The development and validation of HLA transgenic mice

Outline

- MHC molecules. Antigen presentation and Disease association
- HLA transgenic mice. Historical views and the issues of CD4/CD8 and endogenous mouse expression
- Validating studies. Processing and Immunodominance
- Transgenic expressing different alleles. The concept of HLA Superytpes
- Experimental details. Breeding, Testing and Study Design

Disclaimer

- The following slides are an **informal** account of my **personal** experience in developing and using HLA transgenics from the 90's to date
- My **opinions** are not necessarily reflective of Taconics opinions, nor do I necessarily endorse their mice

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Structure of MHC molecules

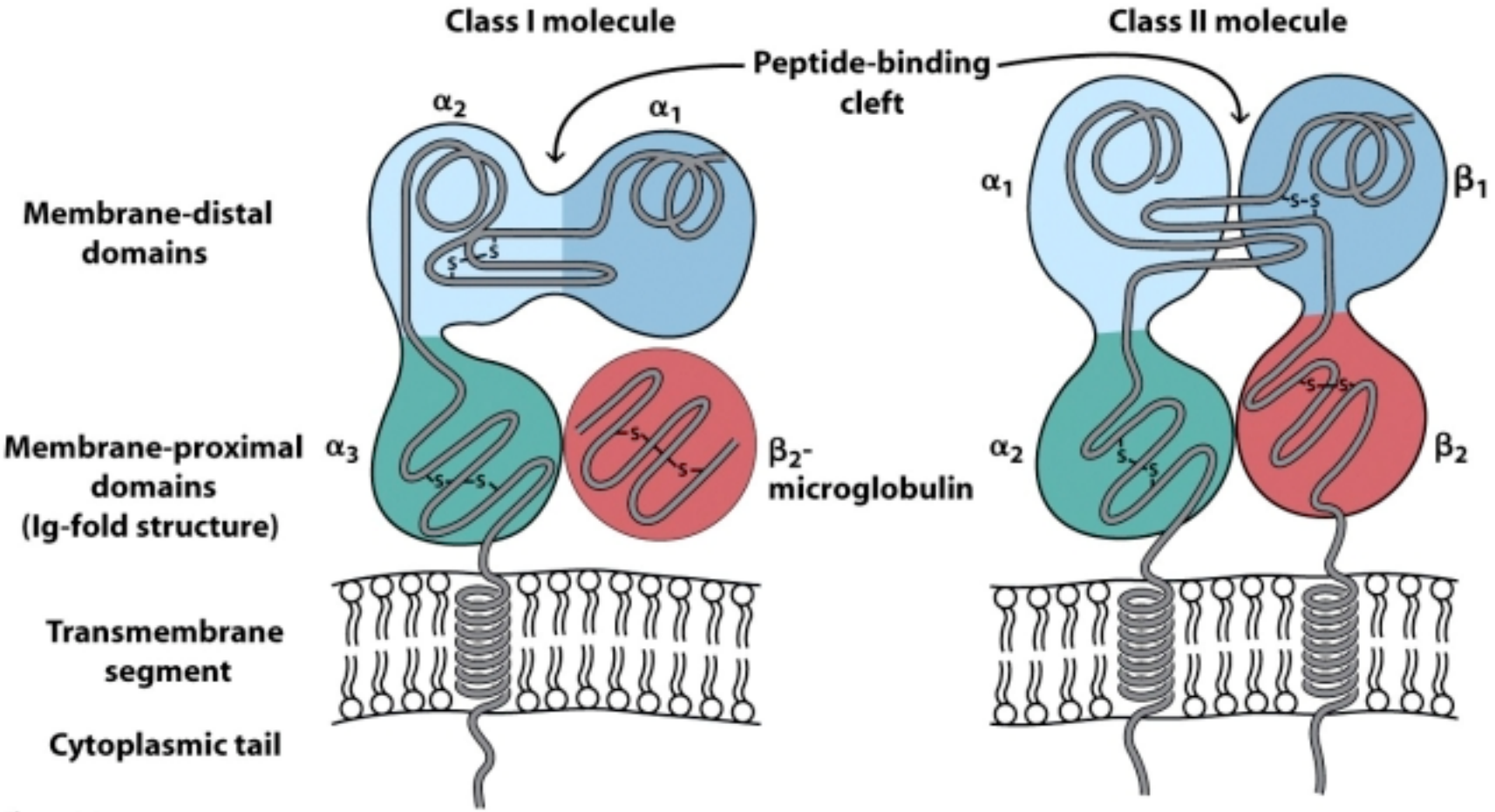
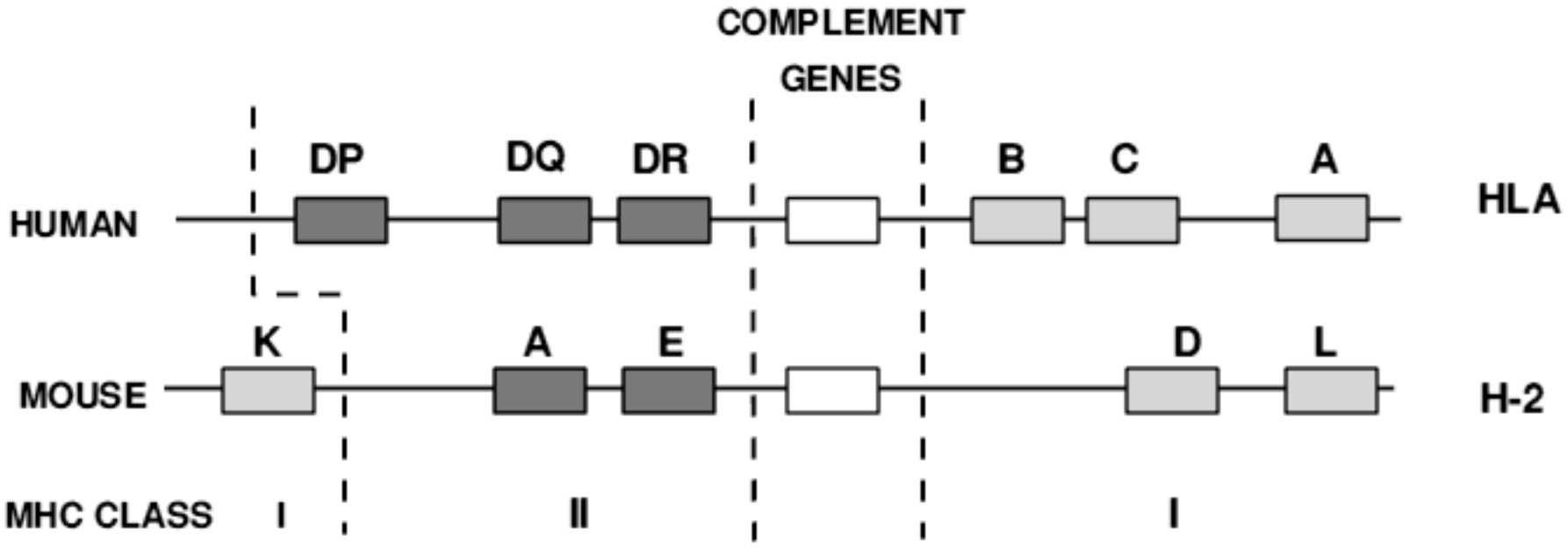


Figure 8-3
Kuby IMMUNOLOGY, Sixth Edition

Class I and Class II MHC genes

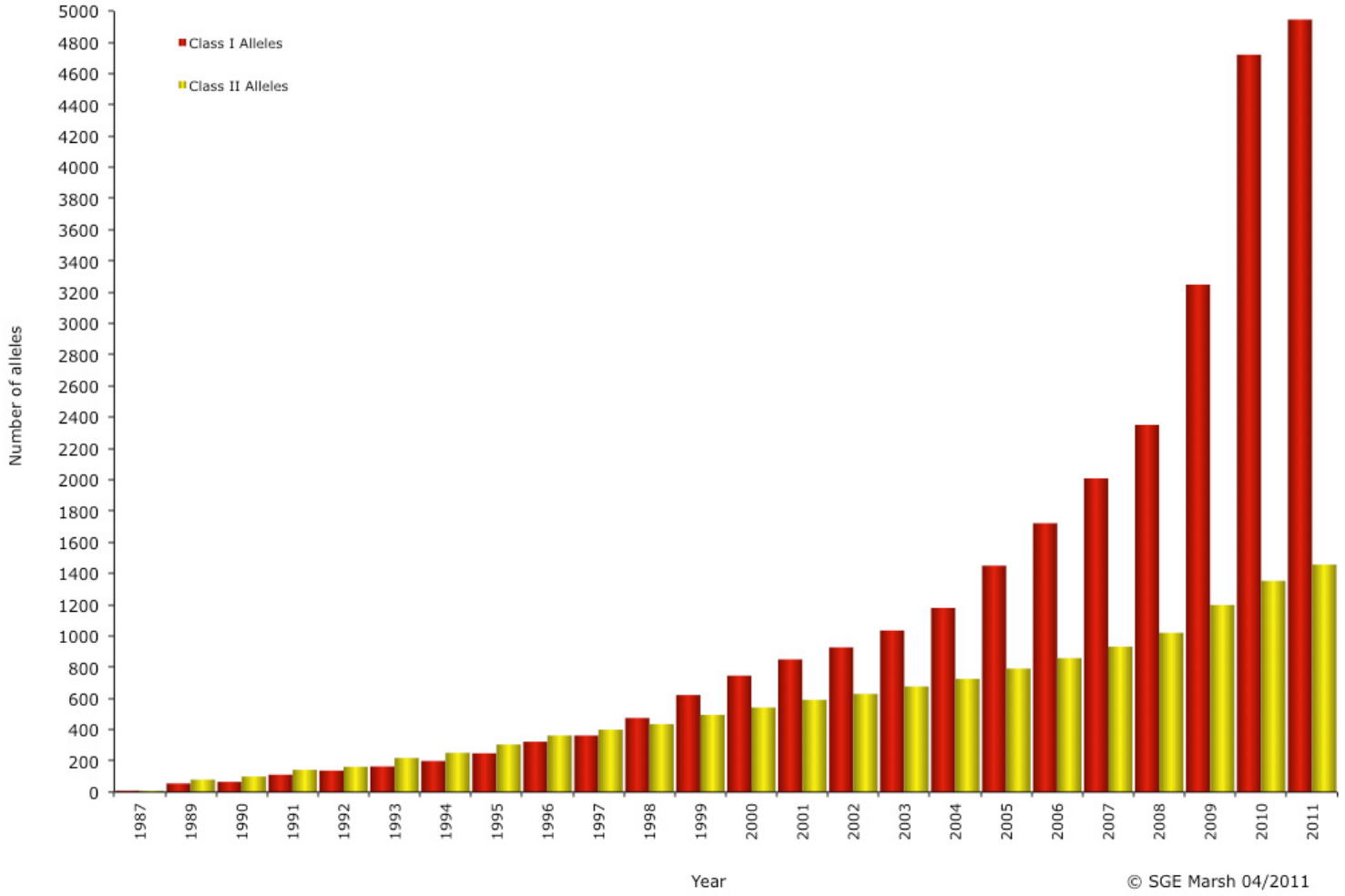


Class I and Class II Differences

- Class I
  - Expressed on surface of all nucleated cells and platelets
  - Present antigens to CD8+ T cells
  - Antigens derived from cytosolic proteins (“endogenous” antigens)

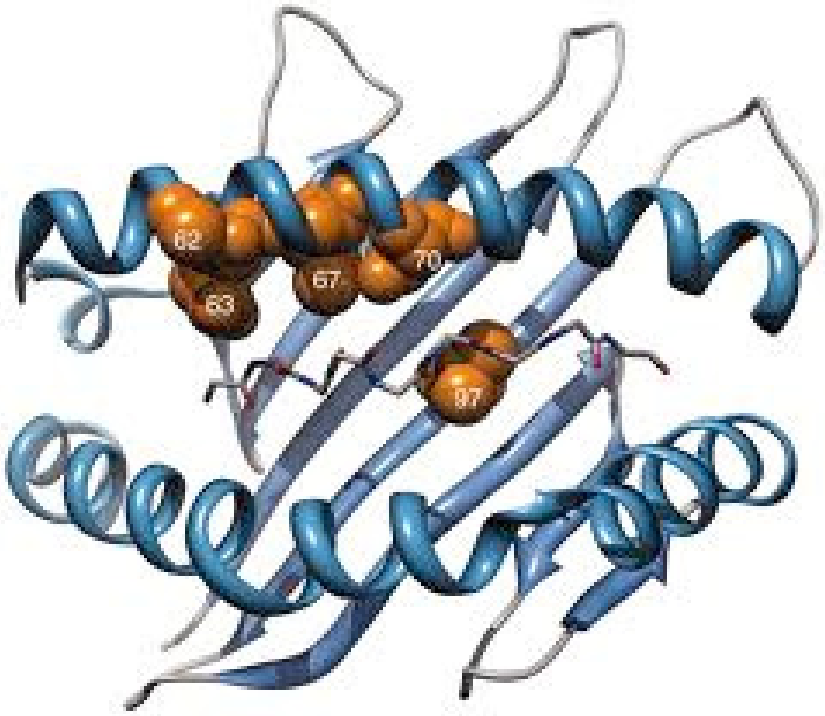
- Class II
  - Expressed mostly on surface of professional antigen presenting cells such as B cells, macrophages and dendritic cells
  - Present antigens to CD4+ T cells
  - Antigens derived from proteins present in endosomes or lysosomes (“exogenous” antigens)

The issue of HLA polymorphism (polygenic, polylocus and allelic)



- http://hla.alleles.org/nomenclature/index.html

The issue of HLA polymorphism (Variation is in the pockets)



sciencemag.org

Associations of HLA Serotype with Susceptibility to Autoimmune Disease

DISEASE	HLA allele	Relative Risk	Sex Ratio (♀:♂)
Ankylosing Spondylitis	B27	87.4	0.3
Acute Anterior Uveitis	B27	10	<0.5
Goodpasture's Syndrome	DR2	15.9	~1
Multiple Sclerosis	DR2	4.8	10
Graves' Disease	DR3	3.7	4–5
Myasthenia Gravis	DR3	2.5	~1
Systemic Lupus Erythematosus	DR3	5.8	10–20
Type I Insulin-dependent Diabetes Mellitus	DR3/DR4 heterozygote	~25	~1
Rheumatoid Arthritis	DR4	4.2	3
Pemphigus Vulgaris	DR4	14.4	~1
Hashimoto's Thyroiditis	DR5	3.2	4–5

ncbi.nlm.nih.gov

HLA molecules and disease resistance

HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Stephen A. Migueles,† et al.

Proc Natl Acad Sci U S A. 2000 March 14; 97(6): 2709–2714.

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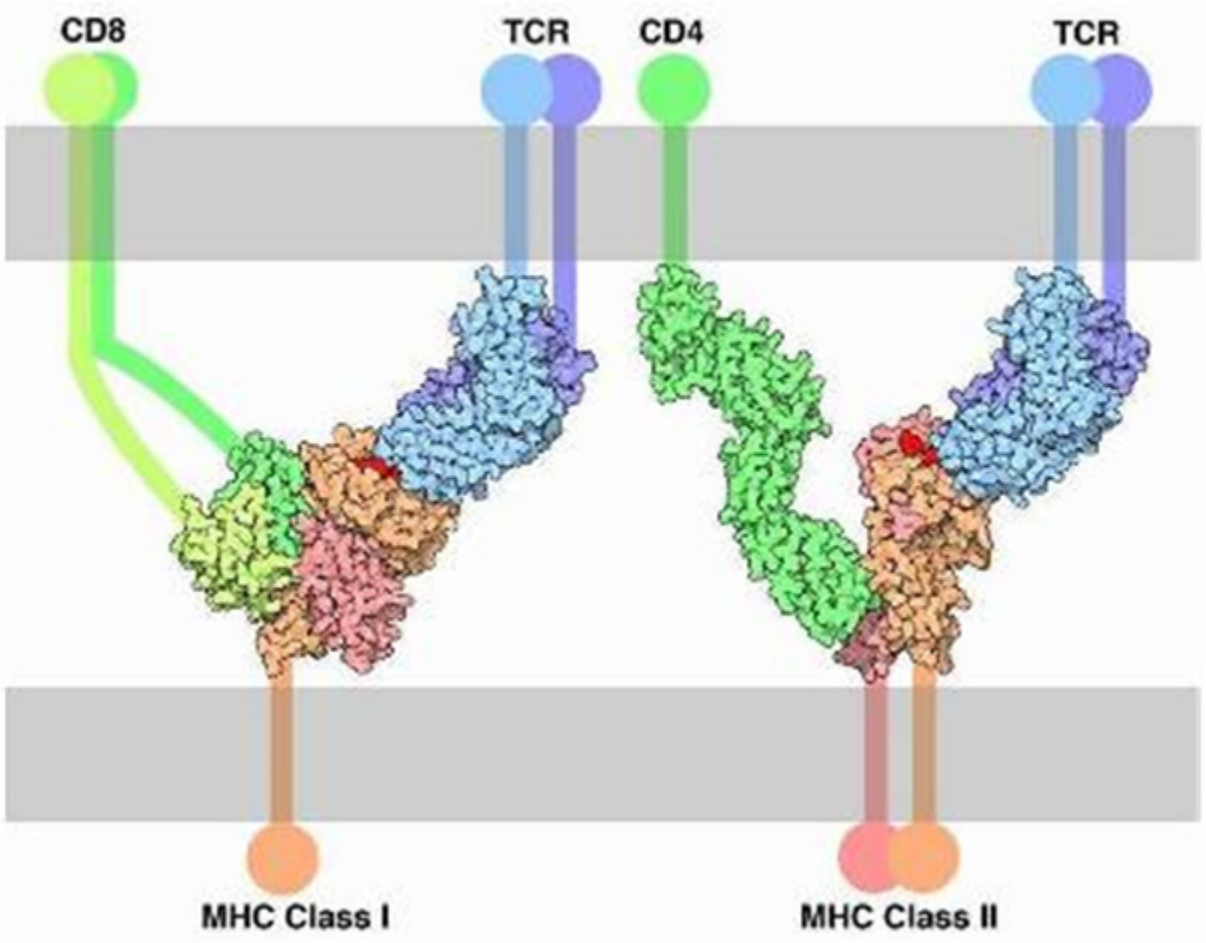
History of HLA transgenic mice

- **Some of the early stuff**
  - **HLA-restricted recognition of viral antigens in HLA transgenic mice 1987 H. Ploegh**
  - **B7 (Chamberlain), Cw3 (Hammerling), A2 (Engelhard); 1988**
- **But….**
  - **“the frequency of HLA-A2.1-restricted, influenza specific CTLp was substantially lower than the frequency of H-2b restricted CTLp, indicating a poor utilization of HLA-A2.1 as a restricting element…”**

The issue of CD4 and CD8



dokuwiki.noctrl.edu

Two solutions

- Make transgenics also for Hu CD8 or CD4
- Make mice expressing chimeric humanA1/A2 but mouse A3 domains
  - Analysis of the HLA-restricted influenza-specific cytotoxic T lymphocyte response in transgenic mice carrying a chimeric human-mouse class I major histocompatibility complex. Vitiello et al. J Exp Med. 1991 Apr 1;173(4):1007-15.

The issue of co-expression of murine MHC molecules

- Murine MHC is generally expressed at higher levels
- Potential for inhibiting HLA restricted responses (immundominance)
- Solution: make mice that express only the HLA transgene (no H2)
- Lemonnier and collaborators pioneered for Class I; Chella David for class II

Some additional considerations and personal viewpoints

- Mice without MHC expression have substantial alterations in thymic education
- Heterozygosity does not seem to impair responses
- In my personal experience lack of expression of murine MHC appears necessary for class II, but not at all for class I

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Validation of the mice

- Expression of HLA does not automatically makes mice a good model for HLA responses
- Is processing the same?
- Is dominance the same?

Is processing the same? It depends…

- Remarkable similarity between human and murine processing apparatuses
- Similar natural processed ligands
  - Falke and coworkers showed this to be the case for HLA A2*01
- In general this is a good approximation but…
  - TAP differences exist between murine and human molecules
  - Positive charged C-termini not efficiently transported by mouse TAP
- Caution with HLA-A11 and other molecules

Is Immunodominance the same? It depends…..

- The issue was experimentally addressed by several studies
- Wenthworth et al compared the repertoire of HLA transgenic mice and humans
  - Wentworth, P.A., Vitiello, A., Sidney, J., Keogh, E., Chesnut, R.W., Grey, H.M., and Sette, A. Differences and similarities in the A2.1-restricted cytotoxic T cells repertoire in humans and human leukocyte antigen transgenic mice. *Eur J Immunol*. 1996 26:97.
- Additional studies for several other strains showing that in general immunogenicity is very similar
- But beware of self/nonself (important for tumor vaccines)
- However…the answer for immunodominance is a bit more complex

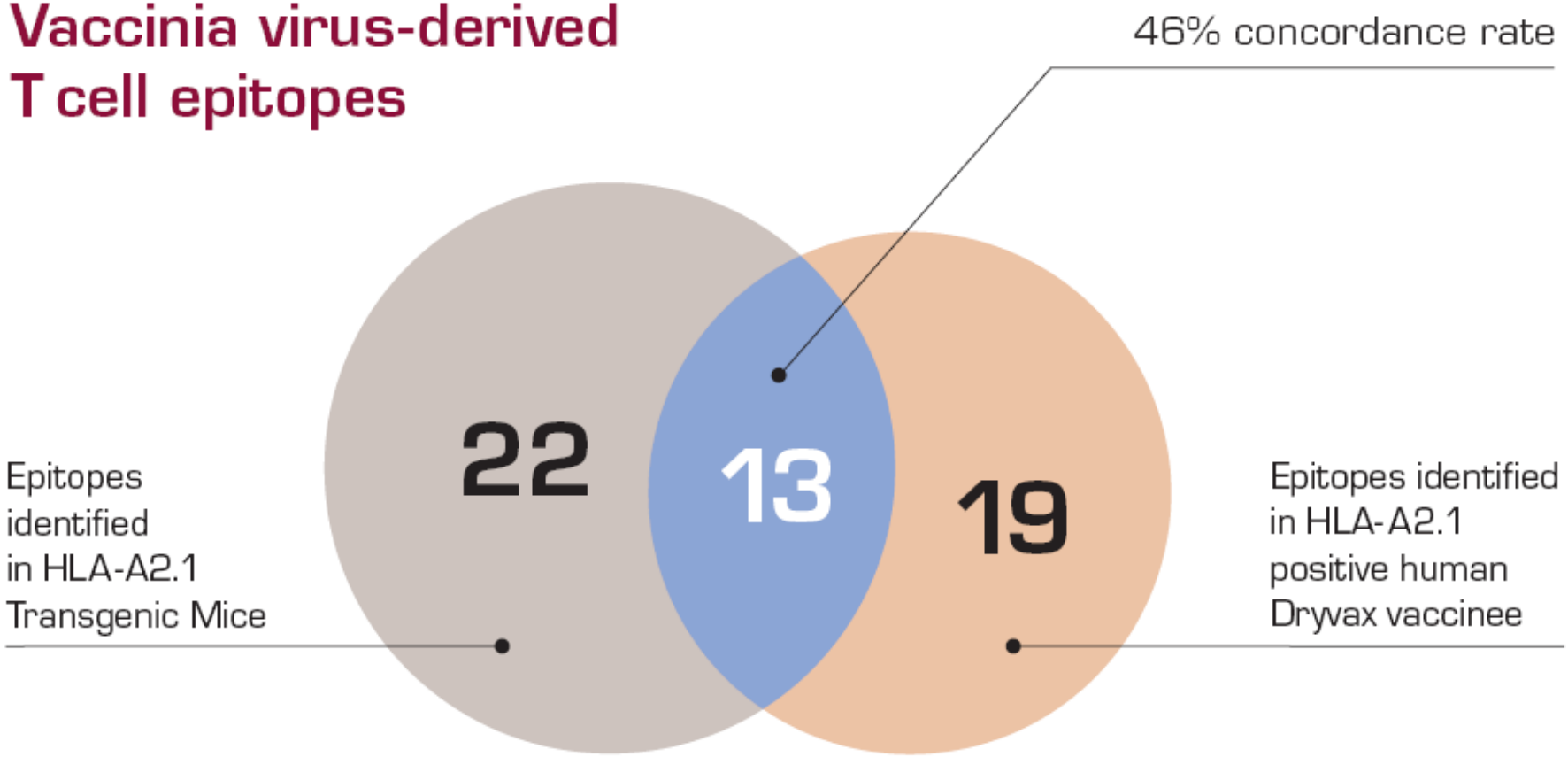
Immunodominance and HLA transgenic mice

- The more complex the system, the more complex the situation
  - Ennis FA, Immunol Lett. 2006, 105(1):97-8
- Also immunodominance in humans is highly variable
- The case of VACV

Immunodominance in VACV

- Kotturi, M.F., Assarsson, E., Peters, B., Grey, H., Oseroff, C., Pasquetto, V., and Sette, A. Of mice and humans: how good are HLA transgenic mice as a model of human immune responses? *Immunome Res*. 2009 Jun 17;5:3. PMCID: PMC2702351

Vaccinia virus-derived T cell epitopes



While transgenic HLA mouse models are suitable to model responses to complex pathogens, care should be taken in interpreting the results obtained.

Immunome Res. 2009; 5:3

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HLA Supertypes

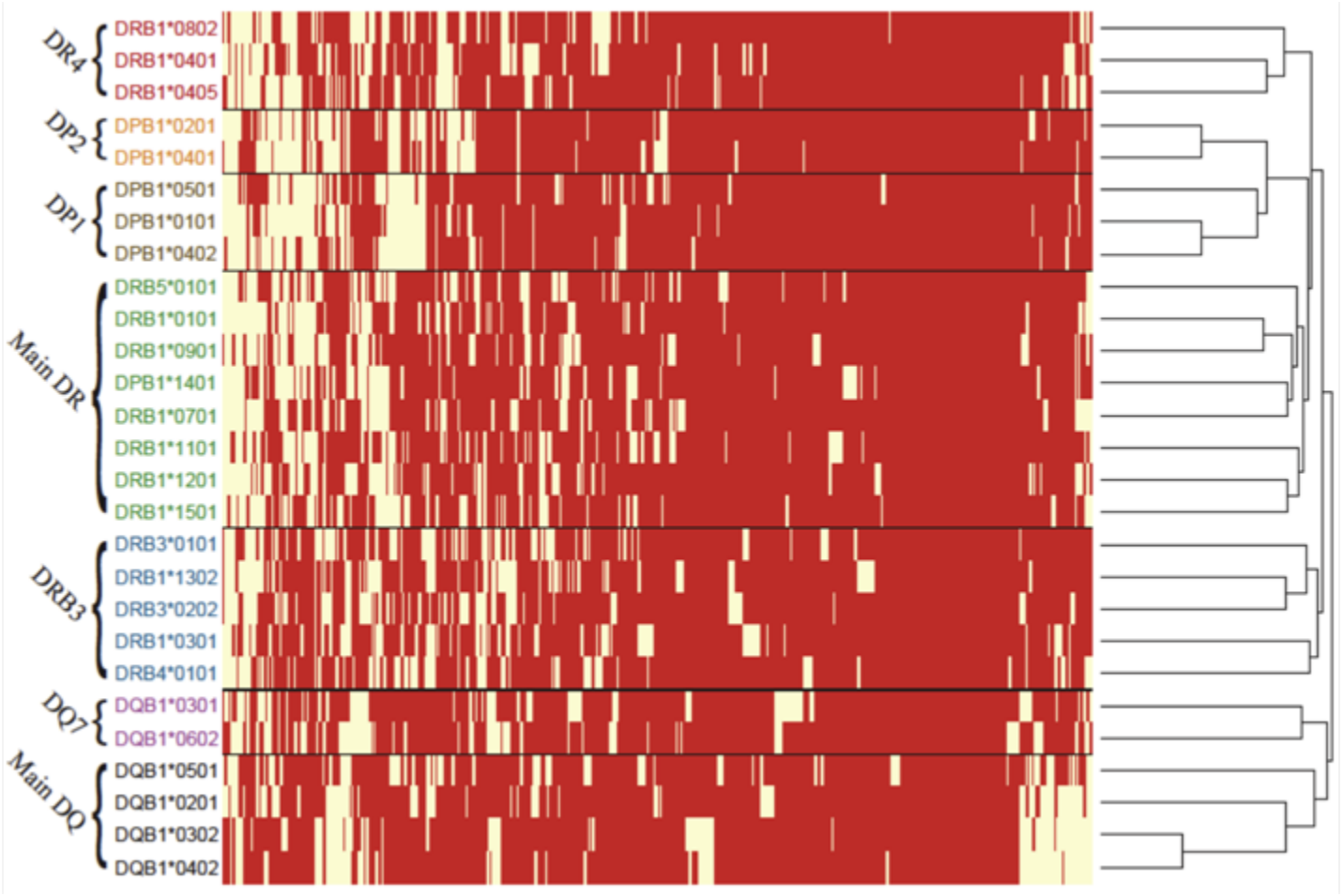
- Defined as families of HLA molecules with overlapping (but distinct) repertoires
- Relevance to diagnostics and vaccines because allow to rationally address the diversity in human populations (and ethnicities)
- Originally described for HLA class I, also apply to HLA class II

Few Major Supertypes Account for the Majority of HLA A and B Types

SPECIFICITY			PHENOTYPIC FREQUENCY *					
Supertype	Position 2	C-terminus	Caucasian	N.A. Black	Japanese	Chinese	Hispanic	Average
A1	TILVMS	FWY	47.1	16.1	21.8	14.7	26.3	25.2
A2	AILMVT	AILMVT	45.8	39.0	42.4	45.9	43.0	43.2
A3	AILMVST	RK	37.5	42.1	45.8	52.7	43.1	44.2
A24	YFWIVLMT	FIYWLM	23.9	38.9	58.6	40.1	38.3	40.0
B7	P	AILMVFWY	43.2	55.1	57.1	43.0	49.3	49.5
B44	E	FWYLIMVA	43.0	21.2	42.9	39.1	39.0	37.0
B27	RHK	FYLWMI	28.4	26.1	13.3	13.9	35.3	23.4
B62	QLIVMP	FWYMIV	12.6	4.8	36.5	25.4	11.1	18.1
B58	ATS	FWYLIV	10.0	25.1	1.6	9.0	5.9	10.3
A1, A2, A3 and A24 only			98.4	94.2	99.9	98.5	97.6	97.8
A1, A2, A3, A24, B7 and B44 only			99.5	98.1	100.0	99.5	99.4	99.3
TOTAL			**99.9**	**99.6**	**100.0**	**99.8**	**99.9**	**99.8**

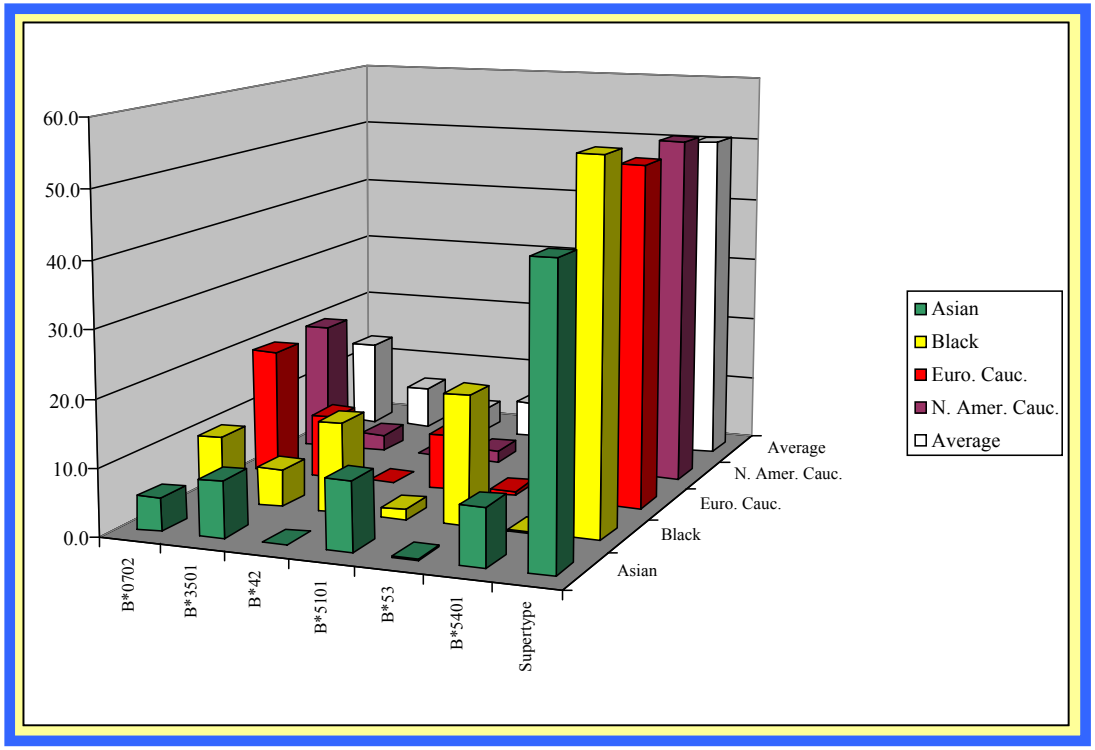
* For derivation of phenotypic frequencies see Sidney et al., 2001.

HLA class II supertpes



Greenbaum et al Immunogenetics 2011

Relevance Of HLA Supertypes for Diagnostics & Epitope Identification



- The frequency of each allele in a supertype may vary dramatically amongst different populations, but the frequency of each supertype is relatively constant.

HLA transgenics and Class I Supertypes

Supertype	*Prototype Allele*	Reference
A1	A*0101	Taconic
A2	A*0201	Taconic
A3	A*1101	Taconic
A24	A*2401	Taconic
B7	B*0702	Taconic
B27	B*2701	C.David; Chamberlain
B44	B*4002	Taconic

It would be nice to have either a B57/58
or a B62 supertype representative (B*1501)

HLA transgenics and Class II Supertypes

Supertype	Prototype Allele	Reference
DR1	B1*0101	Altmann
DR4	B1*0401	Taconics, Sonderstrup, David
DR3	B1*0301	Altmann
DPmain	?	Tsuji K (?)
DP2	?	Tsuji K (?)
DQmain	DQ2 and DQ8	David
DQ7	DQ6	David

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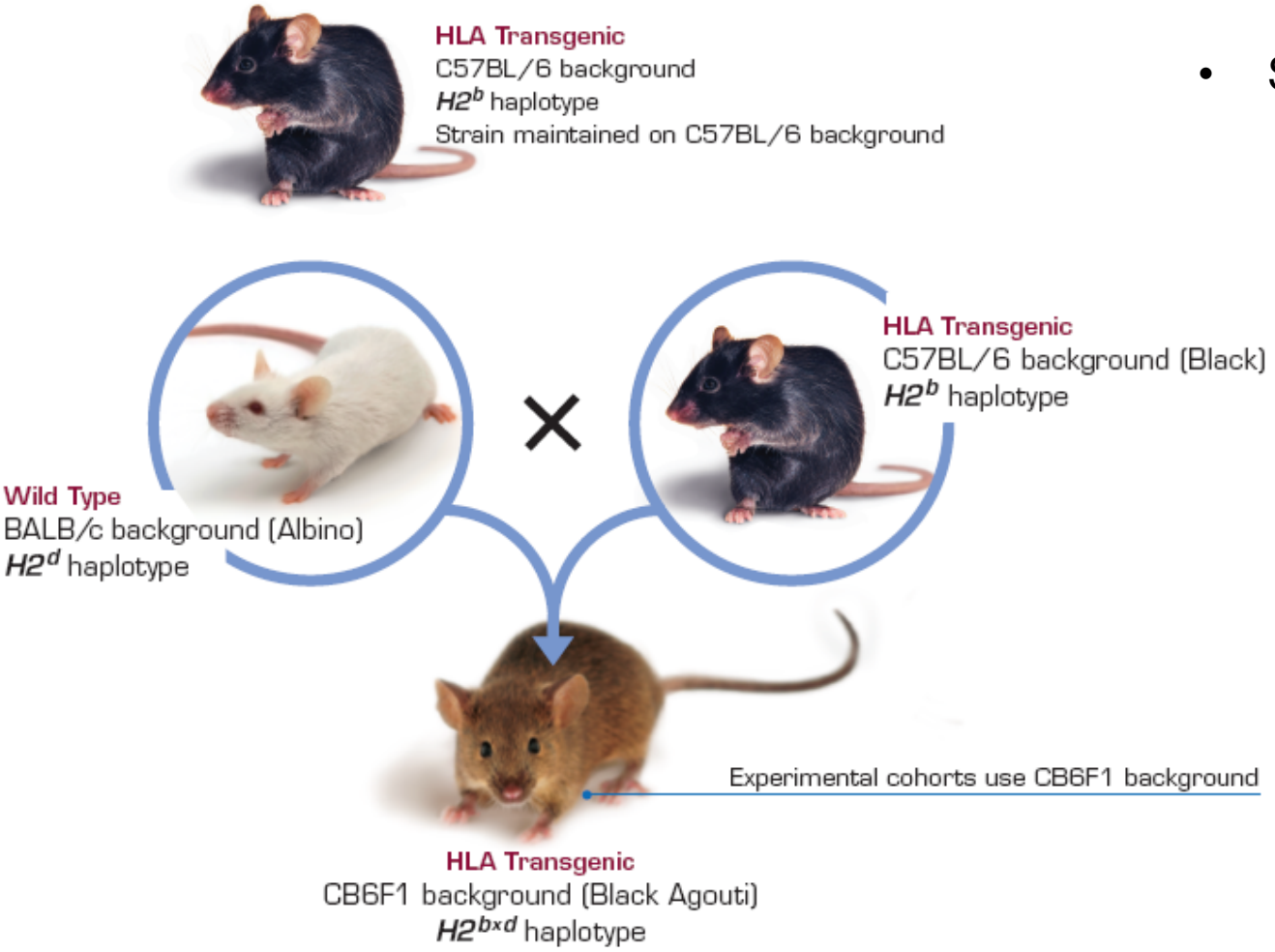
Experimental details. Breeding, Testing and Study Design

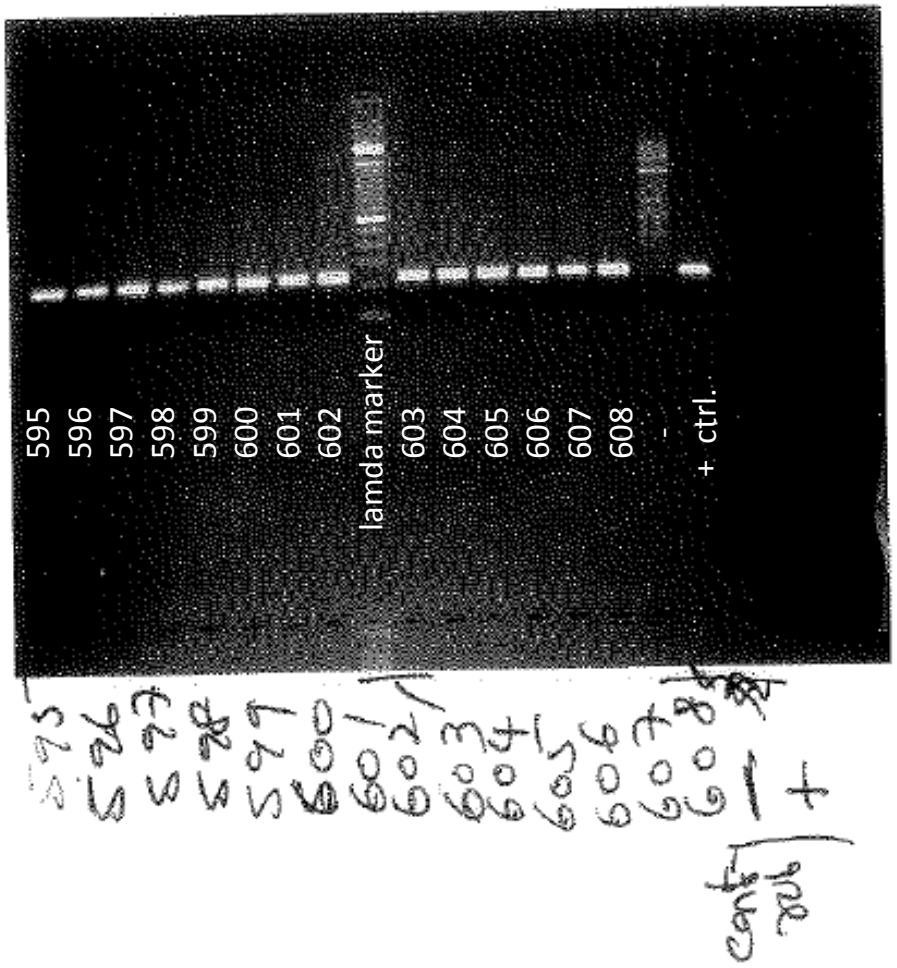


- Strain maintenance:
  - We prefer keeping the breeders on the H2b (C57BL/6) background (either with or without murine expression)
  - But for experimental animals we prefer to use BDF1 (BALB/c x C57BL/6 F1) as they breed better and have better responses

Experimental details. Breeding, Testing and Study Design

- Testing Empiricals
  - Either PCR or FACS work
  - PCR for Class II, FACS for class I
  - But it is best to use FACS for breeders as expression tend to drift lower

Example of testing DRB1*0101 expression by PCR



Experimental details. Breeding, Testing and Study Design

- Study Design Tips
  - In general in the case of mice expressing murine *domains* tetramers do not work well (remember…CD4/8 issues)
  - In the case of mice co-expressing murine *molecules* need to establish restriction
    - Best show that T cells recognize an APC expressing the transgene AND do not recognize one not expressing the transgene
    - Cases of dual restriction exist….

Some specific examples of HLA transgenic mice use

- Vaccine testing
  - Use in testing and releasing human vaccines for which an epitope based response is expected
- Animal models
  - In vivo study of responses against specific human pathogens

HLA transgenic mice can be used to develop and test vaccine constructs

- CTL responses important for vaccines targeting diseases such as hepatitis B and C, HIV and cancer
- Typical vaccines based on killed or inactivated pathogens or recomb. or purified proteins are ineffective at inducing CTL responses
- Potential solution is small multiepitope peptides which can induce CTL and HTL responses

Epitope	Sequence	MHC Restriction
HBVpol149	HTLWKAGILYK	A11
PADRE	AKFVAAWTLKAAA	IA^2
HBVcore18	FLPSDFFPSV	A2.1
HIVenv120	KLTPLCVTL	A2.1
HBVpol551-V	YMDDVVLGV	A2.1
HBVpol455	GLSRYVARL	A2.1
HIVpol476	ILKEPVHGV	A2.1
HBVcore141	STLPETTVVRR	A11
HIVenv49	TVYYGVPVWK	A11
HBVenv335	WLSLLVPFV	A2.1

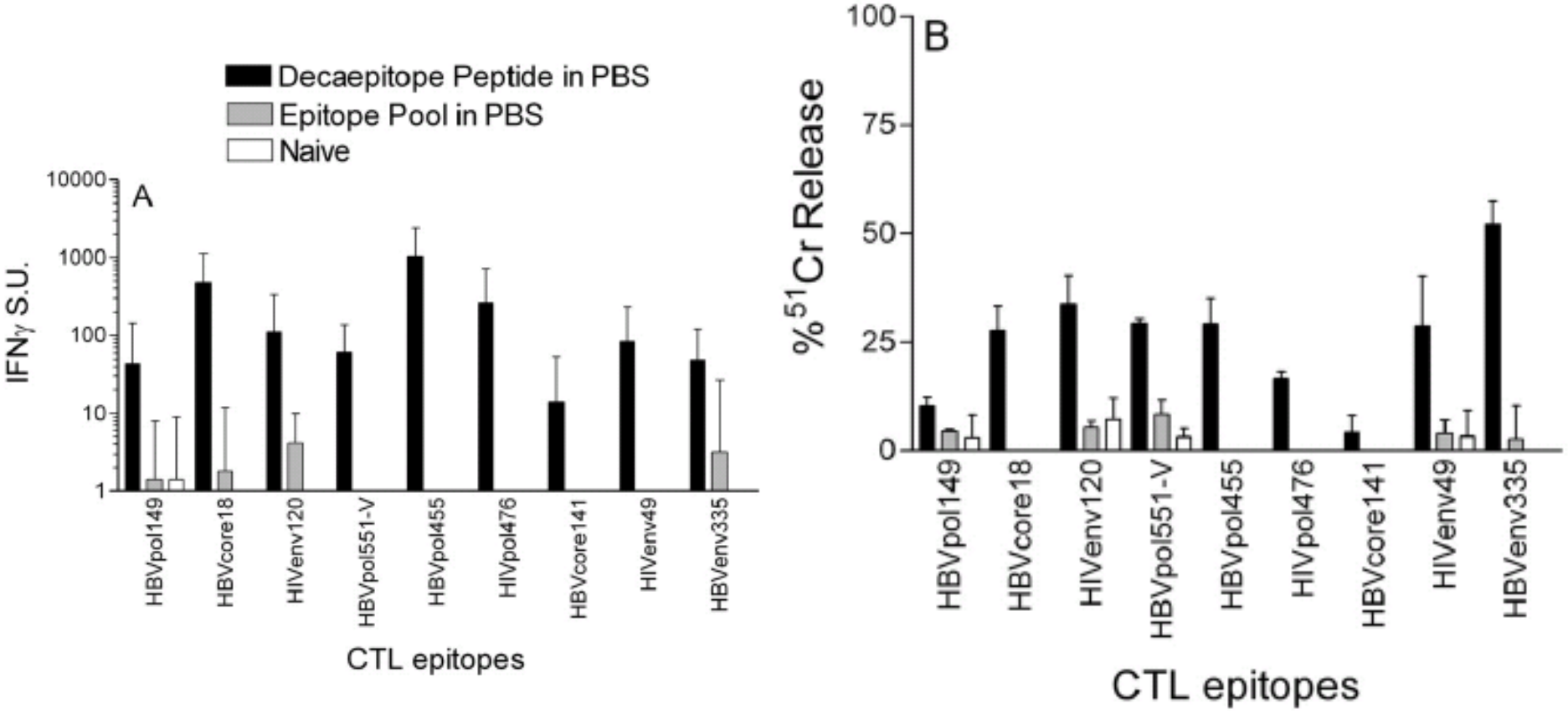
J Immunol. 2002, 168(12):6189-98.

Experimental Setup

- HLA transgenic mice immunized
- splenocytes harvested 11-14 days later and cultured in vitro
- splenocytes stimulated once with the appropriate epitope (A2.1- or A11-restricted)
- IFN-γ production or lysis of peptide-coated cells measured

J Immunol. 2002, 168(12):6189-98.

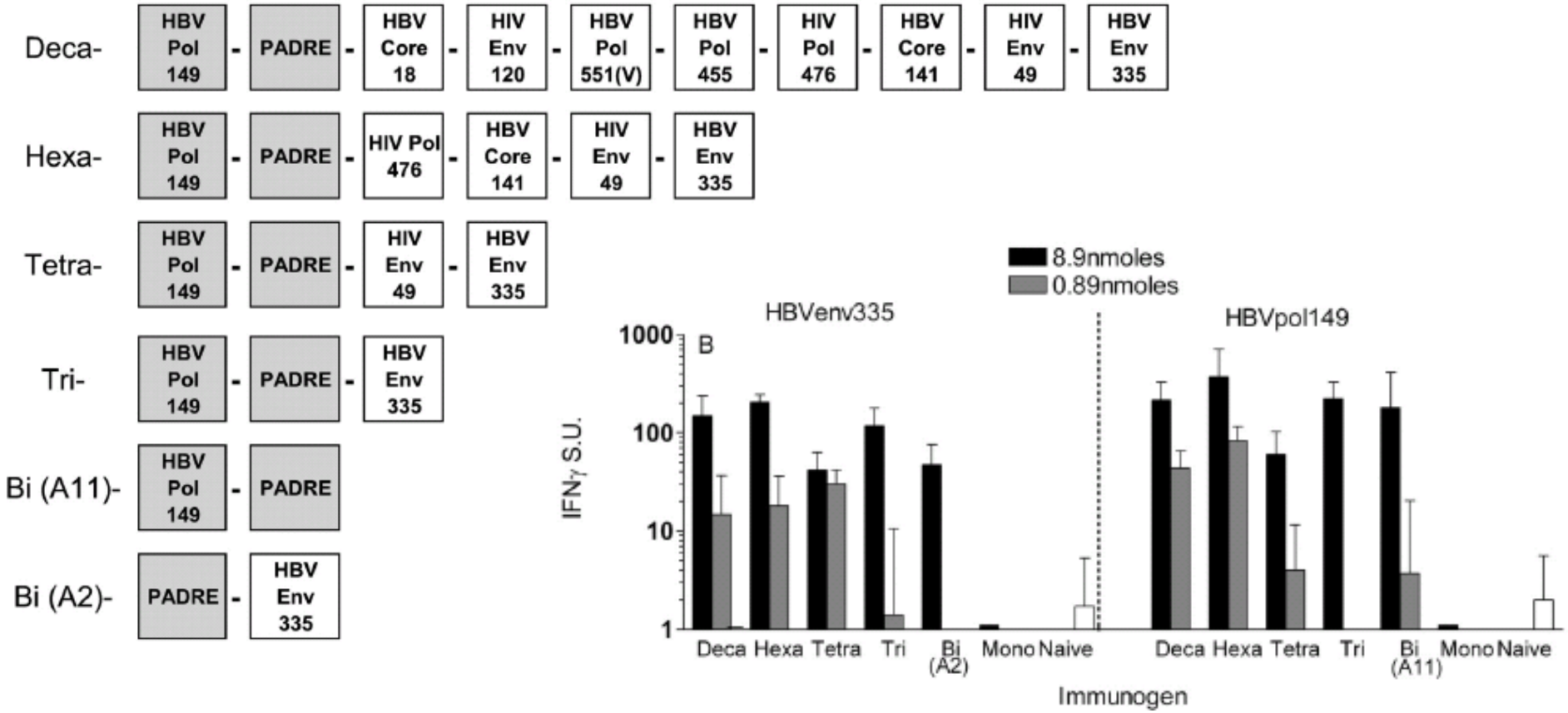
Immunogenicity testing in HLA transgenic mice



Multiepitope peptides are more immunogenic in HLA transgenic mice compared to individual epitopes. Splenocytes from immunized HLA transgenic mice are cytotoxic.

J Immunol. 2002, 168(12):6189-98.

HLA transgenic mice can be used to identify vaccine development strategies



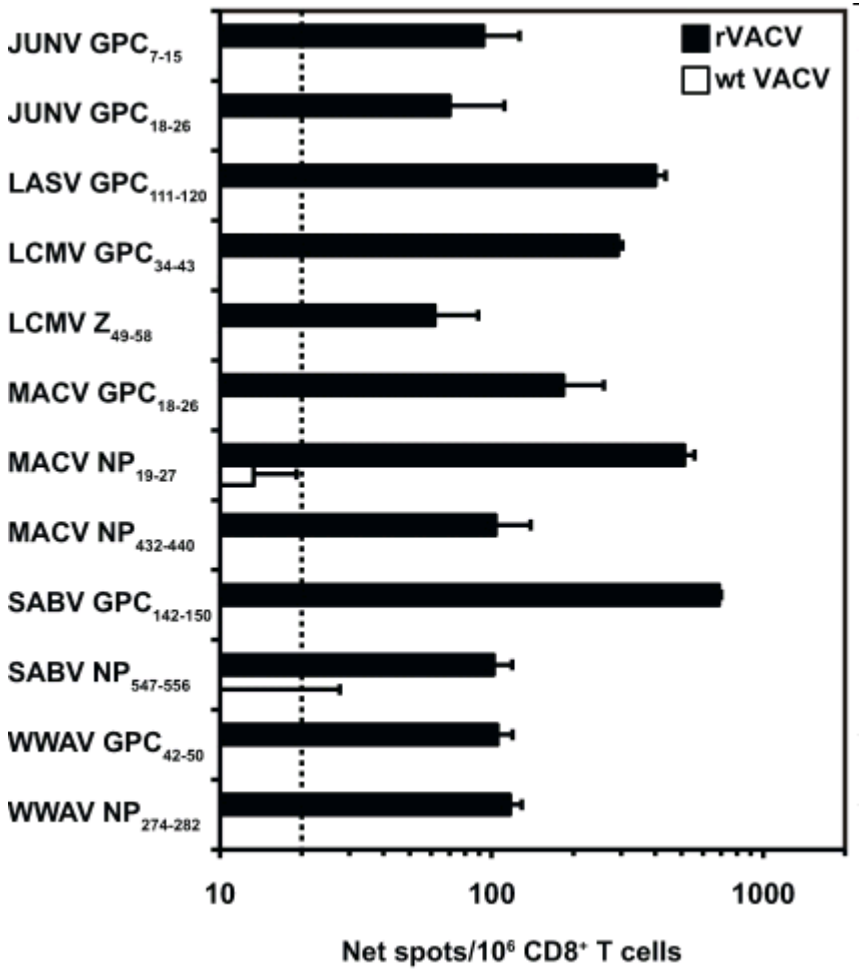
Immunogenicity is a function of both dose and immunogen size.

J Immunol. 2002, 168(12):6189-98.

HLA transgenic mice for use in epitope identification

- HLA transgenic mice can be used to identify epitopes and study protective capacity of human epitopes from lethal challenges
- HLA transgenic mice can be used in combination with epitope predictions and in vitro HLA binding assays for epitope identification
- Choose relevant HLA transgenic mice based on the human ethnic/regional populations of interest

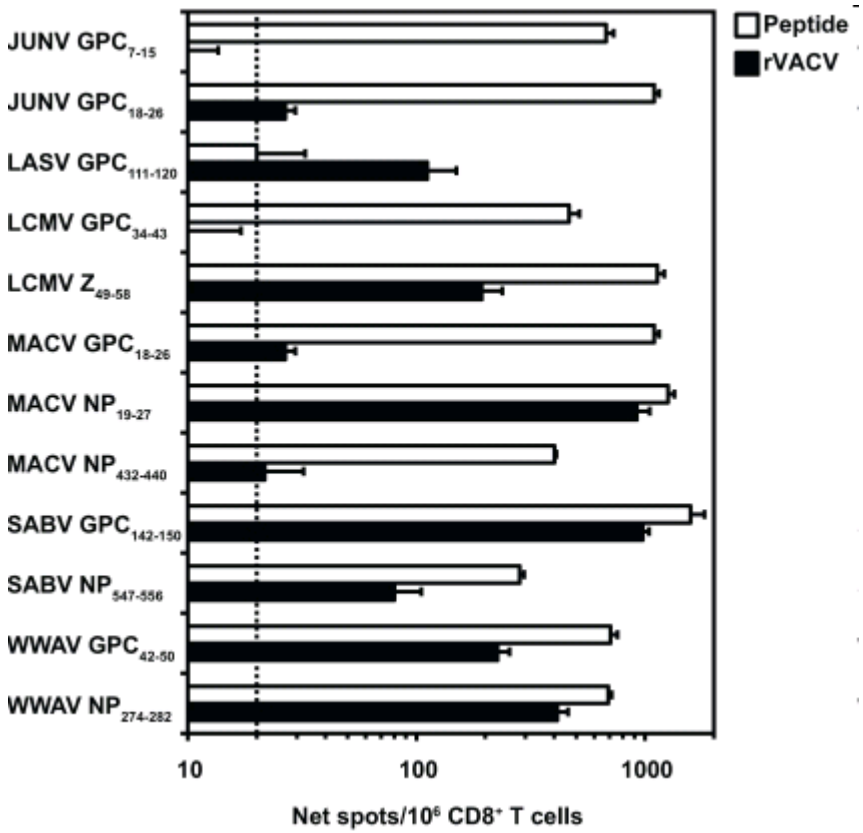
Arenavirus epitope identification using HLA transgenic mice



- Arenavirus protein sequences first screened in silico to identify potential peptides with HLA-A02 affinity of $\leqslant$ 100 nM: 481 peptides identified
- HLA-A2.1 transgenic mice were infected with recombinant vaccinia viruses (rVACV) expressing individual arenavirus proteins or a control wt vaccinia virus
- Purified splenic CD8+ T cells later isolated from these mice and exposed to a human cell line expressing HLA-A2.1, which had been pulsed with one of the 481 identified peptides
- 12 peptides identified as antigenic

PLoS Pathogen. 2009 Dec;5(12):e1000695

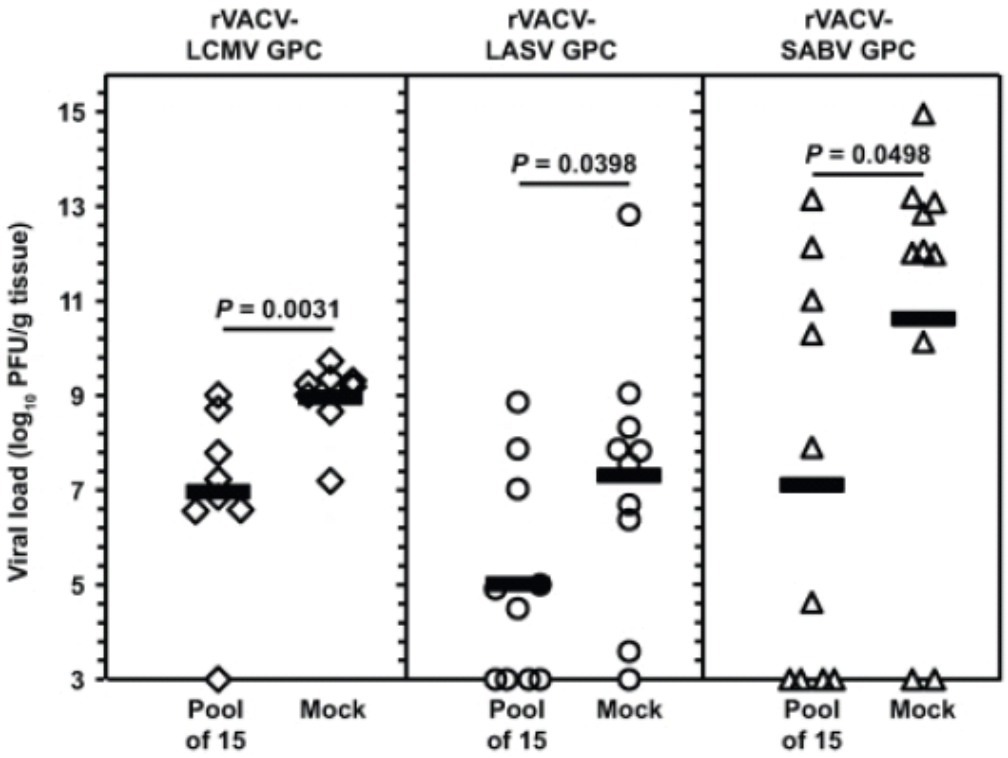
Arenavirus epitope identification using HLA transgenic mice



- HLA-A2.1 mice then immunized with the 12 identified peptides (individually)
- Splenic CD8+ T cells isolated and assayed for recognition of the human target cells expressing the appropriate arenavirus protein antigen
- 7 of the 12 peptides displayed robust response and thus are processed naturally in human APCs

PLoS Pathogen. 2009 Dec;5(12):e1000695

Challenge assays in HLA transgenic mice can confirm utility of identified epitopes



- HLA-A2.1 mice primed with pool of HLA-A2-restricted arenavirus peptides
- Mice then challenged with rVACV expressing arenavirus proteins and viral titer determined

PLoS Pathogen. 2009 Dec;5(12):e1000695

Acknowledgements

- Daisy John
- Carla Oseroff
- Daniela Weiskopf