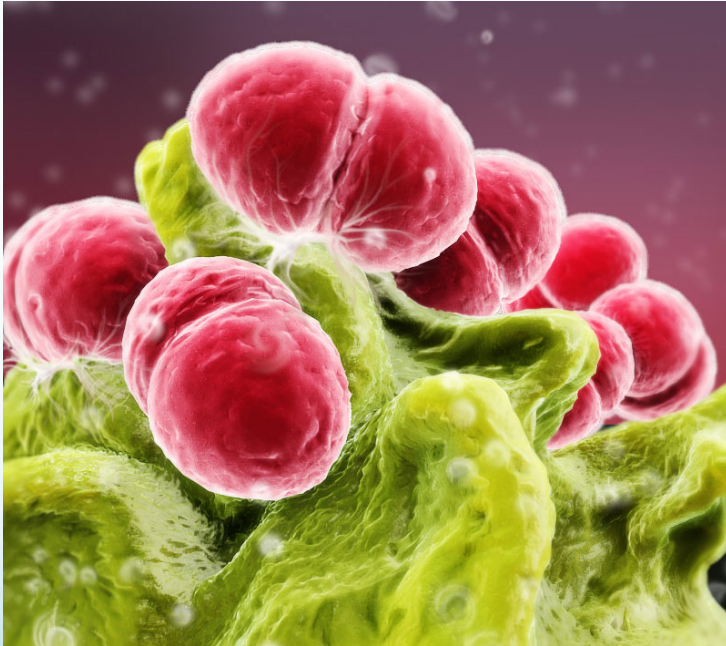


Using genomics to identify virulence factors and new vaccine targets for group A *Streptococcus*

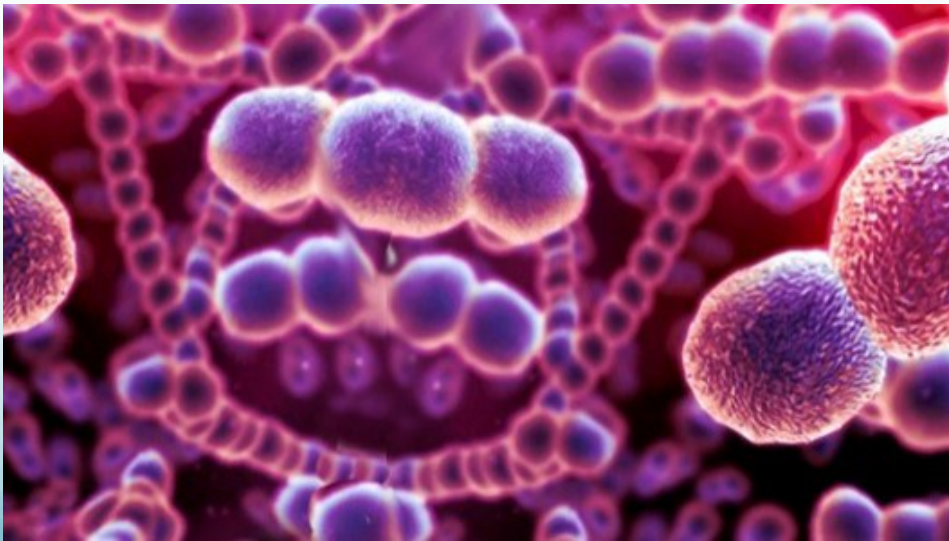


Jason Cole Ph.D.
University of California San Diego
University of Queensland

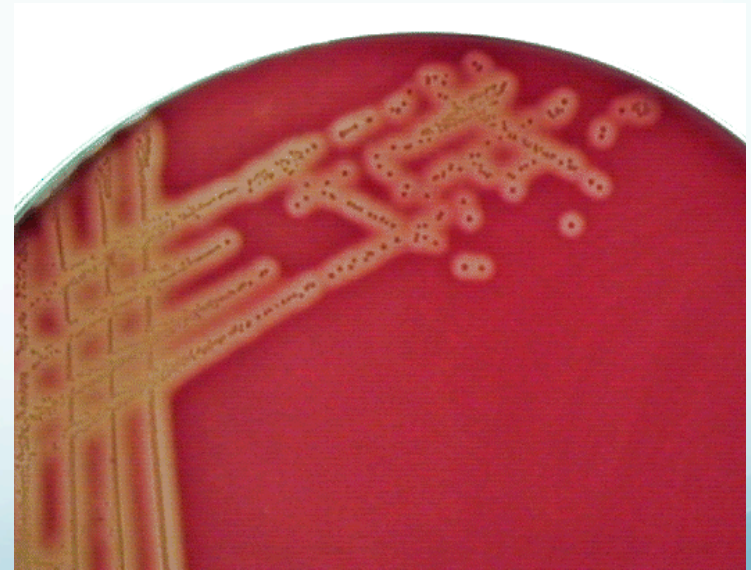
BioVision Alexandria Egypt
8 April 2014

Group A *Streptococcus* (GAS)

- Gram-positive β -hemolytic bacterium found in the throat and skin
- An important exclusively human pathogen
- Mild infections to life-threatening invasive illnesses



gizmag.com



academic.pgcc.edu

Group A *Streptococcus* (GAS)

- Gram-positive pathogen causing 700 million infections annually
 - Pharyngitis (strep sore throat)
 - Impetigo
 - Cellulitis

Pharyngitis



healthcentral.com

Impetigo



utc.edu

Cellulitis



vet.uga.edu

Group A *Streptococcus* (GAS)

- Causes 650,000 invasive infections with 25% mortality
 - Bacteremia
 - Toxic shock syndrome
 - Necrotizing fasciitis (flesh-eating disease)

Necrotizing fasciitis



diseaseworld.com

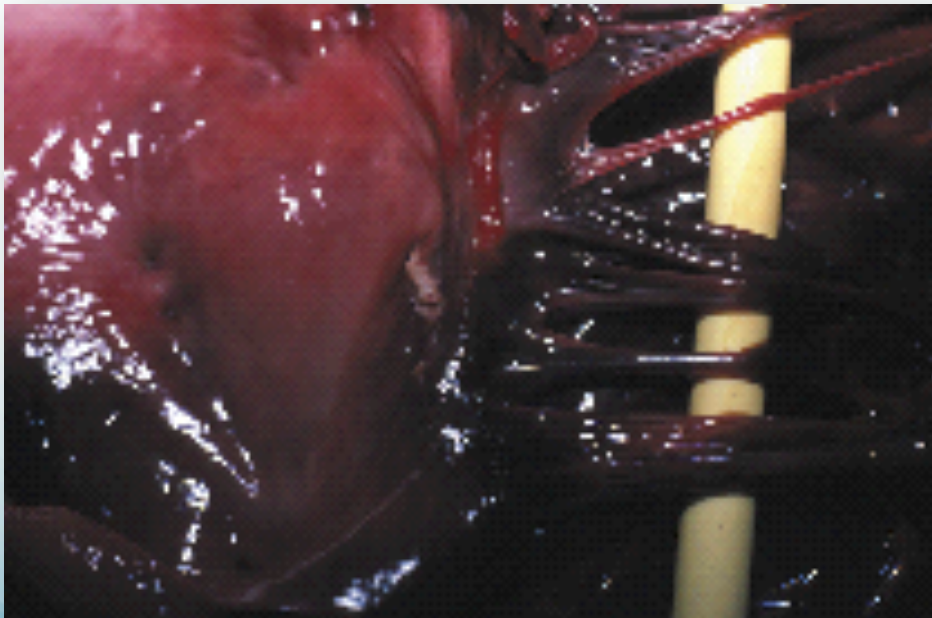


sfghed.ucsf.edu

GAS Causes Rheumatic Heart Disease

- Repeated infections may trigger rheumatic heart disease
 - 15.6–19.6 million cases worldwide
 - 233,000 deaths annually

Rheumatic heart disease (RHD)

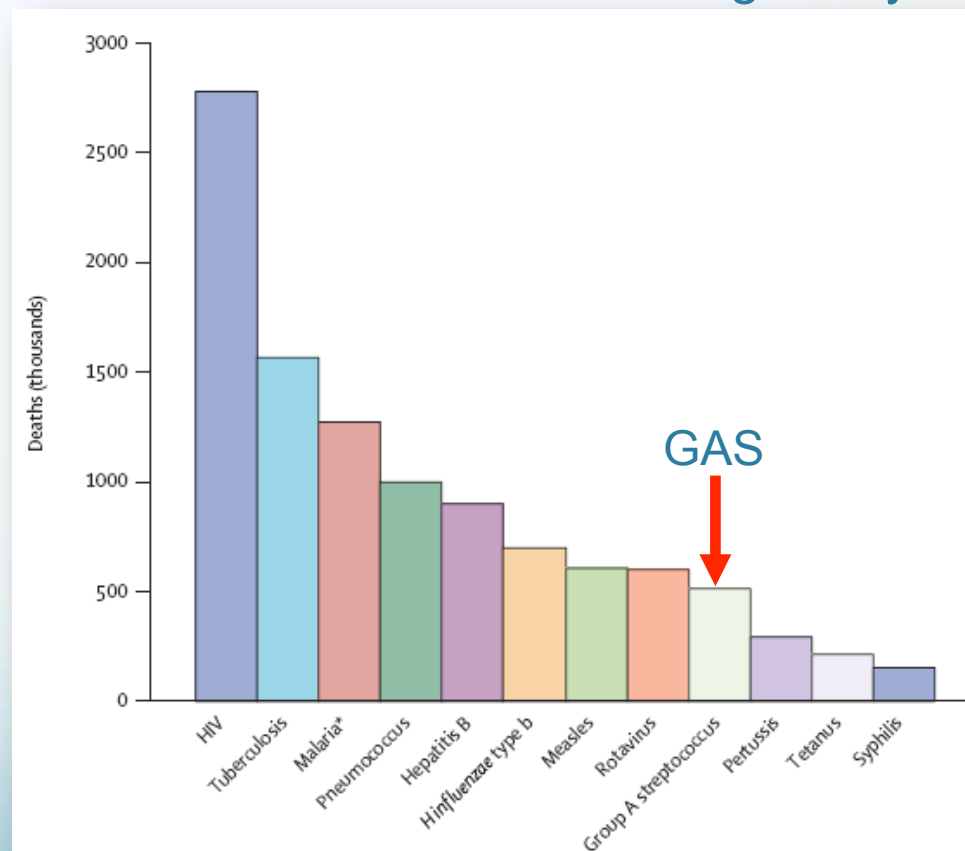


RHD patient heart valve shows thickening & calcification

GAS Mortality Worldwide

- ~700 million human infections annually, resulting in >500,000 deaths

Infection-related deaths globally



Prevention of GAS Diseases

- Prophylactic GAS treatment requires injections of penicillin
- This strategy has limited success due to unavailability of treatment or low levels of patient compliance
- Concerns exist over GAS developing resistance to penicillin



No GAS Vaccine Available

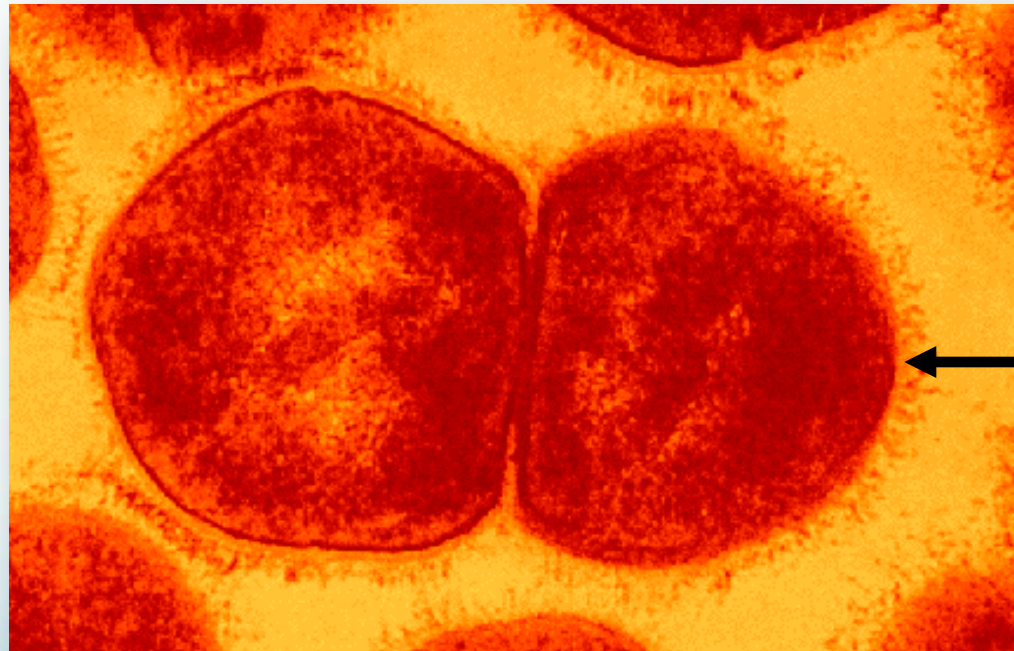
Existing GAS Vaccine Candidates

Characteristics/Deficiencies	M protein	C5a peptidase	FbaA	FBP54	CHO	SfbI	R28	SOF	SpeB	Sib35	Spa	Sse
Highly conserved across serotypes	-	+	-	+	+	-	ND	-	+	ND	ND	-
Ubiquitous expression	+	+	-	-	+	-	ND	-	-	ND	ND	ND
Possible toxicity and/or proteolytic activity	-	+	-	-	-	-	-	+	+	-	-	+
Systemic protection	+	ND	+	+	+	ND	+	+	+	+	+	ND
Intranasal protection	+	+	ND	ND	+	+	ND	-	ND	ND	ND	ND
Subcutaneous protection	+	ND	ND	ND	ND	-	ND	ND	ND	ND	ND	+
Opsonic/Bactericidal antibodies	+	ND	+	+	ND	ND	ND	+	ND	+	+	-
Auto-immune reactivity	+	ND	ND	ND	+	-	ND	ND	ND	ND	ND	ND

Identification of New Vaccine Antigens

- The aim is to map the GAS cell surface proteome to identify novel antigens that prevent infection and do not induce auto immunity

Electron microscopic image of GAS



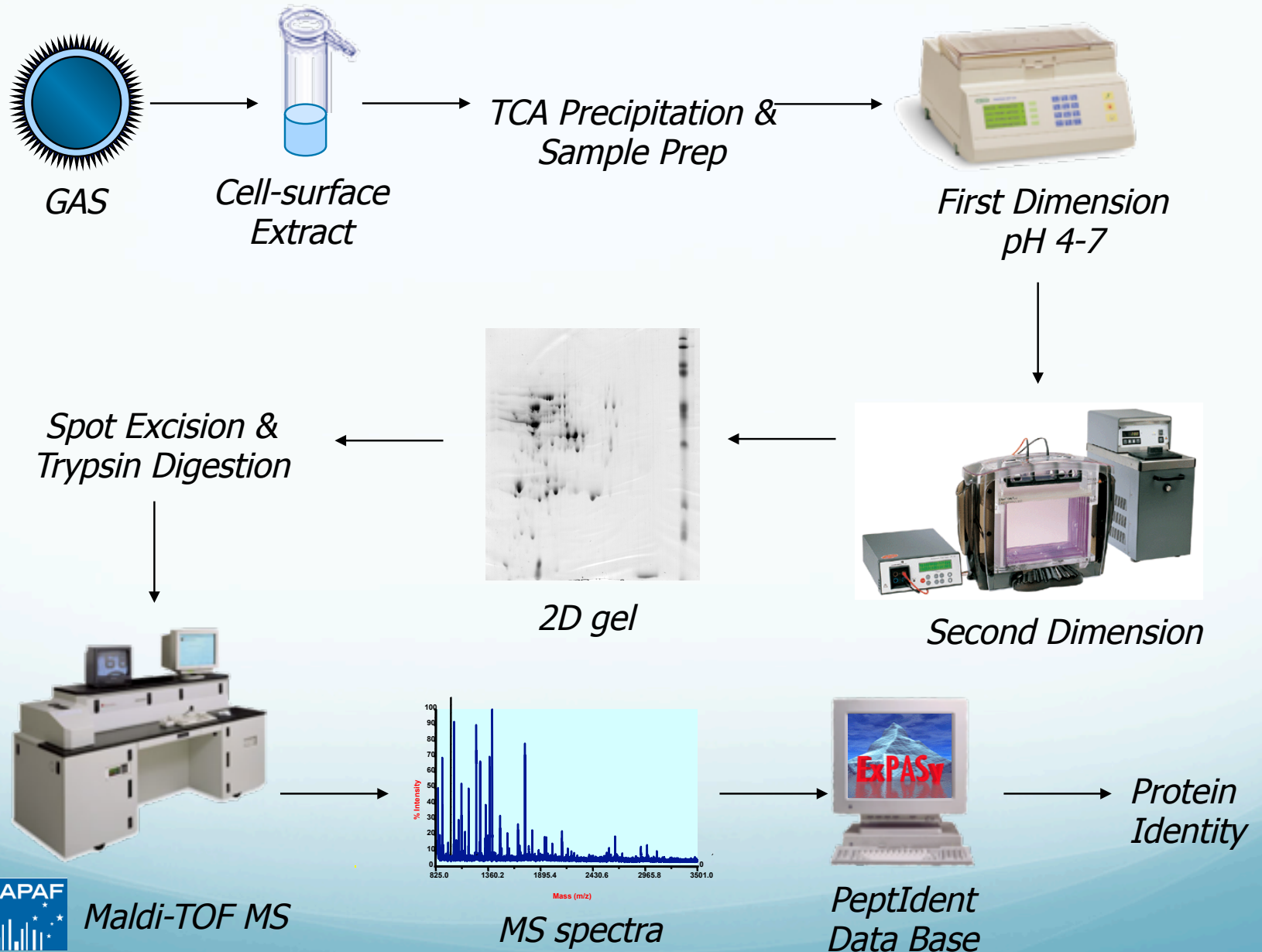
← Cell surface proteins

Criteria for Vaccine Candidate Selection

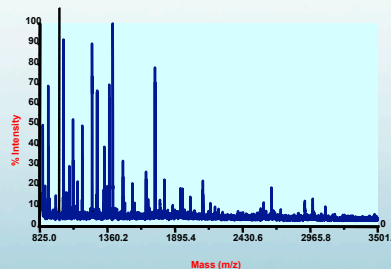
- No human homologue
- Homologues published as cell surface-associated and/or protective in other streptococcal species
- Previously characterized as GAS virulence determinants



Proteomic Analysis of the GAS Cell Surface



Maldi-TOF MS

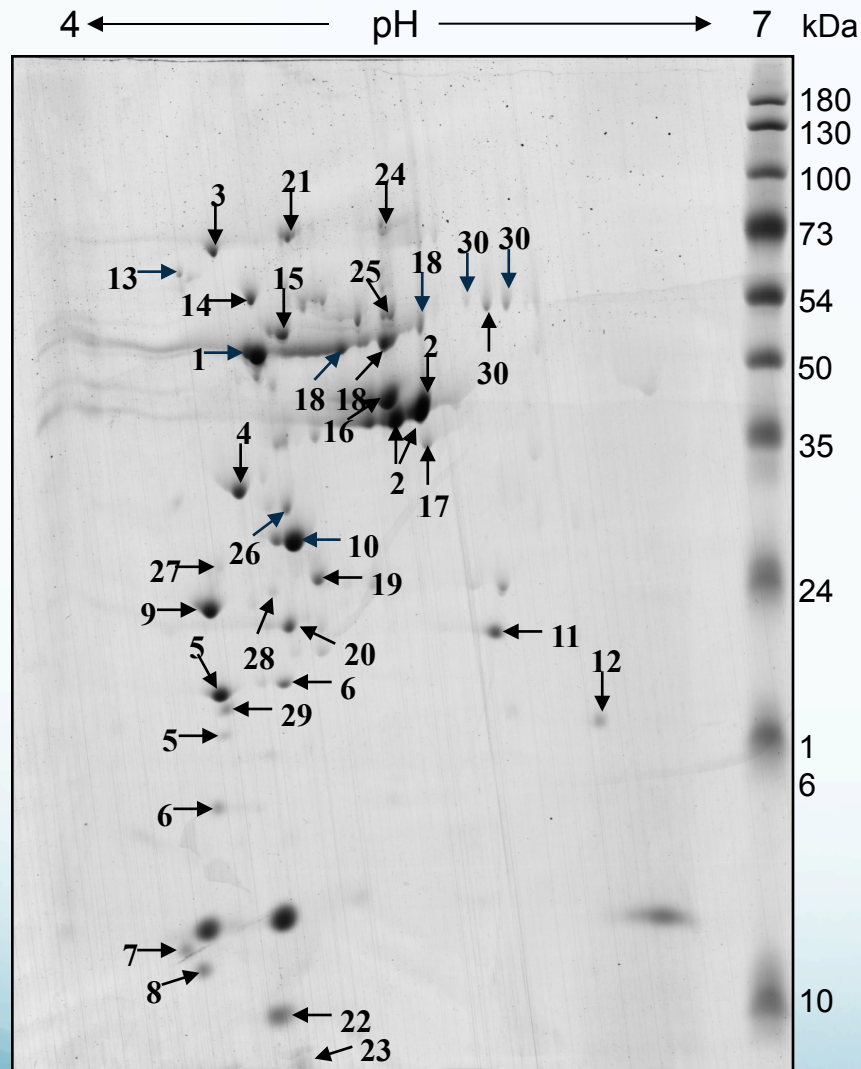


MS spectra



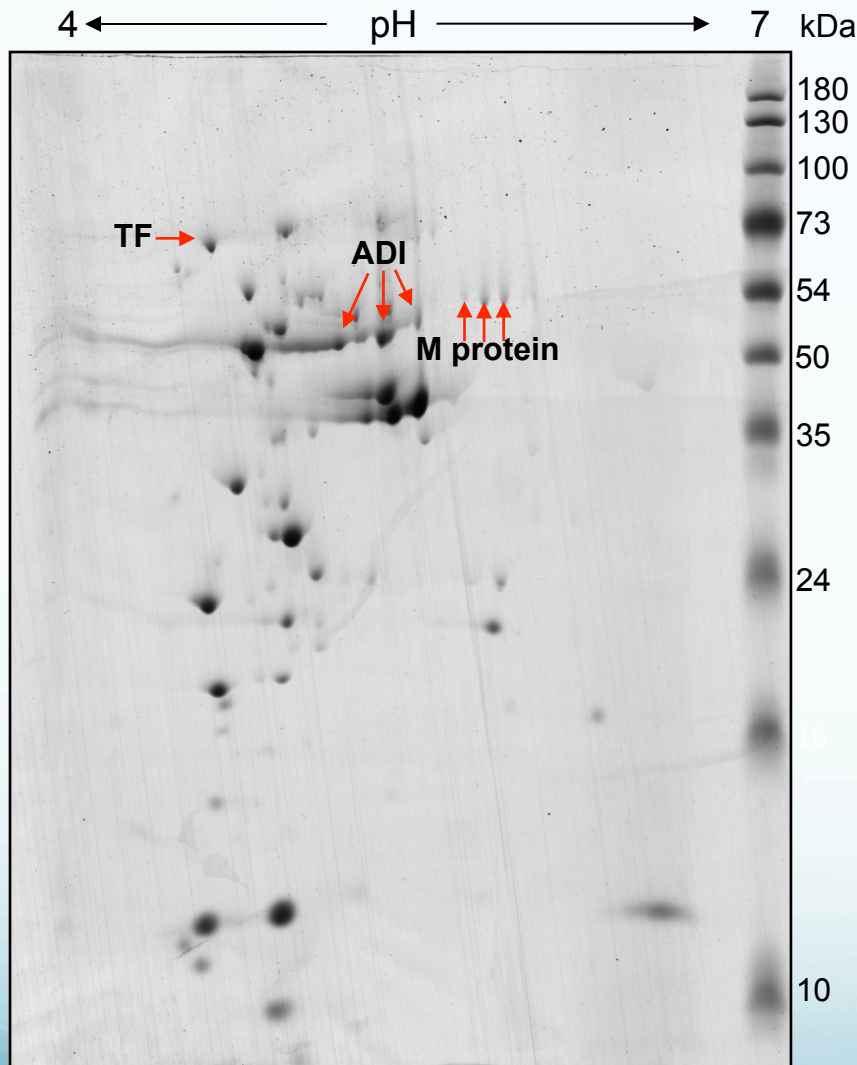
PeptIdent
Data Base

Proteomic Analysis of the GAS Cell Surface



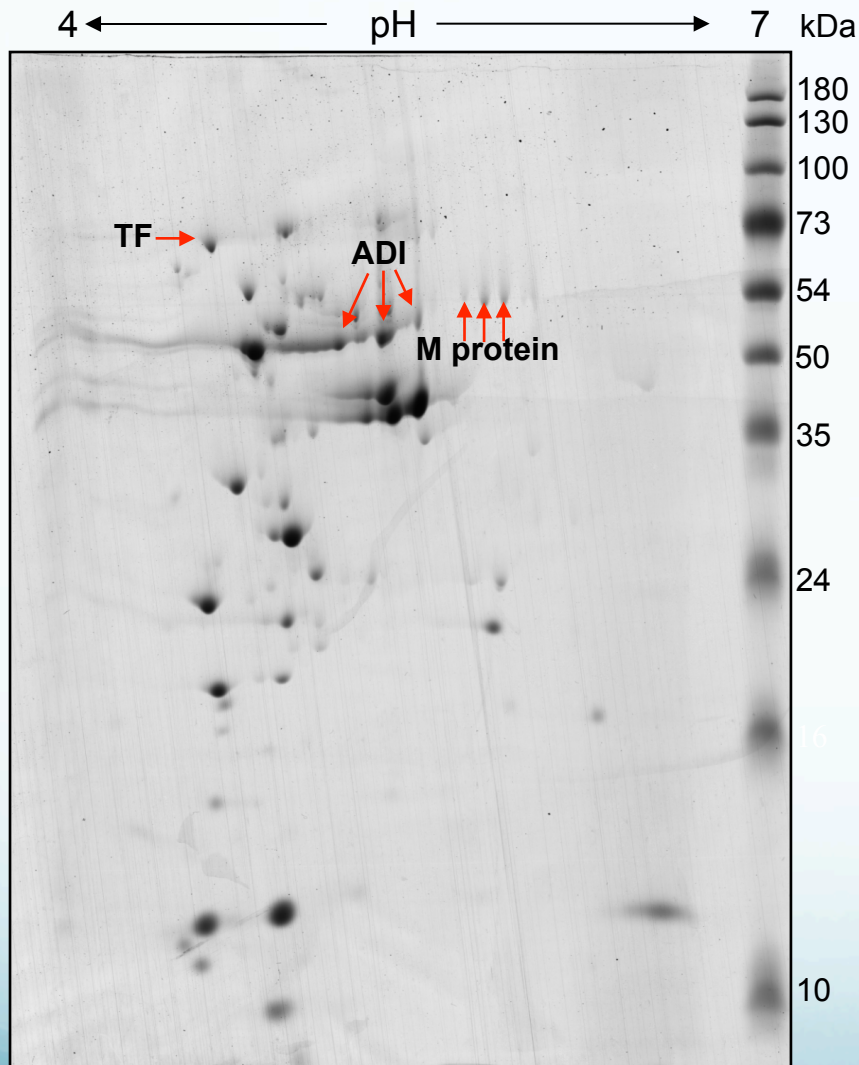
Mutanolysin extract of cell wall proteins from GAS isolate 5448

Proteomic Analysis of the GAS Cell Surface



- Started with ~20 candidates
- Tested candidates for
 - Conservation
 - Surface localization
 - Protection in mice
- Two “hits”
 - Arginine deiminase (ADI)
 - Trigger factor (TF)

Proteomic Analysis of the GAS Cell Surface



- Arginine deiminase (ADI)
 - Arginine degradation

- Trigger factor (TF)
 - Chaperone activity

- ADI and TF are anchorless - no signal sequence and no cell wall anchor motif

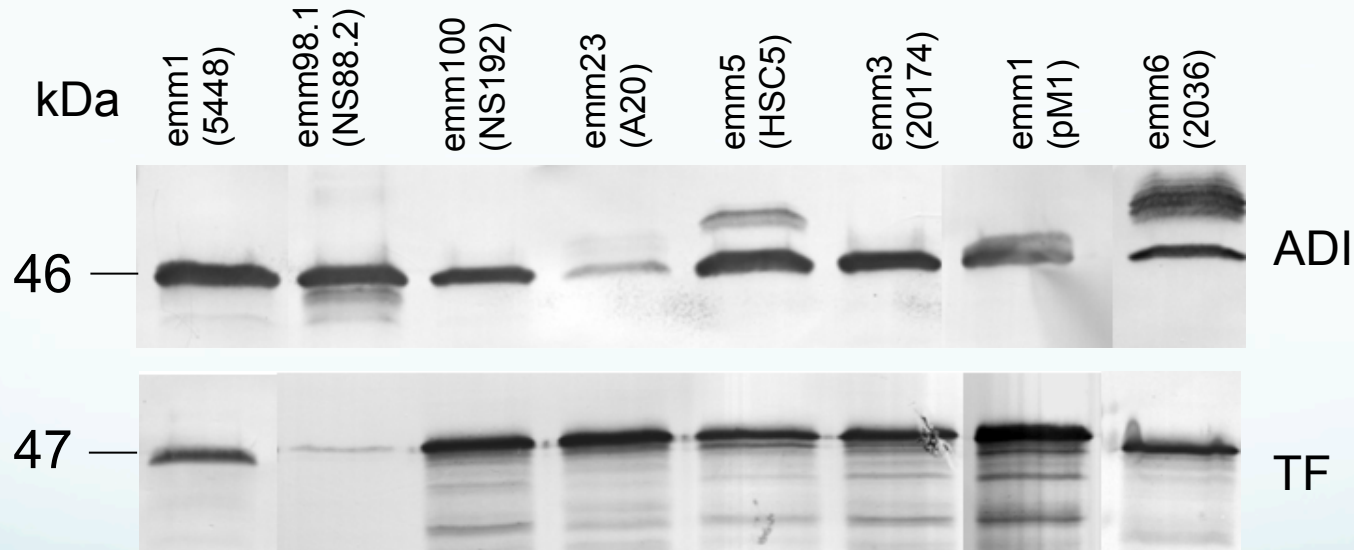
Conservation of Surface Proteins: BlastP

GAS strain	Serotype	Amino acid identity (%)	
		ADI	TF
M1	<i>emm1</i>	99	99
M49 591	<i>emm49</i>	99	100
MGAS10394	<i>emm6</i>	99	100
MGAS315	<i>emm3</i>	100	100
MGAS8232	<i>emm18</i>	99	99
SSI-1	<i>emm3</i>	100	100
MGAS10270	<i>emm2</i>	99	99
MGAS10750	<i>emm4</i>	99	99
MGAS2096	<i>emm12</i>	99	99
MGAS5005	<i>emm1</i>	99	99
MGAS6180	<i>emm28</i>	99	99
Manfredo	<i>emm5</i>	99	99
NZ131	<i>emm49</i>	99	99
MGAS9429	<i>emm12</i>	99	99

Expression of Surface Proteins

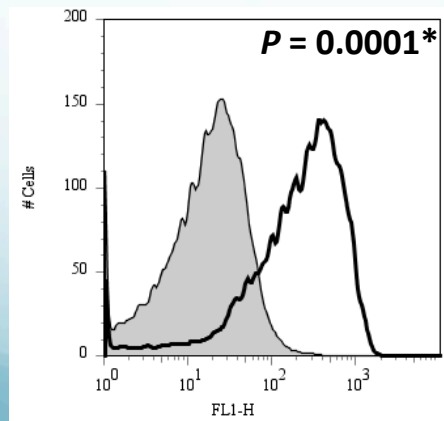
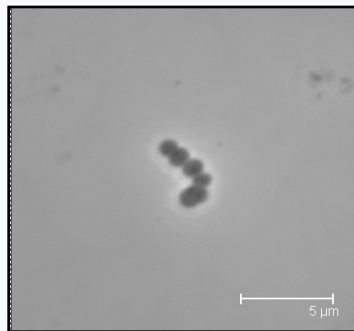
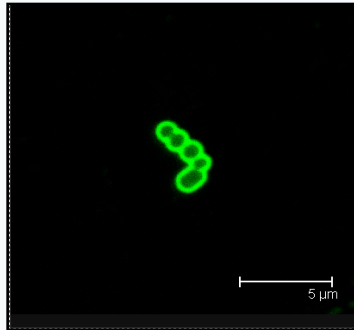
- ADI and TF are expressed in multiple GAS strains

Western blot of cell wall extracts

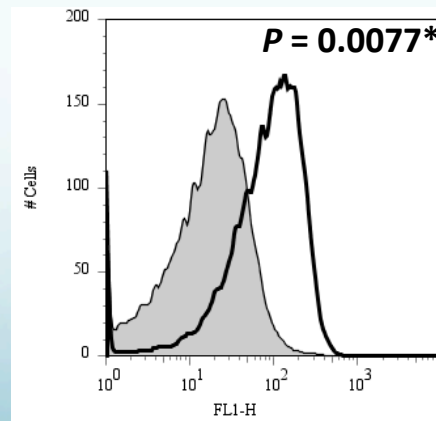
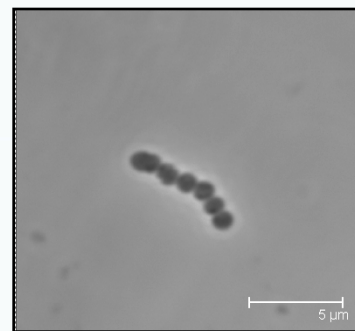


Visualization on the GAS Cell Surface

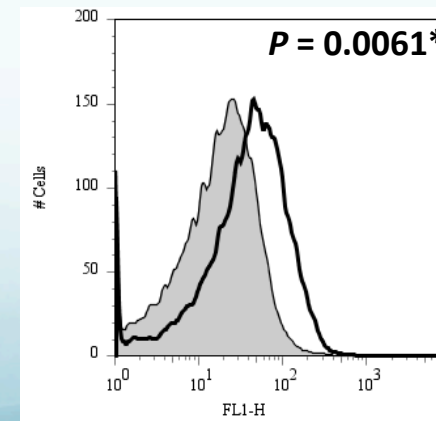
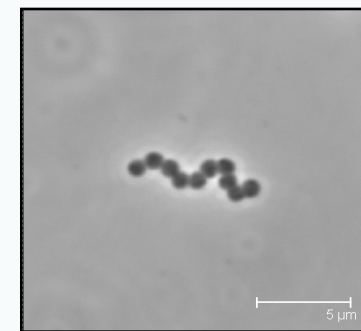
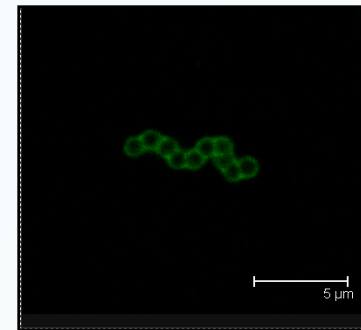
M protein



ADI



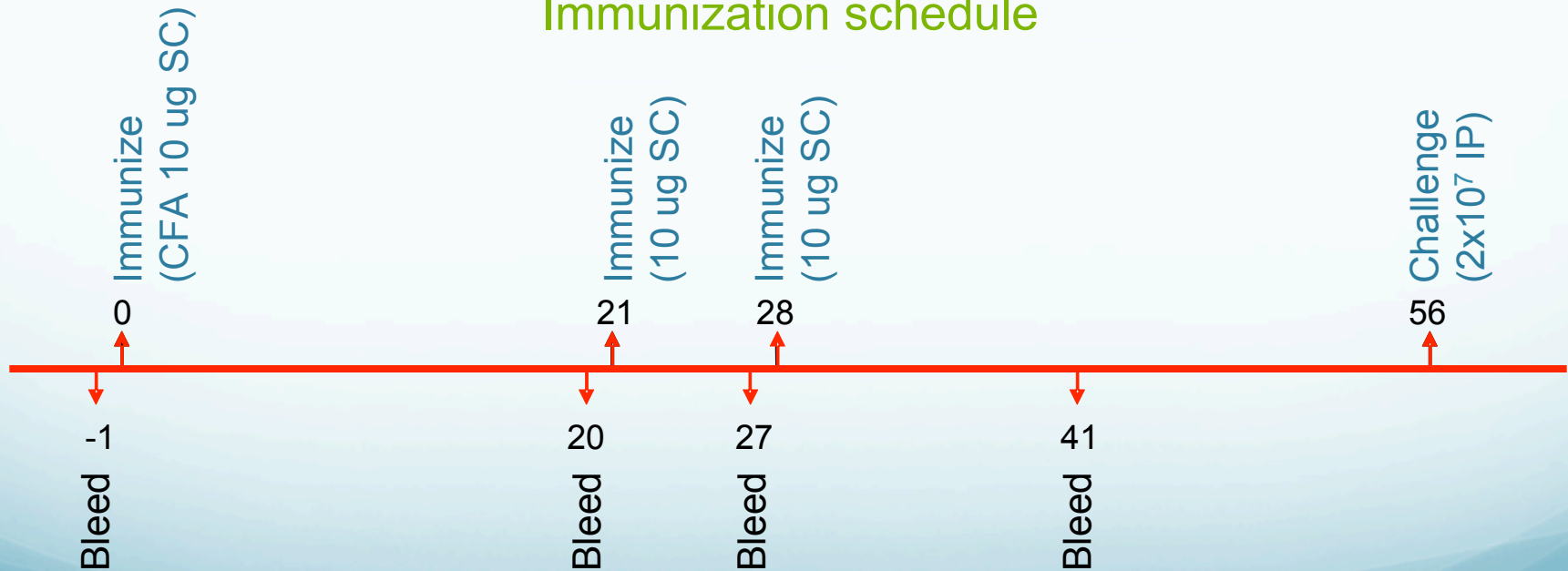
TF



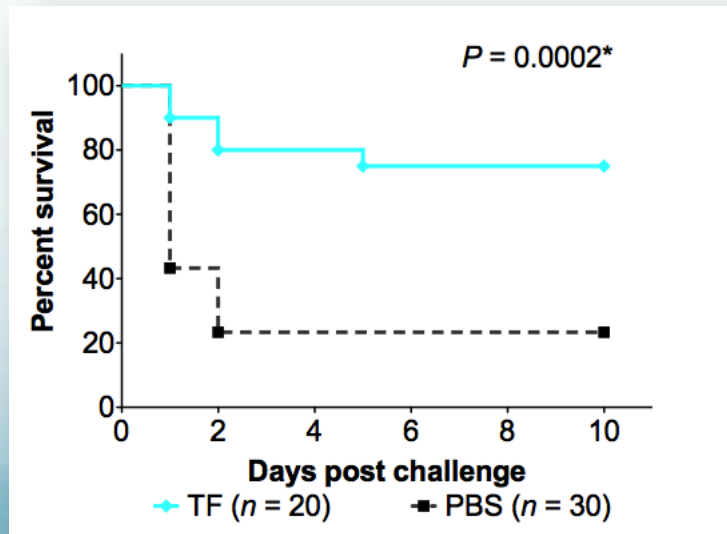
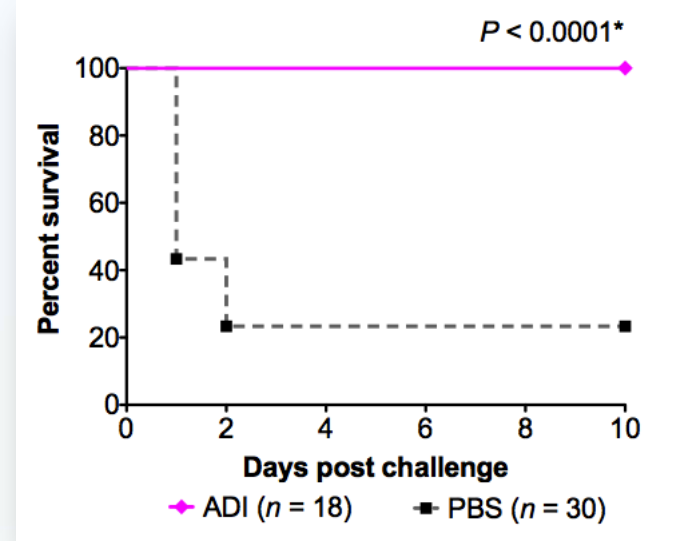
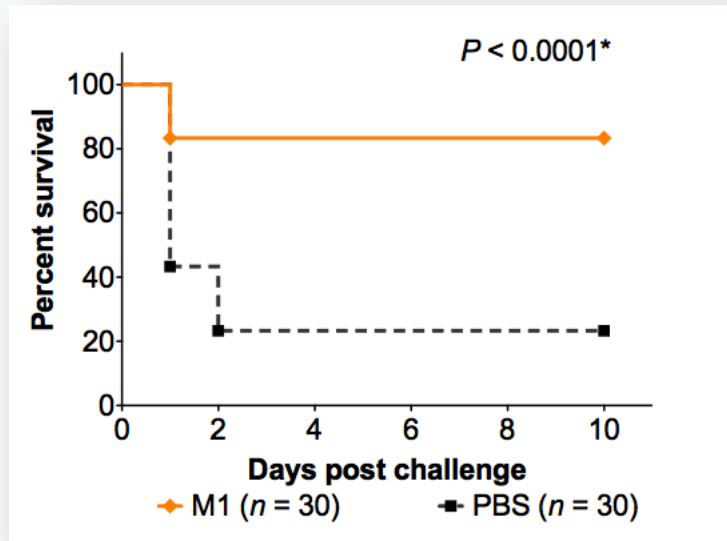
Immunization & Challenge

- Can the highly conserved surface proteins protect mice against lethal systemic challenge?
- Female BALB/c mice 4-6 weeks

Immunization schedule



Protection Following Lethal IP Challenge



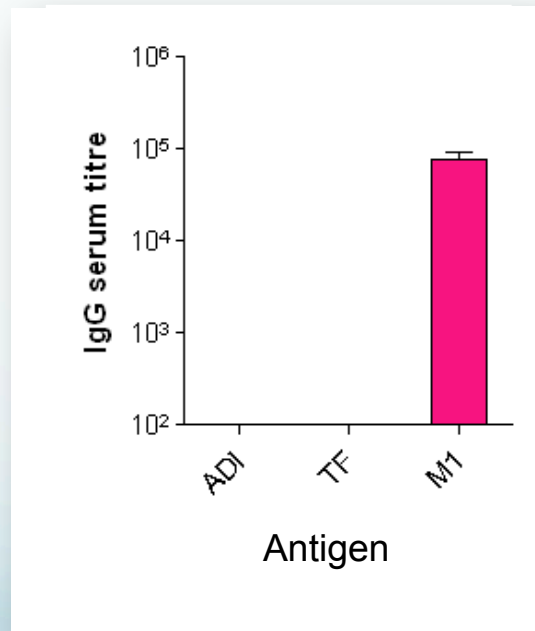
Dose: 2×10^7 cfu/mL pM1 (*emm1*)

Henningham *et al.*
J Mol Med (2012)

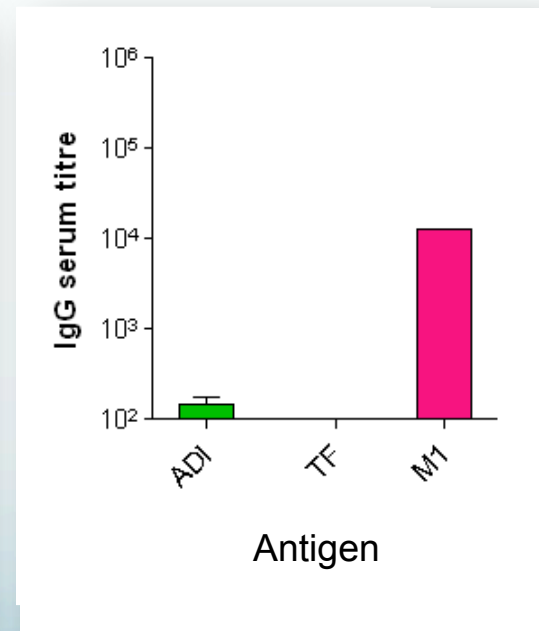
Reactivity of Proteins with Human Serum

- Pooled serum from patients with rheumatic fever and rheumatic heart disease has reduced reactivity to ADI and TF

Rheumatic fever serum



Rheumatic heart disease serum



Conclusion & Future Directions

- ADI and TF protect mice against systemic GAS infection
- Enhance safety of ADI and TF via abrogation of enzyme activity
 - ADI mutant proteins D166A and D277A retained structure, recognition by antisera and immunogenic epitopes, making them ideal for inclusion in GAS vaccine preparations

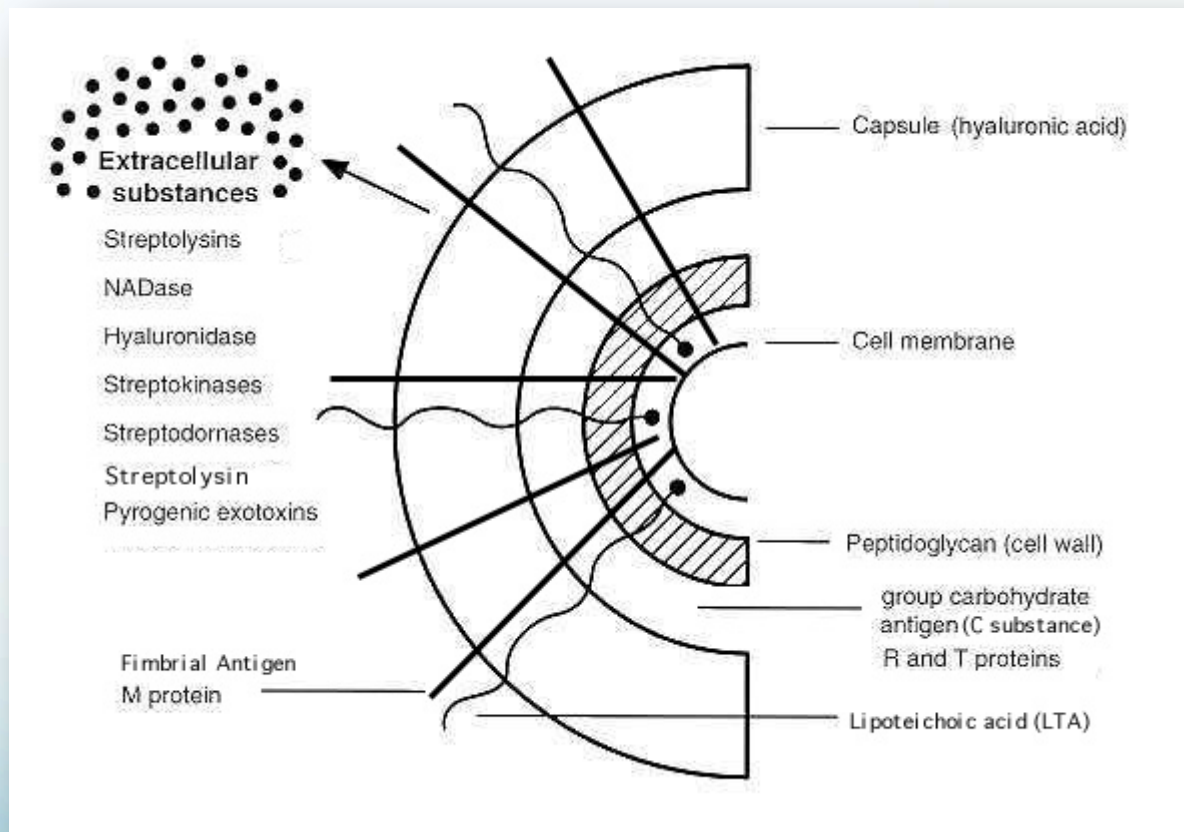
ADI crystal structure



Group A Carbohydrate (GAC)

- Polymer of rhamnose and N-acetylglucosamine (GlcNAc)

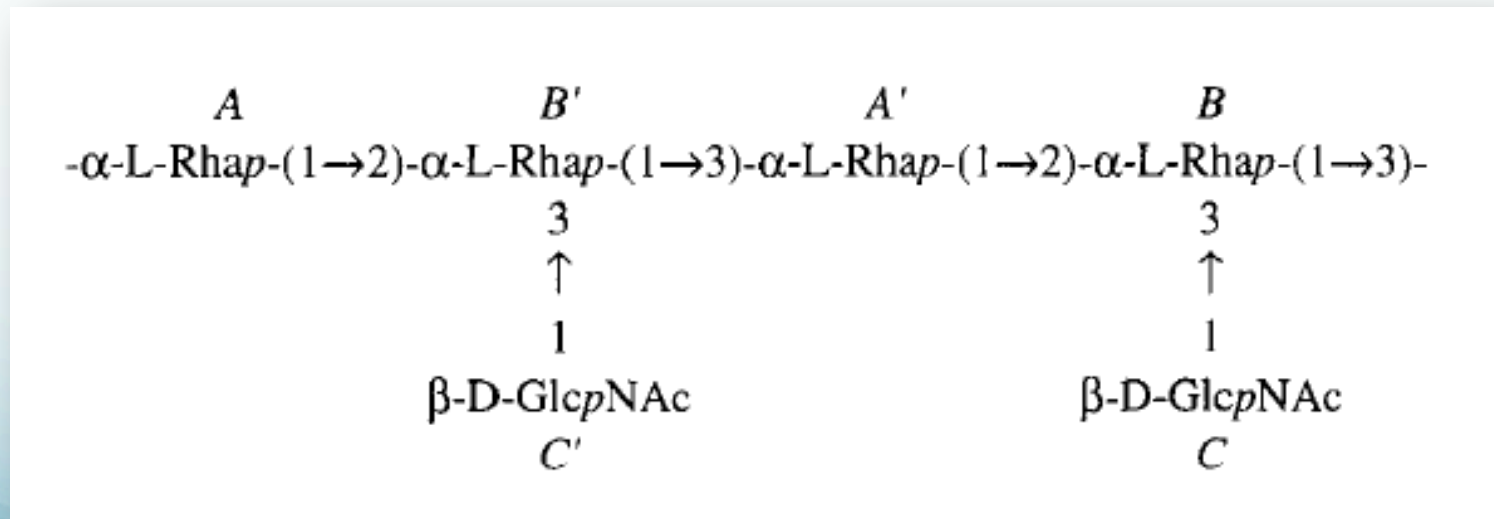
GAS cell wall structure



Group A Carbohydrate (GAC)

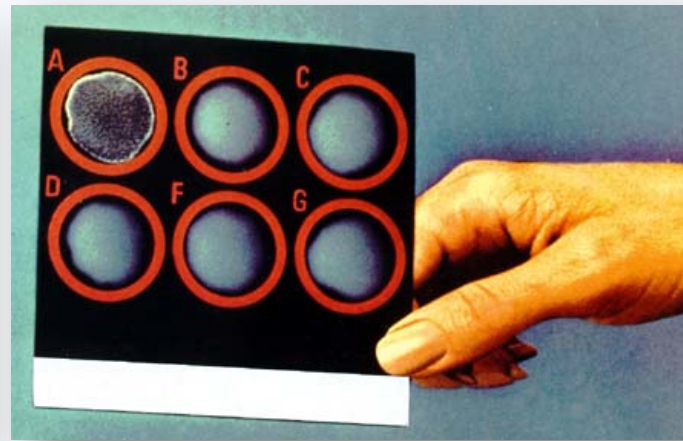
- Polymer of rhamnose and N-acetylglucosamine (GlcNAc)
 - 50% of the cell wall
 - Covalently linked to cell wall
 - Highly conserved among GAS

Group A carbohydrate structure



Group A Carbohydrate (GAC)

- Rapid test kits based on latex agglutination

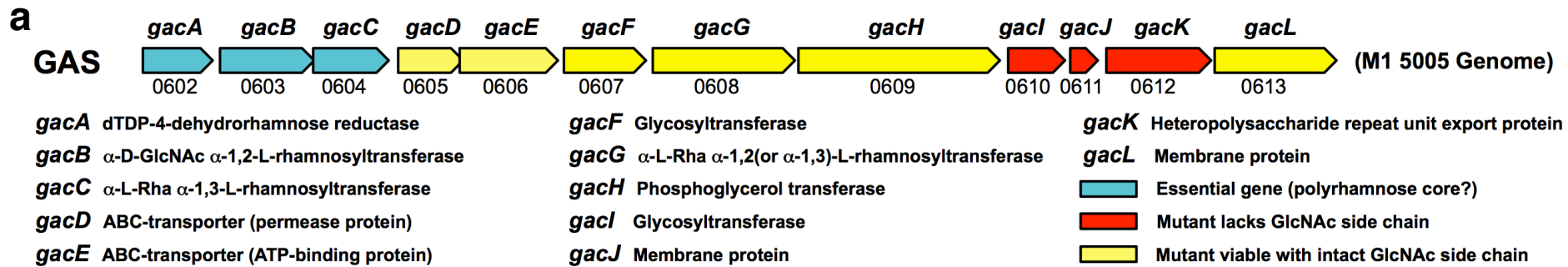


Agglutinating antibodies recognize the GlcNAc side chain

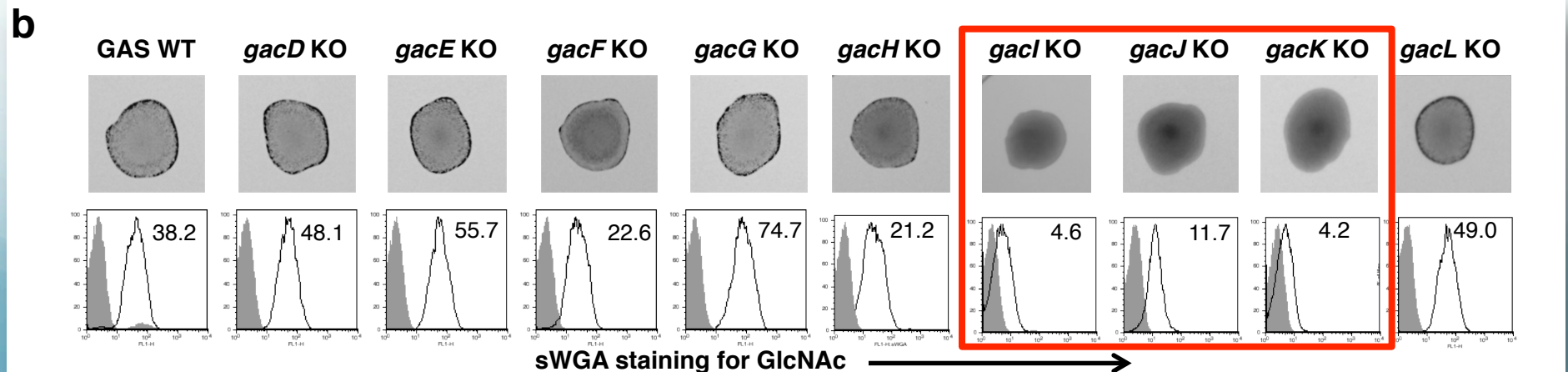
- Antibodies against the GlcNAc side chain may trigger RHD, hampering vaccine development

Identification of GAC Biosynthesis Genes

➤ Putative 12-gene GAC biosynthesis locus (*gacA-L*)



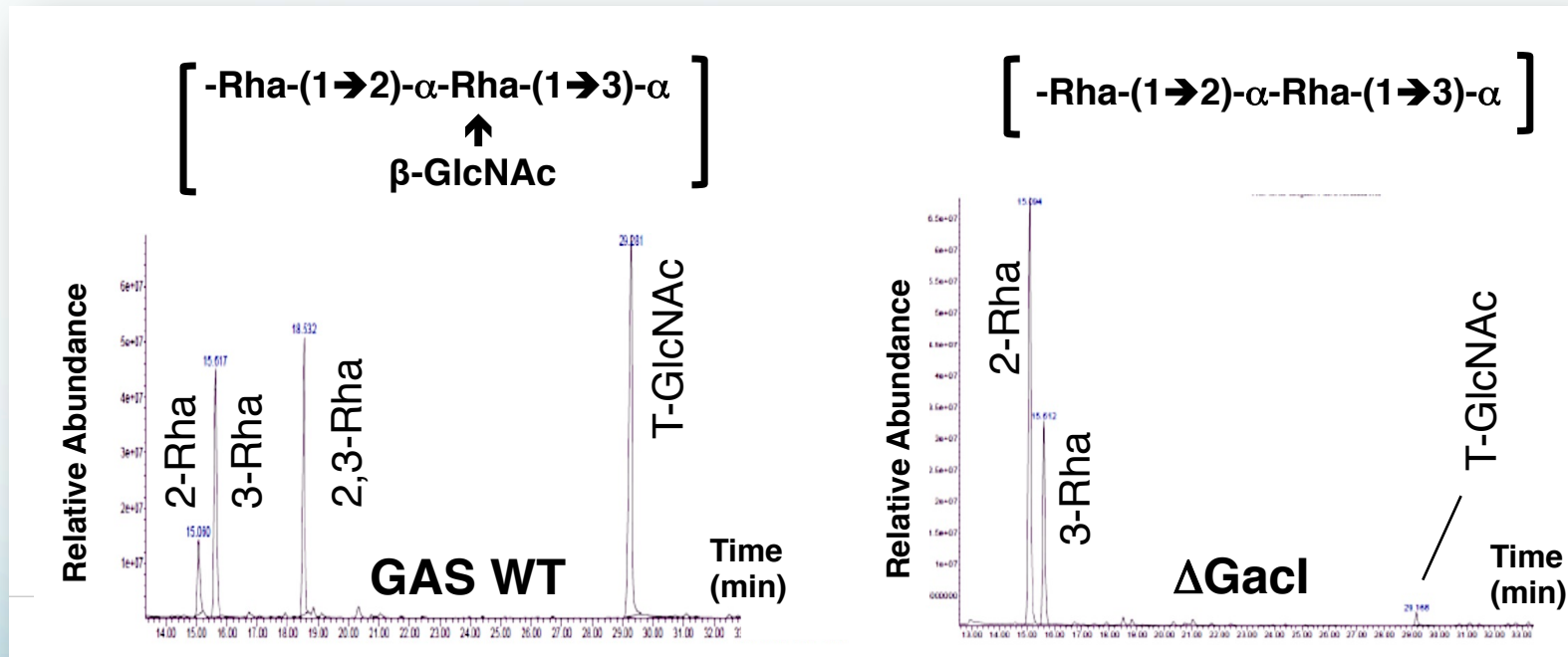
➤ *gacI*, *gacJ* and *gacK* mutants lack GlcNAc side chain



Glycoanalysis of $\Delta gacI$ GAC

- GacI is essential for the GlcNAc side chain of GAC

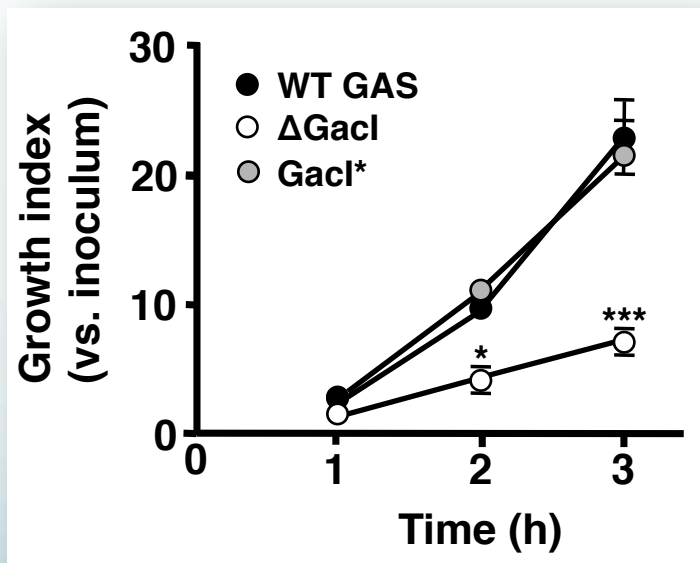
Glycoanalysis



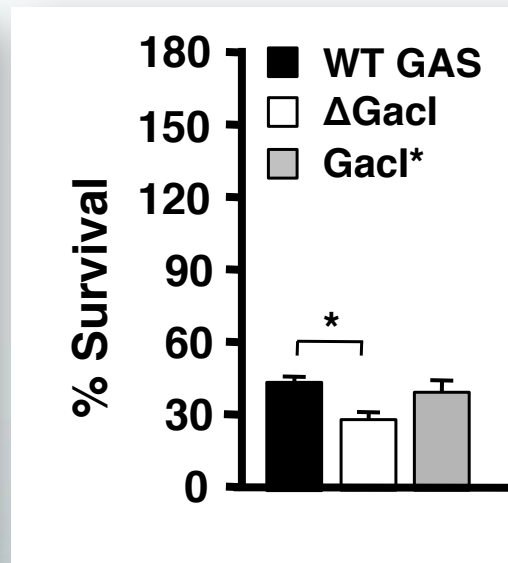
GlcNAc Enhances Innate Immune Resistance

- GlcNAc side chain promotes blood growth & neutrophil resistance

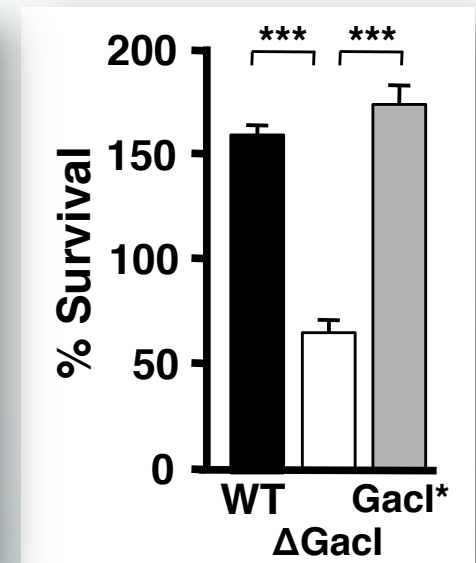
Human blood



Human neutrophils



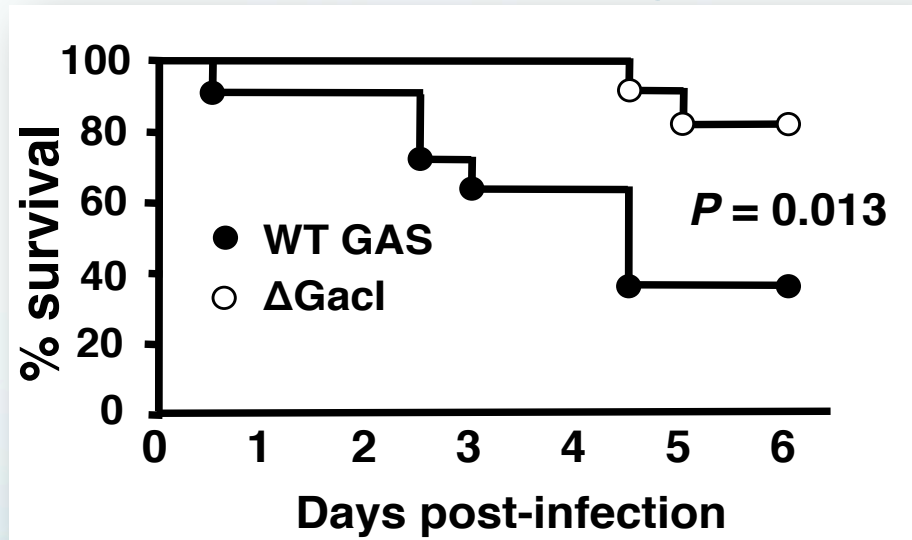
Human NETs



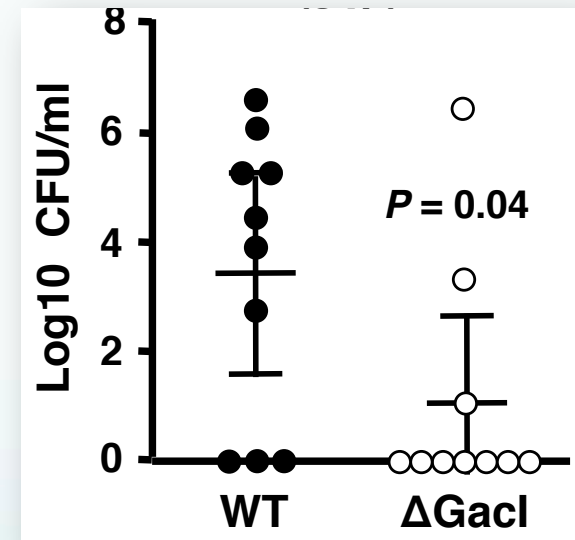
GlcNAc Enhances Virulence

- GlcNAc side chain promotes survival in a mouse infection model

Mouse IP challenge



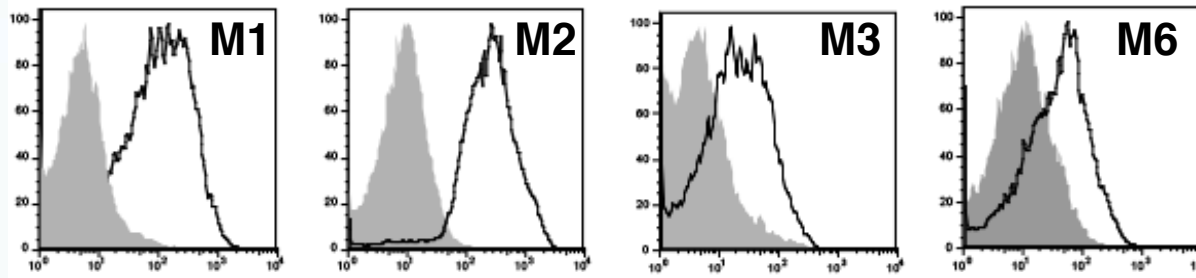
Mouse blood counts



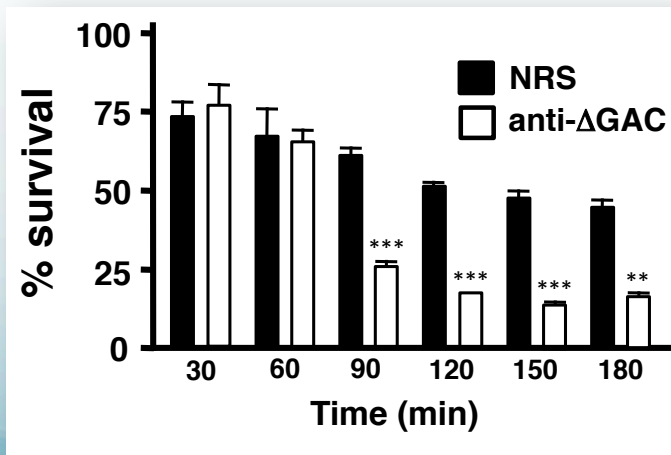
Anti- Δ GAC Antibodies Promote GAS Killing

- Anti- Δ GAC antibodies promote blood and neutrophil clearance

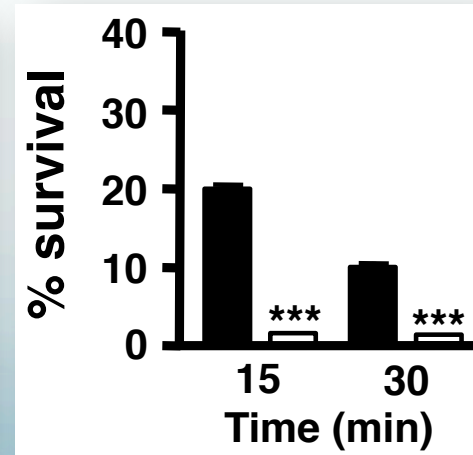
Anti- Δ GAC IgG binding to GAS



Whole blood killing



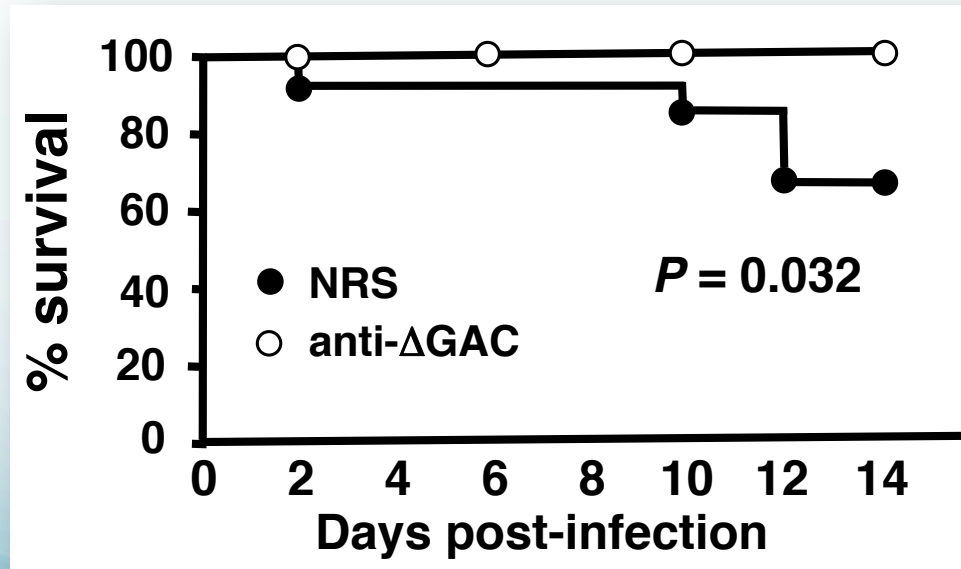
Neutrophil killing



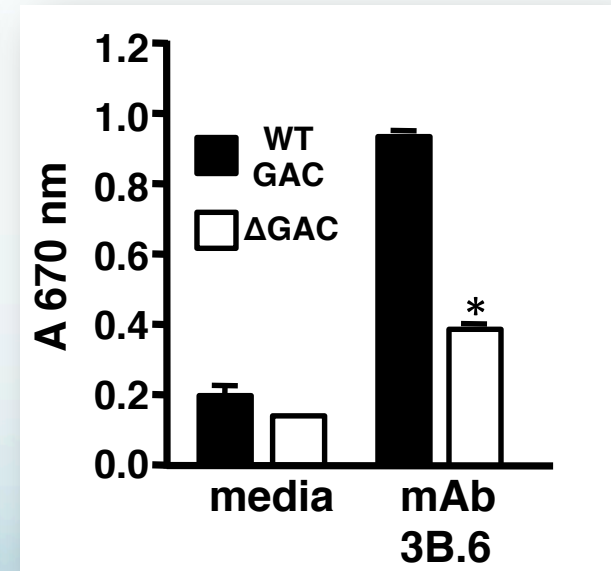
Anti- Δ GAC Antibodies Protect Mice

- Passive immunization with anti- Δ GAC antibodies protects mice
- Absence of GlcNAc may alleviate potential RHD safety concerns

Passive immunization
IP challenge



Reactivity of mAb from
RHD patient



Conclusions

- GAC is encoded by a conserved 12-gene locus (*gacA-L*)
- *Gacl* is essential for the GlcNAc side chain of GAC
- The GlcNAc side chain is a virulence factor
- GlcNAc-deficient GAC warrants further investigation as a vaccine

Future Work

- Complete non-human primate efficacy and safety testing prior to human clinical trials
- Immunize macaque monkeys with cocktail of ADI + Δ GAC
 - Monitor immune response
 - Investigate antiserum cross-reactivity with human heart tissue
 - Pharyngeal challenge with GAS



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BioVision 2014



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