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HTLV III INFECTION IN SERONEGATIVE HAEMOPHILIACS FOLLOWING TRANSFUSION OF FACTOR VIII

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SUMMARY

Fifteen haemophiliacs were observed to acquire anti-HTLVIII during 1984. One batch of factor VIII concentrate was given to all these patients and is presumed to be the cause of the seroconversion. A further 18 patients who received the same batch did not seroconvert. Lymphocyte subset data was available prior to the transfusion of the implicated batch when the patients had reduced T helper cell numbers and T helper/suppressor ratios; neither of these values changed in those who seroconverted. The probability of seroconversion was independently related to the pre-existant low T helper/suppressor ratio, the number of vials of the implicated batch transfused, and to the total annual factor VIII consumption. In addition 10 patients received a batch of factor IX concentrate from the same donor plasma; none of these individuals developed anti-HTLVIII.

INTRODUCTION

Substantial evidence has accumulated to demonstrate that the most likely cause of the acquired immune deficiency syndrome (AIDS) is the virus HTLVIII/LAV. Transfusion of red cells, platelets or factor VIII and IX concentrates may result in the appearance of anti-HTLVIII and a small proportion of these seropositive recipients will develop symptoms of the AIDS-related complex or AIDS (1,2). In haemophiliacs tests for anti-HTLVIII, on stored serum samples, have demonstrated that seroconversion was first detectable in 1978 in the USA (3), and no later than the following year in the UK (RAW, & RST, unpublished observation).

In contrast to the situation elsewhere in the UK, almost all haemophiliacs attending the Edinburgh Haemophilia Centre have received factor VIII and IX concentrates prepared exclusively from locally collected plasma by the Scottish National Blood Transfusion Service (SNBTS). Until recently there were no reported cases of AIDS in Scotland and we therefore considered it possible that our patients might not be exposed to HTLVIII. We did, however, consider it possible that because of the long incubation period between infection and the appearance of AIDS that some asymptomatic carriers of HTLVIII might have donated blood. In an earlier report on immune abnormalities in our patients, we demonstrated both a reduction in the absolute T helper cell numbers and the helper/suppressor ratio and, because of our apparently AIDS-free donor population, we concluded that these immune changes were the result of infusion of factor VIII concentrates per se rather than infection by HTLVIII (4). We have now confirmed by testing stored serum samples, that at the time our previous study was undertaken (4) in the Spring of 1983, all the patients who received solely SNBTS blood products were sero-negative for anti-HTLVIII.

As part of the continuing assessment of our haemophiliacs we have now observed that 16of our patients seroconverted for anti-HTLVIII in 1984 and all but one of these individuals had previously received a common batch of SNBTS factor VIII concentrate. This report describes the lymphocyte subset numbers before and after infusion of this contaminated batch of factor VIII concentrate both in those who developed anti-HTLVIII and those who did not seroconvert. This study also reports the lymphocyte markers and HTLVIII serology in patients with haemophilia B who received the batch of factor IX concentrate prepared from the same pool of donor plasma.

Method

Anti-HTLVIII was detected as described previously (5), on serum samples which had been collected periodically from all patients and stored at -20°C. Lymphocytes were enumerated with a Coulter S Plus. Subsets were quantified by indirect immunofluorescence using T_4 or T_8 specific monoclonal antibodies in the first layer. The technical details have been reported elsewhere (4). Statistical analysis of differences between groups for any variable was by the Wilcoxon Rank Sum Test and the changes within groups was by the Wilcoxon Signed Rank Sum Test. The effect of several variables simultaneously on the probability of seroconversion was investigated using multiple linear logistic regression.

Patients

In this study 34 patients with haemophilia A (29 severe; 5 moderate) of mean age 27.6 years (range 10-49) and 8 patients with haemophilia B (4 severe; 4 moderate) of mean age 26.2 (range 8-56) and one patient with severe von Willebrand's disease of age 56 were studied. Antibodies to factor VIIIC were present in three patients with severe haemophilia A. Serum samples collected in late 1983 and early 1984 were all negative for anti-HTLVIII.

During 1984 all patients in this study were treated on demand with multiple batches of SNBTS intermediate purity factor VIII concentrate. In addition to factor VIII, two patients with haemophilia A with anti-factor VIIIC antibodies, and all the patients with haemophilia B, received SNBTS factor IX concentrate(Defix). The former two patients also received FEIBA(Immuno). Two haemophilia A patients (one with an antifactor VIII inhibitor) also received commercial high-purity factor VIII concentrates.

No patient was known to have other risk factors for developing antibodies to HTLVIII other than their replacement therapy.

Results

Between April and October 1984,16 patients with haemophilia A (Table 1) developed anti-HTLVIII. Scrutiny of the transfusion records of these patients revealed that all but one had received a common batch (A) of SNBTS factor VIII between March and May 1984. Of all the other batches of factor VIII transfused during this period, the next most likely implicated batch (B) was transfused during January 1984 and could only account for 9 of the 16 patients who developed anti-HTLVIII. One patient with severe haemophilia A, who did not receive batch A developed anti-HTLVIII; the source of his infection remains obscure but he did receive batch B. He had an orthopaedic operation in November 1983 which was covered by SNBTS factor VIII but since January 1984 he had only received treatment on 3 occasions with factor VIII from batch B. He had not received any commercial factor VIII.

In addition to the 15 patients who are known to have seroconverted a further 18 patients received the implicated batch A. Serum samples from these latter patients have been collected at least four months and in some instances up to 8 months, after transfusion of batch A and have been found to be negative for anti-HTLVIII. The time between first infusion of batch A to the development of anti-HTLVII has been difficult to determine precisely. The shortest time from first infusion to the presence of detectable antibody was 31 days (6). In Fig. 1 the period over which infusions were given has been related to the time of the first anti-HTLVIII positive serum sample and the number of vials of the implicated batch transfused. For most patients it is difficult to estimate the minimum time between infusion and the development of anti-HTLVIII because, as can be seen in Fig. 1, the patients received repeated infusions of the implicated batch and the serum samples were only taken periodically, mostly every few months. One of the patients who received 81 bottles of the implicated batch was negative at 20 weeks before being detected positive at 40 weeks.

Lymphocyte subsets were measured in 24 of the patients during the Spring of 1983 (4) and again in the Autumn of 1984 (Table 1) after 15 of the 33 recipients of batch A had seroconverted to HTLVIII. The recipients of batch A had reduced T helper cell numbers and T helper/suppressor ratios in 1983 compared with controls.

In 1983 the haemophiliacs who were to develop anti-HTLVIII had a reduced mean T_h/T_s ratio of 1.51 (median 1.24) compared to a ratio of 2.11 (2.10) in the patients who failed to seroconvert and 2.05 (1.93) in controls (Fig. 2). The difference between the two patient groups just failed to reach conventional levels for statistical significance (p=0.06). The 1983 absolute T_h numbers in those who subsequently developed anti-HTLVIII were reduced whereas in those who failed to seroconvert it was no different from controls (Table 1). The T_s concentrations were normal in both groups. When the T_h or T_s numbers or T_h/T_s ratio in 1983 were compared to the values found in 1984 no significant change was noted in the concentrations of these lymphocyte subsets.

The group of 15 patients who seroconverted used a significantly larger number of vials of batch A ($P < 0.01$) and also had a higher annual factor VIII consumption ($p < 0.01$) than the 18 patients who failed to seroconvert, (Table 1, Fig. 3 and 4). When these three factors were assessed simultaneously by applying multiple linear logistic regression analysis to the data from the 24 patients with complete observations, the effect of the T_h/T_s ratio was significant ($p < 0.02$) with the number of vials used in batch A and annual factor VIII consumption achieving significance only at the 10% level (2-tailed test). In the circumstances of this study it could be argued that one-tailed tests would be reasonable for these variables, in which case significance at the 5% level would be achieved. The fitted equation with all 3 terms included was:

$$p = \frac{\exp\,(0.83 - 2.32\ T_h/T_s + 0.074\ n + 0.034\ ann)}{1 + \exp\,(0.83 - 2.32\ T_h/T_s + 0.074\ n + 0.034\ ann)}$$

where n denotes the number of vials in batch A and ann denotes the total annual usage of factor VIII (in thousands of units). The magnitude of the effect of these factors on the seroconversion rate is illustrated by 8 of 9 patients (89%) with a helper/suppressor ratio of less than or equal to 1.5 seroconverting compared to 6 of 15 (40%) patients with higher levels; 8 out of 9 patients (89%) using mroe than 40 vials of batch A seroconverted compared to 7 of 23 (30%) with lower usage; 11 of 13 patients (85%) using more than 75,000 units of factor VIII seroconverted compared to 4 of 19 (21%) using less.

All patients are clinically well; two patients have persistent splenomegaly which appeared after the infusion of batch A. One of these individuals, reported in detail elswhere (6), developed a glandular fever-like illness with lymphadenopathy, splenomegaly, floridly avtoical peripheral blood

lymphocytes, and development of ant-HTLVIII following receipt of batch A.

The batch of SNBTS factor IX concentrate, C_7 prepared from the same source plasma as the factor VIII preparation (A) was identified, and transfusion records revealed that it was given to a total of 10 patients; 8 with haemophilia B and two wtih haemophilia A with anti-factor VIII inhibitors. All ten patients have been tested at least 4 months post therapy and none has detectable antibody to HTLVIII. Lymphocyte subset data was available on relatively few and no conclusions can be drawn on this small number. The average number of vials transfused per patient was only 12.5 (range 3-21).

Discussion

Scotland is one of the few parts of the Western World where the incidence of anti-HTLVIII antibody in patients with haemophilia A at the beginning of 1984 was low. Up till then the development of anti-HTLVIII in our patients could be attributed to the occasional use of commercial blood products in a few patients but no cases were attributable to SNBTS factor VIII. This low prevalence of antibody contrasts markedly with that found in North America. It appears that anti-HTLVIII antibodies first began to appear in haemophiliacs in 1978 in the United States (3) but possibly not until 1979 in English haemophiliacs. During the past 7 years there has been a steady increase in the prevalence of antibodies so that now over 90% of some haemophilia populations have anti-HTLVIII antibodies (7).

It is important to review the evidence for batch A causing the development of anti-HTLVIII in our patients. Of the 16 who seroconverted, 15 received this batch; the next most likely batch (B) would only account for 9 seroconversions. The time course for seroconversions ranged from two weeks to 8 months from the mid point of the duration of the transfusions of batch A or 31 days from first transfusion. There is a dose relationship between the amount of batch A transfused and the likelihood of the patients developing detectable antibody (Fig. 3). The principle evidence against batch A is that one patient who did not receive this has seroconverted. The source of his apparent infection is obscure. The definitive investigation to determine whether batch A could have caused the observed seroconversions will depend upon the future availability of a reliable test to identify blood products which are capable of transmitting the HTLVIII virus.

A unique feature of our study is that the patients had lymphocyte subsets measured during the Spring of 1983 when, as we now know, all patients who had received exclusively SNBTS factor VIII were negative for anti-HTLVIII. We have thus been able to compare lymphocyte subsets data after infection with HTLVIII with that observed a year prior to infection(4). Our observation that there is no change in the absolute number of T helper cells or in the helper/suppressor ratio after infection is of great importance. It is commonly assumed that the reduction in T helper cell numbers is a result of the HTLVIII virus being tropic for T helper cells (8). This current study supports our previous conclusion that the abnormal T lymphocyte subsets are a result of the intravenous infusion factor VIII concentrates per se,

and not HTLVIII infection. It is possible however, that there will be a progressive time dependant fall in T helper cell numbers as a result of HTLVIII infection but only prolonged follow up will reveal this.

The pre-infection lymphocyte subset numbers did identify individuals who would produce antibody to HTLVIII. Of those haemophiliacs with a $T_{h/s}$ ratio $\leqslant$ 1.5 in 1983, 89% seroconverted in 1984 whereas only 40% with a ratio $>$ 1.5 developed anti-HTLVIII. Similarly of the 9 patients who received more than 40 vials of batch A, 8 (89%) seroconverted and 10 of 11 (91%) with an annual factor VIII consumption of greater than 75,000 i.u. seroconverted. Analysis of the data on this relatively small number of patients, has demonstrated therefore that the chance of developing anti-HTLVIII is dependant upon the $T_{h/s}$ ratio, the number of transfused vials of presumed HTLVIII infected factor VIII, and the total annual consumption of factor VIII. Our data demonstrate reduced T_h and $T_{h/s}$ counts in 1983 when the patients were negative for anti-HTLVIII and that those with $T_{h/s} \leqslant 1.5$ were more susceptible to infection a finding in keeping with the hypothesis of Levy and Ziegler (9), that infection by an AIDS virus could be considered as an opportunistic infection in an immunomodulated host.

If our interpretation is correct, that all but one of our seropositive patients developed anti-HTLVIII as a result of the transfusion of a single contaminated batch of factor VIII, then it is of interest that this represents only half of the patients who received this batch of factor VIII concentrate.

One possible explanation could be that these apparently sero-negative patients have in fact developed antibody but its concentration was below the level of detection. Another possibility is that because of the altered immunological status of these patients they did not produce specific antibodies readily. A negative antibody test may reflect the absence of viral infection or replication in the lymphocytes.

The pool of source plasma from which this implicated batch of factor VIII was prepared was identified by the SNBTS. The factor IX batch (C) prepared from this same pool of plasma was therefore known and the 10 patients, 8 with Christmas Disease, and two with haemophilia A with anti-factor VIII inhibitors, were examined for evidence of HTLVIII infection. None of these individuals demonstrated seroconversion when tested up to four months after infusion of this batch. Patients with haemophilia B are known to have fewer lymphocyte subset abnormalities, a lower prevalence of

antibodies to HTLVIII and a lesser chance of developing AIDS (10). It is possible that the HTLVIII virus is preferentially excluded from the factor IX concentrate during its manufacture.

The Scottish Protein Fractionation Centre has developed a programme to study possible methods for eliminating the transmission of viral infections by blood factor concentrates. Although this project was initially conceived to reduce the risk of hepatitis transmission the expertise developed was put immediately into effect, following the finding of HTLVIII antibodies in our Scottish patients. All SNBTS factor VIII concentrates being issued are now heat treated. It is hoped that this will eliminate further HTLVIII infection, but only close follow-up of the patients will provide firm evidence to substantiate this expectation.

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Fig. 1 Relationship between time of infusion of batch A, no. of vials transfused and development of anti-HTLVIII.

Fig. 2 Frequency distribution of 1983 T helper/suppressor ratios in patients with haemophilia A who received batch A. The two lower graphs are for those patients who seroconverted or remained seronegative in 1984 following transfusion of the implicated batch of factor VIII.

Fig. 3 Number of vials of batch A of factor VIII transfused in those who seroconverted and in patients who remained seronegative.

Fig. 4 Total factor VIII consumption per patient during 1984 for individuals who developed anti-HTLVIII and those who remained seronegative.

Table 1 Lymphocyte subsets in patients who received batch A of factor VIII and factor IX (batch C, derived from the same donor source plasma as batch A). The 1983 data was collected prior to the transfusion of batch A and C; the results for 1984 are following transfusion of these implicated batches.

x Patients compared to controls; $p < 0.005$

xx Patients who seroconverted compared to those who remained seronegative; $p < 0.005$

xxx Patients who seroconverted compared to those who remained seronegative; $p < 0.01$

BATCH TRANSFUSED	RECIPIENTS	TOTAL NUMBER	T_h MEAN NUMBER RANGE 1983	T_h MEAN NUMBER RANGE 1984	T_s MEAN NUMBER RANGE 1983	T_s MEAN NUMBER RANGE 1984	$T_{h/s}$ MEAN NUMBER RANGE 1983	$T_{h/s}$ MEAN NUMBER RANGE 1984	No. of bottles of batch A or C Mean Number Range	FVIII or FIX usage in 1984 Mean Number Range
Batch A (FVIII) March-July 1984	Haemophilia A and VWD		0.64[x]	0.62[x]	0.54	0.70	1.51[x]	1.14[x]	44.7[xx]	90,439[xxx]
	a) Seroconverted to HTLVIII	15	14	15	14	15	14	15	15	15
			0.32-1.45	0.14-1.47	0.10-0.90	0.28-1.80	0.58-3.10	0.27-3.30	10-109	16,230-170,79
	b) No sero-conversion	18	0.84	0.77[x]	0.46	0.54	2.11	1.60	17.9[xx]	41,015[xxx]
			10	15	10	15	10	15	17	17
			0.53-1.45	0.30-1.42	0.22-1.23	0.28-0.90	1.18-2.95	0.33-2.40	3-51	7,860-176,790
Batch C (FIX) June-July 1984	Haemophilia B All seronegative	8	0.86	0.78	0.56	0.48	1.70	1.67	14.5	32,350
			4	3	4	3	4	3	6	6
			0.47-1.36	0.78-0.79	0.36-0.87	0.39-0.52	0.73-2.50	1.50-2.00	6.21	3,000-91,200
	Haemophilia A with inhibitors All seronegative	2	-	0.82	-	0.62	-	1.40	6.5	60,500
				2		2		2	2	2
				0.70-0.93		0.51-0.73		0.96-1.83	3-10	22,200-99,000
	Normal Male Controls	22	1.09 0.6-2.01		0.56 0.22-1.21		2.05 1.59-3.93			

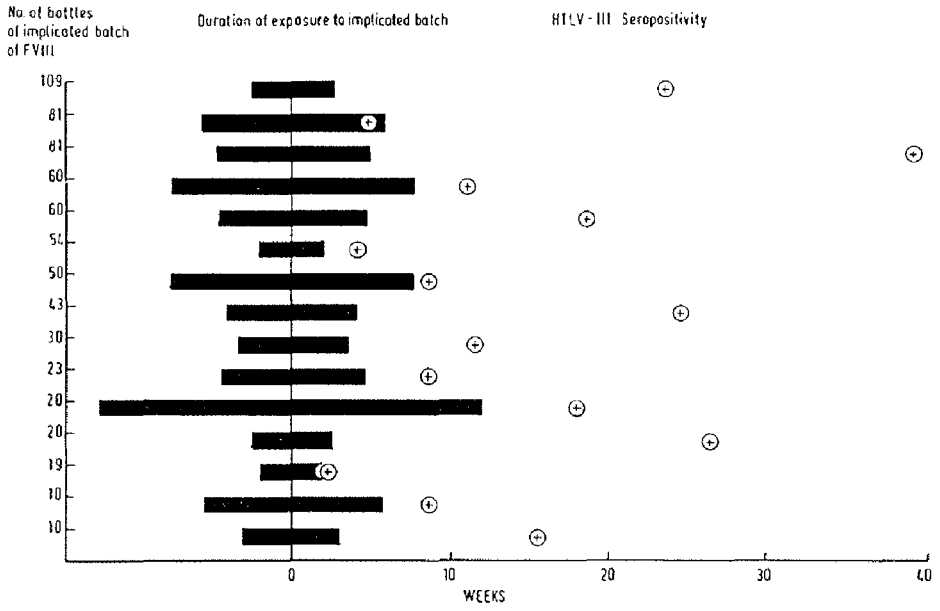
No. of bottles of implicated batch of FVIII
Duration of exposure to implicated batch
HTLV - III Seropositivity
109
81
81
60
60
50
50
43
30
23
20
20
19
10
10
0
10
20
30
40
WEEKS

FIGURE 1

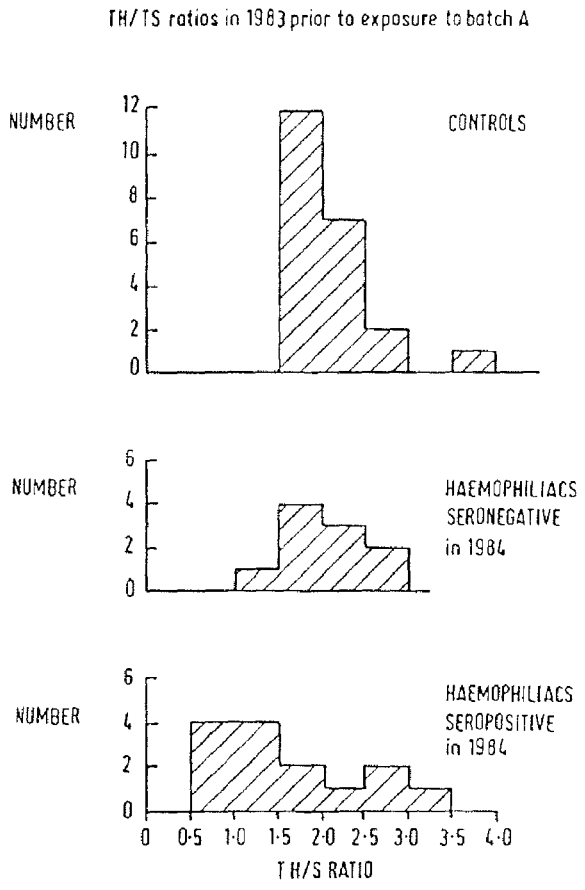
TH/TS ratios in 1983 prior to exposure to batch A
NUMBER
12
10
8
6
4
2
0
CONTROLS
NUMBER
6
4
2
0
HAEMOPHILIACS
SERONEGATIVE
in 1984
NUMBER
6
4
2
0
HAEMOPHILIACS
SEROPOSITIVE
in 1984
0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
4·0
TH/S RATIO

FIGURE 2

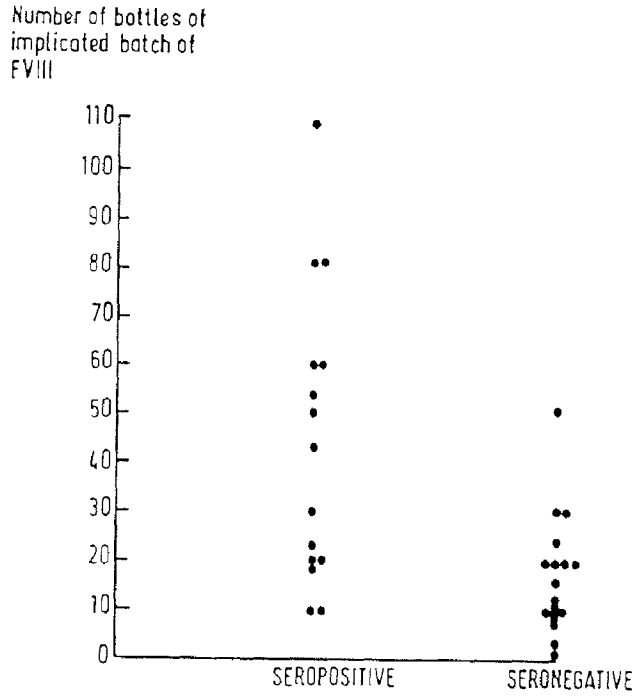

FIGURE 3

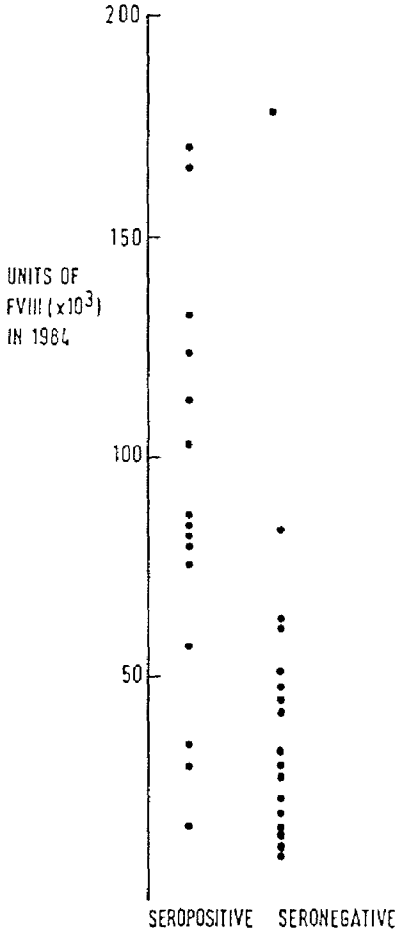

FIGURE 4