Worton cannot be taken as a convincing analogy since such cell lines are especially prone to non-disjunction with frequent chromosome loss and gain resulting in uniparental disomy (personal communication, A. Geurts van Kessel, department of human genetics, Nijmegen). Moreover, in that study only one genetically aberrant cell line was detected whereas the survival of two mutant cell lines would be a prerequisite for twin spotting.

We disagree with the statement that somatic recombination is rare in man. Mitotic crossing-over occurs rather frequently,[3,4] but further research is necessary to establish the contribution of the different mechanisms to the origin of twin spotting.

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Preparation of lymphocytes for autolymphocyte therapy in metastatic renal carcinoma

SIR,—Dr Osband and colleagues (April 28, p 994) report the effect of autolymphocyte therapy on survival and quality of life in patients with metastatic renal-cell carcinoma. The results of this prospectively randomised, controlled, multicentre study are impressive, showing a clear survival benefit of this form of immunotherapy. I would comment on the procedure for preparation of the lymphocytes, and suggest an alternative protocol based on our observations in experimental autoimmune diseases.

We have investigated methods of selecting antigen-specific T-cells without knowledge of the antigen.[1] Our strategy was to culture in various ways heterogeneous populations of lymphocytes in the absence of specific antigen and to look for the resulting change in the frequency and activity of the T-cells responding to specific antigen. On culture of lymphocytes from rats with adjuvant arthritis in medium containing only lymphokines, we found a decrease in the proliferation indices relative to the reactivity detected on extraction from the rat. In contrast, when the mixed population of cells was first activated with concanavalin A and then cultured in medium containing interleukin-2 (IL-2), we found a striking increase in both reactivity (up to 60-fold) and cell frequency (up to 40-fold) of the antigen-specific T-cells. In addition, on repeated mitogen stimulation specific antigen reactivity was lost. Our findings can be explained by the fact that memory T-cells have an accelerated kinetics of expression of IL-2 receptors, and thus when stimulated with naive T-cells they divide earlier than do naive T-cells.[2] Moreover, memory T-cells proved to respond more rapidly to concanavalin A in rats[3] and to anti-CD3 monoclonal antibodies in man.[4]

Osband and colleagues have based their clinical study on animal models, showing that spleen cells from animals with a tumour could mediate a therapeutic effect in mice injected with the same tumour, after the cells were treated with a lymphokine mixture from a supernatant of a mixed lymphocyte culture.[5] Osband et al do not mention investigations that have directly examined various forms of cell preparation on tumour-cell-specific lymphocyte frequencies or cytotoxic capacity. Moreover, lymphokine generated T-cells are highly dependent on exogenous IL-2 and die rapidly in vitro or in vivo without repeated IL-2 supplements,[6] in contrast with antigen-driven T-cells that mediated specific tumour therapy and persisted as long-term functional memory T-cells.[7]

On the basis of our results in adjuvant arthritis, we recommend that the two optional forms of cell preparation (ie, lymphokine or mitogen stimulation) are compared in the animal model both in respect of efficacy and of the change in cell frequency of the anti-tumour T-lymphocytes or anti-tumour reactivity. This modification of the treatment protocol may enhance both the therapeutic benefit and its longlasting effect.

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"Nailpolish sign" in cyanotic heart disease

SIR,—Patients with congenital cyanotic heart disease often try to conceal the external manifestations of their affliction. Although clubbing does not attract much notice unless it is severe, cyanosis (especially of the nails) is often the focus of attention. However, this is easy to conceal with nailpolish. We have noticed that females of all ages with congenital cyanotic heart disease tend to use nailpolish to conceal the cyanotic tinge. The nails of young boys are also often painted by their parents, and we have even seen a few men resort to this measure.

The nail beds are an important site for the detection of cyanosis since the capillary loops are numerous and tortuous, and lie parallel to the surface of the dermal papillae. By contrast only one capillary loop per papilla is present in other areas of the body, with the capillaries lying perpendicular to the skin and therefore contributing less to skin colour than they do in the nail beds.[1]

The practice of painting nails may lead to diagnostic errors, especially in a busy outpatient department. If the doctor does not remove the nailpolish he would be unable to examine the nails adequately, and mild cyanosis and that brought on by exercise may be missed. Awareness of the possible clinical significance of painted nails, and a bottle of acetone to hand, would go a long way to overcome this difficulty.

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Prevention of hepatitis C virus infection in haemophiliacs

SIR,—Dr Makris and colleagues (May 12, p 1117) urge rapid implementation of anti-HCV screening of blood donations. However, before their data can be said to support such a conclusion, several points need clarification.

Which patients received which preparations of heated factor VIII and did any receive one type exclusively as in some recently published studies?[1,2] Various procedures for inactivation of viruses in blood products have different efficacies and the UK heated factor VIII (factor VIII-Y) has an excellent safety record.[3] As far as we are aware, factor VIII-Y has never transmitted non-A, non-B hepatitis.

Why did the haemophiliacs in this study show a low prevalence of anti-HCV relative to other UK studies?[1,4] Were earlier samples of the seronegative patients available? From other reports, some of them are likely to have been positive.[4]

Seropositive haemophiliacs without histological evidence of chronic liver disease may reflect a non-progressive infection or a false-positive result, in the absence of supplementary testing such as recombinant immunoblot assay.[5]

By the same token, histological evidence of chronic liver disease in seronegative patients may reflect false-negativity, a possibility cited by Alter et al[6] in the context of post-transfusion hepatitis studies.

So far, no transmission of NANBH by UK heat-treated factor VIII has been reported,[4] an observation which should reduce some of the urgency for the introduction of new anti-HCV assays whose cost effectiveness is still debatable.

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**This letter has been shown to Professor Preston and his colleagues, whose reply follows.—ED L.

SIR,—19 patients received material heat-treated by a single method and none of those who received exclusively NHS heat-treated products (dry heat at 80°C for 72 h) is anti-HCV positive:

Viral inactivation process	*Patients treated*	*Anti-HCV positive*
Dry heat 80°C, 72 h	10	0
Dry heat 60°C, 32 h	2	1
Wet heat 60°C, 20 h	7	3

This is consistent with reports from the UK haemophilia centre directors[1] and Skidmore et al.[2]

The difference in the reported incidence of anti-HCV may be due to different prescribing policies in respect of both amount and product(s) used, the selection of patients for HCV testing, and the type of test used. We used a radioimmunoassay; the other two studies quoted[2,3] used an ELISA system. We have not tested older samples from patients found to be negative and cannot comment on the findings of Noel and colleagues.[4]

All 29 HCV seropositive haemophiliac patients on whom biopsies were done had histological evidence of chronic liver disease. Although 5 patients did not have the typical histological features of NANBH (non-A, non-B hepatitis) they did have chronic hepatitis, and we suggested possible explanations for this.

We agree that that the absence of anti-HCV in patients with histological chronic liver disease may represent false negativity, especially since Weiner et al have demonstrated HCV sequences in the livers of HCV seronegative patients.[5] However, the biopsy findings in this group were not typical of chronic NANBH.

Our comments on the need to eliminate HCV from clotting factor concentrates were not directed specifically towards UK products. We acknowledge the safety record of heat-treated factor VIII (HT) but several other products are used in the UK and HCV transmission still occurs.[1] There has been an impressive improvement in viral inactivation/elimination procedures for clotting factor concentrates but Dr Bernstein and colleagues' report (June 23, p 1531) of HCV transmission by a wet-heated, highly purified, high purity product with a previous good safety record[6] is a sharp reminder that optimism should be tempered with caution. It seems to us important to ensure safe products through the combined effects of donor selection and viral inactivation/elimination. This dual approach should greatly reduce the risk of unexpected HCV transmission, such as happened with Scottish National Blood Transfusion Service intravenous IgG preparations and with a batch of wet-heated commercial factor VIII concentrate.[7] Although the immunoglobulin was produced by a different manufacturing process, it is likely that the transmission of HCV by these two products resulted from a heavy viral load in the starting plasma.[7,8] In our view, the current safety record of HT should not influence decisions on donor screening in the UK.

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False-positive hepatitis C virus antibody tests in paraproteinaemia

SIR,—In June, 1989, the prevalence of antibody to hepatitis C virus (HCV) was investigated by the French Viral Hepatitis Study Group.[1] Of 1627 donor blood samples tested at our centre by the Ortho Diagnostic Systems immunoassay, 9 were reactive. These 9 donors were interviewed and blood was taken for laboratory testing of virus markers and liver enzymes and for protein electrophoresis. 2 had a history of blood transfusion; none had hepatitis B virus or human immunodeficiency virus markers or raised liver enzymes. 1 symptom-free donor had paraproteinaemia (IgG kappa), subsequently found to be monoclonal gammopathy. This anomaly led us to investigate other sera from patients with paraproteinaemia.

ANTI-HCV RATIOS OF SERA FROM PATIENTS WITH PARAPROTEINAEMIA

Paraprotein	Anti-HCV ratio*		
	<1	1–2	>2
IgG kappa	45	4	4
IgG lambda	34	3	8
IgM kappa	47	5	1
IgM lambda	18	0	1
IgA kappa	8	1	0
IgA lambda	4	0	1
Total	156	13 (7·1%)	15 (8·1%)

*Optical density of sample/optical density of cut-off

184 sera were tested, and 28 (15·2%) were found positive (table). These 28 anti-HCV ELISA-positive sera were then tested by [illegible] recombinant immunoblot assay (RIBA) and only 1 was positive, 3 others being indeterminate. A positive anti-HCV ELISA test in a patient with paraproteinaemia must be regarded with caution since in our experience at least 80% were unconfirmed by RIBA.

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