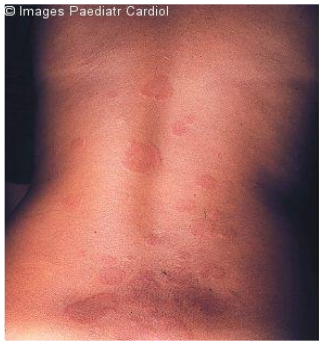
S. pyogenes candidate vaccine

Luiza Guilherme, PhD

Heart Institute (InCor)
University of São Paulo, São Paulo, Brazil
luizagui@usp.br

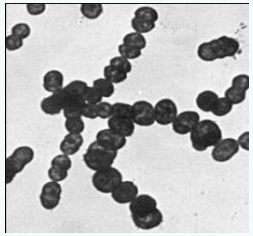
RF and RHD

Polyarthritis (90%)



Eritema *marginatum*

Streptococcus pyogenes



(3 a 4%) **Auto-immune Reations**

Subcutaneous Nodulles

Sydenham´s Chorea

Carditis

RHD

(30-45%)

(Decourt, 1972); (Kaplan, 1979)
Reviewed by Steer et al, 2007

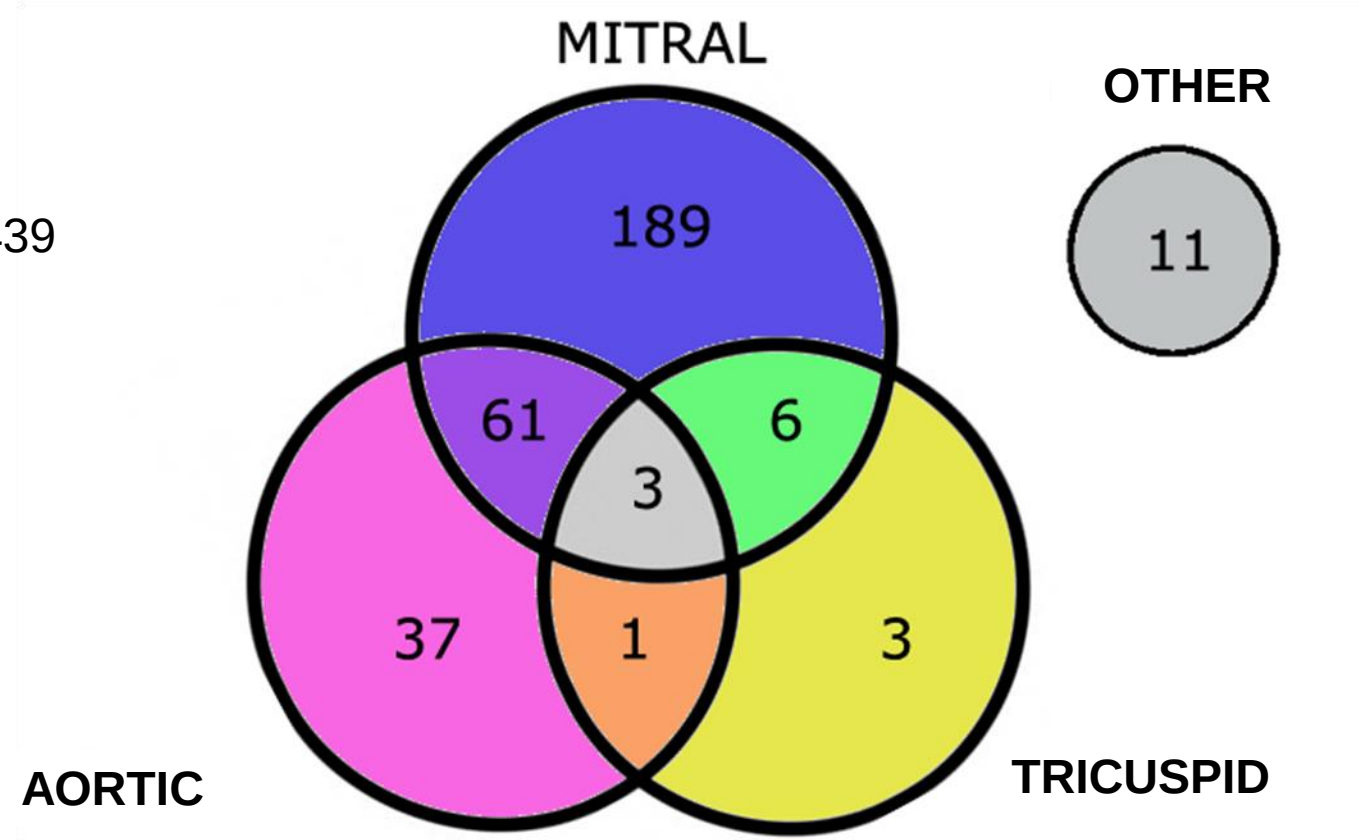
1840: “ Rheumatic fever licks the articulations and bits the heart.” (Jean Baptiste Bouillot)

2013

Pattern of Valvular Lesions of Rheumatic Fever/ Rheumatic Heart Disease Patients Based on Cardiology Auscultation

311 out of 439 patients

63.6%



GROUP of PATIENTS OF RHEUMATIC FEVER

CLINICAL HOSPITAL, SCHOOL of MEDICINE, UNIVERSITY of SÃO PAULO

Spina G., 2013

VALVAR SURGERIES - InCor

1980 - 2004

Rheumatic Fever Surgeries	Number - Patients
Valvar Replacement	3873
Valvar Plastic	1131
Total	**5004**

49.5 % of cardiac surgeries performed in 25 years

Pomerantzeff PMA, Brandao CM , et al.
Valve Reconstruction in the Heart Institute of São Paulo, Brazil.; Semin Thorac Cardiovasc Surg, 2002

Medical Care

- Ambulatory

  2014

  – Following-up: approximately 12000 patients;

  – Average monthly attendance: 1600 patients;

  May/2015:

  - New cases of VHD: 81patients
  - Total: 1604 patients

Medical Care -Teaching

- 28 bed ward for valvular heart disease;
- **In 2014**: 615 surgeries (25% of all surgeries);
- **May 2015**: 57 valvular heart surgeries (20% of all surgeries);

Monthly Activity Report, may 2015 – U.I.M.H.

Latin American / European / North American guidelines have a few diferences and a lot of similarities:

- What is the Latin America (Brazilian) reality?
  - Higher prevalence of rheumatic fever (up to 70% of all surgical cases of valvular disease).
  - Incidence of rheumatic heart disease at school age is : 1 to 7/1000 children in Brazil versus 0.1 to 0.4/1000 in the USA.
  - Higher incidence of valvular disease in young people.

- What are the European and USA realities?
  - Higher prevalence of degenerative valve disease (aortic stenosis and mitral regurgitation)

Rheumatic Fever – InCor
São Paulo - 2011

- ~ 600 outpatients / month
- ~ 2000 patients waiting for valvular surgery
- 38% of surgeries are in young patients

Genetic Susceptibility

Adaptive IR

HLA – Class II alleles

Serology, Mol Biol

Innate IR

SNP

MBL-2- Alleles A / O

TLR-2

FCN-2

FcRII A

Cytokines

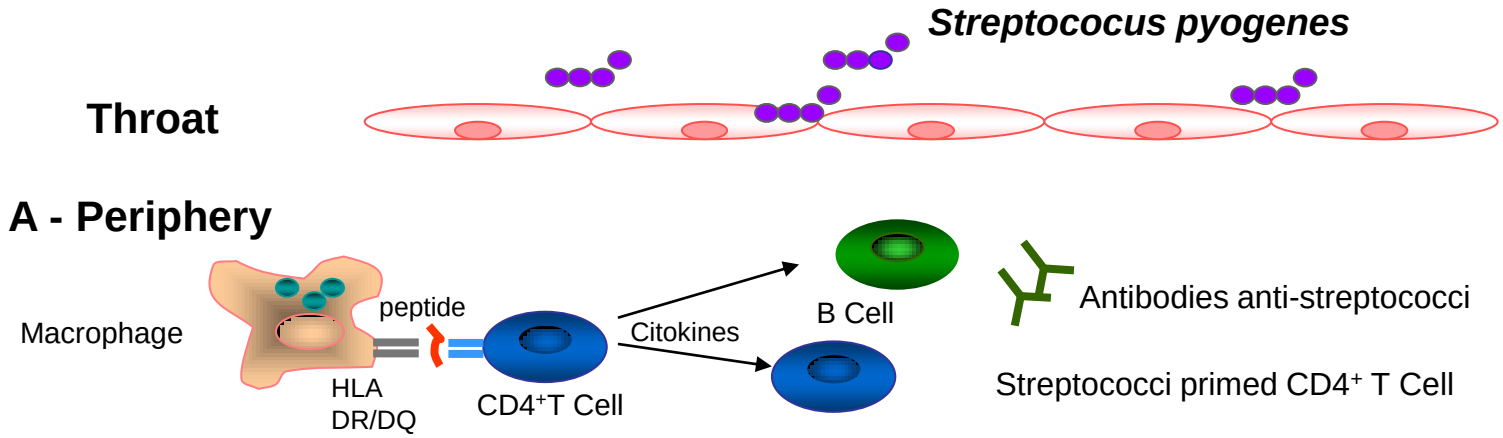
TNF- alfa

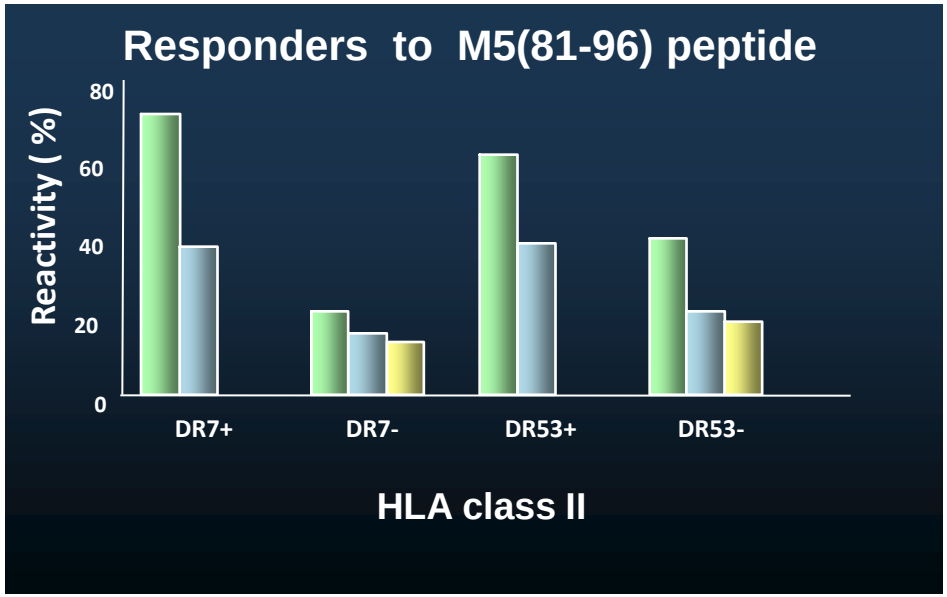
TGF-beta

IL-1 Ra

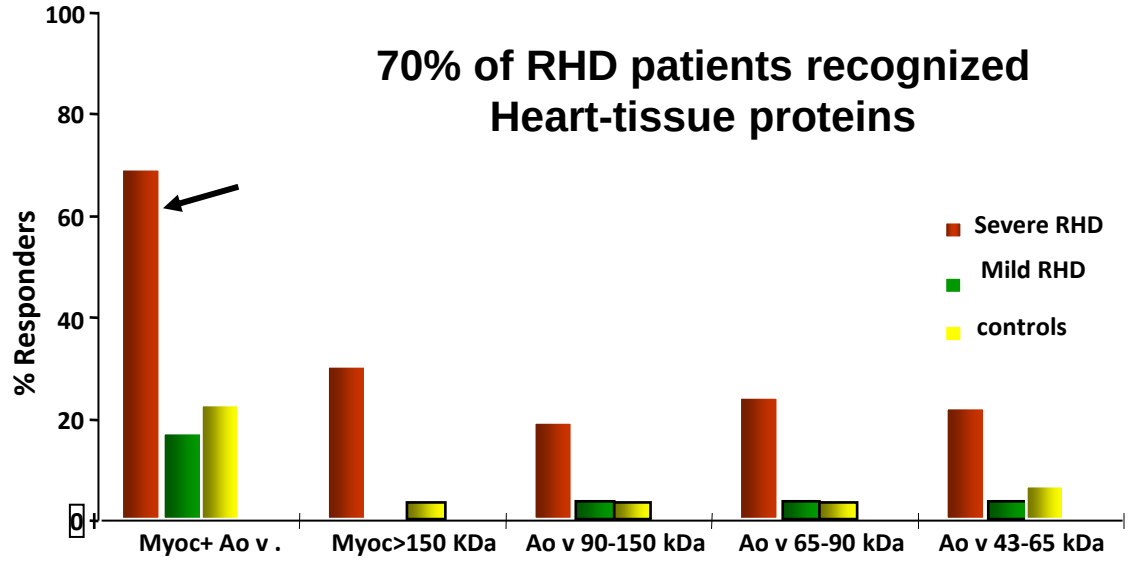
IL-10

RHD- Autoimmune Reactions: Peripheral T-cells and M protein Response



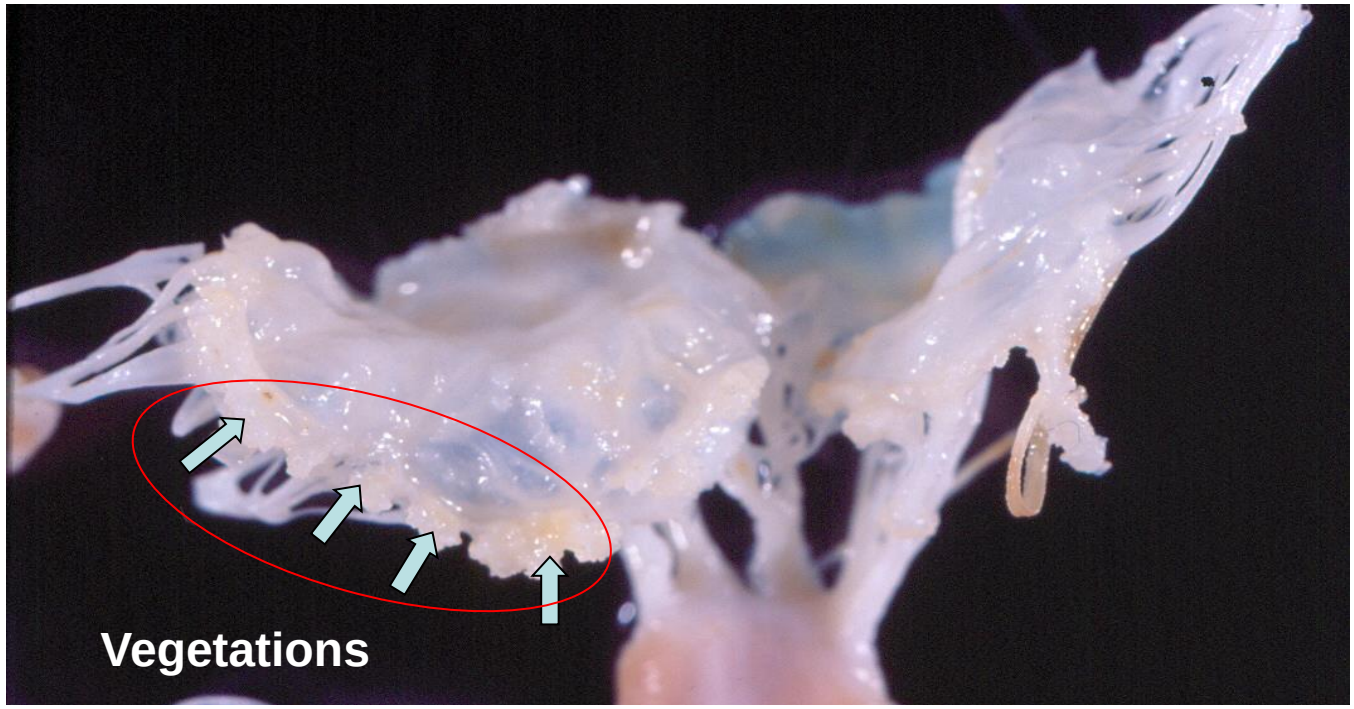


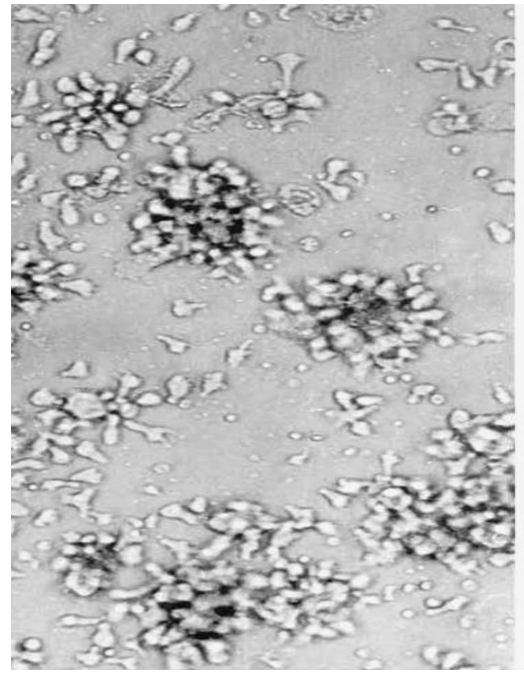
severe / mild RHD Controls



• Guilherme et al, Infect Immun, 2001

Rheumatic Valvulitis –Mitral v.





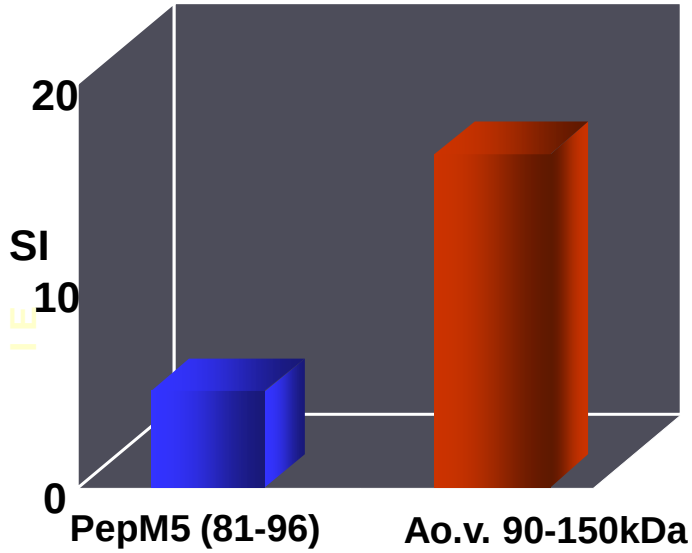
Valve Cross Reactive Proteins

Vimentin, Collagen VI, Lumican

Guilherme,L et al,

Circulation 1995 J. Autoimmunity, 2001

Martins C, Guilherme,L et al, 2014



Intralesional Auto-reactive T cell clones

Acute Phase – 67%

Chronic Phase – 20 to 30%

Cytokines in RHD

Th1

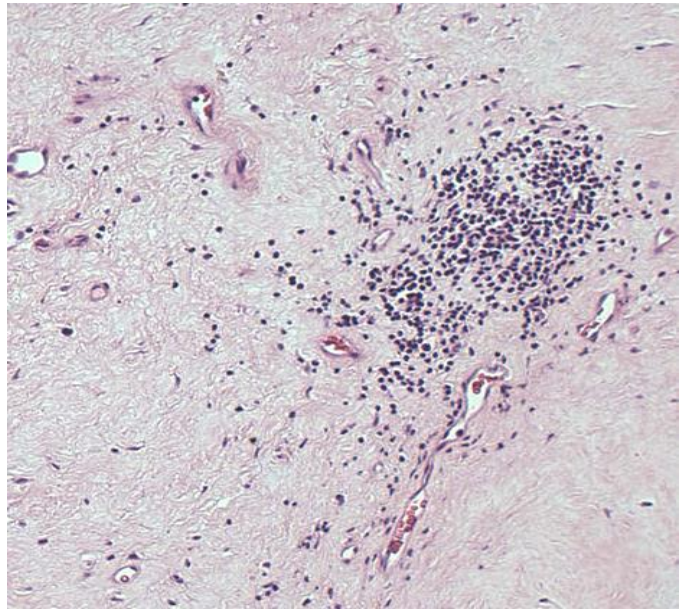
Inflammatory

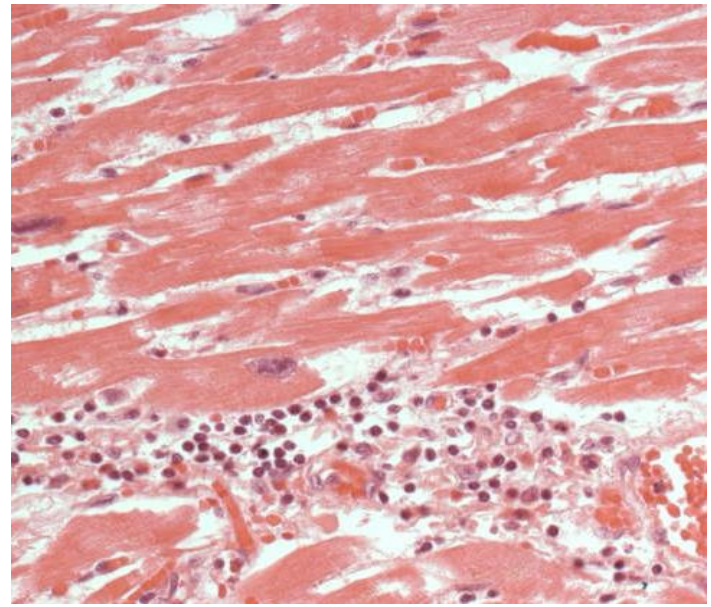
- TNFa , IFNg
- IL-17, IL-23

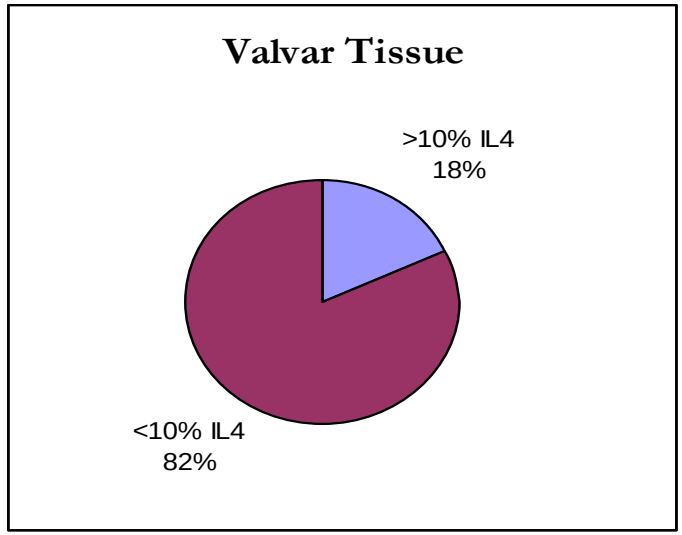
Regulatory

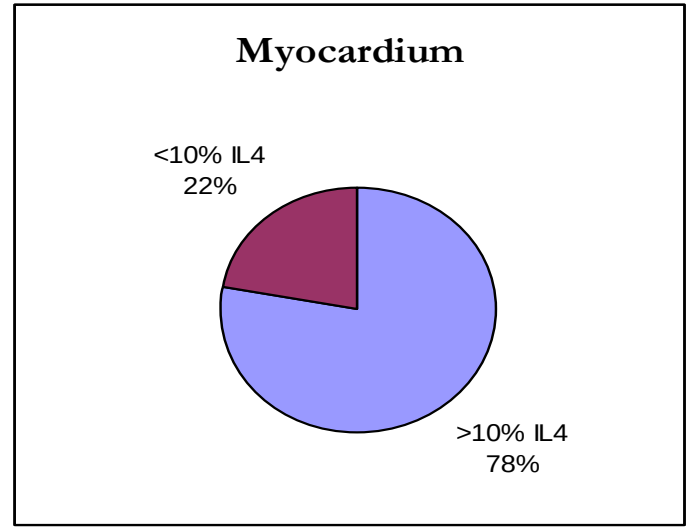
- IL-10
- IL-4 –Low numbers

- **Progression of RHD lesions**
- **Permanent valvular damage**







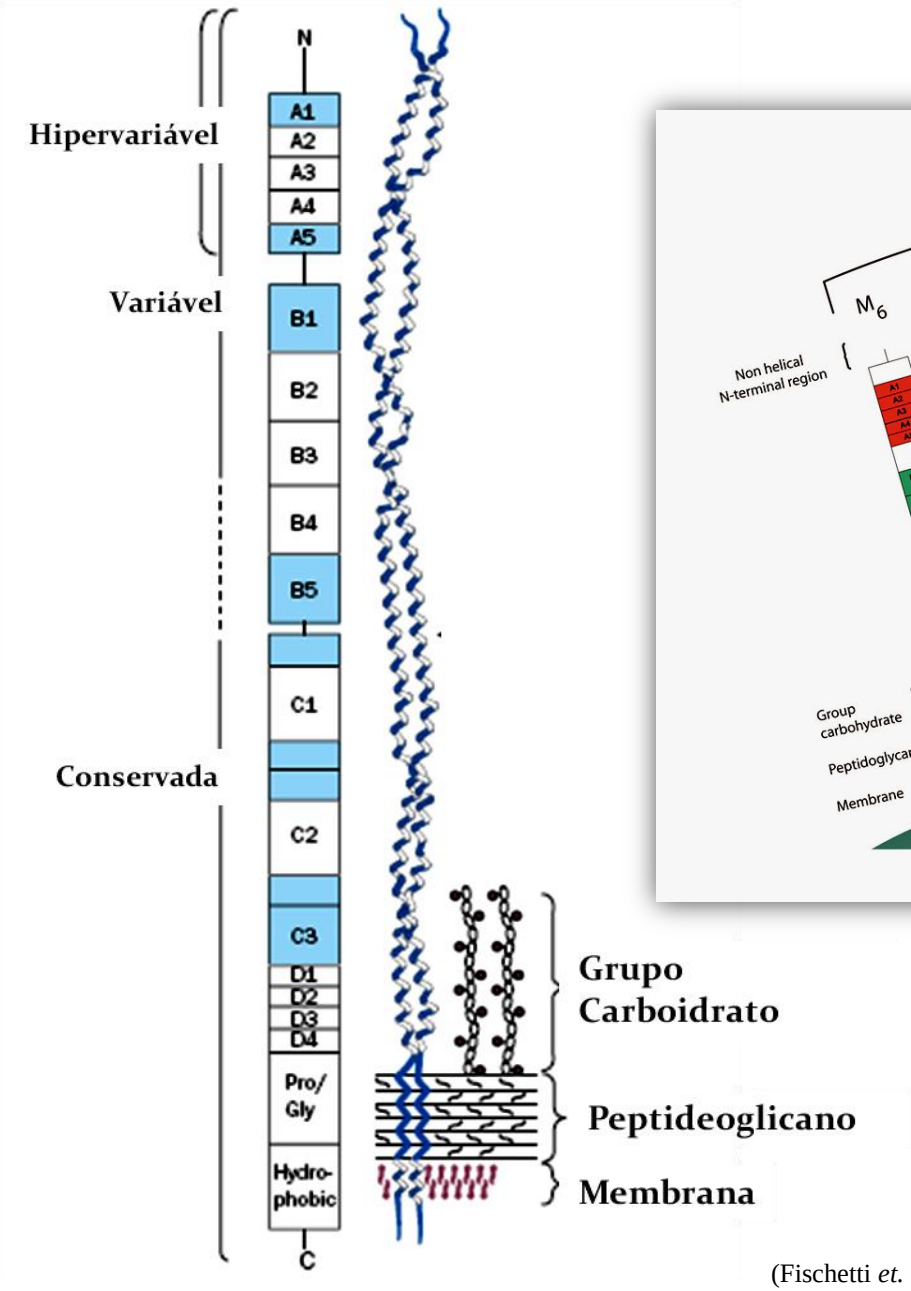


*** P < 0.02; O.R. = 15.8**

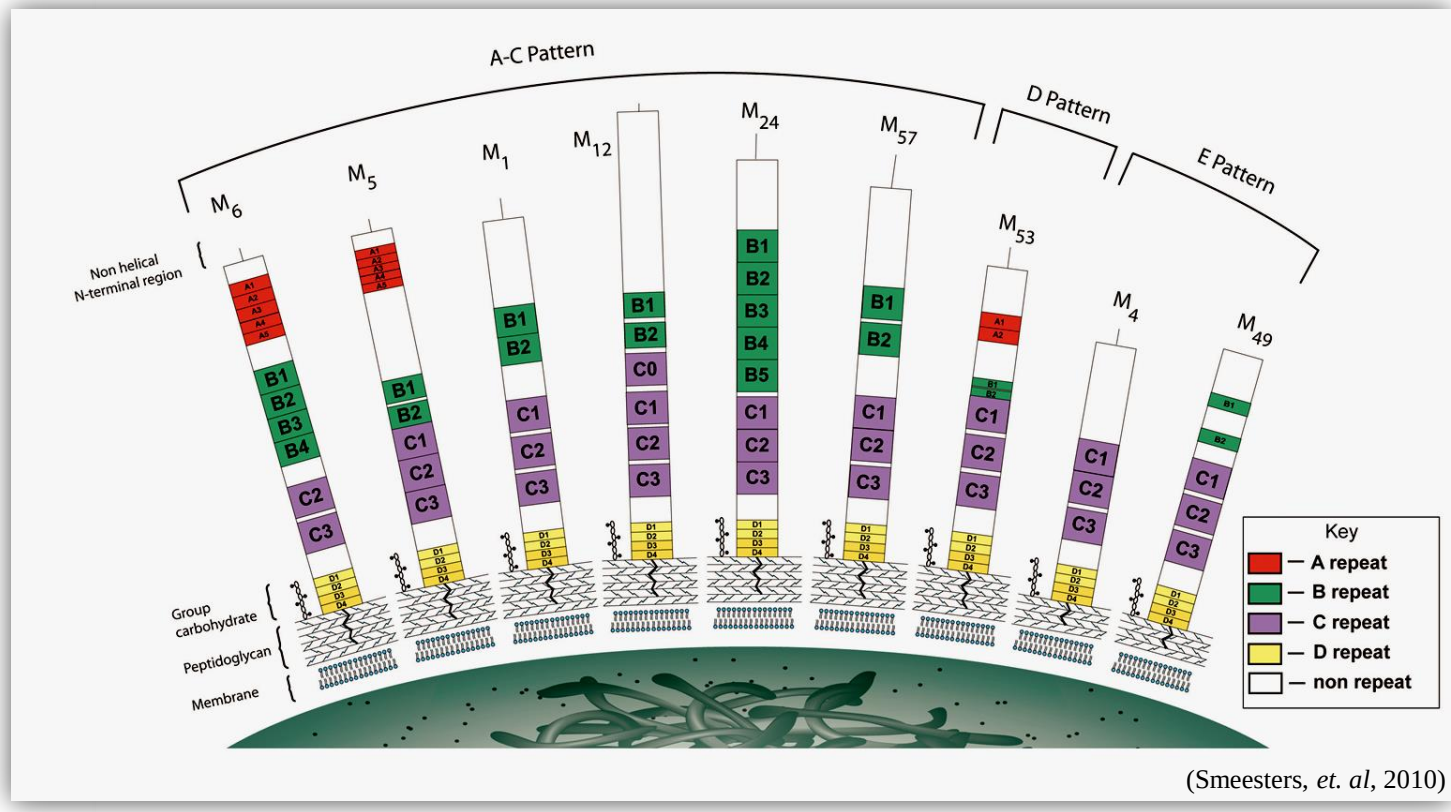
Guilherme L, et al, Am J Pathol,165:1583-91, 2004

M protein

Streptococcus pyogenes



(Fischetti *et. al*, 1991)



(Smeesters, *et. al*, 2010)

More than 200 strains

Vaccine Development
M protein- C-terminal Region: T and B Epitopes

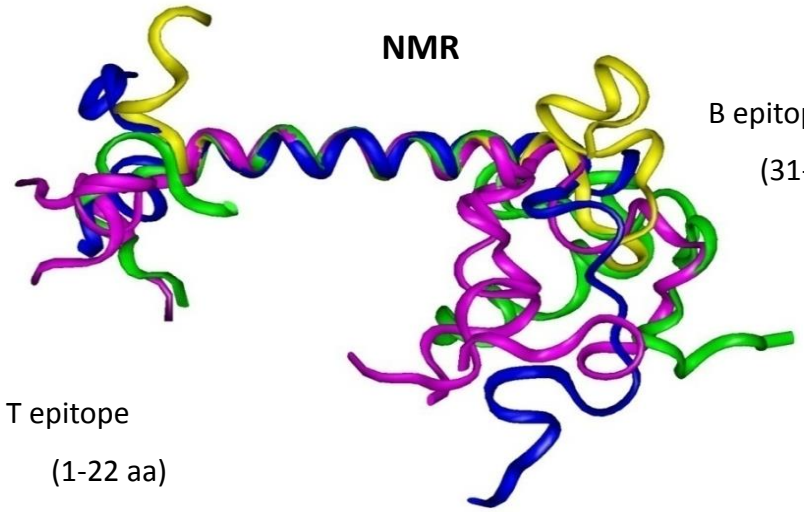
Identical Region

253 KGLRRDLDASREAKKQLEAEQQ 279 **T epitope**

288 EASRKGLRRDLDASREAKKQVEKA 312 **B epitope**

PepVac/Rec.Prot – StreptInCor 55 aa

253KGLRRDLDASREAKKQLEAEQQKLEEQNKISEASRKGLRRDLDASREAKKQVEKA312



Sequence data bank

PDB ID 2KK9

RCSB 101224

Patents

INPI, BR – 0501290 / 0604997-4,

International: China, Korea, Japan, USA

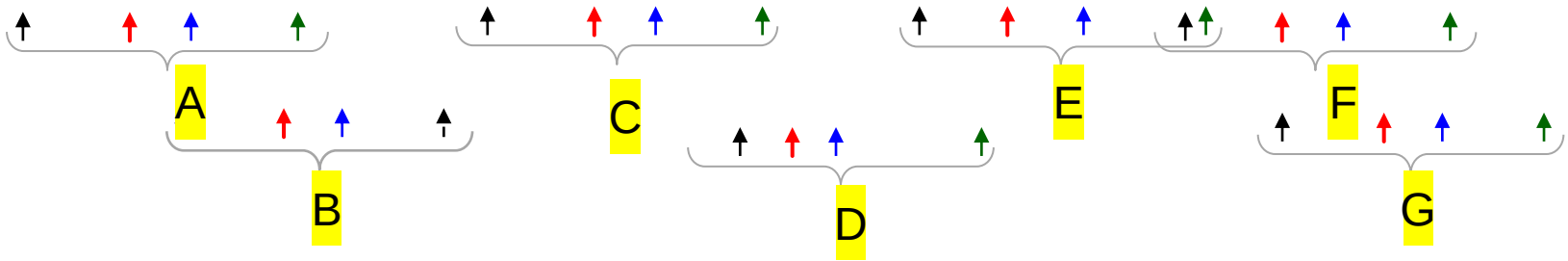
Therapeutic Effect : USA

Guilherme L, Kalil, J, et al, Clin Dev Immunol, 2006, Methods, 2009,

J Biol Chem, 2011

HLA Class II – Binding Prediction

^{253}KGLRRDLDASREAKKQLEAEQQKLEEQNKISEASRKGLRRDLDASREAKKQVEKA^{312}



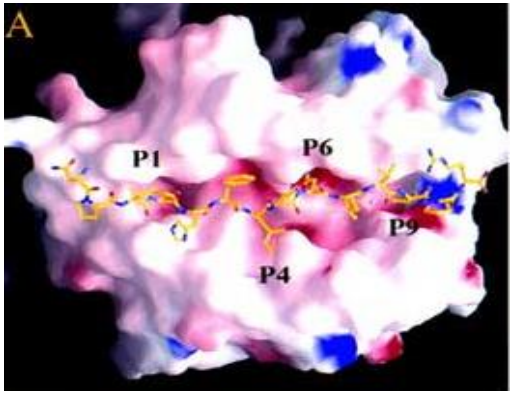
Afinity

P1 - L, I

P4 - D, S, E, A, Q, N

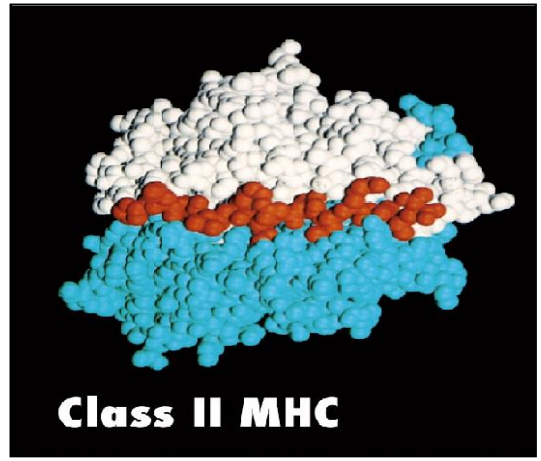
P6 - D, E, Q, R,

P9 - R, K, E, L,

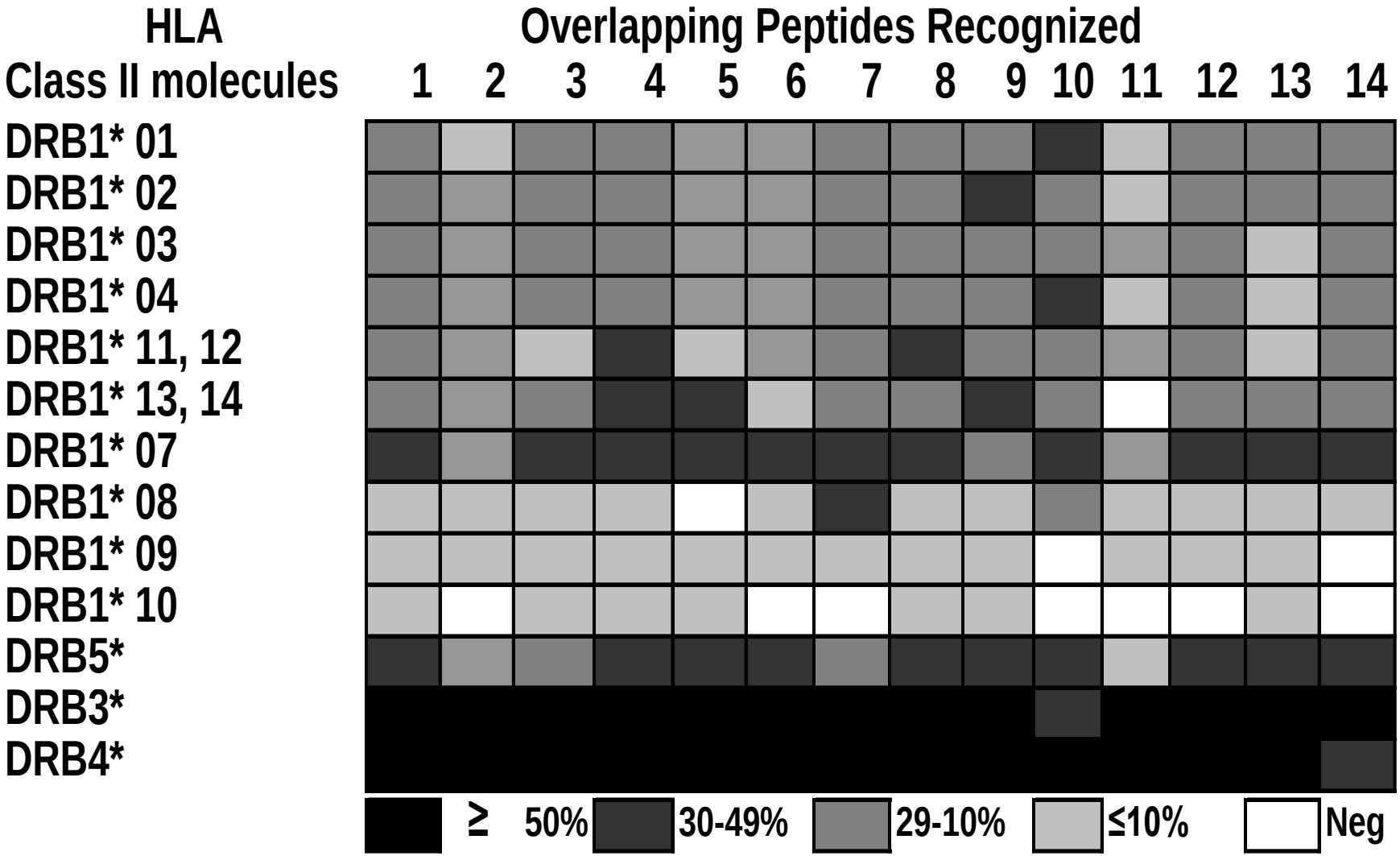


Binding Prediction

Pept.		HLA-II	
A	F	DR-03	DR52
B	G	DR-04,08,09	DR-52, 53
C		DR-04	DR-53
D		DR-06, 07	DR-52, 53
E		DR02, 05	DR-51, 52



Human Humoral and Celular Reactivity



250 PBMC samples

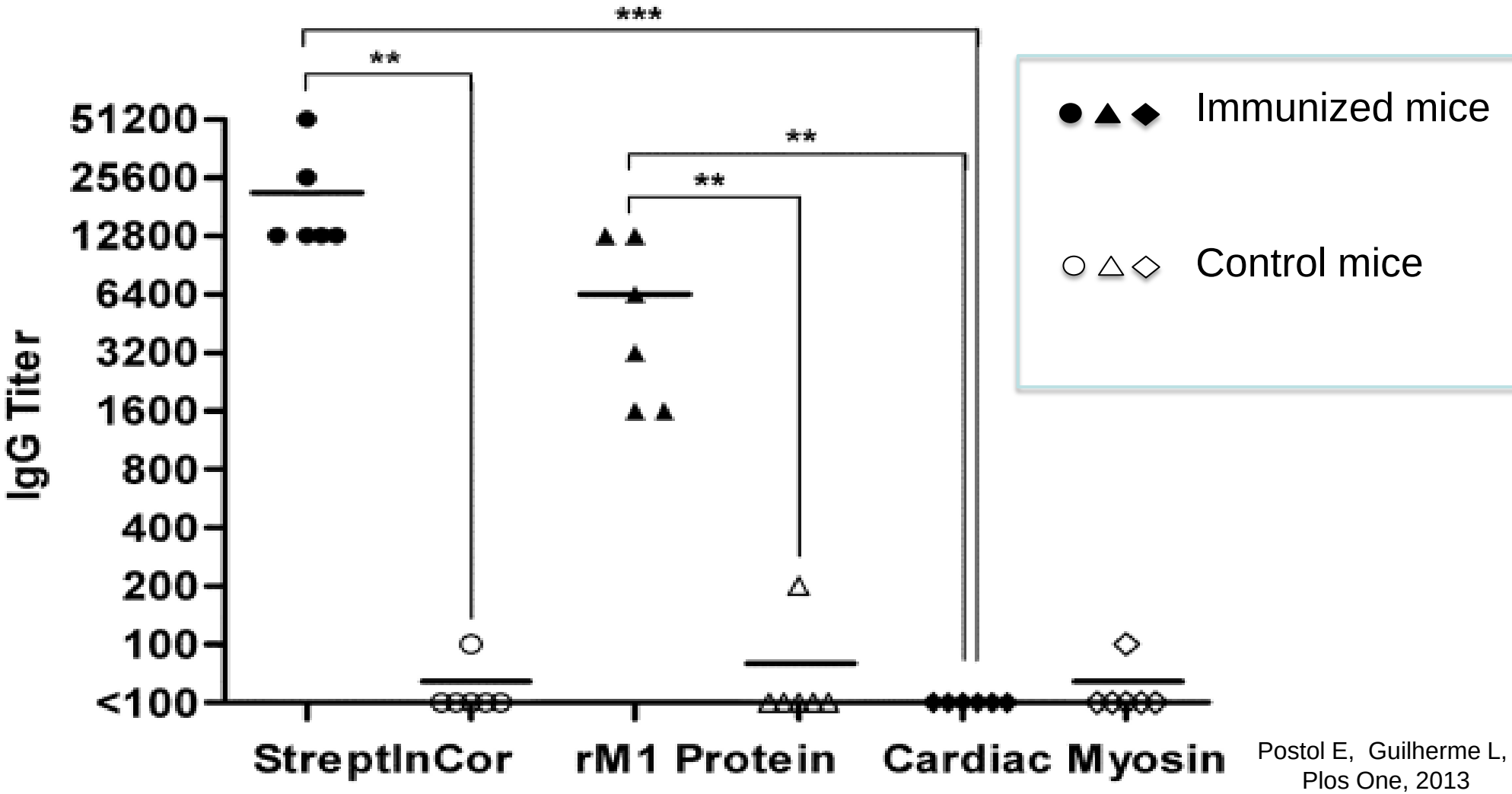
Guilherme et al, J Biol Chem, 2011

Experimental Assays

- Mice (**Balb-C, C57BL6, Swiss, HLA- class II transgenic mice**)
- Mini pigs (25-30Kg)

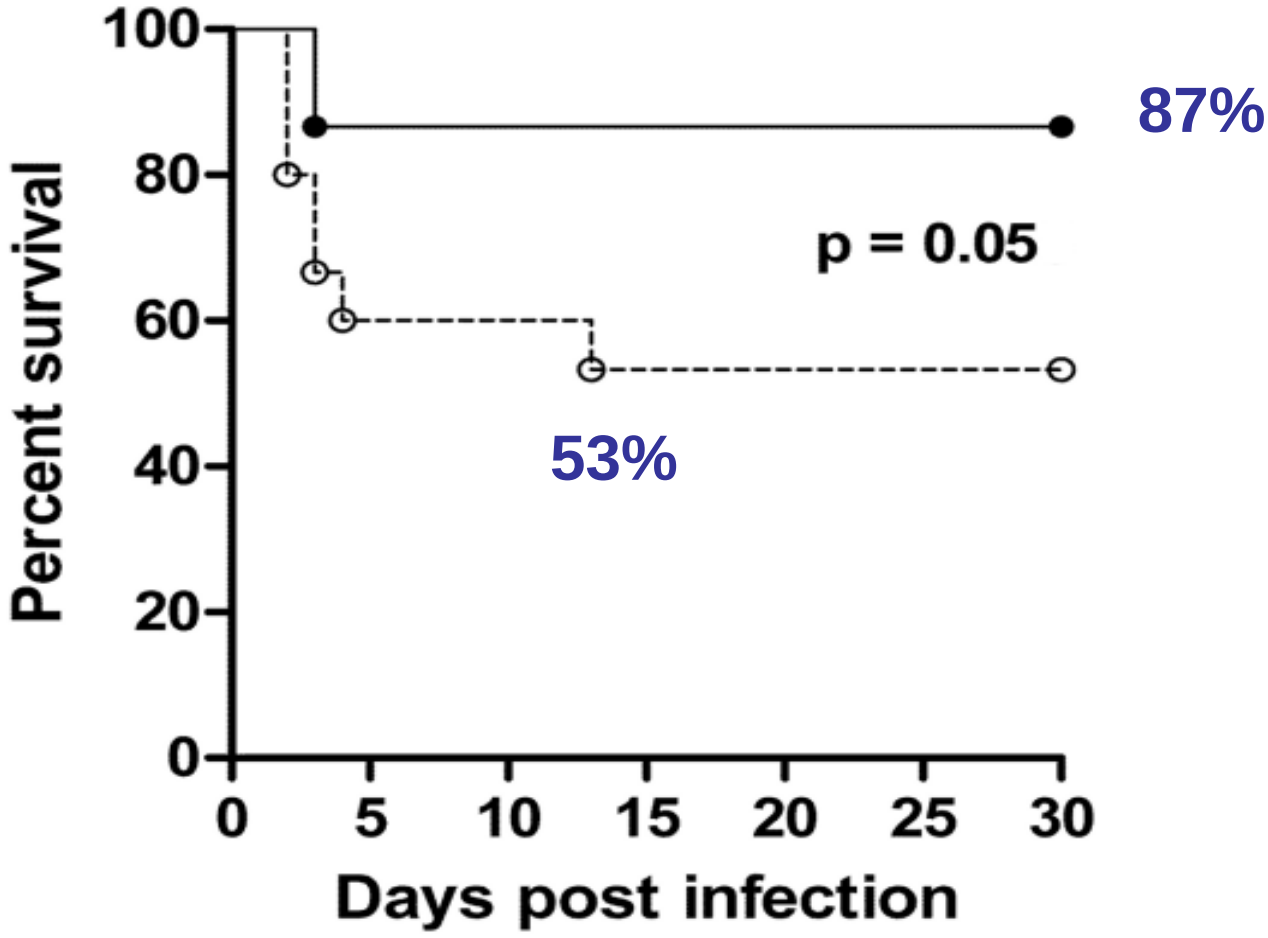
StreptInCor induces high and specific IgG antibodies

No crossreativity against cardiac myosin was observed



Postol E, Guilherme L, Plos One, 2013

Survival after emm1 *S.pyogenes* challange



Immunized mice

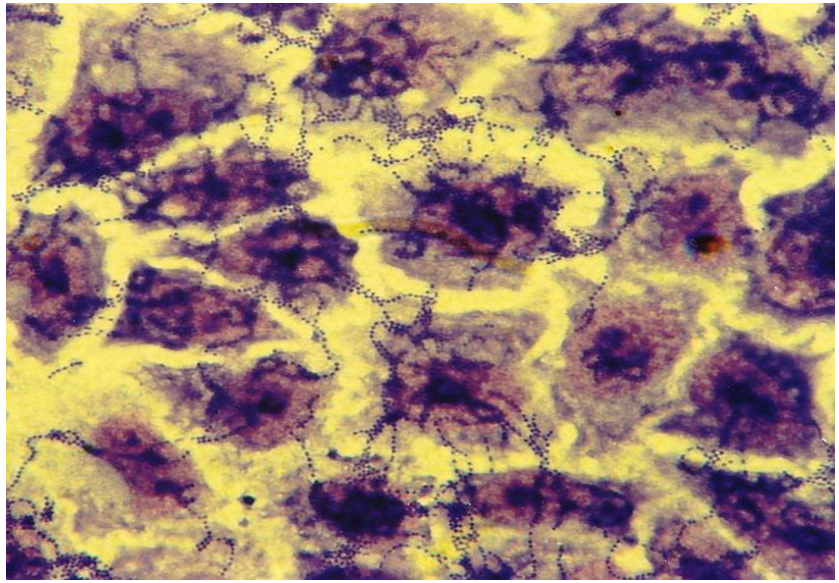
Controls

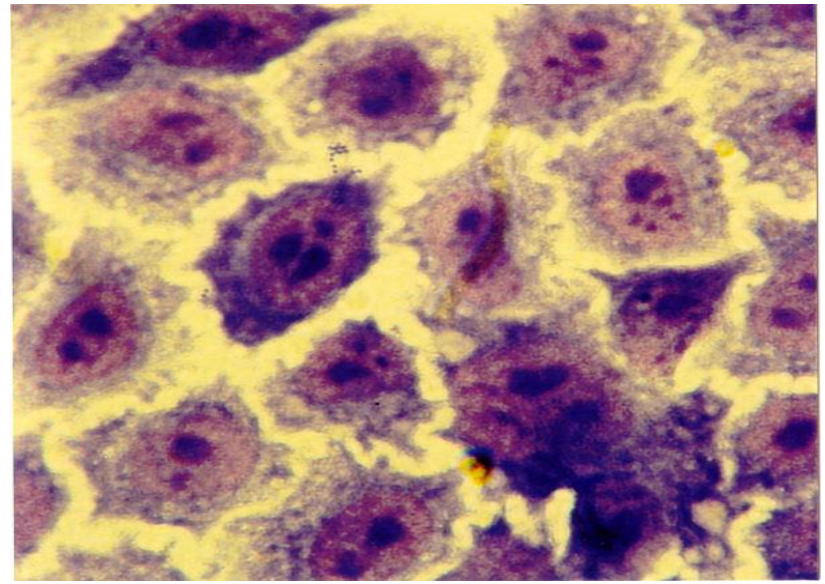
Postol E, Guilherme L, Plos One, 2013/ 2014

Adhesion/Invasion Inhibition – Hep-2 cells

Adhesion - *S. pyogenes*

Sera from StreptInCor immunized mice



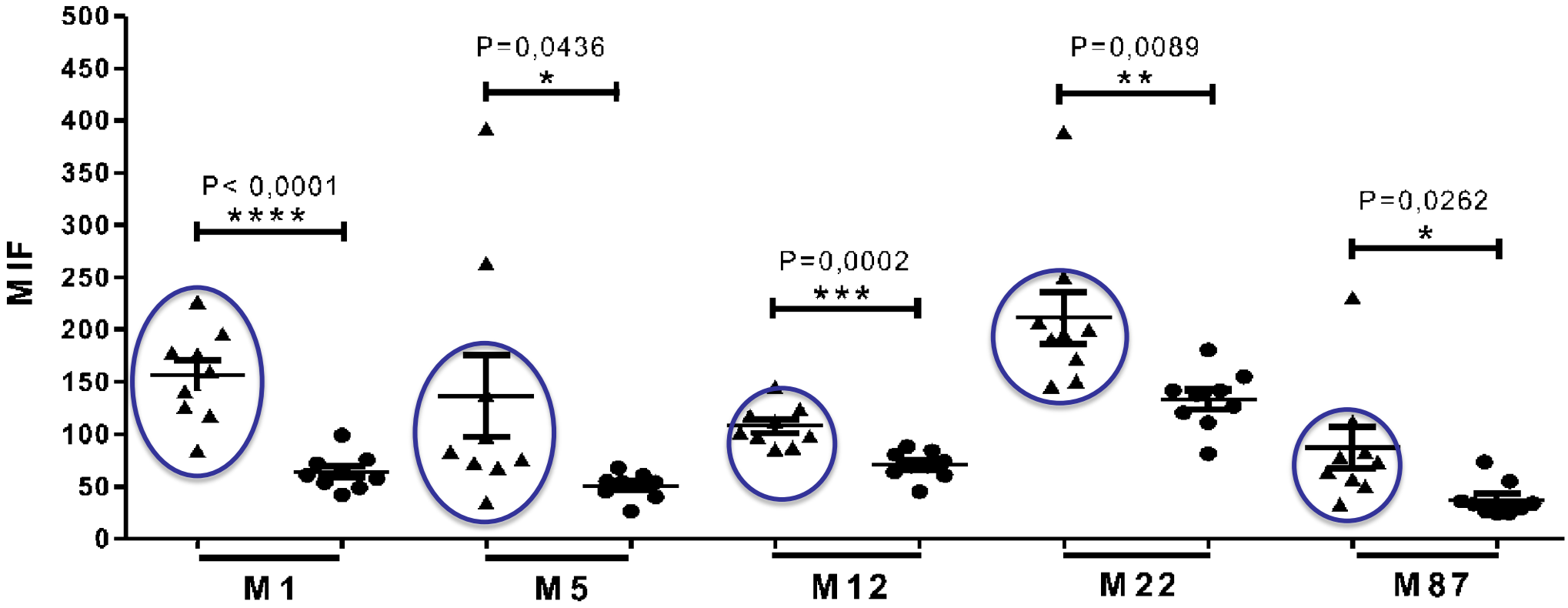


***S. pyogenes* – M1 Adhesion/Invasion Inhibition**

BALB/c (N=5)	**95.0 %**
C57BL6 (N=7)	**92.0 %**
Swiss (N=3)	**98.5 %**

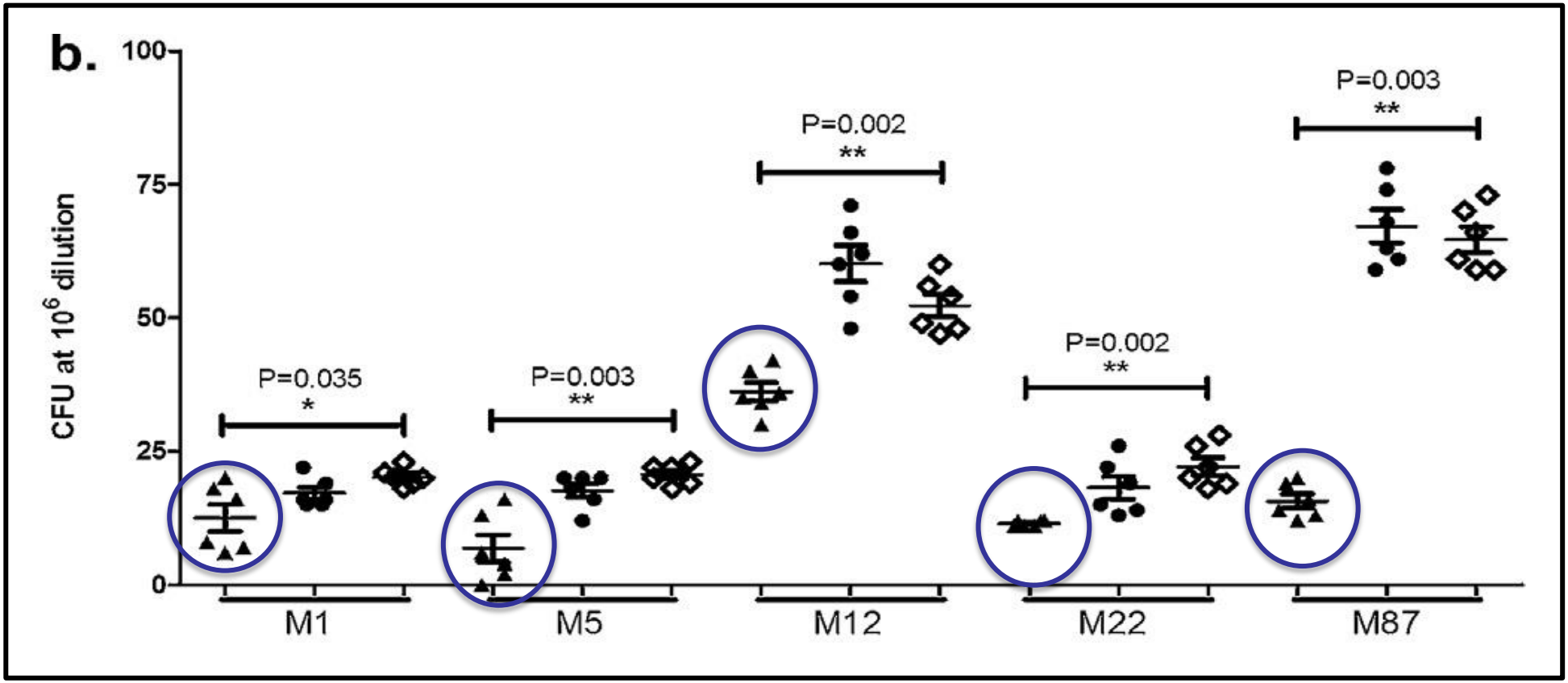
UFC without sera > 200.000

Anti-StreptInCor antibodies induce Neutralization of several *S. pyogenes* strains



De Amicis MK, Guilherme L, Vaccine, 2013/2014

Anti-StreptInCor antibodies induce opsonophagocytose and *S. pyogenes* death



▲ StreptInCor immunized BALB/c sera

● Control sera

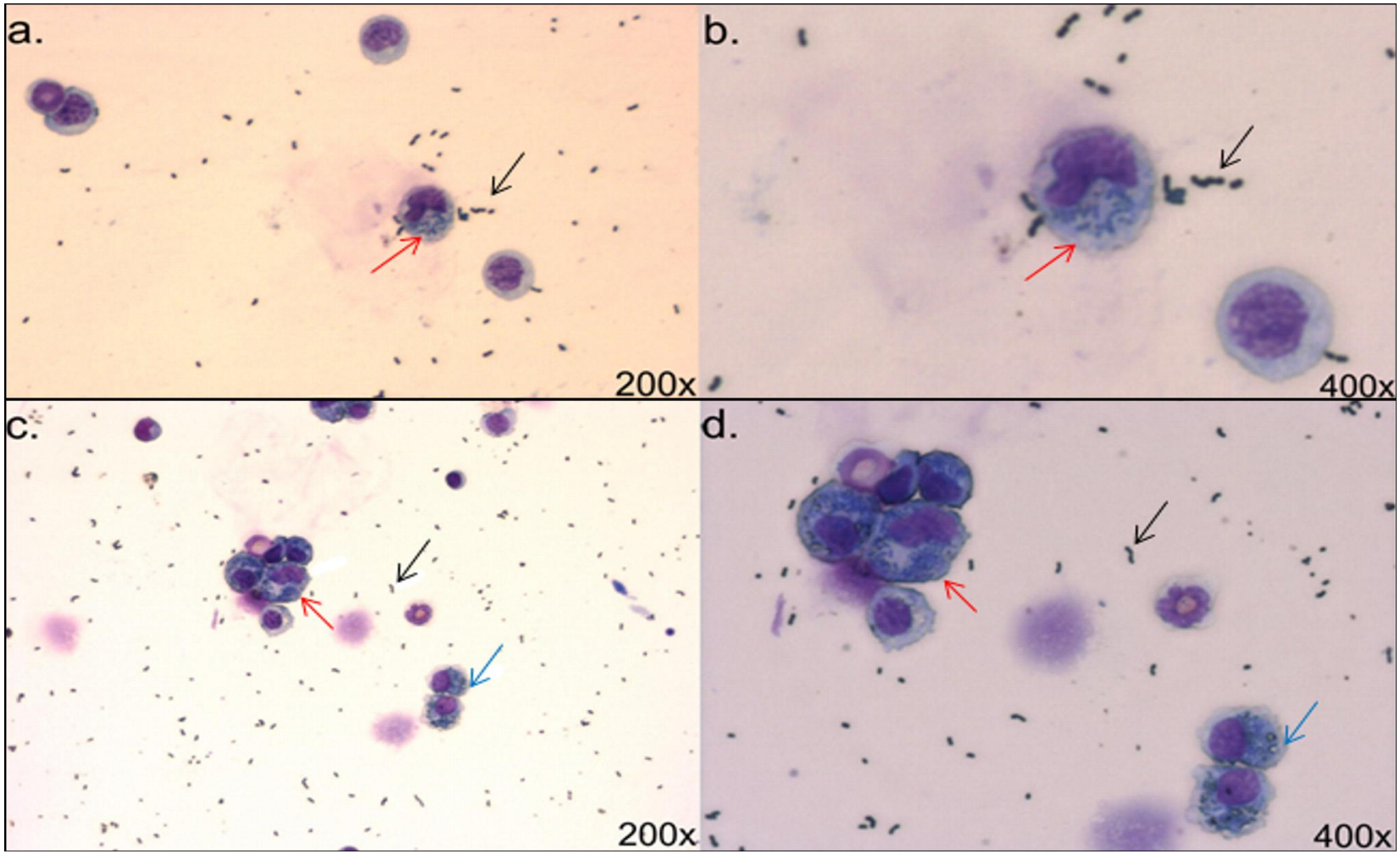
◇ *Pool of* pre-immune mice sera

De Amicis MK, Guilherme L, Vaccine, 2013/14

Opsonization, phagocytose/death of M1 induced by anti-StreptInCor antibodies

Pré-immune sera

Hiperimmune sera



S.pyogenes

Phagocytosis of S.pyogenes by APC

De Amicis MK, Guilherme L, Vaccine, 2013/14

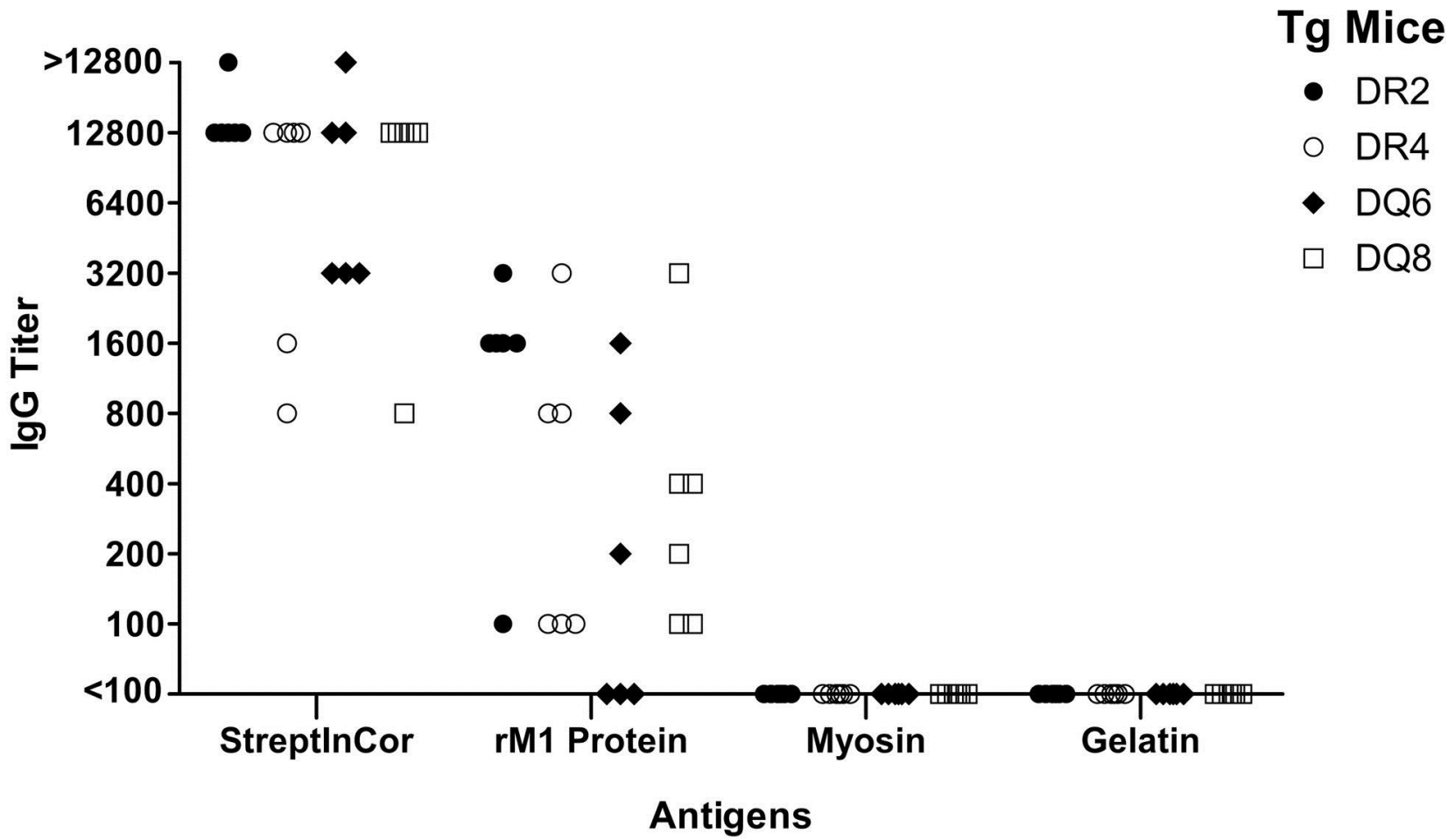
HLA Class II Tg Mice Model

- **DRB1 (DR2, DR4)**
- **DQ6 and DQ8**

Prof Chella David, Clinic Mayo, USA

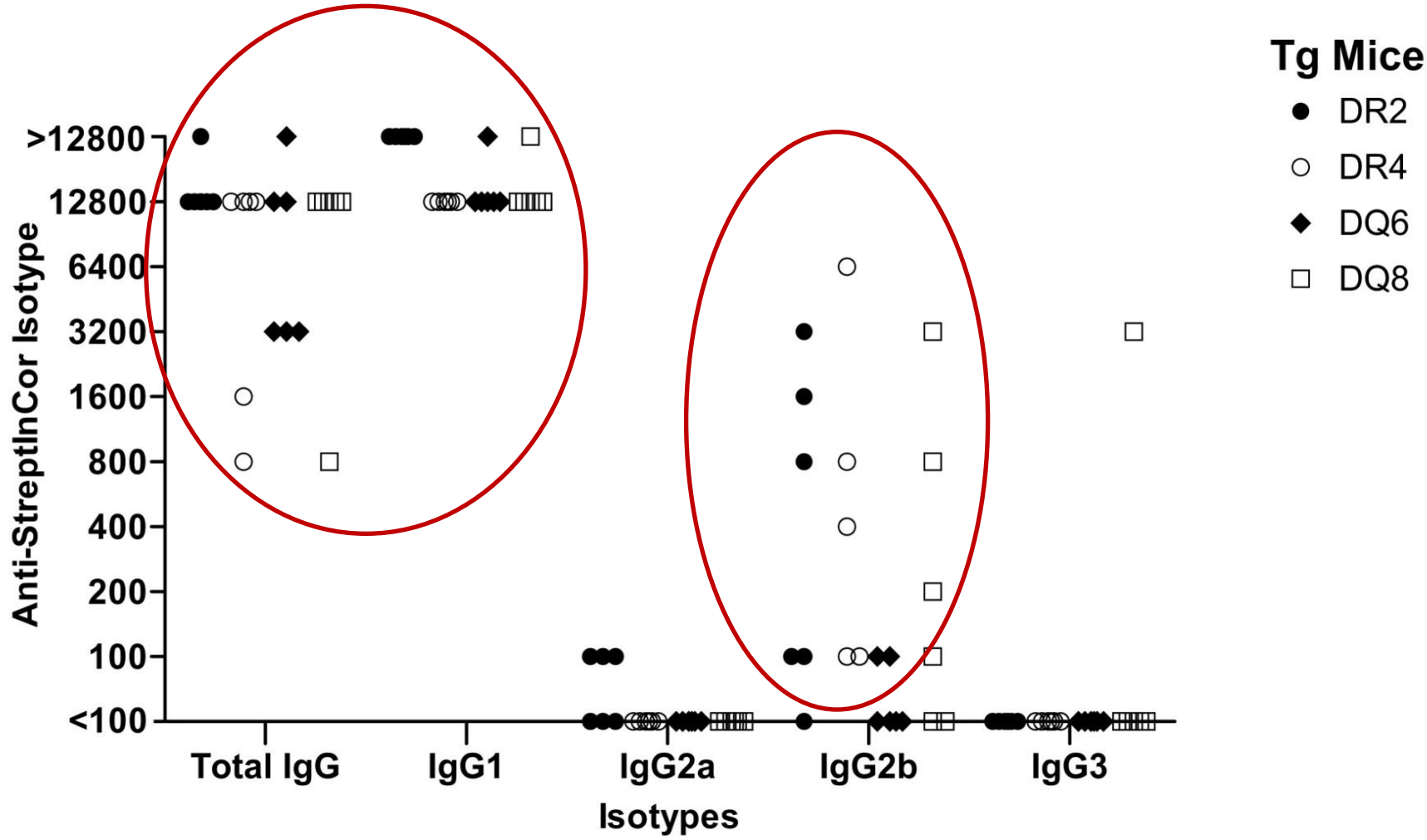
HLA class II Tg Mice

StreptInCor antibodies recognize heterologous protein without crossreactivity against cardiac myosin



Guerino, T; Guilherme L, Vaccine, 2011

HLA-Class II -Transgenic mice: StreptInCor + ALUM



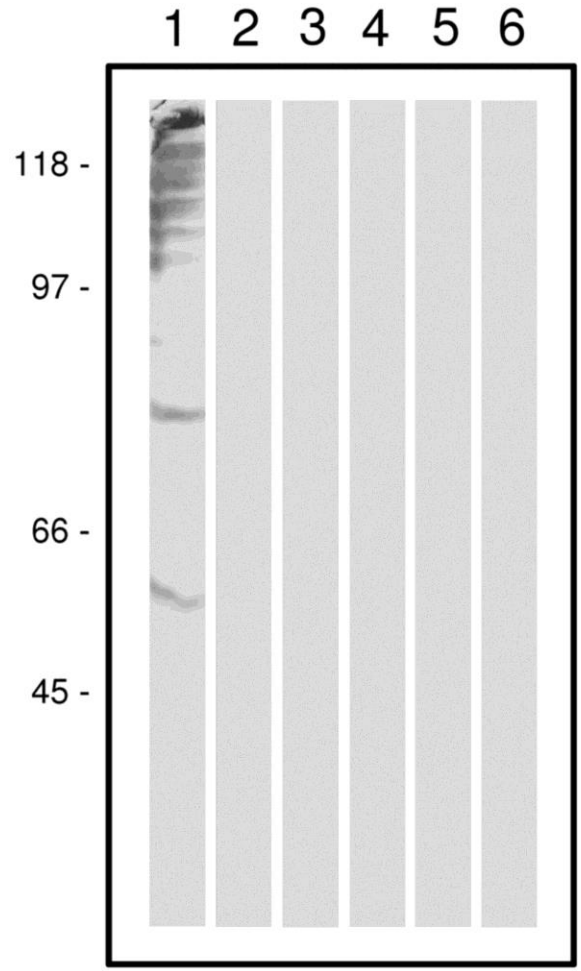
Guerino, T Guilherme et al, Vaccine, 2011

Immune Recognition of Overlapping M Protein C-terminal Peptides

Overlapping Peptides Sequences (20 aa residues)	Transgenic Mice Bearing HLA Class II Alleles Humoral Immune Response (IgG)			
	DR2	**DR4**	**DQ6**	**DQ8**
KGLRRDLDASREAKKQLEAE	$6^{+}/6$	$5^{+}/6$	$2^{+}/6$	$5^{+}/6$
KGLRRDLDASREAKKQVEKA	$5^{+}/6$	$5^{+}/6$	$3^{+}/6$	$4^{+}/6$
GLRRDLDASREAKKQVEKAL	$5^{+}/6$	$5^{+}/6$	$2^{+}/6$	$3^{+}/6$
LDASREAKKQLEAEQQKLEE	$4^{+}/6$	$5^{+}/6$	$3^{+}/6$	$6^{+}/6$
KLEEQNKISEASRKGLRRDL	$5^{+}/6$	$4^{+}/6$	$3^{+}/6$	$5^{+}/6$
KISEASRKGLRRDLDASREA	$5^{+}/6$	$2^{+}/6$	$2^{+}/6$	$3^{+}/6$
SEASRKGLRRDLDASREAKK	$5^{+}/6$	$4^{+}/6$	$3^{+}/6$	$5^{+}/6$
ASRKGLRRDLDASREAKKQV	$4^{+}/6$	$4^{+}/6$	$1^{+}/6$	$2^{+}/6$

Guerino, T; Guilherme L, Vaccine, 2011,

StreptInCor did not Induce Heart-tissue Proteins Crossreactive Antibodies



1 (+) mouse anti-myosin sera

2 (-) non-immunized sera

3 DR2 tg mice sera

4 DR4 tg mice sera

5 DQ6 tg mice sera

6 DQ8 tg mice sera

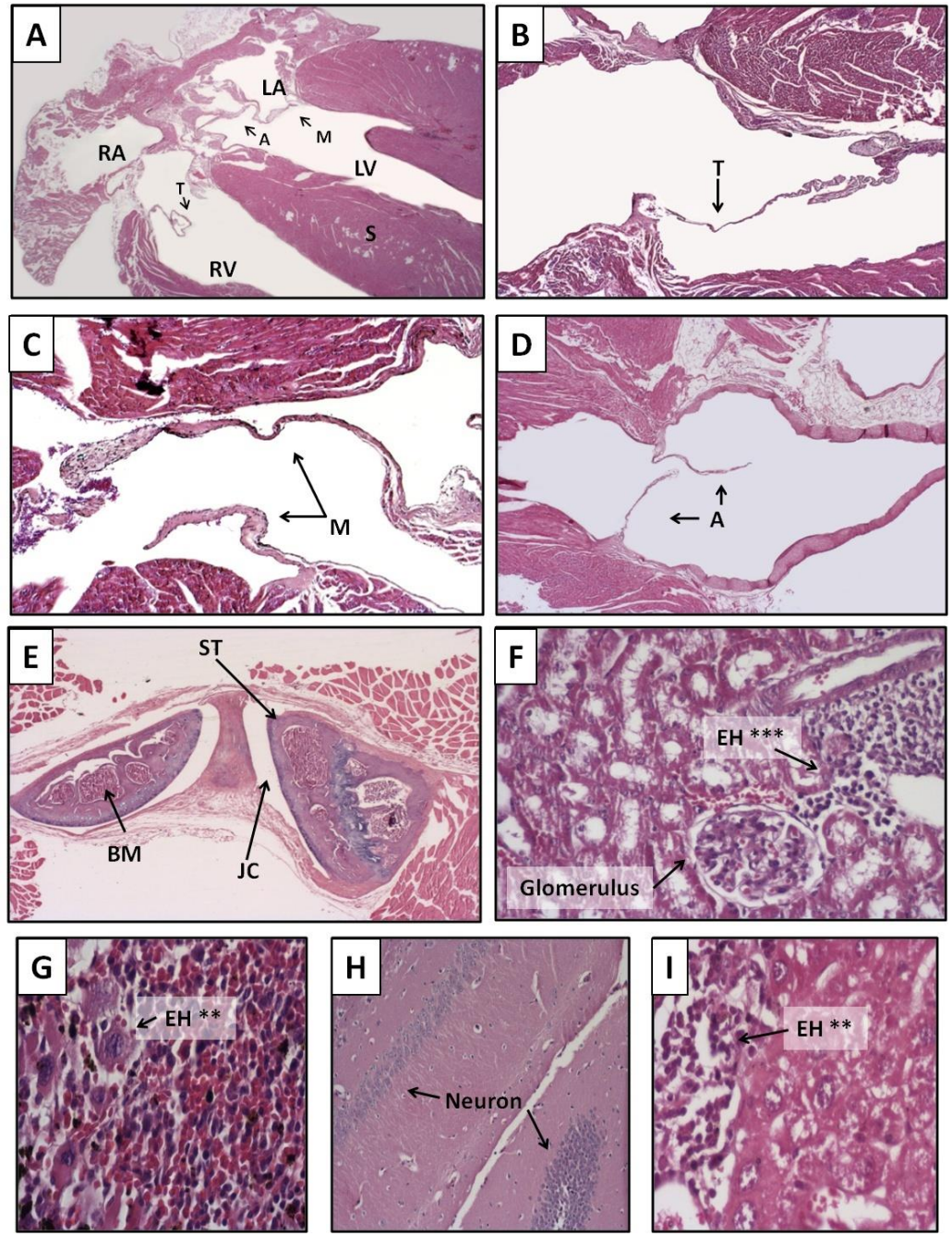
Human myocardium tissue, Cadaveric donor

Guerino, T; Guilherme L, Vaccine, 2011

Animal model HLA-class II Tg Mice

StreptInCor vaccine did not induces autoimmune reactions

1 year post- vaccination



A- Heart

B, C, D –Tri, Mi, Ao valves

E- Articulations

F- Kidney

G - Spleen

H - Brain

I - Liver

Guerino, T; Guilherme L, Vaccine, 2011

Autoimunnity Control
RHD - Heart tissue infiltrating cells

	StreptInCor Several peptides)	T cell lines T = 29 *	%	T cell clones N=49 (5 T cell Lines)	%
Negative	**23/29**	**79.3**		**42/49**	**85.7**
Positive	**6/29**	**20.7**		**7/49** **IL-10**	**14.3**

* 14 valves and 15 myocardium

T-Reg Cells?

Guilherme L, et al, unpublish data

T regulatory (Treg) Cells

Are defined by several cell markers and are important tools

as immunotherapy in organ transplantation and

autoimmune diseases.

C-terminal M protein epitope- StreptInCor has a potential to induce:

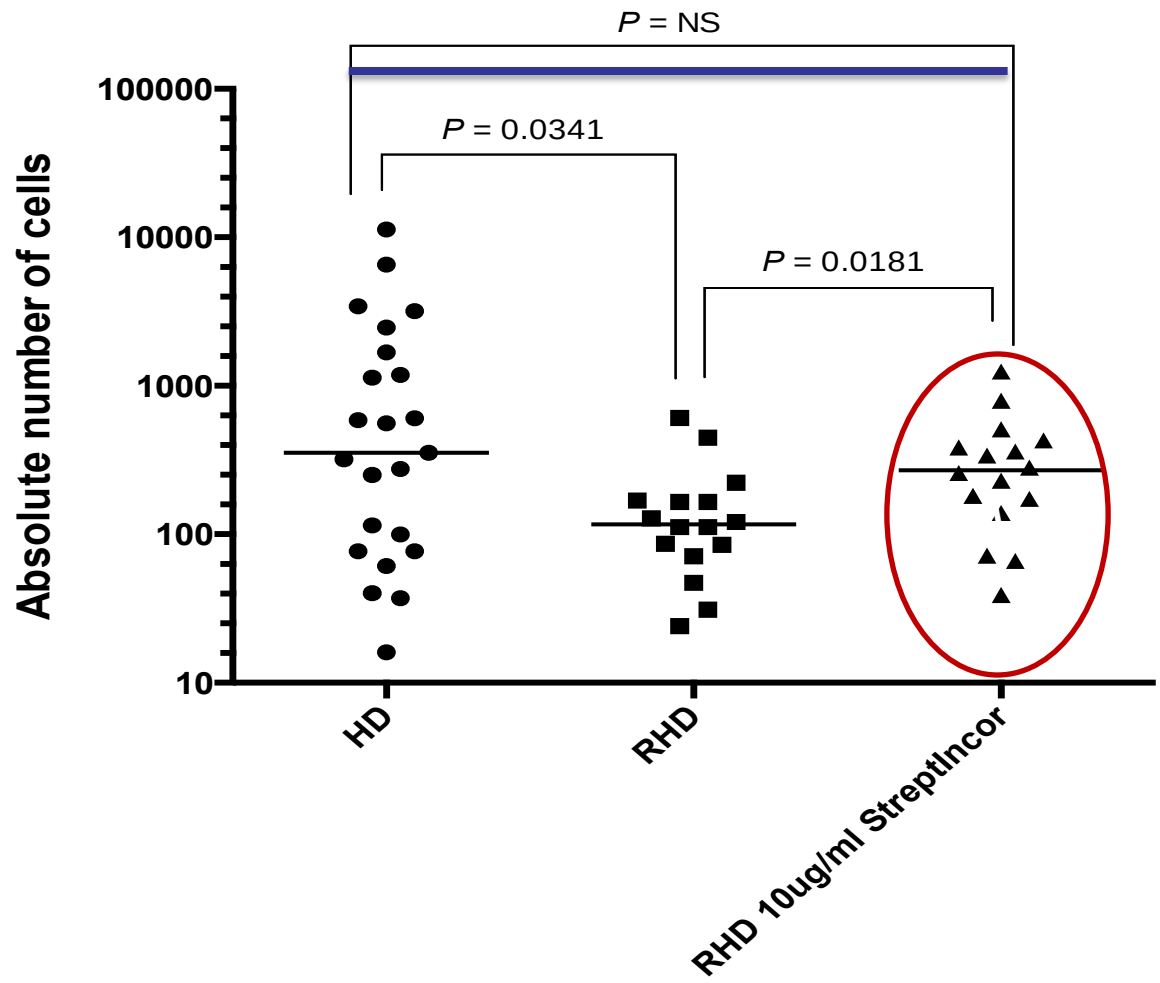
- **Protection against *S. pyogenes* (vaccine)**
- **T reg cells that regulate autoimmune reactions (therapeutic effect)**

StreptInCor : Potential Therapeutic Effect

T regulatory cells: Peripheral blood of RHD patients

Flow Cytometry

$CD3^{+}\ CD4^{+}\ CD25^{high}\ CD127^{-}\ Foxp3^{+}$



StreptInCor

(vaccine candidate epitope**)**

increases the numbers of Natural T Reg cells

Köhler KF, Guilherme L, et al , In Preparation

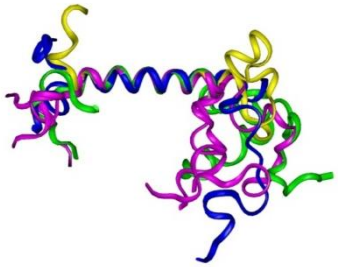
Summary

- **The social-economic impact of RF/RHD in Brazil is still important**

In the last 20 years - our studies lead to:

1. **Better understanding of the autoimmune and inflammatory mechanisms leading to the rheumatic heart lesions**

2. **C-terminal M protein epitope - <u>StreptInCor has a potential to induce</u>:**



- **Protection against *S. pyogenes* (vaccine)**

- **Cells that regulate autoimmune reactions (therapeutic effect)**

Both Properties of StreptInCor
Certainly will contribute to a better life of RF/RHD patients and to prevent new infections.

Clinical Phase I Assays / Design of the Study

- **Clinical Phase I :** random, double-blind, controlled with placebo, sequential dosing of StreptInCor (50 µg, 100µg, 200 µg - 2 doses with 28d interval); 6 months boost .

- **Healthy Volunteer**: individual without confirmed disease diagnosis or infection that would compromise the immune response, with ages between 18 and 45 years old.

Next Steps

- **GMP production**
- **Phase I/IIa Clinical Trials – 2015/2016**
- **ANVISA and FDA registration**

Financial Support
CNPq , FAPESP , BNDES
Brazil.

Prof Jorge Kalil, MD,PhD Director of Immunology Lab – Heart Institute

Prof. Luiza Guilherme, Pharm, PhD, Rheumatic Fever Group´s Leader

RF/RHD Mechanism of Pathogenesis

Kellen Cristhina Faé, PhD
Rajendranath Ramasawy,PhD
Ana Flavia Vigna, PhD
Luciana Nogueira, PhD
Sandra Emiko Oshiro, MSc
Carlo Martins, PhD student
Nathalia Moreira Santos, MsC Student
Selma A Palácios
Simone Santos

Vaccine Development

Claudio R Puschel, PhD – **Peptide Synthesis**
Washington R Silva
Samar F Barros, PhD – **Molecular Biology, Microbiology**
Raquel Alencar, MSc
Karine Marafigo De Amicis, MsC Student
Karen Kohler, PhD **Cellular Immunology**
Leticia Chaves, MSc – **Humoral Response**
Edilberto Postol, PhD – **Experimental**
Daniella Santoro, PhD
Fabio T Higa
Milton T.G. Silva, MsC Student
Luiz R Mundel
Frederico Moraes Ferreira, PhD- **Structural analysis**

Advisors – Lab of Immunology

Prof Edecio Cunha-Neto, MD,PhD
Veronica Coelho, MD,PhD

Heart Institute, 1.Pediatric Cardiology, 2.Valvopathy, 3.Surgical and 4.Pathology Divisions

1. Ana C Tanaka, MD

2. Prof Max Grinberg, MD; Prof Flavio Tarasoutchi, MD; Guilherme Spina, MD; Roney Sampaio,MD

3. Prof Pablo Pommerantzeff, MD, Carlos Brandão, MD

4. Lea Demarchi, MD, Prof Vera D Aiello, MD: Paulo S Gutierrez

Pediatric Rheumatology Division, Clinical Hospital

Prof Maria H Kiss, MD, Clovis A Silva, MD

Saint Louis Hospital, Paris, France –TCR, Treg Cells

Prof Dominique Charron, MD,PhD; Prof Antoine Toubert, MD,PhD

Oklahoma University, Oklahoma, USA – Cardiac Myosin Studies

Prof Madeleine W Cunningham, PhD

Finlay Institute, Havana, Cuba – Mucosal Adjuvant

Prof Oliver Perez Martin, MD; Miriam Lastre, MD; Caridad Zayas

Mayo Clinic Rochester, USA, HLA-class II Transgenic Mice

Prof Chella David, MD,PhD


Thank you!
HOSPITAL DAS CLÍNICAS
INSTITUTO DO CORAÇÃO