HLA Class I protection in HTLV-I infection

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1. Introduction
   - The Virus
   - Why study this virus?
   - Aim
2. Method
   - Overall Scheme
   - Epitope Prediction Software
3. Results
   - Software Validation
   - Binding as a predictor of disease
   - Theory to experiment
   - The role of CTLs and NK cells
4. Conclusion

Human T-lymphotropic virus (Type 1)

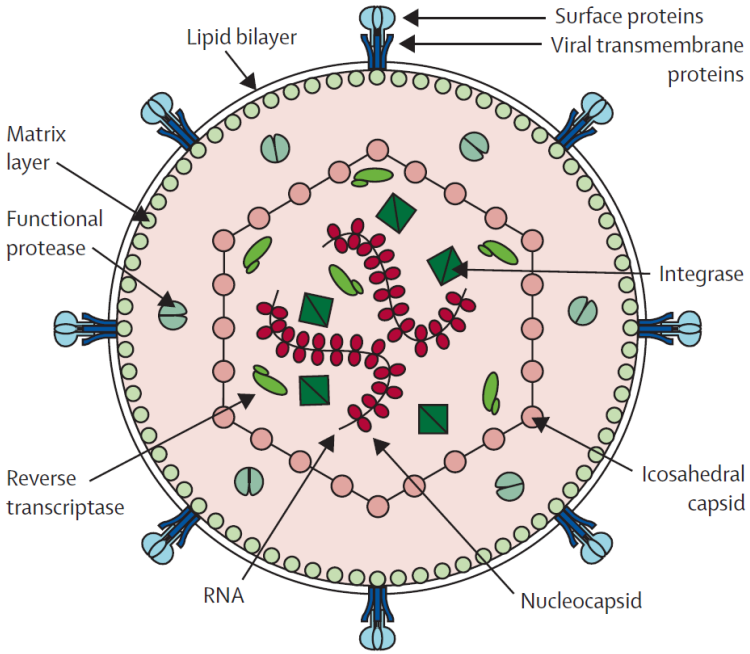

Figure: Stylised Human T-lymphotropic virus

- Family Retroviridae
- Up to 20 million people infected
- Endemic: Japan, South America, Caribbean
- Infects T-cells, mitosis / cell-cell transmission

Individuals Infected with HTLV-I

95%	$\rightarrow$	Asymptomatic Carriers (ACs)
2-3%	$\rightarrow$	Chronic Inflammatory Diseases e.g. HAM/TSP
2-3%	$\rightarrow$	Leukaemia (ATL)

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Question

What determines these different outcomes to infection?

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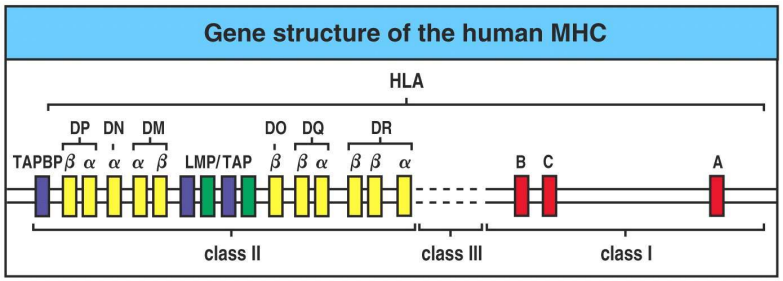
Differences in the host immune response?

- Certain MHC class I alleles confer protection in individuals
- Protective - A*02 and Cw*08
- Detrimental - B*54



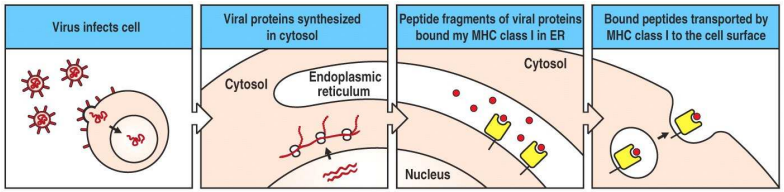


Figure 1-28 Immunobiology, 6/e. (© Garland Science 2005)

What determines these different outcomes to infection?

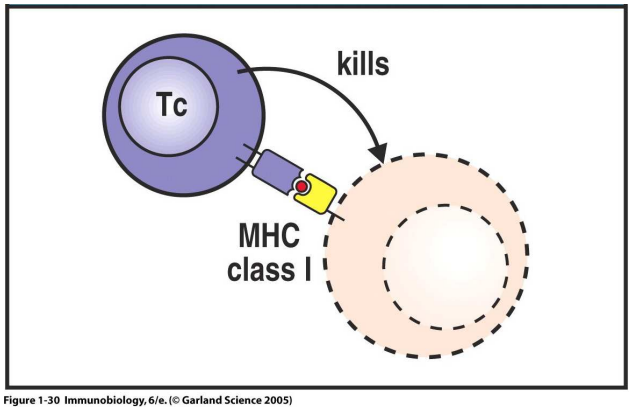


Figure: The T-cell receptor recognizes antigens bound to MHC class I

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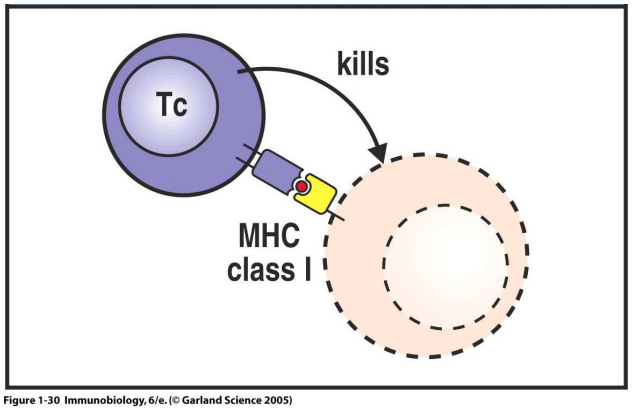


Figure: The T-cell receptor recognizes antigens bound to MHC class I

- Why do specific forms of the MHC class I genotype afford greater protection than others?
- Differences in the cytotoxic T lymphocyte (CTL) immune response that they restrict?

What is the role of the $CD8^+$ T cell response?

- Test and improve existing epitope prediction software in order to predict HTLV-I epitopes
- Test hypotheses about the epitope properties of protective and detrimental alleles
- Model the $CD8^+$ T cell response in terms of its rate of lysis of infected $CD4^+$ T cells
- Further understand the role of CTLs and NK cells in HTLV-I infection

How to answer the question?

Cohort of HTLV-I infected individuals:

- 202 asymptomatic carriers
- 230 HAM/TSP patients
- MHC class I genotype
- Proviral load

$$\begin{array}{lcl} \text{HTLV-I genome} & \rightarrow & \\ & & \text{Predicted Epitopes} \approx \\ \text{MHC Class I} & \rightarrow & \end{array} \quad \begin{array}{l} \\ \\ \text{Proviral Load} \\ \\ \text{Disease Status} \end{array}$$

Software Development

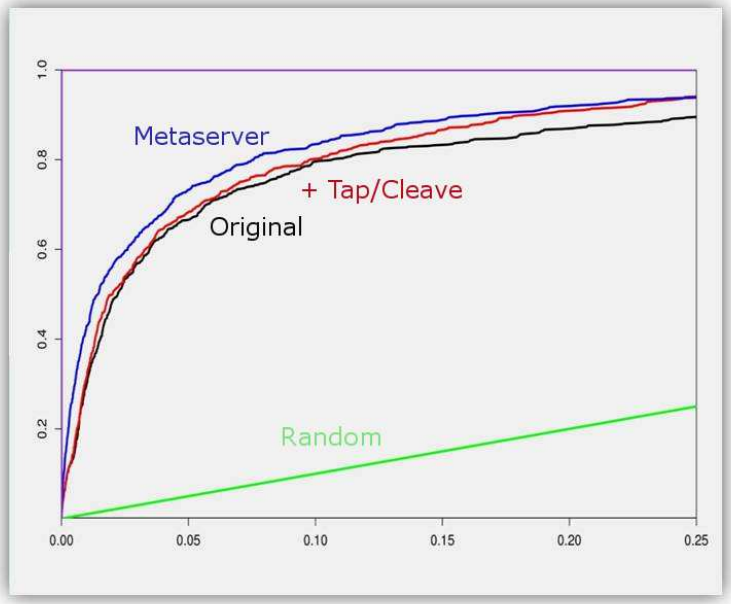


Figure: ROC curve that illustrates epitope prediction performance

Current Software

- NetMHC - predicts peptide-MHC binding
- NetCTL - also predicts TAP and cleavage events
- Normalise predicted binding affinities

Software Development

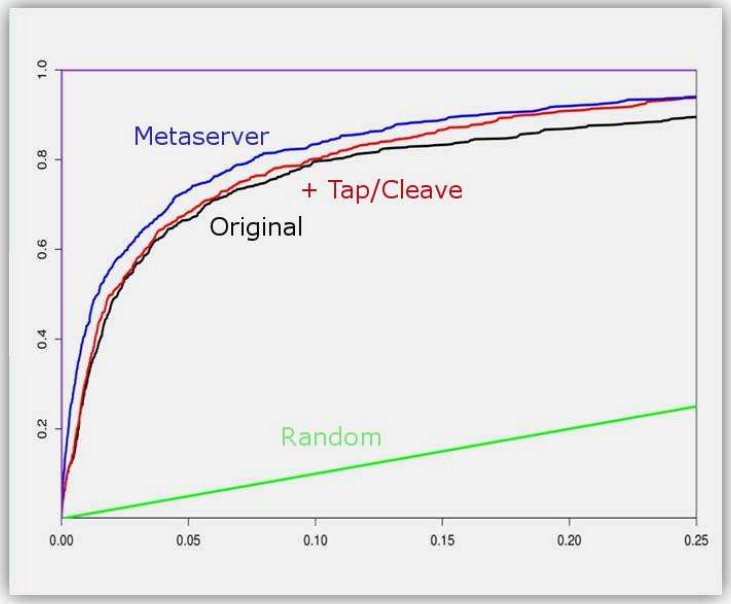


Figure: ROC curve that illustrates epitope prediction performance

Metaserver (MacNamara et al. PLoS Comp. Biol. 2009)

- Removes normalising function
- Significant improvement in accuracy

The Rank Method

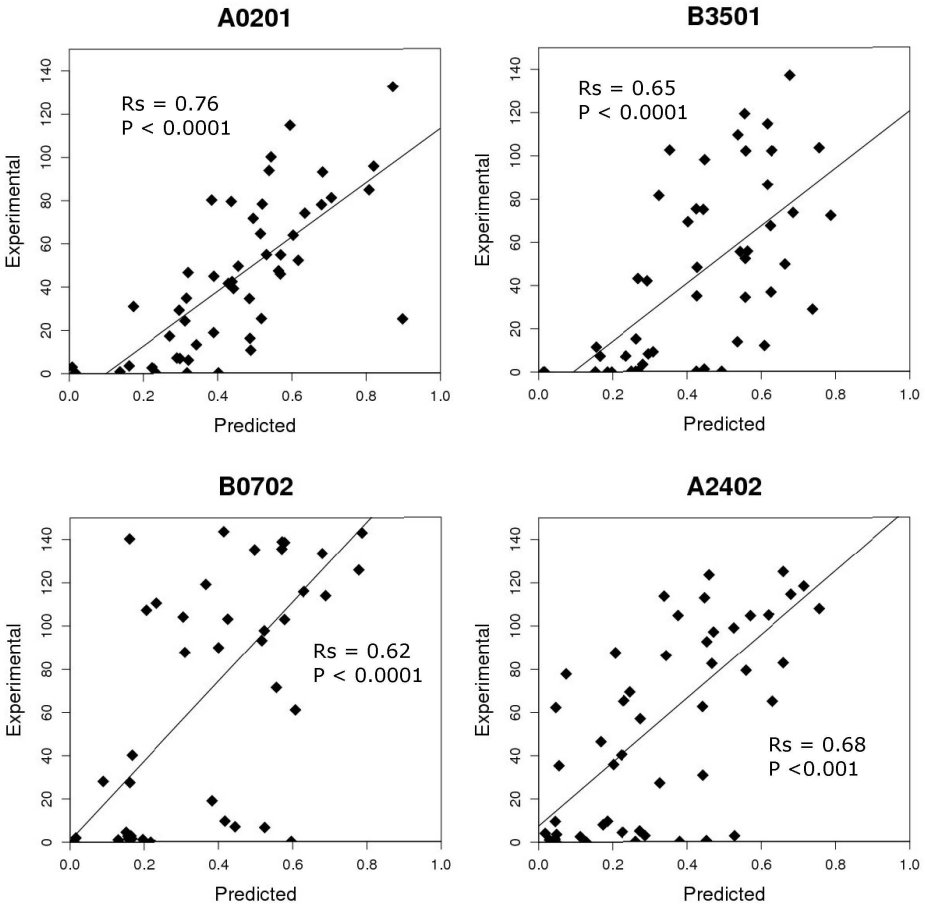
		A*02		B*54		C*08	
Strong	1	Gag	TPKDKTKVL	Tax	LPTTLFQPA	Tax	YLYQLSPPI
	2	Pol	PADPKEKDL	Pro	LPVIPLDPA	Tax	LLFGYPVYV
	3	Rof	RPPPAPCLL	Env	FPFSLLVDA	Pol	ALLGEIQWV
	4	P12	RPPPAPCLL	Pol	MPVFTLSPV	Pol	SLISHGLPV
	5	Gag	NANKECQKL	Rof	LPITMRFPA	Pol	FQPYFAFTV
	6	Gag	ANNPQQQGL	P12	LPITMRFPA	Gag	FMQTIRLAV
Weak	7	Gag	GAPPNHRPW	Pro	LPFRTTPIV	Pol	LTYDAVPTV
	...	...	...	...	...	...	...
	3389	P12	LLLFLLPPS	Tax	DNDHEPQIS	Tax	DNDHEPQIS

The Rank Method

		A*02		B*54		C*08	
Strong	1	Gag	TPKDKTKVL	Tax	LPTTLFQPA	Tax	YLYQLSPPI
	2	Pol	PADPKEKDL	Pro	LPVIPLDPA	Tax	LLFGYPVYV
	3	Rof	RPPPAPCLL	Env	FPFSLLVDA	Pol	ALLGEIQWV
	4	P12	RPPPAPCLL	Pol	MPVFTLSPV	Pol	SLISHGLPV
	5	Gag	NANKECQKL	Rof	LPITMRFPA	Pol	FQPYFAFTV
	6	Gag	ANNPQQQGL	P12	LPITMRFPA	Gag	FMQTIRLAV
Weak	7	Gag	GAPPNHRPW	Pro	LPFRTTPIV	Pol	LTYDAVPTV
	...	...	...	...	...	...	...
	3389	P12	LLLFLLPPS	Tax	DNDHEPQIS	Tax	DNDHEPQIS

- From Borghans et al. 2007
- Defines how well an allele binds a specific protein
- Example: A*02-Gag $\rightarrow$ 1,5,6,7...

Software Validation for HTLV-I



- Predicted versus laboratory binding affinities
- 50 HTLV-I peptides for 4 alleles
- Strong correlation found

Software Validation for HTLV-I

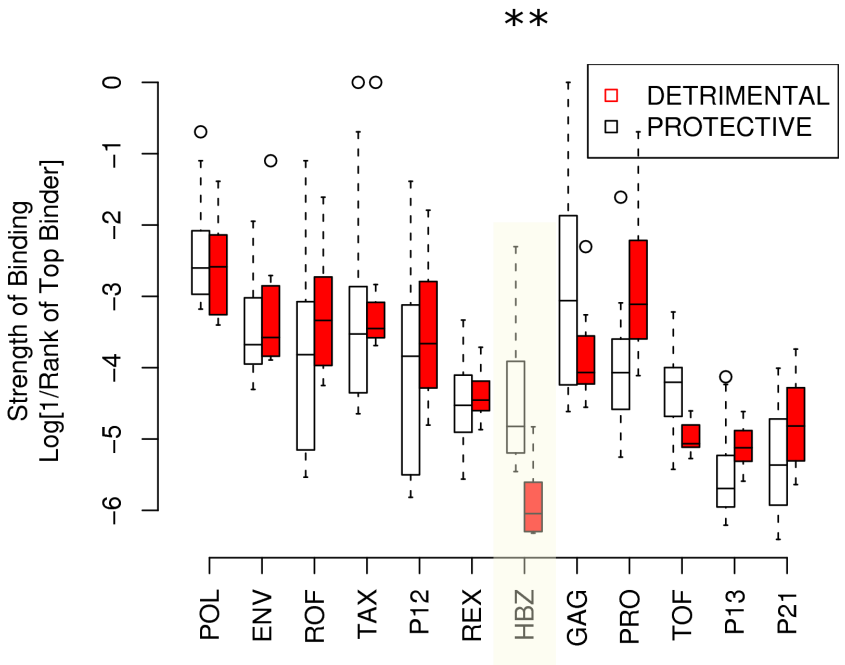
Conclusion

Metaserver and Epipred predict MHC class I binding affinity to HTLV-I peptides with good accuracy

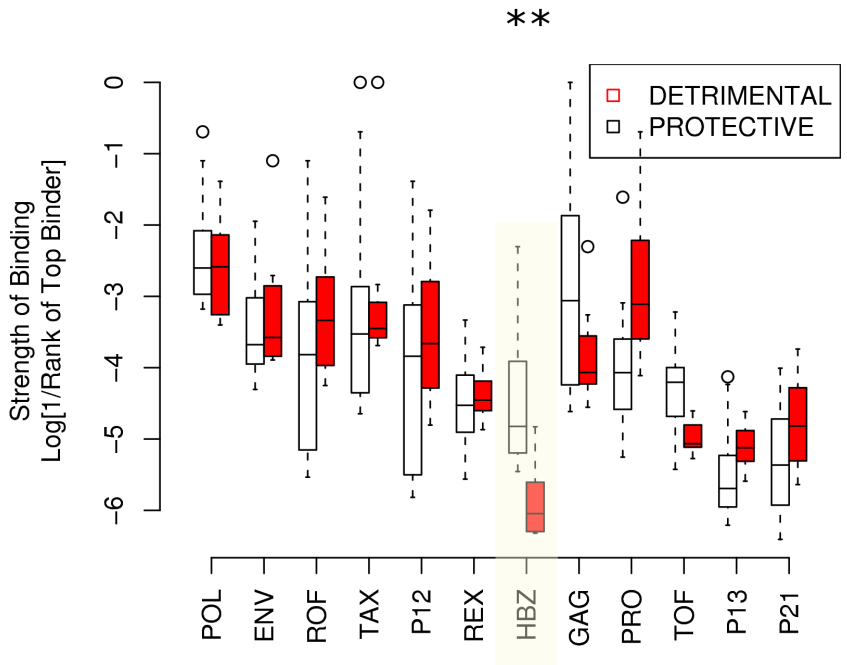
Do protective alleles bind different proteins?

Do protective alleles (A*02 and Cw*08) have a different binding specificity compared to the detrimental allele (B*54)?

Do protective alleles bind different proteins?



Do protective alleles bind different proteins?



Conclusion

Protective alleles (A*02 and Cw*08) bind HBZ significantly more strongly than a detrimental allele (B*54)

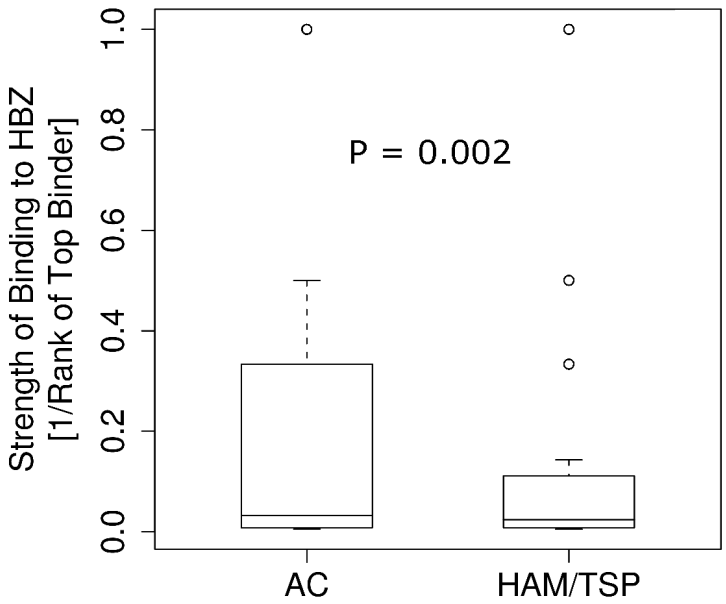
Do ACs and HAM/TSP patients bind different proteins?

202 AC individuals
HLA class I type $\longrightarrow$ Predicted epitopes from each protein

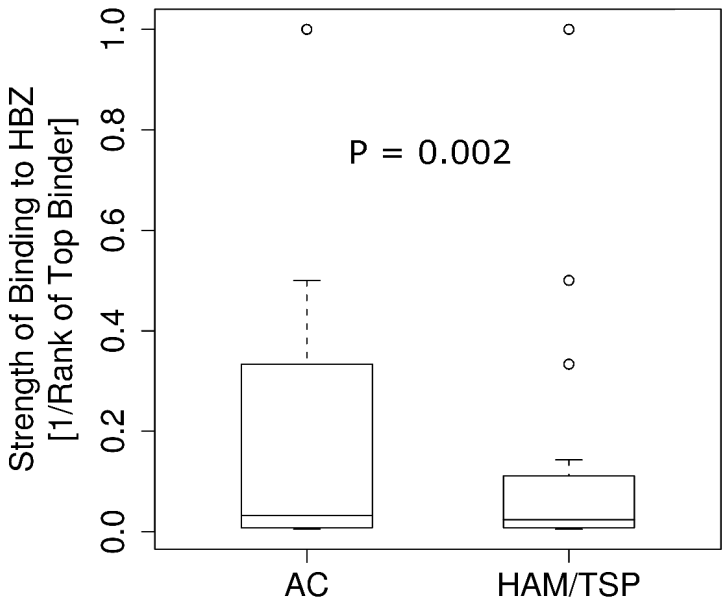
Vs.

230 HAM/TSP individuals
HLA class I type $\longrightarrow$ Predicted epitopes from each protein

Do ACs and HAM/TSP patients bind different proteins?



Do ACs and HAM/TSP patients bind different proteins?



Conclusion

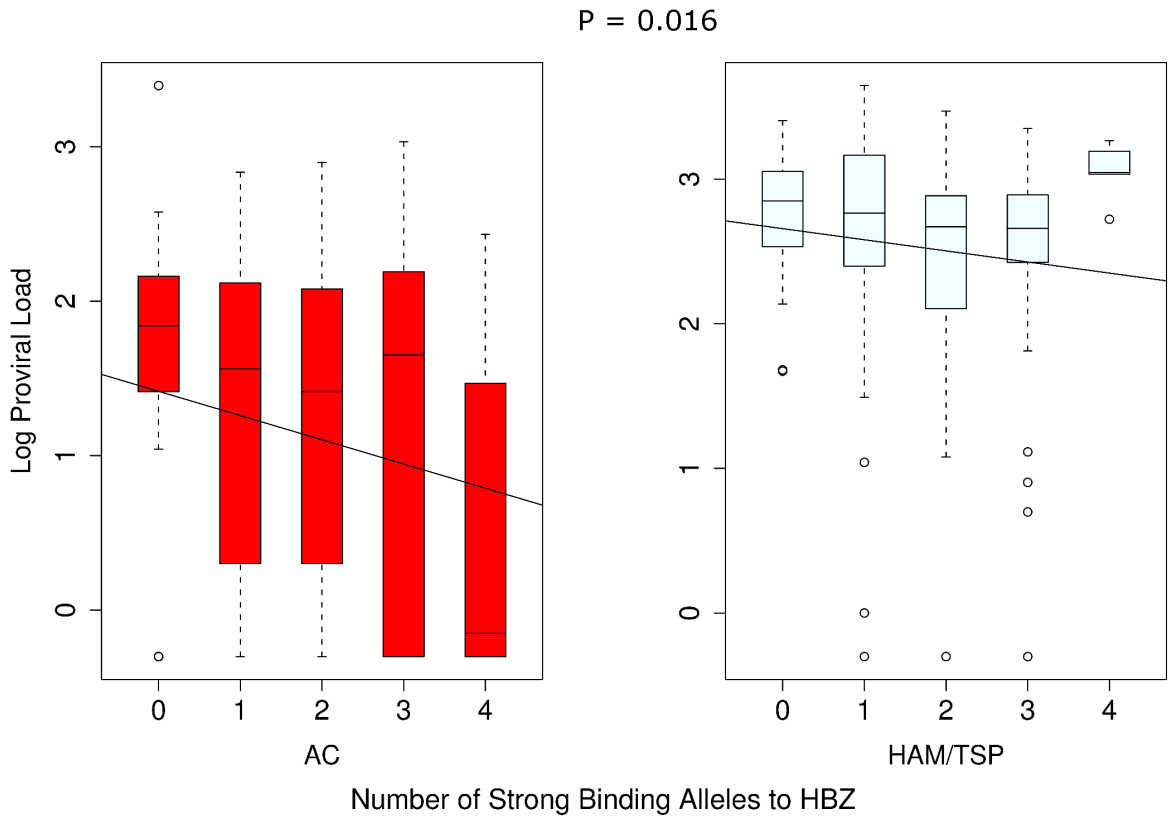
Asymptomatic carriers possess HLA molecules that strongly bind HBZ

Why is binding HBZ protective?

Hypothesis

Binding HBZ is associated with a reduced proviral load

Why is binding HBZ protective?

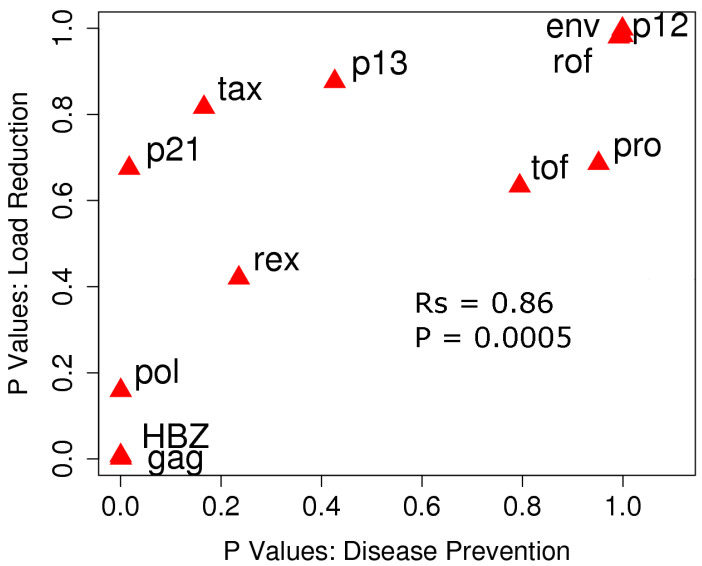


Why is binding HBZ protective?

Conclusion

Binding HBZ is associated with a reduced proviral load

Is There a Link Between risk and proviral load with other proteins?



For each protein 2 hypotheses were tested:

- Binding the protein is associated with reduced HAM/TSP risk
- Binding the protein is associated with reduced proviral load

Is There a Link Between risk and proviral load with other proteins?

Conclusion

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- ACs possess alleles that bind more strongly to proteins associated with a reduced proviral load

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- ACs possess alleles that bind more strongly to proteins associated with a reduced proviral load
- This is not related to immunogenicity

Is There a Link Between risk and proviral load with other proteins?

Conclusion

- ACs possess alleles that bind more strongly to proteins associated with a reduced proviral load
- This is not related to immunogenicity
- Strong evidence of a protective CTL response (MacNamara et al. PLoS Pathogens, 2010)

Can we detect HBZ-specific CTL?

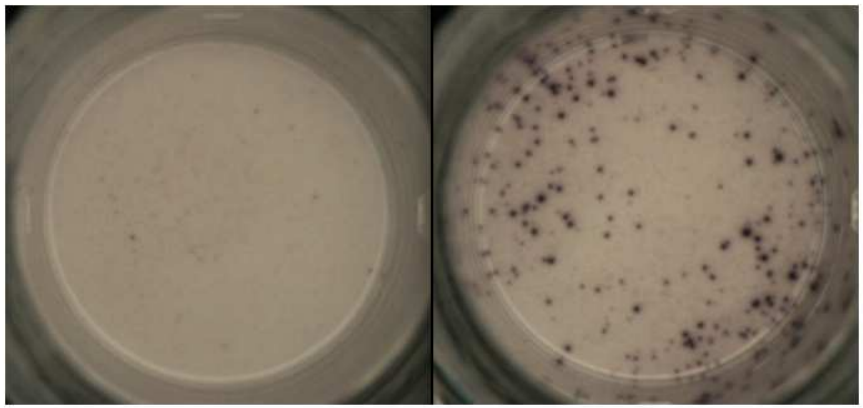


Figure: IFN-γ ELISpot that demonstrates a CTL response against HBZ from a HTLV infected patient (compared to control, left)

- Detection of HBZ-specific $CD8^+$ T cells (IFNγ, CD107a)
- Demonstration of HBZ-specific lysis (B-LCL, naturally infected)

Why HBZ?

HBZ inhibits expression of other HTLV-I genes

$\Rightarrow$Protects cell from immune response

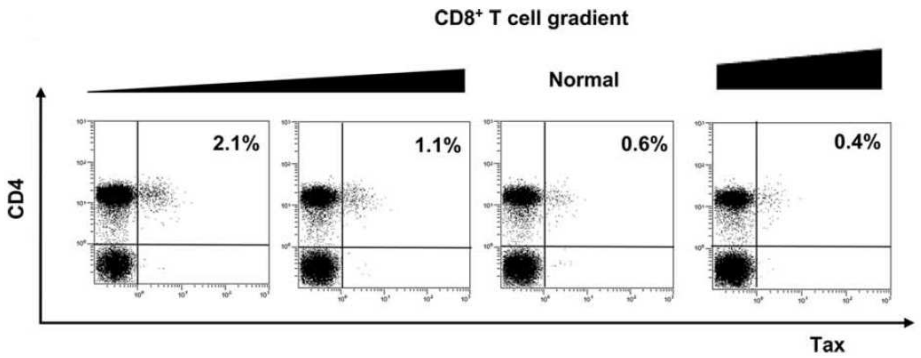
HBZ expression drives infected cell proliferation

$\Downarrow$

Hypothesis

HBZ expressing cells have a survival advantage and blocking this pathway is important

The rate of lysis of infected cells



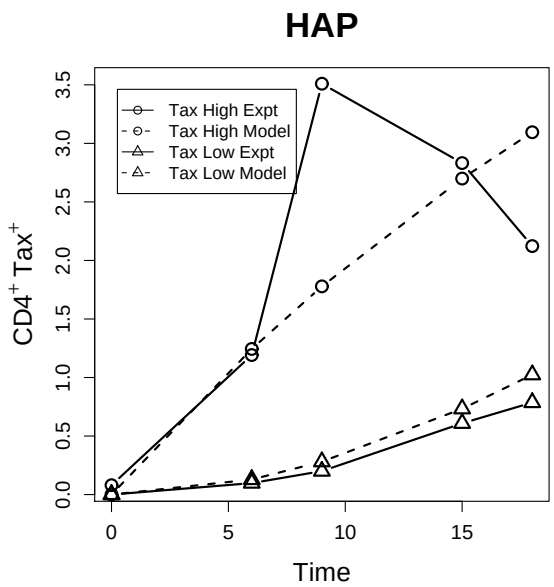
$$\frac{dy}{dt} = c - \epsilon yz$$

- Flow Cytometry detects level of Tax expression
- $CD8^+$ T cells added at increasing concentrations
- The lysis rate measured using model

The rate of lysis of infected cells

Time course of tax expression

Target $CD4^+$ cells divided into Tax^{high} and Tax^{low}



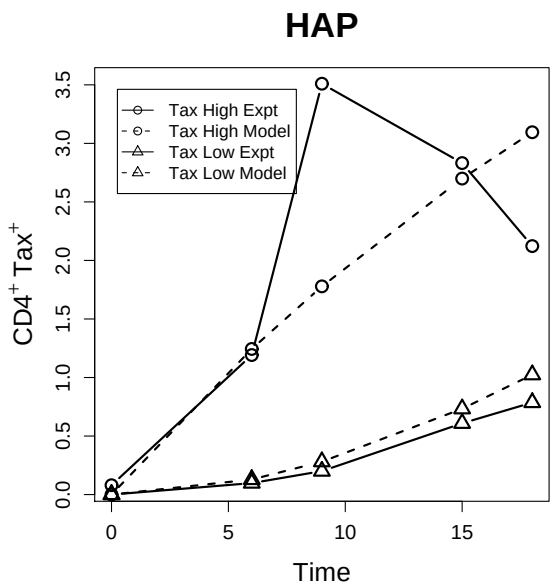
The rate of lysis of infected cells

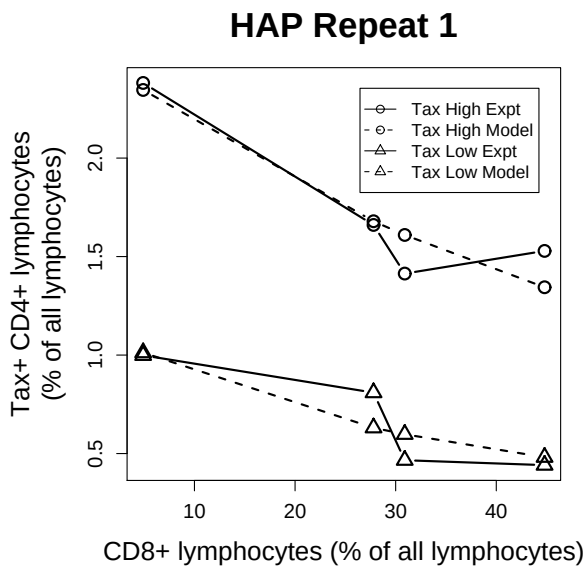
Time course of tax expression

Target $CD4^+$ cells divided into Tax^{high} and Tax^{low}

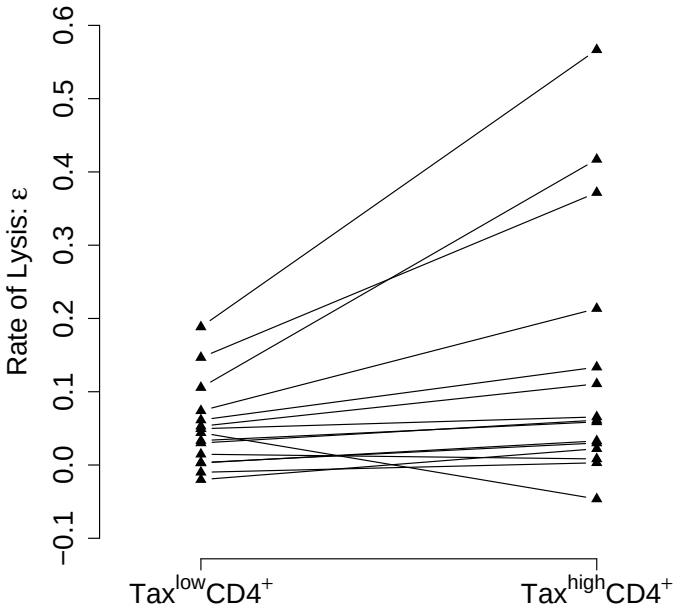
Lysis of $CD4^+$ cells

Rate of lysis measured for Tax^{high} and Tax^{low}



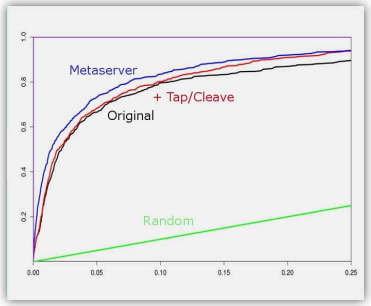


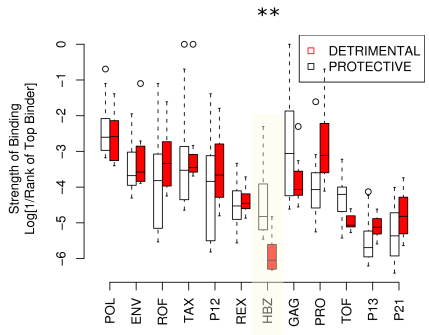
The rate of lysis of infected cells



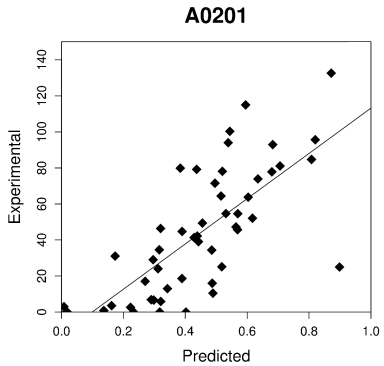
- Target $CD4^+$ cells are killed quicker when expressing higher levels of Tax
- MacNamara et al. J. Immunol. 2009

Summary



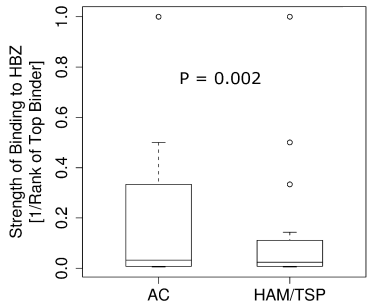


1. Developed accurate epitope prediction software



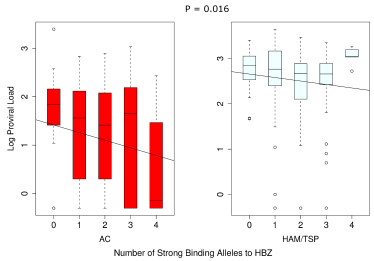
2. Validated software experimentally for 200 HTLV-I peptides

3. Protective alleles bind HBZ more strongly than a detrimental allele

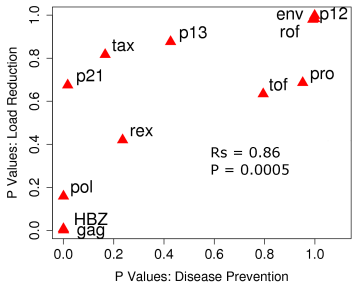


4. ACs have HLA alleles which are specific for HBZ

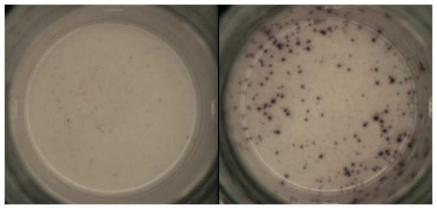
Summary



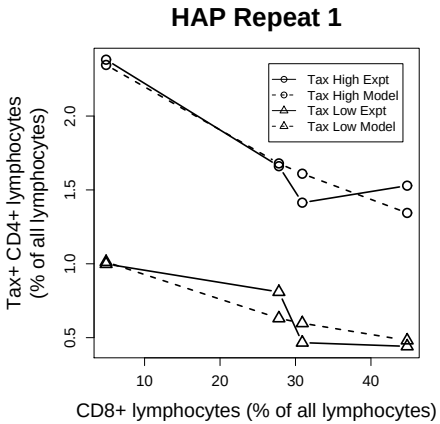
5. Binding HBZ is associated with a reduced proviral load



6. Proteins that are preferentially targeted by ACs are those associated with a greater reduction in load when bound



7. HBZ-specific responses exist



8. Antigen expression levels affect lysis rate and no KIR:HLA associations were found

Primary supervisor: Dr. B Asquith
Secondary supervisor: Prof. Charles Bangham

Collaborators & Data:

- Aileen Rowan, Silva Youshya
- Ulrich Kadolsky

wellcome trust