THE ROLE OF LEUCODEPLETION

IN PREVENTION

OF HTLV TRANSMISSION BY TRANSFUSION

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THE ROLE OF LEUCODEPLETION OF CELLULAR BLOOD COMPONENTS IN THE PREVENTION OF TRANSMISSION OF HTLV-I AND -II.

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Background.

The rationale for consideration of leucodepletion in preventing transfusion-associated HTLV infection is based on the strong association of this group of viruses with the genome of human lymphocytes. This paper will therefore consider:-

1. The evidence for exclusive transmission by lymphocyte-containing blood components.

2. Evidence for a dose-response or threshold effect of lymphocyte contamination.

3. The potential of current technologies for leucodepletion of cellular components.

4. Evidence that these technologies could provide a 'safe' level of leucodepletion with regard to HTLV infection and where relevant evidence is lacking, how this could be obtained.

Note - unless specifically stated, 'HTLV' will be used throughout to encompass both HTLV-I and -II.

1. HTLV transmission by cellular blood components.

Like all retroviruses, the life cycle of HTLV requires integration via reverse transcriptase of a DNA copy of its RNA genome into host genetic material. HTLV targets human T lymphocytes, without subtype selection, with up to 1-10% of circulating lymphocytes infected[1]. Evidence for transmission via transfusion first came from endemic areas of Japan, with a report of infection of 26/41 (65%) recipients of infected red cells and platelets[2]. Strikingly, patients transfused with 'acellular' components (fresh frozen plasma, cryoprecipitate, frozen/washed red cells) remained uninfected. Since this original report, a number of retrospective and prospective studies have confirmed the overall transmission rate from cellular components and the lack of risk with plasma derived components including factor VIII (Table 1). Transmission with cellular components applies equally to HTLV-I and -II[8].

Of importance is the consistent observation that transmission via stored red cells decreases progressively with storage time, such that red cells more than 14 days old have never convincingly been shown to be infectious (Table 2). Additional proof of the red cell storage effect comes from donors from whom both platelets and red cells have been transfused. Of 3 such donors from whom platelets transmitted HTLV to their recipients, 12 day old red cells from 1 donor were infectious, while 22 and 39 day old red cells from the other 2 were not[5]. Since up to 60% of lymphocytes in stored red cells remain present at 21 days, the decline in infectivity with storage may relate to the need for lymphocytes to retain viability and perhaps proliferative capacity if infection is to result. In contrast, the age of stored platelet concentrates does not appear to influence the transmission rate. These observations may explain the apparently greater transmission rate with platelets than red cells, since this difference disappears when only red cells < 14 days old are considered[8]. The need for viable lymphocytes might explain the lack of transmission with FFP, in which the few detectable lymphocytes lack proliferative capacity *in vitro*[9].

2. Evidence for a minimum infective dose of lymphocytes.

The minimum infective dose of lymphocytes from a well HTLV carrier is not known precisely. The observations discussed above concerning the need for viable lymphocytes might lead one to suppose that lymphocyte removal by filtration to a critical 'safe' level might be easily achieved. However, the following epidemiological and experimental data suggest that the minimum infective volume of blood might be very small:-

a) intravenous drug use, in which blood exchange by shared needles is generally of low volume, is a major risk factor for HTLV infection, particularly HTLV-II[10].

b) HTLV can be transmitted to rabbits via a volume of human breast milk or semen containing $< 6 \times 10^7$ lymphocytes[11].

c) the minimum volume of blood able to transmit HTLV to rabbits is 0.01 ml, containing approximately 10^4 lymphocytes[12] . The transmission rate was 100% when 0.1 ml blood was injected.

d) there is an unconfirmed report of transmission to a physician via a needlestick injury (quoted as a personal communication in reference 12)

e) transmission to an infant by a 44 ml red cell transfusion estimated to contain a total 8×10^7 lymphocytes[13] .

Thus the 'safe' level of lymphocytes required to prevent human transmission, if indeed such a level exists, cannot be ascertained with any certainty. The stratified data on aging red cells might suggest, in theory at least, that an unquantifiable, but perhaps only minimal, degree of protection might be achieved by progressive lowering of leucocytes in transfused components.

3. The potential of current leucodepleting technologies.

The UK Guidelines for Transfusion Services (Red Book) define a leucocyte depleted unit of red cells or platelets as one containing $< 5 \times 10^6$ leucocytes/pack. Two methods are in general use for the production of leucocyte depleted components, namely the use of polyester fibre filtration (for red cells and platelets, many manufacturers) and certain apheresis techniques (for platelets only, Cobe Spectra and LRS). Both filtration and apheresis (LRS) can now achieve $< 10^5$ leucocytes/pack under optimal conditions[14]. The filtration process can be adversely affected by variables such as component age, ambient temperature and flow rate. In addition, the loss of intact leucocytes from stored components, particularly red cells, means that leucocyte fragments and debris can escape through the filter[15]. For these reasons, leucodepletion, once almost universally carried out at the bedside, is now increasingly being performed as a part of component processing at blood centres. This also allows quality assurance of the process, either by counting residual leucocytes in every pack, or by the use of statistical process control once a method has been validated. Flow cytometric studies have shown that leucodepleting filters remove granulocytes and lymphocytes with equal efficacy; monocyte removal is particularly efficient. Lymphocytes passing through leucodepleting filters retain viability, as demonstrated by a case of transfusion-associated graft-versus-host disease (TA-GVHD) following transfusion of filtered red cells[16].

Lesser degrees of leucocyte removal can also be achieved using 'bottom and top' (BAT) processing technology in which the residual leucocytes are reduced by 80-90% via removal of the buffy coat from red cells (residual leucocytes 10^8/unit) and by 1 log for platelets to 10^7/unit. This methodology is standard in Holland and Sweden, not used at all in the US, and has been introduced patchily but increasingly across the UK. However, because of its increased cost, the main driver to this technology is the great improvement in platelet yields, with buffy coat depleted red cells as a by-product. No policy decision has yet been taken that all red cells produced in the UK be buffy coat depleted, and the projected figure for London and South East Zone of the NBS is 40-50%.

Clinical use of leucodepleted components.

Because of cost, use of leucodepleted components (as opposed to buffy coat depleted) is currently highly restricted. The only absolute indications arising from the Edinburgh Consensus Conference on Leucodepletion were aplastic anaemia and recurrent transfusion reactions, with acute leukaemia and neonatal/intra-uterine transfusion unproven indications[17]. A further indication is to prevent CMV transmission if CMV seronegative components are unavailable, as discussed below. Less than 5% red cells and no more than 20% of platelets issued are currently leucodepleted (NBS London and South East Components Group, 1995). Thus introduction of leucodepleting technology for, say, all immunosuppressed patients would be a major and costly departure from current practice, with no evidence of benefit in terms of HTLV prevention. However, this could certainly be considered pragmatically for neonates, as currently practised in several NBS centres. Additional advantages of leucodepletion include reductions in cytokine accumulation and HLA alloimmunisation, and perhaps additional protection from CMV(assuming seronegative units remain the 'gold standard').

4. Evidence that HTLV infection could be prevented by leucodepletion.

There are no clinical studies in the literature on HTLV prevention by leucodepletion. By analogy with CMV, a number of different type of studies would be required to satisfactorily demonstrate that leucodepletion can consistently prevent transmission of HTLV, including:-

a) viral genomic detection using PCR in filtered units. However, detected virus might not viable, and conversely the infective dose may be below the detcetion limits of the assay. The PCR method would have to be modified to concentrate/capture viral particles prior to amplification.
Only 1 such study appears to have been performed (quoted in a book[18] but not yet published in a peer-reviewed journal). In this study, Sekiguchi and colleagues filtered red cells from 5 HTLV-I infected individuals using Sepacell 500 filters. All were PCR positive and 4/5 HTLV antigen positive in the supernatants prior to filtration. After 2-4 $\log_{10}$ leucocyte reduction, 2/5 remained PCR positive, with no correlation with the extent of leucoreduction achieved. However, HTLV could not be cultured from these units after filtration, for unclear reasons.

b) a non-randomised study in susceptible individuals to demonstrate of lack of seroconversion by infected filtered components - now not ethical as a prospective study. Surveys of patients receiving only leucodepleted blood (eg aplastic anaemia or thalassaemia) for HTLV positivity are possible, but positive results in those and control patients will be far be too few for meaningful analysis.

c) a randomised study comparing filtration and screening. This been done for CMV in bone marrow transplantation but is neither ethically nor statistically feasible for HTLV.

A final point concerns the possible, but as yet theoretical, benefit of gamma irradiated cellular components in HTLV prevention. The presumed need for viable donor lymphocytes to enable transmission to occur suggests that blood components gamma irradiated for the prevention of TA-GVHD might carry a reduced HTLV risk. However, HTLV transmission by irradiated red cells has been described [13]. Indications for irradiated components include bone marrow transplantation, intrauterine transfusion and Hodgkin's disease, but not acute leukaemia[19]. Thus, although no direct evidence exists, the experimental literature in this area should be kept under review.

Conclusions.

1. On current evidence, leucocyte depletion cannot be recommended for reliable prevention of transfusion-transmitted HTLV infection, although red cells more than 14 days old appear to be safe.

2. The degree of any partial protection afforded by processing strategies which reduce lymphocyte contamination prior to component storage cannot be deduced.

3. If the preliminary evidence on the lack of effect of leucocyte depletion is confirmed, clinical studies will be unnecessary and inappropriate.

References

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Table 1. Transmission of HTLV by cellular and 'cell-free' blood components.

Reference	Country	No. patients	Product	Transmissions
2	Japan	41	RBC/plts	26 (65%)
3	US	88	RBC	11 (12.6%)
		24	Plts	6 (25%)
		19	FFP/cryo	0
4	Jamaica	54	RBC/Plts	24 (44%)
		12	FFP/cryo	0
5	US	36	RBC	10 (28%)
		15	Plts	6 (40%)
		6	FFP/cryo	0
6	US	54	RBC	11 (20%)
		20	Plts	15 (75%)
		4	Frozen/thawed RBC	0
		17	FFP/cryo	0
7	US/France	179	Factor VIII	0
8	Italy	142	Factor VIII	0

Table 2 HTLV transmission by red cell components stratified by age.

Ref	Red cell age (days)	No. patients	Transmissions (%)
2	0-5	15	12 (80)
	6-10	18	10 (56)
	11-14	6	3 (50)
	>14		
3	0-5	5	4 (80)
	6-10	5	2(40)
	>10	9	0 (0)
4	0-6	31	20 (65)
	7-14	20	6 (30)
	>14		
5	0-14	19	9 (47)
	>14	15	0
6	0-5	3	2 (66.6)
	6-10	18	8 (44)
	11-20	17	0
	>20	32	1*