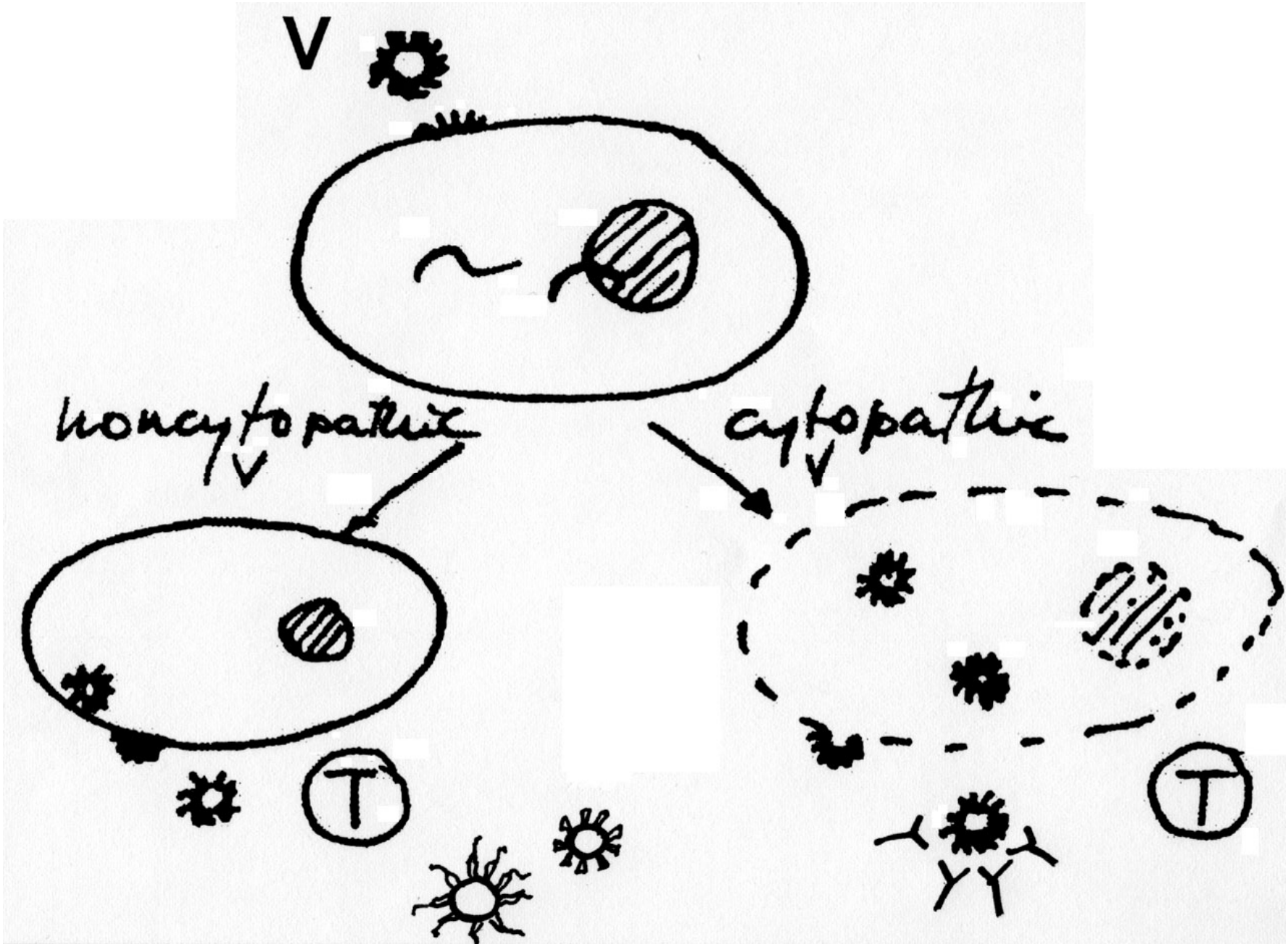
Immunoprotection vs. Immunopathology

Rolf Zinkernagel
University of Zurich, Switzerland


V
noncytopathic
cytopathic
T
T

Cytopathic, acute inf.

Mucosae → viraemia → brain

Skin nAb

e.g. class. epidemic childhood infections

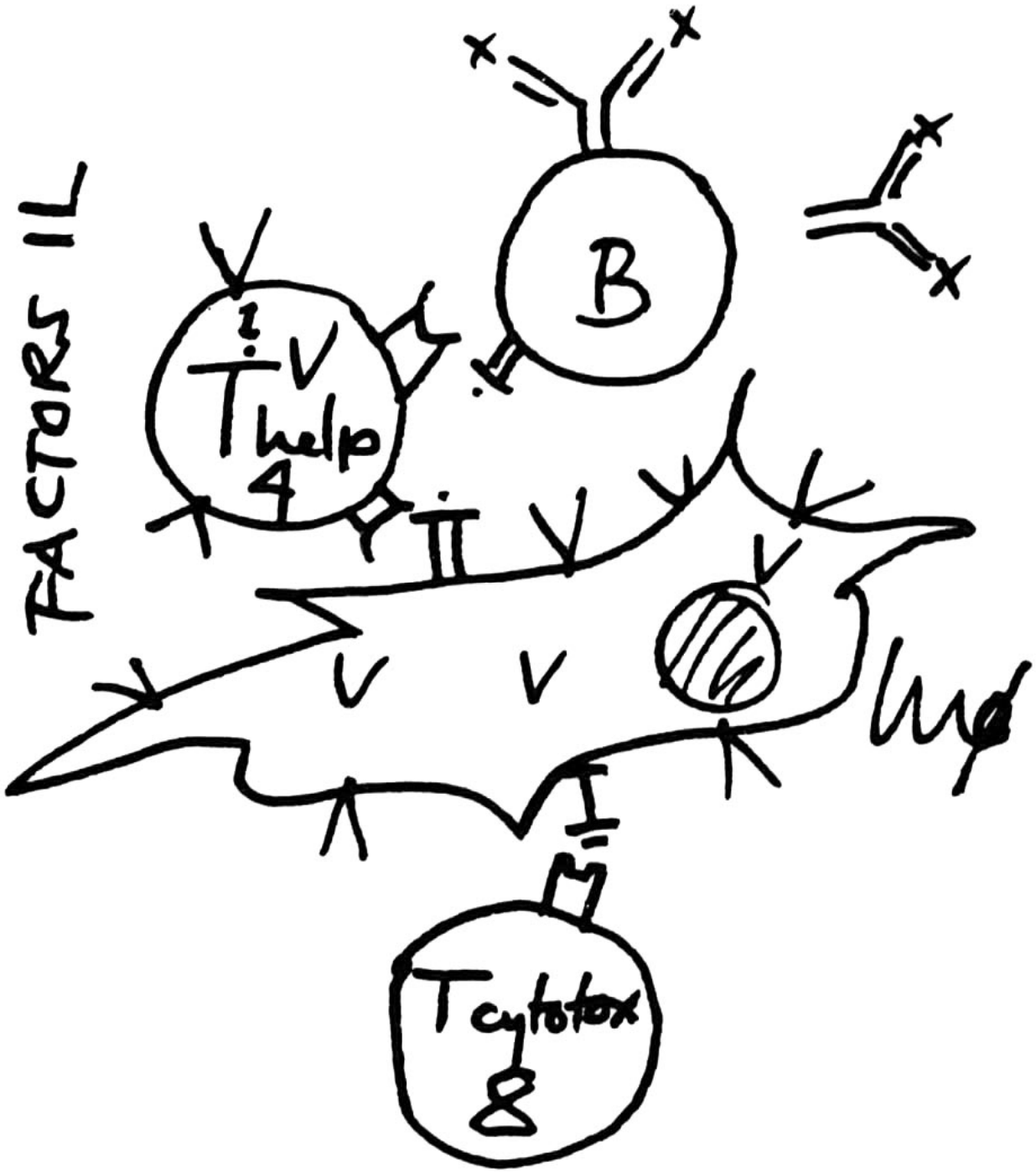
Intermittently cytopathic inf.

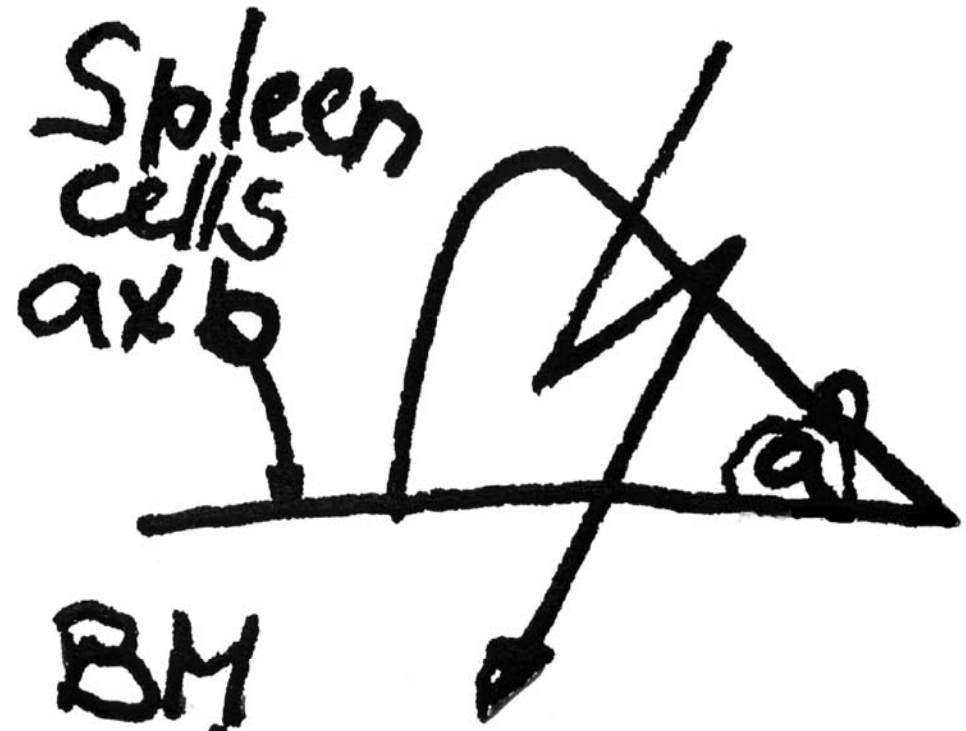
herpes, measles, "coxsackie"

Non-cytopathic persistent inf.

T cell immunopathology

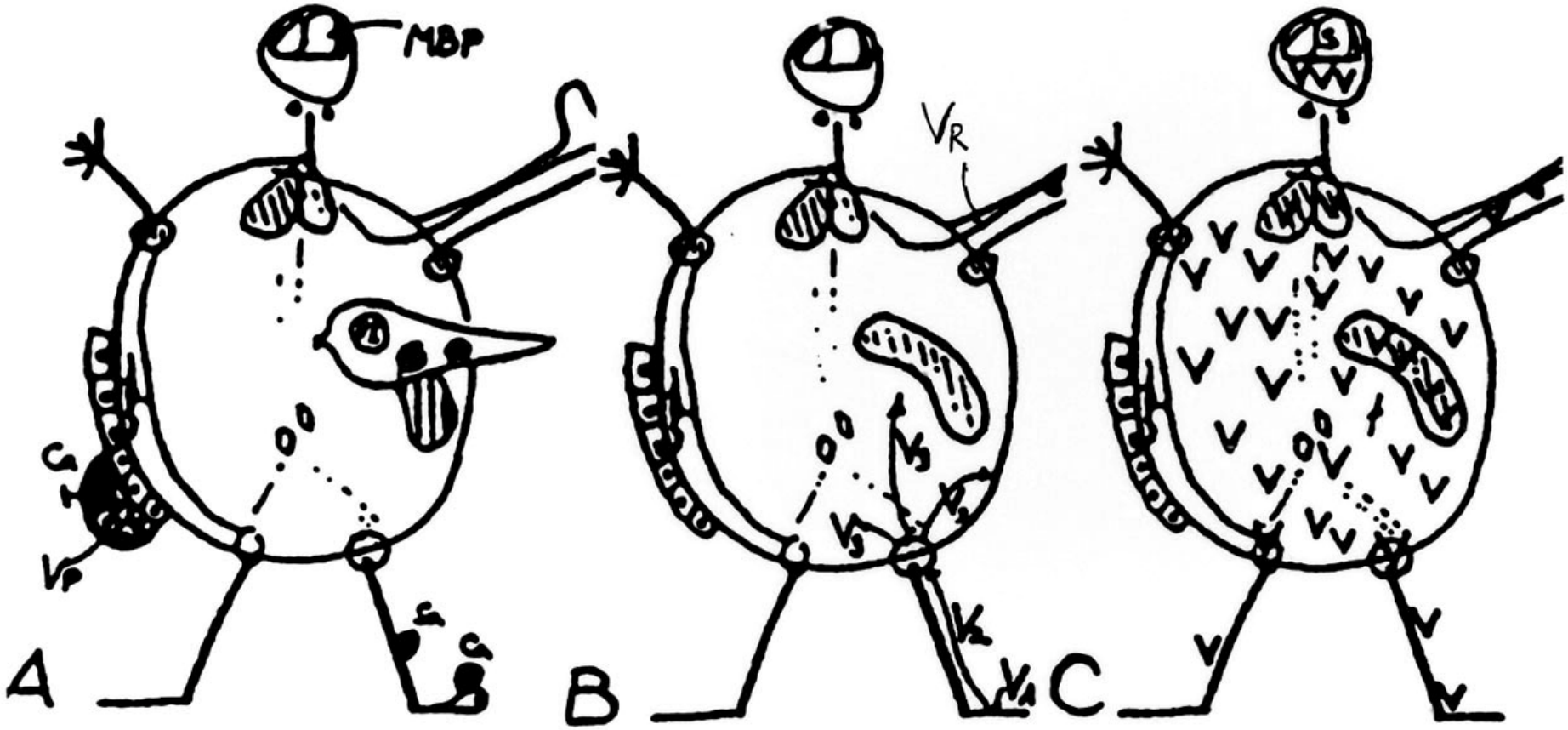
e.g. HBV, HCV, HIV-2

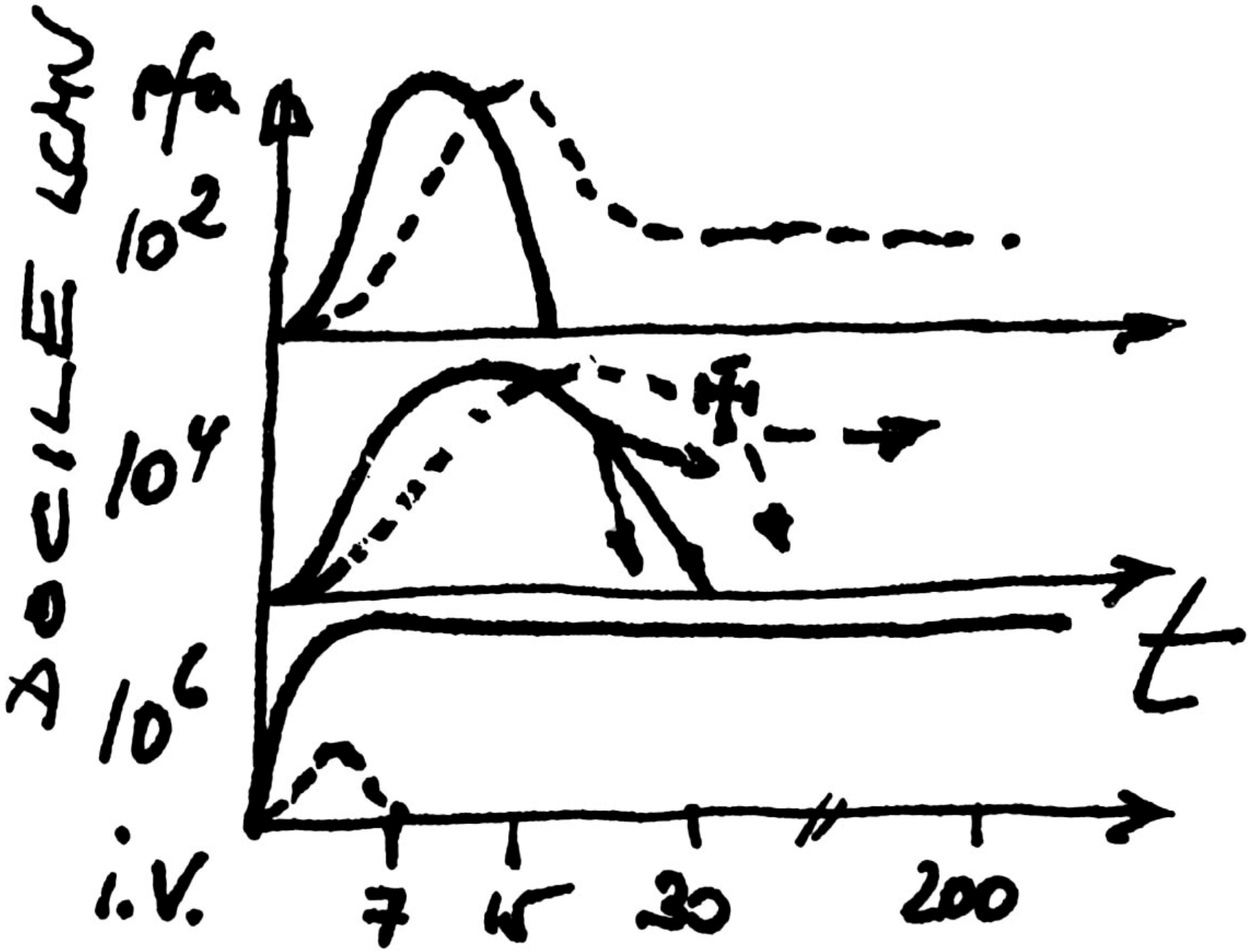

FACTORS IL
B
T help
4
Tcytotox
8

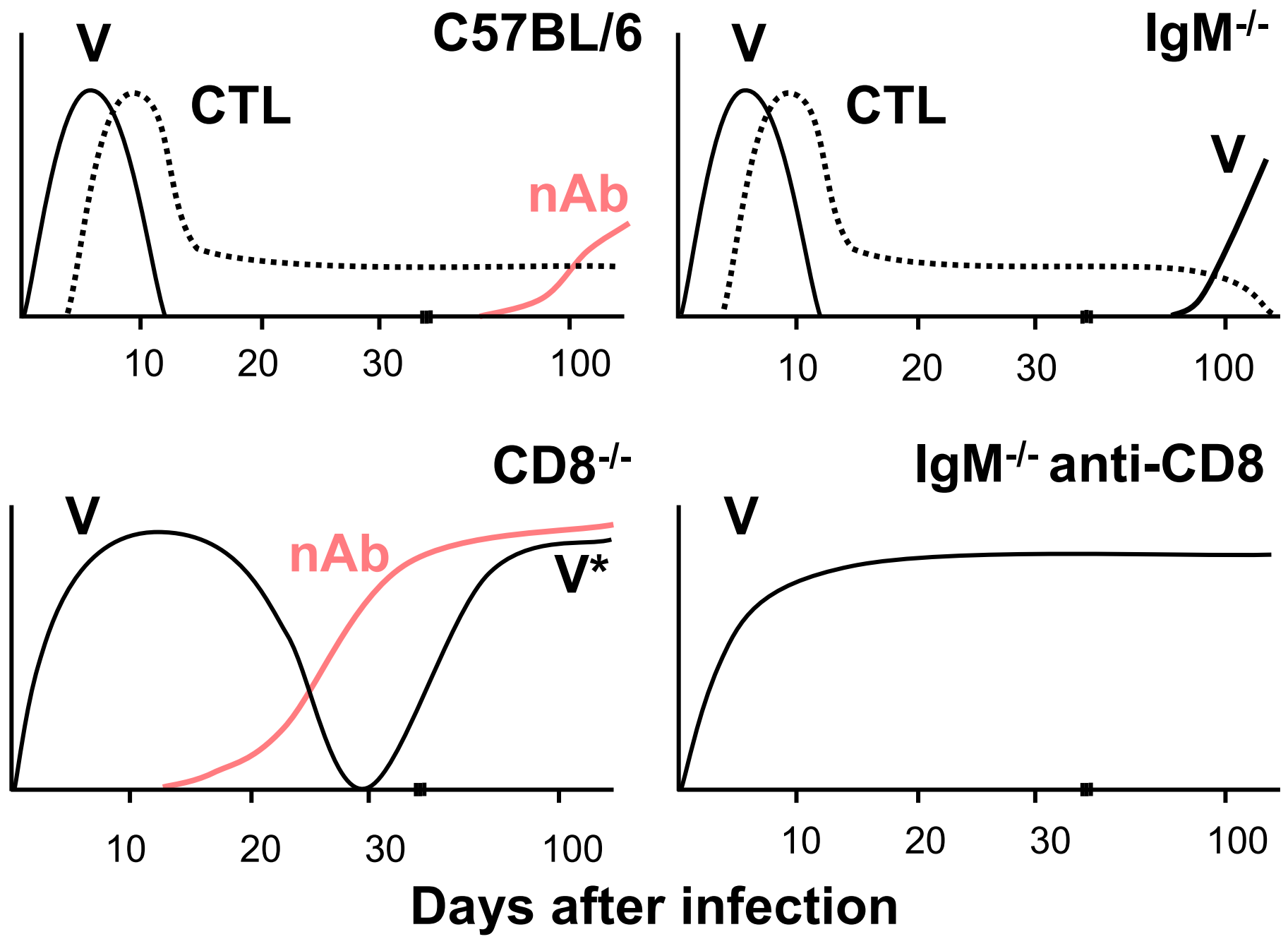


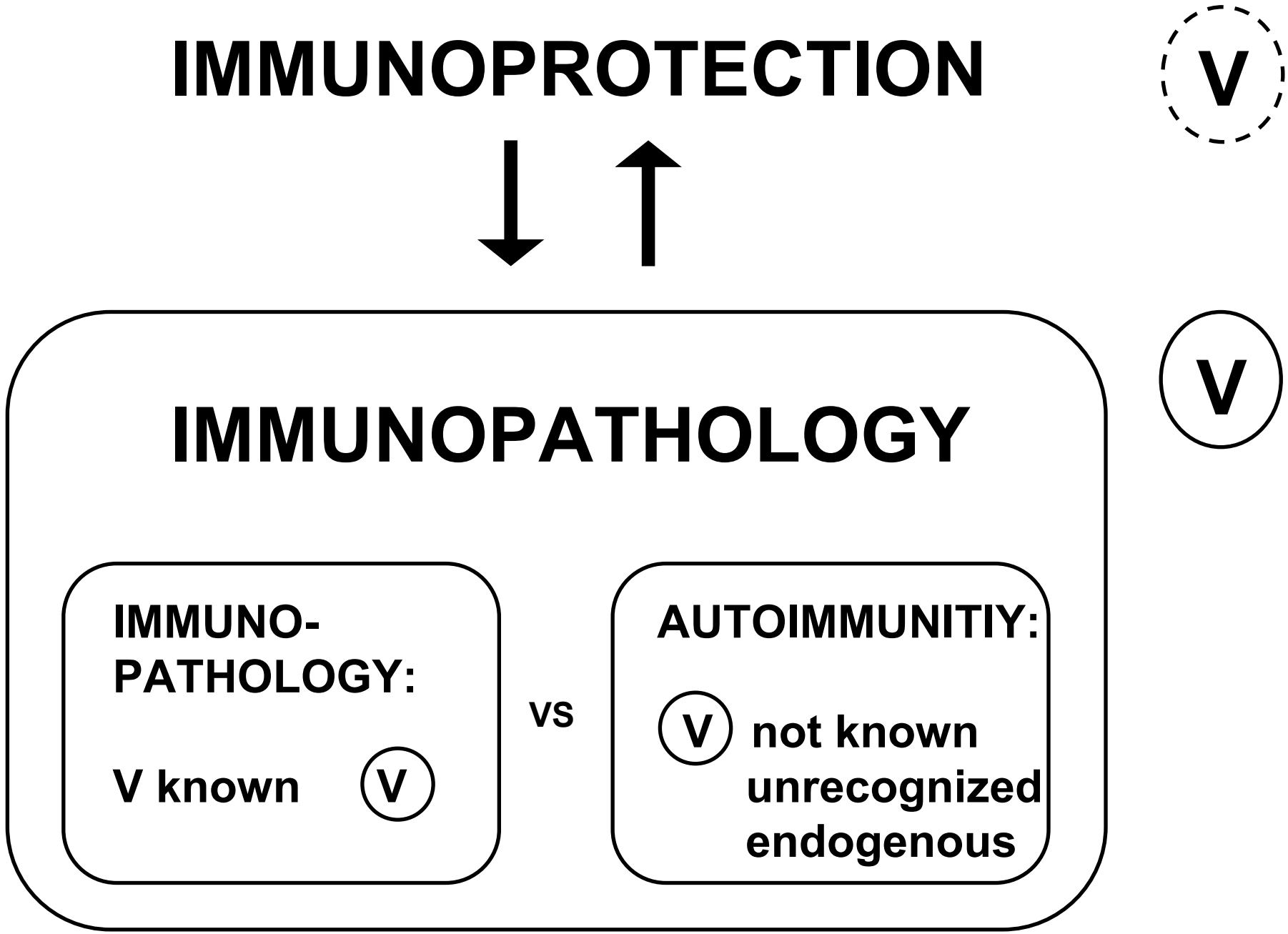
BM
axb

	CD8+CTL a+V	CD8+CTL b+V
Spleen cells axb	+	+
BM axb	+	−
nude	−	+


MBP
V_R
A
B
C


DOSE (cfu)
10^2
10^4
10^6
i.v.
7
15
30
200
t

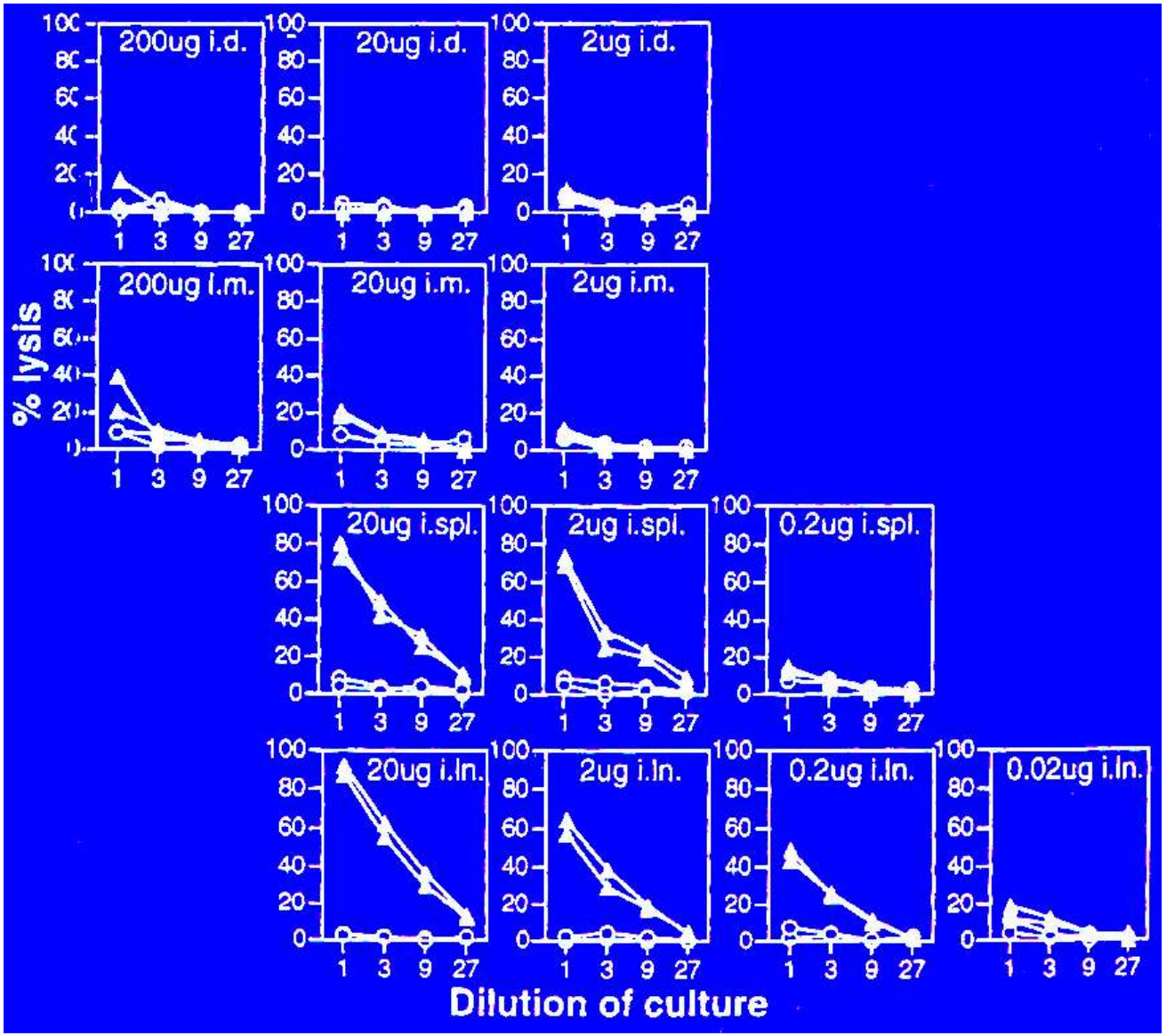

C57BL/6
V
CTL
nAb
10
20
30
100
IgM-/-
V
CTL
V
10
20
30
100
CD8-/-
V
nAb
V*
10
20
30
100
IgM-/- anti-CD8
V
10
20
30
100
Days after infection

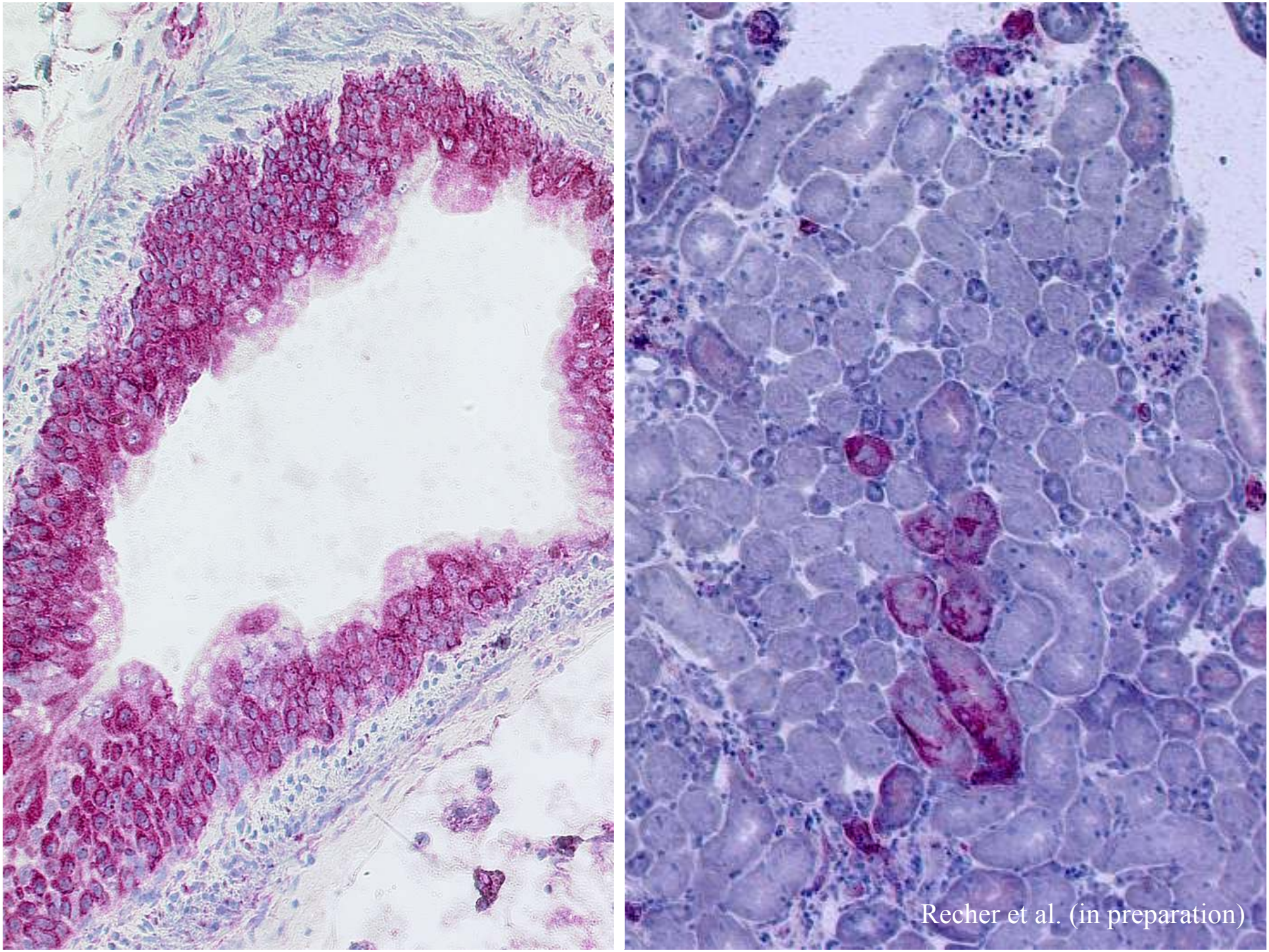

IMMUNOPROTECTION
V
IMMUNOPATHOLOGY
V
IMMUNO-
PATHOLOGY:
V known
V
vs
AUTOIMMUNITIY:
V
not known
unrecognized
endogenous

Antigen (dose/time) regulates immune responses

AG not reaching Lk,	-	ignorance
AG persists in „all“ Lk, spl	-	tolerance
AG reaches init. few Lk, spl	++	immune
> 3d < 20d		response
AG persists		
extra lymphatically widely	+	immunopath.
locally	±	memory

	200μg	20μg	2μg	0. 2μg	0.02μg
Intradermal					
Intramuscular					
Into spleen					
Into lymph node					




Recher et al. (in preparation)

A non-retroviral RNA virus persists in DNA form

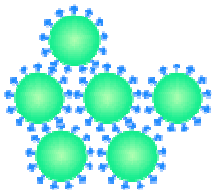
Paul Klenerman, Hans Hengartner & Rolf M. Zinkernagel

Institute of Experimental Immunology, University Hospital, Schmelzbergstrasse 12, 8091 Zurich, Switzerland

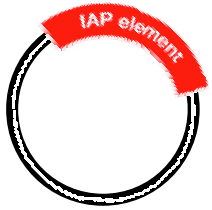
Table 1 Species-specific production of LCMV DNA

Line	Origin	Titre	RT*	LCMV DNA PCR	
				in vitro	*in vivo*
MC57	Mouse	6.9	>200	+	+
L 929	Mouse	7.5	>200	+	+
IC21	Mouse	5.1	>200	+	+
BHK	Hamster	4.6	>200	+	+
CCL 158	Guinea-pig	7.8	>200	–	–
CRL 1405	Guinea-pig	7.5	88	–	–
CCL 100	Gerbil	5.5	9	–	NT
208F	Rat	3.7	4	–	–
MDCK	Dog	4.8	55	–	NT
BSC 40	Cow	5.8	<0.1	–	NT
Vero	Monkey	6.1	<0.1	–	NT
Hela	Human	6.2	<0.1	–	–

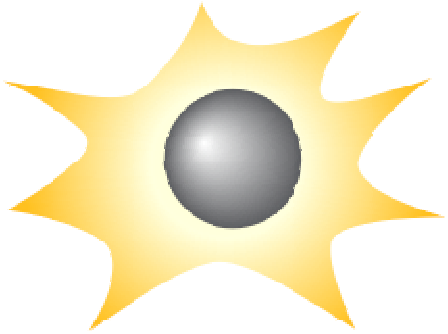
Experiment:



LCMV



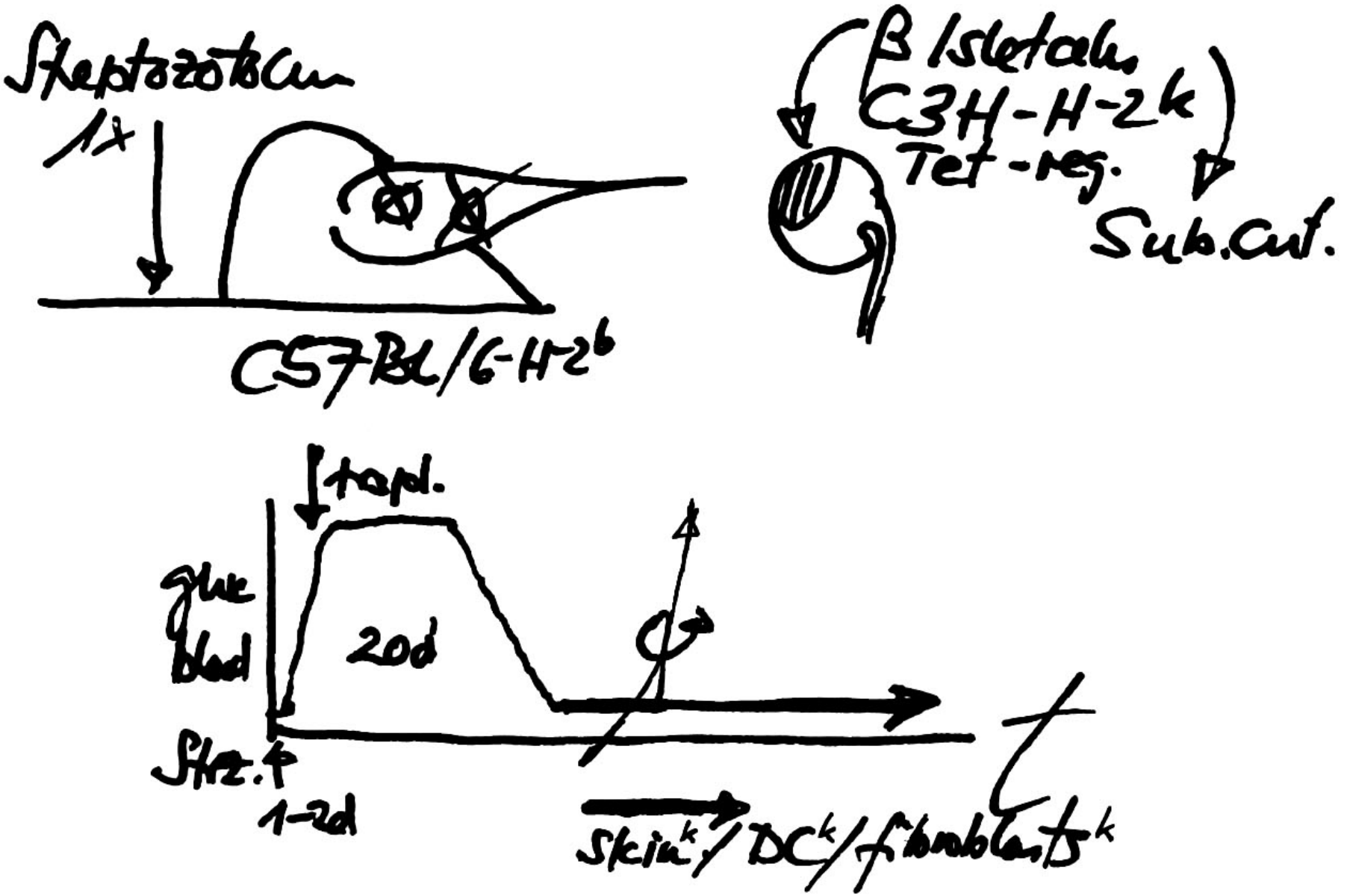
IAP-expressing plasmid

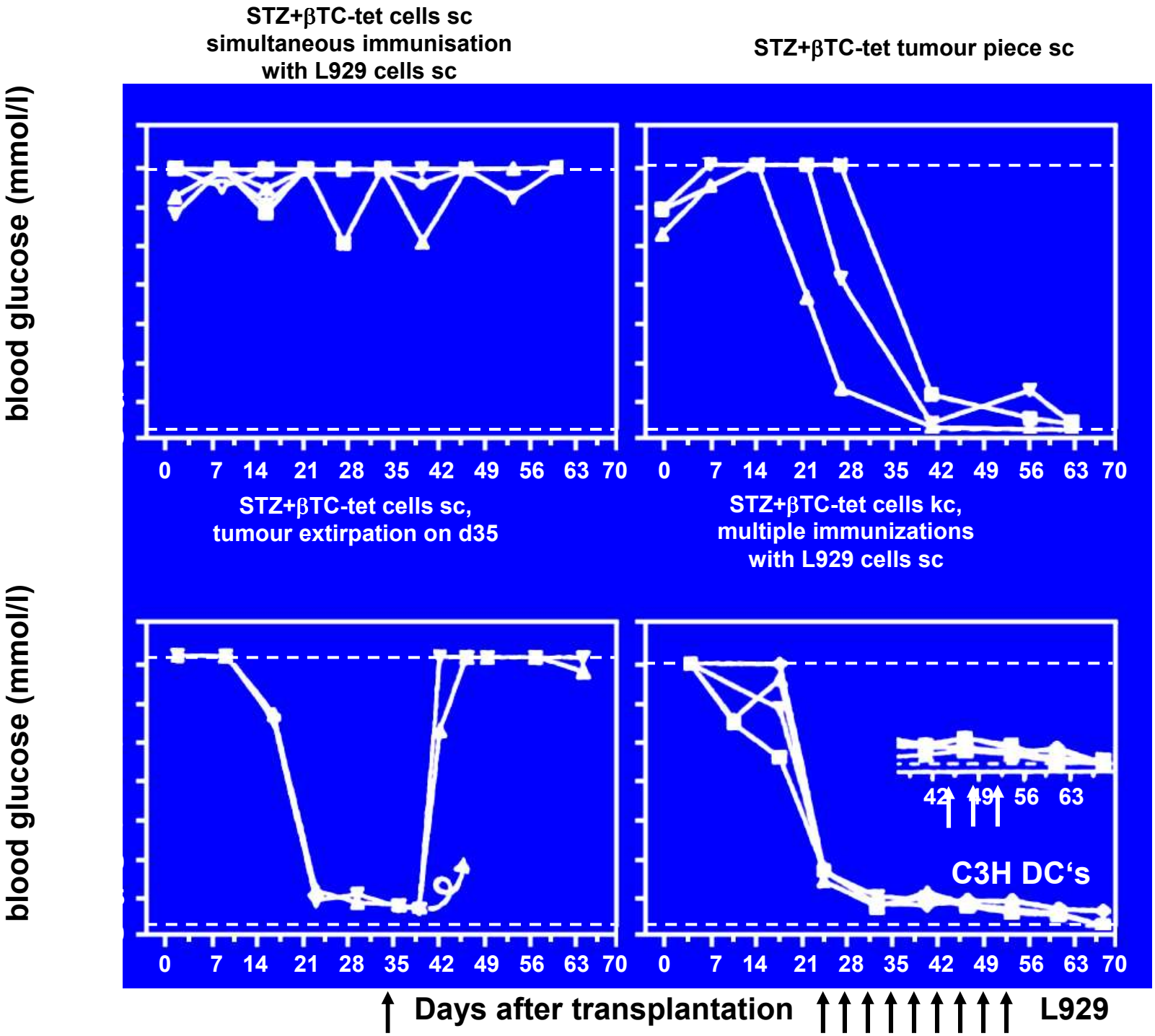


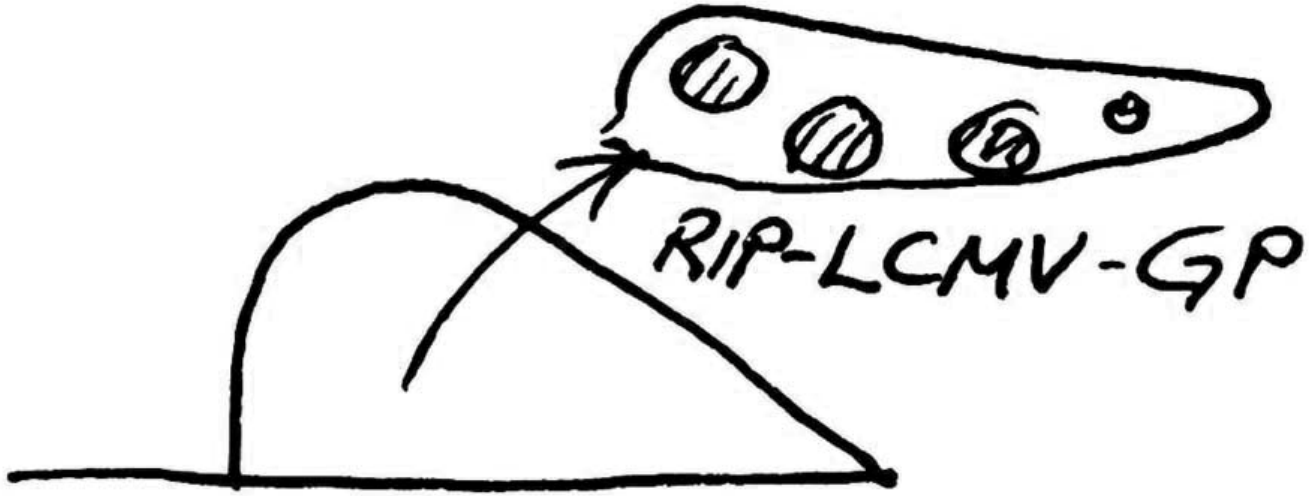
non-murine cell lines

RT $^{\text{negative}}$ and cLCMV-formation $^{\text{negative}}$

cLCMV formation?


Streptozotocin
1x
C57BL/6-H-2b
β Isletcells
C3H-H-2k
Tet-neg.
Sub.cut.
transpl.
gluc
blood
20d
Str.
1-2d
Skin / DC / fibroblasts


STZ+βTC-tet cells sc
simultaneous immunisation
with L929 cells sc
STZ+βTC-tet tumour piece sc
blood glucose (mmol/l)
0 7 14 21 28 35 42 49 56 63 70
STZ+βTC-tet cells sc,
tumour extirpation on d35
STZ+βTC-tet cells kc,
multiple immunizations
with L929 cells sc
blood glucose (mmol/l)
42 49 56 63
C3H DC's
Days after transplantation
L929

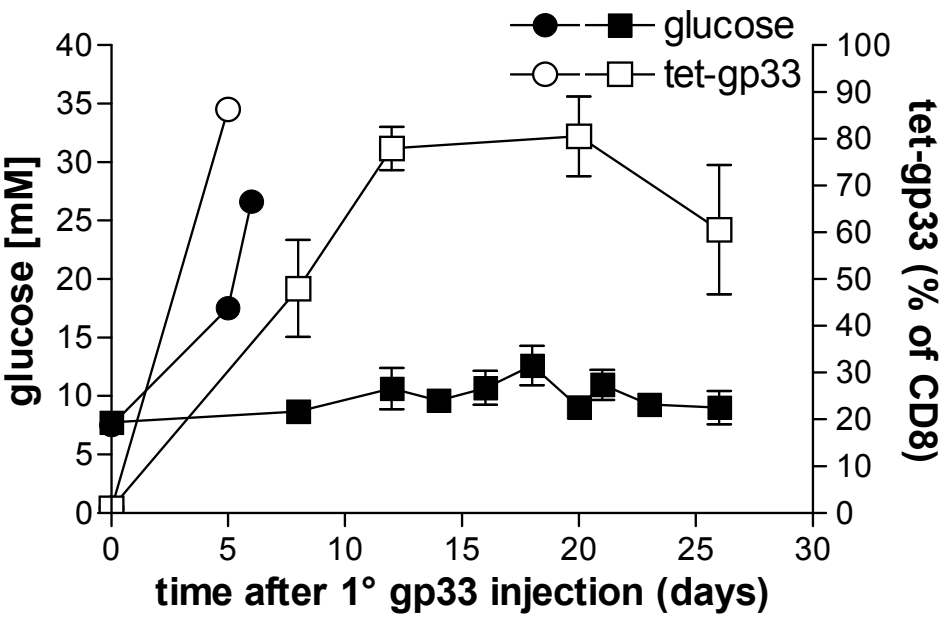


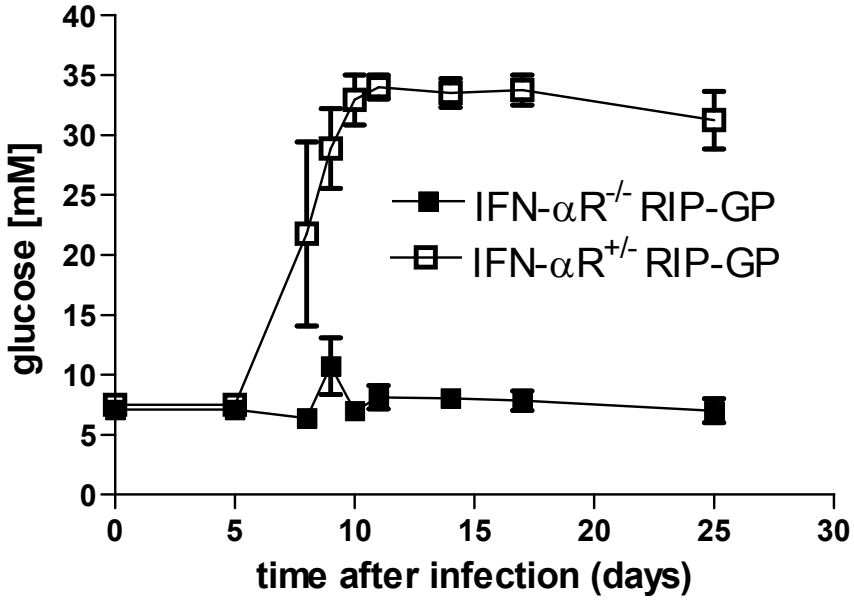
Treatm.	CTLresp.	Diabetes
∅	—	—
LCMV	++	++
vacc-GP	+	—

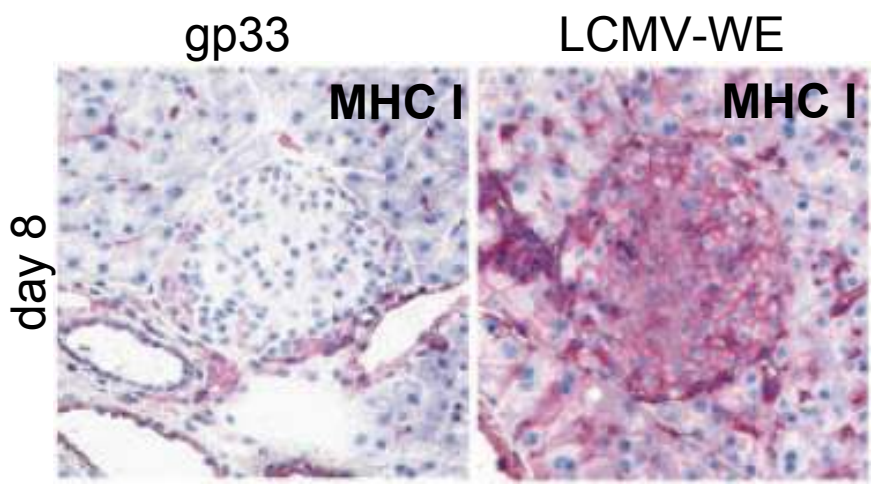
RIP-GP tg mice:

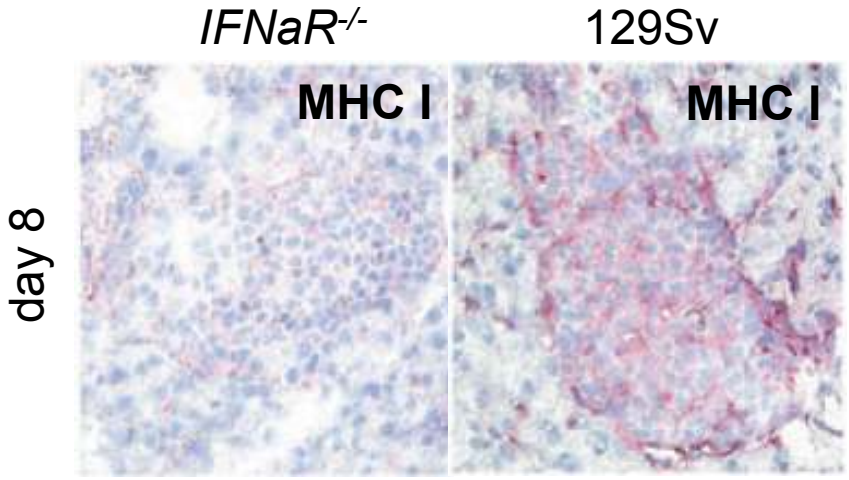
	GP	**GP+B7**	**GP+TCR_{tg}**
No treatment	-	-	-
LCMV	+++	+++	+++
vacc-GP_{LCMV}	-	++	++

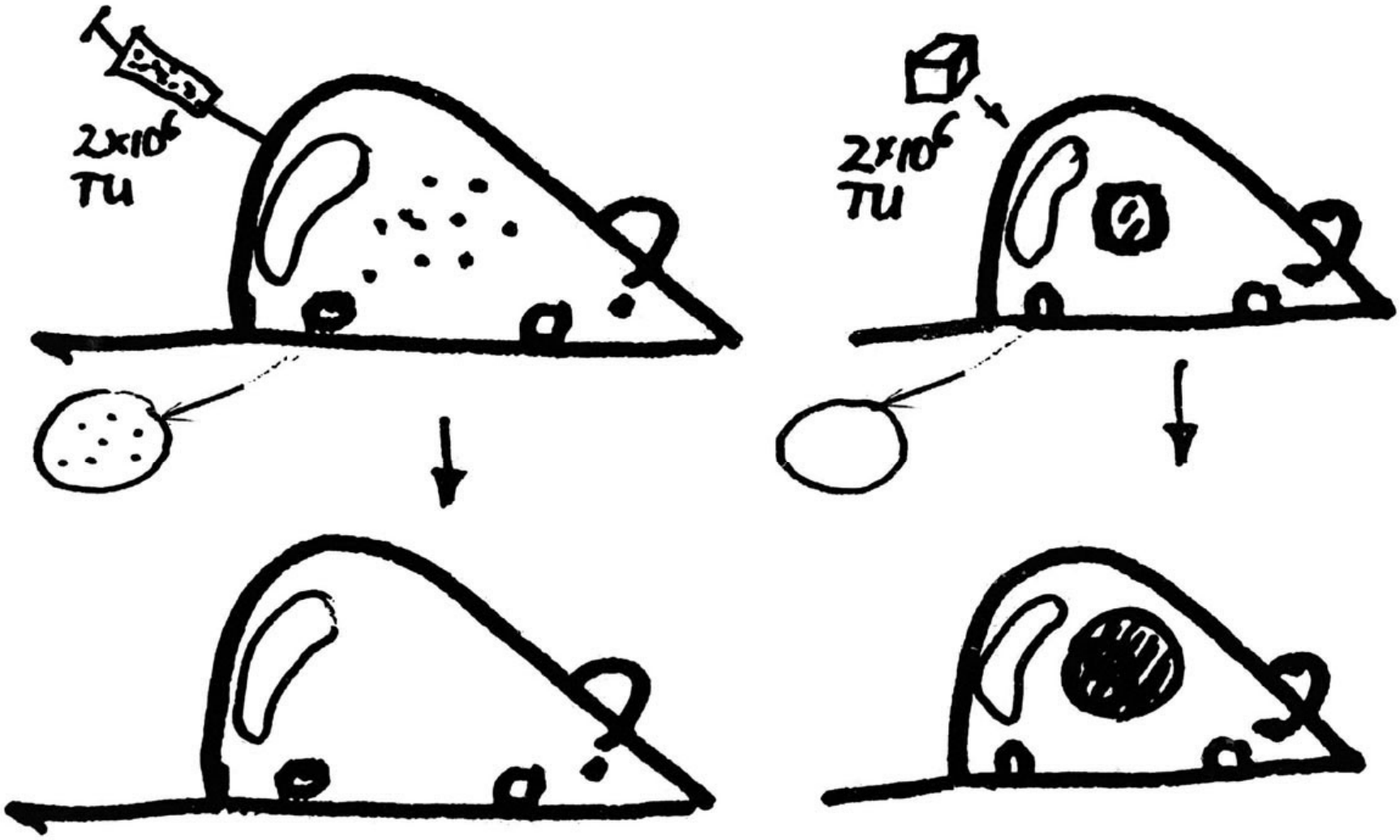
IFN-α is required for induction of diabetes

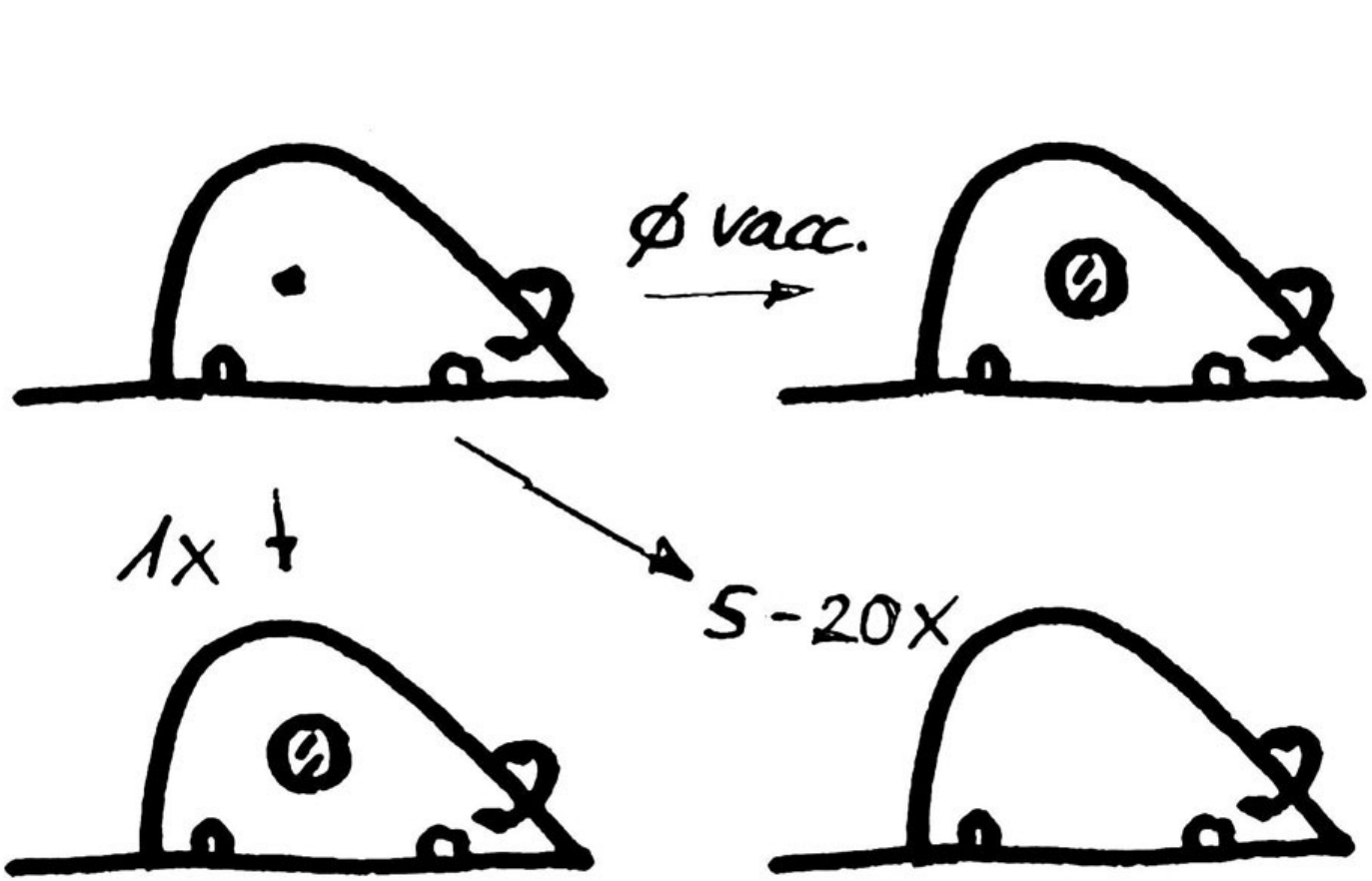


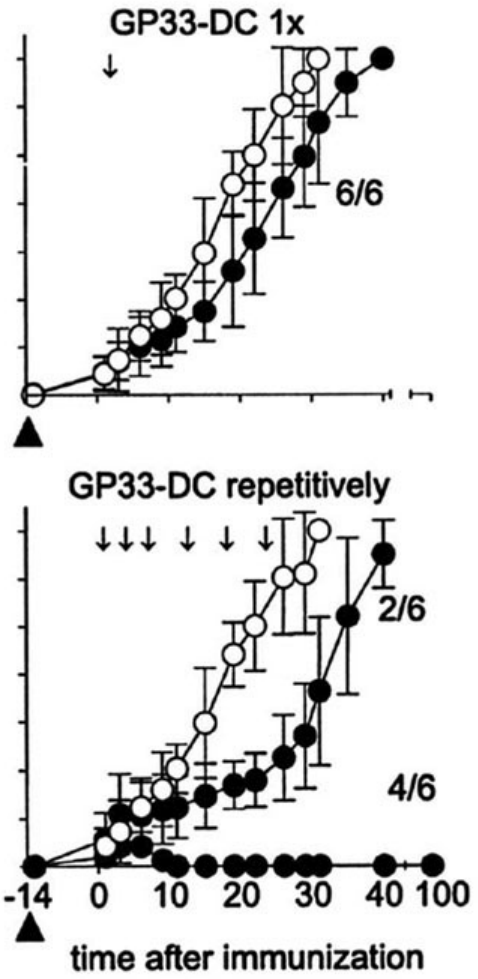


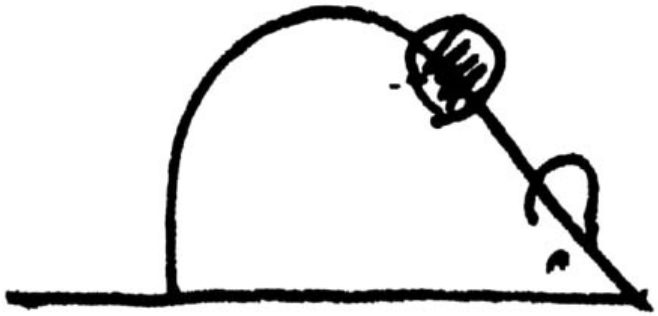





2x10⁶
TU
2x10⁶
TU


ø vacc.
1x
5-20x

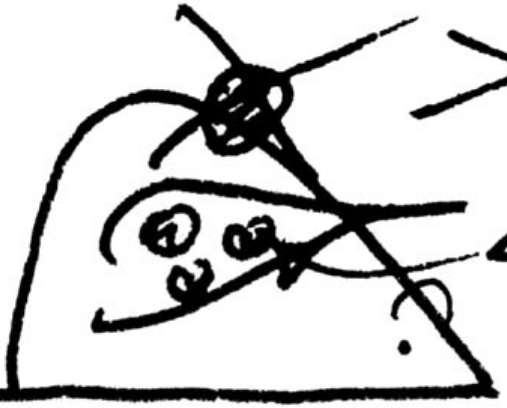

GP33-DC 1x
6/6
GP33-DC repetitively
2/6
4/6
-14
0
10
20
30
40
100
time after immunization



Vacc: DC$_{GP}$

LCMV

Vacc-GP



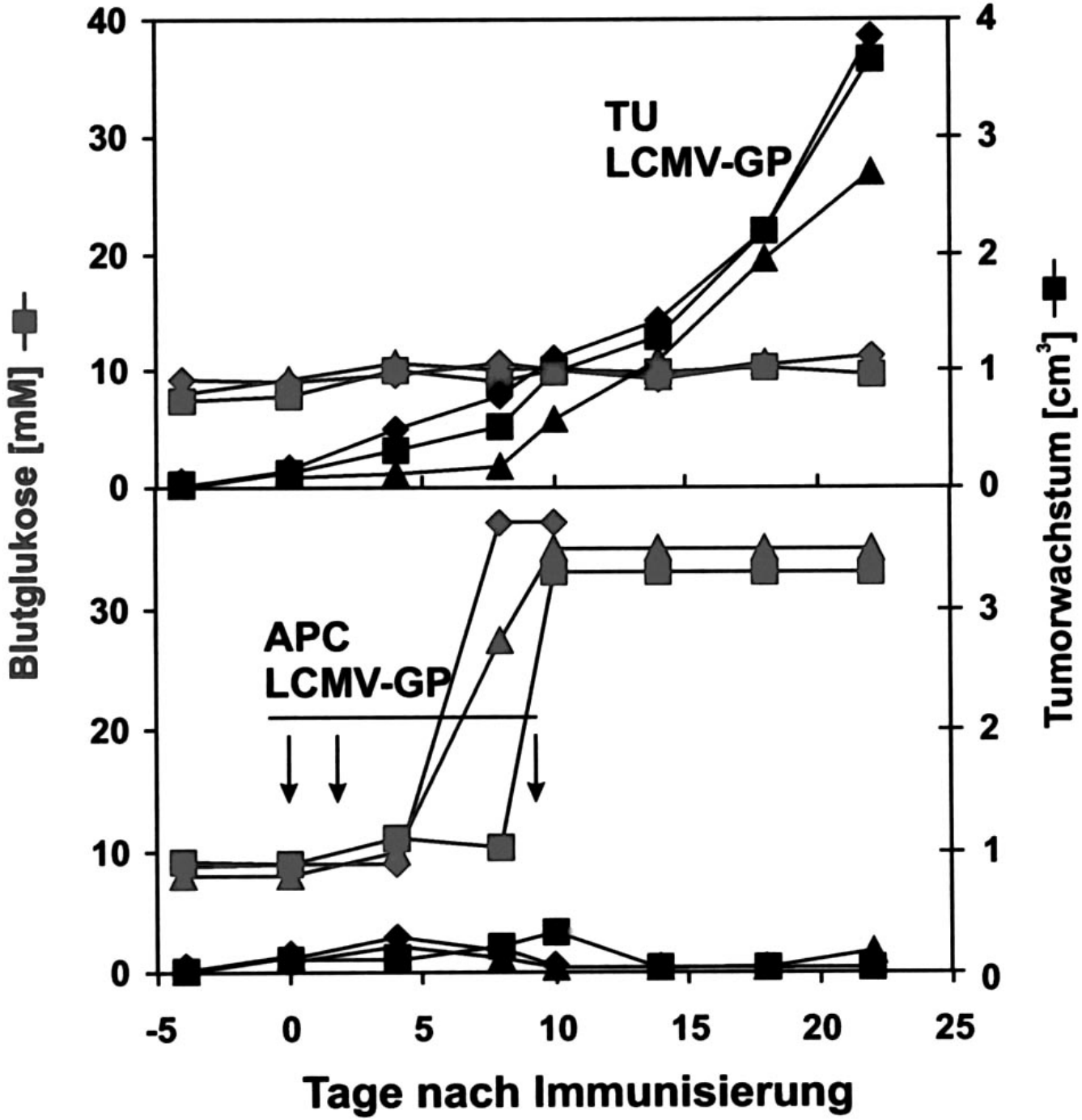
> 99.9%

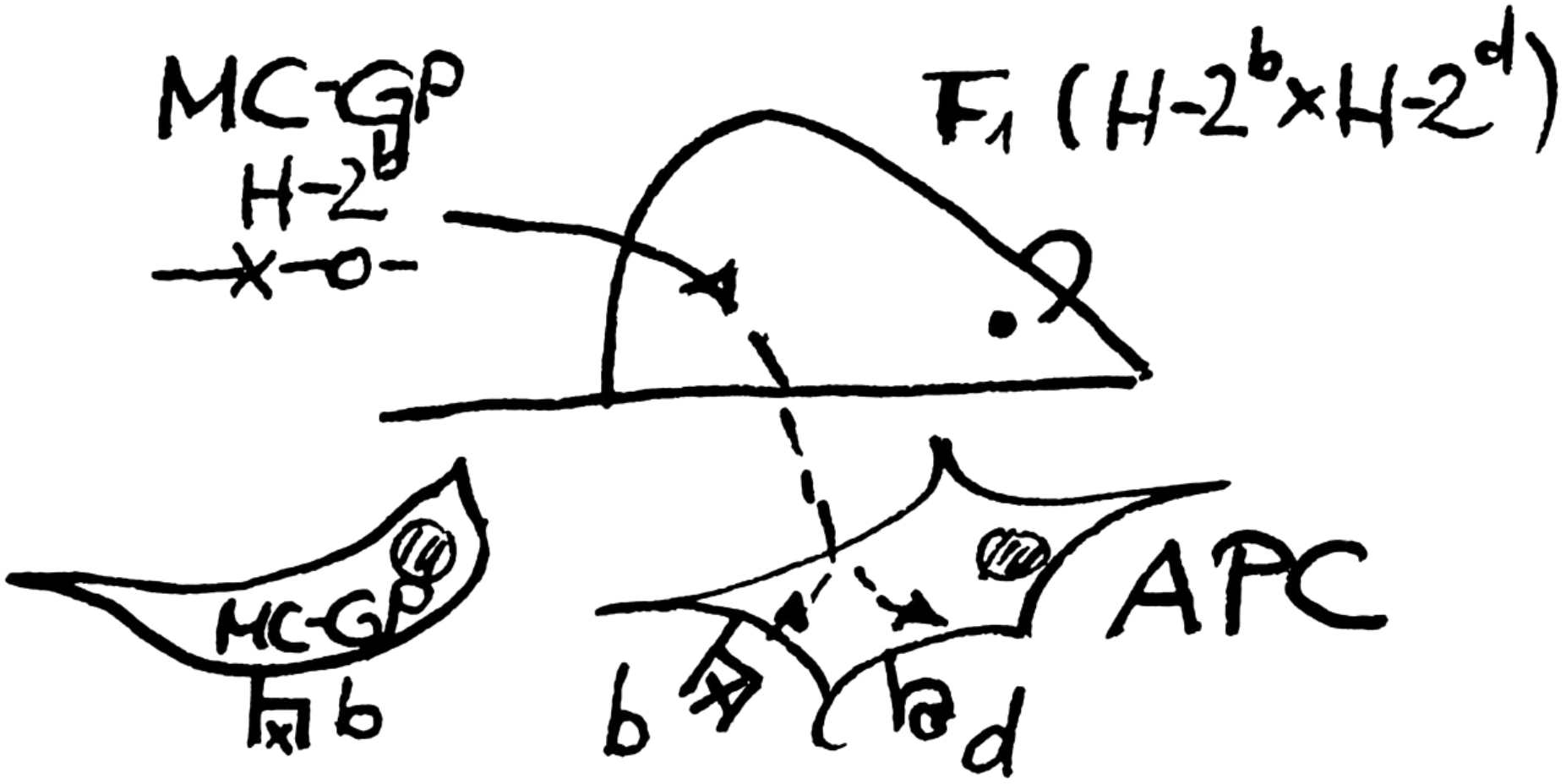
~~99.9%~~

< 90%

no diabetes

?


TU
LCMV-GP
APC
LCMV-GP
Blutglukose [mM]
Tumorwachstum [cm³]
Tage nach Immunisierung
40
30
20
10
0
4
3
2
1
0
-5
0
5
10
15
20
25


MC-GP
H-2b
F1 (H-2b × H-2d)
MC-GP
b
APC
b
d

	BALB/c H-2^d immunised with:	2° CTL NP_{118} L^d	Protection LCMV	Protection vacc-NP
	L929 H-2^k	—	—	—
	L929 H-2^{k}NP	—	—	—
10^6	L929 H-2^{k}NP+L^d	+	+	+
10^7	L929 H-2^{k}NP+L^d freeze thawed	—	—	—

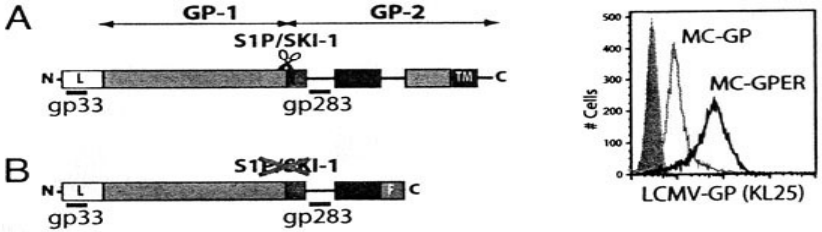
(BALB/c x cBA (H-2^d x H-2^k) F1 same results

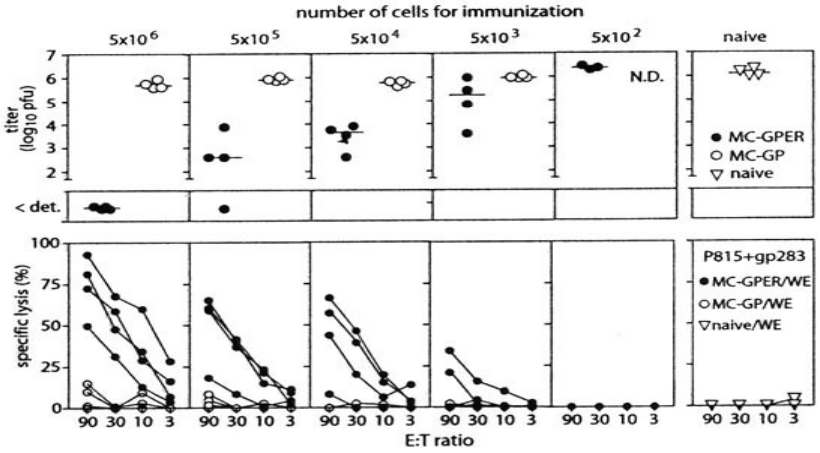
Lindey et al
Science 298, 1990

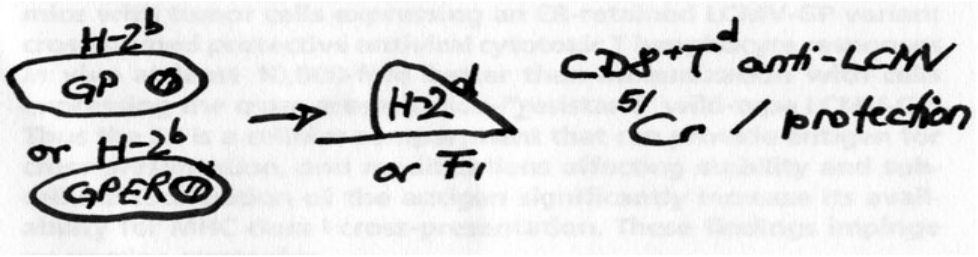
A lymphocytic choriomeningitis virus glycoprotein variant that is retained in the endoplasmic reticulum efficiently cross-primes CD8$^+$ T cell responses

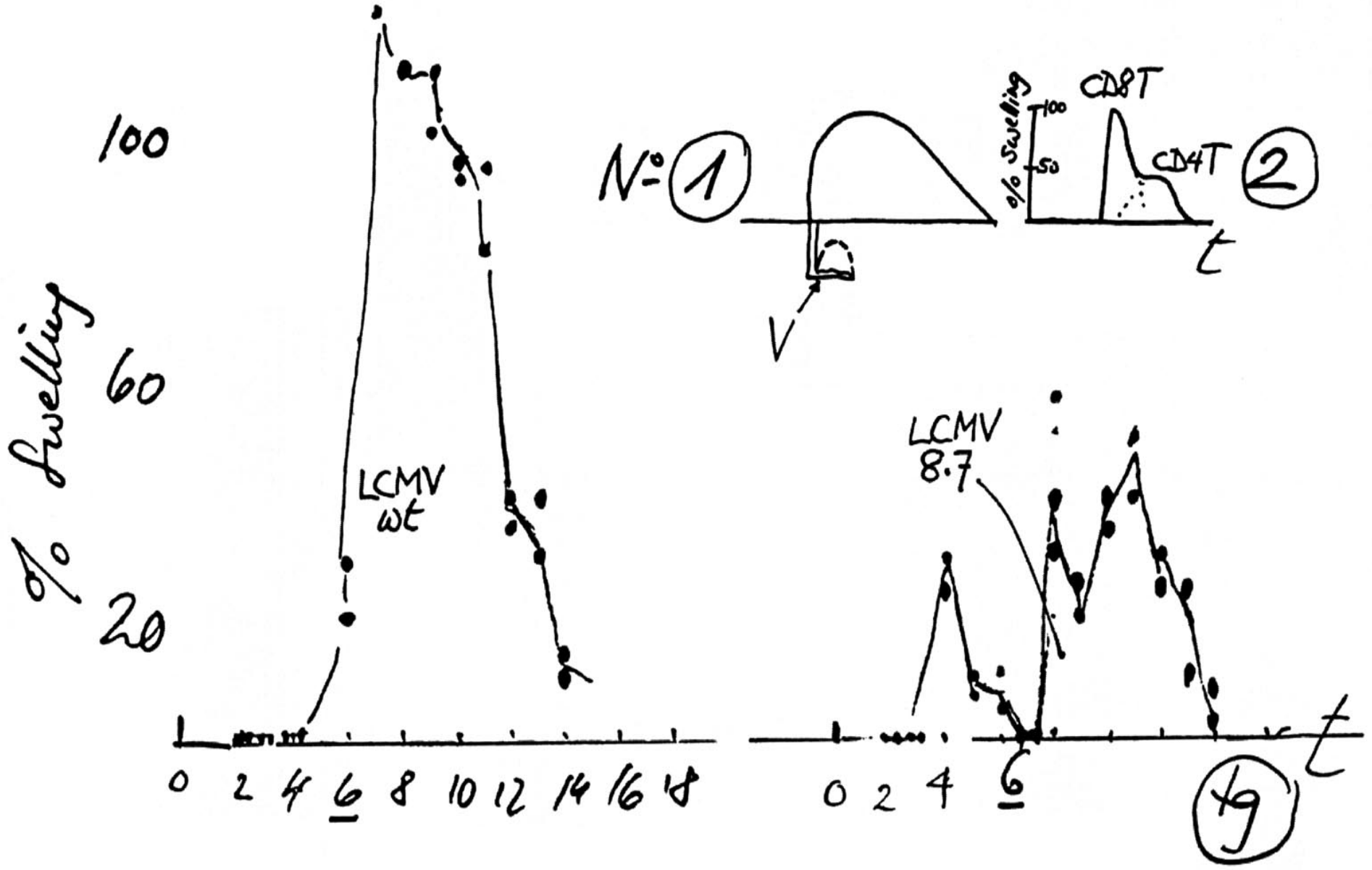
Stefan Freigang*†, Bruno Eschli*, Nicola Harris*‡, Markus Geuking*, Katharina Quirin§, Sabrina Schrempf¶, Raphael Zellweger*, Jacqueline Weber*, Hans Hengartner*, and Rolf M. Zinkernagel*‖

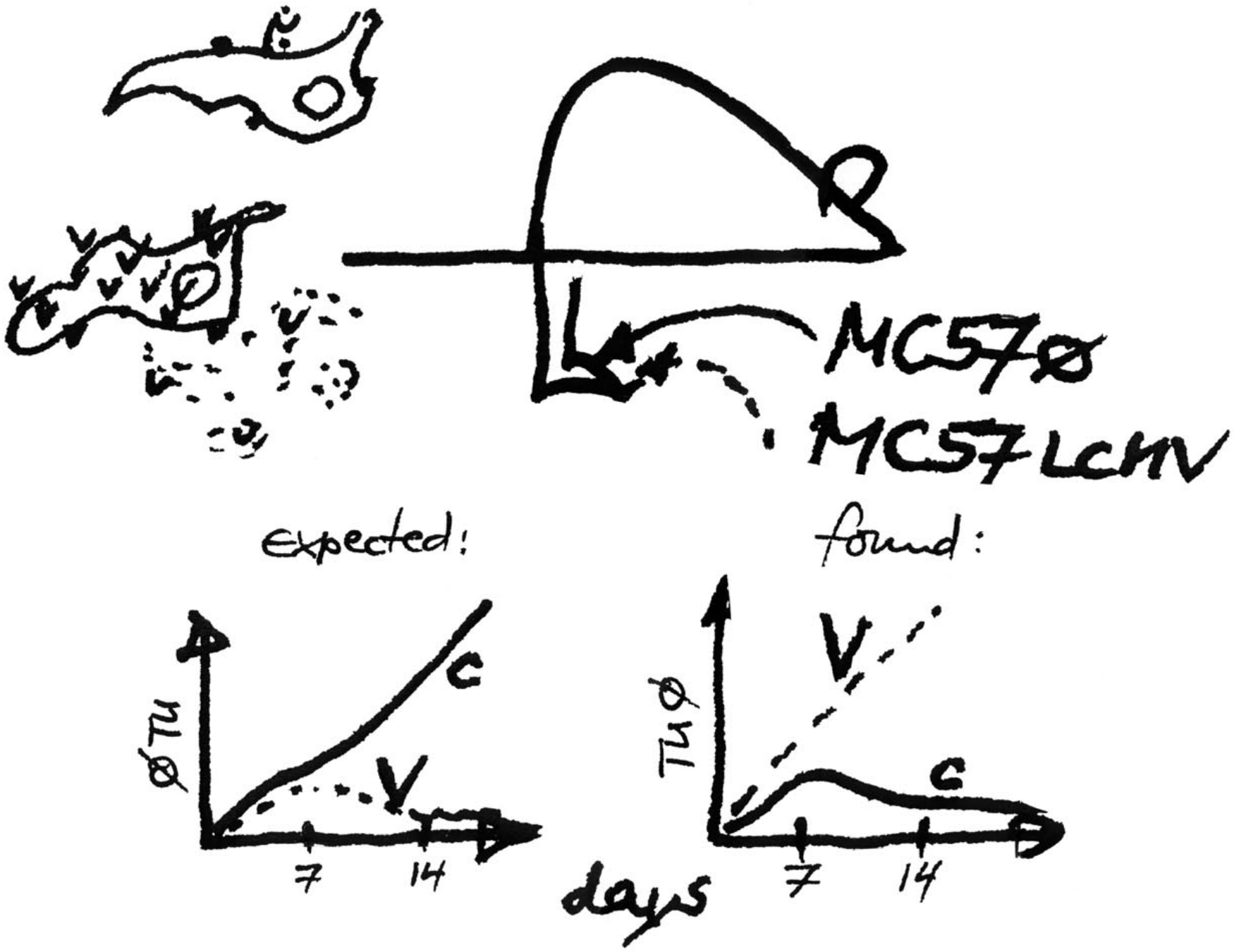
*Institute of Experimental Immunology, Department of Pathology, University Hospital, Schmelzbergstrasse 12, CH-8091 Zurich, Switzerland; ‡Environmental

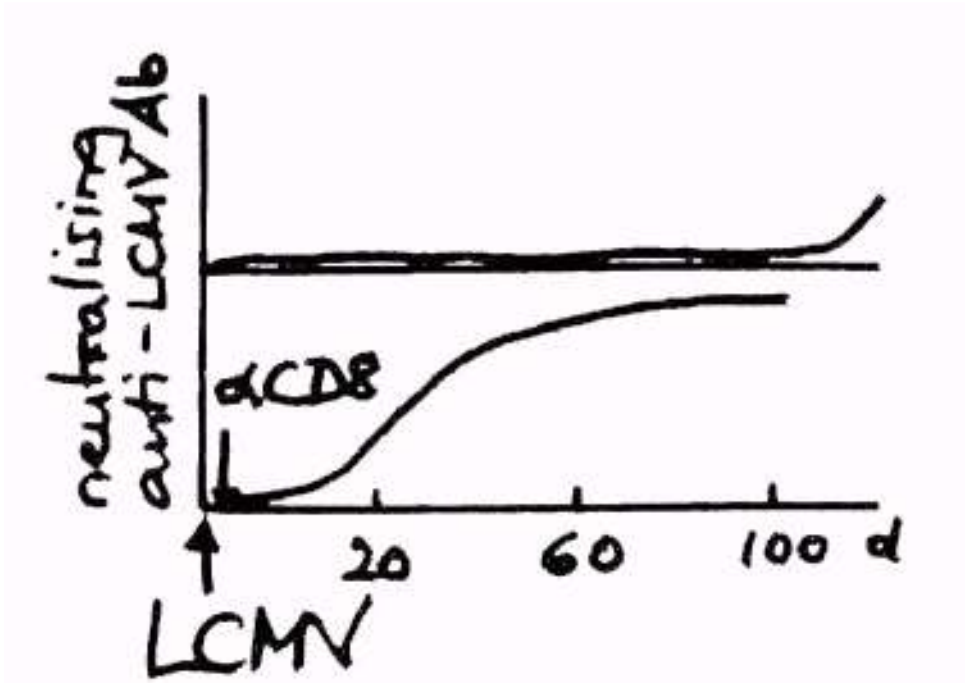


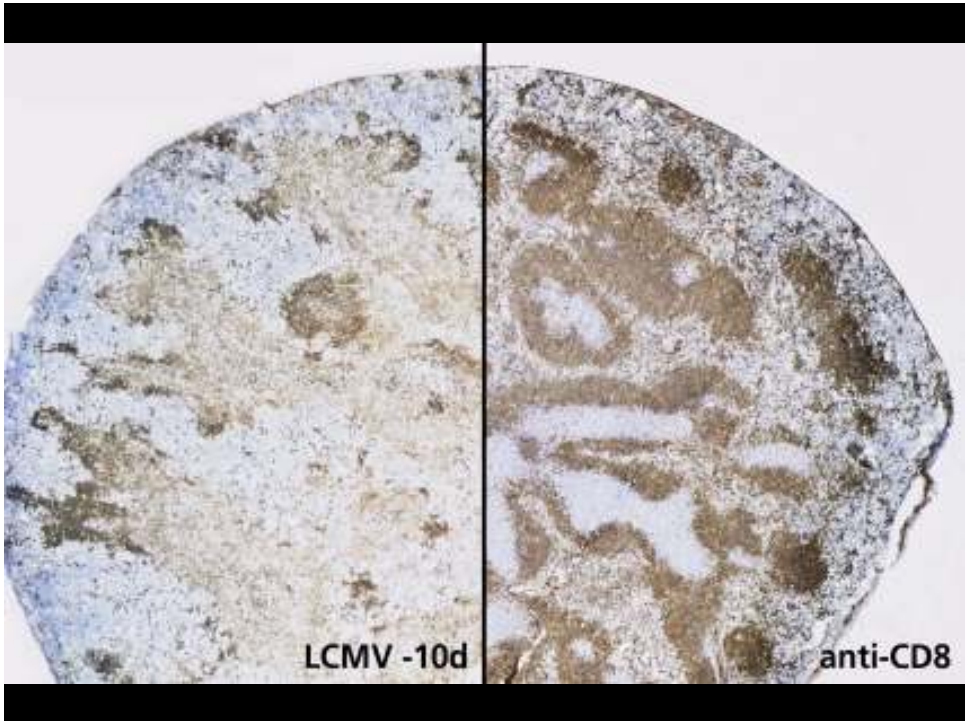




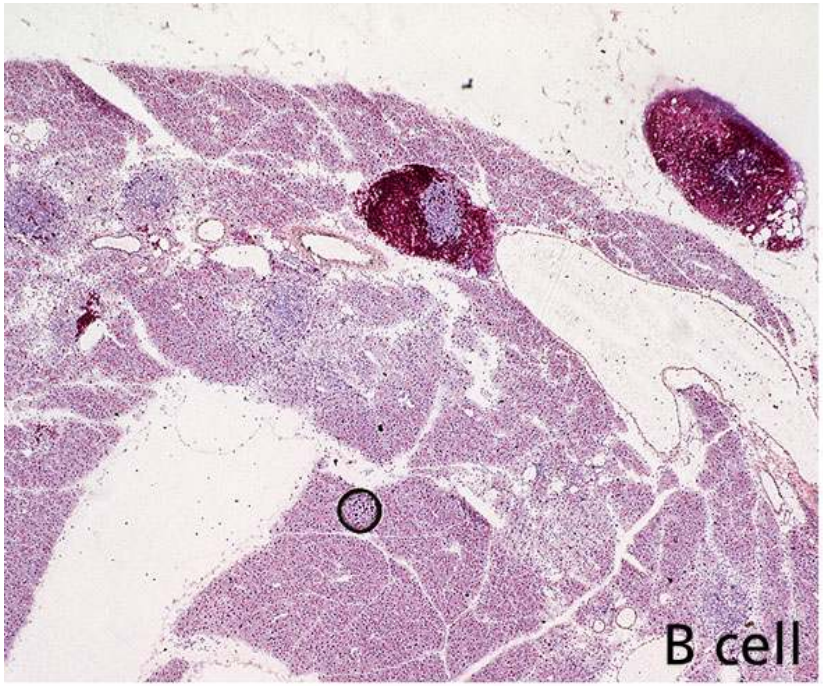

% swelling
100
60
20
LCMV
wt
0 2 4 6 8 10 12 14 16 18
N° 1
V
% swelling
100
50
CD8T
CD4T
2
t
LCMV
8.7
0 2 4 6
t
4g

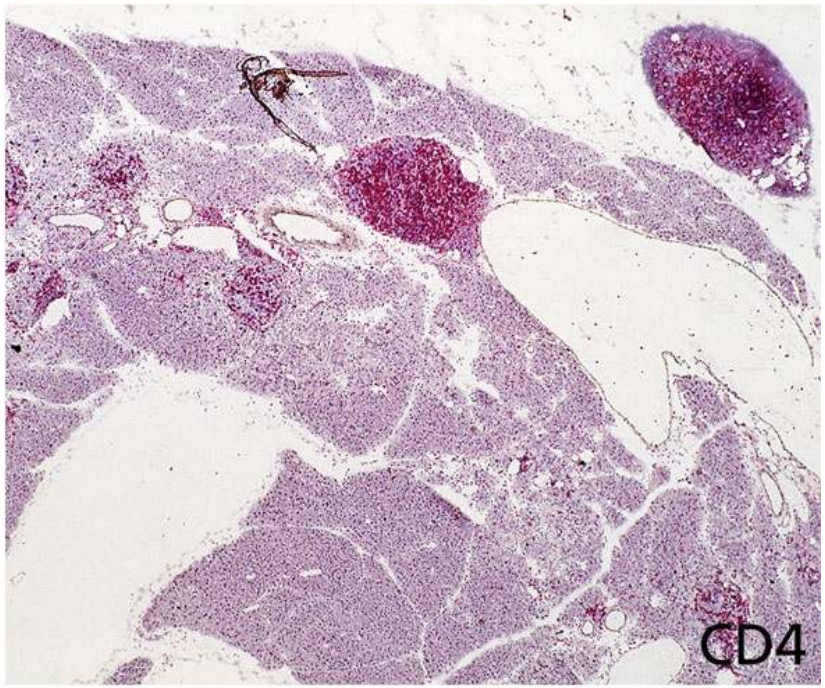

MC57o
MC57LcMV
expected:
found:
TMO
c
V
7
14
days

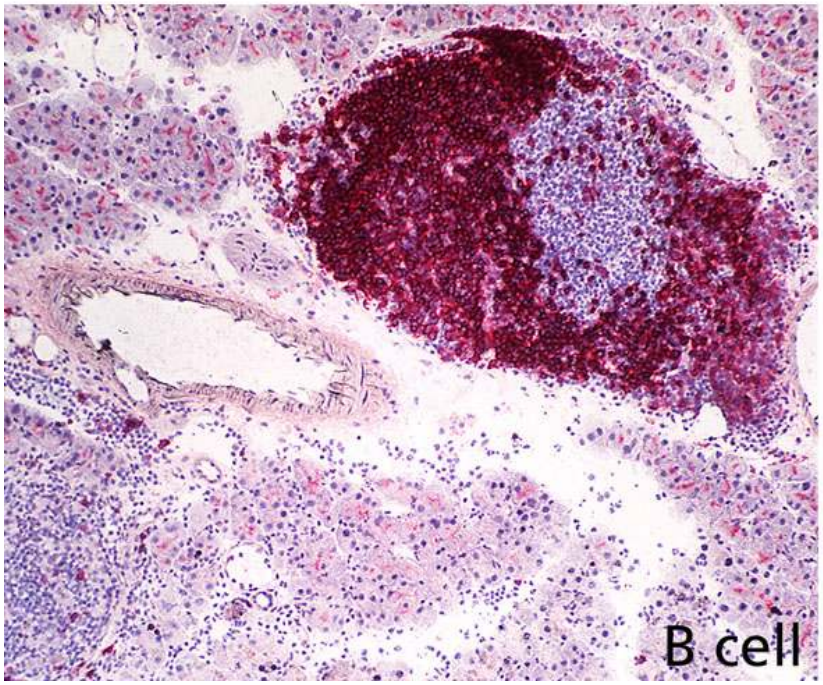

neutralising anti-LCMV Ab
αCD8
20
60
100 d
LCMV

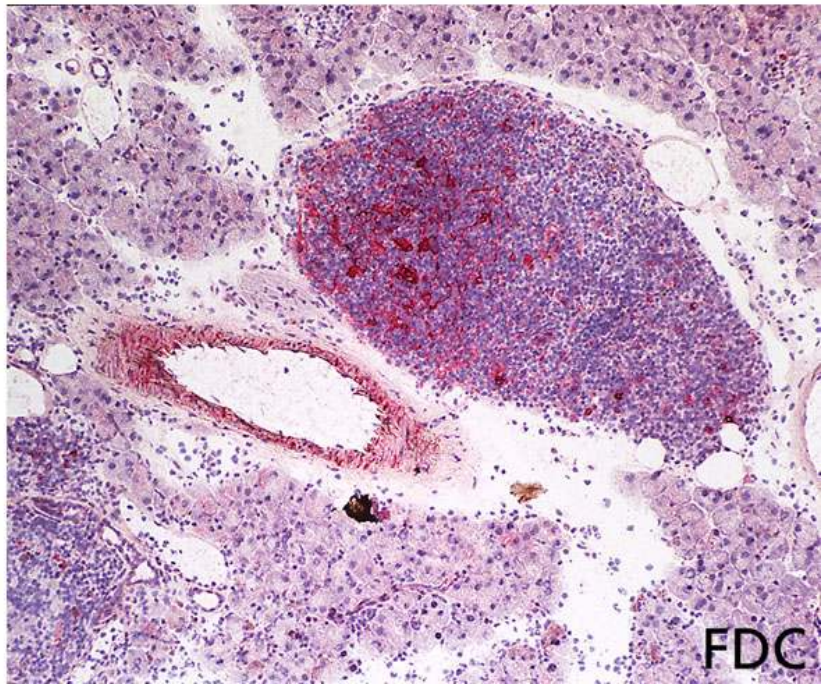

LCMV -10d
anti-CD8

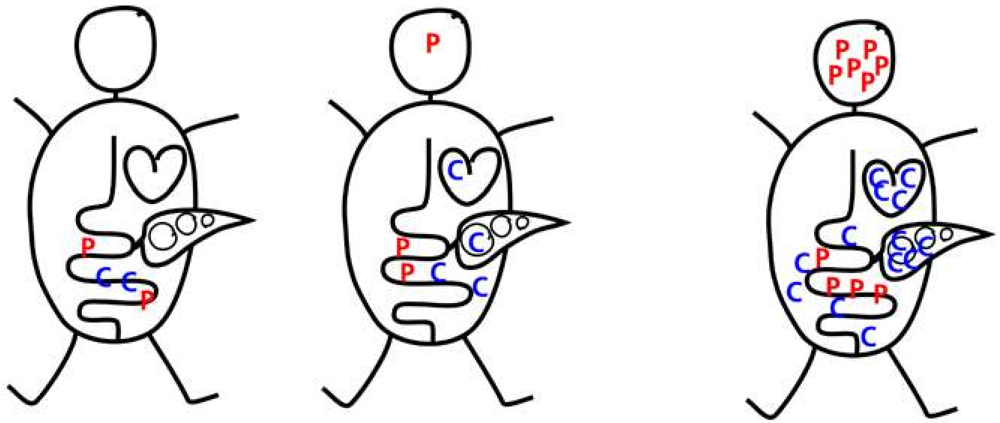
Ag	$\rightarrow$	**LK, spleen**
LK,spl.	$\rightarrow$	**Ag/organ**
		autoimmunity’
		chronic rej.

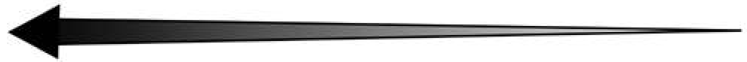
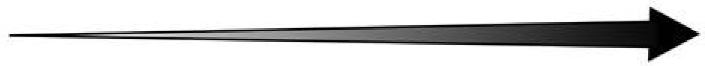

B cell


CD4


B cell


FDC



		+	++	++++
	Virus dose, replication	+	++	++++
A	"natural" or maternal neutralizing antibodies (serum + milk) or vaccination low level TB (infection immunity) act. macrophages + NK cells			
B	MHC-low responder IFN α,β,γ low responder, immune defects immunosupression			

Examples:				
	Polio Virus (P)	diarrhea	transient paralysis	paralysis (death)
	Coxsackie Virus (C)	diarrhea	partial (< 10%, acute) islet cell destruction or few myocytes infected transient autoimmune response	destruction (> 30 - 90%, chronic) by virus and/or immunpathology ^ juvenile diabetes ^ chronic cardiomyopathy

Immunopathology - Autoimmunity

- "Unnoticed" strictly peripheral commensal infections
- Acute cytophathic, early, polio (vaccines)
- Chronic immuno-pathological degen. (late), persistent (years), TB, TU (no vaccines)
- Aetiology of autoimmune disease (Borna, gastr. ulcer, B. burgdorferi)
- True carriers

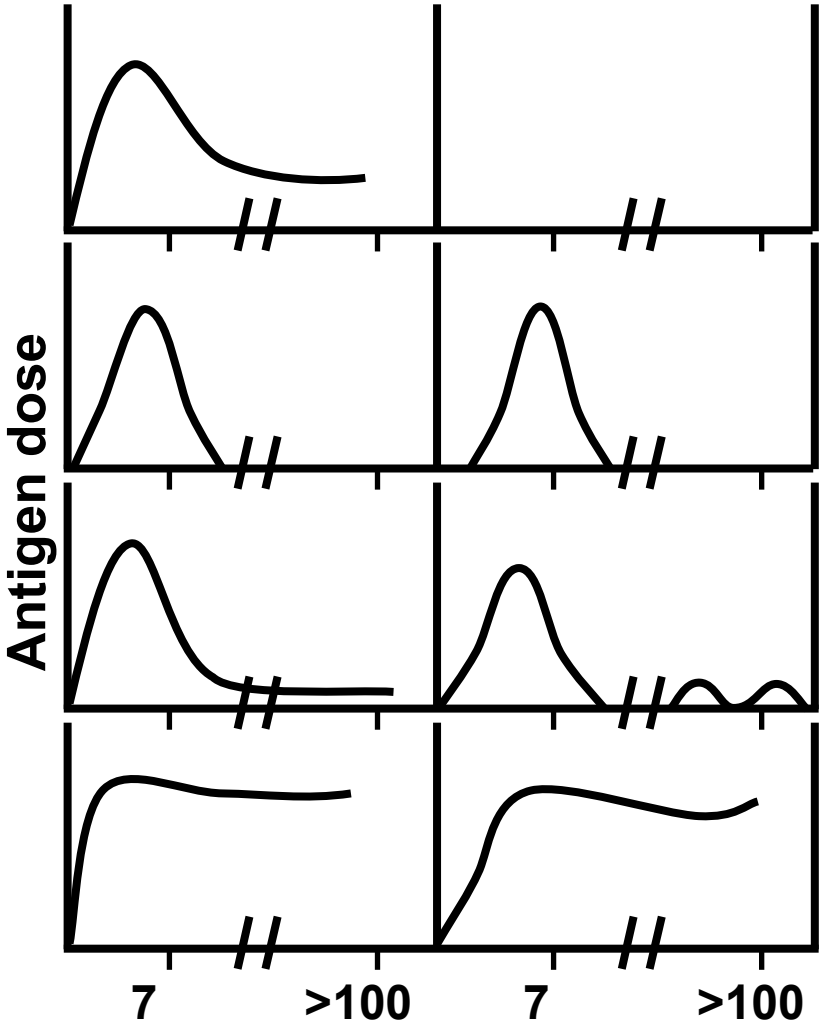
Autoimmunity

- Autoantibodies
- T cell-mediated
- Problems with elimination (Immune complexes, C', etc.)

- chron. infections inflammations
- mimicry
- regulation
- bystander effects

Method of detection

Correlation versus cause

Peripheral solid organs	Peripheral lymphatic organs	Imm. resp.	Trans-plant.	Viruses Bact.
		Ignor.	Corne a	Papill. V perif. self
		Reject .	Skin Heart	Polio V Infl.
		2-way imm. inf.imm.	GVH chron. rej.	TB, HBV, HIV
		Exhaust. deletion	BM chimae-rism	HCV, LDV (HIV, LCMV)

Conclusion

- Co-Evolution explains why no vaccine (yet?) available against HIV, TB, Malaria: Variability, persistence, immunopathology
- Words mislead: Antigen is most important regulator of immune response
- To measure something does not prove biological relevance
- Wrong promises are dangerous also for immunologists
- Protection / reduction of of disease / death are relevant, chance for MD
- To know, learn and act accordingly are most important (incl. prevention, antibiotics, antivirals, vector control, education [measles])

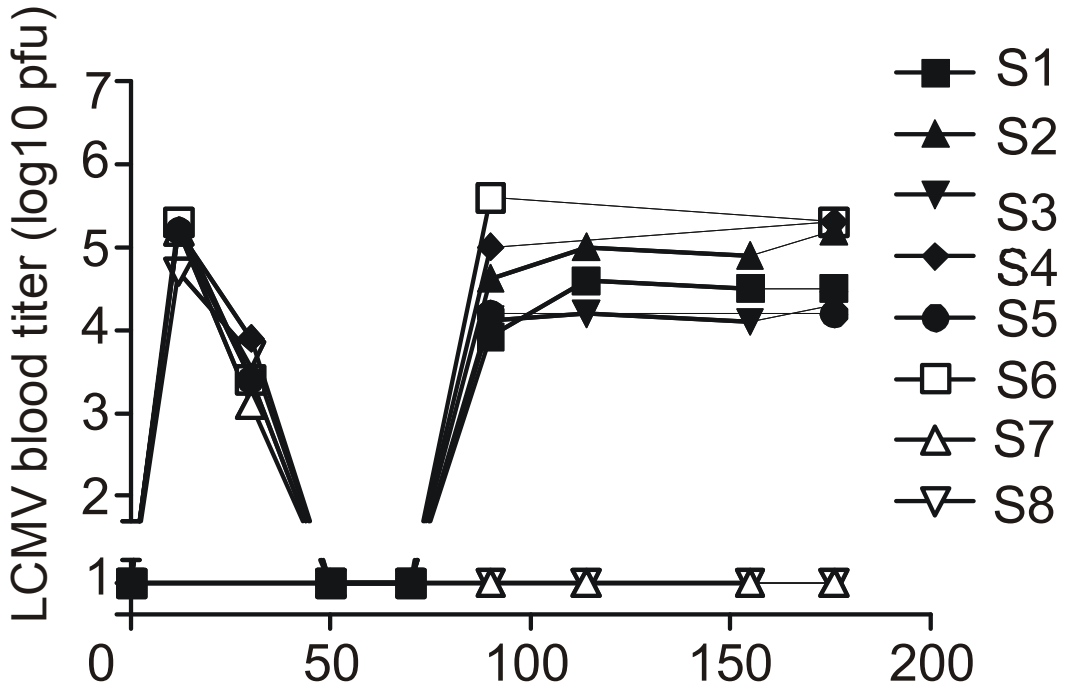
H. Hengartner

A. Althage
M. Bachmann
Th. Kündig
A. Ciurea
U. Karrer
L. Hunziker
M. Recher
D. Pinschewer
C. de la Torre

A. Ochsenbein
B. Ludewig
M. Pericin
HP. Roost
St. Freigang
M. Martinic
Th. Rülicke
B. Odermatt
A. Navarini
K. Lang

Thank you!

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

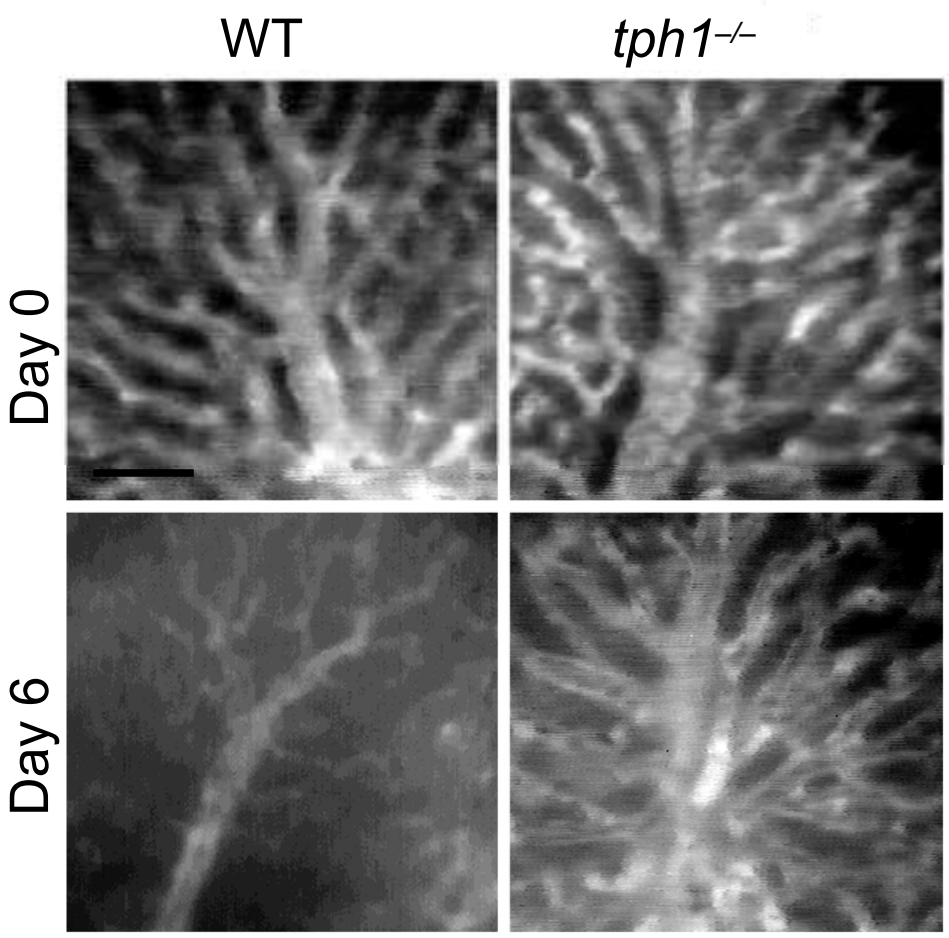


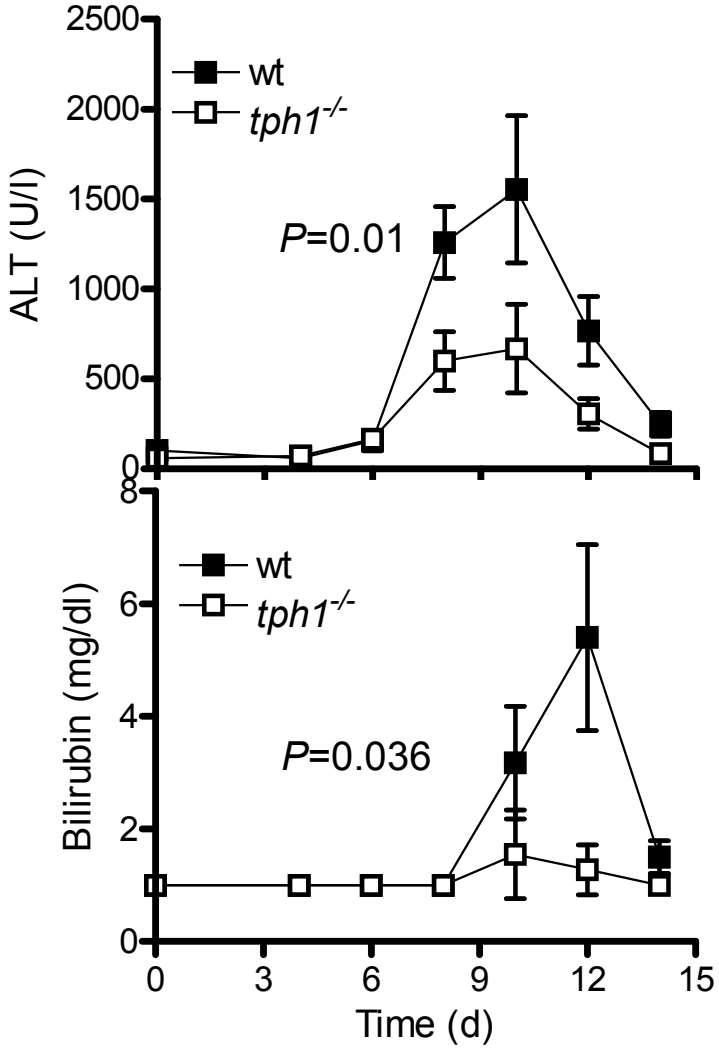
B Ab

- Prevent penetration IgA
- systemic neutr.-opson.IgM/G
- adoptive transferable IgG
- IgM 1-2d, regulated by Ag-dose and structure (no negative selection)

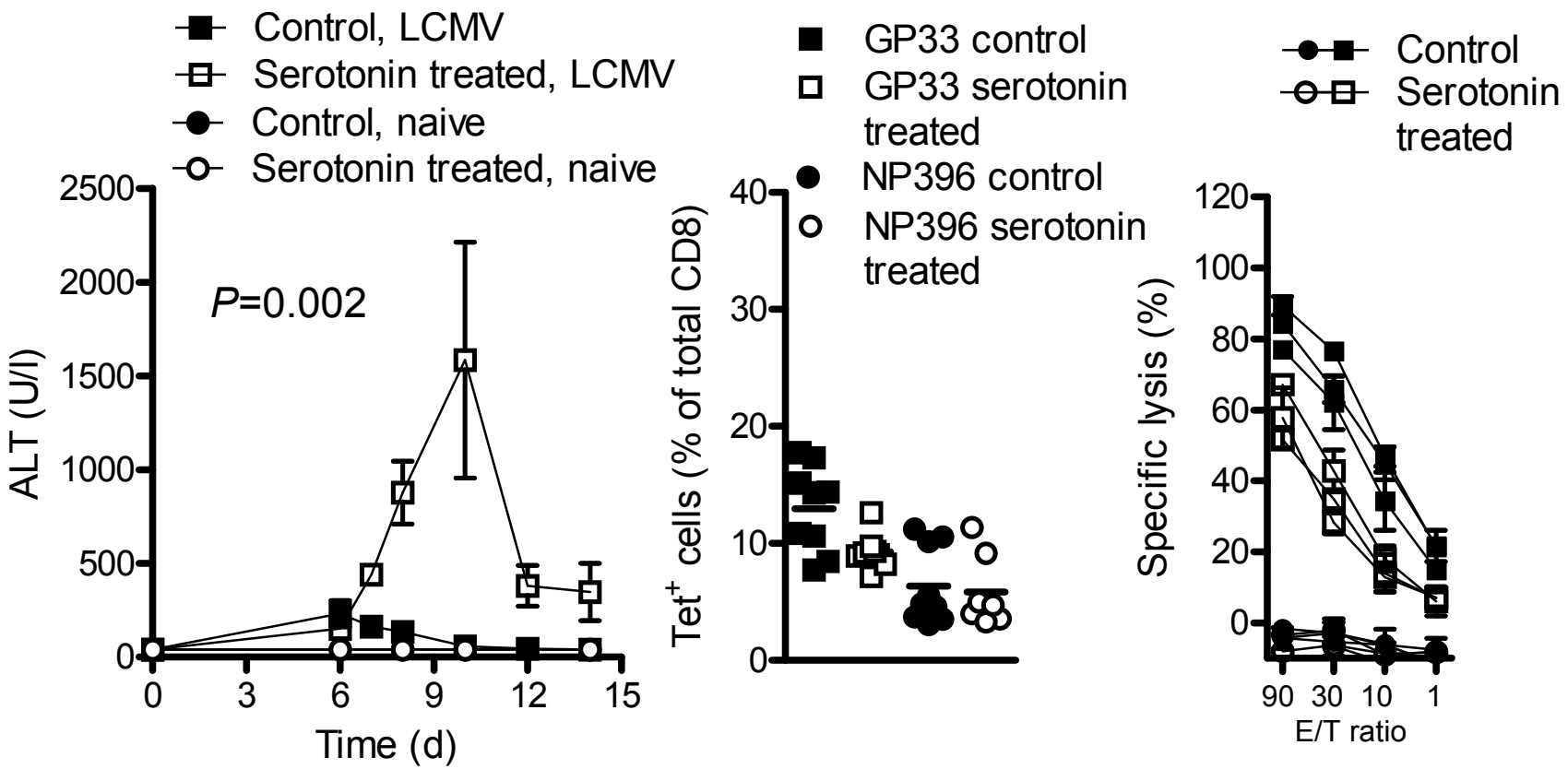
T

- Control-elim. intracell parasites also in solid organs
- regulate longterm IgG
- cause imunopathology (negative selection)
- inertia





Lang et al. Nat Med. 2008 Jul;14(7):756-61.

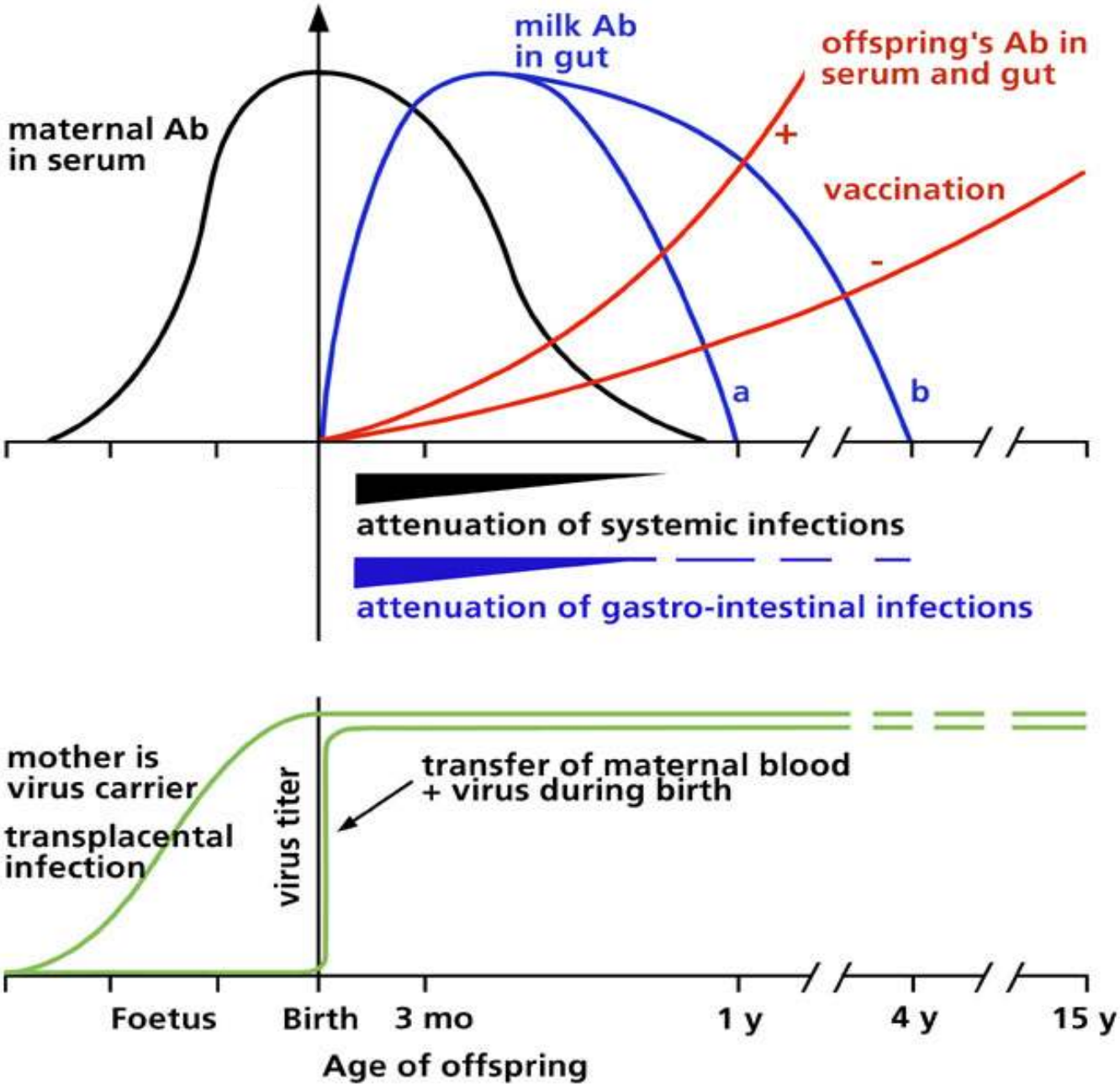

Control, LCMV
Serotonin treated, LCMV
Control, naive
Serotonin treated, naive
2500
2000
1500
1000
500
0
ALT (U/l)
P=0.002
0
3
6
9
12
15
Time (d)
GP33 control
GP33 serotonin treated
NP396 control
NP396 serotonin treated
40
30
20
10
0
Tet+ cells (% of total CD8)
Control
Serotonin treated
120
100
80
60
40
20
0
Specific lysis (%)
90
30
10
1
E/T ratio

Conclusion

- Experimental models representative?
- Correctly measured but do we measure the right parameters?
- Tlr signals may trigger autoimmune disease
- Sometimes 2 hits necessary?

IMMUNITY

- “innate resistance“ > 95 %
- Ab in eggs
- protective memory via Ab (vaccines)
- TB: no vaccine
- autoimmunity > 30 y, female > male
  5 : 1
- tumors > 30 years
- academic memory: earlier + greater


milk Ab
in gut
offspring's Ab in
serum and gut
maternal Ab
in serum
+
vaccination
-
a
b
attenuation of systemic infections
attenuation of gastro-intestinal infections
mother is
virus carrier
transplacental
infection
virus titer
transfer of maternal blood
+ virus during birth
Foetus
Birth
3 mo
1 y
4 y
15 y
Age of offspring

Antigens drive protective antibody titers

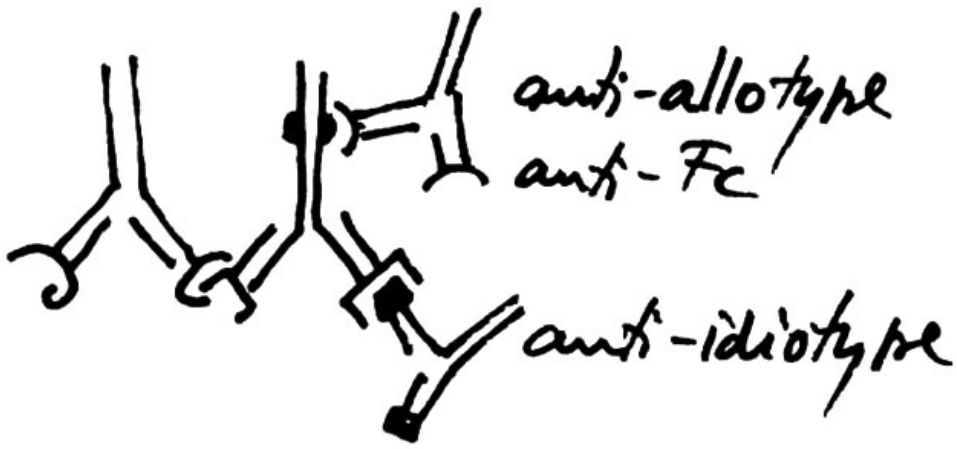
- protection by nAb
- increase of memory B frequency antigen-independent
- maintenance of nAb-titers antigen-dependent
- re-exposure

  1) from within (HIV, HBV, measles)
  2) Ag-Ab-complexes
  3) from outside (Polio) mother $\rightarrow$ offspring

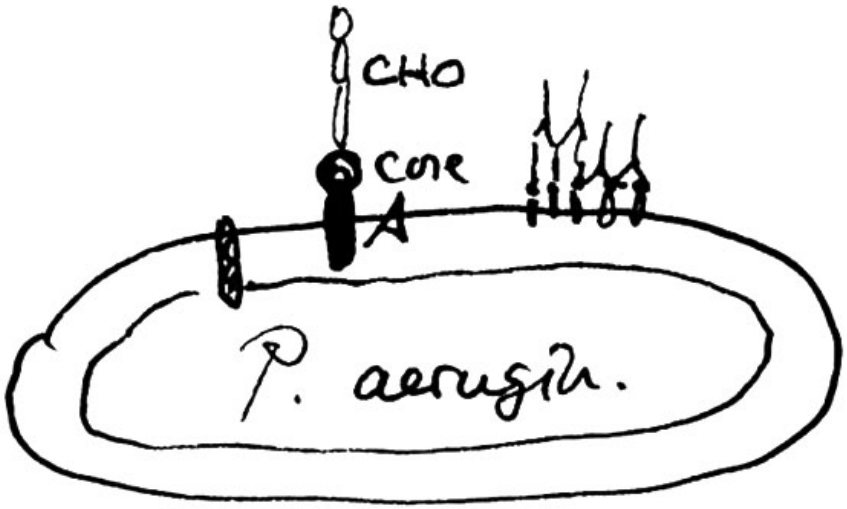
     nAb-transfer for early protection

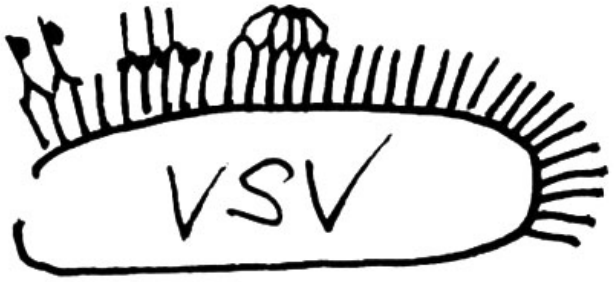
Conclusion

- Maternal nAb attenuate inf. in offspr.: physiol. vaccination
- Shift balance of pers. + chron. infections (Herpes, Coxsackie etc.)
- Vaccinations against autoimmune disease?


anti-allotype
anti-Fc
anti-idiotype

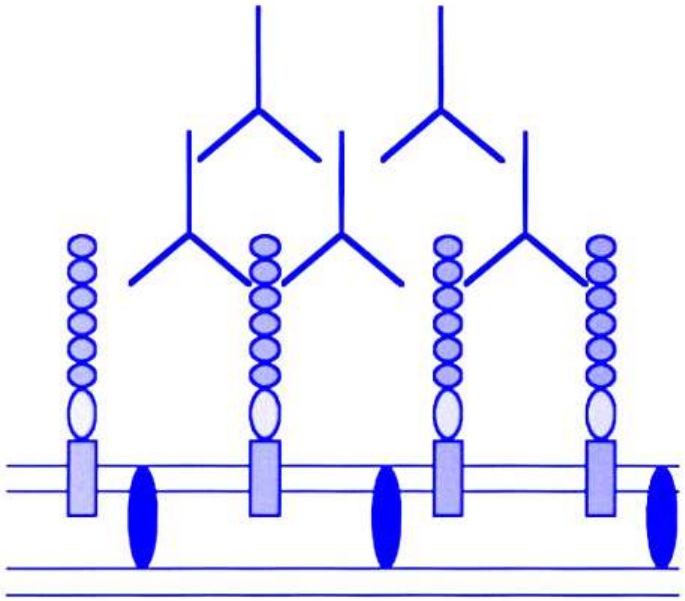

S. typhi

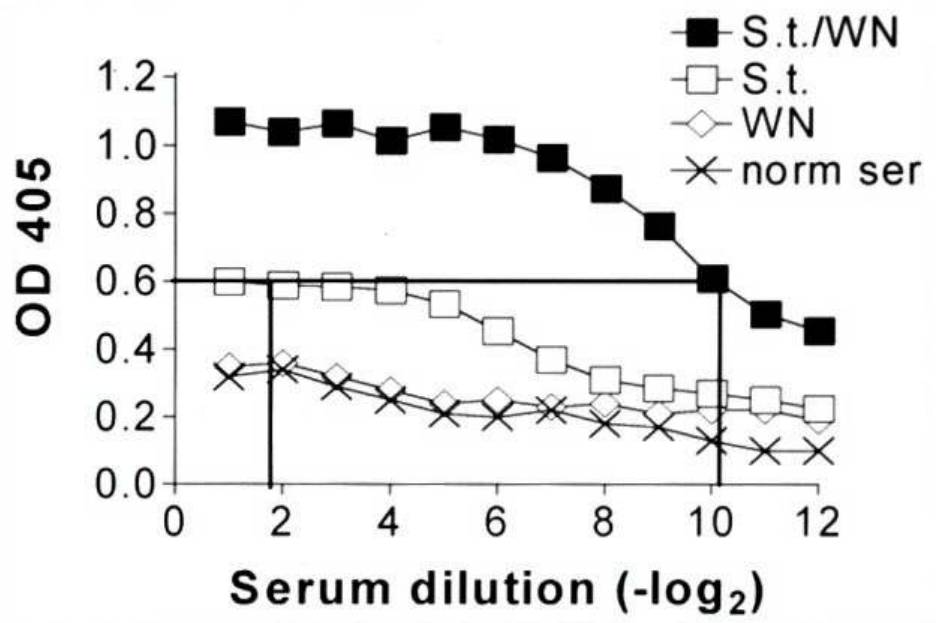

CHO
core
A
P. aerugin.


VSV

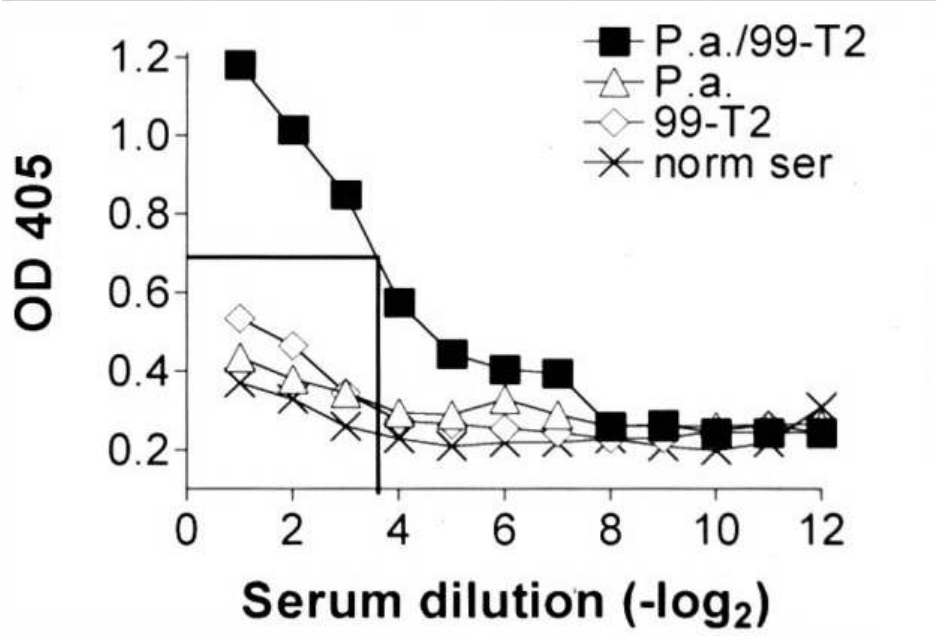
Anti-antibodies/ gramnegative bacteria

Salmonella typhi

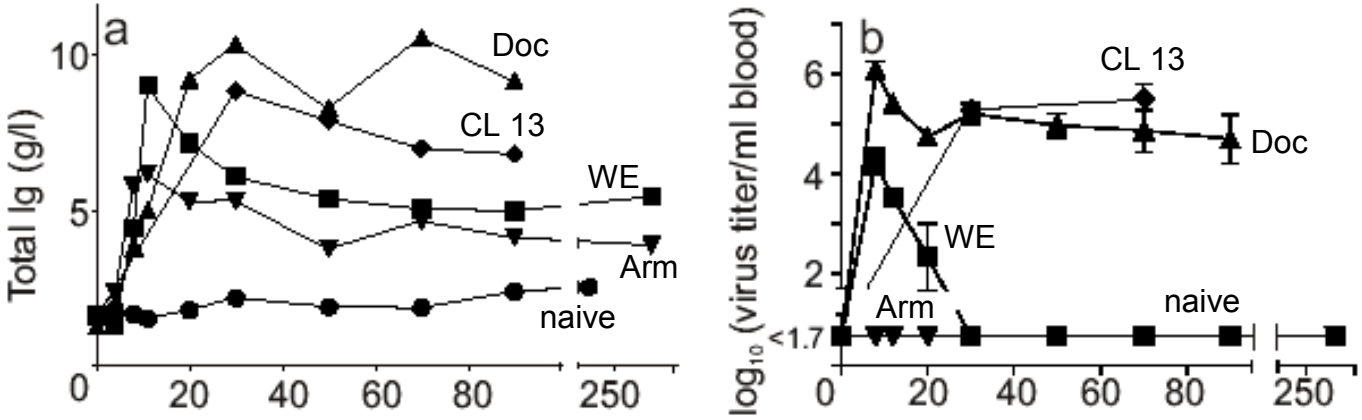




Pseudomonas aeruginosa



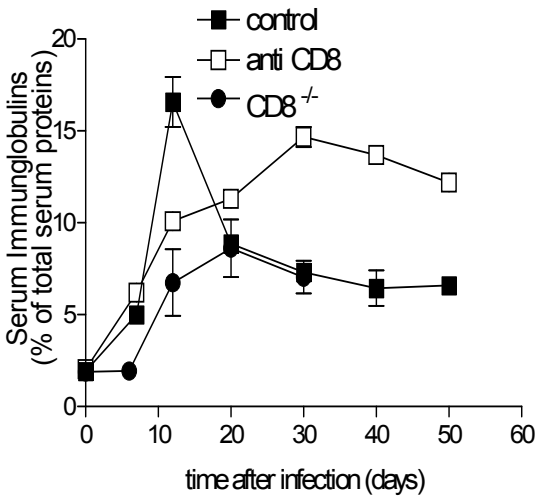
Hypergammaglobulinemia **Virus titer**



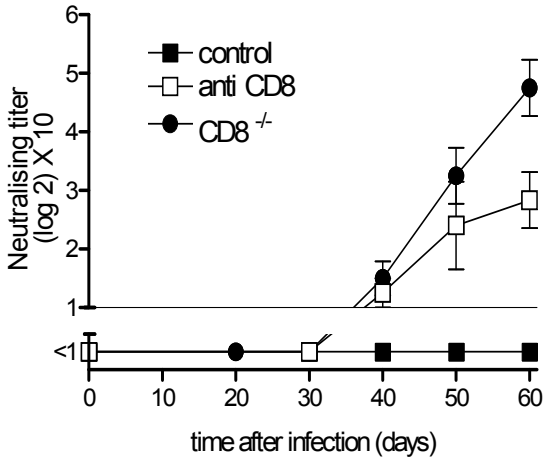
Mouse strain (2^6pfu WE)	Day 0	Day 20
BALB/c	1.5 ± 0.8	7.7 ± 1.3
129/Sv	0.7 ± 0.2	12.3 ± 1.8
DBA/2	1.7 ± 0.7	11.6 ± 2.1
C3H HeJ	0.9 ± 0.2	6.9 ± 1.2
C3H EB/FEJ	1.3 ± 0.5	6.1 ± 1.2
C57BL/6 germ-free	1.6 ± 0.4	7.9 ± 1.4
IFN-α/β receptor$^{-/-}$	1.2 ± 0.3	13.0 ± 2.1
IFN-γ receptor$^{-/-}$	1.1 ± 0.3	10.2 ± 1.9
IL-4$^{-/-}$	1.2 ± 0.2	8.7 ± 1.6
IL-12$^{-/-}$	1.3 ± 0.5	15.3 ± 2.4
C3$^{-/-}$	1.3 ± 0.5	11.5 ± 2.2
CR2$^{-/-}$	1.2 ± 0.2	13.6 ± 2.0
IgM$^{-/-}$	1.4 ± 0.7	8.0 ± 1.8
CD19$^{-/-}$	1.5 ± 0.3	7.8 ± 1.3
FcγRI-III$^{-/-}$	3.8 ± 1.9	17.9 ± 3.2

Hypergammaglobulinemia vs. neutralizing antibodies

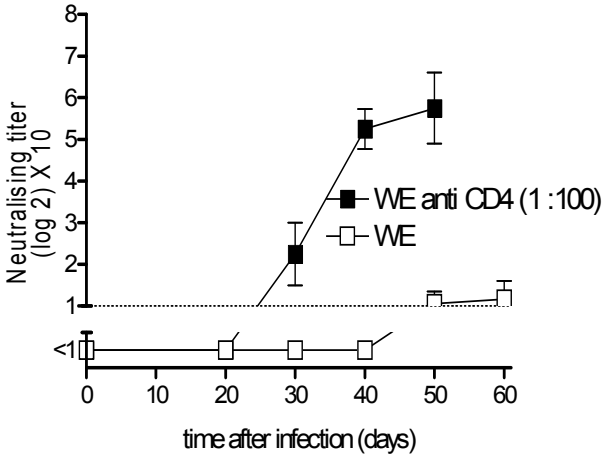
Hypergammaglobulinemia



Neutralising antibodies

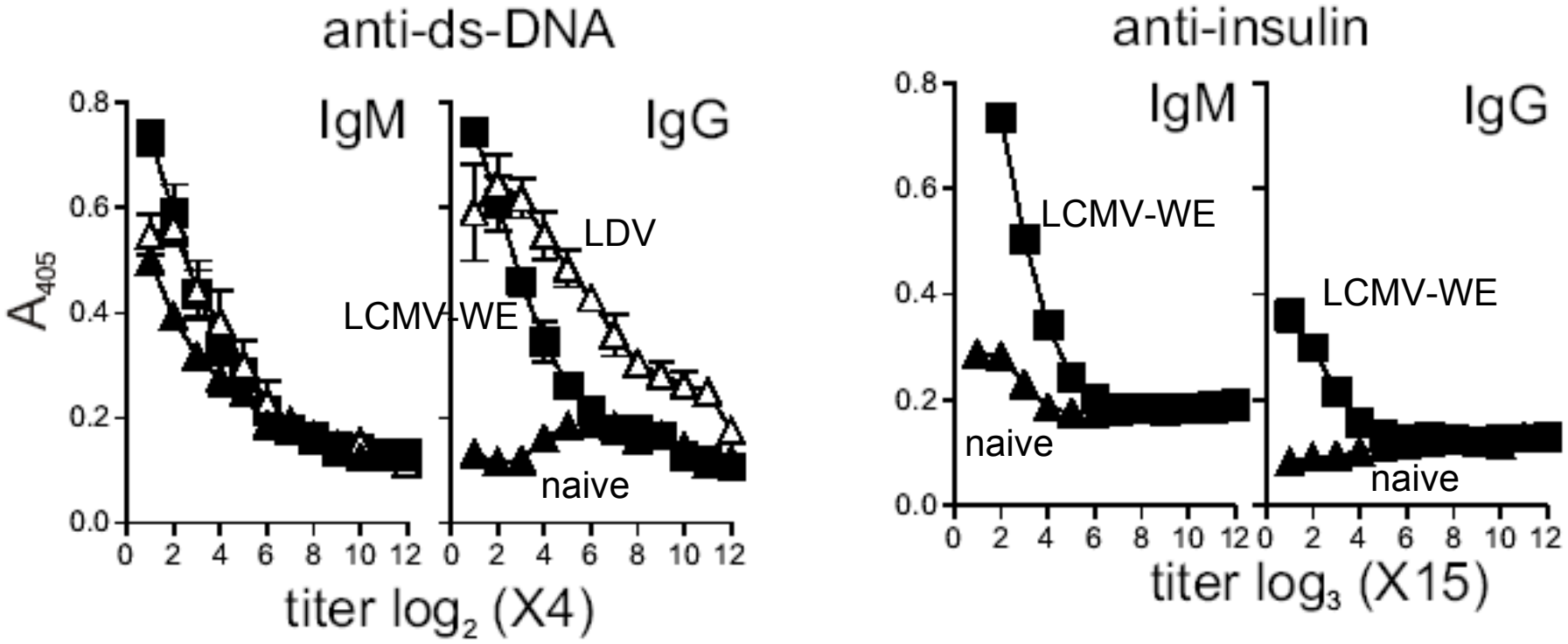


Neutralising antibodies



Recher, Lang, Hunziker 2004

Autoantibodies during LCMV hypergammaglobulinemia

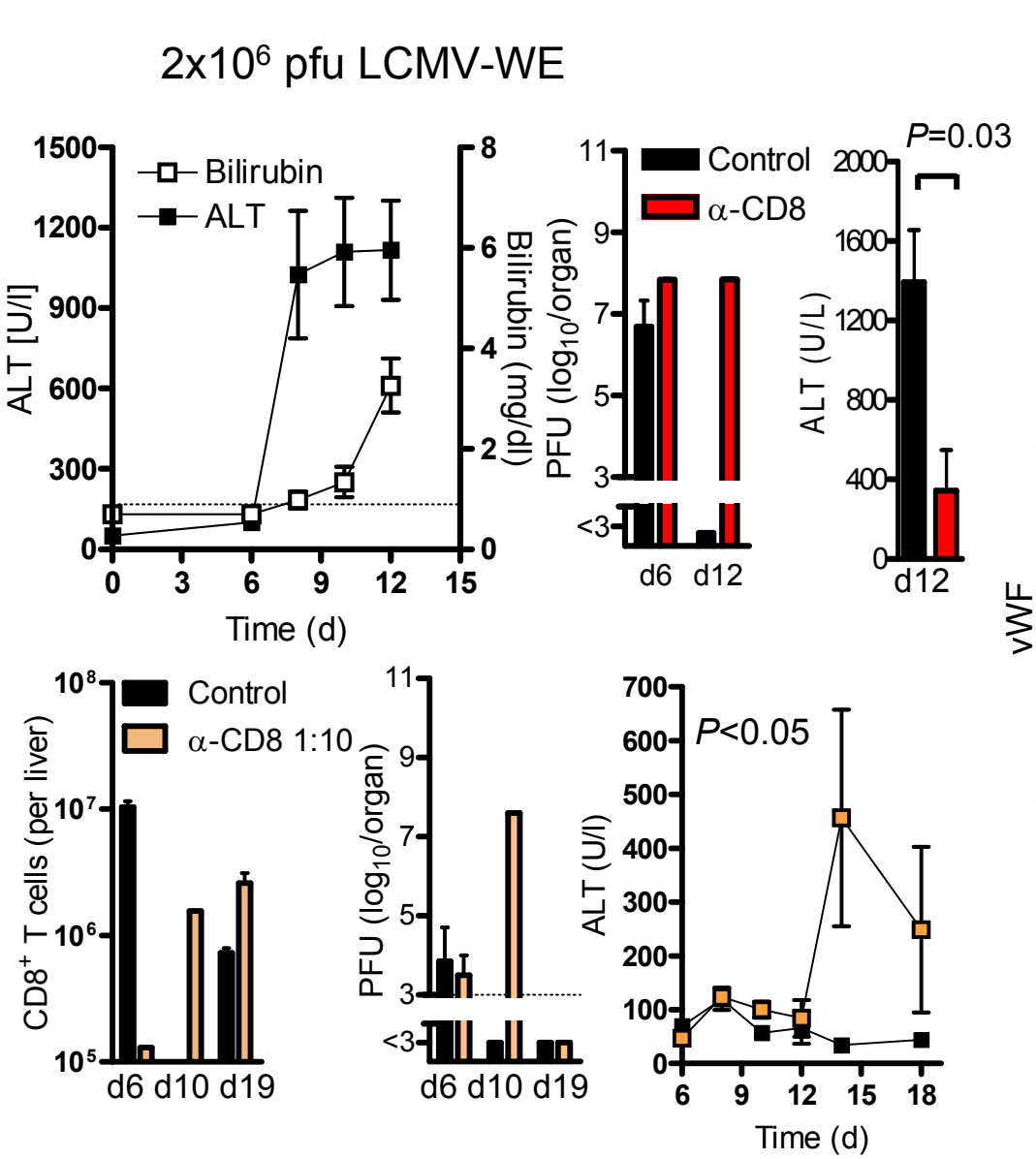


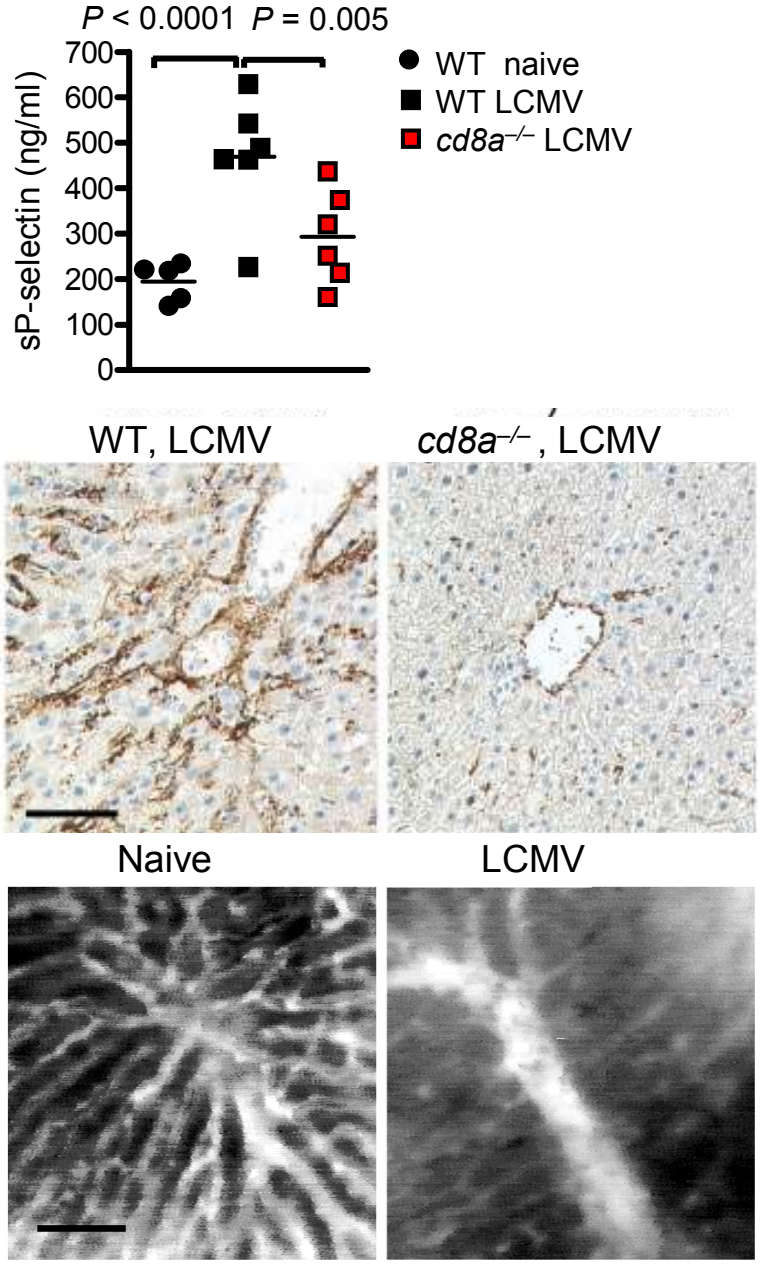
Infections ?
in Auto-Immunopathology

• Rheumat:	Yersinia	
	Salmonella	?
• MS ?		
• Psych./Neur. Dis.:	Borna V	
	Campylobacter	?
• Cardiomyopathies:	Coxsackie	
• Atherosclerosis:	Mycobact.	?
	Chlamydia	
	Herpes	?

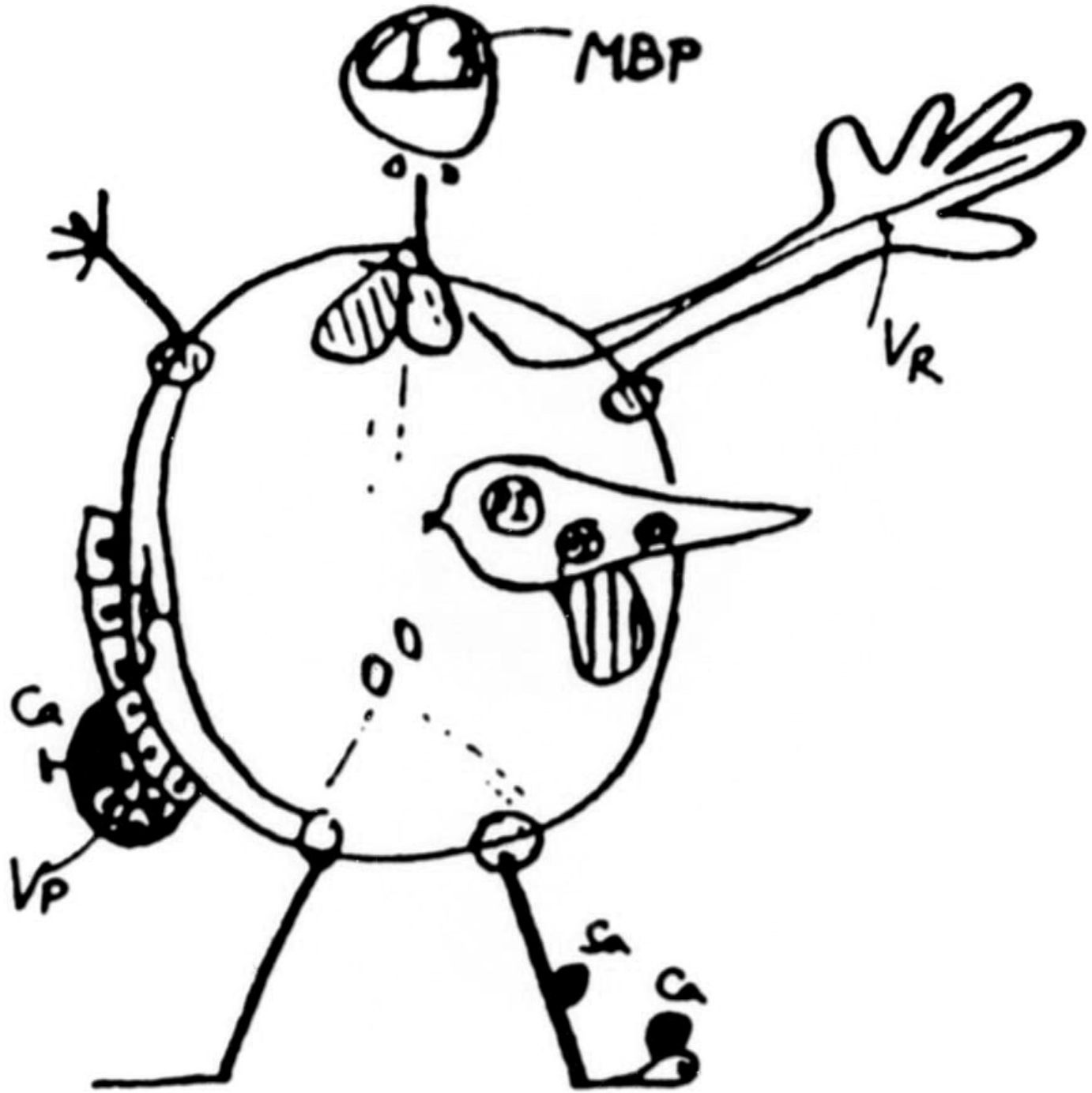
Inflammation, Immunity and Immunopathology

- Immune system
- No T cell tolerance against many self-AG ?
- Chronic infections
  Aetiology of autoimmune disease ?
- No B cell tolerance against many self-AG ?
- Role of maternal antibodies and of chronic infections ?
- What to do ?





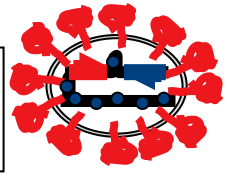
Lang et al. Nat Med. 2008 Jul;14(7):756-61.

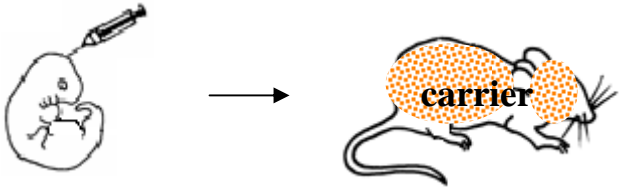

MBP
VR
VP

„Dual viral hit“ model for organ-specific immune disease

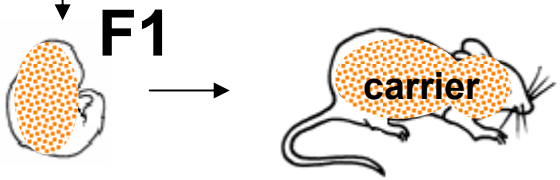
D. Merkler , J.C. de la Torre, D.D. Pinschewer
J. Clin. Invest. doi10.1172/JCI27372

Wild type LCMV strains (ARM / WE)



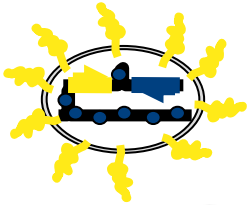


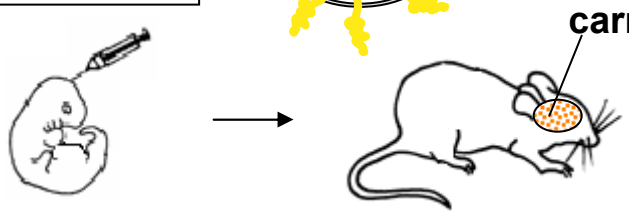
Systemic carrier status
Thymic LCMV infection during ontogeny
-> central antiviral T cell tolerance



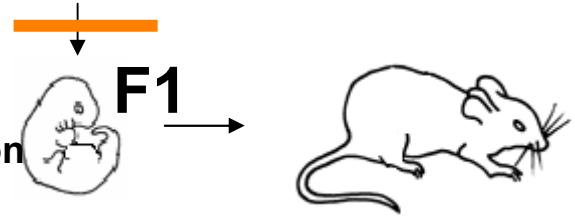
Vertical transmission: natural habitat of LCMV in mouse carrier colonies

rLCMV/INDG





CNS (neuron) restricted carrier status
NO Thymic LCMV infection during ontogeny
-> NO antiviral T cell tolerance but INDIFFERENCE

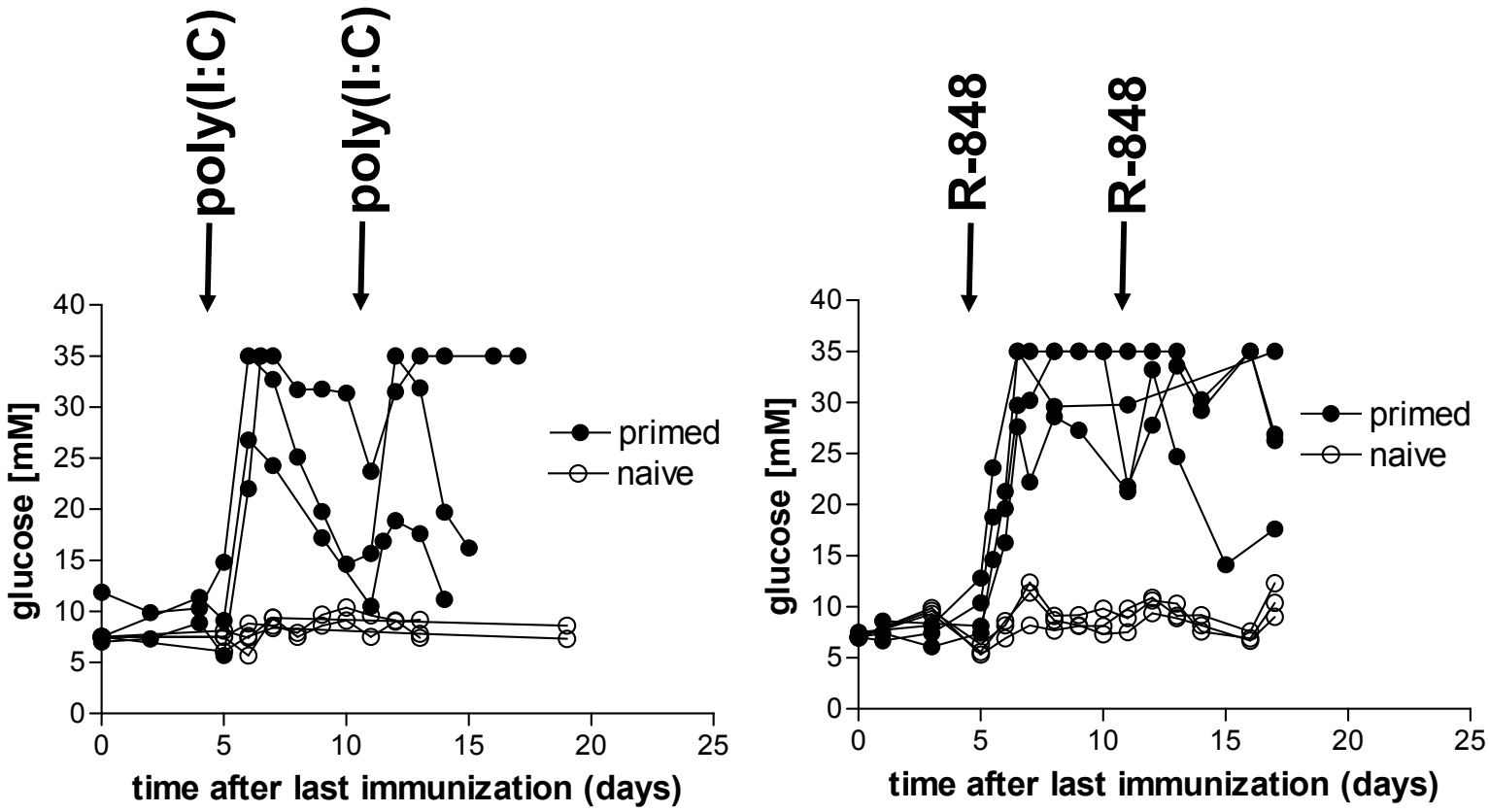


Poliomyelitis – age distribution in Massachusetts 1912 – 1952

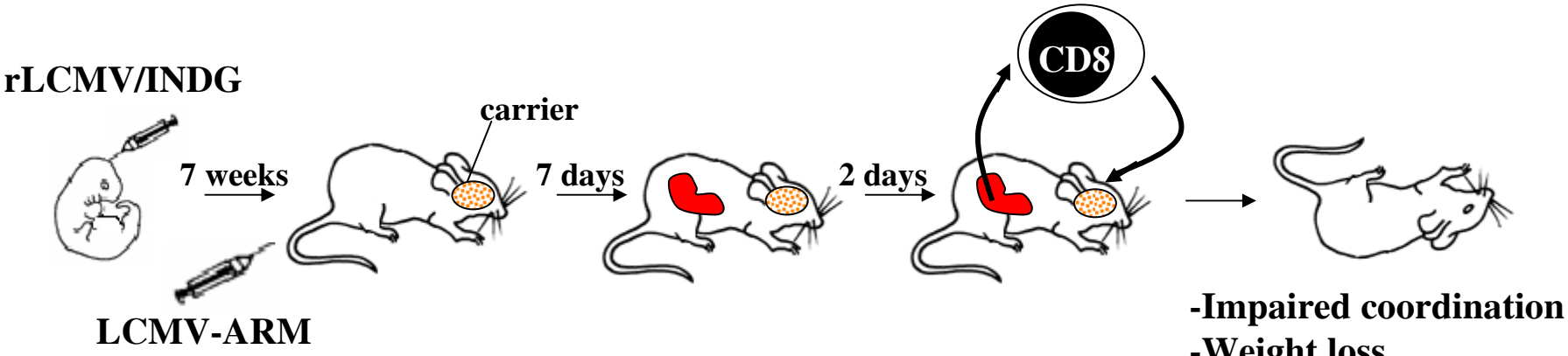
Years	Percent 0 – 4 years	Percent 5 – 9 years	Percent 10+ years
1912-1916	70	18	12
1930-1934	28	38	34
1948-1952	18	27	55

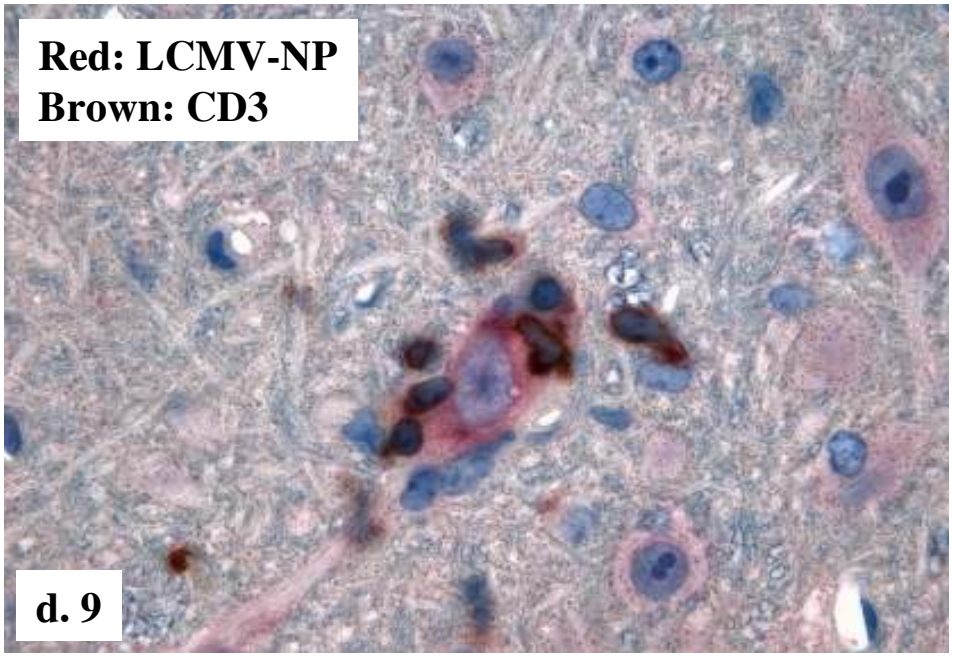
Modified from: Nathanson,N. Am J Epidemiol 1979; 110:672-692.

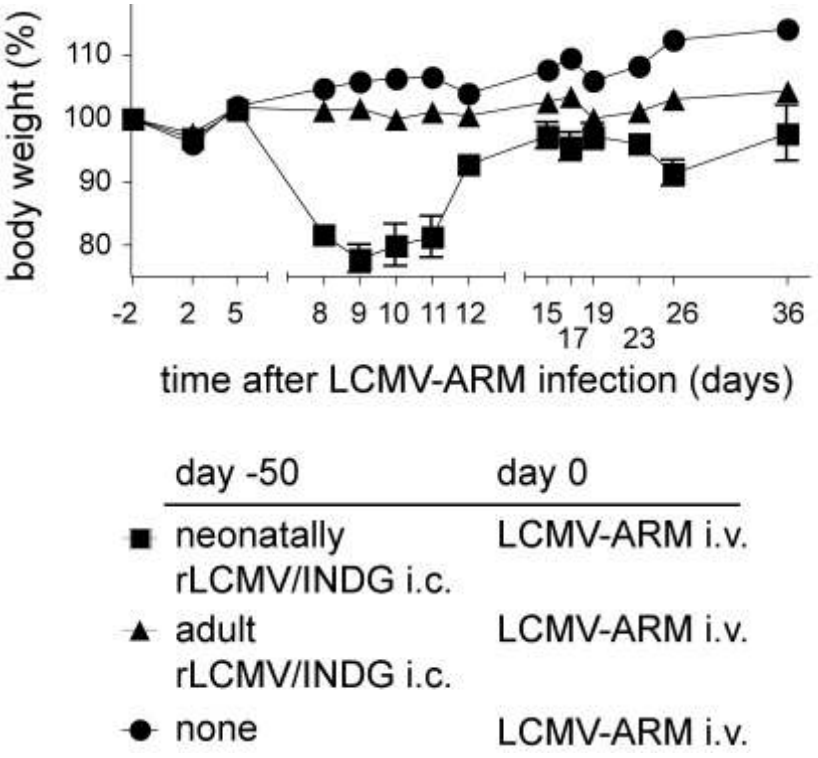
ligands for TLR3 (poly(I:C)) and TLR7 (R-848) can induce diabetes in gp33-primed mice

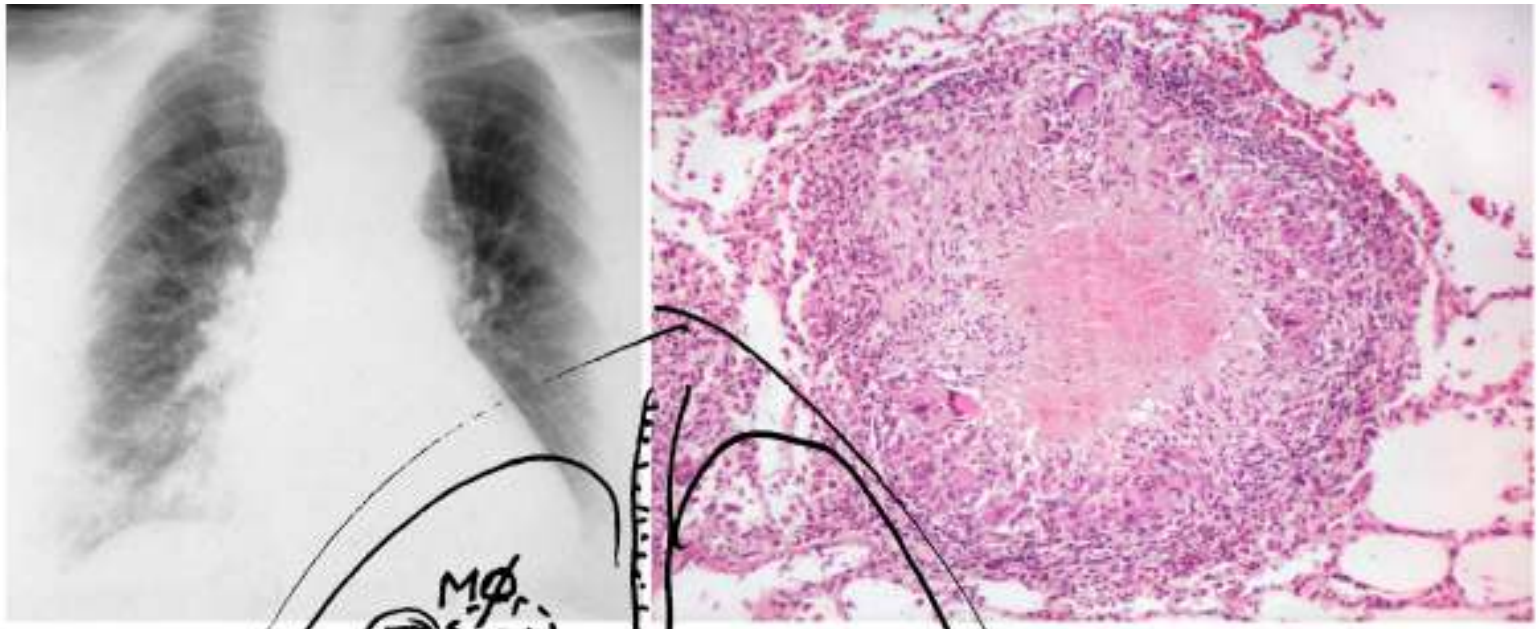


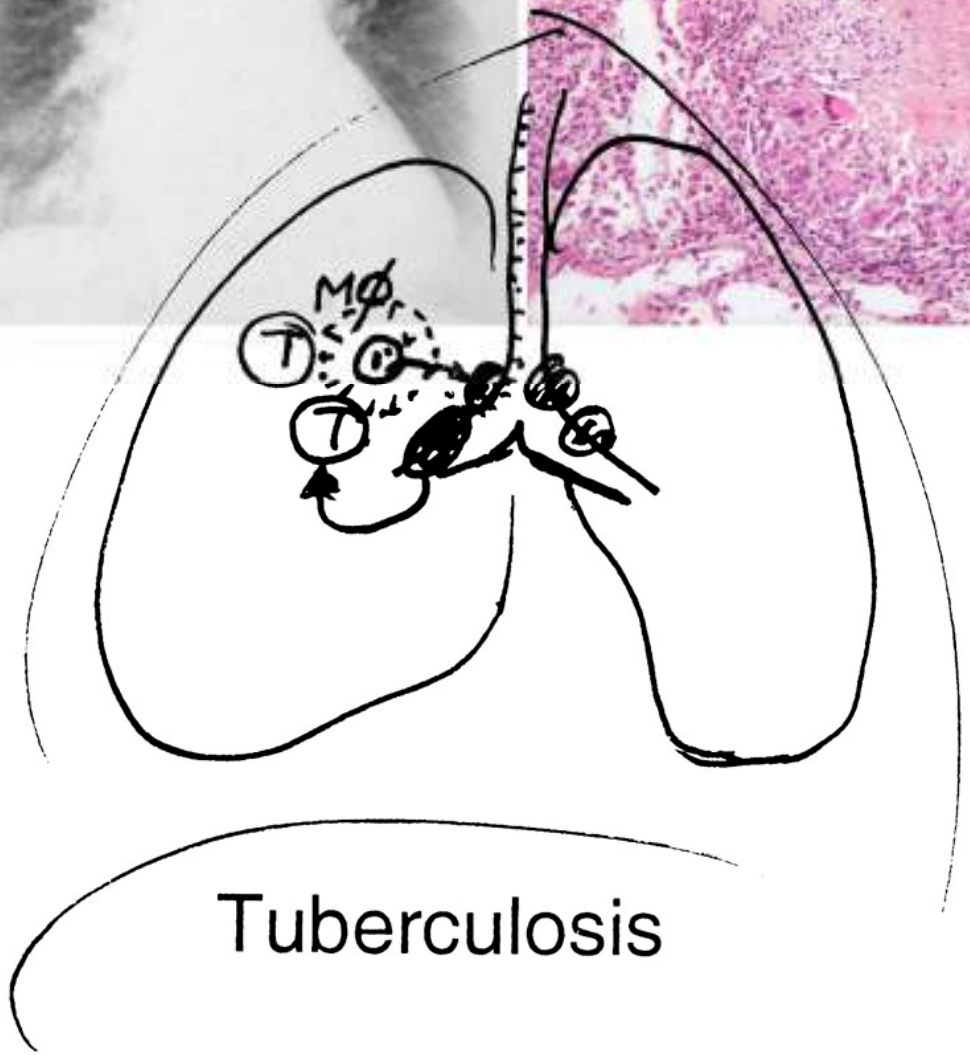
„Dual viral hit“ model of organ-specific immune disease











Tuberculosis

Koch’s postulates

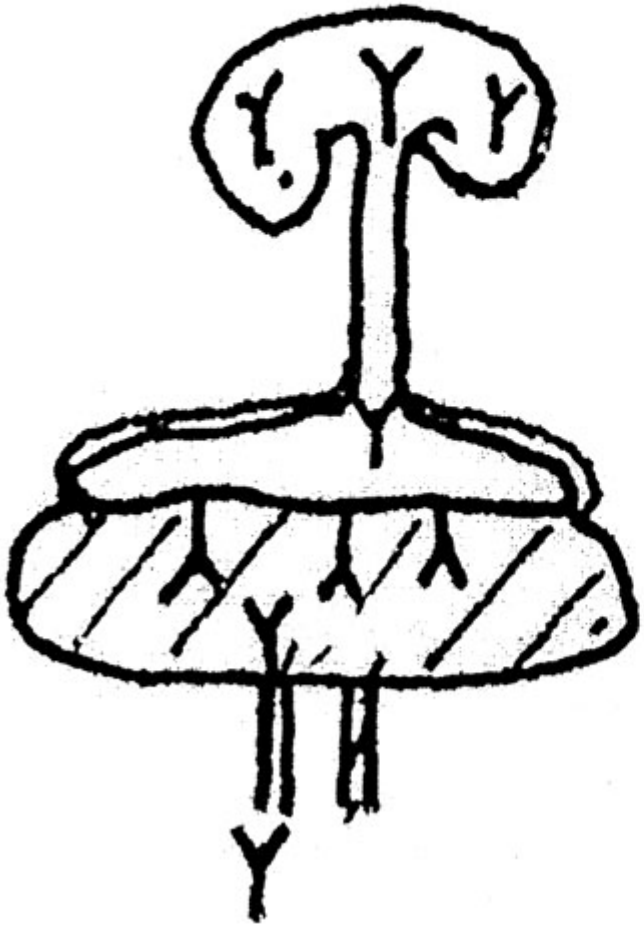
cytopath. org

1. in lesion
2. culturable
3. reproduces dis.
4. reisolated

noncytopath.

5. variable immunopath.
6. variable tumors

Humans



calves