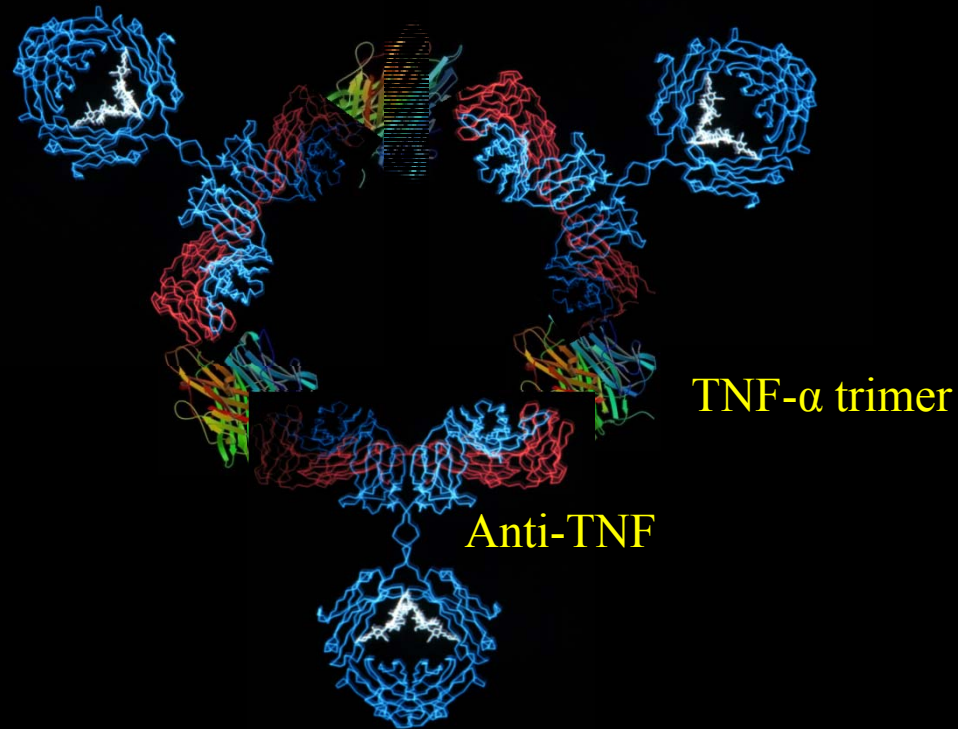


The immunogenicity of biopharmaceuticals: *Aggregates v immune complexes*



Roy Jefferis

School of Immunity & Infection, University of Birmingham UK
European Immunogenicity Platform: Copenhagen February 2012

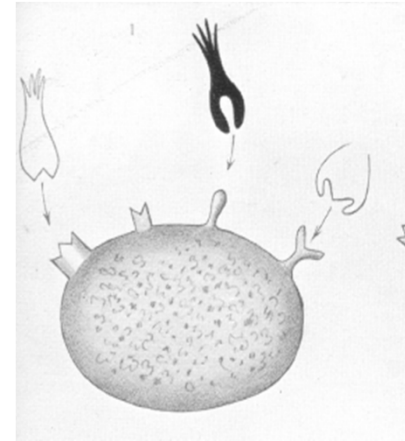
Immunogenicity of biopharmaceuticals:

Product	% Ab incidence
Humira (Western study)	12
(Japanese study)	44
Remicade (CD)	61
Remicade (RA)	21
Campath-1H	63
GM-CSF (1)	74
GM-CSF (2)	95
IL-2	53

Immune responses to recombinant proteins may be equated with auto-immunity



Paul Ehrlich (1854-1915)



Noted for his research in **autoimmunity** calling it "*horror autotoxicus*"

Propounded the **side chain theory** for antibody production

He popularized the **magic bullet** concept

CROONIAN LECTURE 1900

Proceedings of the Royal Society (London) 66, 424-448

Immune responses to recombinant human proteins may be equated with auto-immunity

Mechanisms of auto-immunity:

Altered self: Somatic mutation, PTM changes, inflammation, apoptosis

Loss of tolerance: Treg cell deviation

Molecular mimicry: Exogenous antigen structurally homologous to self molecule(s)

The immunogenicity of biopharmaceuticals: *Aggregates v immune complexes*

Recombinant human proteins

Autoimmunity & tolerance

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Immune complex formation

Potential structural heterogeneties (non-self) within biopharmaceuticals

Post-translational modifications:

glycosylation, γ -carboxylation, β -hydroxyaspartic acid, acetylation, proline isomerisation, N-terminal Met.

Chemical & physical modifications:

atypical conformation, aggregates, fragmentation, oxidation, deamidation, deimination, isoaspartyl residues, glycation

Antibodies to altered self that are diagnostic for autoimmune diseases

Disease	Antigen	Modification
Rheum. Arthritis.	IgG	?????????
	filaggrin	deimination
Coeliac	α -gliadin	deamidation
SLE	α -crystallin	phosphorylation
	SnRNP	isoasp generation
AI enceph	MBP	deimination
	MBP	acetylation

PAD4: peptidylarginine deiminase 4

Qiao S-W et al. J Immunol 187:3064-3071 (2011)

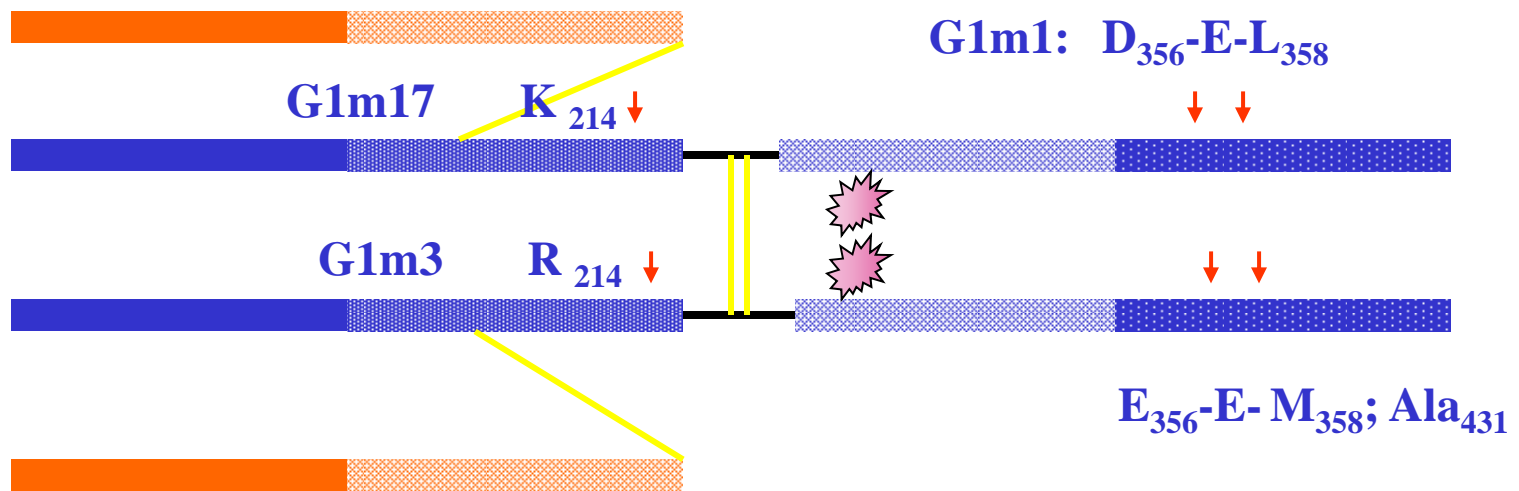
Allotypes of licensed antibody therapeutics

→ Rituxan	G1m(17,1)	Km 3
Zenopax	G1m(17,1)	Km 3
Remicade	G1m(17,1)	Km 3
Campath	G1m(17,1)	Km 3
Humira	G1m(17,1)	Km 3
→ Herceptin	G1m(17)	Km 3
→ Xolair	G1m(17)	Km 3
Simulect	G1m(3)	Km 3
Synagis	G1m(3)	Km 3
Erbitux	G1m(3)	Km 3

Jefferis, R. & Lefranc, M-P. mAbs 1:1-7 (2009)

Carter, P. et al., PNAS 89:4285 (1992)

Sequence correlates for IgG1 heavy chain allotypes



G1m(17) IgG-Fc engineered to remove G1m(1) allotope

Jefferis, R. and Lefranc, M-P. mAbs 1, 332-38 (2009)

Carter, P. et al. Proc Natl Acad Sci. 89:4285-9 (1992)

IgG1 heavy chain-coding gene polymorphism (G1m allotypes) and development of antibodies-to-infliximab

Magdelaine-Beuzelin C. et al. Pharmacogenet Genomics. 19:383-7 (2009).

Surprising negative association between IgG1 allotype disparity and anti-adalimumab formation in RA

Bartelds, GM. et al. Arthritis Research & Therapy 12:R221 (2010)

The G1m(1) allotype and CD4⁺ T-cell responsiveness

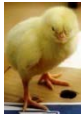
Stickler MM et al. Genes Immun. 12:213-21 (2011)

Glycoprotein production vehicles:

Mammalian: CHO, Sp2/0; NSO; Per.C6; HEK 293 etc



Transgenics: goat; sheep; cows; rabbits; pigs etc



Aves: chickens (eggs)



Yeasts: *Pichia pastoris*; *Saccharomyces cerevisiae*



Insect cells: Sf9 (baculovirus infected)



Plants: tobacco; corn; tomato; potato; moss



Bacteria: *Escherichia coli*; *Bacillus subtilis*

The immunogenicity of biopharmaceuticals: *Aggregates v immune complexes*

Recombinant human proteins

Autoimmunity & tolerance

Aggregates

Antibody therapeutics – anti-self

Immune complex formation

Tolerance:

an active mechanism of self/non-self discrimination



Gonzalez S et al. Self/Nonself 2:19-25 (2011)

Immune responses to recombinant human proteins may be equated with auto-immunity

Induction/loss of tolerance

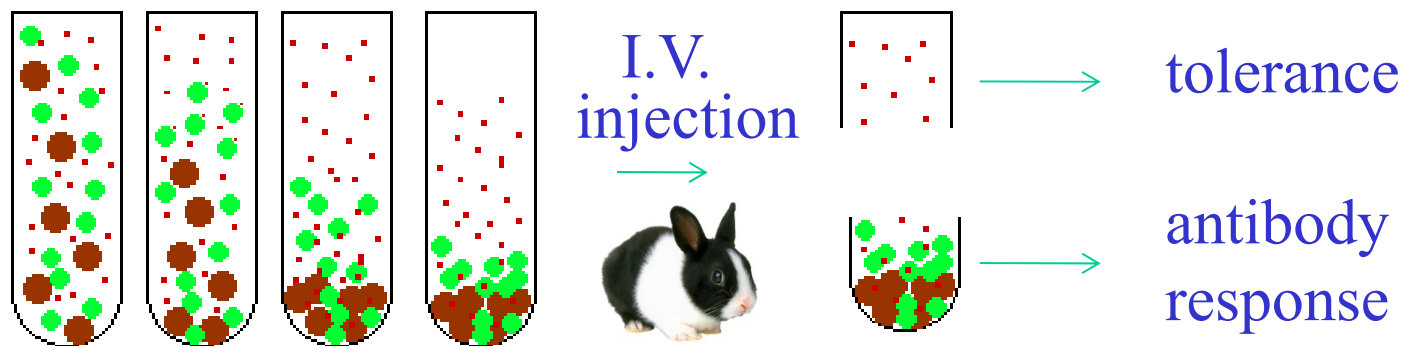
Altered self: Somatic mutation, inflammation, apoptosis

Molecular mimicry: Exogenous antigen structurally homologous to self molecule(s)

Induction of tolerance

Low zone: Repeated low doses of aggregate free protein

High zone: Single injection of aggregate free high dose



Mitchison, N. A.. Proc R Soc Lond B Biol Sci 161: 275–92 (1964)

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Aggregates in MAbs and therapeutic proteins: a regulatory perspective

An important part of protein aggregation studies is evaluating the biological activity of the aggregate.

Differences between monomeric and aggregated protein can profoundly influence the potency of a protein-based drug.

There is no consensus on the maximum allowable limit for protein - based pharmaceutical aggregates.

Cordoba-Rodriguez, RV. Aggregates in MAbs and therapeutic proteins: A regulatory perspective. BioPharm Internat. Nov. 2008

Aggregates of IgG antibodies may:

Differ in biologic effector functions activated

Promote uptake by antigen presenting dendritic cells

Cross link antigen receptors on B cells to:

Induce T cell independent responses

Process antigen and present to T cells

Overlooking sub-visible particles in therapeutic protein products may compromise product quality

The impact of protein aggregates on immunogenicity needs to be elucidated and should include studies of the role of protein class, amount of aggregate, size of aggregates, and protein **conformation in aggregates**.

Pharmaceutical and academic researchers and instrument manufacturers should work to define the quantitative capabilities of current particle counting instruments for particles as small as 0.1 μm and develop new instruments as needed.

Carpenter JF, et al. J Pharm Sci. 98:1201-5 (2009)

The immunogenicity of biopharmaceuticals:

Aggregates v immune complexes

Administration of monomeric antibody results in the formation of immune complexes

What are the differences between aggregates and immune complexes?

The immunogenicity of biopharmaceuticals: *Aggregates v immune complexes*

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Immune complex formation

Induction of high zone tolerance to alemtuzumab

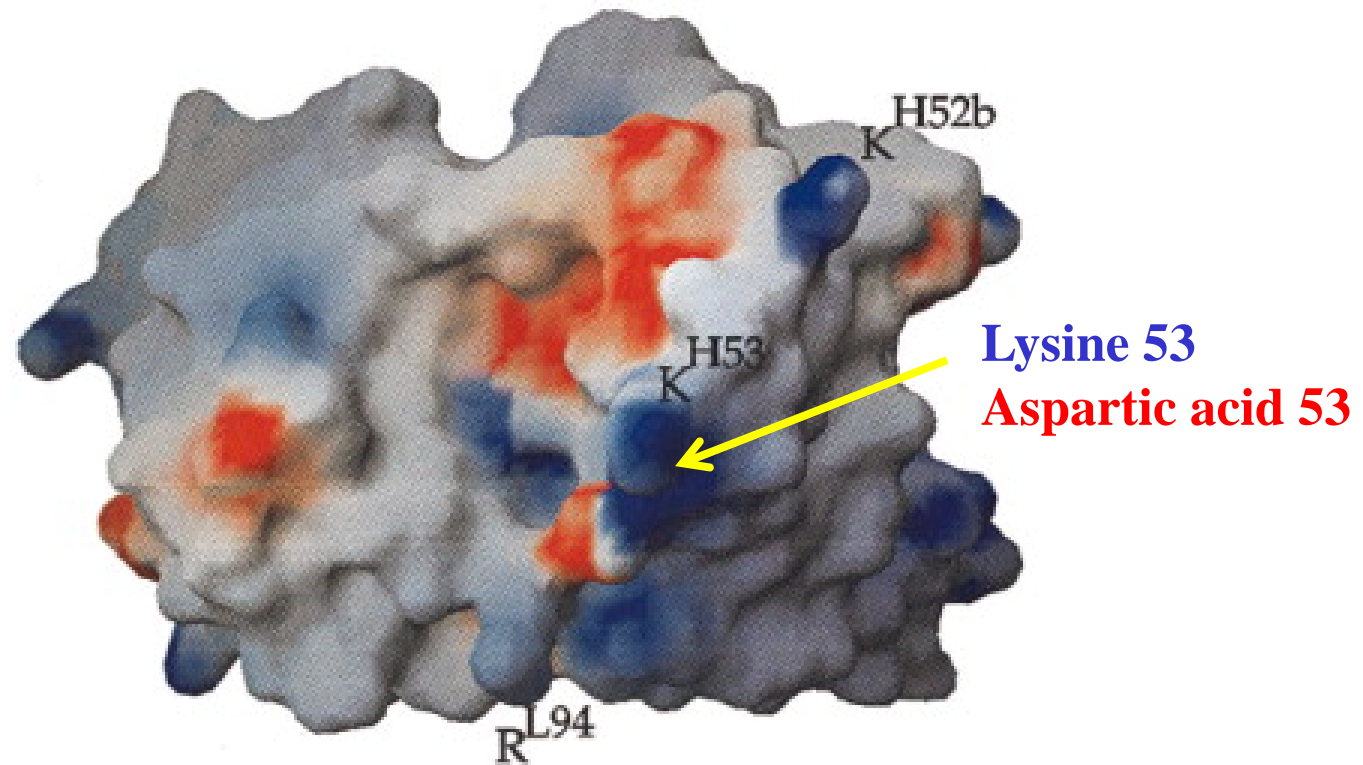
CAMPATH-1H (alemtuzumab) anti-CD52:

A humanised form of the original rat CAMPATH-1G

74 % of patient receiving alemtuzumab developed
anti-drug antibody (ADA)

Somerfield J, et al. Immunol. 185:763-8 (2010)

Crystal structure of Campath-1G Fab and its humanized form Campath-1H

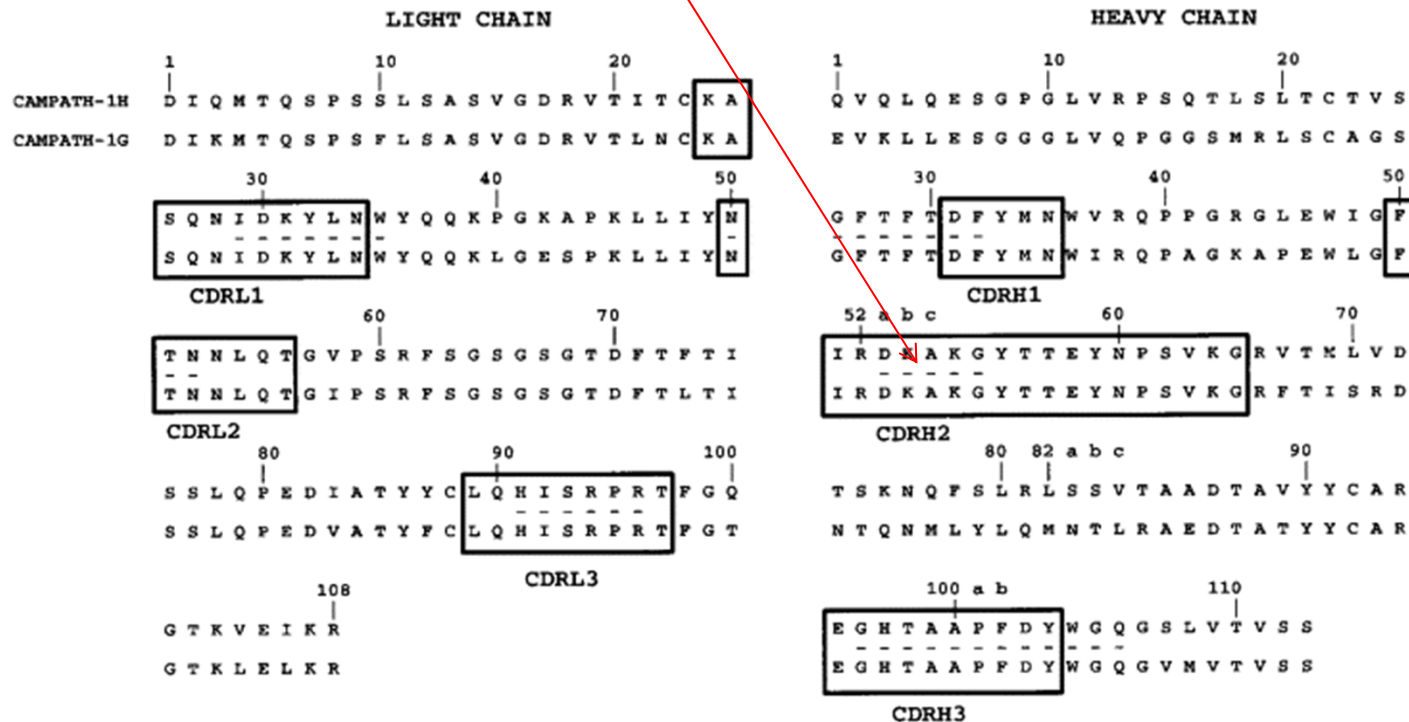


A non-CD52 binding variant, **SM3**, was generated

Cheetam GM et al. J M B 284:85-99 (1999)

Sequence of rat Campath-1G and the humanised form Campath-1H

lysine₅₃ > aspartic acid₅₃ in the heavy chain

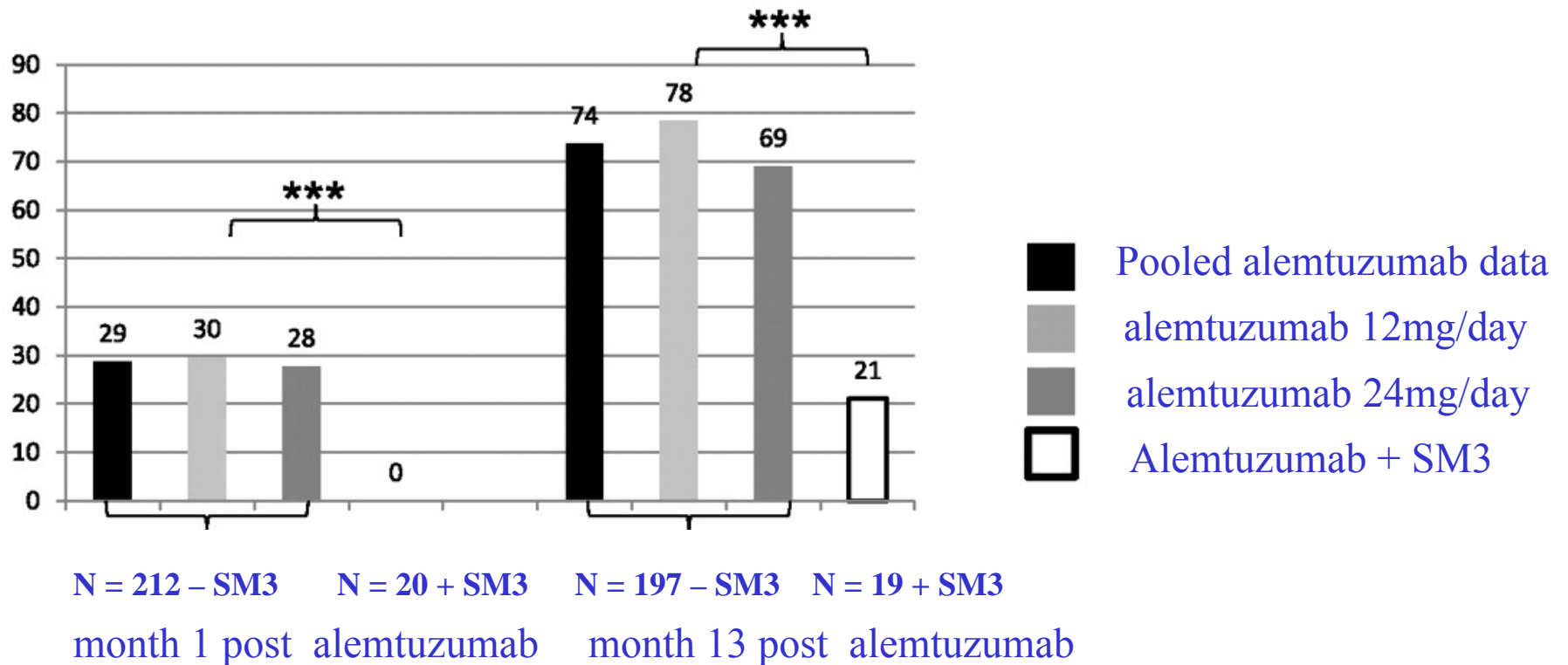


61 CDR residues of SM3 are the same as the original rat Campath-1G

Cheetham GM et al. J M B 284:85-99 (1999)

A strategy to reduce the immunogenicity of biological therapies

Induce tolerance to SM3: lysine/aspartic acid heavy chain variant



Somerfield J, et al. Immunol. 185:763-8 (2010)

Induction of high zone tolerance to alemtuzumab

CAMPATH-1H (alemtuzumab) anti-CD52:

74 % of patient receiving alemtuzumab developed anti-drug antibody (ADA)

21% of patients developed ADA following exposure to SM3

Somerfield J, et al. Immunol. 185:763-8 (2010)

Immune tolerance induction to enzyme replacement therapy in infantile Pompe disease

Infantile Pompe disease resulting from a deficiency of lysosomal acid α -glucosidase (GAA) requires enzyme replacement therapy with rhGAA. Patients develop high-titer antibody to the rhGAA and do poorly.

The combination of rituximab with methotrexate \pm intravenous gammaglobulins induced tolerance induction to rhGAA when instituted in the naïve setting or following antibody development. It should be considered in other conditions in which antibody response to the therapeutic protein elicits a robust antibody response that interferes with product efficacy

Messenger YH et al. Genet Med. 14:135-142 (2012)

The immunogenicity of biopharmaceuticals: *Aggregates v immune complexes*

Recombinant human proteins

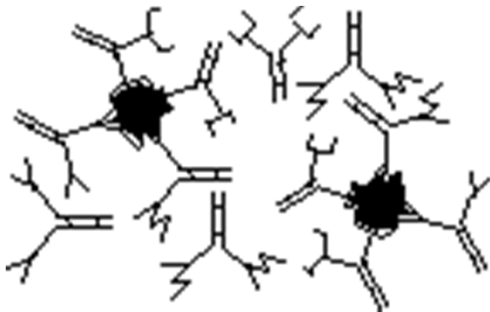
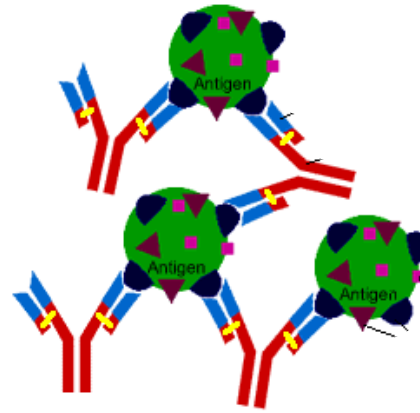
Autoimmunity & tolerance

Aggregates

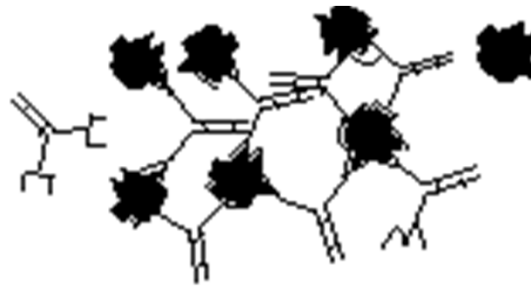
Antibody therapeutics – anti-self

Immune complex formation

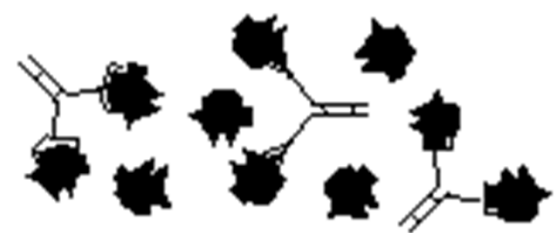
Immune complex formation (aggregation!)



Ab Xs

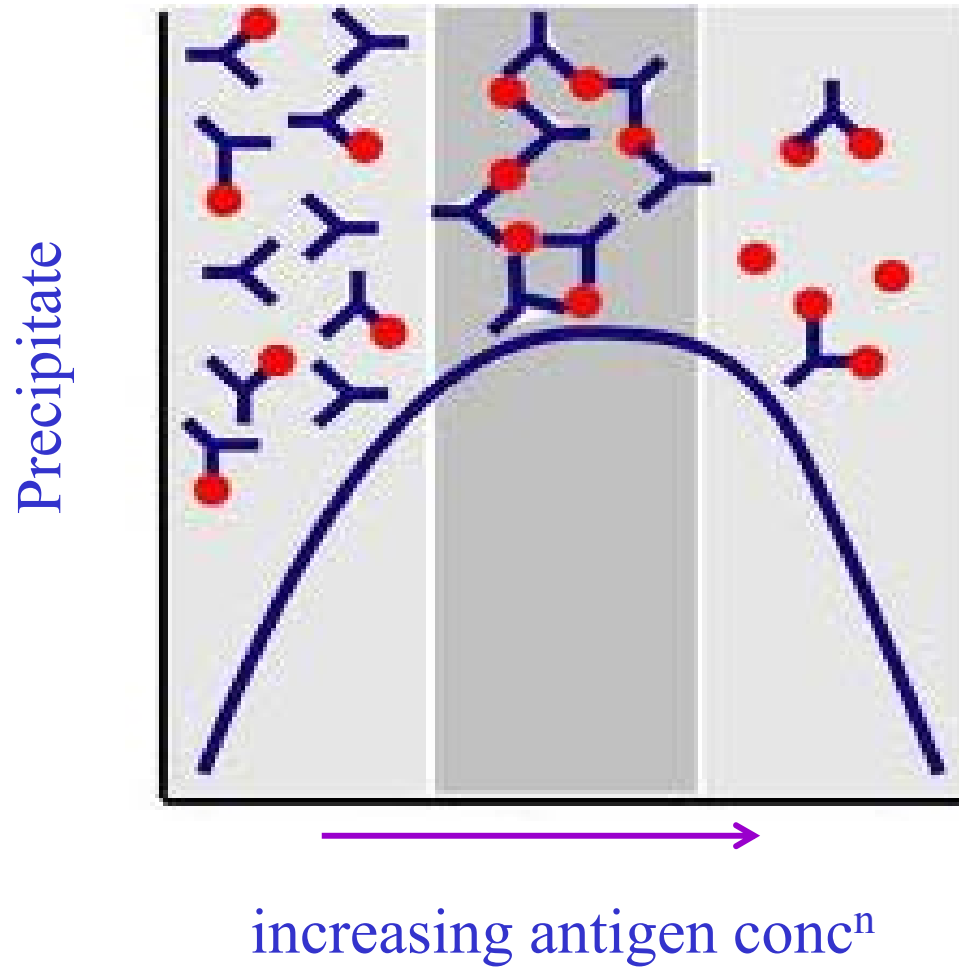


equivalence

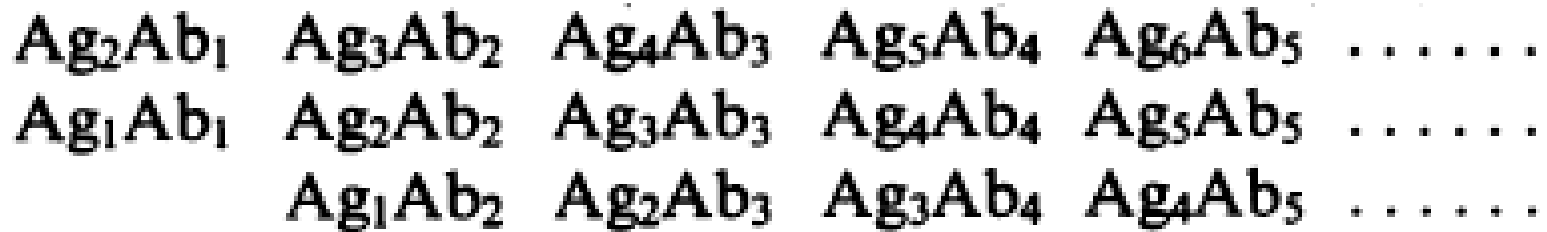


Ag Xs

Titration of antibody with soluble protein antigen



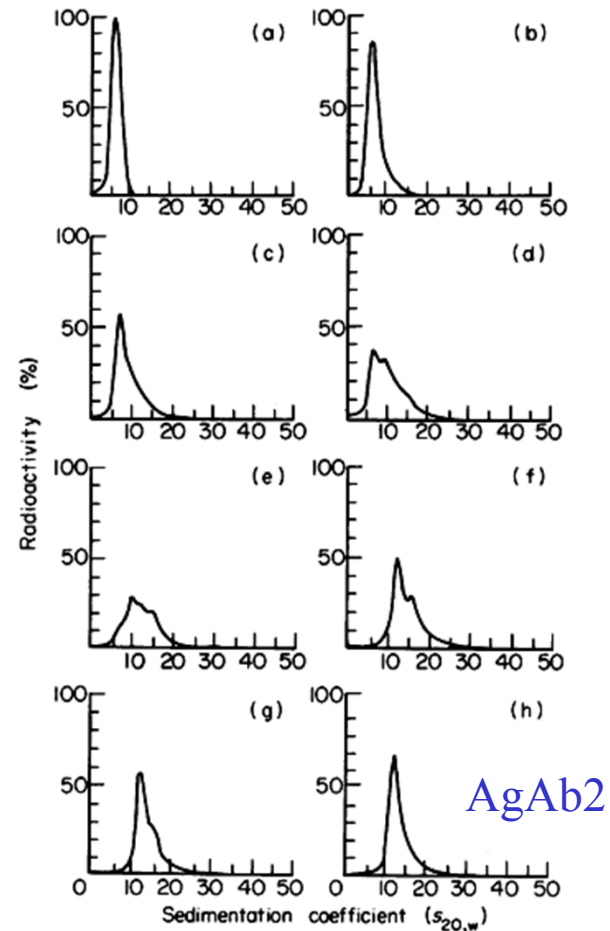
Possible immune complexes formed between divalent mouse IgG antibody and divalent antigen (human IgG)



No. of IgGs in the complexes	1-2	1-3	1-4	1-5	1-6	1-7	1-8
	Equivalent <i>s</i> values (Svedberg units)						
1	8	7	7	6	6	6	5
2	13	12	11	10	10	9	9
3	17	16	14	13	13	12	11
4	20	19	17	16	15	14	14

Steensgaard, J. Jefferis, R. et al. *Immunology*. 46:751-60 (1982)

Immune complexes formed with IgG and anti- κ (6e1)



[Ag] 150 μg ;

[Ab]

a) 0 μg ; b) 25 μg ;

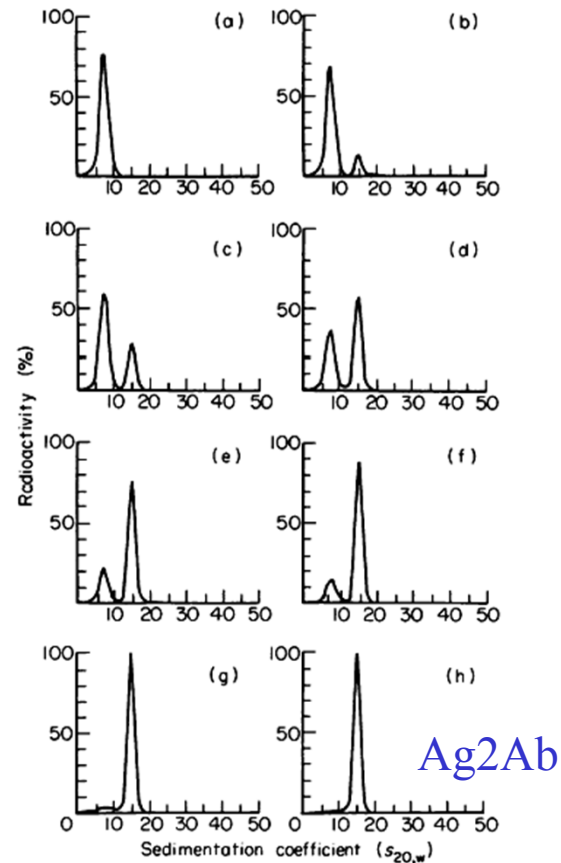
c) 50 μg ; d) 75 μg ;

e) 100 μg ; f) 200 μg ;

g) 400 μg ; h) 800 μg

Steensgaard, J. Jefferis, R. et al. Immunology. 46:751-60 (1982)

Immune complexes formed with IgG and anti-Fc (X3a8)



[Ag] 150ug;

[Ab]

a) 0 ug; b) 160 ug;

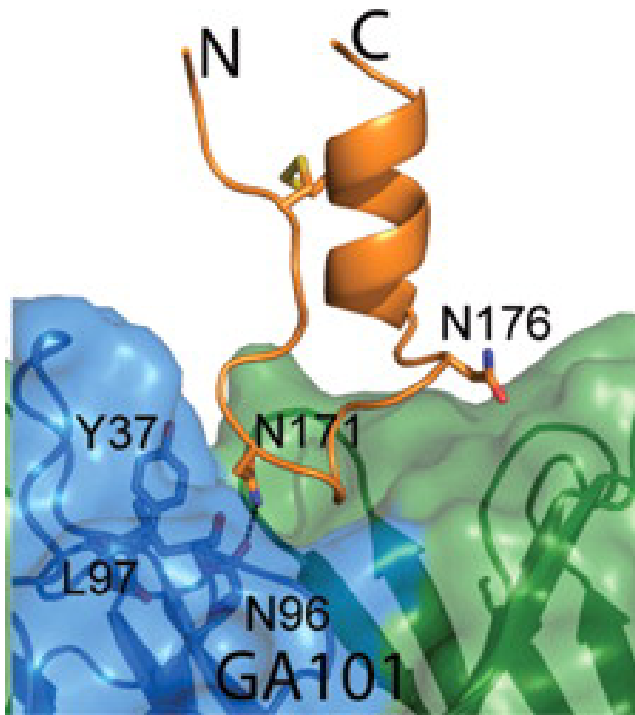
c) 320 ug; d) 640 ug;

e) 800 ug; f) 960 ug;

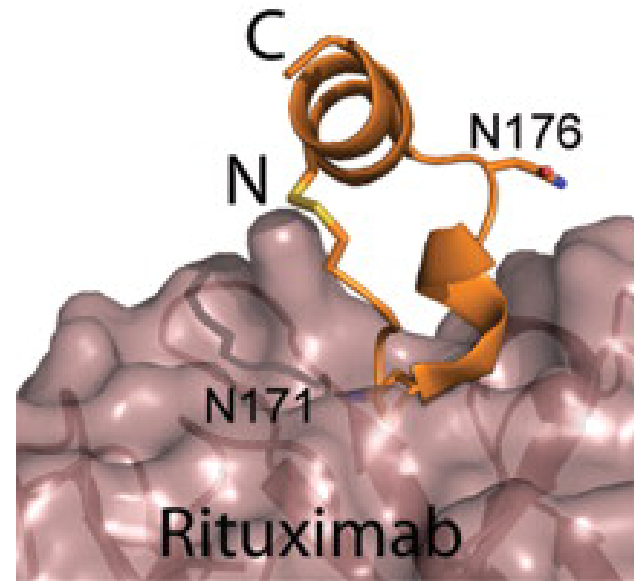
g) 1120 ug; h) 1280 ug

Steensgaard, J. Jefferis, R. et al. Immunology. 46:751-60 (1982)

Paratope/epitope orientations for rituximab (Type I) & obinutuzumab (Type II) anti-CD20 antibodies



Obinutuzumab
(GA101)

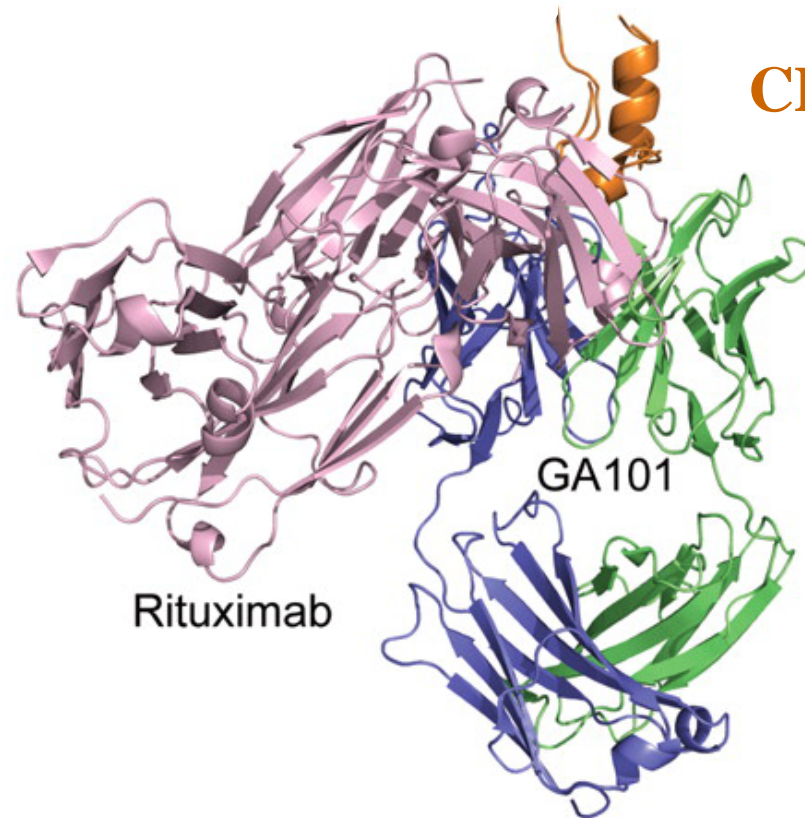


Rituximab

Niederfellner G et al. Blood 118:358-367 (2011)

Anti-CD20 Type I (Rituximab) & Type II (GA101) mAbs

Complement
activating



CD20 peptide

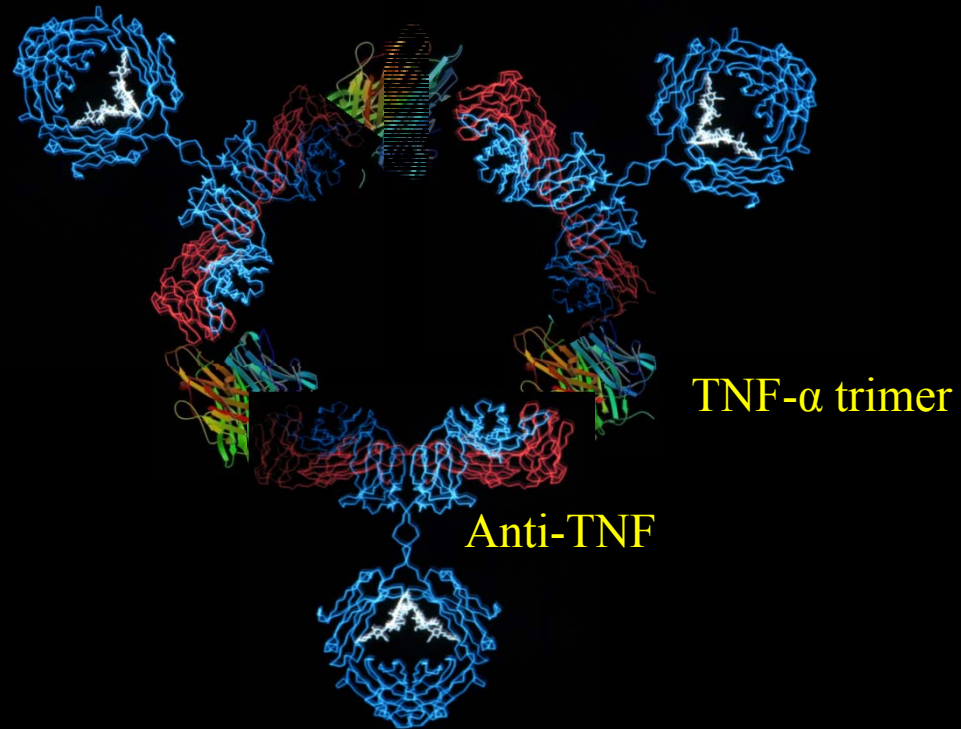
Direct cell
killing

Niederfellner G et al. Blood 118:358-367 (2011)

Take home message

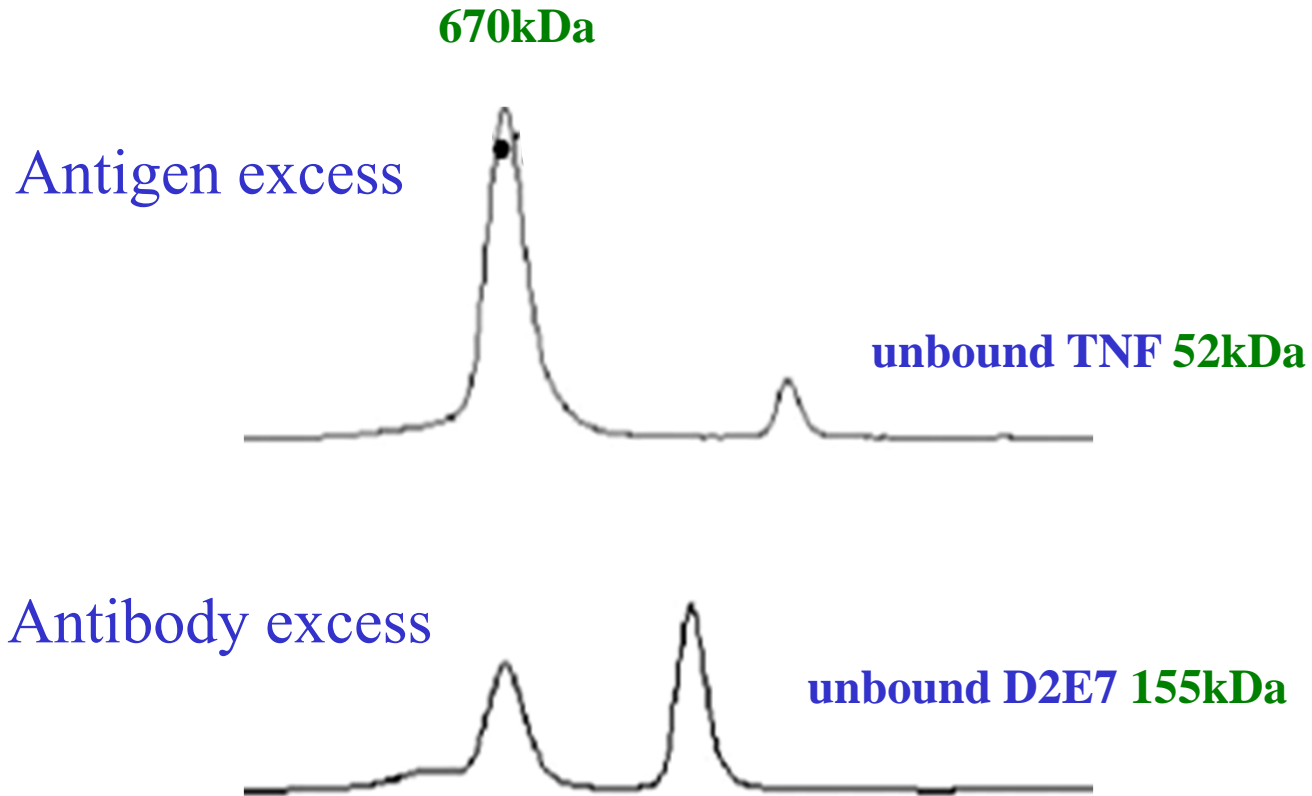
The epitope/paratope specificity of an antibody can influence the physical structure of immune complex and consequently the biological effector mechanisms of IgG-Fc activated

Immune complexes formed between TNF α trimer and anti-TNF MAb D2E7



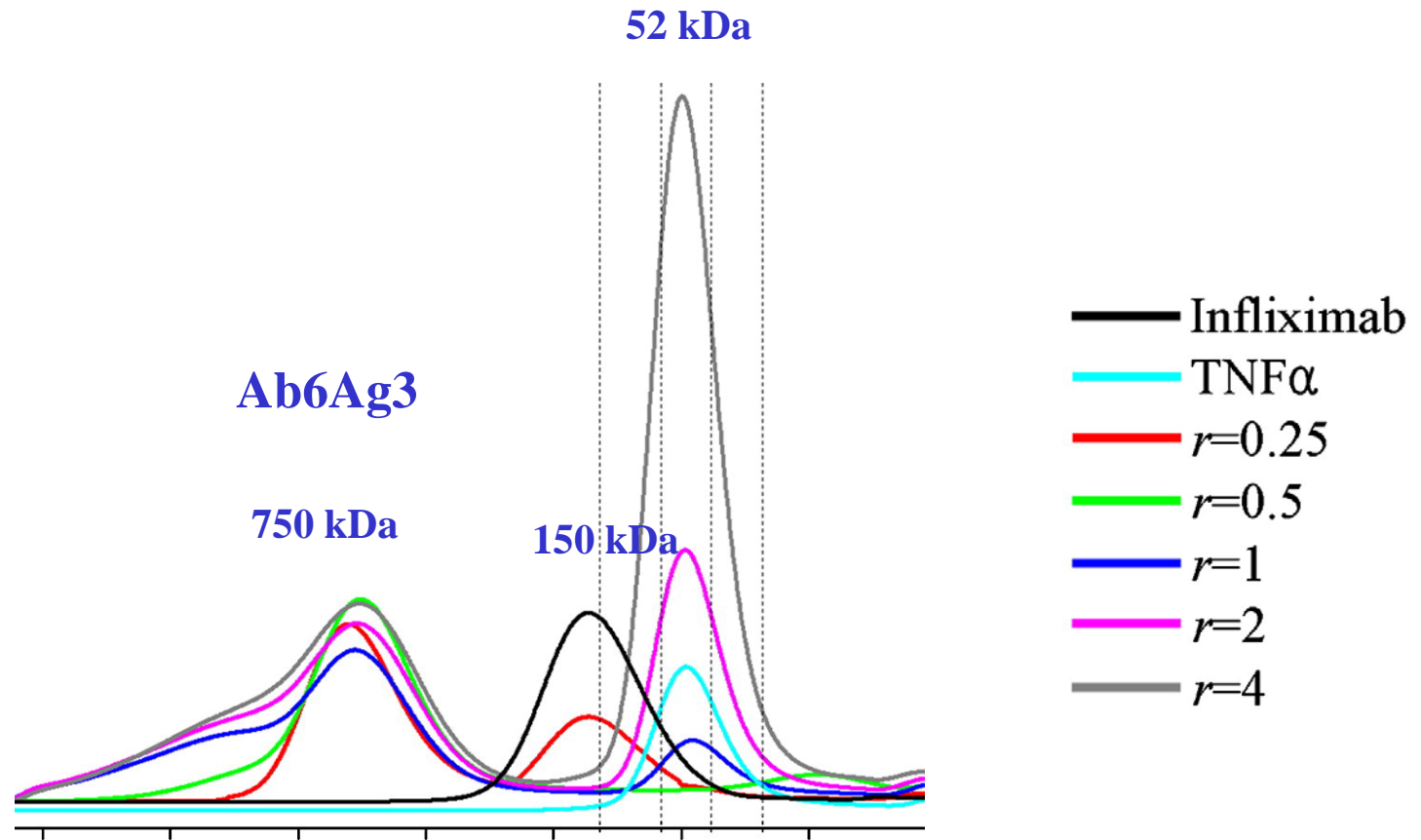
Santora LC et al., *Anal Biochem.* 299:119-29 (2001).

Immune complexes formed between TNF α trimer and anti-TNF MAb D2E7



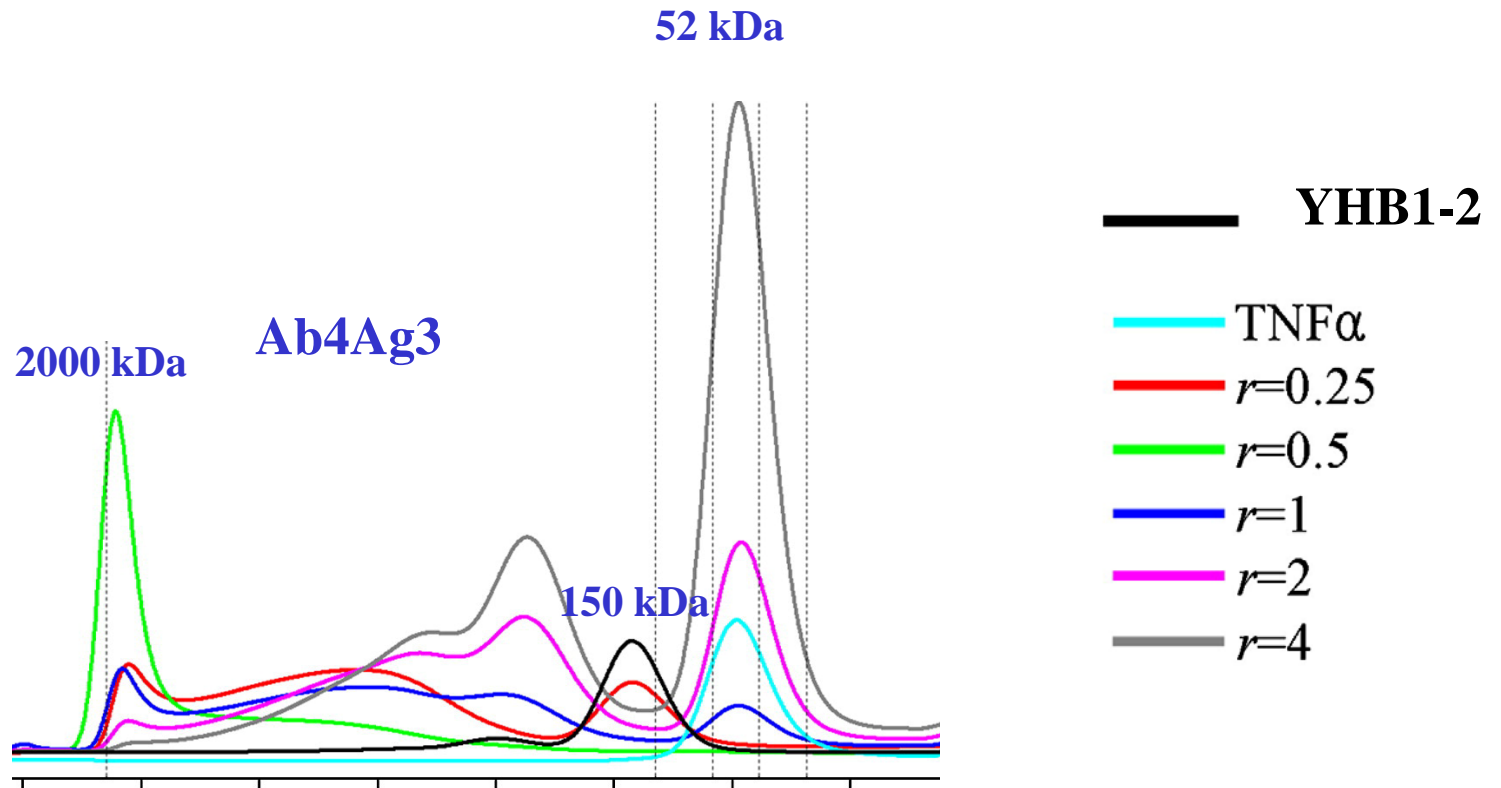
Santora LC et al., Anal Biochem. 299:119-29 (2001).

Immune complexes formed between TNF α & Infliximab



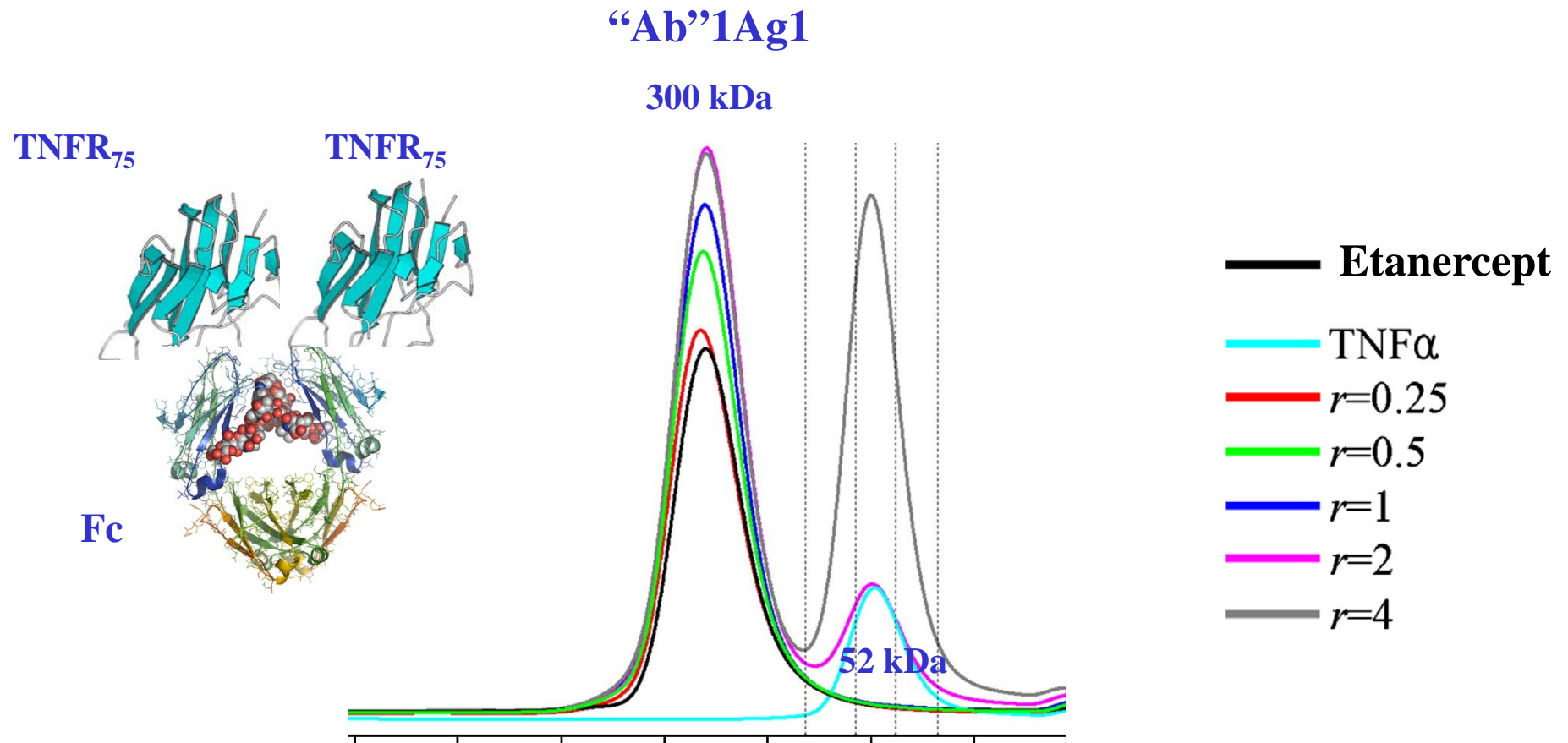
Kim MS, Kim YS et al., J Mol Biol. 374:1374-88 (2007).

Immune complexes formed between TNF α & YHB1-2



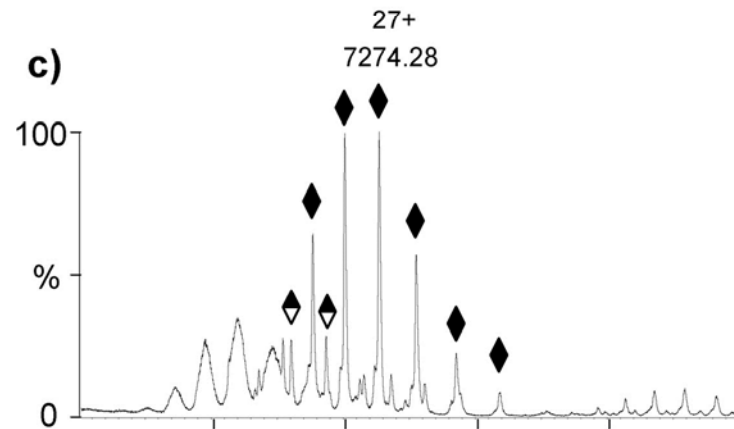
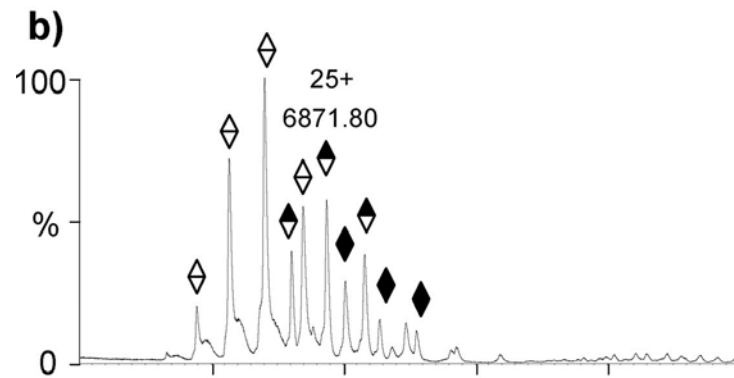
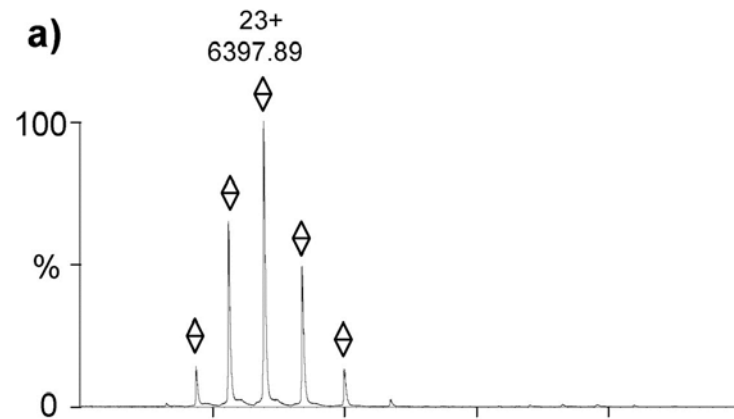
Kim MS, Kim YS et al., J Mol Biol. 374:1374-88 (2007).

Immune complexes formed between TNF α & Etanercept



Kim MS, Kim YS et al., J Mol Biol. 374:1374-88 (2007).

Non covalent ESI-MS



- ◇ 1:0 Ab
- ◇ 1:1 Ab:Ag
- ◆ 1:2 Ab:Ag

Atmanene C. et al., Anal Chem 81:6364-73 (2009)

Aggregates in mAbs and therapeutic proteins: A regulatory perspective

An important part of **protein aggregation** studies is evaluating the biological activity of the **aggregate**.

Differences between **monomeric** and **aggregated protein** can profoundly influence the potency of a **protein-based** drug.

There is no consensus on the maximum allowable limit for protein - based pharmaceutical **aggregates**.

Cordoba-Rodriguez, RV. BioPharm Internat. Nov. 2008

Immune complexes formed by MAbs

An important part of **immune complex** studies is evaluating the biological activity of the **complexes**.

Differences between **monomeric mAb** and **immune complexes** may influence the potency of a **mAb based drug**.

There has been little study of the **immune complexes** formed on administration of therapeutic mAbs

R. Jefferis. mAbs 3:(6) November/December 2011



Buttel I, Jefferis R. et al. *Taking immunogenicity assessment of therapeutic proteins to the next level* *Biologicals* 39:100-109 (2010)



Jefferis, R. & Lefranc, M-P. *Human Immunoglobulin Allotypes: Possible implications for immunogenicity.* *mAbs* 1:1-7 (2009)



Jefferis, R. *Glycosylation as a strategy to improve antibody-based therapeutics.* *Nature reviews: Drug Discovery.* 8: 226-234 (2009)



Magdelaine-Beuzelin, C., Jefferis R et al., *IgG1 heavy chain-coding gene polymorphism and development of antibodies-to-infliximab.* *Pharmacogen Genomics.* **19**:383 (2009).