REVIEW

What's new in haemolytic uraemic syndrome?

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Abstract Recent advances in understanding the aetiology of the disorders that make up the haemolytic uraemic syndrome (HUS) permit a revised classification of the syndrome. With appropriate laboratory support, an aetiologically-based subgroup diagnosis can be made in all but a few cases. HUS caused by enterohaemorrhagic *Escherichia coli* remains by far the most prevalent subgroup, and new insights into this zoonosis are discussed. The most rapidly expanding area of interest is the subgroup of inherited and acquired abnormalities of complement regulation. Details of the pathogenesis are incomplete but it is reasonable to conclude that local activation of the alternative pathway of complement in the glomerulus is a central event. There is no evidence-based treatment for this diagnostic subgroup. However, in circumstances where there is a mutated plasma factor such as complement factor H, strategies to replace the abnormal protein by plasmapheresis or more radically by liver transplantation are logical, and anecdotal successes are reported. In summary, the clinical presentation of HUS gives a strong indication as to the underlying cause. Patients without evidence of EHEC infection should be fully investigated to determine the aetiology. Where complement abnormalities are suspected there is a strong argument for empirical and early plasma exchange, although rapid advances in this field may provide more specific treatments in the near future.

Abbreviations

HUS	Haemolytic uraemic syndrome
TTP	Thrombotic thrombocytopenic purpura
ADAMTS13	A dysintegrin and metalloprotease with thrombospondin type motifs 13
EHEC	Enterohaemorrhagic *Escherichia coli*
STEC	Shiga toxin-producing *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
Stx	Shiga toxin
GFR	Glomerular filtration rate
MCP	Membrane cofactor protein (CD46)
FH	Complement factor H
FI	Complement factor I

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Classification

Haemolytic uraemic syndrome (HUS) is diagnosed on the simultaneous features of microangiopathic haemolytic anaemia, thrombocytopenia, and acute renal failure. It is worth noting that there is an overlap between the clinical presentations of HUS and thrombotic thrombocytopenic purpura (TTP), even though they have clearly different aetiologies. Patients with an acquired or inherited deficiency of the von Willebrand protease, ADAMTS13, are much more likely to present with insidious or fluctuating neurological signs of TTP, rather than the abrupt renal failure that typifies HUS. But this is not absolute.

In 2006, the European Paediatric Study Group for HUS [2] published a revised classification of HUS, TTP, and related disorders, based on a contemporary understanding of causation (summarised in Table 1). In brief, patients can be classified at two levels. In level 1, the cause is well established, even if details of the pathogenesis are incomplete. In level 2, disease or drug associations may be described but causation is not proven. In practice the diagnostic subgroups in level 1 segregate well. However, occasionally an individual patient, or a particular episode in an individual, may have more than one aetiology. The classification permits this. It fits with the idea that HUS can be caused by either, or both, an external environmental trigger and/or an intrinsic inherited risk factor. This is referred to as a "two hits" hypothesis. For example, a patient with a complement factor H mutation might present with an episode of HUS precipitated by infection, perhaps enterohaemorrhagic *Escherichia coli* infection, or pregnancy. With comprehensive investigation, almost all patients can be allocated to an aetiologically-based subgroup diagnosis. However, some of the investigations dealt with below are costly and not easily accessed. A guideline has therefore been proposed that uses clinical presentation in the first instance to indicate which advanced investigations are needed, and what immediate treatment should be considered while awaiting diagnostic results.

Table 1 Classification of HUS based upon causation

Level 1: Aetiology advanced
 1(i) Infection induced
  (a) Shiga and shiga-like toxin producing bacteria, enterohaemorrhagic
    Escherichia coli, Shigella *dysenteriae* type 1
  (b) *Streptococcus pneumoniae*
 1(ii) Disorders of complement regulation
  (a) Genetic
  (b) Acquired
 1(iii) ADAMTS13 deficiency
  (a) Genetic
  (b) Acquired
 1(iv) Defective cobalamine metabolism
 1(v) Quinine-induced
Level 2: Aetiology unknown
 2(i) HIV infection
 2(ii) Malignancy, cancer chemotherapy, ionizing radiation
 2(iii) Calcineurin inhibitors and transplantation
 2(iv) Pregnancy HELLP syndrome, contraceptive pill
 2(v) Systemic lupus erythematosus, antiphospholipid antibody syndrome
 2(vi) Glomerulopathy
 2(vii) Familial not included in level 1
2(viii) Unclassified

Investigation

The diagnosis of HUS caused by enterohaemorrhagic *E. coli* or invasive pneumococcal infections [55] is generally straightforward, based upon the clinical presentation supported by locally available microbiological tests [3, 40]. These two groups account for at least 90% of all childhood HUS [31]. The remainder, commonly referred to as "atypical" for want of a better description, need comprehensive investigation including mutation analysis of specific complement genes [27] and the activity of von Willebrand factor cleaving protease, ADAMTS13 [53]. A subgroup diagnosis can be expected in about 50% of these cases. A list of relevant investigations for the atypical subgroup is given in the guideline and is updated on the website of the European Paediatric Study Group for HUS at www.espn.cardiff.ac.uk/registries. The guideline also gives contact details of specialist laboratories that provide complement gene mutation analysis and investigation of ADAMTS13 activity.

Enterohaemorrhagic *E. coli* infection

Enterohaemorrhagic *E.coli* (EHEC) cause about 90% of all childhood HUS in developed countries [11, 31]. EHEC have various virulence factors implicated in causing haemorrhagic colitis and HUS in humans, but an essential property is the production of certain Shiga toxins (Stx). Not all Stx producing *E.coli* (STEC) are pathogenic in man, and therefore they are not all EHEC [38]. The forms of Stx implicated in human disease are mostly Stx-2 and its variant Stx-2c, and less frequently Stx-1 (Stx-1 is identical to Shiga toxin, the exotoxin of *Shigella dysenteriae* type 1) [17, 19, 26, 29, 30]. EHEC with more than one toxin plasmid, for example, Stx-1 plus Stx-2, are well recognized. Organisms producing Stx 2d had been thought not to cause human disease but recently a toxin variant has been described that is activated by enzymes in intestinal mucus to the form Stx-2d$^{(activatable)}$ which is implicated in HUS [3]. Stx 2e is the toxin associated with Edema disease in piglets, and Stx-2f -producing *E. coli* occur in avians with no evidence to date that they can intoxicate humans.

The majority of EHEC express intimin on their surface and can deliver the intimin receptor into host enterocytes by a type 3 secretion system [13]. This is an important virulence factor and is the property that defines enteropathogenic *E. coli* (EPEC). It permits the organisms to bind tightly to the host surface, and the enterocyte responds by cytoskeletal changes. This induces loss of villi and pedestal formation, the attachment and effacement lesion. The loss of absorptive villi is the explanation for the watery diarrhea induced by EPEC. Understanding the molecular basis for

attachment and effacement and the host signalling mechanisms that subtend it has progressed rapidly [7]. While this is recognised as an important virulence factor for EHEC, probably by allowing exotoxin to be produced in the immediate vicinity of the enterocyte, it is not essential. Thus any strategy to immunize against intimin or other components of the type 3 secretion might reduce but not abolish the risk of EHEC.

Being a zoonosis, EHEC reside in other species, often without causing disease in them. Transmission to humans has been described via a wide range of foods, but only recently has the importance of direct contact with animals, particularly petting farm animals, been shown [56]. It is not known why some individuals develop colitis and HUS after EHEC exposure while others do not. Humans acquire antibodies against Stx [30], and experimental models show that Stx antibody can protect against toxic manifestations [51]. The incidence of HUS is greatest in children between 6 months and 5 years of age and this might be explained by their lack of immunity to toxin. A strategy to prevent human disease by active immunization with relevant Stx toxoids is logical, and novel approaches to the development of such agents are being pursued.

At present there is no proven active treatment for EHEC infection or HUS. The role of antibiotics used during the diarrhoeal phase of the disease remains controversial. A recent meta-analysis has not shown adverse effects of antibiotics [45]. However, the conclusions are weakened because different antibiotics were given, and there was variable correction for severity of disease in the studies. Some antibiotics cause a burst of toxin release from the affected bacteria in culture, while others are thought not to do so [25]. Given that EHEC are typically not enteroinvasive and the infection is self-limiting, there is no direct indication for their use, and with hypothetical reasons why they might be harmful, most paediatric nephrologists do not use them.

Supportive therapy, including careful fluid and electrolyte balance remains the mainstay of treatment of this form of HUS [1]. It is vital that children with confirmed or suspected STEC infection are monitored carefully for their intravascular volume status. The development of oligoanuric renal failure during HUS indicates a poorer long term outcome [18], and a recent study suggests that early intravascular volume expansion with isotonic saline preceding the oligoanuric phase may reduce the incidence of oligoanuric renal failure [1].

Therapeutic proposals to sequester Stx in the intestine have not shown clinical benefit [52]. However, passive immunisation with anti-Stx antibodies has been shown to prevent or arrest disease in laboratory animals and is an attractive idea for humans [33]. Humanised monoclonal antibodies against Stx-1 and Stx 2 (Shiga-mab, Thallion

Pharmaceuticals Inc., Canada) are now being tested in phase 2 trials. The initial studies propose to administer the antibody in the diarrhoeal stage of the illness before HUS occurs to show whether HUS can be prevented. A prerequisite for such studies will be the ability to positively diagnose EHEC infection at an early stage. Selective stool culture techniques with subsequent identification of Shiga-like toxin or Stx bacteriophage are too slow for this strategy. Rapid, near-to-patient techniques are required and under development. If successful this would revolutionize the ability of clinicians to identify and intervene early in EHEC infections.

The mortality from Stx-HUS is now about 2% [31]. It is important to note that although the glomerular filtration rate returns to normal in most patients, there may be permanent nephron loss, which may have longer term sequelae [9, 32]. A recent systematic review and meta-analysis of the intermediate outcome of Stx-HUS described permanent and serious renal sequelae (hypertension, proteinuria, declining GFR) in 5–25% of patients [18].

Disorders of complement regulation

Although cases of HUS with complement abnormalities were described more than 20 years ago, the significance of this was not appreciated until the seminal report of Warwicker et al. [54]. Since then a number of different gene mutations have been described in patients with HUS, all within genes encoding proteins of the alternative complement pathway [5, 6, 12, 15, 16, 35, 36, 39, 43, 44, 50].

The alternative complement pathway

The alternative complement pathway is a network of plasma proteins that provides antibody-independent defence against micro-organisms. The goal of the pathway is to quickly remove invading pathogens by coating them with the opsonin, C3b, and by promoting terminal complement pathway activation on their surface. The activation and regulation of the alternative pathway is shown in Fig. 1.

Since the alternative complement pathway is spontaneously activated by the hydrolysis of fluid phase complement C3, and positively amplified, its regulation is essential for host cell protection. This is provided by fluid-phase and membrane bound complement regulatory proteins. In the fluid phase, C3b is inactivated by the serine protease factor I (F1) which is dependent on cofactor activity from other regulators. Membrane cofactor protein (MCP) is a resident transmembrane protein expressed on almost every human cell except erythrocytes. It is a cofactor for factor I in the
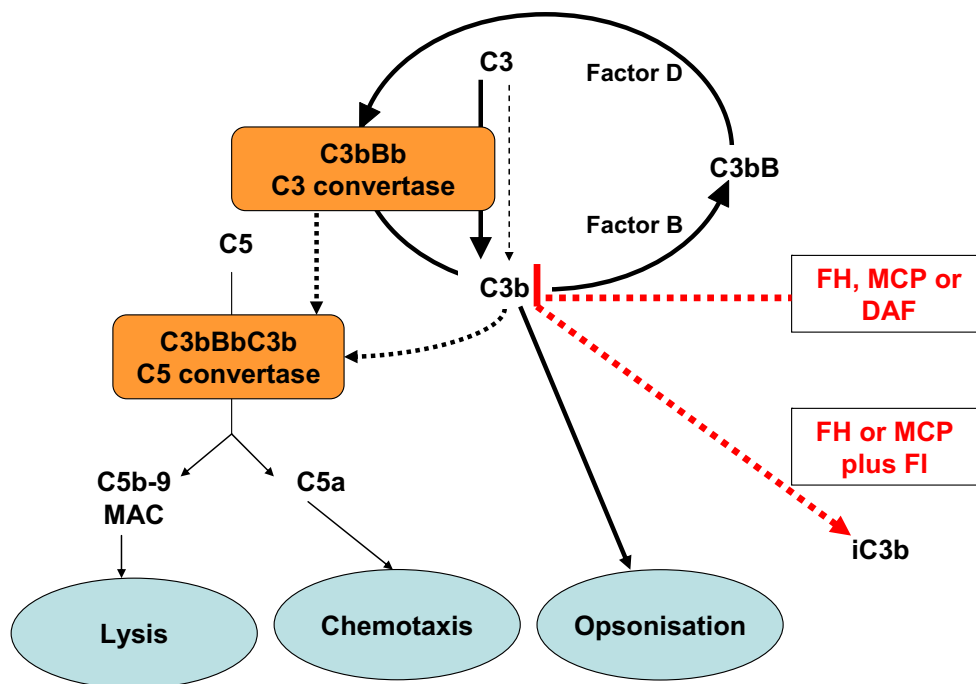
Fig. 1 The alternative complement pathway showing the centrality of C3b. The alternative pathway is continuously activated through the spontaneous hydrolysis of C3 (*black dashed line*) to a form of C3b that is able to combine with factors B and D to form a C3 convertase. This generates further C3b from C3 and a stable C3 convertase (C3bBb) is formed, amplifying the production of C3b. This C3b can participate in further C3 convertase formation and C5 convertase formation (the addition of one C3b molecule to the C3 convertase). Cleavage of C5 results in the C5a fragment (an anaphylotoxin) and the C5b fragment, which combines with C6, C7, C8, and multiple C9 molecules to form C5b-9, the membrane attack complex (MAC). The end results of alternative pathway activation are opsonisation with C3b, chemotaxis through anaphylotoxin C5a, and cell lysis through MAC. Host cells possess membrane-bound complement regulatory proteins (e.g. membrane cofactor protein, MCP, decay accelerating factor, DAF) and are rich in polyanions which promote the binding of plasma regulator factor H (FH). These regulators inactivate C3b (*red dashed line*) or dissociate the C3 convertase (*dotted red line*) so that amplification is limited

cleavage of C3b and C4b (formed during classical and lectin pathway activation) and also promotes the decay of C3 convertases (decay acceleration). Factor H is a fluid-phase regulator that has cofactor activity for the cleavage of C3b by factor I and also has decay acceleration activity. In addition to its role as a fluid phase regulator, FH is the main mechanism by which the alternative pathway discriminates between foreign and host surfaces. Unlike many pathogens, host surfaces are rich in polyanions such as sialic acid and glycosaminoglycans. FH has high affinity for these anionic sites, particularly when C3b is deposited during complement activation on the cell surface.

Mutations in FH, MCP, and FI are all implicated in the pathogenesis of HUS and the incidences of these mutations are summarised in Table 2. There are now reports emerging that mutations in C3 and factor B have also been detected in patients with HUS [15].

Complement gene mutations in HUS

FH mutations associated with HUS are mostly heterozygous missense mutations affecting the C-terminal domain of the protein which is important for host cell recognition [49]. In such cases, plasma FH and C3 concentrations are often normal. Mutant FH proteins exhibit reduced binding to C3b [23, 48], to heparin [22, 23], and to endothelial cells [23]. Most work normally as a cofactor for the cleavage of C3b in the fluid phase but show impaired complement regulation on cell surfaces including endothelial cells and erythrocytes [20, 47]. Other FH mutations affect the structure of the protein causing impaired secretion and low plasma concentration of factor H [37]. Plasma C3 concentration may be normal or low. Patients with FH mutations tend to have early onset of disease (70% onset before 12 years of age), follow a relapsing course, and 70% of cases progress to end-stage renal failure or death [6, 50].

Table 2 Incidence of mutations in proteins of alternative complement pathway in cohorts of patients with non-Stx-HUS

Gene	Incidence of mutations	Reference
Factor H	36 of 220 cases (16%)	[35, 39, 43, 50]
MCP	21 of 173 cases (12%)	[12, 16, 36, 44]
Factor I	13 of 193 cases (7%)	[12]
Factor B	Not yet determined	
C3	Not yet determined	[14–16]

Renal transplantation also has a poor outcome. Graft loss, mostly due to recurrent disease or graft thrombosis, occurs in about 80% of cases [4].

MCP mutations are usually heterozygous and appear to result in heterozygous MCP deficiency or impaired binding to C3b [6]. HUS associated with MCP mutation follows a relapsing course with gradual progression to end-stage renal failure. Recurrence after renal transplantation is rare and it is likely that the donor kidney is protected from recurrence because of donor MCP expression in the vasculature of the graft, although endothelial donor-host chimerism is a potential risk [14].

The FI mutations so far described are heterozygous and associated with either normal or reduced FI concentration and a variable reduction of complement C3. Certain mutant proteins demonstrate reduced inactivation of C3b and C4b [28]. The clinical course is severe with progression to irreversible renal failure. Renal grafts have been lost to recurrent HUS in the majority of those who have undergone renal transplantation [6].

Mutations have also been described in factor B and C3 and these appear to result in gain of function by rendering the C3 convertase more resistant to decay [12, 15]. In addition, some patients have antibodies to FH that impede its regulatory activity [10, 24].

In combination, these findings point to increased activation of the alternative complement pathway as a central phenomenon in this form of HUS. This is supported by animal models in which the terminal complement pathway is essential for developing glomerular thrombotic microangiopathy [34]. However, plasma concentrations of C3 in patients are often normal or only marginally reduced. It is therefore assumed that the escape of complement activation that underlies the disease is seldom systemic but takes place as a local event within the glomerular microcirculation.

Novel therapeutic strategies

Knowing the cause of atypical HUS allows a theoretical approach to therapy even though details of the pathogenesis are unconfirmed. In practice, the complex laboratory investigations needed to confirm aetiology take several weeks, and therefore initial treatment has to be empirical. The European Study Group for HUS argues for plasma exchange, replacing with fresh frozen plasma or a standardised whole plasma product such as Octaplas, as a catch-all, first line treatment. This is suggested on the basis that it would replace mutant complement proteins responsible for the disease and remove autoantibodies to factor H or ADAMTS13. These authors justify the urgency and invasive nature of this approach on the fact that glomerular thrombotic microangiopathy is a destructive process and a first episode of HUS may lead rapidly to end-stage failure. At present there are anecdotes but no evidence to back up this proposal. In patients with MCP mutations, plasma therapy is not expected to have a direct impact on the disease as MCP is a resident membrane protein not a circulating plasma factor.

It seems likely that specific treatments will be needed in different groups once the cause has been identified. In patients with genetic FH abnormalities, and based on the idea that the disease is caused by haplotype insufficiency, it seems logical to give normal factor H. Plasma derived factor H concentrate is being developed commercially with that intention. The same logic applies to HUS associated with FI mutation. Both factor H and factor I are produced in the liver, and successful liver transplantation would theoretically restore normal complement regulation. So far six cases of FH associated HUS have been treated by liver or liver plus kidney transplantation. Poor outcome occurred in the first three cases [8, 41, 42] but the three survivors have good organ function, normal complement activity, and no recurrence of HUS [21, 46].

Summary

The clinical presentation of HUS gives a strong indication as to the underlying cause. EHEC infection remains the most prevalent. The virulence factors of EHEC in humans are increasingly understood, as is the ecology of these organisms in the wider environment. Patterns of transmission are better recognised. New tools for microbiological diagnosis are in production, and there are prospects for early intervention by targeting Stx.

Patients with atypical HUS (familial or relapsing, or not associated with EHEC or *Shigella dysenteriae* or invasive *Streptococcus pneumoniae* infection) should be fully investigated to determine the cause. These patients are difficult to manage and should be transferred to a paediatric nephrology service urgently. There is a strong argument for empirical and early plasma exchange to replace defective proteins or remove causative antibodies. Knowledge of the cause of disease, particularly in the group with abnormal complement regulation, opens up the possibility of specific treatments in the near future.

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