Anti- factor VIII IgG4 and intracellular cytokines profile in T and B cells from hemophilia A patients with complete success, failure, or relapse after immune tolerance induction treatment: longitudinal evaluation with five years follow-up

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INTRODUCTION AND METHODS

Immune tolerance induction (ITI) is the treatment choice for hemophilia A patients with high-responding inhibitor, with 70% of success rate. The mechanism involved in the achievement of factor (F) VIII tolerance and why some patients present relapse or ITI failure are still unclear. The objective of this longitudinal study with five years of follow-up was to evaluate the distribution of anti-FVIII IgG1/IgG4 and intracellular cytokines profile in T and B cells during ITI protocol.

Anti-FVIII inhibitor was determined by Nijmegen-Bethesda assay. Anti-FVIII IgG1/IgG4 was performed by ELISA (Southern-Biotechnology). The intracellular cytokines and surface cell markers were analyzed before and after *in vitro* FVIII stimulation by flow cytometry. The profiles analyzed were (1) $CD4^+$ $IL4^+$,$IL5^+$, $IL10^+$, $IL21^+$, $TGFB^+$; (2) $CD4^+CD25^+FOXP3^+$ (regulatory T cells); (3) $CD19^+CD24^+CD38^+$ (regulatory B cells). Only cells and cytokines that showed reactivity for FVIII, were considered positive. Results were showed as a fold of reactivity between stimulated / no stimulated samples. Cytokines and cells that not presented reactivity were showed as without reactivity (WR).

RESULTS

Four patients were analyzed, with median age of 5.2y (1.9-13.2y) when they started ITI. Analysis included pre and 5 to 9 different time-points with follow-up of 3 to 5 years. Two patients (Patient 3 and 4) achieved ITI success, while one had inhibitor relapse (Patient 1) and the last one ITI failure (Patient 2) (Table 1). For all patients the decrease of inhibitor titer was associated with reduction of anti-FVIII IgG4, which disappeared when patients achieved ITI success (Figures 1 and 2). Anti-FVIII IgG4 persisted in low titer in both patients who failed or relapsed (Figure 1). Two patients that achieved different response for ITI, failure (Patient 2) and success (Patient 3) had cytokines profile and regulatory T and B cells evaluated both pre and post ITI. Patient 2, 243 weeks after the beginning of the first ITI attempt received rituximab at a fixed dose of 100 mg/week (60 mg/m2) during four weeks. After 20 weeks of rituximab he presented a considerable decrease of inhibitor titer (3.38 BU/mL). The analysis at week 20 compared with pre ITI, showed reduction of CD4+IL4+ and CD4+ TGFB+ and increase of CD4+IL21+. Interestingly, we also observed decrease of regulatory T cells, and increase of regulatory B cells in the same proportion. Currently, he maintained with inhibitor < 1 BU/mL. Patient 3 achieved complete success after 14 weeks and this response was sustained for more than 4 years. Comparing the long-term response analysis (week 202) with baseline (pre ITI), the cytokines profile was similar to patient 2, however the CD4+IL10+ increased, together with regulatory T cell.

Patients	1	2	3	4
Age of first inhibitor	2y 2m	3y 11m	1y 10m	1y 2m
Age at start of ITI	5y 9m	13y 2m	1y 11m	4y 7m
Diagnosis (*F8* genotype)	sHA (INV22)	sHA (INV22)	sHA (INV22)	sHA (INV22)
Historical peak titer (BU/mL)	150	56	9.19	54
Pre-ITI titer (BU/mL)	3.7	11	7.8	1.7
ITI status	Relapse	Failure (partial success after rituximab)	Success	Success
Period of follow-up	294 weeks	263 weeks	202 weeks	296 weeks
Initial ITI protocol (pd-FVIII)	35 IU/kg 3x/week	50 IU/kg 3x/week	35 IU/kg 3x/week	35 IU/kg 3x/week

ITI, immune tolerance induction; sHA, severe hemophilia A; pd-FVIII, plasma-derived factor VIII concentrate; ND, not determined; INV22, intron 22 inversion of factor VIII gene.

Table 1: Clinical characteristics of hemophilia A patients submitted to ITI treatment.

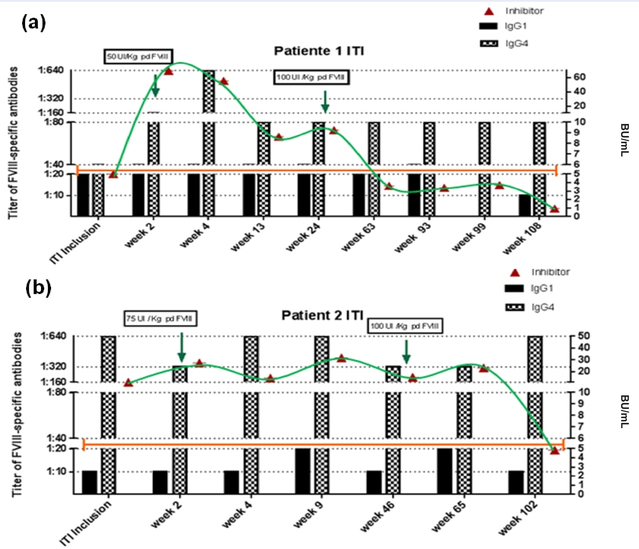

Figure 1. Longitudinal evaluation of Patient 1 with relapse and Patient 2 with failure to the first ITI treatment. a) Despite the low titer (< 1 BU/mL) of inhibitor at week 108, the patient 1 persisted with inadequate pharmacokinetic parameters to FVIII. This was associated with the persistence of anti-FVIII IgG4. **b)** Patient 2 did not achieve success up to week 102 of the ITI protocol. The high inhibitor titer is associated with the persistence of anti-FVIII IgG4.

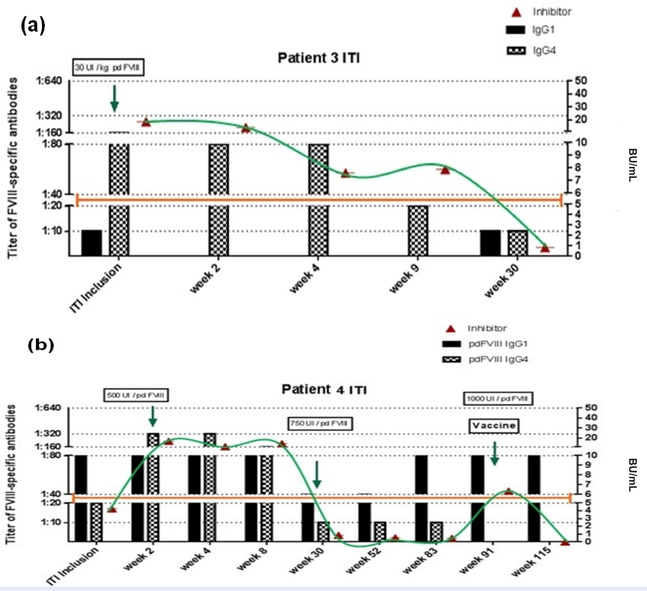

Figure 2. Longitudinal evaluation of Patient 3 and 4 with complete ITI success. a) Patient 3 obtained complete ITI success at week 14, with remarkable reduction of anti-FVIII IgG4 and inhibitor titer, and a low titer of anti-FVIII IgG1 also appeared. **b)** Patient 4 also achieved complete ITI success at week 30, when was observed decrease of the inhibitor and anti-FVIII IgG4 titers. The patient continued to be treated with pdFVIII concentrates with good pharmacokinetic parameters. At week 91 after received vaccine, the inhibitor titer increased with no clinical relevance, and few weeks after was negative again.

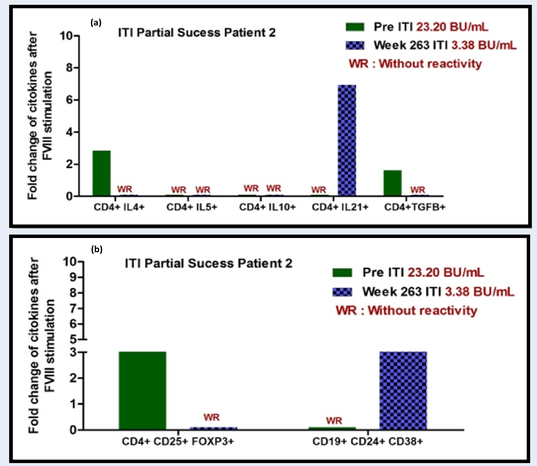

Figure 3. Cytokines and Regulatory T and B cells of samples pre and post ITI protocol from Patient 2 with partial success after rescue with rituximab. Graphs show the fold change pre and after FVIII stimulation.

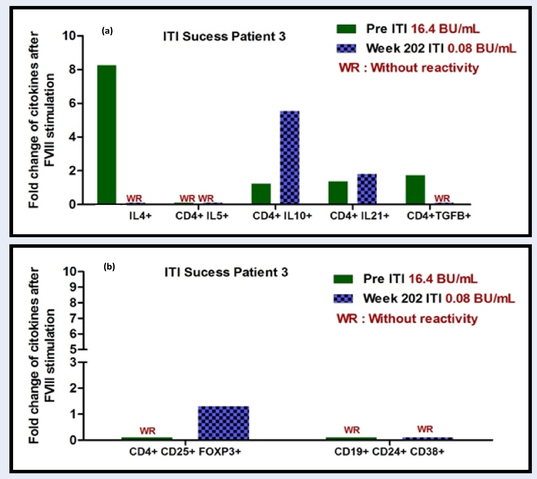

Figure 4. Cytokines and Regulatory T and B cells of samples pre and post ITI protocol from Patient 3 with complete ITI success. Graphs show the fold change pre and after FVIII stimulation.

DISCUSSION AND CONCLUSION

The IgG subclasses have been studied in different cohorts of the hemophilia A patients, including during ITI treatment. In this study, we determined the distribution of the anti-FVIII IgG1 and IgG4, cytokine and regulatory T and B cells profile in response to ITI treatment. The patients that achieved complete ITI success showed anti-FVIII IgG4 decreased with the increase, CD4+IL10+, CD4+IL21+ and CD4+CD25+FOXP3+ (regulatory T cell). The shift from anti-FVIII IgG4 to IgG1 in the ITI context with higher frequency of regulatory T or B cells was showed in this study. The knowledge of the tolerance induction mechanism will contribute to proper new strategies to achieve FVIII tolerance. Furthermore, based on previous and our results, the persistence of anti-FVIII IgG4 may be used as a marker of a poor prognosis in ITI treatment.