39799
P-T-037

The Development of Anti-Factor VIII Antibodies Associated with the Change of Serum BAFF Level in Hemophilia A mice

Shin-Nan Cheng, [1, 2], Ming-Shen Dai, [2, 3], Chiew-Peng Huang, [2], Yeu-Chin Chen, [2, 3]

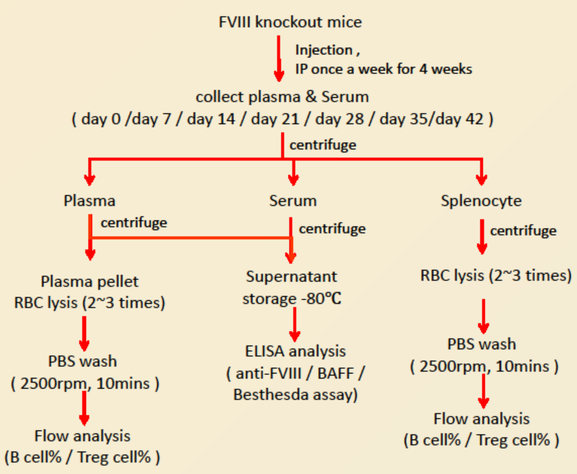
[1]Department of Pediatric, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan
[2]Hemophilia Care & Research Center, Tri-Service General Hospital, Taipei, Taiwan
[3]Division Hematology/Oncology, Tri-Service General Hospital, Taipei, Taiwan

Objectives:

Hemophilia A is an X-linked bleeding disorder, caused by defects in factor VIII (FVIII) gene. FVIII antibodies provide a major challenge of replacement therapy. B cell-activating factor (BAFF) is involved in the survival and maturation of B cell and plays a critical role in most of immune responses. The purpose of this study was to investigate the relationship between the emergence of anti-FVIII antibodies and BAFF level in animal model.

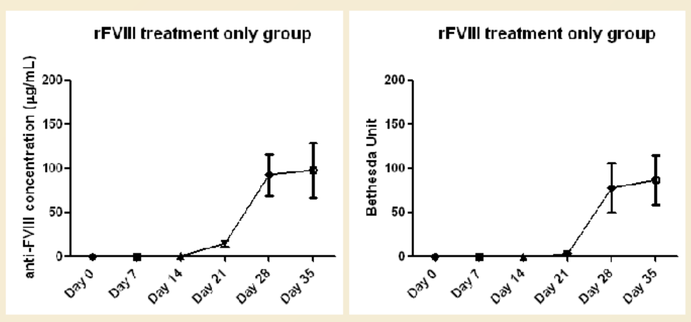
Methods:

Hemophilia mice (C57BL/6 and 129 mix background, Exon 16 knockout) were intraperitoneally injected with 2 IU (~80IU Kg^{-1}) of human recombinant FVIII (rFVIII) (Baxter) diluted in PBS, with/without anti-CD20 antibody treatment at 4 consecutive weeks. The mice serum and plasma were sampled before injection and after 4 consecutive weekly injections. Total anti-FVIII antibody titres and serum BAFF concentration were detected by ELISA. The difference between those experimental groups was evaluated by one-way ANOVA using PRISM 5.



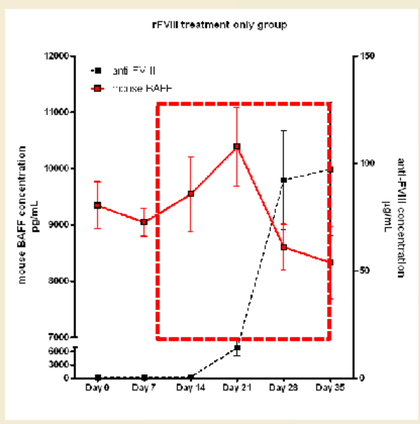
Results:

Figure 1. Recombinant factor VIII (FVIII) induces FVIII antibody formation in the hemophilia A mouse



Anti-FVIII titer ≒ 92-123 μg/mL; anti-FVIII ≒ 78-97 BU,
Repeat times, N > 3 and each group, n > 3

Figure 2. Anti-FVIII inhibitory antibodies formation after BAFF level decline



Fingure 3. Anti-FVIII inhibitory antibodies formation after CD20% rise

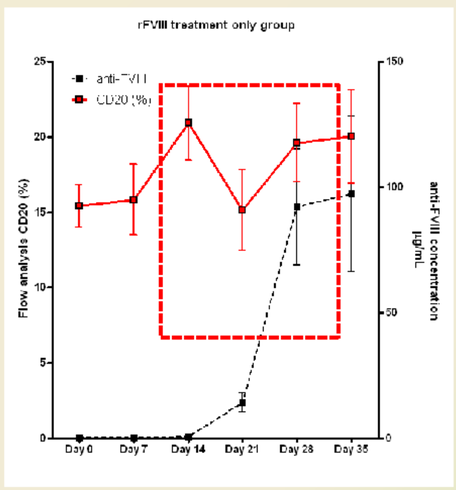


Figure 4 CD20 population change post-FVIII treatment with inhibiter formation

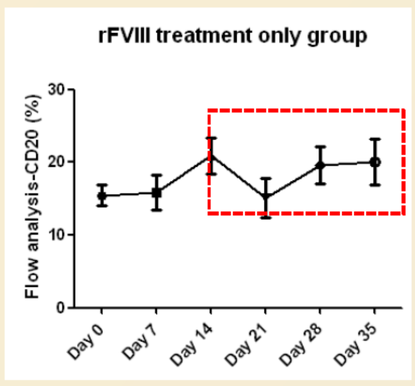


Figure 5. Treg cell population change post-FVIII treatment with inhibiter formation

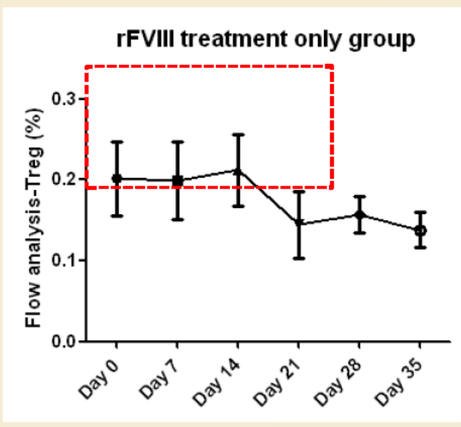
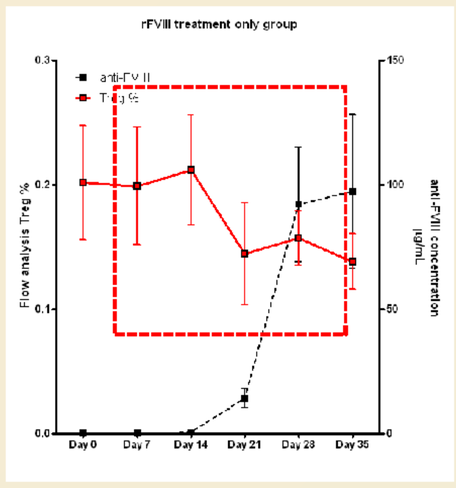


Figure 6. Related Treg cell population change with anti-FVIII post-FVIII treatment with anti-FVIII formation



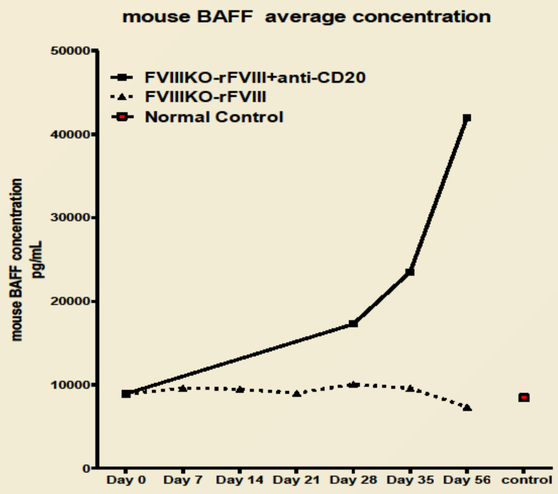
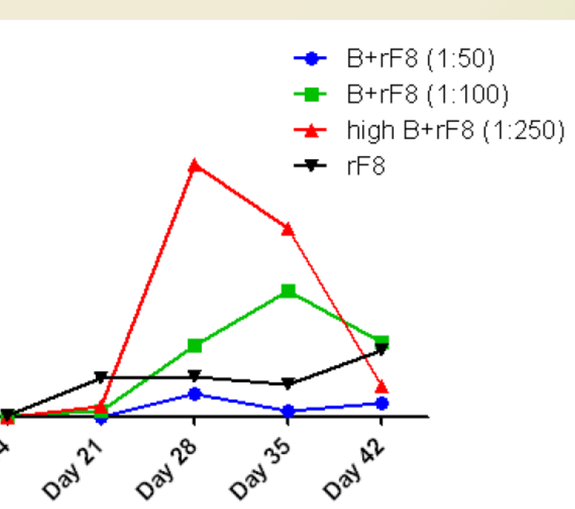
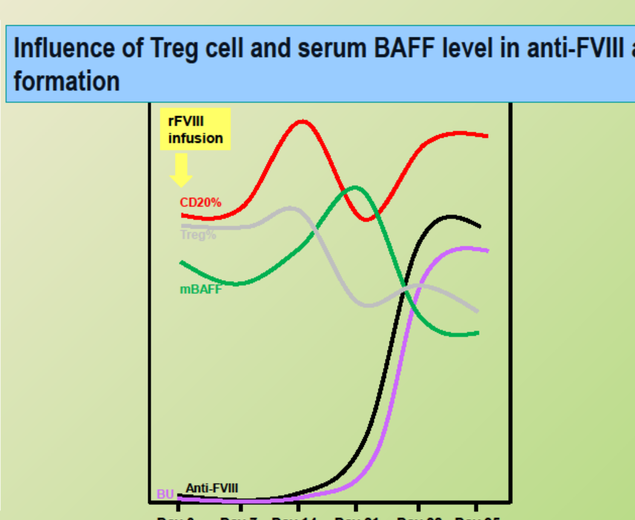


Figure 7. Anti-CD20 + rFVIII injection with related anti-FVIII level



➢P=0.0003, ***



➢P=0.2654, NS

Influence of Treg cell and serum BAFF level in anti-FVIII antibodies formation

Conclusions:

1. BAFF level slightly increased before high-titer anti-FVIII antibodies formation, then the BAFF level decreased while the antibodies significant increased.
2. Interestingly, high-titer anti-FVIII antibodies developed after regulatory T cell population (percentage of Treg) dropped, probably because lost of its tolerance ability.
3. We found CD20% slightly increased before high-titer anti-FVIII antibodies formation, then the CD20% rise again while the antibodies significant increased.
4. BAFF level in anti-CD20 antibody treated group increased probably because of compensatory immune responses . .
5. For the experiment group injection with BAFF-R, it seems delay the formation of antibodies.
6. Our preliminary experiment indicated that B cell may play a role in early anti-FVIII inhibitor formation and therefore BAFF targeting strategy might prevent or reduce its occurrence.

DOI: 10.3252/pso.eu.WFH2014.2014