

**What can pre-clinical testing
of Factor VIII concentrates tell us:
a cautionary tale**

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TS 3

Preclinical testing of FVIII

Clinical
perspective

Evaluate the risk of inducing
antibodies inhibiting FVIII activity



Immunological
perspective

Evaluate the risk of increased
immunogenicity

**FVIII is a foreign protein
to haemophilia A patients**

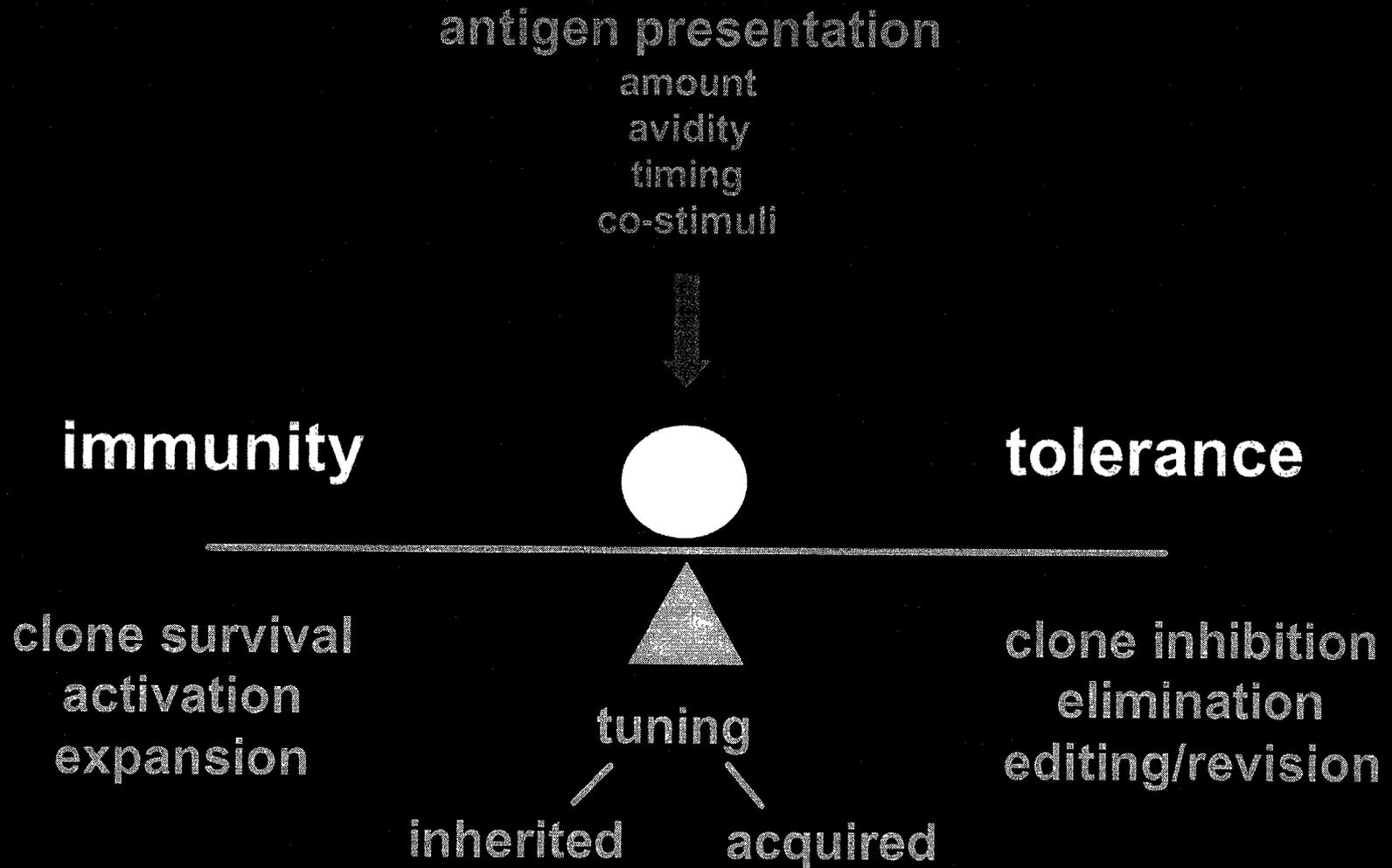
fully



partially

**an immune response
is to be expected**

Balance between tolerance and immunity



**Specific B and T cells
with the potential to react to FVIII exposure
are present in the periphery**



**B cells
continuously produced
by the bone marrow**

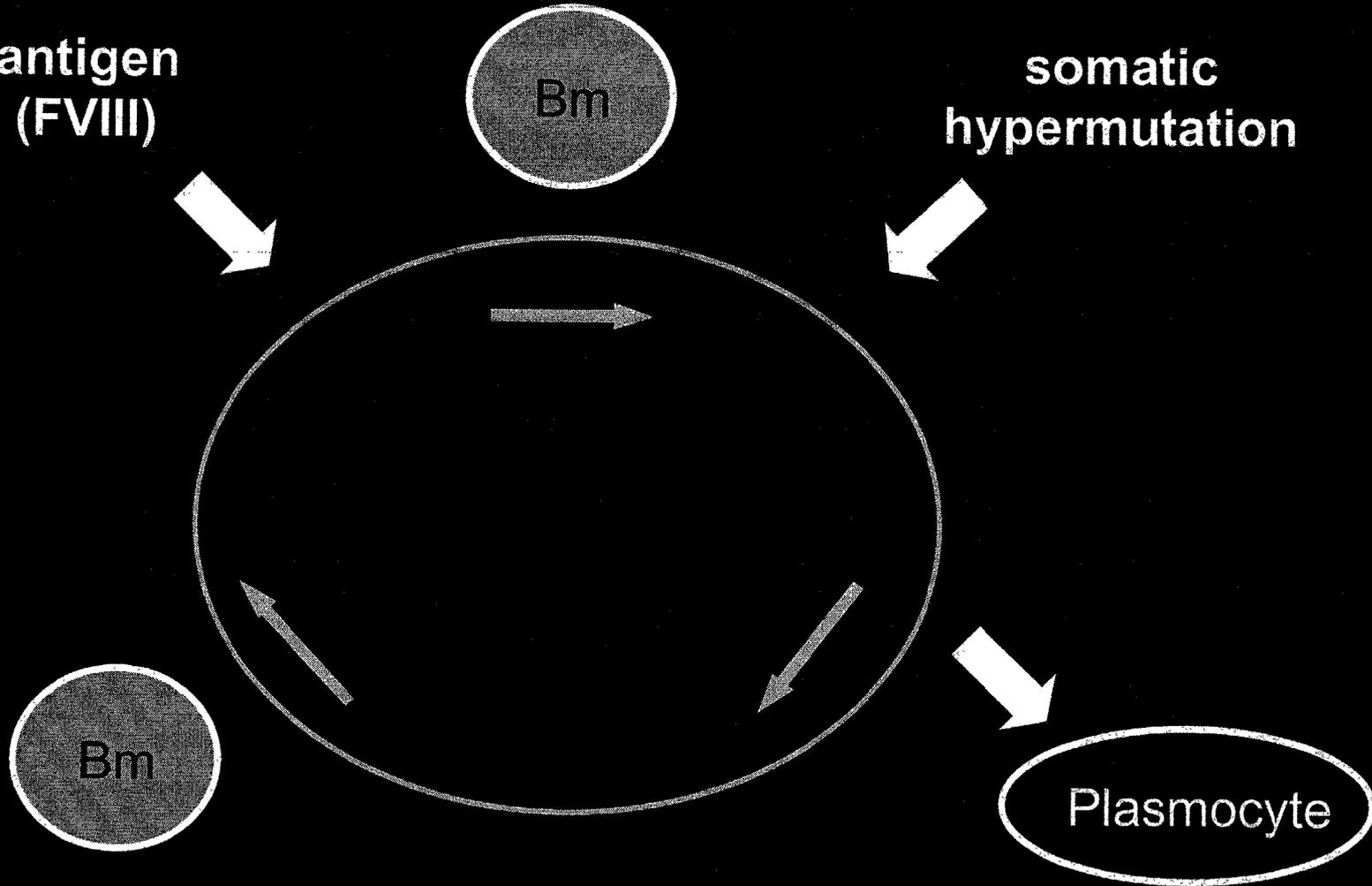


**T cells
from a repertoire
essentially shaped at birth**

Secondary response

antigen
(FVIII)

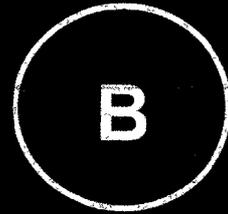
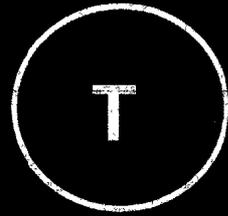
somatic
hypermutation



Bm

Bm

Plasmacyte



**antigen
(FVIII)**

**Short-lived
plasma cells**

**Long-lived
plasma cells**

+

Memory B cells

**Germinal center
independent**

**Germinal center
dependent**

Homeostasis of FVIII immune response

CD4+
T cells



Regul
T cells

FVIII

Mem
B cells

Anti-Id
B cells

Regulatory T cells

CD4+
CD25+
FoxP3+

active selection
in thymus

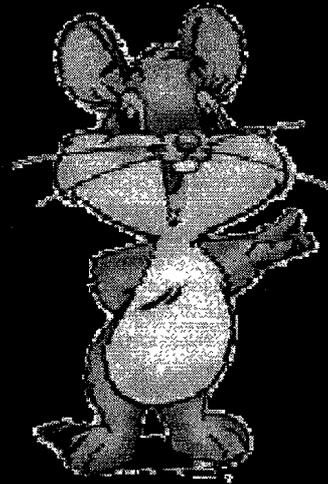
NATURAL

CD4+
CD25(-)
FoxP3(-)

differentiation
in the periphery

ADAPTIVE

FVIII



?

Optimal model criteria

- **identical genetic background**
eliminate variables such as MHC haplotype or TCR
- **deficiency in antigen to be tested**
avoid skewing of response due to auto-antigen
- **possibility to evaluate the immune response at clonal level**
identify new conformational and/or sequential epitopes
- **no or limited use of adjuvants**
prevent distortion of the response due to lipid emulsion

Available mouse models

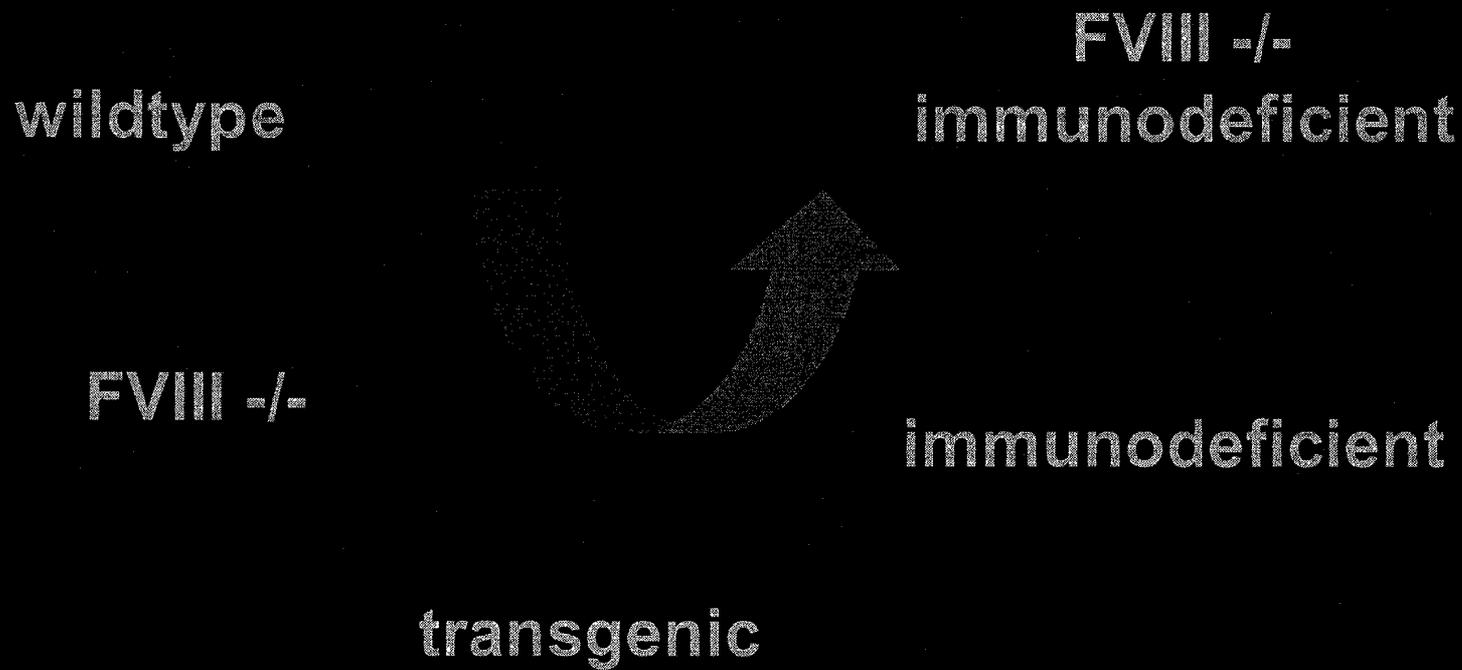
wildtype

FVIII -/-
immunodeficient

FVIII -/-

immunodeficient

transgenic



Wildtype mouse strains (e.g. BALB/c, C57Bl/6)

- **normal level of FVIII**

 - the specific T cell repertoire is purged from T cells
to dominant epitopes

 - the anti-FVIII response is skewed towards determinants
that distinguish self from injected FVIII

- **usefulness:**

 - establish a library of monoclonal antibodies**

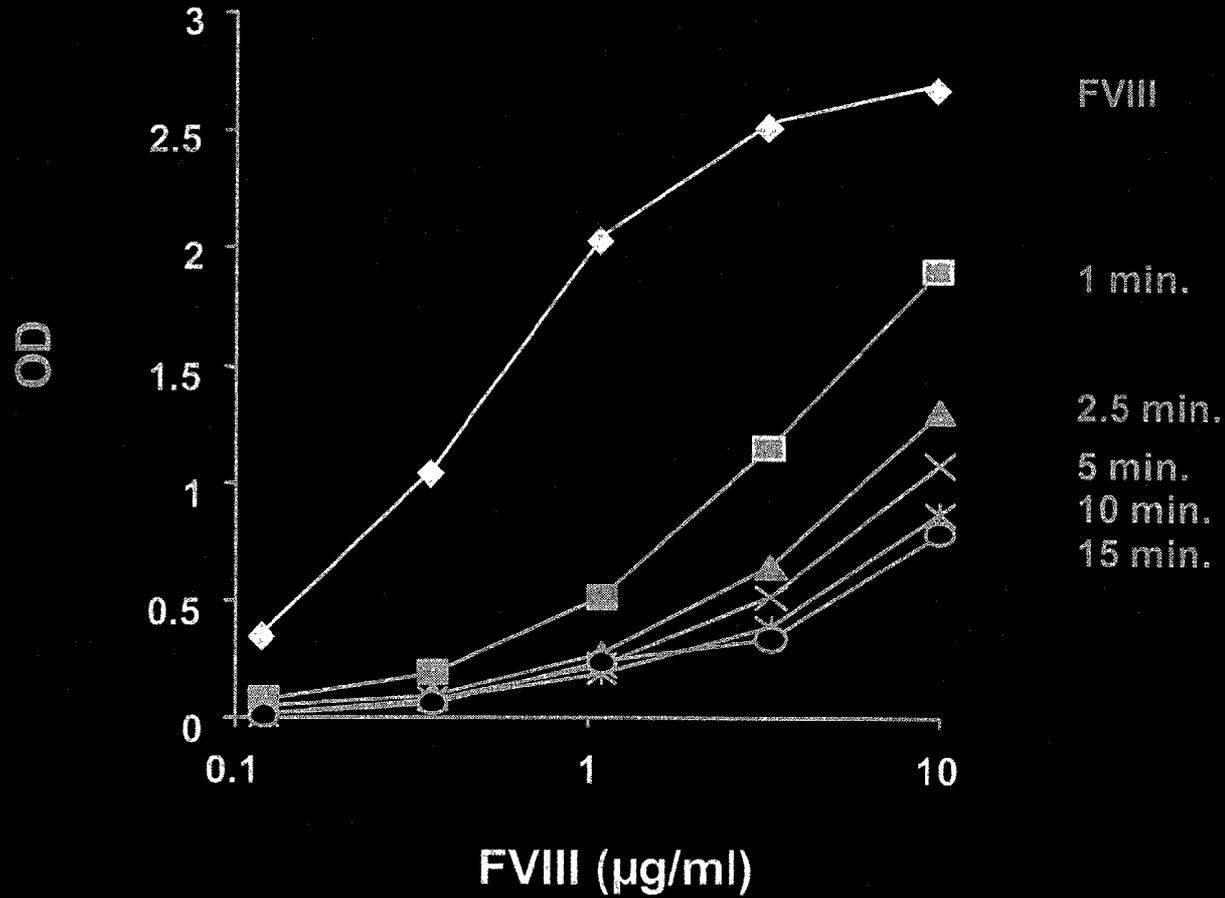
 - mechanism of FVIII inactivation

 - relationship between structure and function

 - comparative antigenicity (e.g. pasteurisation)

Heat-denatured FVIII (63°C)

anti-a3 mAb



FVIII -/- mice

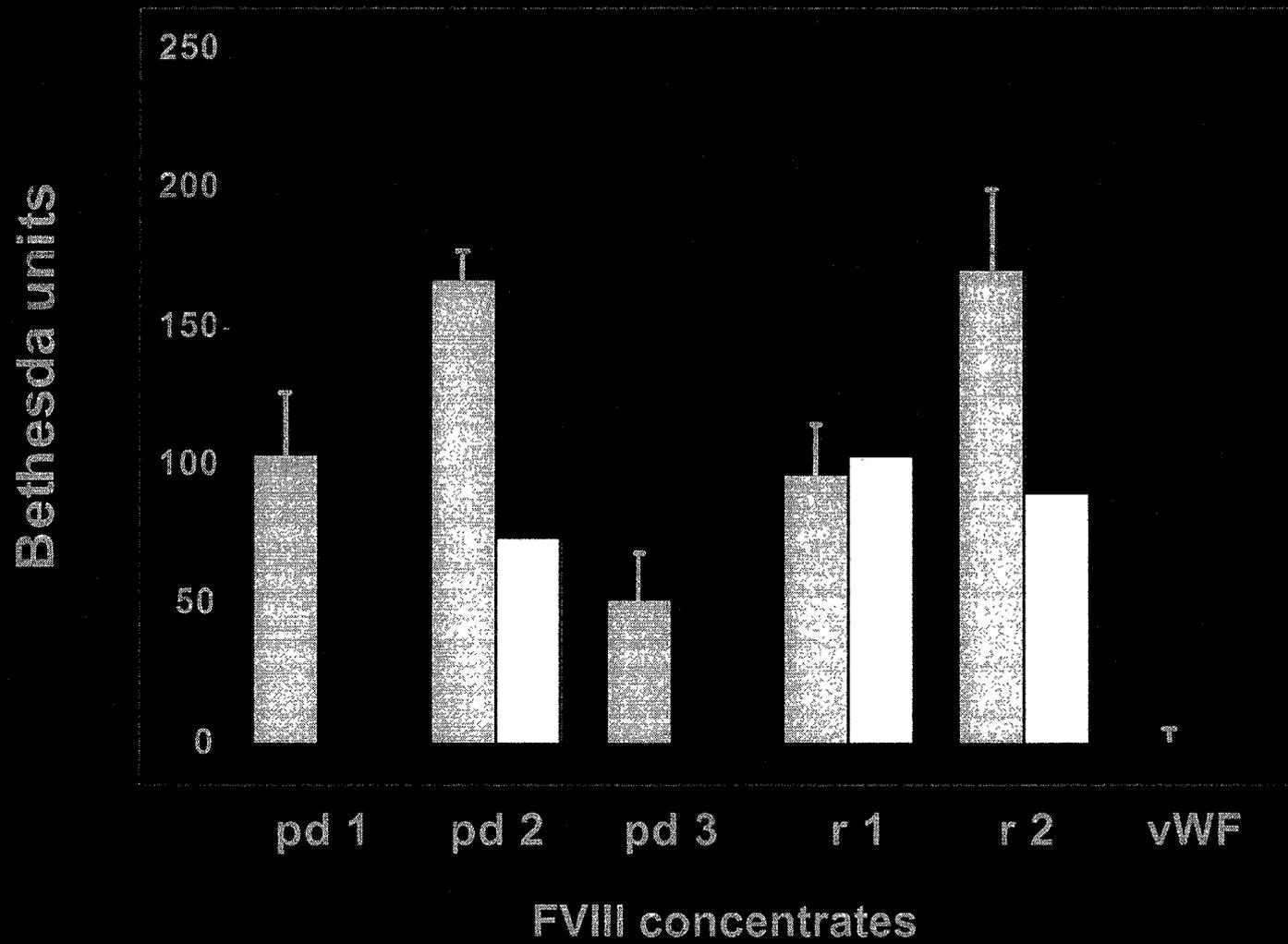
- **no elimination of anti-FVIII T cells**

full immunocompetence towards FVIII
qualitatively normal anti-FVIII response

- **usefulness:**

direct comparison of FVIII immunogenicity
evaluation of the impact of added factors (vWF ...)

Influence of vWF on FVIII concentrate immunogenicity



Immunodeficient mouse strains (SCID ...)

- **absence of functional B and/or T lymphocytes**
no rejection of allografts such as human cells
- **normal level of FVIII**
- **no formation of lymphoid organs**
no primary immune response
- **usefulness:**
 - comparison of FVIII immunogenicity directly on human cells**
 - between individuals
 - between products
 - evaluation of new treatment strategies**



healthy donor
haemophilia A patient

PBMC
 $15 \cdot 10^6$

Severe Combined ImmunoDeficient mice
(SCID)

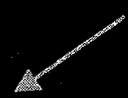


Saline

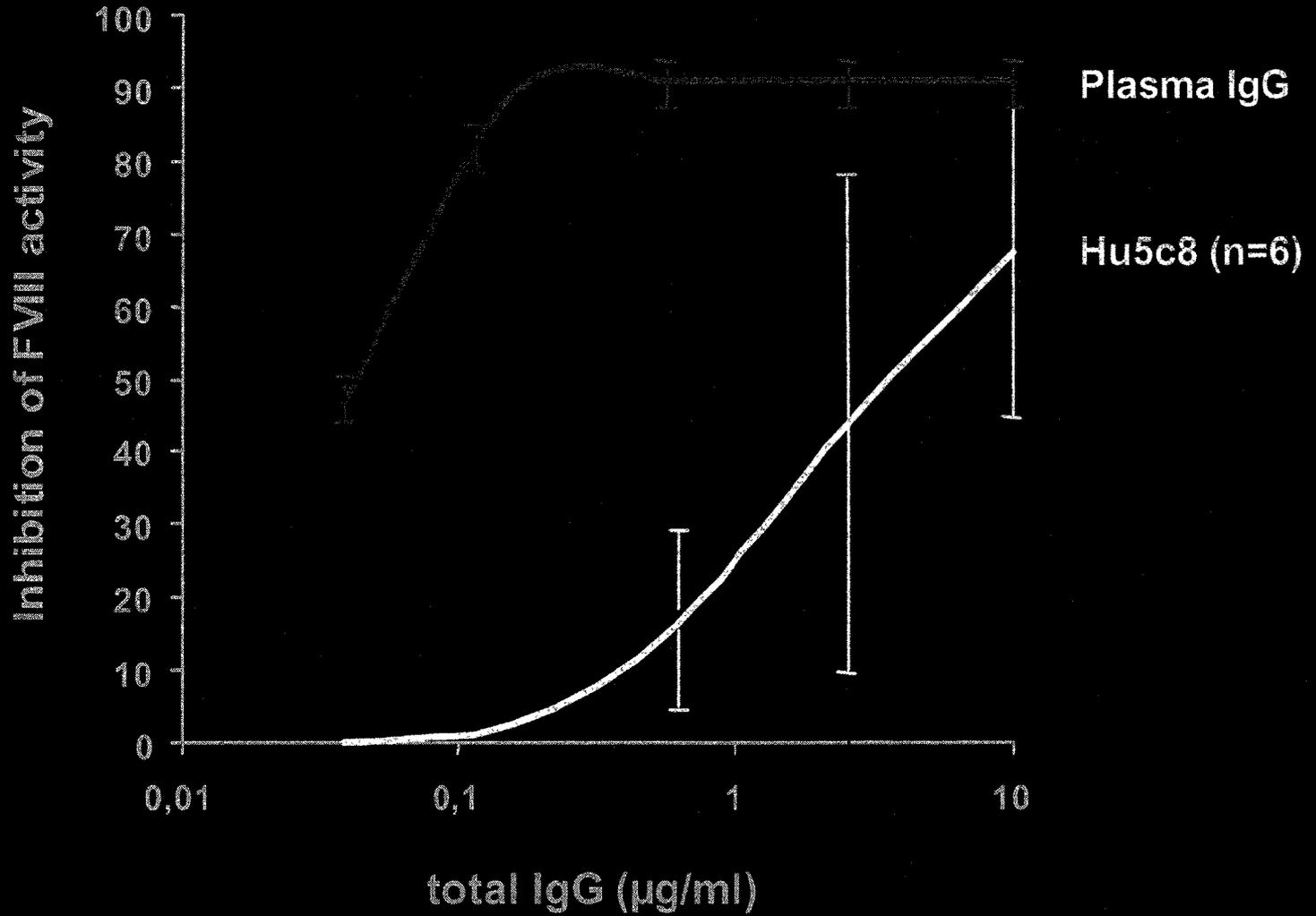


FVIII

bleeding



Inhibition of FVIII activity: Bethesda assay



SCID - FVIII -/- strain

- absence of FVIII
- absence of functional B and T cells

- **usefulness:**

evaluation of the full repertoire of human anti-FVIII
evaluation of vectors for gene therapy

Caveats

- **the number of VH genes in mice is 100-fold higher than in man**
- **MHC-class II determinants are fully distinct in mouse as compared to humans**
- **although highly homologous, mouse and human FVIII show differences in immunogenicity**
- **the effects of inflammation on FVIII processing are overlooked**
- **haemophilia A is heterogeneous**

Transgenic mice

targeted replacement (knock-in) mice

carrying a T and/or B cell receptor for FVIII
 $\pm 50\%$ of cells carry the transgene

specific for murine FVIII

. usefulness:

allows clonal analysis of the anti-FVIII response in vivo
and transfer experiments

evaluation of treatment strategies

evaluation of conditions under which FVIII is administered
(inflammation, SC injections ...)

Preclinical evaluation of FVIII

Comparative immunogenicity of FVIII in FVIII^{-/-} mice

- level of specific antibodies
- level of inhibitors
- epitope mapping

B cell epitope mapping (1)

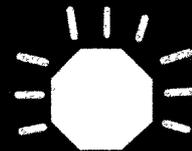
dsDNA template (PIG+R&D vector)

90 min

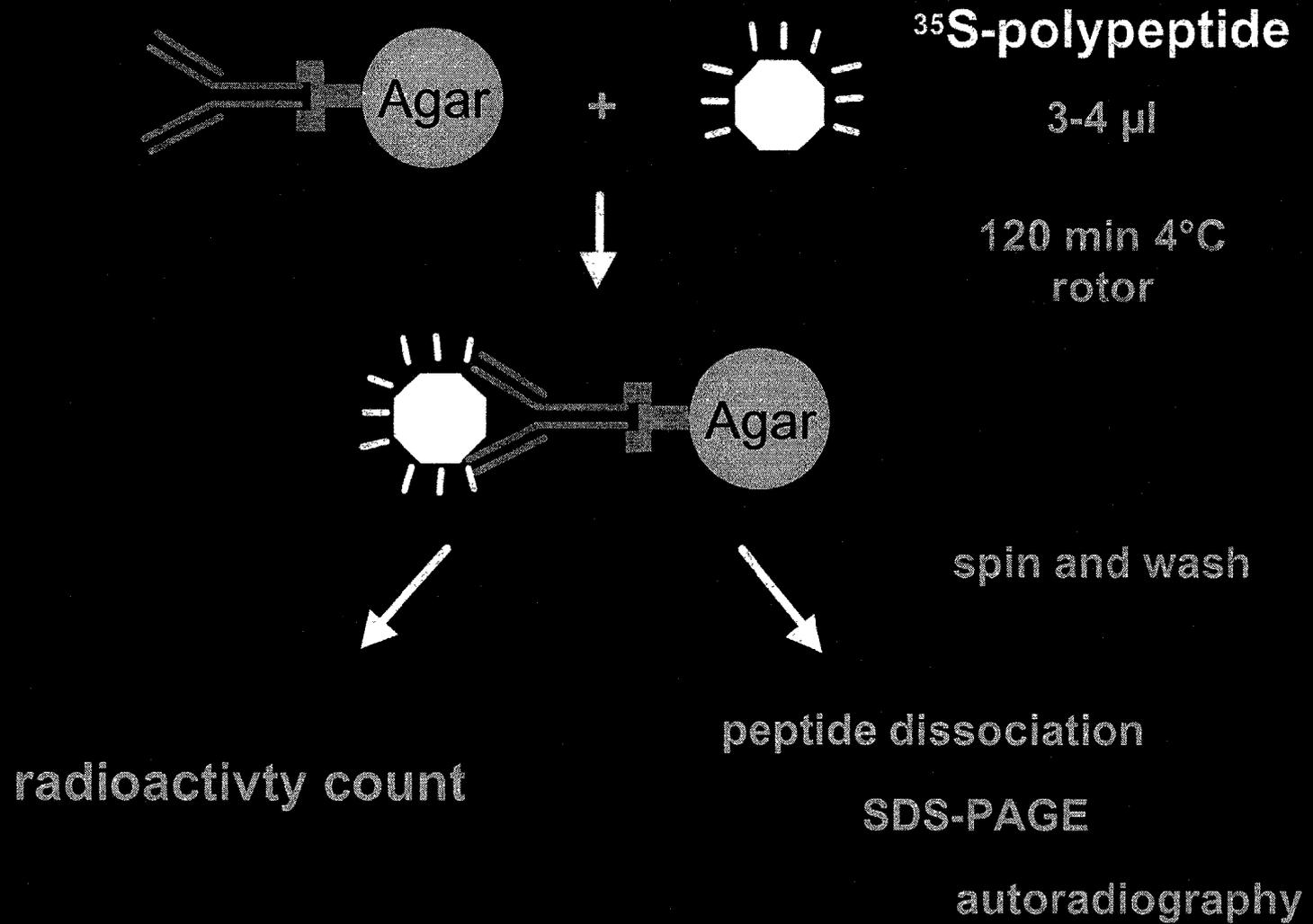
T7 transcriptase
rabbit reticulocyte lysate

aminoacid mixture (less MET)
³⁵S-MET

³⁵S-polypeptide



B cell epitope mapping (2)



Parameters for prospective trials

1. Characteristics of the FVIII concentrates

list of FVIII concentrates to which patients have been exposed
doses received
method of administration

2. Clinical data

race
gene defect
circumstances of administration ...

Parameters for prospective trials (2)

3. Biological evaluation

Antibodies

- inhibitor titre with a panel of FVIII concentrates
- total anti-FVIII antibodies
 - titre (ELISA or immunoprecipitation)
 - capacity to inhibit FVIII binding to vWF and phospholipids
others (such as FIX)
 - presence of catalytic antibodies
- epitope mapping

Parameters for prospective trials (3)

3. Biological evaluation

T lymphocytes

- spectrotype analysis
- proliferation assays with FVIII and polypeptides
- limiting dilution analysis