Factor VIII Product-Dependent Recognition of Anti-Factor VIII Antibodies

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Objective

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, despite numerous studies performed in an attempt to establish such a relationship. In the current study, we evaluated the development of both inhibitory and non-inhibitory anti-FVIII antibodies in 34 HA subjects. Twenty HA subjects were treated with pharmacologic FVIII product A (contains full-length recombinant (r)FVIII) and 14 subjects were treated with other FVIII replacement products.

Methods

Human subjects

Thirty four male individuals (1-55 years old; Table 1) with HA 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 HA subjects or their parents/guardians. All individuals were advised not to withhold replacement therapy and did not need to self infuse with factor VIII from 0.25 to 4 days prior to the blood draw. Twenty HA individuals that required prophylaxis used for the replacement therapy a pharmacologic product (product A, produced in BHK cells) containing full-length rFVIII, two used another pharmacologic product (product B) containing full-length rFVIII, two used product C containing B domain-less rFVIII (both produced in CHO cells), two used cryoprecipitate and 8 used plasma-derived FVIII (pdFVIII). 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 ºC until further use for the total anti-FVIII antibody assay.

Multiplex Fluorescence Immunoassay (MFI) of FVIII antibodies

The procedure for the anti-FVIII antibody MFI was adopted from a previously described and validated assay (1,2). Microsphere beads were coupled to product A, product C and product B. Non-specific binding control microsphere beads were coupled to ovalbumin. For the calibration, human anti-FVIII antibody standard purified from plasmas of two hemophilia A subjects with 64 BU and 280 BU inhibitory anti-FVIII antibody were added at 0.02-8.0 nM to wells containing FVIII- and ovalbumin-coupled microspheres. Test plasma samples were diluted 50-200,000-fold (according to their BU values) and also added to wells containing ovalbumin- and FVIII-coupled beads. The plate was incubated at 25° C for 2 hours. Wells were washed and probed with mouse anti-human IgG-R-PE. Following 1 hr. incubation, wells were washed, beads resuspended and analyzed by Bio-Plex200 instrument (Bio-Rad Laboratories, Hercules, CA). 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 with FVIII inhibitor values of 20 BU and 280 BU (both plasmas with less than <1% FVIII activity). Binding of 8 isotypes of anti-human Ig to rFVIII-coupled beads was comparable with that observed for antibody purified from HA subject's plasma.

Results

All 34 HA subjects of this study developed anti-FVIII antibodies, but with highly variable concentrations (from 50 nM to 570 µM; Table 1). Eleven of 20 HA subjects treated with product A and 5 of 14 treated with other products contained quantifiable inhibitory anti-FVIII antibodies by the Nijmegen assay (0.8-3584 BU). There was a certain correlation between the inhibitory antibody titer and the molar concentration of total antibody (r^2=0.6; Figure 1). Pronounced differences in antibody recognition by three rFVIII products were observed. For example, for the group treated with product A, the antibody titers determined with this product as a capture protein 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; Table 2). For the group of 14 HA subjects treated with products other than product A, only one showed higher antibody titer when measured with this product (tables 1 and 2).

Figure 1

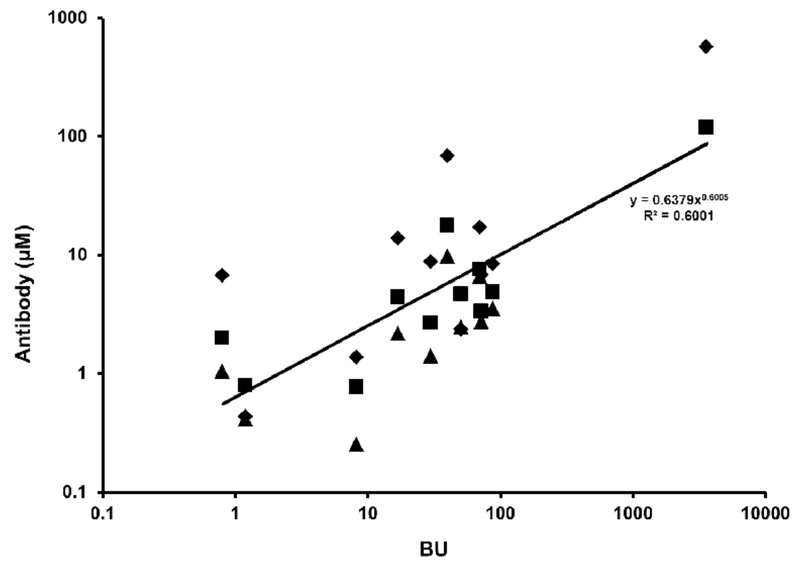

Figure 1. Correlations between the total (µM) and inhibitory (BU) anti-FVIII antibody.
The molar concentration of antibodies was determined in the MFI assay based on their binding to FVIII products A (◆), B (■) and C (▲). Inhibitory antibodies were measured in a standard Nijmegen assay.

Table 1. Anti-FVIII antibody concentrations in severe hemophilia A patients

Subject ID	Age (years)	BU[a]	Treated with	Total antibody (µM)[b] Product A	Product B	Product C	Antibody ratios[c] A/B	A/C	B/C	nM/BU[d] 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	pdFVIII[g]	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	pdFVIII	NQ	0.53±0.19	0.08±0.02	-	-	6.6	-	-	-
3-2	4	NQ	pdFVIII	0.02 ±0.00	0.18±0.01	0.15±0.01	0.11	0.13	1.2	-	-	-
3-6	17	36	pdFVIII	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	pdFVIII	0.60±0.12	0.66±0.08	1.00±0.07	0.91	0.6	0.7	-	-	-
3-12	4	NQ	pdFVIII	1.69±0.33	6.28±0.55	1.81±0.26	0.27	0.93	3.5	-	-	-
3-13	2	NQ	pdFVIII	0.82±0.12	1.86±0.16	1.67±0.30	0.44	0.49	1.1	-	-	-
3-17	1	NQ	pdFVIII	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	-	-	-

[a]Bethesda units
[b]Quantitated by capturing antibodies with indicated FVIII product
[c]Ratios of molar antibody concentrations quantitated with corresponding FVIII product
[d]Concentration of total antibody per 1 BU for each FVIII product tested
[e]not quantifiable (<0.4 BU for inhibitor and <0.001 nM for total antibody)
[f]Cryoprecipitate
[g]plasma-derived FVIII

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

Conclusions

1. For the group of HA subjects treated with rFVIII product A, anti-FVIII antibodies have the highest affinity for this product.
2. A significant fraction of antibodies bind to the B domain of FVIII.

References

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Conflict of Interest

The authors declare no competing financial interests.

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