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The Role of HIV Infectivity and Composition of Factor VIII Concentrates on the Immunity of Haemophiliacs Positive for HIV Antibodies

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Abstract. Forty-six subjects (44 HIV antibody-positive) with some degree of immune deficiency (at least TH/TS ratio below 1) were randomly distributed into 4 treatment groups. Each group was assigned to 1 of 4 products to be used exclusively for a 1-year period: 1 concentrate was of intermediate purity and not heat-treated, and 3 were heat-treated in order to inactivate HIV, 2 of them being of higher purity. At 4–6-month intervals, check-ups, including as markers clinical examination, platelet, lymphocyte and T cell subset counts, IgG levels and delayed hypersensitivity test, were carried out. At entry as well as at the end of the study, groups were not statistically distinguishable. No intra- nor inter-group differences were demonstrable for any of the markers. In contrast, using a scoring system for each marker and the results of check-up at entry as reference, significant differences between groups appeared on subsequent check-ups. Patients receiving intermediate-purity factor VIII, whether heat-treated or not, were mostly steady, while groups receiving heat-treated concentrates of a higher purity significantly worsened. This surprising outcome was no related to differences in anti-HIV titers or specificities. From this study, the potential long-term predictive value of this scoring system could not be established.

Introduction

In Western industrialized countries, some 20,000 haemophiliacs have seroconverted for the human immunodeficiency virus (HIV) over the past 3–4 years. They are presumably carriers of the virus in the form of DNA integrated into cell genomes or replicating in T lymphocytes and central nervous system cells [1–3]. At the end of 1985, approximately 150 of these patients have developed full-blown AIDS, and many more have developed one of the other clinical conditions associated with the virus (AIDS-related complex or generalized lymphadenopathy) [4, 5].

Several non-genetic factors have been suspected of playing a role in the progression from asymptomatic HIV antibody-positive carriers to AIDS. In multitransfused patients such as haemophiliacs, superinfection with HIV present in therapeutic products [5], repeated stimulations of the immune system by isologous proteins and viruses present in blood products [6, 7] and iterative bursts of immune complexes induced by replacement therapy, or intrinsic immunosuppressive properties of factor VIII [7] are potential cofactors of clinical complications.

The availability of a new generation of factor VIII concentrates characterized by a high specific activity and/or heat-induced inactivation of viruses provided the opportunity to assess their effect on the recipient immune system. We report here the results of a 1-year prospective study (1984–1985) comparing the immunologic outcome of HIV antibody-positive haemophilia A patients treated with concentrates of various purities, heat-treated or not.

1 The following members of the AIDS-Haemophilia French Study Group have participated in this study: J.F. Bach, M.F. Torchet, Hôpital Necker, Paris; F. Barre-Sinoussi, S. Chamaret, Institut Pasteur, Paris; A.M. Courouce, J.Y. Muller, CNTS, Paris; Y. Laurian, Hôpital de Bicêtre, Kremlin-Bicêtre; P. Maisonneuve, Hôpital Cochin, Paris; J.P. Revillard, INSERM and Hôpital E. Herriot, Lyon, France.

Table I. Clinical and biological status of a selected group of 46 haemophilia A patients at entry

	Lympha-denopathy	Platelet count $<150 \times 10^3/\mu l$	Lymphocyte count $<1{,}000/\mu l$	T4+ $<600/\mu l$	T8+ $>1{,}200/\mu l$	T4+/T8+ ratio <1	IgG >16 g/l	Anergy[a]
Subjects, n	12	2	3	21	10	39	33	4
Subjects, % (of total)	26	4	6.5	46	22	85	72	14

[a] Twenty-nine patients only were tested.

Materials and Methods

Patients Selection

Forty-six patients with severe haemophilia A were included in the study. Forty-four were selected from a previously reported cross-section study [8] of 299 patients on the basis of a T4+/T8+ lymphocyte ratio below 1.0. Two further patients were selected on the same basis. Thirty-nine had one or more additional clinical or biological abnormalities (table I). All patients were positive for anti-HIV IgG antibodies tested and confirmed by two different ELISA assays, except for patient 3 in group 2 and patient 9 in group 4 who remained negative after repeated testing with ELISA, Western blot and radioimmunoprecipitation techniques on several samples throughout the study.

Study Design

Patients were randomly distributed into 1 of 4 groups defined according to the brands of factor VIII concentrate, each of them exclusively used for the entire duration of the study. Group 1 consisted of 13 patients treated with the non-heated factor VIII concentrate prepared by the 'Centre National de Transfusion Sanguine', Paris, France. Group 2 included 11 patients treated with Hemofil T from Hyland Lab. (Glendale, Calif., USA). Factorate HT from Armour Lab (Kankakee, Ill., USA) was given to the 12 patients of group 3 and Haemate P from Behringwerke (Marburg, FRG) to the 10 patients of group 4. The last 3 products had been submitted to a procedure of viral inactivation at one stage of their production. The design of this prospective study consisted of regular work-ups at approximately 4-month intervals for a total duration of 12.3 ± 1.8 months.

Methods

Lymphadenopathy is defined as 2 extra-inguinal areas with at least one lymph node more than 1 cm in diameter. Complete blood cell counts were performed with the model S Coulter counter. T lymphocyte membrane-associated phenotypes were determined by indirect immunofluorescence using monoclonal antibodies (Ortho, Raritan, N.J., USA) to helper/inducer (OKT4) and supressor/cytotoxic (OKT8) T cell subsets. Absolute numbers of positive cells were calculated. Normal values for adult blood donors were 845 ± 110 and $533 \pm 129/\mu l$ for T4+ and T8+ cells, respectively. Delayed cutaneous hypersensitivity (DH) to 7 antigens were tested with Merieux-Multitest (Lyon, France) [9]. For each antigen, DH was considered positive when the diameter of the induration was at least 2 mm at 48 h. Since nearly all patients had been immunized with BCG, diphtheria and tetanus anatoxins, DH was considered decreased when positive results were recorded for two or less recall antigens.

Replacement Therapy

The therapeutic materials used in this study were selected according to two criteria: specific activity and viral inactivation. Two products (factor VIII concentrate CNTS and Hemofil T) had a relatively low specific activity (0.6 and 0.9 IU/mg of factor VIII protein, respectively) and contained similar contaminating proteins in various proportions. Methods to study proteins in factor VIII concentrates were previously described [10]. Fibrinogen represented 50 and 36% of total proteins; fibronectin 15 and 40%; IgG 10 and 6%; IgM 2 and 1.3%, and albumin 15 and 1%, respectively.

The other 2 products (Factorate HT and Haemate P) had a high specific activity (2 and 3.6 IU/mg protein, respectively) and a radically different protein composition. Factorate HT contained approximately 75% of total proteins as fibrinogen, 2% fibronectin, 4% IgG, 2.4% IgM and 4% albumin. In contrast, Haemate P contained 80% albumin, 17% fibronectin, 3% IgM and virtually undectable fibrinogen and IgG. Given values are the average of 3 consecutive lots of each manufacturer analysed in one laboratory (J.P.A.). Hemofil T and Factorate HT were submitted to heat viral inactivation in dry state at 60 °C for 72 and 90 h, respectively. Haemate P was heat-treated in solution at 60 °C for 10 h during the manufacturing process.

Scoring System

In order to evaluate the evolution of immune deficiency in patients from one work-up to another, we devised a scoring system taking into account 8 parameters which were recognized in our previous studies as being indicative of LAV-related immune deficiency [8, 11]. The values obtained at the initial work-up (before the onset of randomized treatment) were taken as baseline (score 0), and a score from 0 to 4 was established arbitrarily for each parameter according to table II. Positive scores were attributed to improvements and negative scores to worsenings. A cumulative score was calculated for each work-up by adding up scores obtained for individual parameters.

Statistical Analysis

Means were compared by analysis of variance including Levene's test [12]. When appropriate, particularly for scores, analysis was performed using two-sided rank sum tests [12, 13].

Table II. Scoring system for the evaluation of immunology parameters[a]

	Score				
	0	1	2	3	4
Lymphadenopathy	–				+
Platelet count/μl[b]	±50,000	51,000–100,000	101,000–150,000	151,000–200,000	>200,000
Lymphocyte count/μl[b]	±500	501–1,000	1,001–1,500	1,501–2,000	>2,000
T4+ lymphocytes/μl[b]	±200	201–400	401–600	601–800	>800
T8+ lymphocytes/μl	±200	200–400	401–600	601–800	>800
T4+/T8+ ratio[b]	±0.2	0.21–0.4	0.41–0.6	0.61–0.8	>0.8
IgG, g/l[c]	±4	4.1–8.0	8.1–12	12.1–16	>16
Loss or appearance of positive antigen[d]	4	2	3	>3	anergy

[a] Numbers given below correspond to differences from baseline values. Baseline values consist of results of work-up at entry.
[b] Parameters for which a decreased value is scored negatively and an increased value is scored positively.
[c] Parameters for which an increased value is scored negatively and a decreased value is scored positively.
[d] Positive = >2 mm in diameter. Loss of positive antigen(s) is scored negatively; appearance of positive antigen(s) is scored positively.

Table III. Composition of randomized groups

Group	Patients	Age, years		Interval for work-ups, months mean ± SD	Treatment, U/kg/month	
		mean	range		mean	range
1	13	24.6	5–47	4.3 ± 1.5	701	69–2339
2	11	16.6	6–30	4.4 ± 1.6	689	64–3243
3	12	23.9	13–47	4.4 ± 1.4	639	89–1896
4	10	23.1	11–36	4.8 ± 1.9	548	79–1560

Results

Forty-six patients were selected for this prospective study on the basis of a T4+/T8+ ratio below 1.0 at the time of the previously reported initial cross-section [8]. At the time of entry, which took place between April and October 1984 (2–6 months after the cross-section study), most patients had both clinical and biological abnormalities (table II). Twenty-six percent of them had lymphadenopathy in at least two extra-inguinal areas, 46% had a T4+ cell count below 1 SD of a reference group of 145 anti-HIV Ab-negative patients, and 22% had a T8+ cell count above 2 SD of the same reference group [8], which corresponded to a T4+/T8+ ratio below 1.0 in 85% of the total cohort. This discrepancy from the initial entry criteria was due to a normalization of a few borderline ratios between the cross-section check-up and the entry examination. In addition, 72% of the patients had an elevated level of serum IgG and 4 of the 29 subjects tested (14%) were anergic. Eight patients had only an isolated low T4+/T8+ ratio. Two patients were seronegative for HIV antibodies; one had 1,536 T8+ cells/μl, a T4+/T8+ ratio of 0.52 and was positive to only 1 of the 7 skin test antigens; the other had a lymphopenia (800/μl), 294 T4+ cells/μl, a T4+/T8+ ratio of 0.72 and was reactive to 2 skin test antigens.

The population of haemophiliacs was randomly distributed into 4 groups. These 4 groups were quite homogeneous in terms of number of subjects, age distribution, amount of treatment received during the study and interval between examinations (table III). Results of clinical and biological work-ups performed at the time of entry

Table IV. Characterization of 4 randomized groups of patients at entry

	Group 1	Group 2	Group 3	Group 4
Patients, n	13	11	12	10
Patients with lymphadenopathy, n	2	5	3	2
Platelets, $\times 10^3/\mu l$	270 ± 79	264 ± 69	251 ± 79	230 ± 69
Lymphocytes/μl	2,442 ± 1,007	2,719 ± 933	2,241 ± 1,017	2,047 ± 1,061
T4+ lymphocytes/μl	641 ± 236	787 ± 239	681 ± 418	661 ± 415
T8+ lymphocytes/μl	1,079 ± 729	1,101 ± 397	891 ± 370	760 ± 397
T4+/T8+ ratio	0.67 ± 0.31	0.74 ± 0.16	0.75 ± 0.27	0.88 ± 0.32
IgG, g/l	18.2 ± 4.8	18.5 ± 4.3	18.4 ± 5.0	18.9 ± 5.4

Values presented as means ± SD.

Table V. Sum of cumulative scores after 12 ± 1.8 months of follow-up

Patient Nr.	Group 1	Group 2	Group 3	Group 4
1	0	-5	-6	-6
2	3	3	-1	1
3	3	6	-13	-8
4	2	2	-4	-5
5	4	-2	-3	-5
6	-3	0	-3	-1
7	-3	-3	-4	1
8	1	0	1	0
9	-10	1	2	3
10	-3	-1	7	-1
11	1	0	-4	
12	4		-6	
13	3			

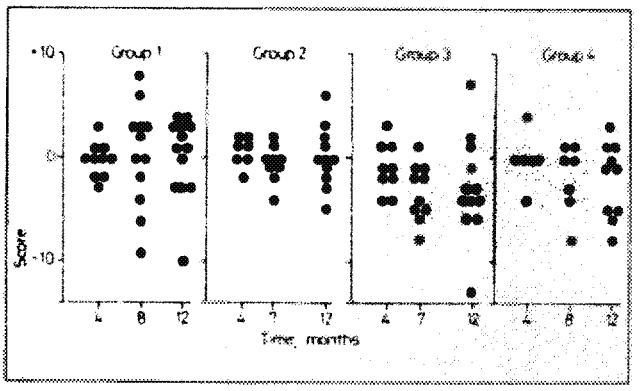

Fig. 1. Sequential scores of patients in groups 1–4 infused with non-heat-treated (group 1) or heat-treated (group 2) intermediate-purity factor VIII concentrate and with heat-treated high-purity concentrates (groups 3 and 4). Thirteen patients distributed in all groups had only 2 check-ups after entry.

are indicated in table IV. None of the clinical, haematologic or immunologic parameters studied differed significantly among the groups.

After 1 year of follow-up, no patient had developed a manifestation indicative of AIDS. The prevalence of abnormalities, as defined in table I, was nearly identical; only lymphadenopathy had increased significantly (<0.05) from 12 to 21 subjects affected. When analysis was performed according to groups, neither means, variances nor ranking sums of each index parameter obtained at the end, during or at entry in the study differed at the 5% probability level.

The scoring system was used in order to assess the follow-up of each individual patient by comparison to the entry work-up. For each prospective work-up, a cumulative score was calculated as the arithmetic sum of positive and negative scores obtained for each parameters. Scores were recorded for individual patients at 3 (and in 13 cases at 2) consecutive examinations performed at 4- or 6-month intervals. Arithmetic sums of the 2–3 cumulative scores obtained for each patient during the study are shown in table V. In the whole population, on the basis of 125 check-ups, changes from baseline affected all parameters, the respective weight in the score evaluation varying from 8 to 18.5%. Lymphadenopathy accounted for 9%, platelet count for 8%, lymphocyte count for 15%, T helper cell count for 12%, T suppressor cell count for 18.5%, T4+/T8+ ratio for 16.5%, IgG level for 9% and DH skin test for 12% of the overall sum of scores. Considering that to a large extent lymphocyte and T cell subset counts

and ratios balanced each other for pluses and minuses, we considered this scoring system gives equal weighing to each of the test parameters. The total number of score changes (corresponding to a score of 1–4) was 8.4, 6.7, 7.3 and 6.6 per patient in groups 1–4, respectively. They were similarly distributed among index parameters. Individual data are given in figure 1.

Ranking tests were applied to results of scores obtained at each work-up (4, 8 and 12 months for 33 patients; 6 and 12 months for 13 patients) of patients distributed according to the purity of products used for treatment or to individual products. Upon analysis of the scores of patients treated with a concentrate of lower (groups 1 and 2) or higher purity (groups 3 and 4), a progressively increasing difference was observed with time; it became significant (<0.05) at the end of the study. Non-parametric analysis of scores obtained at successive check-ups in the 4 treatment groups did not reveal significant intergroup differences. However, at the end of the study, the total cohort was no longer homogeneous (<0.05).

Discussion

Over the past 2 years, some evidence has been provided that factor VIII-containing blood products prepared from small or large pools of plasma drawn from HIV antibody-free donor populations might cause some immunologic disturbances [14–18]. They are essentially decreased T4+ cell counts, increased T8+ cells, low T4+/T8+ ratios and elevated IgG levels. These findings, however, have not been confirmed in a group of Finnish haemophiliacs treated with local cryoprecipitate who did not present with any significant immunologic abnormalities [19] and by our own study of haemophilia patients treated with products of French origin [8]. In these patients, the above mentioned disturbances were indeed found, but they correlated significantly with the presence of HIV antibody.

In the 1-year prospective study presented here, 24 patients (groups 1 and 2) were treated with factor VIII concentrates of a relatively low purity, while 22 patients (groups 3 and 4) received products of a higher purity. On average, the first cohort was given 160 mg of proteins per kilogram of body weight per month, including approximately 15 mg of IgG. The second cohort was treated with a similar amount of factor VIII corresponding to an average of 53 mg/kg/month of proteins and only traces of IgG. If the protein load and/or the protein composition of products was a significant cause of immunological abnormality in this population of HIV-contaminated patients, an improvement of the index immunological parameters was to be expected in the low-protein cohort. No significant difference was found between the 2 cohorts for any of the parameters measured at entry and at the end of the study or for results obtained at two consecutive check-ups. In contrast, when a scoring system was applied to check-ups at 4, 8 and 12 months of follow-up, the differences in rank tests between the 2 cohorts increased progressively, with p values of 0.30, 0.12 and <0.05, respectively. The results suggest that in HIV antibody-positive multitransfused haemophiliacs, neither a 3- to 5-time decrease in protein load nor a 3- to 20-time decrease in IgG load positively affected the course of immunologic abnormalities. If anything, those haemophiliacs who received the less pure concentrate progressively improved as compared to those treated with a purer material. This unexpected result might be interpreted as a consequence of an immunoregulatory role of circulating immune complexes [20]. Several authors have demonstrated the high frequency of circulating immune complexes following replacement therapy with factor VIII concentrates [21–23]. At least part of these complexes are IgG/anti-IgG [24].

The design of our study was addressing a second important question with regard to the treatment of HIV antibody-positive haemophiliacs. Is the use of non-heat-treated factor VIII concentrate harmful to the HIV antibody-positive patient's immunological and clinical condition? Concern about a potential superinfection has been raised [5, 25]. One of the 4 products randomly assigned to 13 patients in this study was not heat-treated and used before October 1985, when both HIV antibody screening of donors and viral inactivation of coagulation factor concentrates became mandatory in France. From a previous study, the presence of infective HIV particles in the French factor VIII concentrate used here was demonstrated, since a yearly prevalence of HIV seroconversion was calculated at 37% in a similarly treated population [10]. Index parameters in this group remained similar to the other 3 groups at each of the 2 or 3 check-ups, and no significant difference was observed between the initial and the final examinations. When results were analysed with the score system, the outcome of patients in the group infused with the non-heat-treated product appeared rather stable (fig. 1). In contrast, patients in the other 3 groups (particularly groups 3 and 4) who received high-purity concentrates tended to slightly worsen over time. However, analysis with ranking tests fell short of

statistical significance ($p<0.1$). Since it was considered unethical to prolong the use of non-heat-treated factor VIII, the comparative study was interrupted, and it is likely that a 1-year follow-up was too short to reach any firm conclusion. From the data collected, no evidence of negative effect of the non-heat-treated concentrate in HIV antibody-positive haemophiliacs was demonstrated. However, it has been shown that non-infective HIV components can induce immunoperturbations in vitro, and such mechanism might play a role in the observed abnormalities [26].

In HIV antibody-positive subjects and haemophilacs in particular, it is of paramount importance to assess potential ways to predict the development of clinical complications (AIDS-related complex or AIDS). Specific markers such as in vitro production of gamma interferon [27] or the urinary concentration of neopterin [28] have been correlated with such occurrence. We attempted to devise a score system including 8 simple, routinely performed, immunologic tests which might be predictive of clinical outcome. In the course of this study, this system was able to discriminate between small groups of patients in the evaluation of the role of the protein load, while actual values of any of the index parameters in each group was not. Considering the lag phase of several years between seroconversion and the occurrence of clinical complications, the 1-year prospective study presented here was insufficient and should be considerably extended in order to achieve full assessment of this approach. However, the fact that the significance of the differences observed between groups tends to increase with time suggests that the score may reflect progressive changes in the immune system at a small group level. Such indicative value at the individual level remains to be demonstrated in long-term studies.

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