Proposed Mechanisms for Aggregate Induced Immunogenicity

Science 262, 1448-1451

Organization:	high	low	absent
Antibody response	+++	+	
Induction of auto-antibodies	+++	-	-

Recombinant Protein Therapy vs Vaccination

Vaccines aim at induction of **maximal** immune responses

⇕
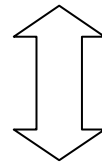
Recombinant protein therapy aims at induction of **minimal** immune responses

→Understanding how vaccines induce maximal immune responses may allow to avoid these features in recombinant proteins therapy
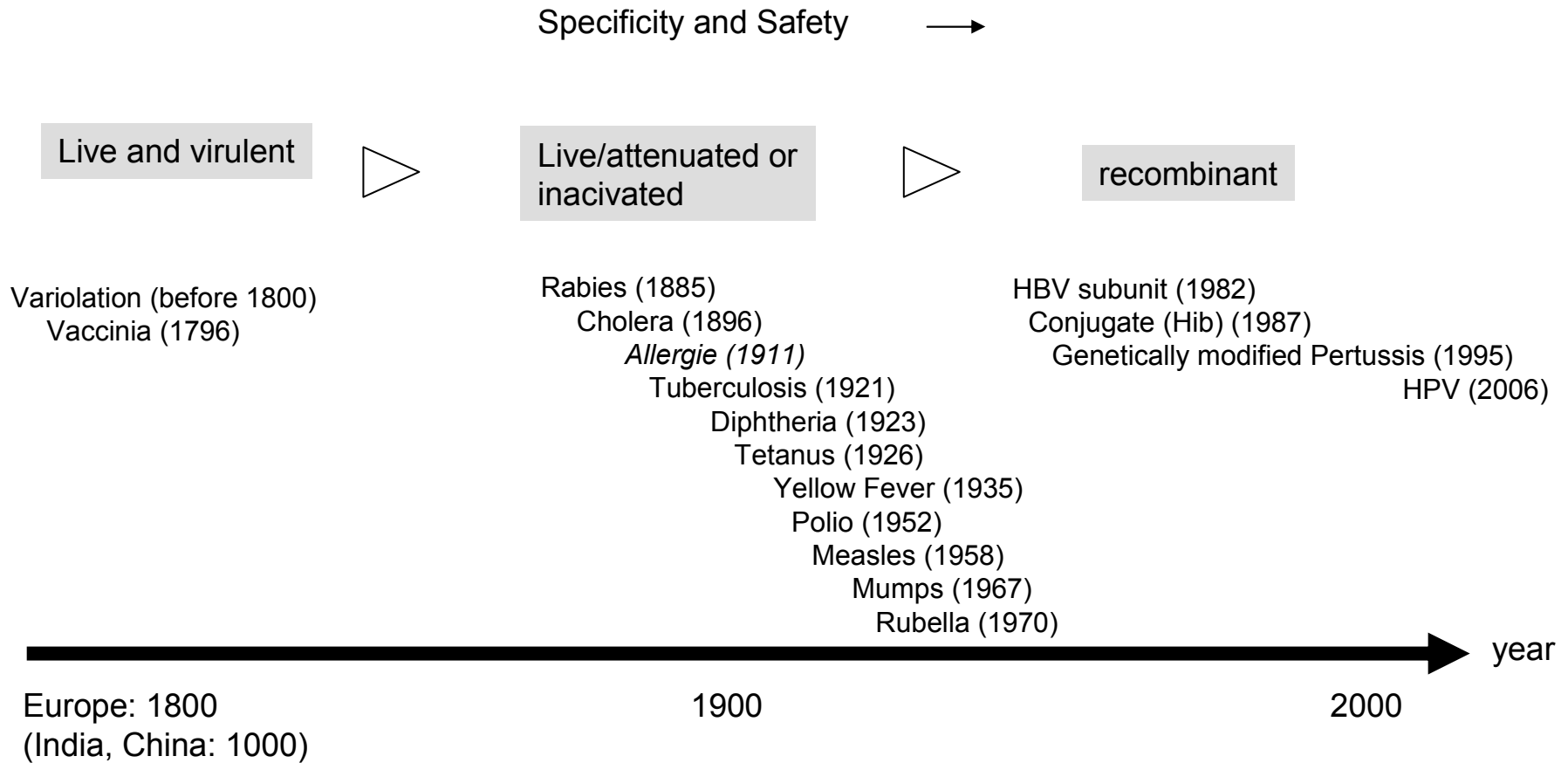
Immunological Background

- Immunological Background

  → History of Vaccines

  → T/B Collaboration

  → Immunogenicity of Aggregates

  → Immune Tolerance

- Self-specific B cell responses

History of Vaccinology

Specificity and Safety ⟶

Live and virulent ▷ Live/attenuated or inacivated ▷ recombinant

Variation (before 1800)
Vaccinia (1796)

Rabies (1885)
Cholera (1896)
Allergie (1911)
Tuberculosis (1921)
Diphtheria (1923)
Tetanus (1926)
Yellow Fever (1935)
Polio (1952)
Measles (1958)
Mumps (1967)
Rubella (1970)

HBV subunit (1982)
Conjugate (Hib) (1987)
Genetically modified Pertussis (1995)
HPV (2006)

⟶ year

Europe: 1800
(India, China: 1000)

1900

2000

4

Source: Bachmann and Dyer, Nature Reviews Drug Discovery, 3, 81 – 88 (2004), WHO
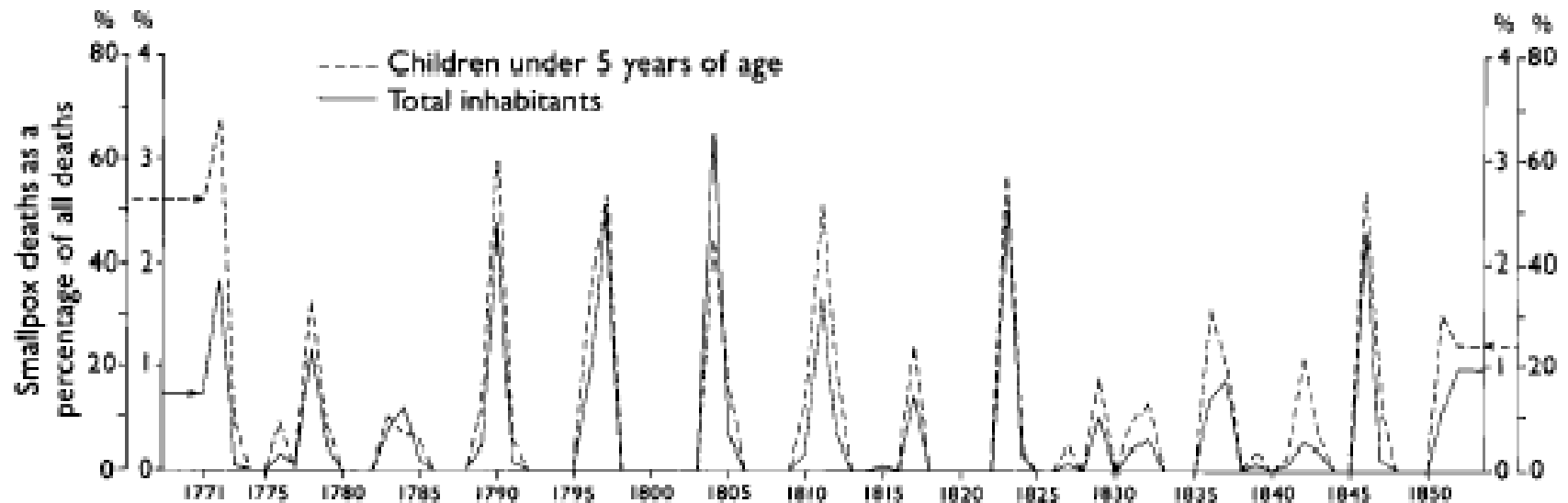
The Toll of Smallpox

Smallpox in Japan

Fig. 5.3. The mortality from smallpox in the Hida district on Honshu Island, Japan, from 1771 to 1851. The population of the district rose from 2677 in 1771 to 3127 in 1851 (lowest 2535 in 1786; highest 3132 in 1834). The solid line indicates deaths due to smallpox as a percentage of all deaths. The broken line (and higher percentage figures) indicates deaths due to smallpox as a percentage of all deaths in children under 5 years of age. The total number in this age group rose from 310 in 1771 to 439 in 1851 (lowest 234 in 1838; highest 444 in 1850). (Data from Suda & Soekawa, 1983.)

Variolation
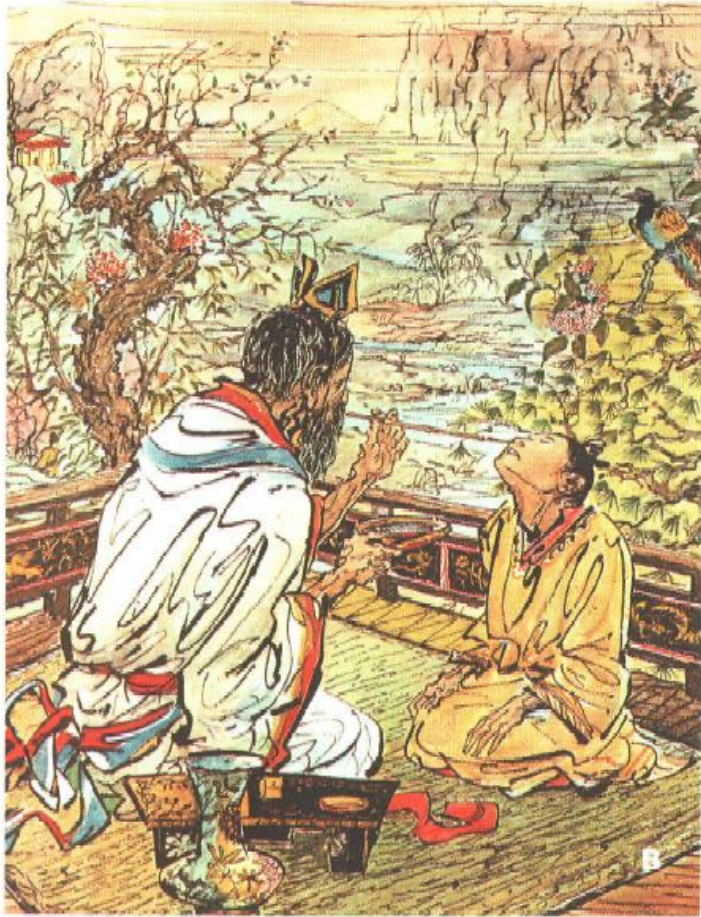
- First vaccination against Smallpox
- Developed in China and India (10th Century)
- Inoculation with life, non-attenuated virus
- 0.5-2% lethality (vs 20-30% of normal smallpox)
- Usually not generalized disease (only local lesion)
- Sometimes cause for local outbreaks of smallpox

Edward Jenner

The Father of the Smallpox Vaccine

What was known:
- Milk-Maids get pox-like disease from cows
- The Milk-Maids are resistant to Smallpox and Variolation

What Jenner did:
- He isolated infectious material from infected milk maid
- Inoculated the son of his servant with the material ("a lad of the name of Phipps")
- Challenged the boy with smallpox
- The boy resisted the disease

Plate 6.4. Edward Jenner. (1749-1823). Pastel portrait by J. R. Smith in 1800.

WELLCOME INSTITUTE LIBRARY, LONDON

Vaccination

Plate 6.14. A: Arm-to-arm vaccination, as practised in Europe. Painting by Charles Desbordes, "La Vaccine", 1822. **B:** Insufflation of powdered smallpox scabs by the intranasal route, as practised with variola virus in China.

MUSÉE DE LA CHARTREUSE

Plate 6.12. Wood engraving from *Harper's weekly*, 23 April 1870, showing a general vaccination day at the Paris Academy of Medicine. Arm-to-arm vaccination had been superseded by vaccination from the cow after the discussions of the Medical Congress of Lyons in 1864.

NATIONAL LIBRARY OF MEDICINE, BETHESDA

Vaccination
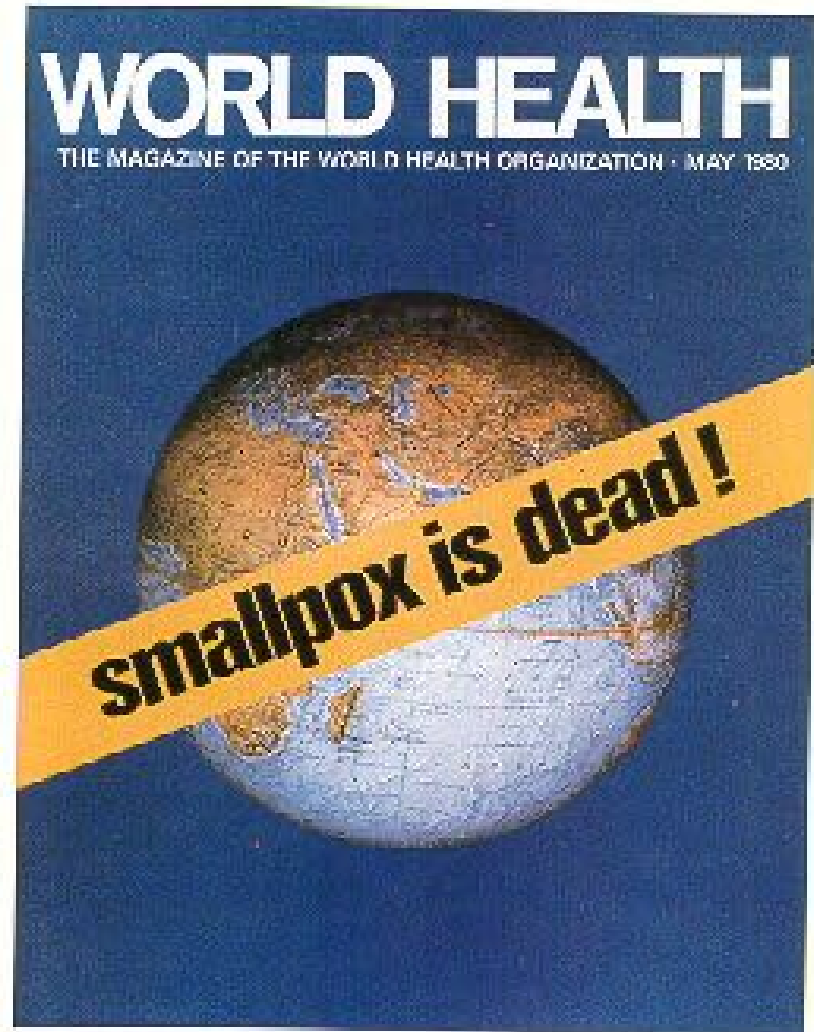
What types of vaccines are currently used or developed?

- Live attenuated (eg Polio Sabine or Rubella)

- Inactivated (eg Polio Salk or Hepatitis A)

- Isolated pathogen-components (eg bacterial conjugate vaccines, Tetanus, Diptheria, Pertussis (toxins))

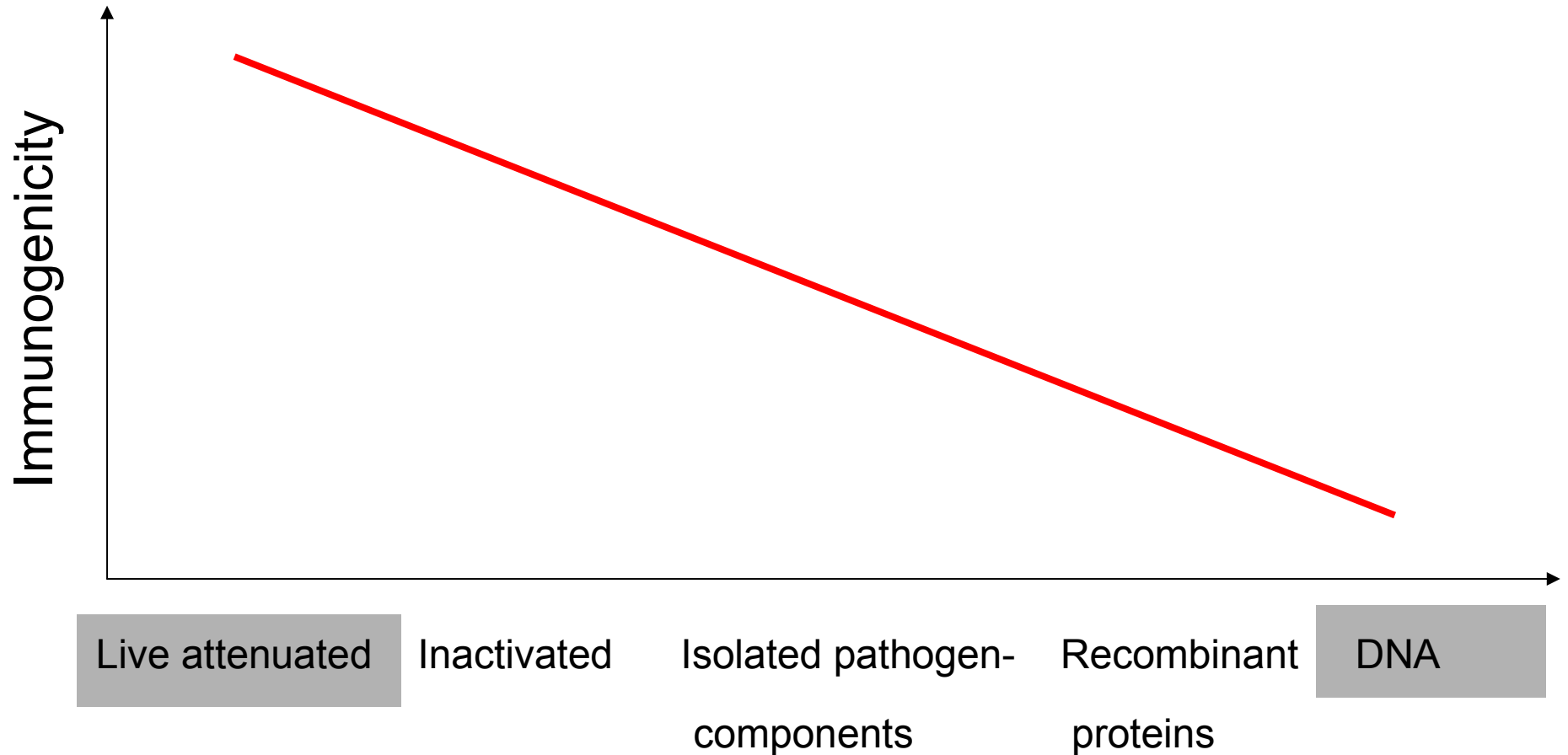- Recombinant (eg Hepatitis B, detoxified version of Diphteria)

- DNA

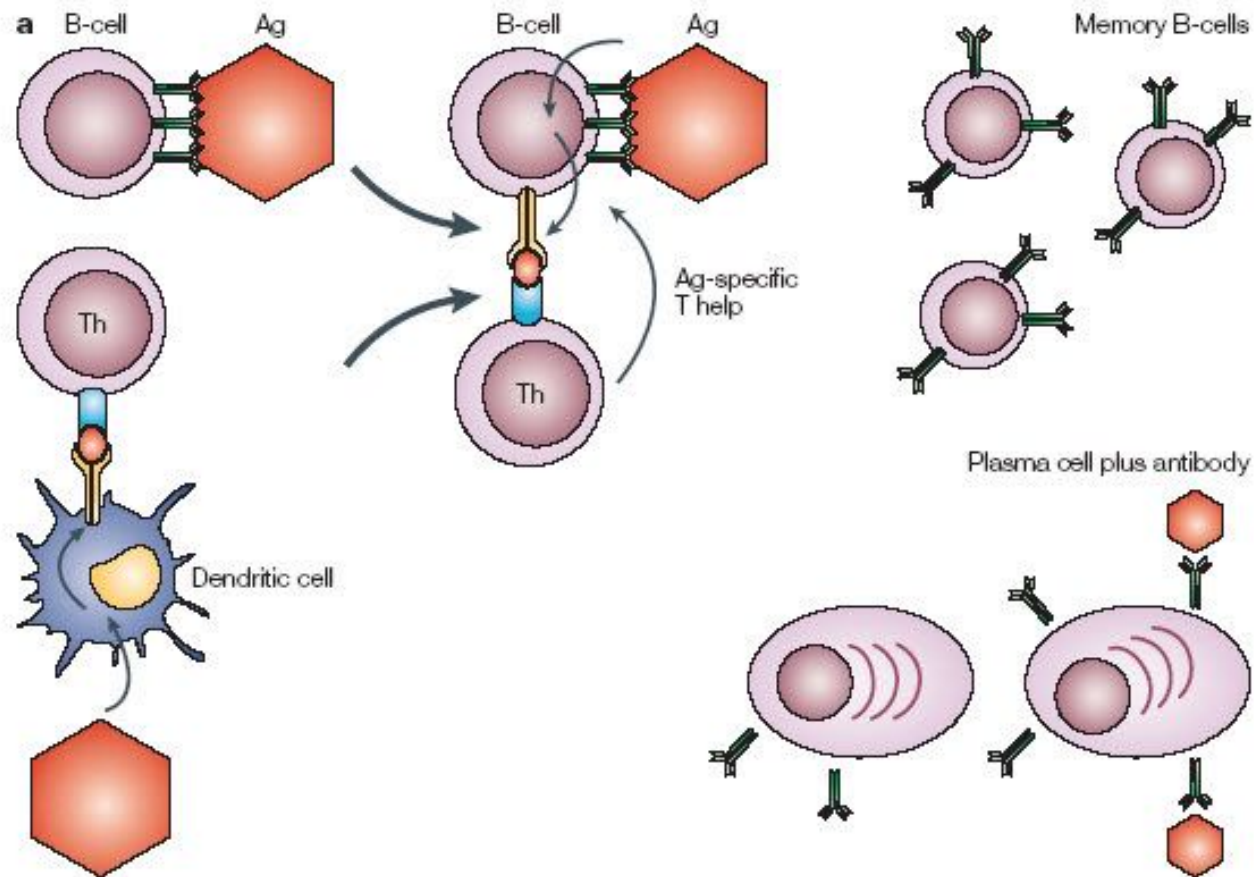# Safety of the various vaccine types

The "less alive" the safer

Safety/Specificity

Live attenuated   Inactivated   Isolated pathogen-components   Recombinant proteins   (DNA)
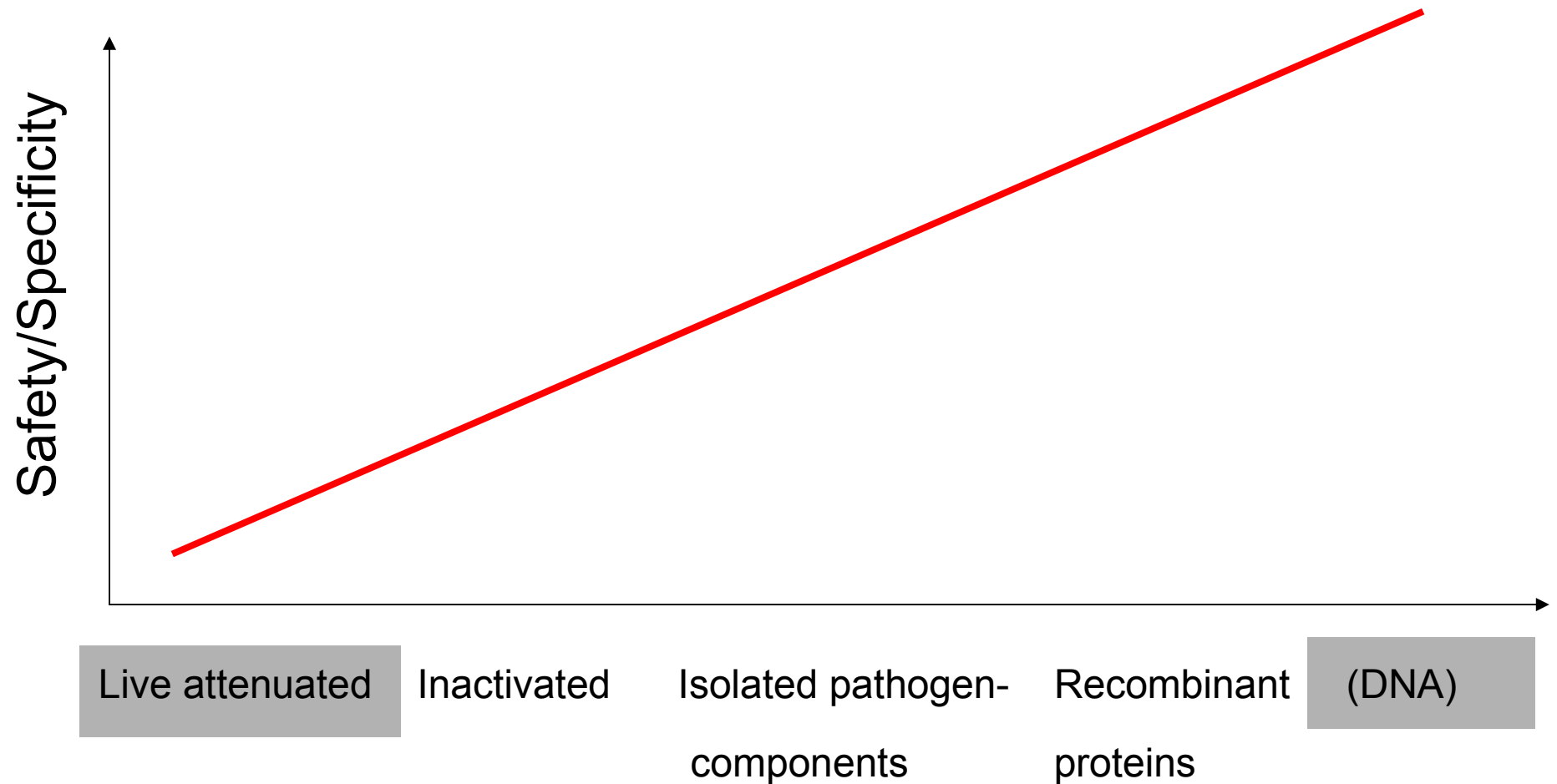
Immunogenicity of the various vaccine types

The "less alive" the less immunogenic

Immunogenicity (y-axis), decreasing from Live attenuated → Inactivated → Isolated pathogen-components → Recombinant proteins → DNA

Immunological Background

Bachmann & Dyer, Nat Rev Drug Disc 3:1-8

Immunological Background

B cells → Recognize native antigen
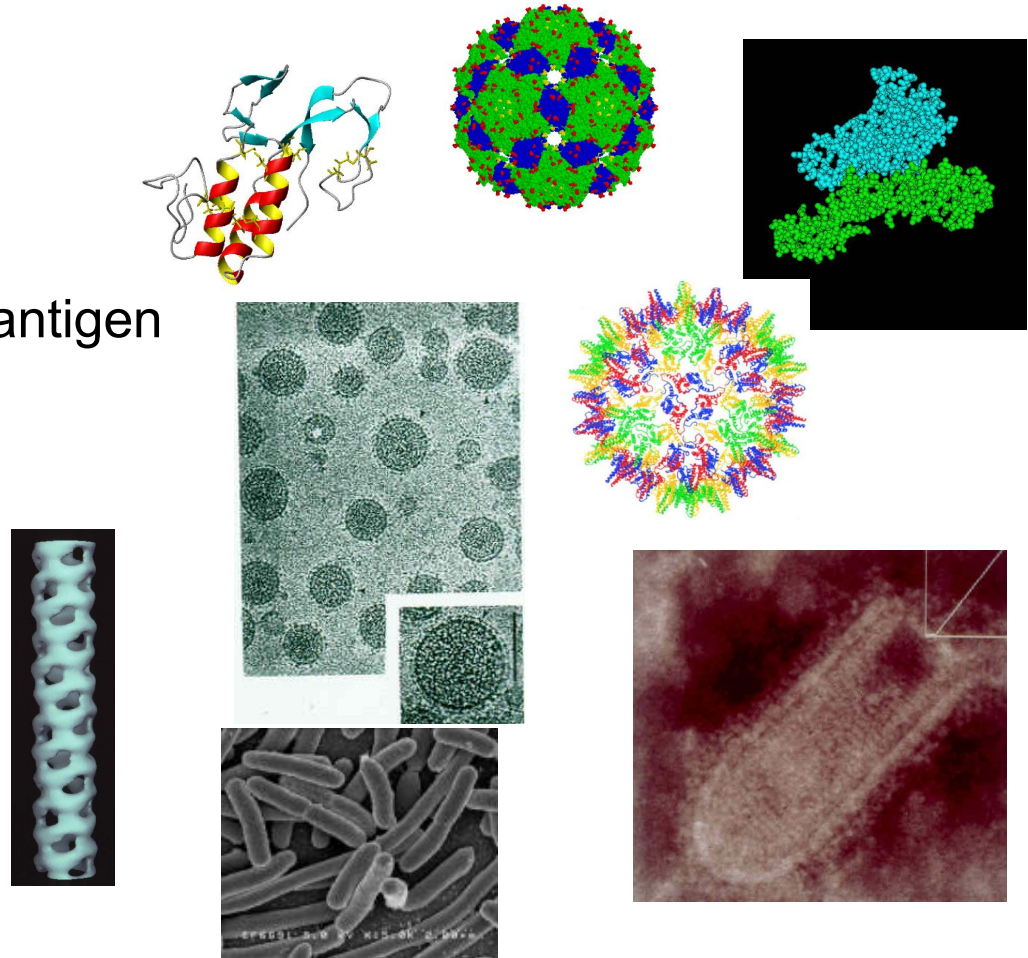
Immunological Background

Th cells → Recognize processed antigen:

Peptides presented on MHC molecules
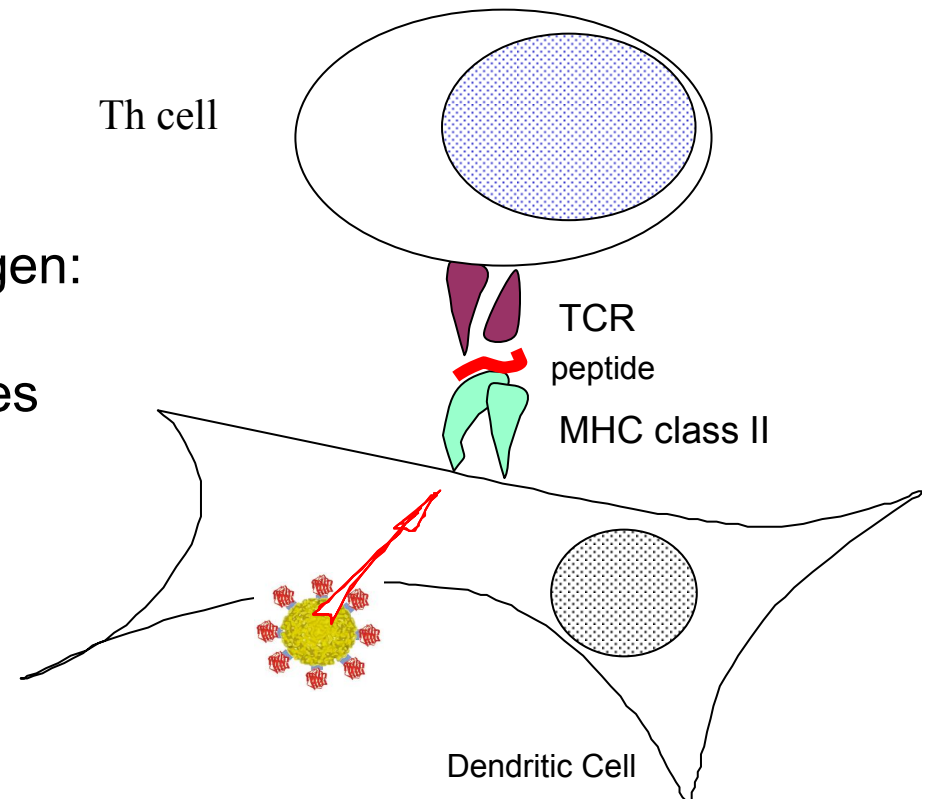
Th cell

TCR

peptide

MHC class II

Dendritic Cell

15

Immunological Background
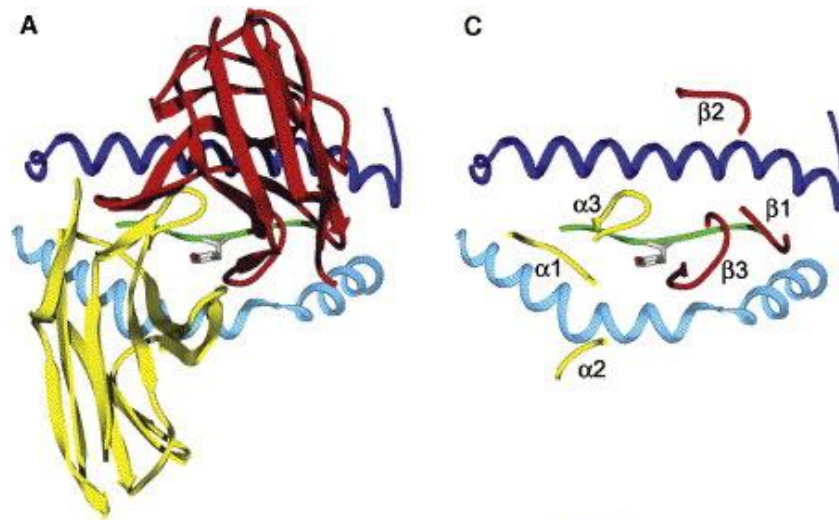
Aggregate Induced Immunogenicity

- Where do aggregates come from?

- Why are aggregates so immunogenic?

Aggregate Induced Immunogenicity

Aggregates are Potent Inducers of Immune Responses

Aggregate Induced Immunogenicity

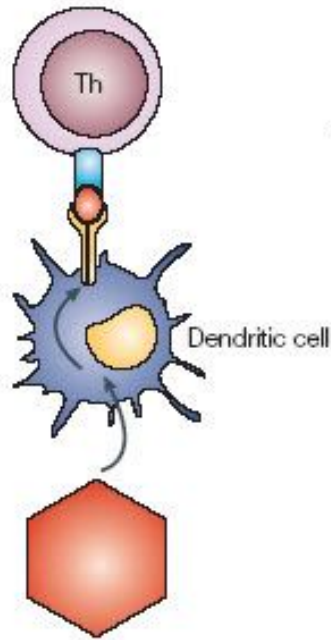**Optimizing Th cell responses**

Parameters that enhance T cell responses

1) Targeting dendritic cells

2) Activation of dendritic cells

3) Long-term T cell stimulation

Th

Dendritic cell

Aggregate Induced Immunogenicity

Optimizing Th cell responses

Parameters that enhance T cell responses

1) Targeting dendritic cells

Aggregates are particulate

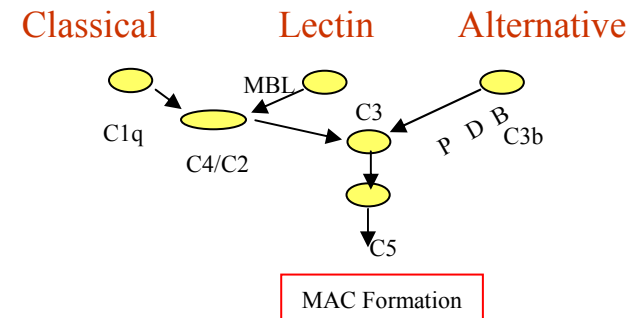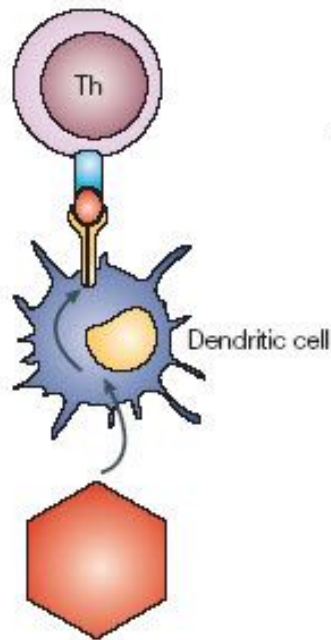→dendritic cells have evolved to efficiently take up and process particulate antigens.

Aggregate Induced Immunogenicity

Optimizing Th ell responses

Parameters that enhance T cell responses

2) Activation of dendritic cells

- Aggregates can bind low affinity/high avidity IgM antibodies more easily
→ Activation of complement (C1q)
- Aggregates may activate the alternative pathway of complement more easily.
→ Activation of C3

Th

Dendritic cell

Classical   Lectin   Alternative

MBL

C1q

C4/C2

C3

P D B C3b

C5

MAC Formation

→Aggregates have the potential to activate the innate immune system including dendritic cells

Aggregate Induced Immunogenicity

Optimizing Th cell responses
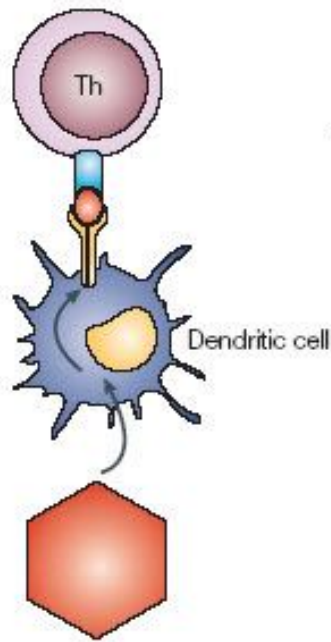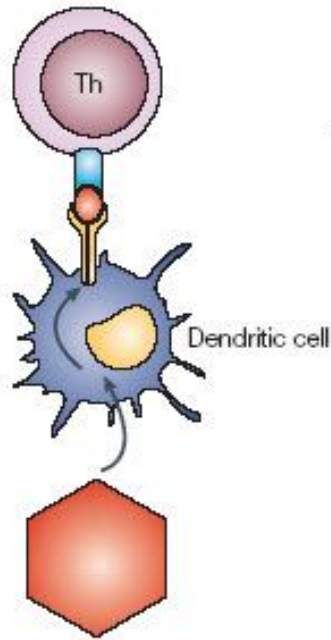
Parameters that enhance T cell responses

3) Long-term T cell stimulation

Aggregates do not persist but protein drugs are continuously injected

Th

Dendritic cell

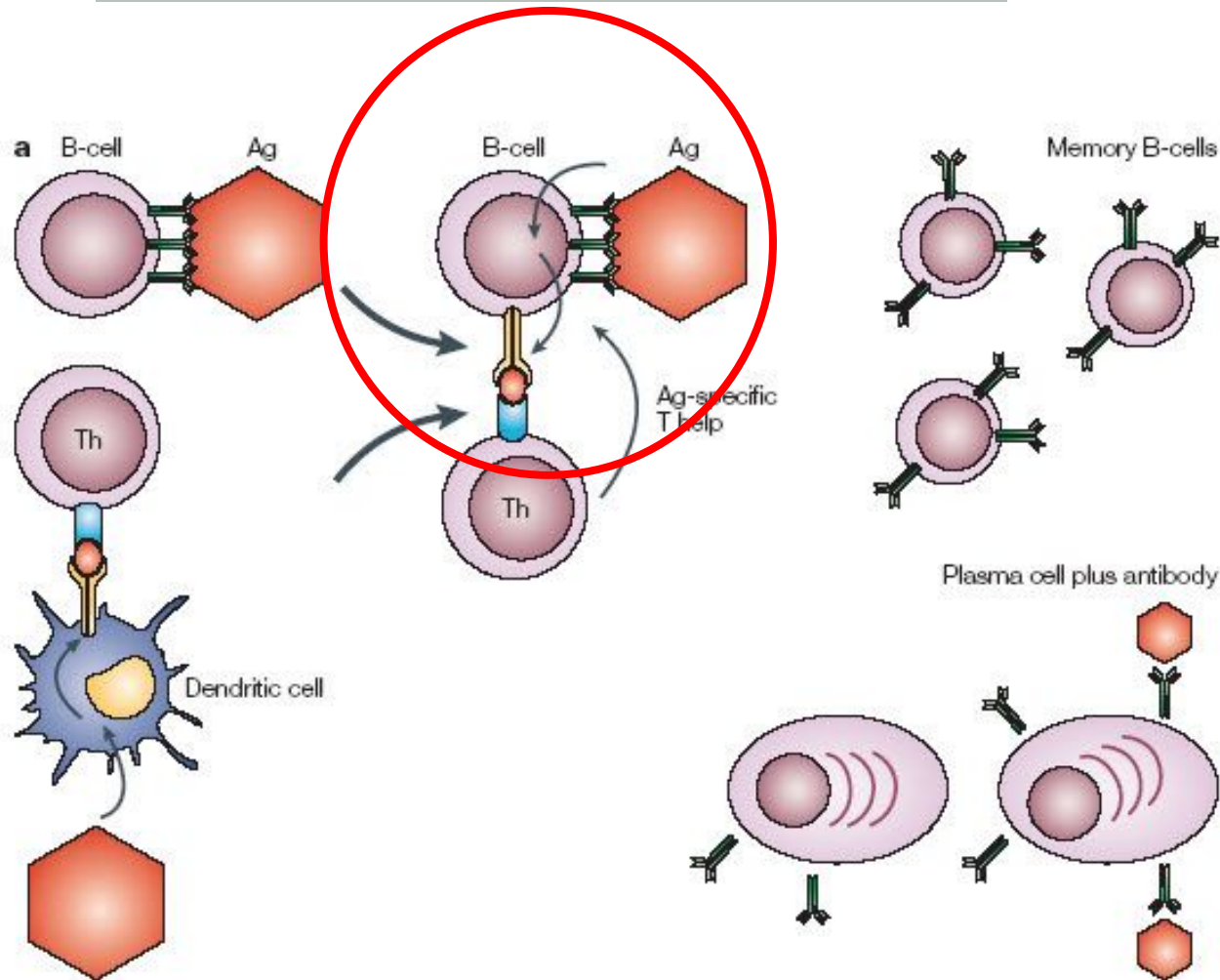# Aggregate Induced Immunogenicity

Optimizing Th cell responses

Parameters that enhance T cell responses

1) Targeting dendritic cells

2) Activation of dendritic cells

3) Long-term T cell stimulation
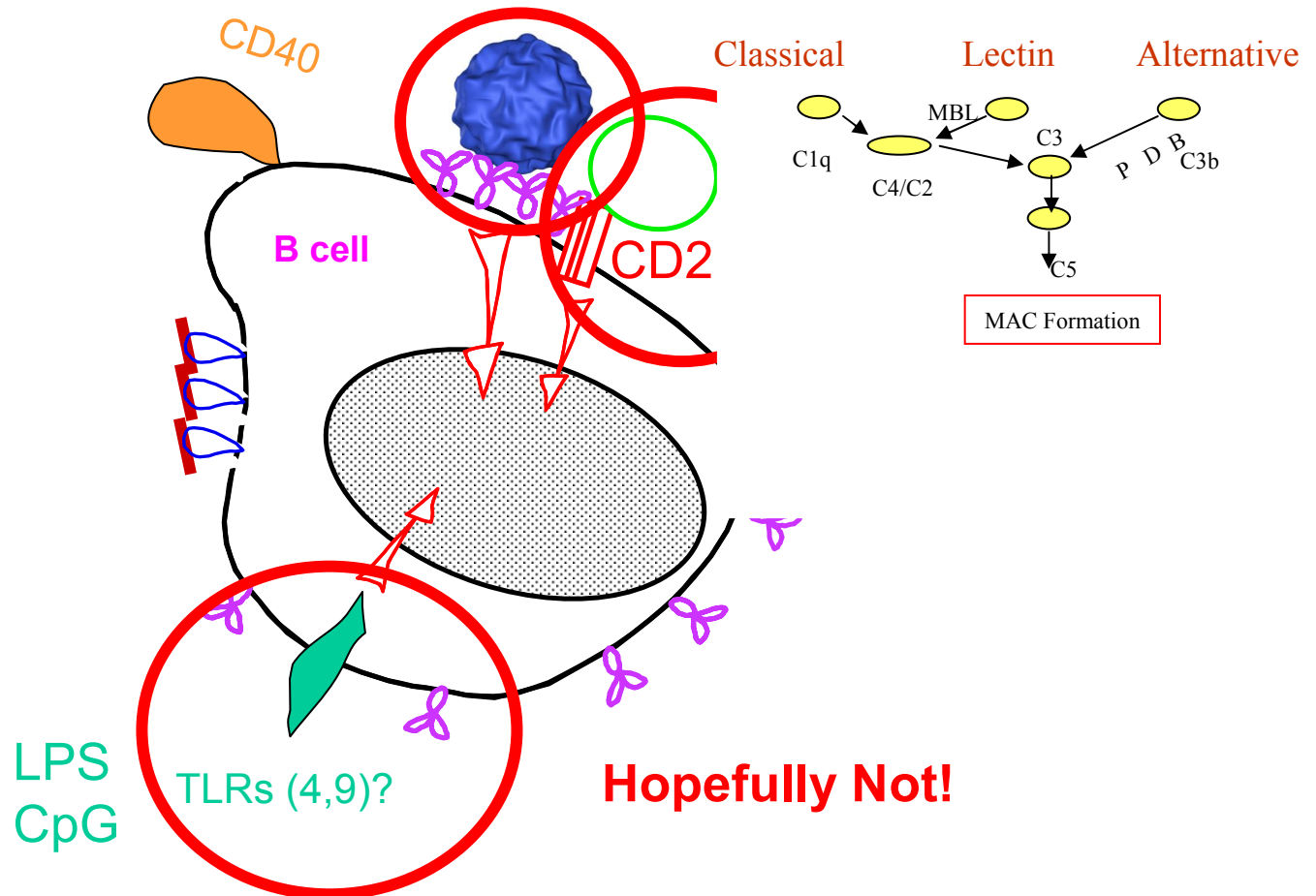
Aggregate Induced Immunogenicity

Aggregate Induced Immunogenicity

Harnessing pattern recognition of B cells

Aggregate Induced Immunogenicity
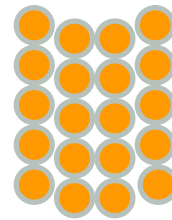
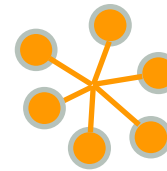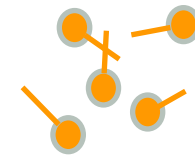**The More Repetitive the More Immunogenic**

Science 262, 1448-1451

Organization:	high	low	absent
Antibody Response	+++	++	-

Aggregates are Potent Inducers of B cell Receptor Signaling
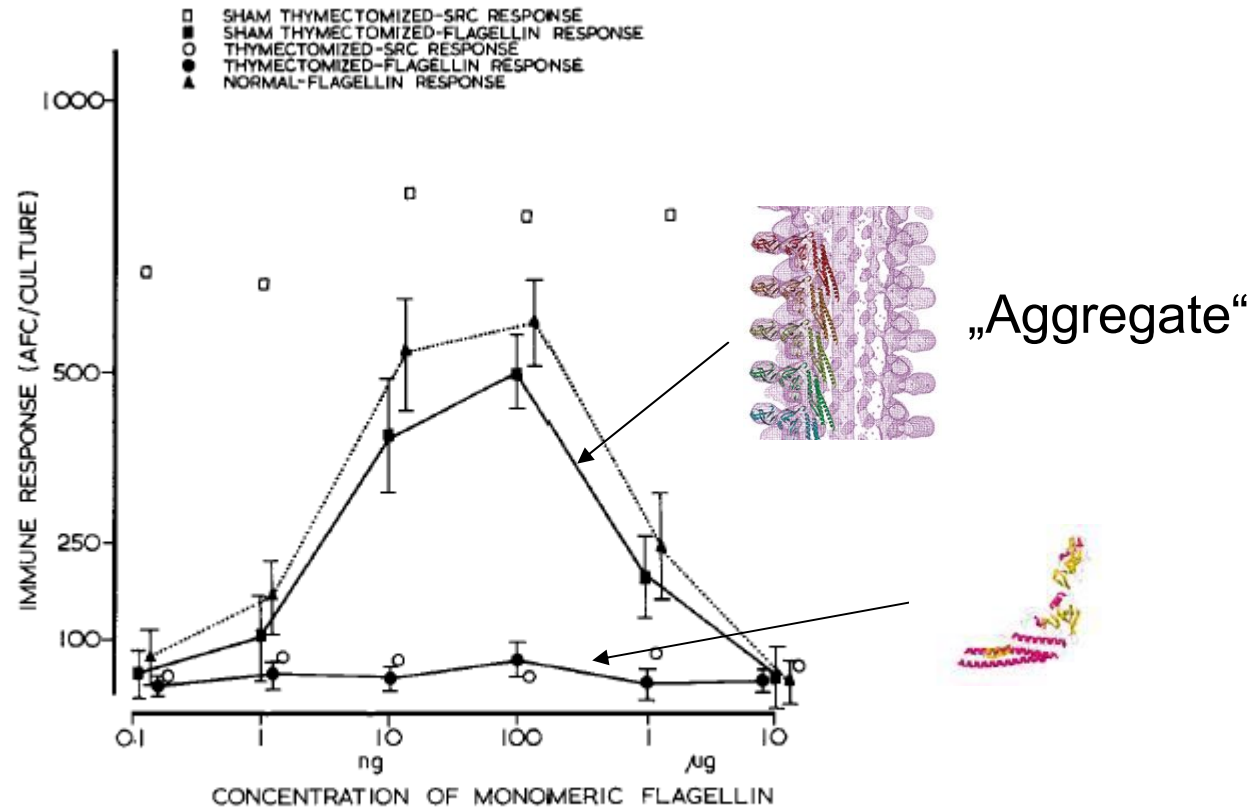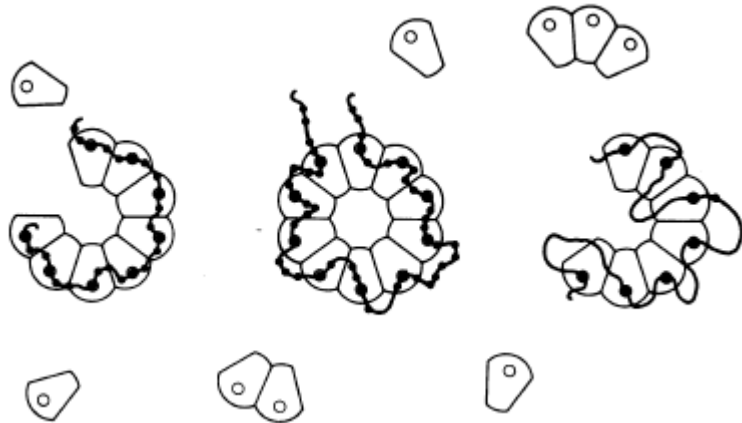
Aggregate Induced Immunogenicity

FIG. 2. The immune response of ATXBM, XBM, and normal spleen cultures to MON. Each point represents the arithmetic mean ± the standard error of the mean of 8–16 cultures. The mean of the response to SRC is indicated by the open symbols.

Feldmann and Basten, JEM 134: 103 (1971)
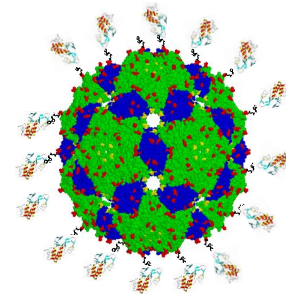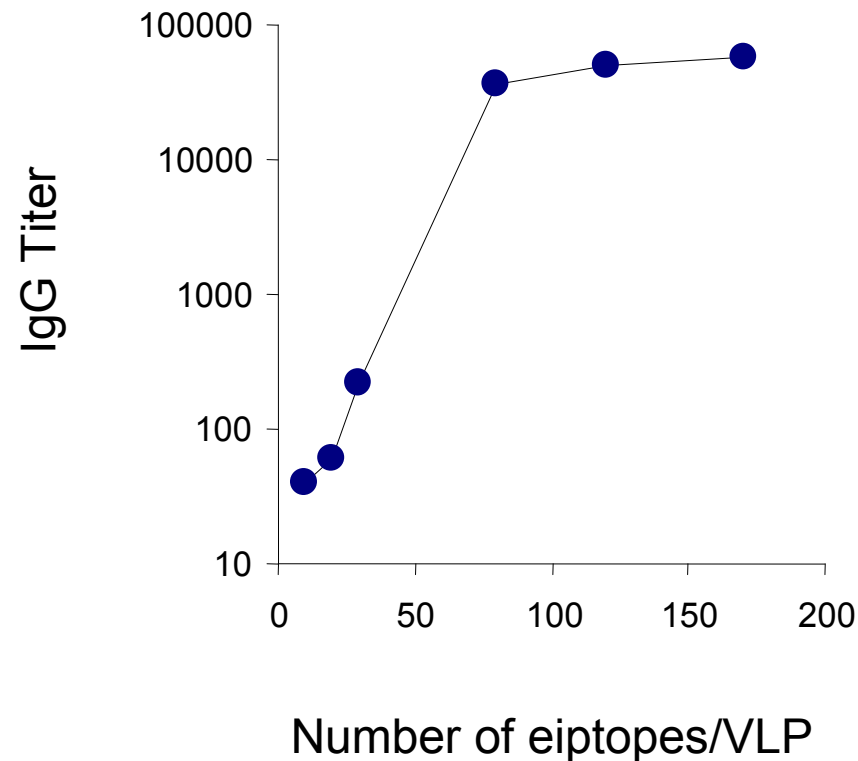
Aggregate Induced Immunogenicity

The Immunon Concept

- At least 15 haptens per molecule
- Optimal spacing is 5-10 nM
- B cell response is quantized in the „Immunon".

Dintzis, Dintzis and Vogelstein, PNAS 73: 3671 (1976)

Role of Epitope Densitiy & Organization

Number of epitopes determines antibody response

Number of eiptopes/VLP

Jegerlehner et al., EJI 32:3305

Aggregate Induced Immunogenicity
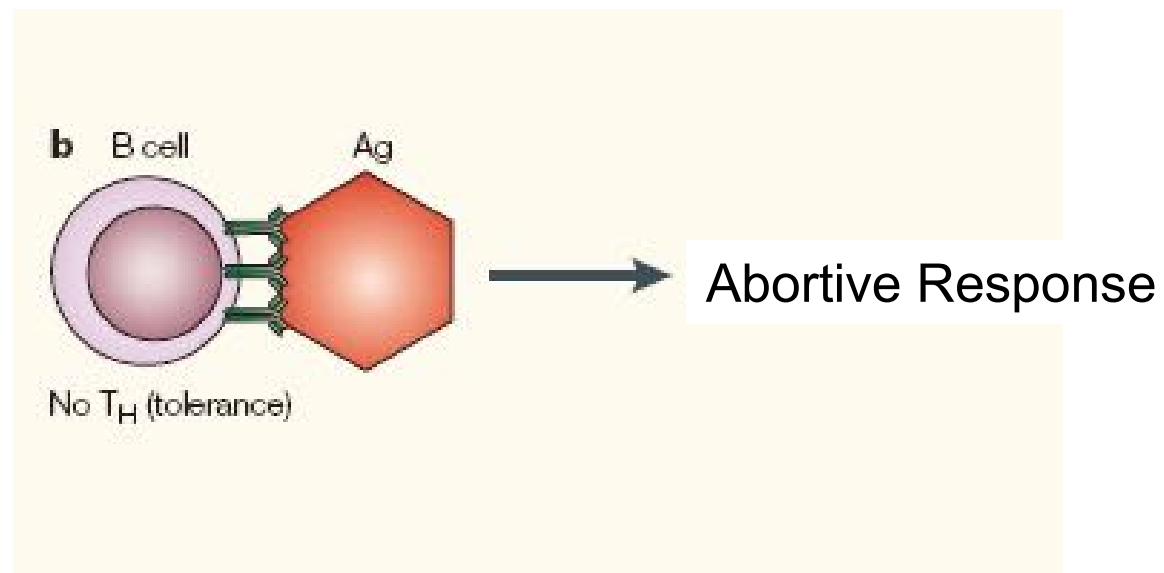
• Aggregates are potent inducers of Th cell responses

• Aggregates are potent inducers of B cell responses
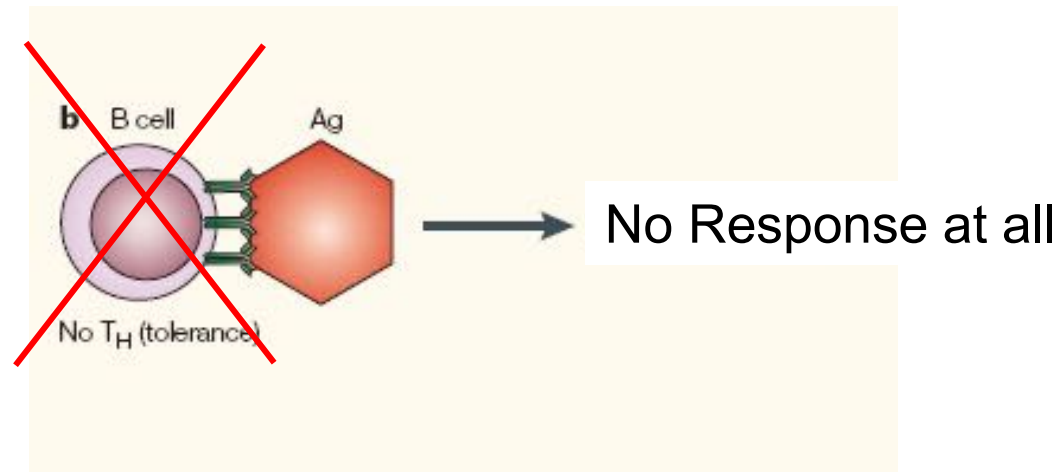
➔ But, how about immunological tolerance?

Immunological Background

Abortive Response

Bachmann & Dyer, Nat Rev Drug Disc 3:1-8

Immunological Background

No Response at all

Bachmann & Dyer, Nat Rev Drug Disc 3:1-8

Tolerance
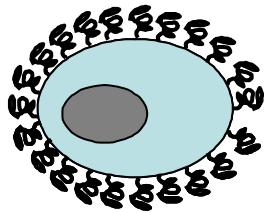
Tolerance

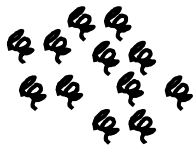|	T cells	B cells	
	++	++	(deletion)
	++	+	(anergy)
	++	-	
	++	-	
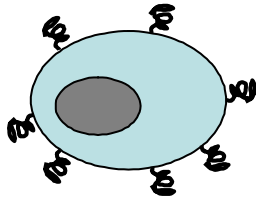
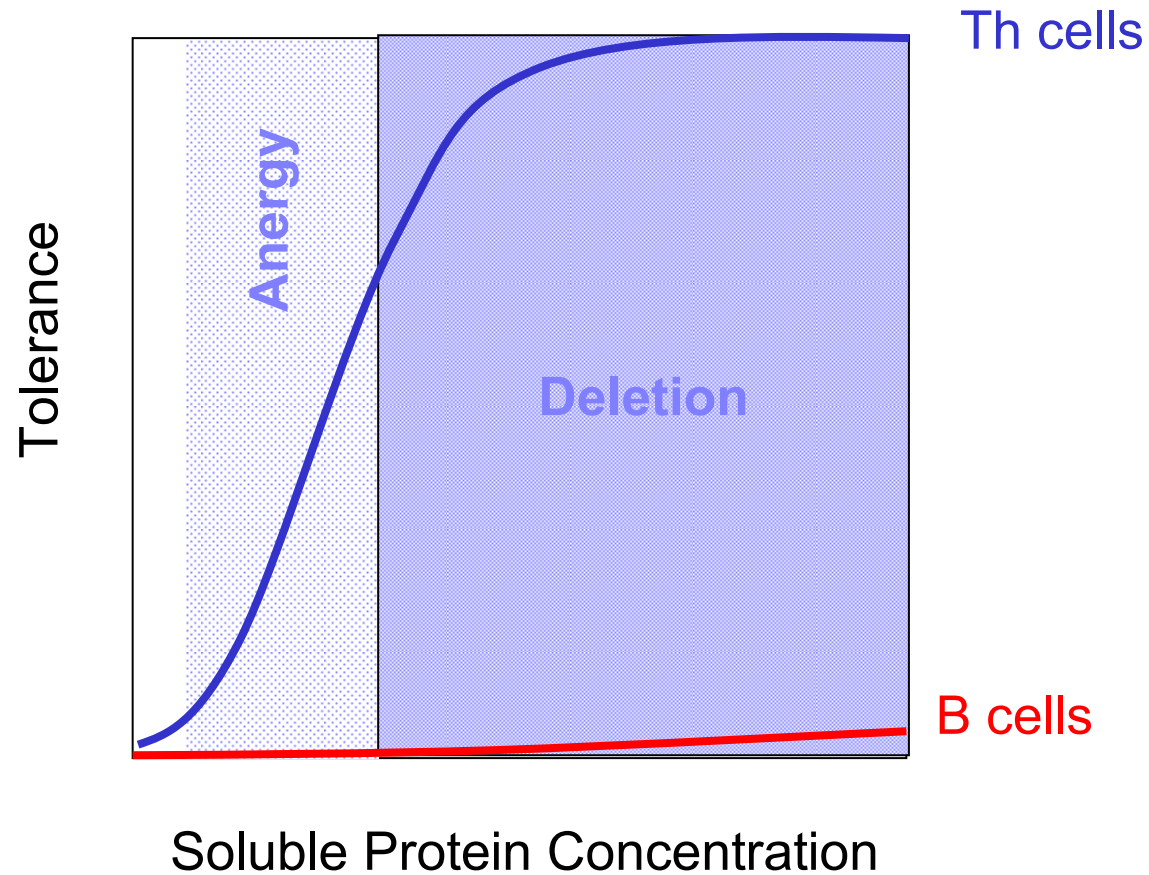- membrane bound proteins induce more solid tolerance than soluble proteins

- Th cell tolerance more solid than B cell tolerance

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Tolerance

Tolerance

Th cells

B cells

Tolerance

Anergy

Deletion

Membrane Protein Concentration/
Aggregates
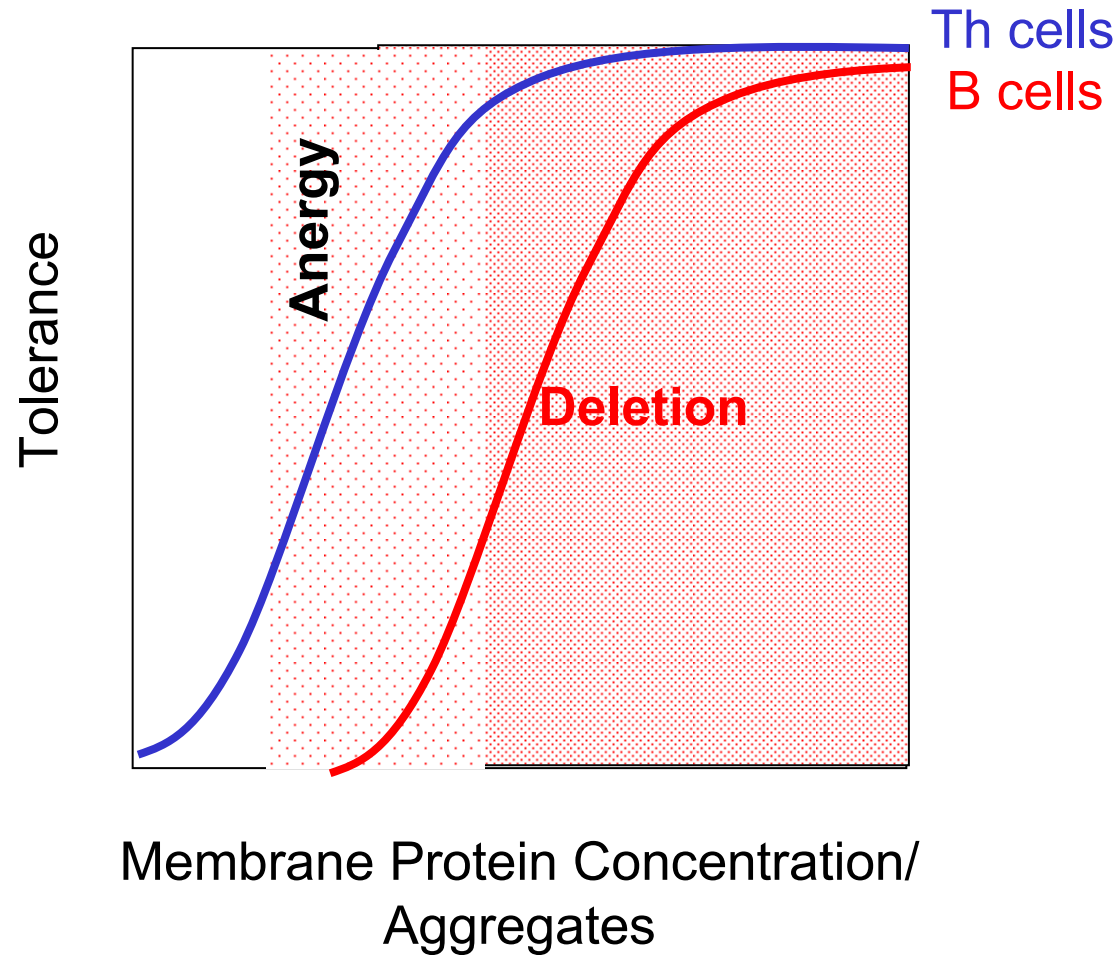
Tolerance

- Strong Th cell tolerance for all proteins

- No B cell tolerance for soluble proteins

→ How can self-specific B cell responses be induced?

Immunological Background

b B cell   Ag

No T_H (tolerance)

→ Abortive Response

Bachmann & Dyer, Nat Rev Drug Disc 3:1-8
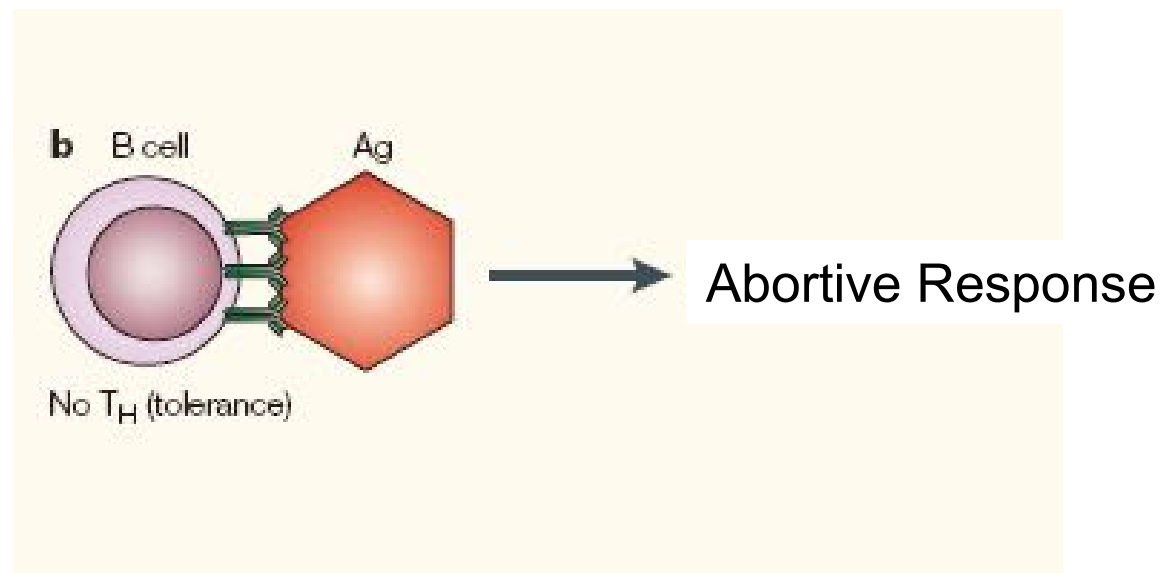
Self-specifc B cell responses

Th cell tolerance may be circumvented: linked Th
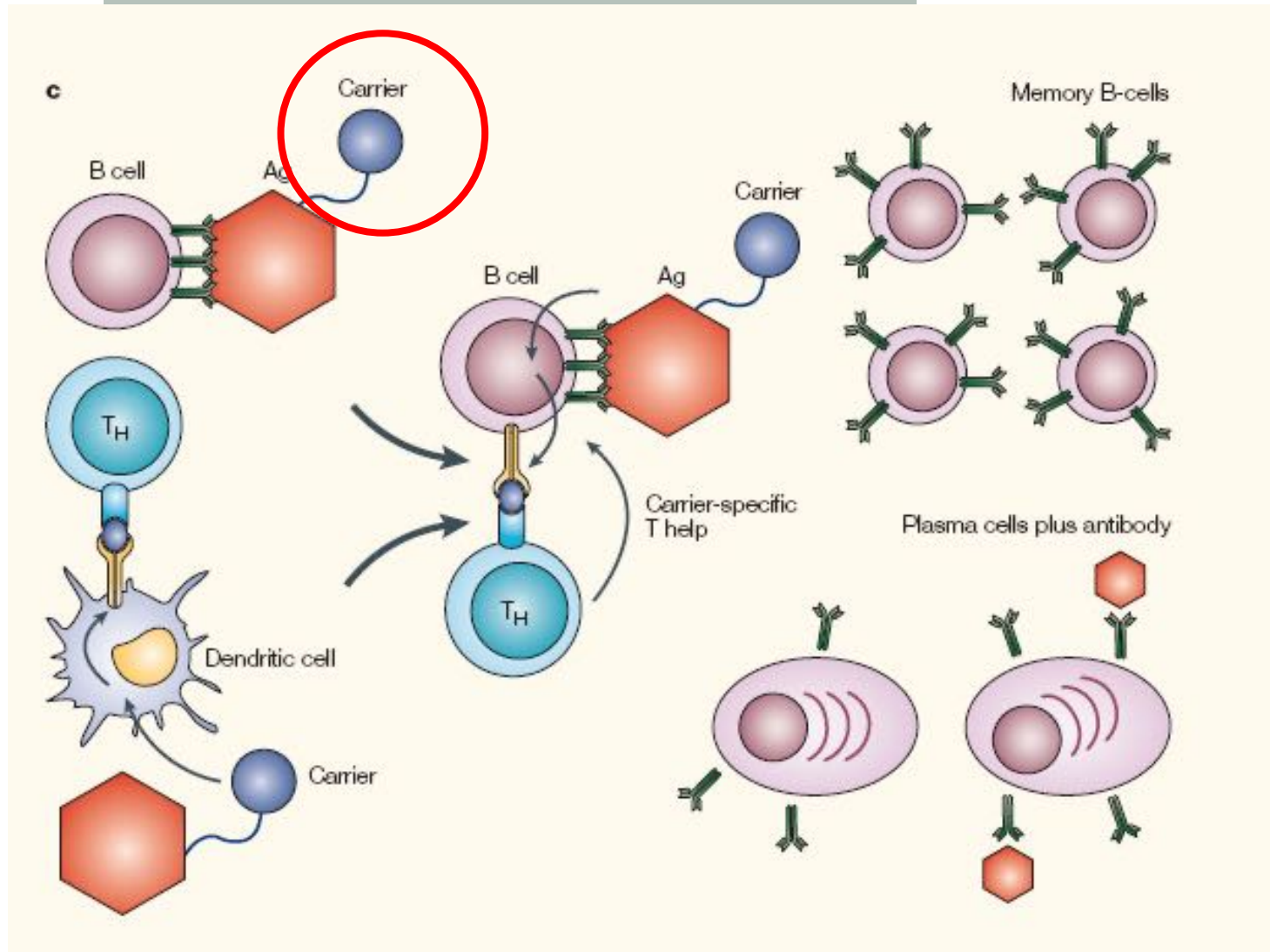
CYT006-AngQb

Phase IIa Results

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b i o t e c h n o l o g y

Hypertension Vaccine

Modulate the Action of Angiotensin II

Renin substrate (angiotensinogen)

(Renin inhibitors)

Renin

Angiotensin I

ACE inhibitors

Angiotensin converting enzyme

Angiotensin II

AIIR blocker

AT_1 Receptor on arterioles

AT_1 Receptor on adrenal cortex
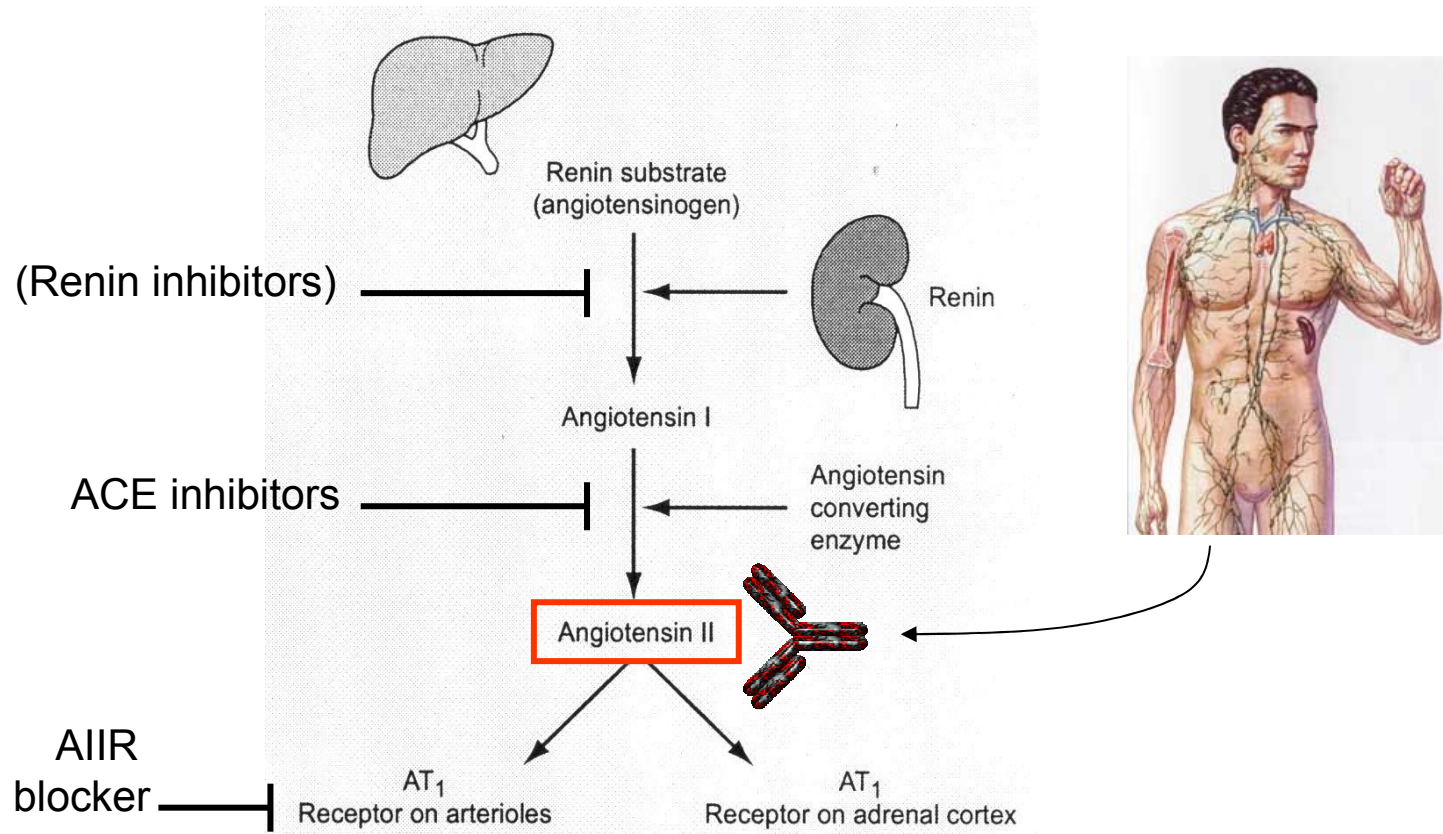
CYT006-AngQb

CGGDRVYIHPF
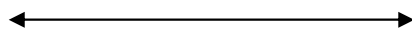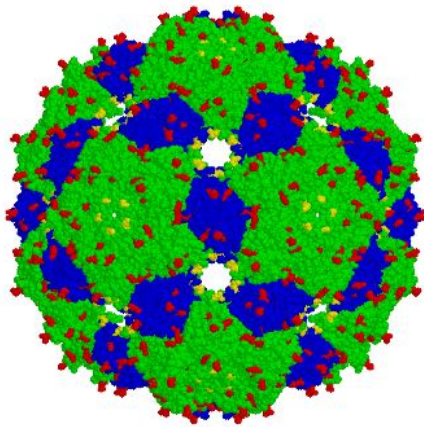
Angiotensin II

30 nm

Qbeta
virus-like particle
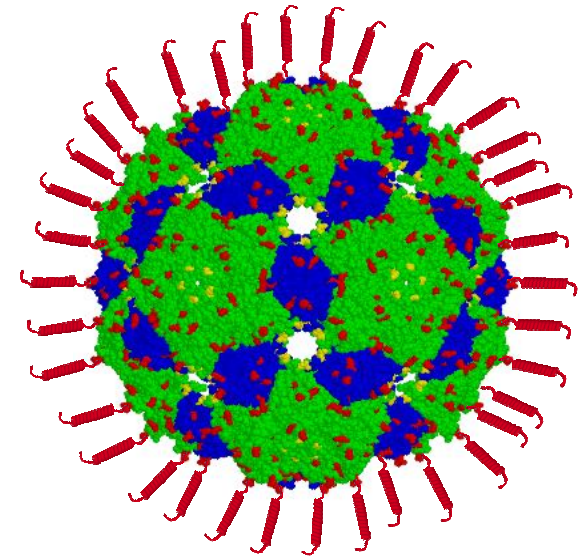
CYT006-AngQb

Preclinical Results (1)
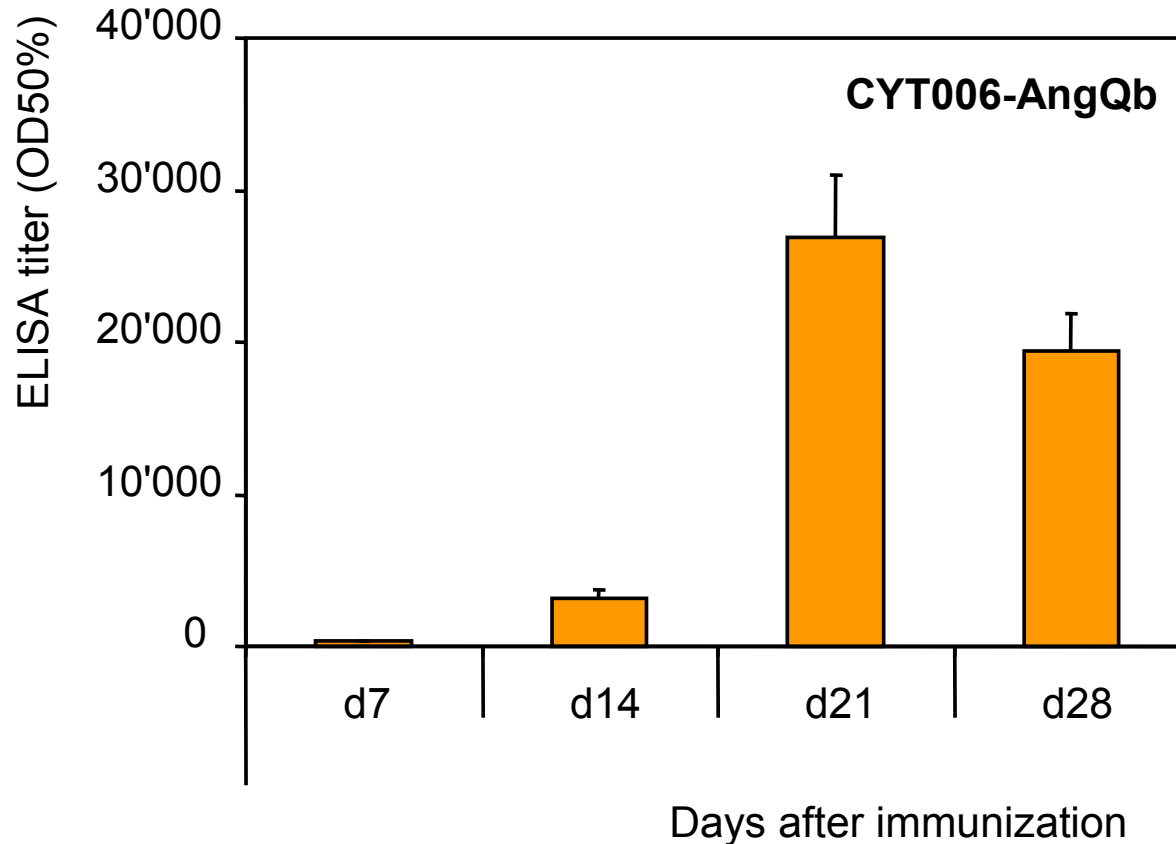
SHR rats immunised on d0, d14 and d28
with 400 ug CYT006-AngQb in Alum

Phase I Results

- Anti-angiotensin II IgG Titers
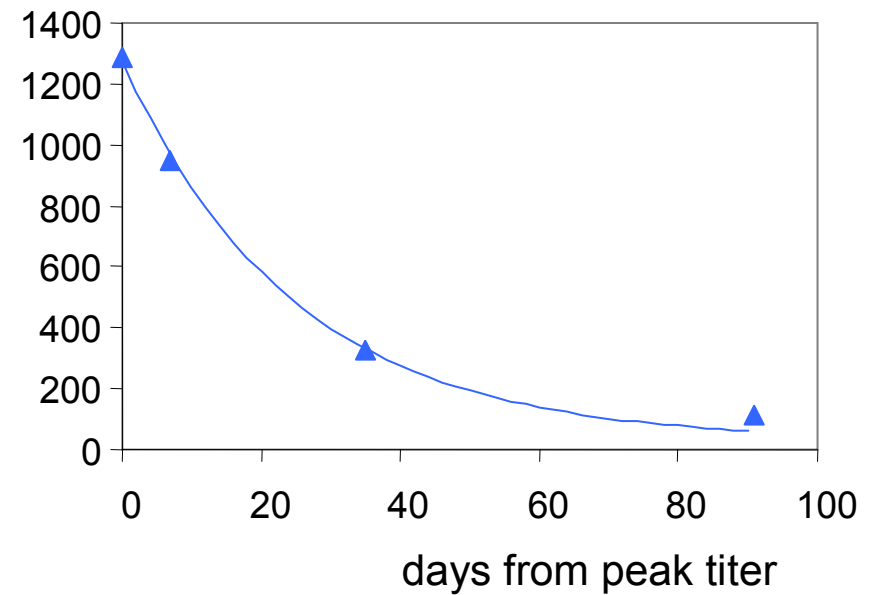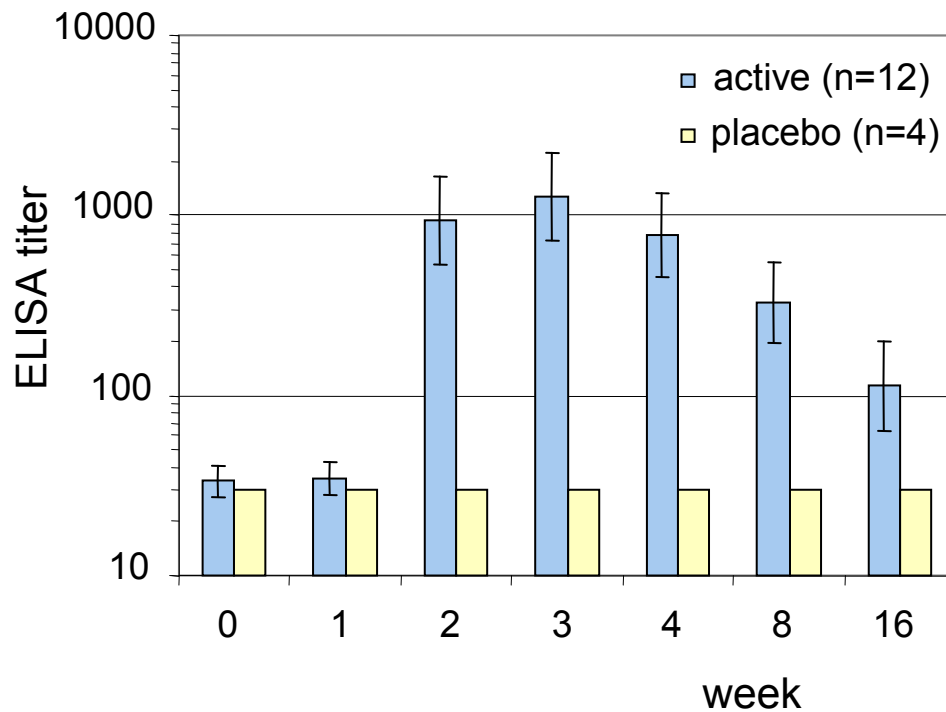
Results (4)
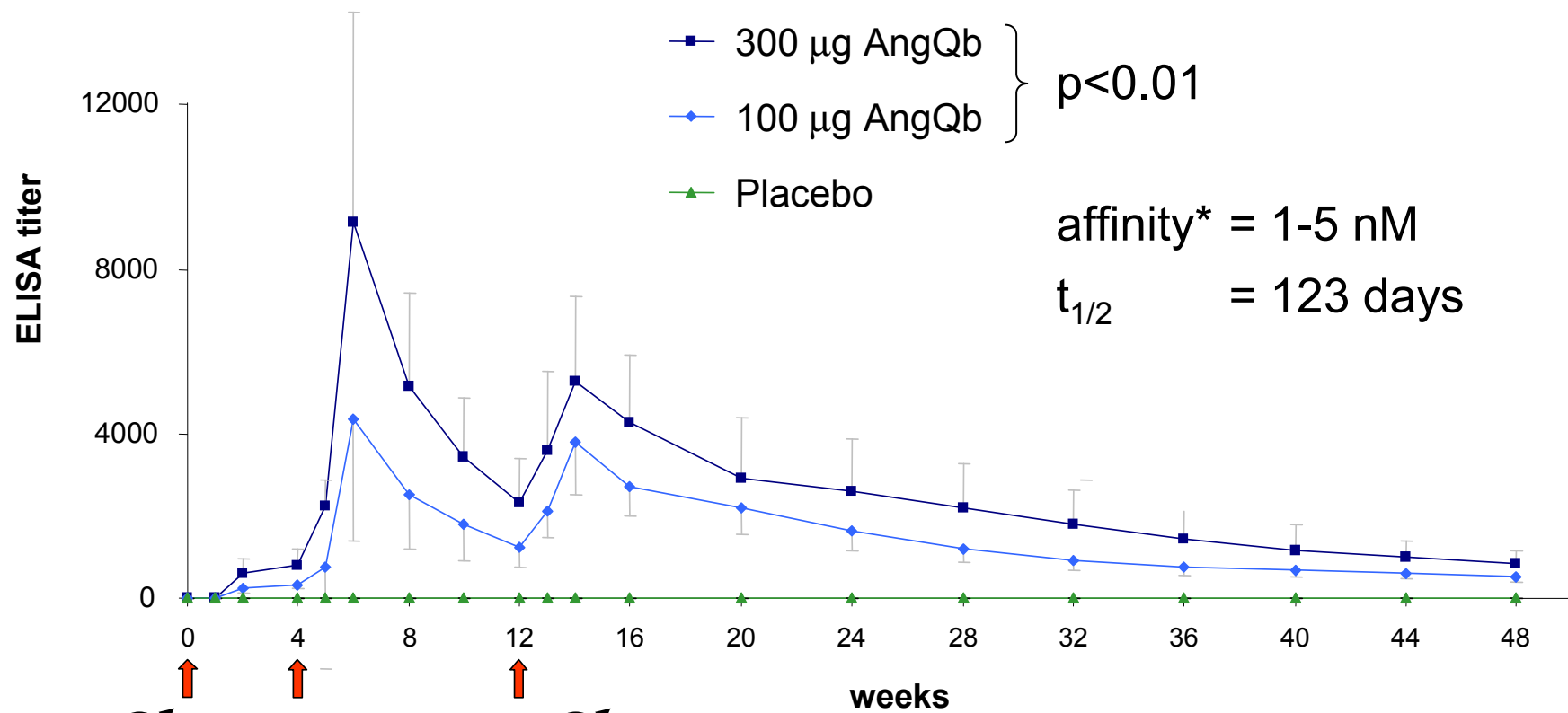
Legend:
- 300 µg AngQb
- 100 µg AngQb
} p<0.01
- Placebo

affinity* = 1-5 nM

$t_{1/2}$ = 123 days

Y-axis: ELISA titer (0, 4000, 8000, 12000)

X-axis: weeks (0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48)

The Lancet, 371 (2008) 821-827

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*range from 10 patient samples

Study Outline (2)

The Lancet, 371 (2008) 821-827

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The Lancet, 371 (2008) 821-827
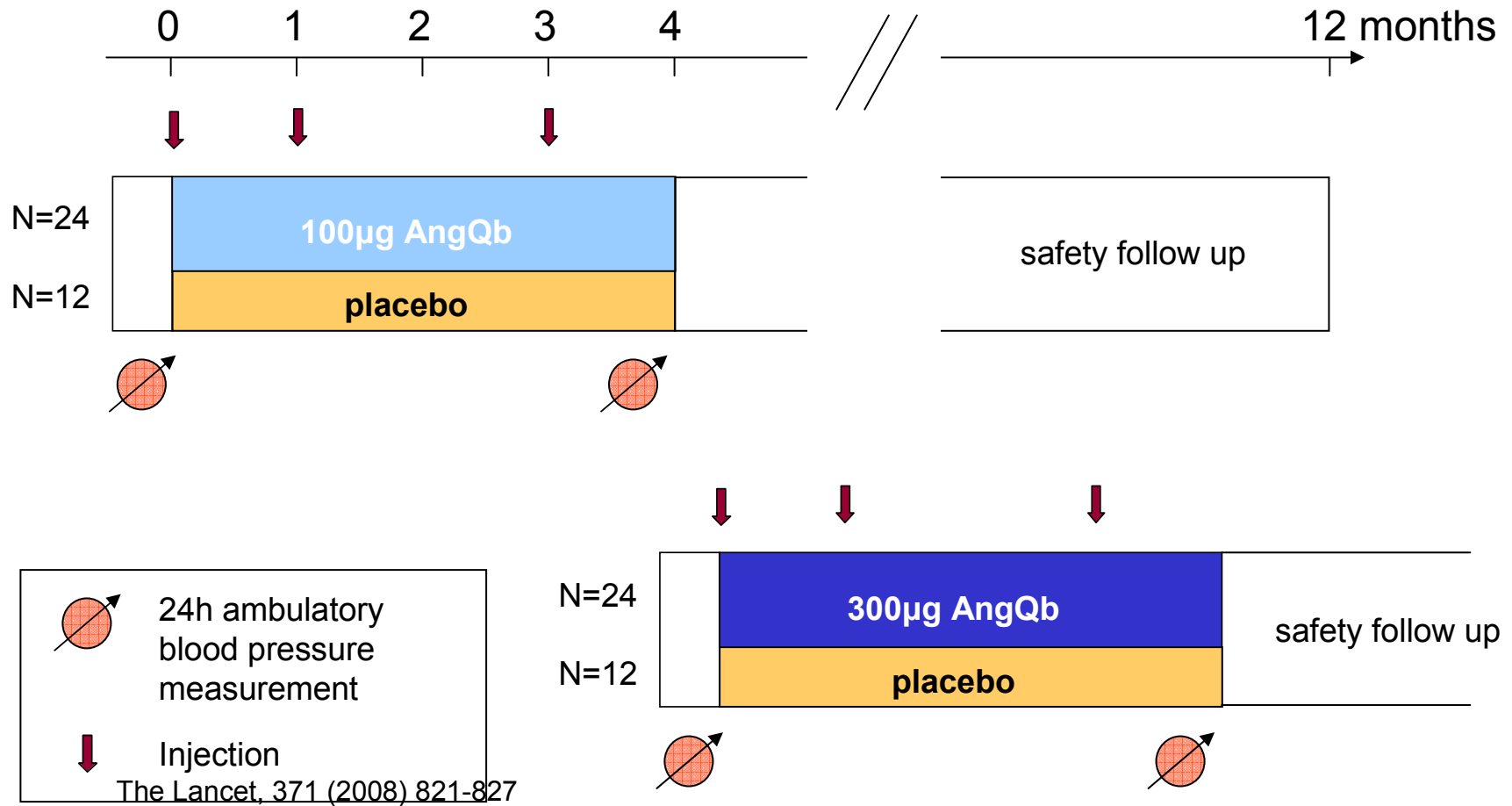
Results (3)

Day-time Blood Pressure	-9.0 / -4.0 mm Hg	p=0.015 / p=0.064
at 8am	-25 / -13 mm Hg	p<0.0001 / p=0.0035

The Lancet, 371 (2008) 821-827

Self-specifc B cell responses

- Linked Th is able to overcome B cell unresponsiveness

- Linking self-antigens to Th cell epitopes is able to cause

  self-specific antibody responses in mice and humans

→ However: Such antibody responses are reversible!

→Unless Th cell tolerance is also broken, antibody responses

  will be reversible and NOT permanently imprinted

Self-specifc B cell responses

2 Important Remaining Questions

- How can aggregates ocvecome Th cell tolerance?

- How long-lived are aggregate-induced antibdy responses?

Self-specifc B cell responses

Is the antibody response really permanently imprinted?

→Did anyone ever wait until the response declined or was the antibody response always maintained by continuous injection of aggregates?

Self-specifc B cell responses

What is the source of linked help?

→ Chemically modified proteins (eg chemicals released from uncoated rubber stoppers)?
→ Additional proteins within the aggregates?
→ Non-specific stimulators of B cells
  (LPS, DNA etc)

Self-specifc B cell responses

Does it make a difference that many patients are immunocompromised?

→ Reduced activity of regulatory T cells?
→ Rapid replenishment of the peripheral T
    cell repertoire may lead to inefficient tolerance
    induction.

Self-specifc B cell responses

Induction of protein-drug-specific antibodies is a rare event; did the few patients have a pre-existing antibody response?

Auto-antibodies against self-molecules

… a common phenomenon in healthy individuals

Protein	Frequency	Clinical Symptom	Reference
IL-6	0.1 %	none	EJI 34:3267
IL-1α	up to 25%	protection from Rheumatoid arthritis	Ann Rheum Dis 61:598
CCR5	up to 10%[*]	Reduced infection with HIV	JI 164:3426
IL-10	<3%	none	Immunol. Today 5: 209
IFNα	<3%	none	Immunol. Today 5: 209

* In HIV- partners of HIV+ individuals

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Auto-antibodies against self-molecules

Also Against Ghrelin, a Hormone Regulating Appetite

Auto-antibodies against self-molecules

Also Against Ghrelin

Blood Donor 34
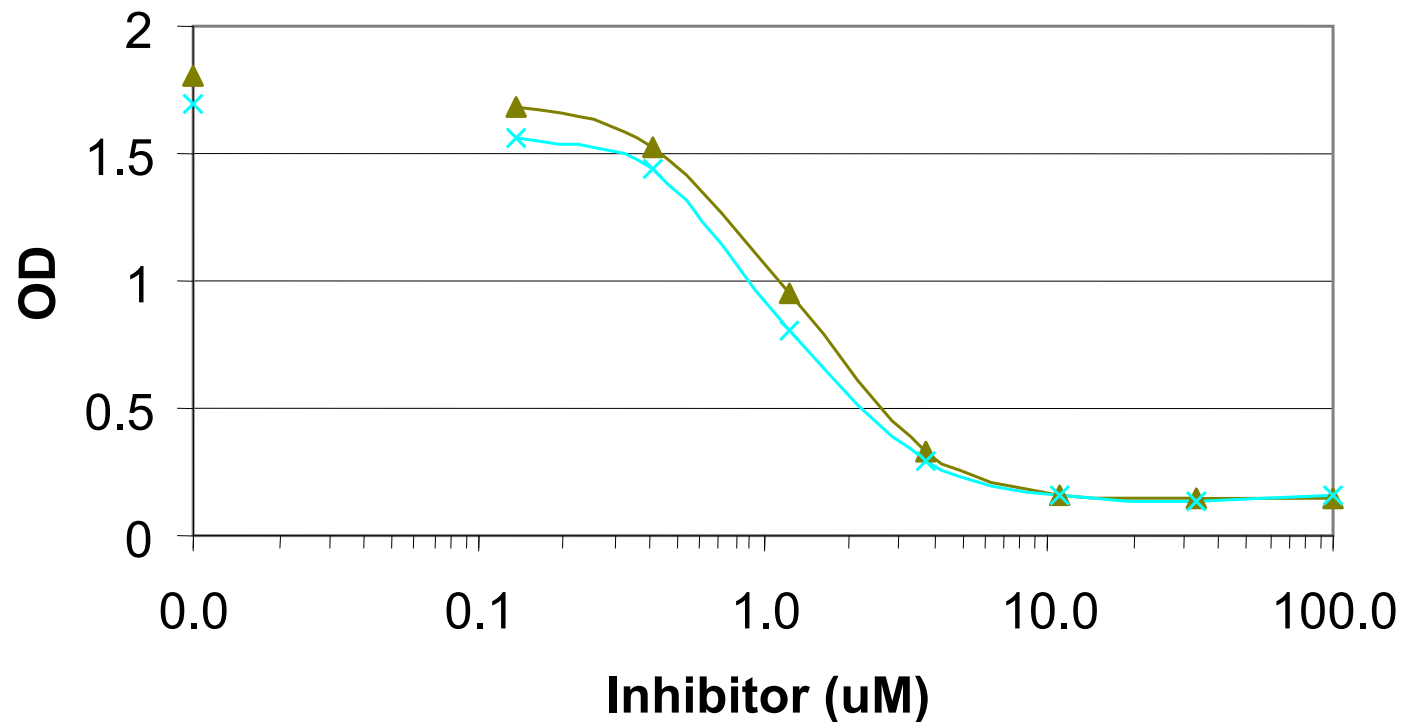
c y t o s
b i o t e c h n o l o g y

Aggregate Induced Immunogenicity

- Aggregates are potent inducers of Th cell responses (particulate)

- Aggregates are potent inducers of B cell responses (repetitive)

- Soluble proteins induce Th cell but no B cell tolerance

- For induction of antibody responses against cytokines etc, Th cell tolerance but not B cell tolerance has to be overcome

- Multiple but yet to be defined mechanisms may be responsible for break of Th cell tolerance; these include:

  → Conjugated Th Cell Epitopes

  → Reduced Regulatory T cell activity

  → Reduced Tolerance Induction

  → Pre-existing Immunity

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