Principles of Antibody Engineering and Therapy

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Antibodies recognize foreign substances (antigens)

examples: bacteria
viruses
cancer cells
pollen (allergies)

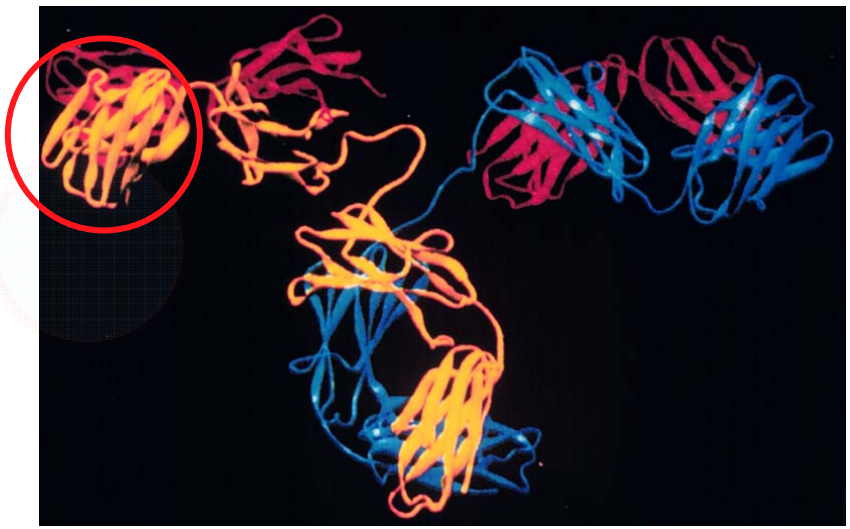
They have the ability to recognize millions of different antigens

Carry out “effector functions”

Examples: kill bacteria
prevent viral attachment to cells
neutralize toxins
destroy cancer cells

How are they able to do all of this?

Antibodies are remarkable molecules with a division of labor



They have variable regions that are the part of the molecule that binds antigen.
There are literally millions of different possible variable regions so antibodies can recognize millions of different antigens.

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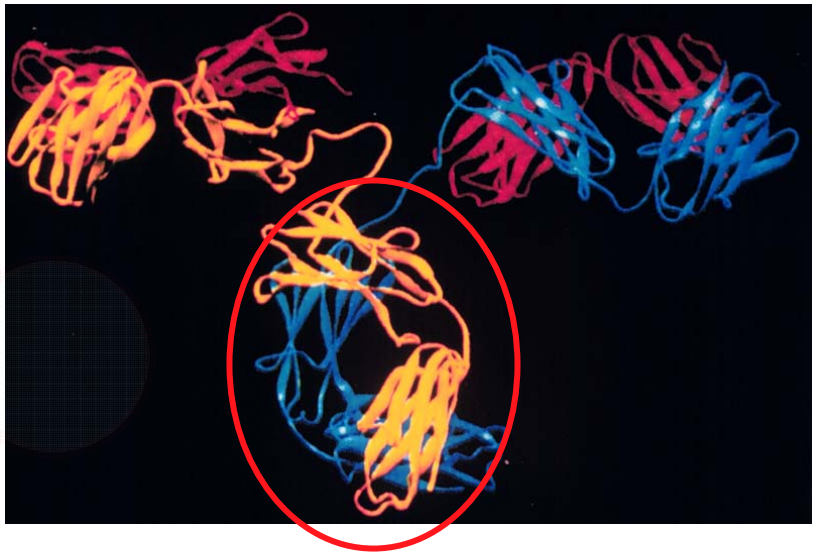
How are they able to do all of this?

Antibodies are remarkable molecules with a division of labor



They have a relatively constant region.
It is this region that is responsible for carrying out the limited number of different effector functions.

Considerations when choosing or making an Ab

Specificity: epitope
affinity

Determined by the Variable Region

Functional properties: Half-life
Fc receptor binding
Complement activation
Tissue penetration

Determined by the Constant Region

The Ab can be divided into different functional regions and Ab fragments have many useful properties.

However the focus of this presentation will be on intact Abs

QuickTime™ and a
None decompressor
are needed to see this picture.

Original Source of Antibodies Was Murine Hybridomas

Advantages: Many precisely characterized specificities
Homogeneous
Available in virtually unlimited quantites
Single constant region with associated effector functions

Disadvantage: IMMUNOGENICITY

A solution was to produce chimeric Abs with the variable region from the mouse Ab joined to a human constant region.

Mouse Antibody

Chimeric Antibody

Mouse Antibody

QuickTime™ and a
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are needed to see this picture.

Chimeric Antibody

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Variable region from mouse recognizes the same antigen

Constant region from human human effector functions

Chimeric Antibody

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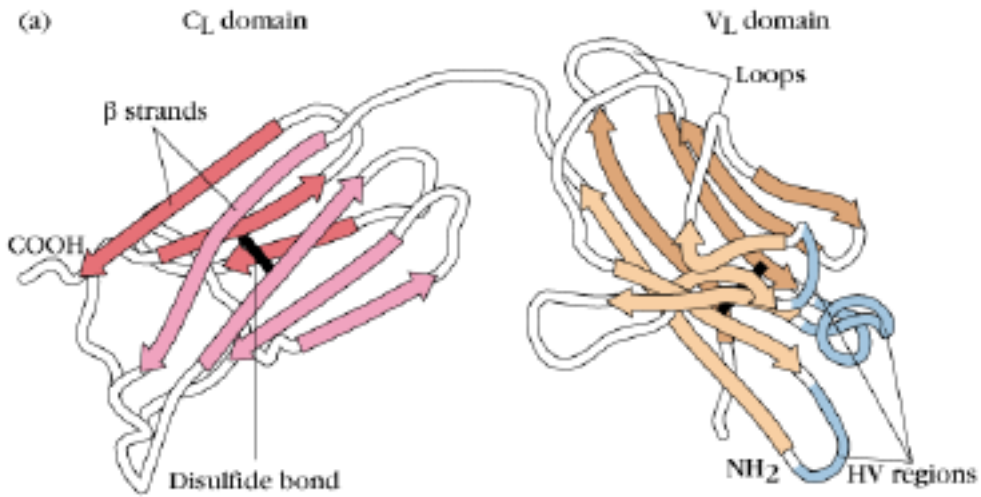
Since this antibody is mostly human it is usually not recognized as foreign

Examples in the clinic: Remicade (treat arthritis)
Rituxin (treat lymphoma)

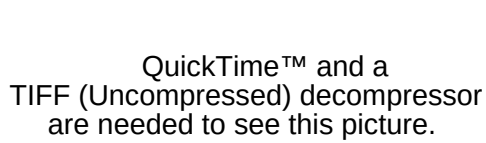
The **CDRs are loops** extending from the variable regions so that they are easily accessible for interaction with Ag. The other amino acids in the variable region are the "**framework**" amino acids and provide a scaffold to maintain the CDRs in the proper orientation.



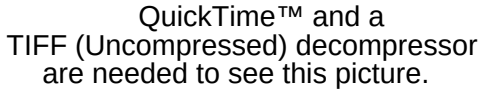
It is the CDRs that determine the binding specificity of the antibody

It is possible to transfer the CDRs from a mouse variable region to a human variable region

Chimeric Antibody



CDR (loop)-grafted Antibody



Recognizes the same antigen
Almost completely human

CDR (loop)-grafted Antibody

QuickTime™ and a
TIFF (Uncompressed) decompressor
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TIFF (Uncompressed) decompressor
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Recognizes the same antigen
Almost completely human

Examples in the clinic: Herceptin (breast cancer)
Synagisis (RSV in infants)

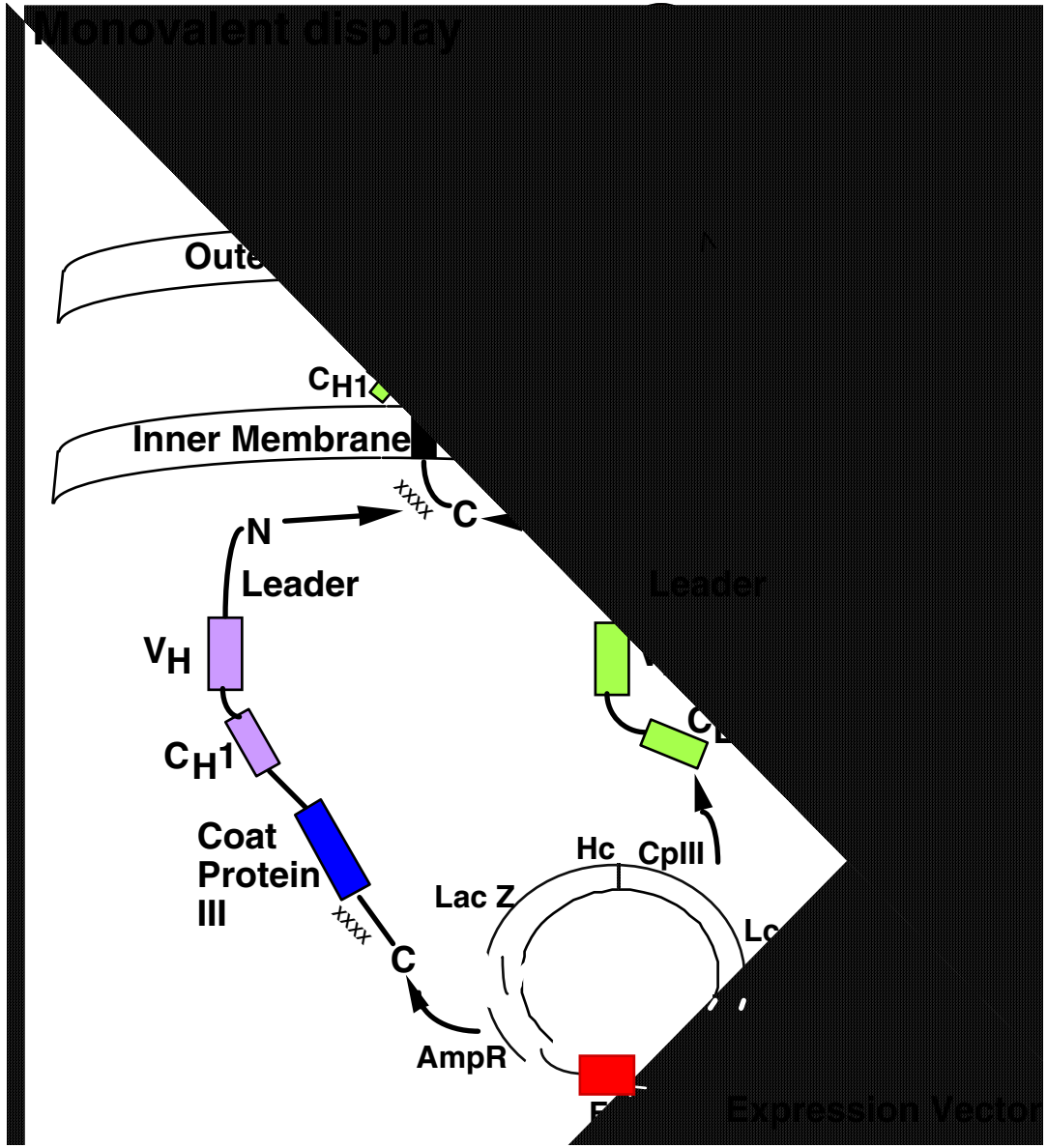
It is possible to immunize a mouse and obtain human Abs

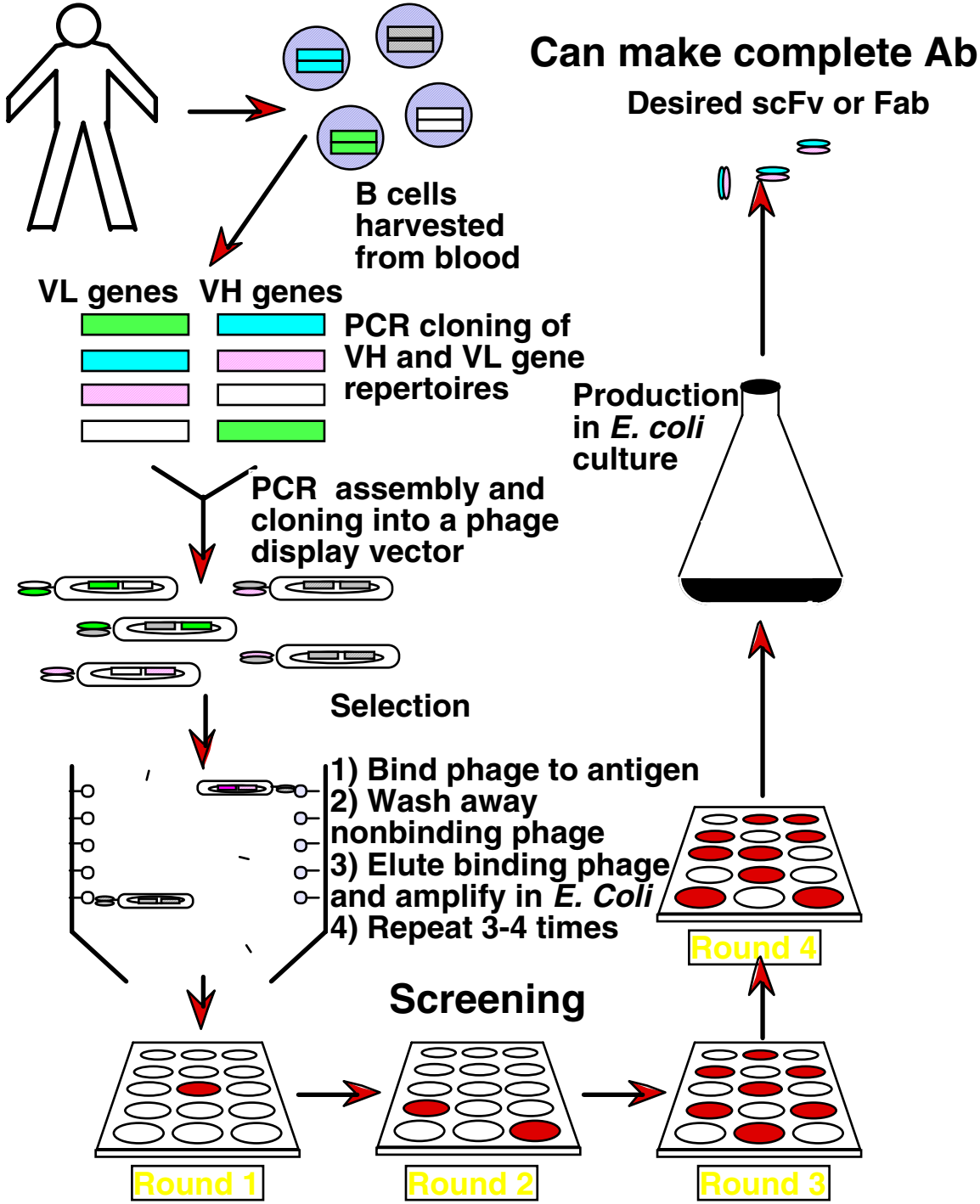
The Xenomouse™ has the murine Ig loci disrupted and contains the information to make a human Ab

It is also possible to obtain specific antibodies without using an animal.

Antibody binding specificities can be expressed on the surface of bacteriophage (bacterial viruses) and selected using phage binding to antigen.

VH and VL can be obtained from either naïve or immunized animals of diverse species including man.



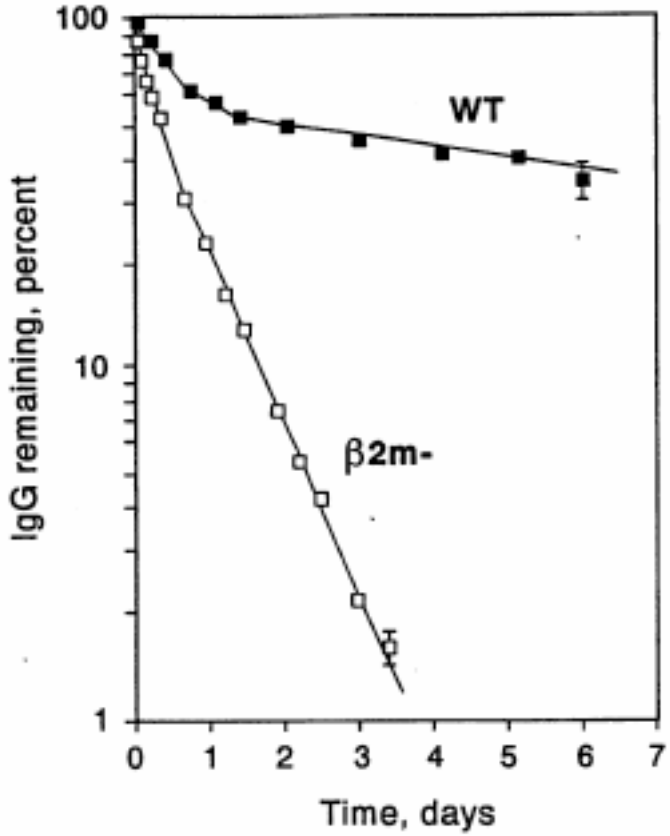

Can make complete Ab
Desired scFv or Fab
B cells harvested from blood
VL genes
VH genes
PCR cloning of VH and VL gene repertoires
PCR assembly and cloning into a phage display vector
Production in E. coli culture
Selection
1) Bind phage to antigen
2) Wash away nonbinding phage
3) Elute binding phage and amplify in E. Coli
4) Repeat 3-4 times
Screening
Round 1
Round 2
Round 3
Round 4

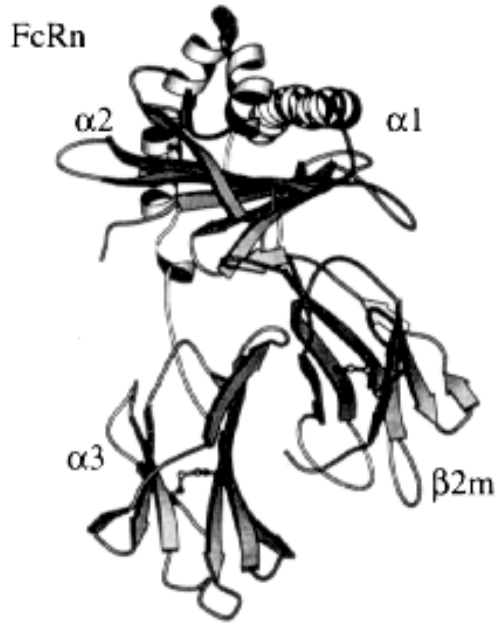
Using the Techniques of Antibody Engineering it is Possible To Produce Abs with the Desired Functional Properties

Half-life
Fc receptor Binding
Complement Activation

One important question is what determines the *in vivo* persistence of antibodies

An important role for FcRn has emerged



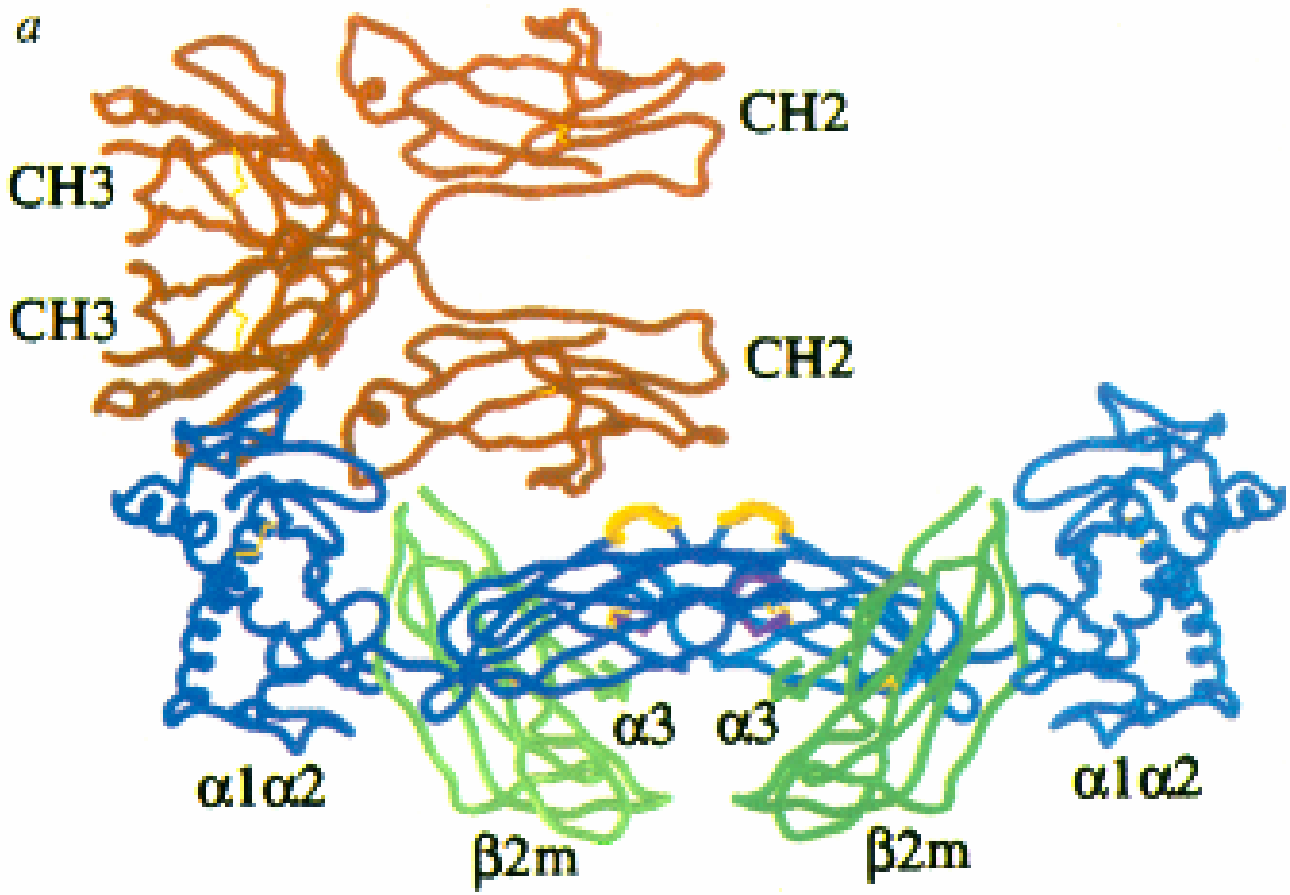


Selective depression of plasma IgG concentration in β2m-/- mice
PNAS 93:1996

	IgG	IgA
Wild-type	**2200 ± 100**	**110 ± 20**
Mutant	**260 ± 30**	**110 ± 20**
Ratio	**8.4:1 ± 0.9**	**1.0:1 ± 0.2**

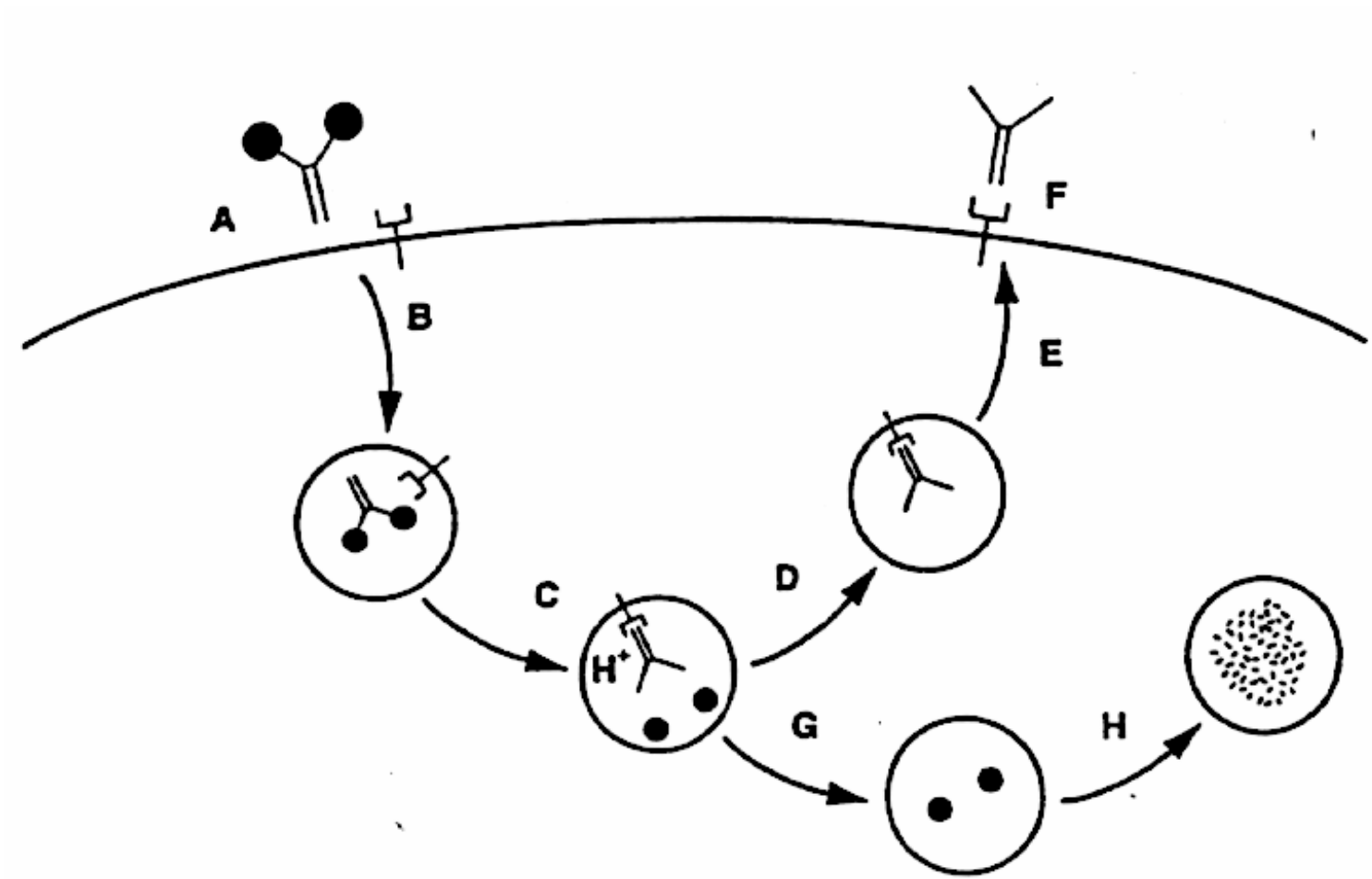
Junghans & Anderson, PNAS 93:5512

FcRn Binds IgG at the CH2/CH3 Interface



Nature 1994 372:379-83

Model for Role of FcRn



Junghans & Anderson, PNAS 93:5512

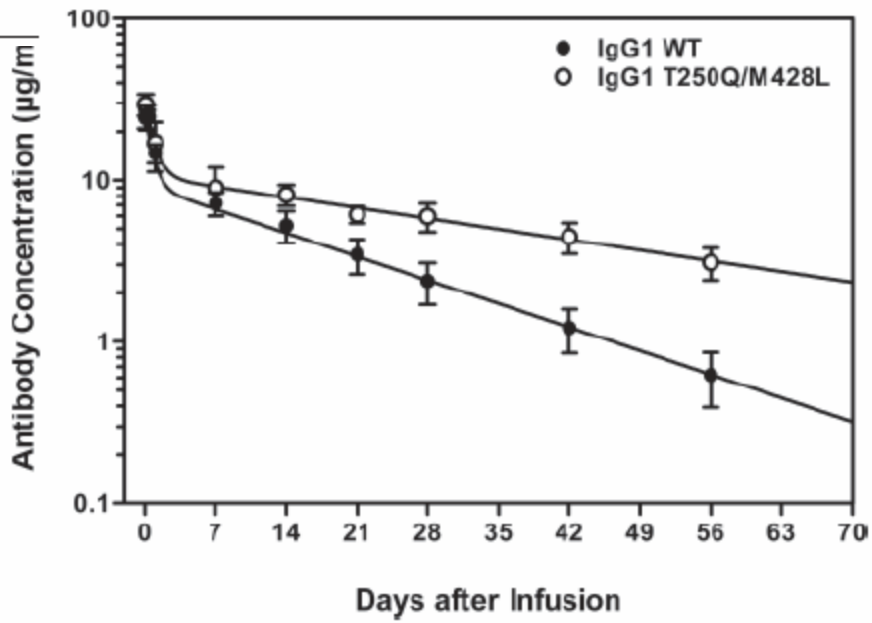
An example of increasing FcRn binding and half-life

Table II. *Binding of OST577 Abs to human FcRn*[a]

OST577	*n*	IC_{50} (μg/ml)	Relative Binding
IgG1 WT	5	10.3 ± 2.8	
IgG1 T250Q	5	3.14 ± 0.86	3.3
IgG1 M428L	5	0.896 ± 0.304	11
IgG1 T250Q/M428L	5	0.351 ± 0.144	29

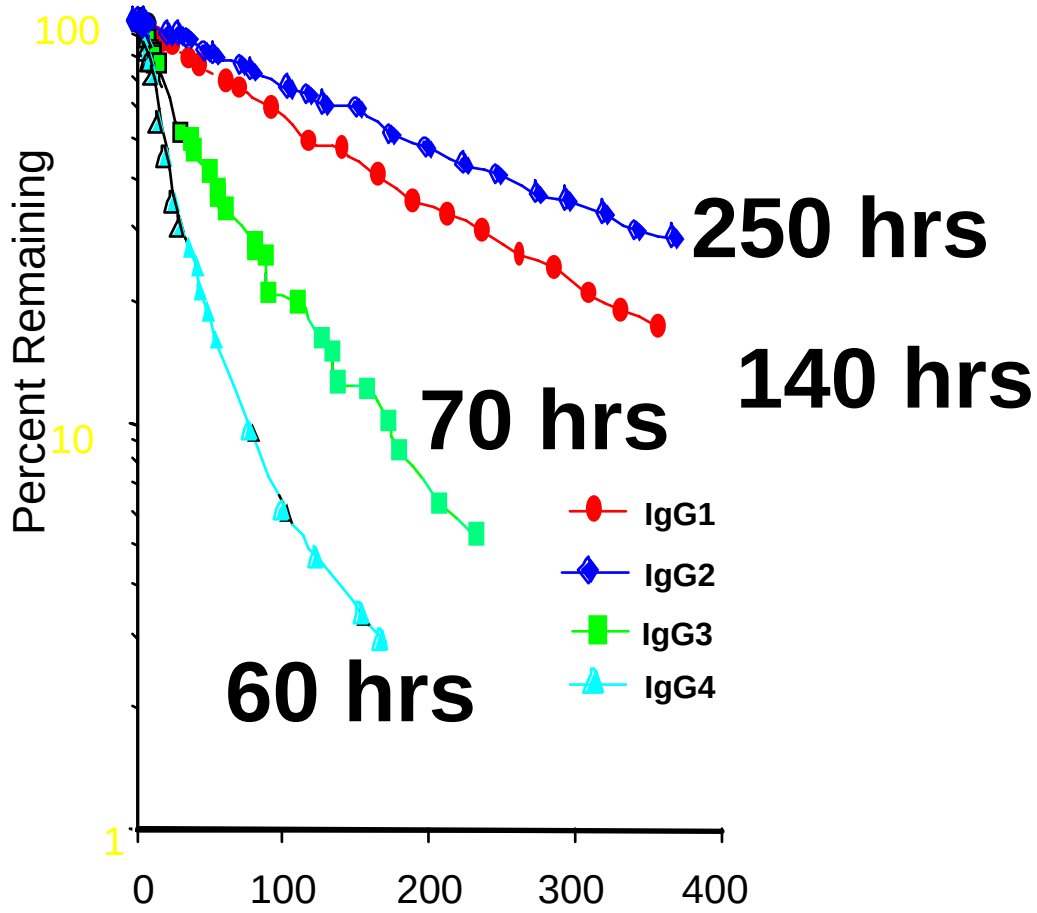
Table III. *Binding of OST577 Abs to rhesus FcRn*[a]

OST577	*n*	IC_{50} (μg/ml)	Relative Binding
IgG1 WT	3	8.86 ± 0.52	
IgG1 T250Q	3	2.97 ± 0.59	3.0
IgG1 M428L	3	0.629 ± 0.060	14
IgG1 T250Q/M428L	3	0.236 ± 0.013	37



Hinton et. al J. Immunol 176:346-356, 2006

Half-Life of Chimeric Antibodies in Mice

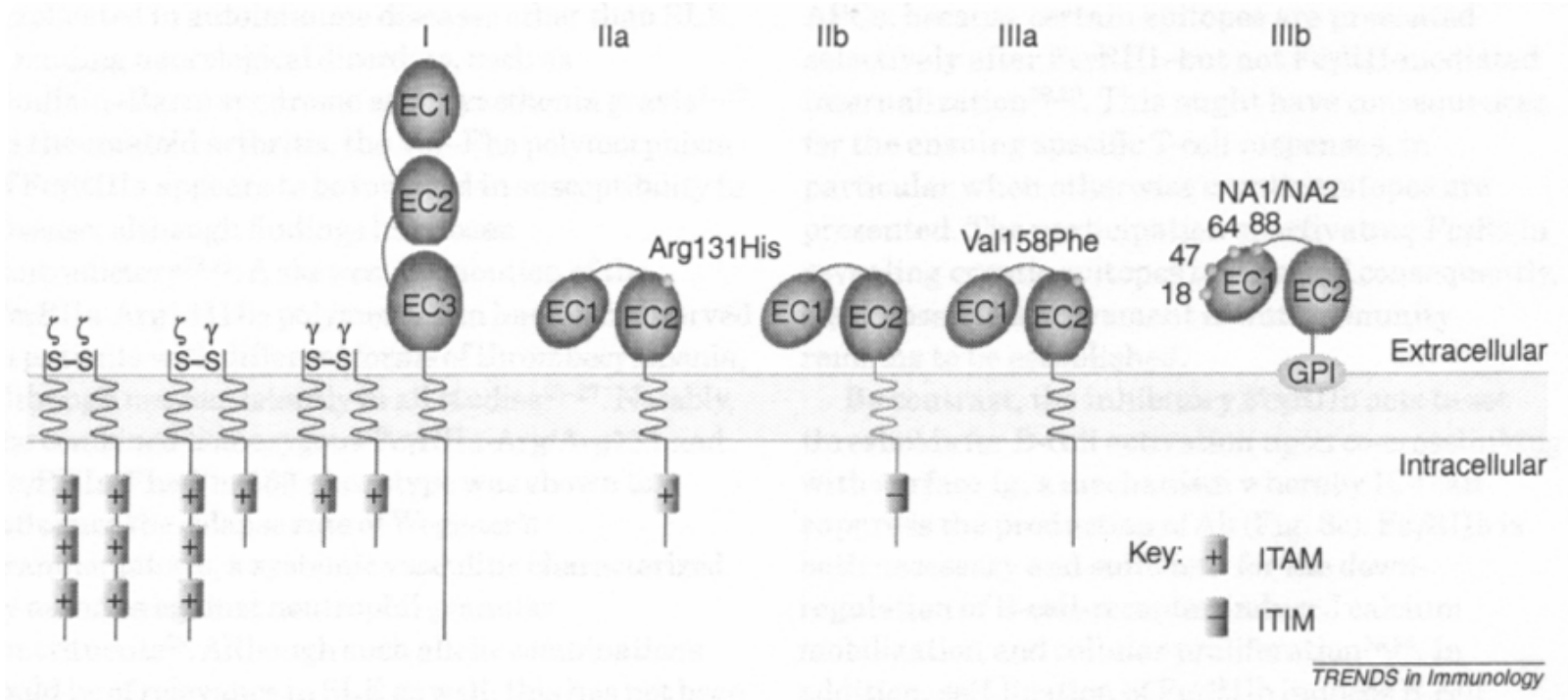


However, in this case we find no direct correlation of FcRn affinity with half-life

One important question is what determines the *in vivo* persistence of antibodies

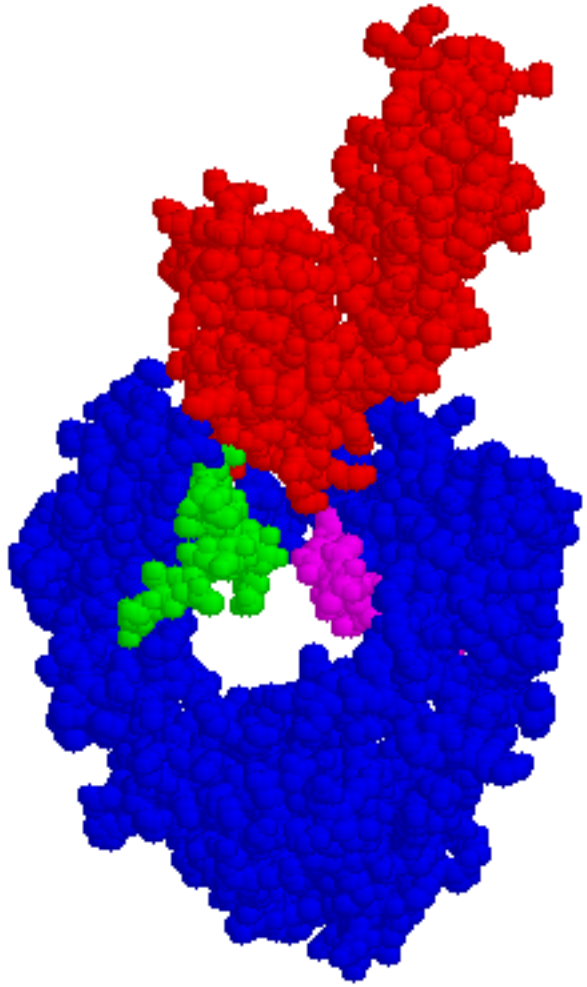
An important role for FcRn has emerged

While it is clear that expression of FcRn is important for a long serum half-life for IgG and that altering the affinity of an Ab for FcRn can alter its half-life in some cases, it remains unclear what other factors contribute to the observed differences *in vivo* persistence of different Abs

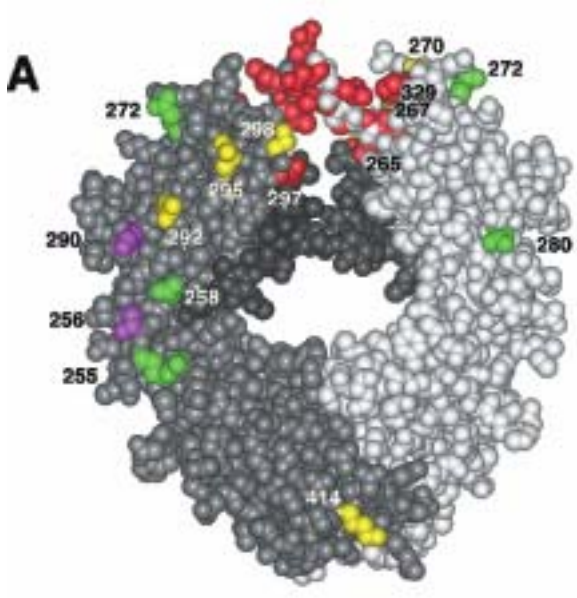


The FcγRs CD16, CD32 and CD64 play important roles in phagocytosis and ADCC. The inhibitory receptor, FcγRIIb plays a very important role in immune modulation. The affinity of an Ab for FcγRs can play an important role in its efficacy.

Complex of FcγRIII with Antibody (human IgG1)



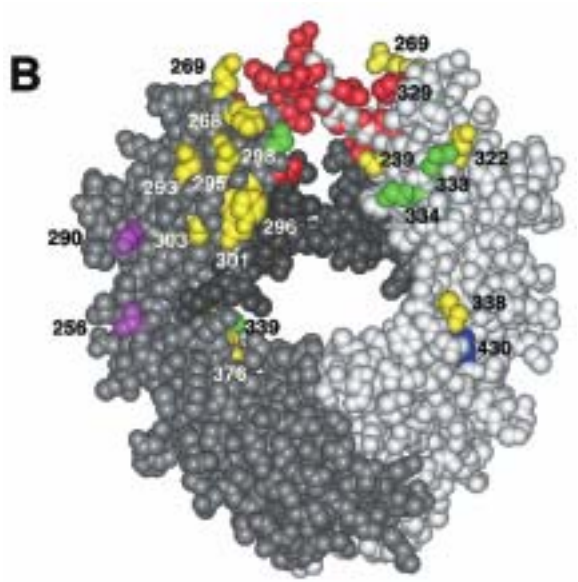
Nature 406:267, 2000



IgG1 residues identifed by site-directed as important for FcγR binding (alanine scanning)

Red: affected binding to all three receptors. The FcγRI site is comprised only of red residues

Magenta: improved binding to FcγRII and FcγRIIIA



Green: A. improved binding to FcγRII
B. improved binding to FcγRIIA

Yellow: A. reduced bind to FcγRII
B.reduced binding to FcγRIIIA

Although the FcγRs bind a similar region they are not identical

Shields et. al., JBC 276:6591-6604, 2001

Site-directed mutagenesis has been used to produce Abs with altered affinity for the different FcγRs

Table 1. FcγR affinity enhancements of Fc variants

	Alem AS [LOG(IC_{50}) (M)] fold		Tras AS [LOG(IC_{50}) (M)] fold				Tras SPR [K_D] fold
Variant	V158 IIIa	F158 IIIa	V158 IIIa	F158 IIIa	IIb	IIIa:IIb*	V158 IIIa
WT	[−7.60 ± 0.02] 1	[−6.90 ± 0.06] 1	[−6.42 ± 0.06] 1	[−6.61 ± 0.05] 1	[−7.23 ± 0.07] 1	1	[252 ± 89 nM] 1
S298A/E333A/K334A†	[−8.71 ± 0.13] 13	[−8.01 ± 0.10] 13					
S239D	[−8.72 ± 0.12] 13	[−7.72 ± 0.06] 7	[−7.65 ± 0.06] 17	[−7.55 ± 0.06] 9	[−8.06 ± 0.07] 7	2	
I332E	[−8.61 ± 0.08] 10	[−7.89 ± 0.09] 10	[−7.22 ± 0.05] 6	[−7.23 ± 0.07] 4	[−8.00 ± 0.06] 6	1	[30 ± 7 nM] 8
S239D/I332E	[−9.44 ± 0.08] 70	[−8.70 ± 0.10] 63	[−8.83 ± 0.05] 254	[−8.10 ± 0.06] 31	[−9.07 ± 0.05] 69	4	[2 ± 2 nM] 126
S239D/I332E/A330L	[−9.66 ± 0.07] 115	[−9.12 ± 0.05] 169	[−8.99 ± 0.05] 370	[−8.38 ± 0.08] 58	[−8.84 ± 0.07] 41	9	

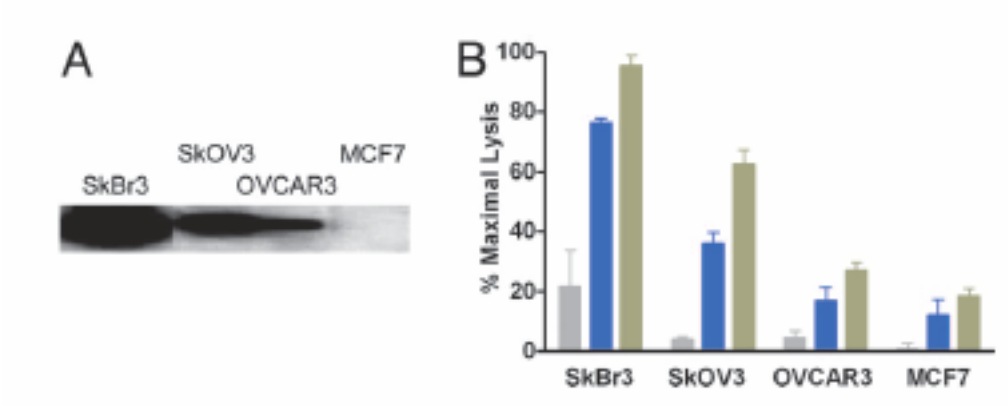
Alemtuzumab (Alem) and trastuzumab (Tras) AlphaScreen (AS) or SPR data provide LOG(IC_{50}) or K_D [bracketed] values followed by folds relative to WT. Fold = $IC_{50_{variant}}/IC_{50_{WT}}$.
*IIIa:IIb = fold V158 FcγRIIIa/fold FcγRIIb for trastuzumab.
†Generated in a previous study (14) and used here for comparison.

In this example there has been an alteration in the relative binding to the activating receptor FcγRIII and the inhibitory receptor FcγRII

PNAS 2006;103;4005-4010;

The increased affinity for FcγRIII translates into more effective ADCC

Cell Based ADCC Against Cell Expressing Different Levels of Her2/*neu*

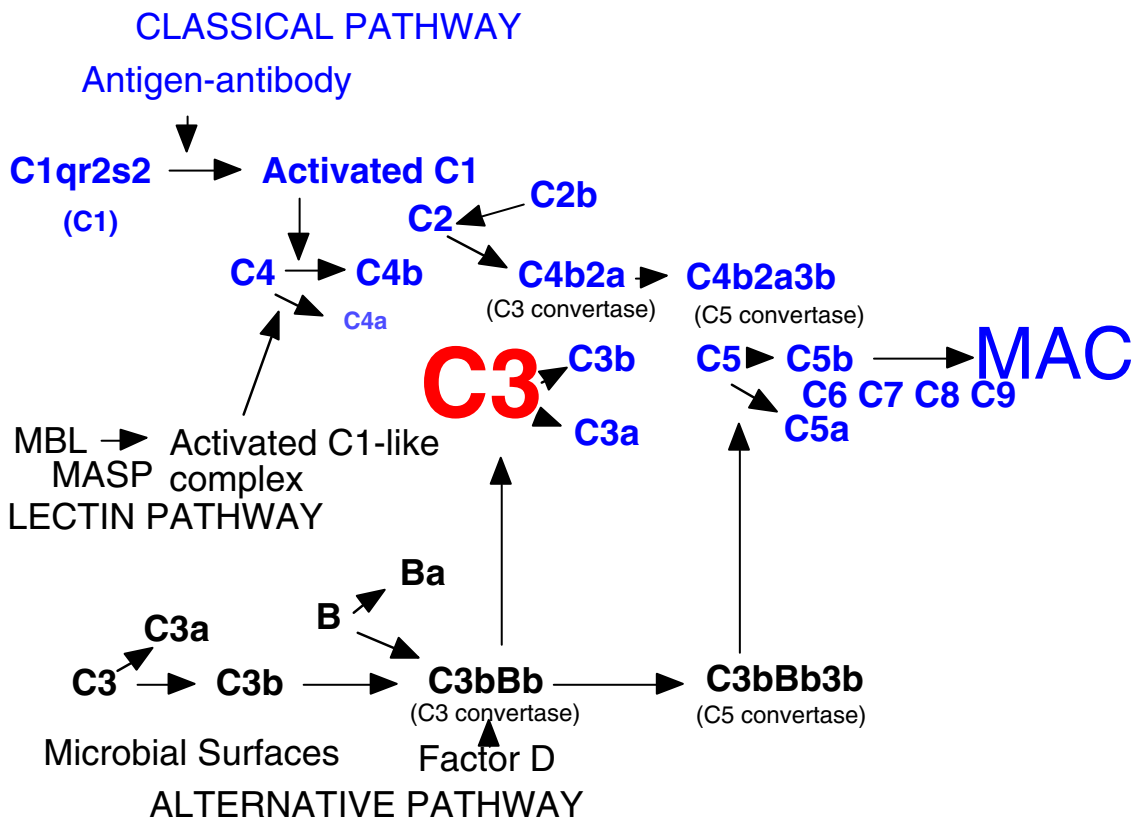


Gray: Wt Trastuzumab
Blue S293D/I332E
Tan S293D/I332E/A330L

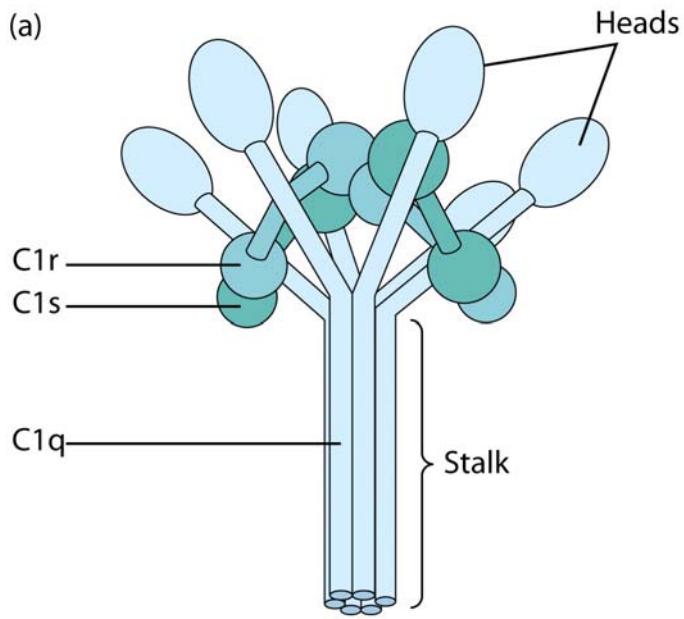
PNAS 2006;103;4005-4010;

Complement activation is also an important component of the antibody-mediated inflammatory response

Antibody mediated complement activation contributes to many effects including cell lysis and opsonization



Complement is activated when C1q binds to two adjacent Fcs of IgG. The C1q binding site is located in the CH2 domain.



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C1q binding site of hIgG1

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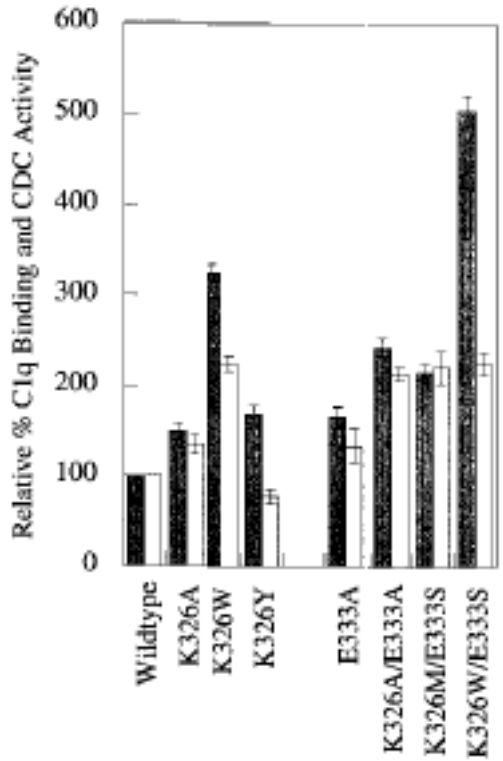
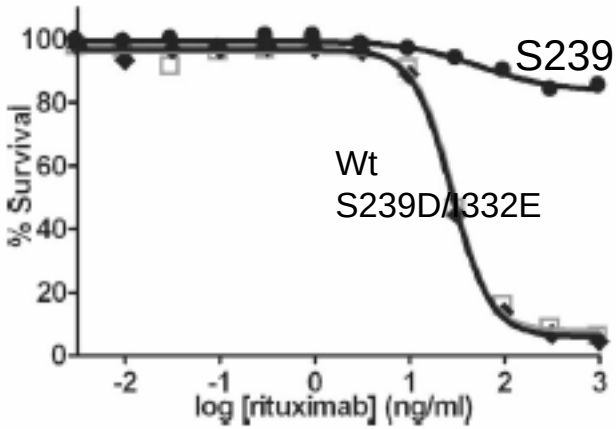


Mutations at K326 and E333 in C_H2 alter C1q binding (filled) and CDC (open)

Idusogie et. al, J. Immunol. 166:2571-2575, 2001

CDC of WIL2-5 lymphoma cells with human complement



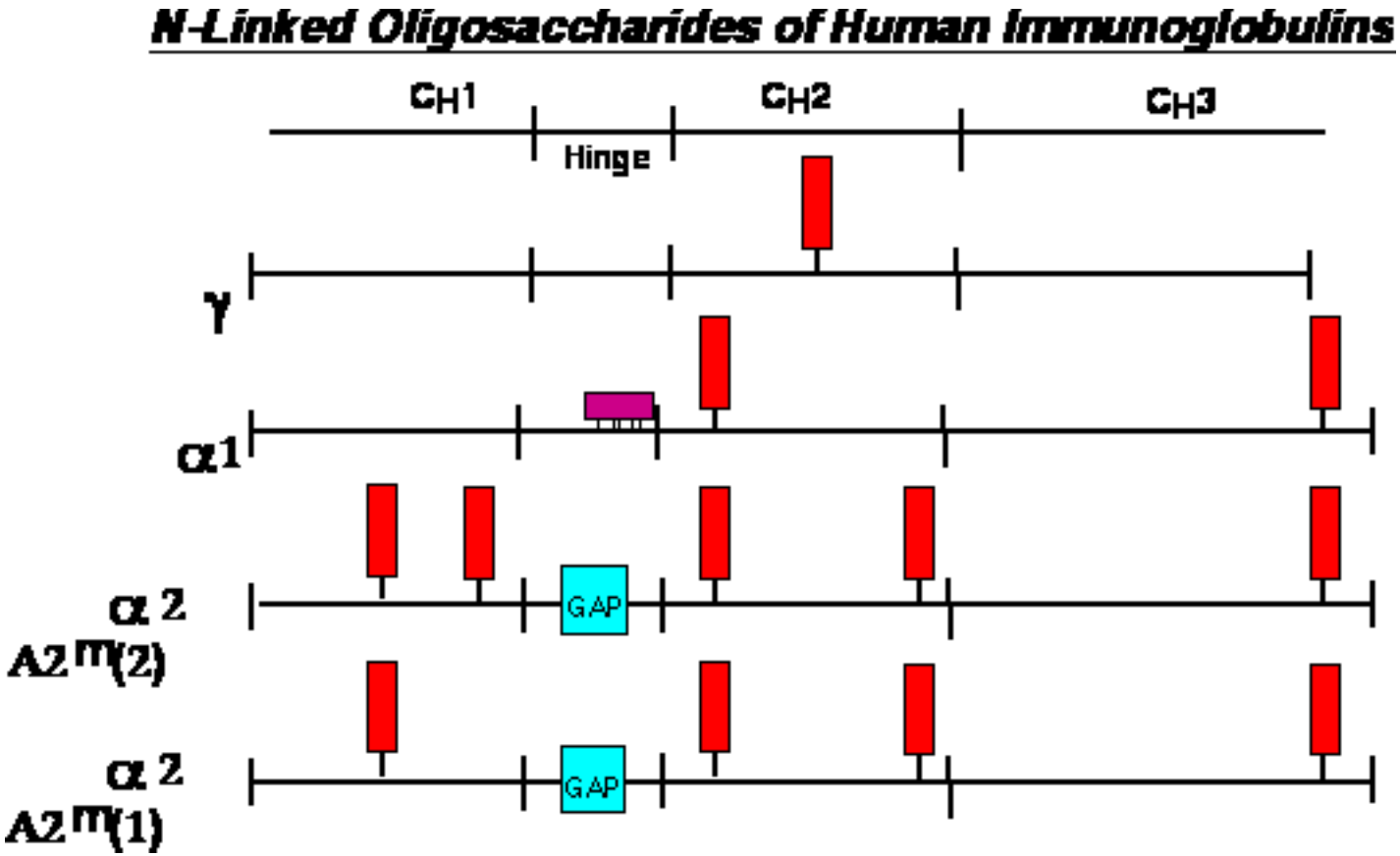
PNAS 2006;103;4005-4010;

Mutations in the C1q binding site can also be made to eliminate complement activation

Summary

Mutagenesis of the constant region of Abs can be used to produce Abs with altered functional properties including half-life, FcγR binding and complement activation

All antibodies are glycoproteins and contain at least one N-linked carbohydrate



Properties of antibodies are determined by both their amino acid sequence and their associated carbohydrate

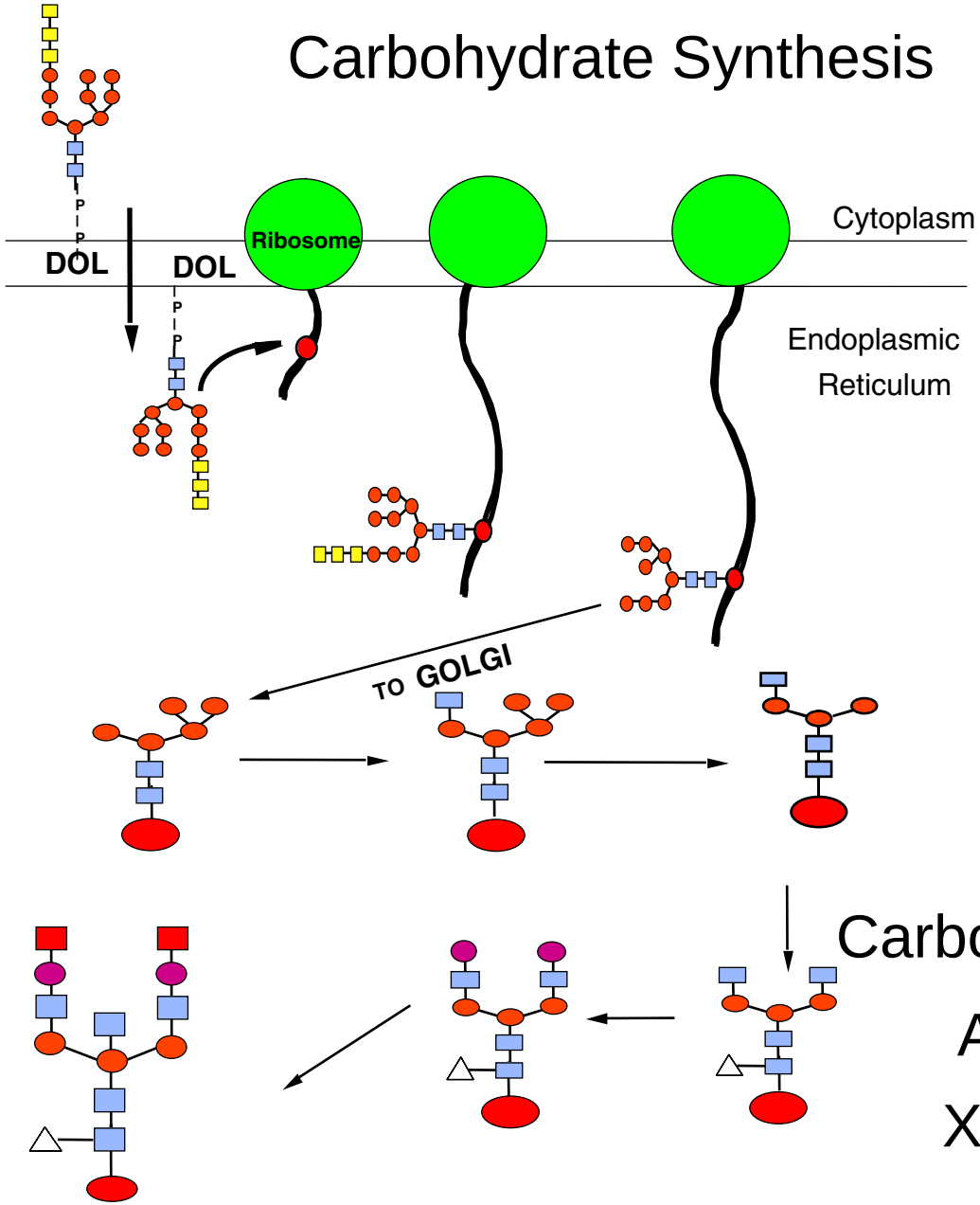
Glycosylation of IgG

2.8 N-linked oligosaccharides per IgG

2 are associated with the Fc region

The remainder are associated with the variable region

Carbohydrate Synthesis



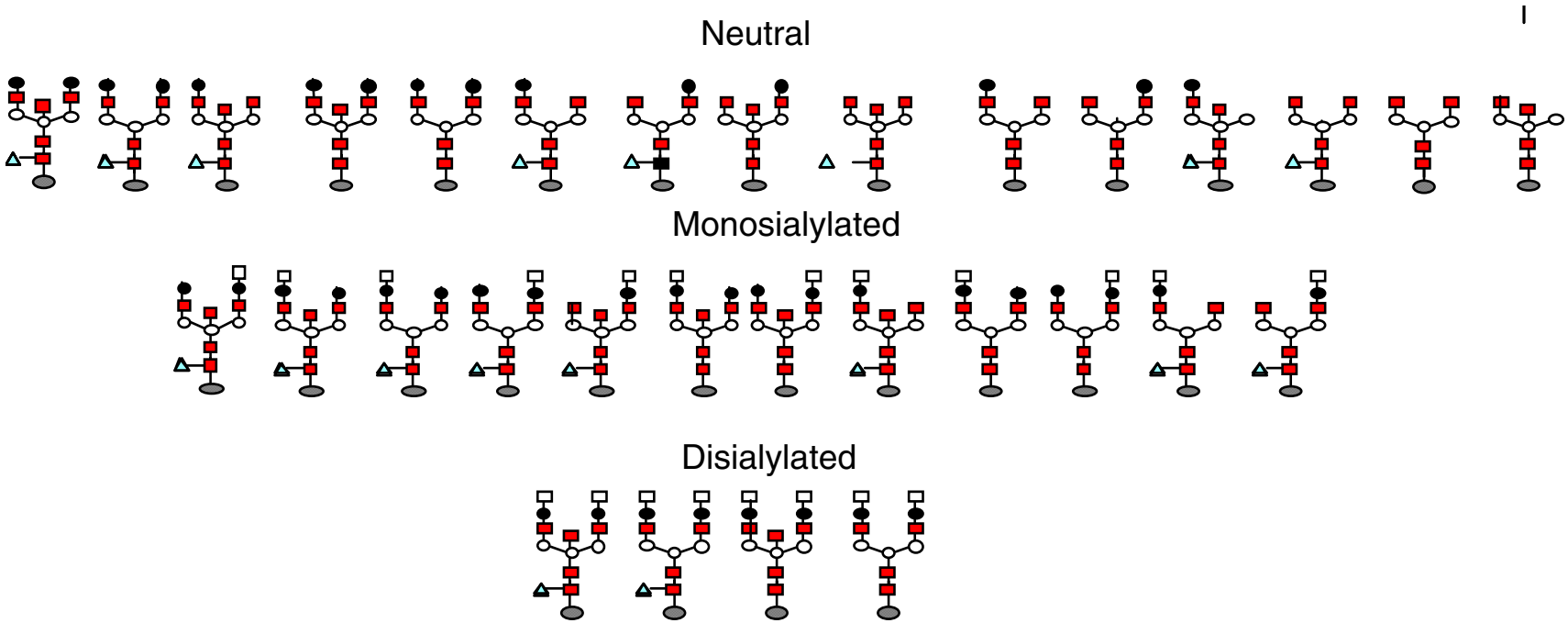
- Glucose
- Mannose
- N-Acetyl Glucosamine
- Asparagine
- Galactose
- Sialic Acid
- Fucose

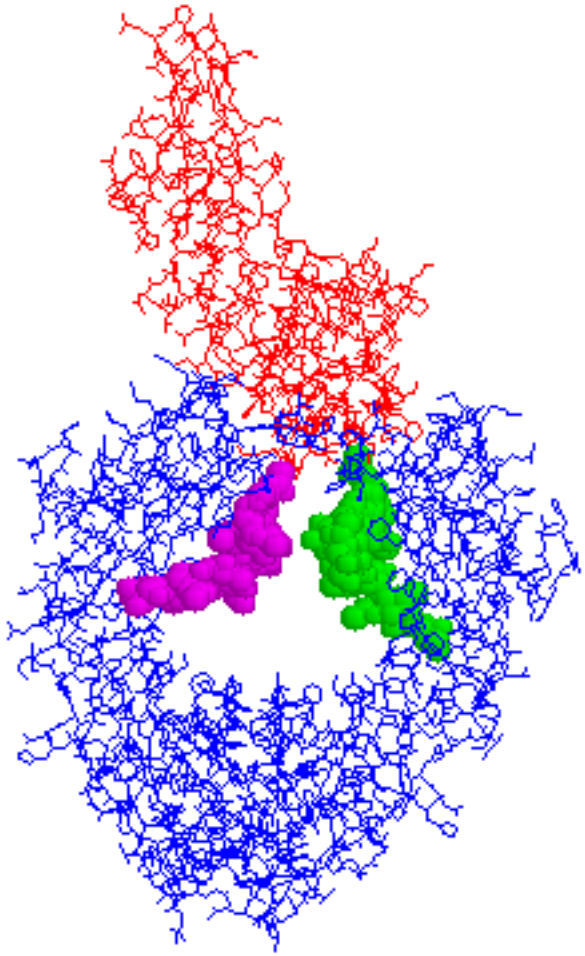
Carbohydrate Addition Site

Asn X Ser/Thr

X cannot be Pro

As a consequence of this synthetic pathways, many different glycoforms are associated with antibodies

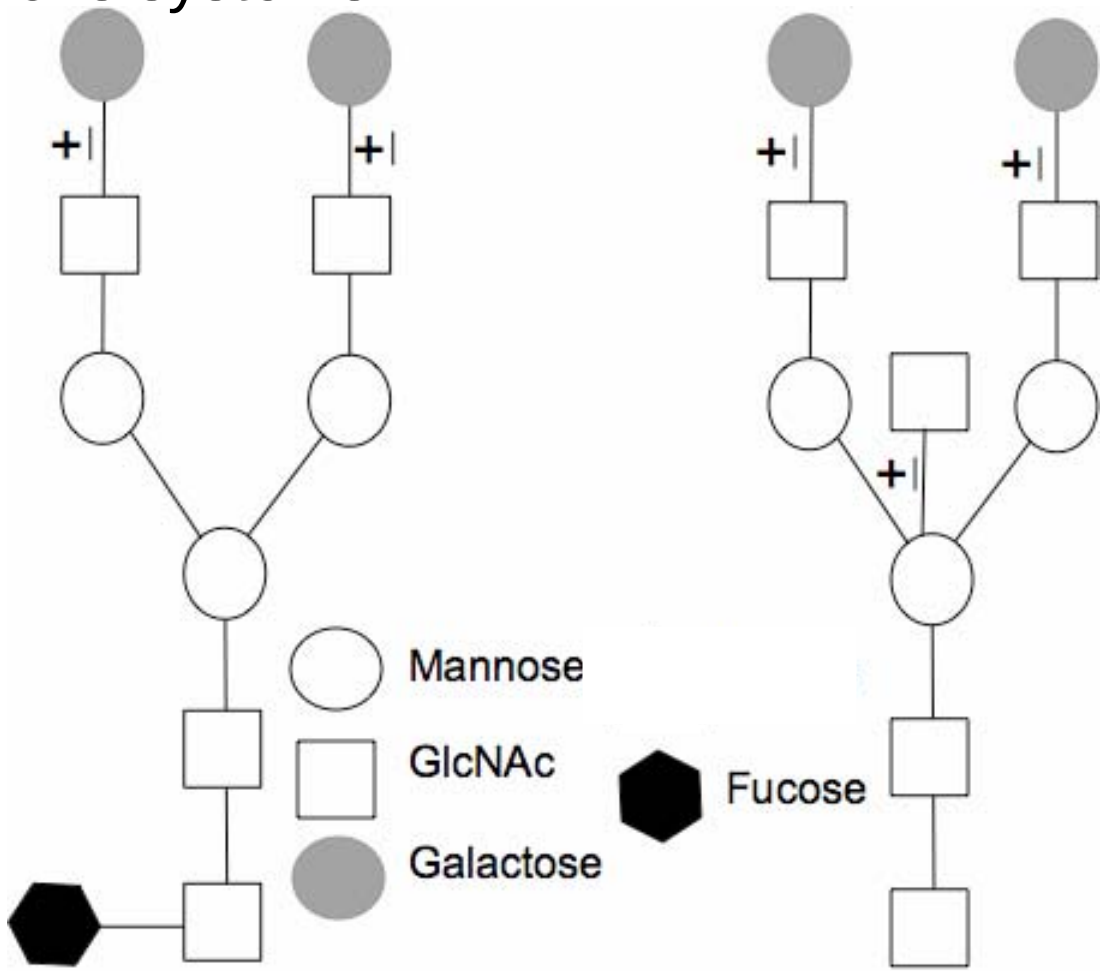




The carbohydrate plays an important role in binding to FcγRs
IgG lacking carbohydrate does not bind.

The structure of the glycan can also influence the properties of the Ab.

Carbohydrates of different structure are added to the same Ab by different expressions systems

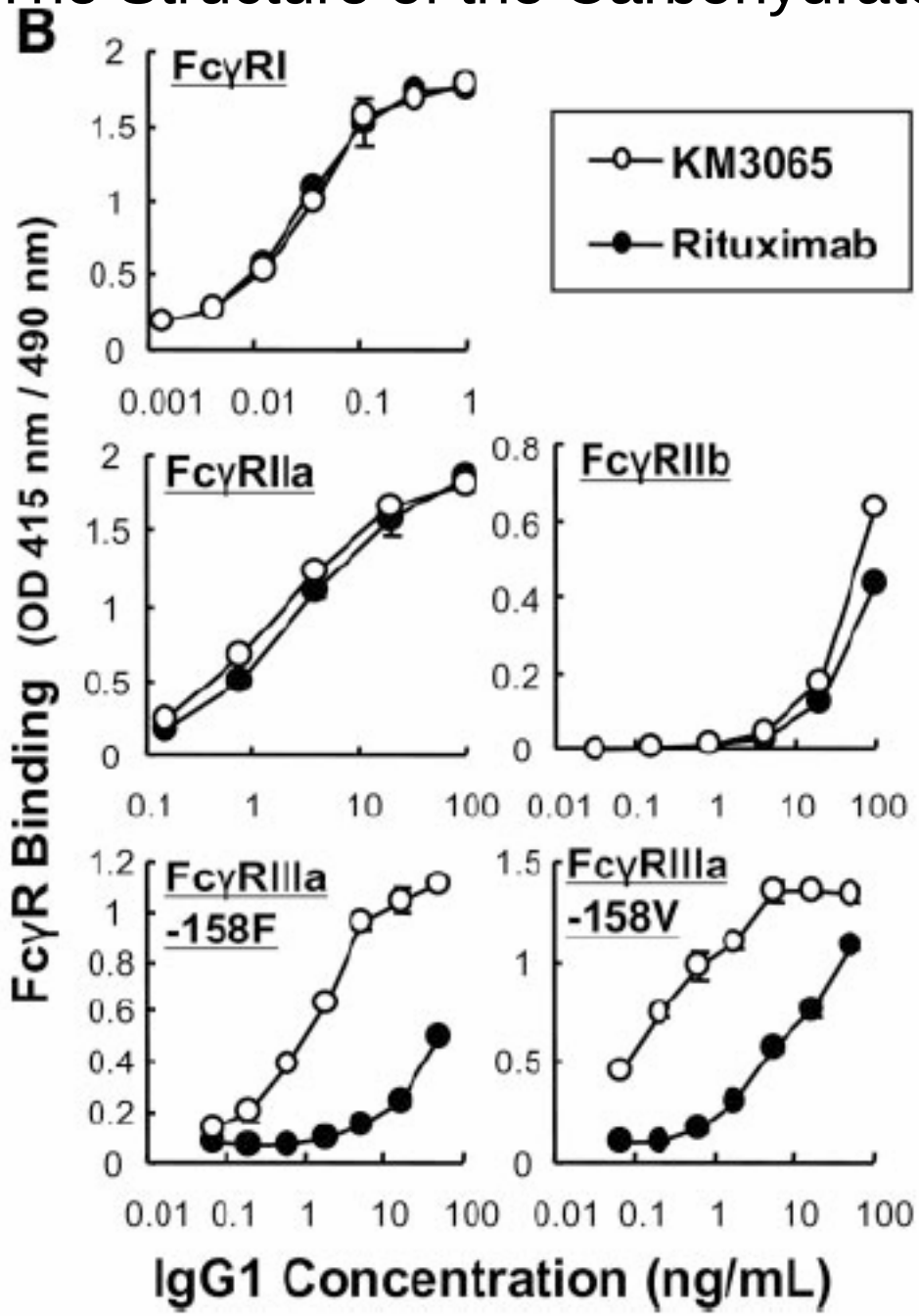


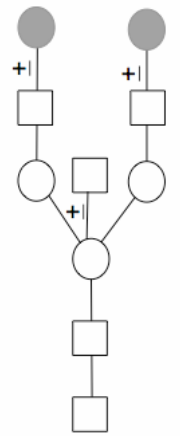
Monosaccharide composition of IgGls

IgGl	Cell line	Fuc	Gal	GlcNAc	Man[a]
KM3065	YB2/0	0.08	0.31	4.41	3
Rituxan™	CHO	0.94	0.54	3.98	3

[a] Molar ratios calculated *versus* 3 mannoses.

The Structure of the Carbohydrate Influences FcγR Binding





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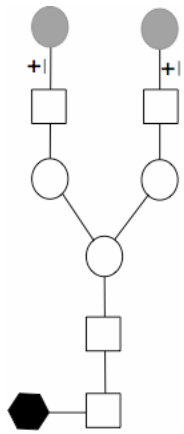
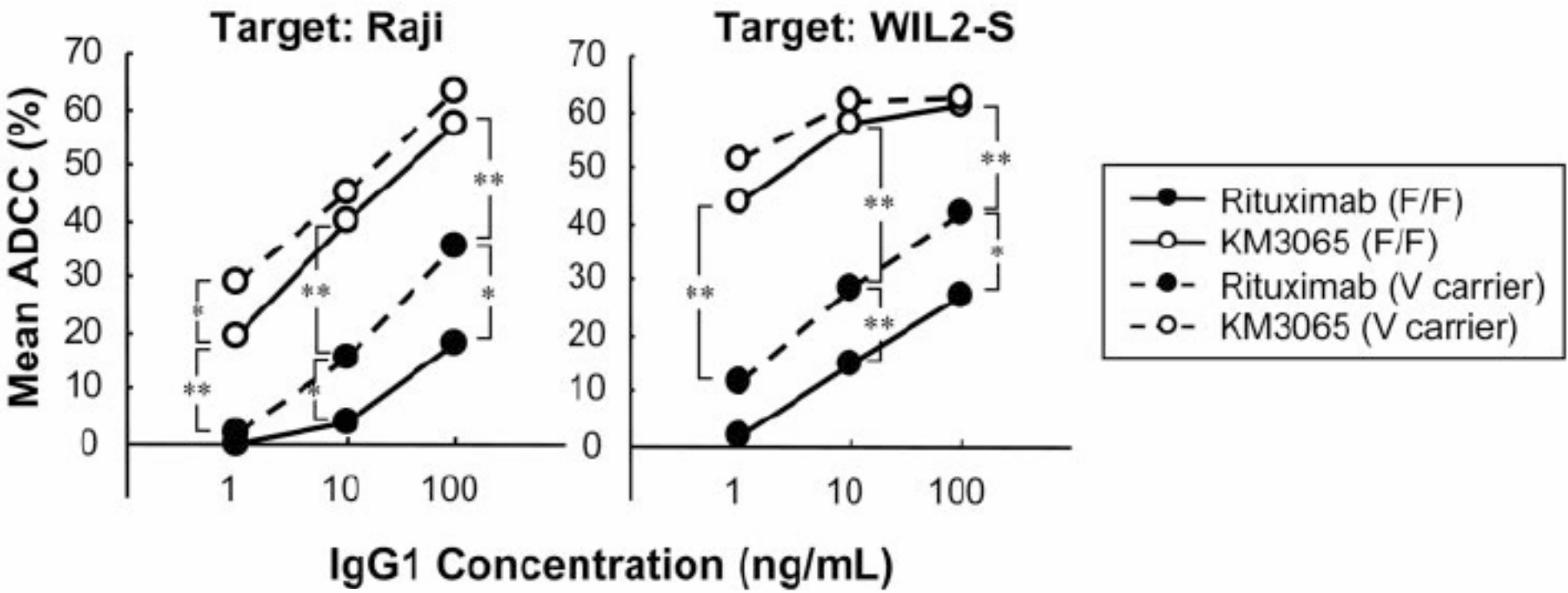
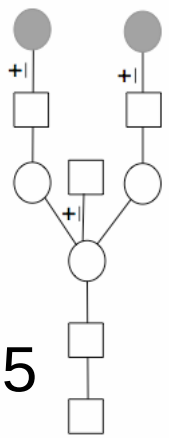


Rituximab
(CHO)

The structure of the carbohydrate influences ADCC





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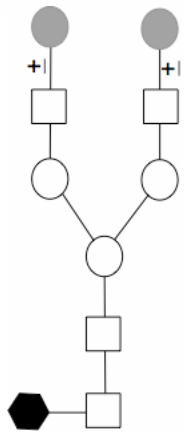
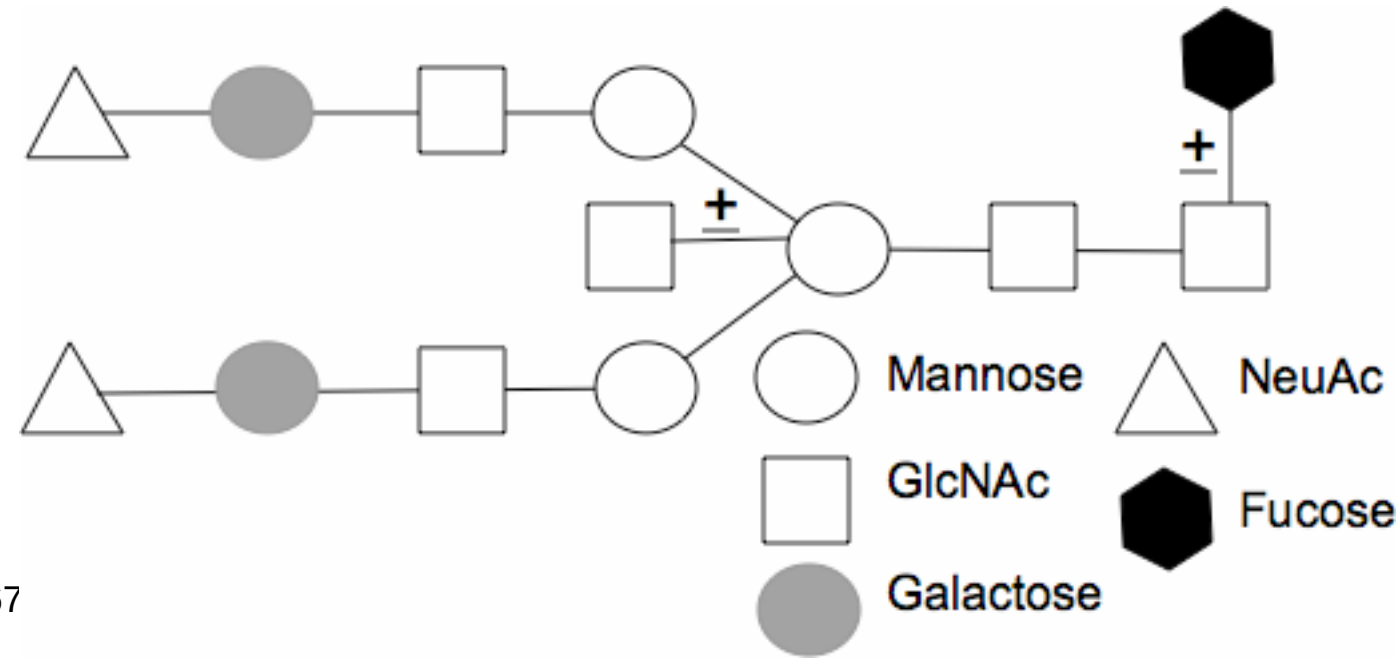


Rituximab (CHO)

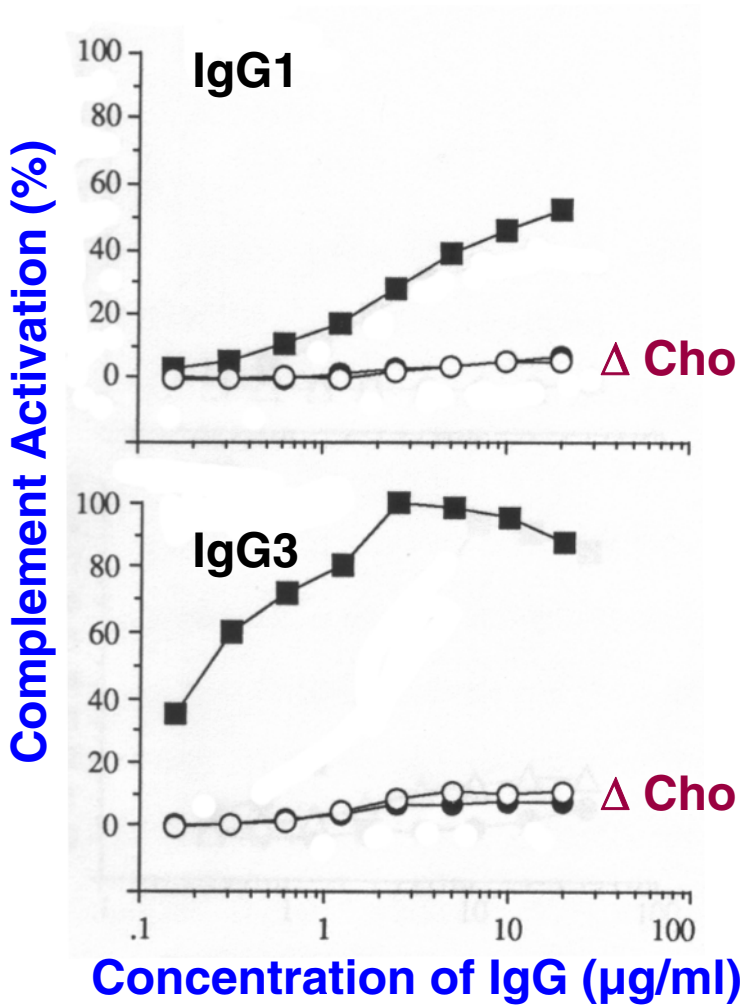
The presence of terminal sialic acid can also influence FcγR binding

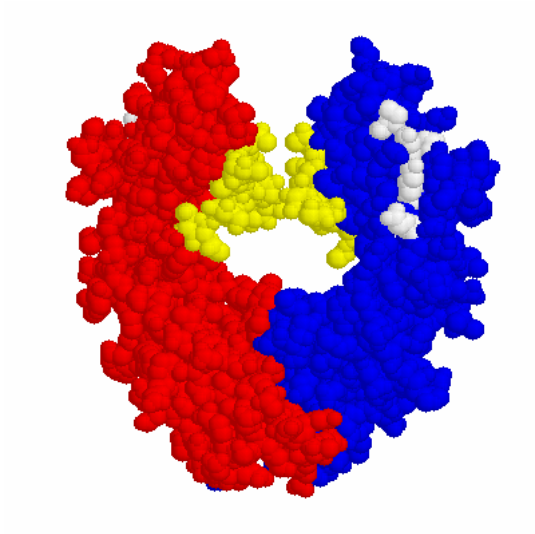
	FcγRIIB (K_A)	FcγRIII (K_A)	FcγRIV (K_A)
6A6-IgG1	4.0×10^6	**5.0×10^5**	n.b
6A6-IgG1 SA	3.9×10^5	**0.7×10^5**	n.b
6A6-IgG2b	3.9×10^6	1.1×10^6	**2.9×10^7**
6A6-IgG2b SA	2.6×10^5	0.5×10^5	**3.3×10^6**



Science 313:67

The presence of carbohydrate in C_H2 is also required for complement activation





QuickTime™ and a
None decompressor
are needed to see this picture.

Production Systems for Recombinant Antibodies

Mammalian Cell Lines: e.g. CHO and murine myelomas

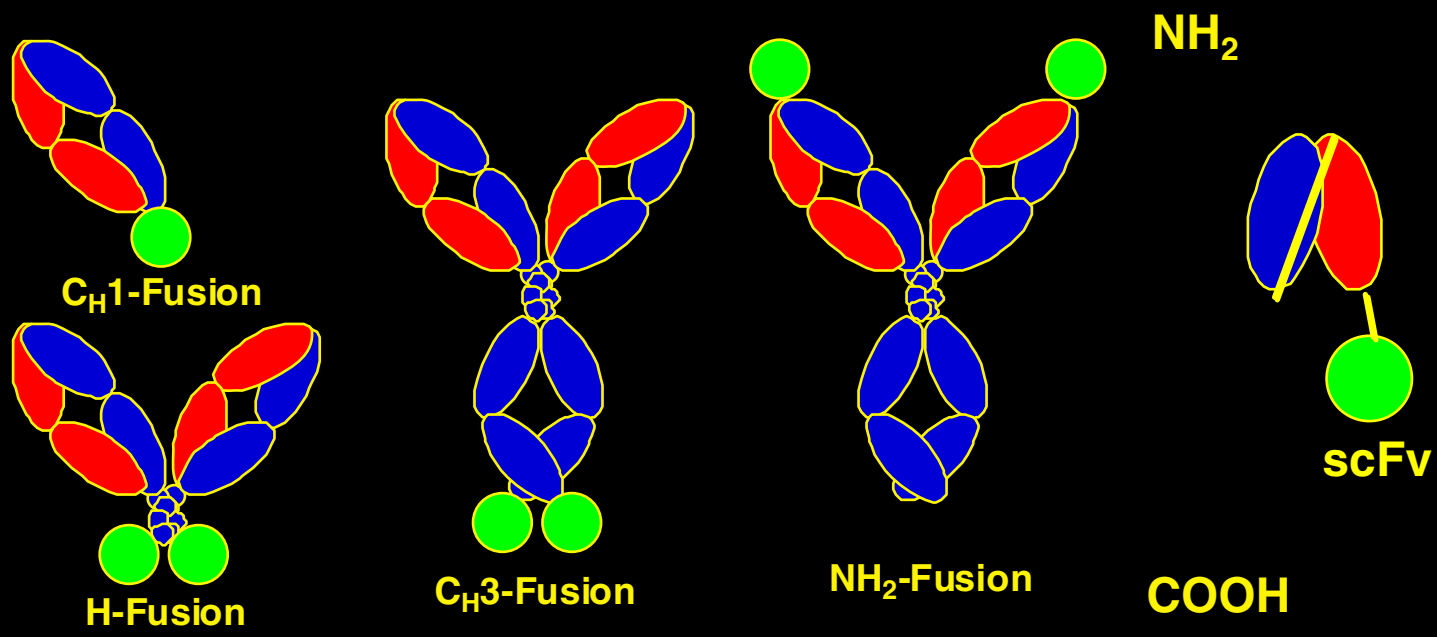
Transgenic Animals
- Cattle
- Chickens (eggs)

Yeast

Bacteria (fragments)

Antibody like proteins can also be produced for many applications

Antibody Fusion Proteins



Drug delivery

Targeting molecules to sites such as tumors

Summary

It is possible to produce recombinant Abs with diverse properties

Half-life
ADCC
Complement Activation

This can be approached by changing either the amino acid sequence or the structure of the attached carbohydrate

Novel molecules such as Ab fusion proteins can also be made

A challenge remains to identify the best Ab for the desired application