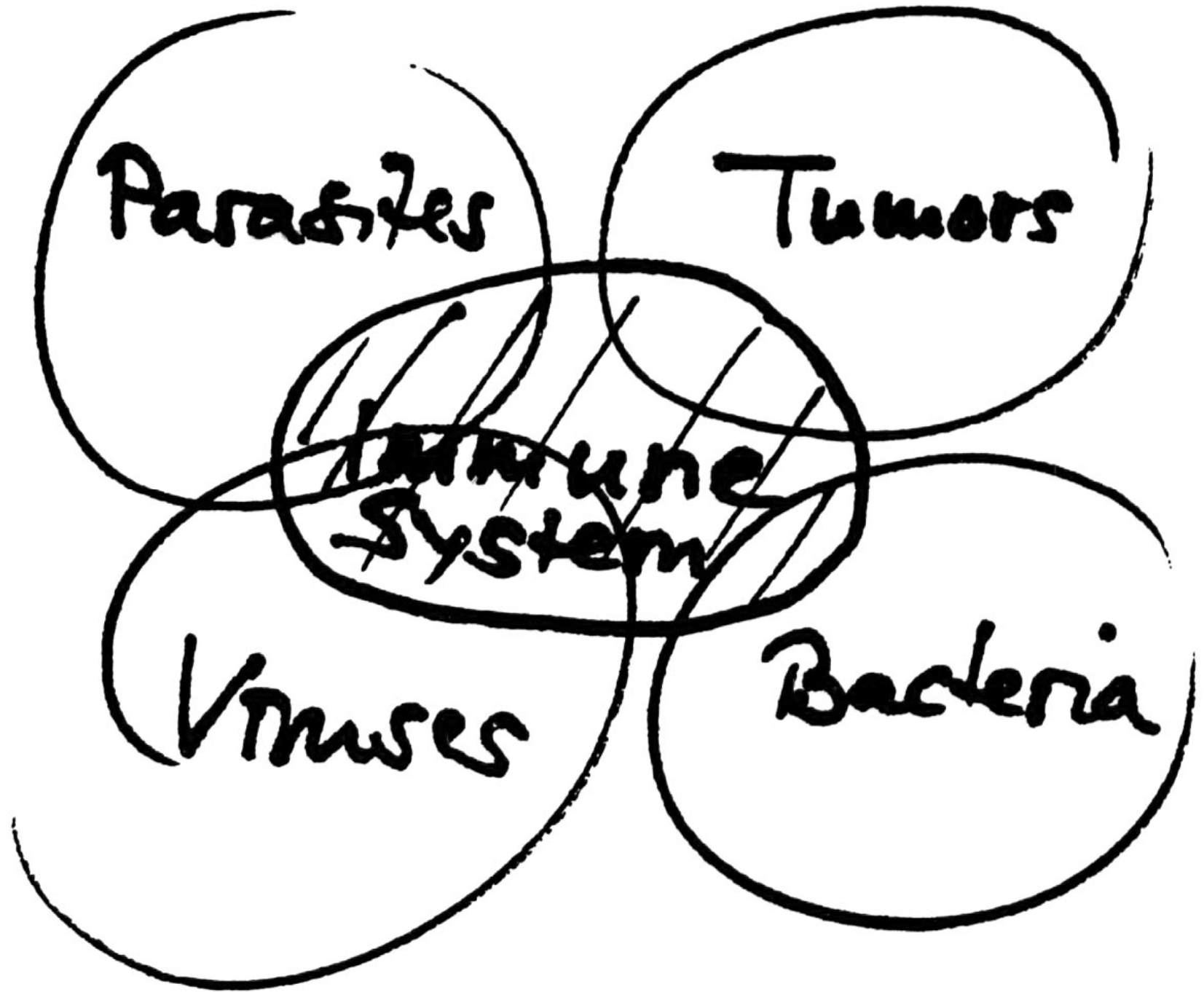


Anti-Viral Antibody and Immunological Memory Responses

Rolf Zinkernagel

University of Zurich, Switzerland



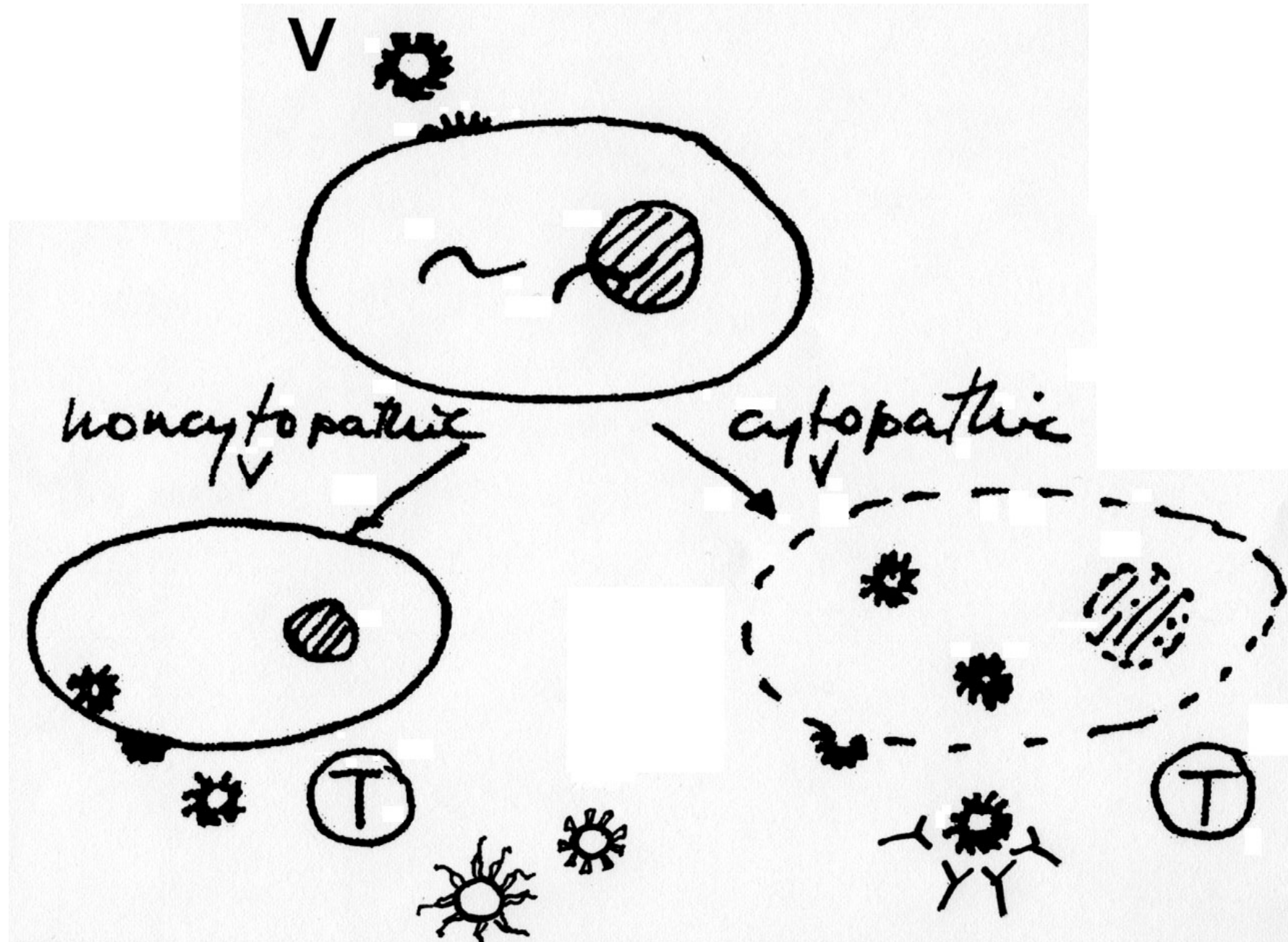
Parasites

Tumors

~~Immune System~~

Viruses

Bacteria



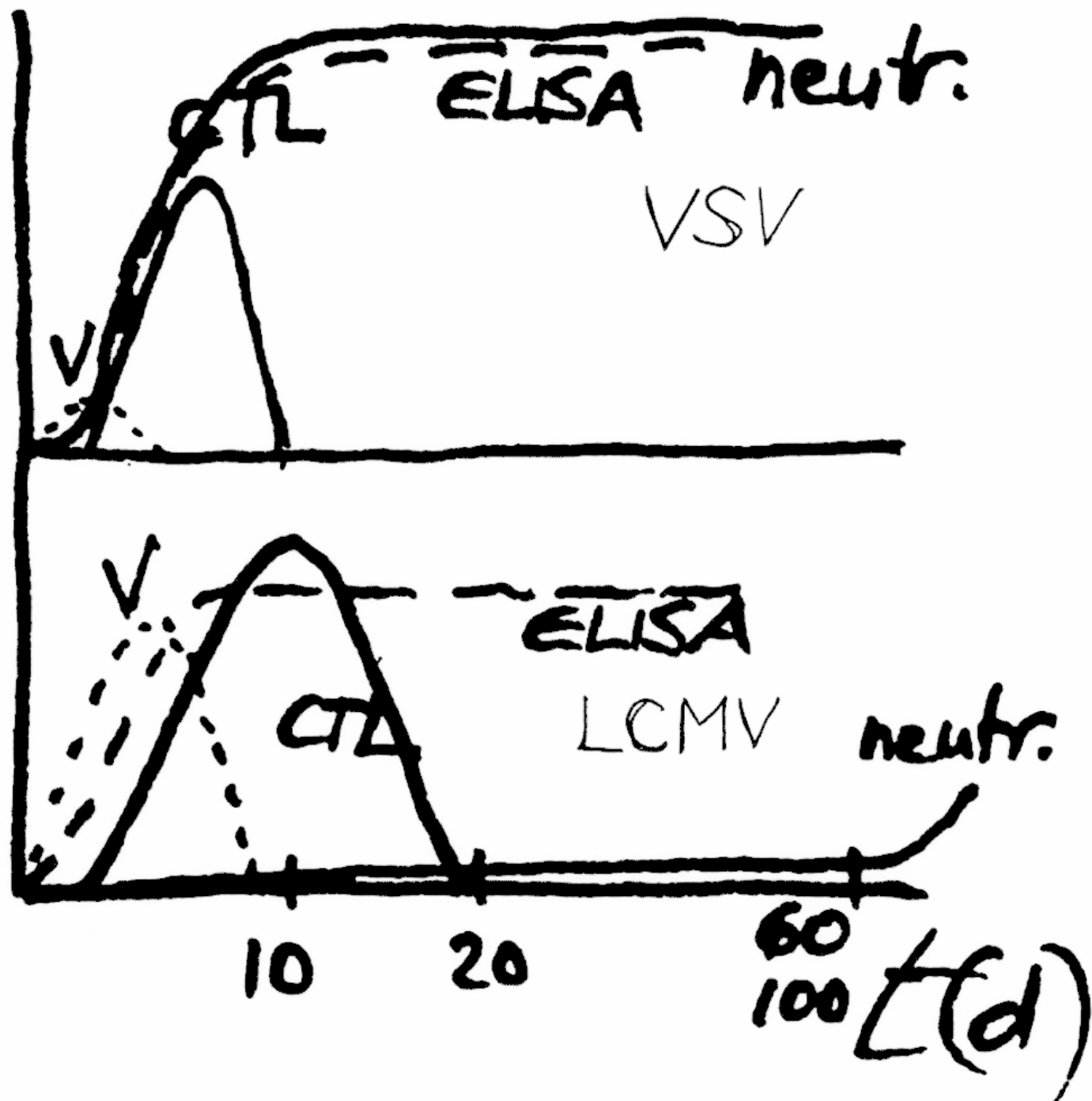
B cells

react:

- Polymers
- (Polymeric self-antigens: collagen, DNA etc.)
- Monomers + LPS (CpG)
- 2° lymphoid organs, GC

not:

- No B cells (low frequency or deleted)
- Monomers, no T help
- No follicular structure (IgA!)



nAb

natural
Ab

IgM
T indep.

IgG
T dep.

VSV

1/20

d2-4

d6-8

LCMV

<1/2

?

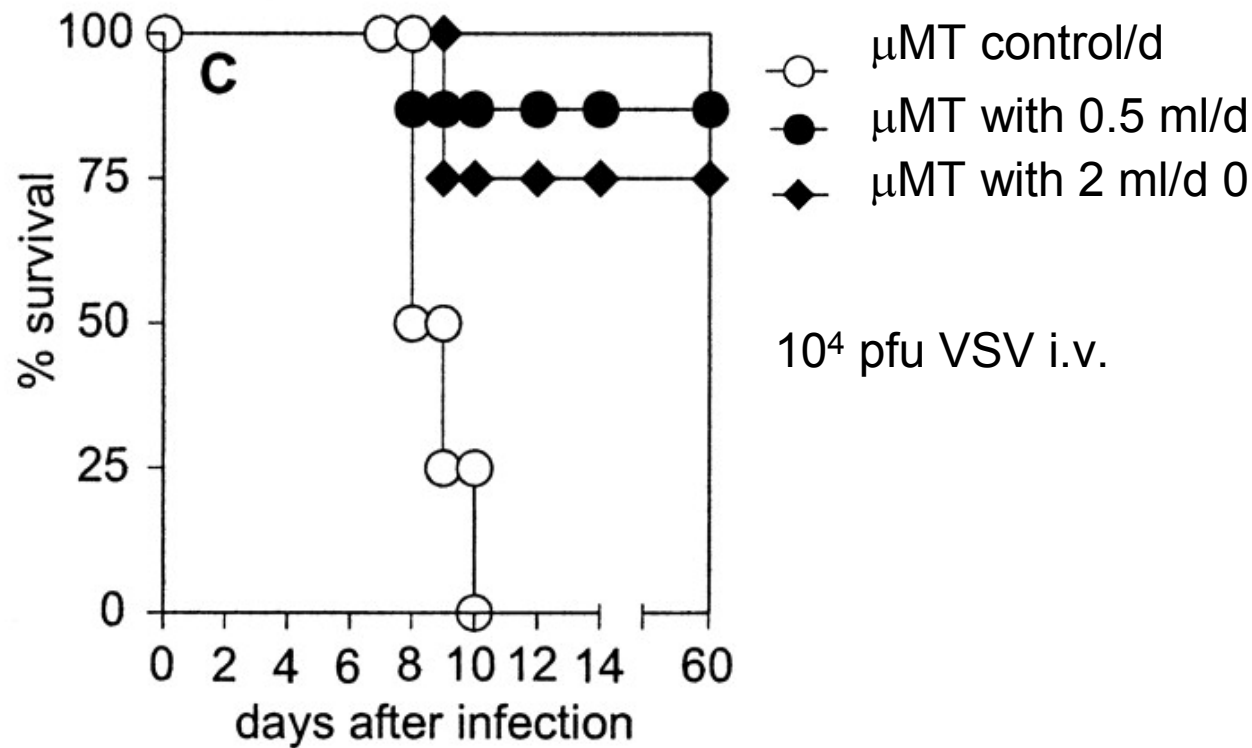
d70-150

tgH25

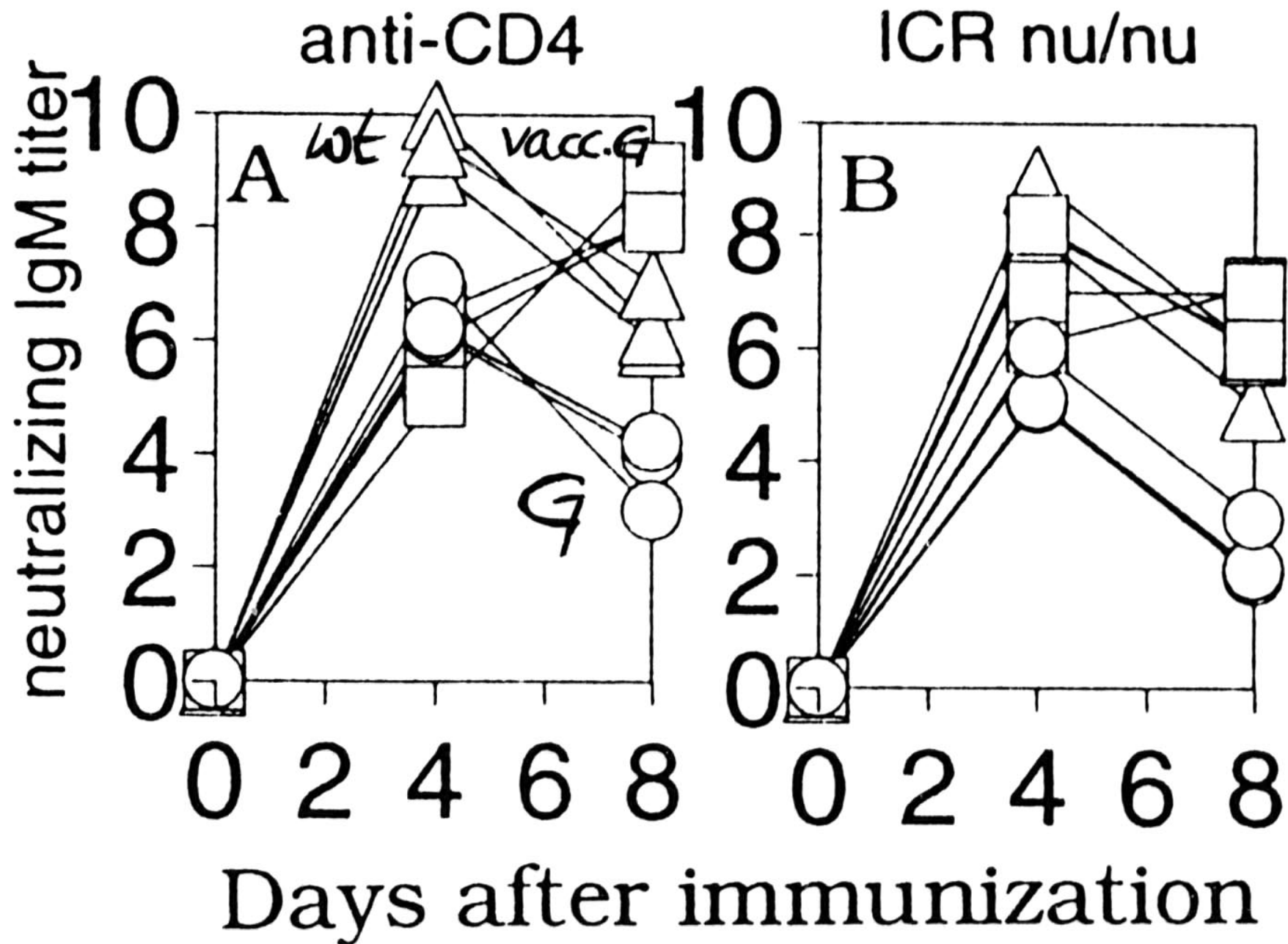
1/40

d2-4

d6-10

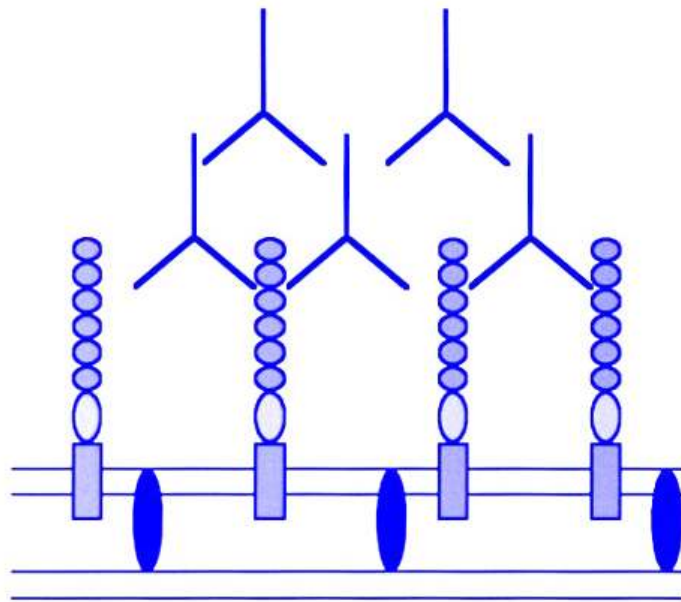


*Hengartner
Ochsenbein
Hangartner
Zellweger*

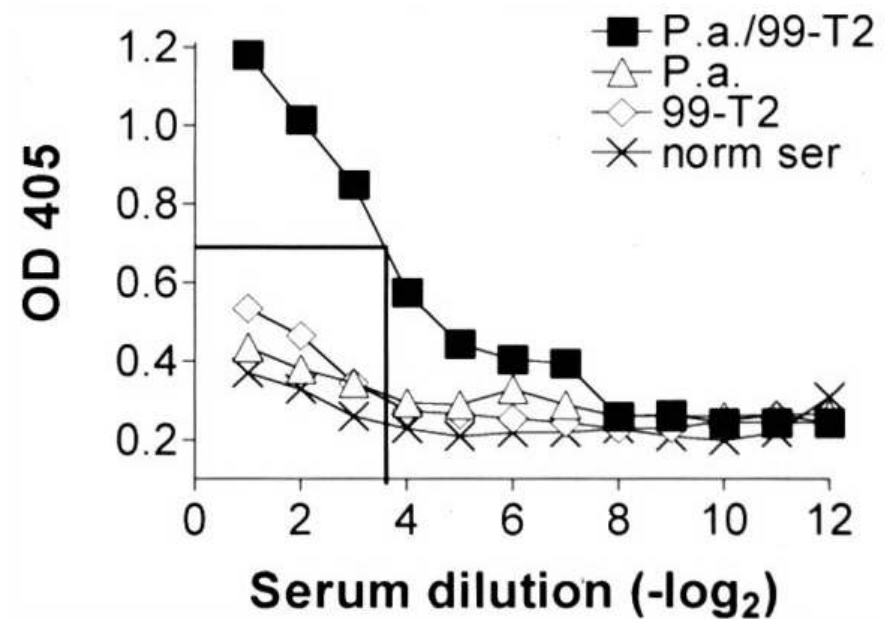
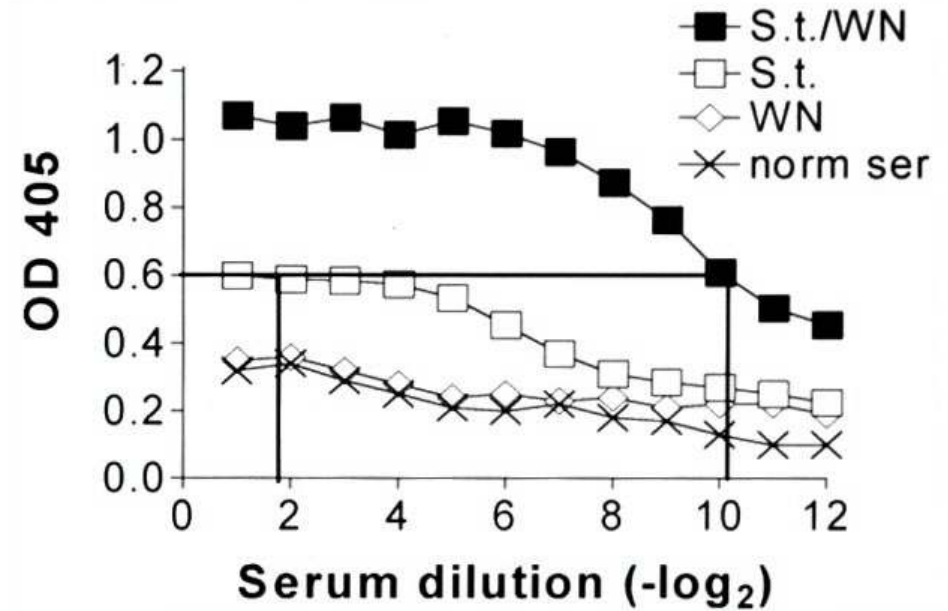


Anti-antibodies/ gramnegative bacteria

Salmonella typhi



Pseudomonas aeruginosa

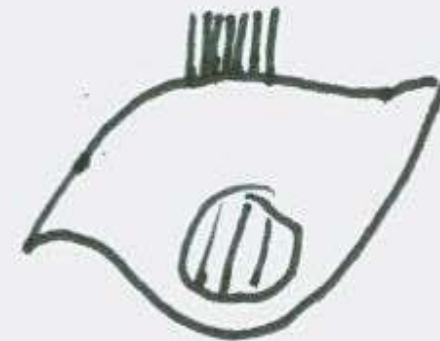
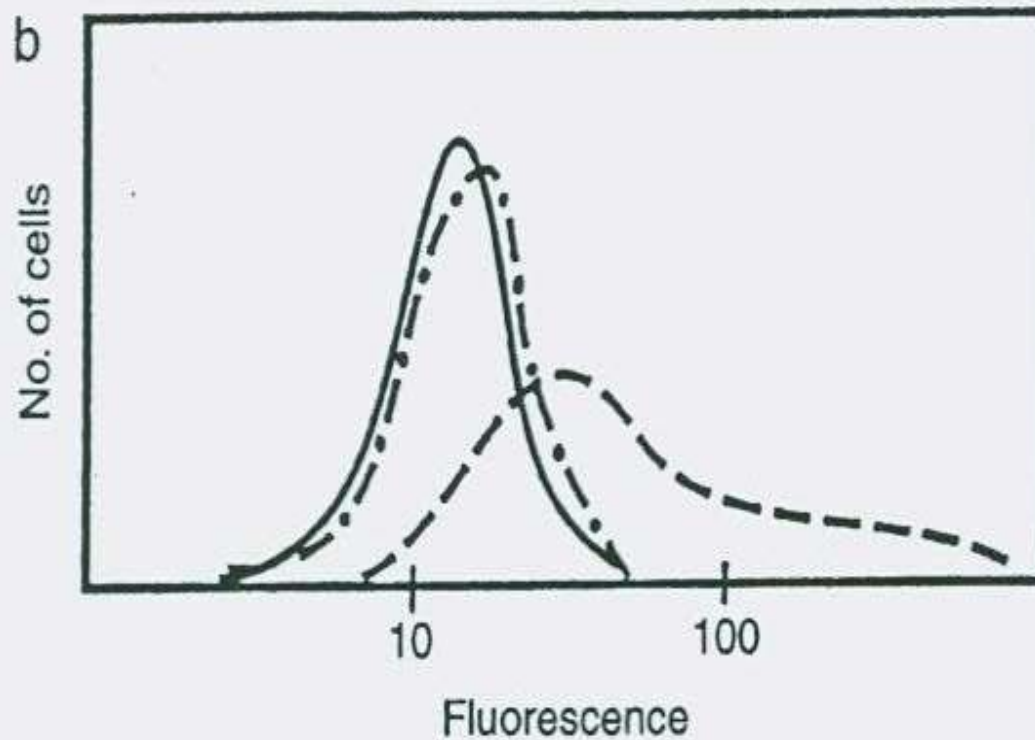


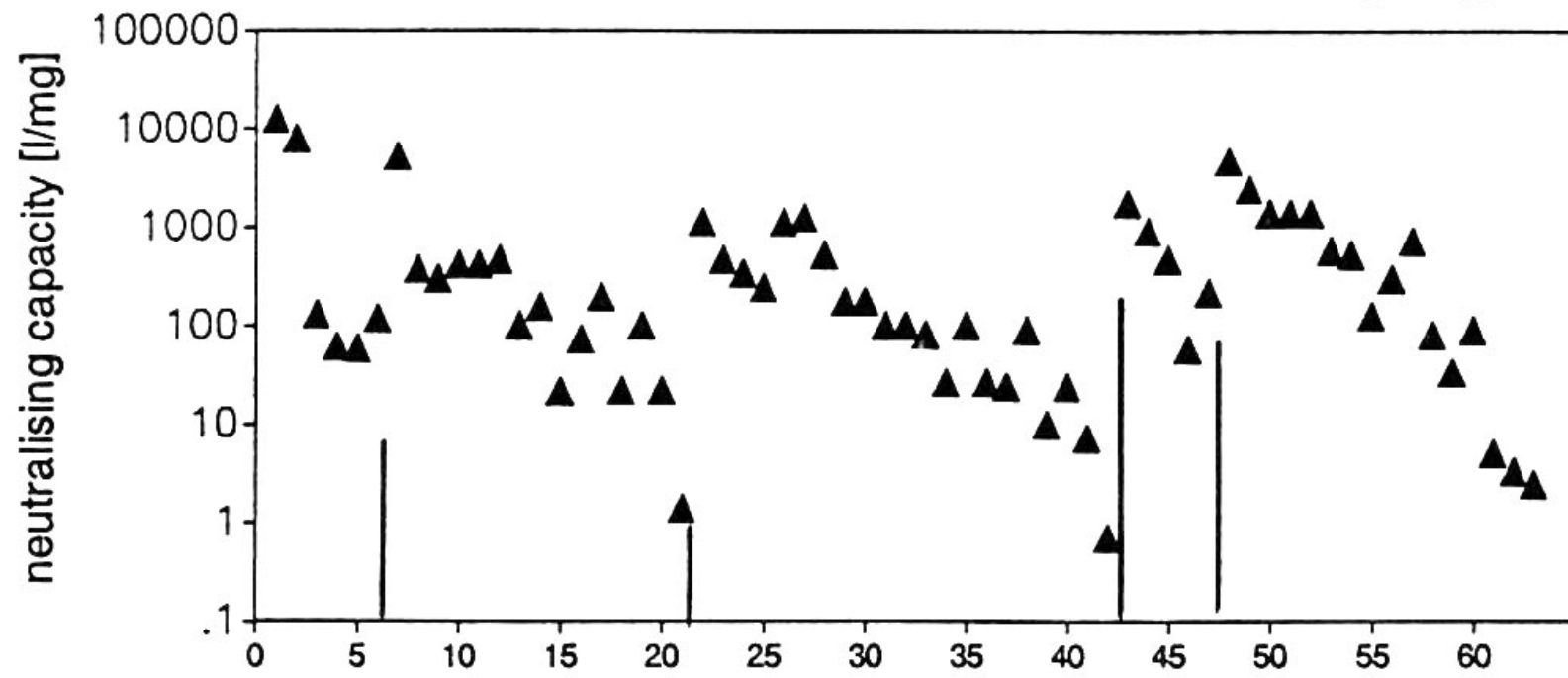
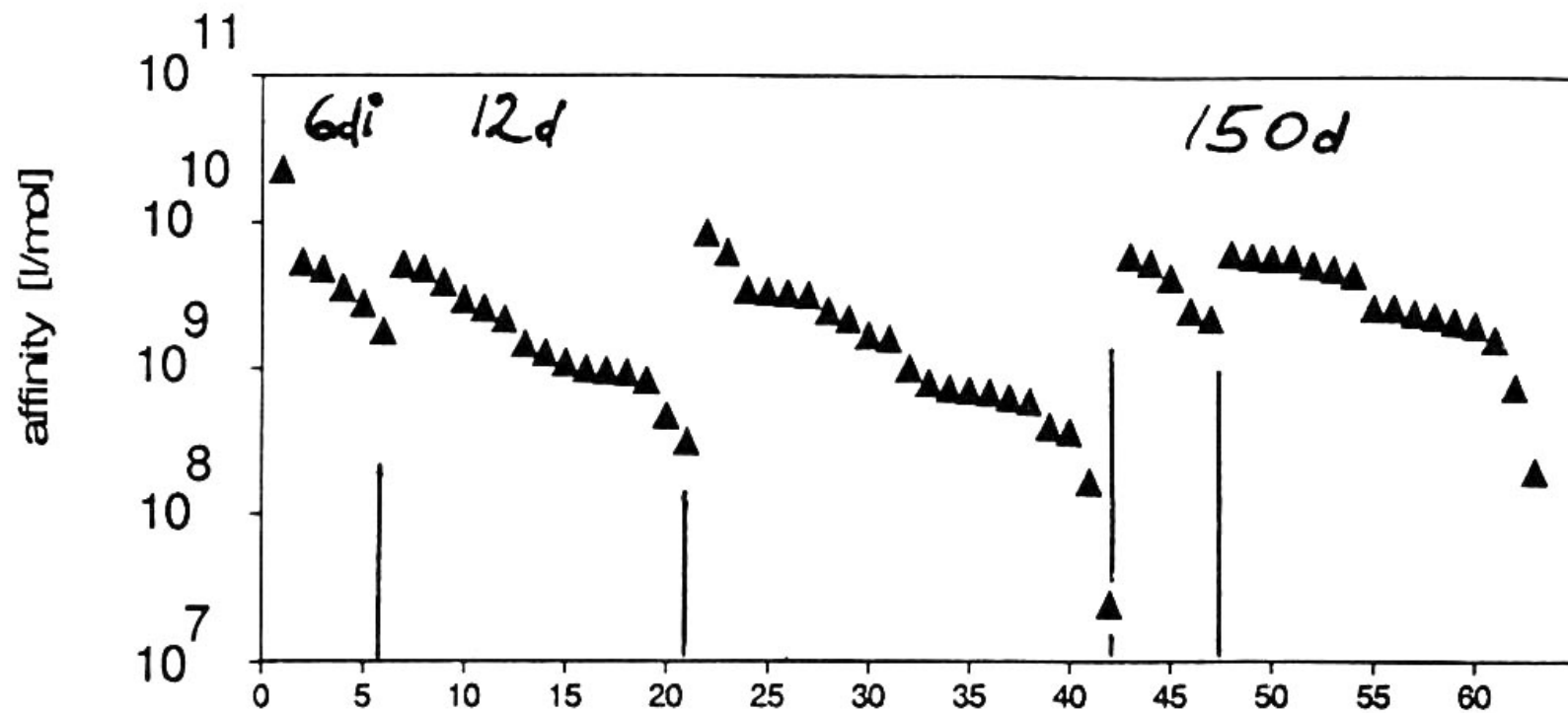
a

Competing antibodies		Detected antibodies, no./no. tested
mAb	Subclass	
VI 7	IgG1	30/33 mouse IgG2a mAb
VI 24	IgG1	28/31 rat IgG mAb
VI 22	IgG2a	28/31 rat IgG mAb
VI 48	IgG2a	28/31 rat IgG mAb
VI 48	IgG2a	95% of a rat day 8 polyclonal antiserum



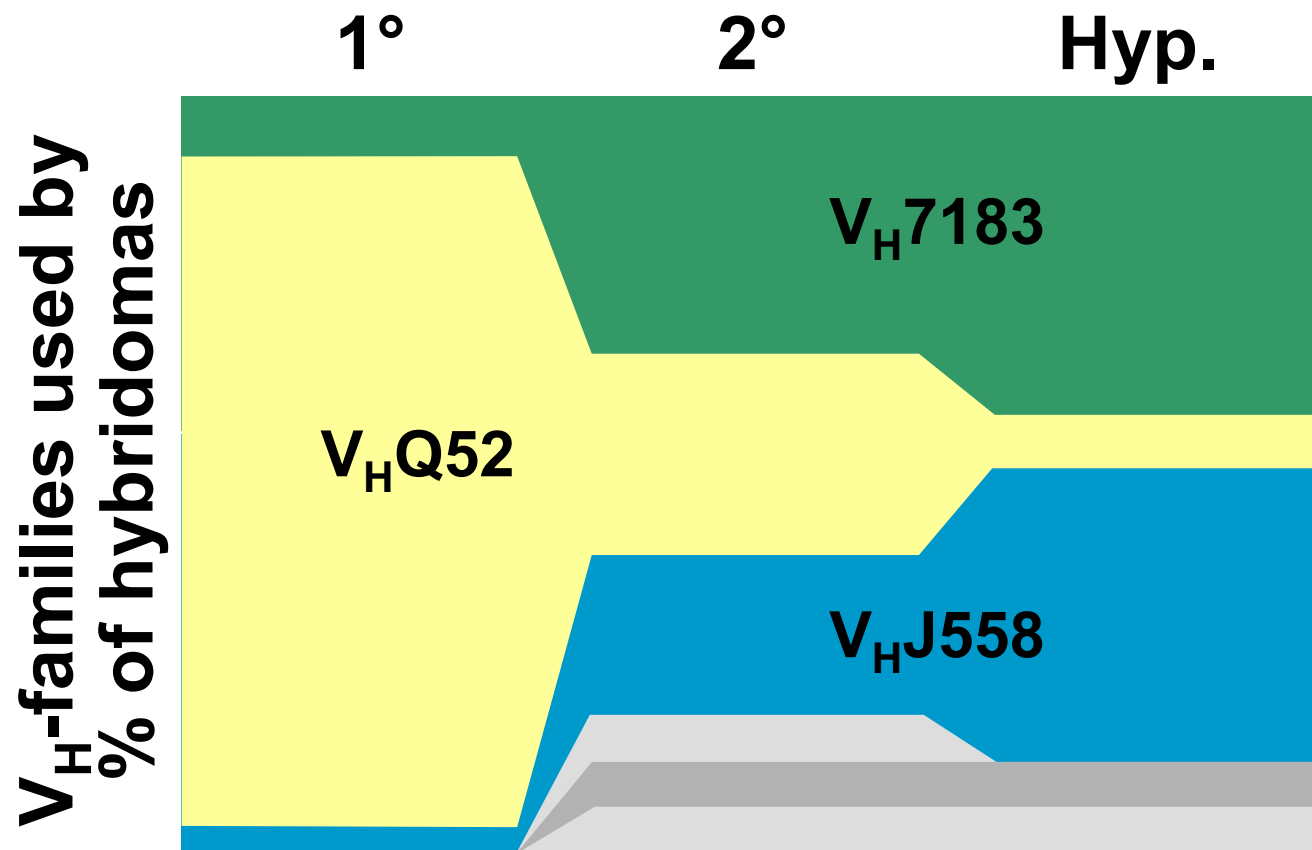
b





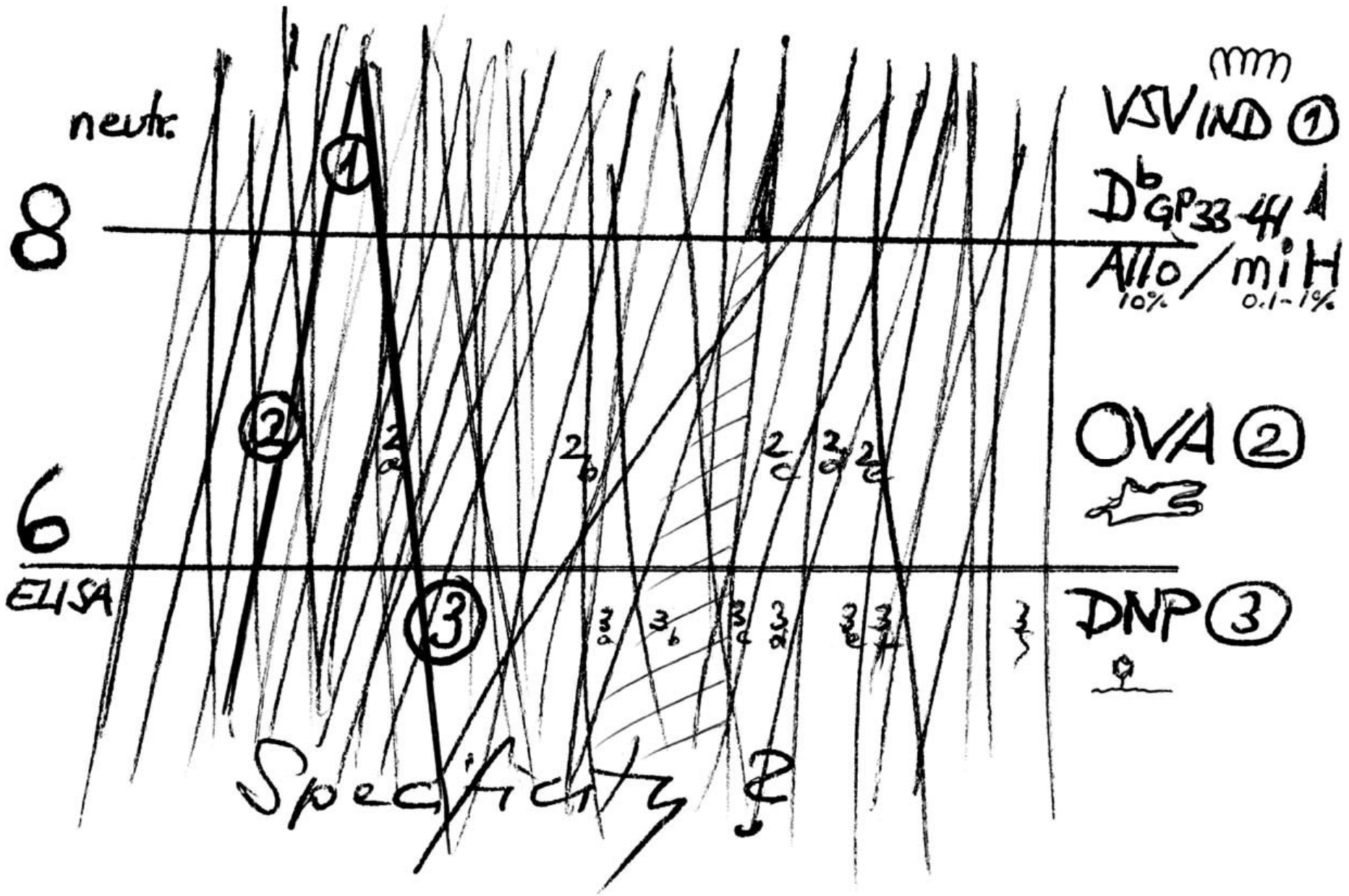
Variable region diversity of VSV-IND neutralizing IgGs

from primary (day 4/5), secondary (day 12), and hyperimmune responses (day 150)



In vivo neutralization is dependent on a minimal serum Ab concentration

Clone	Subclass	Avidity (M ⁻¹)	<u><i>in vitro</i></u> Neutralizing capacity (1μg/ml) (dilution factor)	<u><i>in vivo</i></u> 50% protective concentration (μg injected)
G7G9E4	IgM	7x10 ⁹	15000	4
VI 22	IgG2a	9x10 ⁹	1200	1
VI 7	IgG1	5x10 ⁹	3300	1
25G9	IgG2a	3x10 ⁹	291	1
VI 29	IgG1	2x5x10 ⁹	520	6
VI 40	IgG3	4x10 ⁸	177	1
VI 41	IgG2a	2x10 ⁸	7	3
VI 42	IgG2a	2x5x10 ⁷	1	> 100
VI 30	IgG2a	< 10 ⁷	n.d.	> 70



mm
VSV IND ①
DGP33-41
Allo/miH
10% 0.1-1%

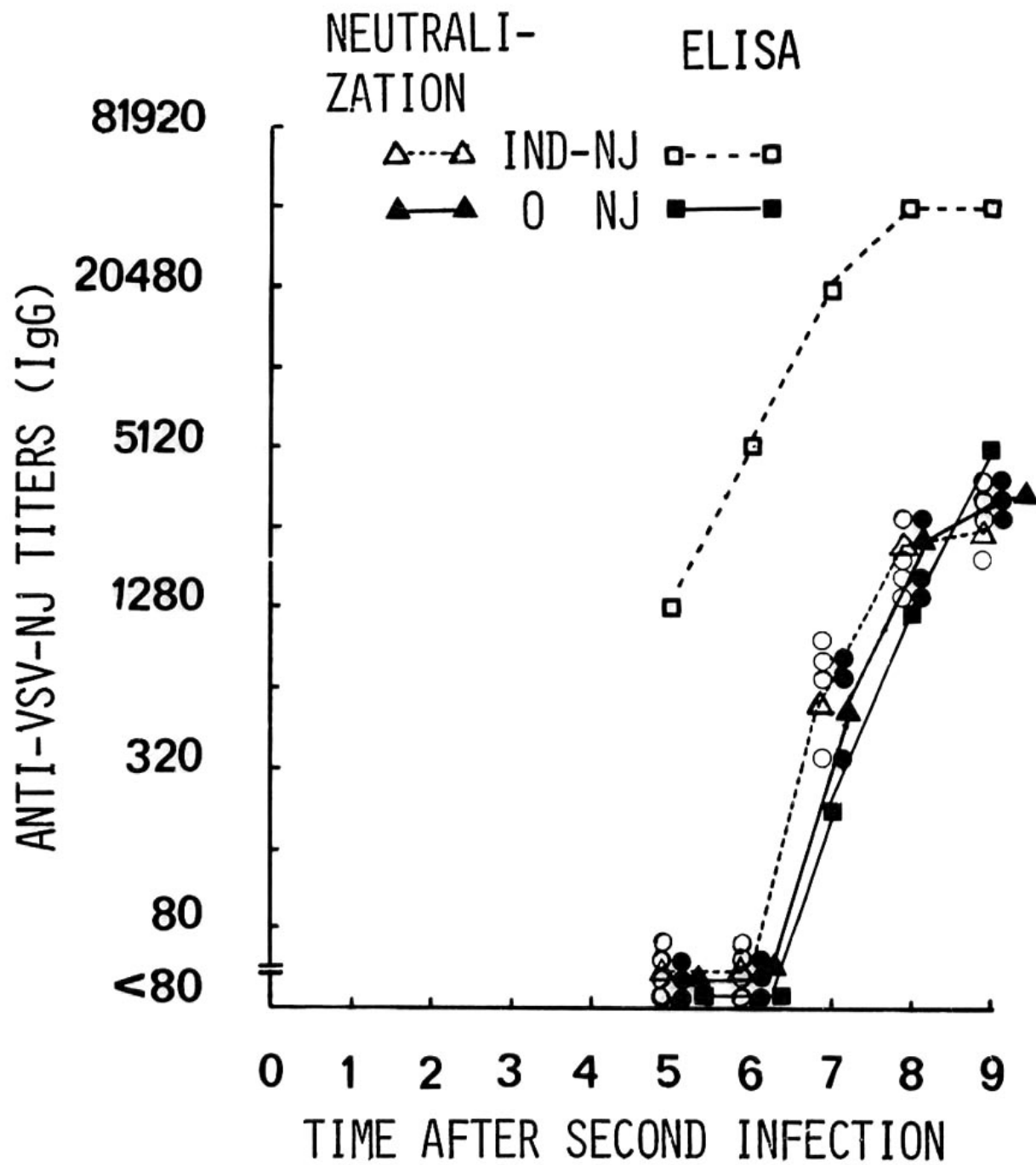
OVA ②

DNP ③

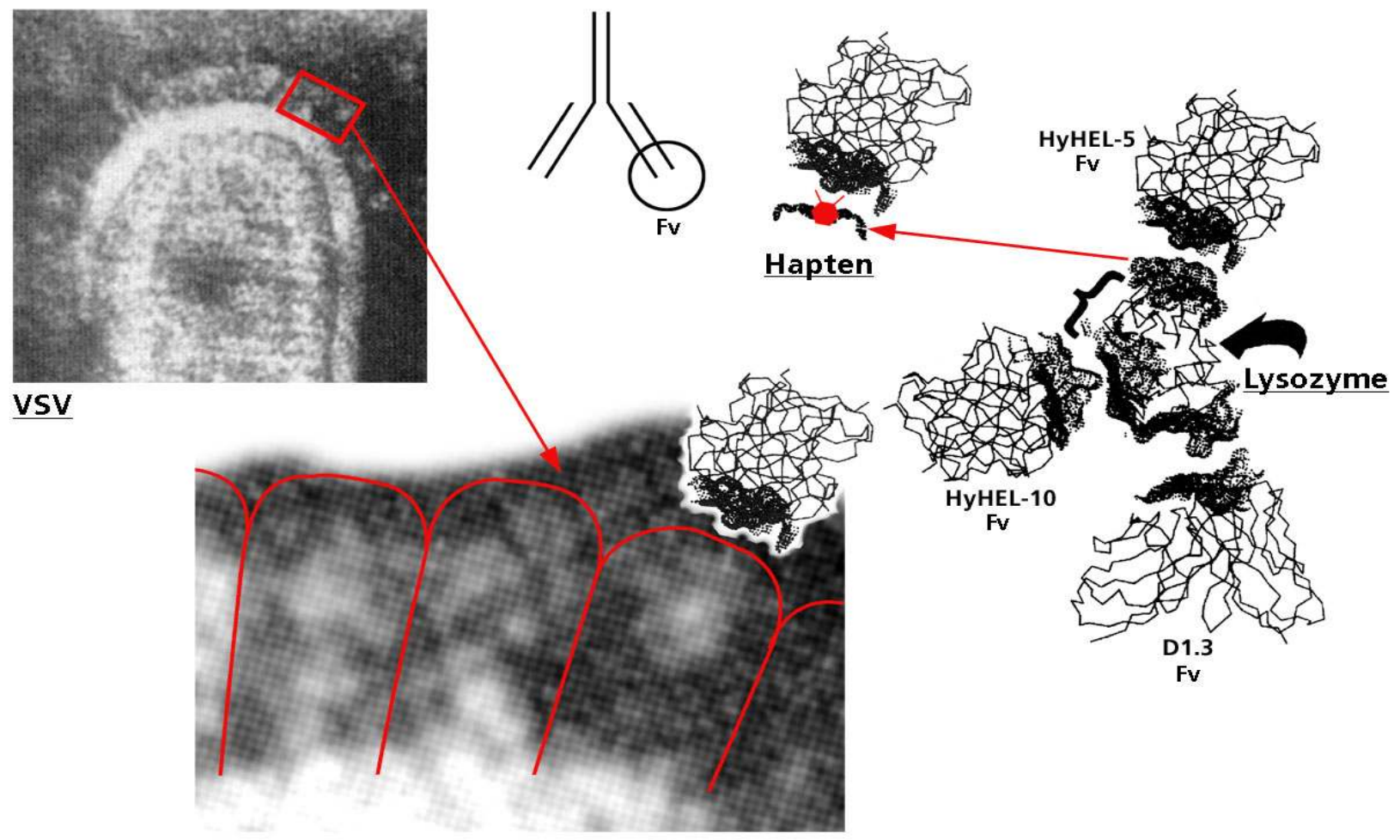
Antibody response: ELISA vs neutr.

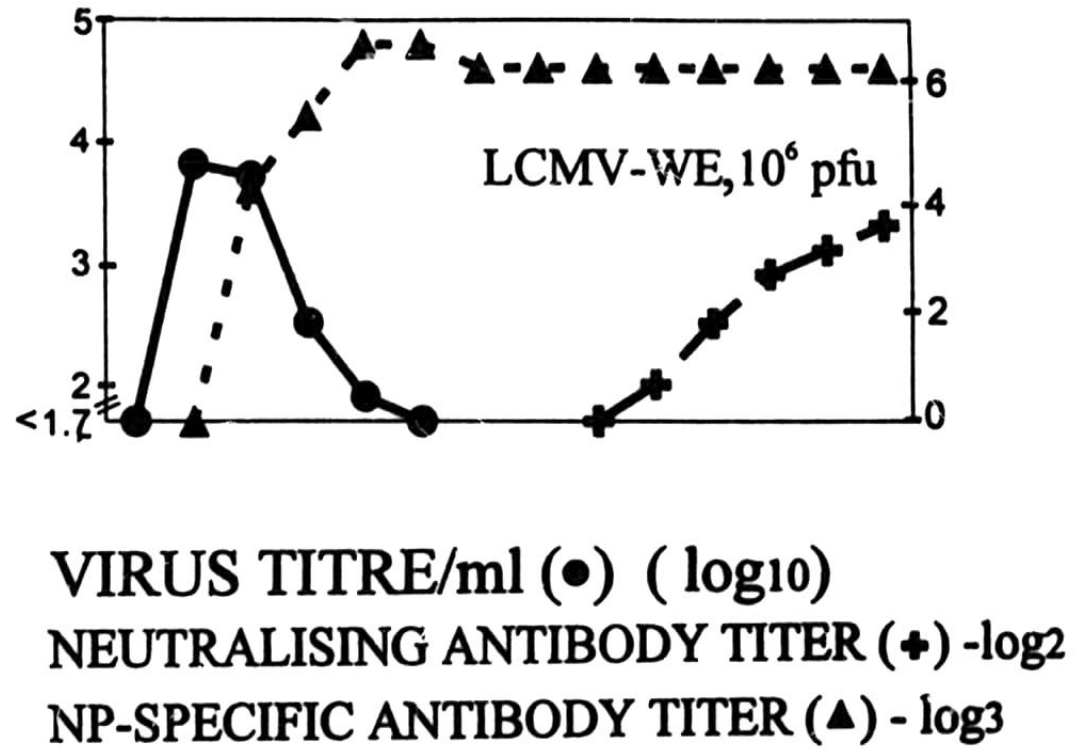
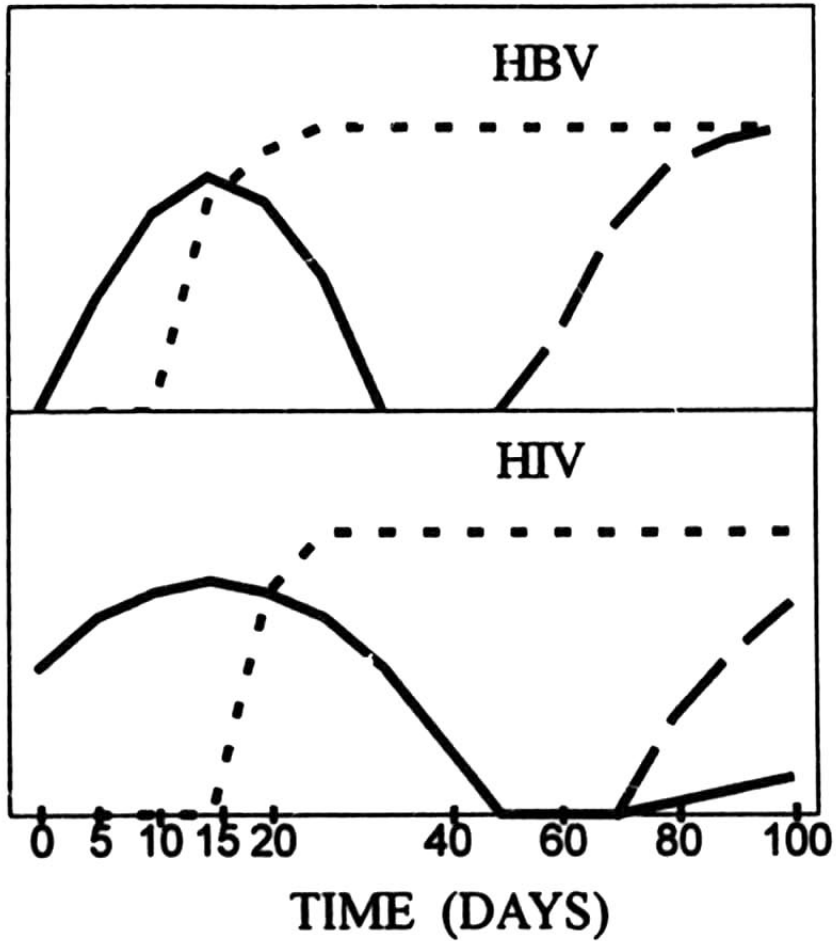
1°	2°	neutr. a IND	ELISA a VSV NP a IND GP	ELISA a DNP
BSA	BSA-DNP	—	—	2°
OVA	BSA-DNP	—	—	1°
VSV-NJ	IND-DNP	1°	2°	2°
—	IND-DNP	1°	1°	1°

nAb: B limiting, DNP: T help limiting

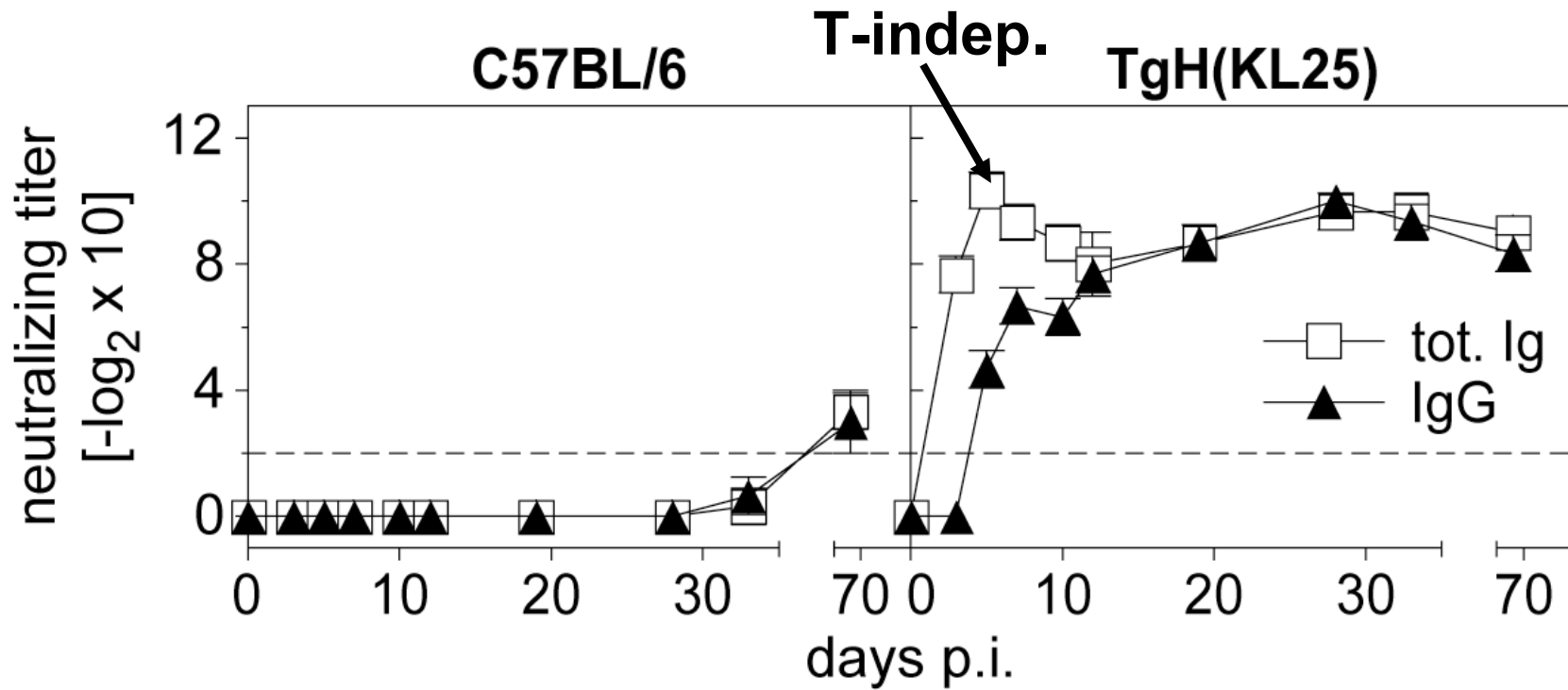


B Frequ.	10^{-5}	10^{-2}	10^{-3-4}
IgG Affin. / Av. M^{-1}	10^9	10^5	$>10^6$



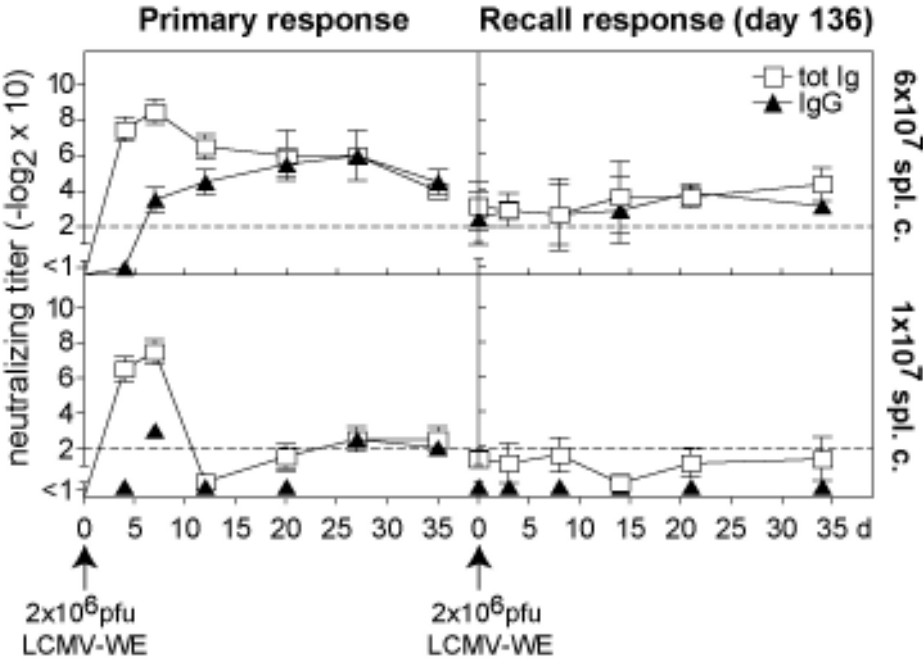


LCMV-neutralizing titers (KL 25 nAb, H-chain tg)

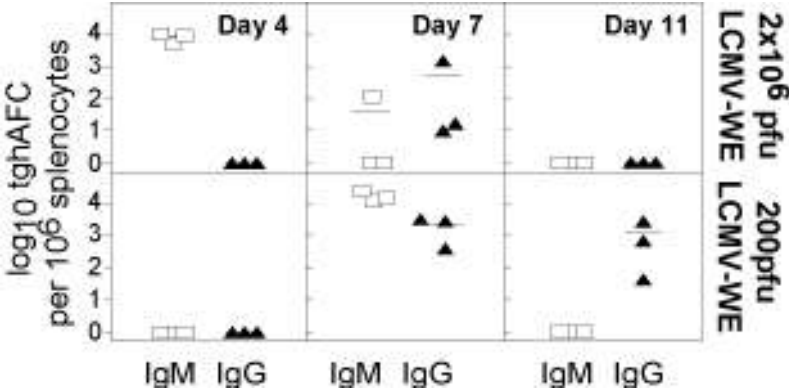


Hangartner, Hengartner

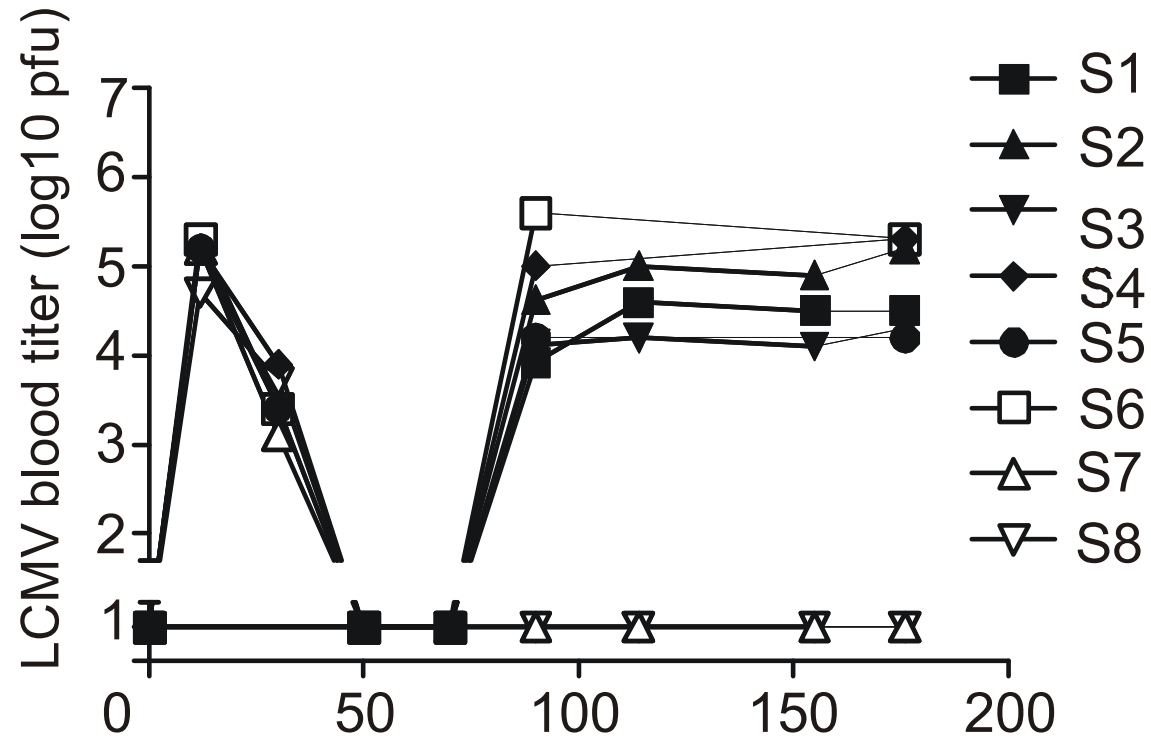
Adoptive transfer (d-1) of TgH(KL25) spl. cells



Adoptive transfer (d-1) of 3×10^7 TgH(KL25) spl. cells



Time dependence of LCMV-viraemia: long-term control or escape



- polymeric AG: IgM
- affinity / avidity
- variability of AG
- pB

Role of T help

	pB	Th	where ?
IgA	mucosal IgA	no role (byst.)	lam.propr.
IgM		no role (byst.)	extra follicular
IgG	DNP 10^{-2-3}	limiting	follicular
	nAb 10^{-5}	not limiting*	follicular
IgE	ectoparasite spec.	T dependent	unclear
	hyper IgE serum	T independent	unclear

*orig. antigenic sin!



CD4⁺ T help?
B cells?

IgM IgG

G purified

—

—

G ~ carrier
protein

—

++

VSV IND wt

++

++

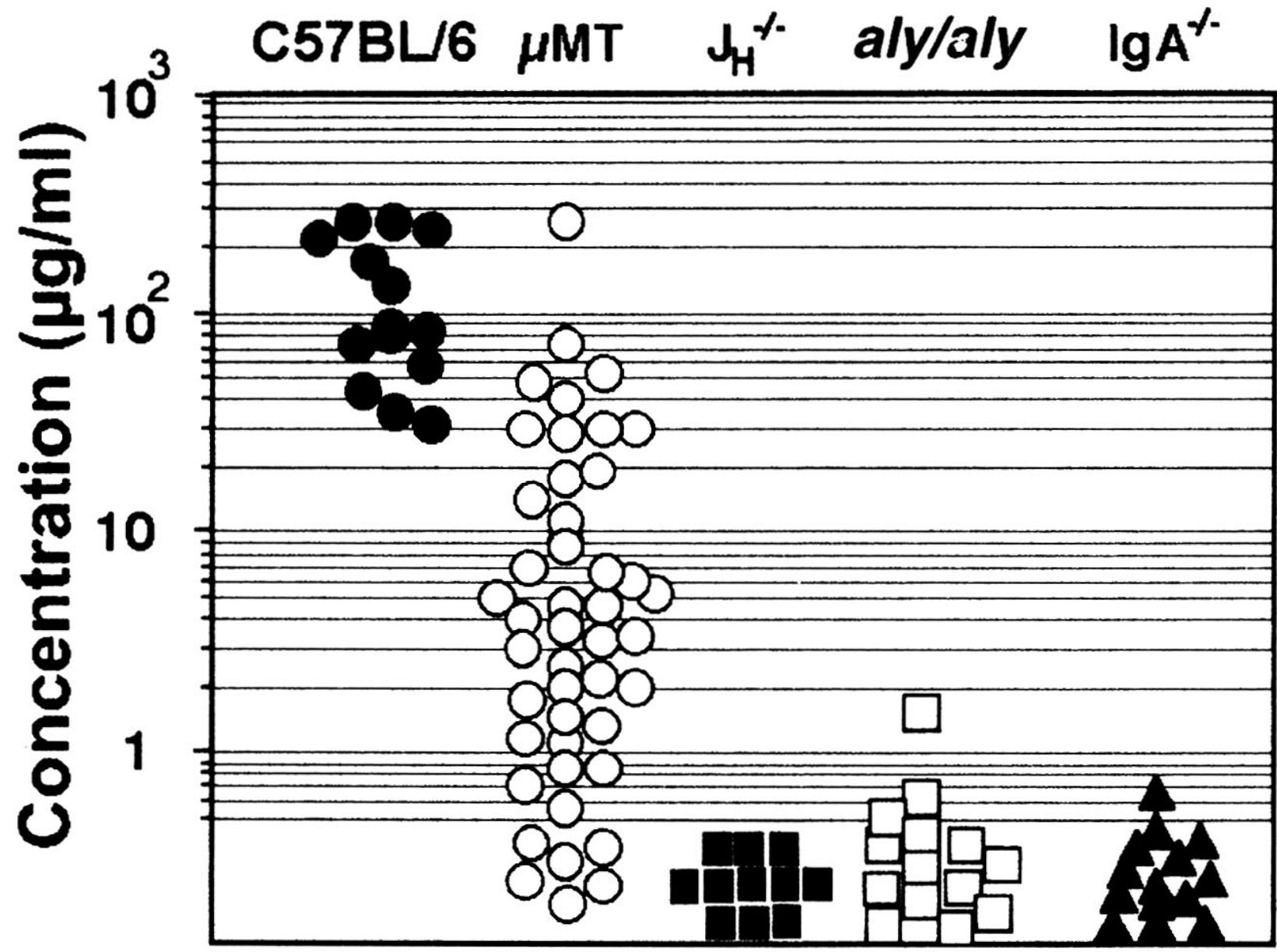
B cells

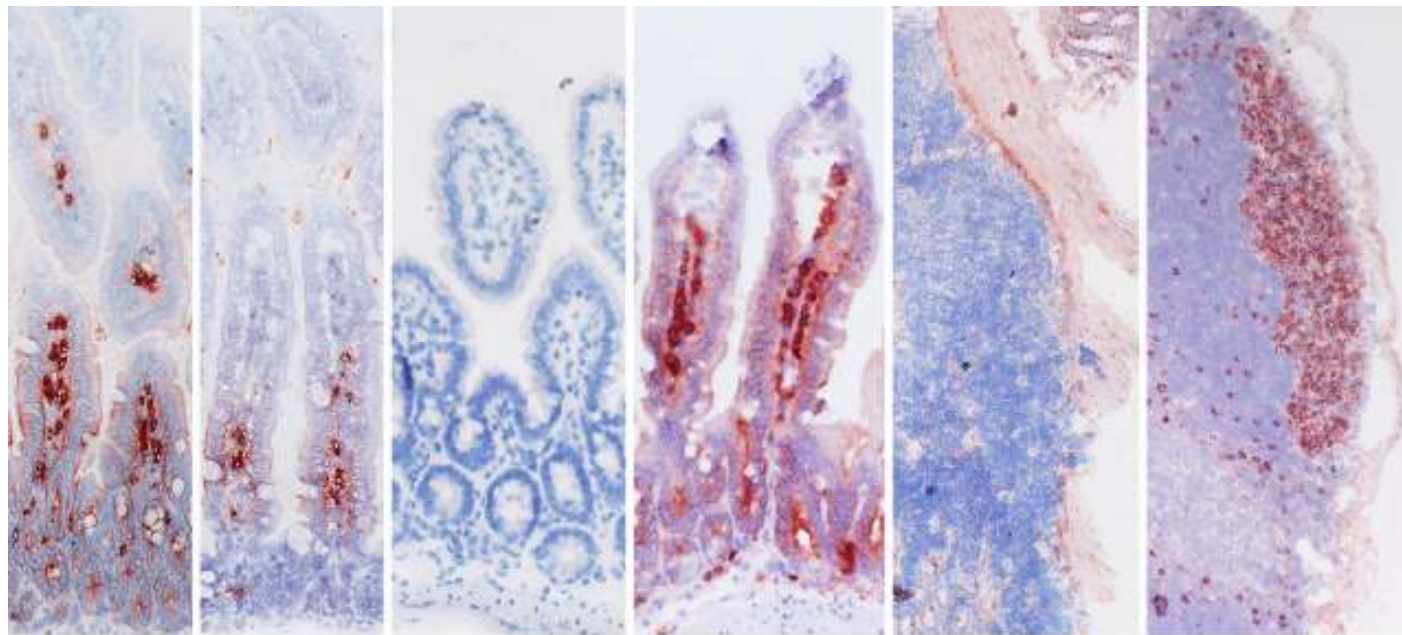
react:

- Polymers
- (Polymeric self-antigens: collagen, DNA etc.)
- Monomers + LPS (CpG)
- 2° lymphoid organs, GC

not:

- No B cells (low frequency or deleted)
- Monomers, no T help
- No follicular structure (IgA!)





C57BL/6

TCR $\beta^{-/-}\delta^{-/-}$

Aly/Aly

TNFR-I^{-/-}

TNFR-I^{-/-}

C57BL/6

IgA

PNA

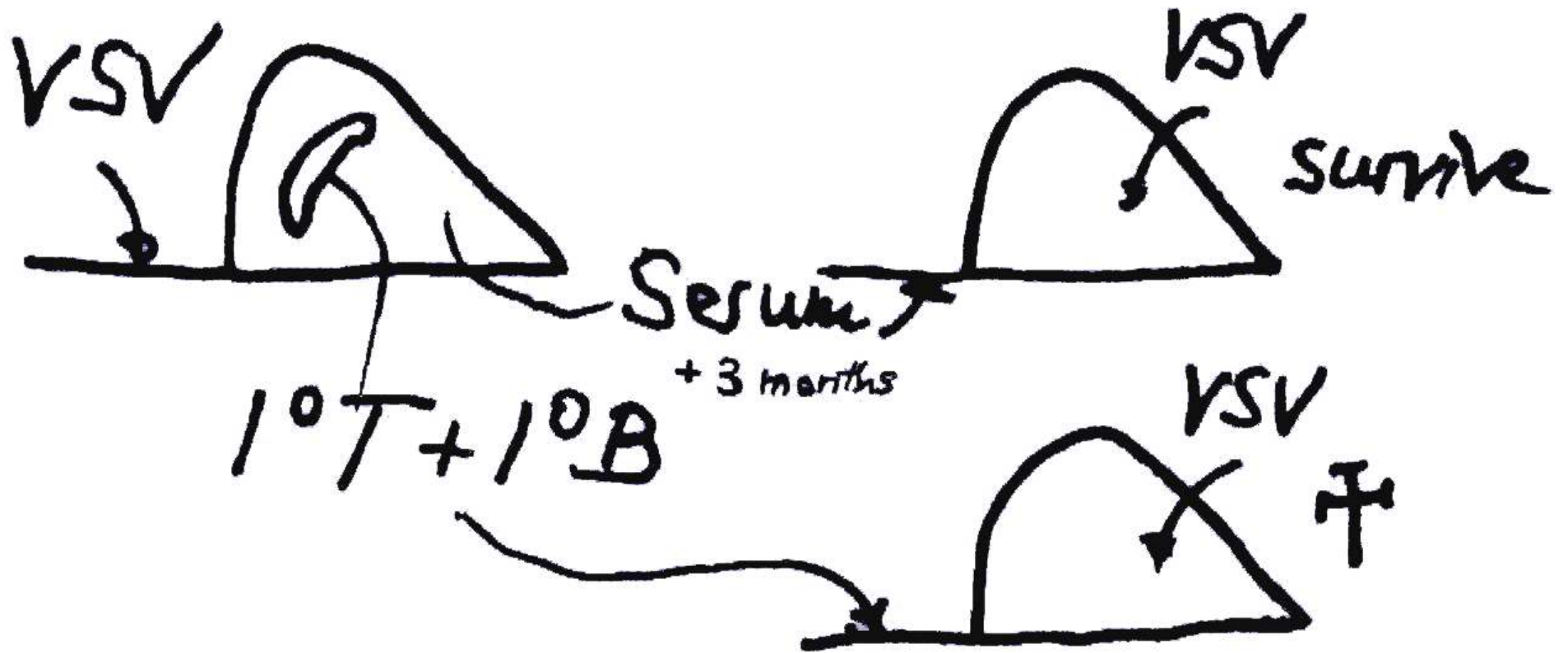
Mouse strain	Housing conditions	IgA-secreting cells (no. per 10^5 lymphocytes)		IgA concentration ($\mu\text{g/ml}$)	
		Intestinal lamina propria	Spleen	Serum	Intestinal wash
C57BL/6	SPF	11,600 \pm 1,500	62 \pm 16	115 \pm 59	18 \pm 4.3
TCR $\beta^{-/-}\delta^{-/-}$	SPF	3,900 \pm 1,600	11 \pm 4	55 \pm 33	6.0 \pm 3.3
C57BL/6 <i>nu/nu</i>	SPF	2,800 \pm 1,700	49 \pm 13	43 \pm 20	5.5 \pm 3.8
CD4 ^{-/-}	Conventional	9,100 \pm 930	86 \pm 27	93 \pm 26	15 \pm 4.5
TNFR-I ^{-/-}	SPF	9,500 \pm 540	52 \pm 26	153 \pm 99	14.8 \pm 7.1
<i>aly/aly</i>	SPF	<1	<0.1	<0.4	<0.4
LT $\alpha^{-/-}$	Conventional	<10	<0.1	<0.4	<0.4
C57BL/6	Germ-free	1,600 \pm 860	14 \pm 1	22 \pm 4	1.5 \pm 0.74

B + T memory vs protection

Quicker + better pB, pT dependent
AG – independent

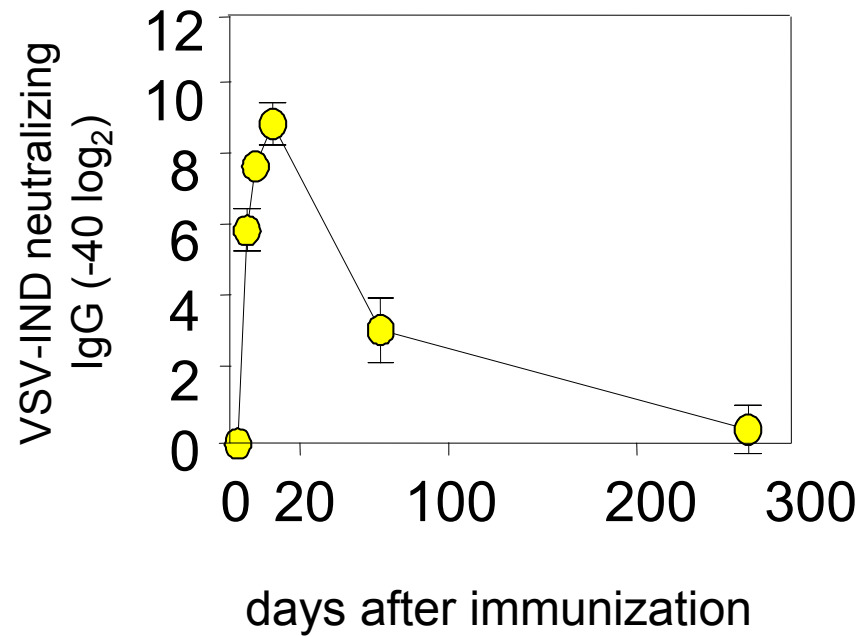
High Ab titer AG – dependent
plasmocytes <10d
bystander act. not sufficient
Co-evolutionary role
(matern. Ab)

Memory versus protection

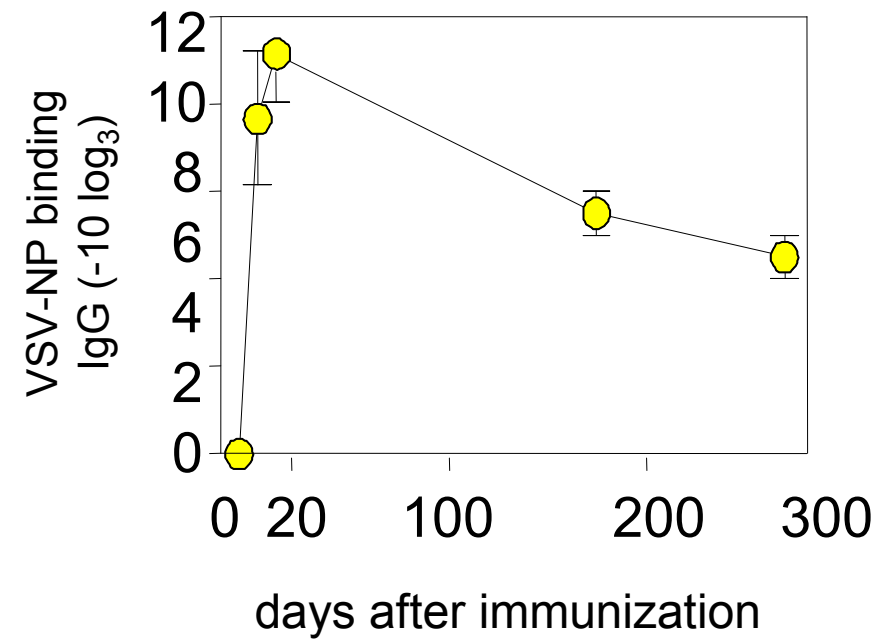


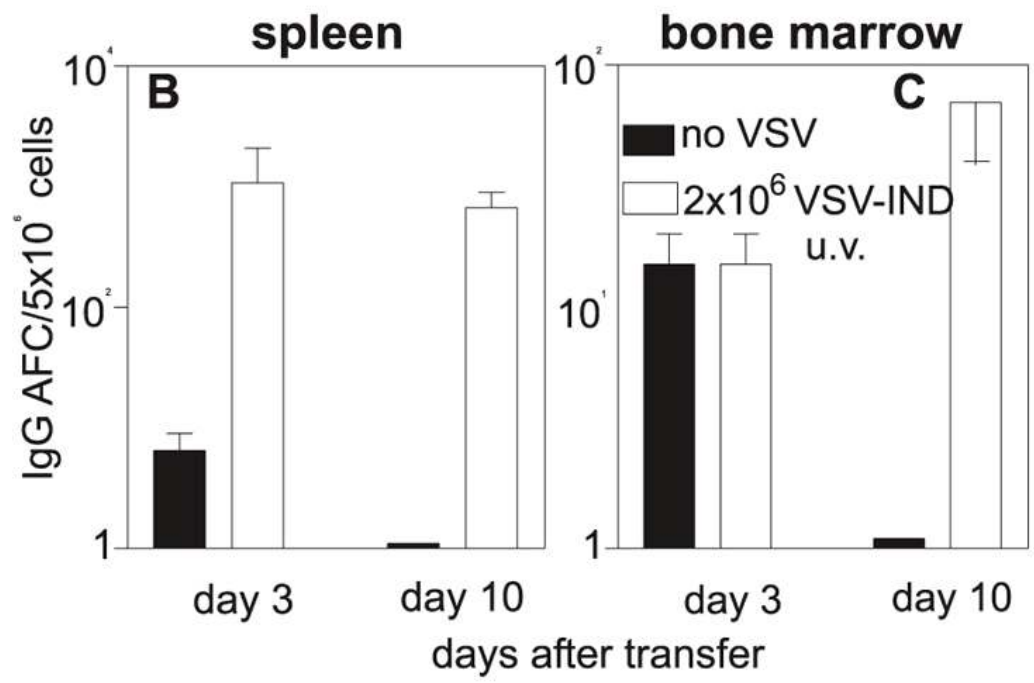
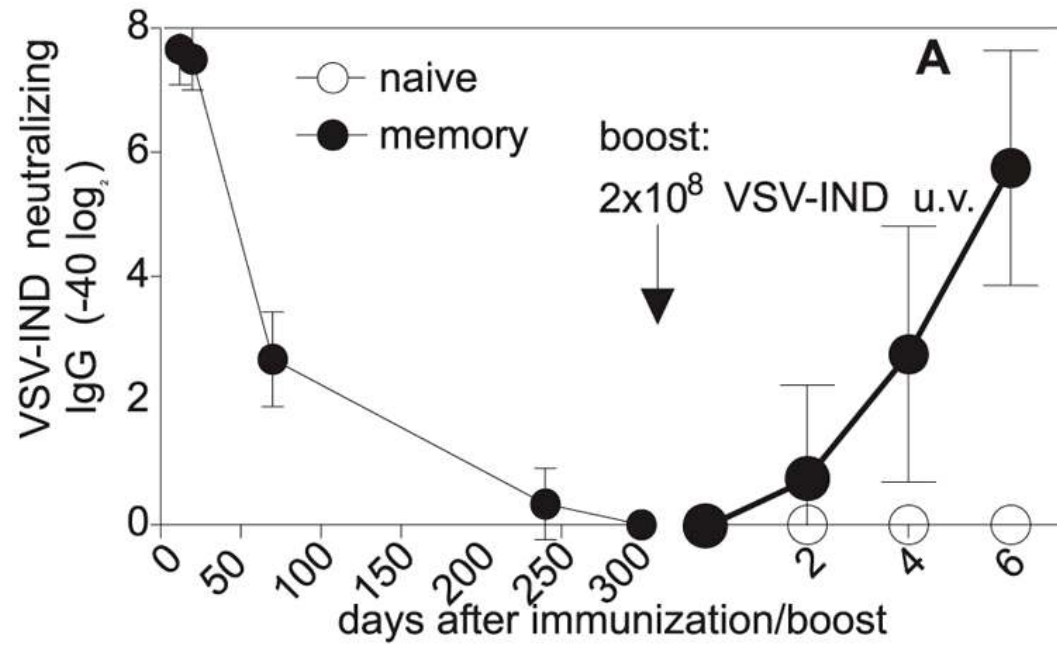
Antibody Memory after immunization with VSV

a) neutralizing antibodies

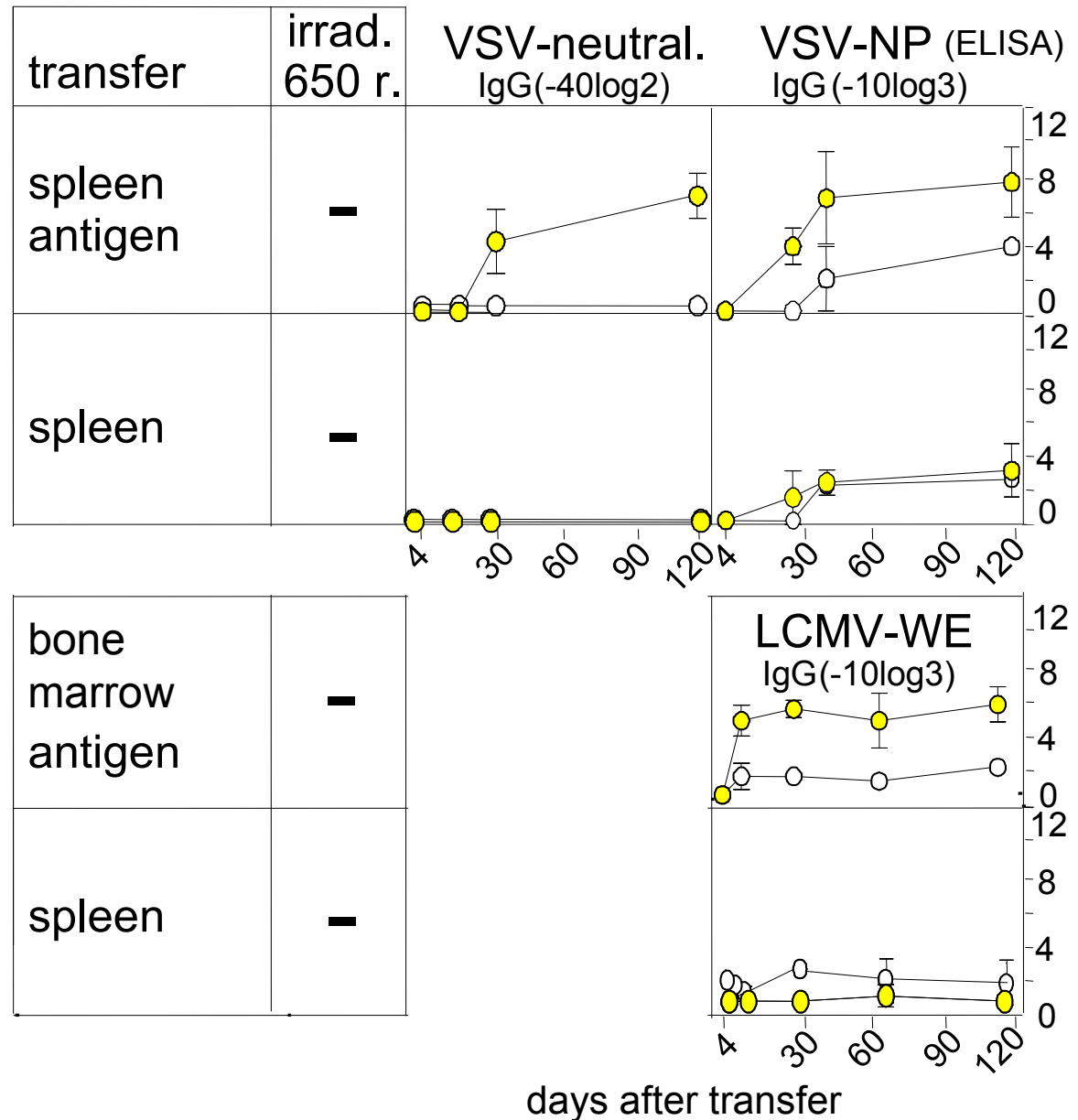
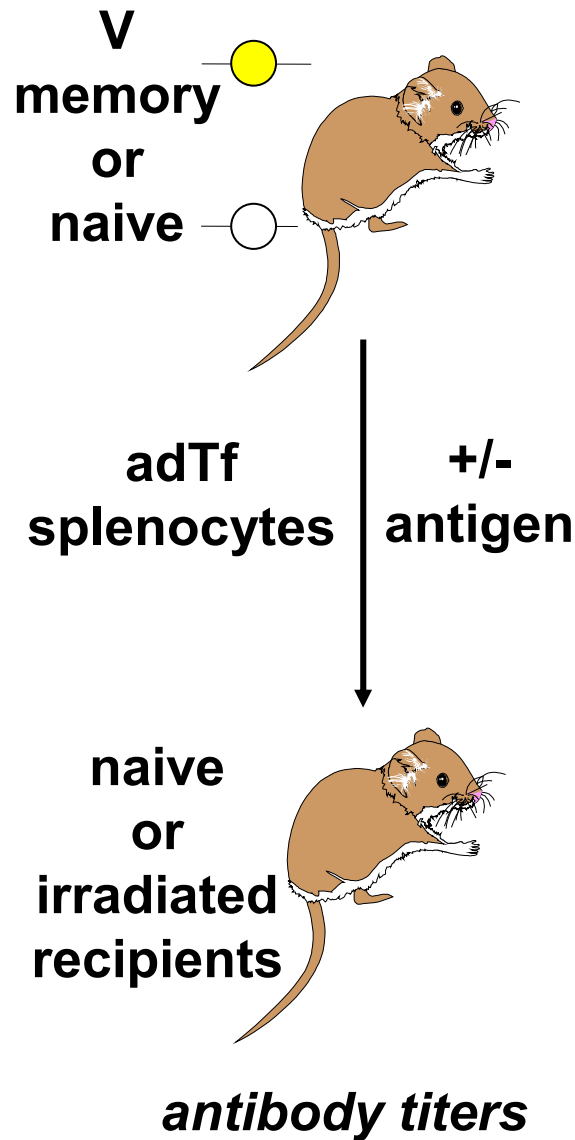


b) ELISA





Adoptive Transfer of "Memory" Cells



Neutral. Ab

- natural Ab → Ag-spleen
- high affinity in germline
- protection: $>10^8$ I/Mol, >1 $\mu\text{g/ml}$ (T help)
- maternal Ab
- B cells not tolerant?
- monomer / multimer (crosslinking)
- protection: antigen +T dependent

Manipulation of B response

- Protective AG site (serotype not shared)
- Shared epitopes not protective
- Live vs protein dose
- AG-dependence of Ab titer
epidemiology (Sabin)
- vs kinetics and cytopathogenicity of V

Institute of Experimental Immunology ETH and University of Zurich

A. Althage

M. Bachmann

Z. Ciurea

K. Fink

S. Freigang

L. Hangartner

L. Hunziker

U. Kalinke

M. Kohler

K. Lang

Th. Leist

M. Martinic

D. Moskophidis

A. Navarini

A. Ochsenbein

B. Odermatt

D. Pinschewer

HP. Pircher

M. Recher

T. Rütlicke

P. Seiler

B. Senn

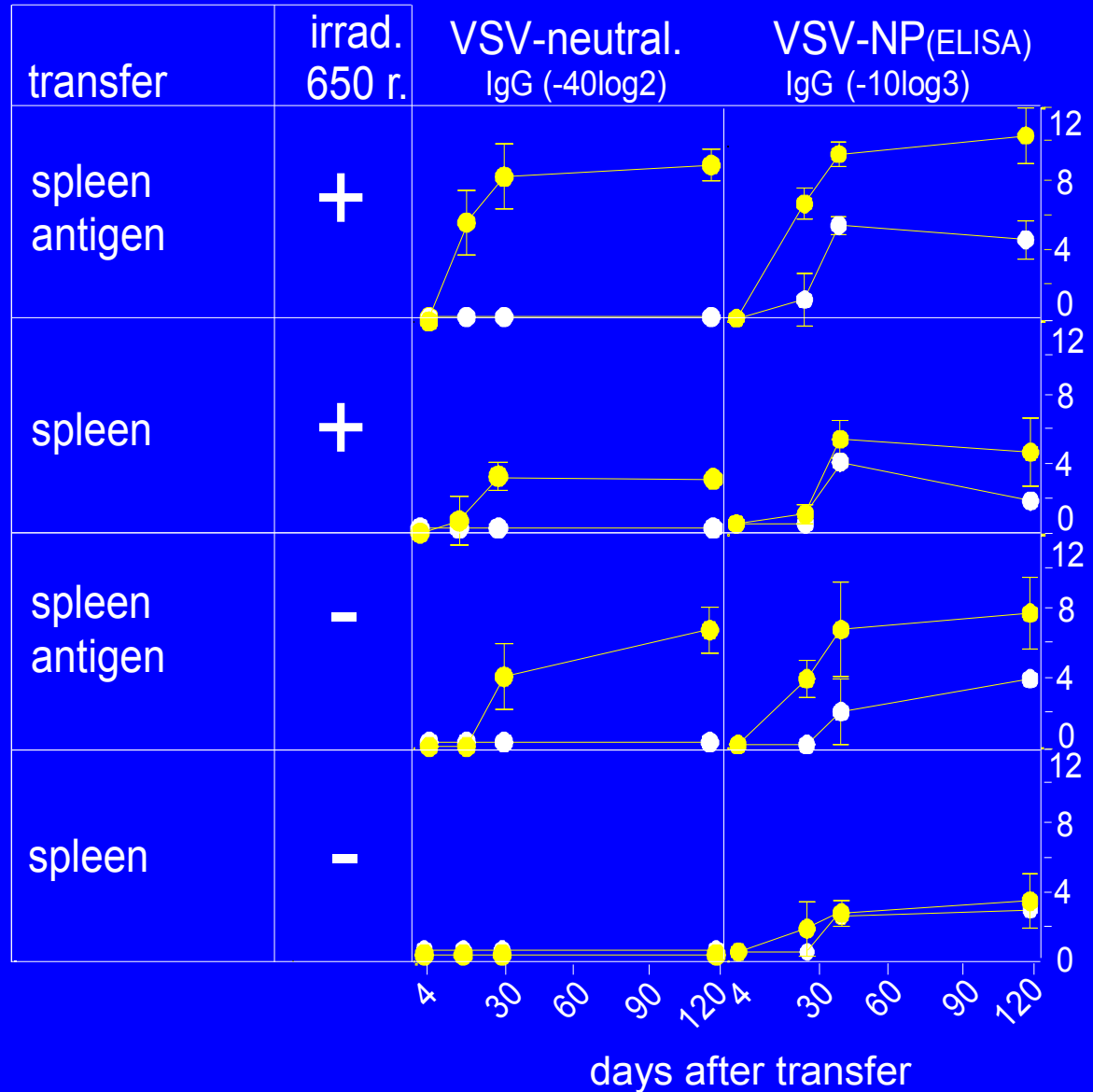
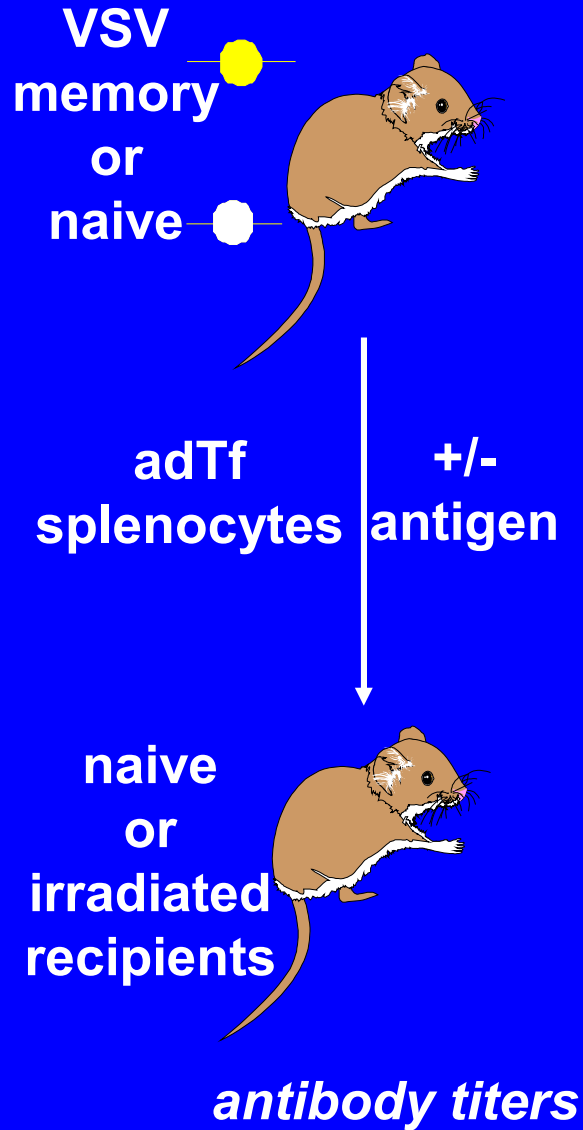
U. Steinhoff

R. Zellweger

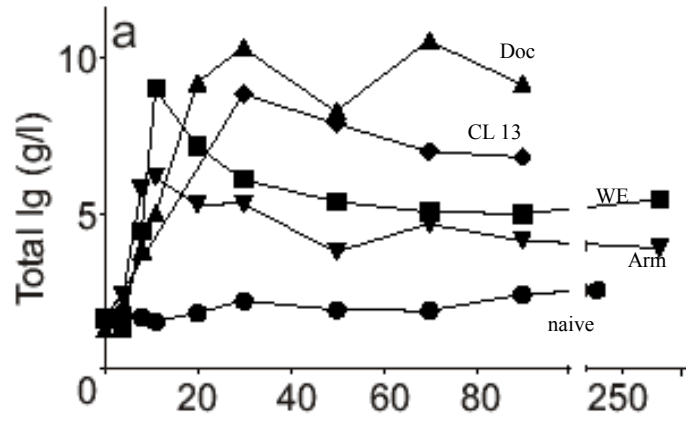
H. Hengartner

Thank you!

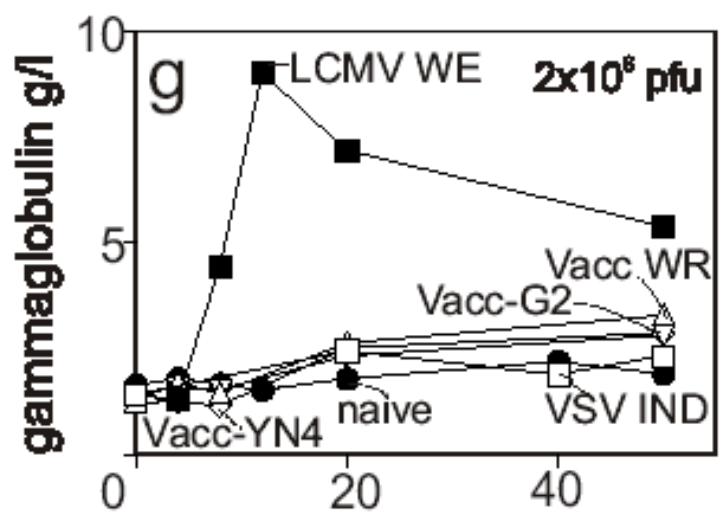
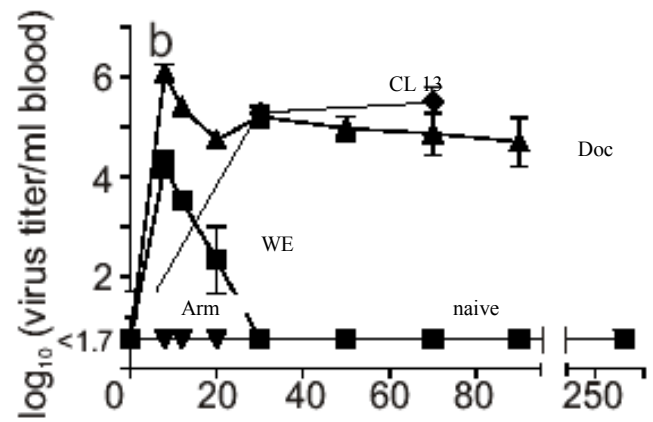
Adoptive Transfer of Spleen Cells: VSV



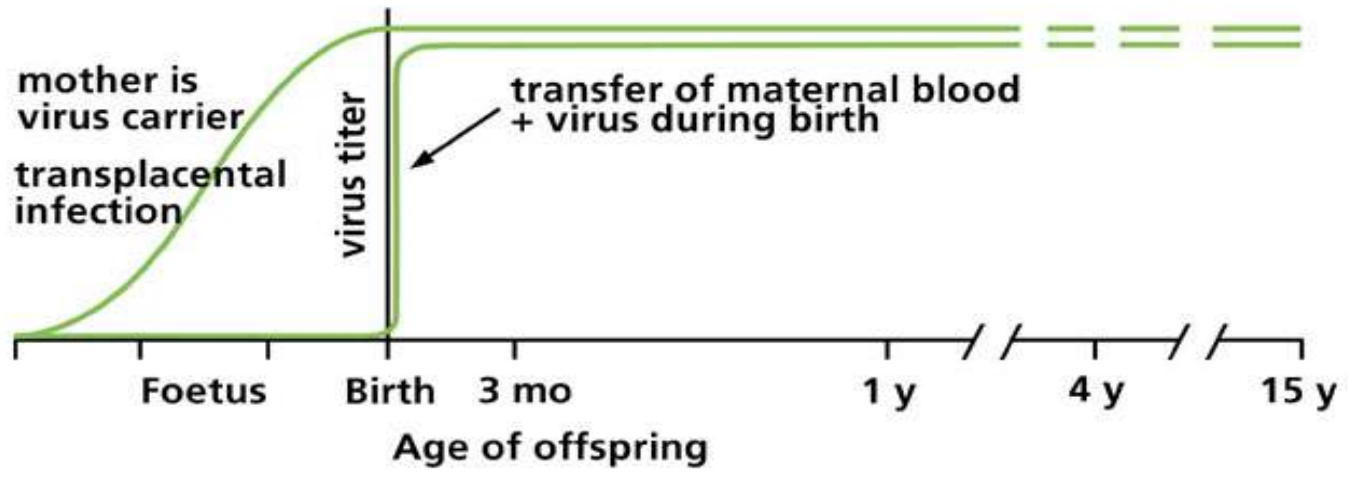
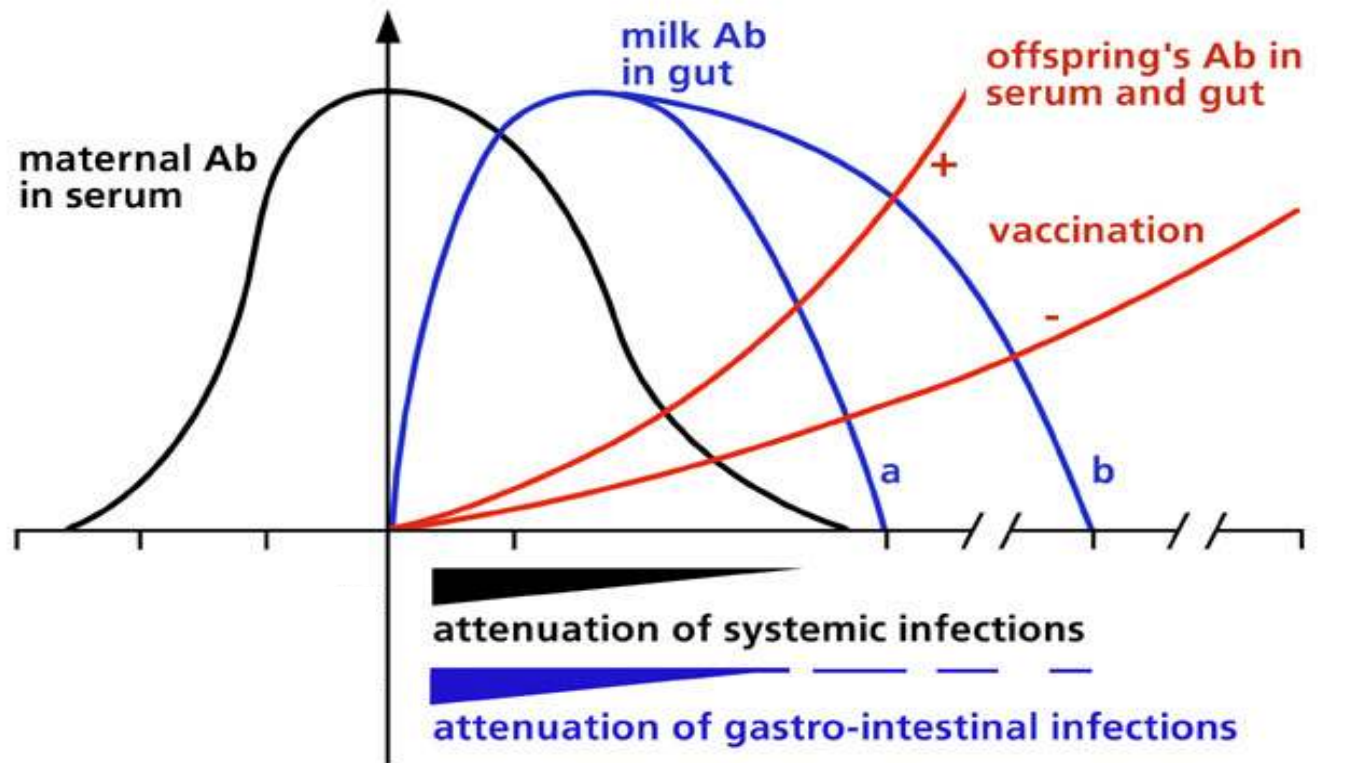
Hypergammaglobulinemia



Virus titer



Hypergammaglobulinemia correlates with virus replication and virus persistence



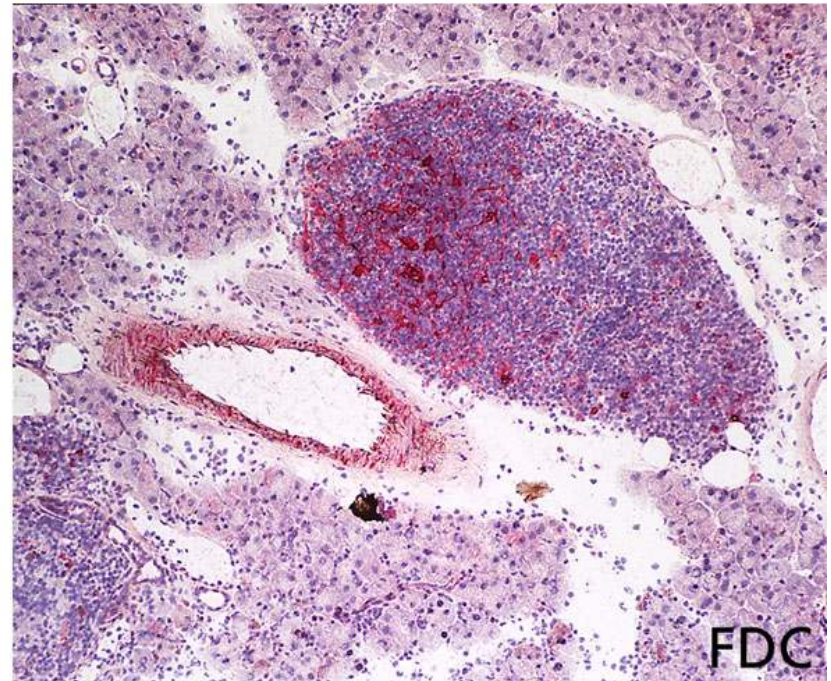
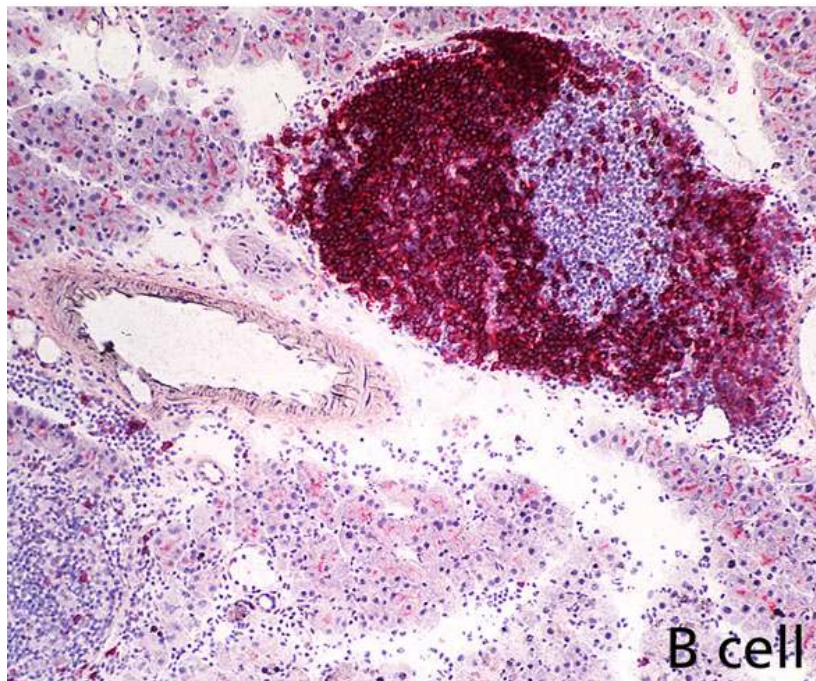
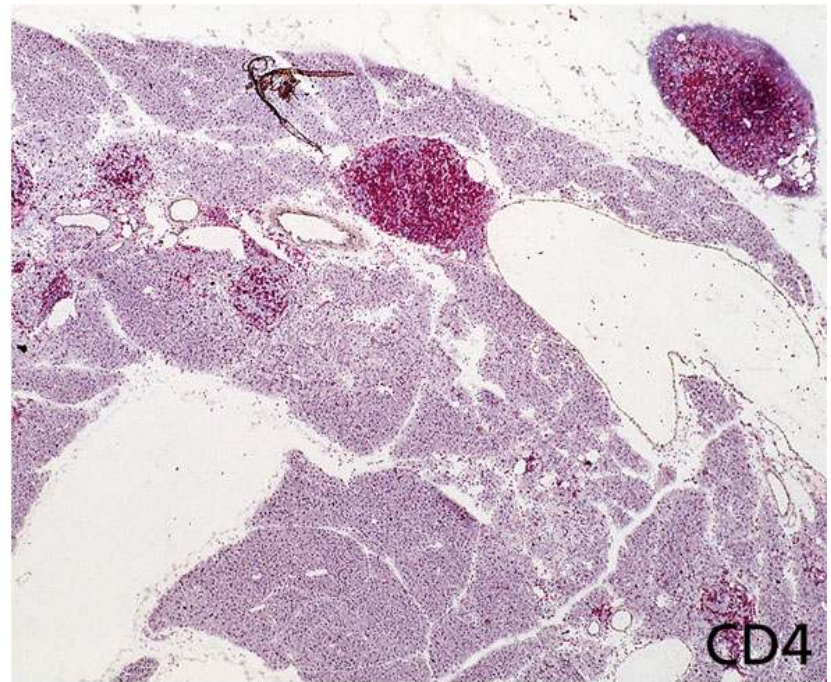
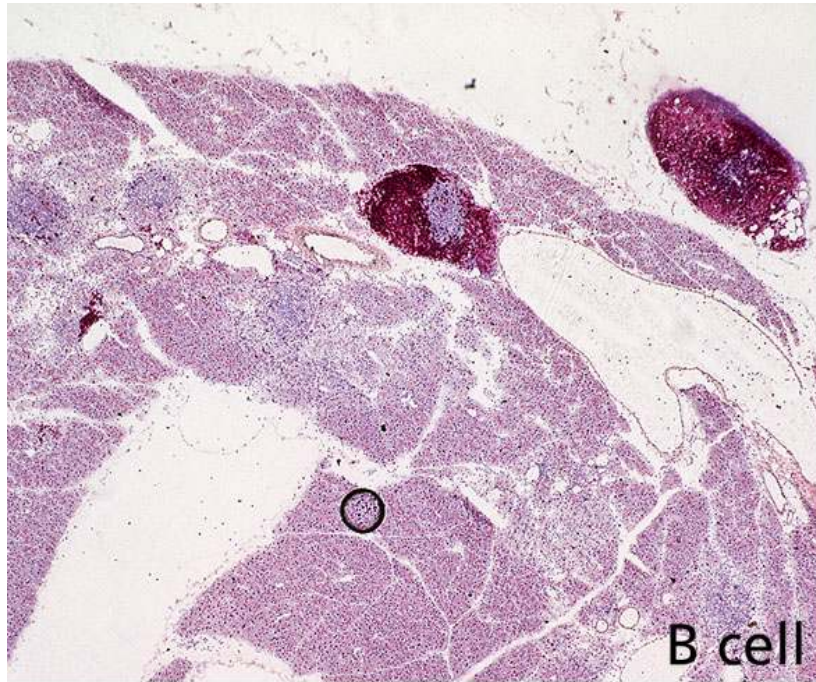
Protection / Mothers / Herds / Newborn

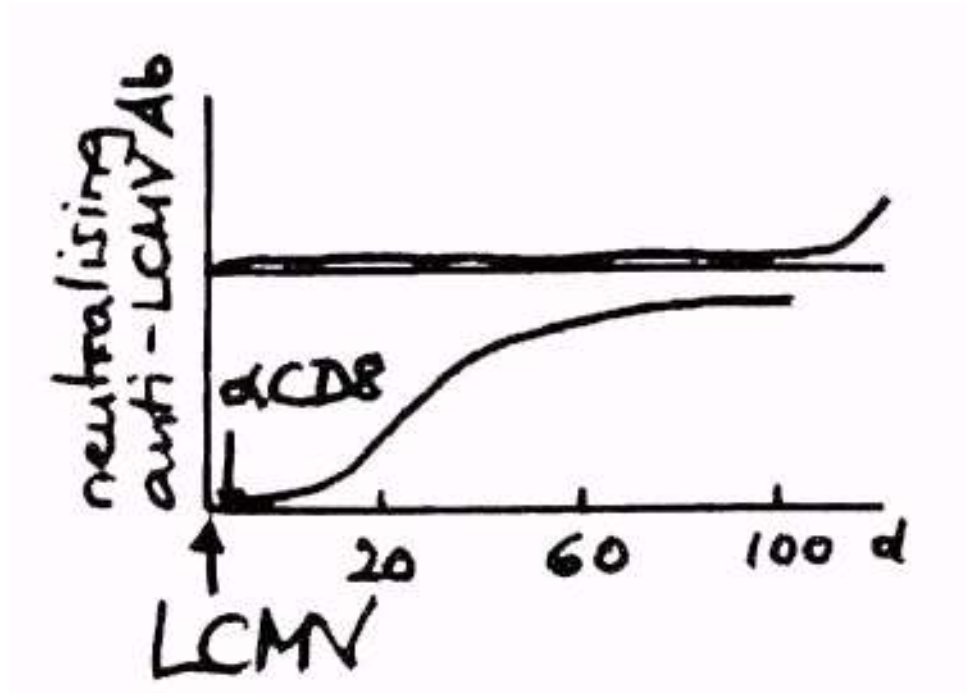
- Memory "idea": earlier + higher (AG-) (pB, pT)
- nAb -: abortions / malformations
- nAb +: herd immunity + transfer to offspr.
physiological – artificial vaccinations
escape by variability
- HIV, TB: slow, T cell immunity AG driven
immunopathology, no vaccines: antibiotics
antivirals
- Emerging infections (virulence – host
resistance – nAb transfer)

B cell repertoire nAb

	natural Ab	IgM T indep.	IgG T dep.
VSV ind	$1/20$	d2-4	d6-8
LCMV	$<1/2$?	d70-150
tgH25	$1/40$	d2-4	d6-10

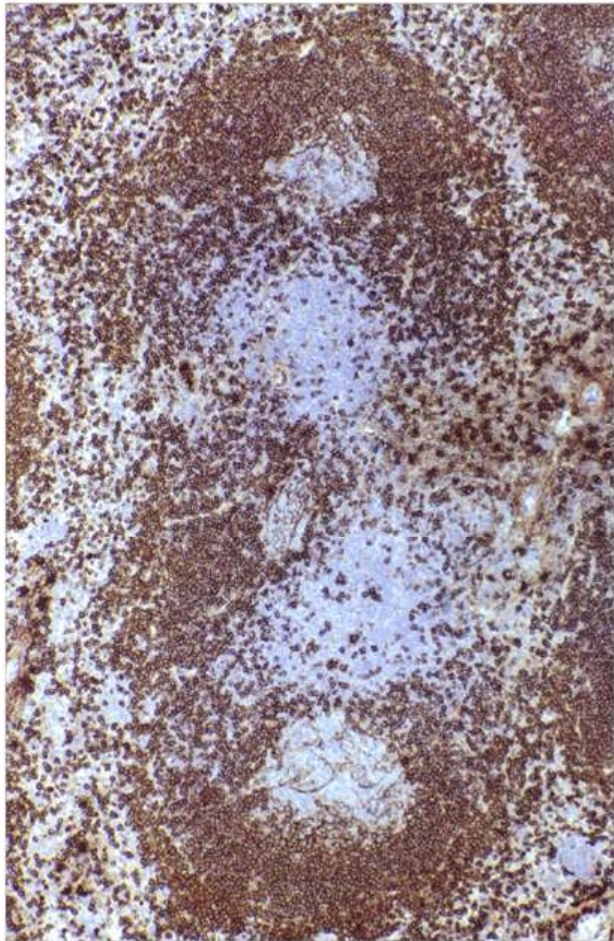
L. Hangartner, R. Zellweger





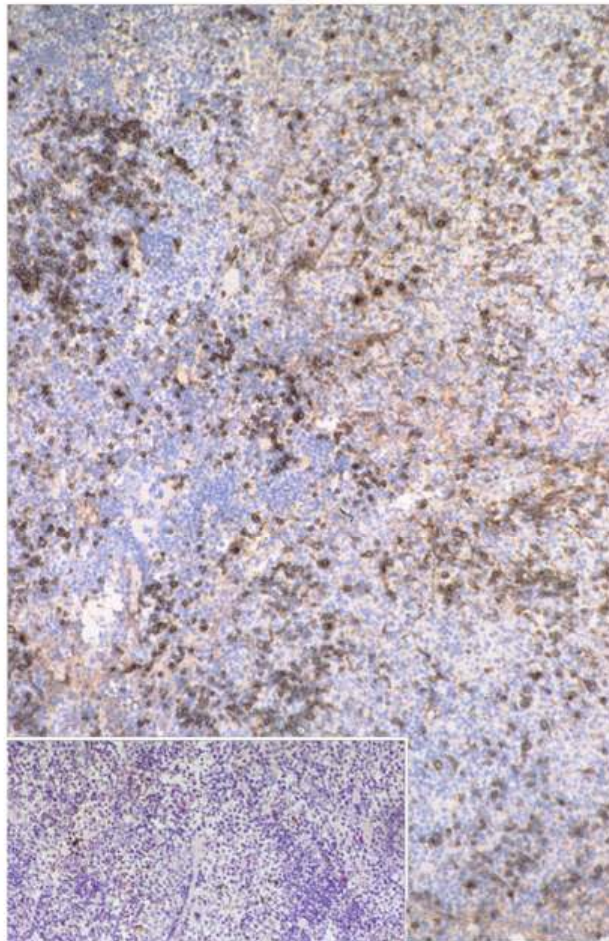
Leist
Odermatt

Kontrolle



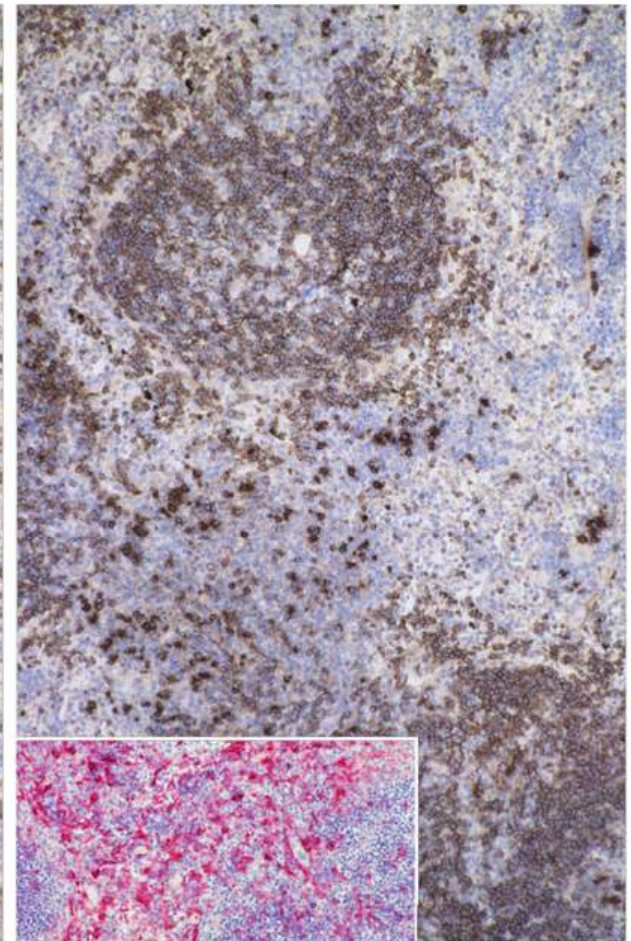
IgM

LCMV

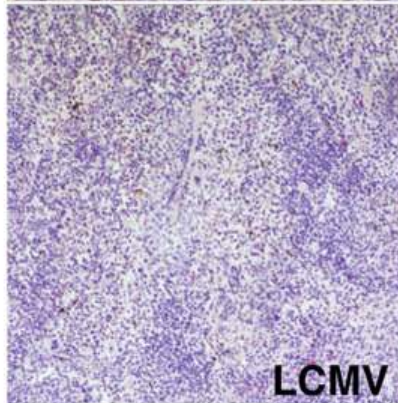


IgM

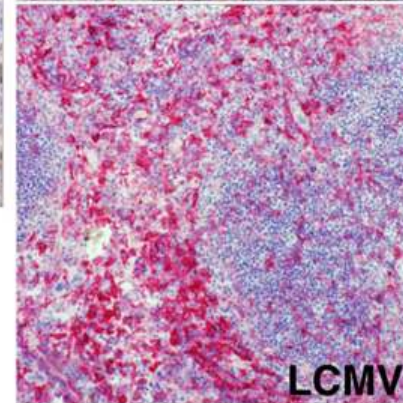
LCMV + Anti CD8



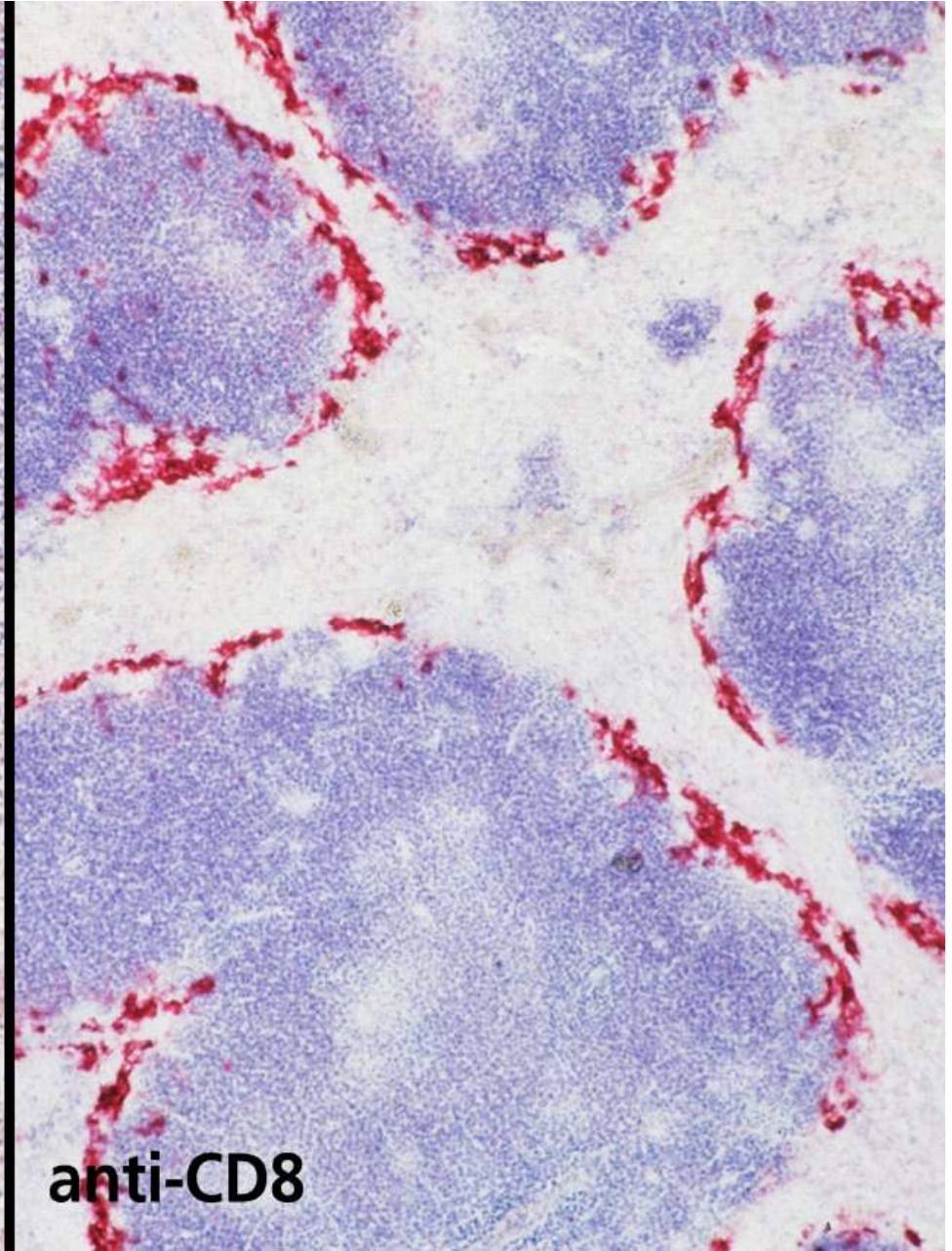
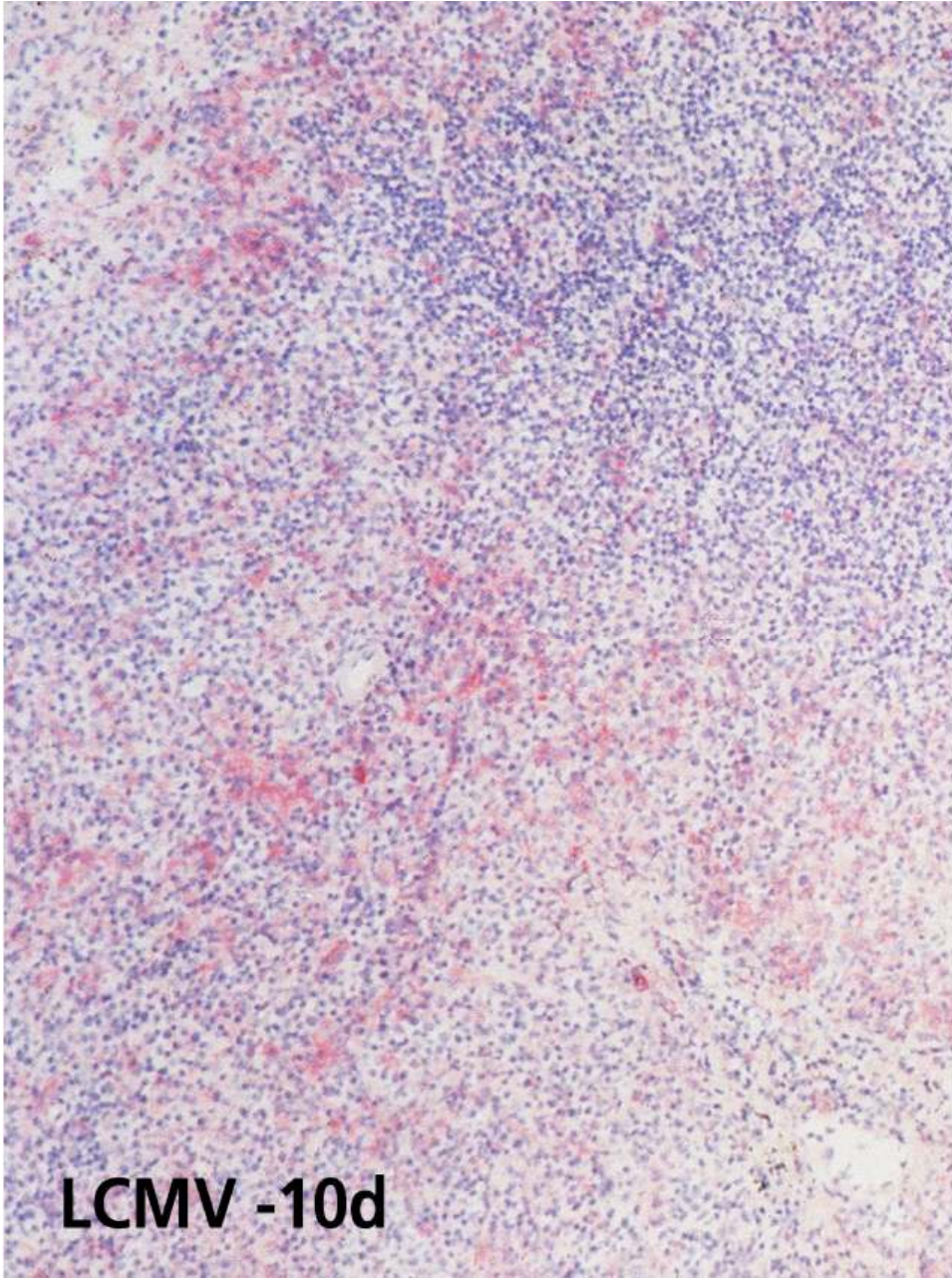
IgM

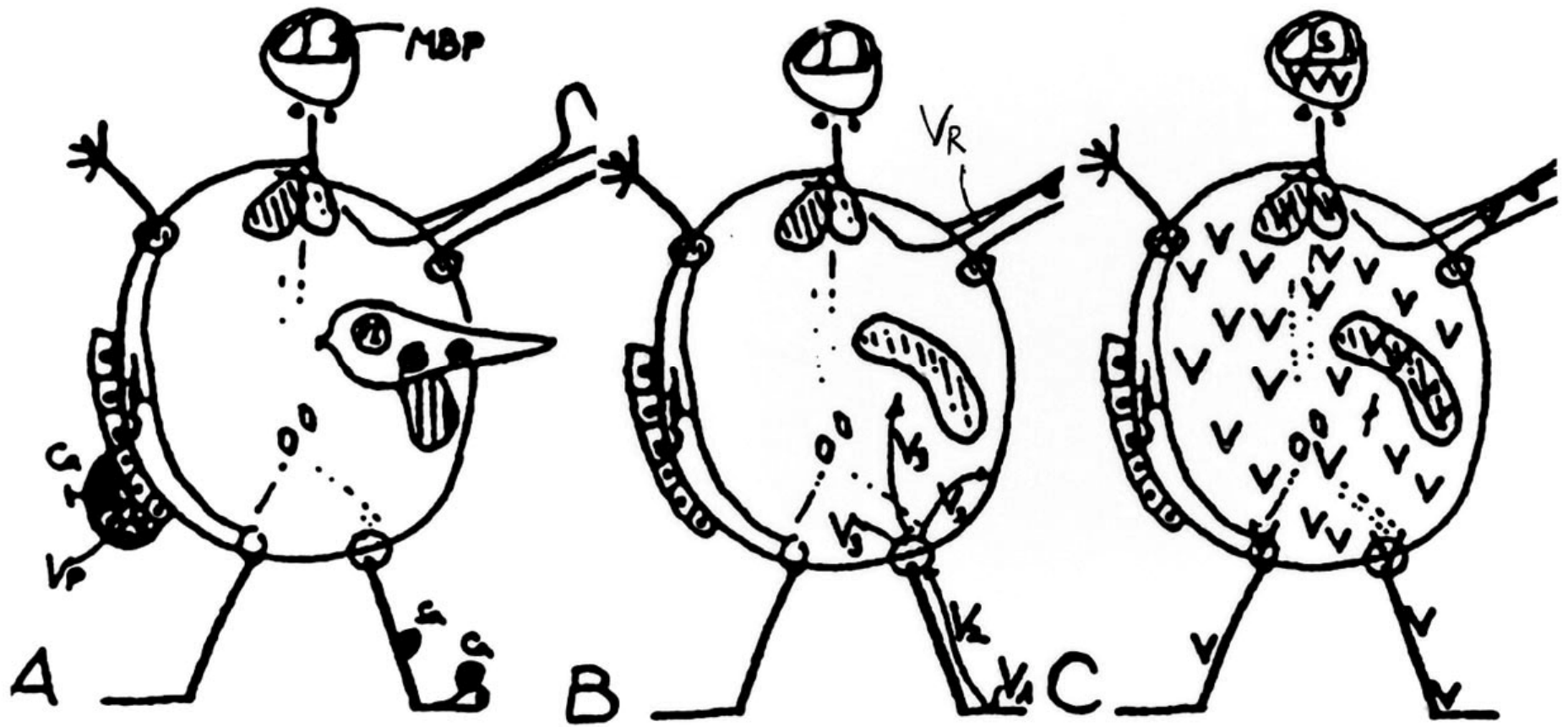


LCMV

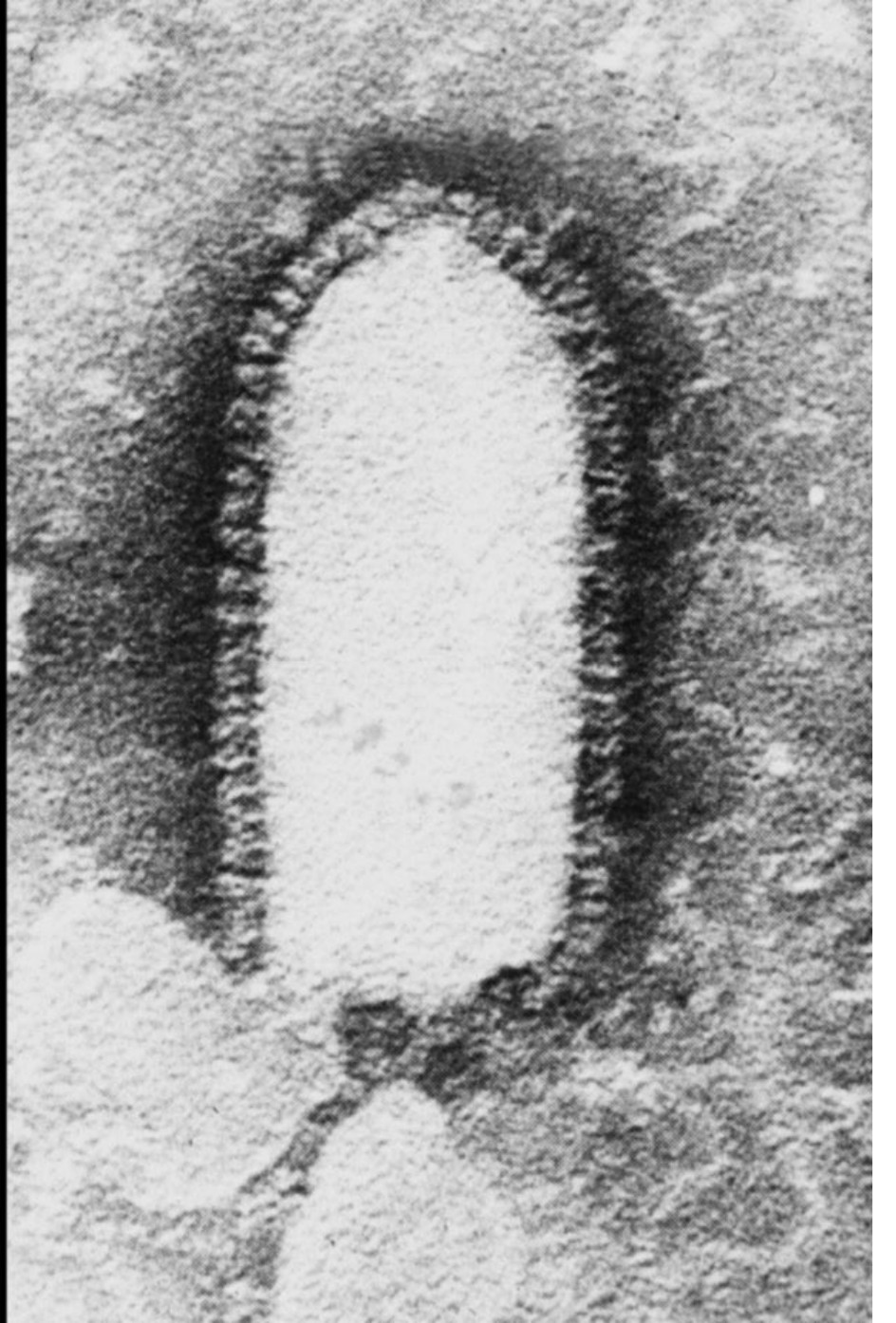
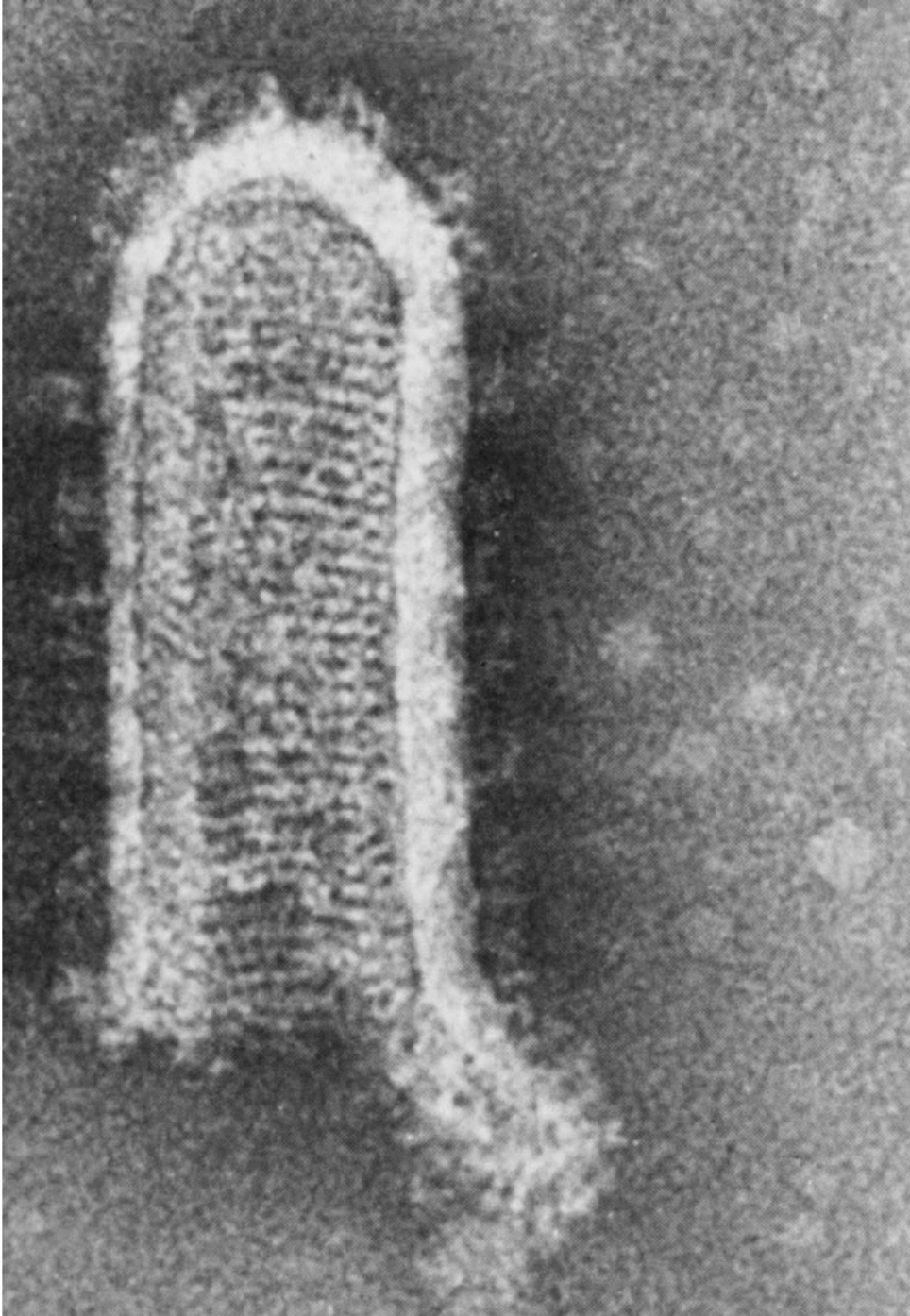


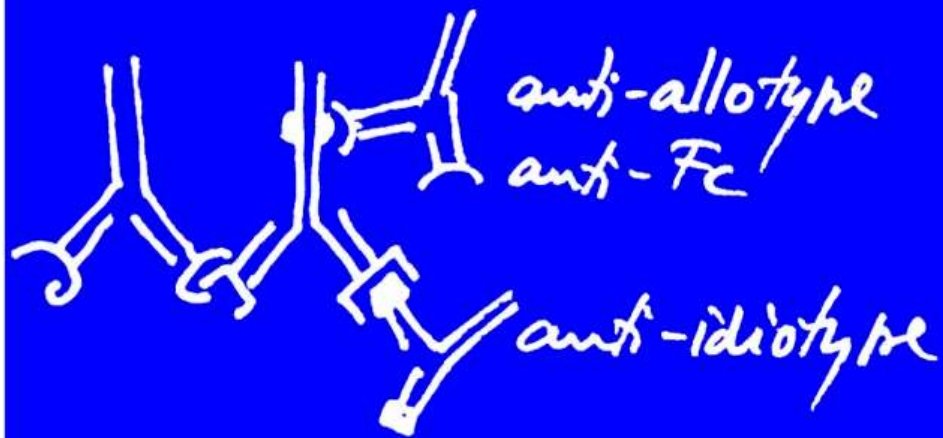
LCMV

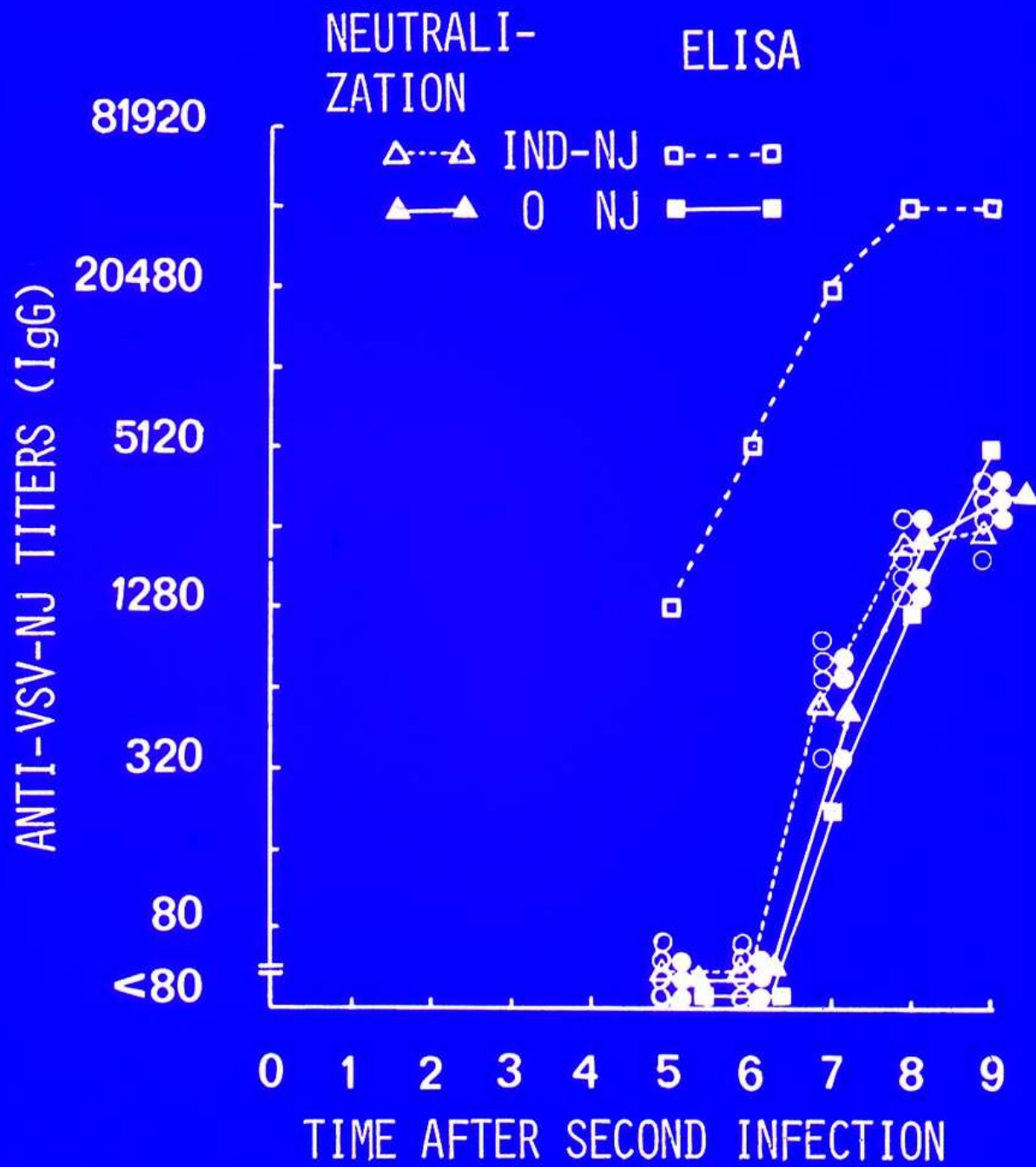




- Specificity
- Memory
- Reactivity
- Protection







Intradermal

Intramuscular

Into spleen

Into lymph node

