IgG4 subclass of anti-FVIII antibodies is correlated to high-titre inhibitor, whereas IgG1 subclass is related to low-titre inhibitor in hemophilia A patients

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INTRODUCTION

The presence of anti-factor VIII alloantibodies, also called inhibitor, in hemophilia patients compromises the replacement therapy effectiveness. The characterization of immunoglobulin (Ig) subclass has been showed an important role to identify the reactivity of factor (F) VIII concentrate used in the treatment of patients with hemophilia A (HA). Recent studies showed a strong correlation between high levels of anti-FVIII antibodies of subclass IgG4 with high-titre inhibitor and immune tolerance induction (ITI) failure. The objective of this study was to evaluate the characteristics of anti-FVIII IgG subclasses in HA patients with inhibitor.

METHOD

We analyzed in this study samples from hemophilia A patients with inhibitor (> 0.6 BU/mL). The anti-FVIII inhibitor was determined by Bethesda assay with the Nijmegen modification. The antihuman-IgG specific for subclasses IgG1, IgG2, IgG3 and IgG4 (Southern Biothechnology Inc., USA) were performed by ELISA assay using two different plasma-derived FVIII (pdFVIII) concentrates, commonly used in Brazil I) Octavi SD Optimum, (Octapharma, France), and II) 8Y (Bio Products Laboratory, UK).

RESULTS

Twenty-six patients with congenital hemophilia A with inhibitor were included in this study. The mean age was 23y (range 8 - 49y), and twenty-four patients had the diagnosis of severe HA, one moderate HA, and one mild HA. The anti-FVIII results by modified Bethesda assay and ELISA assay are summarized in table 1. Twenty among twenty-six (77%) were high-responder inhibitors patients, and four of them had low-titre inhibitor (< 5 BU/mL) at the moment of the analysis. IgG1 subclass was positive in 23/26 (88%) patients, and in six low-titer inhibitor patients was the only IgG subclass present. In contrast, IgG4 was positive in all high-titer inhibitor patient, and in only one low-titer moderate HA patient. Furthermore, we also observed a correlation between the signal intensity of IgG4 and the inhibitor titer (figure 1). No correlation between IgG1 positive reaction and the inhibitor titer was observed.

We also included in our analysis two different pdFVIII concentrates, one with 100 IU/mg of protein (Octavi SD Optimum, Octapharma), and another intermediate purity pdFVIII concentrate (2 IU FVIII per mg) with high levels of von Willebrand factor (8Y, Bio Products Laboratory). It was observed a considerable reduction in the reactivity with FVIII concentrate 8Y.

Table 1. Evaluation of anti-FVIII IgG subclasses in HA patients with inhibitor.

Patient number	Inhibitor titre (BU/mL)	Inhibitor	IgG1		IgG2		IGg3		IgG4	
Group A (< 5 UB/mL)			I	II	I	II	I	II	I	II
1	0.6	Low-responder	-	-	-	-	-	-	++++	-
2	0.7	Low-responder	++++	-	+	-	-	-	-	-
3	0.75	Low-responder	++	-	+	-	-	-	-	-
4	0.95	Low-responder	+++	-	-	-	-	-	-	-
5	1.4	Low-responder	+	-	-	-	-	-	+	-
6	2.8	Low-responder	++	-	-	-	-	-	-	-
7	0.9	High-responder	-	+++	-	-	-	-	-	-
8	1.9	High-responder	++++	-	-	-	+	-	+	-
9	3.15	High-responder	+	+++	-	-	-	-	-	-
10	2	High-responder	++++	-	-	-	-	-	-	-
11	4.9	High-responder	++	-	-	-	-	-	-	-
Group B (> 5 UB/mL)										
12	7.4	High-responder	++++	-	-	-	-	-	++++	-
13	8	High-responder	+++	-	-	-	-	-	+	-
14	8.4	High-responder	+++	-	+++	-	-	-	+	-
15	11	High-responder	++++	-	-	-	-	-	++++	-
16	11.5	High-responder	++++	-	++++	-	-	-	++	-
17	12	High-responder	+	-	+	-	-	-	++	-
18	12.5	High-responder	+	-	-	-	-	-	+	-
19	14.5	High-responder	-	-	-	-	-	-	+	-
20	16	High-responder	++	-	+++	-	-	-	+	-
21	25	High-responder	++	-	-	-	-	-	+++	-
22	35	High-responder	-	-	-	-	++++	-	++++	-
23	40	High-responder	++++	-	++++	-	++	-	++++	-
24	58	High-responder	-	-	-	-	-	-	++++	-
25	155	High-responder	+	-	+	-	-	-	+++	-
26	384	High-responder	+	+	-	-	-	-	+	-

Two different FVIII concentrates were used as target antigen in the ELISA: (I) Octavi SDOptimum (Octapharma, France), (II) 8Y (Bio Products Laboratory, UK).
The results are presented as a positive reaction obtained with the following dilutions of the tests sample: -, negative, +,1:10, ++1:40, +++, 1:80, ++++, 1:320.

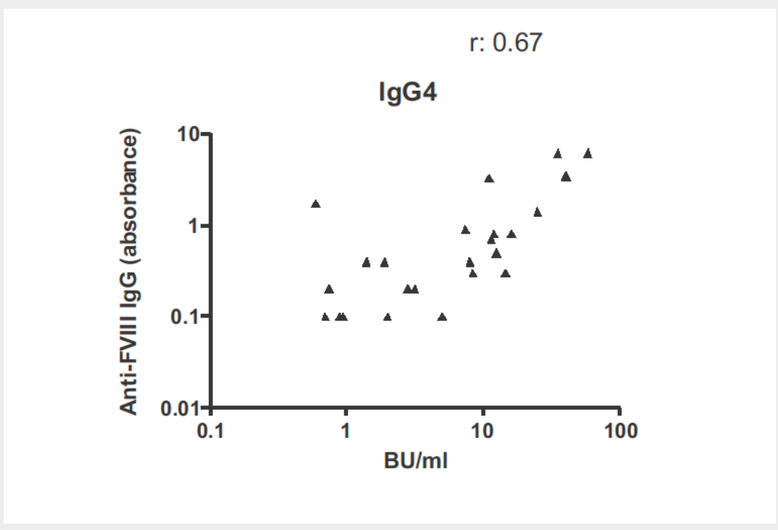

Figure 1. Correlation between levels of anti-FVIII IgG4 and inhibitor titre as measured by the modified Bethesda assay. Anti IgG4 (absorbance) are plotted against Bethesda titre (BU/ml). Correlation coefficients (r) were calculated using non-parametric correlation analysis (Spearman Rank).

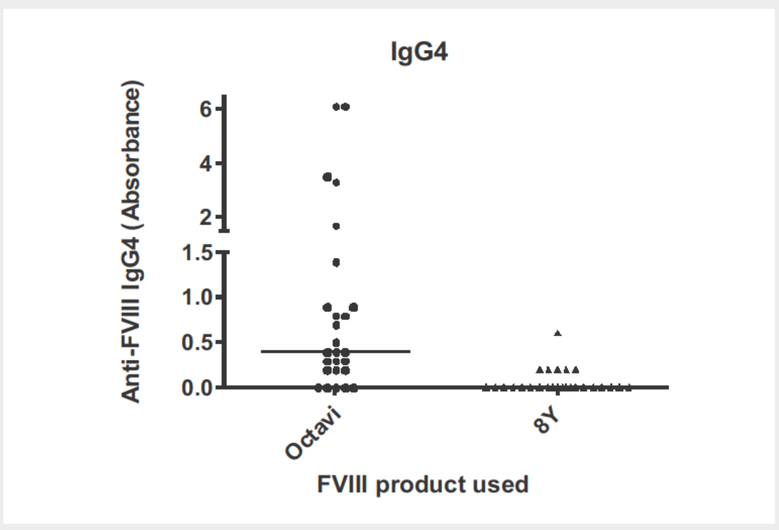

Figure 2. Anti-FVIII IgG4 positive reaction using two pdFVIII concentrates. It was observed a considerable reduction in the reactivity with intermediate purity pdFVIII concentrate (8Y, Bio Products Laboratory), when compared to high purity pdFVIII concentrate (Octavi SD Optimum).

CONCLUSION

Our results demonstrated that IgG4 subclass of anti-FVIII antibodies is correlated to high-titre inhibitor, whereas IgG1 subclass is related to low-titre inhibitor in hemophilia A patients. We also observed a correlation of IgG4 positive reaction and high-titre of inhibitor. The presence of IgG4 anti-FVIII antibodies is related to Th2 immune response to FVIII, what can interfere in the success of immune tolerance induction (ITI) as previous reported. Therefore, the evaluation of IgG subclasses in patients with inhibitor may be useful alternative for monitoring the presence of inhibitor, and the decision of ITI treatment and outcome.

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