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Medicines and Healthcare Technologies Department  
UMR 0496

FVIII-specific regulatory and conventional CD4  
T cells share common epitopes in healthy  
individuals.

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# Examples of immunogenic Self: low abundant proteins

Examples of Immunogenic Proteins					
Protein Category	Protein	Type and Producer Cells	Binding Antibodies	Neutralizing Antibodies	Clinical Consequences
Nonhuman	Insulin	Natural	Yes	Yes	Loss of efficacy uncommon
Human	Glucocerebrosidase	Natural	Yes	Yes	Loss of efficacy
	Factor VIII	Natural	Yes	Yes	Loss of efficacy
	Follicle-stimulating hormone	Natural	No	No	—
Homologous to native protein	IFN- $\alpha$ 2a	rDNA	Yes	Yes	Loss of efficacy
	GM-CSF	rDNA	Yes	Yes	Loss of efficacy
		rDNA	Yes	No	No loss of efficacy
	G-CSF	rDNA	No	No	No loss of efficacy
	IFN- $\beta$	rDNA	Yes	Yes	Loss of efficacy
	Epo	rDNA	Yes	Yes	Cross-reacted with endogenous protein and caused adverse effects
	IL-2	rDNA	Yes	Yes	Loss of efficacy
Sequence variants	IFN- $\beta$	rDNA	Yes	Yes	Loss of efficacy
	IFN- $\alpha$ Con 1	rDNA	Yes	No	Loss of efficacy not reported

- **EPO and PRCA:** autoimmune disease (Casadevall, 2002 & 2007)
- **IFN- $\beta$ :** in multiple sclerosis (Peh et al, 2019)
- **IFN- $\alpha$ :** HCV patients (Scagnolari et al 2013)
- **GM-CSF:** colon cancer (Raghammar, Blood, 1994)

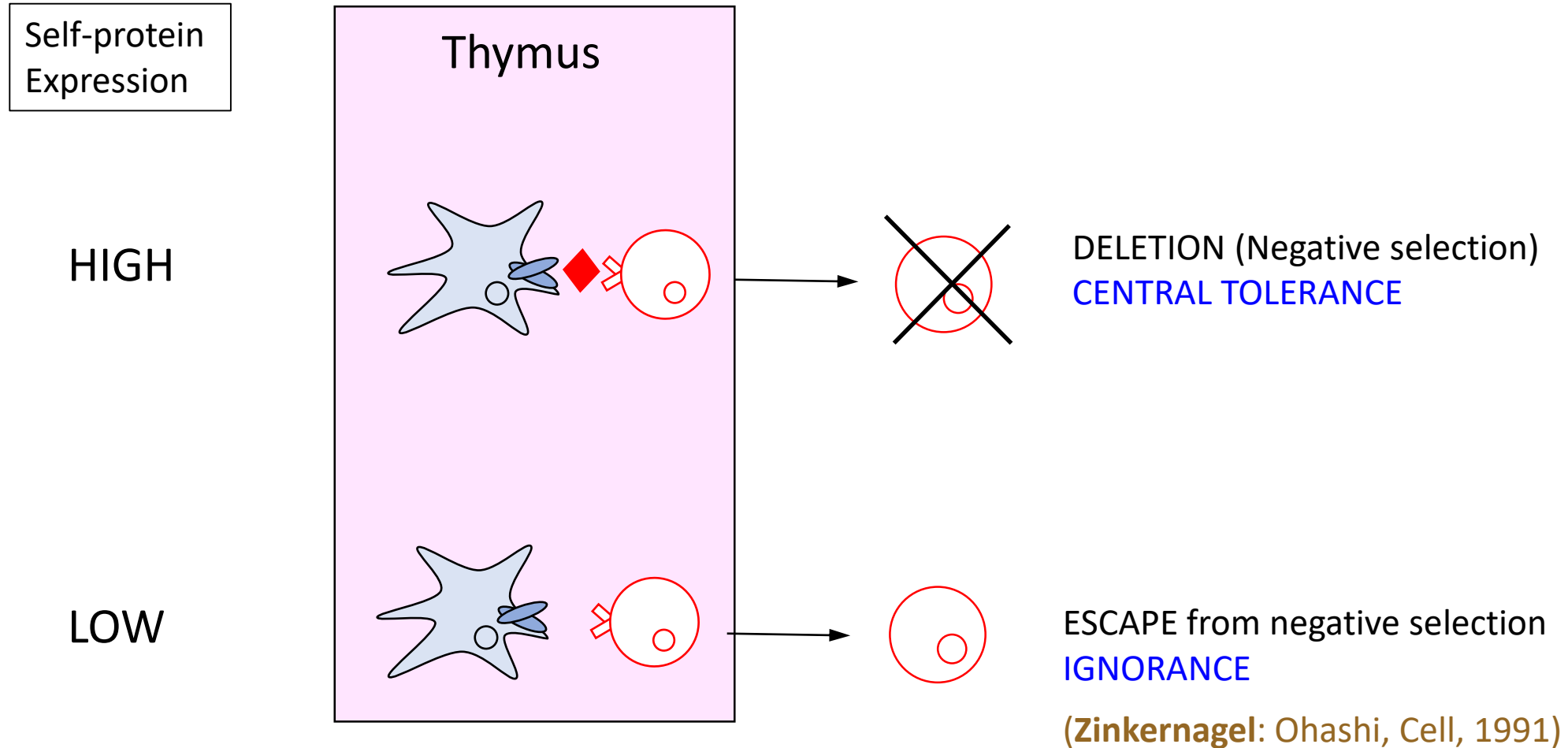
- **Spontaneous onset**

(not linked to protein infusion)

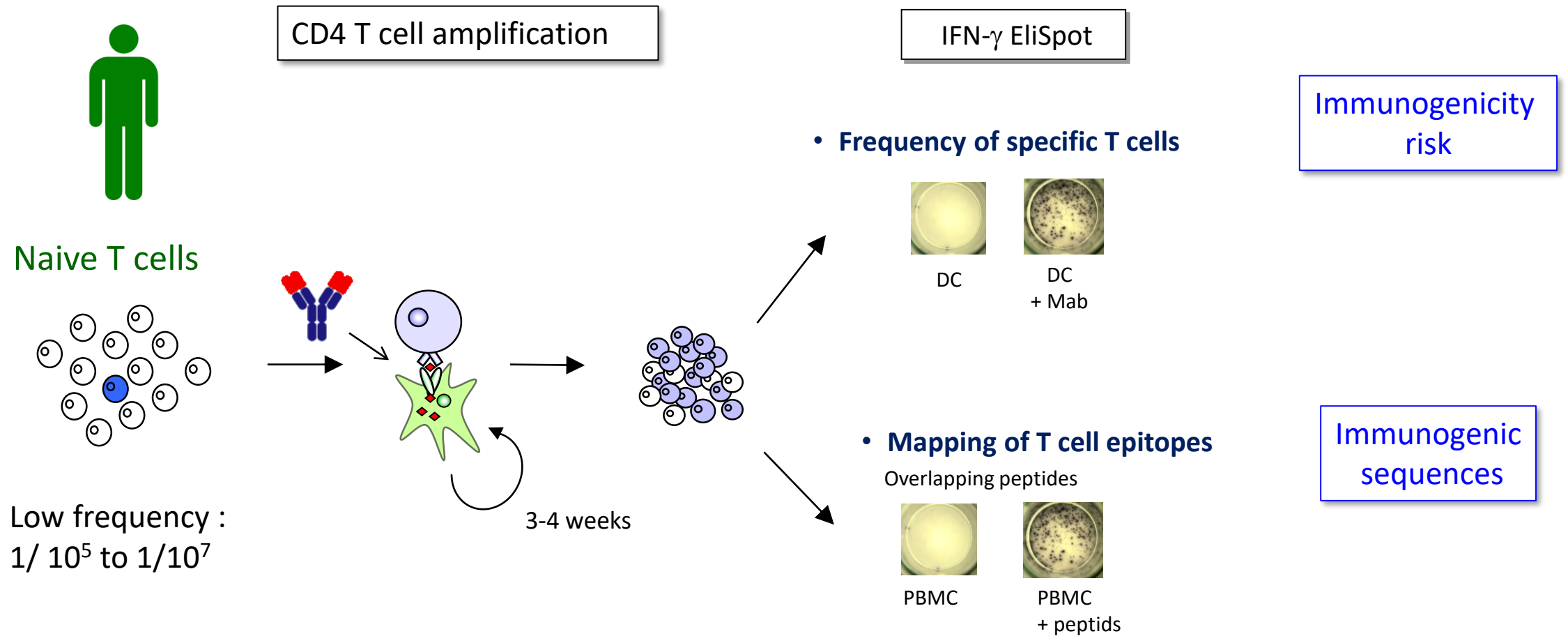
- **IFN- $\alpha$  :** Critical pneumonia Covid-19 (Zhang et al, JEM, 2022)
- **IFN- $\gamma$  :** Mycobacterial infection (Shih et al, JEM, 2022)
- **GM-CSF:** Pulmonary alveolar proteinosis (Ushida, Blood, 2004)

Wardhwa and Thorpe, 2007

# Self expression and T cell selection

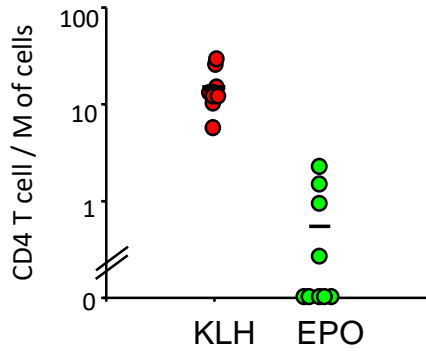


# T cell amplification assay



# CD4 T cells specific for low abundant self-proteins

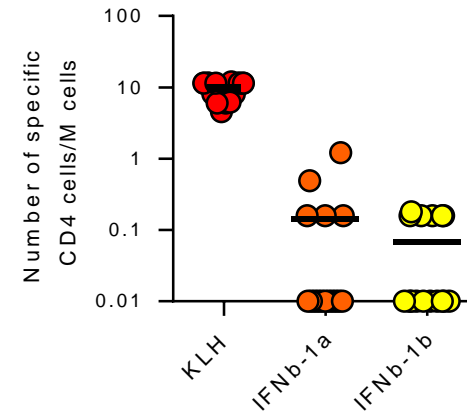
## EPO



- Altered batches of Epo
- Rare events of PRCA

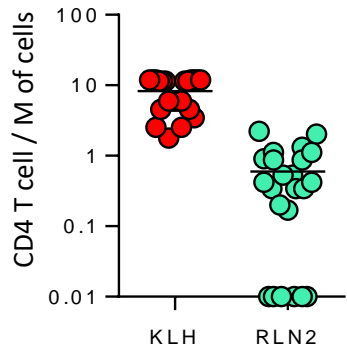
Delluc et al, Blood, 2010

## IFN- $\beta$



- Approved drugs in multiple sclerosis
- IFN- $\beta$ -1a: 2-28% ADA  
IFN- $\beta$ -1b : 28-47% ADA
- T cell detected but at the same level

## H2-Relaxin



- insulin-like structure
- Anti-inflammatory properties
- Multiple injections lead to ADA (Seabold, 2000)

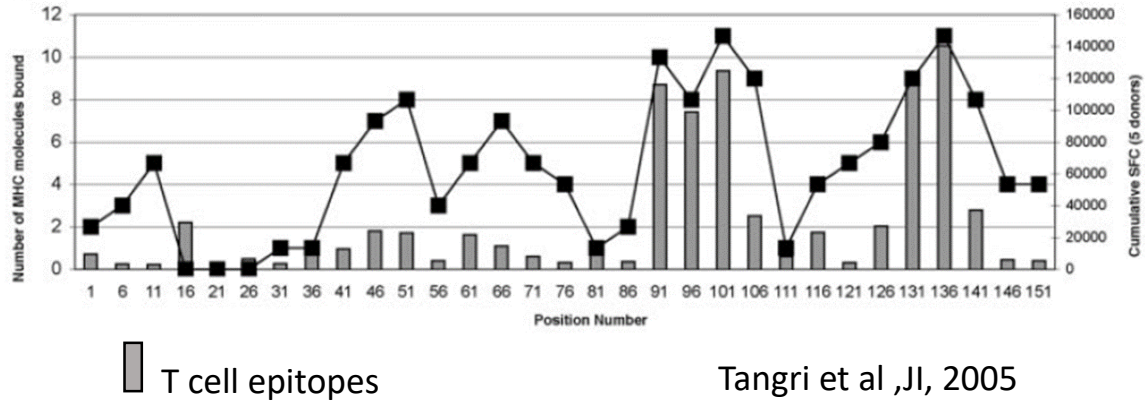
Azam et al , JI, 2019



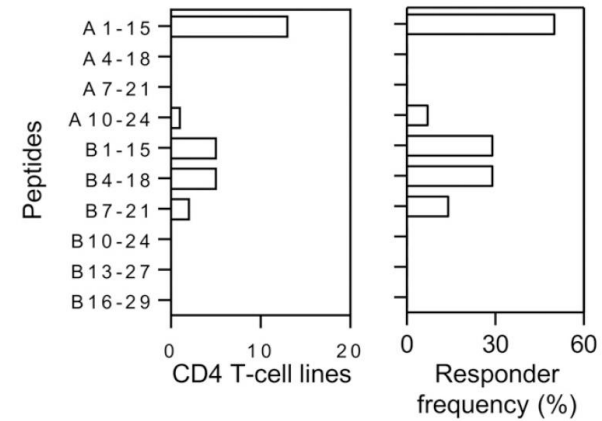
T cells specific for immunogenic proteins are detected in the repertoire of healthy donors

# T cell epitopes of low abundant biopharmaceuticals

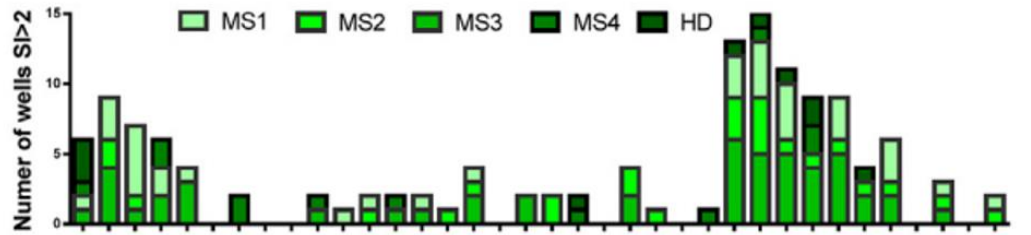
## EPO



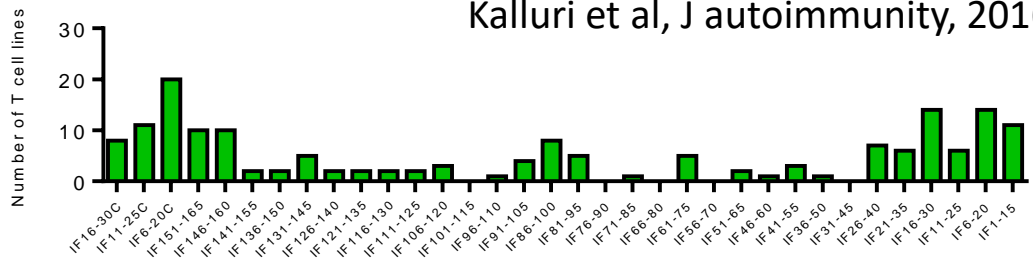
## H2-Relaxin



## IFN-β



Kalluri et al, J autoimmunity, 2016

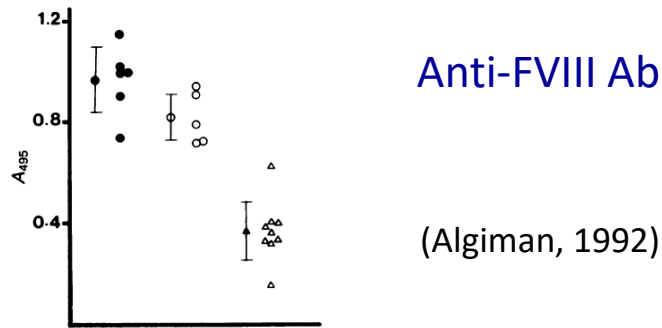


Maillere et al, unpublished

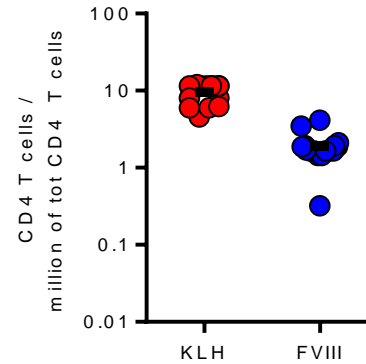
T cell epitopes of low abundant biopharmaceuticals are **not mutated**

# Immunogenicity and T cell response to FVIII in healthy donors

- Immunogenicity of FVIII
  - Important issue for HA patients
  - Immunogenicity : Severe HA (30%) >> moderate
  - endogenous FVIII expression
- Healthy donors:
  - 20% anti-FVIII antibodies (Wheelan, 2013)
  - Acquired hemophilia



## Factor VIII



T cells specific for FVIII are detected in the repertoire of healthy donors

Meunier et al, Blood Adv, 2017

frontiers | Frontiers in Immunology

The self-reactive FVIII T cell repertoire in healthy individuals relies on a short set of epitopes and public clonotypes

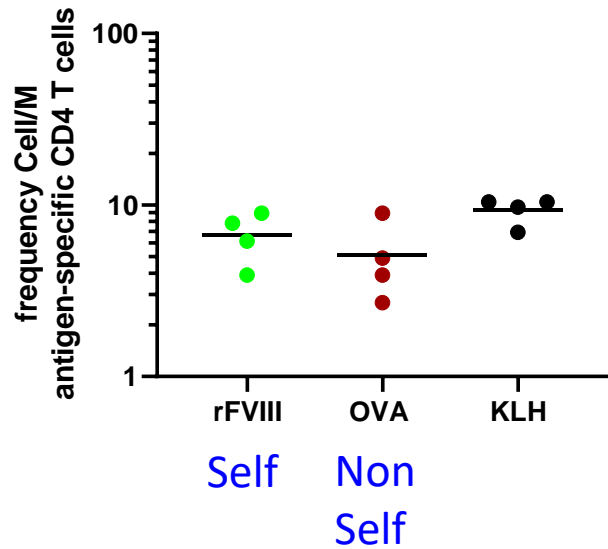
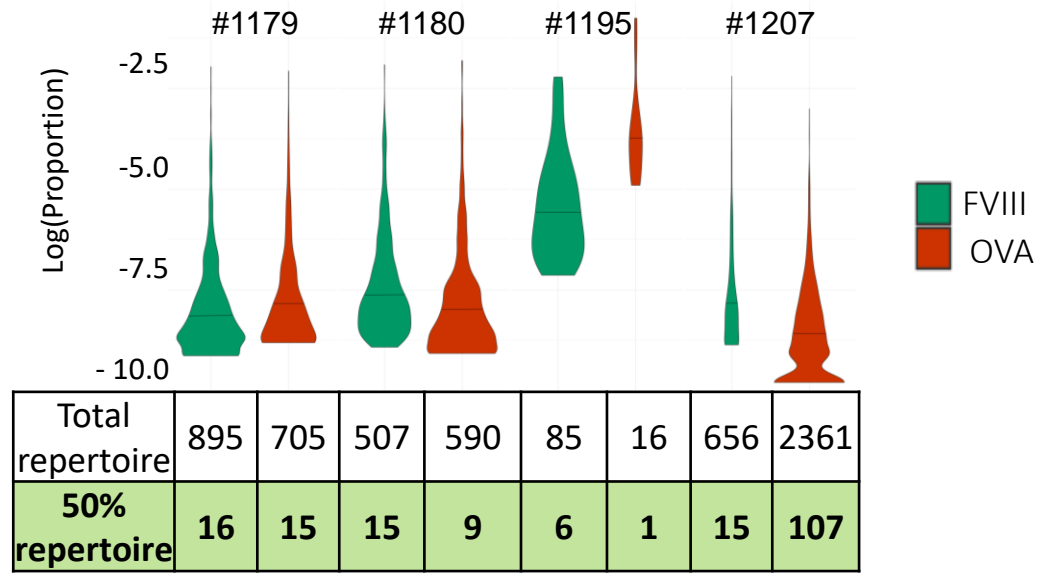
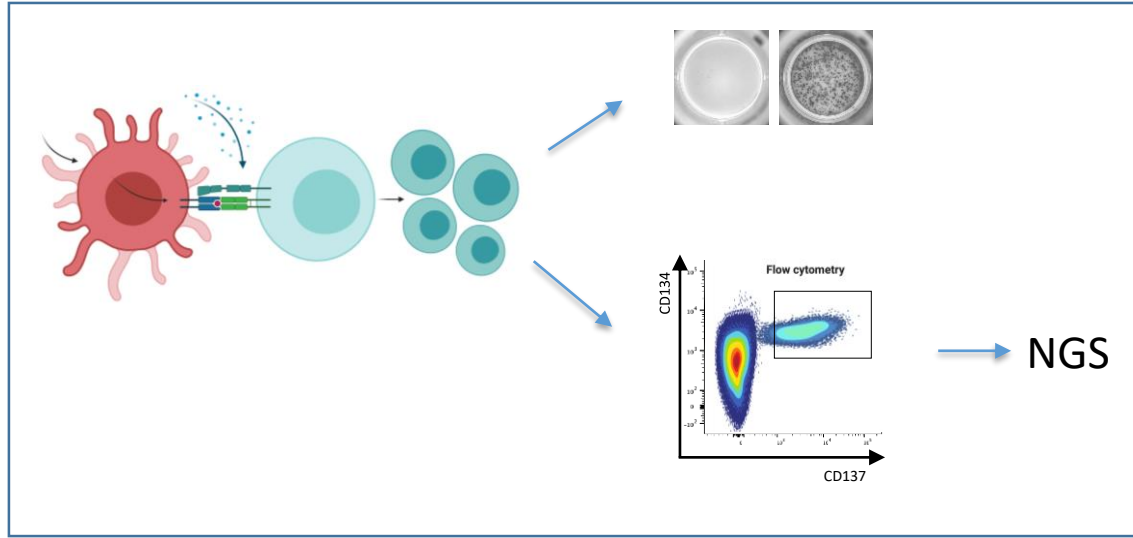
Valeria Porcheddu\*, Gautier Lhomme, Rémi Giraudet, Evelyne Correia and Bernard Maillère

Patient type	Sample size	Prevalence of positive individuals, % (95% CI)
Healthy	600	19 (16-22)
Severe hemophilia A without inhibitor (HA-no/INH)	77	34 (24-45)
Severe hemophilia A after successful ITI (HA-ITI)	23	39 (22-59)
Severe hemophilia A with inhibitor (HA-INH)	20	100 (84-100)
Acquired hemophilia A (Acqu-HA)	9	100 (70-100)

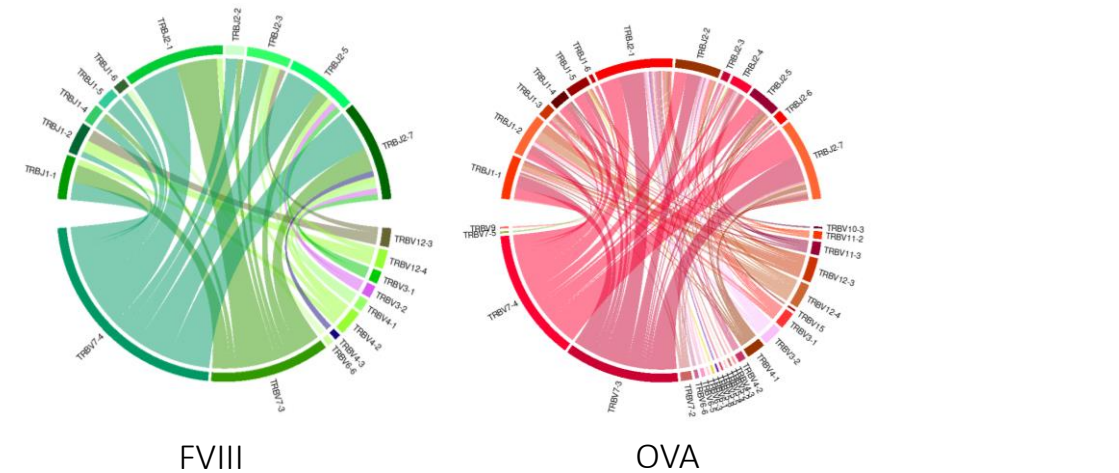
(wheelan, 2013)



# Comparison of the T cell repertoires specific for OVA et FVIII

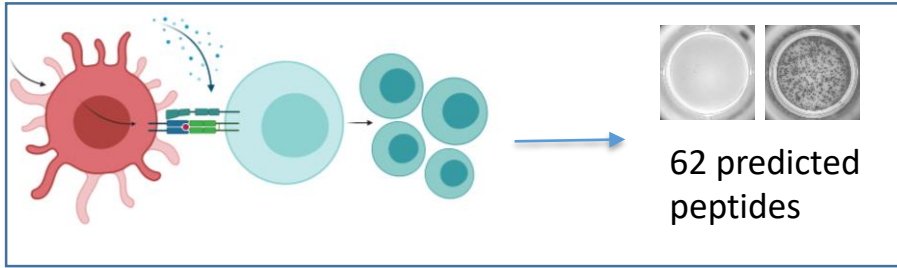


FVIII and Ova specific T cells exhibit similar cell frequencies and clonotype distribution

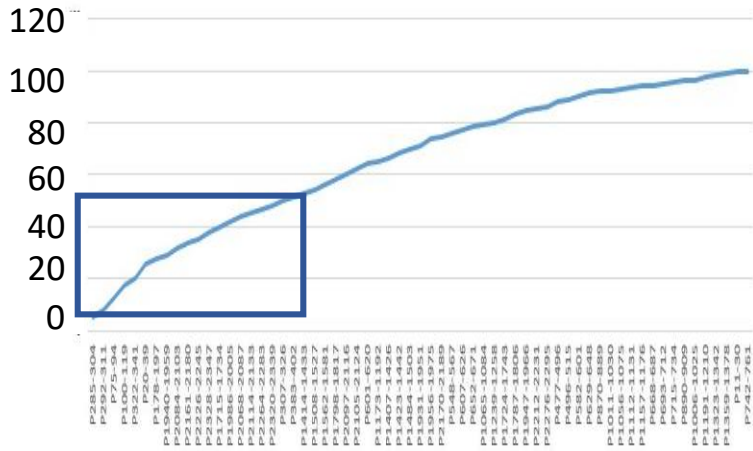




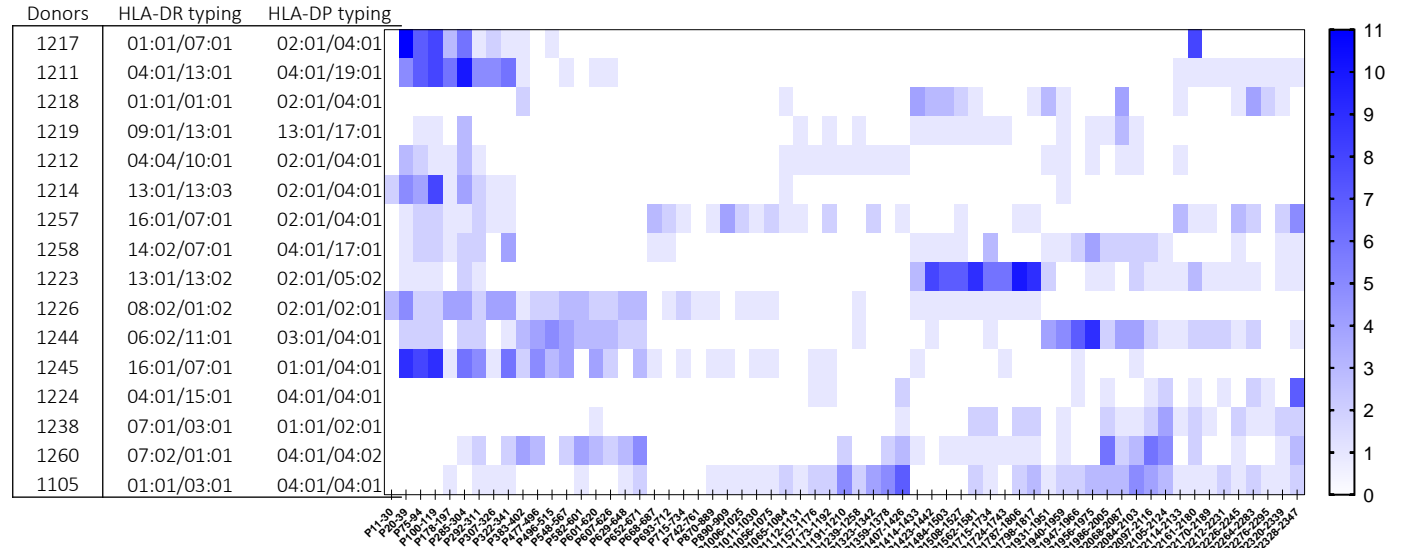
# T cell epitopes of FVIII in healthy donors



Cumulative % specific T cell lines



T cell epitope map



18 peptides cover 50% of the total T cell response

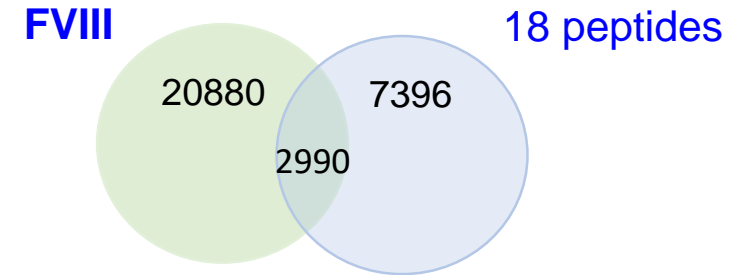
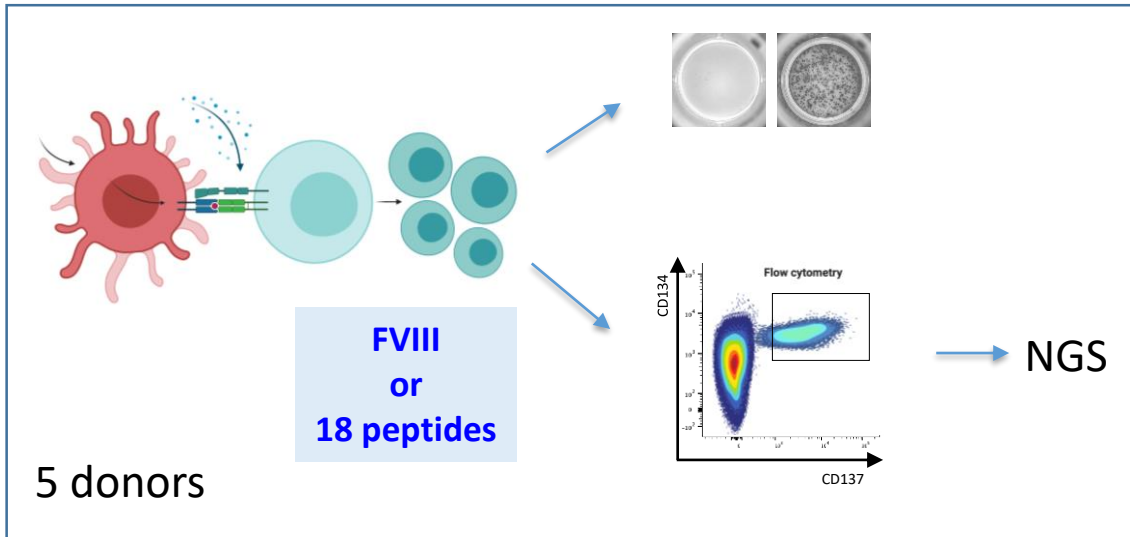
# T cell epitopes of FVIII in healthy donors and HA patients

Positions	Sequence	FVIII Domain	T cell response in HA patients
P285-304	VHSIFLEGHTFLVRNHRQAS	A1	/
P292-311	GHTFLVRNHRQASLEISPIT	A1	/
P75-94	DHLFNIKPRPPWMGLLGPT	A1	/
P100-119	YDTVVITLKNMASHPVS LHA	A1	/
P322-341	LGQFLLFCHISSHQHDGMEA	a1	/
P20-39	ATRRYYLGAVELSWDYMQSD	A1	/
P178-197	LSHVDLVKDLNSGLIGALLV	A1	[1]
P1940-1959	INGYIMDTLPGLVMAQDQRI	A3	/
P2084-2103	KEPFSWIKVDLLAPMIIHGI	C1	[2]
P2161-2180	PPIIARYIRLHPHYSIRST	C1/C2	[3]
P2226-2245	KARLHLQGRSNAWRPQVNNP	C2	[1], [4], [2], [5], [6], [7]
P2328-2347	HPQSWVHQIALRMEVLGCEA	C2	/
P1715-1734	RHYFIAAVERLWDYGMSSSP	A3	/
P1986-2005	KKEEYKMALYNLYPGVFETV	A3	/
P2068-2087	KLARLHYSGSINAWSTKEPF	C1	/
P2114-2133	SLYISQFIIMYSLDGKKWQT	C1	[8], [9], [10], [11]
P2264-2283	TQGVKSLTSMYVKEFLISS	C2	/
P2320-2339	LLTRYLRIHPQSWVHQIALR	C2	[1], [2], [5], [7]

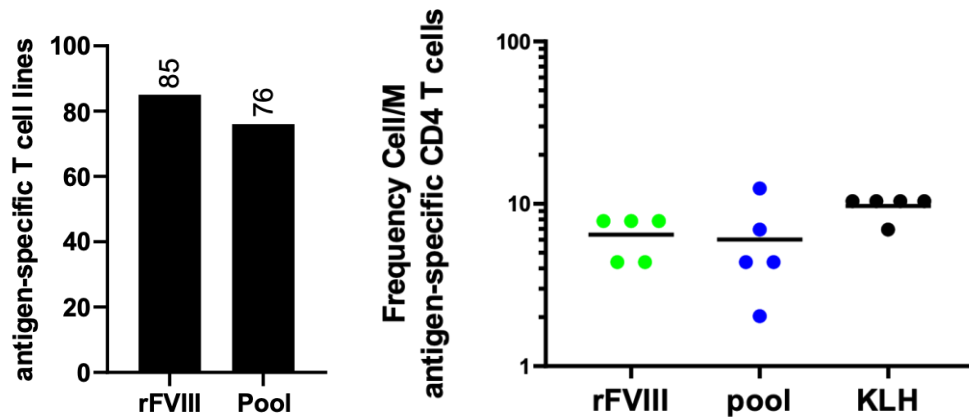
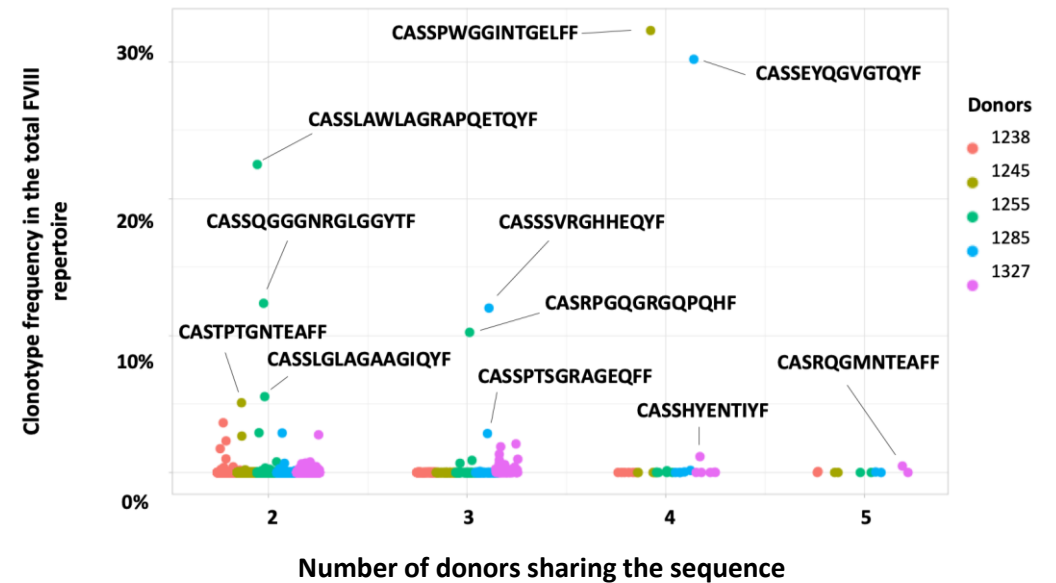
1. Gunasekera D et al. 2023 Front Immunol
2. Ettinger RA et al. 2016 Blood
3. Garnier A et al. 2016 Immunol Cell Biol
4. Ettinger RA et al. 2018 Blood Adv
5. Ettinger RA et al. 2010 Haemophilia
6. Ettinger RA et al. 2009 Blood
7. James EA et al. 2007 J Thromb Haemost
8. Diego VP et al. 2020 J Thromb Haemost
9. Peyron I et al. 2018 Haematologica
10. van Haren SM et al. 2011 Mol Cell Proteomics
11. Jones TD et al. 2005 J Thromb Haemost

**HLA-DR and DP** restricted T cell epitopes  
(inhibition experiments with HLA mAb)

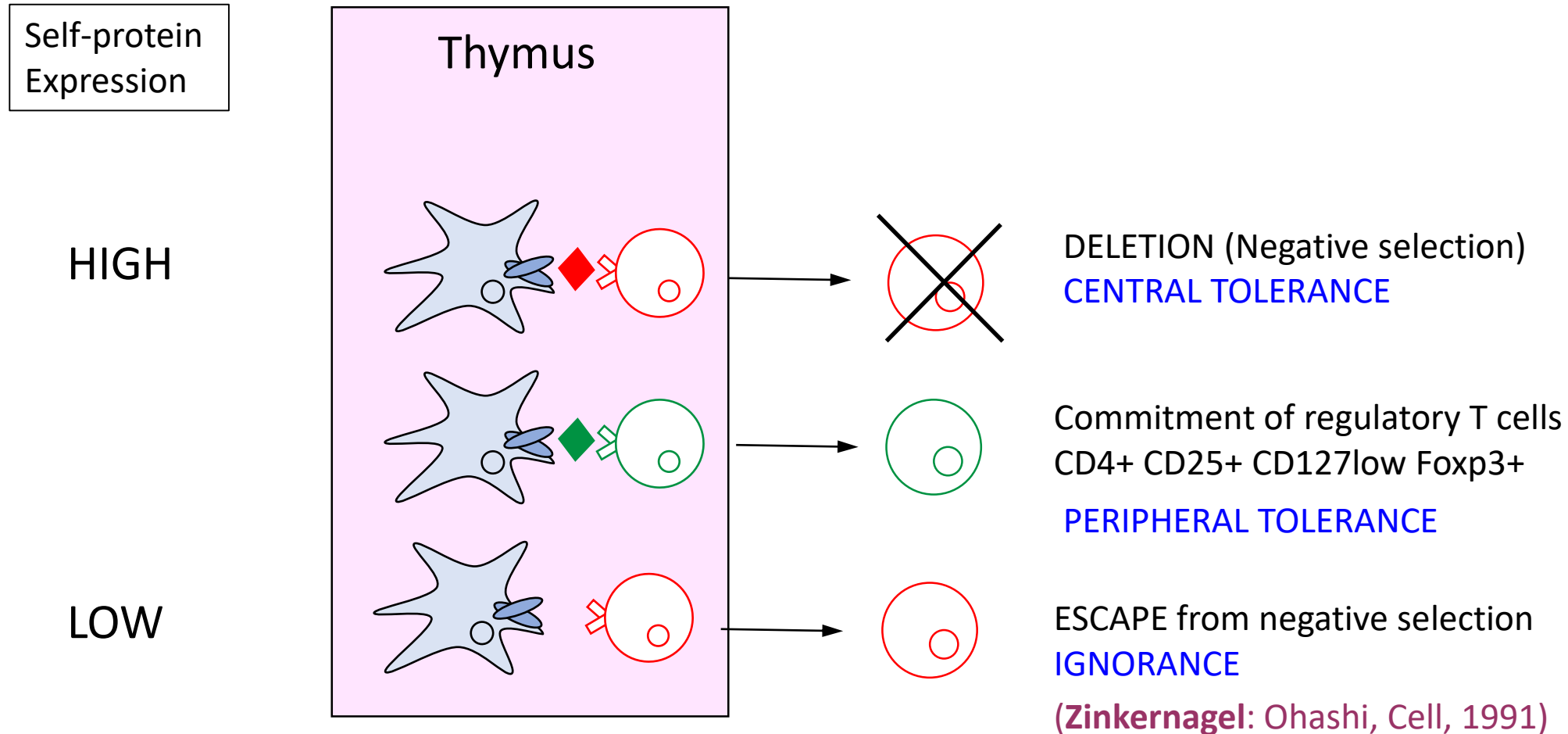
# 18 immunodominant FVIII peptides induce the majority of the T cell response to FVIII



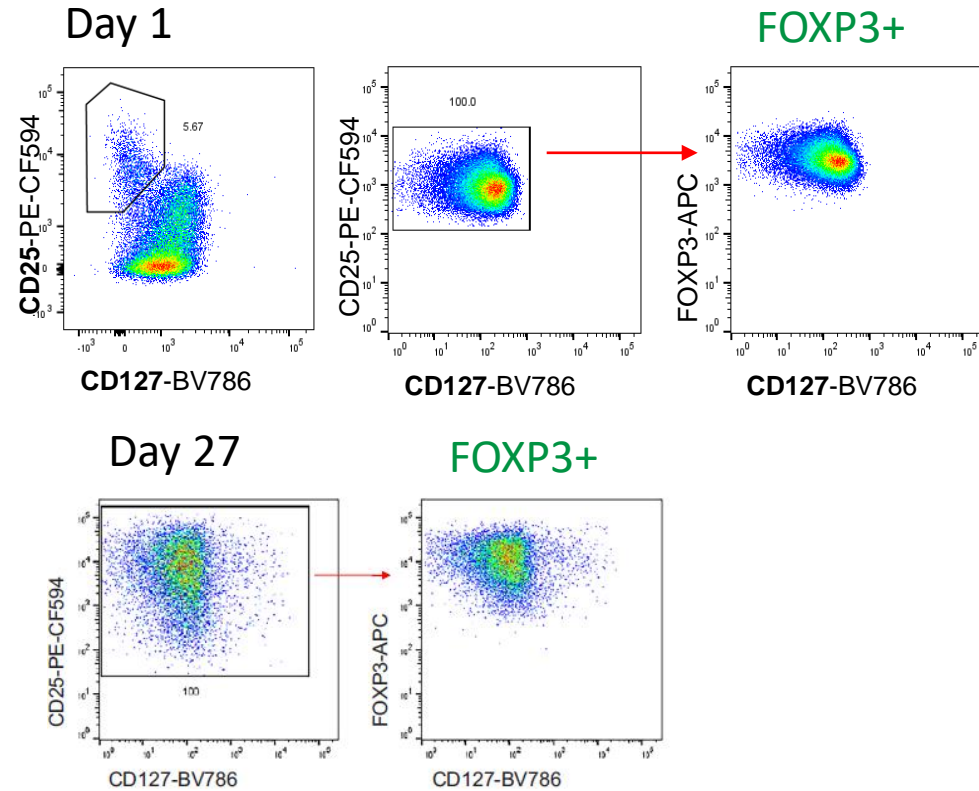
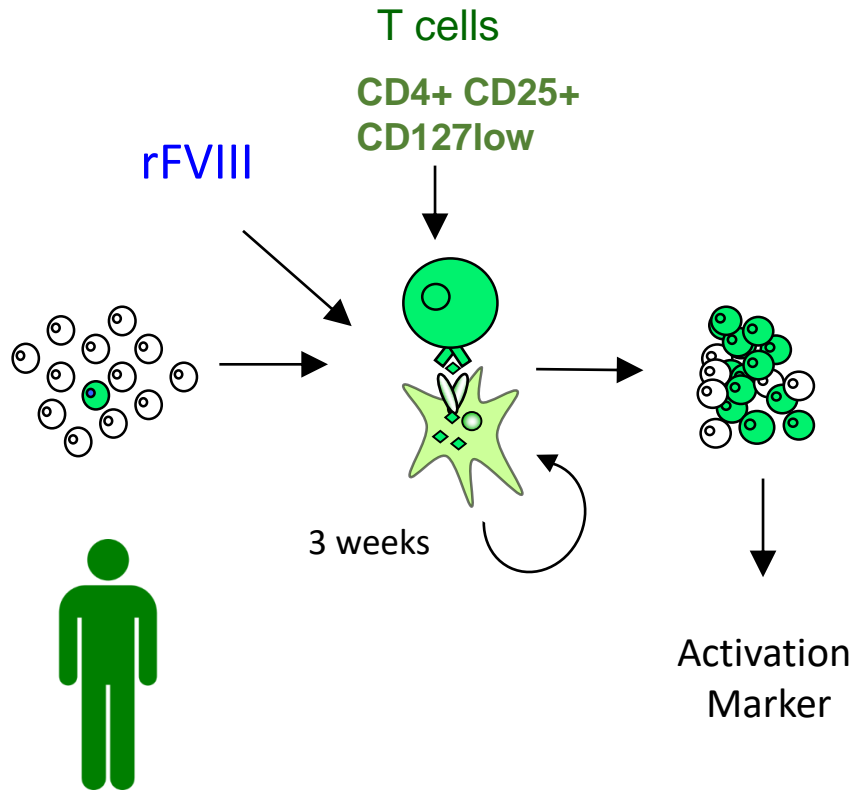
## Public clonotypes among multiple HLA-unrelated donors



# Self expression and T cell selection



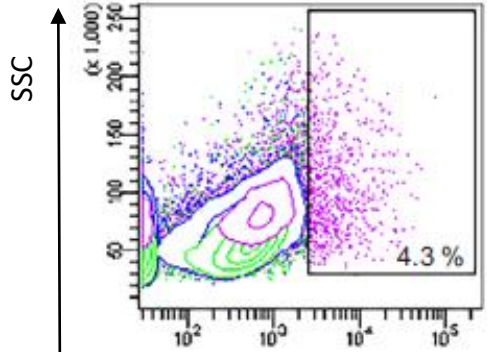
# Culture of FVIII-specific Treg cells



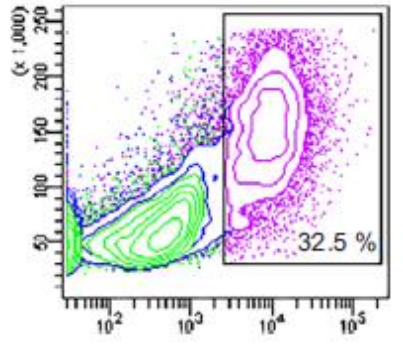
- CD4+ CD25+ CD127low T cells cultured with FVIII remained Foxp3+
- They are TDSR neg

# Frequency of FVIII-specific Treg cells

None

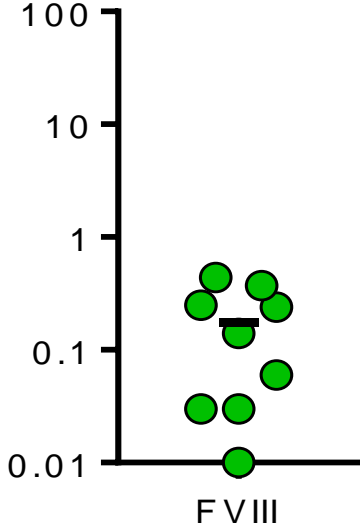


+ FVIII

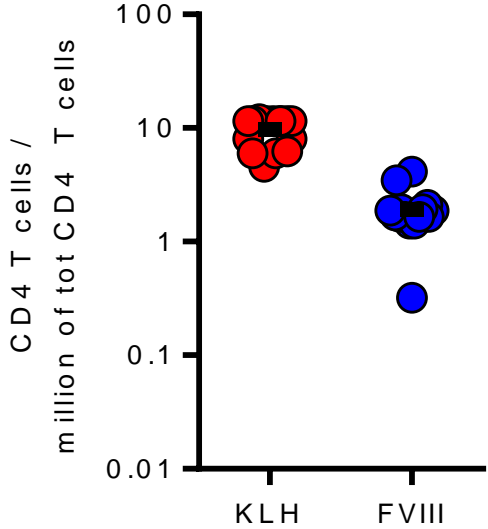


CD137

nb of Treg cells in  
1 million of total CD4<sup>+</sup> T cells



0.2 cells/M



Meunier et al, Blood Adv, 2017

1.7 cells/M

<<  
10X

Menier et al, EJI, 2024

# Conclusion:

- biopharmaceuticals such as cytokines, hormones, hematopoietic factors correspond to **low abundant self proteins** and can be immunogenic
- T cells specific for immunogenic proteins are detected in the repertoire of healthy donors and recognize non mutated sequences
- **FVIII-specific conventional CD4 T cells** Porcheddu et al, Front Immunol, 2024
  - FVIII-specific T cell repertoire is dominated by few clonotypes but is very large
  - **18 T cell epitopes** cover 50% of the *in vitro* T cell response to FVIII
- **FVIII-specific Regulatory CD4 T cells** Menier et al, EJI, 2024
  - **FVIII-specific Treg cells** have been detected from healthy donors and quantified after in vitro amplification. 10 fold less frequent than conventional T Cells
  - The **same epitopes** elicit FVIII-specific Treg cells (CD4+ CD25+ CD127low Foxp3+)

Conventional T cells and Treg cells can share **the same T cell epitopes**



# Acknowledgments



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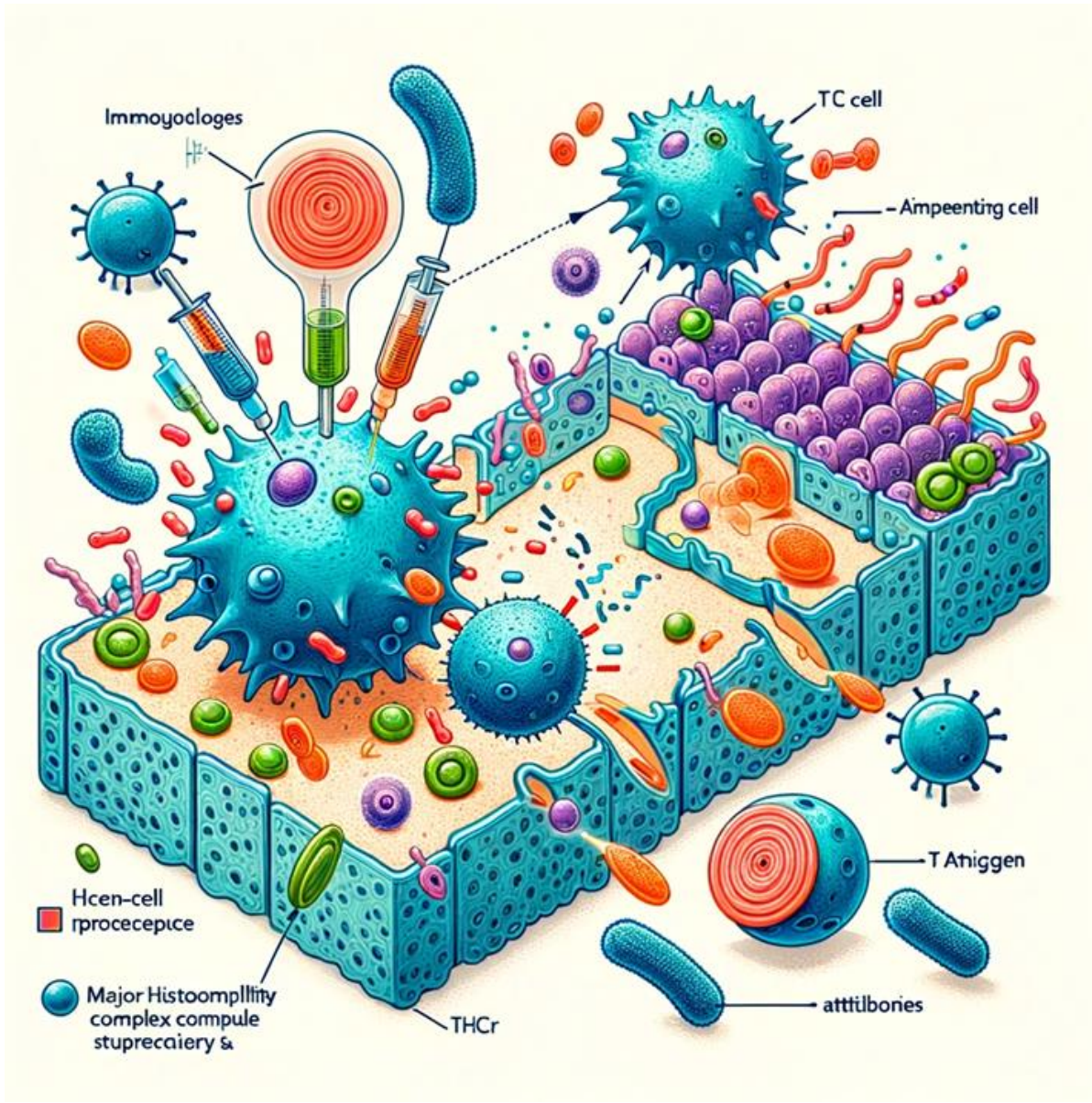
<http://www.abirisk.eu/>

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T and B cell cooperation  
seen by ChatGPT