical importance than focal as they were associated with higher creatinine concentrations at any given time after transplantation, and prognosis was poorer for grafts showing persistence of this feature. It is nevertheless noteworthy that some patients with focal infiltrates in both groups were considered to be undergoing rejection episodes on clinical grounds. Conversely, a considerable number of patients with diffuse infiltrates had stable renal function. This has been documented by others.¹⁸ The relation between cellular infiltration and so called clinical rejection episodes is thus not clear cut, and the problem as to what degree of cellular infiltration, if any, indicates the need for additional immunosuppressive treatment has yet to be resolved. Some infiltrates may resolve spontaneously, whereas others persist despite intravenous methylprednisolone. In practice decisions have to be based on the combination of clinical and pathological findings rather than on histology alone. More definitive changes are interstitial haemorrhage, vascular fibrinoid necrosis, and glomerular capillary thrombosis, which were all associated, although not inevitably, with imminent graft failure, confirming previous findings.¹⁹ The increased incidence of interstitial haemorrhage in the group treated with azathioprine and prednisolone reflected the poorer graft survival of these patients in the trial as a whole.²⁰

Several recent studies suggested that interstitial fibrosis is an important feature of chronic cyclosporin nephrotoxicity.^{11 12} In our investigation no difference was found in the quantity of interstitial fibrosis after 90 days of cyclosporin or azathioprine and prednisolone. Fibrosis in both groups was associated with preceding diffuse cellular infiltration of the interstitium and severe changes in arterial vessels, indicating that it was due to chronic rejection. Possibly, kidneys already rendered fibrotic by other factors such as chronic rejection, or cardiovascular disease might be more susceptible to the effects of cyclo-

sporin, although the difference in mean creatinine fall after conversion for grafts with severe and minimal fibrosis was not significant. Aetiology of fibrosis can only be adequately assessed when sequential biopsies have been taken, and it is worth noting that diffuse interstitial cellular infiltration was often clinically "silent." "Semiquantitative" assessment of features such as interstitial fibrosis on a 0 to +++ scale should be interpreted with caution, as substantial overlap was found between these groups following more rigidly objective measurement. This study cannot exclude the possibility that interstitial fibrosis may be produced by therapeutic administration of cyclosporin for periods longer than 90 days. The importance of the present results is that they indicate that fibrosis induced by cyclosporin should not be a problem when short term administration of the drug is contemplated. This corresponds with the clinical finding of complete reversibility of raised serum creatinine concentrations after this time.¹⁵

The question often posed to the pathologist is whether a given deterioration of renal function can be attributed to rejection or cyclosporin toxicity. This investigation has indicated that it is not usually a case of either or, but a question of degree. In the days immediately after a renal transplantation a multiplicity of factors may operate to impair renal function. Renal biopsy may be helpful in assessing the severity of the rejection reaction; severity of cyclosporin toxicity can only be diagnosed by exclusion, or empirically by reducing the dose.

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