DDICC Meeting Minutes June 26, 2001

Digestive Diseases Interagency Coordinating Committee (DDICC)

Hemolytic-Uremic Syndrome
Meeting Summary

National Institutes of Health
Building 1, Room 151
Bethesda, MD

Participants

Chair:

Jay Hoofnagle, M.D.	National Institute of Diabetes and Digestive and Kidney Diseases

Members:

Josephine Briggs, M.D.	National Institute of Diabetes and Digestive and Kidney Diseases
Dennis Lang, Ph.D.	National Institute of Allergy and Infectious Diseases
Kenneth Gruber, Ph.D.	National Institute of Dental and Craniofacial Research
Frank Hamilton, M.D., M.P.H.	National Institute of Diabetes and Digestive and Kidney Diseases
Stephen James, M.D	National Institute of Diabetes and Digestive and Kidney Diseases
Thomas Kresina, Ph.D.	National Institute of Allergy and Infectious Diseases
James A. Lindsay, Ph.D	United States Department of Agriculture
Clare Schmitt	Center for Scientific Review
Gladys Hirschman	National Institute of Diabetes and Digestive and Kidney Diseases

(Two additional attendees from NIDDK and two from NICHD)

Speakers:

Phillip Tarr, M.D.	University of Washington
Howard Trachtman, M.D.	Albert Einstein College of Medicine

Guest:

Marcia Ciol	University of Washington

Welcome

Jay H. Hoofnagle, M.D., Director of the Division of Digestive Diseases and Nutrition at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and Chair of the Digestive Diseases Interagency Coordinating Committee (DDICC), welcomed the participants to this meeting on hemolytic-uremic syndrome (HUS).

***E. coli* 0157:H7 and the Pathophysiology of HUS**

Phillip Tarr, M.D., Professor of Pediatrics and Microbiology at the University of Washington, discussed his research on HUS, a nonimmune, microangiopathic process that occurs mainly in children. HUS consists of hemolytic anemia, thrombocytopenia, and thrombi in small vessels throughout the body, but in childhood, primarily in the kidneys. In the Pacific Northwest and elsewhere in the world where HUS is common, *E. coli* 0157:H7 is the predominant and often the exclusive precipitant of postdiarrheal HUS. The disease is largely endemic, sporadic, and rural.

In a typical HUS case, diarrhea starts 3 days following ingestion of *E. coli* 0157:H7, and the diarrhea turns bloody after 1 to 3 days. The advent of bloody diarrhea prompts parents to bring their child to an emergency room, about 2.5 days following the beginning of diarrhea.

In the hospital, a sorbitol MacConkey agar culture substantiates infection with *E. coli* 0157:H7. Of those children who are infected with this organism and develop bloody diarrhea, 10 to 15% progress to HUS, usually on day 6.5 following the beginning of diarrhea, and 85 to 90% of the cases resolve spontaneously.

Dr. Tarr's group has established an HUS surveillance system throughout Oregon, Washington, and Idaho to identify *E. coli* 0157:H7-infected children as soon as possible after they present in the emergency room. The system includes 40 hospital-based and 8 private laboratories and 34 institutional review boards (IRBs).

Dr. Tarr reported data on the HUS surveillance system. Patient eligibility for enrollment in the study includes children 10 years old or younger with either a positive culture for *E. coli* 0157:H7 or acute bloody diarrhea. An informed consent form is provided to the patients' parents. Blood, urine, and stool samples are obtained, and a standardized questionnaire is administered as soon as possible after admission.

The definition of HUS used in the study includes: hematocrit less than 30%, hemolysis observable on a peripheral blood smear, platelet count under 150,000/mm^3, and creatinine above normal levels by age. Between April 1997 and September 2000, 129 infected subjects were enrolled; 18 subsequently developed HUS. Two control groups were also enrolled—children undergoing operations for noninflammatory disorders, such as hernias, and children with acute bloody diarrhea caused by a pathogen other than *E. coli* 0157:H7. Because HUS pathogenic characteristics can be detected as early as 5 days after initiation of diarrheal illness, researchers restricted their analyses to children enrolled on or before day 4 of illness in order to avoid misidentifying a disease outcome as a disease predictor.

Dr. Tarr reported that, contrary to other studies, no evidence was found that expression of P1 or ABO blood group antigens protects against HUS development. Previous studies had suggested that these antigens, which bind Shiga toxins *in vitro*, might sequester these toxins *in vivo*, thereby preventing them from affecting endothelial cells. Also, Dr. Tarr's group found no evidence that common host prothrombic alleles play a role in HUS development in infected children. Prothrombic allele frequencies were similar among subjects who progressed to HUS compared with a control group and published North American controls.

Dr. Tarr said that *E. coli* 0157:H7's Shiga toxin genotype seems to play a role in HUS development, but the relationship is complex. *In vitro* and *in vivo* animal data suggest that Shiga toxin 2 (Stx2) is the more potent of the two toxins, but epidemiologic and retrospective data paradoxically show that *E. coli* 0157:H7 that express genes encoding both Shiga toxin 1 (Stx1) and Stx2 are less likely to be associated with HUS than those containing Stx2 alone. The data from Dr. Tarr's group also suggested a trend toward a higher frequency of HUS development among children infected with Stx2 isolates compared with children infected with isolates containing both toxins. However, the researchers also identified the first North American isolate—and only the second isolate ever—of an Stx1-producing *E. coli* 0157:H7 strain that did not contain an Stx2 gene, and the strain was isolated from a child who developed HUS.

Expecting a positive correlation between the titer of free fecal Shiga toxin in stool samples and the risk of developing HUS, the investigators instead found a negative correlation. Children with uncomplicated infections had higher free Shiga toxin titers than those who progressed to HUS, and two of the three children who had appreciable titers prior to developing HUS had the mildest forms of the disease. Most of the children who had undetectable free fecal Shiga toxin had detectable *E. coli* 0157:H7. Dr. Tarr concluded that free fecal Shiga toxin should not be used as an index of host injury or therapy.

Because thrombotic thrombocytopenic purpura (TTP), like HUS, involves low platelet counts, the researchers examined the blood von Willebrand factor (vWF) metalloproteinase (MP) levels, since this MP is deficient in TTP. vWF is secreted from endothelial cells as a large, flexible, disulfide-linked polymer. As vWF courses through small blood vessels, it is unfolded into a form that stimulates platelet aggregation but is soon cleaved by vWF MP.

The researchers found that children with *E. coli* 0157:H7 infection, children with HUS, and normal controls all had similar levels of vWF MP activity in their blood but that the normal size of the circulating vWF multimer decreased both before and during HUS. Dr. Tarr's group hypothesized that between the time of presentation in the emergency room and the development of HUS, thrombi increasingly occlude small blood vessels, causing vWF multimers to be sheared to sizes that are smaller than normal and less able to stimulate platelet aggregation.

The researchers also discovered abnormally high fibrin levels in thrombi in HUS, in contrast to TTP, in which minimal or no fibrin is found in thrombi. Also in contrast to TTP, in which abnormally high levels of vWF occur in the blood vessel lumen, normal intraluminal vWF levels were found in HUS. The researchers concluded that no theoretical support exists for plasma therapies in Stx-related HUS.

The investigators found no significant differences in cytokine levels in the urine or blood among pre-HUS children, children with uncomplicated infections, or children with HUS. Usually, the values were normal.

Dr. Tarr suggested that the disease process leading to HUS begins when Shiga toxin, and possibly other proteins secreted by *E. coli* 0157:H7, injure blood vessel endothelial cells, thereby triggering coagulation. Prothrombin is converted to thrombin at a high rate, as indicated by elevated blood levels of Fragment 1+2 (F1+2), the peptide cleaved from prothrombin during its conversion. Prior to the development of HUS, while hematocrit, platelet count, and creatinine values are still normal, F1+2 levels are as high as in life-threatening septic syndromes. Circulating F1+2 levels are also significantly high in *E. coli* 0157:H7-infected children who do not progress to HUS.

In addition to increased coagulation, Dr. Tarr noted that HUS also involves a defect in fibrinolysis, the process that limits the size of blood clots. In fibrinolysis, tissue-type plasminogen activator (t-PA)—the main circulating anticoagulant—converts plasminogen to plasmin, which breaks down fibrin multimers in the clot to D-dimers. t-PA is primarily inhibited by plasminogen activator inhibitor type 1 (PAI-1), the levels of which are elevated prior to the development of HUS. When t-PA is complexed to PAI-1, fibrinolysis does not occur, so abnormally high PAI-1 levels result in vascular occlusion by blood clots.

Dr. Tarr indicated that t-PA levels are elevated prior to the development of HUS, but because PAI-1 levels are also elevated, the t-PA is complexed to PAI-1 and is therefore inactive. D-dimer levels in the blood are also elevated prior to the development of HUS, as well as during HUS and in uncomplicated infection. However, Dr. Tarr hypothesized that the elevated D-dimer levels represent elevated fibrin levels due to the large numbers of blood clots rather than because of net fibrinolysis.

Dr. Tarr reported that coagulation activation during HUS development occurs prior to renal injury. Prior to developing HUS, the majority of children in the study who had provided urine samples had normal beta-2 microglobulin levels in their urine, indicating the absence of renal injury. When HUS developed, and urine samples could be obtained,

the samples contained dramatically elevated beta-2 microglobulin levels. Dr. Tarr conjectured that these levels were probably higher in children who were not producing urine.

In a multicenter study peripheral to this NIDDK-funded study, researchers found that nephrologic resolution, as indicated by resumption of urination and falling creatinine levels, does not occur until PAI-1 levels start to decrease. This indicates that until the blockage on fibrinolysis is removed, the kidneys do not resume proper functioning.

Dr. Tarr indicated that over the next several years, his research group will try to better define the coagulation lesion. To demonstrate increased fibrin formation in the pre-HUS state, researchers will measure blood fibrinopeptide A (FPA) levels, since thrombin cleaves FPA from fibrinogen during coagulation. They will also look for intact soluble fibrin, which is not useful in hemostasis and, therefore, could be used as an indicator of dysregulated fibrin generation. Also of interest is the question of whether PAI-1 elevation is specific to *E. coli* 0157:H7 infection or is a nonspecific host stress response.

To better determine when renal injury occurs during HUS development, investigators will collect daily urine samples to determine at what point urinary enzymes start to rise. Dr. Tarr suggested that until urinary enzyme levels increase, the kidneys remain uninjured and are amenable to thrombolytic treatment.

The 24- to 48-hour window between the time of patient presentation in the emergency room to the time of HUS development represents an opportunity to prevent HUS. Dr. Tarr concluded by stating that one potential treatment for HUS is administration of an antithrombin hirudin analog such as loperidine. Another possible treatment might be a drug that will prevent PAI-1 inhibition of t-PA.

Multicenter Trial of SYNSORB Pk in *E. coli*-Related HUS

Howard Trachtman, M.D., Professor of Pediatrics at Albert Einstein College of Medicine, discussed results from the largest clinical trial so far attempted for HUS. The trial tested the efficacy of SYNSORB Pk, an adsorbent agent composed of a platform molecule—diatomaceous silicon dioxide—covalently linked to Pk—the trisaccharide that comprises the Shiga toxin receptor on the endothelial cell surface. The theory was that SYNSORB Pk, by binding Shiga toxin, would limit its absorption from the gastrointestinal tract, thereby preventing continued injury to the kidney.

The trial was conducted from July 1997 through April 14, 2001, when it was terminated. Thirty centers participated. The administrative center was at Schneider Children's Hospital in Long Island, NY; the microbiology core laboratory was at Tufts New England Medical Center; and the data coordinating center was at Children's Hospital of Philadelphia.

The primary endpoints were to: (1) reduce the combined endpoint of death and serious extrarenal events from 20% to 5%; and (2) reduce the need for dialysis from 50% to 25%.

Children enrolled in the study exhibited the clinical triad of HUS, including bloody diarrhea. Researchers elected to do a 2:1 randomization scheme to facilitate recruitment. Researchers enrolled 150 children with HUS prodrome. The racial breakdown of subjects reflected the fact that HUS is predominantly a disease of Caucasians—80% of subjects were white; the other 20% were mostly Asian. Only two subjects were African-American.

Subjects in the treatment group received 500 mg/kg/day of SYNSORB Pk every 8 hours. The study was conducted in two phases: a hospital phase of variable duration, depending on the severity of illness, and a 60-day outpatient followup period. Dialysis was initiated only in children who had oligoenuria for 72 hours, which allowed time for SYNSORB Pk to demonstrate efficacy.

Of the 120 children who completed the entire followup period, 77 received SYNSORB and 43 received a placebo. The incidence of the first endpoint was 17% in the SYNSORB-treated group and 21% in the placebo group, and no statistical difference in dialysis rates was found between the two groups. On the basis of these results, the study was terminated.

Almost half of the children in the study had clear evidence of a Shiga toxin-producing strain in their intestines. Almost all isolated strains produced either Stx2 or a combination of Stx1 and Stx2. Less than 5% produced only Stx1.

Dr. Trachtman indicated that interleukin-8 (IL-8), interleukin-1 alpha (IL-1α), and tumor necrosis factor alpha (TNFα) levels were measured in stool, urine, and plasma samples. IL-8 levels were acutely elevated within the first day of sampling. IL-1α levels were also elevated early in the disease, although less than IL-8. Virtually no samples were positive for TNFα, in contrast to reports from other studies. TNFα has been eliminated from the conclusion phase of the analysis.

Basic fibroblast growth factor (bFGF) levels rose dramatically during the first 5 days of illness and normalized rapidly during the convalescent phase. Unlike the other cytokines measured, bFGF levels in plasma did not change in parallel with bFGF levels in urine and stool. Dr. Trachtman suggested that bFGF levels might be used to assess the severity of illness, time the recovery, and prognosticate for the future. Plasma bFGF levels later in the disease process might serve as an index for recovery of organs other than the kidney, and urinary bFGF levels might serve as an index for renal recovery.

Urinary HMG1 (a macrophage-derived cytokine) levels have been measured in 13 patients. In studies with mice, HMG1 has been found to be released 24 to 36 hours after the injection of lipopolysaccharide (LPS). Administering anti-HMG antibodies to mice lowers the mortality rate following LPS injection, while antibodies for all other cytokines have no impact on mortality from LPS endotoxemia. Researchers found that of the 13 children, 5 had peak HMG1 values over 2 days after HUS onset, suggesting that a therapeutic intervention aimed at HMG1 might ameliorate the disease.

Dr. Trachtman concluded that the study's endpoints were too ambitious. For endpoint 1, a more realistic goal would be to lower the combined rate of death and serious extrarenal events from 18% to 12%. Regarding endpoint 2, the original estimate of 50% of patients requiring dialysis was too high, the actual rate being closer to 40%. A realistic endpoint would be to reduce that rate by 27 to 33%.

Dr. Trachtman reported that $1.6 million was spent on the trial: 25% at the administrative center, 20% for patient care, 25% in the data coordinating center, and 30% for scientific studies.

Regarding treatments for HUS, Dr. Trachtman suggested that oral treatment with Stx-binding agents may not be worth investigating further because Shiga toxins may no longer be in the intestinal lumen by the time the agents are administered to patients. A potentially more effective approach might be to block cytokines with delayed activation, such as HMG1. Another possible therapeutic approach would be to inject monoclonal antibodies directed against Stx2 to protect against neurological symptoms and to prolong survival. This has been demonstrated in a model with piglets, which develop CNS disease and have increased mortality.

In closing, Dr. Trachtman noted that a genetic marker for children who are likely to develop HUS might be the allele that codes for a mutation in the Short Consensus Repeat 20 (SCR 20) domain of factor H, a regulatory compound in the alternative complement cascade. Factor H deficiency has been associated with HUS susceptibility.

In discussion, Dr. Tarr indicated that HUS might be transmitted from one family member to another. In families in which one case of HUS occurs, an additional instance is reported in 7 to 10% of cases. However, these subsequent cases could be due to coprimary or secondary infections. He said that children admitted to the hospital with bloody diarrhea are placed under contact precautions involving the use of gowns, gloves, and masks. Because these items are unlikely to be used in the home, he has concluded that all children with bloody diarrhea should be admitted to the hospital.

Dr. Tarr also said that only a minority of people who are colonized with *E. coli* 0157:H7 develop symptoms. In the widely reported "Jack in the Box" outbreak, researchers estimated that for every child who ate an infected hamburger and developed symptoms, 300 ate infected hamburgers and did not develop symptoms. Similar numbers have been reported in other outbreaks. Farm families have been found in which children have appreciable numbers of *E. coli* 0157:H7 in their stools and yet develop no symptoms. Also, studies indicate that under 1% of food service workers carry the organism at detectable rates.

The Committee discussed studies in which bacterial toxins induced cytokine responses within the first few hours after toxin administration, but showed diminished responses after 24 hours. This early cytokine response might be why Dr. Tarr detected few changes in cytokine levels in his study.

Dr. Tarr was asked about interventions he considered promising, particularly interventions earlier in the disease. He replied that children with *E. coli* 0157:H7-related HUS who received parenteral hydration had a lower dialysis rate than those who did not receive this treatment. Thus, he recommends that the standard of care for the control group should be parenteral hydration and hospitalization.

Research Portfolios Funded by NIH

Frank Hamilton, M.D., M.P.H., discussed the NIDDK Division of Digestive Diseases and Nutrition research portfolio. He said that in 1994, NIDDK, the National Institute of Allergy and Infectious Diseases (NIAID), the U.S. Department of Agriculture (USDA), and the Department of Defense (DoD) produced a consensus conference on the role of emerging *E. coli* 0157:H7. As a result of this conference, NIDDK allocated $1.5 million for this activity and issued an RFA. Forty applications were received, and 12 grants were funded. In FY1998, Congress requested a consensus on medical treatment for *E. coli* and other food-borne illnesses. NIDDK produced a Congressional report that highlighted research efforts by NIAID and NIDDK. Identified research needs were:

- transmission reservoirs
- pathogenesis of HUS
- development of an animal model of HUS
- mechanism by which *E. coli* causes endothelial injury
- mechanisms by which *E. coli* colonizes the gastrointestinal (GI) tract.

Another RFA was released in 1999 and subsequently funded in FY2000. This RFA funded 45 grants, 5 of them by NIAID. NIDDK is currently funding six new grants related to the gastrointestinal tract. NIDDK's budget for current research on *E. coli* 0157:H7 infection is $1.8 million, which funds 12 investigators.

Gladys Hirschman, M.D. then discussed the Division of Kidney, Urologic, and Hematologic Diseases research portfolio. She said that the first RFA on *E. coli* 0157:H7,released in 1996, supported research on:

- renal cell dysfunction
- the genetics of human glomerular endothelial cell receptors and its relationship to the development of HUS
- development of a baboon model of HUS
- SYNSORB Pk treatment of HUS.

Most of the projects supported in response to the second RFA issued in FY2000 emphasize studying the pathophysiology of HUS. New grantees are studying:

- the role of inflammation and thrombosis in the development of HUS
- the role of the renal angiotensin system at the level of the proximal renal tubule leading to HUS
- the effect of Stx1 on brain endothelial cells
- the role of endothelial cell glycolipid receptors in HUS pathology
- the role of thrombin and other coagulation factors in HUS
- recombinant Stx-specific human antibodies.

Dennis Lang, Ph.D, discussed the research portfolio of the Enteric Diseases Program in NIAID's Division of Microbiology and Infectious Diseases. He said that he prepares an annual report on NIH spending on food- and waterborne illnesses for the Secretary of the Department of Health and Human Services. The major food-borne disease organisms addressed by the report are *Campylobacter* (the leading cause of diarrheal disease in the U.S.), several strains of *E. coli*, *Salmonella, Shigella, Listeria, Clostridium, Staphylococcus,* and *Bacillus*. The major waterborne disease organisms addressed by the report are *Vibrio cholera* and *Aeromonas hydrophila*. The major viruses addressed by the report are caliciviruses, rotavirus, astrovirus, and some parvoviruses.

Dr. Lang indicated that NIH currently funds 386 projects on these organisms. The amount of funding for research on food- and waterborne illnesses will probably continue to increase.

Research Portfolios Funded by USDA

James A. Lindsay, Ph.D., National Program Leader of the Food Safety Program in the Agricultural Research Service (ARS) of USDA, presented an overview of USDA research on food safety. He said that the budget for the food safety program in ARS, the research arm of the USDA, has increased by $45 million within the past 5 years, particularly for projects involving *E. coli* 0157:H7, *Listeria*, and *Campylobacter*. The annual budget is currently $92 million. Thirty-eight projects involve *E. coli* 0157:H7, and funding for these projects totals nearly $12 million.

Dr. Lindsay stated that the Food Safety Program involves both preharvest and postharvest projects. Current preharvest projects include:

- developing methods to trace flow of enterohemorrhagic *E. coli* (EHEC) reservoirs
- investigating transmission between and within herds
- identifying strategies to control EHEC in cattle
- studying whether transportation increases shedding of pathogen prior to slaughter
- developing an RT-PCR method for differentiating EHECs

- studying the effectiveness of vaccination in cattle
- studying the colonization and shedding of strains lacking *Stx* and *tir* genes
- developing a whole-carcass fecal detector for studying fecal contamination in cattle
- developing a method for eliminating pathogens in cattle that involves adding sodium chlorate to their drinking water. This method eliminates pathogens, including *E. coli* 0157:H7 and *Salmonella*, but has no effect on beneficial bacteria.

Current postharvest projects include:

- studying whether the acid washes used on carcasses affect the outer membrane structure and virulence of *E. coli* 0157:H7
- developing a 96-volt format for *E. coli* 0157:H7 biosensors
- developing automated inspection of fruit and vegetables
- developing high-pressure processing strategies for meat
- studying whether ethnic slaughtering methods, such as kosher slaughter, affect *E. coli* 0157:H7 levels
- developing improved safety methods, such as radiation and thermal inactivation
- studying the host stress response to bacterial pathogens
- developing multiplex PCRs that allow simultaneous measurements of *E. coli* 0157:H7, *Salmonella*, and *Listeria*
- developing a pathogen-modeling program that predicts growth of any organism under a variety of conditions
- developing methods of onsite decontamination of fresh fruit and vegetables
- studying the incorporation of bacteriophages into packaged fruit and vegetables
- studying whether *E. coli* 0157:H7 encapsulation in protozoal cysts is related to seasonal fluctuations in outbreaks
- studying factors that affect dairy production, including *E. coli* 0157:H7 and other pathogens.

Cooperative research projects conducted with the National Alliance for Food Safety, a consortium consisting of ARS plus various universities, include:

- studying factors contributing to the presence of *E. coli* 0157:H7 in feed lots

- studying the prevalence of antibiotic resistance in cattle.

In the discussion period, Dr. Lindsay noted that the President's Food Safety Initiative has resulted in increased awareness among agencies of each other's projects on food safety. A project inventory lists each of the grants in this field. Dr. Lindsay said that there is a potential for interaction among the USDA, NIH, and FDA that did not exist previously.

Adjournment

Dr. Hoofnagle indicated that potential topics for the next meeting include bioimaging in digestive diseases and celiac disease.

Dr. Hoofnagle thanked the speakers and other participants and adjourned the meeting.

Approved by: _____ Date: _____

Jay H. Hoofnagle, M.D., Chairman
Digestive Diseases Interagency Coordinating Committee, NIDDK

Approved by: _____ Date: _____

Thomas F. Kresina, Ph.D., Executive Secretary
Digestive Diseases Interagency Coordinating Committee, NIDDK **[please confirm]**