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Relationship of Partially Purified Factor Concentrates to Immune Tests and AIDS

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We evaluated those members of a cohort of 203 hemophilic men providing all necessary information at their 1984 and 1985 evaluations, to determine whether non-heated or <80°C dry heat-treated partially purified factor products were associated with 1) the development of AIDS in human immunodeficiency virus (HIV)-infected persons or 2) abnormal immune test results in participants seroconverting or remaining HIV seronegative. We found no relationship between type of factor (VIII vs. IX) (n = 162), frequency of usage (≥once a week vs. <once a week) (n = 141), or yearly doses of non-heated or dry heat-treated partially purified factor (n = 118) and the development of AIDS. Seroconverting participants using partially purified factor products ≥once a week in 1984 had lower T-helper cell numbers in 1985 than those receiving factor less frequently (median 515 vs. 772/mm^3, n = 9), as did those using factor ≥once a week in 1985 (median 515 vs. 726/mm^3, n = 10). A similar relation was seen between 1984 frequency and 1985 T-helper cell numbers of seronegative participants (median 741 vs. 1037/mm^3, n = 31). The yearly dose of heat-treated products was not associated with any immune test result or changes in results between years. These findings suggested that frequency of administration of partially purified factor materials may have had an effect upon the immune system, but that total yearly dose did not. Previously used partly purified, dry heat-treated factor concentrates were not associated with the development of AIDS.

Key words: HIV, hemophilia, factor, cofactors

INTRODUCTION

Factor VIII and IX products are critical for prevention or termination of bleeding episodes in persons with clotting factor deficiencies. The preparations most widely used throughout the world in the 1980s were derived from U.S. plasma donations that were processed through a sequence of crude purification steps (referred to herein as "partially purified"). These products were thereby enriched in factor VIII or IX, but also contained a wide variety of other plasma proteins. In addition, they may be contaminated with a number of viruses with which some donor(s) had been infected, including hepatitis B virus (HBV), non-A,non-B hepatitis virus(es), and human immunodeficiency virus (HIV). In fall of 1984, in vitro studies showed that HIV inoculated into these preparations was readily inactivated by heating them in either a wet or a dry (lyophilized) state [1]. On the basis of these findings, partially purified, <80°C dry-heated preparations became the preferred therapy for hemophilia, since these were both much safer from HIV (although not safe from non-A, non-B hepatitis) transmission and also were more readily available and less expensive than wet-heated partially purified products [2,3].

Between 1987 and 1988, all but 1 manufacturer discontinued using partial purification/<80°C dry heat-treatment processes and all manufacturers preferentially marketed any of a variety of other both new and previ-

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ously available virally inactivated preparations, including those 1) partially purified and inactivated by heating in a wet state, pasteurizing, or detergent-treating and/or 2) purified by anti-factor VIII monoclonal antibody techniques combined with dry-heating or detergent-treating [3]. Manufacturers claim that factor VIII yields from plasma processed for some of these preparations are up to 3-fold lower than for partially purified, <80°C dry heat-treated preparations. In addition, only one or two companies are currently marketing each type of preparation, all of which currently cost from 2- to 7-fold more than partially purified, <80°C dry-heated preparations. This has caused a crisis in both supply of and cost coverage for factor products [3–8]. As of June 1988, there has been a severe and clinically significant shortage of factor concentrate products in countries supplied by U.S. plasma product manufacturers or dependent upon plasma from U.S. donors [3–8].

This shift from partially purified, dry-heated products has been arguably justified on three fronts. First, although there has been worldwide total of only eight confirmed instances of HIV seroconversion on donor-screened, partially purified, <80°C dry heat-treated preparations, these eight instances have led some patients to distrust the materials despite overwhelming evidence of their large margin of safety vis-a-vis HIV transmission [1,2]. Second, data strongly support that other products are safer from hepatitis transmission, compared to those made by the <80°C dry heat-treatment techniques [2]. (Products dry-heated at >80°C may also be safe and less expensive, but these are unavailable in the United States [2,4,9].) Third, it has been theorized that products which are purer in their factor composition (i.e., have less "contamination" with antigens other than factor VIII or IX) are less immunosuppressive than partially purified preparations. It has also been suggested that heat-treatment might render these non-factor antigens even more immunosuppressive. Thus, purer preparations might prevent or impede advancement of HIV-related immunodeficiency implicitly related to partially purified and/or heat-treated preparations [4,10,11].

Possible immune effects of factor products have been discussed in the literature, specifically in regard to 1) the periodic "antigenic boluses" received intravenously when any factor products are administered, 2) non-factor VIII or IX alloantigens present in partially purified concentrated materials [11], and 3) possible presence of these alloantigens in greater amounts or more immunosuppressive forms in heat-treated materials [4,10,12–19]. Data to support these hypotheses, however, are sparse and contradictory [10,17,18,20]. Furthermore, the influence of these hypothesized immune effects on the progression of HIV infection is equally theoretical [10,12,17].

Between approximately 1980 and 1984, the majority of hemophilic patients worldwide used U.S.-produced, non-virally treated, partially purified factor products and are now infected with HIV. These patients are now confronted with the possibility of developing AIDS and are concerned about whether the use of these products might influence or have influenced their rate of HIV disease progression. We therefore examined a cohort of hemophilic persons from throughout the United States, to assess the effects of frequency of partially purified factor therapy, partially purified factor dose, factor type (VIII or IX), and <80°C dry heat-treatment of partially purified factor on the following: development of AIDS and the immune test results of HIV-infected and non-infected persons. We did not compare non-heated to heated products, since the former are no longer available and thus this comparison would have no clinical relevance. Since length of infection is a critical factor in HIV-related immune deterioration [9,21,22], for immune tests, we included only those cohort members for whom we could document either seronegativity or the year of HIV seroconversion.

PARTICIPANTS AND METHODS

Participants were members of a cohort study described in detail elsewhere [23]. All cohort members were enrolled in 1984 and were asked to return for evaluation in 1985. Each evaluation included the participant's and his health care provider's completion of questionnaires concerning the patient's yearly blood product usage and clinical status. Laboratory immune testing of blood samples taken at each evaluation was done at the Centers for Disease Control (CDC). This study has been approved by human subjects review committees, and informed consent was obtained from all participants. The analyses are based on 150 participants who were HIV seropositive at enrollment, 11 seroconverting between 1984 and 1985, and 42 who remained seronegative throughout evaluation. These 42 also were negative for HIV-1 DNA in their peripheral blood mononuclear cells (PBMCs), by the polymerase chain reaction (PCR) amplification technique [24]. Only participants providing accurate information on factor dose or usage (in the form of complete and dated medical, pharmacy, or log book recording) were included in the pertinent analyses. Partially purified factor dose information is referred to as follows: usage in the 3 years before enrollment, "1981–1983 usage"; usage in the year before enrollment, "1983 usage"; usage in the year prior to the second evaluation, "1984 usage." Material used before 1984 was not virally inactivated by any technique, including heat. Material referred to herein as "heat-treated" was partially purified and prepared using <80°C dry heat-treatment methods.

Individuals who were seropositive at enrollment were included only in AIDS vs. non-AIDS comparisons, since we did not know year of infection for these participants and length of HIV infection has been shown to be associated with both amount of factor used and with the degree of immune deterioration of infected hemophilic men [12,21,22]. Information concerning development of AIDS was complete as of April 1989 reporting to CDC.

Serum specimens were tested for antibody to HIV by Western blot analysis as previously described [23]. Serologic reactions with the 41 kd protein, or with the 25 kd protein in association with a reaction to any other HIV protein (18 kd, 53 kd, 55 kd, 65 kd, or 110 kd) were scored as positive. Lymphocyte subpopulations were quantitated by indirect immunofluorescence on a fluorescence-activated cell sorter with commercial monoclonal antibodies: OKT4A for T-helper/inducer cells and OKT8 for T-suppressor/cytotoxic cells (23). Laboratory normal ranges are T-helper cells, 408–1,583/mm^3; T-suppressor cells, 190–820/mm^3; T-helper/T-suppressor ratios, 1.0–3.9. Lymphocyte transformation responses were quantitated by a micromethod, with the mitogens phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM), as previously described [23]. Results were "normalized" by comparison with a single normal control donor whose mononuclear cells had been collected, frozen down in bulk, and used as a control each time patients cells were studied [23]. The formula used was

$$\frac{\text{Stimulated} - \text{unstimulated (patient)}}{\text{Stimulated} - \text{unstimulated (control)}} \times 100 \quad (1)$$

Laboratory normal ranges results are phytohemagglutinin, >86%; Con A, >61%; pokeweed mitogen, >41%. Immunoglobulins G, A, and M were quantitated by nephelometry in 1984 and using a DuPont[1] discrete clinical analyzer in 1985. Laboratory normal ranges are IgG, 786–1,647 mm/dL; IgA, 94–420 mg/dL; and IgM, 54–290 mg/dL.

These analyses include all persons who provided information on partially purified factor usage in either 1984 or 1985, had definitive HIV antibody results, and had the described immune tests performed. Partially purified factor information included the following: type of factor used (factor VIII vs. IX) (n = 203); frequency of usage in 1984 (n = 174); frequency of usage in 1985 (n = 152); amount used in 1983 (n = 151); amount used in 1981 through 1983 (n = 116); amount used in 1984 (n = 147); proportion of factor used in 1984 that was heat-treated (n = 147). Numbers for subgroup evaluations are given in text or tables, where appropriate.

Group comparisons were made using either the Fisher's exact test (2-tailed) or the Wilcoxon rank-sum test, as appropriate [25]. A Spearman's rank correlation coefficient (r_s) was used to measure the strength of association of 1984 and 1985 immune test results with factor dose in various years or proportion of factor used in 1984 that was heat-treated [25]. Significance levels were set at .05.

TABLE I. Partially Purified Factor Usage, by Development of AIDS in HIV-Seropositive Hemophilic Men: Longitudinal Hemophilia Cohort Study, 1988*

	AIDS	Non-AIDS
Proportion using factor at least once a week (%)		
In 1984 (n = 141)	17/22 (77)	76/119 (64)
In 1985 (n = 119)	10/19 (53)	51/100 (51)
At the most recent evaluation before AIDS diagnosis (n = 24)	12/24 (50)	—
Median No. of units of factor used in		
1981–1983 (n = 97)[a]	218,740	221,714
1983 (n = 120)	65,935	77,550
In 1984, median No. of units used of (n = 118):		
Heat-treated factor	11,766	24,120
Non heat-treated factor	53,787	41,320
No. using factor VIII/No. using factor IX (% using factor VIII)	20/6 (77)	104/32 (76)

*Individual who seroconverted and developed AIDS following his last examination is included in this group (see text for details); therefore, total here is 162, not 161.
[a]Nos. providing accurate information.

RESULTS

Effects of Frequency of Administration and Quantity of Concentrate Used

a. On development of AIDS. Twenty-six participants (16% of seropositive participants) had developed AIDS as of April 1989. Twenty-one of these were HIV seropositive at enrollment, four seroconverted between 1984 and 1985, and one seroconverted after his 1985 evaluation, following use of partially purified, non-heat-treated factor IX concentrate for elective surgery. The proportion of those developing AIDS who used partially purified factor at least once a week in the latest evaluation before the date of their AIDS diagnosis did not differ from the proportion without AIDS who used factor at least once a week in 1984 (P = .22) or 1985 (P = .90) (Table I). Those developing AIDS did not use more par

[1]Use of trade names and commercial sources is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

TABLE II. Median 1984 and 1985 T Cell Subset Results for HIV-Seroconverting and HIV-Seronegative Hemophilic Men: Longitudinal Hemophilia Cohort Study, 1988*

	Seroconverting		Seronegative	
	1984	1985	1984	1985
T-helper cells/mm^3	706	661	1068	899
Range	376–1,181	100–990	288–2,277	252–1,523
No. tested	11	10	41	39
T-suppressor cells/mm^3	446	654	608	614
Range	163–1,407	346–1,610	196–1,298	156–1,848
No. tested	11	10	41	39
T-helper/T-suppressor	1.5	1.2	1.6	1.4
Range	0.4–2.3	0.3–1.5	0.8–3.1	0.5–2.5
No. tested	11	11	42	42

*Laboratory normal ranges are as follows: T helper cells, 408–1,583/mm^3; T-suppressor cells, 190–820/mm^3; T-helper/T-suppressor ratio, 1.0–3.9.

tially purified factor in 1981–1983, (P = .84); 1983 (P = .75); or 1984 (P = .68).

b. On immune test results. *Participants seroconverting between 1984 and 1985.* T cell subset results for these participants are shown on Table II. Persons using partially purified factor at least once a week in 1984 had significantly lower T-helper cell numbers in 1985 (median 515/mm^3, range 100–649, n = 4) than did those using factor less than once a week (median 772/mm^3, range 615–990, n = 5) (P = .037); this was also the case for those using factor at least once a week in 1985 compared to those using factor less than once a week in that year (median 515/mm^3, n = 4 vs. median 726/mm^3, n = 6) (P = .025). The amount of partially purified factor used in 1981–1983 was negatively associated with 1985 T-suppressor cell numbers (median 654/mm^3) (r_s = −.94, P = .005, n = 6) and with responses to PHA (median 217%) (r_s = −.83, P = .042, n = 6).

Seronegative participants. T cell subset results for these participants are shown in Table II. Table III shows that persons using partially purified factor at least once a week in 1984 had significantly lower T-helper and T-suppressor cell numbers in 1985. This relation is not seen between 1984 usage and 1984 testing (data not shown, P = .329 and P = .598, respectively). Results differed for 1985 usage and 1985 testing. T-suppressor cell numbers in 1985 were actually higher for those using partially purified factor at least once a week in that year, compared to those using factor less frequently in that year (median 806/mm^3, n = 6 vs. median 603/mm^3, n = 25) (P = .026). The 1983 dose of partially purified factor was positively associated with 1984 T-helper cell numbers (median 1,068/mm^3) (r_s = .36, P = .047, n = 31). The amount of partially purified factor used in 1981 through 1983 was negatively associated with IgG levels in 1984 (median 1,205 mg/dL) (r_s = −.73, P = .0009, n = 17) and 1985 (median 1,150 mg/dL) (r_s = −.71, P = .003, n = 15) and with 1984 responses to PWM (median 97%) (r_s = −.86, P = .014, n = 7).

TABLE III. Relationship Between Frequency of Partially Purified Factor Usage in 1984 and Median 1985 T Cell Subset Results in HIV-Seronegative Hemophilic Men: Longitudinal Hemophilia Cohort Study, 1988*

	At least once a week		Less than once a week
T-helper cells/mm^3	741		1037
Range	334–1,159		252–1,468
No. tested	8		23
		P = .036	
T-suppressor cells/mm^3	486		650
Range	337–1,136		156–1,848
No. tested	8		23
		P = .036	
T-helper/T-suppressor cell ratio	1.7		1.4
Range	0.5–2.3		0.7–2.5
No. tested	9		25
		P = .532	

*Laboratory normal ranges are as follows: T-helper cells, 408–1,583/mm^3; T-suppressor cells, 190–820/mm^3; T-helper/T-suppressor ratio, 1.0–3.9.

Effects of Type of Factor Used and Heat Treatment/Non-Heat Treatment of Factor

a. On development of AIDS. The proportion of those with AIDS using factor VIII, as opposed to using factor IX, did not suffer from the proportion without AIDS using factor VIII (vs. IX) (Table I). A higher proportion of the partially purified factor used in 1984 by patients developing AIDS was not heat treated (medians 76% vs. 58%), but this difference was not significant (P = .18).

b. On immune test results. *Participants seroconverting between 1984 and 1985.* In 1985, participants using factor VIII had higher responses to PHA, ConA, and PWM than did those using factor IX, but the numbers tested were small and the difference between factor VIII and IX users' responses to PWM was not significant (Table IV). The amounts of partially purified, non-heat-treated factor used in 1983 and 1984 were positively associated with IgG and IgA levels in 1984 and/or 1985,

TABLE IV. Relationship Between Type of Factor Used and 1985 Mitogen Responses in Hemophilic Men Seroconverting to HIV Between 1984 and 1985: Longitudinal Hemophilia Cohort Study, 1988*

Mitogen	Type of factor used		Factor IX
	Factor VIII		
Phytohemagglutinin			
Median	273%		93%
Range	209–547%		8–217%
No. tested	5		4
		$P = .037$	
Concanavalin A			
Median	612%		129%
Range	271–783%		22–296%
No. tested	5		4
		$P = .037$	
Pokeweed			
Median	345%		33%
Range	0–452%		30–37%
No. tested	5		2
		$P = .333$	

*"Normalized" by comparison with a single normal control donor whose mononuclear cells had been collected, frozen down in bulk, and used as a control each time patients' cells were studied [23]. Formula used was

$$\frac{\text{Stimulated} - \text{unstimulated (patient)}}{\text{Stimulated} - \text{unstimulated (control)}} \times 100$$

Laboratory normal ranges are as follows: phytohemagglutinin, >86%; concanavalin A, >61%; pokeweed mitogen, >41%.

TABLE V. Association of Immunoglobulins G and A Levels With Partially Purified Factor Usage, by Year, in Hemophilic Men Seroconverting to HIV Between 1984 and 1985: Longitudinal Hemophilia Cohort Study, 1988*

	1984		1985	
	IgG	IgA	IgG	IgA
Units used in 1983[a]				
r_s	.70	.80	.48	.88
p	.036	.010	NS[b]	.004
n	9	9	8	8
1984, non-heat treated				
r_s	.73	.81	.56	.88
p	.011	.003	NS	.0008
n	11	11	10	10
Proportion non-heat treated (1984)				
r_s	.74	.75	.64	.87
p	.008	.007	.048	.001
n	11	11	10	10

*Median values were as follows, in mg/dL: For 1984, IgG, 1,290 and IgA 184; for 1985, IgG 1,375 and IgA 126.
[a]All material used in 1983 was non-heat treated.
[b]NS, non significant.

as was the proportion of partially purified factor used in 1984 that was non-heat treated (Table V). The proportion of factor used in 1984 that was non-heat treated was also negatively associated with responses to PHA in 1984 (median 217%) ($r_s = -.79$, $P = .036$, n = 7). The amount of partially purified, heat-treated factor used in 1984 was not related to any immune test results, nor to changes in any immune test results between 1984 and 1985.

Seronegative participants. For participants remaining negative through 1985, the type of factor (factor VIII vs. IX) used was not significantly related to the results of any immune tests (data not shown). In 1985, responses to the T-cell mitogens PHA and Con A were positively associated with the proportion of partially purified factor used in 1984 that was non-heat treated (PHA median 152%, $r_s = .47$, $P = .027$, n = 22) (Con A median 221%, $r_s = .47$, $P = .026$, n = 22); responses to PWM were not. For seronegative participants, as for those seroconverting, the amount of partially purified, heat-treated factor used in 1984 was not related to any immune test results, or to changes in any immune test results between 1984 and 1985.

DISCUSSION

We evaluated members of a cohort of hemophilic persons to determine the effects of frequency of partially purified factor therapy, partially purified factor dose, factor type (VIII or IX), and <80°C dry heat-treatment of partially purified factor on development of AIDS and the immune test results of HIV-infected and non-infected persons. Assessment of available data to determine whether partially purified factor products might be cofactors in HIV disease outcome is complicated by two facts: 1) the amount of partially purified factor used is directly related to length of HIV infection on a population basis—i.e., those receiving more factor tended to have been infected earlier [12,21,22]—and 2) immune function tends to decline as the length of HIV infection increases [12]. Since the date of infection for any given individual is usually not known, it is difficult to differentiate the effects of length of HIV infection from the possible effects of partially purified factor concentrate dose. We have dealt with this difficulty in two ways. First, we compared all seropositive participants with and without AIDS, since the above confounding would produce a bias away from a null hypothesis of no effect and toward an alternative hypothesis that partially purified concentrates are cofactors for the development of AIDS. In fact, we found no association between the development of AIDS and any of the following: frequency of therapy, partially purified factor doses, type of factor used, or heat treatment. Second, we assessed the immune function of those few study participants for whom we knew the year of seroconversion. For those serocon-

verting, those using partially purified factor at least once a week in 1984 or 1985 had lower T-helper cell numbers than those receiving factor concentrates less frequently. From these data, we cannot assess whether this would have been the case if solely heat-treated materials were used. Relationships between partially purified factor dose and immunoglobulin G and A levels of those seroconverting were similar to the relationships previously found for red blood cell product recipients and are thus not surprising [26]. We cannot explain why these relationships were not found for seronegative participants. These immunoglobulin findings are very non-specific; their clinical relevance is unclear. Our data concerning participants seroconverting between 1984 and 1985 showed differences between factor VIII and factor IX users' responses to T cell mitogens. These findings cannot be explained by the relatively higher doses of factor usually taken by factor VIII recipients, since factor dose was negatively related to PHA responsiveness. They may indicate a series of chronic stimulation in those using factor VIII; certainly they are not indicative of "suppression." But as with immunoglobulin levels, mitogen responsiveness is extremely nonspecific, and non-antigen-specific T cell activation has been associated with increased HIV production [27]. Thus, although it is extremely difficult to determine the clinical relevance of mitogen responsiveness, these findings may have clinical importance in terms of HIV infection. Most important, for those seroconverting, the dose of partially purified, heat-treated factor had no relation to any immune test result or to changes in immune test results from one year to another.

The difficulty in assessing the effects of partially purified factor products on the immune system, independent of HIV infection, is that most persons in the United States who receive these products are infected with HIV. Within our study group, we had 42 persons who were HIV-seronegative at the initiation of the study and who remained seronegative over a 12- to ≥24-month period. All these individuals were also negative for HIV-1 proviral DNA in their PBMCs, by the PCR [24]. We thus could use the seronegative members of this cohort to assess the effects of partially purified factor products on the immune system, independent of HIV infection. As with those seroconverting, HIV-seronegative participants using partially purified factor at least once a week in 1984 tended to have lower T-helper cell numbers than those receiving factor concentrates less frequently. This trend was not seen for seronegative participants' 1985 usage. Rather, 1985 usage at least once a week was positively associated with 1985 T-suppressor cell numbers. This temporal variation in associations is consistent with the elegant "checks and balances" interaction of T cell subsets and may have short-term immune implications that cannot be addressed in this study. The only relation found between dose of partially purified factor and T cell subset numbers or T-helper/T-suppressor ratios was a positive one, between 1983 factor dosage and 1984 T-helper cell numbers. Unlike those who seroconverted, these non-infected participants showed a positive association between the proportion of partially purified factor used in 1984 that was non-heat-treated factor and T cell mitogen responses. As with participants seroconverting to HIV, the dose of heat-treated factor had no relation to any immune test result or to changes in immune test results from one year to another.

In summary, we find no evidence to date for an adverse effect of previously used partially purified, dry heat-treated products on either the development of AIDS or on immune test results. (Indeed, they almost certainly would have prevented AIDS in one of our participants who had received partially purified, non-heat-treated factor IX products in late 1985.) These findings do offer some support to the idea that frequent "boluses" of partially purified, non-heat-treated factor may have an effect upon the immune system; however, we cannot assess whether this will be the case when only virus-inactivated products are being given.

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