

# Immune Response Mechanisms in Haemophilia A

Amina Abdelmageed<sup>1</sup> Clivia Lisowski<sup>1</sup> Janine Becker-Gotot<sup>1</sup> 

<sup>1</sup>Institute of Molecular Medicine and Experimental Immunology, University Clinic Bonn, University of Bonn, Bonn, Germany

**Address for correspondence** Janine Becker-Gotot, Dr. rer. nat., University Clinic Bonn, University of Bonn, Venusberg Campus 1, 53127 Bonn, Germany (e-mail: jbecker-gotot@uni-bonn.de).

Hamostaseologie 2026;46:17–23.

## Abstract

Haemophilia A (HA) is an X-linked bleeding disorder caused by the deficiency or dysfunction of coagulation factor VIII (FVIII). Substitution therapy with recombinant or plasma-derived FVIII effectively restores haemostasis but carries the risk of eliciting FVIII-specific neutralizing antibodies known as inhibitors. Inhibitor formation reflects the absence of central immune tolerance, driven by complex interactions among antigen-presenting cells, B cells and CD4 T cell subsets. Recent studies highlight the essential roles of T cell polarization, costimulatory signalling and cytokine networks in shaping the immunogenic or tolerogenic outcome of FVIII exposure. This review summarizes mechanistic insights into how distinct CD4 T cell subpopulations, including Th1, Th2, Th17, follicular helper (Tfh), follicular regulatory (Tfr) and regulatory T cells (Tregs), influence inhibitor development in HA. Furthermore, it discusses emerging immunological concepts and cellular pathways that could be targeted to achieve durable tolerance towards FVIII, with emphasis on translational strategies that align mechanistic understanding with clinical need.

## Keywords

- ▶ haemophilia A
- ▶ inhibitors
- ▶ factor 8
- ▶ CD4 T cell subsets
- ▶ regulatory T cells

## Introduction

Haemophilia A (HA) affects approximately one in every 5,000 male live births and results from reduced levels or functional deficiency of coagulation factor VIII (FVIII). The absence of FVIII impairs thrombin generation and fibrin formation, compromising haemostasis. FVIII replacement therapy, whether recombinant or plasma-derived, effectively prevents spontaneous and trauma-induced bleeding. However, repeated administration of FVIII exposes the immune system to exogenous epitopes that are perceived as foreign. Particularly in individuals lacking endogenous FVIII or expressing truncated variants, critical epitopes evade central tolerance mechanisms and trigger the formation of FVIII-specific antibodies that neutralize its coagulant activity.<sup>1</sup> In addition to the foreign-epitope phenomenon, factors such as the underlying *F8* mutation, host HLA class II alleles, and the inflammatory context<sup>1,2</sup> at the time of exposure can tune antigen presentation. This influences downstream T cell priming and provides a rationale for precision approaches to minimize immunogenicity in high-risk patients.

Inhibitors, targeting catalytic or binding domains and thereby abrogating FVIII's activity, develop in approximately 20 to 30% of patients with severe HA and represent the most serious complication of therapy. These antibodies render FVIII treatment ineffective, resulting in increased bleeding tendency, arthropathy and morbidity.<sup>1</sup> The development of inhibitors reflects a failure of both central and peripheral immune tolerance and depends on interactions between antigen-presenting cells (APCs), B cells and helper T cell subsets.<sup>2</sup> Although novel therapeutic approaches such as emicizumab and gene therapy have been introduced, FVIII replacement remains indispensable for achieving haemostatic control during surgical procedures or acute trauma.<sup>1</sup> Patients undergoing these procedures may encounter exposure to FVIII for the first time during acute treatment under extremely inflammatory conditions, which might heighten the risk of renewed or uncontrolled inhibitor formation.<sup>1</sup> Thus, modulating the immune response to FVIII remains a central objective in the management of patients with HA. Investigations into T cell subsets and whether they favour or oppose inhibitor formation during active inflammation or in different treatment settings are limited. Here, we review past

received

October 31, 2025

accepted after revision

November 17, 2025

© 2026. Thieme. All rights reserved.

Georg Thieme Verlag KG,

Oswald-Hesse-Straße 50,

70469 Stuttgart, Germany

DOI <https://doi.org/10.1055/a-2748-8775>.

ISSN 0720-9355.

ISSN 0720-9355.

ISSN 0720-9355.

and recent knowledge on the involvement of T cells in HA and the usage of this knowledge to redefine or establish alternative therapeutic approaches.

### **FVIII Immunogenicity and Antibody Formation**

FVIII is a large glycoprotein that circulates in plasma complexed with von Willebrand factor (vWF). Its immunogenicity has been widely studied to better understand and prevent inhibitor formation.<sup>3</sup> Although FVIII is an endogenous self-protein, it also exhibits immunogenic potential in healthy individuals. Centrally tolerized individuals harbour low frequencies of FVIII-specific B cells that, possibly upon exposure to inflammatory or danger signals, can produce low-affinity, non-neutralizing anti-FVIII antibodies.<sup>4</sup> FVIII inhibitors may also develop in non-haemophilic individuals, leading to acquired haemophilia A, a rare but severe autoimmune disorder that remains difficult to predict.<sup>5</sup> Together, these observations suggest that the FVIII-specific B cell repertoire is present at low frequency in healthy humans and can be driven towards pathogenicity by the combination of antigen availability and proinflammatory cues.

Comparative analyses of neutralizing and non-neutralizing antibodies in patients with severe or non-severe HA, as well as in healthy individuals, revealed distinct IgG subclass profiles correlated with inhibitor titers.<sup>6</sup> IgG4 was the predominant subclass in patients with inhibitors but was virtually absent in healthy subjects and non-inhibitor HA patients. In contrast, anti-FVIII IgG1 and IgG3 antibodies predominated in individuals without inhibitors or with low inhibitor titers. Neutralizing IgG4 and IgG1 antibodies exhibited approximately 100-fold higher affinity for FVIII than non-neutralizing antibodies.<sup>6</sup> This indicates a strong dependence on somatic hypermutation and affinity maturation, both germinal center (GC)-driven processes,<sup>7</sup> and underscores the need for early strategies that interrupt T cell-dependent pathways before high-affinity neutralizing responses consolidate.

### **T Cell Involvement**

The contribution of T cells to the immune response against FVIII was recognized in the 1990s. Stimulation of peripheral blood mononuclear cells with FVIII-induced T cell proliferation in HA patients with high inhibitor titers but not in those with no or low inhibitor titers indicates a pivotal role of T cells in inhibitor development.<sup>8</sup> The importance of CD4 T cells in generating FVIII-specific antibodies was further demonstrated in 1993. HA patients with a history of high-titre inhibitors, who experienced CD4 T cell depletion due to HIV-1 infection, also exhibited a loss of inhibitors, even after re-exposure to FVIII.<sup>9</sup> Moreover, FVIII-specific naive and memory CD4 T cells were later identified in healthy individuals, suggesting that a degree of central tolerance escape exists for FVIII-specific clones. However, the lack of their expansion *in vivo* indicates that inhibitory mechanisms, likely regulatory T cell-mediated suppression, prevent overt immune activation.<sup>10</sup> These observations in humans prompted rigorous investigation of how particular T cell subtypes and effector T cell differentiation can determine

the trajectory from exposure to either tolerance or immunogenicity.

Experimental data from murine models of HA support the essential role of T cells in the anti-FVIII immune response. Following exposure to recombinant human FVIII (rhFVIII), enhanced T cell activation was observed in the murine spleen, coinciding with anti-FVIII antibody production and inhibitor formation.<sup>11</sup> Depletion or functional inactivation of T cells using anti-CD3 antibodies abolished FVIII-specific antibody responses.<sup>12</sup> Collectively, these findings underscore that activated T cells are indispensable for inhibitor formation and that murine models can provide valuable insights into T cell subtypes that modulate tolerance or immunogenicity towards FVIII.

### **Antigen Presentation and T Cell Priming**

Following infusion, FVIII is internalized by dendritic cells, macrophages and B cells within secondary lymphoid organs such as the spleen. Within 30 minutes of administration, FVIII accumulates in the splenic marginal zone and is subsequently transported to the white pulp, where antigen presentation occurs.<sup>13</sup> FVIII-derived peptides are processed and presented to T cells through MHC class II molecules by B cells and other antigen-presenting cells, mainly dendritic cells.<sup>14</sup> Several T cell-dependent FVIII epitopes have been identified as immunodominant regions that are responsible for inhibitor development.<sup>15</sup> Apart from MHC recognition, T cell activation additionally requires costimulatory signalling and cytokine-mediated support.<sup>16</sup> Adequate co-stimulation drives T cell proliferation and differentiation into effector and memory subsets, while its absence leads to anergy.<sup>16</sup> The necessity of T cell-APC cooperation was further confirmed by CD40L blockade experiments, in which HA mice co-treated with FVIII and anti-CD40L exhibited reduced FVIII antibody titers and impaired T cell activation.<sup>17</sup> Moreover, innovative approaches have been explored to suppress T cell-dependent antibody responses. One example is the use of CRISPR-Cas9-engineered high-affinity FVIII-specific 'decoy' B cells, which cannot differentiate into plasma cells because they lack the transcription factor Blimp1. These 'decoy' B cells outcompete host FVIII-specific B cells in germinal centers by abrogating T cell co-stimulation. As a result, cognate anti-FVIII IgG production is diminished by 6-fold.<sup>18</sup>

Alternatively, polarization of activated CD4 T cells into specific T cell subsets favours non-inhibitory antibody formation.<sup>19</sup> Upon activation, the resulting CD4 T cell subtype is determined by the surrounding cytokine milieu and dictates B cell fate by modulating immunoglobulin class switching, somatic hypermutation, affinity maturation, and differentiation.<sup>7</sup> These four processes are key events for improving the quality and quantity of antibodies and thereby regulate their neutralizing capacity. Class switching is considered an 'extra-follicular' process that occurs after B cell activation at the T-B cell border and is dependent on cross-talk with T cells. Secretion of characteristic T cell subtype-specific cytokines and chemokines instructs B cells to produce specific immunoglobulin isotypes.<sup>19</sup> This shapes the outcome of the humoral response to FVIII. Somatic hypermutation (SHM)

occurs within the GC, where B cells rapidly proliferate in the dark zone and introduce random point mutations into the variable region of the BCR and subsequently antibodies. This process is the initial diversification step that drives affinity maturation and enhances the antigen-binding capacity. Affinity maturation follows SHM in the GC light zone as a selection step. Within that process, B cells with high-affinity BCRs are better equipped to capture antigen from follicular dendritic cells and receive survival signals from Tfh cells, whereas B cells with low affinity are eliminated. This iterative cycle of mutation and selection leads to antibodies with increased affinity. Selected B cells go on to differentiate into long-lived plasma cells to secrete high-affinity antibodies or memory B cells to provide a robust response upon re-exposure.<sup>20</sup> In summary, antibody class and affinity are determined by the quality of T cell help, particularly through germinal center reactions mediated by T follicular helper (Tfh) cells.

The predominance of high-affinity IgG4 antibodies in patients with inhibitors indicates a Th2-skewed, GC-dependent maturation process.<sup>21</sup> Recently, a study has suggested that inhibitor formation can occur independently of a GC reaction following weekly FVIII injections. However, further investigation is necessary to assess the contribution of the extrafollicular response in promoting inhibitor production.<sup>22</sup> This T cell dependence might pave the way for innovative therapeutic approaches to modulate antigen dose and timing, co-stimulation, and the cytokine milieu to steer immune responses away from pathogenic affinity maturation in the context of inhibitors by targeting T cell subsets.

## CD4 T Cell Subsets in FVIII Immunogenicity

### Th1 Cells

Th1 cells, defined by T-box expressed in T cells (T-bet) transcription factor expression and secretion of IFN $\gamma$ , promote cellular immunity and support the production of opsonizing IgG subclasses. In haemophilia models, Th1 responses correlate with lower inhibitor titers and may contribute to protective immunity.<sup>21,23,24</sup> Although IFN $\gamma$  promotes class switching to IgG1 antibodies, the presence of Th1 cells is associated with non-neutralizing antibody responses or low inhibitor titers in HA.<sup>6</sup> Th1 cells have been associated with the early response to FVIII, but not in maintaining long-term antibody synthesis. This indicates that although other T cell subtypes may be more detrimental to inhibitor development, Th1 cells may still play a role in the antibody response to FVIII.<sup>23</sup> Although Th1 dominance is not universally protective, it might skew antibody quality away from high-affinity, neutralizing IgG4 isotypes towards less neutralizing IgGs in high-titer individuals.

### Th2 Cells

Th2 cells, driven by GATA-binding protein 3 (GATA3) and the cytokines IL-4, IL-5 and IL-13, promote humoral immunity and are central to class-switch recombination. IL-4 induces switching to IgG4 in humans (IgG1 in mice) and has been directly associated with the generation of inhibitory anti-

FVIII antibodies.<sup>25</sup> Clinically, a high Th2:Th1 ratio correlates with unfavourable outcomes in HA patients.<sup>21</sup> In murine models, anti-CD3 therapy redirected immune polarization towards Th1 predominance and concomitantly reduced inhibitor formation,<sup>12</sup> supporting the notion that Th2 bias enhances FVIII immunogenicity. It has since been proposed that some FVIII-responsive cells previously categorized as Th2 cells may, in fact, have been Tfh cells, as both express IL-4.<sup>26</sup> This historical mislabelling underscores the need for precise phenotyping when interpreting legacy datasets.

### Th17 Cells

Th17 cells, characterized by RAR-related orphan receptor gamma t (ROR $\gamma$ t) expression and secretion of IL-17A, IL-6 and IL-21, bridge inflammation and adaptive immunity. The presence of Th17 cells seems to promote inhibitor production in the initial stage of the response.<sup>23</sup> Furthermore, Th17-derived IL-6 and IL-21 can additionally enhance GC formation and antibody affinity maturation.<sup>19</sup> The IL-21-STAT3 pathway is particularly relevant in that process. It enhances Tfh differentiation and sustains B cell help, thereby indirectly supporting inhibitor development.<sup>19</sup> Moreover, elevated IL-6 levels have been associated with inhibitor risk and may serve as prognostic indicators.<sup>27</sup> Thus, dampening the Th-17-associated IL-6/IL-21 axis at the right time point might curtail the generation of high-affinity neutralizing antibodies.

### Follicular Helper T Cells (Tfh)

Tfh cells are specialized providers of B cell help within GCs. Defined by CXCR5, PD-1, Bcl-6, ICOS and CD40L expression, they secrete IL-21, IL-4 and CXCL13, which attract B cells and promote SHM and affinity maturation.<sup>19</sup> FVIII immunization in HA mice increases Tfh frequencies in parallel with GC expansion and inhibitor persistence.<sup>28</sup> Tfh differentiation requires IL-6 and IL-21 signalling as well as suppression of IL-2, which otherwise inhibits Bcl-6 expression.<sup>19</sup> Thus, cytokine balance between IL-2 and IL-21 is critical to control the magnitude of FVIII-specific responses and might offer a way to fine tune the immune reaction. Clinically, chemokines such as CXCL13, the ligand for CXCR5 and essential for organizing the GC structure, may serve as soluble biomarkers of active GC help.<sup>29</sup> This provides a non-invasive window into the immunologic state during therapy.

### Regulatory T Cells (Tregs)

Tregs are characterized by expression of the transcription factor Forkhead Box P3 (FOXP3). It was observed that Treg transfer prevented inhibitor formation, demonstrating that they are essential to diminish inhibitor responses and sustain tolerance to FVIII.<sup>30</sup> Tregs modulate effector cells by utilizing sophisticated, multifaceted approaches that include contact-dependent and -independent mechanisms. The latter includes secretion of inhibitory cytokines, such as IL-10 and TGF- $\beta$ , which have been shown to suppress the activation of other T cells or APCs. Additionally, Tregs can starve cells of essential growth factors, for example, by sequestering IL-2 via the IL-2 receptor (CD25). Moreover, they can create a toxic metabolic environment by converting extracellular ATP

into adenosine, which delivers a potent inhibitory signal to effector T cells. Tregs can also directly induce death in target cells via cytotoxic mechanisms such as perforin/granzyme-mediated killing. Furthermore, through contact-dependent mechanisms, Tregs express checkpoint molecules that inhibit immune cell activation by competing with co-stimulatory signals.<sup>31</sup> FVIII-specific natural Tregs (nTregs) exist in healthy individuals,<sup>32</sup> indicating physiological tolerance, while induced Tregs (iTregs) can develop following high-dose FVIII administration.<sup>33</sup> In HA mice, repeated FVIII infusions expand FVIII-specific induced Tregs (iTregs) that suppress B cell activation via PD-1/PD-L1 signalling.<sup>33</sup> Moreover, oral application of FVIII has revealed an alternative expansion of latency-associated peptide positive (LAP<sup>+</sup>) Tregs. These cells were able to prevent inhibitor formation, suggesting that mucosal delivery may be a more effective route to promote tolerance, pending further investigation.<sup>34</sup> Loss of Treg function or numerical imbalance may facilitate inhibitor formation. Thus, treatments aiming to induce Tregs and maintain their stability may incur long-term tolerance to FVIII. The incorporation of suicide genes into Tregs may enable their controlled elimination, thereby mitigating potential adverse events and ensuring patient safety. This is particularly important, as global immunosuppression can promote tumorigenesis and heighten patient susceptibility to infection.

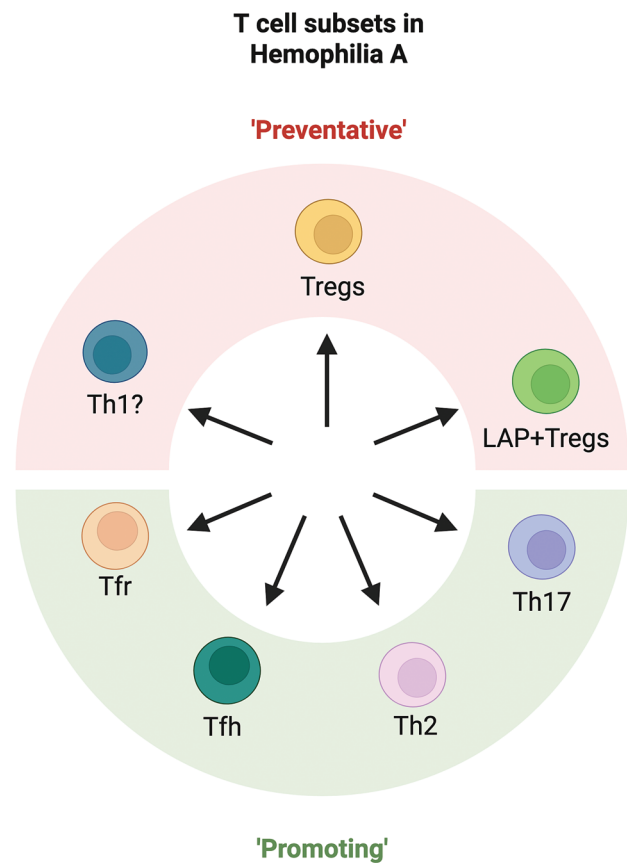
#### Follicular Regulatory T Cells (Tfr)

Tfr cells share phenotypic features of both Tfh and Treg lineages (CXCR5<sup>+</sup>FOXP3<sup>+</sup>) and regulate GC reactions. Although generally thought to suppress auto-antibody formation,<sup>35</sup> emerging data suggest that Tfr cells enhance FVIII-specific responses. FVIII immunization promotes Tfr activation and expansion and is correlated with inhibitor titers in HA mice, whereas the depletion of Tfr cells mitigates inhibitor production.<sup>36</sup> Possible mechanisms might include sequestration of IL-2 to sustain Tfh survival and modulation of antigen presentation within GCs; this warrants further investigation.<sup>35</sup> Furthermore, to effectively restrain Tfr function, it is essential to elucidate the origin of Tfr cells. Specifically, further studies are needed to clarify whether they derive from Tfh or Treg lineages and to define the extent of their phenotypic and functional plasticity. Tfr cells seem to play a role in determining FVIII immunogenicity, and although difficult to achieve, a cell type-specific depletion might provide a future therapy goal.

In conclusion, modulating FVIII-specific T cell subsets by favouring and stabilizing tolerogenic phenotypes (→Fig. 1) is a key goal for future therapy to avoid inhibitor onset or improve inhibitor eradication.

#### Immune Tolerance Induction as a Strategy to Modulate T Cell Responses

Nowadays, immune tolerance induction (ITI) is the standard clinical approach to eradicate inhibitors by repeated high-dose FVIII administration. Its mechanism is multifactorial and includes the deletion of FVIII-specific memory B cells through (i) induction of apoptosis via Fas-FasL pathways<sup>37</sup> and (ii) expansion of antigen-specific Tregs.<sup>33</sup> In addition, the



**Fig. 1** CD4 T cell subsets and their role in modulating the inhibitor response in haemophilia A. Several CD4 T cell subsets have been implicated in shaping the immune response to FVIII. Preventative subsets, including regulatory T cells (Tregs) and potentially Th1 cells, promote immune tolerance by suppressing FVIII-specific effector responses and maintaining peripheral immune regulation. In contrast, Th2, T follicular helper (Tfh), Th17, and T follicular regulatory (Tfr) cells are associated with the stimulation of B cell activation and antibody production, thereby contributing to the development and persistence of FVIII inhibitors. The overall outcome, tolerance versus inhibitor formation, depends on the dynamic balance between these regulatory and effector CD4 T cell subsets. (Created with BioRender.com.)

treatment favours differentiation of tolerogenic PD-L1<sup>+</sup> IDO<sup>+</sup> dendritic cells, which further promotes Treg differentiation.<sup>38</sup> Treg-mediated initiation of B cell apoptosis via PD-L1 has emerged as a key mechanism underlying tolerance induction in mice and humans, indicating that the PD-1–PD-L1 axis could be a valuable target for therapy improvement.<sup>33</sup> In clinical practice, ITI success and durability are influenced by baseline inhibitor titers, the time from inhibitor detection to ITI and concomitant inflammation.<sup>39</sup> These factors likely modulate the same cellular pathways. Overall, successful ITI correlates with increased frequencies of FVIII-specific Tregs and a concomitant reduction in effector cytokines.<sup>40</sup> Consequently, further studies are required to elucidate the mechanisms underlying durable tolerance and to develop strategies that sustain long-term immune regulation in HA. Pragmatically, pairing antigen exposure with environment-shaping interventions (e.g., Treg-favouring cytokine support) may prove necessary to drive and maintain tolerance in difficult-to-treat patients.

## Translational Perspectives for Intervention

### Cytokine Regulation

Application of an IL-2/anti-IL-2 monoclonal antibody complex markedly reduced FVIII antibody titers, primarily through a 7-fold expansion of Tregs. In contrast, depletion of Tregs with anti-CD25 antibodies targeting the IL-2 receptor  $\alpha$ -chain abolished this protective effect.<sup>41</sup> At the same time, IL-2 signalling can suppress the differentiation of Tfh cells,<sup>42</sup> which may be detrimental in patients with concurrent infections due to broad inhibition of humoral immune responses. To mitigate these effects, the approach has recently been optimized to selectively target IL-2 signalling in Tregs, thereby minimizing off-target modulation of other lymphocyte subsets.<sup>43</sup>

Selectively increasing IL-10 or TGF- $\beta$  production in FVIII-specific Tregs may dampen APC activation and foster induction of additional Tregs. Additionally, IFN $\gamma$  could potentially oppose Th2 dominance and shift the immune response towards non-inhibitory antibody production. Diminishing GC reactions by restraining IL-21, which is indispensable for high-affinity antibody production,<sup>19</sup> might be an alternative strategy. Targeting cytokines that modulate tolerance to FVIII is conceptually attractive. However, these interventions must be directed to FVIII-specific immunity to prevent systemic suppression.

### Inflammation Control and Cell-based Tolerance

Reducing inflammatory stimuli following FVIII administration has been observed to enhance tolerance. Dexamethasone induced long-lasting effects (up to 18 weeks) in E17KO<sup>h</sup>MHC mice by decreasing FVIII-specific B cells and increasing FOXP3<sup>+</sup>CD4<sup>+</sup> thymic T cells.<sup>44</sup> Transfer of tolero-

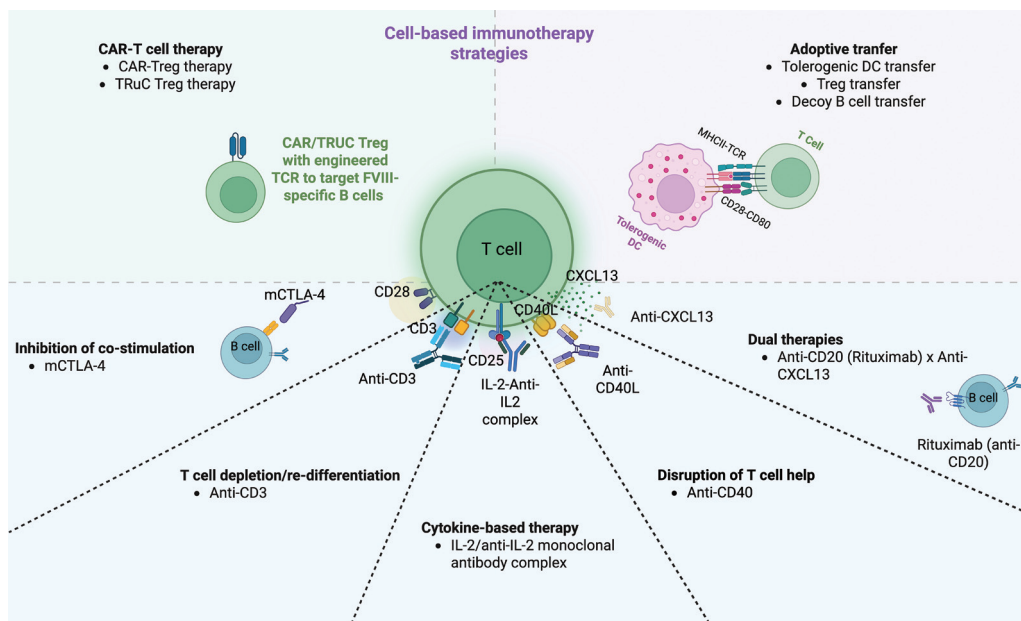
genic dendritic cells likewise promoted inhibitory T cells that suppress humoral responses to FVIII.<sup>45</sup> More broadly, anti-CD3 treatment induces T cell anergy and promotes iTreg differentiation,<sup>12,46</sup> while rapamycin favours metabolic reprogramming towards Treg stability and diminishes effector T cell responses.<sup>47</sup> Rituximab (anti-CD20), although effective for B cell depletion, spares long-lived plasma cells. Combined with anti-CXCL13, it reduced Tfh cells, enhanced CD25<sup>+</sup> Treg expansion and lowered anti-FVIII levels in HA mice.<sup>29</sup> Nevertheless, despite a mechanistic appeal, many of these approaches lack antigen specificity and can cause broad immunosuppression. Thus, they should be applied exclusively in the short term.

### Costimulatory Checkpoints

Modulation of T cell pathways can also be achieved by targeting costimulatory molecules such as CD28, ICOS and CD40L, which synergize to promote T cell activation. Moreover, CTLA-4 engagement has been shown to reduce the initial response to FVIII. It also prevents subsequent increases in anti-FVIII antibody titers by directly blocking CD80/CD86 on B cells and other APCs. This blockage disrupts their interaction with T cells and suppresses immune activation.<sup>48</sup> These pathways highlight actionable checkpoints where short, well-timed interventions might tip the balance towards tolerance without incurring prolonged systemic immunosuppression.

### Adoptive Regulatory Cell Therapy

Although promising, most interventions induce broad immunosuppression. Antigen-specific Tregs thus represent a precision alternative. High-affinity FVIII-specific CAR-Tregs have been engineered to suppress the FVIII-directed



**Fig. 2** Potential therapeutic strategies to manage inhibitor formation in haemophilia A. This diagram illustrates conceptual mechanisms through which the FVIII-specific immune response can be modulated to prevent or eradicate inhibitor development. Approaches include strategies that suppress or reprogramme FVIII-specific effector CD4 T cells, enhance regulatory T cell (Treg) activity, promote tolerogenic antigen presentation or exploit immune checkpoint pathways to restore tolerance. Collectively, these interventions aim to rebalance helper and regulatory T cell functions, thereby mitigating anti-FVIII antibody production and supporting durable immune tolerance. (Created with BioRender.com.)

response. Yet in vivo, they showed loss of suppressive function, likely due to transcriptional instability of the regulatory programme.<sup>49</sup> Modifying intracellular signalling motifs or forcing IL-10 expression did not restore durable suppression.<sup>49</sup> To improve phenotypic integrity and control activation thresholds, a single-chain variable fragment (scFv) was fused to TCR signalling domains to generate a TCR-fusion construct (TRuC). This design aims to stabilize Treg signalling and to prevent re-differentiation into effector phenotypes.<sup>49</sup> Building on this concept, Doglio et al. developed CXCR5<sup>+</sup> FVIII-specific TRuC- and CAR-Tregs capable of homing to secondary lymphoid organs, where GC responses to FVIII occur. This approach enhanced local immunoregulation while reducing off-target effects. Future clinical trials will be essential to assess their safety, stability and therapeutic efficacy.<sup>50</sup> Incorporating lymphoid-homing cues and Treg-stabilizing circuits may be decisive for translating these platforms into the clinic.

## Outlook and Conclusion

T cell subsets are central to the immune response against FVIII. Their differentiation and interaction with B cells determine whether FVIII exposure results in inhibitor formation or tolerance. The intricate cytokine networks, particularly IL-2, IL-6, IL-10, IL-21 and TGF- $\beta$ , act as molecular switches shaping these outcomes. Future research should elucidate how the spatial organization of Tfh-B cells and Treg-APC interactions within lymphoid tissues governs FVIII immunogenicity. Understanding these mechanisms will improve next-generation therapies that aim for antigen-specific immune modulation (**-Fig. 2**). Overall, durable tolerance to FVIII in HA might become an achievable therapeutic reality by (i) aligning the dose, timing and inflammatory context with the patients' genetic risk, (ii) by restoring the balance between effector and regulatory T cell pathways, and (iii) by increasing the specificity and stability of Tregs.

### Conflict of Interest

The authors declare that they have no conflict of interest.

### Acknowledgements

The study was funded by the Deutsche Forschungsgemeinschaft (German Research Foundation) grants Excellence strategy EXC2151 project number 390873048. The graphical summary was created with BioRender.com.

## References

- Schep SJ, Schutgens REG, Fischer K, Boes ML. Review of immune tolerance induction in hemophilia A. *Blood Rev* 2018;32(04):326–338
- Astermark J. FVIII inhibitors: pathogenesis and avoidance. *Blood* 2015;125(13):2045–2051
- Lacroix-Desmazes S, Voorberg J, Lillicrap D, Scott DW, Pratt KP. Tolerating factor VIII: recent progress. *Front Immunol* 2020;10:2991
- Whelan SFJ, Hofbauer CJ, Horling FM, et al. Distinct characteristics of antibody responses against factor VIII in healthy individuals and in different cohorts of hemophilia A patients. *Blood* 2013;121(06):1039–1048
- Yousphi AS, Bakhtiar A, Cheema MA, Nasim S, Ullah W. Acquired hemophilia A: a rare but potentially fatal bleeding disorder. *Cureus* 2019;11(08):e5442
- Hofbauer CJ, Whelan SFJ, Hirschler M, et al. Affinity of FVIII-specific antibodies reveals major differences between neutralizing and nonneutralizing antibodies in humans. *Blood* 2015;125(07):1180–1188
- Sun L, Su Y, Jiao A, Wang X, Zhang B. T cells in health and disease. *Signal Transduct Target Ther* 2023;8(01):235
- Singer ST, Addiego JE Jr, Reason DC, Lucas AH. T lymphocyte proliferative responses induced by recombinant factor VIII in hemophilia A patients with inhibitors. *Thromb Haemost* 1996;76(01):17–22
- Bray GL, Kroner BL, Arkin S, et al. Loss of high-responder inhibitors in patients with severe hemophilia A and human immunodeficiency virus type 1 infection: a report from the Multi-Center Hemophilia Cohort Study. *Am J Hematol* 1993;42(04):375–379
- Meunier S, Menier C, Marcon E, Lacroix-Desmazes S, Maillère B. CD4 T cells specific for factor VIII are present at high frequency in healthy donors and comprise naïve and memory cells. *Blood Adv* 2017;1(21):1842–1847
- Qian J, Borovok M, Bi L, Kazazian HH Jr, Hoyer LW. Inhibitor antibody development and T cell response to human factor VIII in murine hemophilia A. *Thromb Haemost* 1999;81(02):240–244
- Waters B, Qadura M, Burnett E, et al. Anti-CD3 prevents factor VIII inhibitor development in hemophilia A mice by a regulatory CD4<sup>+</sup>CD25<sup>+</sup>-dependent mechanism and by shifting cytokine production to favor a Th1 response. *Blood* 2009;113(01):193–203
- Navarrete A, Dasgupta S, Delignat S, et al. Splenic marginal zone antigen-presenting cells are critical for the primary allo-immune response to therapeutic factor VIII in hemophilia A. *J Thromb Haemost* 2009;7(11):1816–1823
- Kaczmarek R, Piñeros AR, Patterson PE, et al. Factor VIII trafficking to CD4<sup>+</sup> T cells shapes its immunogenicity and requires several types of antigen-presenting cells. *Blood* 2023;142(03):290–305
- Steinitz KN, van Helden PM, Binder B, et al. CD4<sup>+</sup> T-cell epitopes associated with antibody responses after intravenously and subcutaneously applied human FVIII in humanized hemophilic E17 HLA-DRB1\*1501 mice. *Blood* 2012;119(17):4073–4082
- Shah K, Al-Haidari A, Sun J, Kazi JU. T cell receptor (TCR) signaling in health and disease. *Signal Transduct Target Ther* 2021;6(01):412
- Rossi G, Sarkar J, Scandella D. Long-term induction of immune tolerance after blockade of CD40-CD40L interaction in a mouse model of hemophilia A. *Blood* 2001;97(09):2750–2757
- Pitner RA, Chao JL, Dahl NP, et al. Blunting specific T-dependent antibody responses with engineered “decoy” B cells. *Mol Ther* 2024;32(10):3453–3469
- Olatunde AC, Hale JS, Lamb TJ. Cytokine-skewed Tfh cells: functional consequences for B cell help. *Trends Immunol* 2021;42(06):536–550
- Mesin L, Ersching J, Victora GD. Germinal center B cell dynamics. *Immunity* 2016;45(03):471–482
- Reding MT, Lei S, Lei H, Green D, Gill J, Conti-Fine BM. Distribution of Th1- and Th2-induced anti-factor VIII IgG subclasses in congenital and acquired hemophilia patients. *Thromb Haemost* 2002;88(04):568–575
- Patel SR, Lundgren TS, Baldwin WH, et al. Neutralizing antibodies against factor VIII can occur through a non-germinal center pathway. *Front Immunol* 2022;13:880829
- Ettinger RA, James EA, Kwok WW, Thompson AR, Pratt KP. Lineages of human T-cell clones, including T helper 17/T helper 1 cells, isolated at different stages of anti-factor VIII immune responses. *Blood* 2009;114(07):1423–1428

- 24 Hu GL, Okita DK, Diethelm-Okita BM, Conti-Fine BM. Recognition of coagulation factor VIII by CD4<sup>+</sup> T cells of healthy humans. *J Thromb Haemost* 2003;1(10):2159–2166
- 25 Wu H, Reding M, Qian J, et al. Mechanism of the immune response to human factor VIII in murine hemophilia A. *Thromb Haemost* 2001;85(01):125–133
- 26 Crotty S. T follicular helper cell biology: a decade of discovery and diseases. *Immunity* 2019;50(05):1132–1148
- 27 Fan MN, Shen T, Konkle BA, et al. Exploration of biomarkers for inhibitor development in persons with hemophilia A. *Res Pract Thromb Haemost* 2025;9(04):102877
- 28 Jing W, Chen J, Cai Y, et al. Induction of activated T follicular helper cells is critical for anti-FVIII inhibitor development in hemophilia A mice. *Blood Adv* 2019;3(20):3099–3110
- 29 Zheng Q, Lin K, Zhang N, Shi Q, Wu Y, Chen Y. Anti-mCD20 in combination with  $\alpha$ -mCXCL13 monoclonal antibody inhibits anti-FVIII antibody development in hemophilia A mice. *Int Immunopharmacol* 2024;139:112735
- 30 Miao CH, Harmeling BR, Ziegler SF, et al. CD4<sup>+</sup>FOXP3<sup>+</sup> regulatory T cells confer long-term regulation of factor VIII-specific immune responses in plasmid-mediated gene therapy-treated hemophilia mice. *Blood* 2009;114(19):4034–4044
- 31 Dikiy S, Rudensky AY. Principles of regulatory T cell function. *Immunity* 2023;56(02):240–255
- 32 Menier C, Meunier S, Porcheddu V, et al. Frequency of natural regulatory T cells specific for factor VIII in the peripheral blood of healthy donors. *Eur J Immunol* 2024;54(04):e2350506
- 33 Becker-Gotot J, Meissner M, Kotov V, et al. Immune tolerance against infused FVIII in hemophilia A is mediated by PD-L1<sup>+</sup> Tregs. *J Clin Invest* 2022;132(22):e159925
- 34 Kwon KC, Sherman A, Chang WJ, et al. Expression and assembly of largest foreign protein in chloroplasts: oral delivery of human FVIII made in lettuce chloroplasts robustly suppresses inhibitor formation in haemophilia A mice. *Plant Biotechnol J* 2018;16(06):1148–1160
- 35 Sage PT, Sharpe AH. The multifaceted functions of follicular regulatory T cells. *Curr Opin Immunol* 2020;67:68–74
- 36 Jing W, Schroeder JA, Kumar S, et al. The T follicular helper/T follicular helper regulatory pathway in FVIII immune responses in mice. *Blood* 2025;146(08):998–1010
- 37 Hausl C, Ahmad RU, Sasgary M, et al. High-dose factor VIII inhibits factor VIII-specific memory B cells in hemophilia A with factor VIII inhibitors. *Blood* 2005;106(10):3415–3422
- 38 Qadura M, Othman M, Waters B, et al. Reduction of the immune response to factor VIII mediated through tolerogenic factor VIII presentation by immature dendritic cells. *J Thromb Haemost* 2008;6(12):2095–2104
- 39 Antun A, Monahan PE, Manco-Johnson MJ, et al. Inhibitor recurrence after immune tolerance induction: a multicenter retrospective cohort study. *J Thromb Haemost* 2015;13(11):1980–1988
- 40 Wang X, Terhorst C, Herzog RW. In vivo induction of regulatory T cells for immune tolerance in hemophilia. *Cell Immunol* 2016;301:18–29
- 41 Liu CL, Ye P, Lin J, Djukovic D, Miao CH. Long-term tolerance to factor VIII is achieved by administration of IL-2/IL-2mAb complexes and low dosages of factor VIII. *J Thromb Haemost* 2014;12(06):921
- 42 Ballesteros-Tato A, León B, Graf BA, et al. Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation. *Immunity* 2012;36(05):847–856
- 43 Rana J, VanDyke D, Muñoz-Melero M, et al. An engineered Treg selective immunocytokine induces sustained immune modulation in a preclinical model of hemophilia A. *J Thromb Haemost* 2025;23(06):1800–1809
- 44 Georgescu MT, Moorehead PC, van Velzen AS, et al. Dexamethasone promotes durable factor VIII-specific tolerance in hemophilia A mice via thymic mechanisms. *Haematologica* 2018;103(08):1403–1413
- 45 Su RJ, Epp A, Feng J, et al. Suppression of the immune response to FVIII in hemophilia A mice by transgene modified tolerogenic dendritic cells. *Mol Ther* 2011;19(10):1896–1904
- 46 Peng B, Ye P, Rawlings DJ, Ochs HD, Miao CH. Anti-CD3 antibodies modulate anti-factor VIII immune responses in hemophilia A mice after factor VIII plasmid-mediated gene therapy. *Blood* 2009;114(20):4373–4382
- 47 Moghimi B, Sack BK, Nayak S, Markusic DM, Mah CS, Herzog RW. Tolerance induction to factor VIII by transient co-administration with rapamycin. *J Thromb Haemost* 2011;9(08):1524
- 48 Qian J, Collins M, Sharpe AH, Hoyer LW. Prevention and treatment of factor VIII inhibitors in murine hemophilia A. *Blood* 2000;95(04):1324–1329
- 49 Rana J, Perry DJ, Kumar SRP, et al. CAR- and TRuC-redireted regulatory T cells differ in capacity to control adaptive immunity to FVIII. *Mol Ther* 2021;29(09):2660–2676
- 50 Doglio M, Rana J, Stucchi A, et al. CXCR5 engineered human and murine Tregs for targeted suppression in secondary and tertiary lymphoid organs. *Front Immunol* 2025;16:1513009