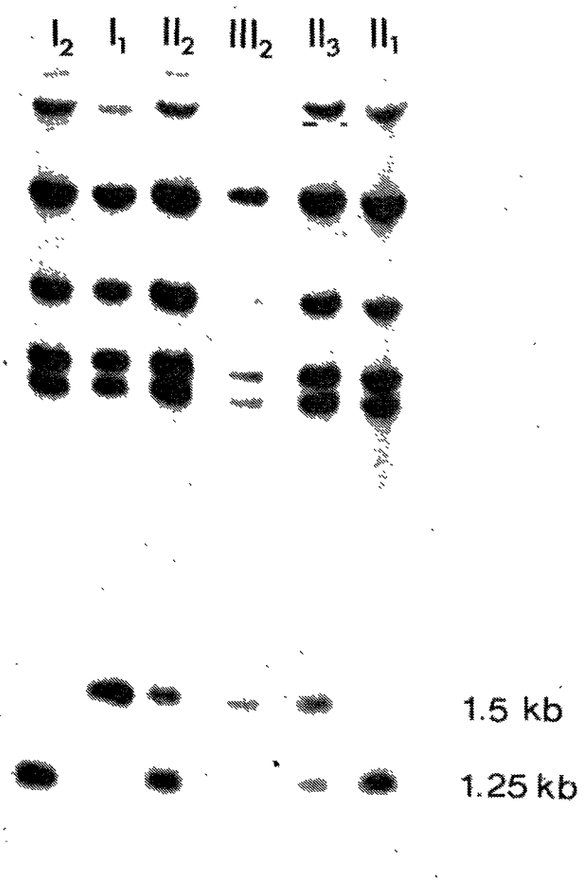

Fig 2—Southern blot hybridisation with the ^{32}P-labelled FVIII cDNA probe.

Figures refer to family members in fig 1. (To improve clarity fig 2 is a composite of two different exposures of the same Southern blot.)

Thus our cDNA probe and the genomic probe of Gitchier et al most probably detect the same polymorphism.

A woman was referred to us in her second pregnancy at 6 weeks of gestation. She was an obligate carrier of haemophilia A, having a maternal uncle (I, fig 1) who had died at the age of 2 years from a severe haemorrhage, a FVIII:CAg of 50%, and a history of induced abortion of a male fetus (III_1) with a FVIII:CAg level below 0·01% after a fetal blood sampling at 20 weeks' of gestation.

The woman was offered first-trimester chorionic biopsy but preferred amniocentesis, and this was done at 15 weeks of pregnancy and amniotic fluid cells were cultivated. The karyotype was male.

DNA was isolated from the cultured amniocytes and from blood samples from the pregnant woman, her parents, and her two siblings. The DNA yield from the amniocytes was considerably lower than from the blood samples. These six DNA samples were cut with *Bcl* I and subjected to Southern blot analysis using the FVIII cDNA probe (fig 2). The pregnant woman (II_2) is heterozygous for the 1·25 kb and 1·5 kb *Bcl* I fragments, while her healthy father (I_1) is hemizygous for the 1·5 kb allele and her carrier mother (I_2) is homozygous for the 1·25 kb allele. The fetus (III_2) carries the same *Bcl* I allele as its healthy grandfather, so the analysis strongly indicates that the fetus is not affected. The pregnancy is therefore continuing.

The family pedigree and the restriction fragment pattern described above is depicted in fig 1, together with the result of a similar analysis with the non-FVIII gene probe DX13, which is very closely linked to haemophilia A.[3] The DX13 polymorphism is revealed by the presence of either a 2·8 kb or a 5·8 kb *Bgl* II fragment. In this family there is co-segregation of the 1·5 kb *Bcl* I fragment with the 2·8 kb *Bgl* II fragment, and of the 1·25 kb *Bcl* I fragment with the 5·8 kb *Bgl* II fragment. Recombination between these two loci has not occurred. Even though we do not know the relative location of the disease locus to the markers (ie, whether the disease locus is located between the two markers or distal to both), this result strengthens the likelihood that the disease locus and the markers do co-segregate.

Gitschier et al have described the use of a FVIII genomic DNA probe to exclude haemophilia A, and our studies confirm the value of this approach. In the future several FVIII gene RFLPs may become available, and these will increase the number of families for which informative RFLPs exist and allow several RFLP analyses to be done, helping to reveal possible recombination events.

We would emphasise the importance of studying DNA from family members in such cases, especially the father of an obligate carrier. DNA analysis of at-risk families should ideally be done before conception because prenatal diagnosis is increasingly likely to be attempted in the first trimester, leaving little time for family studies.

We thank the Danish Medical Research Council (grants 12–4562, 12–5223, 12–3923), the Nordisk Insulin Foundation, and the Novo Foundation for their support and Dr Kay Davies (Oxford) for access to the DX13 probe.

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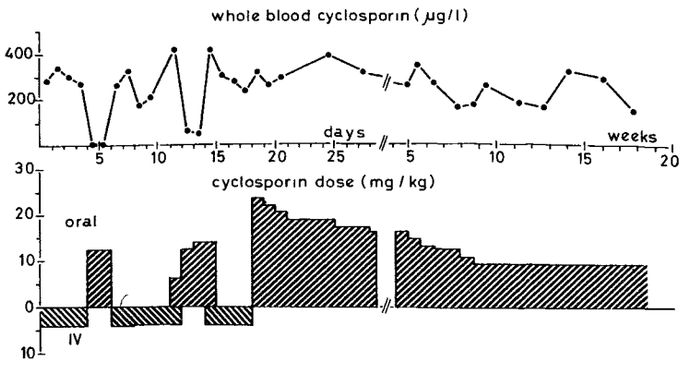
CHANGING ROUTE OF CYCLOSPORIN ADMINISTRATION: NEED FOR CLOSE MONITORING

SIR,—We would again like to stress the importance of cyclosporin monitoring, especially when changing the route of administration. This drug shows considerable between and within individual variation in absorption, and its bioavailability ranges from 4 to 60%.[1] We measure whole blood trough levels[2] daily and adjust the dosage accordingly, taking into account biochemical data and clinical observations. Therapeutic levels are defined as trough blood levels between 100 and 300 μg/l.

A 28-year-old renal allograft recipient was put on intravenous cyclosporin for the first 4 days post transplantation at daily doses of 4·2 mg/kg, in two doses. Trough levels were 270–340 μg/l. On days 5 and 6 the drug was administered orally (12·5 mg/kg), resulting in trough levels of less than 25 μg/l. Since the patient was on no other drug apart from prednisone 10 mg/day and drug intake was supervised we suspected that he was absorbing less cyclosporin than usual. In sixteen renal transplant patients a mean intravenous dose of 5·2±1·6 mg/kg for 3–4 days and a subsequent oral starting dose of 12·4±2·5 mg/kg resulted in therapeutic blood levels (ratio oral/intravenous 2·52±0·58). From day 7 to day 12 cyclosporin was again administered intravenously (3·9 mg/kg), and trough levels were 210–260 μg/l. When on day 13 the drug was again given orally (12·5 mg/kg) the trough level was 60 μg/l. Increasing the dose to 14·1 mg/kg did not increase the trough level.

After another 3 days of intravenous administration (3·9 mg/kg, blood levels 180–300 μg/l) an oral dose of 23·4 mg/kg/daily was administered. The blood level now remained therapeutic at 320 μg/l. The next day the dose was reduced to 21·9 mg/kg, with a blood level of 260 μg/l. Over the next 12 days, the doses were tapered to 16·2 mg/kg, with therapeutic blood levels. Dose reduction to 9·2 mg/kg resulted in blood levels of around 170 μg/l (figure).



Cyclosporin blood concentration and dose profile.

The bioavailability of the drug in this patient seems to have gradually improved, an effect we have observed in several other patients. This case demonstrates how close monitoring of the blood levels can avoid unpleasant surprises. A low, but statistically not improbable, bioavailability with subsequent subtherapeutic blood levels might have gone unheeded until signs of rejection had appeared—and the remarkable increase in (presumably) absorption could have led to excessive blood levels if the dosage had not been adjusted according to monitoring results.

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ALTERNATE DAY CYCLOSPORIN AND AZATHIOPRINE PLUS STEROIDS

SIR,—A major advantage of cyclosporin is that maintenance corticosteroids can often be avoided or kept to a low dose.[1] However, some patients have rejections that are uncontrollable with cyclosporin and steroids or cyclosporin nephrotoxicity develops, and therapy has to be changed. Both categories of patient have been changed to daily azathioprine and steroids, sometimes with success, but in other patients rejection progresses or occurs for the first time. Furthermore cushingoid features or other side-effects usually develop.

Because the side-effects of these agents are dose dependent and because their action on the immune system differs, therapy with cyclosporin and azathioprine plus steroids administered on alternate days may be advantageous in this highly selected group of patients who have a poor prognosis.

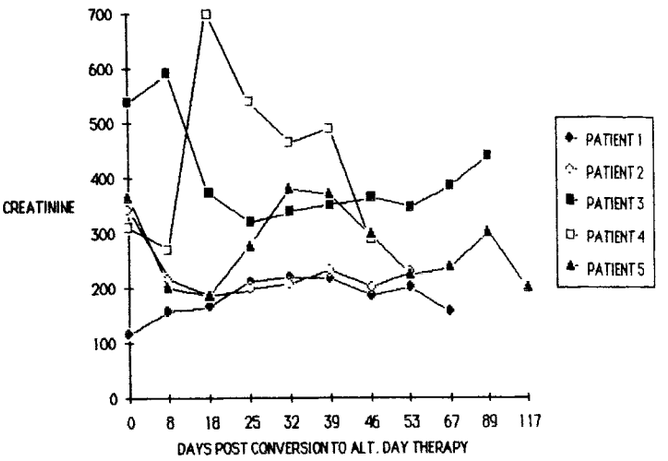
Five patients have been converted 14–111 days after renal transplantation to alternate-day therapy with cyclosporin and azathioprine plus steroids and have been followed up on this treatment for between 47 and 117 days. Four patients received cadaver renal allografts and one patient had a paratopic pancreas and renal allograft from the same cadaver donor. All patients were on cyclosporin at first,[2] and were converted to alternate-day therapy for the reasons given in the table. The alternate-day dosage of azathioprine was started at 1–1·5 mg/kg plus prednisone 0·5–1·25 mg/kg; cyclosporin was maintained at 6/20 mg/kg.

CADAVER ALLOGRAFT RECIPIENTS CHANGED TO ALTERNATE DAY THERAPY WITH CYCLOSPORIN (C) AND AZATHIOPRINE/STEROIDS (A/S)

Case	Age, sex	Day changed	Reason for change	Current renal function
1	60, F	61	Rejection on C; fluid retention on A/S	Stable*
2	54, M	34	C toxicity; neutropenia on A/S	Stable†
3	56, M	111	Four rejection episodes not responding to bolus methylprednisolone	Improved
4	23, F	33	Two rejection episodes not responding to bolus methylprednisolone	Improved and stable
5	51, M	14	Rejection in presence of high C levels; no response to bolus methylprednisolone	Improved and stable

*Oedema resolved. †Neutropenia resolved.

All patients tolerated the change well and have only slight cushingoid changes. Allograft function has improved although the serum creatinine is slowly increasing in patient 3 (figure).



Renal function after change to alternate day therapy.

Creatinine levels in μmol/l.

Cyclosporin, azathioprine, and steroids have different mechanisms of action and thus combinations may be advantageous, but because of the risk of overwhelming infections or lymphoma they have been combined in low doses. The therapeutic index of chemical immunosuppressive agents may not be enhanced by combining agents in low doses, such as cyclosporin, azathioprine, and steroids. Threshold high peak blood levels may be necessary. We have tested this idea in patients in whom either serious side-effects developed after conversion to azathioprine plus steroids or rejection continued on cyclosporin despite bolus steroid therapy and high blood levels of cyclosporin with nephrotoxicity. These patients comprise a highly selected group with a poor prognosis whose management is difficult, and our early experience is encouraging.

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RESTRICTION ENZYME ANALYSIS AND HERPES SIMPLEX INFECTIONS

SIR,—Identification of herpes simplex viruses (HSV) and studies of strain epidemiology have benefited from the introduction of restriction enzyme analysis of HSV DNA into clinical virology.[1] Unfortunately, Dr Smith and her colleagues (April 27, p 979) appear to cloud the molecular basis for interpretation of this test and draw conclusions based on insufficient clinical and social data.

Restriction enzymes can clearly distinguish the DNA of HSV type 1 from that of HSV type 2 because homology between the respective genomes is limited: they can detect the presence or absence of enzyme cleavage sites in the DNA of epidemiologically unrelated viruses within each serotype; and they can highlight reiterative sequences detected as size heterogeneity in certain DNA fragments without alteration in the number of relative position of