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PEARLS OF LABORATORY MEDICINE

Clinical Applications of Complement Testing

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CCTC Clinical Chemistry Trainee Council

Learning Objectives

- List the types of diseases associated with complement testing
- Understand the role of functional and antigen testing in differentiating primary complement defects
- Provide guidance to physicians about selecting the appropriate complement test(s)

What is the Normal Role of the Complement System?

Innate Immunity

- Opsonization of microorganisms
- Neutralization of viruses
- Signals inflammatory cells

Waste Disposal

- Clearance of apoptotic cells
- Clearance of immune complexes

Diseases Related to Complement Testing

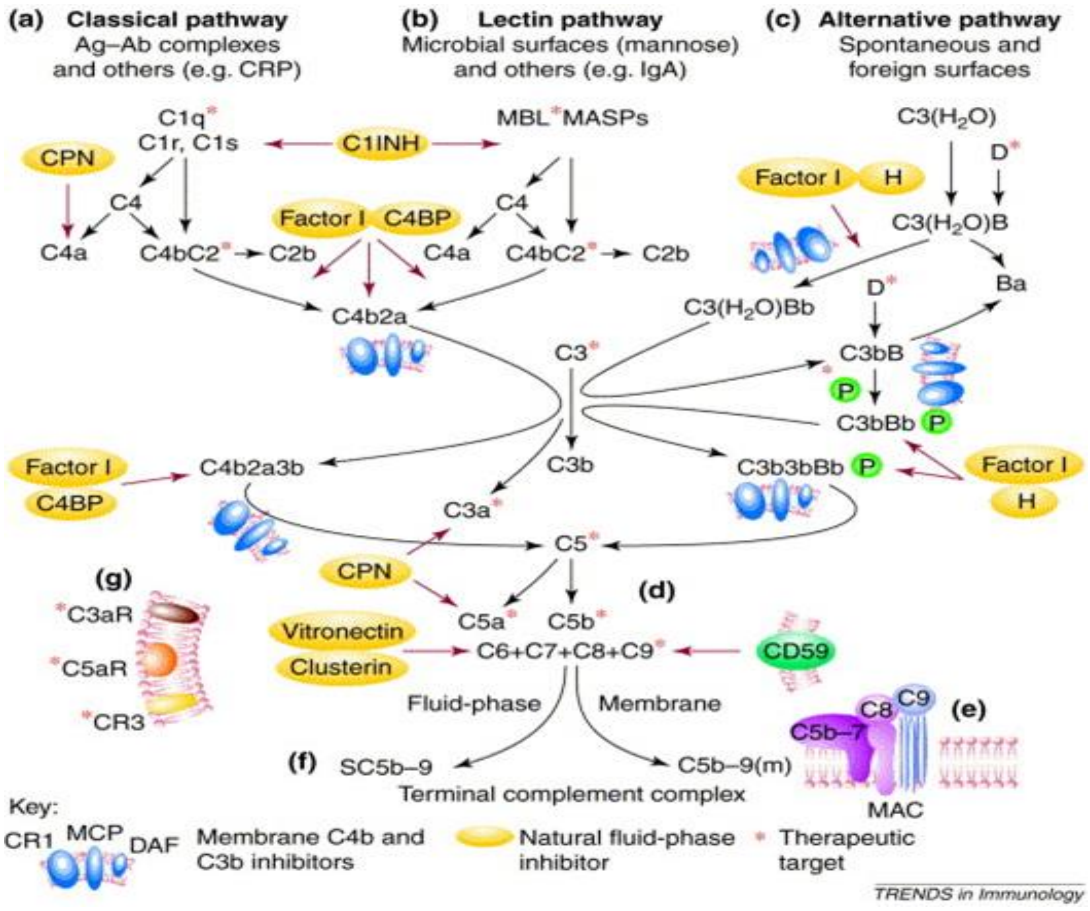
Low Activity

- Immunodeficiency – recurrent infections (Neisseria)
- Unable to clear immune complexes – early autoimmune diseases (SLE)

Unregulated Activity

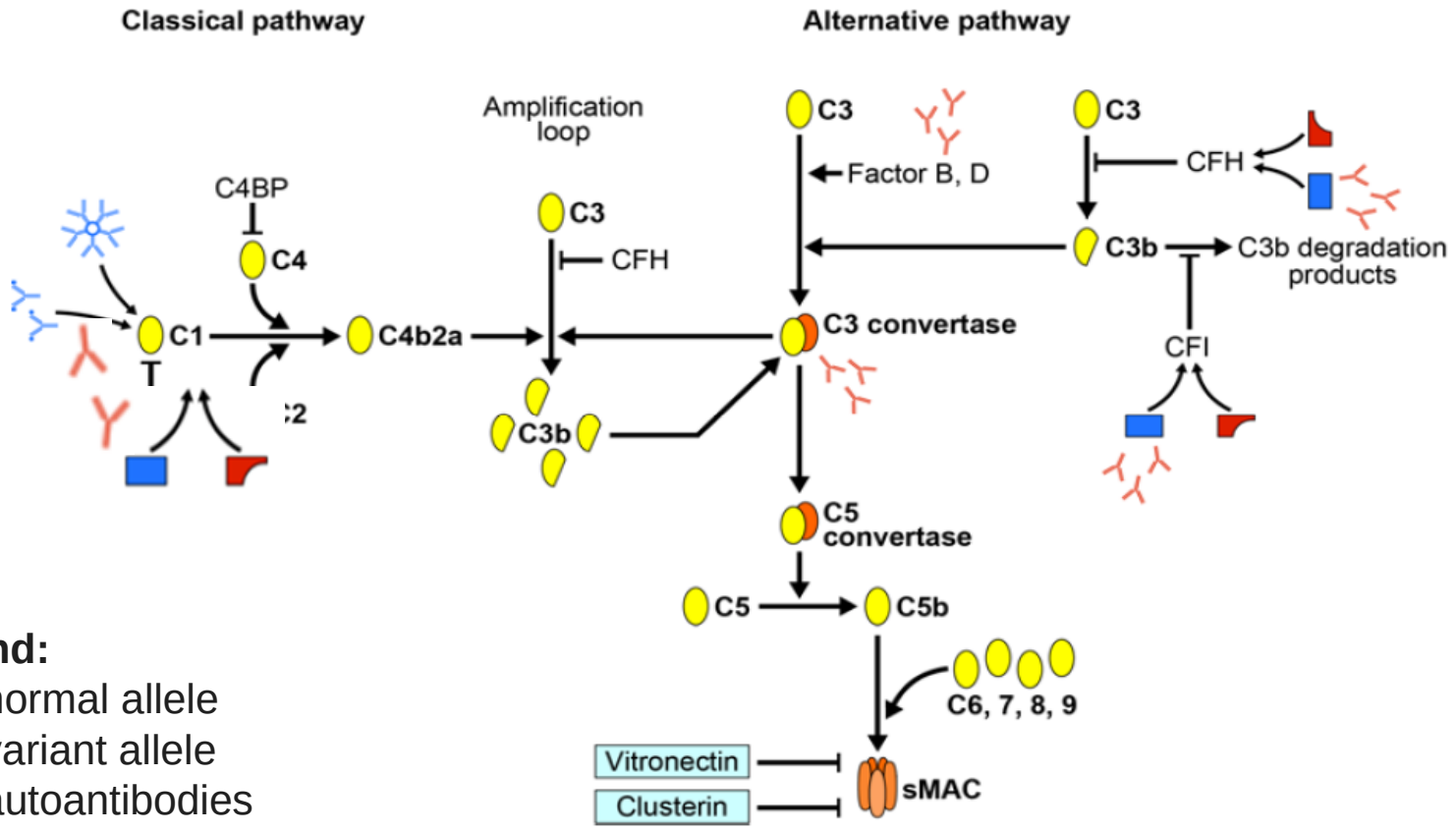
- Increased cellular destruction
  - Red cells – Paroxysmal Nocturnal Hemoglobinuria (PNH)
  - Renal glomerulus – atypical HUS, dense deposit disease, C3-glomerulonephritis
- Increase in vasoactive peptides
  - Angioedema (Hereditary or Acquired)

Complement: A Well-Regulated System



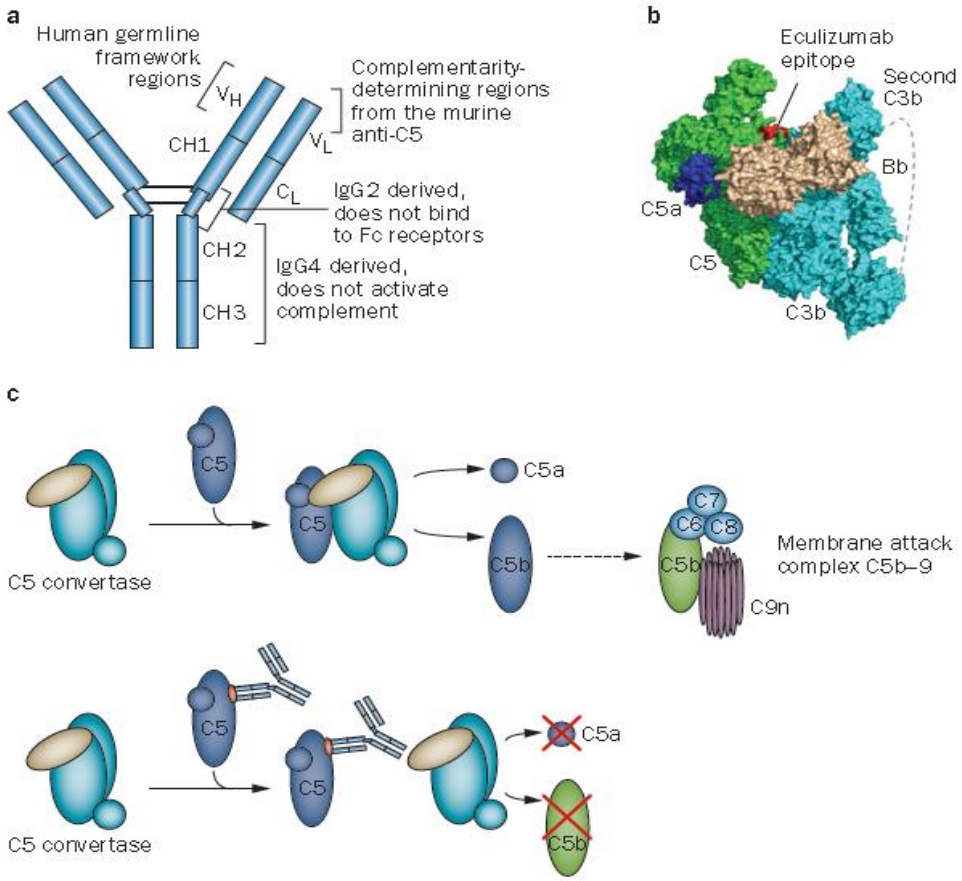
Tom Mollnes, et al. Complement in inflammatory tissue damage and disease. Trends in Immunology 2002; 23: 61-4 *(reproduced with permission).*

Primary Complement Defects



Sethi S, Fervenza F. Membranoproliferative Glomerulonephritis: A New Look at an Old Entity. NEJM 2012; 366: 1119-31 *(adapted and reproduced with permission).*

Eculizumab: Anti-C5 Monoclonal Antibody



Zuber J, et al. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. Nat Rev Nephrol 2012; 8: 643 (*reproduced with permission*).

General Overview of Primary Complement Defects

Protein Defect		Regulator Protein Defect		Factor Proteins Defect	
		Alternative Pathway	Classical Pathway	Alternative Pathway	Classical Pathway
Germ Line (Peds)	Target System	Renal (TMA) Eyes	Endothelium Red Cells	Innate Immunity	
	Diseases	DDD,aHUS,C3 –GN, MD	PNH, HAE	Rare	URIs, Otitis Media, SLE,RA
Acquired (Adult; Auto-Antibody)	Target System	Renal	Endothelium	Renal	Rare
	Diseases	C3 GN	Acquired angioedema	C3 Nephritic Factor	?

TMA – Thrombotic microangiopathy, DDD – Dense Deposit Disease, aHUS – atypical hemolytic uremic syndrome,

PNH – Paroxysmal Nocturnal Hemoglobinureia, HAE – Hereditary Angioedema, MD – Macular Degeneration, C3 GN – C3 Glomerulonephritis

Two Major Categories of Complement Tests

Quantitation
How much protein?

Examples:
C3, C4, C1q, Factor H, Factor B

Results in mg/dL

Functional Testing
Is the protein working?

Testing the Cascade

CH50 – Classical Pathway
AH50 - Alternative Pathway
sC5-C9 (sMAC)

Individual Factors

Examples:
C3, C4, C9, Factor H

Results in IU or percent

Pre-Analytical Variables and Specimen Collection Are Critical

- Compounds which would stabilize complement are not routinely available
- The complement system is easy to activate post-blood draw
  - Samples should be frozen as soon as possible after removing cellular components
- Specimens from patients post-apheresis or who are on complement inhibitors are unacceptable
- Abnormal results should be repeated

Please see "Basics of Complement Testing" Pearl by Dr. Maria Willrich, available at www.traineecouncil.org.

Testing for Complement Defects

Primary Symptoms – Immunodeficiency

- **Pediatrics**
  - **Presentation:** children with recurring infections
  - **Screening:** total hemolytic or CH50 assay
  - **Follow-up:**
    - C1, C4, C5, C6, C7, C8, and C9 levels and function
    - Consider genetic testing of complement proteins
- **Adults**
  - **Presentation**: association with complement is rare
  - **Screening:** total hemolytic or CH50
  - **Follow-up:** same as pediatrics

Testing for Complement Defects

Primary Symptoms - Systemic Lupus Erythematosus

- **Pediatrics**
  - **Typical presentation:** malar rash, positive dsDNA antibodies
  - **Screening:** Total hemolytic or CH50 assay and C1q, C2 and C4 levels
  - **Follow-up:** C3 and C4 levels
- **Adults**
  - **Typical presentation:** malar rash, positive dsDNA antibodies
  - **Screening and Follow-up:** C3 and C4 levels

Testing for Complement Defects

Primary Symptoms - Thrombotic Microangiopathy (TMA) with Renal Disease

- **Pediatrics**
  - Dense Deposit Disease, complement-mediated HUS
  - **Screening:**
    - CH50
    - AH50
    - C3 and C4
    - SC5-C9 (sMAC)
    - Split products (Bb, C4d)
    - Factor H and B
  - **Classical Findings:**
    - Low AH50, Factor H, Factor B
    - Elevated SMAC, Bb
    - Normal C4

Testing for Complement Defects

Primary Symptoms - Thrombotic Microangiopathies (TMAs) with Renal Disease

- **Adults**
  - TTP vs. diarrhea-associated HUS vs. complement-related HUS vs. C3-glomerulonephritis
  - **Screening:**
    - ADAMTS-13 negative
    - Shiga Toxin negative
      - AH50, CH50, C3, C4, Factor H, Factor B
- **Collect specimen before apheresis!**

Classical Atypical-HUS Findings

- AH50 activity – below normal
- Factor H and/or B – below normal
- C4 protein level and C4d – normal
- Factor Bb and sMAC - elevated

Follow-up Testing for Alternative Pathway Defects

- Antibodies to Factor H
- C3 Nephritic Factor
- Genetic testing of complement proteins

Testing for Complement Defects

Primary Symptoms – Recurrent Angioedema without Uticaria or Pruritis (Upper Airway)

- Hereditary Angioedema (HAE)
  - Screening:
    - C4 level, C1INH level, C1INH function
    - Not on treatment
  - During attack and recovery:
    - C4 should be low during attack
    - C1INH level normal and function are low suggests genetic abnormality (<40 years, acquired)
    - C1INH level is low and > 40 years, acquired

Testing for Complement Defects

Paroxysmal Nocturnal Hemoglobinurea (PNH)

- Loss of complement inhibitors (CD 59 and CD 55) on the surface of red cells
- Red cell destruction leads to hemoglobinurea
- Diagnosis made by evaluation of red and white blood cells via flow cytometry

Summary

- Complement-associated diseases are less common in practice and the appropriate tests to order is not widely understood by physicians
- Keep a complement cascade chart readily accessible to help guide utilization
- **Proper specimen collection is KEY to accurate results**

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Disclosures/Potential Conflicts of Interest

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