Romana's Sign of the Times: Molecular Approaches for Protective *T. cruzi* Vaccines

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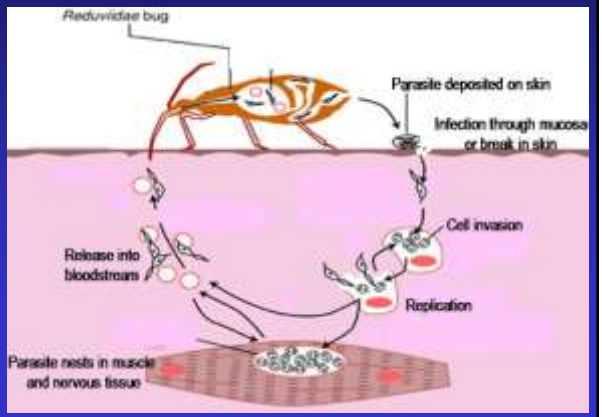
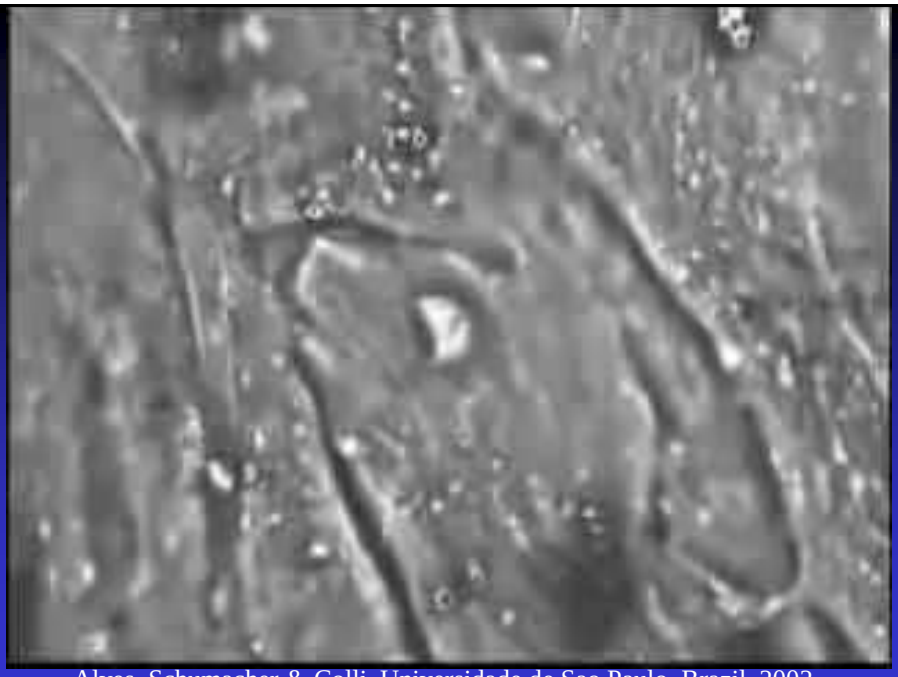
Trypanosoma cruzi: A Mucosally Invasive, Chronic Intracellular Protozoal Pathogen

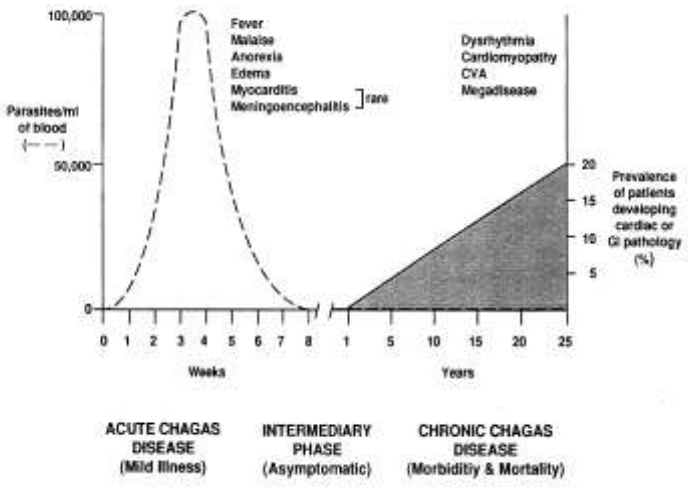
-8 to 13 million infected
-more than 20,000 deaths/y
-80-300,000 infected in US
-Acute contaminative infection
-Chronic intracellular infection
-10-40% develop chronic disease
-Common cause of sudden death
-Chemotherapy not ideal
-No available vaccines





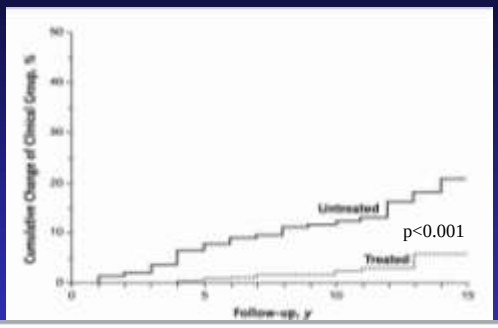
Alves, Schumacher & Colli, Universidade de Sao Paulo, Brazil, 2002
http://www.medictube.com

Inverse Relation Between Parasite Load/Disease Initially Considered Evidence for Autoimmunity



Best Evidence Against Autoimmunity

Viotti et al, Long-Term Cardiac Outcomes of Treating Chronic Chagas Disease with Benznidazole, Annals of Internal Medicine, 2006, 144:724-734.



Outcome	Treated Patients, n/n (%)	Untreated Patients, n/n (%)	Adjusted Hazard Ratio (95% CI)	P Value
Change of clinical group	12/283 (4)	40/283 (14)	0.24 (0.10–0.59)	0.002
New electrocardiographic abnormalities*	15/283 (5)	45/283 (16)	0.27 (0.13–0.57)	0.001
3+	130/218 (60)	177/212 (83)	0.55 (0.44–0.70)	<0.001
3−	32/218 (15)	12/212 (6)	2.1 (1.06–4.06)	0.034

* 3+ = persistence of positive results on 3 tests; 3− = negative seroconversion of 3 serologic tests.

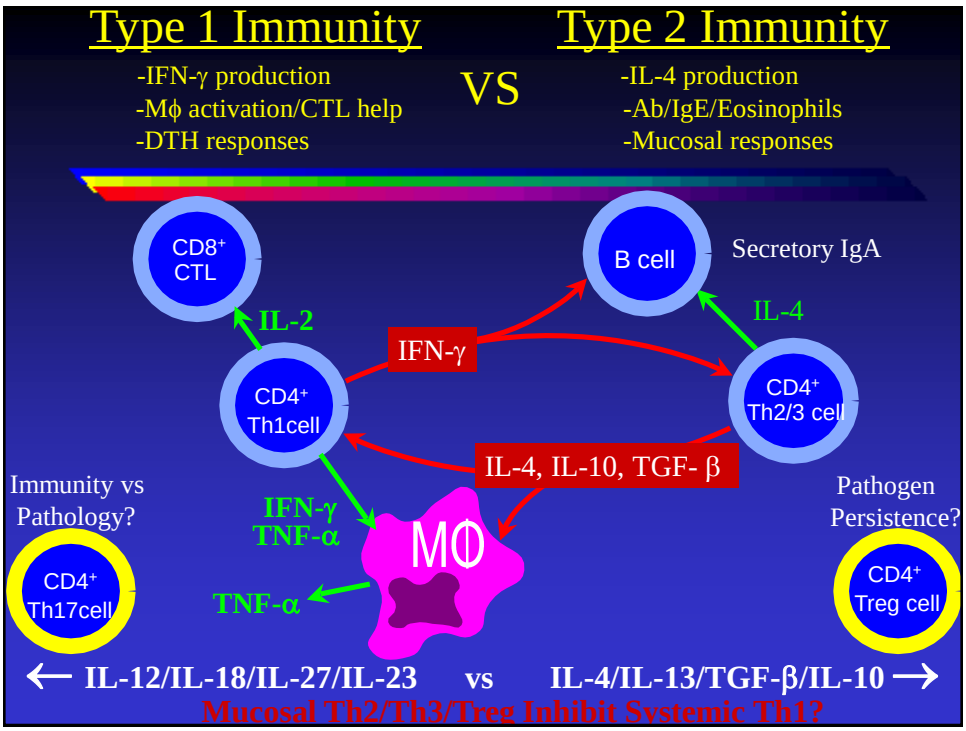
∴Lowering parasite load protects against Chagas dx progression (parasite-directed immunopathology, not autoimmunity)

Potential Vaccine Strategies

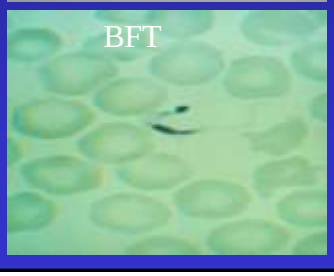
- Mucosal vaccinations to prevent infection
- Prophylactic vaccines to prevent/reduce disease
- Immunotherapies to prevent disease post-infection
- Antibody protection against infection/parasitemia
- T cell protection against intracellular parasites

Other Reasons for Immunologist to Study *T. cruzi*

- Model of mucosally invasive, intracellular pathogen
- Model for studies of differential trafficking
- Elucidate host-pathogen persistent interactions
- Elucidate fundamental parasite evasion strategies
- Protection requires CD4+ T, CD8+ T & B cells



Dipetalogaster maximus Infected Insects Produce IMT/Mouse Blood Source of BFT

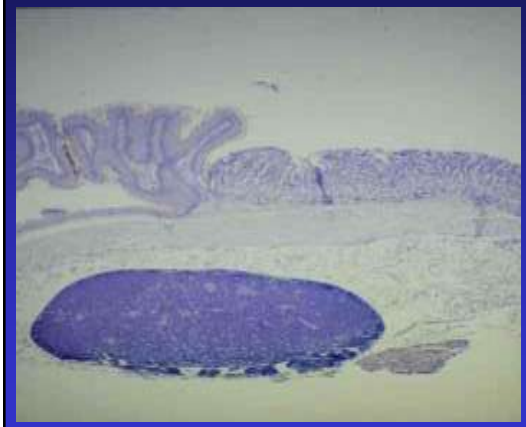


-IMT mucosally invasive

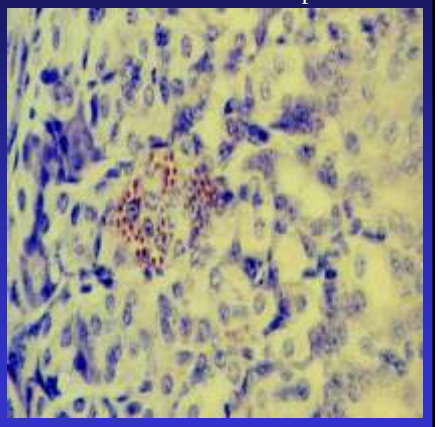
-Parenteral BFT lethal

Gastric Invasion after Oral *T. cruzi* Challenge

H&E Stain of Proximal Gastric Mucosa



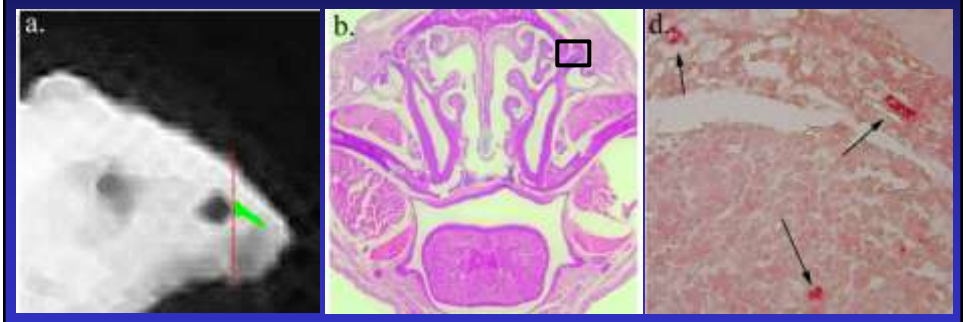
T. cruzi Immunostain of Epithelium



Parasites swallowed and infect epithelia lining proximal gastric mucosa.

Mucosal Invasion after Conjunctival Challenge

Coronal Section | H&E Stain | *T. cruzi* Immunostain



Parasites infect epithelia lining nasolacrimal duct/nasal cavity

Investigations of Mucosal, Cutaneous And Systemic Immunity

- Gastric RT-PCR/quantitative cultures
- Ocular/nasal epithelial RT-PCR/cultures
- "Natural" SQ challenge model studies
- Draining lymph node PCR/cultures
- Parasitemia/survival post-systemic challenge

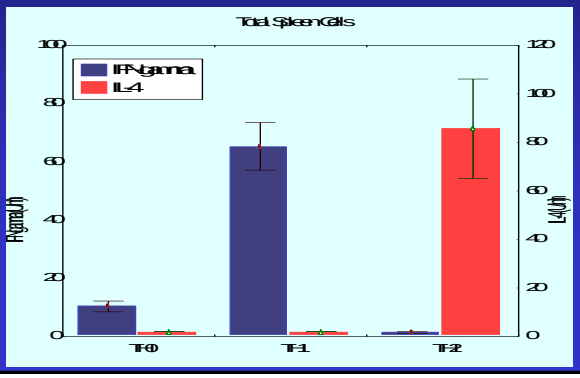
Molecular & Cellular Requirements for *T. cruzi* Mucosal & Systemic Immunity

- Immune spleen cells transfer mucosal/systemic protection
- Both CD4+ and CD8+ T cells required for 1º immunity
- Purified memory $CD8^+$ T cells alone transfer protection
- Immune T cells from infected mice most protective

Hypothesis: Th2 and Th1 responses will induce optimal mucosal and systemic protection, respectively.

IN Th Bias Immunization Model

- Th0: TcAg + CT only
- Th1: TcAg + CT + IL-12/αIL-4
- Th2: TcAg + CT + IL-4/αIFN-γ

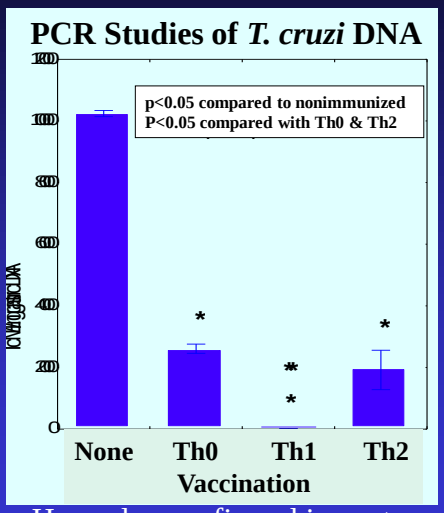


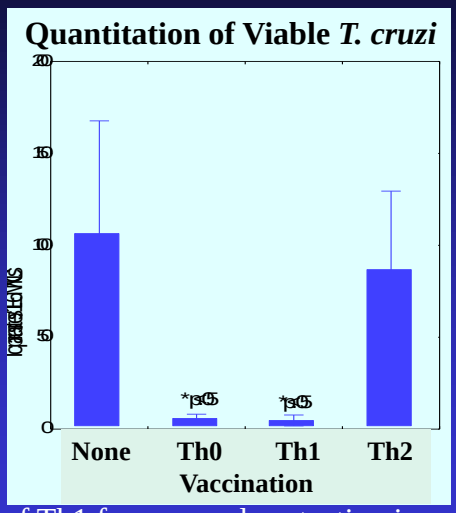
Systemic Protection After Th Bias Immunizations

Immunization	Survival after BFT
None	0/9
Th0	2/7
Th1	6/6*
Th2	1/8

*P<0.05 by Fisher's exact test

Detection of Mucosal *T. cruzi* Replication After PO IMT Challenge





Have also confirmed importance of Th1 for mucosal protection in a 2nd model *T. cruzi* system (secondary infection of knockout mice).

Conclusions Regarding Type 1 & 2 Responses for *T. cruzi* Immunity

- Type 1 immunity (IFN-γ) essential for:
  - -Development of immune memory
  - -Both mucosal & systemic protection
- Vaccines can focus on Type 1 induction, not differential mucosal & systemic responses

Development of Molecular Trans-sialidase Vaccines Protective Against Mucosal & Systemic *T. cruzi* Infection

- Highly conserved virulence factor
- Neuraminidase activity/transfer of sialic acid
- TS enzymatic activity required for infection
- Induces mucosal & systemic protection
- Successful in versatile expression systems
  - -(DNA, rec. protein + CpG, rSalm./rBCG/rAdeno)

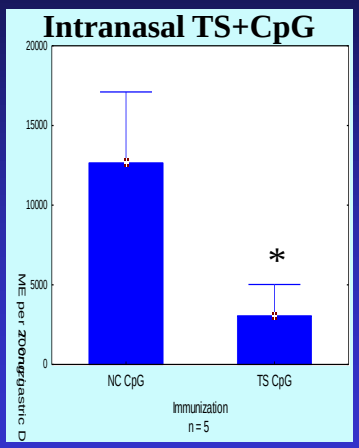
Systemic Protection Against Tulahuén Induced by TS DNA/Protein/rSalmonella*

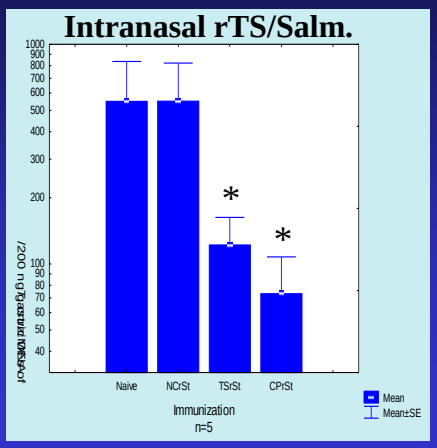
Immunization	NC DNA/rP/rSt	TS DNA/rP/rSt
DNA IM X 4	1/11 (9%)	10/10 (100%)†
rP + CpG IM X 2	0/5 (0%)	7/7 (100%)†
rP + CpG IN X 2	1/8 (13%)	8/8 (100%)†
rSalmonella IN X 3	0/5 (0%)	5/5 (100%)†

*Survival > 3 months after 5,000 *T. cruzi* BFT SC.

†$p<0.05$ by Fisher's exact test

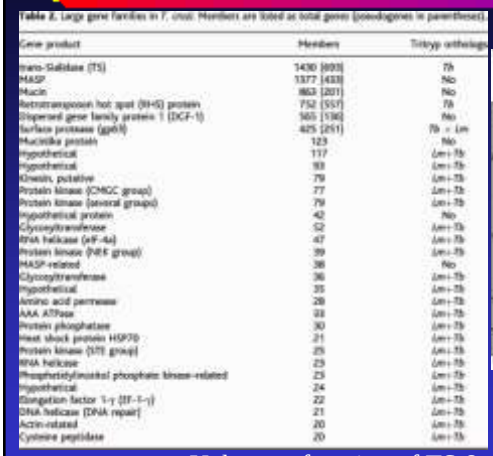
TS Induced Mucosal Protection





*$p<0.05$

Sequencing of *T. cruzi* Genome in 2005 (El-Sayed et al, Science) Identified TS Superfamily



Gene product	Members	Tritryp orthologs
trans-Sialidase (TS)	1430 [693]	7b
MASP	1377 [433]	No
Mucin	863 [201]	No
Retrotransposon hot spot (RHS) protein	752 [557]	7b
Dispersed gene family protein 1 (DGF-1)	565 [136]	No
Surface protease (gp63)	425 [251]	7b + Lm
Mucinlike protein	123	No
Hypothetical	117	Lm+7b
Hypothetical	93	Lm+7b
Kinesin, putative	79	Lm+7b
Protein kinase (CMGC group)	77	Lm+7b
Protein kinase (several groups)	79	Lm+7b
Hypothetical protein	42	No
Glycosyltransferase	52	Lm+7b
RNA helicase (eIF-4a)	47	Lm+7b
Protein kinase (NEK group)	39	Lm+7b
MASP-related	38	No
Glycosyltransferase	36	Lm+7b
Hypothetical	35	Lm+7b
Amino acid permease	28	Lm+7b
AAA ATPase	33	Lm+7b
Protein phosphatase	30	Lm+7b
Heat shock protein HSP70	21	Lm+7b
Protein kinase (STE group)	25	Lm+7b
RNA helicase	23	Lm+7b
Phosphatidylinositol phosphate kinase–related	23	Lm+7b
Hypothetical	24	Lm+7b
Elongation factor 1-γ (EF-1-γ)	22	Lm+7b
DNA helicase (DNA repair)	21	Lm+7b
Actin-related	20	Lm+7b
Cysteine peptidase	20	Lm+7b

Subfamily	Domains	Genes	Function
Active TS	SAPA	12	TS activity
	catalytic site		binding to sialic acid/galactose
	lectin-like		binding to CD43
TS-like*	lectin-like	725	Cell adhesion–laminin binding
			Complement regulation
			interaction with (1)-adrenergic receptor
			binding to sialic acid/galactose
TS pseudogenes		691	
Total		1430	

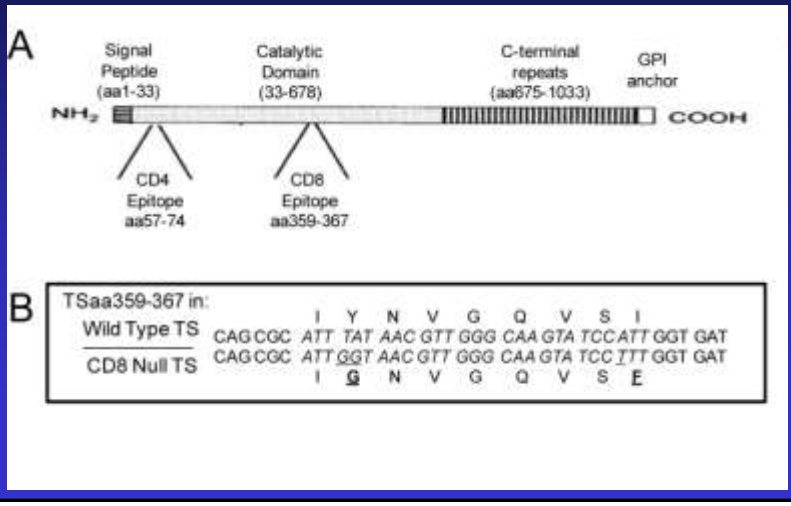
- Unknown function of TS & other large gene families
- Could TS superfamily have evolved for immune evasion?
- Homologous but nonidentical T cell epitopes present
- "Altered peptide ligands" could dampen T cell responses

Key Reasons to Pursue TS Vaccine Development

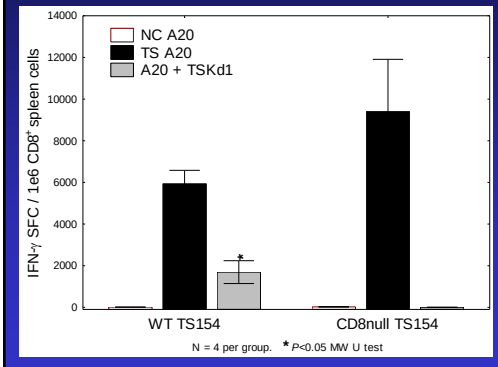
- TS-specific responses active during infection
- TS escape mutants not detectable
- TS best antigen tested in Hoft lab over 20y
- Humans mount robust TS-specific immunity

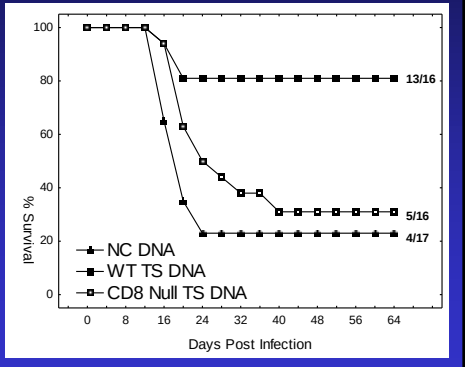
Note: reagents <u>needed</u> for careful study of affects of chronic infection on TS immunity.

Mapping Immunodominant TS-specific H2^{d}-restricted T cell Epitopes



Immunodominant TSKd1 Epitope Required for Protection

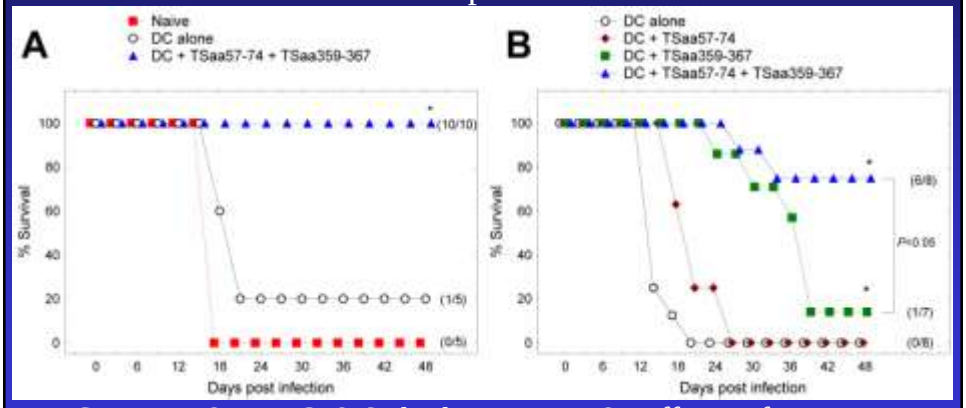




∴ Tsaa 359-67 (TSKd1) necessary for protective immunity
(Also, additional CD8+ T cell epitopes present in TS)

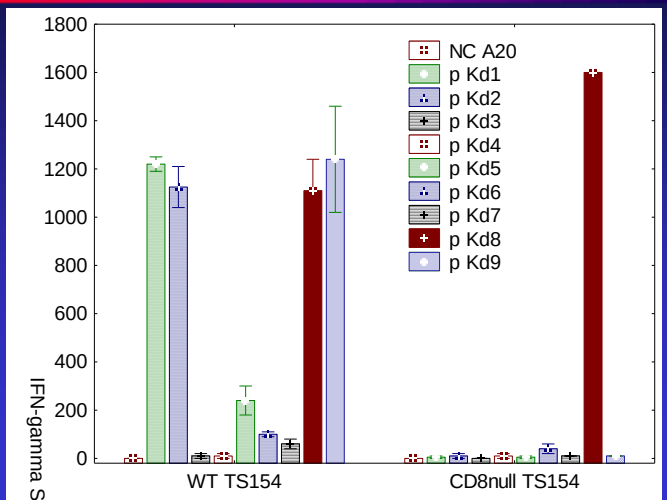
Single Pair of CD4 (TSaa57-74) & CD8 (TSaa359-67) Inducing Peptides Protective

- Dendritic cells (DC) purified from flt3-ligand treated mice
- TSaa57-74 & TSaa359-67 pulsed DCs used to vaccinate



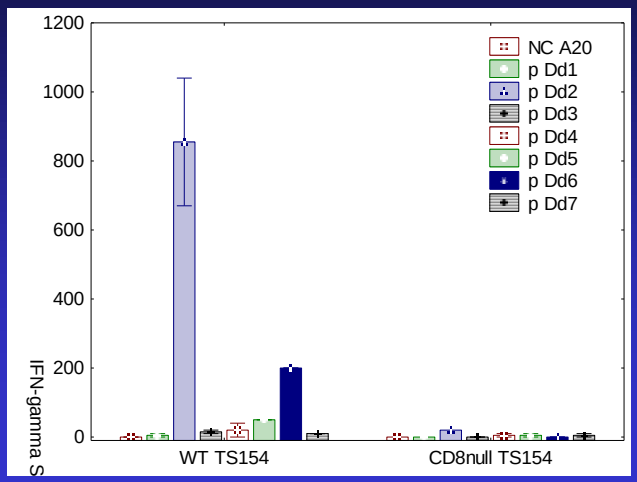
∴ TSaa57-74 & Tsaa 359-67 both necessary & sufficient for protection

Vaccine-induced CD8 null vs WT TS DNA Response to Predicted TS-K^d restricted Epitopes



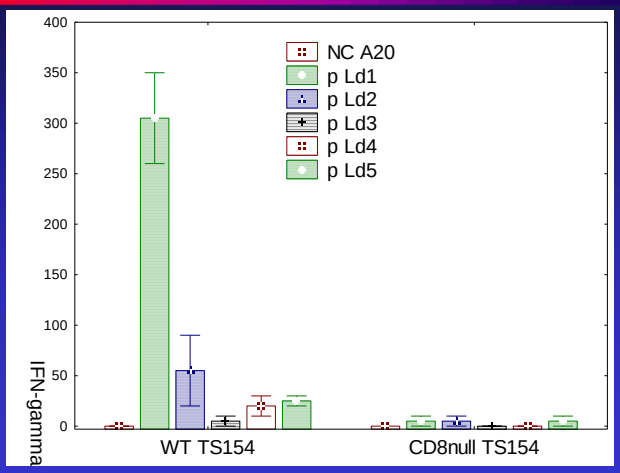
∴ Responses to TSKd1, 2, 5, 6, 7, 8 & 9 vs. only to TSKd8

Vaccine-induced CD8 null vs WT TS DNA Response to Predicted TS-D^d restricted Epitopes



∴ Responses to TSDd 2, 5, 6 vs none

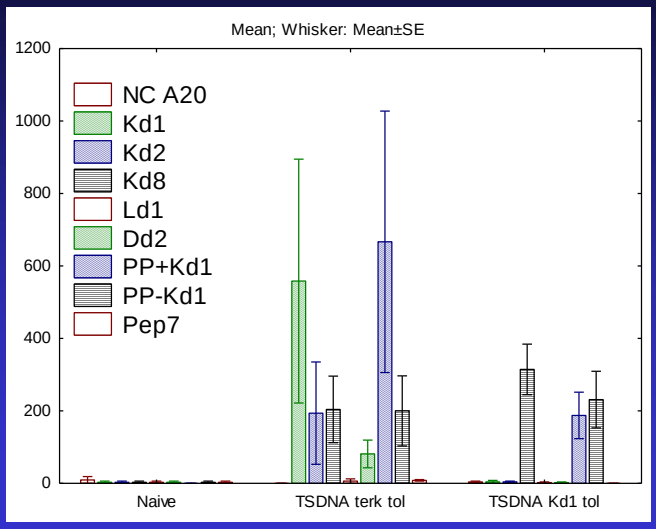
Vaccine-induced CD8 null vs WT TS DNA Response to Predicted TS-L^d restricted Epitopes



∴ Responses to Ld 1 & 2 vs none

Tolerization Confirms Immunodominant Helper Effects

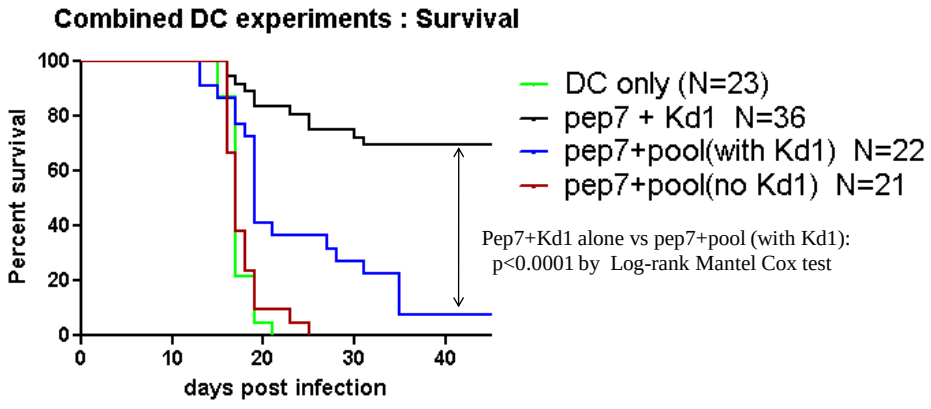
(TSKd1 peptide IV beginning 1 wk prior to WT TS DNA vaccination)



∴ As before Kd1,Kd2, Kd8, Ld1 & Dd2 responses vs Kd8 alone responses

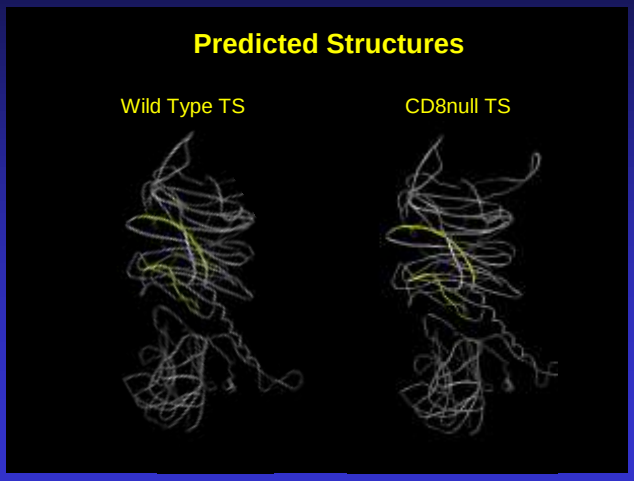
Immunodominant Helper Effects: Good or Bad?

(Vaccinations with DCs pulsed with peptides)



∴ TSKd1 immunodominant helper effects advantageous for parasite?

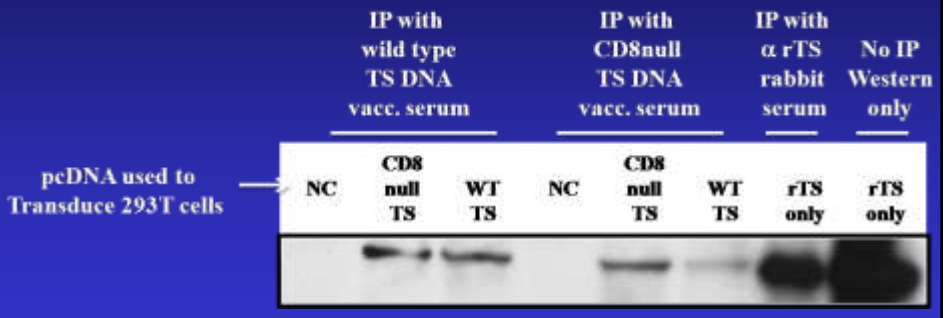
Mechanistic studies: Structural Differences?



http://swissmodel.expasy.org

Mechanistic studies: Structural Differences?

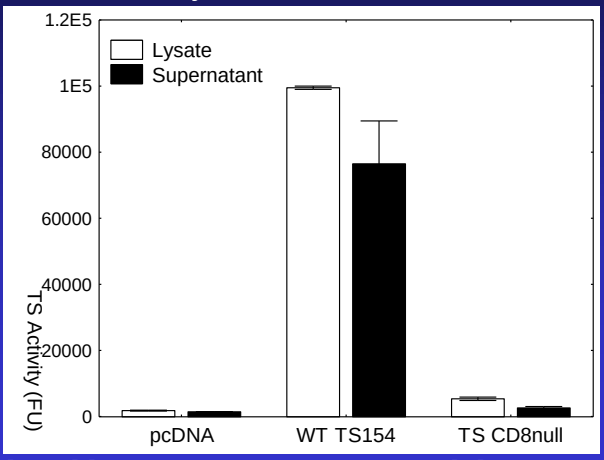
Immunoprecipitations with heterologous WT vs CD8 null TS-specific serum:



∴ Antibodies induced by each protein express reciprocal recognition

Differences in TS enzymatic activity

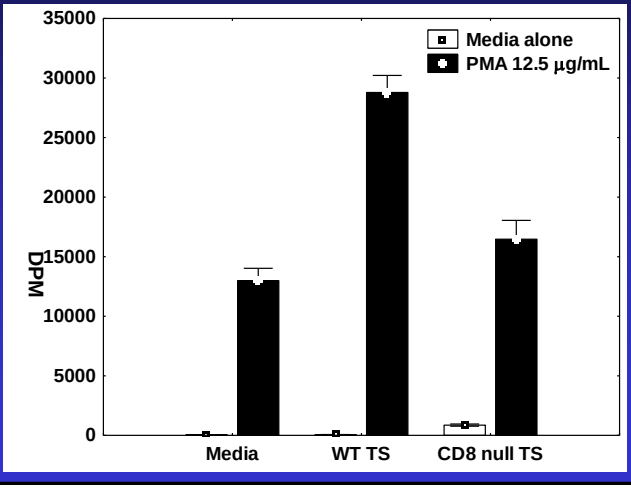
Transiently transfected SNs/lysates tested for sialic acid transfer activity:



∴Only wild type TS expresses trans-sialidase activity:

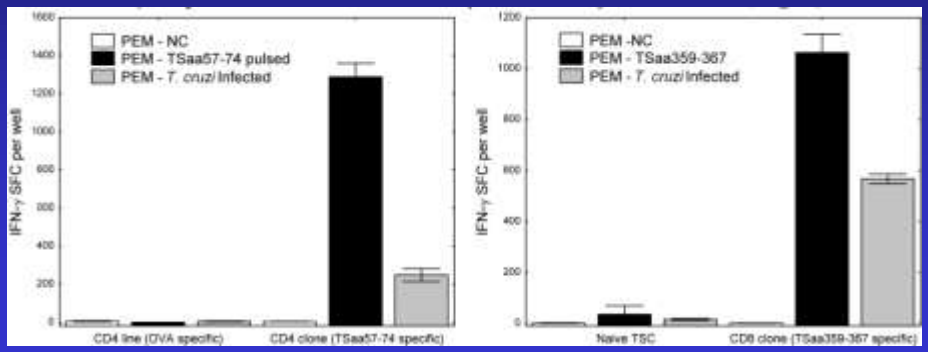
Wild Type TS but not CD8 null TS expresses costimulatory activity

(Proliferation of naive $CD8^+$ T cells stimulated with PMA ± WT/CD8 null TS protein)

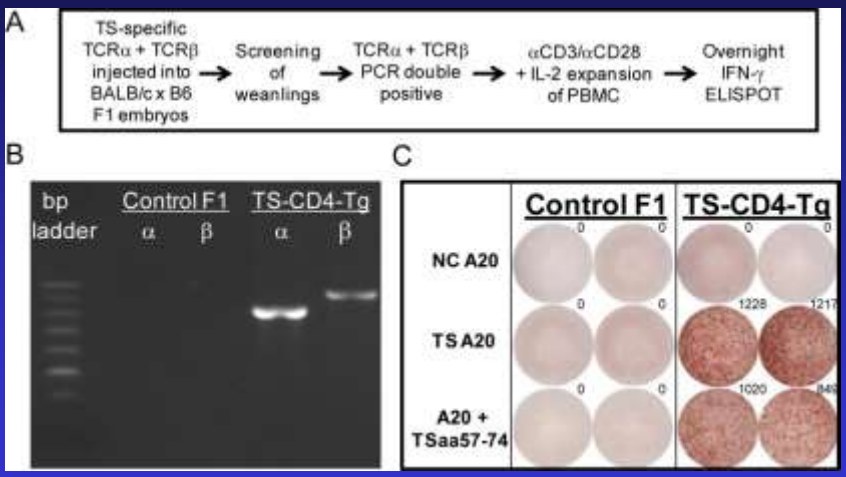


Cloning of CD4/CD8 T cells & TCR

- TSaa57-74/359-67 specific Tc cloned by limiting dilution
- TCRα/TCRβ chains recovered from representative clones
- TCRα/TCRβ transduction reconstitutes TCR specificity

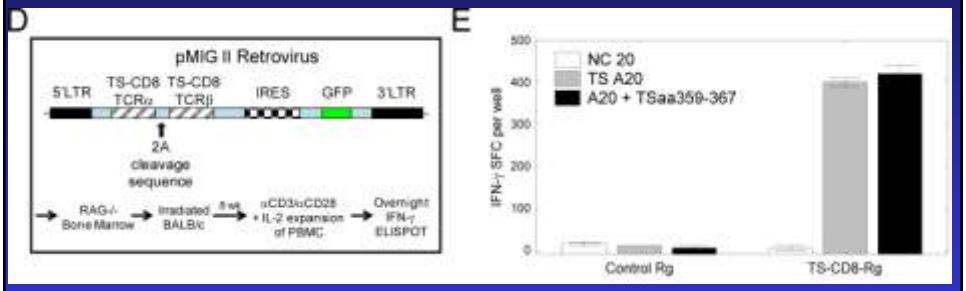


Construction of TCR Transgenic Mice



Breeding TCR Tg mice now in DRC.

Construction of TCR Retrogenic Mice



Ongoing experiments with TCR Rg mice in DRC.

Now Poised to Address Key Questions

- Frequency of TS-specific CD4+/CD8+ T cells required?
- Immune mechanisms required for optimal protection?
- Can T cells prevent/clear chronic *T. cruzi* infection?
- Are TS-specific T cells down regulated by infection?

New Collaboration with EpiVax

- Separate TS genes into functional vs nonfunctional sets
- Map HLA restricted immunogenic consensus sequences
- Test ICS immunity in HLA-DR/A2 dual transgenic mice
- Prepare TS functional vs nonfunctional TS ICS vaccines
- Assess vaccine-induced protection in HLA-DR/A2 Tg mice
- Evaluate if *T. cruzi*-infected humans respond to TS ICS
- Consider clinical development of new TS ICS vaccines

Summary of Presentation

- Chagas disease remains major public health problem
- Potential for prophylactic & immunotherapeutic vaccines
- TS-specific *T. cruzi* vaccines in pre-clinical development
- TS superfamily may have role in parasite persistence
- Novel immunodominance phenomenon aids parasite?
- State-of-the art reagents recently developed to address mechanisms of immunoprotection/immunoevasion
- Immunogenic concensus-based vaccines in development

T. cruzi Collaborators

Hoft Lab:
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Cade Lawrence
Nelson Salazar
Anita Schnapp
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Mauricio Rodrigues

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Roy Curtiss/Donata Sizemore

Wash U/Pathology & Immunology:
Paul Allen/Dave Donermeyer

EpiVax, Inc:
Anne De Groot/Bill Martin
Matt Ardito/Lenny Moise