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ILLUSTRATED REVIEW

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Development of inhibitors in hemophilia A: An illustrated review

Letícia Lemos Jardim MSc, PhD[1,2] | Daniel Gonçalves Chaves MSc, PhD[3] | Suely Meireles Rezende MD, PhD[1]

[1]Faculty of Medicine, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

[2]Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands

[3]Hemocentro de Belo Horizonte, Fundação HEMOMINAS, Belo Horizonte, Brazil

Correspondence
Suely Meireles Rezende, Faculty of Medicine, Universidade Federal de Minas Gerais, Avenida Alfredo Balena, 190, room 255, Belo Horizonte 30130-100, MG, Brazil.
Emails: suely.rezende@uol.com.br; srezende@medicina.ufmg.br

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Abstract

This illustrated review focuses on the development of inhibitors in patients with congenital hemophilia, which is the most serious treatment-related complication in these patients. Hemophilia A (HA) is an inherited X-linked bleeding disorder affecting 1:5000-10 000 newborn males worldwide. It results from the deficiency of coagulation factor VIII (FVIII), due to mutation(s) in its coding gene (*F8*). Treatment requires administration of FVIII-containing products either on demand or as prophylaxis, which can induce inhibitor development in 20%-35% of patients. Inhibitors are alloantibodies that neutralize the procoagulant activity of exogenous FVIII. During the initial administration of FVIII-containing products, patients with HA can develop a proinflammatory immune response with synthesis of anti-FVIII IgG1, which has no FVIII inhibitory activity. However, in patients with inhibitors, immune response shifts toward an anti-inflammatory/regulatory pattern favoring the synthesis of anti- FVIII IgG4 antibodies. Patients with inhibitors present with bleeding episodes that are difficult to control, and they have reduced response to FVIII replacement. Currently, immune tolerance induction is the available treatment for eradication of persistent high-titer inhibitors. Despite the clinical relevance, the immunological mechanisms for inhibitor development in patients with HA remains unexplained.

KEYWORDS

antibody, factor VIII, hemophilia, immune response, inhibitor

Essentials

- Hemophilia A (HA) is a bleeding disorder caused by the deficiency of coagulation factor VIII (FVIII).
- The main treatment-related complication in patients with HA is the development of inhibitor.
- Inhibitors are alloantibodies that neutralize the procoagulant activity of infused FVIII.
- The reasons why only 20%-30% of the patients with HA develop inhibitors remain a challenge.

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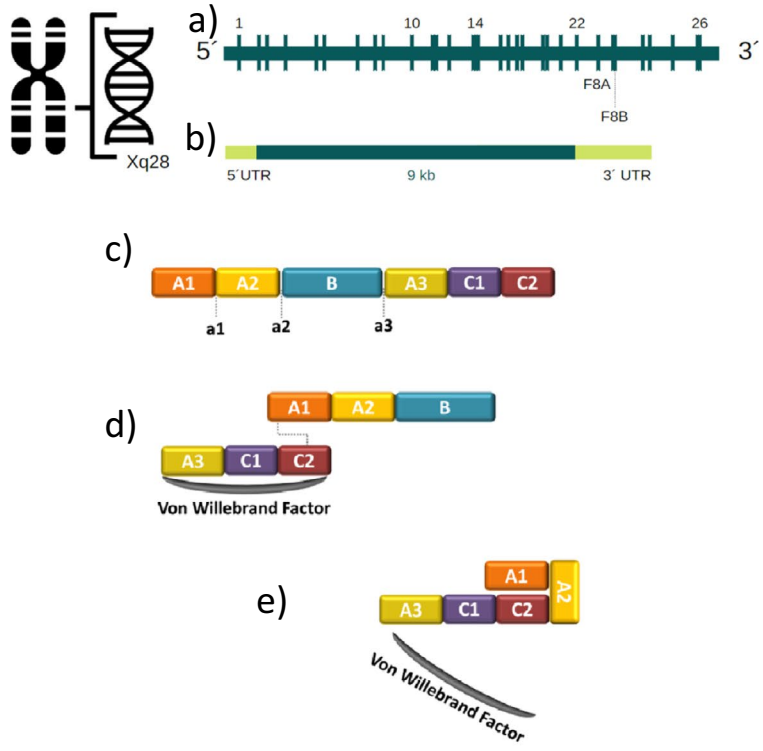
Factor VIII - Gene and protein[1-2]



a) Structure of factor VIII gene (*F8*);

b) Transcription of messenger RNA, with 2 noncoding regions (5´UTR and 3´UTR);

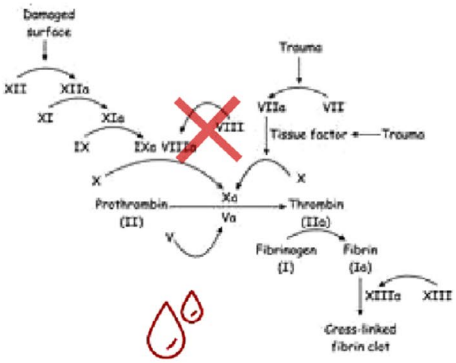
c) Primary structure of FVIII, representing the domains and the breakpoints in acidic regions a1, a2, and a3;

d) Inactive FVIII associated with von Willebrand factor. Acidic regions a1, a2, and a3 contains interaction sites recognized by FX and thrombin;

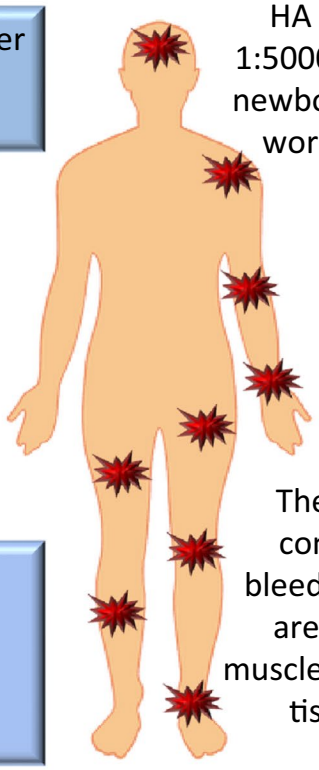
e) Protein activation after thrombin cleavage and dissociation of von Willebrand factor.

Hemophilia A[1,3-4]

Hemophilia A (HA) is an inherited X-linked bleeding disorder caused by the deficiency of coagulation FVIII due to mutations in *F8*.



HA affects 1:5000-10 000 newborn males worldwide.



The most common bleeding sites are: joint, muscles and soft tissues.

The main determinant of the bleeding phenotype is the residual level of FVIII:

FVIII <1%:	FVIII 1%-5%:	FVIII >5%-40%:
SEVERE	MODERATE	MILD

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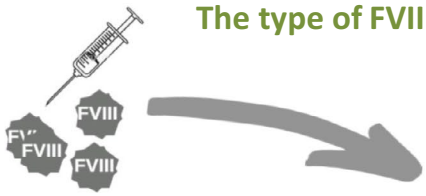
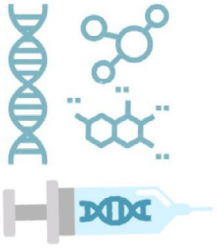
Treatment of hemophilia A



The type of FVIII concentrate can be:

OR



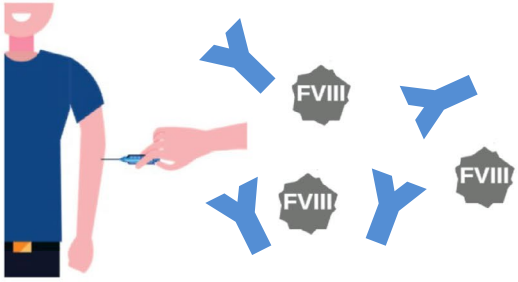
PLASMA-DERIVED (pdFVIII)

RECOMBINANT (rFVIII)

Treatment of bleeding episodes requires intravenous infusion of FVIII products either on demand or on a prophylactic basis.[3]

Inhibitors in hemophilia A

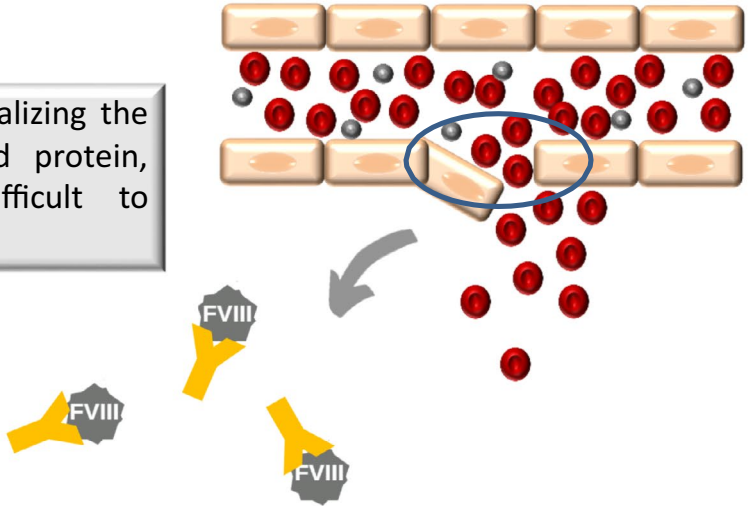
Neutralizing alloantibodies (inhibitors) are the main treatment-related complication in patients with severe HA.[5-6]



During initial administration of FVIII-containing products, the immune system develop a pro-inflammatory response which involves synthesis of antibodies against FVIII.[7-10]

Inhibitors bind FVIII epitopes, neutralizing the therapeutic activity of the infused protein, leading to bleeding that is difficult to control.[7,11]

Cumulative incidence of inhibitor development in patients with HA is 20%-35%.



In about 95% of patients with HA who develop inhibitors, it occurs within the first 75 exposure days to FVIII replacement.[12]

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Inhibitors in hemophilia A[11]

An inhibitor is confirmed when there is an antibody titer above 0.6 Bethesda Unit (BU)/mL in two consecutive plasma measurements. Then, inhibitor is classified as:

High-titer inhibitors:
Titer ≥5 BU/mL

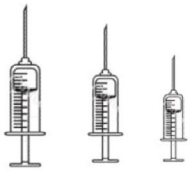
Low-titer inhibitors:
Titer <5 BU/mL

Risk factors for inhibitor development[4,13-14]

Mutations in genes related to immune response

Family history of inhibitors

Type of *F8* mutation



Intensity of FVIII infusions

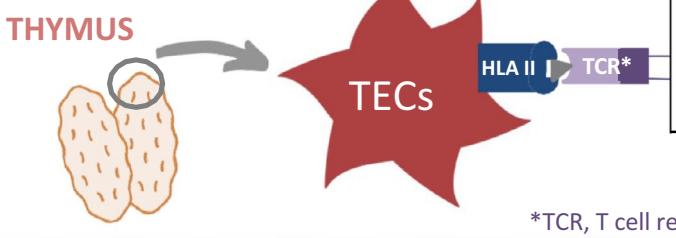
Younger age at first exposure to FVIII

Type of FVIII concentrate used (pdFVIII or rFVIII)

Immunology of inhibitors in hemophilia A

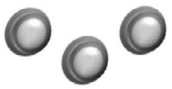
Part I: Central tolerance for the deficient protein[6,15]

Immature T cells enter the thymus and interact with thymic epithelial cells (TECs)



*TCR, T cell receptor

T cells that do not recognize "self antigens" presented by class II human leukocyte antigen (HLA)



Signaling for survival

T cells that recognize "self-antigens" presented by class II HLA

Signaling for apoptotic death

As a result of their genetic mutation, some patients with HA are unable to express large portions or any amount of FVIII. These patients are not able to eliminate high-affinity FVIII-reactive T cells. These T cells will then populate peripheral tissues and may induce the development of inhibitors when patients are treated with FVIII.

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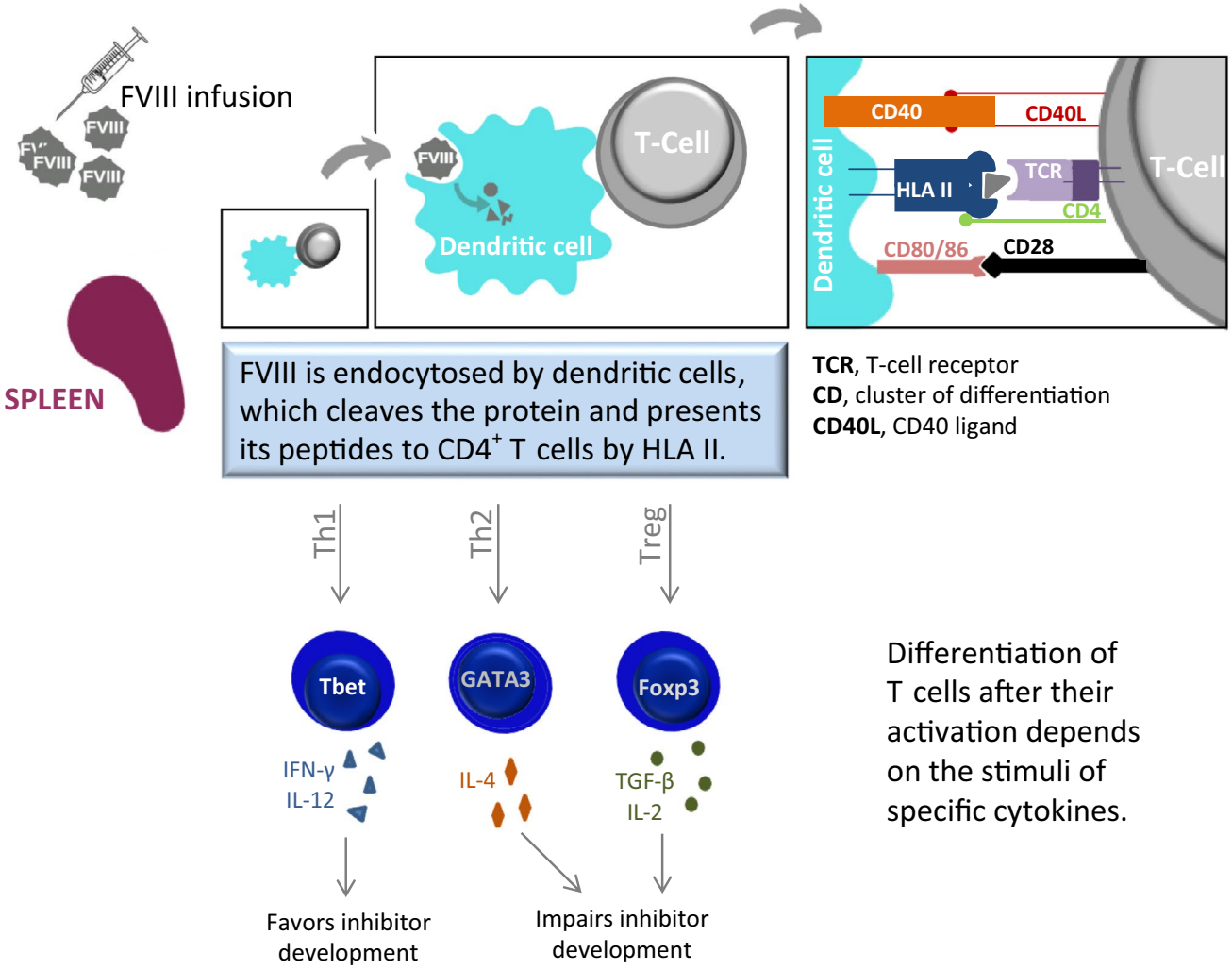
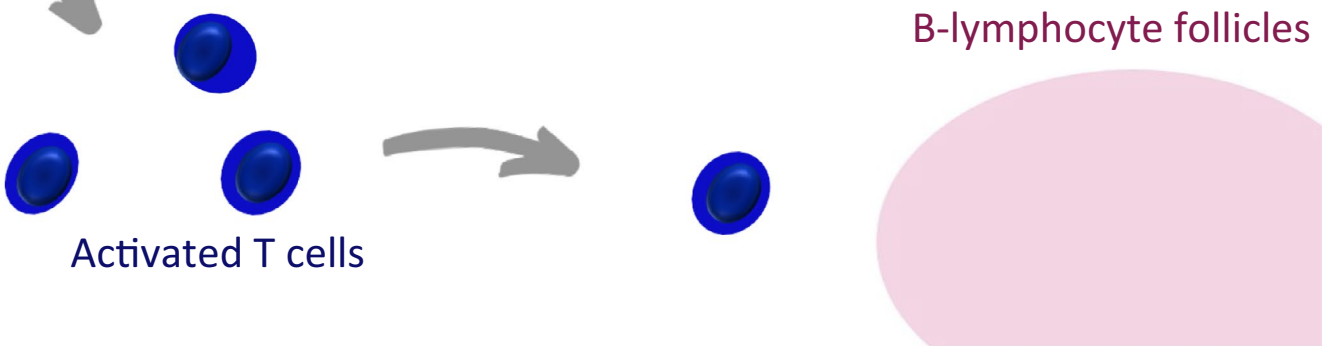
Part II: Primary response[6-9,15-17].



Transcription factors: Tbet, cell-specific transcription factor / GATA3, zinc finger transcription factor 3 / Foxp3, Forkhead box p3

These interactions result in a clonal expansion of the active T cells which migrate to the B-lymphocyte follicles in the spleen.



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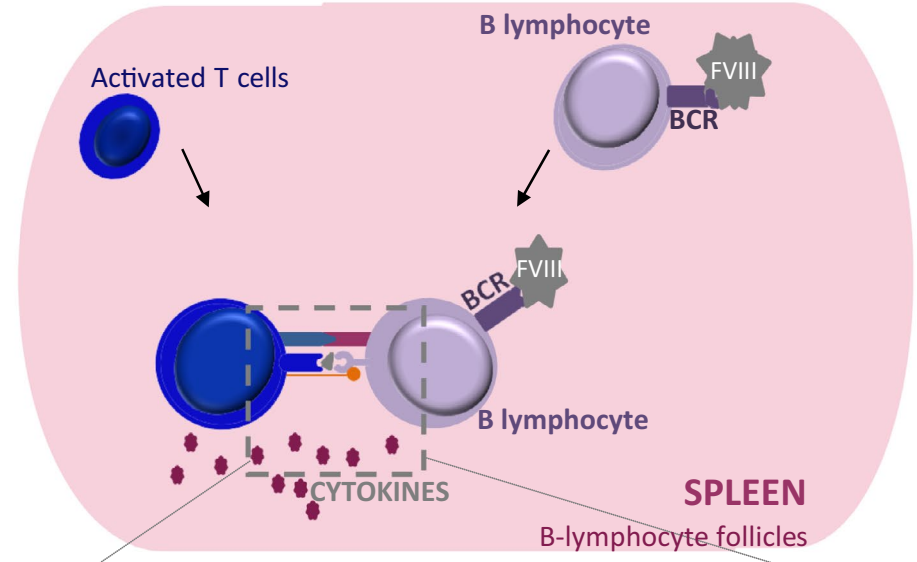
Immunology of inhibitors in hemophilia A

Part III: Primary response[6,15-17].



Follicular B lymphocytes that have been primed by infused FVIII and have internalized FVIII through the BCR present FVIII peptides via class II HLA on the membrane surface in the B lymphocyte follicle.

Interaction between activated T cells and B lymphocytes through TCR-HLA, co-stimulatory molecules, and cytokine stimulation triggers the differentiation of follicular B lymphocytes.

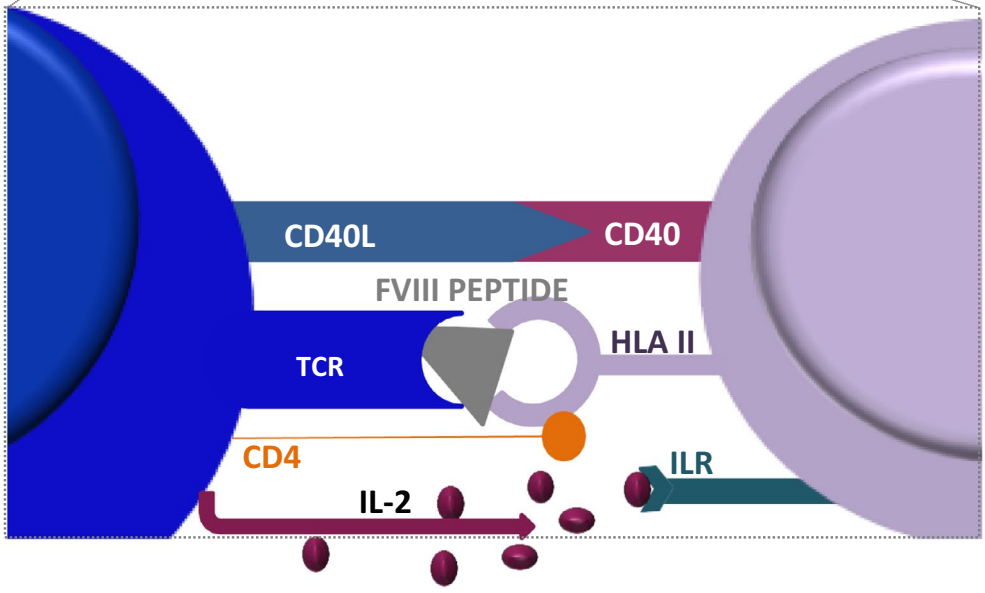
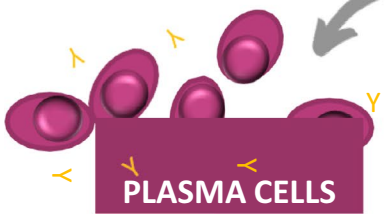


Figure legend:

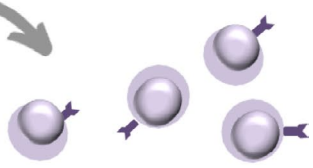
BCR, B-cell receptor
TCR, T-cell receptor
HLA II, class II human leukocyte antigen
IL, Interleukin
ILR, Interleukin receptor

Follicular B lymphocyte can differentiate into:

OR



Involved in long-lasting antibody production



MEMORY B LYMPHOCYTES

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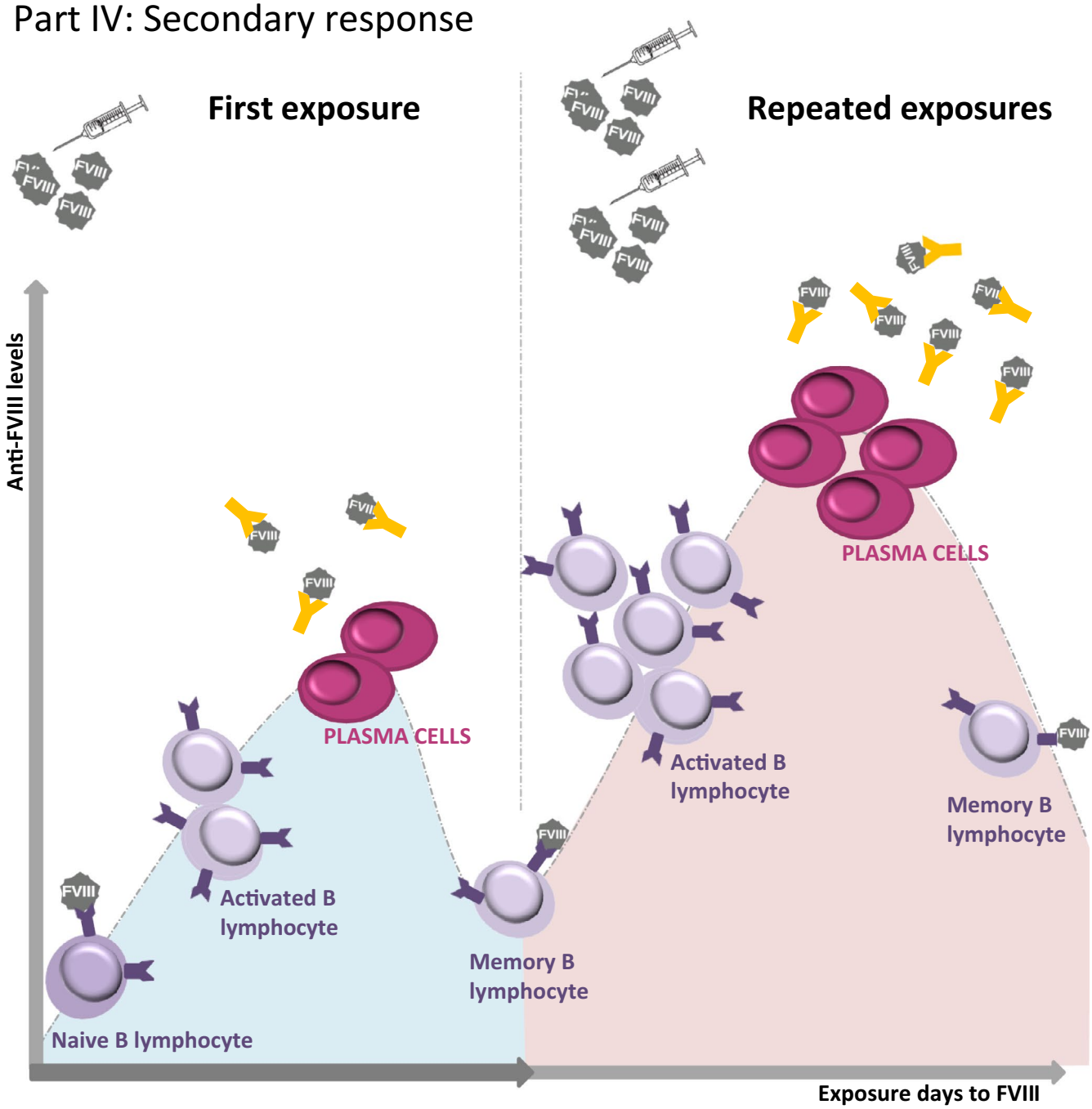
Immunology of inhibitors in hemophilia A

Part IV: Secondary response



Memory B lymphocytes can develop a faster and stronger immune response against FVIII than naive B lymphocytes, with production of high-affinity neutralizing antibodies.[15]

Anti-FVIII antibodies, mostly IgG1, IgM, and IgA, have been reported in healthy individuals. Anti-FVIII IgG4 is predominantly found in patients with HA who developed inhibitors, especially those with high titer.[17-19]

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Inhibitors in hemophilia A

Treatment[5]

Bypassing agents such as activated prothrombin complex concentrate and activated recombinant factor VII are needed to treat/prevent bleeding episodes in patients with HA and high-titer inhibitors.

Emicizumab is a nonfactor therapy recently approved for the prevention of bleeding in patients with HA and inhibitors.

Immune tolerance induction (ITI)[20]

Immune tolerance induction (ITI) is the unique available treatment for eradication of persistent high-titer inhibitors in HA. ITI is effective in 60%-80% of treated patients with HA.

However, ITI is a demanding and high-cost treatment and requires frequent infusions of FVIII for months to years

Final Remarks

Inhibitor development is the main complication of HA, affecting about 30% of patients. ITI can eradicate inhibitors but is costly and not successful for all patients. Furthermore, inhibitors can recur. Therefore, a better understanding of biological mechanisms, epidemiology, and risk factors for inhibitor development is needed.

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RELATIONSHIP DISCLOSURE

The authors state that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

LLJ, DGC, and SMR created the capsules and the conceptual design and wrote the paper. All authors critically revised the manuscript and approved the final version.

ORCID

Letícia Lemos Jardim https://orcid.org/0000-0003-3358-0075

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