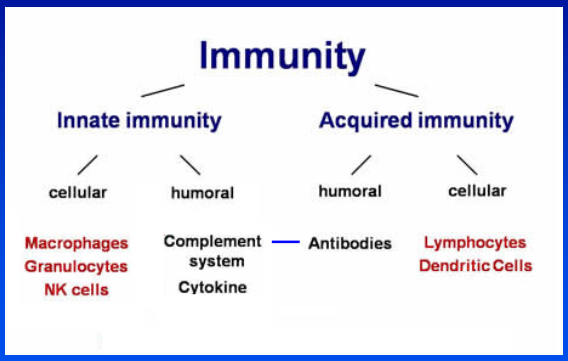
Komplement och immunbrist - en uppdatering

Lennart Truedsson

Inst. för Laboratoriemedicin, Lund, avd. MIG
Lunds Universitet

Klinisk Immunologi och Transfusionsmedicin
Labmedicin Skåne

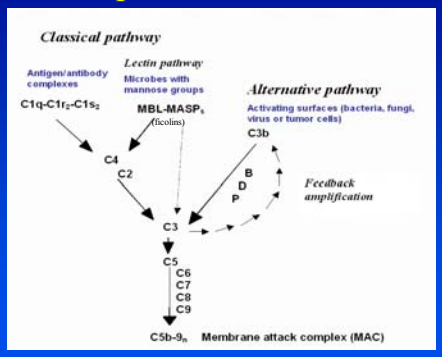
Complement is part of the immune system



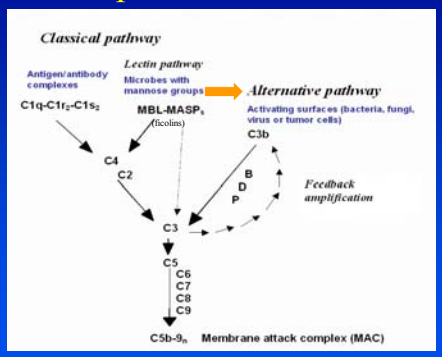
The Complement System

- ~ 35 plasma proteins and membrane proteins
- Several complement proteins present in proenzyme form
- Three main activation pathways

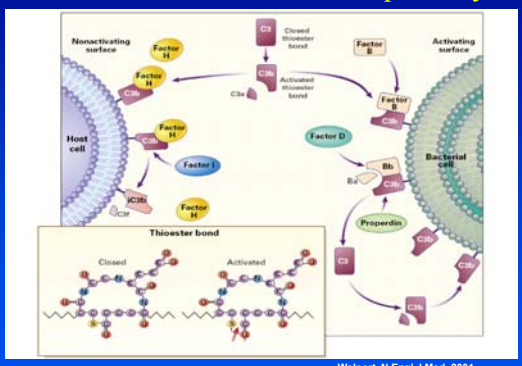
Complement activation



Complement activation



Activation of the alternative pathway



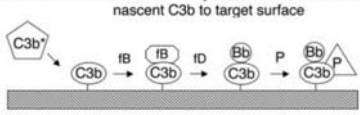
Walport, N Engl J Med, 2001

Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly.

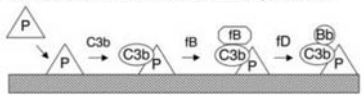
Spitzer D, Mitchell LM, Atkinson JP, Hourcade DE.

Division of Rheumatology, Department of Medicine, School of Medicine, Washington University, St. Louis, MO 63110, USA.

A Standard model: AP initiated by covalent attachment of nascent C3b to target surface

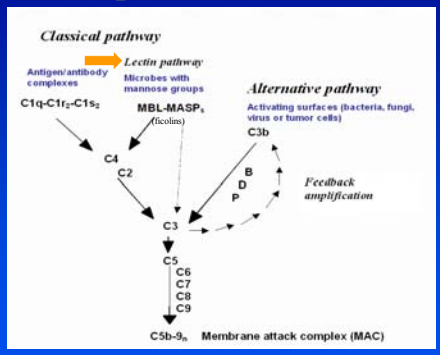


B Proposed properdin-directed model: AP initiated by non-covalent attachment of properdin to target surface



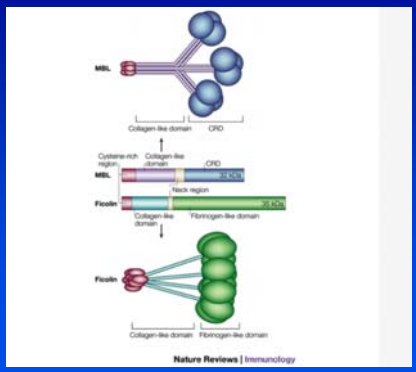
Spitzer et al, J Immunol, 2007

Complement activation

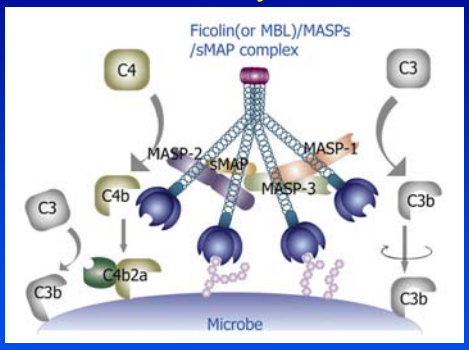


MBL and ficolins

Teizo Fujita, Nature Reviews Immunology 2, 346-353 (May 2002)

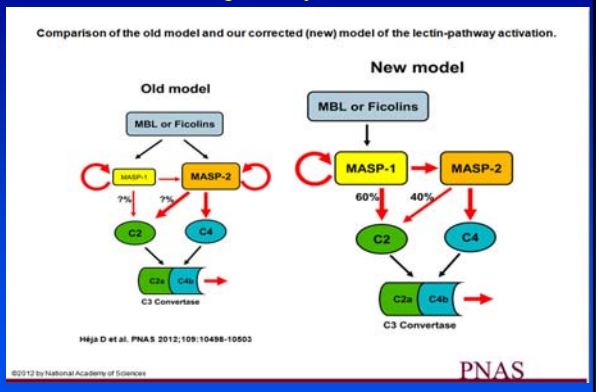


Lectin Pathway activation



Fujita et al, Immunol Rev, 2004

Lectin pathway activation



Lectin pathway activation

J Innate Immun. 2012 Dec 4. [Epub ahead of print]

Collectin-11/MASP Complex Formation Triggers Activation of the Lectin Complement Pathway - The Fifth Lectin Pathway Initiation Complex.

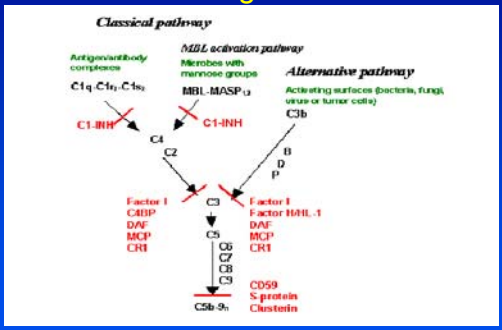
Ma YJ, Skjoedt MO, Garred P.

Laboratory of Molecular Medicine, Department of Clinical Immunology, Rigshospitalet, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

Molecules forming complexes with MASPs:

- MBL
- Ficolin-1
- Ficolin-2
- Ficolin-3
- Collectin-11

The complement system: activation and regulation



Complement protein synthesis

- Monocytes are able to produce most (all) of the complement proteins.
- Hepatocytes in the liver produce the majority of the complement protein molecules found in serum (e. g. C4, C3, factor B and C1-INH).
- Epithelial cells, fibroblasts are able to produce several complement proteins e. g. C3.
- C1q is produced by bone-marrow derived cells
- Factor D (adipsin) is produced by fat cells

Biological effects of complement

• Scavenger effect	Clearance of apoptotic cell material by binding to C1q
• Lymphocyte activation	Binding of antigen-bound C3d to CR2 on B cells enhance antibody production
• Virus neutralisation	Coating of virus with e. g. C3b prevents cell contact

Biological effects of complement

• Opsonisation	Coating of microbes with mainly C3b and/or iC3b increases phagocytosis
• Elimination of immune complexes	Coating of IC with C3b and/or C4b, and binding to CR1 on erythrocytes and transport to the liver and the spleen
• Inflammatory response	Anaphylatoxins (C5a, C3a), Chemotaxis (C5a, C3a).
• Cytolysis	Activation and formation of the Membrane attack complex (MAC)

Biological effects of complement

- Protective

  Scavenger effect, opsonisation, inflammatory response, lysis …

- Harmful

  Scavenger effect, opsonisation, inflammatory response, lysis …

Complement deficiencies

- Inherited complement deficiencies
  - Complete or subtotal deficiency or dysfunction
  - Autosomal recessive or X-linked basis

  One exception: C1 inhibitor deficiency or dysfunction, which causes hereditary angioedema (HAE) in the heterozygous state.

- Acquired complement deficiencies
  - Autoantibodies to complement proteins

Complement deficiencies

Table 3 | Summary of complement deficiencies in humans

Complement component involved	Frequency	Main disease association(s)	Reference(s)
C1q	50–100 reported cases	SLE; glomerulonephritis; infections	Skattum et al.[7]
C1r or C1s	10–50 reported cases	SLE; glomerulonephritis	Wu et al.[106]
C2	Estimated prevalence 1/20,000*	SLE; infections	Skattum et al.[7]
C3	20–50 reported cases	SLE; glomerulonephritis; recurrent infections	
C4	20–50 reported cases	SLE; glomerulonephritis; infections	
MBL	Estimated prevalence 1/10*	Susceptibility to infections	
Factor D	<20 reported cases	Neisserial infections	
Factor B	1 reported case	Neisserial infections	Slade et al, 2013
Properdin	50–100 reported cases	Meningococcal disease	Fijen et al.[107]
C5, C6, C7 or C8	20–100 reported cases	Usually healthy; recurrent neisserial infections	Skattum et al.[7]
C9	<10 reported cases Estimated prevalence 1/1,000‡	Usually healthy	Skattum et al.[7] Witzel-Schlömp et al.[105]
C1Inh	Estimated prevalence 1/50,000§	Hereditary angioedema	Skattum et al.[7]

*Estimated prevalence in white populations. ‡Rare deficiency in white populations, but common in the Japanese population (prevalence of about 0.1%). §Heterozygous deficiency. Abbreviations: C1Inh, plasma protease C1 inhibitor; MBL, mannose-binding lectin; SLE, systemic lupus erythematosus.

Adapted from: Sturfelt & Truedsson, Nature Rheumatol., 2012

Infections in complement deficiency: principal reasons

- Impaired antibody responses
- Impaired serum bactericidal activity
- Impaired opsonization

Classical pathway deficiencies and association with SLE

- Decreased capacity for immune complex handling
- The waste-disposal hypothesis (C1q)
- Aberrant tolerance induction
- Influence on cytokine regulation (C1q)

Complement deficiencies in Sweden 2014

Population 9 milj.

Deficiency (n)	Identified (n)	Estimated (n)
C1q	8	<5
C4	1	<5
C2	63	450
C3	1	<5
C6	2	?
C7	18	?
C8	3	?
C9	1	<5
Properdin	9	?
MBL	>200	>500,000
MASP-2	1	?
C1 inhibitor	~130	180
Factor I	2	?
Factor H	1	<5

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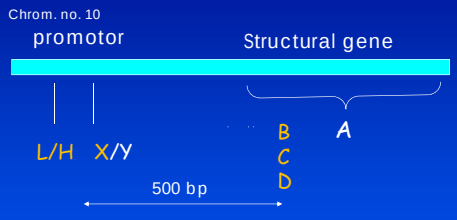
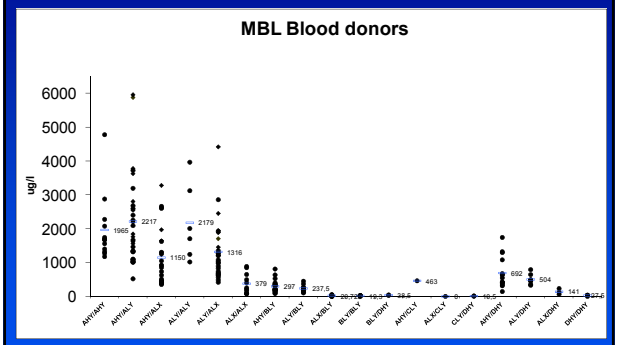
The MBL gene



Structural gene variants: A/B/C/D
Promotor region variants: L/H
Promotor region variants: X/Y



MBL deficiency – a disease modifier

MBL def.: Serum conc. <100 ng/mL.
Increased risk for infections reported in several disease states:

- Hypogammaglobulimenia
- Severe immunosuppresion
- Cystic fibrosis
- SLE
- Possible association with atherosclerosis
- May modify inflammatory disease such as rheumatoid arthritis

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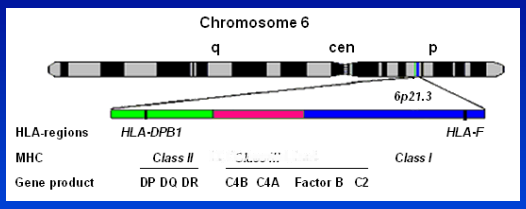
C2 genetics



Yu CY. et al., Exp Clin Immunogenet, 1998.

Nearly all cases of C2D (90%) are caused by a 28-bp deletion in the C2 gene, a mutation associated with the *HLA-B*18,S042,DRB1*15* haplotype – C2 deficiency type I

Principal manifestations of C2D in a Swedish study

- Invasive infections 57%
- Systemic lupus erythematosus 25%
- Cardiovascular disease 15%

C2 deficiency type I

- The predominant form of C2 deficiency (>95%)
- No C2 synthesis due to a 28 bp deletion in the C2 gene located in the MHC haplotype:
  *HLA-A*25, HLA-B*18, S042, DRB1*15*
- Prevalence of heterozygous carriers about 1.4% in Western countries
- Prevalence of homozygous deficiency about 1/20,000 in Western countries

C2 deficiency type II

- C2 secretion defect
- Small amounts of circulating C2 can be found
- Rare variants have been described
- Various MHC haplotypes

Clinical Immunology (2012) 144, 214–227

available at www.sciencedirect.com

Clinical Immunology

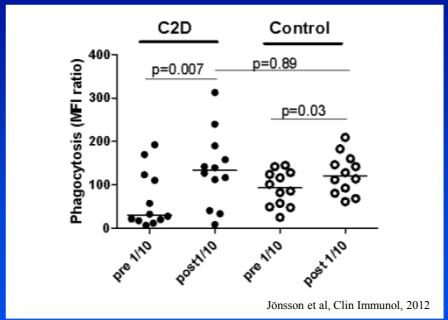
www.elsevier.com/locate/yclim

ELSEVIER

Vaccination against encapsulated bacteria in hereditary C2 deficiency results in antibody response and opsonization due to antibody-dependent complement activation

Göran Jönsson [a,*,1], Christian Lood [b,1], Birgitta Gullstrand [b], Eva Holmström [b], Barbro Selander [b], Jean Henrik Braconier [a], Gunnar Sturfelt [c], Anders A. Bengtsson [c], Lennart Truedsson [b]

Phagocytosis response in C2 deficiency



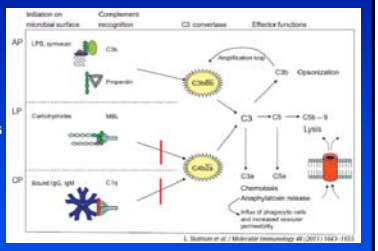
Jönsson et al, Clin Immunol, 2012

Increased phagocytosis of pneumococci by neutrophils in post vaccination serum

Complement activation in C2 deficiency

Blocked pathways:

- Classical pathway
- MBL and ficolin pathways (C4b2a)



C2 bypass activation

1: J Immunol. 1989 Oct 1;143(7):2256-61.

Lysis of sensitized sheep erythrocytes in human sera deficient in the second component of complement.

Knutzen Steuer KL, Sloan LB, Oglesby TJ, Farries TC, Nickells MW, Densen P, Harley JB, Atkinson JP.

The Journal of Clinical Investigation http://www.jci.org Volume 116 Number 5 May 2006

Related Commentary, page 1215 Research article

Mannan-binding lectin activates C3 and the alternative complement pathway without involvement of C2

Barbro Selander,[1] Ulla Mårtensson,[1] Andrej Weintraub,[2] Eva Holmström,[1] Misao Matsushita,[3] Steffen Thiel,[4] Jens C. Jensenius,[4] Lennart Truedsson,[1] and Anders G. Sjöholm[1]

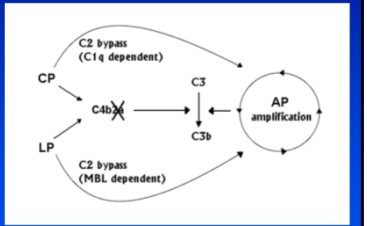
Complement activation in C2 deficiency

Blocked pathways:

- Classical pathway
- MBL and ficolin pathways (C4b2a)

Available pathways:

- Alternative pathway
- C1q-dependent C2 bypass
- MBL-dependent C2 bypass



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Adapted from: Sturfelt & Truedsson, Nature Rheumatol., 2012

Properdin deficiency

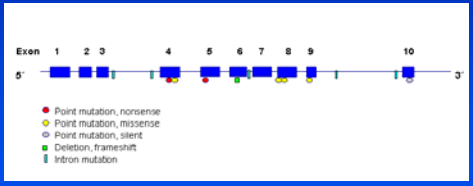
Three deficiency types

- type I: complete deficiency
- type II: low serum concentration (~10%)
- type III: dysfunctional properdin molecule

- X-linked inheritance
- >80 cases from 24 families are reported
- Increased risk for Nesserial infection

Properdin deficiency – heterogenous genetic background

- Type I – 10 different mutations found (exon 4, 5, 6, 7 and 8)
- Type II – 2 different mutations found (exon 4 and 8)
- Type III – 1 mutation in a Dutch family (exon 9)



Alternative pathway deficiencies

- Properdin, factor D, factor B
- Increased risk for Nesserial infection but not for bacterial infections in general
- Explanation - properdin as activation initiator?

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Assembly of C1q

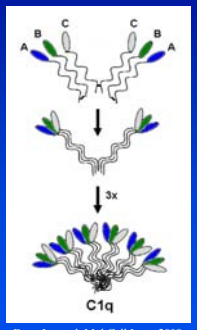
C1q 460 kD

C1q chains A, B and C

A/B heterodimers and C/C homodimers

Heterotrimeric unit

C1q with 18 chains



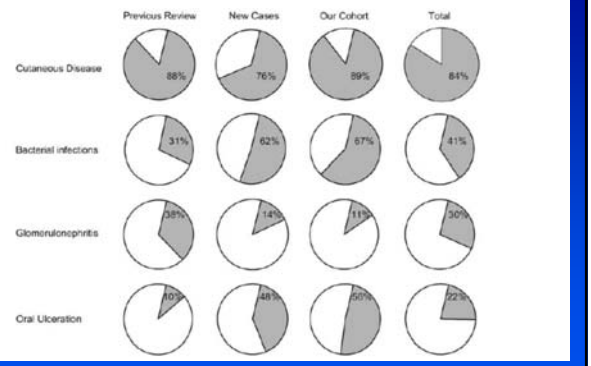
From Lu et al, Mol Cell Imm, 2008

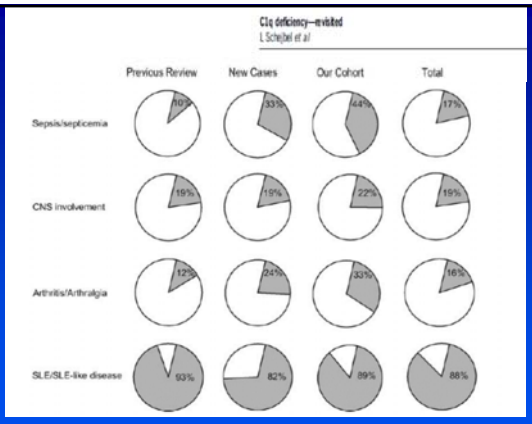
C1q deficiency – different mutations

Table 1 Mutations reported causing C1q deficiency

C1q chain	Mutation[a]	Systematic names	Old nomenclature[b]	Total number of families described	Origin of families
C1qA	**g.6149delG**	**Glu53fs**	Glu12fs	1	**Sweden** (this paper)
	g.7235C>T	**Gln64X**	**Gln42X**		
C1qA	**g.7667C>T**	**Gln208X**	**Gln186X**	8	**Iraq** (this paper) Turkey[6–8] Slovak Republic[16] Cyprus[17]
C1qA	**g.7693G>A**	**Trp216X**	Trp194X	1	**SuaLan** (this paper)
C1qB	g.11393G>A	Gly42Asp	Gly15Asp	1	Morocco[15]
C1qB	g.12965C>T	Arg 177X	Arg150X	1	Mexico[18]
C1qB	**g.13166G>A**	**Gly244Arg**	Gly217Arg	1	**Inuit**[14]
C1qC	g.5499G>A	Gly34Arg	Gly6Arg	5	Germany[19] India[14] Saudi Arabia[20] Caucasian[21] Arabian[14]
C1qC	g.5564delG	Gly55fsX83		1	Pakistan[22]
C1qC	**g.8626C>T**	**Arg69X**	**Arg41X**	2	**Kosova** (this paper) Yugoslavia[14]
C1qC	g.8633delC	Gln71fsX137	Gln43fs→108X	1	England[14]
C1qC	**g.8647G>A**	**Gly76Arg**	**Gly48Arg**	1	**Turkey**[23]

[a]According to NCBI reference sequence NG_007282 (C1qA), NG_007283 (C1qB) and NG_007565 (C1QC).
[b]Codon numbers according to original publications or Sellar et al.[4]
Mutations and origin of patients identified in our laboratory are in bold.





C1q deficiency in Sweden

8 known cases

5 children with C1q deficiency

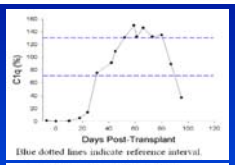
Infection proneness and SLE-like disease

Varying clinical symptoms

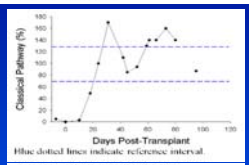
Treatment by hematopoietic stem cell transplantation has been used (2 cases)

ALLOGENEIC BONE MARROW TRANSPLANTATION RESTORES COMPLEMENT FUNCTION IN HUMAN C1q DEFICIENCY

Richard Olsson[1], Stefan Hagelberg[2], Olle Ringdén[1], Lennart Truedsson[3], Anders Ahlin[4]
[1]Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institutet, [2]Department of Pediatrics, Karolinska University Hospital, [3]Department of Laboratory Medicine, Section of Microbiology, Immunology and Glycobiology, Lund University, [4]Department of Pediatrics, Sachs' Children's Hospital, Sweden.



Blue dotted lines indicate reference interval.



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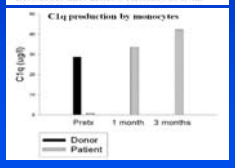


Autoantibodies disappeared

After the treatment with HCT the SLE-related autoantibodies disappeared as shown in the table

	Pre-transplant	Post-transplant 2 months	Post-transplant 3 months
IgG (g/L)	9	16	8
ANA (titer)	1600	Neg	Neg
Anti-Sm/RNP (IE/mL)	1.4	<0.1	<0.1
Anti-Sm (IE/mL)	3.8	1.2	<0.1

C1q deficiency in Sweden

A 9-year-old boy and a 12-year-old girl with refractory SLE.

C1q production was restored and decreased the severity of SLE symptoms decreased post-transplant.

The boy developed post-transplant lymphoproliferative disease and died from multiple organ failure four months post-transplant.

The girl is alive and well 24 months post-transplant, and all clinical symptoms of SLE have resolved.

Conclusions: Allo-HSCT can cure SLE in human C1q deficiency and should be considered early in patients with severe disease.

Olsson RF et al.: Allogeneic Haematopoietic Stem Cell Transplantation in the Treatment of Human C1q Deficiency: the Karolinska Experience. Transplantation, *in press*.

Successful cure of C1q deficiency in human subjects treated with hematopoietic stem cell transplantation

To the Editor:

Hematopoietic stem cell transplantation (HSCT) is used to cure a number of primary immunodeficiency diseases but has never been attempted for patients with complement deficiencies because most complement factors are produced in the liver rather than the bone marrow. Inherited deficiency of the C1q component of the classical complement pathway is a severe autosomal

J Allergy Clin Immunol 2014 Jan;133(1):265-7

J Autoimmun. 2015 Aug;62:39-44. doi: 10.1016/j.jaut.2015.06.002. Epub 2015 Jun 26.

Marked variability in clinical presentation and outcome of patients with C1q immunodeficiency.

van Schaarenburg RA[1], Schejbel L[2], Trouw LA[3], Topaloglu R[4], Al-Mayouf SM[5], Riordan A[6], Simon A[7], Kallel-Sellami M[8], Arkwright PD[9], Ahlin A[10], Hagelberg S[10], Nielsen S[11], Shayesteh A[12], Morales A[13], Tam S[14], Genel F[15], Berg S[16], Ketel AG[17], Merlijn van den Berg J[18], Kuijpers TW[19], Olsson BE[16], Huizinga TW[1], Lankester AC[20], Trouw LA[21].

Author information

Abstract

OBJECTIVE: Globally approximately 60 cases of C1q deficiency have been described with a high prevalence of Systemic Lupus Erythematosus (SLE). So far treatment has been guided by the clinical presentation rather than the underlying C1q deficiency. Recently, it was shown that C1q production can be restored by allogeneic hematopoietic stem cell transplantation. Current literature lacks information on disease progression and quality of life of C1q deficient persons which is of major importance to guide clinicians taking care of patients with this rare disease.

METHODS: We performed an international survey, of clinicians treating C1q deficient patients. A high response rate of >70% of the contacted clinicians yielded information on 45 patients with C1q deficiency of which 25 are published.

RESULTS: Follow-up data of 45 patients from 31 families was obtained for a median of 11 years after diagnosis. Of these patients 36 (80%) suffer from SLE, of which 16 suffer from SLE and infections, 5 (11%) suffer from infections only and 4 (9%) have no symptoms. In total 9 (20%) of the C1q deficient individuals had died. All except for one died before the age of 20 years. Estimated survival times suggest 20% case-fatality before the age of 20, and at least 50% of patients are expected to reach their middle ages.

CONCLUSION: Here we report the largest phenotypic data set on C1q deficiency to date, revealing high variance; with high mortality but also a subset of patients with an excellent prognosis. Management of C1q deficiency requires a personalized approach.

Treatment of infection susceptibility in complement deficiency

- Increased awareness (antibiotics etc).
- Substitution therapy.
  - Plasma infusion (C1q, C2 and C3)
  - Purified plasma MBL has been tried as treatment (cystic fibrosis).
  - Recombinant MBL has been developed (tested in volonteers).
  - Recombinat C2 has been developed, not tested in humans.
- Vaccination.

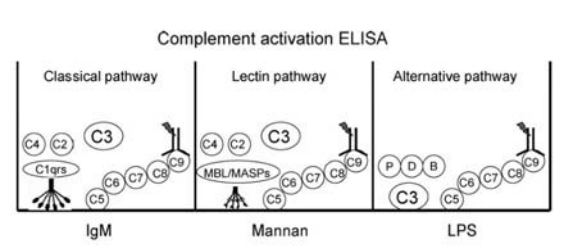
How to find the complement deficient patients

- Clinical symptoms indicating immunodeficeincy
- Complement analysis with screening for deficiency

Analysis of complement function to detect deficiency

- CH50, AP50
- Hemolysis in gel (HIG)
- ELISA

Analysis of complement function



Mollnes et al, Mol Immunol, 2007

Analysis of complement function

Table 1
Complement deficiency and detection by complement activation ELISA

Impaired function/deficiency	Activity in ELISA		
Component	Classical	Lectin	Alternative
C1q, C1r, C1s	Low	Normal	Normal
C4, C2	Low	Low	Normal
MBL, MASP2	Normal	Low	Normal
B, D, P	Normal	Normal	Low
C3, C5, C6, C7, C8, C9	Low	Low	Low

Mollnes et al, Mol Immunol, 2007

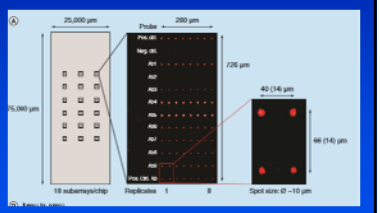
Andra metoder –

arrays?

Miniaturization of multiplexed planar recombinant antibody arrays for serum protein profiling.

Petersson L, Coen M, Amro NA, **Truedsson** L, Borrebaeck CA, **Wingren** C.

Bioanalysis. 2014 May;6(9):1175-85. doi: 10.4155/bio.13.342.

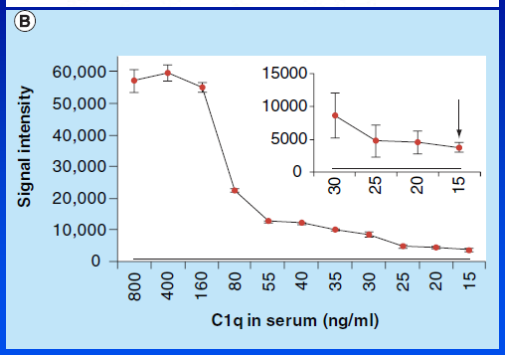


SLIPI, 2015

Bioanalysis. 2014 May;6(9):1175-85. doi: 10.4155/bio.13.342.

Miniaturization of multiplexed planar recombinant antibody arrays for serum protein profiling.

Petersson L[1], Coen M, Amro NA, Truedsson L, Borrebaeck CA, Wingren C.



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Heat differentiated complement factor profiling

Carl Hamsten [A,b], Lillemor Skattum [c], Lennart Truedsson [c], Ulrika von Döbeln [d], Mathias Uhlén [e], Jochen M. Schwenk [e], Lennart Hammarström [f], Peter Nilsson [e], Maja Neiman [e,*]

ARTICLE INFO

ABSTRACT

Complement components and their cascade of reactions are important defense mechanisms within both innate and adaptive immunity. Many complement deficient patients still remain undiagnosed because of a lack of high throughput screening tools. Aiming towards neonatal proteome screening for immunodeficiencies, we used a multiplex profiling approach with antibody bead arrays to measure 9 complement proteins in serum and dried blood spots. Several complement components have been described as heat sensitive, thus their heat-dependent detectability was investigated. Using sera from 16 patients with complement deficiencies and 23 controls, we confirmed that the proteins C1q, C2, C3, C6, C9 and factor H were positively affected by heating, thus the identification of deficient patients was improved when preheating samples. Measurements of C7, C8 and factor I were negatively affected by heating and non-heated samples should be used in analysis of these components. In addition, a proof of concept study demonstrated the feasibility of labeling eluates from dried blood spots to perform a subsequent correct classification of C2-deficiencies. Our study demonstrates the potential of using multiplexed single binder assays for screening of complement components that open possibilities to expand such analysis to other forms of deficiencies.

C. Hamsten et al. / Journal of Proteomics 126 (2015) 155–162

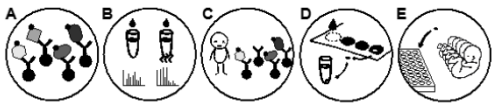

Figure 1 – Overview of heat differentiated complement factor profiling.

A) Parallel detection of complement factors is enabled by using antibody bead arrays and direct labelling of serum or plasma proteins.
B) A preanalytical heating of diluted and labelled serum was found to influence complement measurements.
C) Sera from patients with complement deficiencies were analysed and used to evaluate the assay.
D) Eluates from dried blood spots was labelled and profiled in the assay which enables
E) large-scale neonatal screening for complement deficiencies.

- Works with blood spots from Guthrie cards?

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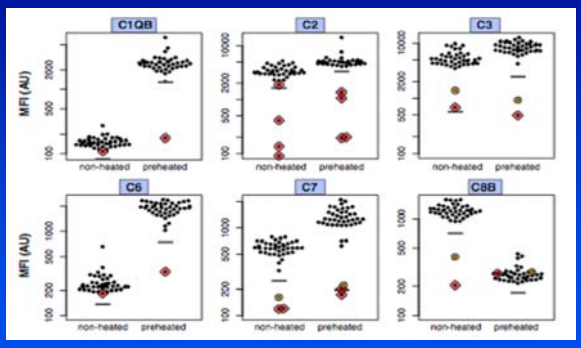


Complement deficiency – take home message

- The consequence varies from almost none as in C9D to serious disease/infections as in e g C1q D.
- Classical pathway def. but no other complement def. are associated with SLE and SLE-like disorders.
- Classical pathway def. confer increased susceptibility for infections with encapsulated bacteria.
- Alternative and terminal pathway def. confer increased susceptibility for infections with Neisseria species.
- Lectin pathway def. is associated with many types of infections in presence of a concomitant immune deficiency.
- The identification of complement def. states is important to ensure optimal prevention and treatment.
- Vaccination against the bacteria commonly causing the infections is important.
- Supplementation of the missing component has so far been tried in very few cases, but should hopefully have a place in the treatment of these patients in the future.
- HSCT may be an option in treatment of complete C1q deficiency.

Factor H deficiency

Results in desreased C3 concentration

Associated with atypical hemolytic uremic syndrome (aHUS)

Recurrent pyogenic infections (*N. meningitidis, H. influenzae*)

Typ I phenotype
Factor H mutation causing a structurell defect - low factor H concentration.

Typ II phenotype
Factor H mutation giving rise to a functional defect.

Factor I deficiency

Results in desreased C3 concentration

Recurrent infections mainly of the respiratory tract

Various immune-complex related diseases (glomerulonephritis, vasculitis, SLE)

Clearance of apoptotic cells and SLE

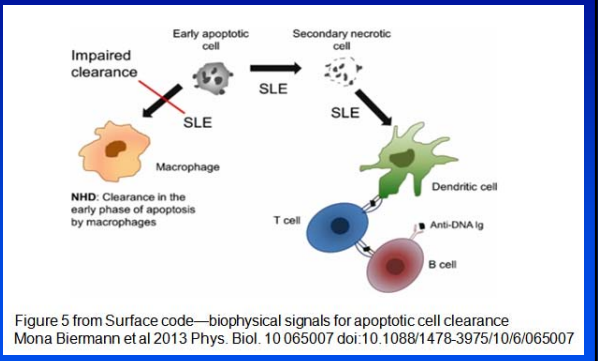
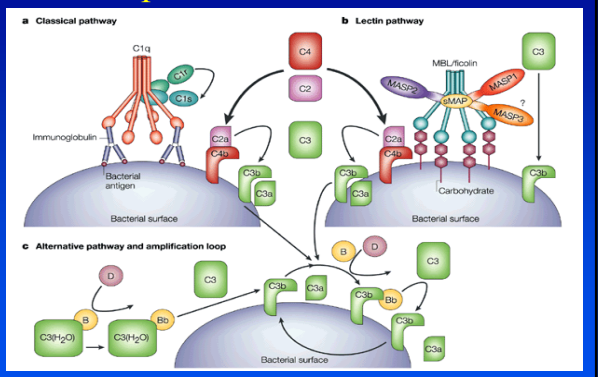


Figure 5 from Surface code—biophysical signals for apoptotic cell clearance
Mona Biermann et al 2013 Phys. Biol. 10 065007 doi:10.1088/1478-3975/10/6/065007

Complement activation -> C3



Nature Reviews Microbiology 3, 948-958 (December 2005)

Infections in C2 deficiency: prophylaxis

- Long-term antibiotic treatment
- Vaccination against infection with encapsulated bacteria (*S. pneumoniae*, *N meningitidis and H. influenzae* type b).

Complement genes

Genes for complement proteins in different chromosomes, examples

Chromosome	Complement protein
1p	C1q (3 chains: C1qA, C1qB, C1qC) C8 (alpha and beta chain)
1q (RCA gene cluster)	CR1, CR2, C4bp, DAF, MCP, H (all regulatory proteins)
6p (MHC class III)	B, C2, C4 (exists as two isotypes, C4A and C4B)
9q	C8 (gamma chain)
X	Properdin

- Several of the complement proteins exist in different genetic variants.
- The complement proteins encoded by genes located in the MHC class III region display a high degree of polymorphism.