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Product-dependent anti-factor VIII antibodies

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Abstract

Introduction—The development of anti-factor (F)VIII antibodies in hemophilia A (HA) subjects undergoing replacement therapy has been well-documented. The correlation between antibody development and the FVIII product used for replacement therapy remains a subject of discussion.

Aim—To evaluate the presence of anti-FVIII antibodies toward three commercial rFVIII products in 34 HA subjects' plasmas.

Methods—Antibodies were quantitated by a Multiplex Fluorescence Immunoassay.

Results—All plasmas contained anti-FVIII antibodies at variable concentrations ranging from 50 nM to 570 μ M. Eleven of 20 HA subjects treated with one (r)FVIII product contained inhibitory anti-FVIII antibodies (0.8–3584 BU). The inhibitory antibody titer and the molar concentrations of total antibody were mildly correlated ($r^2=0.6$). Pronounced differences in antibody recognition with the three rFVIII products were observed. For the group treated with Product “A”, the titer toward this product was 2.4-fold higher than that observed with another full-length rFVIII-containing product (Product “B”) and almost 4-fold higher than that measured with a B domain-less rFVIII product (Product “C”). For the group of 14 HA subjects treated with FVIII other than Product “A”, only one showed higher antibody titer when measured with this product.

Conclusions—Our data suggest that the development of anti-FVIII antibodies is biased toward the product used for treatment and that a significant fraction of antibodies bind to the B domain of FVIII.

Keywords

Hemophilia A; Anti-factor VIII antibodies; Immunoassay; Factor VIII products; Bethesda assay; Factor VIII inhibitors

Introduction

Hemophilia A (deficiency in factor (F)VIII) is the most common bleeding disorder which affects one in 5,000 male newborns worldwide.[1] Treatment is based on replacement therapy, accomplished by injecting the missing protein derived from natural or recombinant

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Authorship

Contribution: S.B. participated in designing experiments, analyzed and interpreted the data and wrote the manuscript; J.K.-A. designed and performed MFI experiments; G.E.R. provided plasma samples and clinical data for the study subjects and helped with editing the manuscript; K.G.M. participated in experimental design, data interpretation and manuscript edition.

Conflict-of-interest disclosure

Saulius Butenas has research support from Baxter. Kenneth Mann has research support and is consultant for Baxter. He has received honoraria from Bayer and is Chairman of the Board of Haematologic Technologies.

sources. Plasma-derived, monoclonal antibody-purified virally inactivated FVIII [2, 3] and recombinant (r)FVIII produced by a variety of cell lines both full length and truncated are approved and available for clinical use.[4–7]

The most serious complication associated with replacement therapy is related to the immune response to the infused protein, i.e. the generation of inhibitory antibodies.[8–13] This response complicates therapy and substantially increases morbidity and mortality of hemophilia patients. In hemophilia A, the incidence of inhibitory antibodies has been reported to be as high as 30%.[14–16] To circumvent this inhibitory immune response, alternative products are used, such as rFVIIa or plasma-derived protein concentrates or a combination of both.[17–19]

Several factors influencing development of FVIII antibodies have been suggested, including genetics, treatment-related and environmental risk factors[10, 11][20]. The influence of the FVIII product used for the replacement therapy on antibody development has been discussed in numerous inconclusive studies which have compared plasma-derived FVIII with rFVIII proteins, both full-length and missing the B-domain.[7, 9, 14–16] However, no clear answers relating anti-FVIII antibody development with the source and structure of FVIII product used for hemophilia A treatment has been clearly identified.

Due to the complications caused by inhibitory anti-FVIII antibodies, primary attention has been directed towards their development and eradication. However, the sensitivity of inhibitory antibody quantitation by standard Bethesda and Nijmegen assays is poor with detectability limit not exceeding 30–50 nM.[21] There has been an increasing interest in total anti-FVIII antibodies, i.e. those undetectable by standard assays.[8, 22–24] The significance of non inhibitory antibodies in hemophilia A treatment and their effect on hemostasis is not understood, although up to 50% of adult hemophilia A subjects contain detectable levels of such antibodies.[8, 23] It has been observed, however, that non inhibitory anti-FVIII antibodies influence the half-life of transfused FVIII[25] or have an effect on FVIII activation/inactivation and *in vivo* clearance from circulation.[26]

In the present study, we quantitated total molar anti-FVIII antibody concentrations in hemophilia A subjects and compared it with the titer of inhibitory anti-FVIII antibodies. We also evaluated the recognition of anti-FVIII antibodies by three different rFVIII products and established a strong dependence between the antibody titer and rFVIII product used for antibody recognition.

Materials and Methods

Human subjects

Thirty four male individuals (1–55 years old; Table 1) with hemophilia A were recruited and advised according to a protocol approved by the Centre Hospitalier Universitaire Sainte-Justine (Montreal, Canada). Informed written consent was obtained from all hemophilia A subjects or their parents/guardians. Twenty hemophilia A individuals on prophylaxis used a pharmacologic product “A” containing full-length rFVIII, two used another pharmacologic product “B” containing full-length rFVIII, two used a product containing B domain-less rFVIII (product “C”), 8 used plasma-derived FVIII (pdFVIII; product “D”) and two used cryoprecipitate. Sixteen of 34 individuals had quantifiable inhibitors present (Table 1). At the blood draw, citrate plasma was collected, frozen and stored at –80°C until used to measure factor VIII:C by a one stage clotting assay and inhibitor titer by the Nijmegen assay. The residual plasma was frozen and stored at –80 °C until further use for subsequent anti-FVIII antibody assays.

Materials

Albumin-free rFVIII produced in Chinese Hamster Ovary (CHO) cells was a gift from Baxter Health Care Corp. (Duarte, CA). This rFVIII has been characterized extensively in our laboratory[27] and used as the calibrator (standard) in all assays. The concentration of this product (0.62 mg/ml) was established by the absorbance at 280 nm using an extinction coefficient $E^{0.1\%}$ value of 1.3. Product “A” containing full-length rFVIII produced in Baby Hamster Kidney (BHK) cells, product “B” containing full-length rFVIII and product “C” containing B domain-less FVIII (both produced in CHO cells) were provided by Dr. Rivard. The FVIII concentration in all products was determined in an in-house immunoassay.[27] Human anti-FVIII antibody standards were purified from plasmas of two hemophilia A subjects with 64 BU and 280 BU inhibitory anti-FVIII antibody and quantitated as described previously.[8] At equimolar concentrations, the anti-FVIII antibodies purified from both plasmas were recognized identically. Na_3PO_4 , ovalbumin, Tween 20, CaCl_2 and NaCl were purchased from Fisher (Pittsburgh, PA). Microsphere beads Luminex classification numbers 030, 035, 038 and 044 with predefined 658:712 nm emission ratios were purchased from Luminex Corp (Austin, TX). Multi-screen filter plates containing 96 wells covered with 1.2 μm PVDF membrane (part # MABV-N1250) were purchased from Millipore Corp (Billerica, MA). Mouse anti-human IgG-R-phycoerythrin was purchased from SouthernBiotech (Birmingham, AL). Different Ig isotypes were purchased from Sigma (Atlanta, GA).

Multiplex Fluorescence Immunoassay (MFI) of FVIII antibodies

The procedure for the anti-FVIII antibody MFI was adopted from a previously described and validated assay.[8] Microsphere beads with Luminex classification numbers 030, 035 and 038 were coupled to Product “A”, Product “C” and Product “B”, respectively. The coupling concentration of FVIII used for each product was 10 $\mu\text{g}/\text{mL}$. Non-specific binding control microsphere beads (# 044) were coupled to ovalbumin. For the calibration, 50 μL of human anti-FVIII antibody standard (0.02–8.0 nM) in PBST/ovalbumin buffer (Phosphate Buffered Saline containing 150 mM NaCl, 5 mM Na_3PO_4 pH 7.4/ 0.1% ovalbumin / 0.05% Tween 20) were added to wells containing 50 μL of FVIII- and ovalbumin-coupled microspheres (100 beads/ μL). Test plasma samples without detectable inhibitors were diluted 50-fold or according to their BU values (range from 100- to 200,000-fold) in PBST and also added to wells containing ovalbumin- and FVIII-coupled beads. The plate was incubated at room temperature (25°C) on a shaker (500 rpm) for 2 hours. Wells were washed three times with 125 μL of PBST and probed with mouse anti-human IgG-R-PE at 5 $\mu\text{g}/\text{mL}$ in PBST/ovalbumin (100 μL per well). Following one hour incubation, wells were washed three times with PBST, beads were resuspended in the same buffer and analyzed by Bio-Plex200 instrument (Bio-Rad Laboratories, Hercules, CA). The readings were recorded as mean fluorescence intensity units (MFIU). Data were analyzed by the subtraction of the MFIU of the non-specific control ovalbumin-coupled beads from the MFIU of the FVIII-coupled beads. Positive and negative controls were included in each assay plate analyzed. The negative control consisted of healthy 10 donor plasma pool in which the signal of binding to FVIII beads was significantly lower than the signal of binding to ovalbumin beads. The positive control consisted of commercial plasma samples (from George King) with a FVIII inhibitor values of 20 BU and 280 BU (both plasmas with less than <1% FVIII activity). Binding of 8 isotypes of PE-labeled anti-human Ig to rFVIII-coupled beads was measured by this procedure as well. Their binding to rFVIII was comparable with that observed for antibody purified from hemophilia A subject’s plasma.

Results

Hemophilia A subjects

Thirty four hemophilia A subjects were enrolled into this study. They were divided into 2 groups, i.e. those treated with product “A” (n=20) and those treated with other FVIII replacement products (n=14). Eleven of 20 treated with product “A” developed inhibitory antibodies against FVIII (Table 1). All 11 hemophilia A subjects were young children (ages from 1 to 6 years) at the time of the study. Their titer of the inhibitor varied in a quite wide range from 0.8 BU for the subject 2-18 to 3584 BU for the subject 2-49. The number of exposures to product “A” leading to the inhibitor development varied from 3 for subjects 2-1, 2-5 and 2-23 to 22 for the subject 2-49. No correlation between the number of exposures or the age of subjects and the inhibitor titer was observed. Five of 14 subjects treated with products other than product “A” developed inhibitors.

Recognition of anti-FVIII antibodies by three different rFVIII products

All 34 hemophilia A subjects contained quantifiable concentrations of anti-FVIII antibodies, although only 16 of them had detectable inhibitor (Table 1). For 30 subjects, antibody was recognized by all three rFVIII products tested. For subjects 2-51, 3-1 and 3-17 total antibody was recognized by products “B” and “C” and not recognized by the product “A”. For subject 3-7 total anti-FVIII antibody was recognized by this product only. The concentration of total anti-FVIII antibodies varied over an extremely wide range from 10 nM for several subjects to 570 μ M for subject 2-49 (when quantitated with product “A”).

There were pronounced differences in antibody recognition by three rFVIII products. Fifteen of 20 hemophilia A subjects treated with product “A” showed the highest antibody concentration when this product was used in the immunoassay to capture anti-FVIII antibodies. On average, total antibody concentration determined using product “A” was 2.45 ± 1.80 (S.D.)-fold higher than that determined using another product (“B”), also containing full-length rFVIII (Table 2). That ratio was independent of the presence or absence of inhibitory antibodies in plasma samples tested. An even more pronounced difference in antibody concentration (3.84 ± 2.37 -fold) was observed when the results obtained with product “A” were compared with those determined with the B domain-less rFVIII-containing product “C”. Sixteen of 20 subjects showed higher values for antibody concentration with product “A” than product “C”. A comparison of products “B” and “C” led to the results indicating that total anti-FVIII antibody was better recognized by the full-length rFVIII-containing product “B” than by the truncated rFVIII-containing product “C” in 13 hemophilia A subjects and the antibody concentration ratio (“B” vs. “C”) for the entire group of 20 subjects was 1.52 ± 0.57 . Overall, in the group of hemophilia A subjects treated with product “A”, the titer of total antibody measured was higher in 15 of 20 subjects when this full-length rFVIII-containing products was used for the antibody quantitation and 10 of 20 when another full-length rFVIII product “B” was used in comparison with values observed with the B domain-less rFVIII product “C”. These data suggest that some antibodies developed in subjects treated with product “A” are directed towards the B domain of FVIII.

For the group of 14 hemophilia A subjects treated with replacement products other than product “A”, there was no prevalence in antibody recognition by this product, i.e. only one subject (3-8; Table 1) showed higher total antibody titer with product “A” than with other two products. For 11 of 14 subjects, higher total anti-FVIII antibody titers were observed with either product “B” or product “C” or both than with product “A” (Tables 1 and 2). A comparison of antibody recognition by products “B” and “C” for this group of 14 subjects showed that on average the full-length rFVIII-containing product “B” led to a higher

antibody titer than B-domainless product “C”, although the difference was less pronounced than for the group of 20 subjects treated with product “A”. Six of 14 subjects had a similar total antibody concentration quantitated with both product “B” and product “C”, for 5 subjects antibody was better recognized by product “B” and for 2 subjects by product “C”.

Correlation between total anti-FVIII antibody and the inhibitor titer

A comparison of total anti-FVIII antibody concentration for hemophilia A subjects with inhibitors (16 of 34) with those without inhibitors (n=18) showed that almost all subjects in the former group had significantly higher antibody concentrations than the latter group, independent of rFVIII product used for the analyses. On average, total anti-FVIII antibody concentration in the inhibitor-containing subjects (subject 2-49 was excluded as an outlier due to an extremely high inhibitor titer and total antibody concentration) detected with all three products was 6.27 μM , whereas antibody concentration for subjects with no inhibitors was 0.50 μM (Table 1). However, there were several exceptions. For example, subject 3-12, with no quantifiable inhibitor, had total anti-FVIII antibody concentration ($6.28 \pm 0.55 \mu\text{M}$ for product “B”) comparable with that observed for two subjects with 72 BU ($6.8 \pm 0.27 \mu\text{M}$ with product “A” for subject 1-14) and 88 BU ($8.3 \pm 1.3 \mu\text{M}$ with product “A” for subject 2-10). Subjects 3-13 and 3-15 had their antibody concentrations in the μM range, i.e. comparable with those detected for the subjects with <10 BU inhibitor titers.

The correlation between molar concentration of total anti-FVIII antibody corresponding to one BU varied over a wide range for the plasma samples analyzed (from 24 nM for the subject 3-8 when analyzed with product “C” to 8375 nM for the subject 2-18 when analyzed with product “A”). High variability in antibody concentration per 1 BU was observed for all 34 subjects enrolled into the study, independent of the product used for their treatment. Despite the high variability in molar concentration of total antibody per 1 BU, there was certain correlation between these two parameters for all 3 FVIII products used in the antibody quantitation (Figure 1). The R^2 varied between (0.58) for the product “A” and (0.74) for the product “C”.

Discussion

The data of this study indicate that: 1) all 34 severe hemophilia A subjects enrolled and treated with various replacement products developed anti-FVIII antibodies recognized by at least one of three rFVIII proteins used for the analysis; 2) antibodies were more frequently recognized by the rFVIII protein used for treatment (or by structurally similar rFVIII proteins) than by other rFVIII proteins tested; 3) a significant fraction of anti-FVIII antibodies are presumed to be toward the B region; 4) while there is correlation between the inhibitory activity and molar concentration of anti-FVIII antibodies, their concentrations per 1 BU varies over a wide range.

The development of inhibitory antibodies upon replacement therapy in severe hemophilia A is a major problem for individuals with this disease, which impairs their quality of life and well being and significantly increases costs of treatment. It is usually assumed that most inhibitory anti-FVIII antibodies develop after relatively few FVIII-containing product exposures and that young children, especially those under 6 months of age, are the most susceptible to antibody development.[28, 29] While the development of inhibitory anti-FVIII antibodies is well documented, the role of various FVIII products in this process remains controversial. Several studies suggested that the rate of inhibitor development is higher in hemophilia A subjects treated with rFVIII vs. those treated with pd FVIII,[30–32] whereas data from other studies indicated that the nature, structure and formulation of FVIII product used for the replacement has no effect on the product’s immunogenicity.[33–36]

Inhibitory anti-FVIII antibodies have been at the center of attention of hemophilia community due to health problems caused by them, whereas the subject of “non-inhibitory” antibodies has been somewhat under-investigated. This can be explained by the lack of strong evidence suggesting their clinical relevance for hemophilia A subjects. However, some preliminary data indicate that non-inhibitory antibodies could influence the half-life of the transfused FVIII[25] through formation and clearance of immune complexes.[37] It is also possible that non-inhibitory antibodies could be predictors for the development of inhibitors.[38] Additional consideration for the quantitation of non-inhibitory antibodies is related to different mechanisms of FVIII activation in the Bethesda assay and in tissue factor-triggered processes. The Bethesda assay is virtually a modification of the physiologically-irrelevant APTT assay with the majority of FVIII being activated by FXIa. [39] In physiologically-relevant blood coagulation the initiator of the reaction is tissue factor with thrombin being the key activator of FVIII.[40] As a consequence, antibodies not detectable in the Bethesda assay could have an effect on tissue factor-triggered thrombin generation and clot formation *in vivo*. Another shortcoming of the Bethesda assay is relatively low sensitivity. Based on the data of Table 1 and on those published previously,[8] an (inhibitory) antibody will be recognized in the Bethesda assay if the concentration of antibody exceeds 30–100 nM, i.e. higher by almost two orders of magnitude than physiologic concentration of FVIII.

It has been suggested in several publications that the prevalence of B domain-binding antibodies do not exceed 20% of total antibodies detected in hemophilia A subjects with no inhibitors,[22, 41–43] and in those with inhibitors the prevalence increases to 54%.[44] Differences in antibody recognition by the full-length rFVIII product “B” and B-domain-less product “C” (both produced in CHO cells) suggest that in this study 50% (9 of 18) of hemophilia A subjects without detectable inhibitors and 81% (13 of 16) of those with inhibitors contain antibodies directed towards the B domain. The higher frequency of such antibodies observed in this group of severe hemophilia A subjects could be related either to the higher sensitivity of the immunoassay used for the quantitation of antibodies or to the study population or both. Similar discrepancies between published data and the results of this study were observed for the frequency of total anti-FVIII antibodies. It was predictable, based on a higher sensitivity ($>10^2$) of the immunoassay over the Bethesda assay, that all inhibitor-containing plasma samples will be positive for anti-FVIII antibodies in the immunoassay and all would be recognized by all three rFVIII products used for their capture. Somewhat unexpected were the results for inhibitor-negative subjects. All contained quantifiable concentrations of anti-FVIII antibody recognized by at least one of three rFVIII products used in the assay, with 78% (14 of 18) of subjects containing antibodies recognized by all three rFVIII products. Once again, this high prevalence of non-inhibitory anti-FVIII antibodies suggests either “uniqueness” of the study population or, most likely, the higher sensitivity of the immunoassay used in comparison with those used in other studies. It is hard to compare existing immunoassays with the assay used in this study, because the former are non-quantitative with respect to the molar concentration of anti-FVIII antibodies.[38, 45–47]

While the difference in anti-FVIII antibody recognition by the full-length rFVIII and by the B domain-less protein can be explained by the presence of antibodies directed towards the B domain, the difference in antibody recognition by two full-length rFVIII proteins must be driven by a distinct mechanism. Fifteen of 20 hemophilia A subjects who developed antibodies (both inhibitory and non-inhibitory) while treated with rFVIII product “A”, showed higher antibody concentration when product “A” was used for capture relative to the other full-length rFVIII product “B”. Although both rFVIII products are produced in mammalian cells, product “A” is expressed in BHK cells and product “B” in CHO cells. One difference in FVIII proteins expressed in those cells is related to distinct

posttranslational modifications, particularly to the structure of N-linked carbohydrates.[48, 49] Hironaka *et al.*[50] detected the presence of Gal(α 1–3)Gal carbohydrate structure on rFVIII derived from BHK cells, which was not observed on plasma-derived FVIII and rFVIII derived from CHO cells.[48] Overall, the carbohydrate structure of plasma-derived FVIII and that of the recombinant counterpart expressed by CHO cells is quite similar, whereas that of rFVIII produced in BHK cells is distinct from both.[48, 51] We hypothesize that these differences in glycosylation may be the cause of different antibody recognition by two full-length rFVIII products. This hypothesis is supported by the observation that in 12 of 14 hemophilia A subjects treated with plasma-derived FVIII (or cryoprecipitate containing plasma-derived FVIII) and rFVIII products produced in CHO cells (products “B” and “C”), the anti-FVIII antibodies developed were better recognized by products “B” and “C” than by rFVIII product “A”. The underlying mechanism regulating antibody development/recognition by carbohydrate structure of FVIII proteins is not clear, but one of the possible explanations is the recognition of carbohydrate chains of different structure or the restricted exposure of different peptide epitopes of FVIII.

This study has several shortcomings. First, to prove the concept that anti-FVIII antibodies have prevalence for that FVIII product, which was used for treatment, larger groups of subjects treated with different FVIII products (including products “A” and “B”) are needed. Second, it would be of interest to map binding epitopes on FVIII for non-inhibitory antibodies, primarily those recognized by the B domain-less rFVIII.

Conclusion

Our data suggest that anti-FVIII antibody recognition by FVIII could be regulated by posttranslational modifications of FVIII, particularly by glycosylation. This knowledge could lead to the development of more efficient replacement therapy for the treatment of hemophilia A subjects with antibodies.

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Figure 1. Correlations between the total (μM) and inhibitory (BU) anti-FVIII antibody
The molar concentration of antibodies was determined in the MFI assay based on their binding to FVIII products “A” (◆), “B” (■) and “C” (▲). Inhibitory antibodies were measured in a standard Nijmegen assay.

Table 1

Anti-FVIII antibody concentrations in severe hemophilia A patients

Subject ID	Age (years)	BU ^a	Treated with	Total antibody (μM) ^b			Antibody ratios ^c			nM/BU ^d		
				Product "A"	Product "B"	Product "C"	"A/B"	"A/C"	"B/C"	"A"	"B"	"C"
1-14	4	72	Product "A"	6.8±0.27	3.3±0.04	2.7±0.46	2.1	2.5	1.2	94	46	37
2-1	1	8.2	Product "A"	1.4±0.14	0.76±0.13	0.25±0.05	1.8	5.6	3.0	171	93	30
2-3	5	70	Product "A"	17±1.6	7.5±0.51	6.5±0.55	2.3	2.6	1.2	243	107	93
2-5	2	1.2	Product "A"	0.43±0.01	0.79±0.03	0.41±0.03	0.54	1.0	1.9	358	658	342
2-10	4	88	Product "A"	8.3±1.3	4.8±1.5	3.5±1.3	1.7	2.4	1.4	94	55	40
2-18	1	0.8	Product "A"	6.7±0.37	2.0±0.26	1.0±0.25	3.3	6.7	2.0	8375	2500	1250
2-23	5	30	Product "A"	8.7±0.29	2.7±0.21	1.4±0.09	3.2	6.2	1.9	290	90	47
2-33	3	50	Product "A"	2.3±0.36	4.6±0.55	2.4±0.15	0.50	0.96	1.9	46	92	48
2-39	3	40	Product "A"	68±1.4	18±1.8	9.6±0.83	3.8	7.1	1.9	1700	450	240
2-47	4	17	Product "A"	14±1.1	4.4±0.67	2.1±0.01	3.2	6.7	2.1	824	260	124
2-49	6	3584	Product "A"	570±35	120±13	120±15	4.7	4.7	1.0	159	33	33
1-1	4	NQ ^e	Product "A"	0.38±0.02	0.26±0.05	0.27±0.06	1.5	1.4	0.96	-	-	-
2-7	5	NQ	Product "A"	0.12±0.02	0.08±0.02	0.06±0.01	1.5	2.0	1.3	-	-	-
2-51	6	NQ	Product "A"	NQ	0.10±0.03	0.20±0.13	-	-	0.50	-	-	-
2-54	39	NQ	Product "A"	0.05±0.01	0.03±0	0.03±0.01	1.7	1.7	1.0	-	-	-
2-56	39	NQ	Product "A"	0.06±0.01	0.01±0	0.01±0	6.0	6.0	1.0	-	-	-
2-57	27	NQ	Product "A"	0.05±0	0.56±0.02	0.32±0.01	0.089	0.16	1.7	-	-	-
2-55	39	NQ	Product "A"	0.44±0	0.07±0.01	0.05±0	6.3	8.8	1.4	-	-	-
2-59	28	NQ	Product "A"	0.36±0.01	0.15±0	0.08±0	2.4	4.5	1.9	-	-	-
2-60	20	NQ	Product "A"	0.01±0.01	0.75±0.02	0.68±0.02	0.013	0.014	1.1	-	-	-
1-9	44	46	Cryoprec. ^f	2.0±0.92	3.7±0.70	3.9±0.55	0.54	0.51	0.95	43	80	85
2-30	55	36	Cryoprec.	6.3±1.0	9.1±0.10	8.8±0.50	0.69	0.72	1.0	175	253	244
2-32	19	6.0	Product "D"	0.85±0.09	1.2±0.04	0.70±0.11	0.71	1.2	1.7	142	200	117
3-1	6	NQ	Product "D"	NQ	0.53±0.19	0.08±0.02	-	-	6.6	-	-	-
3-2	4	NQ	Product "D"	0.02 ±0.00	0.18±0.01	0.15±0.01	0.11	0.13	1.2	-	-	-
3-6	17	36	Product "D"	1.33±0.18	1.41±0.12	1.05±0.13	0.94	1.3	1.3	37	39	29
3-11	1	NQ	Product "D"	0.60±0.12	0.66±0.08	1.00±0.07	0.91	0.6	0.7	-	-	-

Subject ID	Age (years)	BU ^a	Treated with	Total antibody (µM) ^b			Antibody ratios ^c			nM/BU ^d		
				Product "A"	Product "B"	Product "C"	"A/B"	"A/C"	"B/C"	"A"	"B"	"C"
3-12	4	NQ	Product "D"	1.69±0.33	6.28±0.55	1.81±0.26	0.27	0.93	3.5	-	-	-
3-13	2	NQ	Product "D"	0.82±0.12	1.86±0.16	1.67±0.30	0.44	0.49	1.1	-	-	-
3-17	1	NQ	Product "D"	NQ	0.40±0.04	0.33±0.02	-	-	1.2	-	-	-
3-8	2	160	Product "B"	19.17±0.23	6.53±0.49	3.78±0.32	2.94	5.1	1.7	120	41	24
3-19	36	NQ	Product "B"	0.05±0.00	0.06±0.00	0.02±0.00	0.83	2.5	3.0	-	-	-
3-7	16	NQ	Product "C"	NQ	NQ	0.84±0.19	-	-	-	-	-	-
3-15	4	NQ	Product "C"	0.40±0.05	0.99±0.19	1.20±0.13	0.40	0.33	0.82	-	-	-

^aBethesda units

^bQuantitated by capturing antibodies with indicated FVIII product

^cRatios of molar antibody concentrations quantitated with corresponding FVIII product

^dConcentration of total antibody per 1 BU for each FVIII product tested

^enot quantifiable (<0.4 BU for inhibitor and <0.001 nM for total antibody)

^fCryoprecipitate

Table 2

Anti-FVIII antibody titer ratios for three FVIII products.

Groups	Pr. "A"/Pr. "B"	Pr. "A"/Pr. "C"	Pr. "B"/Pr. "C"
I. Treated with Product "A"			
1. With inhibitor	2.47±1.32	4.22±2.38	1.83±0.56
2. Without inhibitor	2.44±2.43	3.07±3.10	1.21±0.42
For the entire Product "A" group	2.45±1.80	3.84±2.37	1.52±0.57
II. Treated with other Products	0.68±0.75	0.99±1.36	1.77±1.66