

A role for complement and immune complexes in immune responses to therapeutic antibodies?

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1. The role of complement and immune complexes in induction of immune responses in general
 - Theoretical considerations about responses to biopharmaceuticals
 - Experimental data
2. Adverse effects induced by complement-activating immune complexes following treatment with rituximab
 - Clinical and experimental data

Is complement important in the induction of humoral immune responses?

Classical study from Doug Fearon's group

C3d of Complement as a Molecular Adjuvant: Bridging Innate and Acquired Immunity

Paul W. Dempsey, Michael E. D. Allison, Srinivas Akkaraju, Christopher C. Goodnow, Douglas T. Fearon*

An optimal immune response should differentiate between harmful and innocuous antigens. Primitive systems of innate immunity, such as the complement system, may play a role in this distinction. When activated, the C3 component of complement attaches to potential antigens on microorganisms. To determine whether this alters acquired immune recognition, mice were immunized with a recombinant model antigen, hen egg lysozyme (HEL), fused to murine C3d. HEL bearing two and three copies of C3d was 1000- and 10,000-fold more immunogenic, respectively, than HEL alone. Thus, C3d is a molecular adjuvant of innate immunity that profoundly influences an acquired immune response.

The decision of the acquired immune system to respond to an antigen may be based not only on what is non-self, but also on what is infectious and of potential danger to the host (1). How the immune system makes this latter determination is not clear because the antigen receptors that are distributed among different lymphocyte clones generally cannot distinguish between noxious and innocuous antigens.

Systems of innate resistance to infection evolved before acquired immunity and are triggered by certain biochemical

characteristics that are shared by microorganisms but not by higher forms of life. Complement is a plasma protein system of innate immunity that is activated by microorganisms in the absence of antibody (2). One consequence of activation is the covalent attachment of fragments of the third complement protein, C3, to the activator, and two of these fragments, C3dg and C3d, bind to CR2 (CD21) on B lymphocytes. CD21 may have B cell-stimulating functions because it associates with CD19, a B cell membrane protein that amplifies B cell activation (3) and is required for normal T cell-dependent, B cell responses (4). In support of this, depleting mice of C3 or blocking the binding of ligand to CD21 raises by approximately 10-fold the threshold dose of antigen required to elicit antibody (5, 6). Therefore, the complement system may be an innate immune system that provides information to the acquired immune system in an at-

tempt to classify antigens according to their potential hazard.

To determine whether the immunity-enhancing function of complement is mediated solely by the attachment of C3d to antigen and, if so, the magnitude of this effect of C3d, we prepared recombinant model antigens of hen egg lysozyme (HEL)

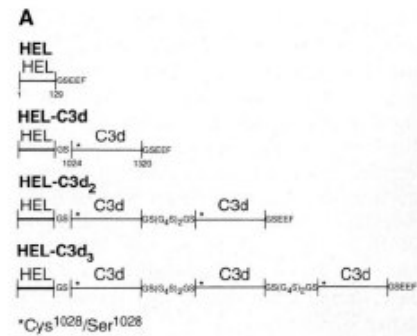
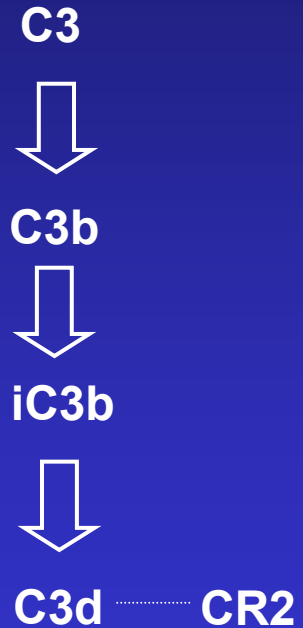


Fig. 1. Recombinant HEL and HEL-C3d fusion proteins. **(A)** Recombinant proteins comprising amino acids 1 to 129 of HEL alone and fused to one, two, or three copies of amino acids 1024 to 1320 of the C3d region of murine C3 were prepared (7). Amino acids in addition to those present in the native proteins are Gly (G), Ser (S), Glu (E), Phe (F), and Ser that was substituted for Cys (C) at position 1028 of C3 to avoid the presence of a free sulfhydryl in the recombinant proteins. **(B)** The recombinant proteins were purified from culture supernatants of transfected cells and assessed by SDS-polyacrylamide gel electrophoresis and Coomassie blue staining. Size markers are on the left in kilodaltons.

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Effect of coupling antigen with C3:

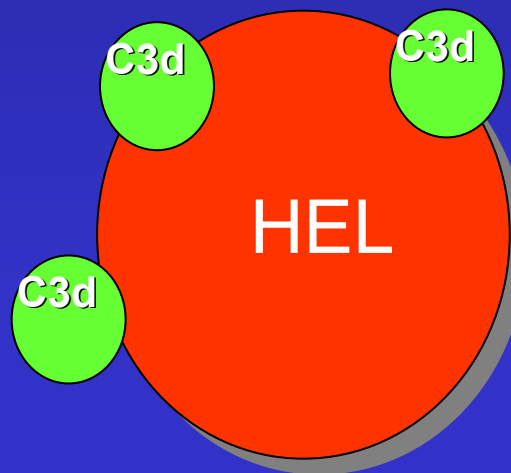
- Attachment of C3 to antigens enhances their immunogenicity (by up to 10,000-fold)

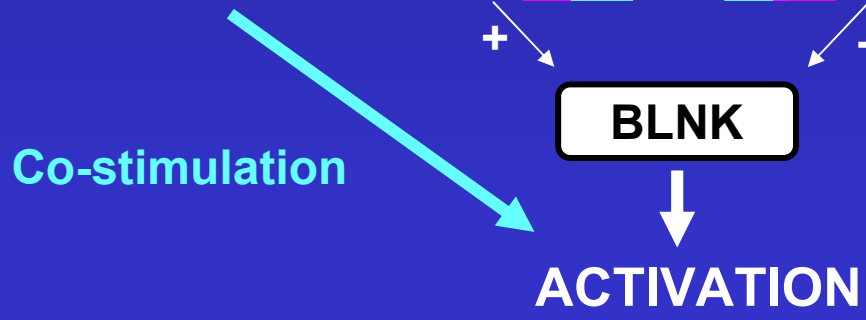
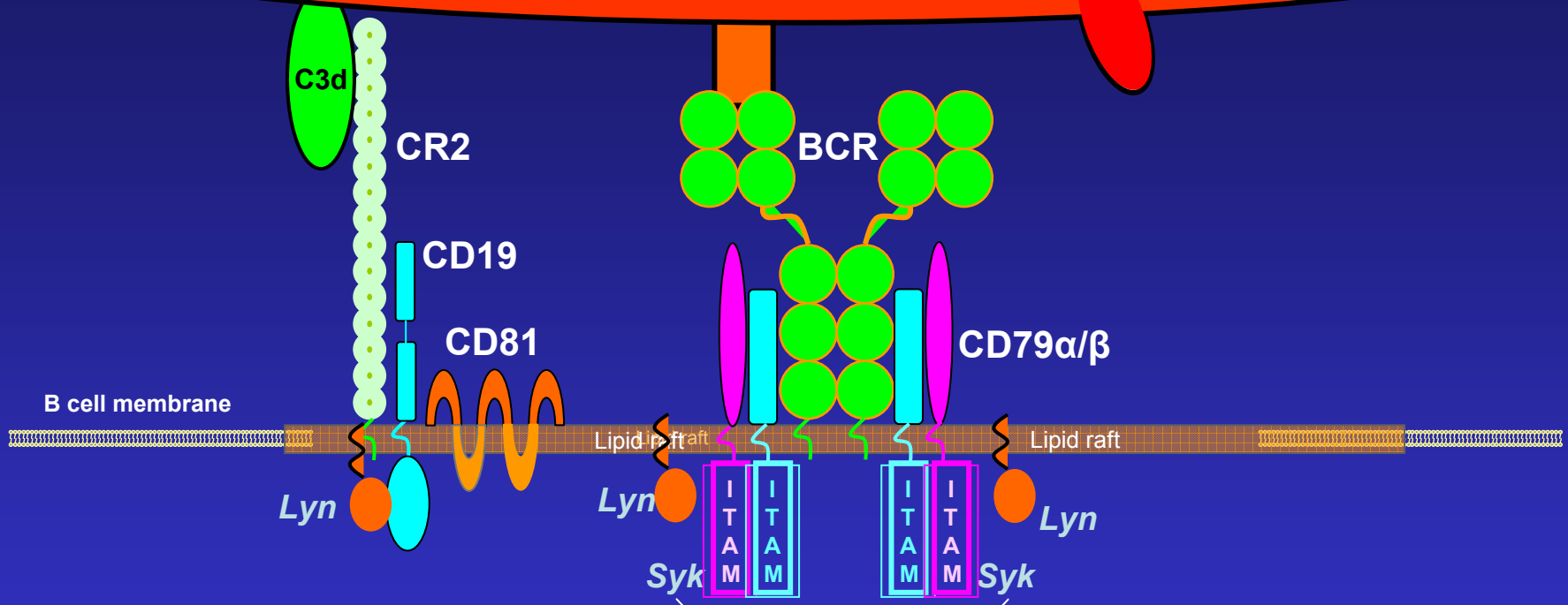
(Dempsey et al., Science, 1996)

2 copies: 1,000-fold

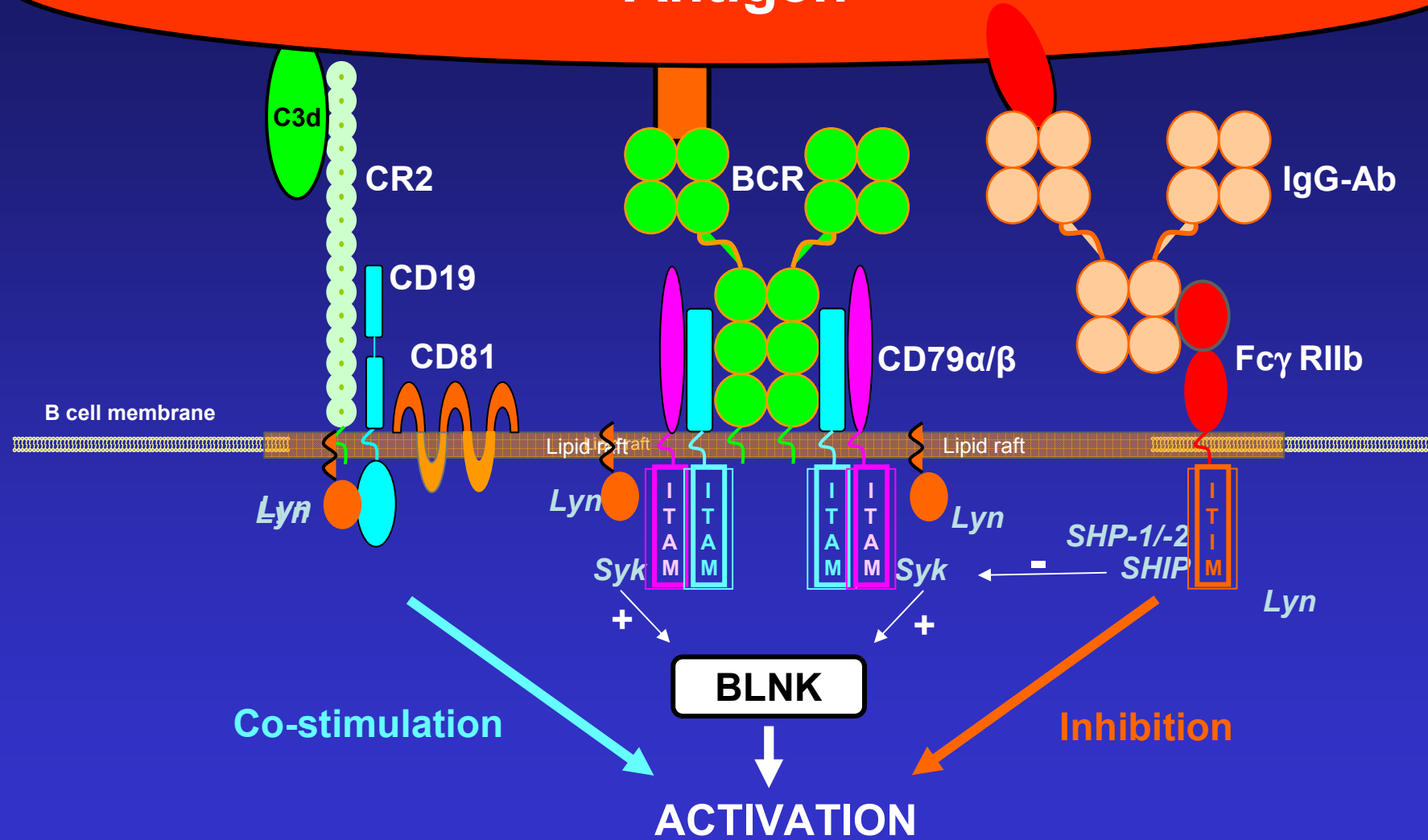
3 copies: 10,000-fold

100-fold more potent than complete Freund's adjuvant





Antigen



IgG antibodies may enhance or inhibit immune responses:

- Inhibit the response against large, particular antigens like RBCs (Rhesus prophylaxis) and malaria parasites
- Enhance the response against soluble antigens, such as KLH, bovine serum albumin (BSA), ovalbumin and (presumably) immunoglobulins

Physiological ligands for the receptor triad:

Antigen alone

Engages BCR only

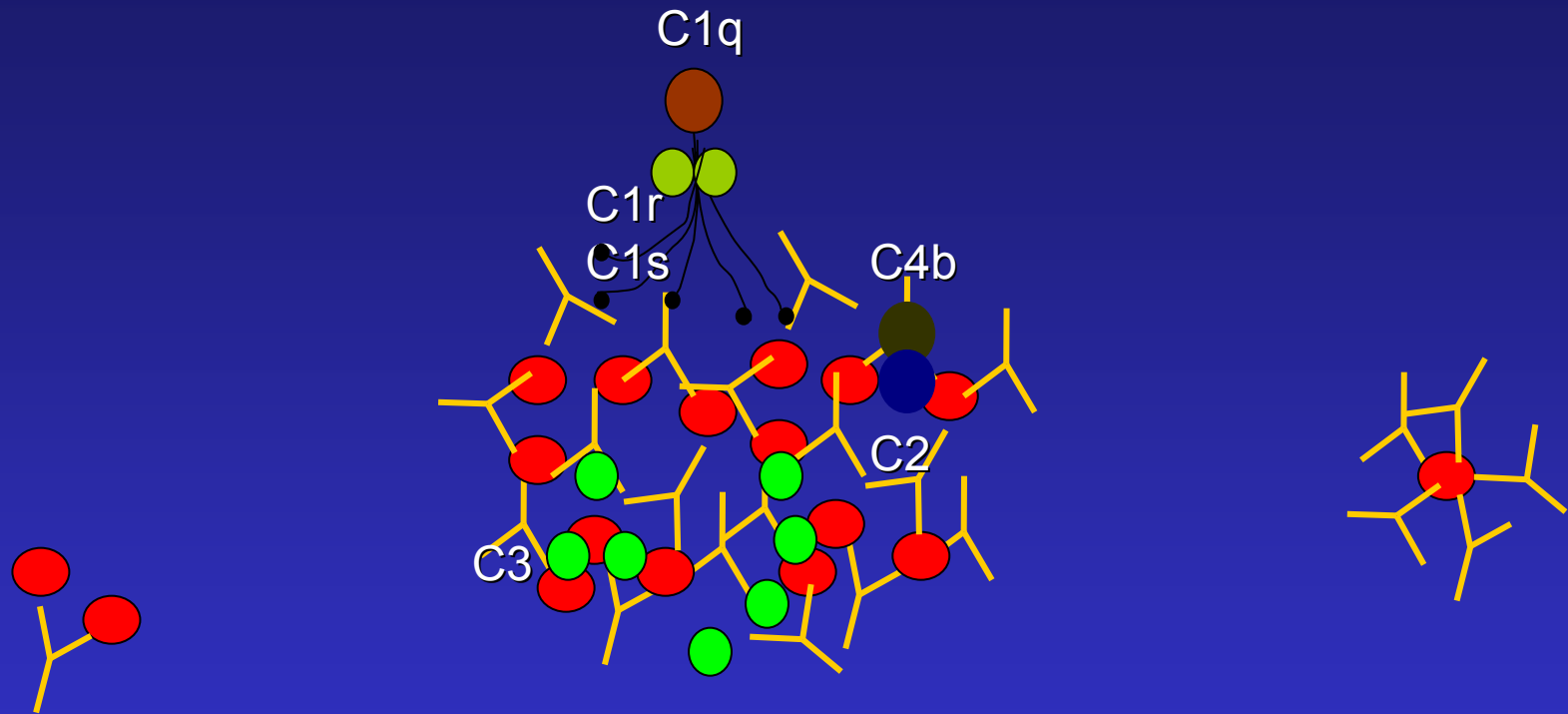
Antigen opsonised with component component 3 (C3) fragments

Engages BCR and CR2/CD19

Antigen in complex with Abs and complement (immune complexes)

May engage the full triad BCR/CR2/ Fc γ RIIb

Immune complexes



Complement activation by antigens

Some antigens activate complement directly via the AP or LP

Examples:

AP: *P. aeruginosa*, group B streptococci, *K. pneumonia*

LP: *S. typhimurium*, *E. Coli*

Antigens that do not (therapeutic mAbs) may activate complement via the CP by

1. Forming **aggregates**
2. Forming immune complexes with **natural (auto)antibodies**
3. Forming immune complexes with pre-existing **acquired antibodies**

Formation of immune complexes with
natural (auto)antibodies



Natural antibodies

- Present without previous immunization
- Germ line configuration (No somatic hypermutation)
- Usually IgM, but also IgG exist
- Low affinity, broad reactivity – "Sticky"
- Often self-reactive (natural autoantibodies)

Complement-activating immune complexes promote antigen-uptake by B cells and subsequent T-cell responses

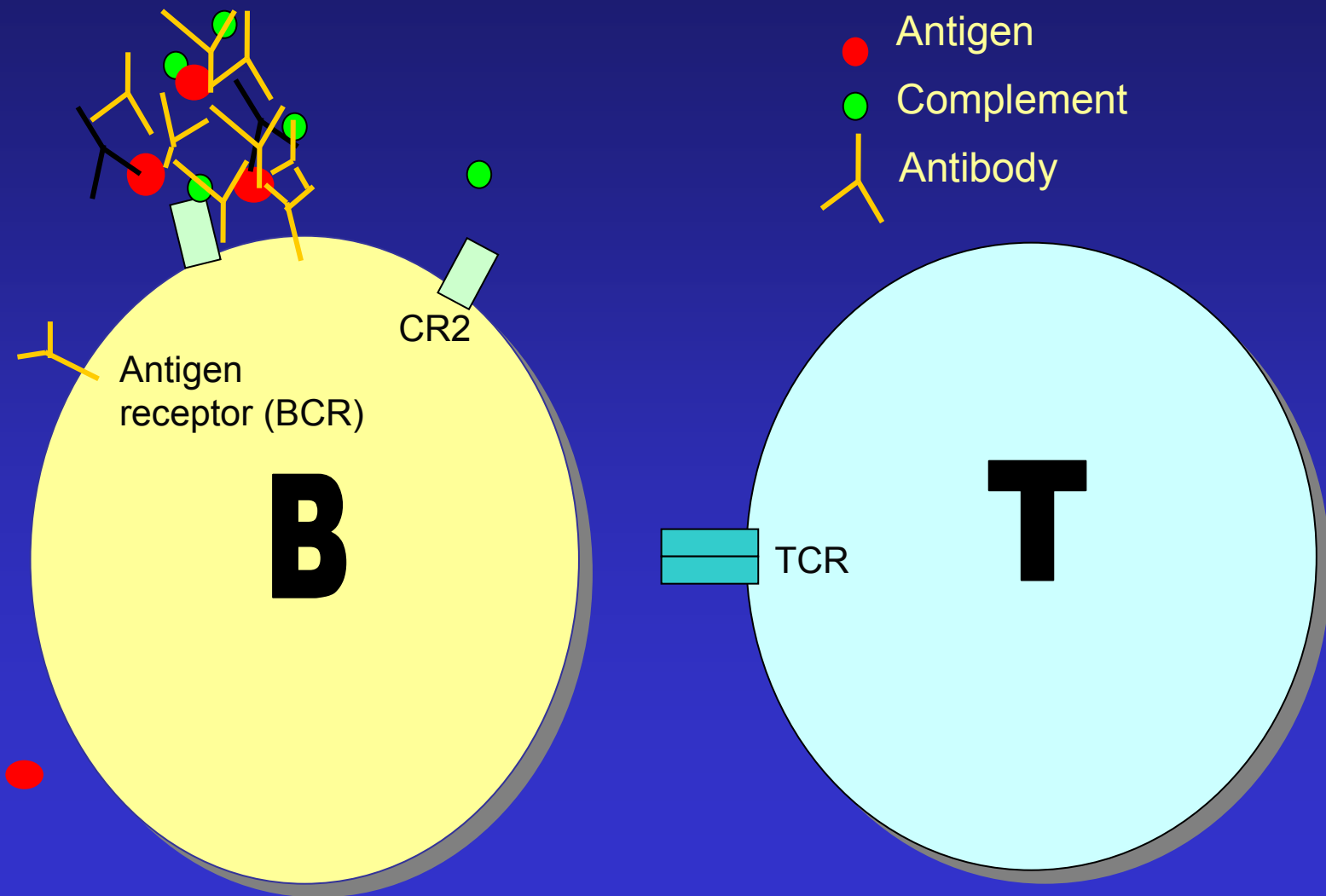
Primary foreign antigen:

Natural antibodies and complement promote uptake of primary antigens (KLH) by B cells and subsequent T-cell responses (Thornton et al., J Immunol 1994)

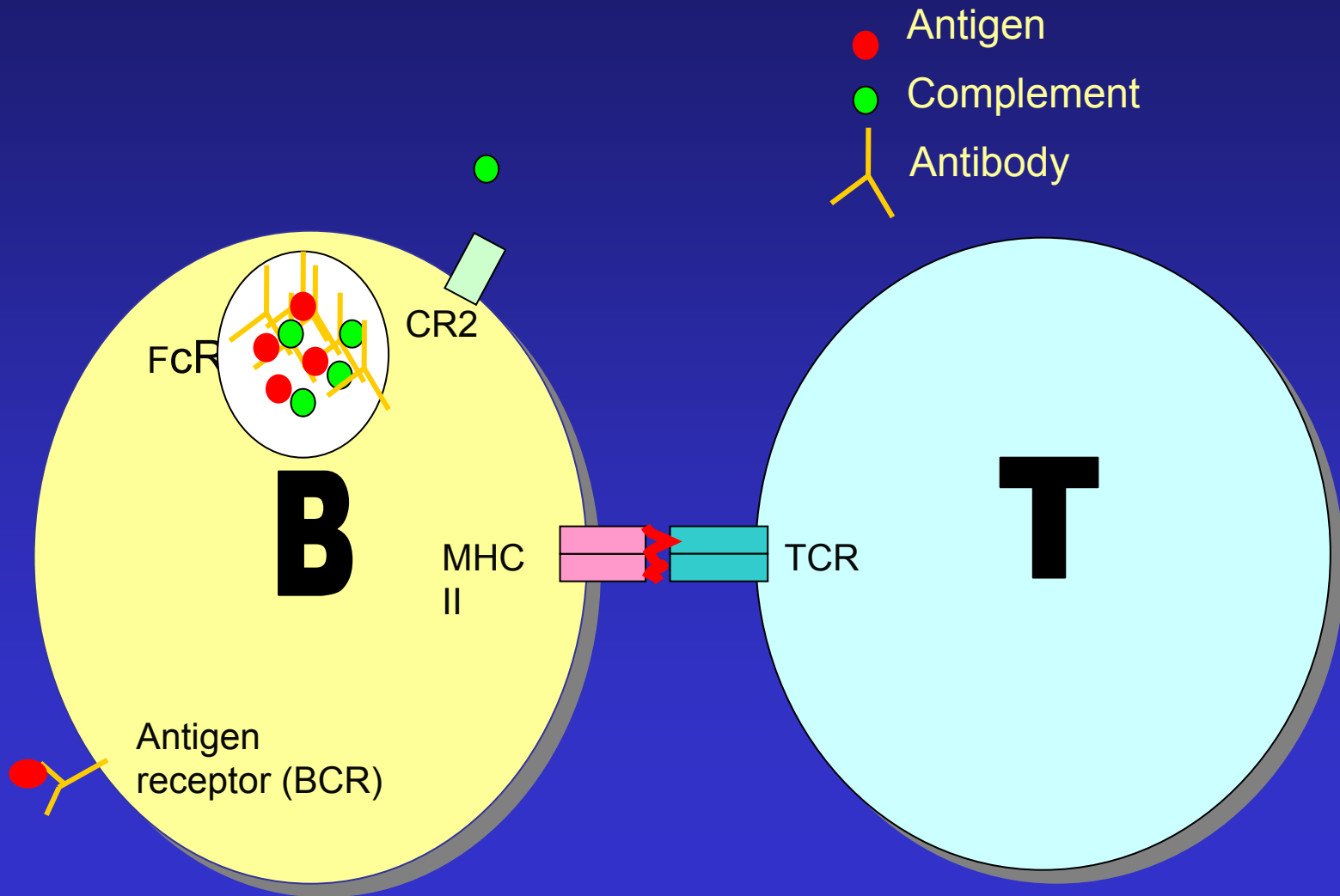
Self-antigen:

Natural autoantibodies and complement promote uptake of a self-antigen, thyroglobulin, by B cells and subsequent T-cell responses (Nielsen et al., Eur J Immunol 2001)

Antigen presentation by specific and non-specific B cells

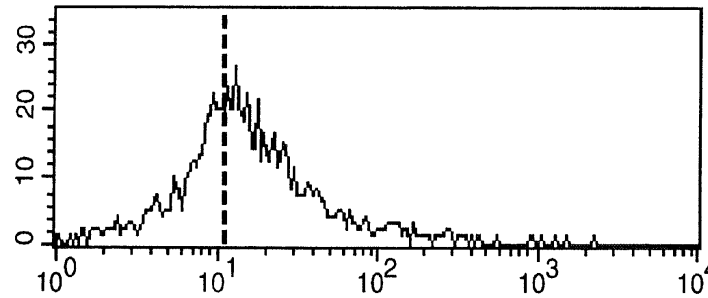


Antigen presentation by specific and non-specific B cells

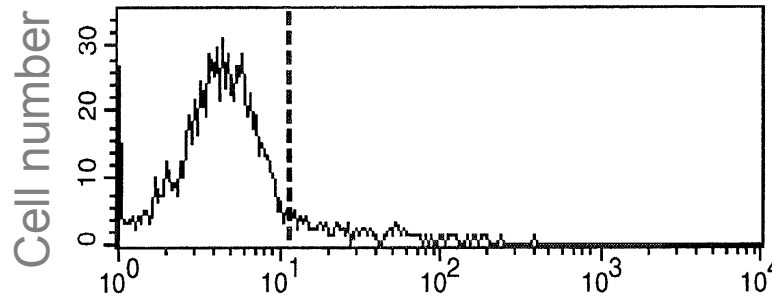


Serum promotes binding of self-antigen (thyroglobulin) to B cells

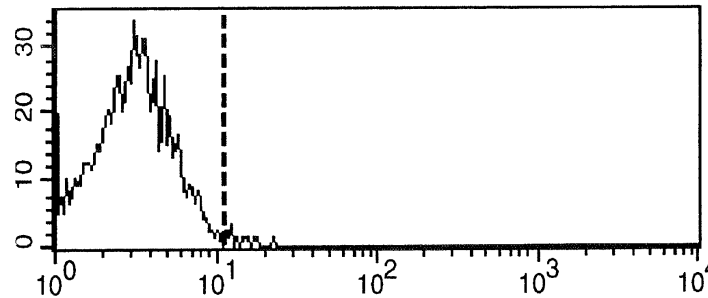
FITC-Tg
+ 80% serum



FITC-Tg
+ PBS

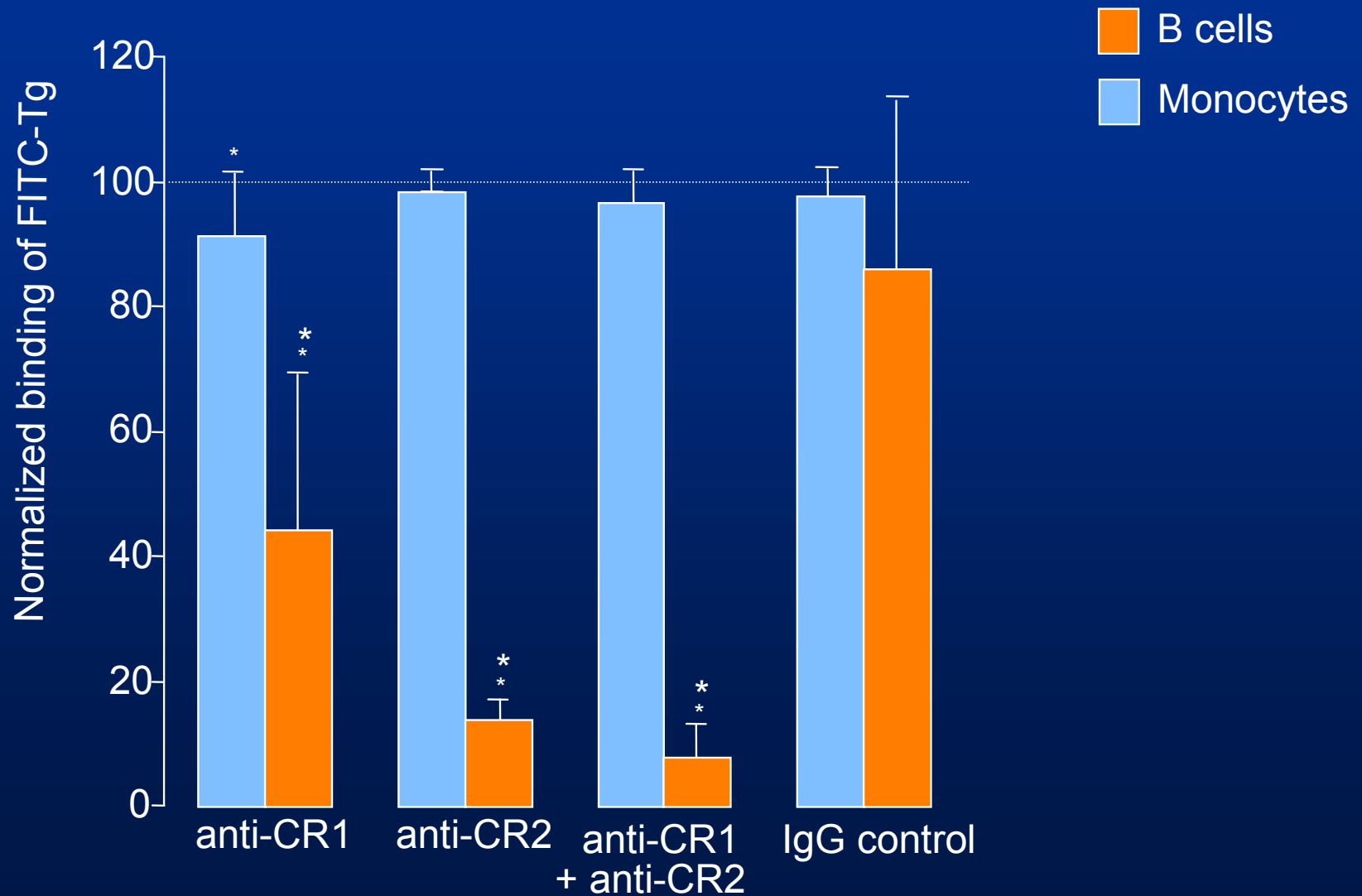


No Ag



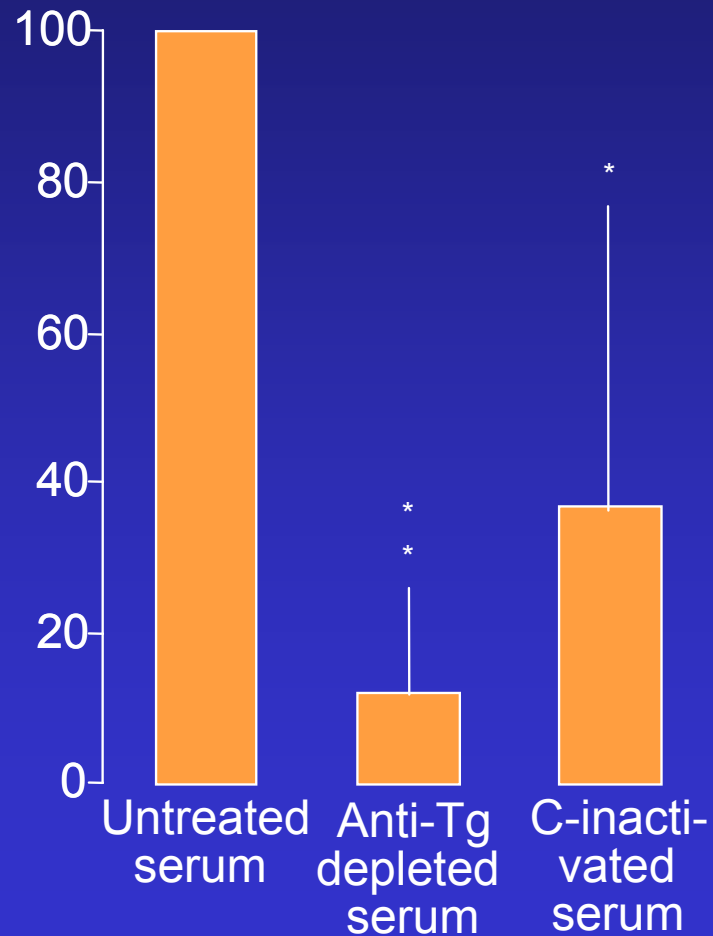
Tg-binding [FL-1]

Complement receptor blockade inhibits Tg-uptake by B cells



Tg-induced CD4⁺ T cell proliferation depends on natural autoantibodies and complement

Normalized CD4⁺ T cell proliferation



Relevance to therapeutic antibodies

Autoantibodies against IgG

1. IgM anti-Fc γ antibodies (or classical RF)

Found in RA and other patient groups

2. Anti-Fab and F(ab)₂ antibodies

Anti-Fab antibodies increase following immunization of volunteers with KLH (Birdsall and Rossen, Clin Exp Immunol. 1983 Aug;53(2):497-504)

Natural antibodies against the **F(ab)₂ portion of IgG** are present in healthy persons (Nasu et al. Clin Exp Immunol. 1980 Nov;42(2):378-86)

- May be anti-idiotypic nature, or
- May interact with **hinge peptides**

Pre-existing antibodies to murine IgG (relevant for chimeric antibodies)

(known as false-positive reactions in e.g. ELISAs)

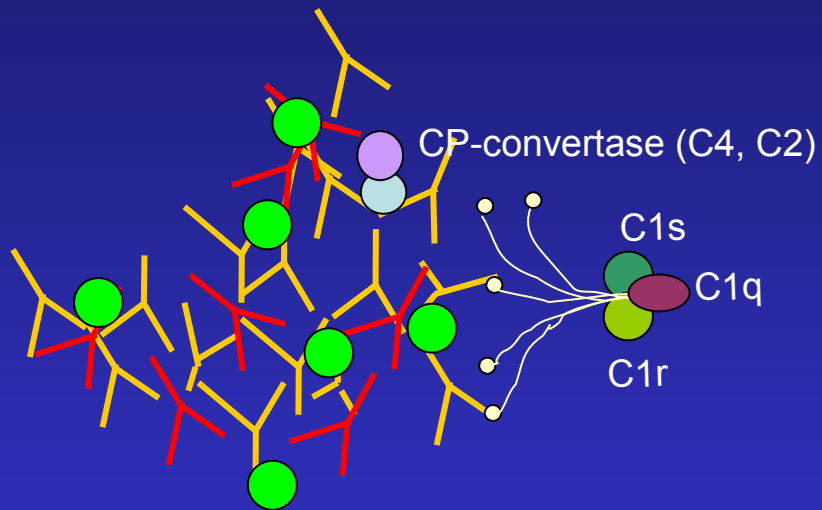
Prevalence 1-80% (!)

Two different groups of naturally occurring heterophilic antibodies (IgG-type) (Hennig et al., J Immunol Methods. 2000 Feb 21;235(1-2):71-80):

Against the **Fab region** of IgG (from goat, mouse, rat, horse, and bovidae, but not rabbit).

Against the **Fc region** of IgG (from mouse, horse, bovidae, and rabbit - but not goat or rat)

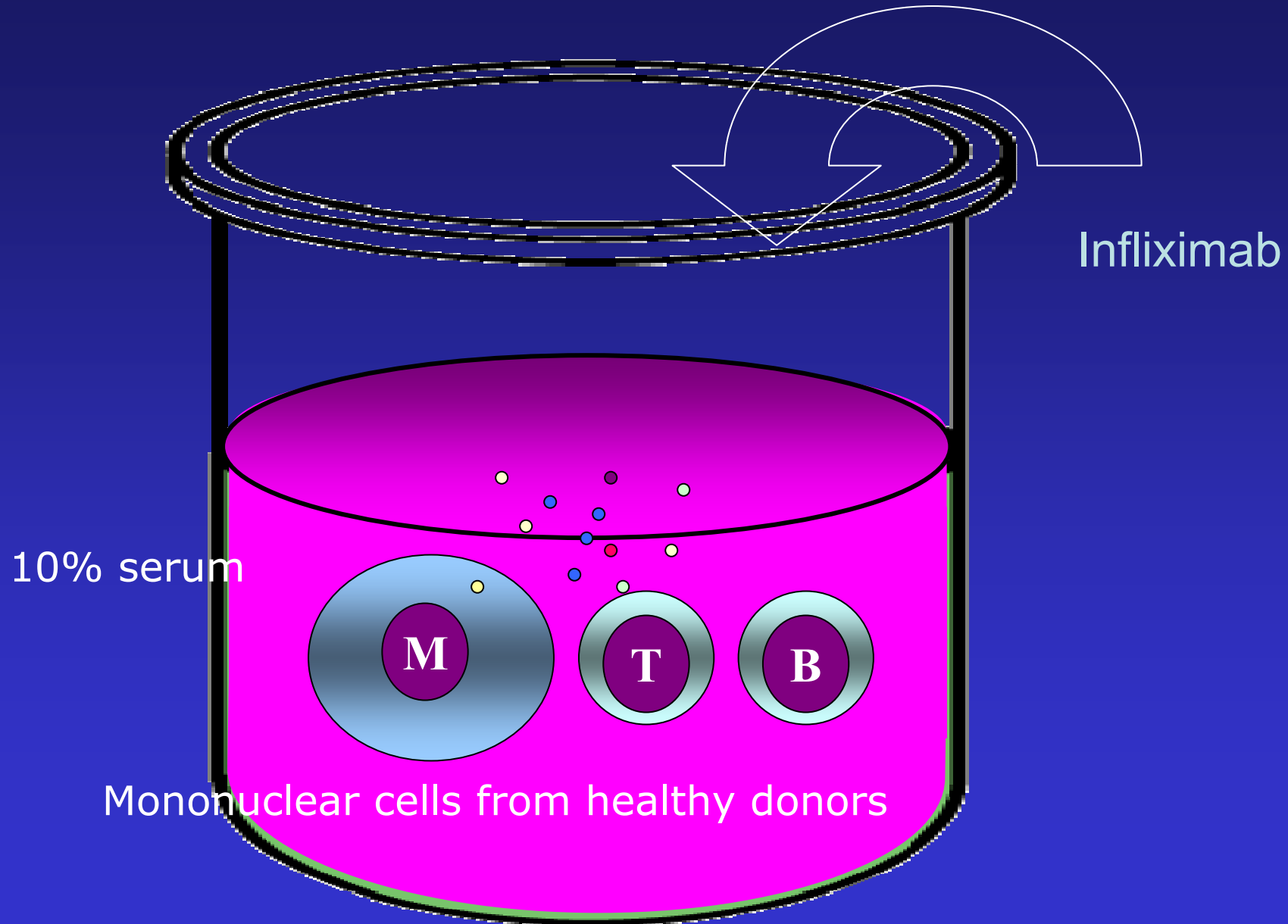
Theoretically, immune complexes may be formed with therapeutic antibodies of IgG type



Incorporation of C3 fragments

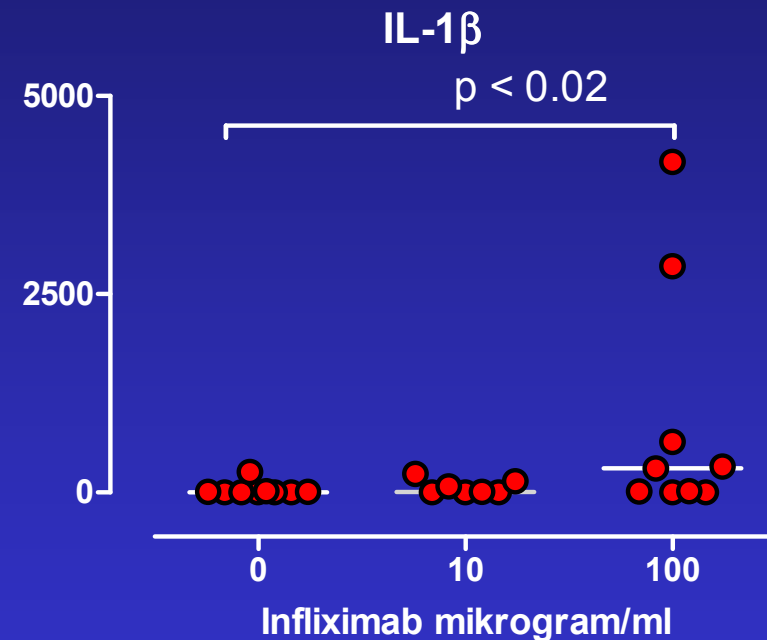
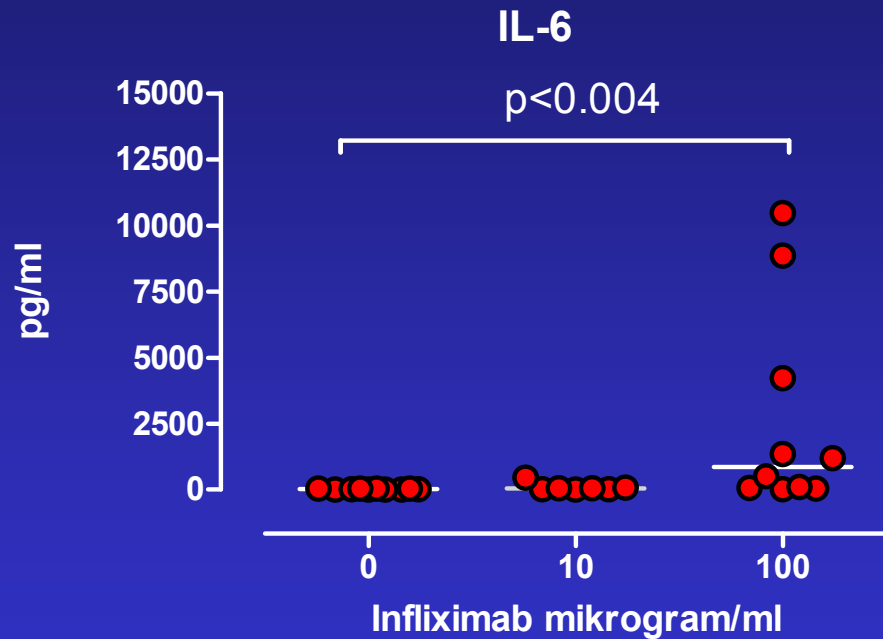
Immunogenicity may be enhanced

A role for complement in induction of immune responses to infliximab?

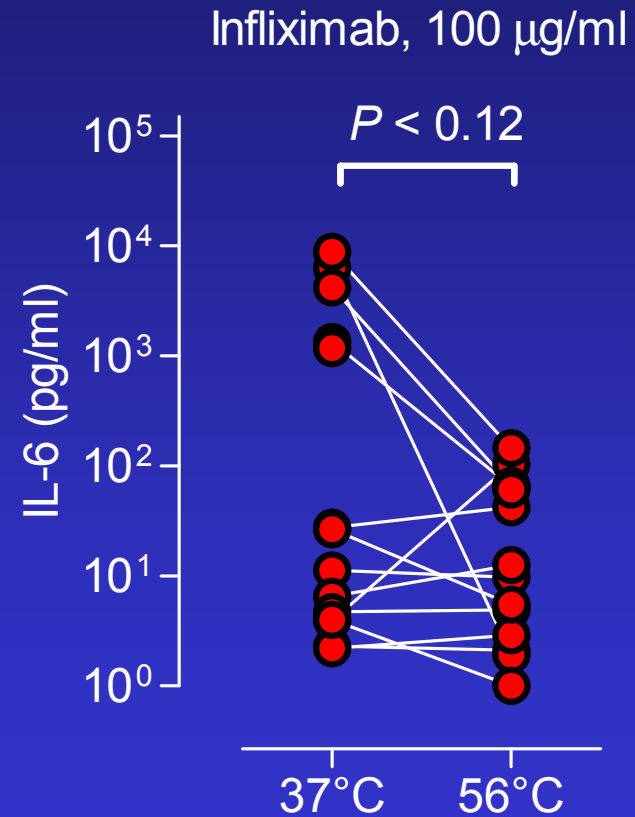
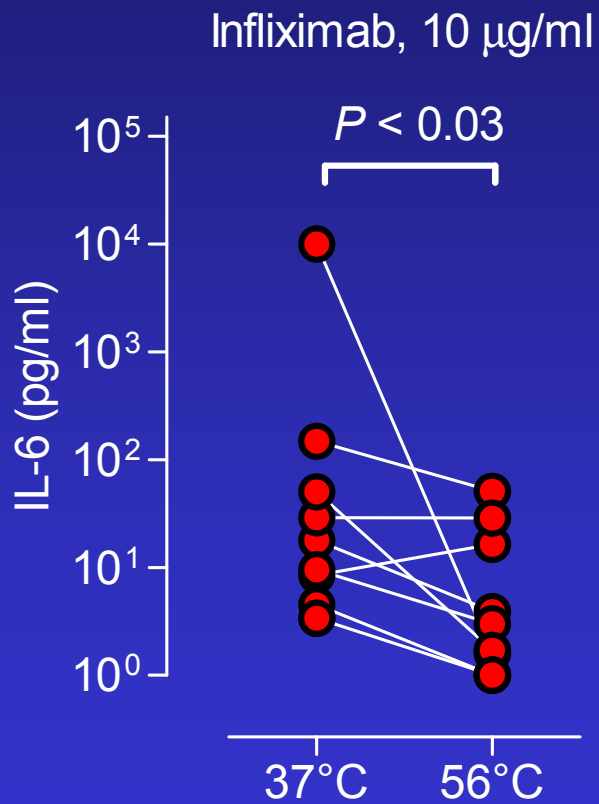


Induction of immune responses to infliximab in PBMC cultures (in presence of 10% autologous serum)

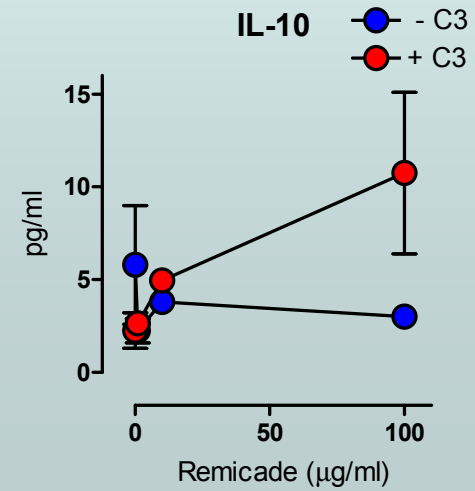
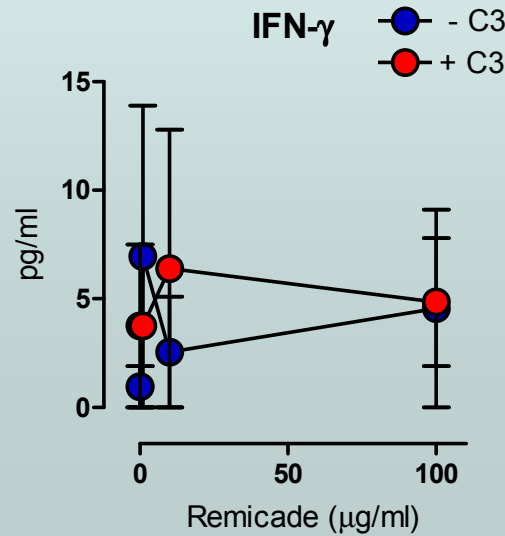
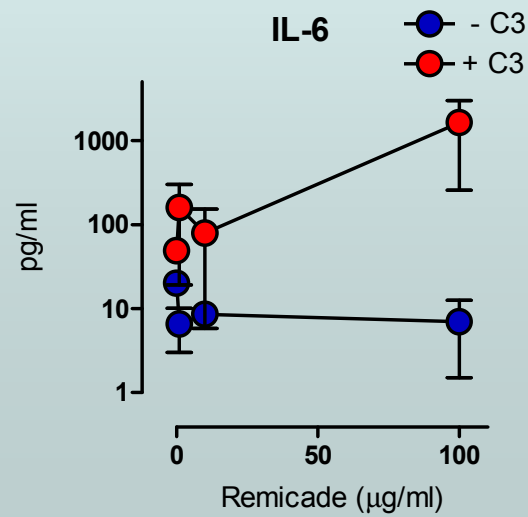
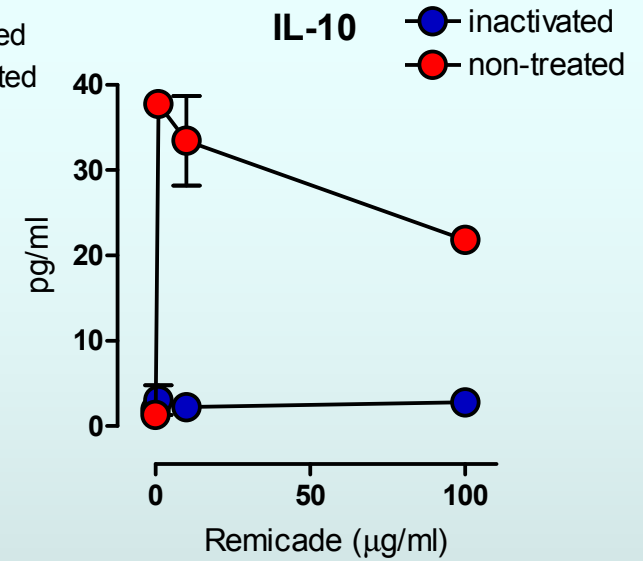
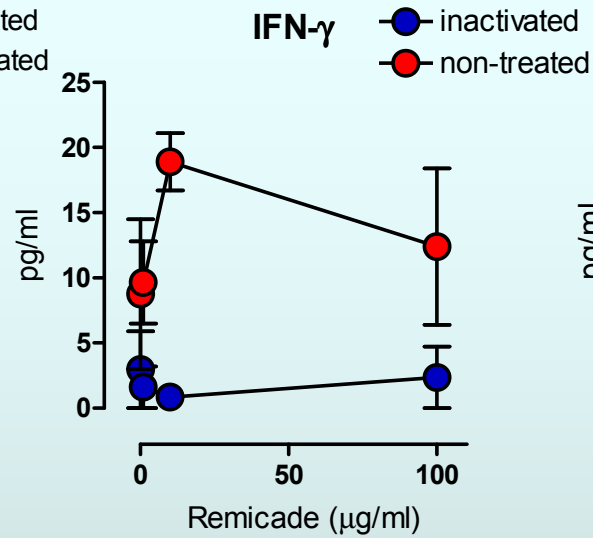
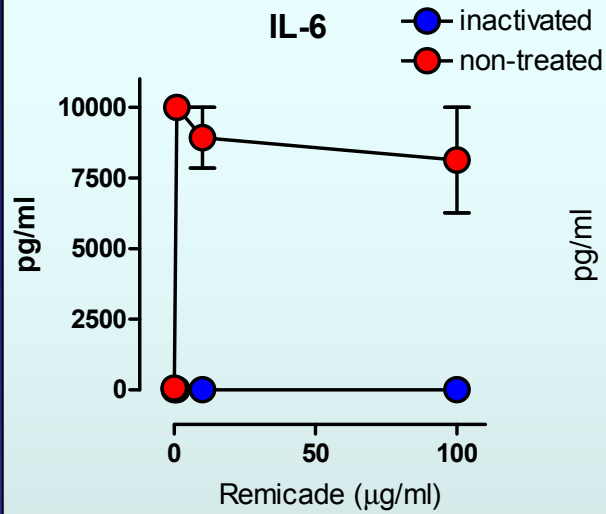
Preliminary data



The production of pro-inflammatory cytokines depends on heat-labile factors



Role of complement in the induction of cytokine responses to infliximab



Conclusions

C3-fragments are extremely potent molecular adjuvants

Complement and (natural) antibodies promote uptake of (self-) antigens by B cells

Complement and (natural) autoantibodies promote T-cell responses to self-antigens (Tg, TPO, MBP)

Complement promotes proinflammatory cytokine responses to infliximab

1. Role of C and ICs in inducing immune responses

- Theoretical considerations
- Experimental data

2. Adverse effects induced by complement-activating ICs following treatment with rituximab

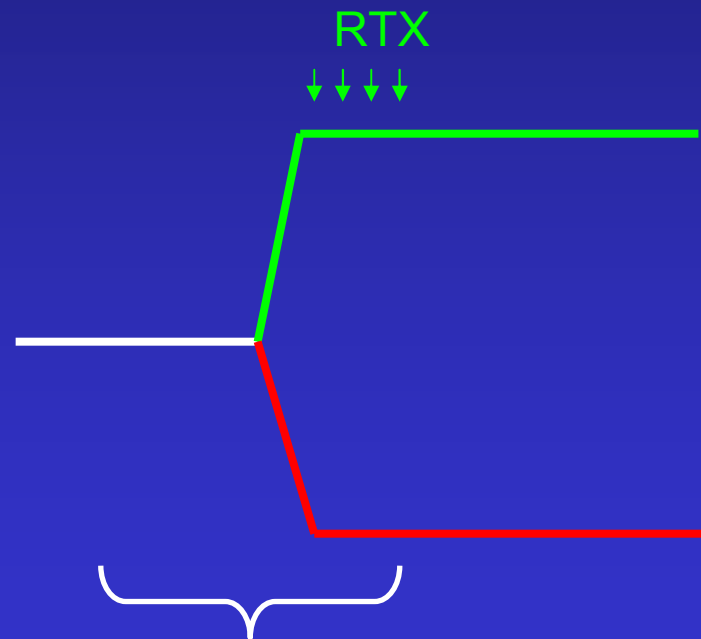
The appearance of serum sickness-like reactions/complement-opsonized immune complexes during treatment with the therapeutic antibody rituximab

- In malignant diseases human anti-chimeric antibodies (HACA) are formed in approx. 1%.
- In a study on Sjögrens disease HACAs were formed in 4 of 8 patients, 3 of these developed SSLR (Pijpe J, Arthritis Rheum 2005)
- In children with ITP, RTX caused SSLRs in 5 out of 60 (Bennet et al. Blood 2006; Wang et al., J Pediatr 2005).
- In a study on Graves' disease 3 of 10 patients developed SSLR (el Fassi et al.,submitted)

Clinical trial, Graves' disease

Prospective, controlled study

- 10 +RTX GD patients
- 10 -RTX GD controls



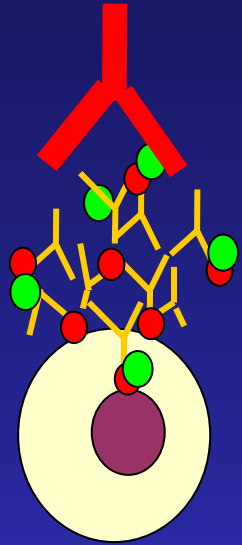
Approx. 4 months of MMI

Adverse effects of 10 GD patients treated with rituximab

Patient	Infusion related adverse events at first Rituximab infusion	Articular adverse events	Other autoimmune manifestations
1	-	-	
2	Hypotension (syst. BP 95 mmHg)	-	
3	-	At day 11: headache, nausea, paraesthesias in the extremities and unilateral leg and hip-pain.	recurrent cases of iritidocyclitis Evaluated for inflammatory bowel disease due to change in bowel habits
4	Hypotension (syst. BP 105 mmHg), fever (38.7 °c)	-	
5	Hypotension (syst. BP 102 mmHg), near-fainting, nausea, chills	At day 11: fever (up to 39.5 °C), migratory polyarthritis. Increased CRP Persisting shoulder pain.	
6	-	-	
7	Hypotension (syst. BP 70 mmHg)	-	
8	-	-	
9	Sinus tachycardia, nausea	-	
10	-	At day 31: fever (up to 39 °c), symmetric polyarthritis and skin exanthema. Persisting joint pain more than two years after treatment	Ulcerative colitis diagnosed day 67. Persisting joint pain more than two years after treatment

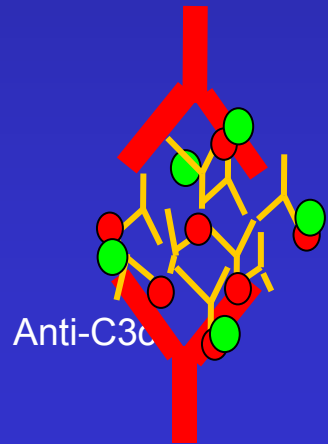
Anti-IgG/IgA/IgM/C3

Circulating complement-opsonized immune complexes

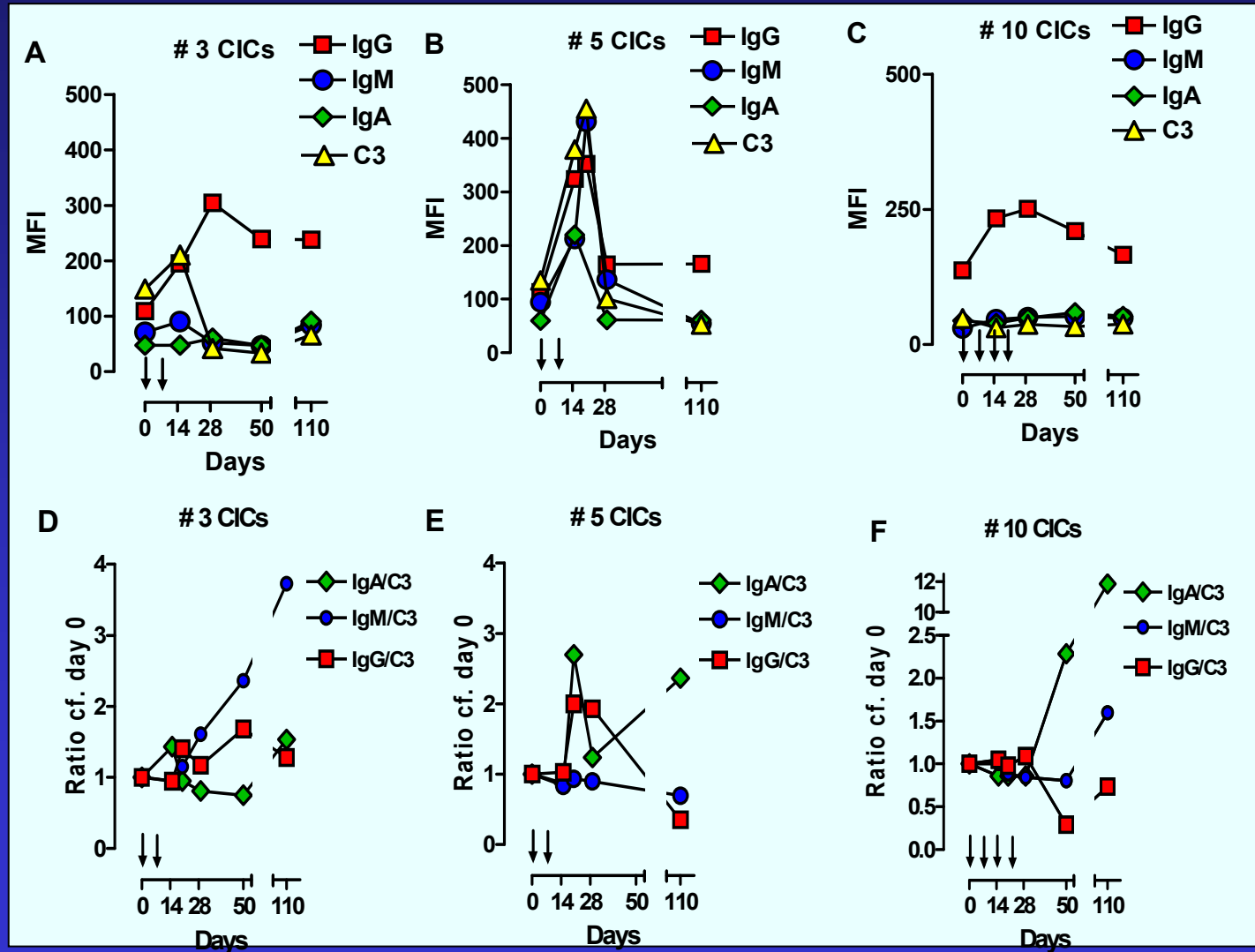


Monocytes

Anti-IgG/IgA/IgM



Anti-C3



Two lupus patients with serum-sickness

Patient 1 (LEN):

47 yr. Female. Discoid lupus/secondary ITP

No previous joint symptoms

Treated with RTX (375 mg/m²) days 0 and 7.

At day 11 (4 days after 2nd infusion, she develops:

- Flu-like illness
- Joint pain: pains in the hands, sensation of swelled fingers/hands, symmetrical pains in ankles, knees, hips.
- Fever, 38.4 °C
- Erythema: pettechia at crura, maculopapulous rash, primaily on the thighs
- Sore throat
- Thrombocytopenia: < 3 billions/L versus 131 billions/L on day 7.
- Hematuria

Complement function:

- Classical pathway: 1 % of control (↓)
- Lectin pathway: 0 % of control (↓)
- Alternative pathway: 84 % of control

Two lupus patients with serum-sickness

Patient 2 (LI):

32 yrs. Female. SLE/secondary ITP

No previous joint symptoms

Treated with RTX (375 mg/m²) days 0, 7 and 14.

At day 22 debutes with SSLR

Complement function:

- Classical pathway: 61 % of control (↓)
- Lectin pathway: 42 % of control
- Alternative pathway: 105 % of control



Assay for anti-rituximab

Tracer ^{125}I -RTX, 4-5.000 CPM

Addition of serum, 0-4%

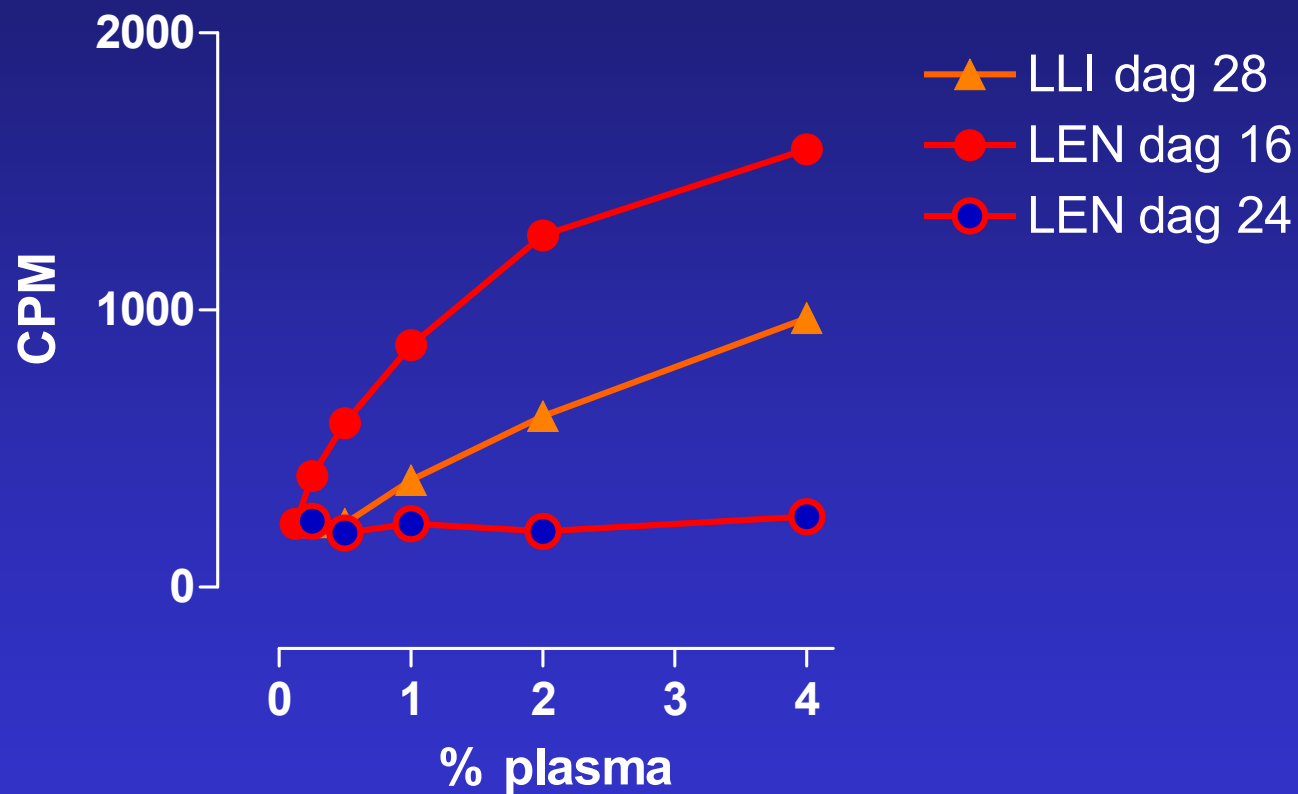
Incubation 18 hours

Incubation with anti-human lambda chain, 3 hours

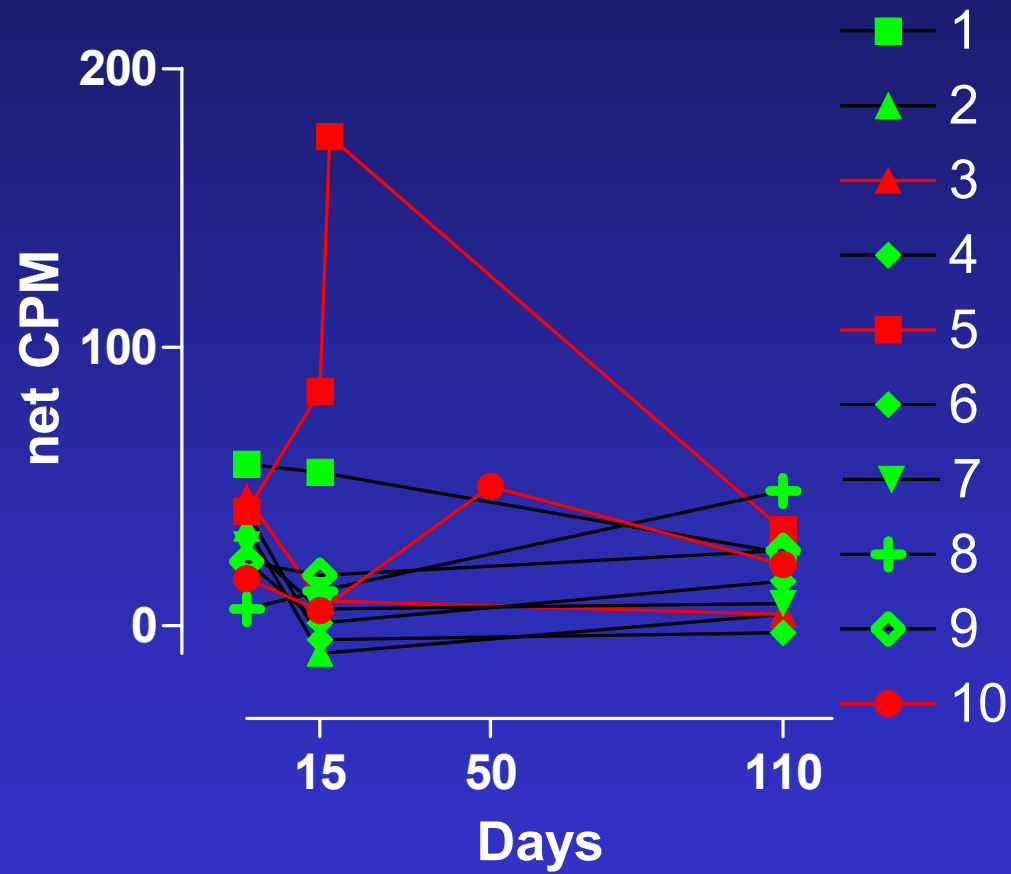
Centrifugation 4000 RPM, 4°C



Anti-RTXactivity



Anti-RTX



Are RTX-binding antibodies present prior to RTX treatment?

Content of IgA (and IgG) in the complexes

Kinetics (SSLR/circulating immune complexes on day 11)

Absence of B cells

Conclusions II

Complement-activating immune complexes are formed in some patients receiving rituximab, and give rise to serum-sickness

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