INACTIVATION OF HTLV-III/LAV DURING PLASMA FRACTIONATION

SIR,—The HTLV-III/LAV is thought to survive the processing of factor VIII from contaminated plasma. However, the viability of the virus in cold ethanol fractionation and the potential infectivity of other plasma products is unknown. Spire et al[1] found that reverse transcriptase activity of LAV is lost rapidly when the virus in buffer is exposed to 19% ethanol. We report here our results on the stability of HTLV-III infectivity to ethanol.

We began by measuring the residual infectivity (median infectious dose, ID_{50}[2]) of HTLV-III seeded into RPMI-1640 medium or plasma (pH 7·4±0·2) and exposed to ethanol for 10 min. ID_{50} reductions with 20% ethanol varied. The ID_{50} in medium at 23°C was reduced 10^3-fold. In one single-donor plasma sample at 23°C, infectivity was reduced 10^2-fold but in three donor plasma samples at −5°C infectivity was reduced by $10^{2·7}$ and $10^{0·7}$ in two and not reduced in the third. These results suggest that HTLV-III is more stable to 20% ethanol in plasma than in medium and that some characteristic(s) of the plasma, possibly lipid content or protein composition, can stabilise the virus to ethanol. Lower temperatures may also stabilise the virus. 50% ethanol has consistently reduced virus titre to levels below the detection limit of our assay when exposure was at 23°C in either culture media or plasma (untreated ID_{50}, $10^{3·7}$–$10^{5·7}$; treated ID_{50}, $<10^1$). However, at −5°C in plasma, titre reductions were only 10^1 and $10^{1·4}$.

Our next experiment was designed to simulate as closely as possible on a small scale the precipitation of fractions I + II + III by 20% ethanol at pH 6·9 and −5°C, as in a common manufacturing procedure.[3] A sample of cryoprecipitate-poor plasma from a pool of about five thousand donations previously found not to contain antibodies to HTLV-III was seeded with the virus. Samples were drawn periodically after addition of ethanol to a final concentration of 20%, then immediately diluted 1:10 in RPMI-1640 medium and frozen to stop the inactivation. Virus in the control plasma (no ethanol) lost very little infectivity over a 2 h period (table). In an identical sample containing 20% ethanol the ID_{50} fell by more than $10^{3·5}$ in 5 min. Since most cold ethanol precipitation steps last for 10 h it is reasonable to expect inactivation of HTLV-III by at least $10^{3·5}$-fold during each plasma fractionation step.

HTLV-III is rapidly inactivated at pH below 7 and above 10. Compared with a pH 7·0 control, virus infectivity is reduced 10^3-fold in 10 min at pH 5·7.[4] Fractionation procedures[5] include precipitation steps at pH values as low as 4·8, in both the presence and absence of ethanol. Although plasma fractionation conditions differ somewhat from those reported on by Martin et al[4] these steps at low pH should provide an extra margin of safety in the fractionation process.

INFECTIVITY* OF HTLV-III IN CRYOPRECIPITATE-POOR PLASMA AS FUNCTION OF TIME

Time	ID_{50}	
	No ethanol	20% ethanol†
0	$10^{4·51}$	$10^{4·50}$
0	$10^{4·19}$	..
1 min	..	$<10^1$
2 min	..	$10^{2·09}$
5 min	..	$<10^1$
10 min	..	$<10^1$
1 h	..	$<10^1$
2 h	$10^{4·43}$	$<10^1$
10 h	..	$<10^1$
20 h	..	$<10^1$

*Corrected for 1:10 dilution in RPMI-1640 medium to quench inactivation at timed intervals.
†Denatured alcohol from a plasma fractionation facility was used. Composition 90% ethanol, 5% methanol, 5% water.

These results indicate that steps in large scale plasma fractionation, especially ethanol at −5°C and low pH, reduce the risk of plasma products being infectious should the source material be contaminated by HTLV-III/LAV.

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