

**ACTIVE vs PASSIVE IMMUNIZATION:  
ADVANTAGES AND DISADVANTAGES OF EACH APPROACH**

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Albert Einstein College of Medicine  
Bronx, New York**

# DEFINITIONS

- **ACTIVE IMMUNIZATION = VACCINES**
- **PASSIVE IMMUNIZATION = PREFORMED ANTIBODY ADMINISTRATION**

# ACTIVE IMMUNIZATION - VACCINES

## ADVANTAGES

- **LONG PROTECTION**
- **LESS COSTLY (TO ADMINISTER)**

## DISADVANTAGES

- **DEPENDENT ON HOST IMMUNE SYSTEM**
  - NOT EVERYONE MAY BE PROTECTED
- **PROTECTIVE RESPONSE TAKES TIME**
- **MAY BE IMPRACTICAL FOR NOSOCOMIAL INFECTION**
- **LONG DEVELOPMENT TIME**
- **? EFFECTS ON MICROFLORA**

# PASSIVE IMMUNIZATION

## ADVANTAGES

- IMMEDIATE IMMUNITY
- PHARMACOLOGIC CONTROL
- RAPID DEVELOPMENT TIME

## DISADVANTAGES

- COSTLY
- TEMPORARY IMMUNITY
- REQUIRES IV ADMINISTRATION
- DOSE-RESPONSE UNCERTAINTY

# REAGENTS FOR PASSIVE THERAPY: mAbs

## ADVANTAGES

- HIGH SPECIFIC ACTIVITY
- PRECISE PHARMACOLOGIC DEFINITION
- SINGLE ISOTYPE
- LOT TO LOT CONSTANCY

## DISADVANTAGES

- COST
- SINGLE SPECIFICITY
  - SELECTION FOR ESCAPE MUTANTS
  - EFFECTIVENESS  $\propto 1/\text{ANTIGENIC DIVERSITY}$
- SINGLE ISOTYPE
  - LIMITED EFFECTOR FUNCTION
  - LIMITED FcR ENGAGEMENT

# REAGENTS FOR PASSIVE THERAPY: POLYCLONAL SERA

## ADVANTAGES

- MULTIPLE SPECIFICITIES
- MULTIPLE ISOTYPES

## DISADVANTAGES

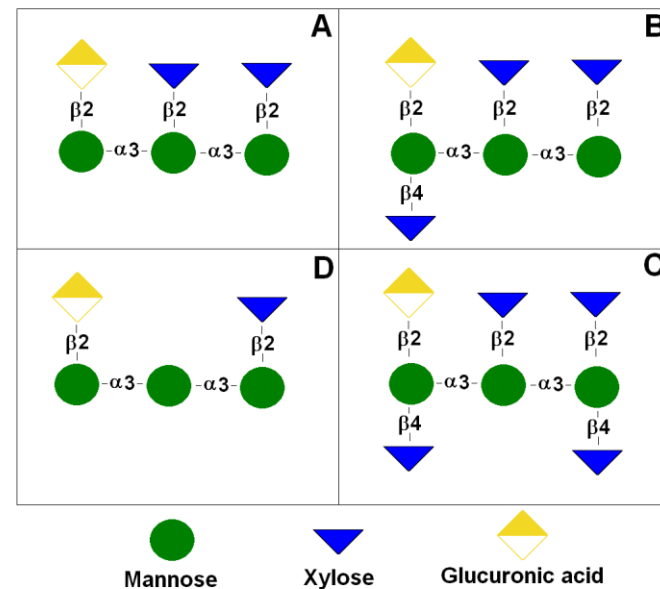
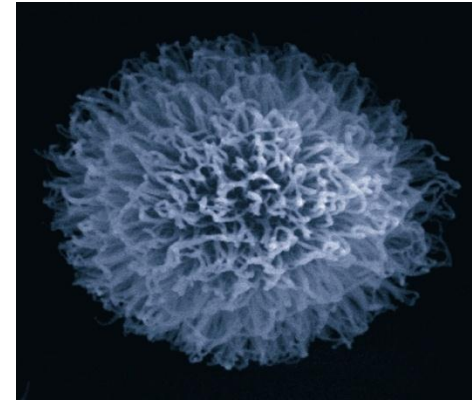
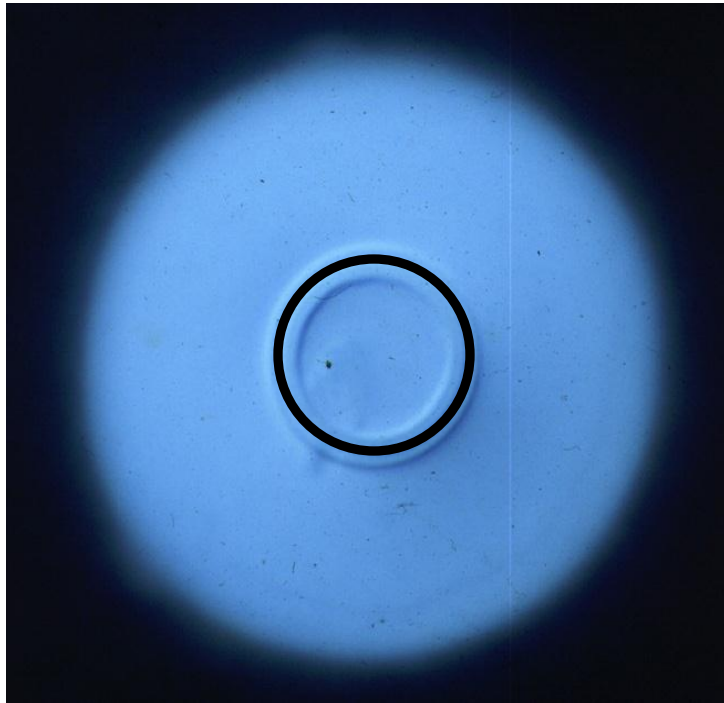
- LOW SPECIFIC ACTIVITY
- LOT TO LOT VARIATION
- REQUIREMENT FOR IMMUNIZED DONORS
- COST
- CONCERN ABOUT INADVERTENT TRANSMISSION OF DISEASE

# **NEW INSIGHTS INTO ANTIBODY-MEDIATED PROTECTION FROM STUDIES WITH A FUNGAL PATHOGEN**

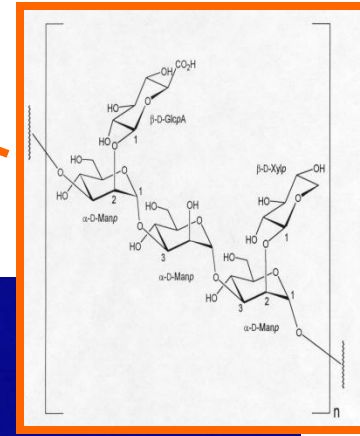
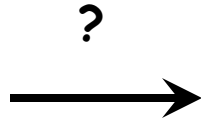
**Or 20 years of work in 18 minutes**

***C. NEOFORMANS* – ONLY ENCAPSULATED EUKARYOTIC PATHOGEN OF HUMANS  
CAPSULE IS THE MAJOR VIRULENCE FACTOR**

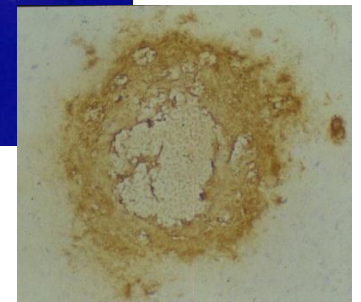
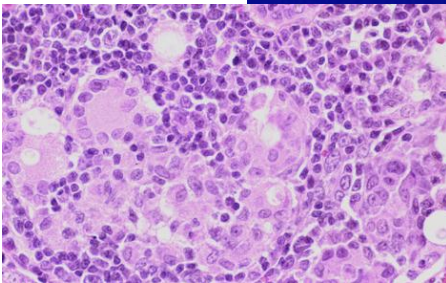
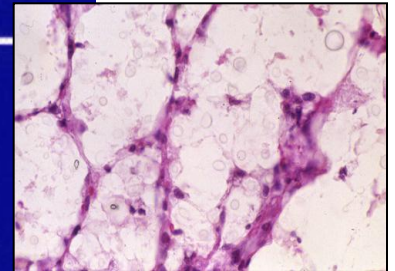
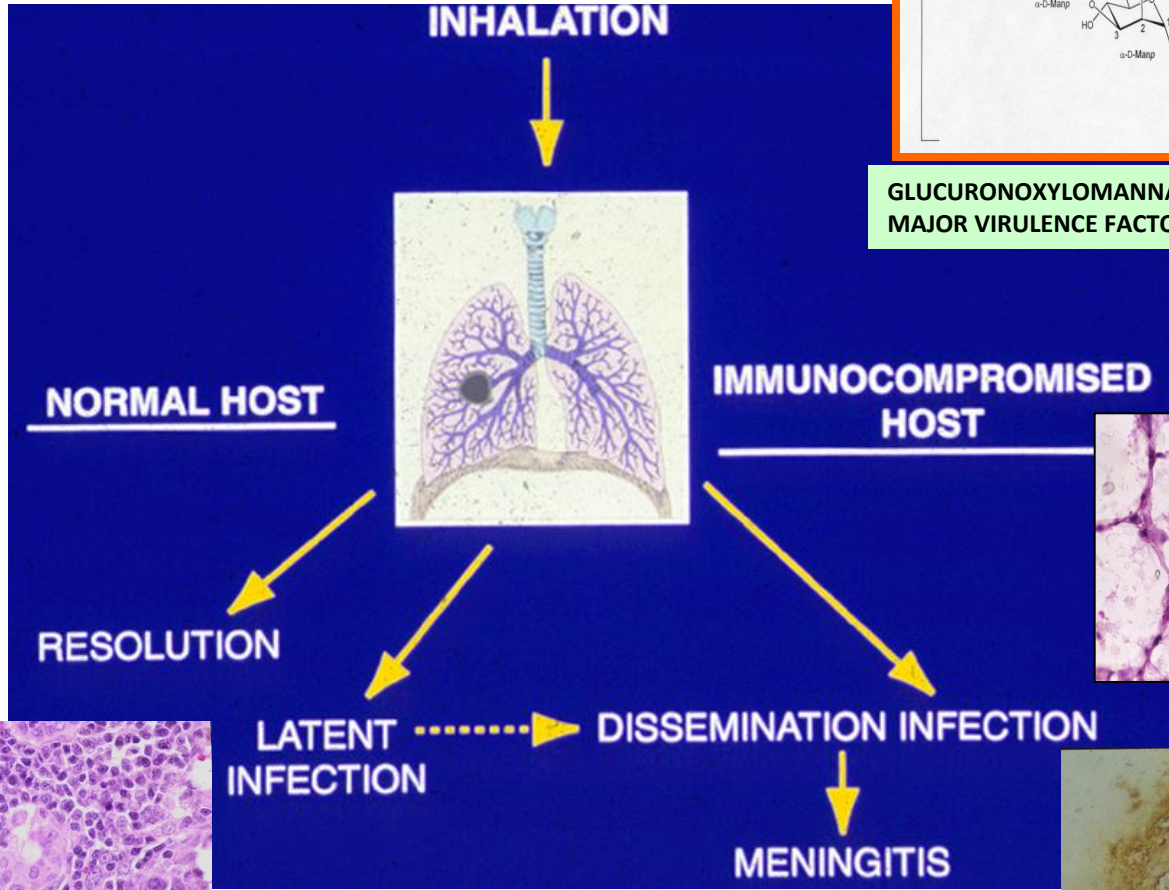
**POLYSACCHARIDE CAPSULE**







**GLUCURONOXYLOMANNAN  
MAJOR VIRULENCE FACTOR**



## WEILER HOSPITAL, NEW YORK CITY SUMMER 1988

**30-SOMETHING CAMBODIAN MAN WITH *C. NEOFORMANS* MENINGITIS DIES AGONIZING DEATH DESPITE RECEIVING LARGE DOSES OF AMPHOTERICIN B DURING A PERIOD OF 3 MONTHS...**

**COULD ANTIBODY THERAPY BE DEVELOPED?**

**CIRCA 1990**

- WORK ON RE
- IF YOU WANT
- GET A DIFFERE  
WE I



# THE GREAT IMMUNOLOGICAL CATASTROPHE

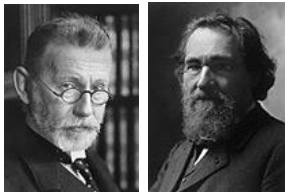
1891-1910

1910-1940

1940-1960s

1970-1990s

HUMORAL  
VS  
PHAGOCYtic  
THEORIES



ANTIBODY  
PROTECTS  
AGAINST  
ALL MICROBES



GALEN

FOUR  
'HUMORS'



'HUMORAL  
IMMUNITY

HUMORAL IMMUNITY



CD8<sup>+</sup> Cells Enhance Resistance to Pulmonary Serotype 3 *Streptococcus pneumoniae* Infection in Mice

Sarah E. Weber,\* Haijun Tian,<sup>†</sup> and Liise-anne Pirofski\*<sup>†</sup>

HUMORAL IMMUNITY

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THE EFFICACY OF A *SALMONELLA TYPHI* Vi CONJUGATE VACCINE  
IN TWO-TO-FIVE-YEAR-OLD CHILDREN

FENG YING C. LIN, M.D., M.P.H., VO ANH HO, M.D., HA BA KHIEM, M.D., DANG DUC TRACH, M.D., PH.D., PHAN VAN BAY, M.D., TRAN CONG THANH, M.D., ZUZANA KOSSACZKA, PH.D., DOLORES A. BRYLA, M.P.H., JOSEPH SHILOACH, PH.D., JOHN B. ROBBINS, M.D., RACHEL SCHNEERSON, M.D., AND SHOUSUN C. SZU, PH.D.

# METHODS FOR ESTABLISHING USEFULNESS OF ANTIBODY-MEDIATED PROTECTION

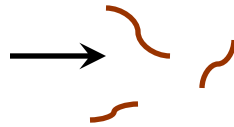
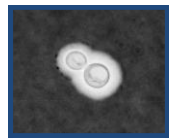
## 1. PASSIVE TESTS

**LOGICAL ERROR:  
JUST BECAUSE THE ASSAY SUGGESTED THAT ANTIBODY WAS NOT  
PROTECTIVE  
DID NOT MEAN THAT ANTIBODY COULD  
NOT BE PROTECTIVE**

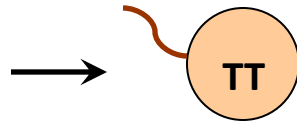
***ERROR INVOLVED MAKING A POSITIVE INFERENCE FROM  
NEGATIVE DATA***

# PROBLEM: EXPERIMENTS WITH POLYCLONAL SERA ARE INCONCLUSIVE

## A DIFFERENT APPROACH



GXM



IMMUNIZE

↓  
YYYYYY

MAKE  
mAbs

PROTECTION STUDIES

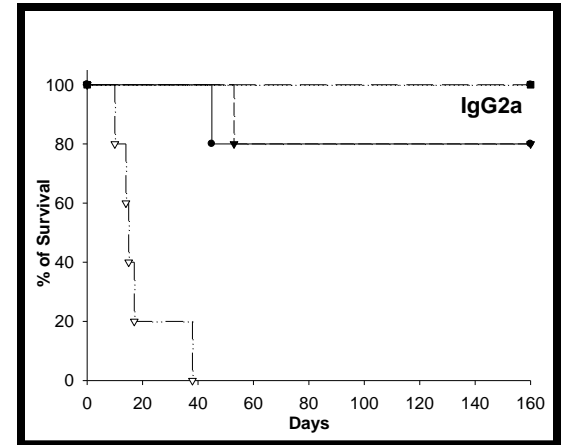


NON-PROTECTIVE mAb

YYYYYYY

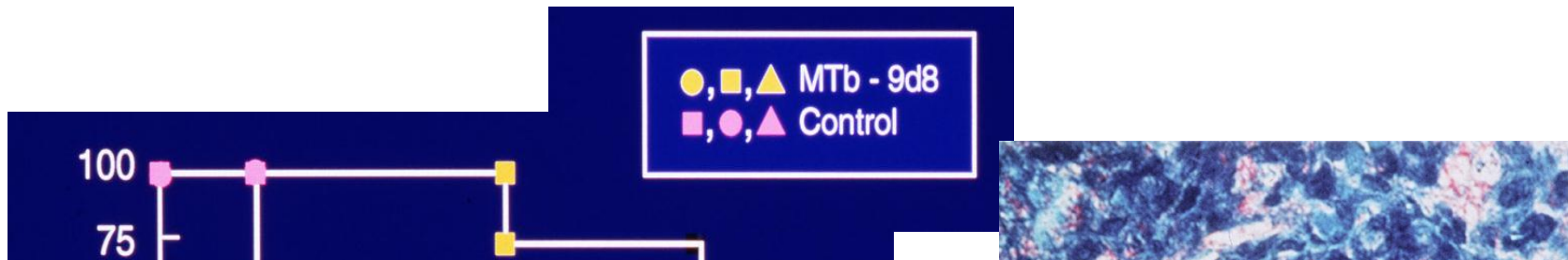
PROTECTIVE mAb

YYYYYYY



NON-PROTECTIVE mAbs INHIBITED EFFICACY OF PROTECTIVE mAbs

## ANTIBODY-MEDIATED PROTECTION AGAINST *M. TUBERCULOSIS*



The Journal of Immunology

## A Novel Human IgA Monoclonal Antibody Protects against Tuberculosis

Sucharitha Balu,\* Rajko Reljic,<sup>†</sup> Melanie J. Lewis,<sup>‡</sup> Richard J. Pleass,<sup>§,¶</sup> Richard McIntosh,<sup>¶</sup> Cees van Kooten,<sup>||</sup> Marjolein van Egmond,<sup>#</sup> Stephen Challacombe,\* Jenny M. Woof,<sup>‡</sup> and Juraj Ivanyi\*



### CONFIRMATION (5 independent labs):

PETHE ET AL. NATURE 2001:412:190  
HAMASUR ET AL. VACCINE 2003:20:4081  
WILLIAMS ET AL. IMMUNOLOGY 2004:111:328  
CHAMBERS ET AL. FEMS IMMUNOL.MED.MICROBIOL. 2004:41:93  
HAMESURE ET AL. CLIN.EXP.IMMUNOL 2005: 138:30-8

# THE CMI vs. HUMORAL PARADIGM UNTENABLE

## HUMORAL IMMUNITY

VIRUSES  
TOXINS  
ENCAPSULATED BACTERIA

## NEW APPROACH

USE MAbs

## CELLULAR IMMUNITY

MYCOBACTERIA  
FUNGI  
SOME BACTERIA  
VIRUSES  
TOXINS (SUPERANTIGENS)

## PROTECTIVE MAbs MADE TO:

*CRYPTOCOCCUS NEOFORMANS* (FUNGUS)

*CANDIDA ALBICANS* (FUNGUS)

*LISTERIA MONOCYTOGENES* (INTRACELLULAR BACTERIA)

*HISTOPLASMA CAPSULATUM* (INTRACELLULAR FUNGUS)

*MYCOBACTERIUM TUBERCULOSIS* (INTRACELLULAR BACTERIA)

*EHRlichia CHAFFENSIS* (INTRACELLULAR BACTERIA)

*LEISHMANIA MEXICANA* (INTRACELLULAR PARASITE)

## TWO EMERGING EXPLANATIONS

### 'GOOD & BAD'

*C. neoformans*  
*C. albicans*  
*M. tuberculosis*

### INADEQUATE AMOUNTS

*L. monocytogenes*  
*H. capsulatum*

# WHY IS IT SO HARD TO PROTECT AGAINST SOME MICROBES WITH ANTIBODY?

## THE THINGS THAT MATTER....

- SPECIFICITY
- ISOTYPE
- AMOUNT
- DEPENDENCE ON HOST
- CELL-MEDIATED IMMUNITY
- GENETICS

	IgM		IgG	
	PROTECTIVE	NON-PROTECTIVE	PROTECTIVE	NON-PROTECTIVE
PATTERN	ANNULAR	PUNCTATE	ANNULAR	ANNULAR
OPSONIC	YES	NO	YES	YES
INHIBITS PS RELEASE	YES	NO	YES	YES
ISOTYPE	NA	NA	IgG1, IgG2a, IgG2b	IgG3
NEED C3	YES	NO	NO	NO

## TWO STORIES:

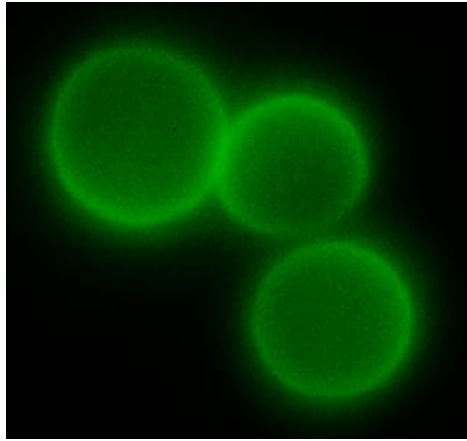
**IgM – EFFICACY REQUIRES ‘RIGHT’ SPECIFICITY AND COMPLEMENT**

**IgG - EFFICACY REQUIRES ‘RIGHT’ ISOTYPE AND HOST**



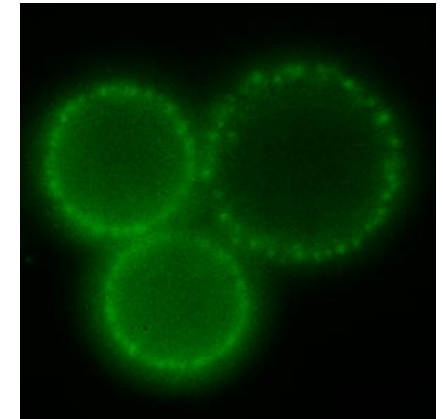
# WHAT IS THE DIFFERENCE BETWEEN PROTECTIVE AND NON-PROTECTIVE ANTIBODIES?

mAb 12A1



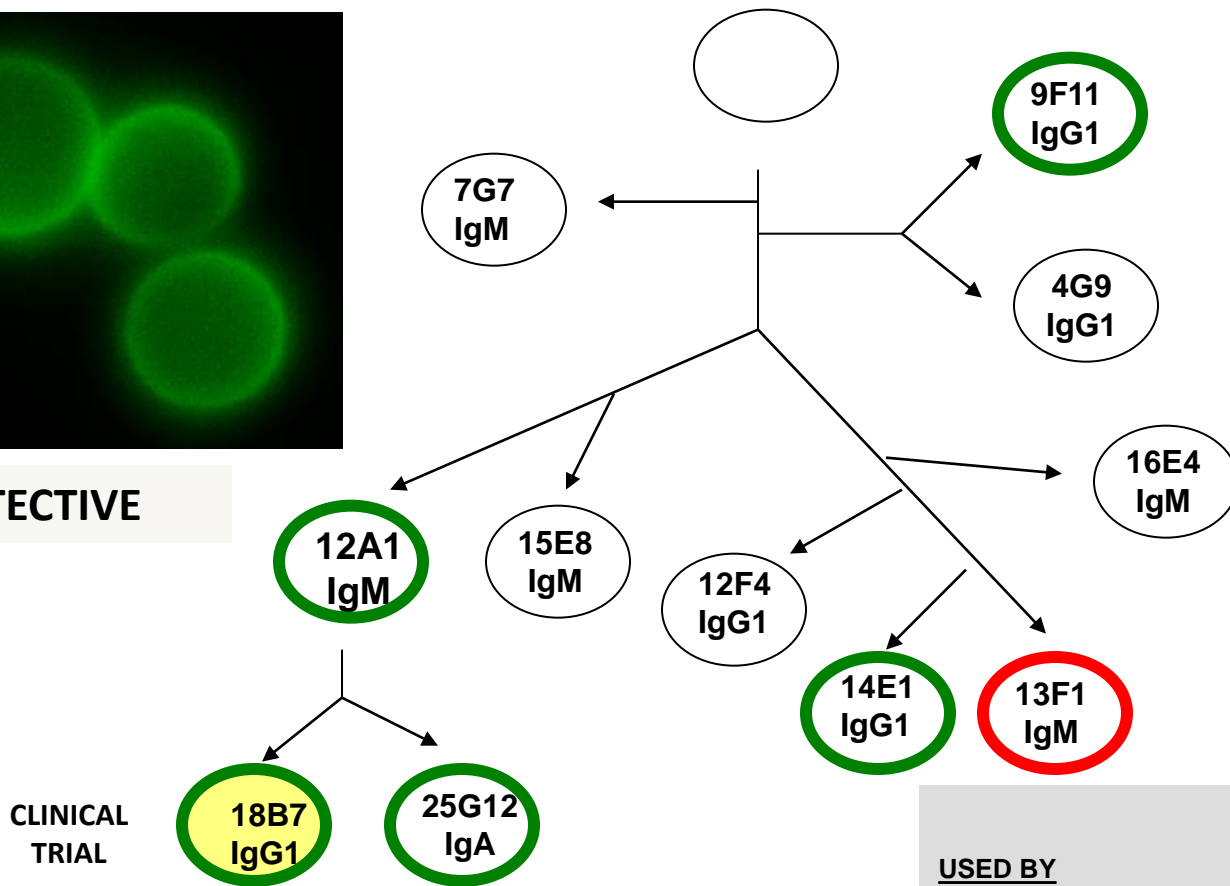
PROTECTIVE

mAb 13F1



NON-PROTECTIVE

PARENTAL  
B CELL



CLINICAL  
TRIAL

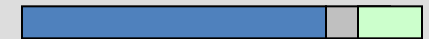
USED BY  
BALB/c  
NZB/NZW  
C3H/He

HUMAN USE V<sub>H</sub>3

VH7183

JH2

7 aa D

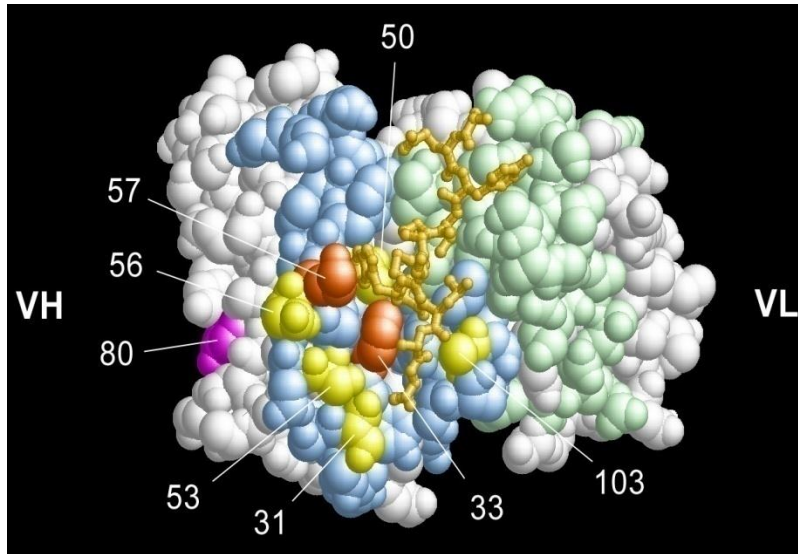


Vk5.1

Jk1

Casadevall & Scharff J Exp Med. 1991;174(1):151-60  
 Mukherjee et al J Exp Med. 1993;177(4):1105-16  
 Mukherjee et al J Exp Med. 1995 Jan 1;181(1):405-9  
 Nussbaum et al J Exp Med. 1997 Feb 17;185(4):685-94.

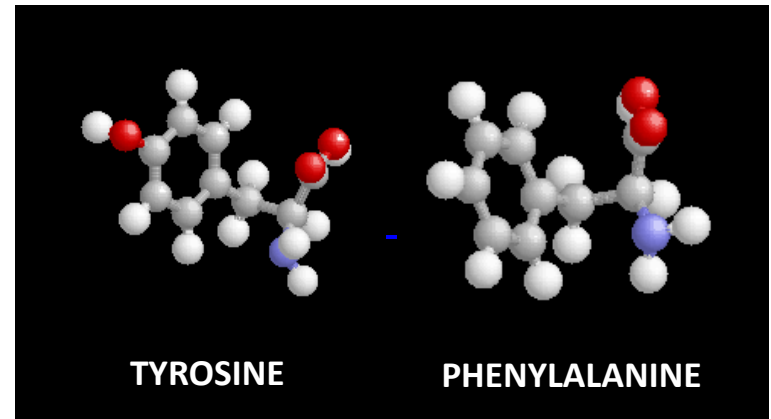
# POSITIONS 33 AND 57 IN PROTECTIVE AND NON-PROTECTIVE CLASS II MAbS TO *C. NEOFORMANS* POLYSACCHARIDE



mAb	33	57	PROTECTIVE	IF
3B10	F	N	YES	ANNULAR
2D10	F	R	YES	ANNULAR
2H1	F	K	YES	ANNULAR
3E5	F	N	YES	ANNULAR
18G9	F	K	YES	ANNULAR
10F10	F	S	YES	ANNULAR
12A1	F	N	YES	ANNULAR
17E12	F	K	YES	ANNULAR
18B7	F	K	YES	ANNULAR
471	L	K	YES	ANNULAR
3C2	L	K	YES	ANNULAR
13F1	Y	S	NO	PUNCTATE
21D2	Y	S	NO	PUNCTATE

## ANTIBODY BINDING SITE FROM X-RAY CRYSTAL STRUCTURE

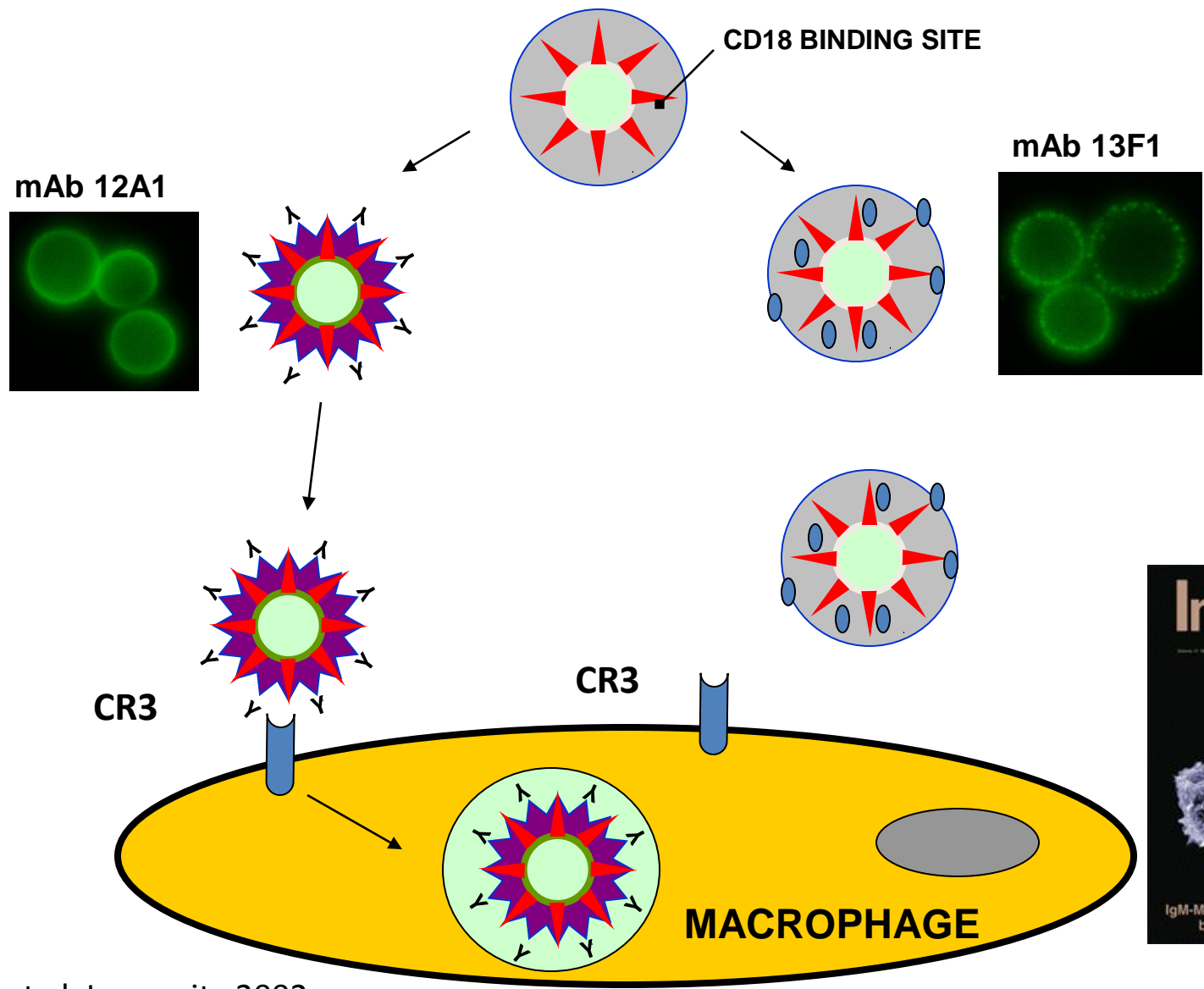
12A1	N Y F M S W V	M I N I N G N N T Y Y P D T V K G D	R D G T F G N Y Y A M	ANNULAR
S31N	S - - - - -	- - - - -	- - - - -	ANNULAR
F33Y	- - Y - - - -	- - - - -	- - - - -	ANNULAR
M50A	- - - - -	A - - - -	- - - - -	ANNULAR
I53S	- - - - -	- - S - - - -	- - - - -	ANNULAR
D56G	- - - - -	- - - G - - -	- - - - -	ANNULAR
N57S	- - - - -	- - - S - - -	- - - - -	ANNULAR
D80Y	- - - - -	- - - - -	- - - Y - - -	ANNULAR
G103Y	- - - - -	- - - - -	- - - Y - - -	ANNULAR
F33Y, N57S	- - Y - - - -	- - S - - - -	- - - - -	PUNCTATE
13F1	S - Y - - - -	A - - - - G S - - - - -	Y - - - - Y - - - -	PUNCTATE



Young et al. J. Mol. Biol. 1997

Nakouzi et al Infect Immun. 2001;69(5):3398-409

# COMPLEMENT-INDEPENDENT IgM-MEDIATED PHAGOCYTOSIS OF *C. NEOFORMANS*



**PROTECTIVE AND NON-PROTECTIVE IgM mAbs  
MEDIATE C3 LOCALIZATION IN DIFFERENT PARTS OF CAPSULE**

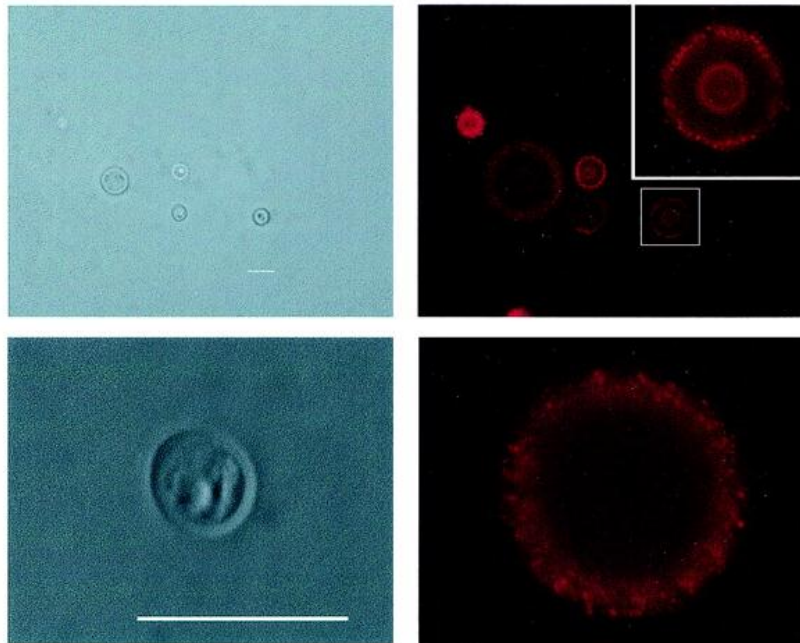
**LIGHT**

**IgM**

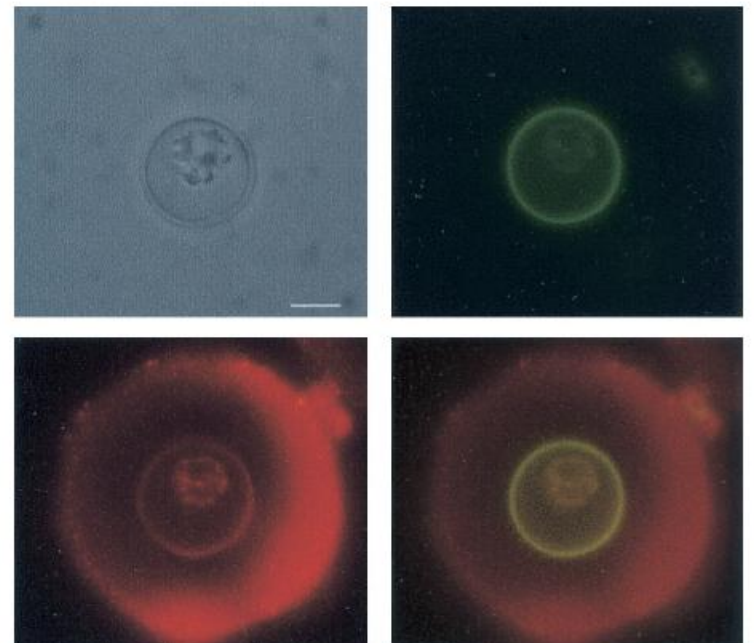
**C3**

**MERGE**

**FUNGAL CELLS IN VIVO COATED WITH  
IgM IN 'PUNCTATE PATTERN**

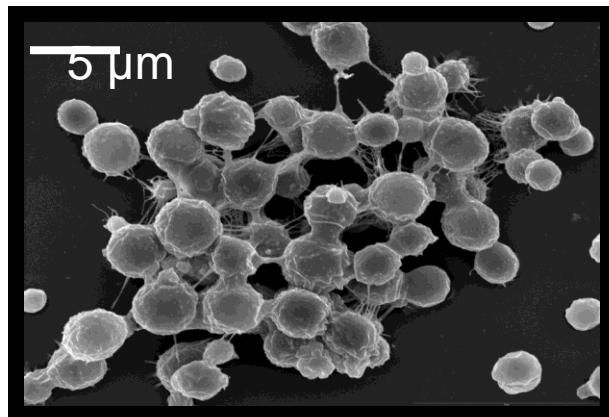
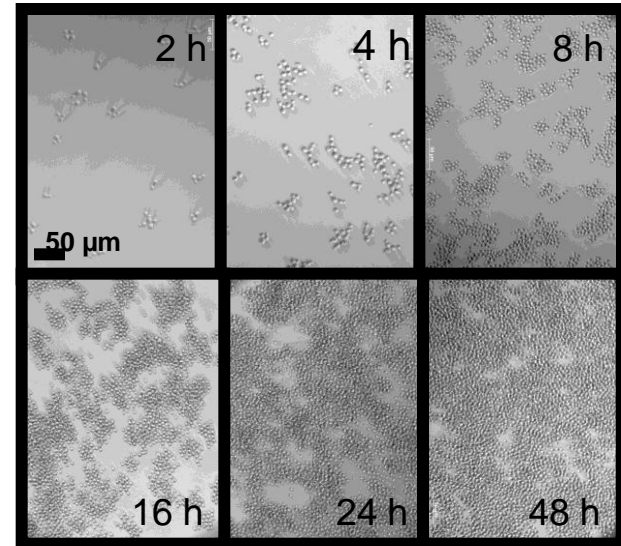
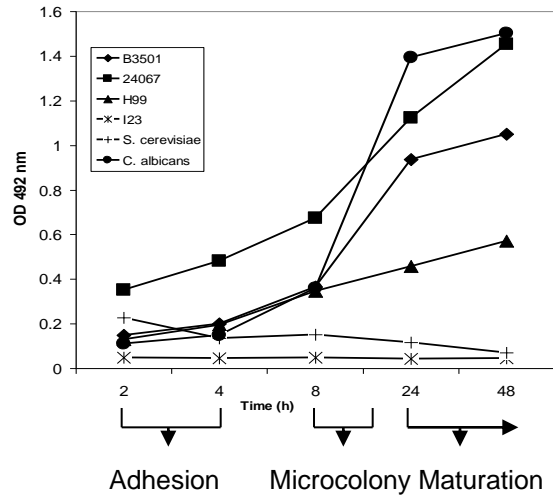


**COMPLEMENT IS DEPOSITED  
IN VIVO AWAY FROM SURFACE**

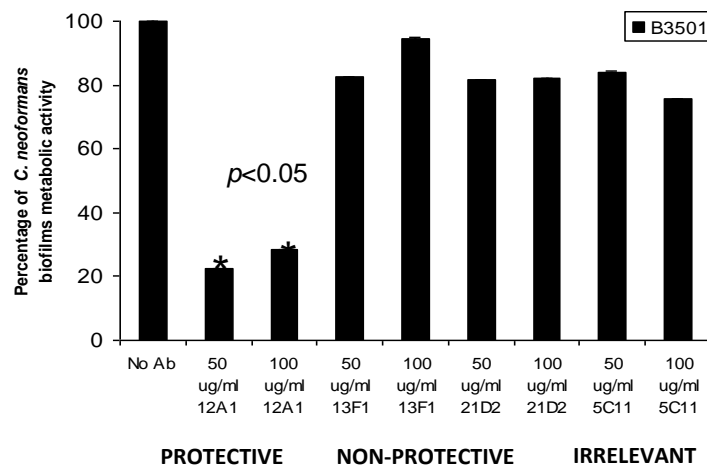
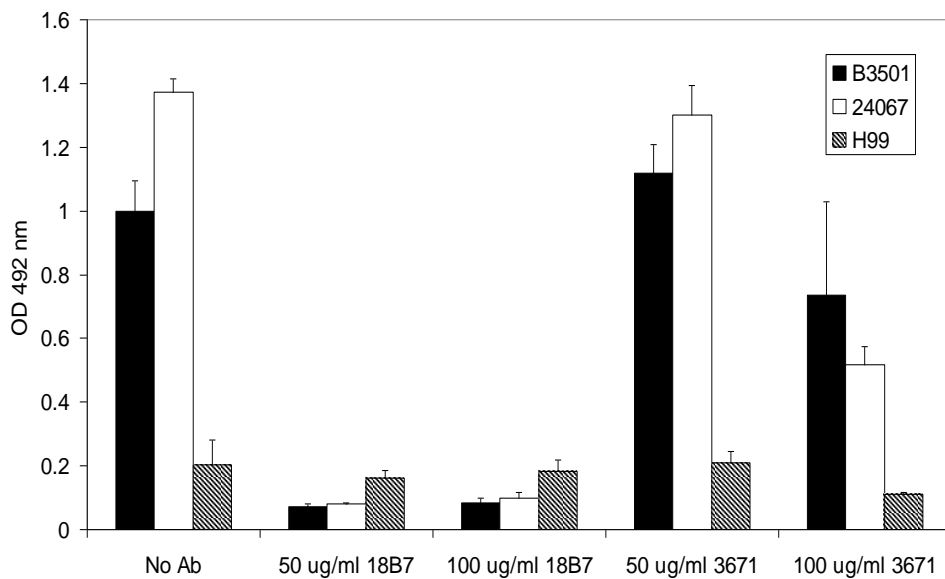
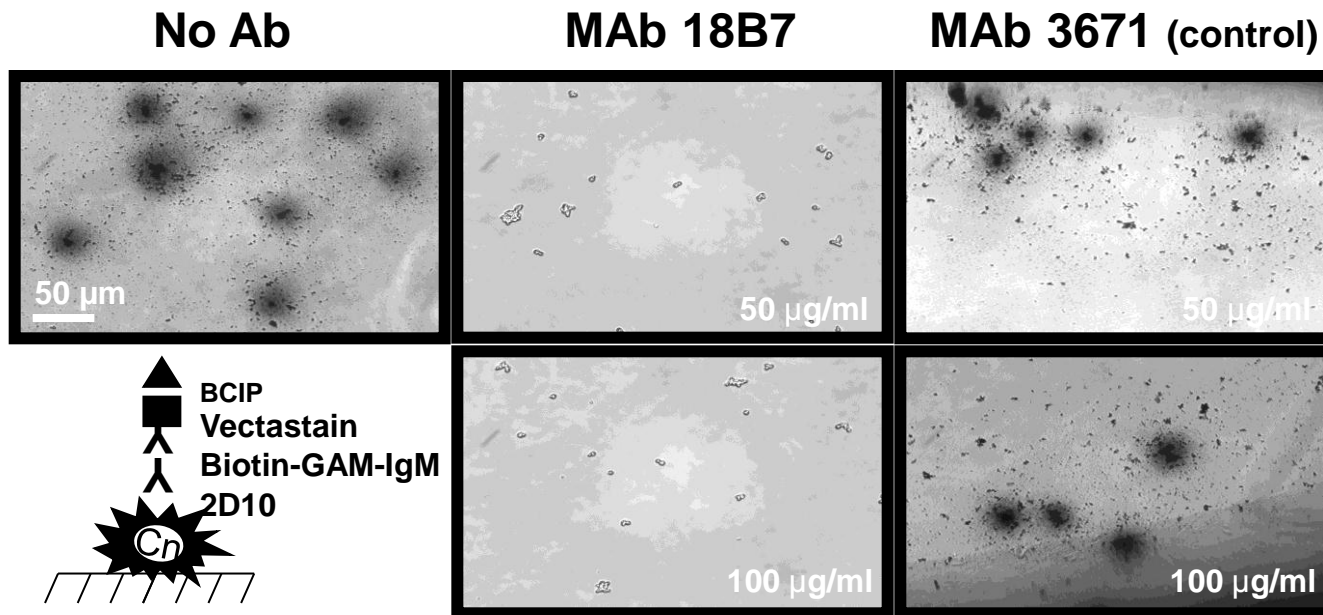


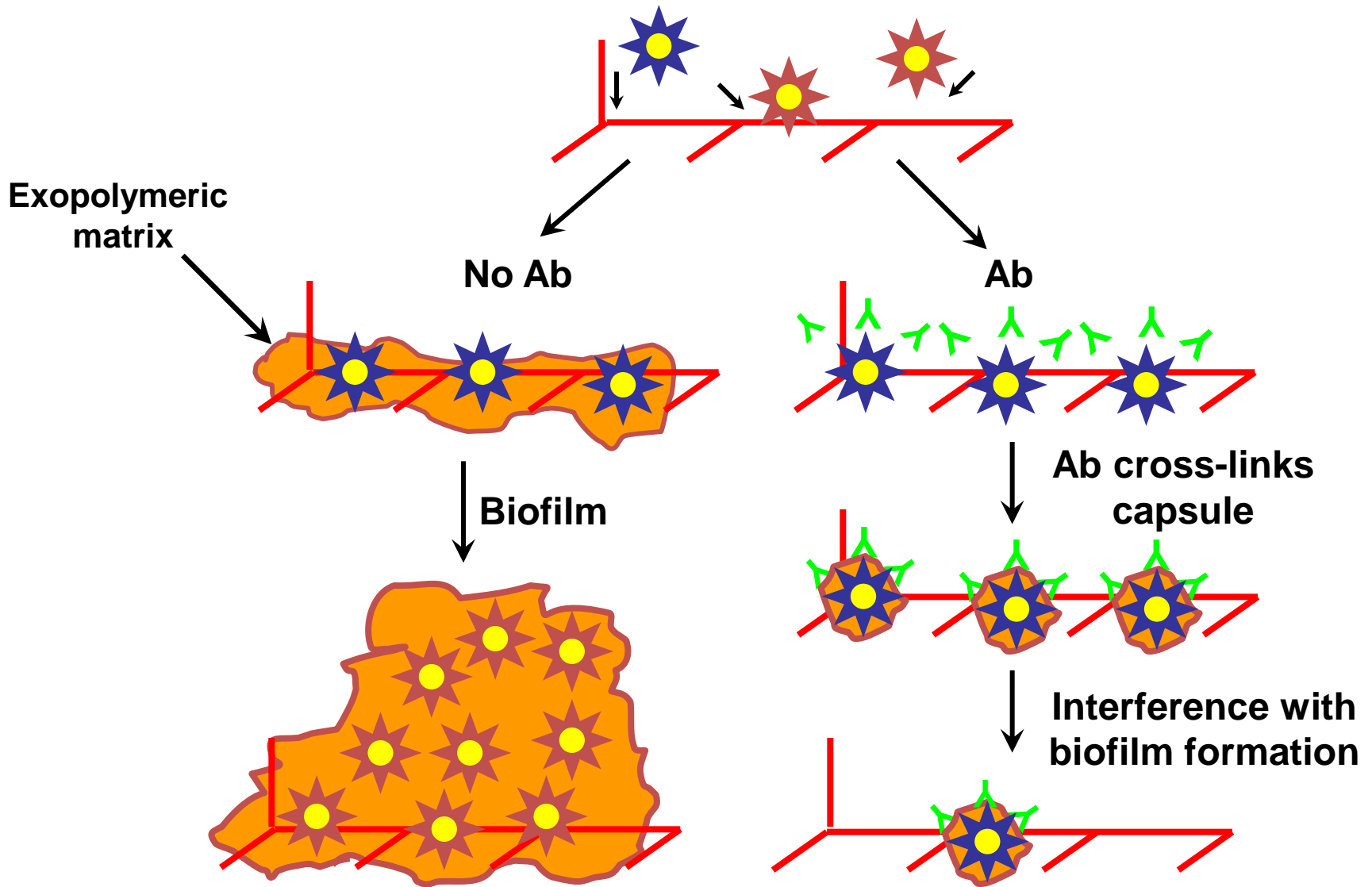
**Zaragoza et al Infect Immun 2004**

# *C. neoformans* BIOFILM FORMATION



# PROTECTIVE ANTIBODIES BLOCK BIOFILM FORMATION

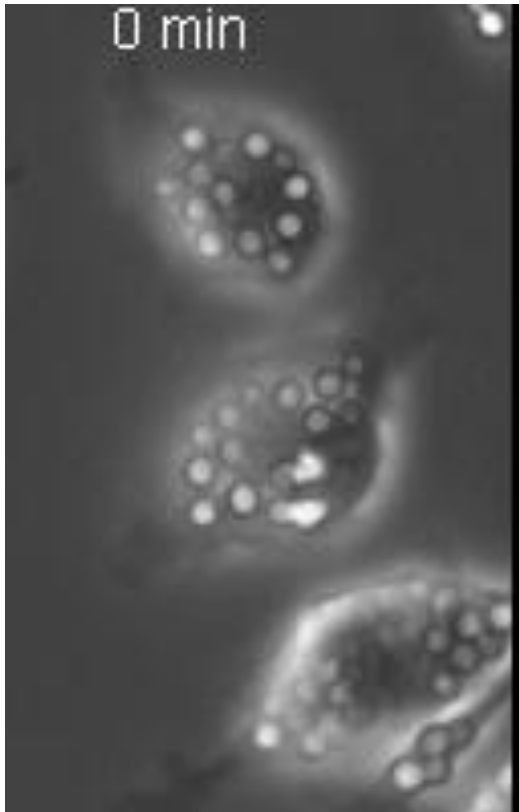




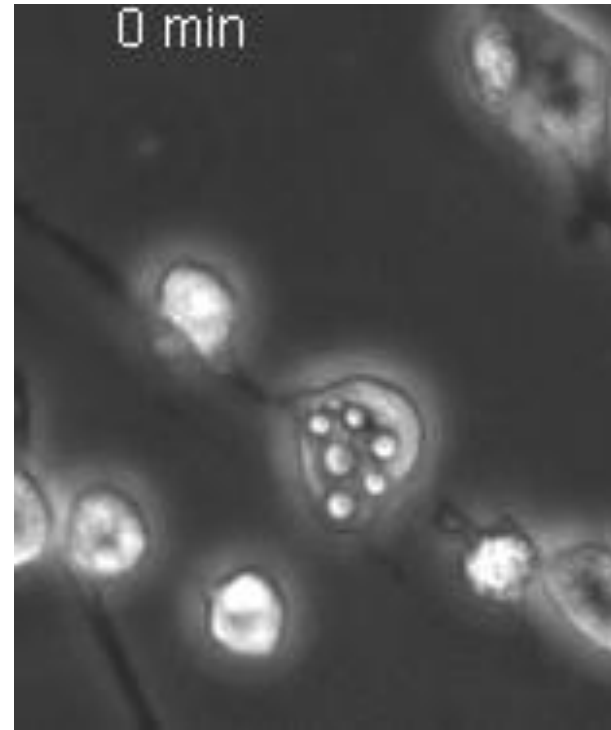
**HOW DOES ANTIBODY INTERFERE WITH FUNGAL PHYSIOLOGY?**

# IgG AND C3 OPSONIZATION HAVE DIFFERENT OUTCOMES IN EXIT DISPERSION

## IgG OPSONIZATION

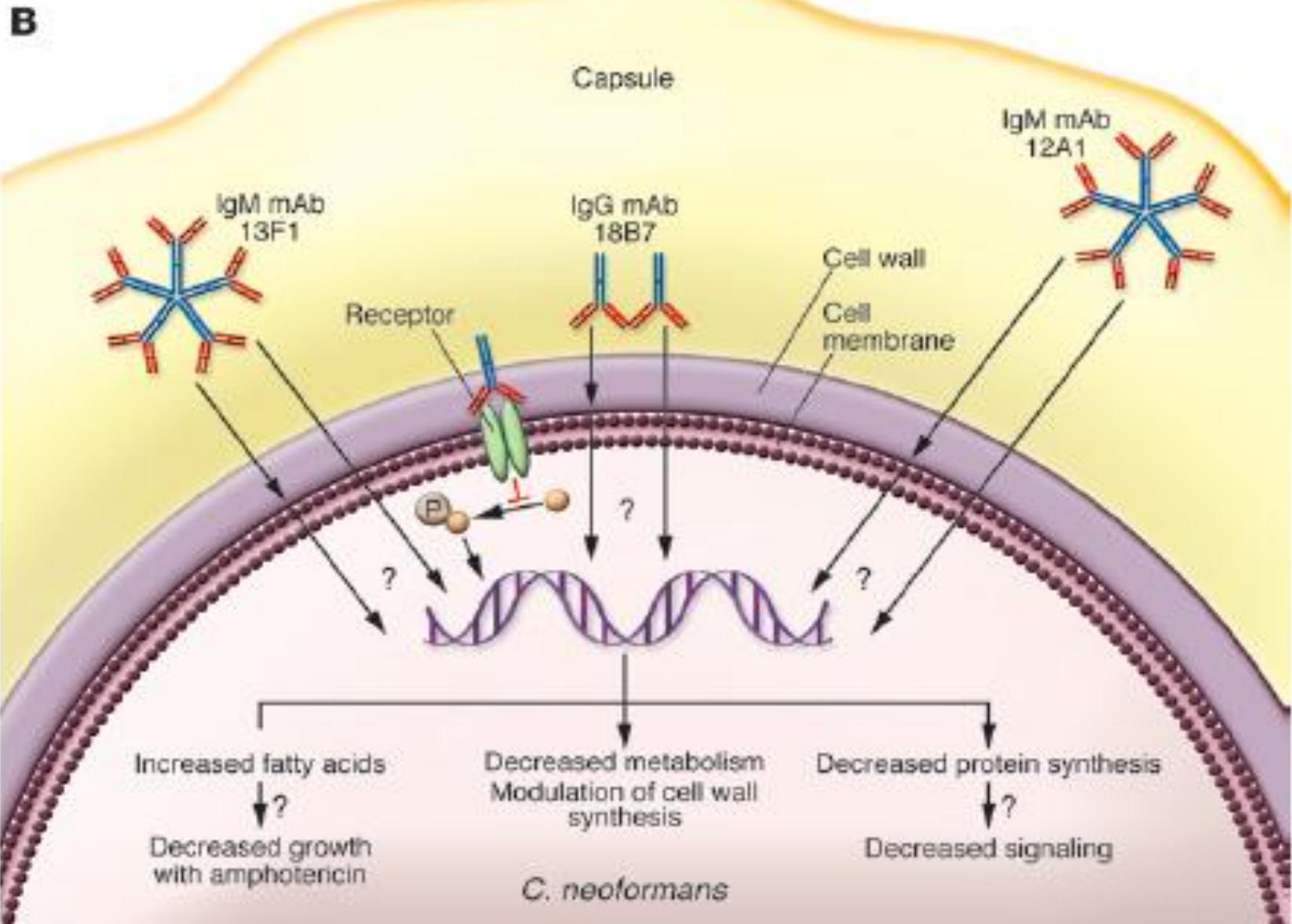


## C3 OPSONIZATION





# ANTIBODY BINDING CAN ALTER MICROBIAL GENE EXPRESSION



# MTb RESPONSE UPON CAPSULAR MAB TREATMENT



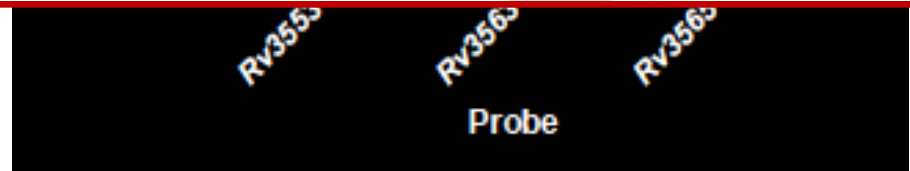
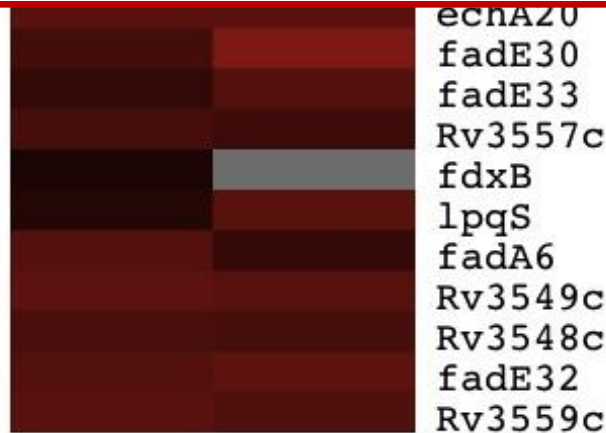
## qRT-PCR Analysis of Selected Transcripts

(fold) 32

## Antibodies to *Streptococcus pneumoniae* Capsular Polysaccharide Enhance Pneumococcal Quorum Sensing

Masahide Yano,<sup>a</sup> Shruti Gohil,<sup>b</sup> J. Robert Coleman,<sup>a</sup> Catherine Manix,<sup>a</sup> and Liise-anne Pirofski<sup>a,b</sup>

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York, USA<sup>a</sup>; and Division of Infectious Diseases, Department of Medicine, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York, USA<sup>b</sup>



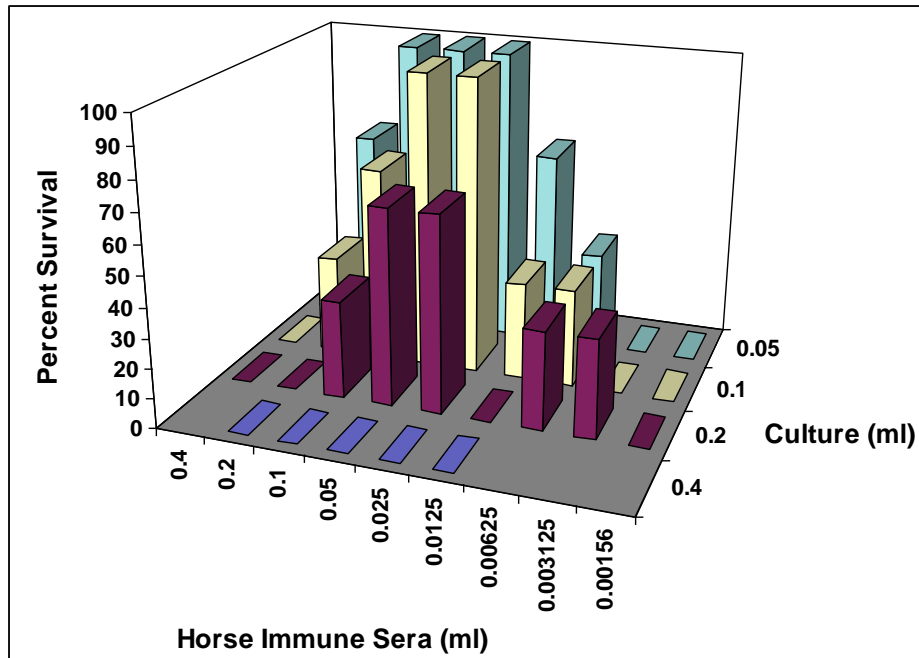
Mid-log phase H37Rv defined MM culture

10 µg/ml Mab

45 min

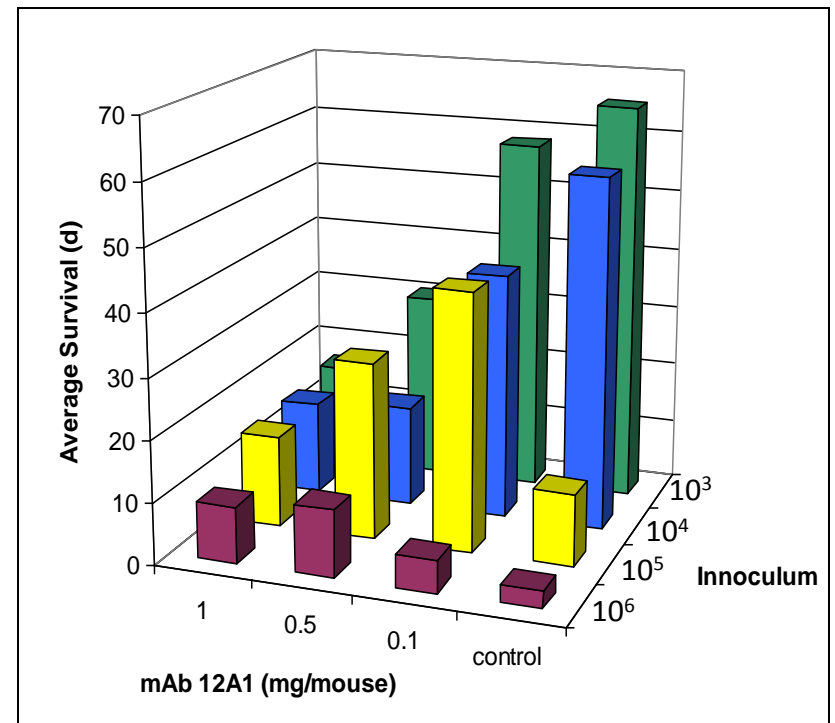
Expression ratio: Capsular Mab/isotype IgG

# MORE IS NOT BETTER: PROZONE-LIKE EFFECTS



**PNEUMOCOCCUS 1935**

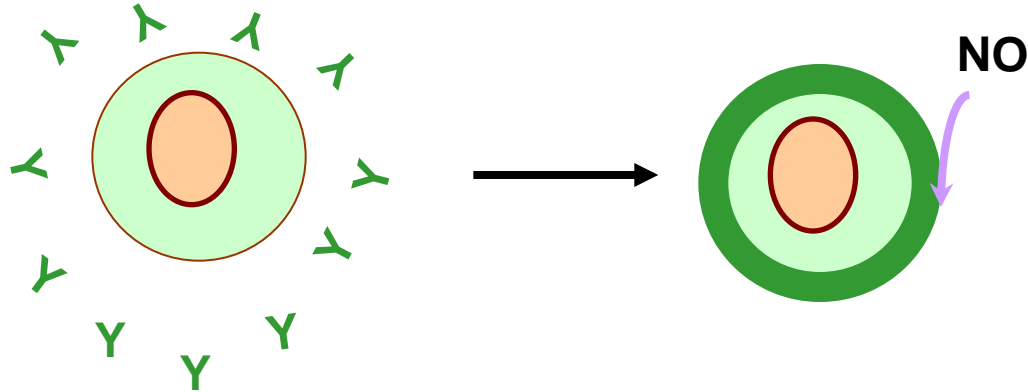
**A SINGLE mAb CAN BE PROTECTIVE,  
NON-PROTECTIVE OR DISEASE  
ENHANCING DEPENDING ON  
ITS CONCENTRATION AND INOCULUM**



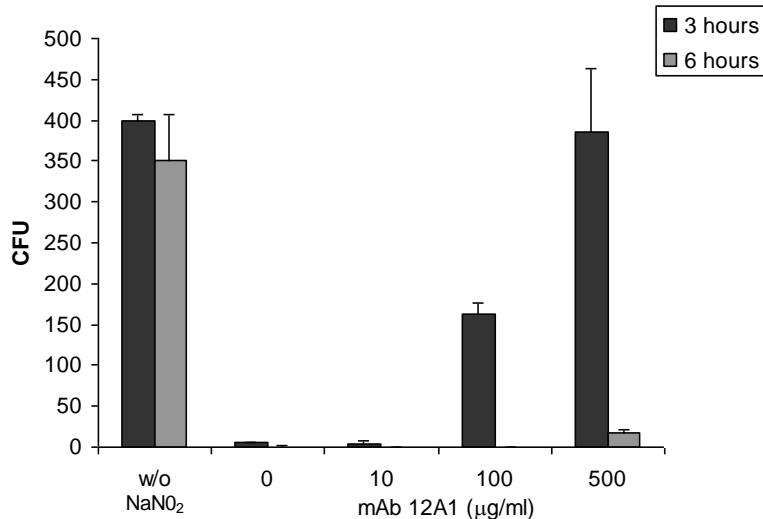
**C. NEOFORMANS 2001**

# LARGE AMOUNTS OF ANTIBODY BOUND TO CAPSULE PROTECT AGAINST NITRIC OXIDE FUNGICIDAL ACTIVITY

**EXCESS ANTIBODY**



## MECHANISM 1: REDUCED KILLING BY MICROBICIDAL OXIDANTS IN VITRO



## MECHANISM 2: DIFFERENCES IN CYTOKINE EXPRESSION IN HIGH AND LOW Ab CONDITIONS

**Ab PROTECTION**

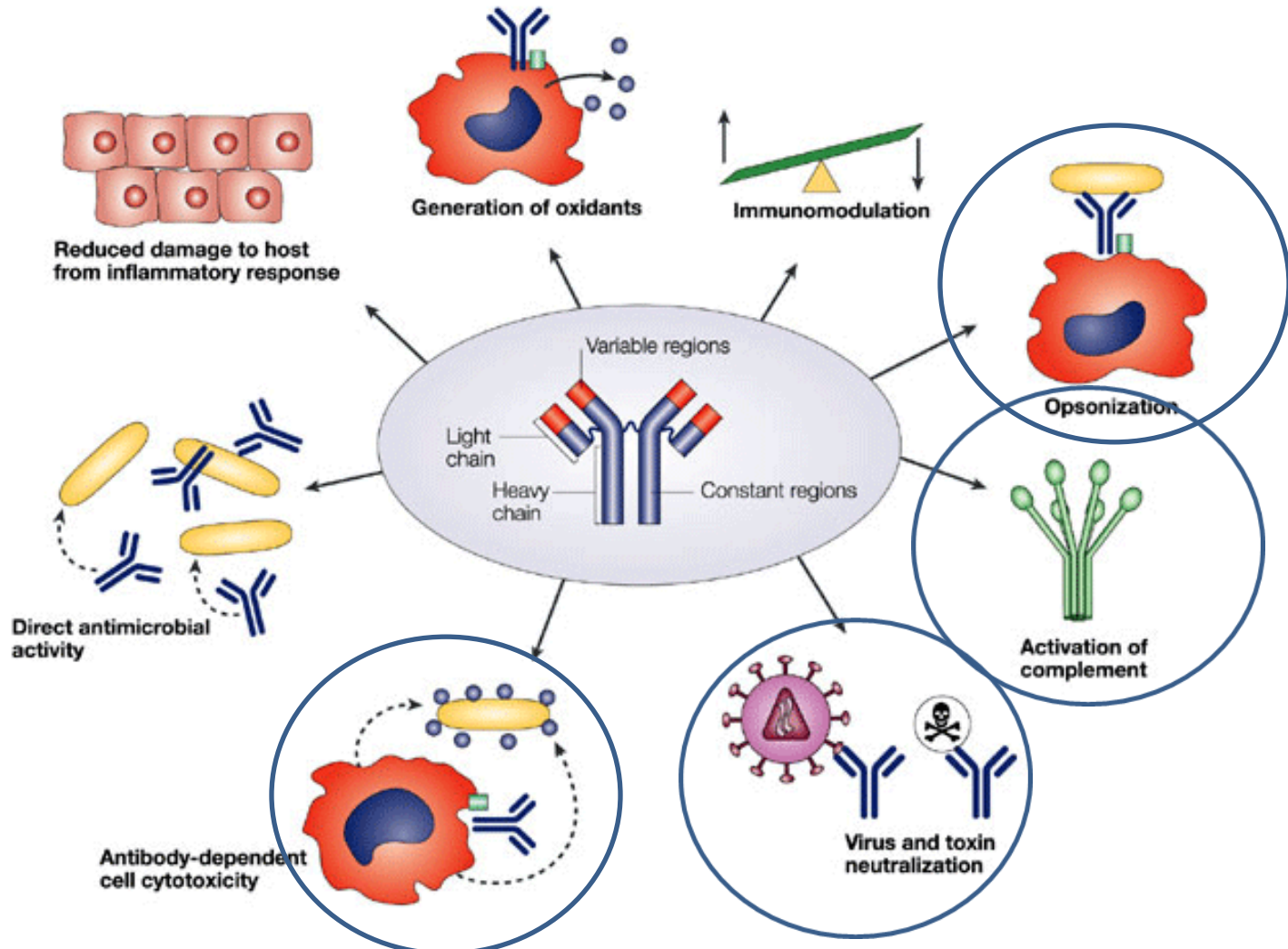
HIGH IL-4, IL-6, IL-12, TNF- $\alpha$

**NO PROTECTION**

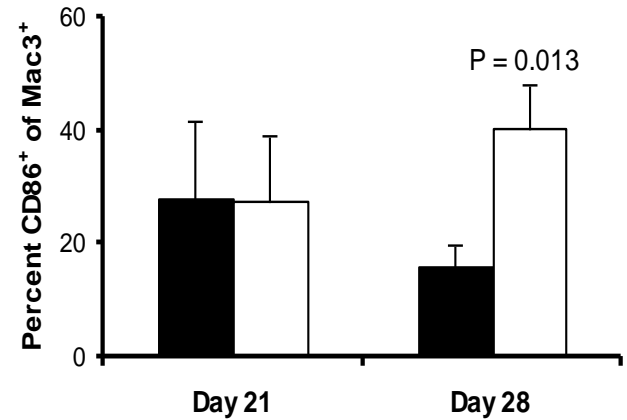
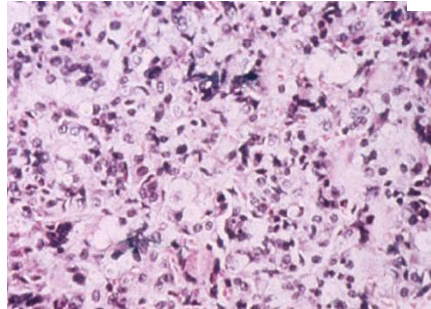
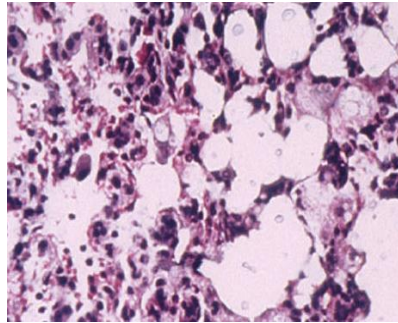
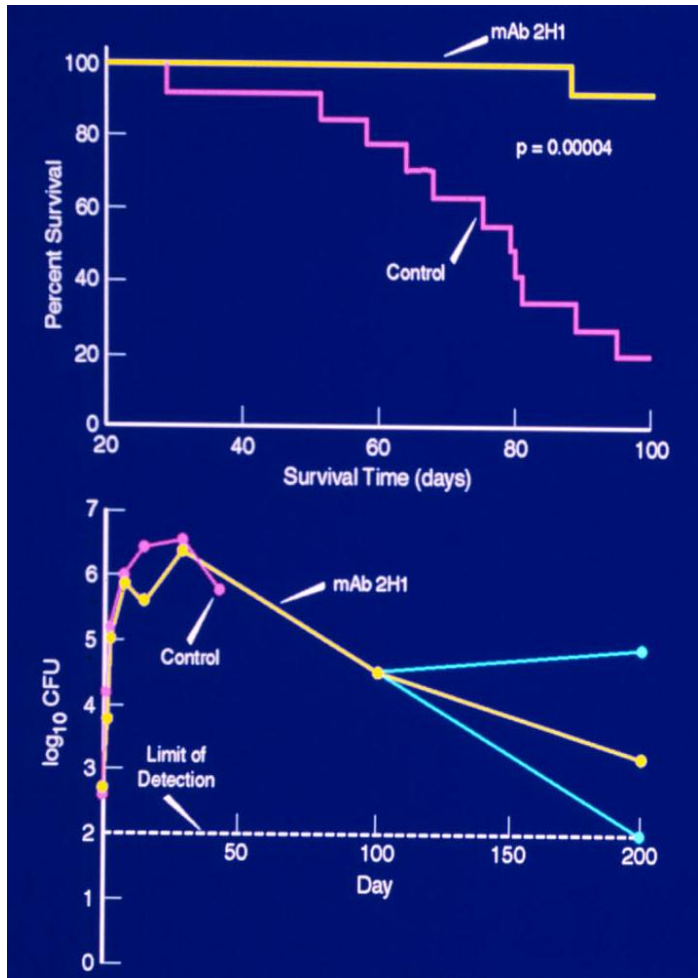
LOW IL-6 AND IFN- $\gamma$ ;  
HIGH IL-10 AND IL-12

## MECHANISM 3: DIFFERENCES IN C3 DEPOSITION IN HIGH AND LOW Ab CONDITIONS

# HOW DO ANTIBODIES WORK?



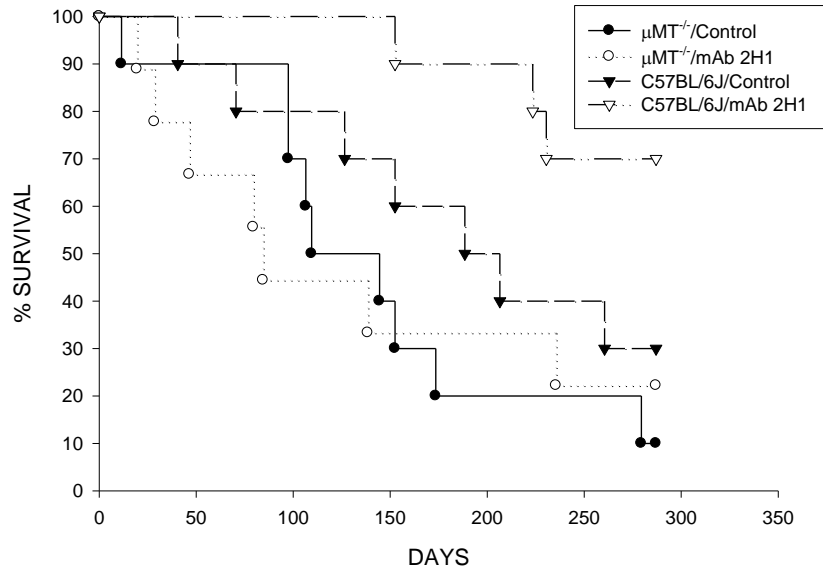
# ANTIBODY-MEDIATED PROTECTION ASSOCIATED WITH CHANGES IN INFLAMMATORY RESPONSE



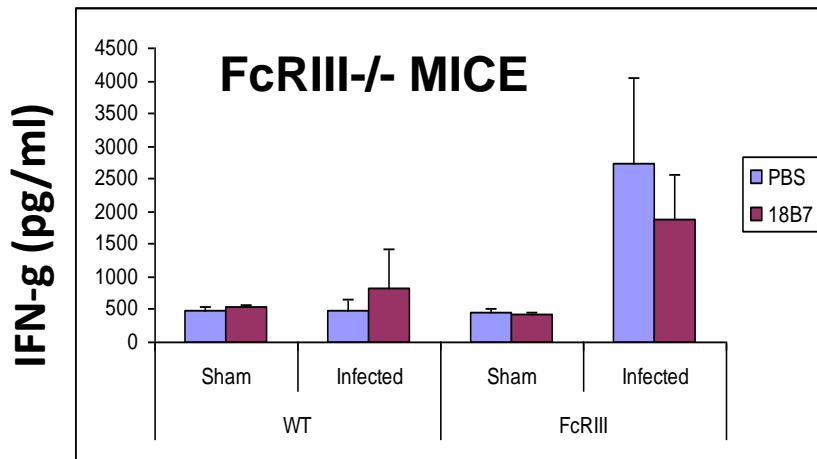
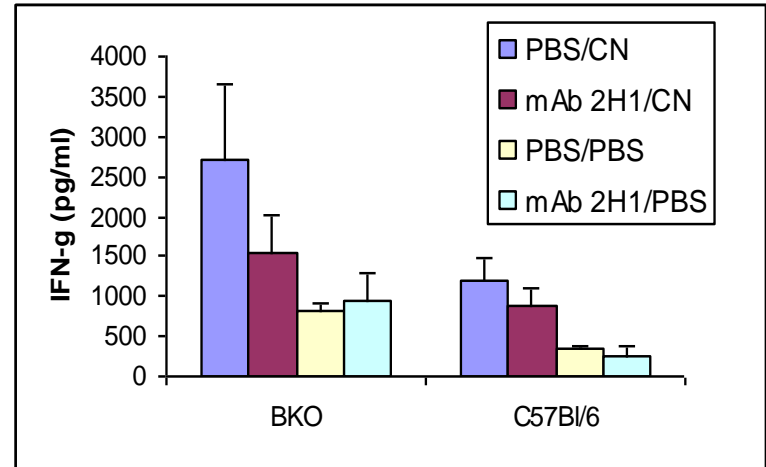
- REDUCED IFN-GAMMA
- INCREASED IL-10
- REDUCED IL-4
- INCREASED B7-2
- INCREASED % GRANULOCYTES

**NET RESULT: DAMAGE**

# CRYPTOCOCCOSIS IN B CELL DEFICIENT AND FcRIII-/- MICE



## B CELL DEFICIENT MICE



ACTION	EFFECT	CONSEQUENCE	OUTCOME
PASSIVE Ab	ADD Ab	LESS IFN-g	PROTECTION
B CELL KO	NO Ab	MORE IFN-g	NO PROTECTION
FcRIII <sup>-/-</sup>	NO FcRIII	MORE IFN-g	NO PROTECTION

Rivera et al. Infect.Immun. (2005)

# **SEVERAL NEW MECHANISMS FOR ANTIBODY-MEDIATED PROTECTION**

- **MEDIATING PHAGOCYTOSIS THROUGH A NON-Fc AND C3 INDEPENDENT MECHANISM**
- **INHIBITING BIOFILM FORMATION**
- **MODULATING INFLAMMATION TO IMPROVE GRANULOMA FORMATION**
- **DIRECT PHYSIOLOGICAL EFFECTS ON MICROORGANISMS**



# PROTECTIVE ANTIBODIES

YYYYYYYYYYYYY

YYYYYYY

**DIRECT  
EFFECTORS**

YYYYYYY

**INDIRECT  
EFFECTORS**

**INDEPENDENT OF CMI**

TOXIN NEUTRALIZATION  
PHAGOCYTOSIS & KILLING  
COMPLEMENT ACTIVATION  
VIRAL NEUTRALIZATION  
ADCC

**DEPENDENT ON CMI**

CYTOKINE CHANGES  
CELL ACTIVATION  
ANTIGEN PRESENTATION  
CLEARANCE OF ANTIGENS

*S. PNEUMONIAE*  
*C. DIPHTHERIA*  
*H. INFLUENZAE*  
WEST NILE VIRUS  
*B. ANTHRACIS*  
VARIOLA VIRUS

**'EASY'**

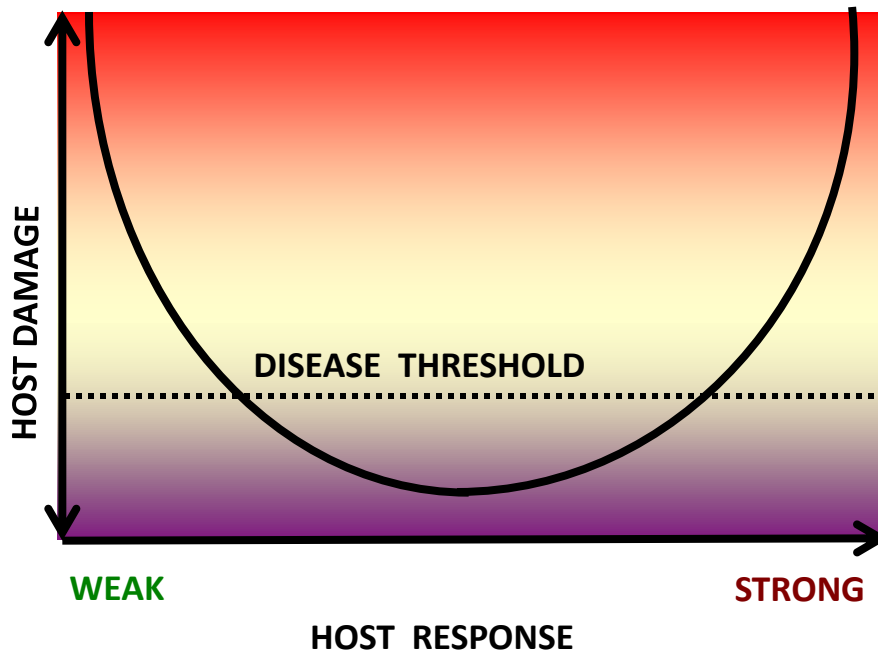
REDUCE MICROBIAL INNOCULUM

REDUCE HOST DAMAGE

*C. NEOFORMANS*  
*M. TUBERCULOSIS*  
*H. CAPSULATUM*

**'HARD'**

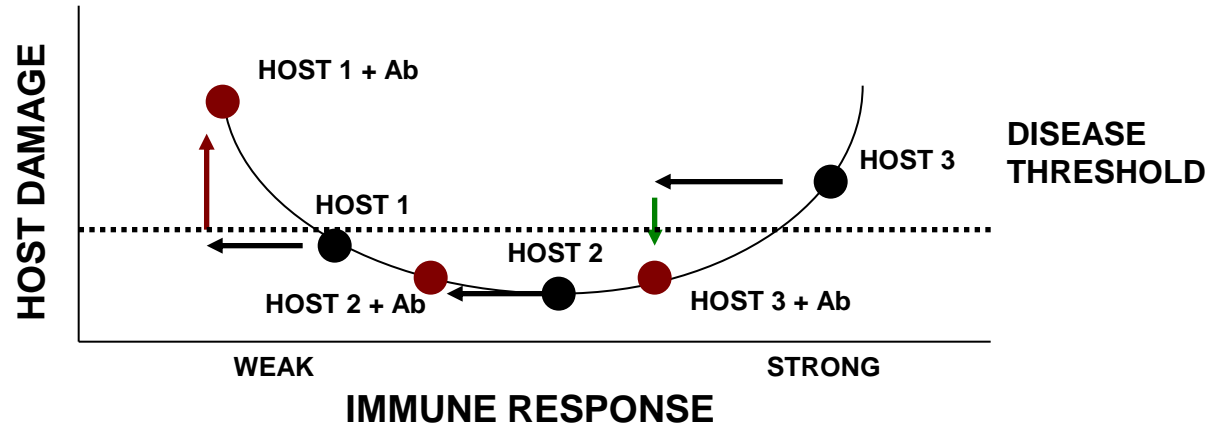
# BASIC RELATIONSHIP FOR 'DAMAGE-RESPONSE FRAMEWORK'



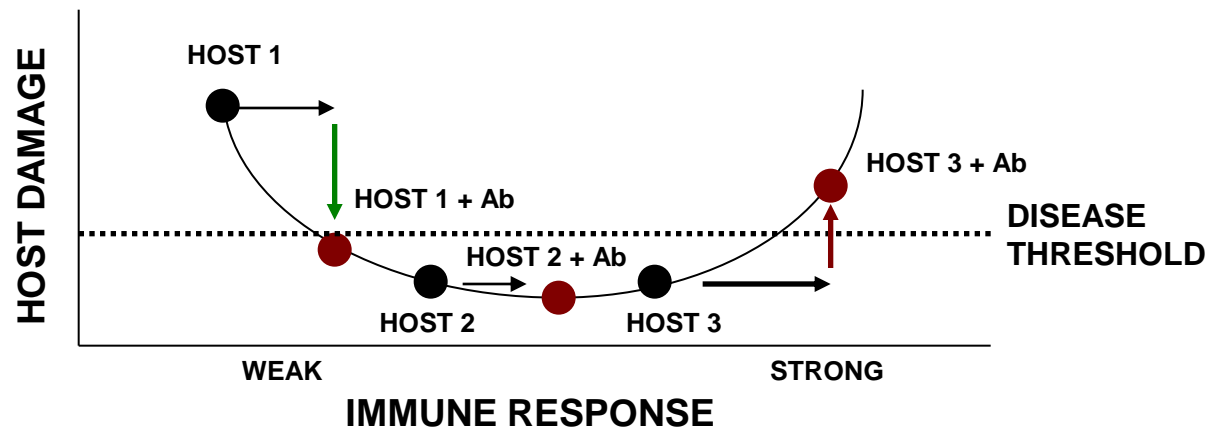
Dr. Liise-anne Pirofski  
PROFESSOR  
CHIEF, ID DIVISION  
ALBERT EINSTEIN/MONTEFIORE

# ANTIBODIES ARE NOT INTRINSICALLY GOOD OR BAD: FUNCTION DEPENDS ON THE CONTEXT OF THE HOST RESPONSE

## A. SITUATION FOR AN ANTI-INFLAMMATORY Ab



## B. SITUATION FOR A PRO-INFLAMMATORY Ab



## SOME TAKE HOME MESSAGES

**FORGET ABOUT THE OLD CELLULAR VS HUMORAL DIVIDE: THINK ONLY IN TERMS OF PROTECTIVE vs. NON-PROTECTIVE**

**HOWEVER... 'PROTECTIVE' AND 'NON-PROTECTIVE' ARE RELATIVE TERMS THAT DEPEND ON HOST AND OTHER FACTORS**

**ANTIBODY EFFICACY IS A COMPLEX FUNCTION**

*PROTECTIVE EFFICACY = F(SPECIFICITY)(AMOUNT)(ISOTYPE)(HOST GENETICS)*

**CONSTANT REGION CAN INFLUENCE ANTIBODY FINE SPECIFICITY**

**ISOTYPE Ab FUNCTION IS PROBABLY PATHOGEN SPECIFIC**

*FOR C. NEOFORMANS EFFICACY: IgG2a > IgG1 > IgG2b >>> IgG3*

*FOR M. TUBERCULOSIS EFFICACY: IgG3 >> IgG2a*

# FINAL THOUGHTS...

**A NEGATIVE EXPERIMENT/TRIAL IS JUST A NEGATIVE EXPERIMENT – AVOID LOGICAL ERRORS BY NOT MAKING POSITIVE INFERENCES FROM NEGATIVE DATA**

**YOU CAN PROBABLY MAKE A PROTECTIVE ANTIBODY AGAINST ANY PATHOGEN**

**EVERY ANTIBODY AND EVERY PATHOGEN ARE DIFFERENT – ALL THOUGH SOME THEMES ARE GENERALIZABLE EACH ANTIBODY-PATHOGEN SYSTEM IS UNIQUE**

**CONSIDERING THE COMPLEXITY I HAVE TOLD YOU ABOUT – IT IS AMAZING, BUT VERY ENCOURAGING THAT WE HAVE ALREADY SUCCEEDED IN MAKING SOME USEFUL VACCINES**