

CROSS-REACTIVITY , HETEROLOGOUS IMMUNITY AND EPSTEIN BARR VIRUS INFECTION

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THE MANY FACES OF T CELL CROSS-REACTIVITY

- 1. T CELL CROSS-REACTIVITY IS COMMON BETWEEN UNRELATED PATHOGENS AND ALTERS T CELL IMMUNODOMINANCE IN SEQUENTIAL OR SIMULTANEOUS INFECTIONS (Laurie Kenney)
- 2. T CELL CROSS-REACTIVITY CAN ALTER THE EFFICIENCY OF THE EFFECTOR RESPONSE AND THUS INFLUENCE PROTECTIVE IMMUNITY, IMMUNOPATHOLOGY, RESULTING IN EITHER BENEFICIAL OR DYSREGULATED HETEROLOGOUS IMMUNITY
- 3. T CELL CROSS-REACTIVITY CAN LEAD TO NARROWING OF THE T CELL REPERTOIRE, GIVING RISE TO VIRAL ESCAPE MUTANTS
- 4. CROSS-REACTIVE TCR PRIVATE SPECIFICITY DETERMINES INDIVIDUAL'S DISEASE OUTCOME RESULTING IN REPERTOIRE NARROWING, PROTECTIVE IMMUNITY, OR IMMUNOPATHOLOGY
- 5. THE SIZE OF THE T CELL CROSSREACTIVE RESPONSE CAN DIRECTLY CORRELATE WITH THE SEVERITY OF IMMUNO-PATHOLOGY AND BE TOLERIZED, OR BLOCKED WITH ANTI-IFNg (Myriam Wlodarczyk)
- 6. MUTATION OF VIRAL PATHOGENIC CROSSREACTIVE EPITOPES PREVENTS IMMUNOPATHOLOGY (Myriam)
- 7. T CELL CROSSREACTIVITY INCREASES WITH AGE

Therapeutic intervention in preventing heterologous immune mediated immunopathology

PB1 and PA224 Flu-memory CD8 responses which induce lung pathology by cross-reactivity with LCMV peptides GP34 and GP276 could be tolerized.

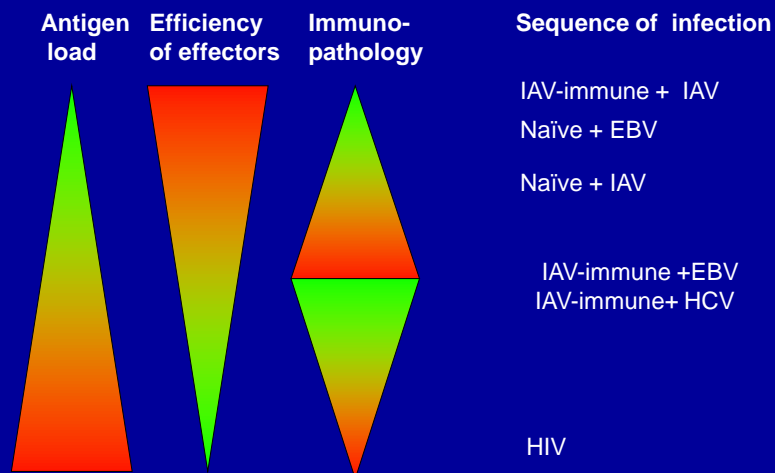
The severity of the lung pathology could be predicted as it is dependent on the burst size of the cross-reactive FLU-specific memory cells before LCMV infection

Blocking IFN γ could prevent lung pathology during LCMV infection in Flu-immune mice.

Immunization with Flu virus with mutations in pathogenic epitopes prevented immunopathology upon LCMV challenge

Myriam Wlodarczyk

Disease outcome during a viral infection is dependent on a balance between antigen load and efficiency of effector function

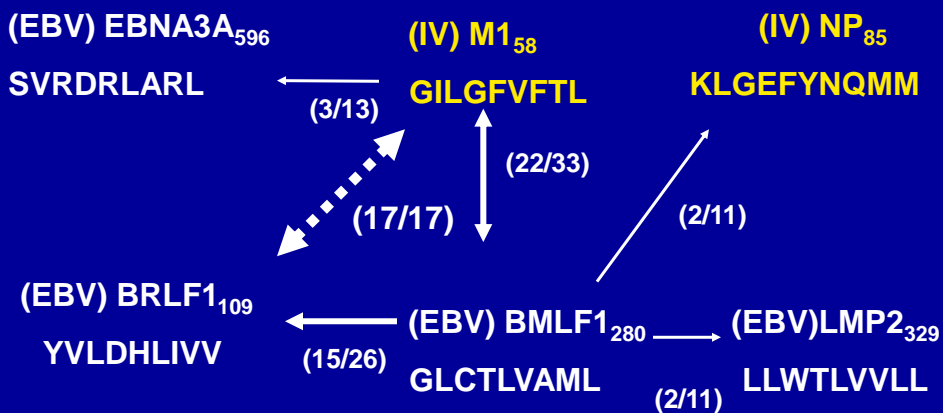


Heterologous immunity and cross-reactive CD8+ T-cells can participate in:

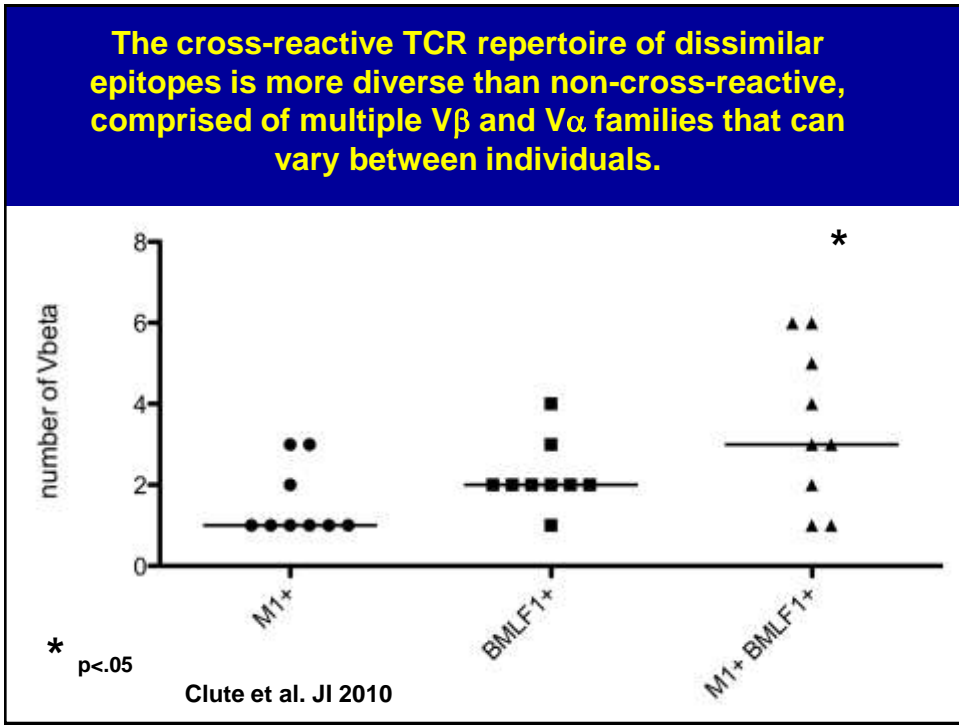
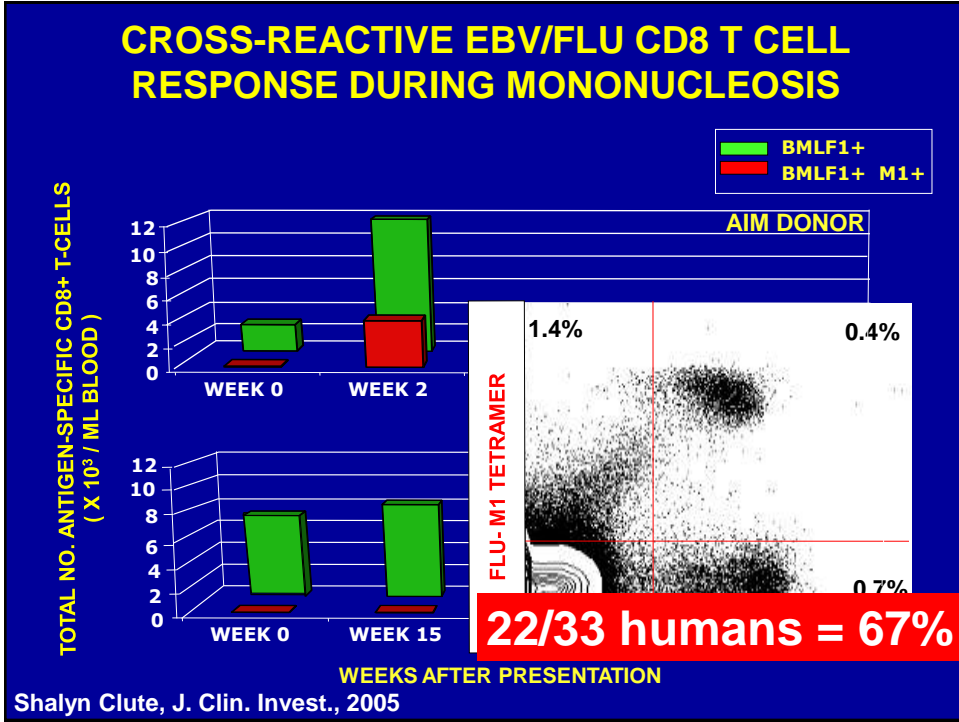
- 1) Mediating immuno-pathology
i.e. infectious mononucleosis
- 2) Mediating protective immunity

MATRIX OF CD8 T CELL CROSS-REACTIVITY

BETWEEN INFLUENZA (IV) AND EBV EPITOPES



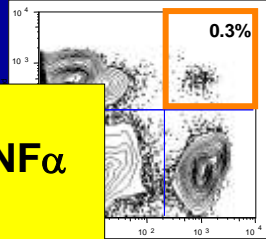
Cornberg, Clute et al. JI 2010



The **M1+ BMLF1+** subset of a T cell line stimulated with both peptides simultaneously responds to both M1 and BMLF1

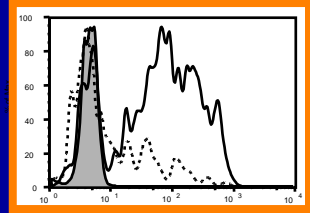
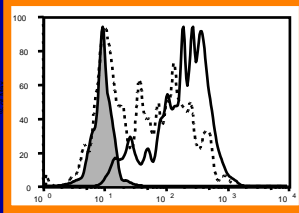
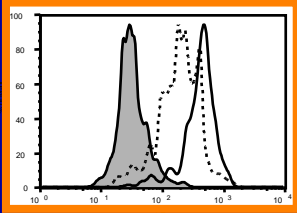
Stimulation
 M1
 — BMLF1
 ■ tyrosinase

MIP-1 β > IFN γ > TNF α



M1+BMLF1+ SUBSET:

BMLF1 tetramer →



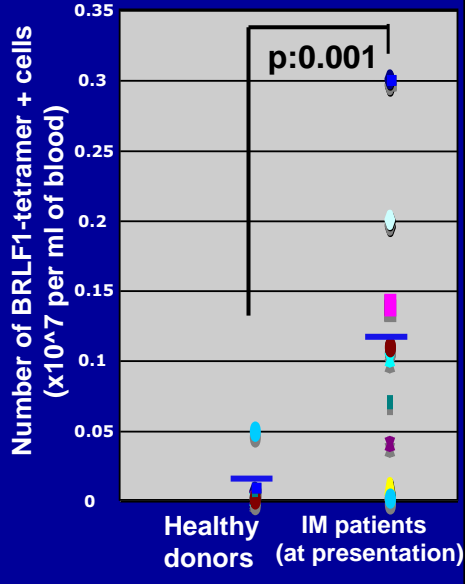
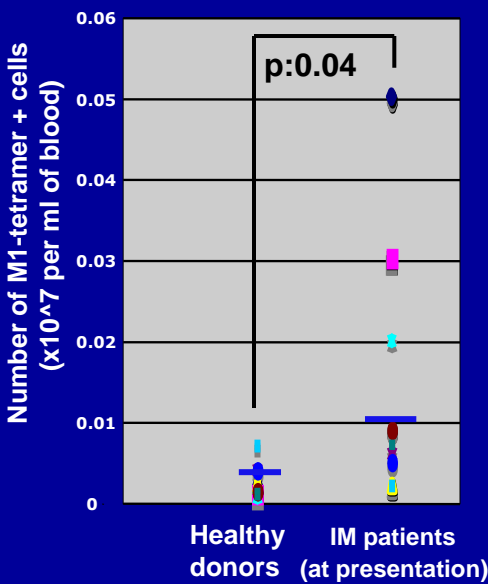
MIP-1 β →

IFN γ →

TNF α →

Shalyn Clute, J. Clin. Invest., 2005

There is an expansion of Flu-M1-specific memory cells as well as the expected EBV-specific T cells in the blood of IM patients



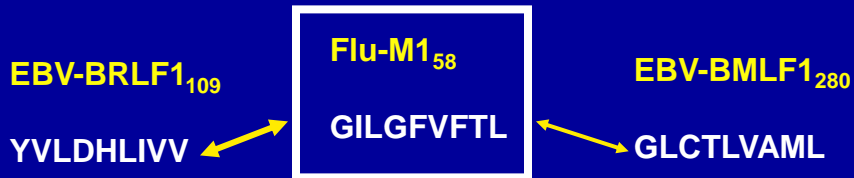
Novel crossreactive epitope

EBV-BRLF1₁₀₉: YVLDHLIVV

- Lytic, immunodominant
- Primary Phase
 - 80-100% of HLA-A2+ people
 - 0.5 to 34% of CD8 T cells at presentation with IM
- Memory Phase
 - 80-100% of HLA-A2+ people
 - 0.02 to 0.7 % of CD8 T cells

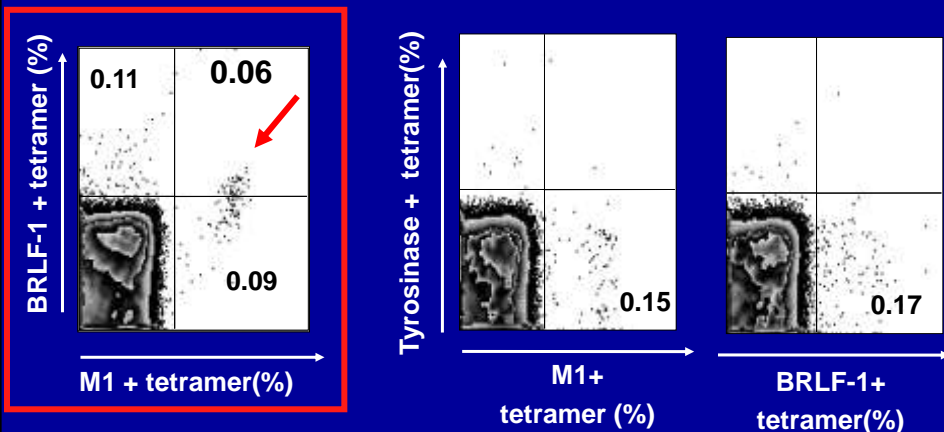
Flu-M1₅₈: GILGFVFTL

- Immunodominant
- Memory Phase
 - 75-100% of HLA-A2+ people
 - 0.01 to 0.3% of CD8 T cells



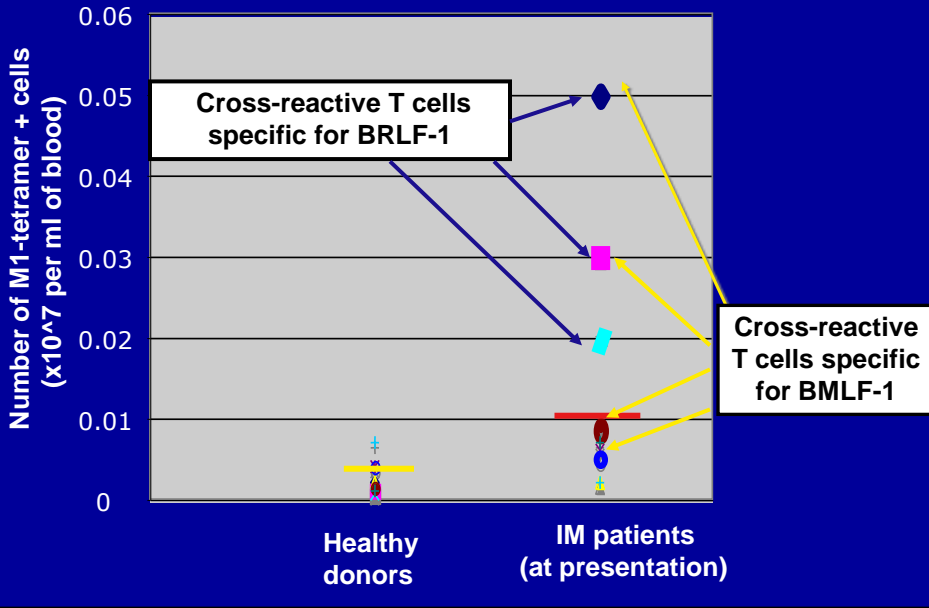
Cross-reactivity between Flu-M1 and EBV-BRLF-1 specific memory T cells is a novel and common event in healthy memory donors (5/5)

direct ex vivo tetramer staining



Nuray Aslan

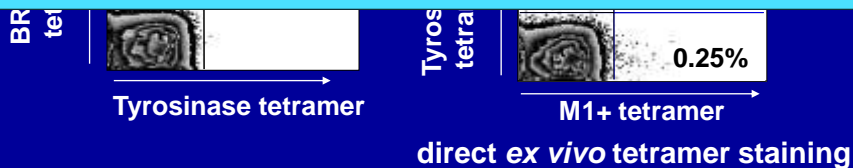
Expanded Flu-M1-specific memory T cells can be crossreactive with either EBV-BRLF-1 or EBV-BMLF-1



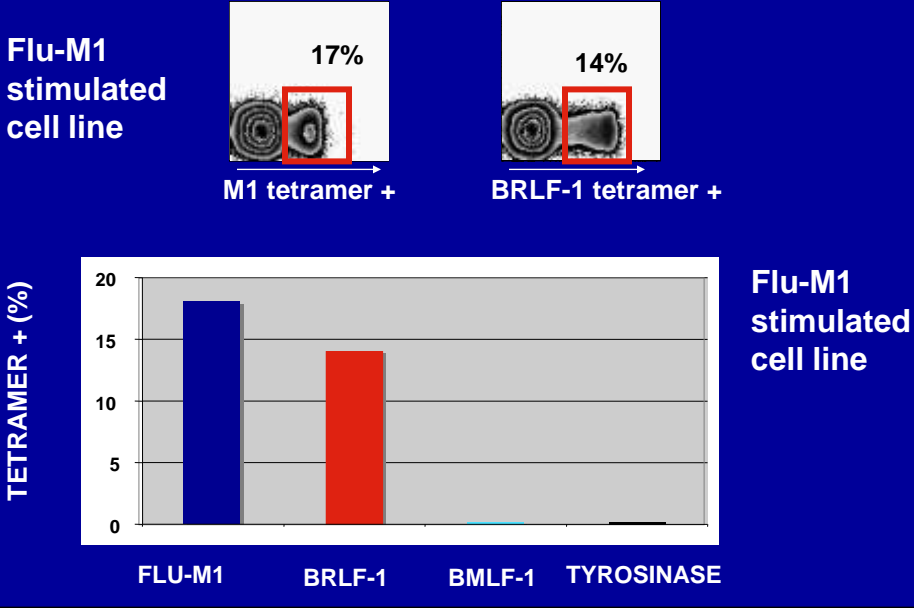
Cross-reactive T cell responses between Flu-M1 and EBV-BRLF-1 epitopes in IM patient (day 14)



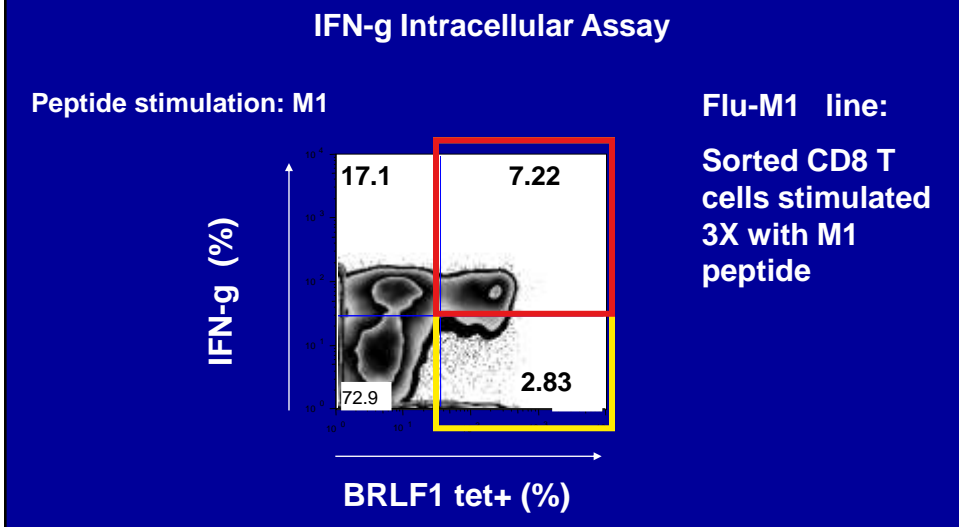
Cross-reactive T cell responses between Flu-M1 and EBV-BRLF-1 epitopes have been detected in 17/17 IM patients



EBV-BRLF-1+ cells from an IM patient proliferate in response to Flu-M1 peptide



EBV-BRLF-1+ and Flu-M1+T cells are functionally crossreactive in IM patients: BRLF-1 specific cells proliferate and produce IFN-g to M1 peptide stimulation in Flu-M1 line

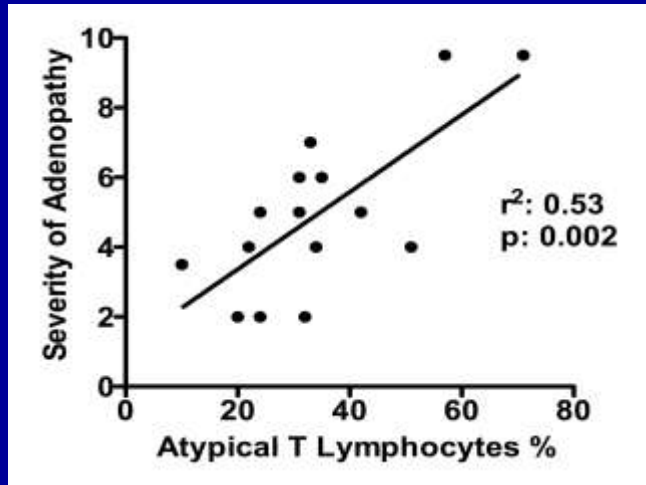


Does this expansion of the crossreactive Flu-M1-specific memory cells or the EBV-antigen specific T cells correlate with IM severity?

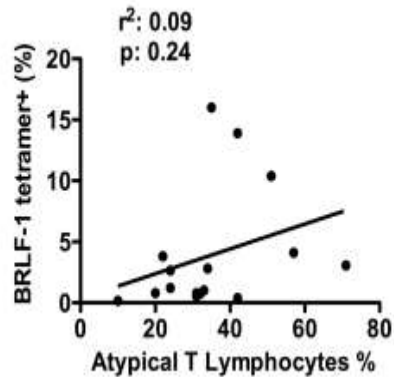
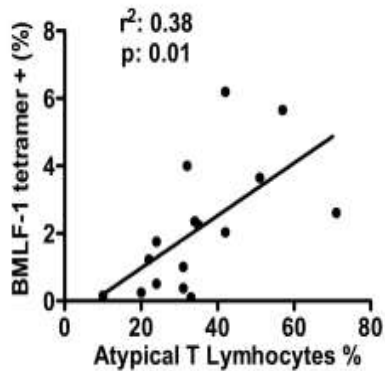
1-Severity of IM correlates with increased CD8 expansion as demonstrated by decreased CD4/CD8 ratio, and clinically by increased lymphadenopathy

2- Both of these pathognomic features of IM directly correlated with the percentage of the classic atypical lymphocytes seen in the PBMC's of IM patients

Severity of IM as measured by lymphadenopathy directly correlates with the percentage of peripheral blood atypical T lymphocytes (pathognomic of IM)

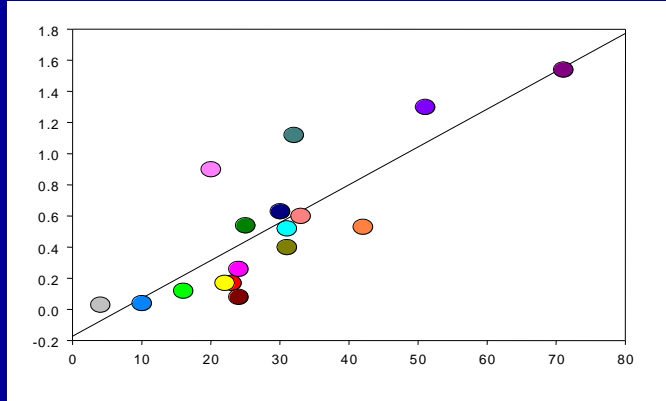


Severity of IM directly correlates with % of EBV-BMLF1 epitope-specific responses but not EBV-BRLF-1



The percentage of Flu-M1-specific CD8 T cells directly correlated with disease severity as assessed by % of atypical T lymphocytes

% M1 tetramer + cells
(in PBMC)

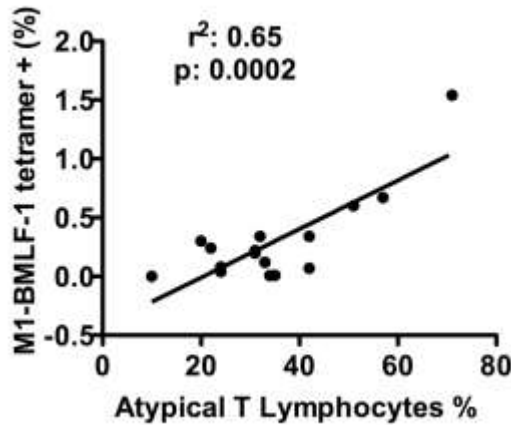


(ex vivo)

Atypical T Lymphocytes % $r^2: 0.69$ $p:0.0001$

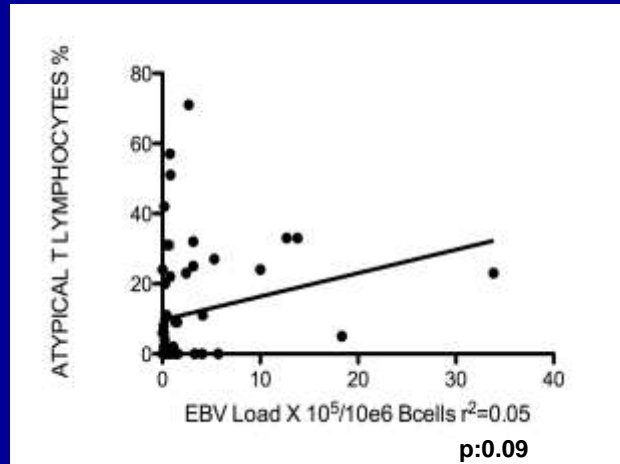
Aslan et al.

The percentage of CXR Flu-M1/EBVBMLF1-specific CD8 T cells directly correlated with disease severity as assessed by % of atypical T lymphocytes



Nuray Aslan

Disease severity of IM did not correlate with EBV-viral load



HETEROLOGOUS IMMUNITY AND CROSSREACTIVE T CELL RESPONSES MAY MEDIATE INFECTIOUS MONONUCLEOSIS

- Cross-reactive CD8 T cell responses commonly occur during EBV infection.
- Cross-reactivity between Flu-M1 and EBV-BMLF1/BRLF1 specific T cells is a novel and common event in IM patients.
- EBV-BMLF-1/BRLF1 and Flu-M1 T cell responses were functionally crossreactive.
- Disease severity of IM correlated with percentage of CXR Flu-M1/ BMLF1-specific CD8 T cells but did not correlate with Flu-M1/EBV-BRLF1 specific responses.

CROSS-REACTIVE MEMORY CD8 T CELLS CAN BE ACTIVATED BY EBV INFECTION AND PROMOTE INFECTIOUS MONONUCLEOSIS.

- **OLDER INDIVIDUALS ARE LIKELY TO HAVE MORE EXTENSIVE REPERTOIRES OF MEMORY T CELLS AND ARE MORE PRONE TO MONONUCLEOSIS UPON INFECTION WITH EBV THAN ARE YOUNG CHILDREN.**
- **CROSS-REACTIVE REPERTOIRES MAY BE BROADER IF THE CROSS-REACTIVE EPITOPES ARE DISSIMILAR AS THERE IS LESS LIKELIHOOD OF A HIGH AFFINITY OLIGOCLONAL EXPANSION**
- **CROSS-REACTIVE T CELLS FUNCTIONALLY RESPOND TO EACH LIGAND DIFFERENTLY**
- **CROSS-REACTIVE MATRICES INCREASE THE LIKELIHOOD THAT CROSS-REACTIVE RESPONSES PARTICIPATE IN MEDIATING INFECTIOUS MONONUCLEOSIS**

Heterologous immunity and cross-reactive CD8+ T-cells can participate in:

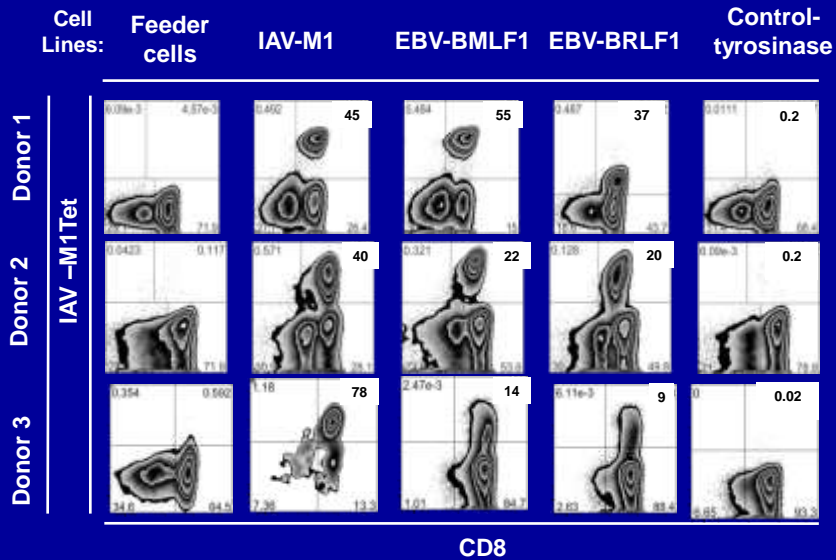
- 1) **Mediating immuno-pathology
i.e. infectious mononucleosis**
- 2) **Mediating protective immunity**

CD8 T cell cross-reactivity as the rule rather than an exception?

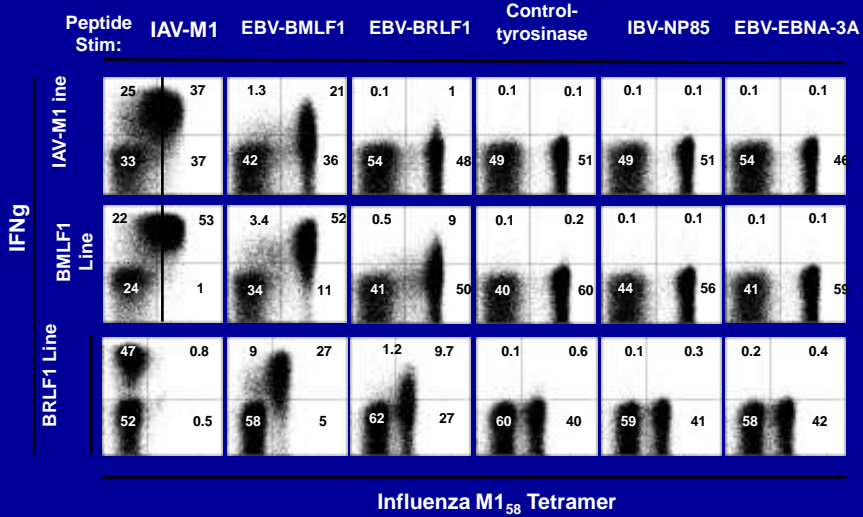
If cross-reactive T cell responses are beneficial, they are likely to go unnoticed, perhaps resulting in asymptomatic or sub-clinical human viral infections.

If cross-reactive T cell responses are harmful to the host, their influence on the outcome of a infection may become more evident, perhaps resulting in an altered disease state.

Three EBV seronegative individuals have CXR responses between IAV-M 1 and EBV-BMLF1 and BRLF1 as demonstrated by expansion of MI tetramer+ cells in cell lines stimulated these two EBV peptides.

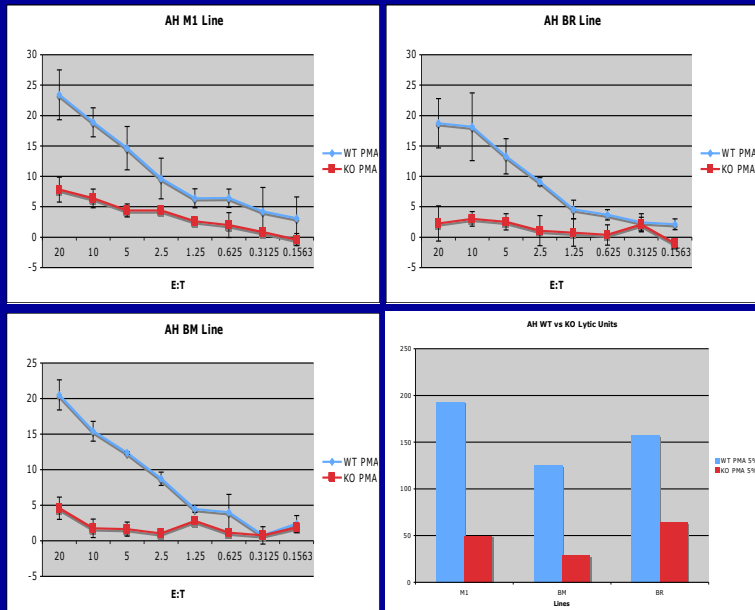


Adult EBV-seronegative individuals have functional antiviral crossreactive responses between influenza M1 and two EBV lytic epitopes BMLF-1 and BRLF-1



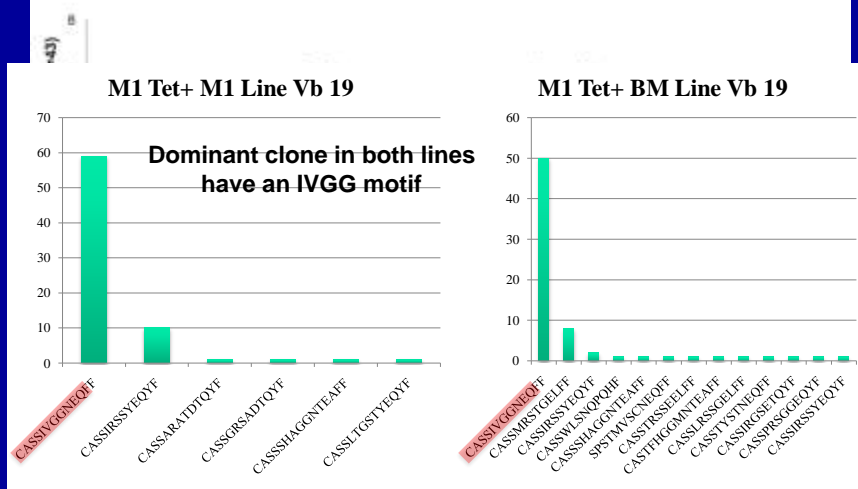
Levi Watkin

FLU-M1, EBV-BMLF-1 and BRLF-1 stimulated cell lines in EBV seronegative donor lyse EBV-infected targets



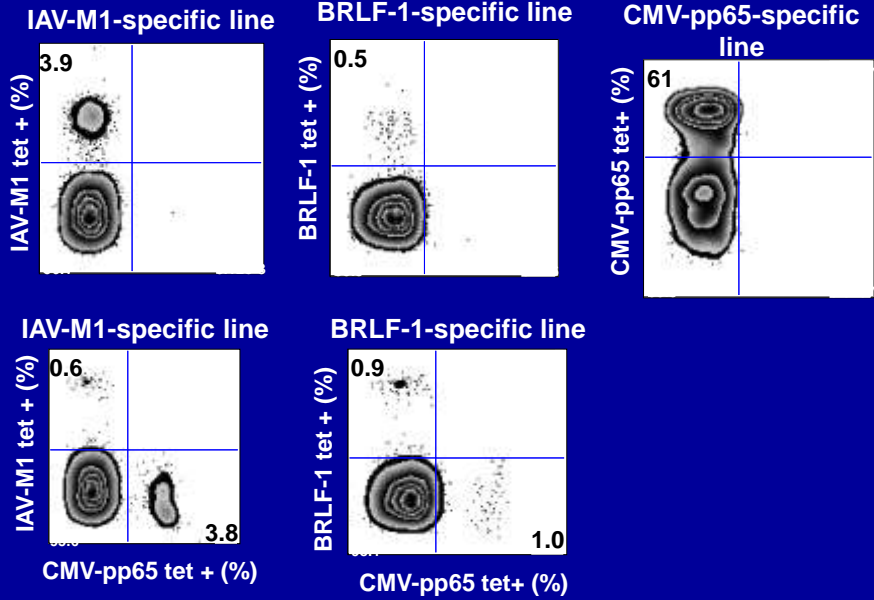
Levi Watkin

UNLIKE OTHER HEALTHY DONORS THE VB17+ (ARDEN), VB19 (IMGT) FLU-M1-SPECIFIC TCR REPERTOIRE IS OLIGO-CLONAL

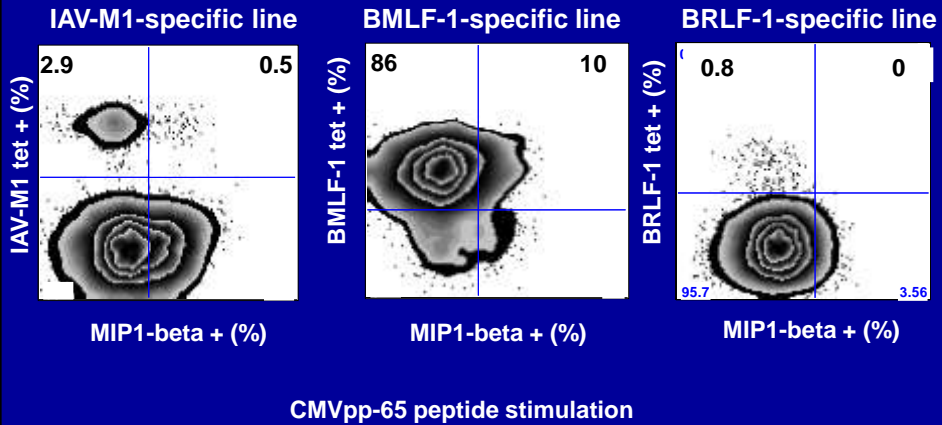


IT IS IMPORTANT TO KEEP IN MIND THAT ANIMAL MODELS OFTEN DEMONSTRATE THE PROTECTIVE ASPECTS OF HETEROLOGOUS IMMUNITY, AND DURING HUMAN INFECTIONS THOSE PROTECTIVE CAPACITIES WILL USUALLY OFTEN GO UNNOTICED.

Elderly donor (85 yrs old) demonstrates promiscuous CMV pp65 crossreactivity

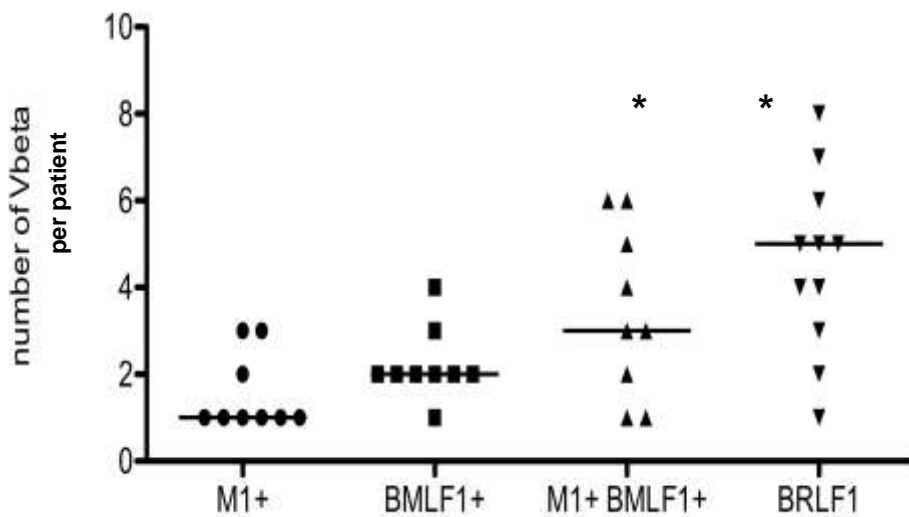


Elderly donor demonstrates cytokine production to CMV pp65 from subset of IAV-M1+ and EBV-BMLF1+ cells



Since naïve T cells are decreased in the elderly,
they may
heavily rely on crossreactive memory T cell responses

The EBV-BRLF1 TCR V β repertoire is more diverse
than non-cross-reactive either Flu-M1 or EBV-BMLF1



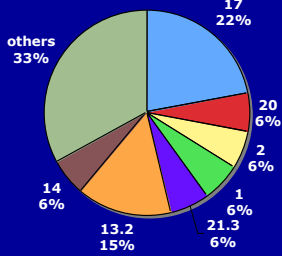
BRLF-1-specific Vbeta repertoire is diverse in both IM patients and healthy memory donors

ex vivo IM patients

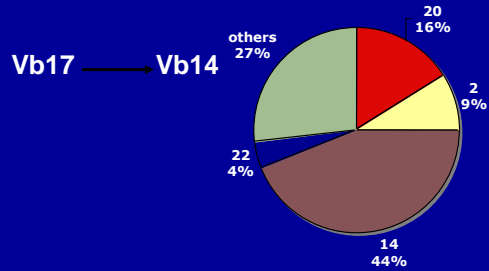
VBeta	Frequency BR%
3	52
2	43
13.2	32
5.1	29
1	26
17	20
20	20
21.3	20
14	17
22	9
9	5
13.1	2

EBV-BRLF-1 TCR Vbeta usage changes over the time course of IM

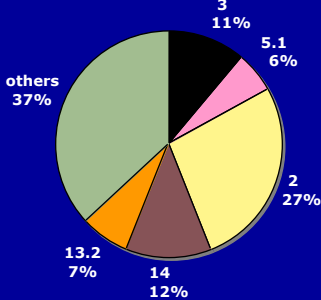
Patient 1 V1



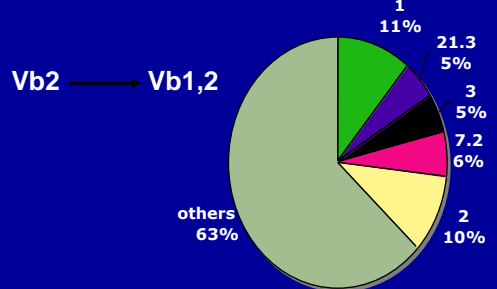
Patient 1 V6



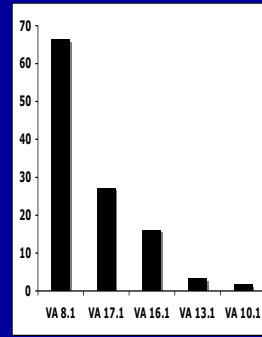
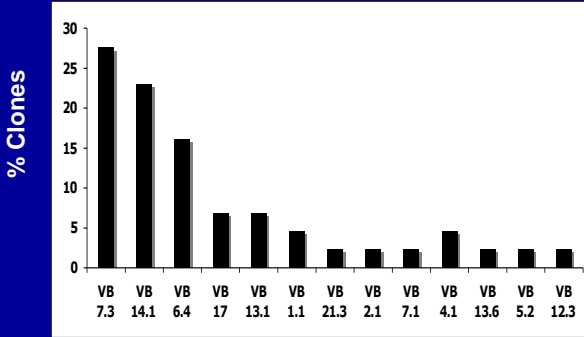
Patient 2 V1



Patient 2 V6



BRLF-1-specific Vb repertoire is diverse while Va usage is oligoclonal suggesting BRLF1 TCR response is restricted by Va usage not Vb



- Diverse Vb usage
- No common CDR3 motif
- Diverse Jb usage

- Oligoclonal
- Va usage dominated by VA 8.1,
- Ja usage dominated by JA 22.1

A cross-reactive TCR expresses a unique combination of M1- specific and BRLF1-specific α - and β -chains

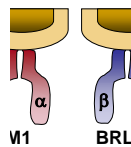
Cross-reactive-Flu-M1-specific TCR repertoire (in vitro):

BRLF-1-specific TCR repertoire (in vitro):

Model 1

Vb17+:
Jb: 2.7
CDR3B: **X-RS-X**
IRSS

VA12.3+:
JA: 42
CDR3A: **X-GGS-X**



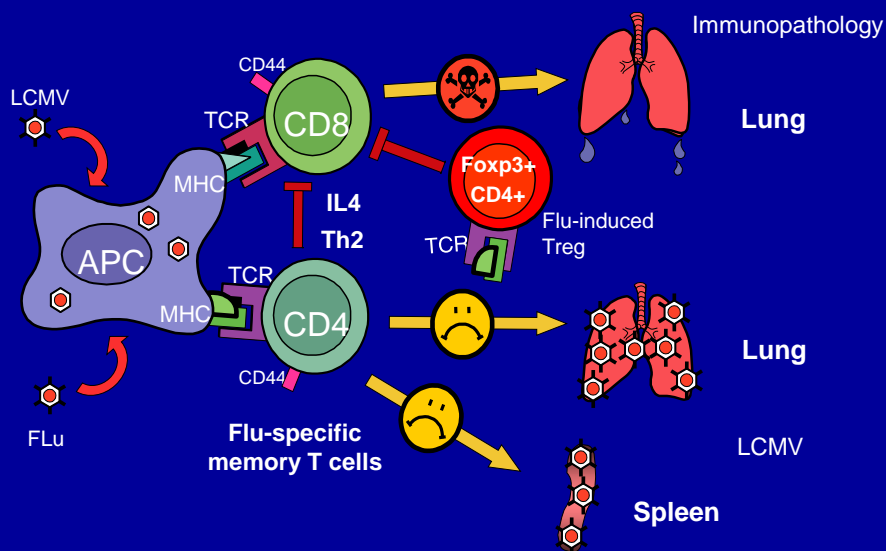
Vb repertoire:
Vb:7.3, 14.1, 6.4, 17, 13.1,1.1, 21.3, 2.1, 7.1, 4.1, 13.6, 5.2, 12.3

VA 8.1+ : JA: 22.1
CDR3A: **XX-TDKLIF**

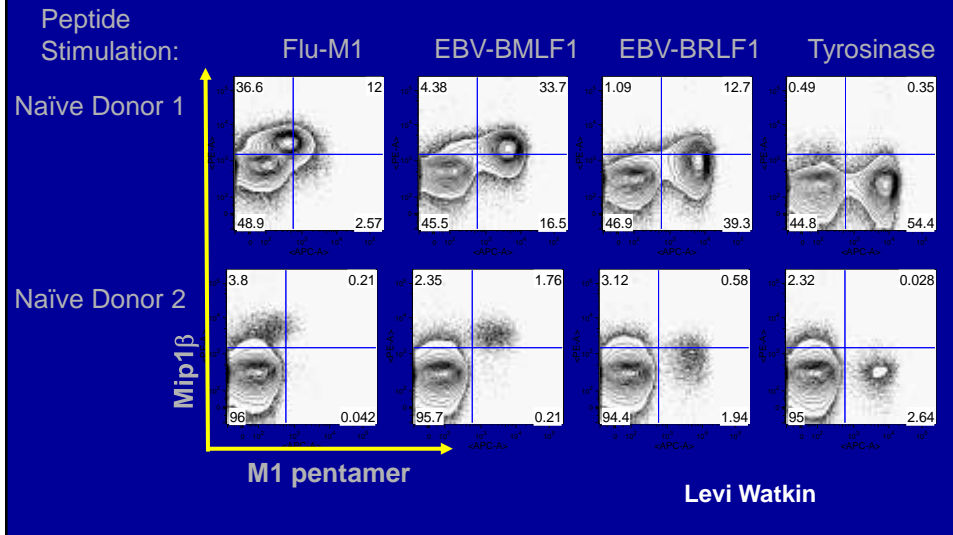
Plasticity and Diversity of EBV-BRLF-1 Vbeta TCR repertoire

- 1- Uses multiple Vbeta families
 - a- that vary between individuals
 - b- that vary during the time course of IM in the same patient
 - c- that vary overtime in healthy EBV- BRLF-1 TCR repertoires include multiple V β families and usage
- 2- Strong contrast with Flu-M1 (Vb 17) and EBV-BMLF-1 (Vb 2, 4, 22, 1,16) which use fewer Vbeta TCR families that are stable overtime
- 3- EBV-BRLF-1 and Flu-M1 Vbeta TCR repertoires in IM patients differ from crossreactive EBV-BRLF-1 repertoire, but are also diverse and change overtime
- 4-BRLF-1-specific TCR appears to be of higher avidity than Flu-M1 or BMLF-1
- 5-Could the enhanced diversity in BRLF-1 Vb usage enhance crossreactivity?
- 6- Could the high propensity of EBV-BRLF-1 to crossreact with many epitopes lead to the constant change in TCR Vbeta usage?

Memory T cells to a heterologous virus can result in enhanced immunopathology during a second infection



Two adult EBV-seronegative individuals have crossreactive responses between influenza M1 and two EBV lytic epitopes BMLF-1 and BRLF-1



OBSERVATIONS ON T CELL CROSS-REACTIVITY

T CELL CROSS-REACTIVITY IS COMMON BETWEEN UNRELATED PATHOGENS AND ALTERS T CELL IMMUNODOMINANCE

T CELL CROSS-REACTIVITY ALTERS PROTECTIVE IMMUNITY, IMMUNOPATHOLOGY, AND IS A CAUSE OF HETEROLOGOUS IMMUNITY

T CELL CROSS-REACTIVITY CAN LEAD TO **NARROWING** OF THE T CELL REPERTOIRE, GIVING RISE TO VIRAL **ESCAPE MUTANTS**

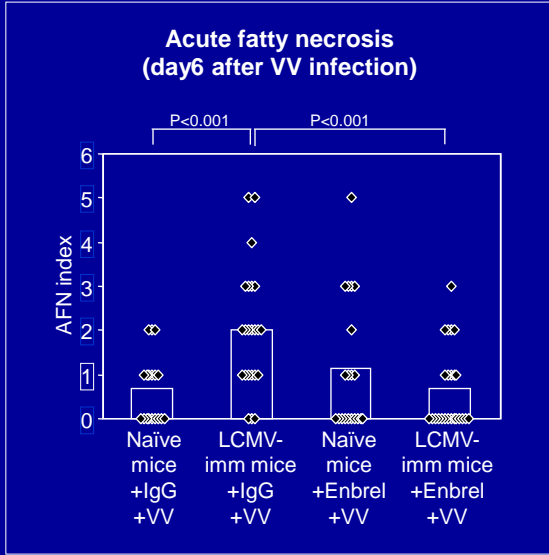
CROSS-REACTIVE TCR PRIVATE SPECIFICITY DETERMINES DISEASE OUTCOME RESULTING IN REPERTOIRE NARROWING, PROTECTIVE IMMUNITY, AND IMMUNOPATHOLOGY

Discussion

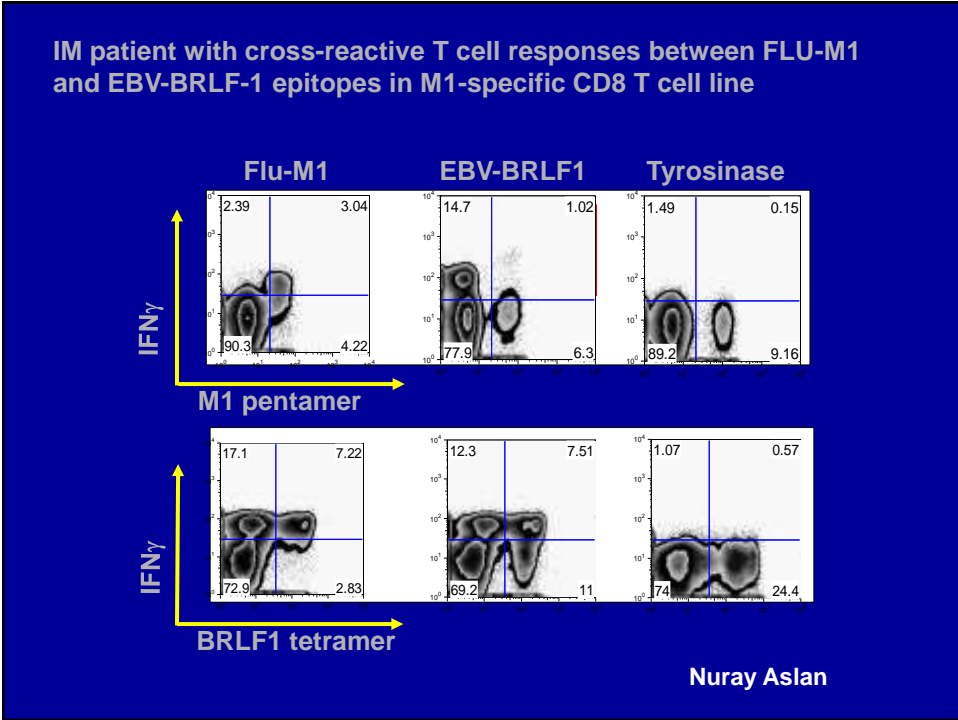
	Male	Female
Normal LCMV memory	Yes	Yes
Normal VV response	Yes	Yes
Skewing of hierarchies	Yes	Yes
Expansion and recruitment of LCMV T cells	Yes	Yes
Heterologous protection	Yes	Yes
Size of visceral fat	large	small
# of LCMV-specific CD8 T cells in FP	10⁵	10⁴
Acute fatty necrosis	High	Low

There is evidence that men and women have different fat distribution, which is positively associated with vascular disease risk. It has also been suggested that vascular disease (i.e. artery plaque formation) may be induced by immunological responses. It is interesting that the heterologous immunity is different in the visceral fat tissue between male and female.

TNF mediates panniculitis: Enbrel treatment significantly decreases acute fatty necrosis in LCMV-immune mice infected with VV



Siwei Nie



Unlike other healthy donors the Vb17+ Flu-M1-specific TCR repertoire has a unique oligo-clonal CDR3 motif

Other Healthy Donors: 40 unique Vb17 sub-clones (90% use the 'xRSx' motif)
64 total CDR3 β sequences analyzed

Flu-M1 (+) Sort

CLONE ID	CDR3 SEQ	FREQUENCY	
2.3	BMP-6	IRSA	14
	BMP-19-1	ARAS	2
	BMP-31-1	IRSA	1
	BMP-1-1	TRSA	1
2.1	BMP-11-6	IVGG	9
	BMP-14-1	RSS	1
	BMP-22-1	IVGG	1
2.2	BMP-38-2	MRSIT	2
2.7	BMP-41-1	IRSA	1
	BMP-35-1	MFHT	1
	BMP-12-1	IRSA	1
	BMP-47-1	QRSS	1
	BMP-39-1	TRSS	1
1.1	BMP-26-1	STAGGDT	1

14 unique Vb17 sub-clones (64% use the 'xRSx' motif)
37 total CDR3 β sequences analyzed

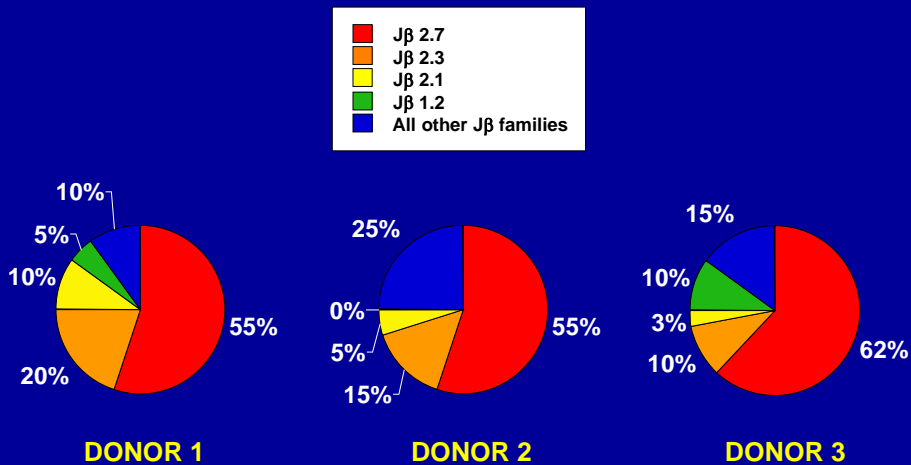
CLONE ID	CDR3 SEQ	FREQUENCY	
2.1	M1P-44-22	IVGG	23
	M1P-44-22-5m	IVGG	1
	M1P-44-22-43m	IVGG	1
	M1P-13-1	IVWG	1
	M1P-42-1	VAGGRS	1
2.2	M1P-25-6	MRSIT	6
	M1P-16-1	QRST	1
2.7	M1P-37-1	IRSA	1
	M1P-39-1	RSS	1
2.3	M1P-38-1	RRST	1
	M1P-26-1	SRSA	1
1.2	M1P-21-1	VGLY	1

12 unique Vb17 sub-clones (50% use the 'xRSx' motif)
39 total CDR3 β sequences analyzed

THE PRIVATE SPECIFICITIES OF A CD8 T CELL MEMORY POOL CAN DETERMINE EPITOPE SPECIFICITY AND PATTERNS OF HETEROLOGOUS IMMUNITY BETWEEN UNRELATED VIRUSES

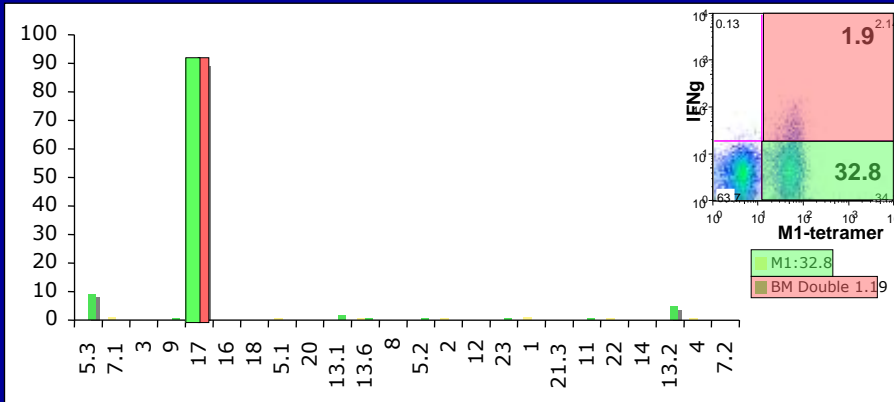
BUT ALL IS NOT RANDOM, AS
NP205>GP34>GP118>NP396=GP276

M1-specific Vb17+ sub-clones can be organized based
on a conserved hierarchy of Jb family usage
(public specificity)



Yuri Naumov, J. Immunol. 2006

EBV sero-negative donor demonstrates high Flu-M1 memory response (0.7%) with strong cross reactivity to EBV-BMLF1: Stimulation of a CD8+ T-cell culture with EBV-BMLF1 peptide results in an expansion of Flu-M1 specific cells



BMLF1 Stimulated Line

Levi Watkin

DISCUSSION POINTS

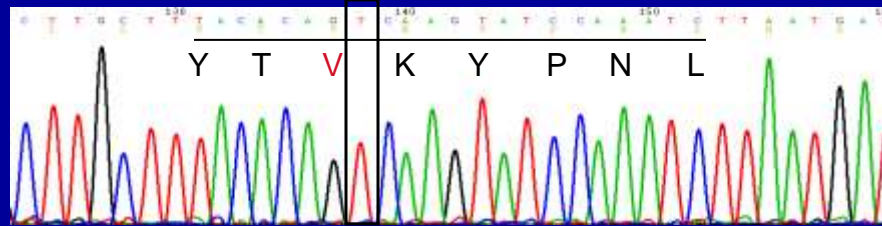
HETEROLOGOUS IMMUNITY INFLUENCES VIRAL CLEARANCE AND IMMUNOPATHOLOGY AND MAY BE A CONSEQUENCE OF CROSS-REACTIVE T CELLS, WHICH MAY ALTER T CELL IMMUNODOMINANCE

T CELL CROSS-REACTIVITY CAN LEAD TO NARROWING OF THE TCR REPERTOIRE AND DEVELOPMENT OF VIRAL ESCAPE MUTANTS

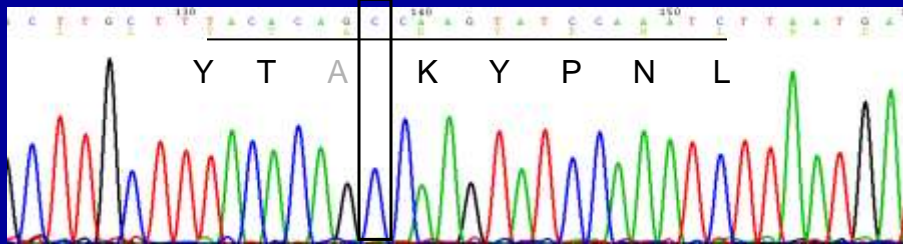
A TCR REPERTOIRE IS UNIQUE TO EACH HOST, AND PATTERNS OF CROSS-REACTIVITY BETWEEN VIRUSES MAY REFLECT THE **PRIVATE SPECIFICITIES** OF THE T CELLS FROM AN INDIVIDUAL HOST AND IMPACT DISEASE OUTCOME

Dominant narrowed NP205 TCR repertoire in PV-immune mouse infected with LCMV clone 13 led to generation of LCMV NP205 (V-A) escape mutant

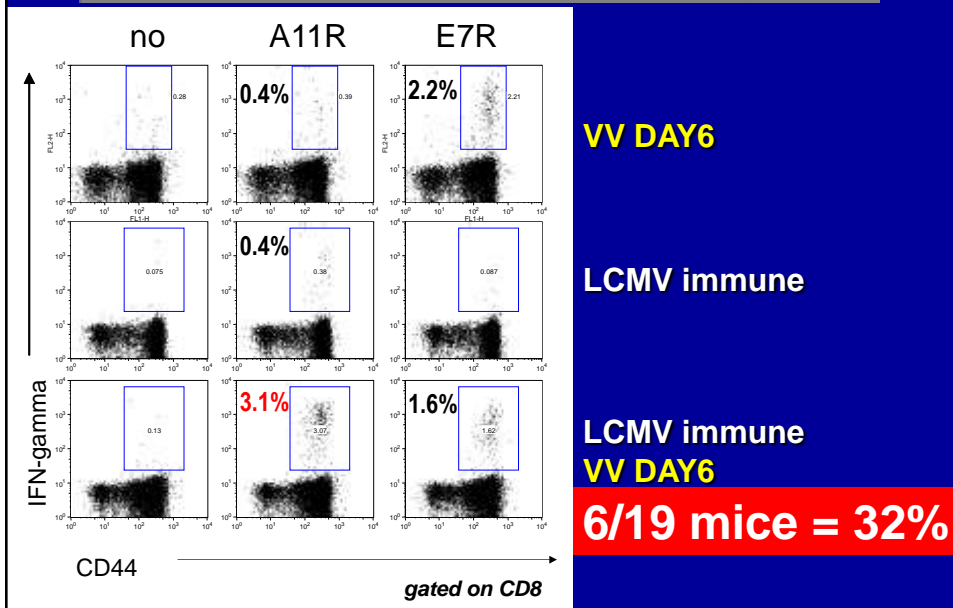
Wildtype NP205 epitope

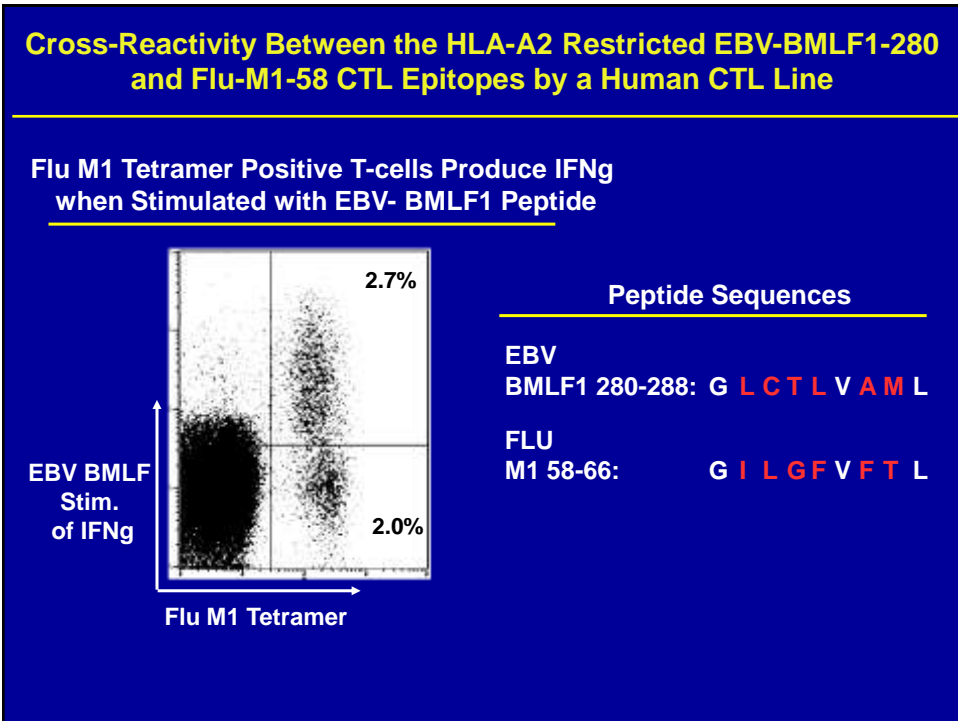
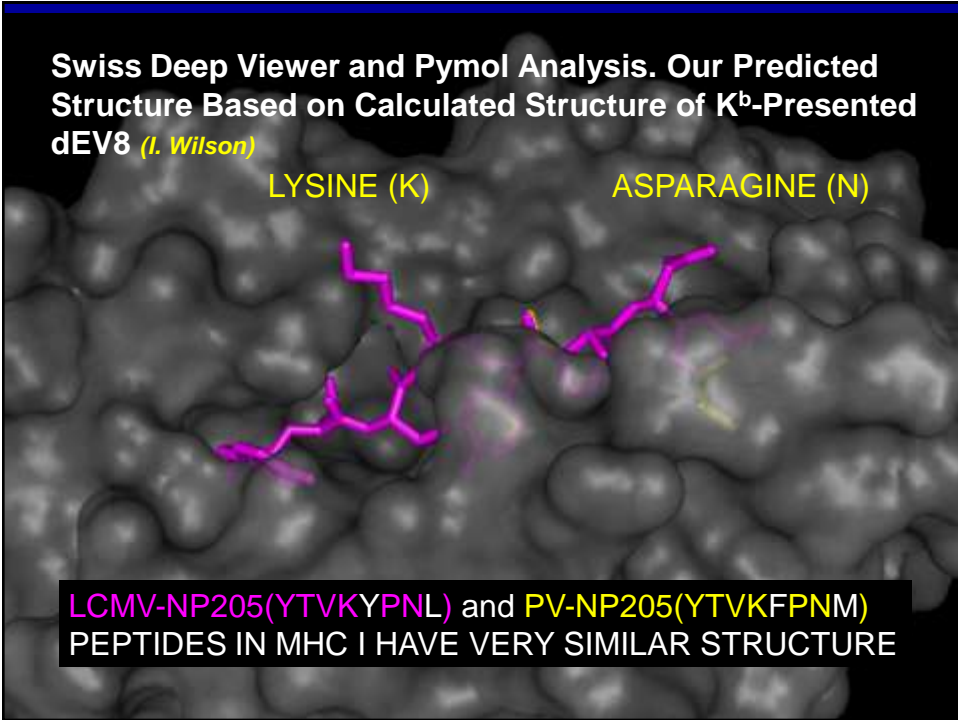


NP205 (V→A) variant clone

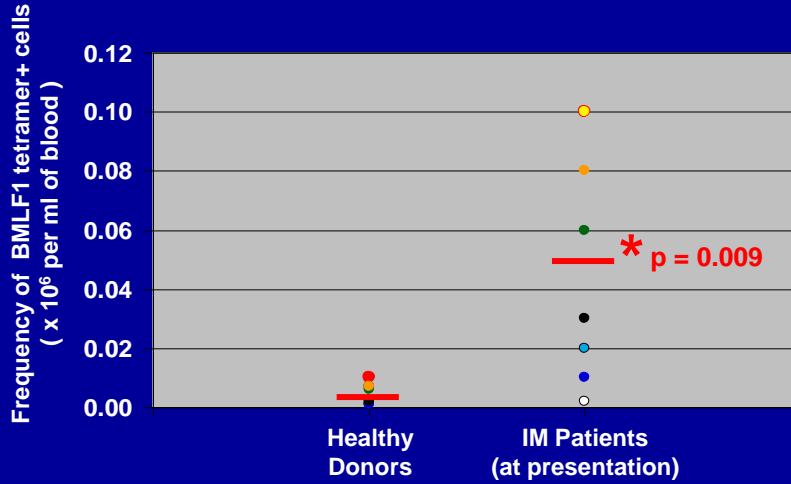


Change of VV-immunodominance after heterologous viral challenge



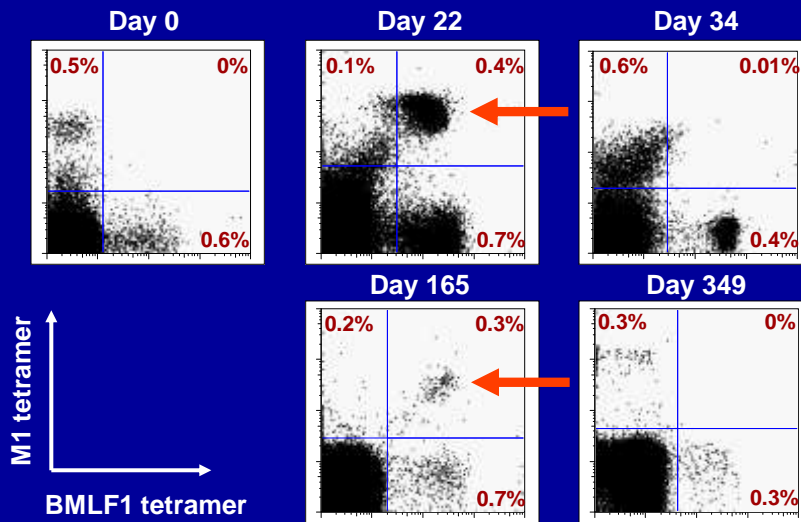


One hallmark of acute EBV infection in HLA-A2+ IM patients is an increase in the number of BMLF1 tetramer+ cells in the blood



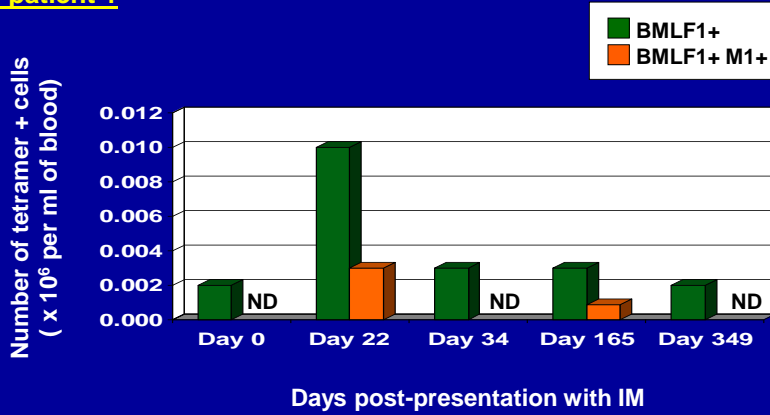
Cross-reactive T cells that co-stained with M1- and BMLF1-loaded tetramers were in the blood during EBV infection

IM patient 1

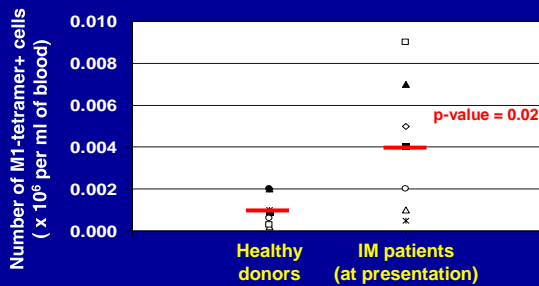


The total number of cross-reactive T cells that co-stained with M1- and BMLF1-loaded tetramers increased during acute EBV infection

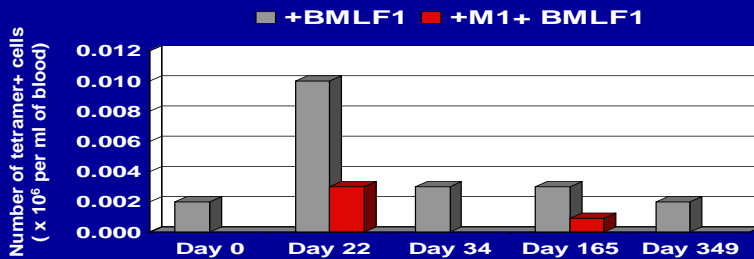
IM patient 1



Cross-reactive memory M1-specific T cells participate in the massive lymphoproliferation characteristic of IM



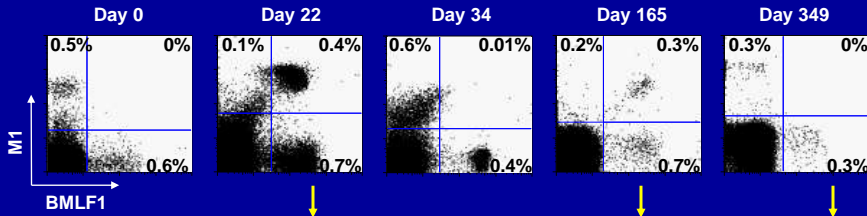
IM patient E1101:



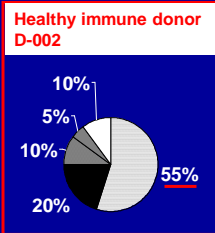
The M1-specific Vβ17+ TCR repertoire was markedly skewed in IM patients compared to healthy immune donors

IM patient E1101

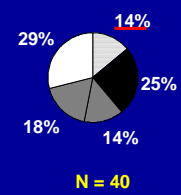
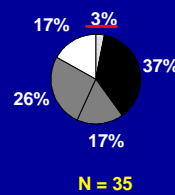
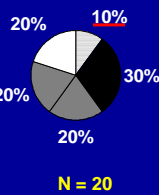
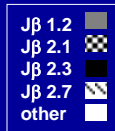
CD8+ T cells isolated ex vivo:



M1-specific cell line cultured for 3 weeks, cDNA synthesized, CDR3 regions of Vβ17+ cells sequenced

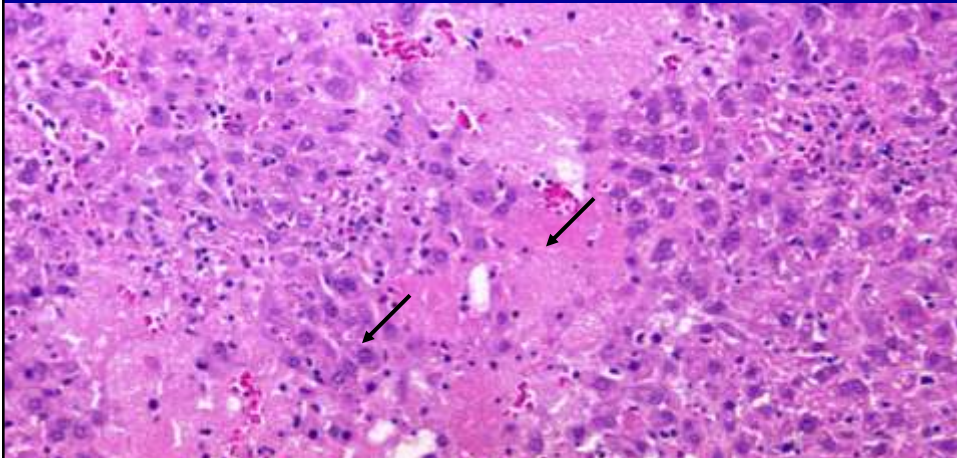


Diversity of Jβ usage:



LCMV INFECTION

LCMV induces fulminant hepatitis with areas of complete necrosis but also hyperregenerative areas (mitotic cells) similar to liver pathology in severe viral infection, septic-shock, or acute toxicity.



Features of the (IV) M1₅₈-specific TCR repertoire

GILGFVFTL

Moss et al. PNAS 1991

Lehner et al. J Exp Med 1995

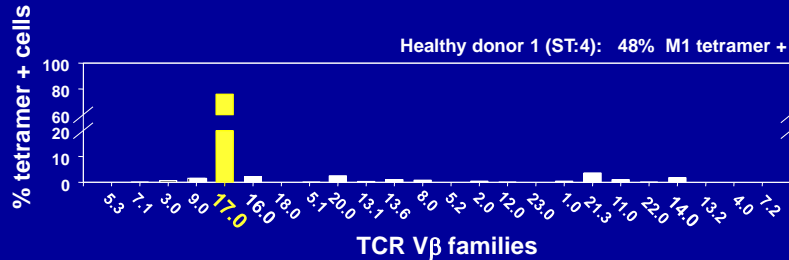
Stewart-Jones et al. Nat Immunol 2003

Public specificity

- M1-specific T cells predominantly express Vβ17
- M1-specific T cells predominantly express the CDR3β amino acid motif “xRSx”

(IV) M1-specific TCR repertoires are focused predominantly on the Vβ17 family

M1-specific T cell lines, gated on the M1 tetramer+ T cells



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Public specificity

Naumov et al. J Immunol 1998
Naumov et al. J Immunol 2003

- Vβ17+ T cell repertoire is made up of many unique T cell clones
 - Within the M1-specific T cell population of one healthy immune individual, 501 CDR3β loops were sequenced and 141 of those sequences were unique (Vβ17+ sub-clones)

Private specificity

The Vβ17+ M1-specific TCR repertoire is highly diverse but focused on common CDR3 motifs

40 unique Vβ17 sub-clones (90% use the 'xRSx' motif)
64 total CDR3β sequences analyzed

	CLONE ID	CDR3 SEQ	FREQUENCY		CLONE ID	CDR3 SEQ	FREQUENCY	
Jβ 2.7	43.27	MRSS	6	Jβ 2.3	1.23	GRST	3	
	17.27	IRSS	5		5.23	PRSG	1	
	23.27	IRSS	3		10.23	SRST	1	
	28.27	IRSS	3		16.23	QRST	1	
	6.27	IRSS	2		18.23	QRST	1	
	25.27	IRSS	2		19.23	QRST	1	
	36.27	TRSS	2		25.23	TRSS	1	
	51.27	VRSS	2		30.23	IRST	1	
	72.27	LRSS	2		32.23	TRSA	1	
	73.27	IRAA	2		33.23	MRTG	1	
	84.27	IRSG	2					
	86.27	FRSA	2					
	1.27	IRSS	1	Jβ 2.1	2.21	IGTG	2	
	3.27	IRSS	1		1.21	GRAG	1	
	19.27	IRSS	1		7.21	YRSS	1	
	22.27	IRSS	1					
	29.27	IRSS	1					
	35.27	TRSS	1					
	38.27	TRSS	1					
	39.27	TRSS	1					
40.27	TRSS	1						
41.27	TRSS	1						
49.27	VRSS	1						
59.27	SRSA	1						
60.27	SRSA	1						
85.27	MRSA	1						
91.27	IRSA	1						

DISCUSSION POINTS

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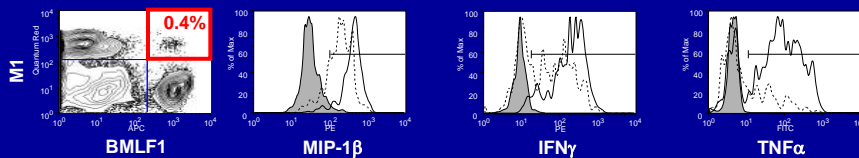
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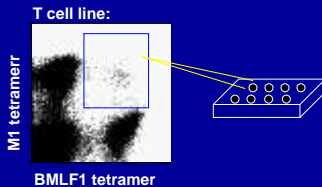
Cross-reactive T cell populations and clones that co-stain with M1 and BMLF1 tetramers are functionally heterogeneous in their response to each peptide

T cell line derived from donor D-002

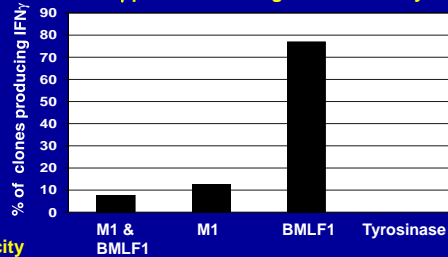
M1+ BMLF1+ tetramer gate:



Sort M1+ BMLF1+ (tetramer) cells at 1 cell per well:

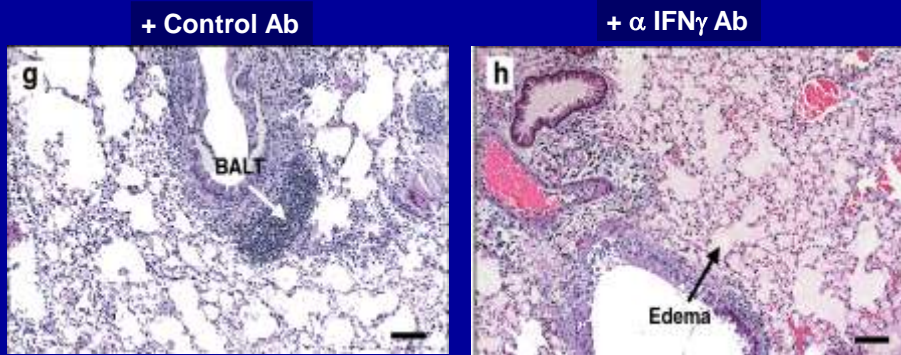


IFN γ production using ELISPOT assay



Culture clones for 2 weeks and test functional specificity

In Vivo Depletion of IFN- γ Results in Severe Edema and No Induction of BALT in VV-infected LCMV-immune Mouse Lung



CROSS-REACTIVE EPITOPES

LCMV NP-205

PV NP-205

VV P24

Rev Imm, 04

FLU A M1-58

EBV BMLF1-280

Rev Imm, 04

FLU A NA-231

HCV NS3-1073

JV, 01

HPV16 E7-11

CORONA NS2-52

03

HIV GP120

M. TB 19kd

Imm, 03

SIN NOMBRE N-421

SEOUL V N-421

IV, 00

YTVKYPNL

YTVKFPNM

AIVNYANL

Welsh, Ann

GILGFVFTL

GLCTLVAML

Welsh, Ann

CVNGSCFTV

CVNGVCWTW

Wedermeyer,

YMLDLQPET

TMLDIQPED

Nilges, JV,

VPTDPNPPEV

VLTDGNPPEV

Hohn, Eur J

ISNQEPLKL

ISNQEPMKL

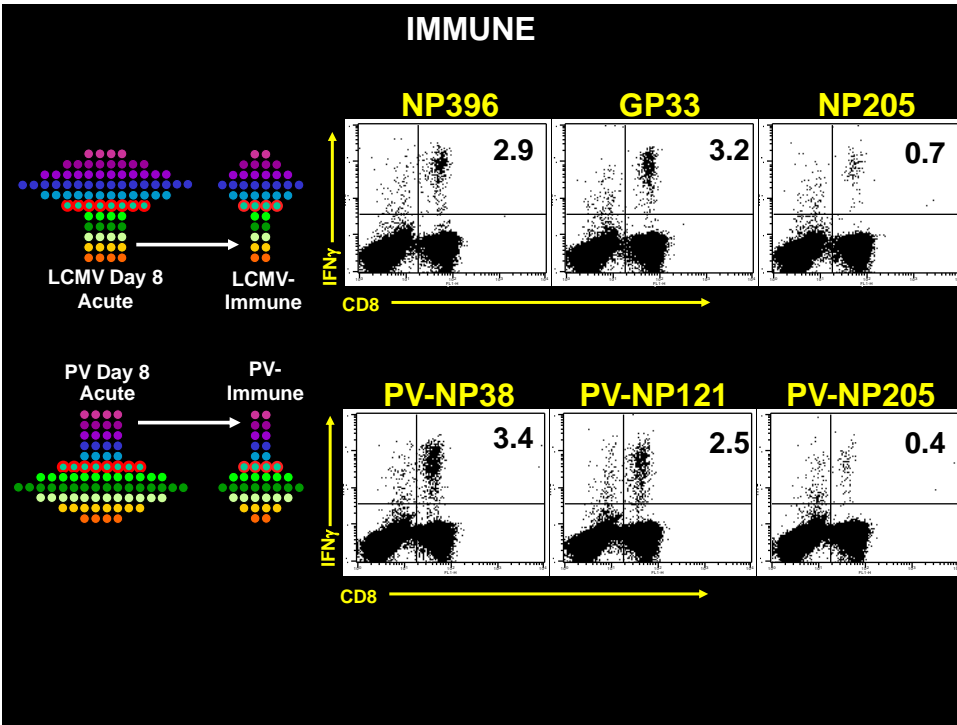
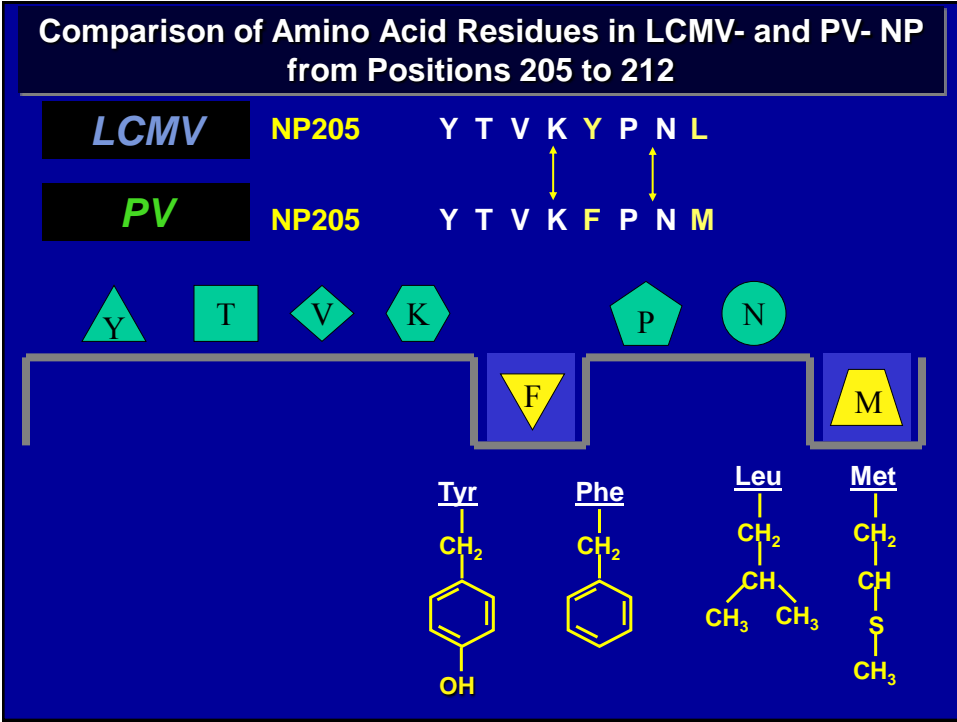
Van Epps,

VP-24 can be cross-reactive with all known LCMV specific H2-Kb epitopes and VP-10

CROSS-REACTIVITY												
	1	2	3	4	<u>5</u>	6	7	<u>8</u>	LCMV VP24	VV VP24	VV VP10	
L-NP205	Y	T	V	K	<u>Y</u>	P	N	<u>L</u>	✓			
VP10	S	T	L	N	<u>F</u>	N	N	<u>L</u>		✓	✓	
VP24	A	I	V	N	<u>Y</u>	A	N	<u>L</u>	✓	✓	✓	
L-GP118	I	S	H	N	<u>F</u>	C	N	<u>L</u>	✓	✓		
L-GP34	A	V	Y	N	<u>F</u>	A	T	<u>C</u>	✓	✓		

Heterologous Immunity Between Viruses

Immunizing Virus	Challenge Virus			
	LCMV	PV	MCMV	VV
LCMV	ND	-1.1	-0.4	-1.5
PV	-0.4	ND	-0.6	-1.8
MCMV	-0.8	-0.5	ND	-1.5
VV	-0.3	-0.1	0.0	ND



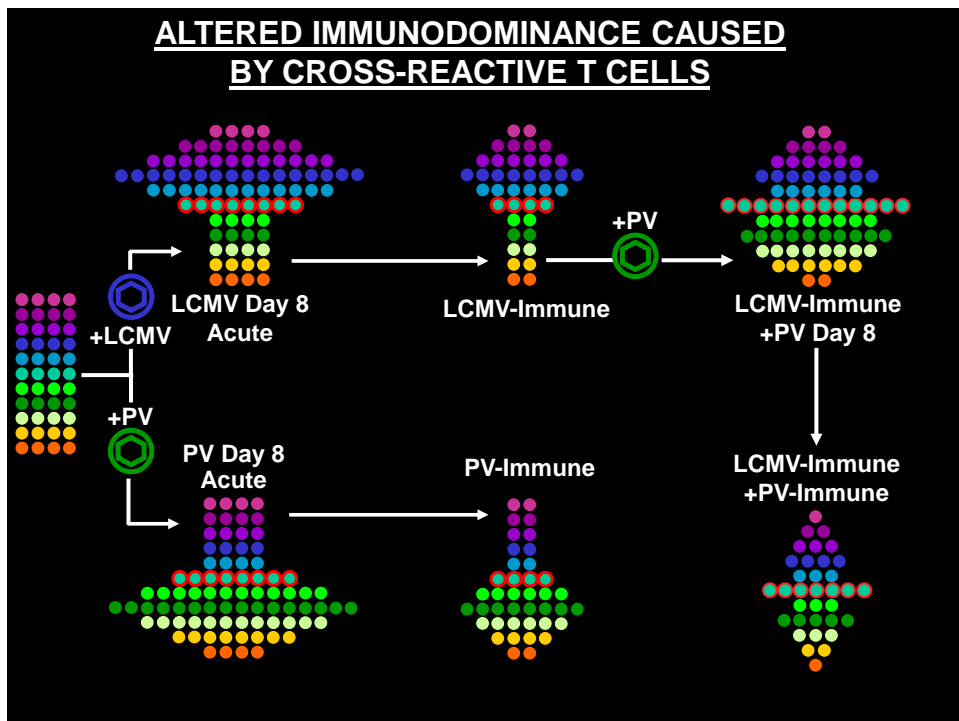
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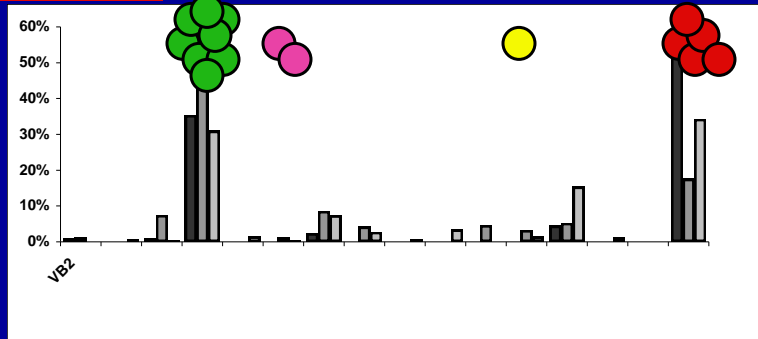
NON CROSS-REACTIVE T CELLS ARE DELETED DURING INFECTION, RESULTING IN A LOSS IN MEMORY

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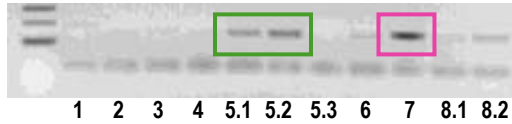
TCR V-beta repertoire of IFN-gamma (PV NP205) positive CD8 T cells

PV infection

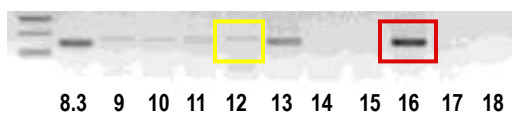


C57BL/6 - D
PV NP205 sorted

V-beta



V-beta



POSSIBLE REASONS FOR THE LOSS IN CD8 MEMORY

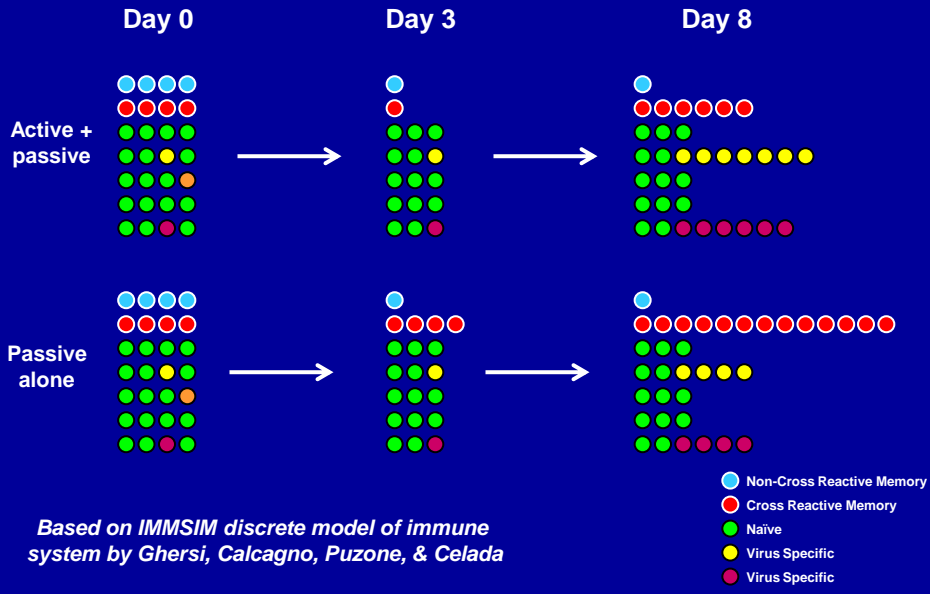
PASSIVE MODEL:

DISPLACEMENT BY NEWLY FORMED MEMORY T CELLS
RESPONDING TO THE MOST RECENT INFECTION

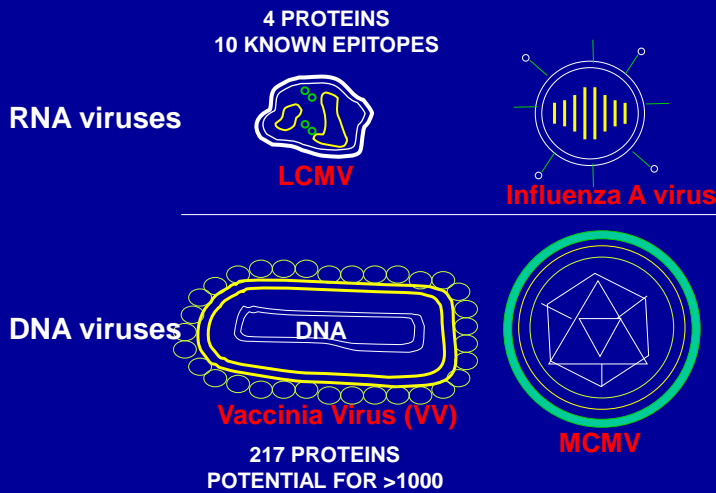
ACTIVE MODEL:

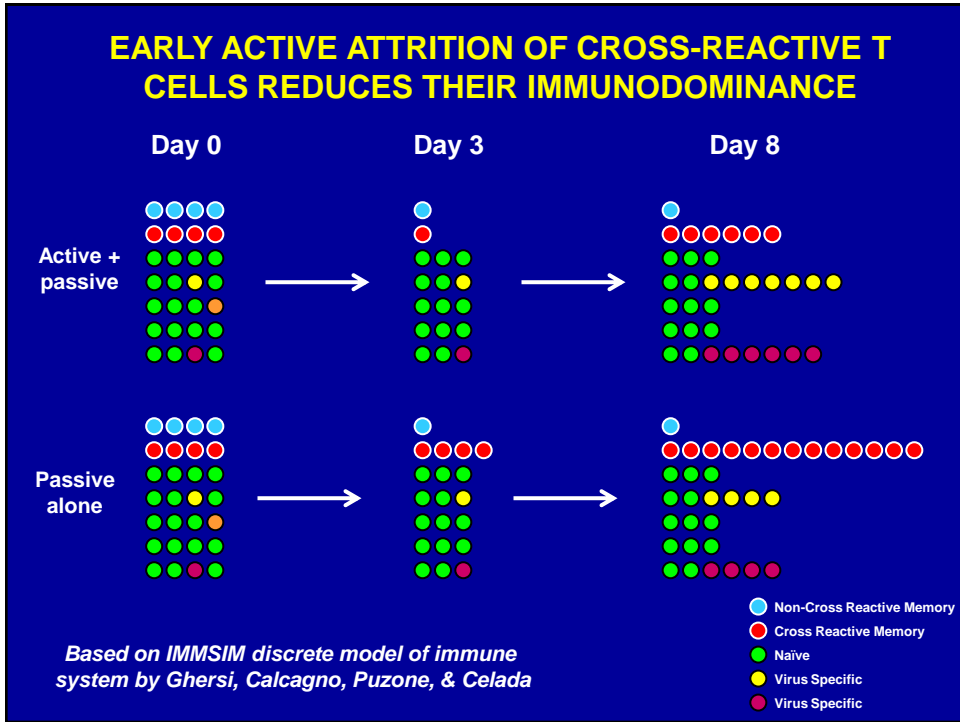
MEMORY CD8 T CELLS ARE KILLED OFF BY FACTORS
LIBERATED DURING INFECTION AND THEN FAIL TO
RETURN TO THEIR FORMER NUMBERS

EARLY ACTIVE ATTRITION OF CROSS-REACTIVE T CELLS REDUCES THEIR IMMUNODOMINANCE



HETEROLOGOUS IMMUNITY IN THE LUNG





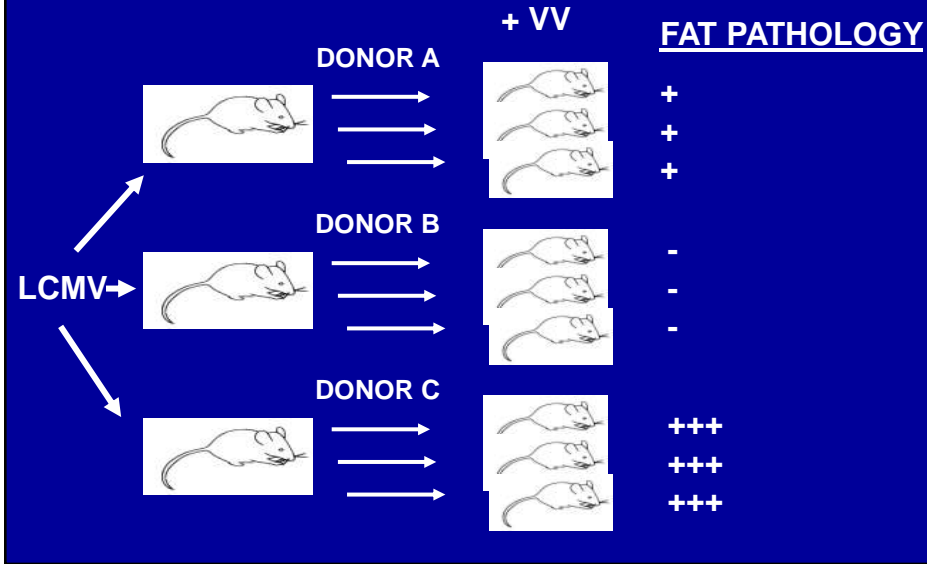
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NON CROSS-REACTIVE T CELLS ARE DELETED DURING INFECTION, RESULTING IN A LOSS IN MEMORY

IMMUNOPATHOLOGY IS A CONSEQUENCE OF A HOST'S UNIQUE IMMUNE SYSTEM



CD8 MEMORY T CELLS: CROSS-REACTIVITY AND HETEROLOGOUS IMMUNITY

Liisa K. Selin, MD, PhD, Associate Professor
U. Mass. Medical School

COLLABORATORS

Raymond M. Welsh, PhD, Professor

MOUSE SYSTEMS:

Michael A. Brehm, PhD, Post-Doc
Markus Cornberg, MD, Post-Doc
Hong D. Chen, Grad Student
Keith A. Daniels, Tech.
Sung-Kwon Kim, PhD, Post-Doc
Brian Sheridan, Tech.
Frances Saccoccio, Tech.
Xiaoting Z. Wang, Grad Student

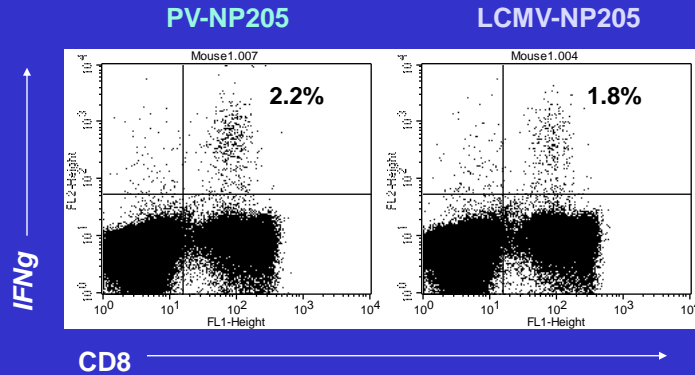
HUMAN SYSTEMS

Yuri Naumov, MD, PhD, Instr.
Shalyn Clute, Grad Student
Levi Watkin, Tech.
LeeAnn Wilkinson, Tech

Katherine Luzuriaga, MD
John Sullivan, MD
Franco Celada, PhD (NYU)

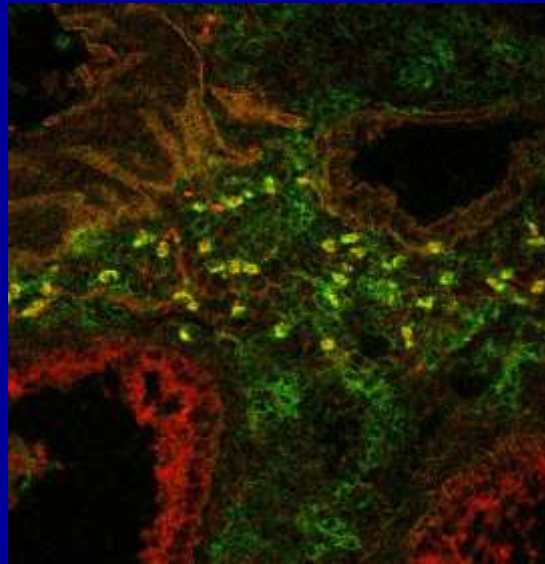
During acute PV infection the majority of the PV-NP205 specific CD8 T-cell population produce IFN-gamma to LCMV-NP205

Acute PV infection



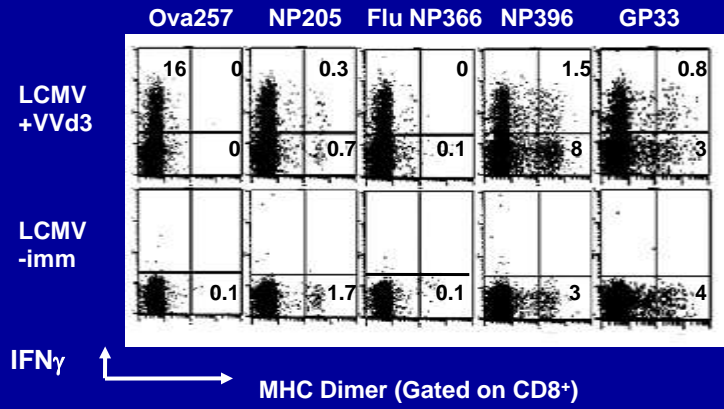
DO THEY GENERATE IDENTICAL TcR REPERTOIRES ?

LCMV NP396-Specific CD8 T Cells in the Lung of VV-Infected LCMV-Immune Mice



Chen, Nat. Imm.
1999

LCMV-SPECIFIC CD8 T CELLS PRODUCE IFN γ IN VIVO IN RESPONSE TO VV INFECTION (Day 3)



Lung

IS THIS WIDESPREAD CROSS-REACTIVITY OR A NON-SPECIFIC ACTIVATION?

Enbrel treatment significantly decreases acute fatty necrosis in LCMV-immune mice infected with VV

LCMV+VV DAY 6
+ IgG-Fc

LCMV+VV DAY 6
+ Enbrel

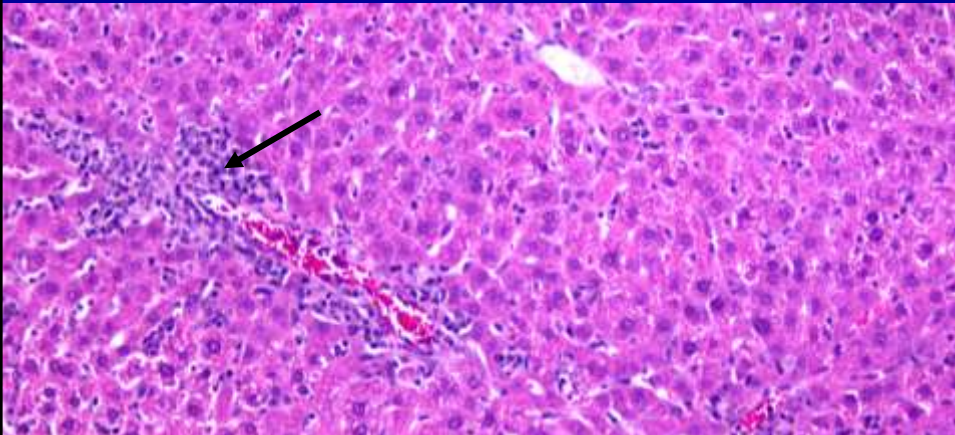


**GENETICALLY IDENTICAL HOSTS USE DIFFERENT
TCR (PRIVATE SPECIFICITY) TO GENERATE SIMILAR
EPIOTOPE-SPECIFIC RESPONSES (PUBLIC
SPECIFICITY)**

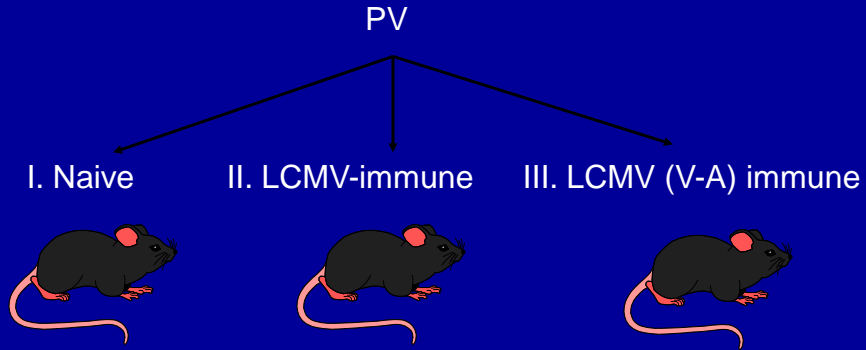


PV+LCMV INFECTION

Mild portal inflammatory activity including piece-meal necrosis similar to mild activity in viral hepatitis.



Partial loss of heterologous protective immunity in the absence of the cross-reactive NP205 response.



- Day 4 post-infection harvest
- Plaque assay to access PV viral load
- FACs analysis to monitor T cell responses

No One is Naive

Virus

Parasite

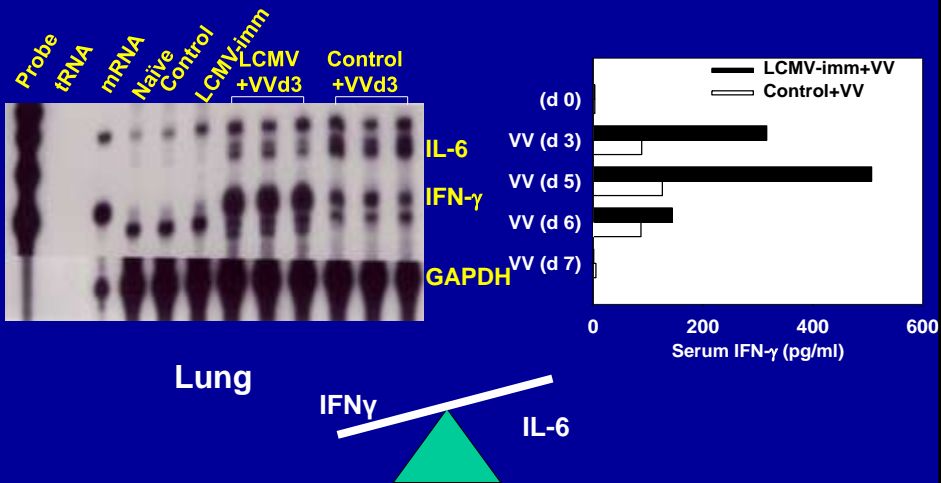
Bacteria

Allergen

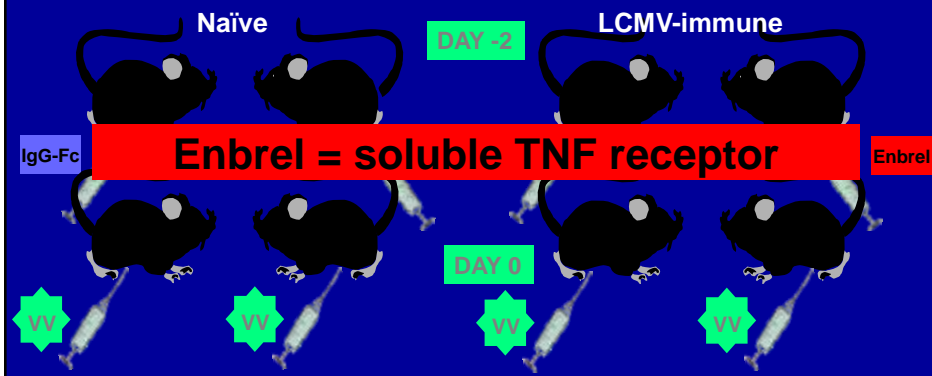
Fungus

Autoimmune antigen

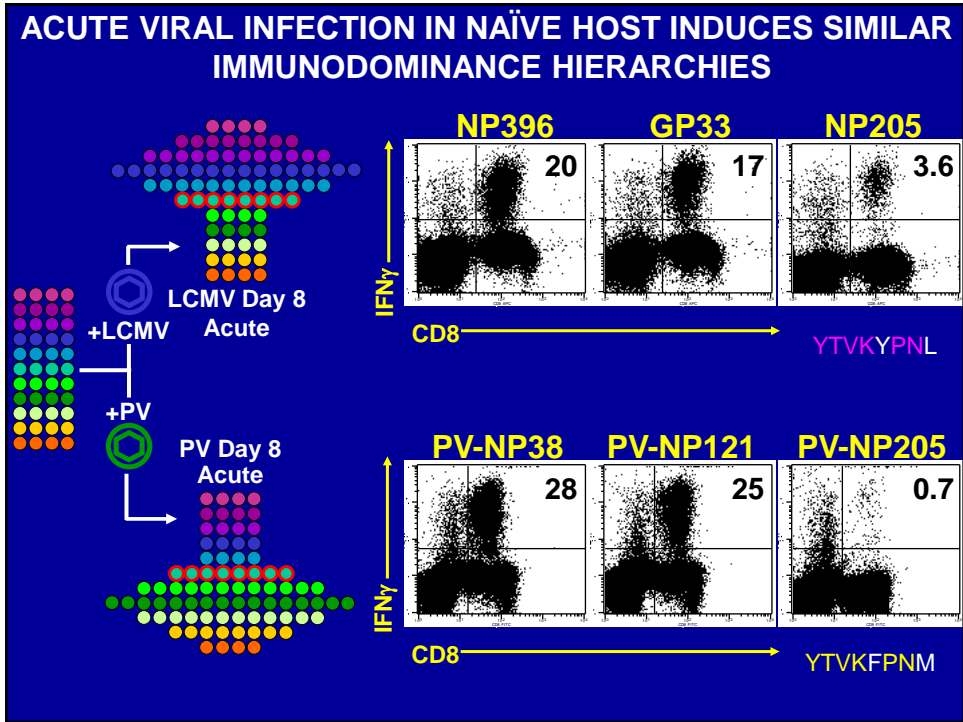
Enhanced IFN- γ and Reduced IL-6 Production in LCMV-immune Mice Early after VV Challenge



TNF signaling plays a role in mediating panniculitis (AFN) in LCMV-immune mice infected with VV



Siwei Nie



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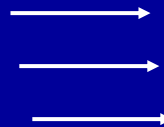
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SYSTEM TO EXAMINE THE PROLIFERATIVE EXPANSION OF VIRUS-SPECIFIC MEMORY CD8 T CELLS AFTER HOMOLOGOUS OR HETEROLOGOUS ANTIGENIC CHALLENGE

Thy 1.1
PV-immune

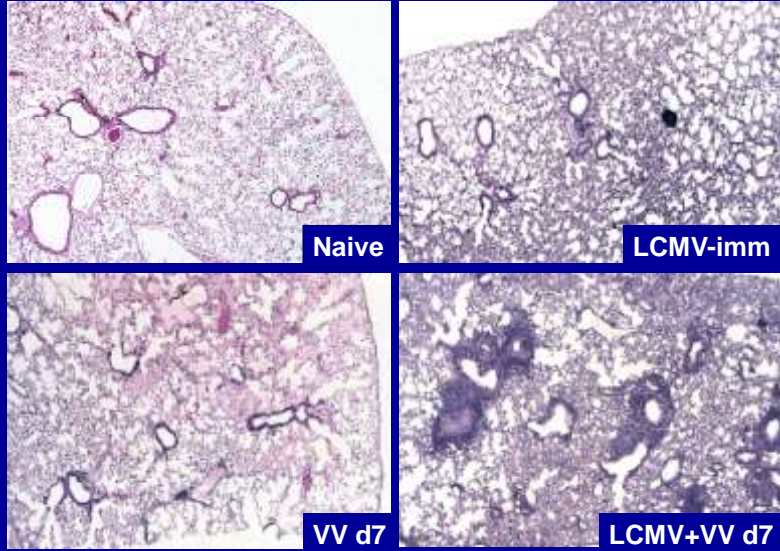
Thy 1.2 Naive

CFSE-labeled splenocytes
adoptive transfer



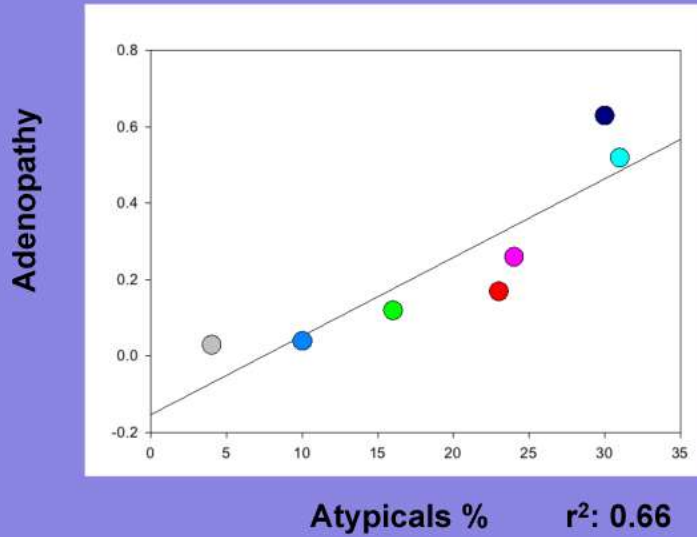
+ LCMV

Altered Immunopathology in the VV-infected LCMV-immune Mouse Lung



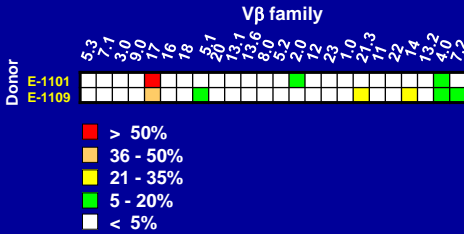
Hong Chen, Nature Immunol. 2001

Severity of IM as measured by lymphadenopathy directly correlates with the percentage of peripheral blood atypical lymphocytes (pathognomic of IM)

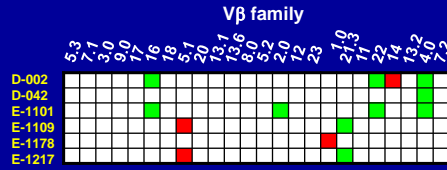


The cross-reactive TCR repertoire can appear more diverse than non-cross-reactive, comprised of multiple Vβ and Vα families that can vary between individuals.

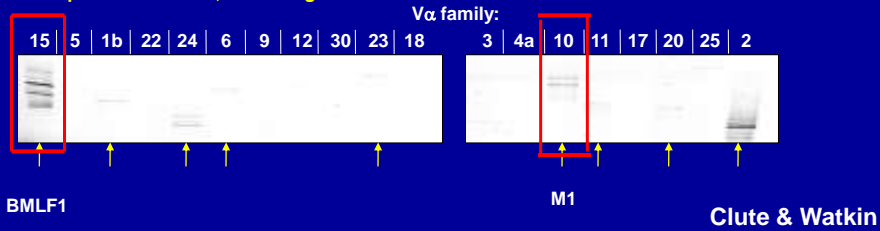
- M1-specific lines, M1+ BMLF1+ gate



- M1-specific lines, M1- BMLF1+ gate



- M1-specific T cell line, BMLF1+ gate



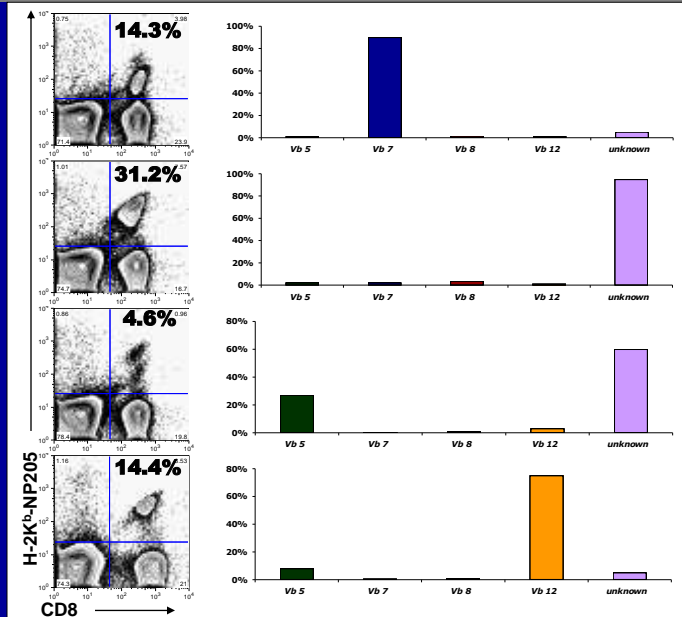
Individual PV+LCMV mice have highly skewed and variable cross-reactive NP205-specific TCR V-beta repertoires

Mouse 1

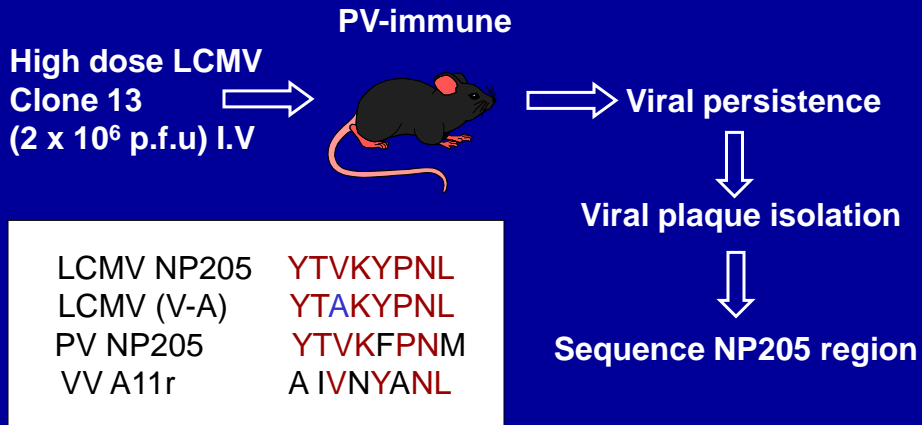
Mouse 2

Mouse 3

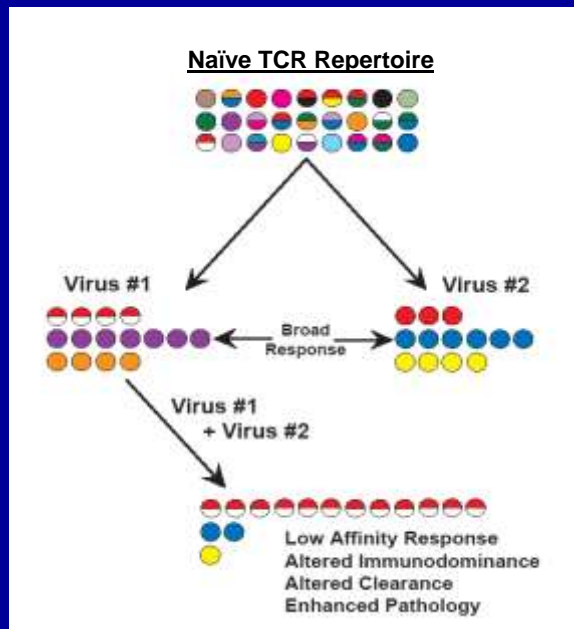
Mouse 4

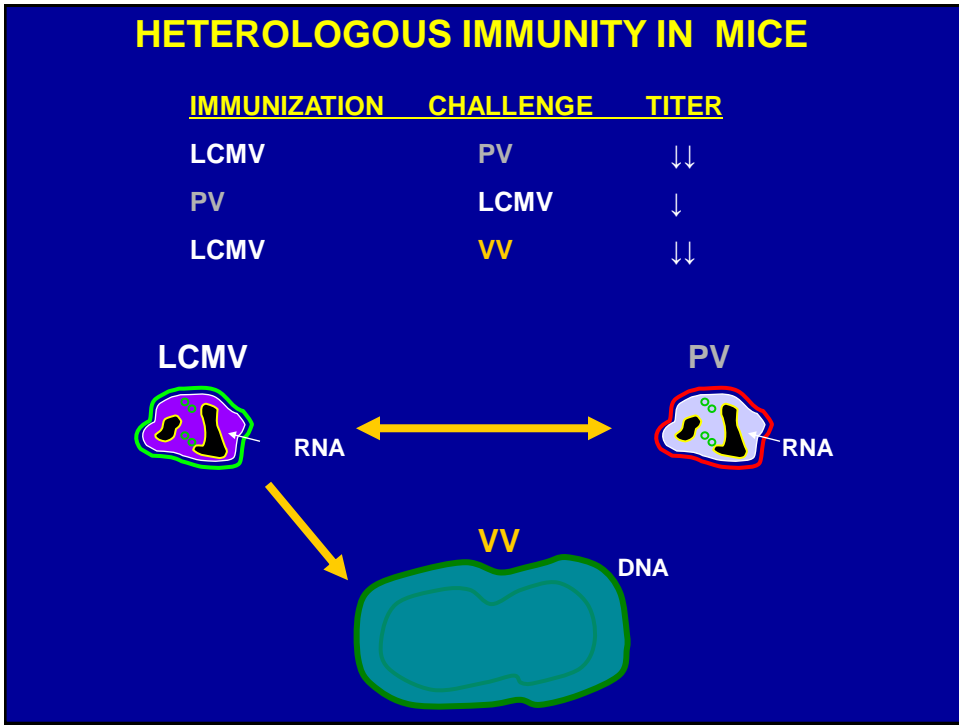
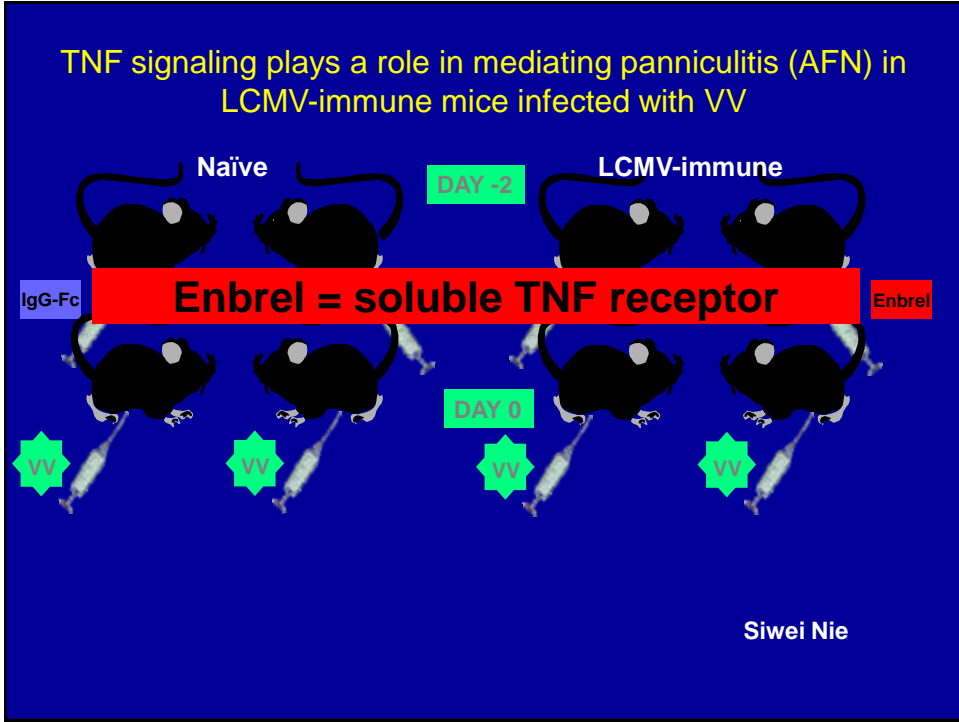


Strategy for selecting LCMV NP205 variants under conditions of a narrow dominant NP205 response



CROSS-REACTIVE T CELLS MAY SKEW TCR REPERTOIRES





NP205 specific CD8 T cell response dominates on day 8, whereas the PV specific NP38 response increases until day 134 after PV infection in LCMV-immune mice

Blood, same mouse is followed overtime

Anke Kraft

Naïve B6 Mice infected with PV

LCMV-Immune B6 Mice infected with PV

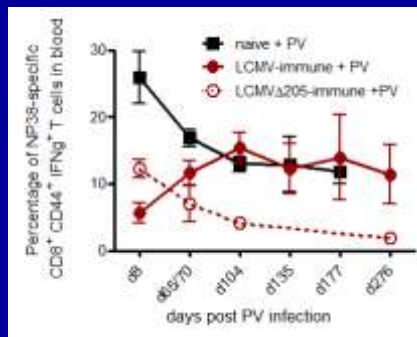
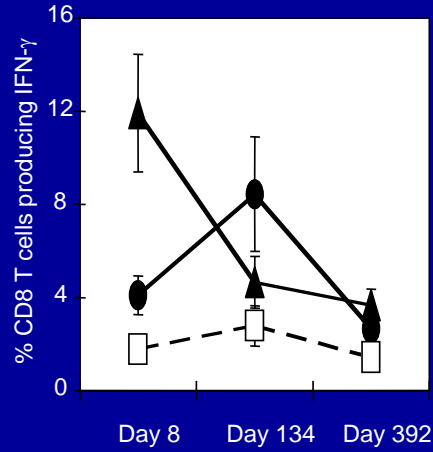
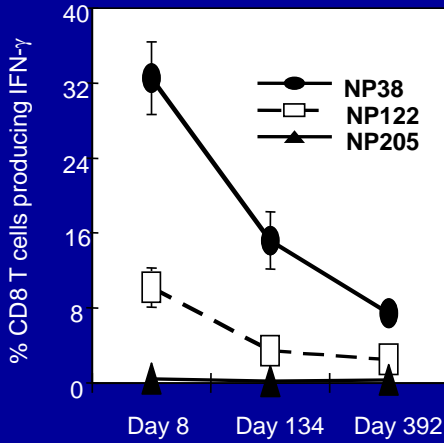


Figure 5: LCMV-immune mice lacking the cross-reactive NP205 epitope did not develop an inflationary NP38-specific CD8⁺ T cell response
 Naive, LCMV or LCMVΔ205-immune mice were infected with PV and blood was collected sequentially at the indicated time-points post infection. PBMCs were stimulated with NP38-specific CD8⁺ T cell peptides and stained intracellularly for IFN γ . Data are cumulative from 1-3 separate experiments. Error bars represent the mean \pm SEM with n=4-12 mice per group.

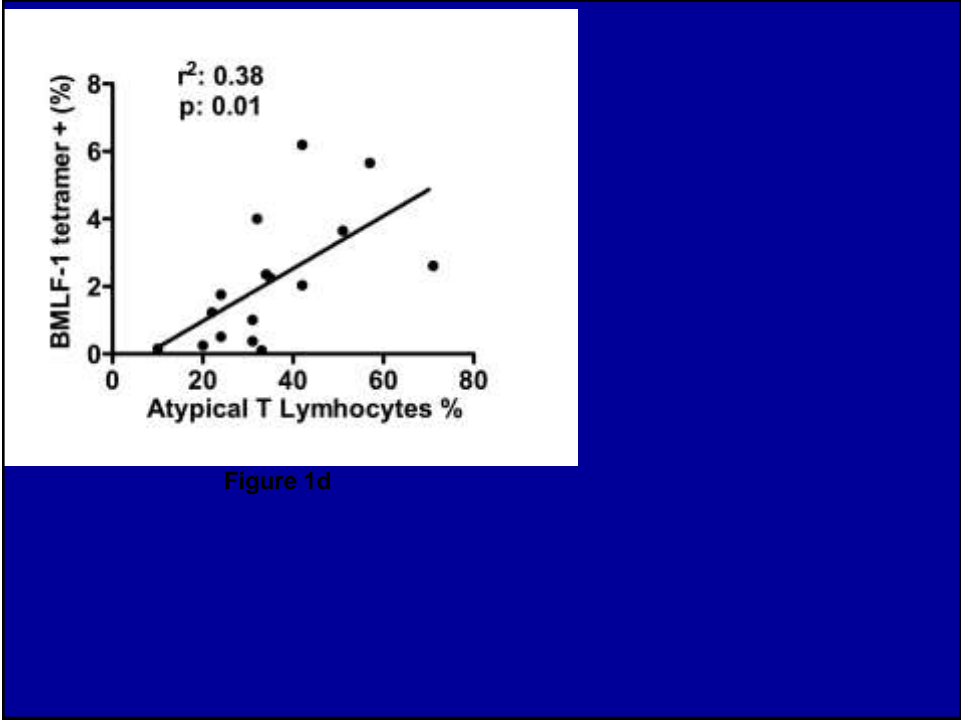


Figure 1d

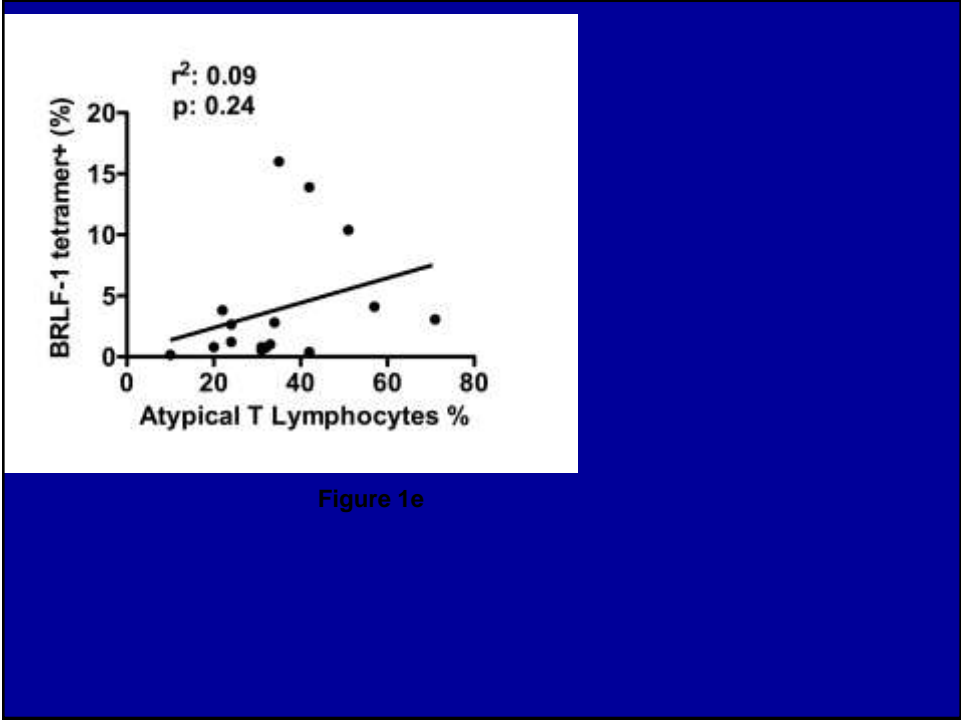


Figure 1e

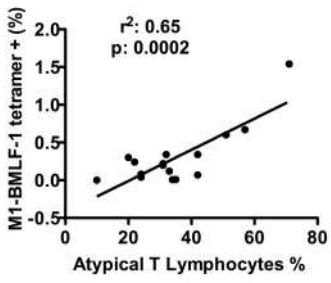


Fig 3 Frequency of CXR M1/BMLF1 tet+ CD8 cells in vivo directly correlated with the severity of IM.

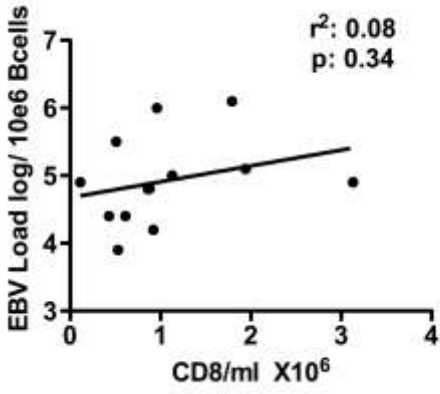


Figure 1g

