# Hemolytic Uremic Syndrome

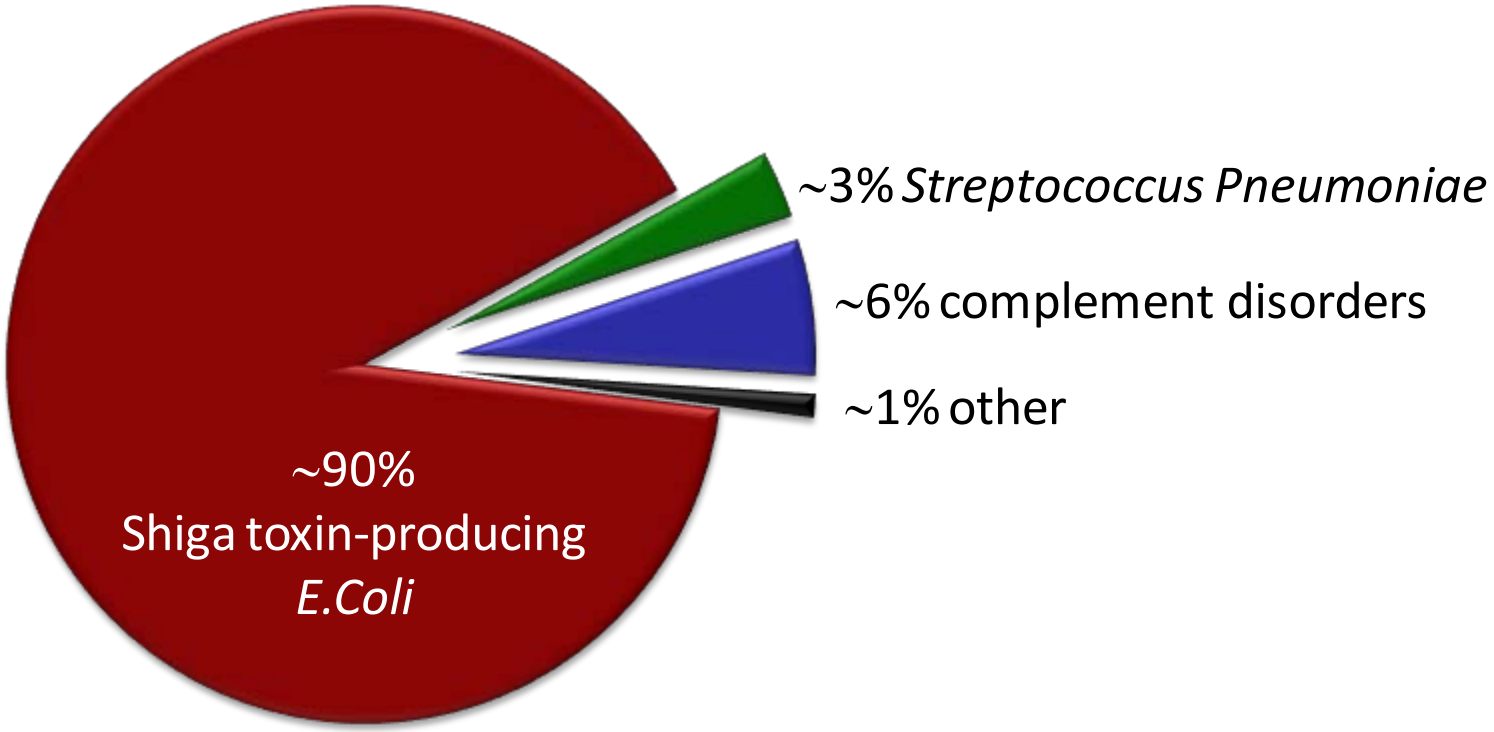
Francesco Emma

*Division of Nephrology and Dialysis*
*Bambino Gesù Children's Hospital, IRCCS*
*Rome, Italy*

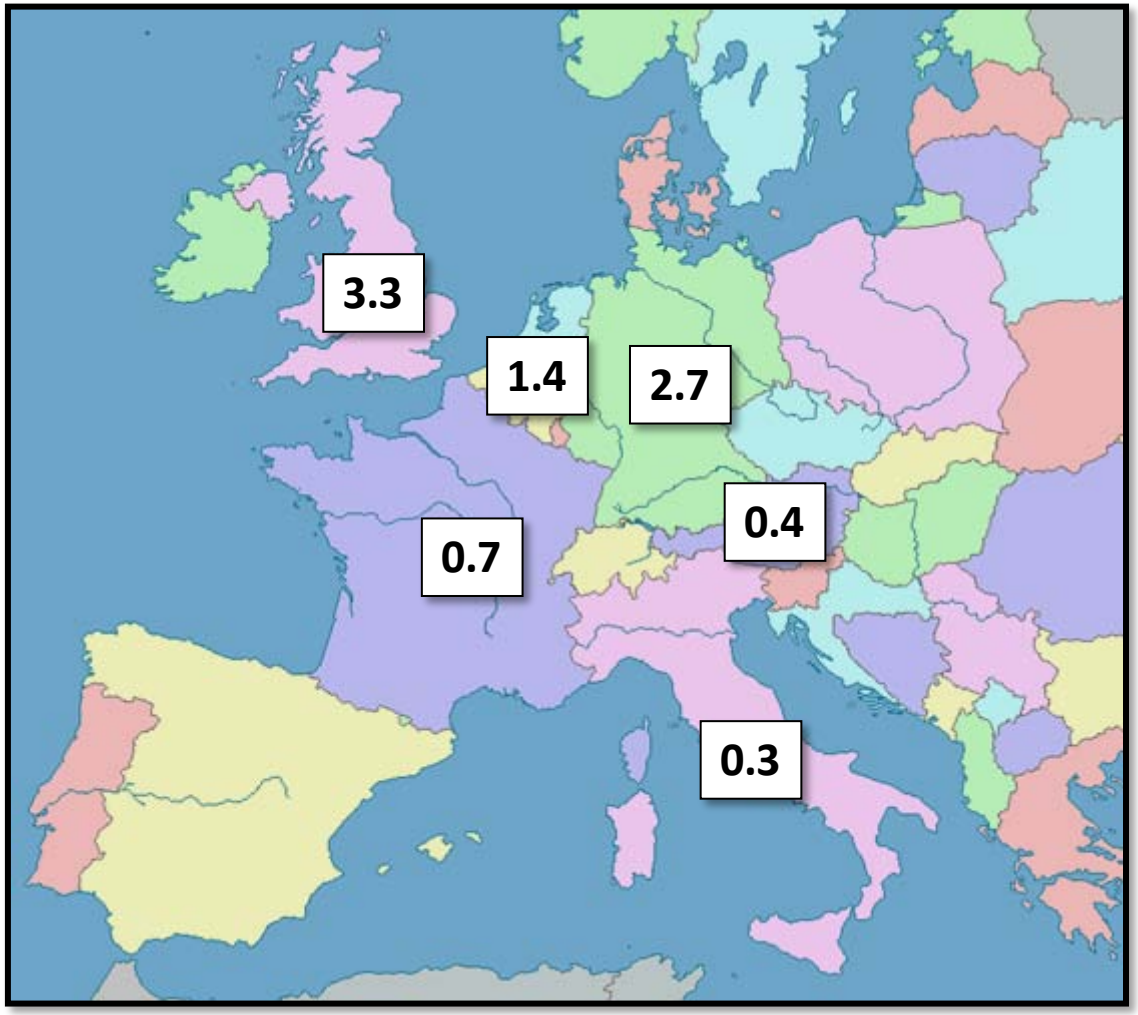
Bambino Gesù
OSPEDALE PEDIATRICO

# Hemolytic Uremic Syndrome (HUS)

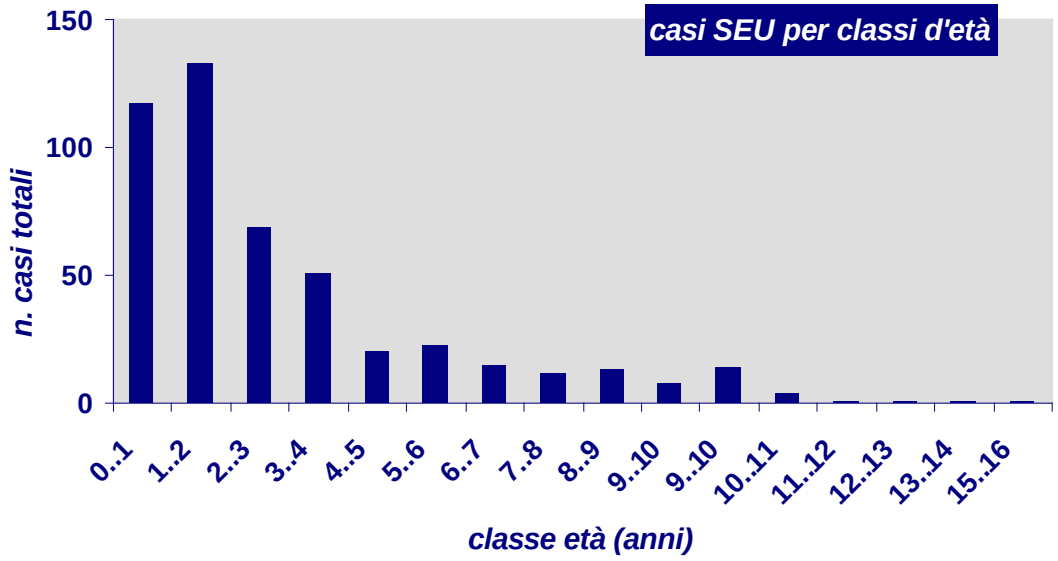
microangiopathic hemolytic anemia
thrombocytopenia
acute nephropathy

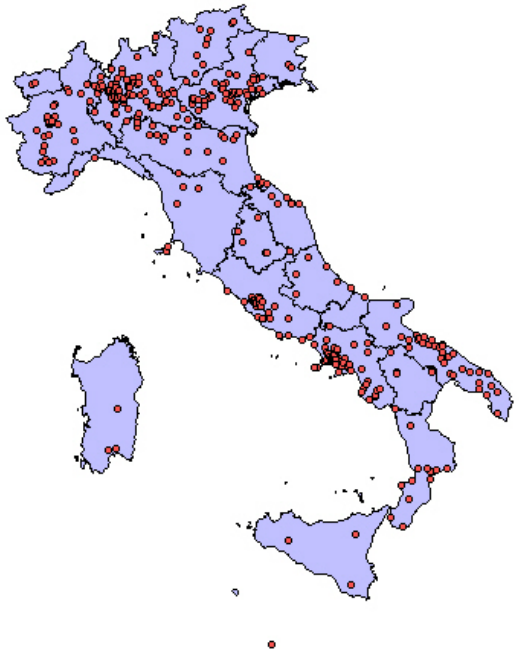


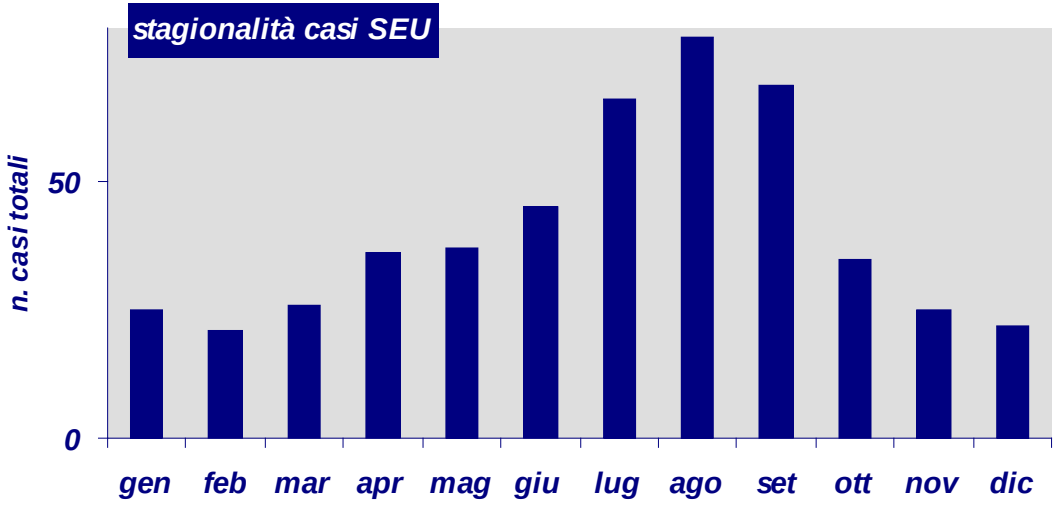
# HUS: incidence (cases / 100,000 x year)



# Italian Registry of Hemolytic Uremic Syndrome






N=481

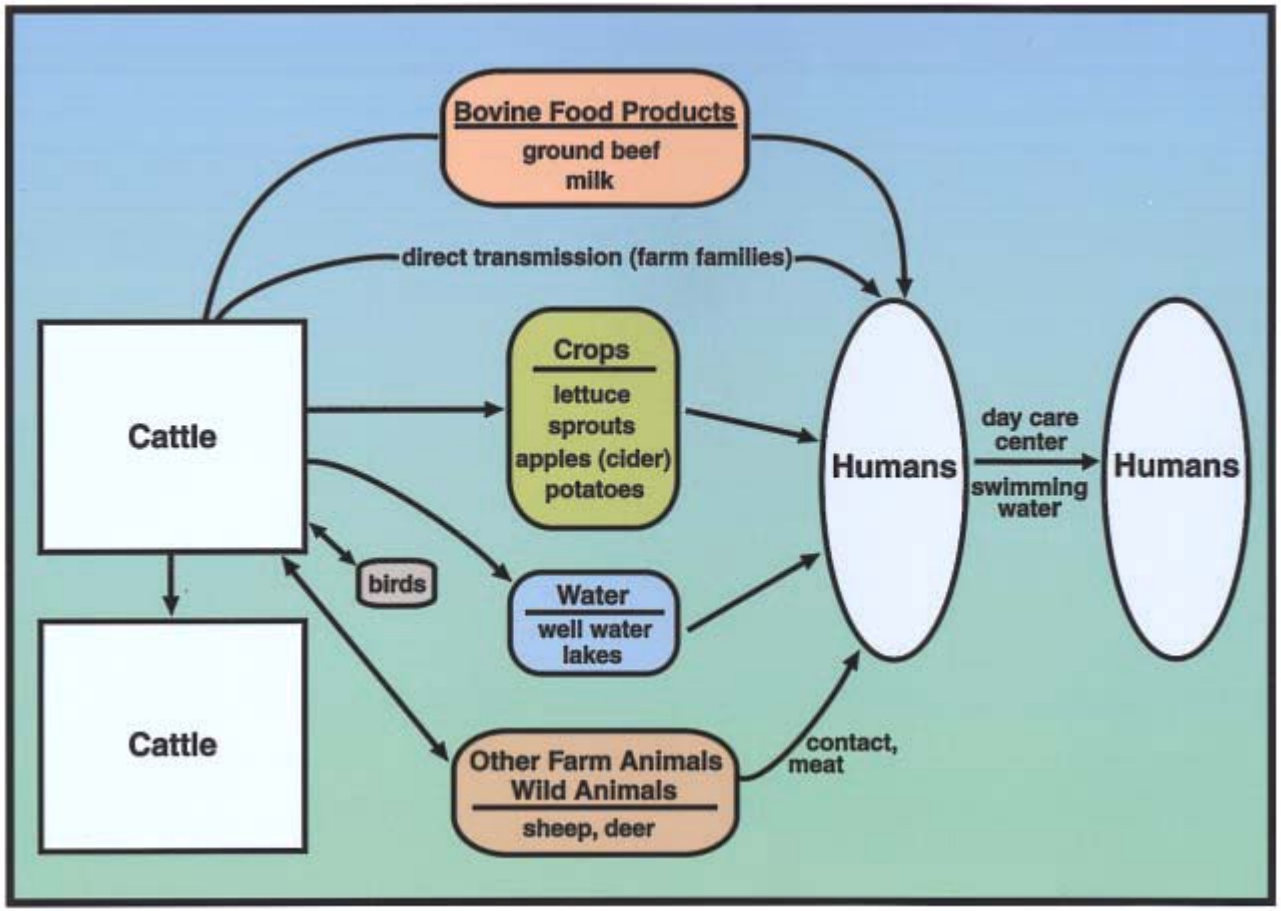
53:47 M:F

Median age : 23 months

< 5 years = 80% of cases

< 2 years = 41% of cases

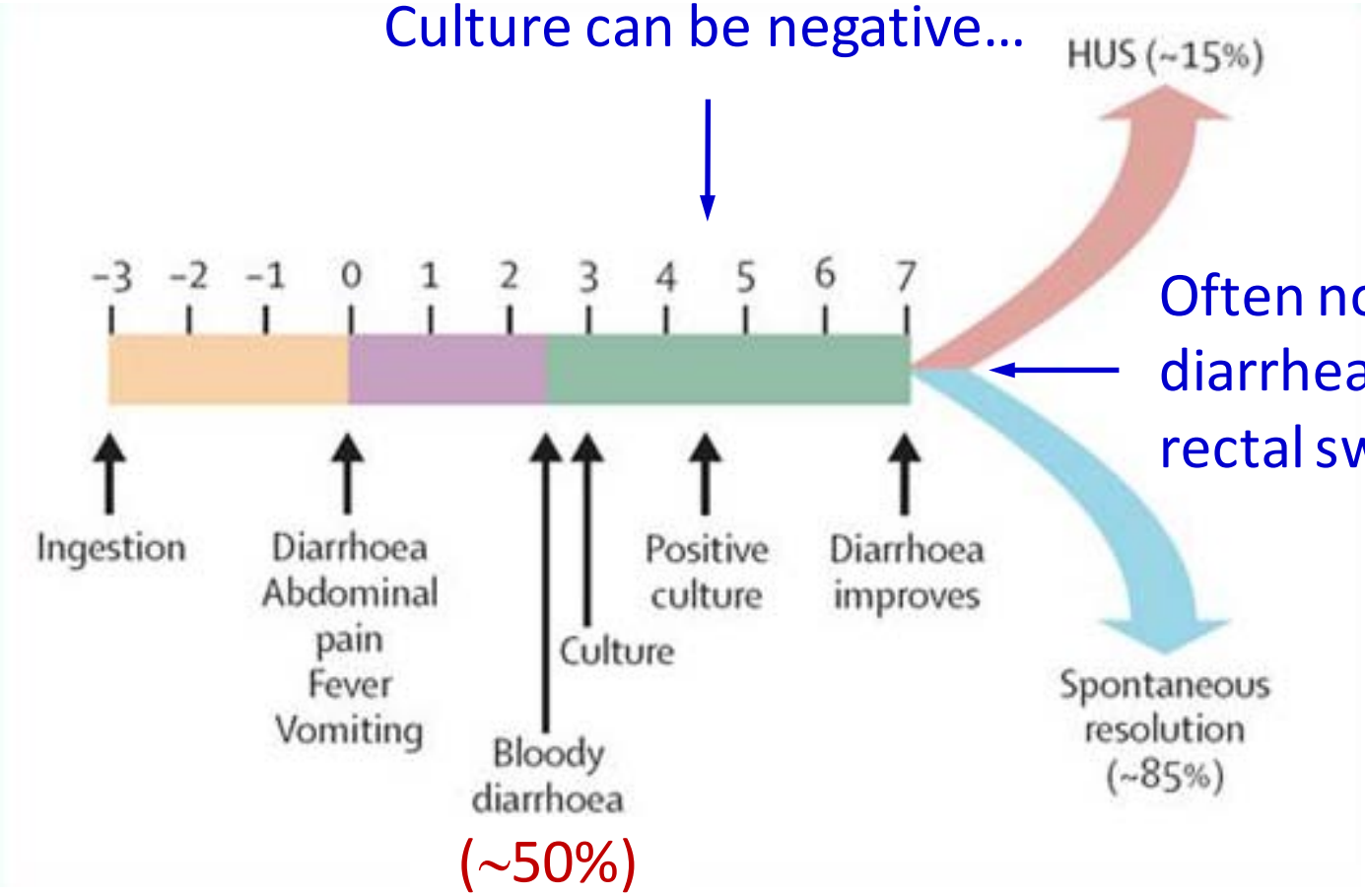
# Model of transmission of *E. Coli* O157:H7 from cattle to humans




Gansheroff, and O'Brien, Proc Natl Acad Sci USA, 2000

# E. coli O157:H7 Infection: time course

Often antibiotics
Culture can be negative…




(~50%)

Often no longer diarrhea; obtain a rectal swab…

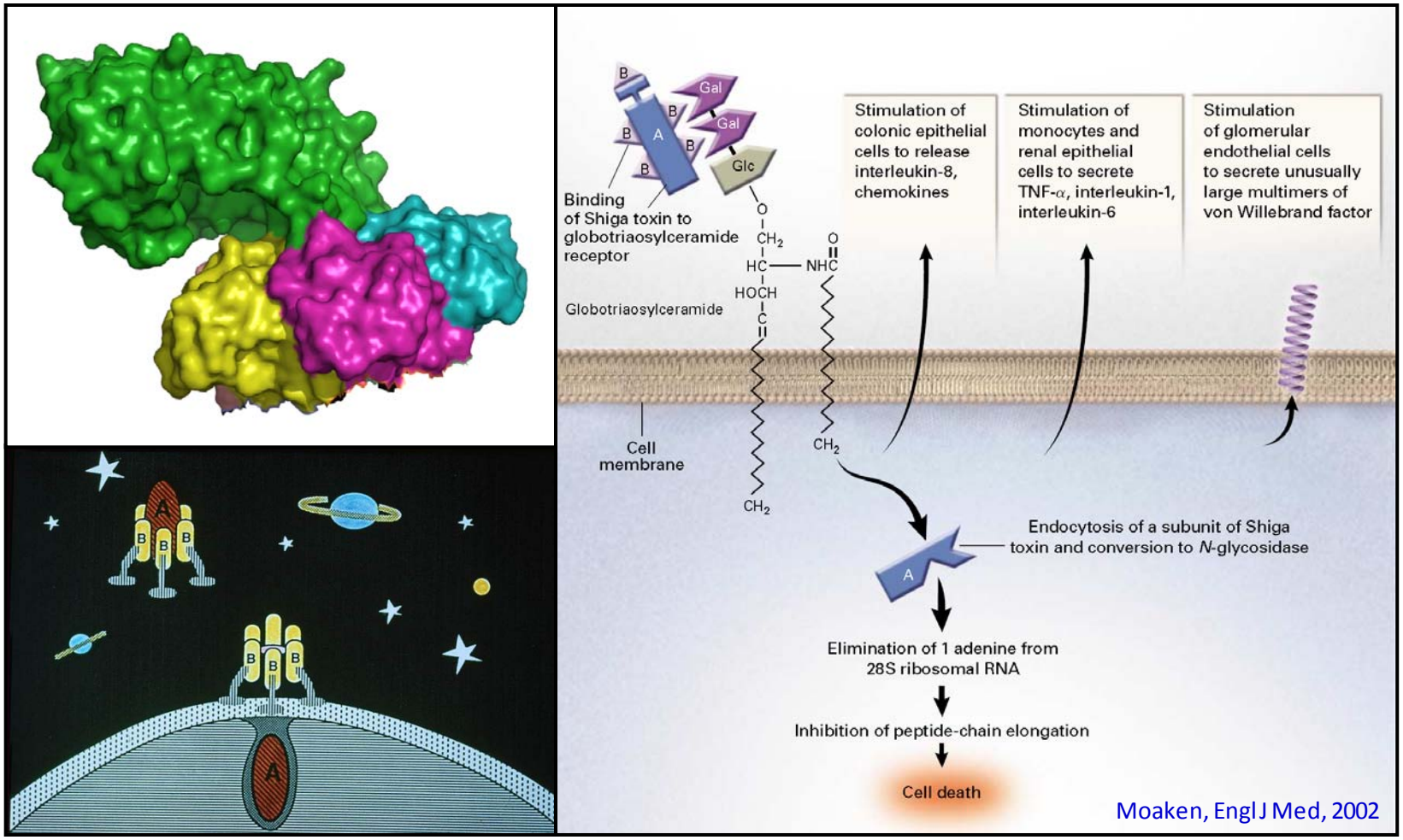
Tarr P, Lancet 2005

# Shiga toxins

- ST1: identical to toxin of Shigella
- ST2: 56% homology; 30X more potent
- E.coli O157:H7 ~100% ST2; 25-80%ST1
- Non O157 STEC
  - O111:H8 (Colitis)
  - O26:H11
  - O103:H2 (UTI)
  - O103:H3
  - other
- Single A subunit, 5 B subunits

Brooks JT et al, Clin Infect Dis, 2004; Tarr PI et al, NEJM,1996

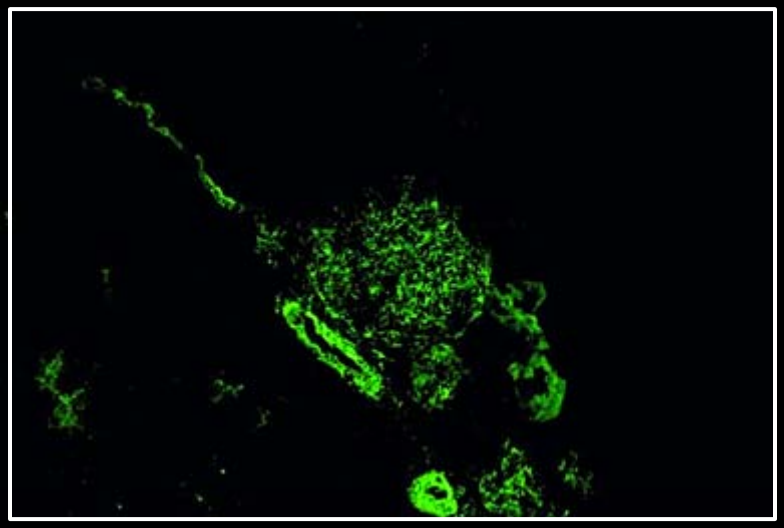
# Shiga toxins



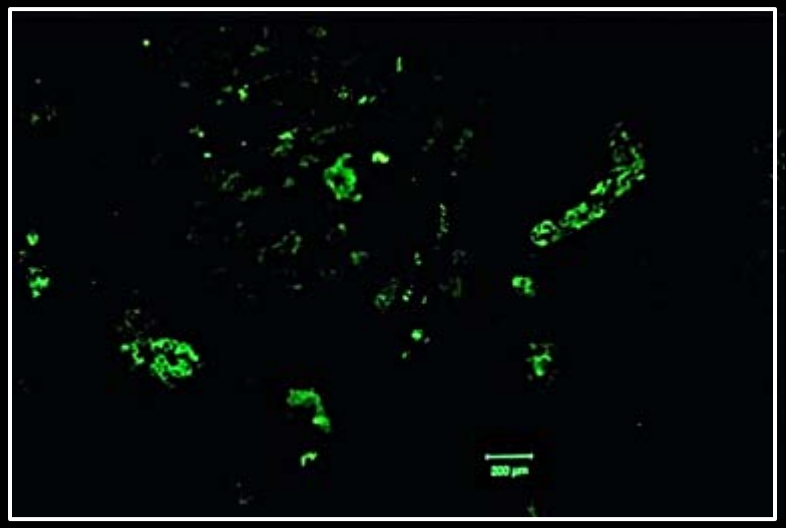

Moaken, Engl J Med, 2002

# Gb3 expression in human kidneys

Pediatric

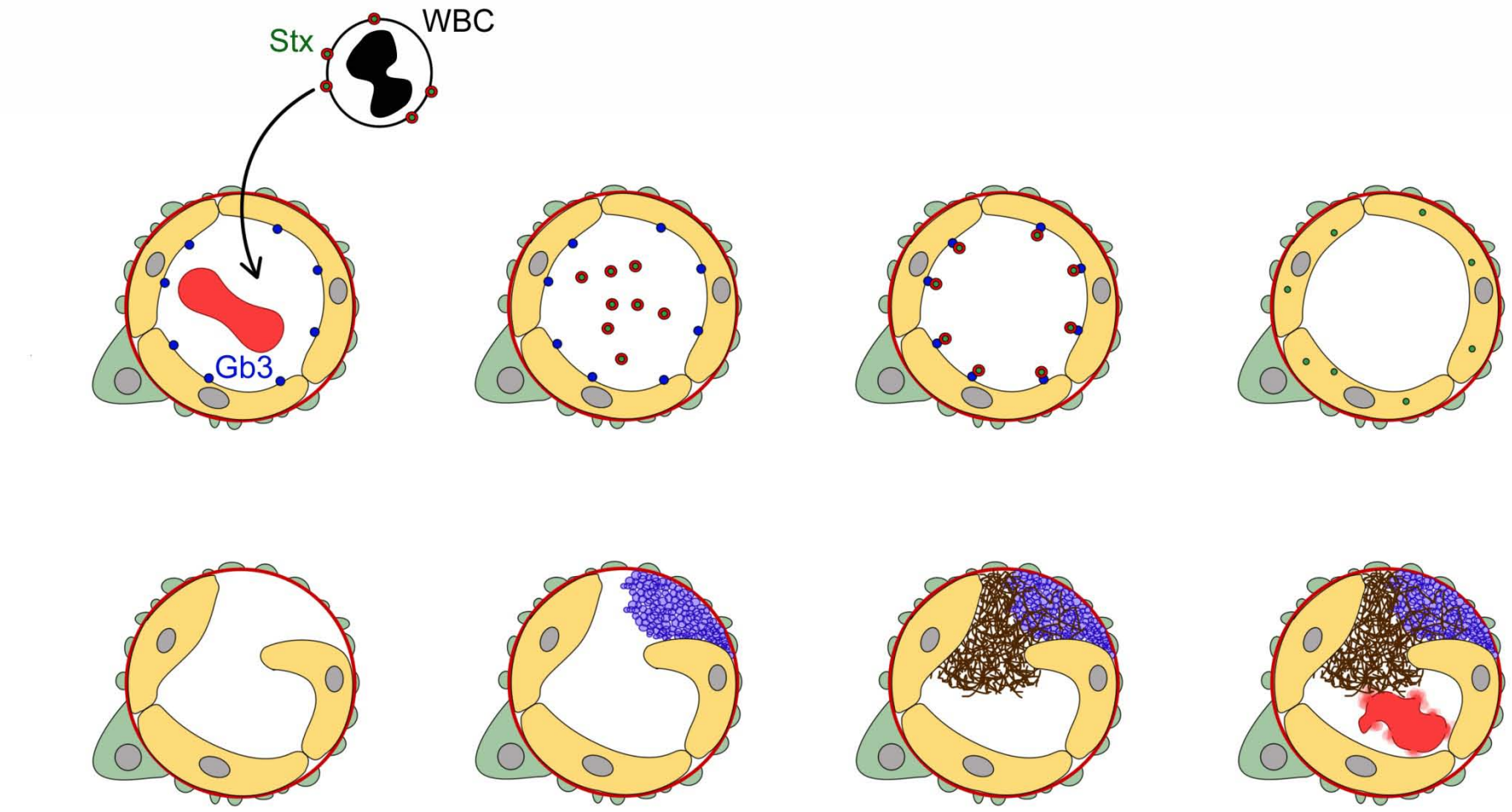


Adult

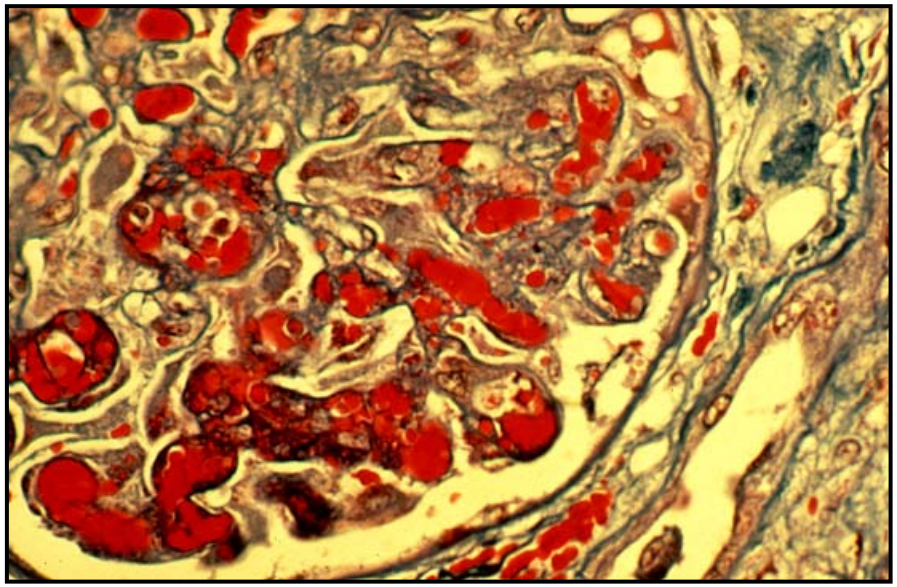


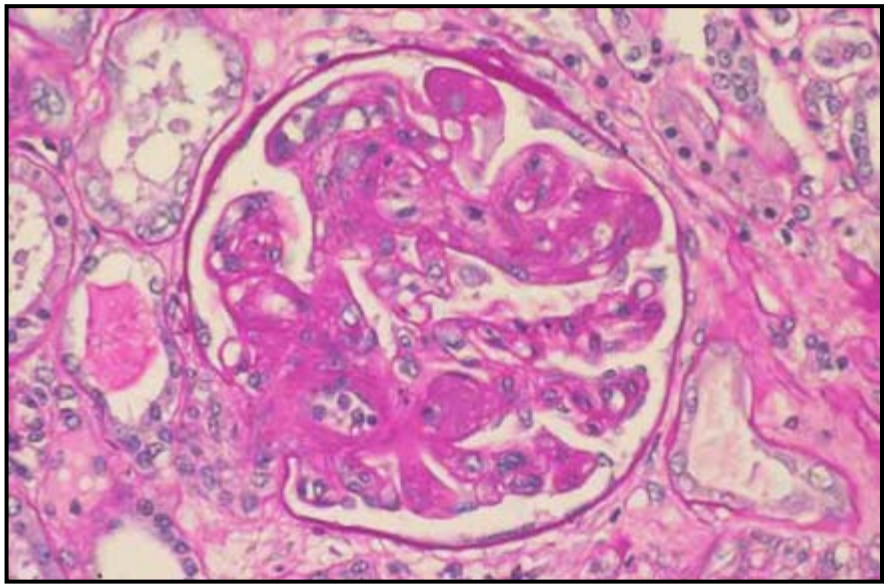

Lingwood CA, 2000

# STEC-HUS

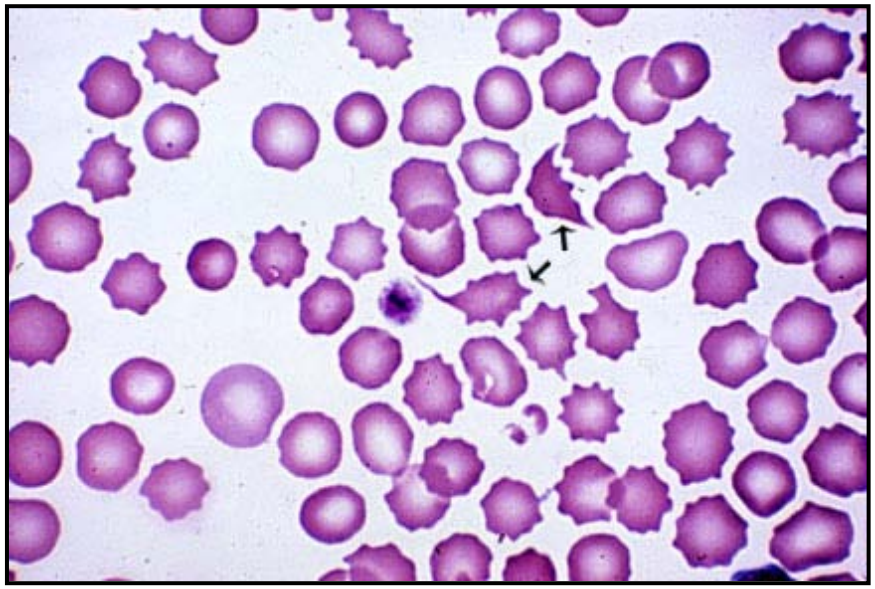

Stx
WBC
Gb3
cell swelling and death
endothelial cell detachment
basement membrane exposure
platelet activation
fibrin deposition
RBC destruction

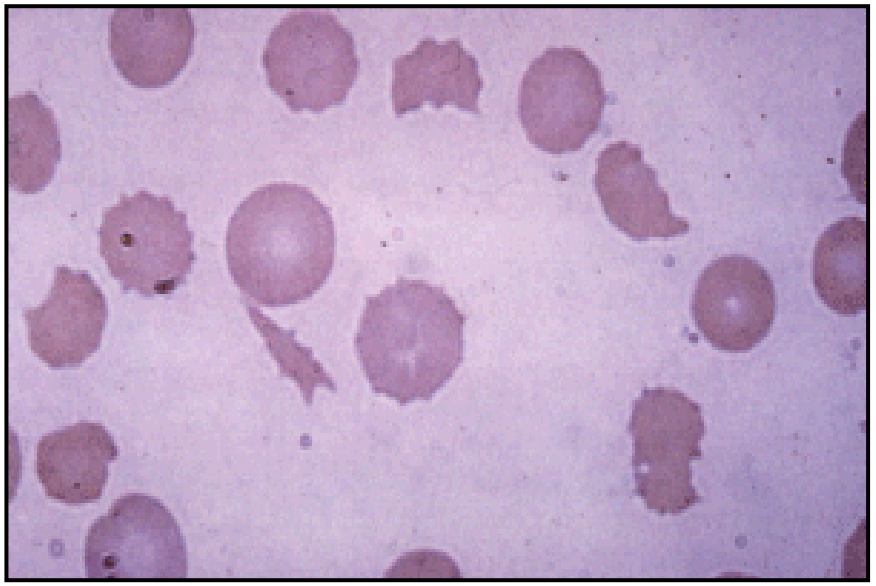
# Renal pathology: acute





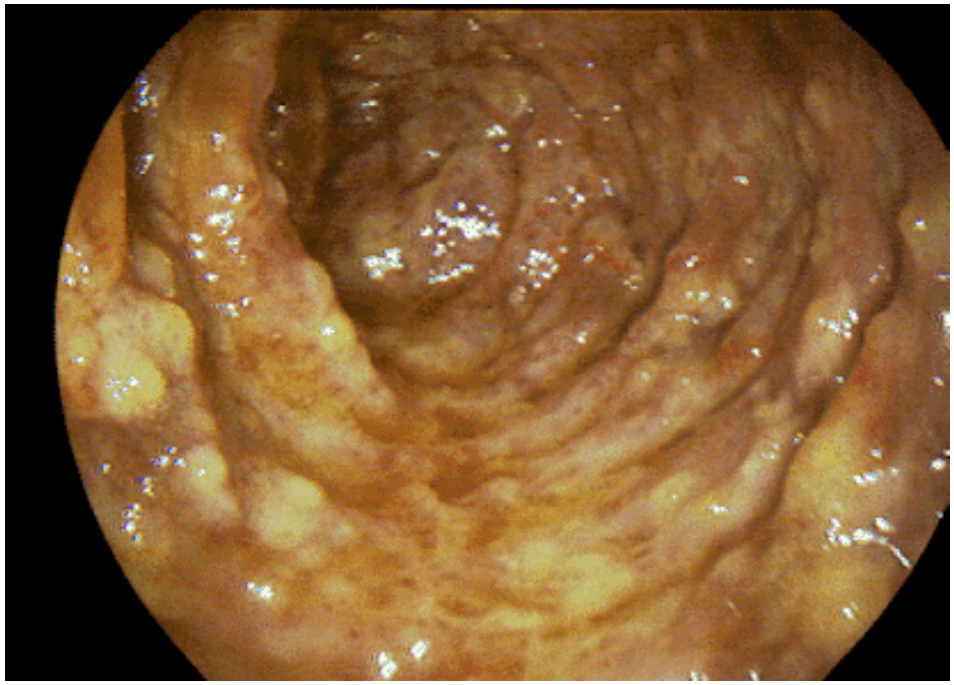
# Microangiopathic hemolysis





- Poor correlation with the degree of ARF
- High LDH

# Gastrointestinal involvement



- Colitis, perforation (2%)
- Prolapse (10%)
- Pancreatitis (20%)
- Diabetes mellitus (8%)
- Elevated LFT's (40%)

Siegler, RL. J.Peds, 1994

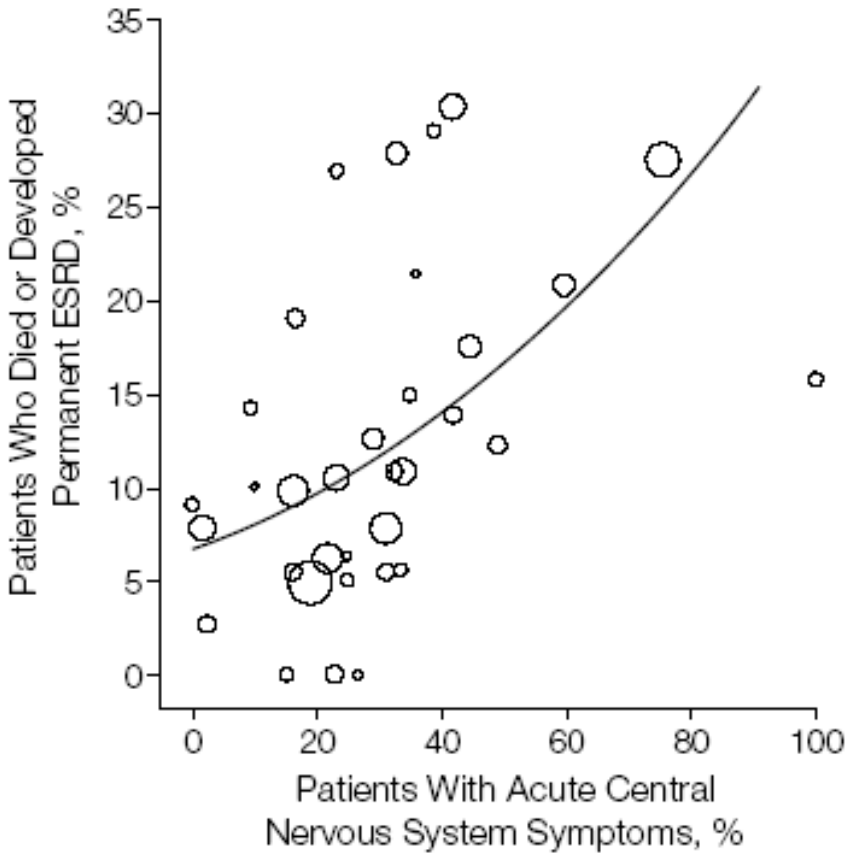
# CNS involvement

- **Symptoms**
  - Seizures
  - Coma
  - Stroke
  - Cortical Blindness
- **Etiology**
  - metabolic (high BUN, low Na+, low Ca++)
  - HTN
  - TMA

Siegler RL, J Peds, 1994; Cimolai N, Pediatrics 1992

# CNS involvement

**Figure 2.** Studies With a Higher Proportion of Patients With Central Nervous System Symptoms (Coma, Seizures, or Stroke)




JAMA 290:1360-1370, 2003

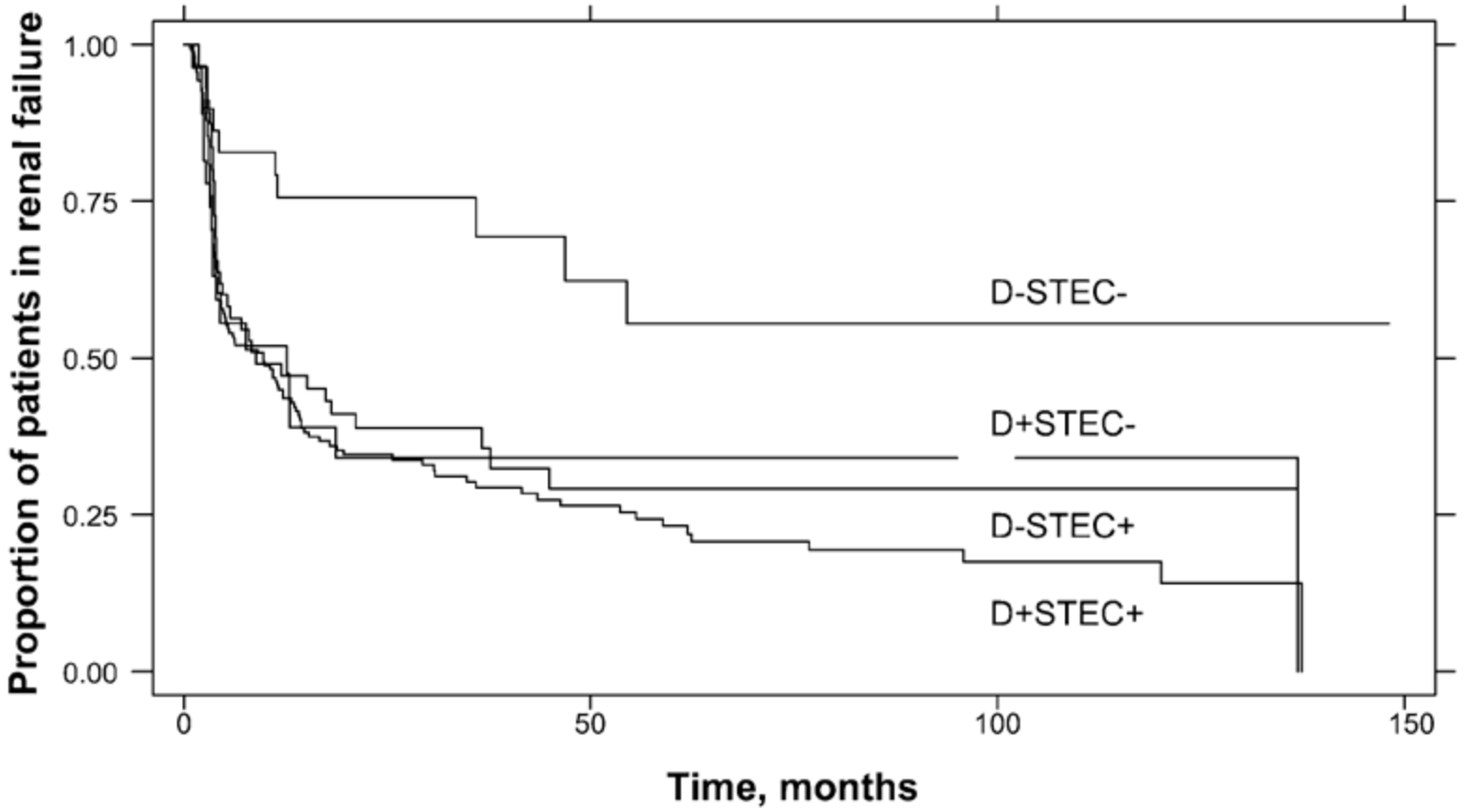
# Reported risk factors for poor outcome

| Reference | N | Oliguria | CNS | age | ↑ WBC | D - |
|---|---|---|---|---|---|---|
| Trompeter et al 1983 | 72 | | | older | | + |
| Loirat et al 1984 | 67 | > 8 d | | > 3 y | | - |
| Walters et al 1989 | 79 | | | | + | |
| Coad et al 1991 | 74 | | | | + | |
| Siegler et al 1991-96 | 118 | >15 d | | < 2 y | + | ± |
| Lopez et al 1992 | 509 | | | | - | + |
| Robson et al 1993 | 71 | > 10 d | | | | |
| Kelles et al 1994 | 95 | no | + | no | | - |
| Tönshoff et al 1994 | 89 | > 14 d | + | no | - | + |
| Renaud et al 1995 | 42 | | | | | + |
| Spizziri et al 1997 | 312 | > 7 d | | no | - | |
| Huseman et al 1999 | 149 | > 7 d | - | | | |
| Gianviti et al 2003 | 387 | | - | no | - | + |

# Supportive management of STEC-HUS

- Fluid resuscitate if dehydrated
- Avoid volume overload
- Treat hypertension
- Transfuse if Hb < 6-7 or earlier if symptomatic
- Avoid platelet transfusions
- Dialysis as needed
- Plasmapheresis ???
- Nutrition (TPN)

# Italian Surveillance Study 1988-2002




| Group | No. patients | Total proportion recovered (95%CI) | No. deceased patients |
|---|---|---|---|
| $D^+STEC^+$ | 157 | 75.8% (68.2–82.1) | 1 |
| $D^-STEC^+$ | 27 | 66.7% (46.0–82.8) | 0 |
| $D^+STEC^-$ | 55 | 65.4% (51.3–77.4) | 1 |
| $D^-STEC^-$ | 29 | 34.5% (18.6–54.3) | 3 |

# Atypical HUS

- Other pathogens
  *S. Pneumoniae*
  HIV, Q fever, CMV, Staphylococcus, Hantavirus

- Drugs
  cyclosporine, tacrolimus
  bleomycin, cisplatin, mitomycin

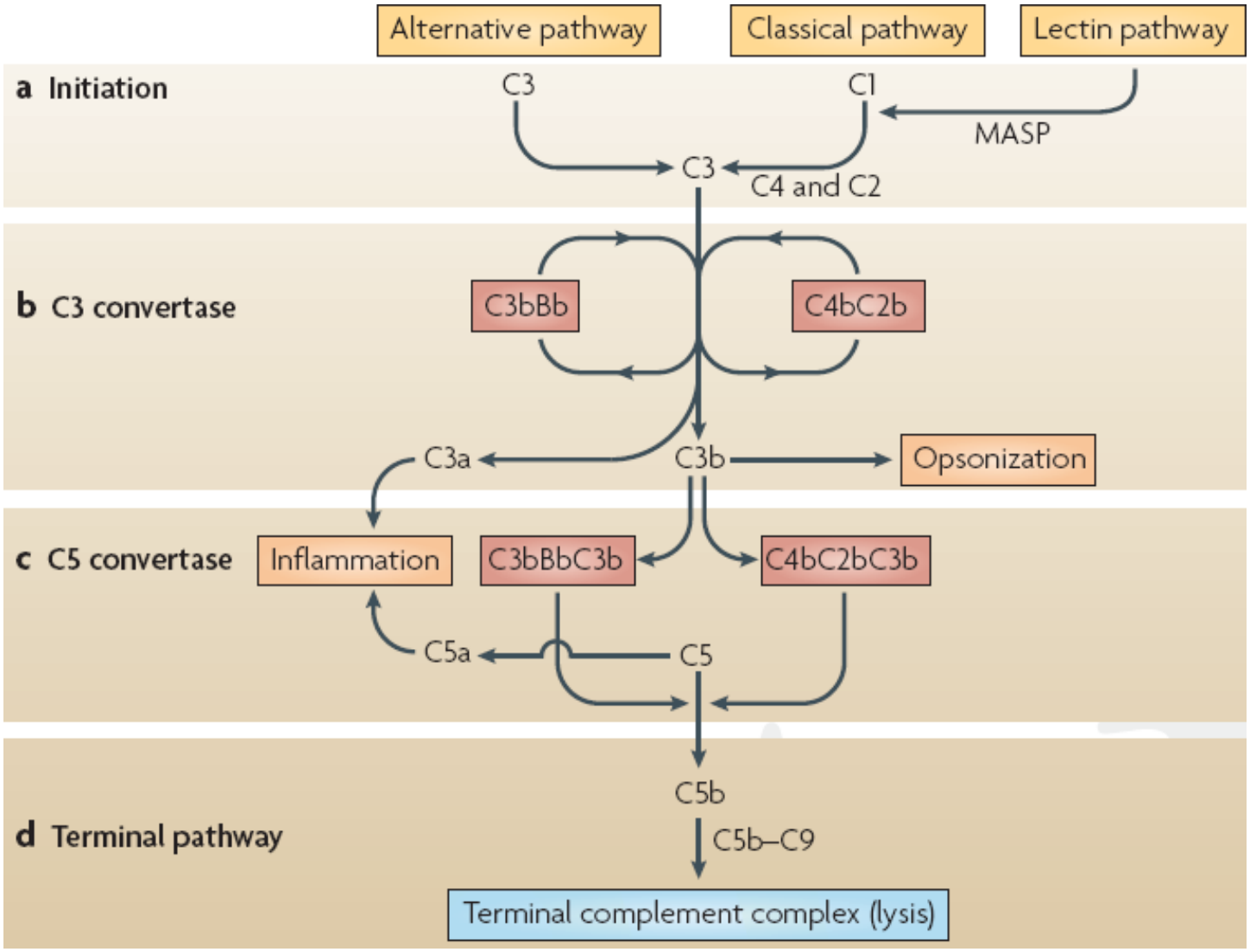
- Underlying genetic defects
  complement factors deficiency
  cobalamin-C defect (methylmalonic aciduria and homocystinuria)

# *Streptococcus Pneumoniae*-associated HUS

- Nearly always invasive disease
  - ± 60% empyema
  - ± 30% meningitis
- Overall mortality: 13% (30% if meningitis)
- Acute phase more severe than D+HUS
  - more dialysis (84%)
  - more transfusions (98%)
- Long term renal sequelae similar to D+HUS
  - protU and/or CRF 20-25%

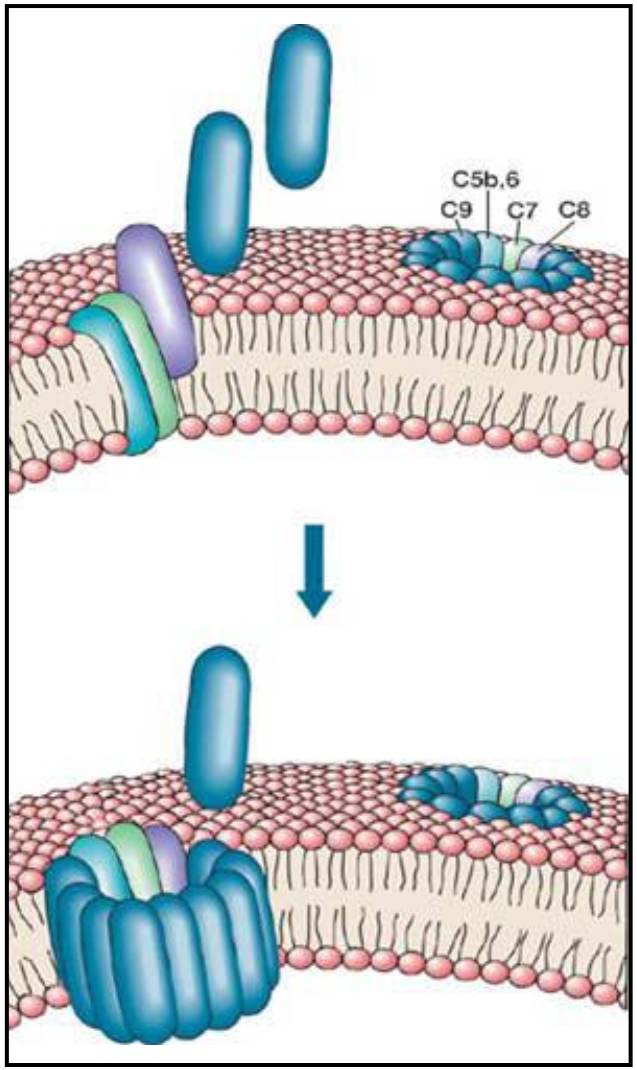
Novak 1983, Martinot 1889, Mc Graw 1989, Eber 1993, Pan 1995, Sajjannar 1996, Mizuwa 1996, Cabrera 1998, Nathanson 2001, Brandt 2002, Waters 2007, Waters 2007

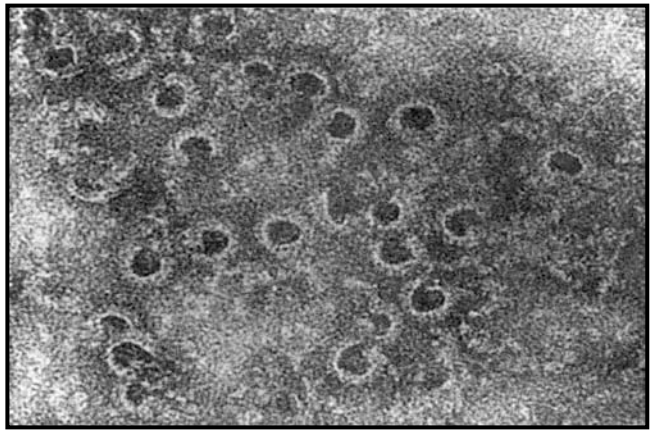
# A simplified view of the complement system

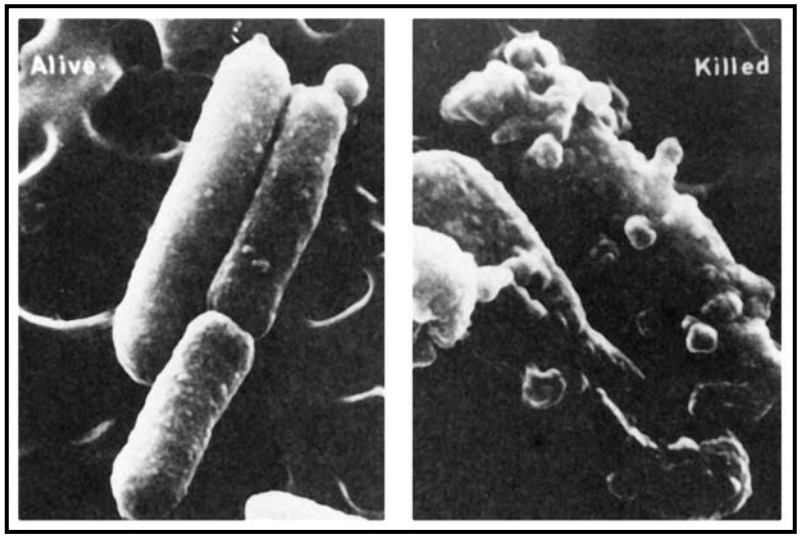



Zipfel and Skerka, Nat Rev Immunol 2009

# Membrane Attack Complex (MAC)



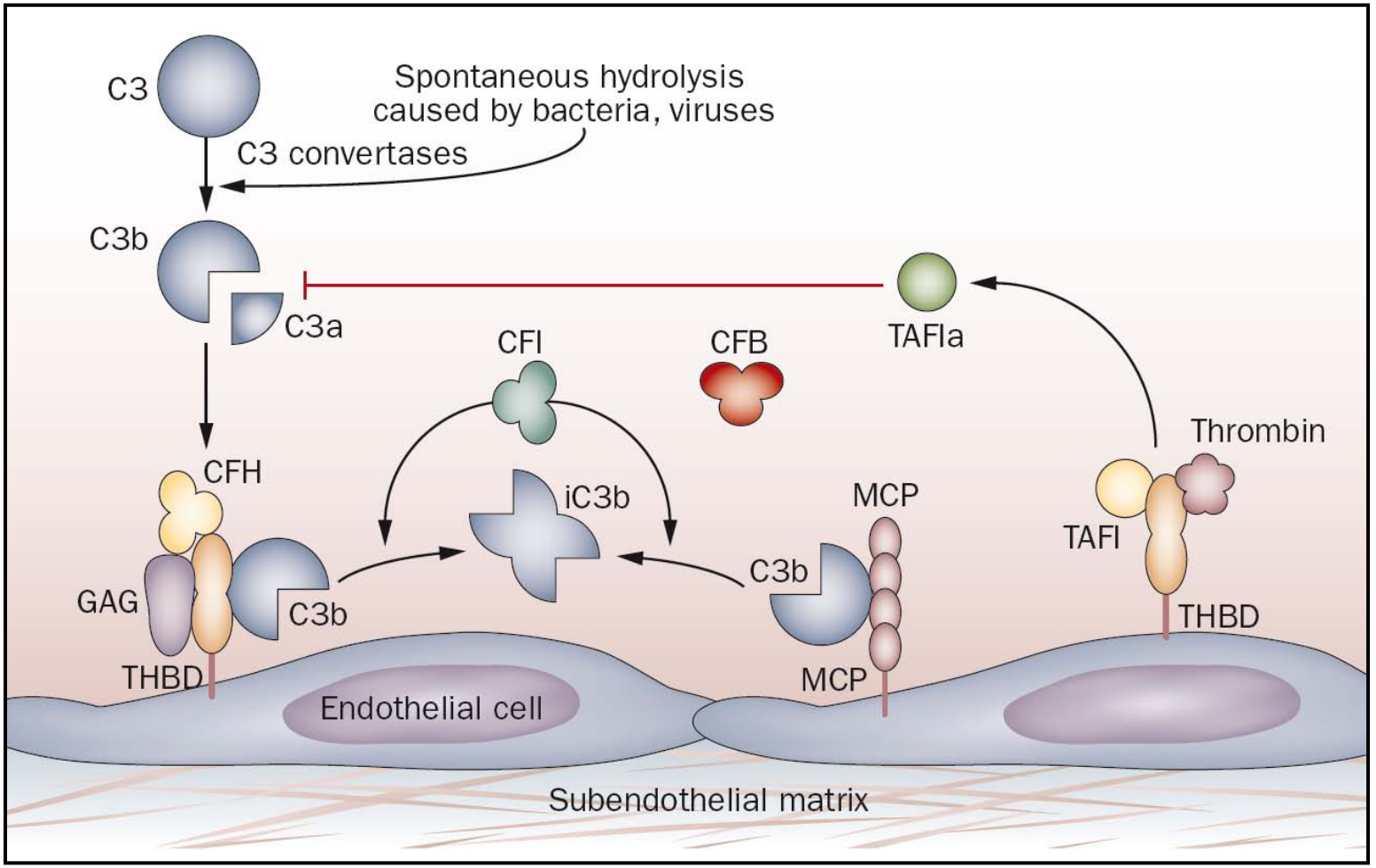





# The area of complement….

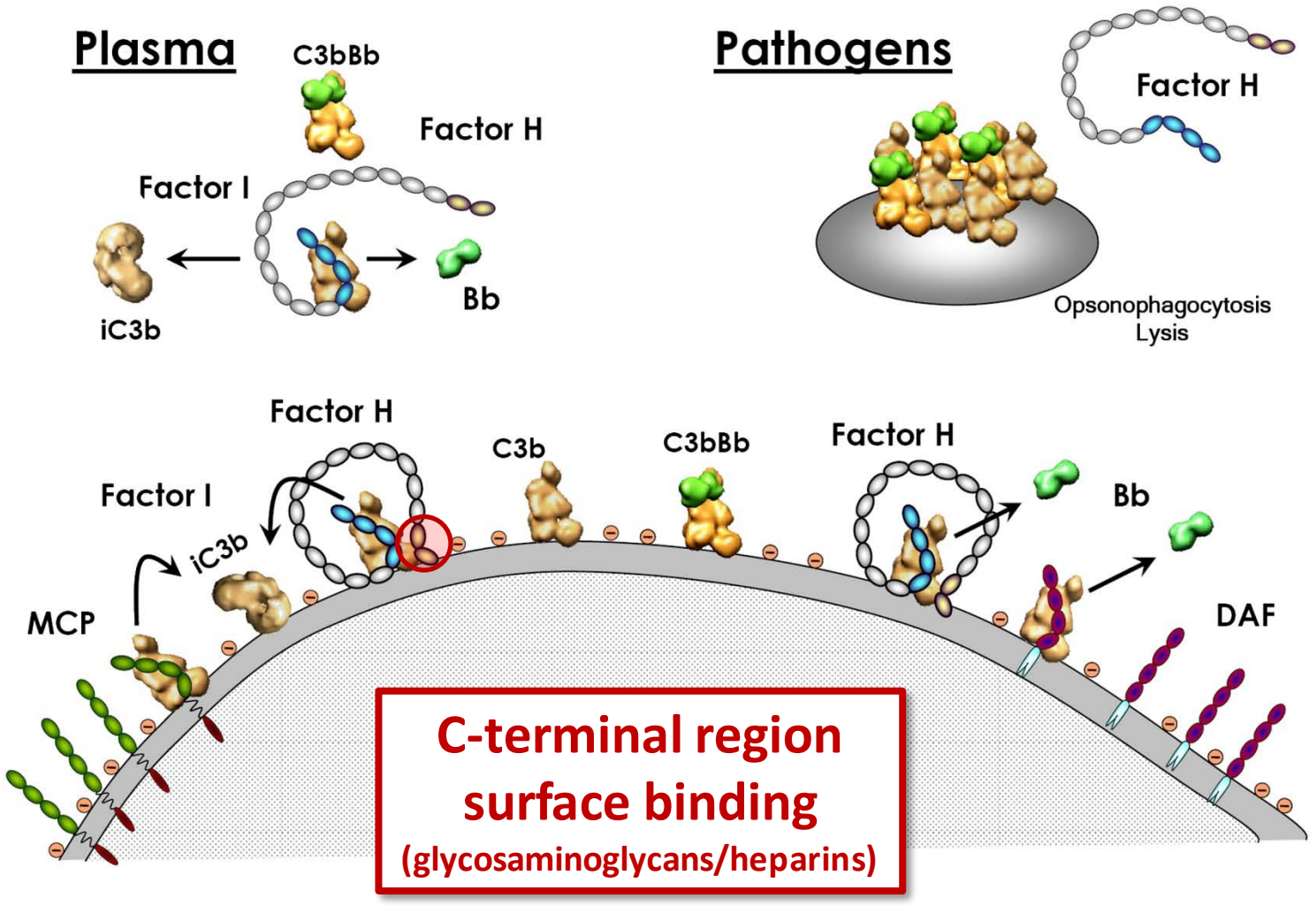
- Erroneous activation or insufficient regulation of the complement cascade may lead to an attack by the immune system against self-tissue.
- Many autoimmune, inflammatory and ischemia/reperfusion injury-related diseases are connected with complement.
- Some of pathological conditions are localized to specific tissues, but many are systemic.

# Endothelial cells with functional complement regulation



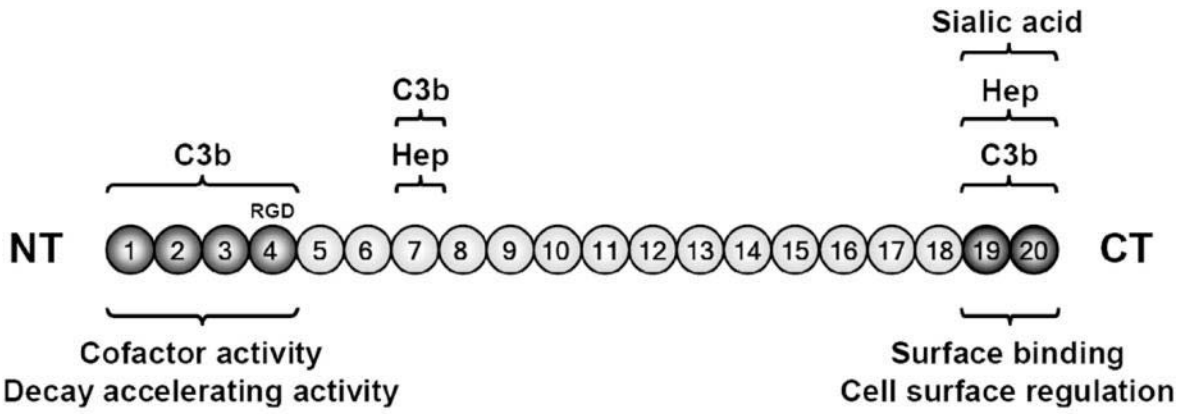

Chiang and Inagi, Nat Rev Nephrol, 2010

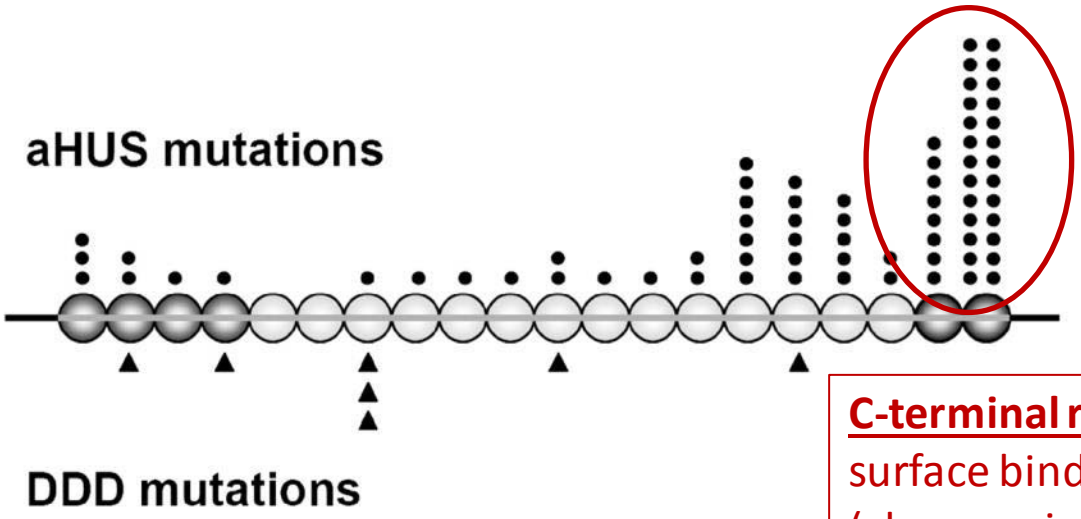
# The AP can be activated on cell surfaces or in the fluid-phase




Rodríguez de Córdoba S et al., Biochem Biophys Acta, 2011

# CFH domain mutations in aHUS and DDD

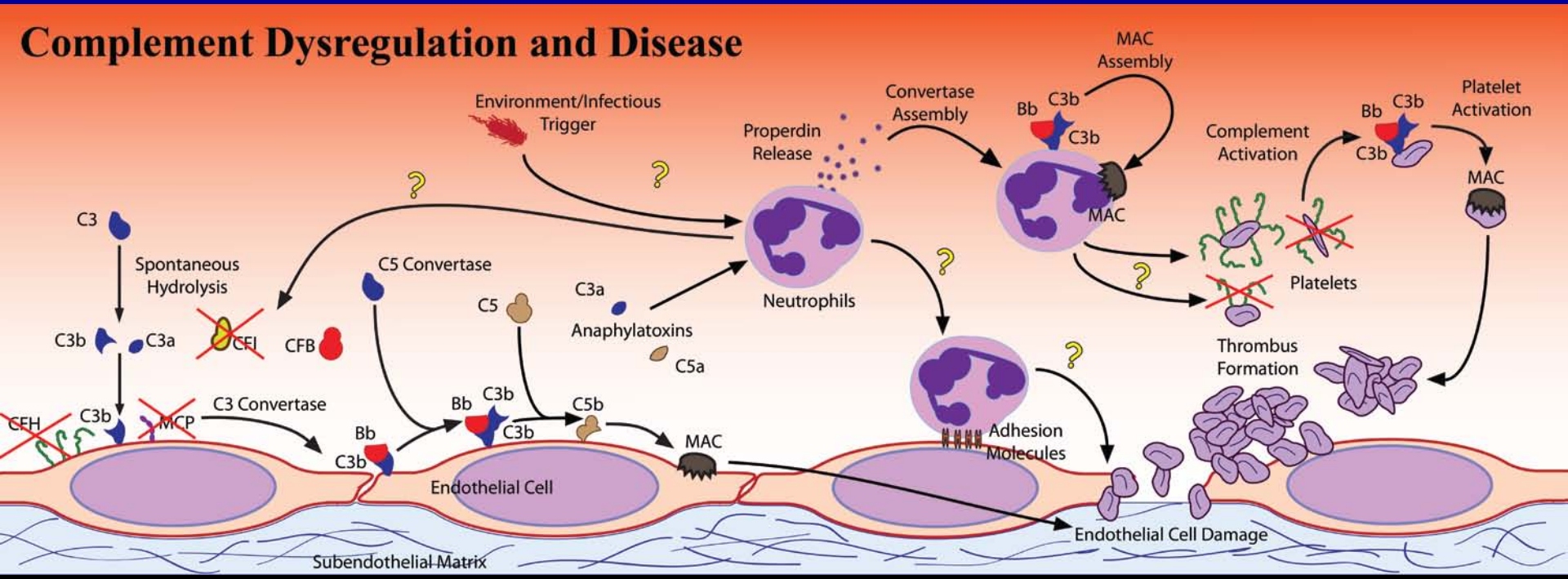



**C-terminal region**
surface binding
(glycosaminoglycans/heparins)

Rodríguez de Córdoba S et al., Biochem Biophys Acta, 2011

# Thrombotic microangiopathy



# aHUS related complement mutations

- Loss of function mutations:
  - Factor H (CFH)
  - Factor I (CFI)
  - Membrane cofactor protein (MCP; CD46)
  - Thrombomodulin (THBD; CD141)

- Gain of function mutations:
  - Factor B (CFB)
  - C3

- Autoantibodies:
  - CFH autoantibodies ± CFHR3-CFHR1 deletion (DEAP-HUS)

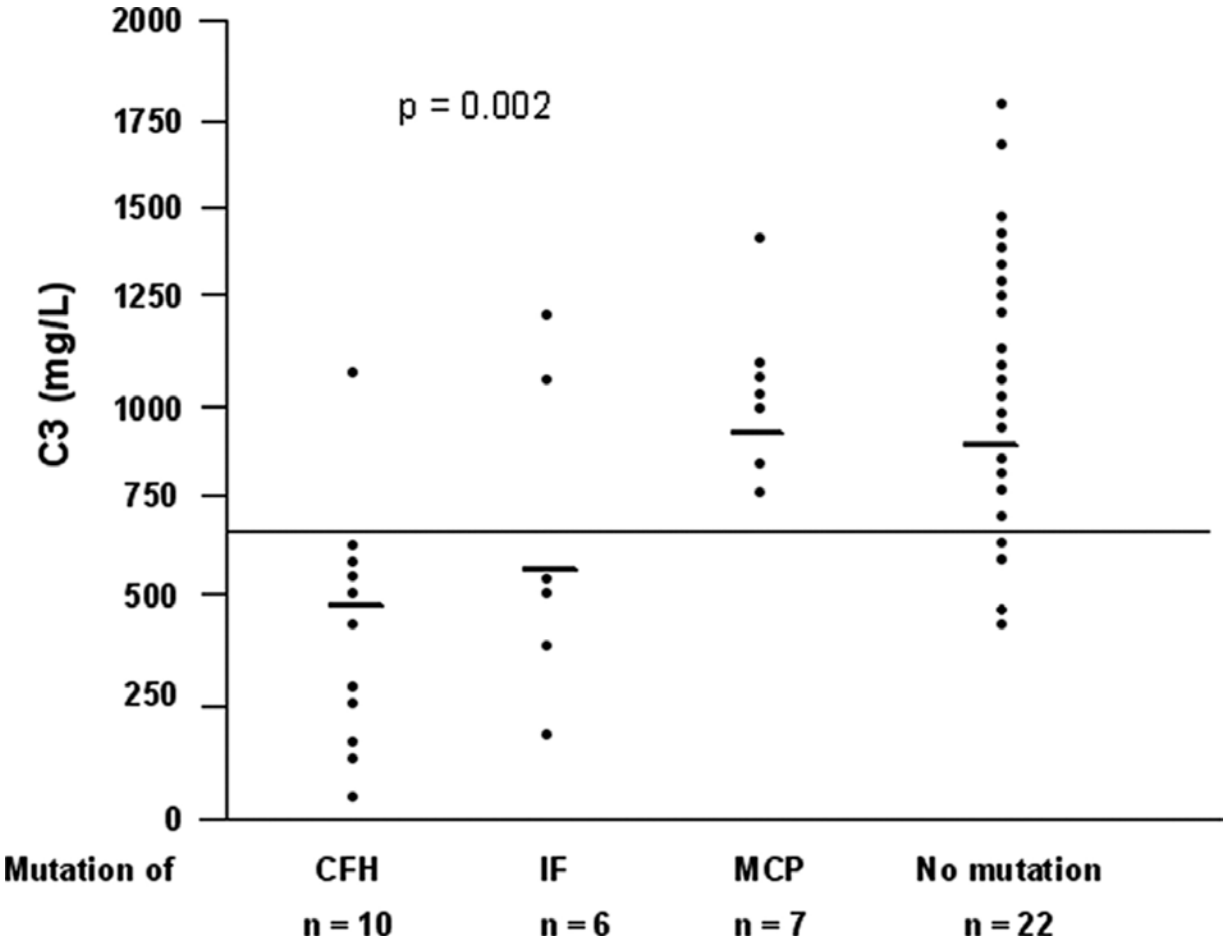
Courtesy of Christoph Licth

# Genotype – phenotype correlation

| Gene | Frequency | Response to plasma therapy [Remission] | Long-term outcome [Death or ESKD] | Outcome after kidney transplantation [Recurrence] |
|---|---|---|---|---|
| CFH | 20-30% | 60% | 70-80% | 80-90% |
| CFH Abs | 6(-10)% | 70-80% (PE + IS) | 30-40% ESKD | 20% |
| MCP | 10-15% | Not indicated | <20% | 15-20% |
| CFI | 4-10% | 30-40% | 60-70% | 70-80% |
| CFB | 1-2% | 30% | 70% | Recurrence in 1 case |
| C3 | 5-10% | 40-50% | 60% | 40-50% |
| THBD | 5% | 60% | 60% | Recurrence in 1 case |

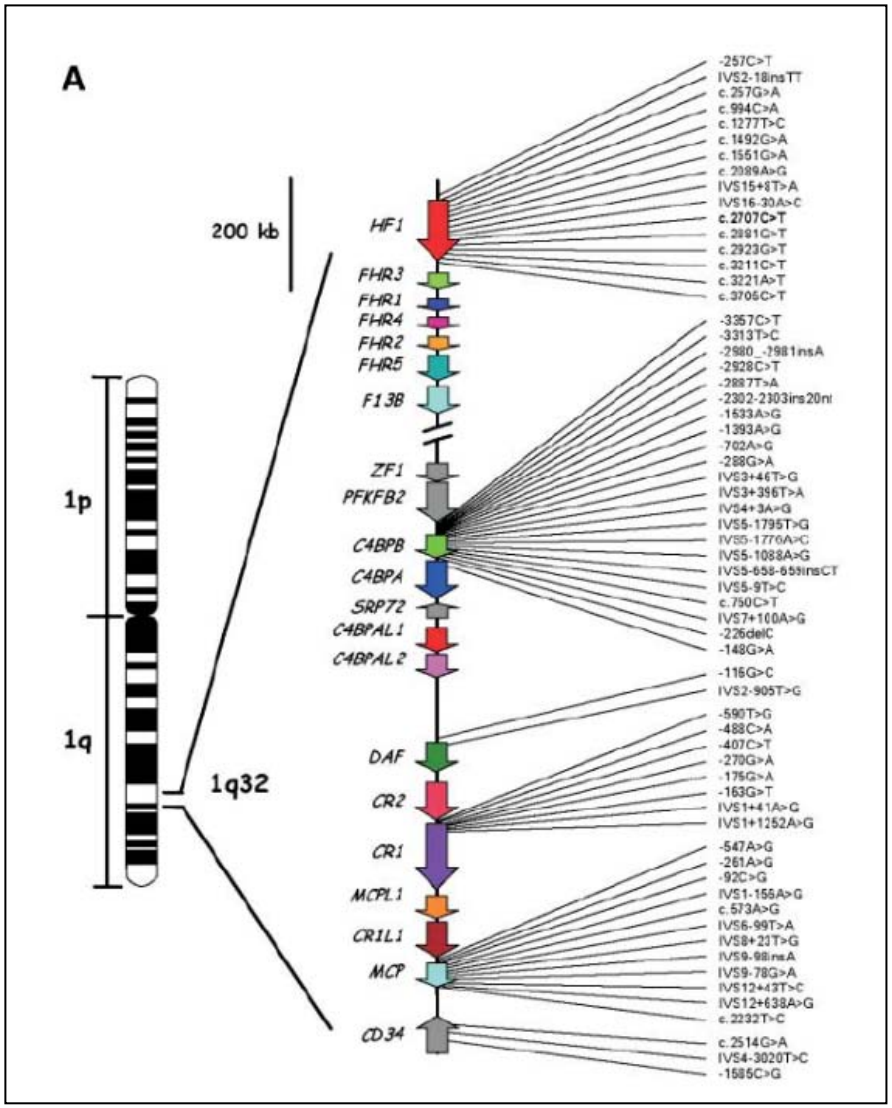
Noris and Remuzzi, N Engl J Med 2009

# C3 levels by mutation




Sellier-Leclerc J Am Soc Nephrol 2007

# Risk aplotypes in aHUS



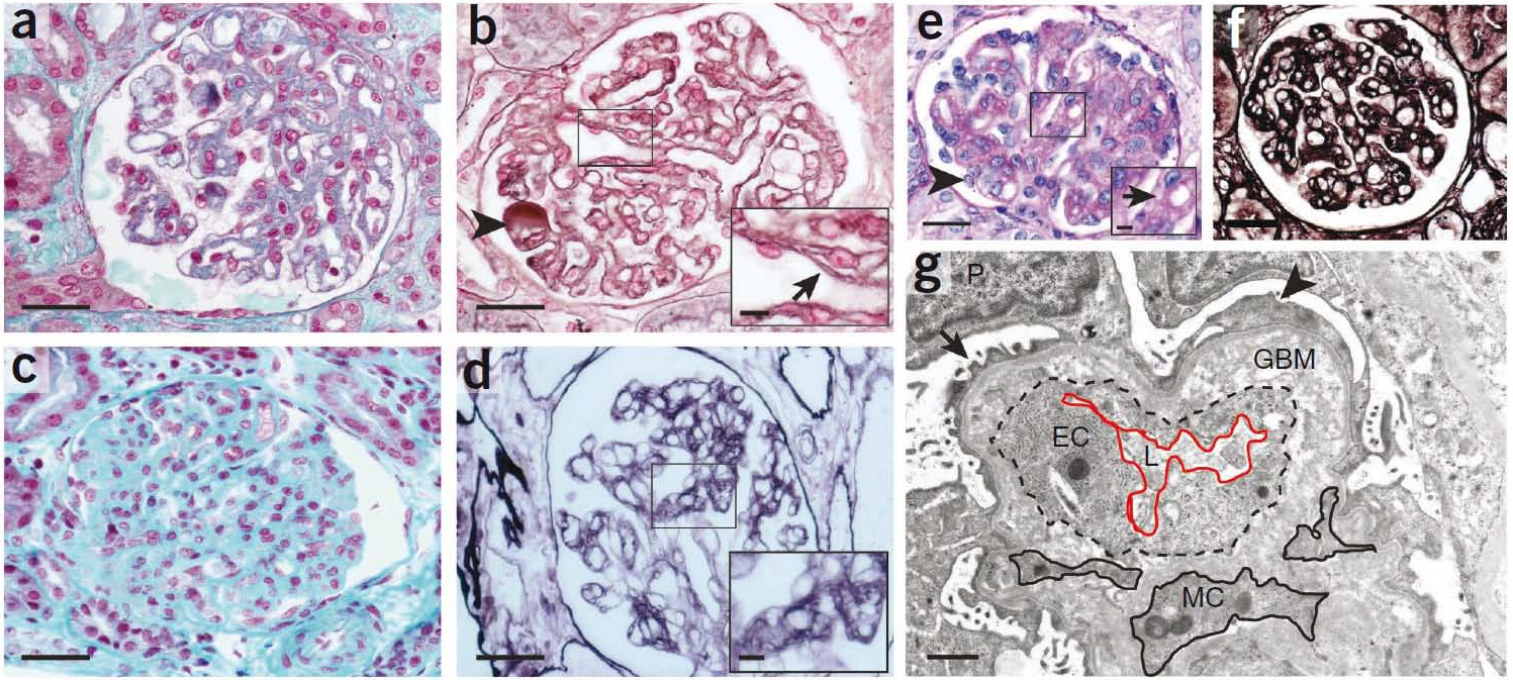

Esparza-Gordillo et al, Hum Mol Genet 2005

# DGKE (diacylglycerol kinase ε) mutations

## Recessive mutations in *DGKE* cause atypical hemolytic-uremic syndrome

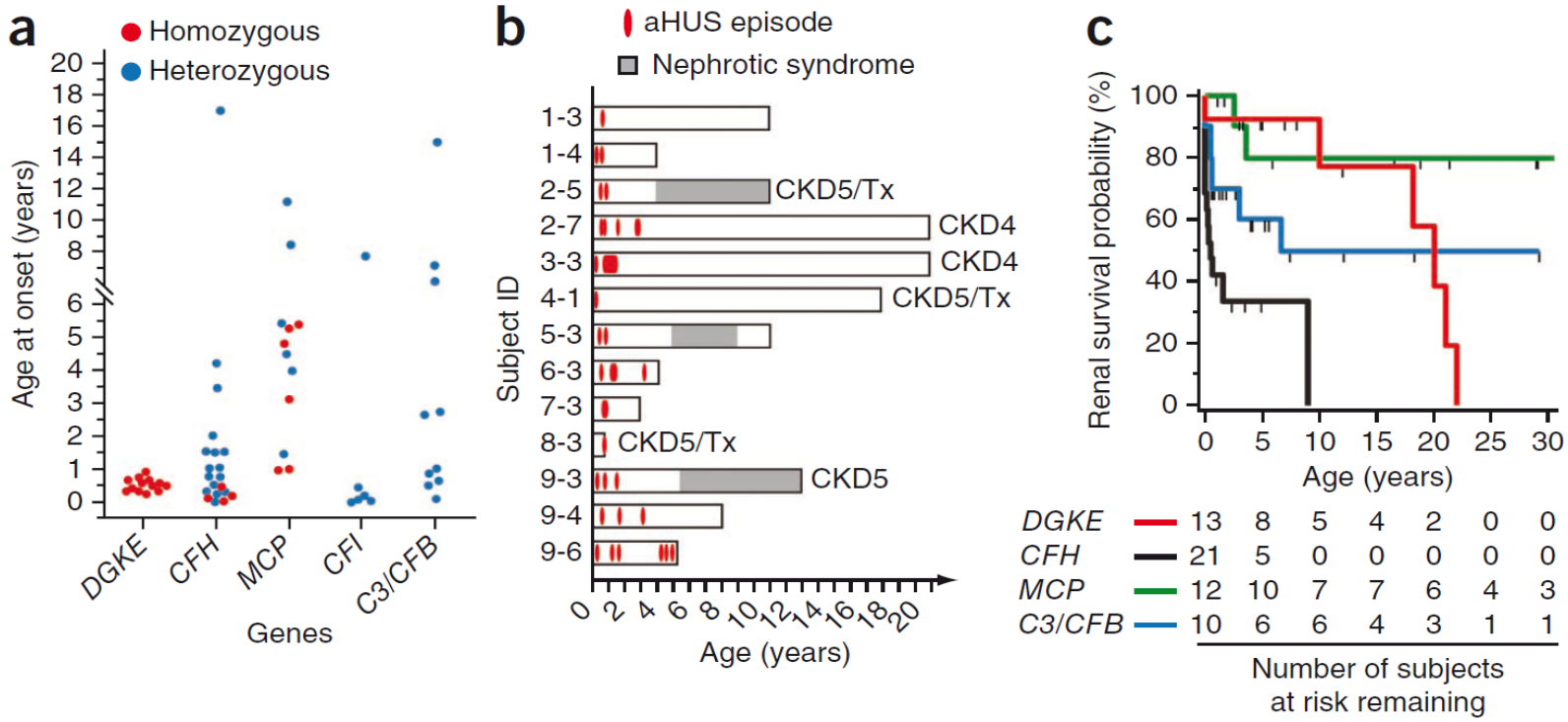
nature genetics

Mathieu Lemaire[1,2,24], Véronique Frémeaux-Bacchi[3,4,24], Franz Schaefer[5,6], Murim Choi[1,2,7], Wai Ho Tang[8], Moglie Le Quintrec[4], Fadi Fakhouri[9], Sophie Taque[10], François Nobili[11], Frank Martinez[12], Weizhen Ji[1,2], John D Overton[1,7], Shrikant M Mane[1,7], Gudrun Nürnberg[13], Janine Altmüller[13], Holger Thiele[13], Denis Morin[14], Georges Deschenes[15], Véronique Baudouin[15], Brigitte Llanas[16], Laure Collard[17], Mohammed A Majid[18], Eva Simkova[18], Peter Nürnberg[13,19,20], Nathalie Rioux-Leclerc[21], Gilbert W Moeckel[22], Marie Claire Gubler[23], John Hwa[8], Chantal Loirat[15] & Richard P Lifton[1,2,7]




Lemaire et al, Nature Genetics 2013

# DGKE (diacylglycerol kinase ε) mutations



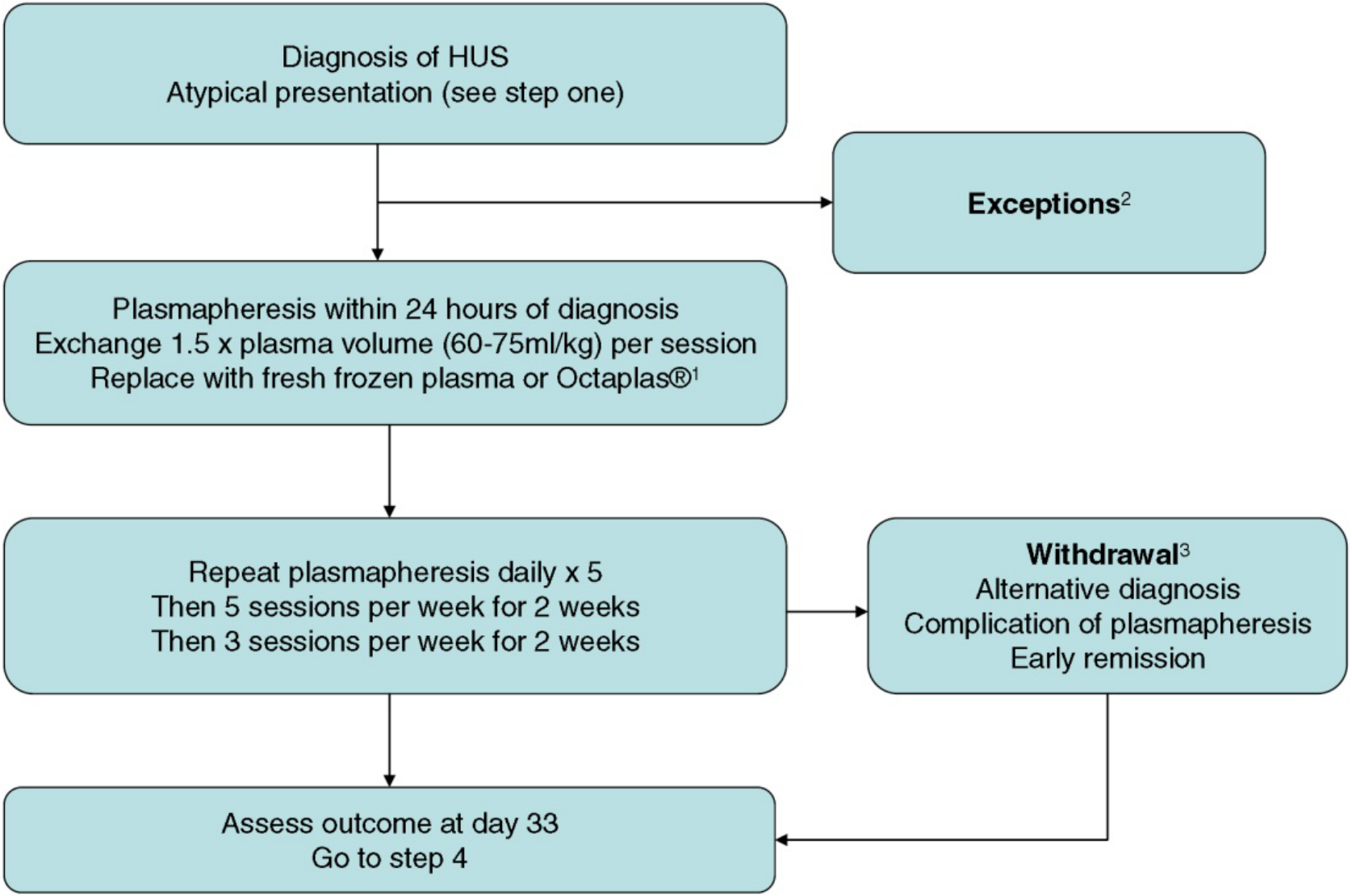

Lemaire et al, Nature Genetics 2013

# DGKE (diacylglycerol kinase ε) mutations

- DGKE is expressed in endothelium, platelets and podocytes
- DGKE inactivates arachidonic acid signaling through diacylglycerols (DAG) (activates thrombosis)
- First gene implicated in aHUS that is not an integral component of the complement cascade
- Symptoms: hypertension, microhematuria and proteinuria and unique finding of nephrotic syndrome
- Relapse of aHUS in subjects while receiving anti-C5
- Renal transplantation could be efficacious and safe

Lemaire et al, Nature Genetics 2013

# Plasmapheresis for aHUS




Ariceta et al, Ped Neprol 2009

# Liver-kidney transplantation

SPECIAL ARTICLE www.jasn.org *J Am Soc Nephrol* 20: 940–949, 2009.

## Liver-Kidney Transplantation to Cure Atypical Hemolytic Uremic Syndrome

Jeffrey M. Saland, Piero Ruggenenti, Giuseppe Remuzzi, and the Consensus Study Group

### Eligibility for combined liver and kidney transplant or liver transplant alone

| |
|---|
| *CFH or CFI* mutation with the exceptions listed in Table 7 |
| Less than 10% normal CFH levels in plasma |
| Patients who have identified mutations of genes encoding and<br>have aHUS recurrence after isolated kidney transplantation or<br>have a family member who had the same mutation and had aHUS recurrence after isolated kidney transplantation |
| HUS recurrence after isolated kidney transplantation in patients with identified mutations of genes that may have both hepatic and nonhepatic sites of expression and protein synthesis (see Table 7) |

### Criteria for which currently available information is insufficient to indicate a specific recommendation for or against liver transplantation or combined liver and kidney transplantation versus isolated kidney transplantation

| |
|---|
| *C3* mutation[a] |
| *CFB* mutation[a] |
| Combined mutations among *MCP*, *CFI*, and *CFH*[b] |
| *CFHR1-CFHR3* deletion without anti-FH autoantibody[b] |
| Low-risk *CFH or CFI* mutation as defined by mutations already reported to the registry as not being associated with recurrence after isolated renal transplantation[b] |

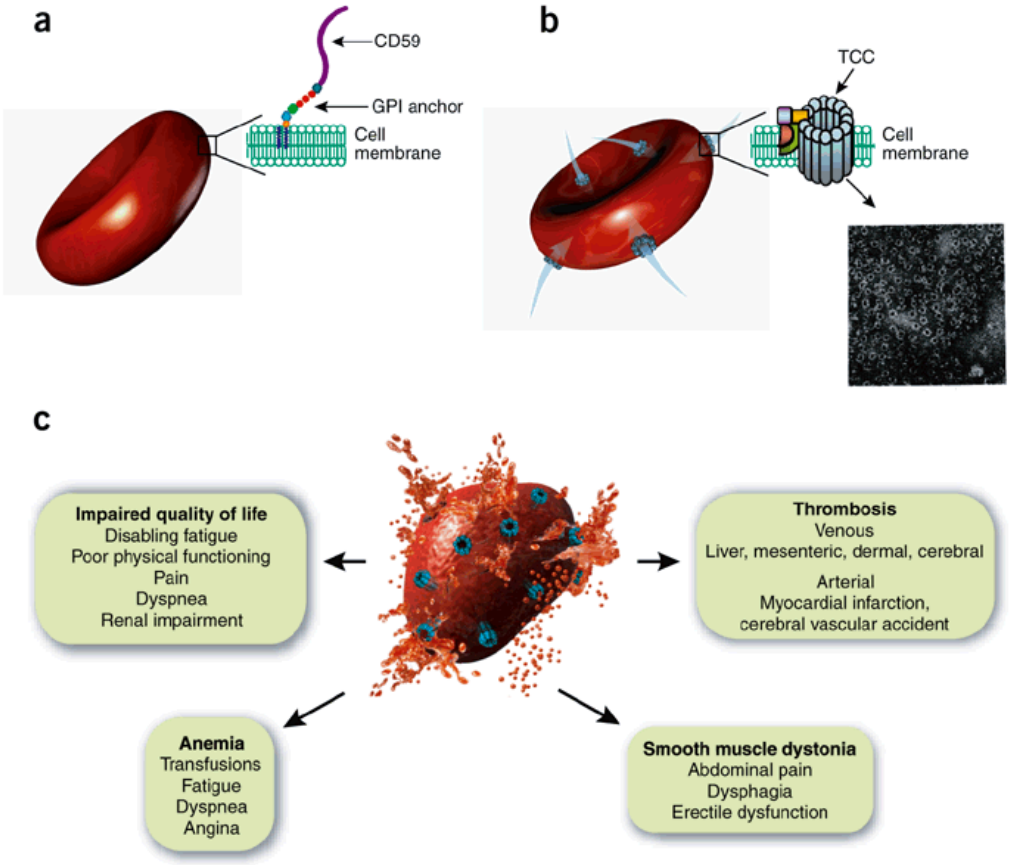
[a]Current knowledge is insufficient to presume liver transplantation would correct complement regulation.
[b]Evidence is sufficient to support liver or combined liver-kidney transplantation for such individuals if they or a family member with the same mutation experienced aHUS recurrence after isolated kidney transplantation.

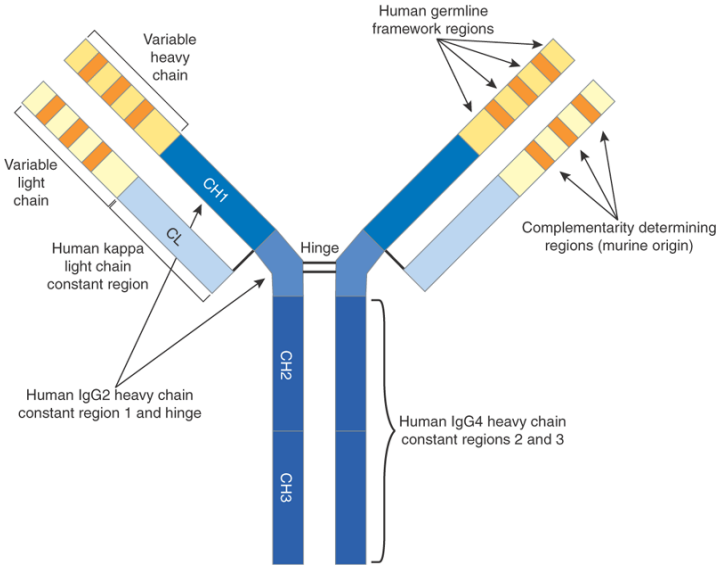
### Eligibility for isolated kidney transplantation

| |
|---|
| No evidence of *CFH*, *CFI*, *CFB*, or *C3* gene mutations |
| *MCP* mutation |
| Mutations associated with successful isolated kidney transplantation in affected family members[a] |
| Anti–factor H autoantibodies |

[a]Conversely, combined liver and kidney transplantation is recommended (see Table 5) for patients who have certain gene mutations and (*1*) have aHUS recurrence after isolated kidney transplantation or (*2*) have a family member who had the same mutation and had aHUS recurrence after isolated kidney transplantation.

# Eculizumab for paroxysmal nocturnal hemoglobinuria




Rother et al. Nature Biotechnology 2007

# Eculizumab for aHUS

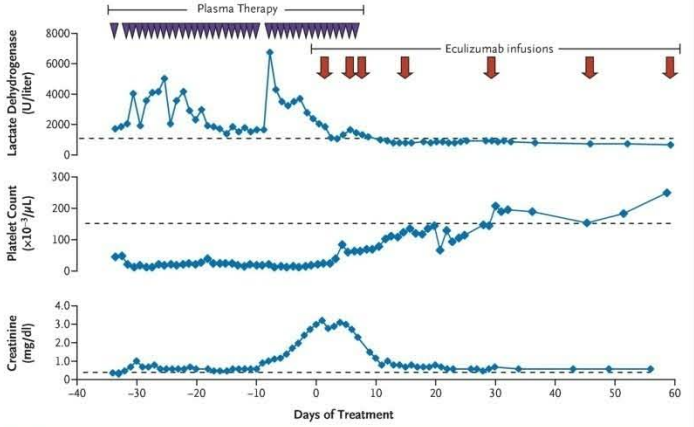



**Figure 1. Response to Eculizumab Therapy in a Patient with Congenital Atypical Hemolytic–Uremic Syndrome.**
Eculizumab therapy was initiated in an infant with atypical hemolytic–uremic syndrome for the treatment of a thrombotic microangiopathic event that was unresponsive to 32 consecutive days of plasmapheresis. During the period before treatment with eculizumab, lactate dehydrogenase levels (as a measure of hemolysis) ranged from 1400 to 6800 U per liter (upper limit of the normal range, 920 U per liter [dashed line]) and platelet counts ranged from 11,000 to 38,000 per microliter (lower limit of the normal range, 135,000 per microliter [dashed line]). Creatinine levels began to increase 10 days before the initiation of eculizumab treatment and reached a level of 3.0 mg per deciliter (upper limit of the normal range, 0.4 mg per deciliter [dashed line]). Shortly after eculizumab therapy was initiated, hemolysis decreased (as assessed by levels of lactate dehydrogenase), platelet counts increased, and creatinine levels decreased. Within 1 week after initiation of eculizumab treatment, plasma therapy was discontinued. By day 20, the level of lactate dehydrogenase and the platelet count were normal, and the creatinine level was near normal (0.6 mg per deciliter). Clinical remission was maintained through day 60 with ongoing eculizumab treatment.

Gruppo et al. N Engl J Med 360; 544, 2009

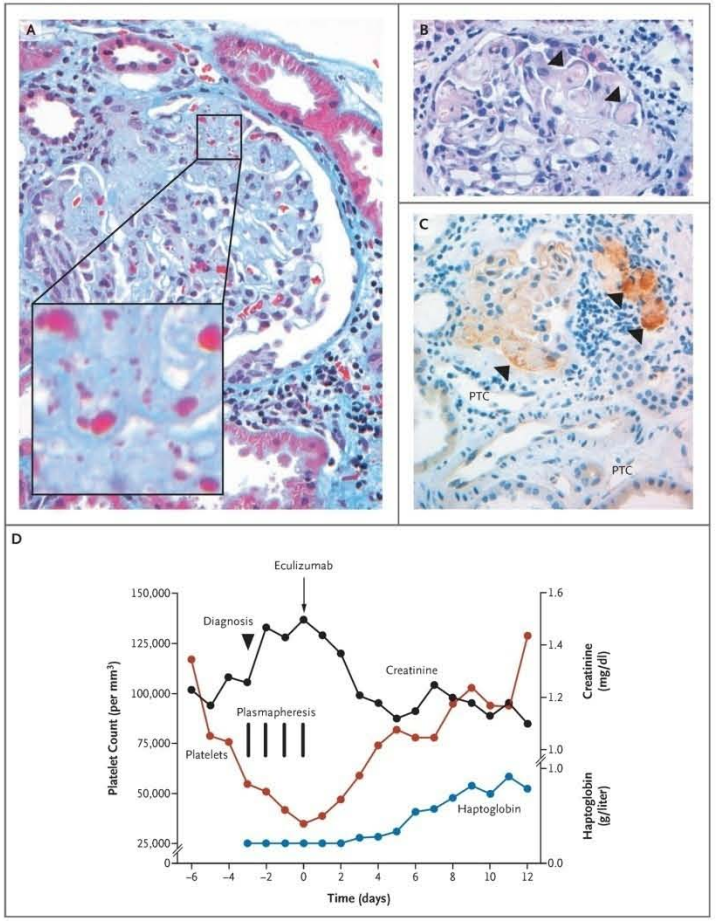
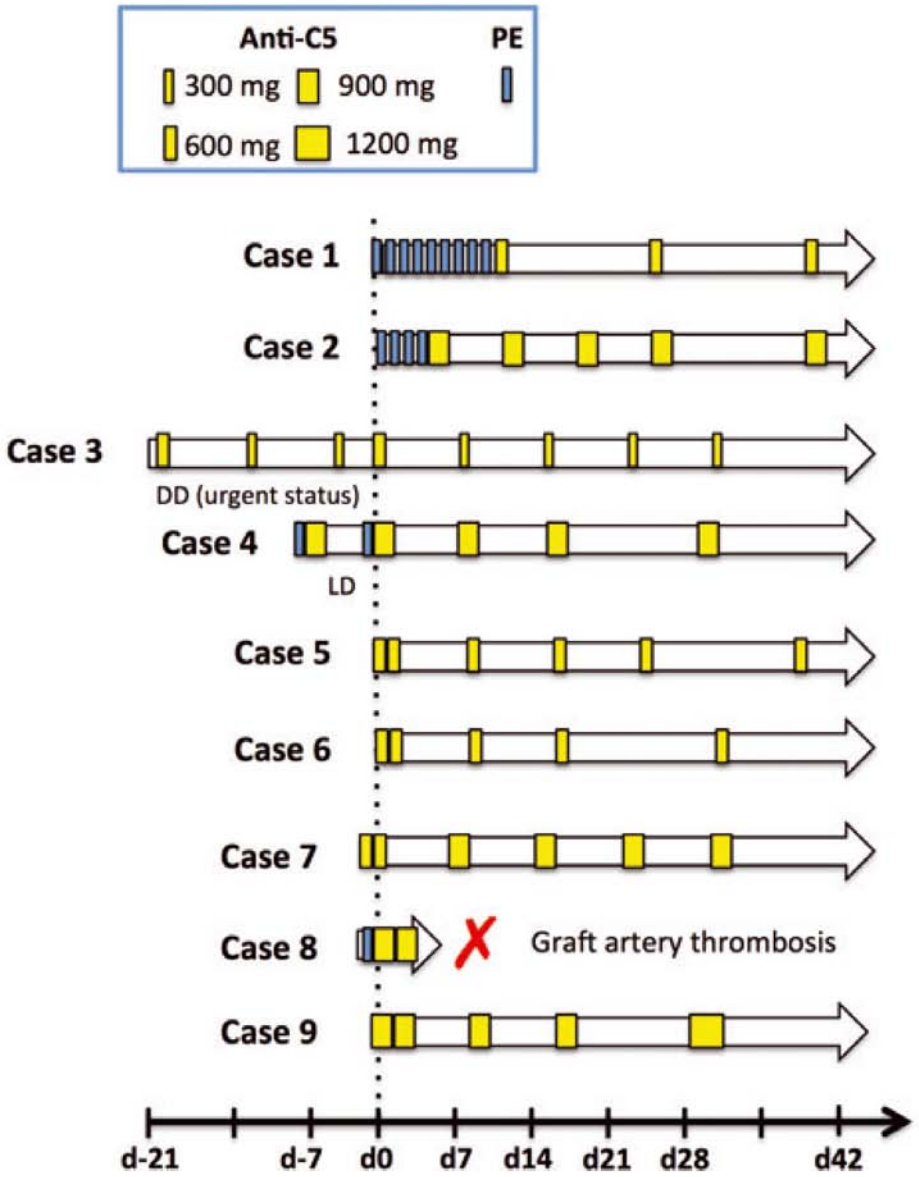



**Figure 1 (facing page). Pathological Findings and Laboratory Values.**
In Panel A, elastic Masson's trichrome stain shows thrombotic microangiopathy with numerous intracapillary erythrocytes. The inset shows magnification of the area with red-cell fragments. In Panel B, hematoxylin and eosin stain shows hyaline microthrombi that occlude glomerular capillaries (arrowheads). In Panel C, immunostaining for complement protein C4d was positive in glomerular capillaries and preglomerular arterioles (arrowheads), indicating an activation of the complement system. C4d staining of peritubular capillaries (PTC), an indicator of humoral rejection, was negative. Panel D shows the time course of the platelet count and laboratory values used to determine the occurrence of hemolysis in the patient who underwent renal transplantation (eculizumab was administered at 0 days). When atypical hemolytic–uremic syndrome was diagnosed (arrowhead), tacrolimus was discontinued and plasma exchange was performed four times (vertical black bars indicate treatments). Despite this treatment, atypical hemolytic–uremic syndrome was aggravated, as indicated by the progressive decrease in platelets to 35,000 per cubic millimeter and the deterioration of transplant function indicated by an increase in the creatinine level to 1.5 mg per deciliter. A single dose of 600 mg of eculizumab was administered (arrow), and measurements of total complement activity (data not shown) showed that eculizumab completely blocked the complement system. After treatment with eculizumab, haptoglobin levels normalized after 8 days, the platelet count increased, and the creatinine level decreased, indicating recovery of transplant function.

Nürnberger et al. N Engl J Med 360; 542, 2009

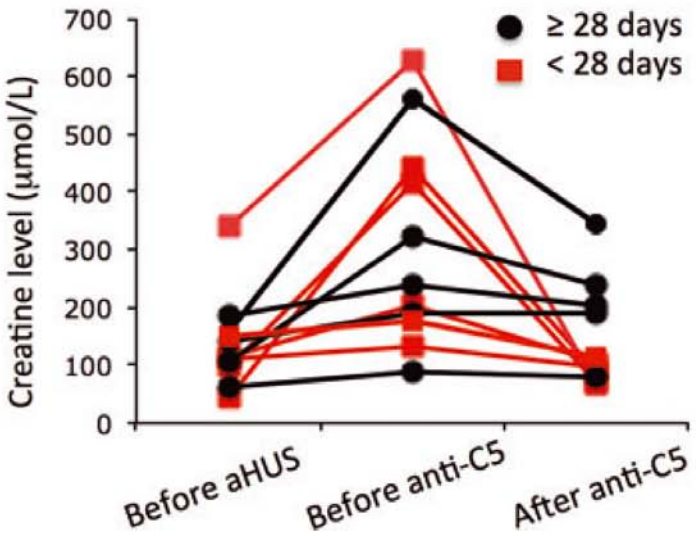
# Prophylactic anti-C5 therapy

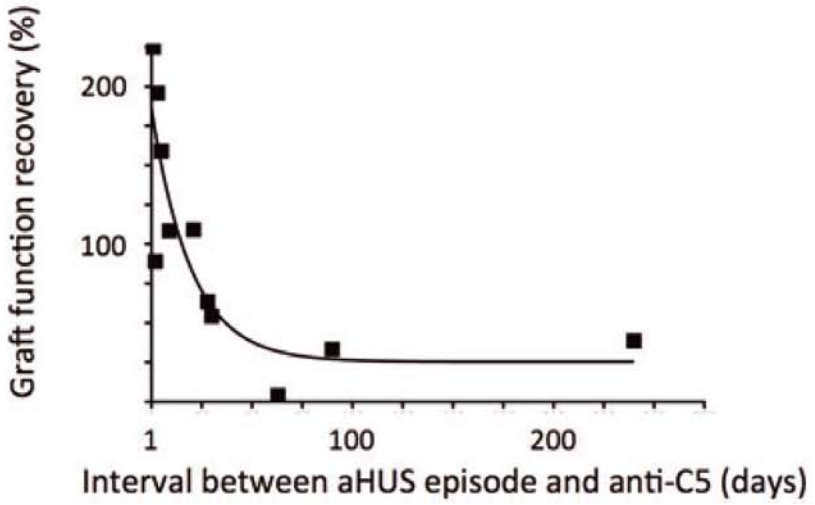



Zuber et al, Am J Transplant, 2012

# Anti-C5 therapy for post transplant recurrence

*Early initiation of anti-C5 therapy is associated with better recovery of renal function.*




Zuber et al, Am J Transplant, 2012

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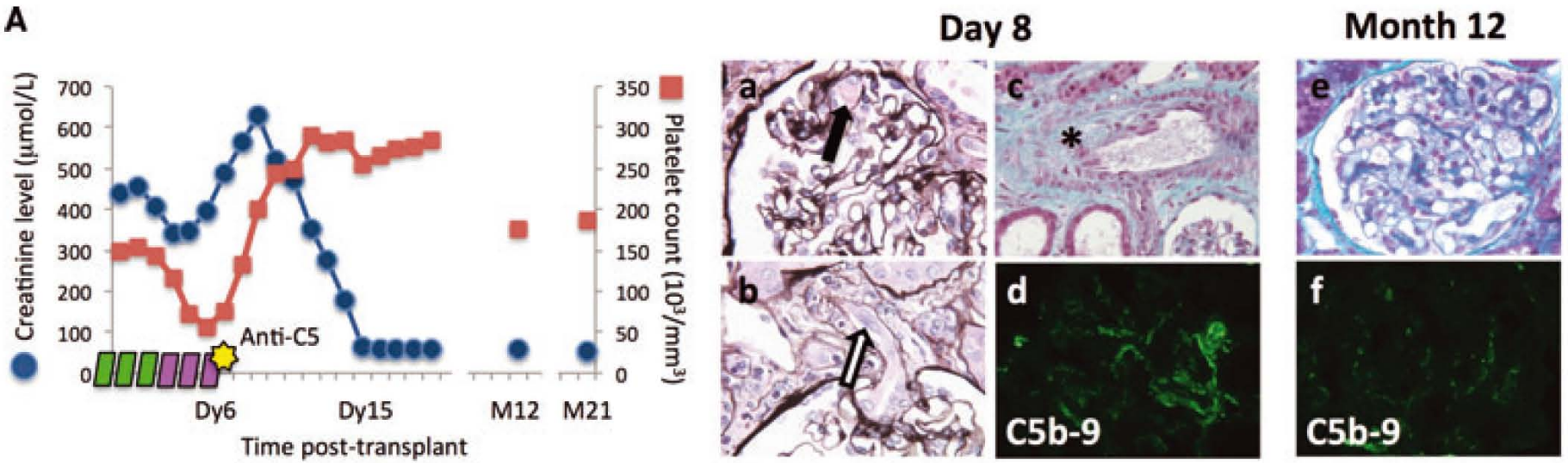


Zuber et al, Am J Transplant, 2012

# What we have learned in the past years

- Posttransplant aHUS recurrence is a strong predictor of early graft failure
- Anti-C5 efficiently prevents aHUS posttransplant recurrences
- Prophylactic plasma therapy may fail to prevent aHUS recurrences
- Prophylactic plasma therapy may mask subclinical, yet progressive, aHUS recurrences
- With the advent of anti-C5 therapy, combined kidney-liver transplantation is no longer a first line treatment for aHUS

# High risk of recurrence

- recurrence in a previous graft
  mutations associated with a risk of recurrence > 80%
  (CFH, CFH-CFHR1 hybrid gene, gain of function mutations in C3, CFB)

- prophylactic therapy (1 additional dose on day 1 post-tx)

Zuber et al, Am J Transplant, 2012

# Low risk of recurrence

- mutations in MCP and anti-CFH antibodies
- treatment if recurrence

Zuber et al, Am J Transplant, 2012

# Moderate risk of recurrence

- All other patients
- prophylactic therapy (1 additional dose on day 1 post-tx)
- attempt to discontinue treatment 1 year after transplantation

Zuber et al, Am J Transplant, 2012

# If post-transplant recurrence…

- Prompt initiation of anti-C5 treatment
- Same treatment for full-blown and incomplete aHUS recurrence
- No discontinuation of anti-C5 treatment

Zuber et al, Am J Transplant, 2012

# aHUS in patients with STEC infections

**Two Patients With History of STEC-HUS, Posttransplant Recurrence and Complement Gene Mutations**

Alberti et al, Am J Transplant 2013

- Two patients with a clinical history of STEC-HUS that lost their graft for HUS recurrence
- Heterozygous CFI mutation in patient #1
  Heterozygous MCP mutation in patient #2 , and in her mother who donated the kidney
- aHUS triggered by STEC infection on a genetic background of impaired complement regulation
- Complement gene sequencing should be performed before kidney transplantation in patients who developed ESRD following STEC-HUS
- Genetic analysis of donors is mandatory before living-related transplantation to exclude carriers of HUS-predisposing mutations