Prevention of Hemolytic Uremic Syndrome (HUS) Caused by Infection with Shiga Toxin-Producing Escherichia coli (STEC) with Monoclonal Antibody Therapy

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Background: Hemolytic-uremic syndrome (HUS) is characterized by thrombocytopenia, nonimmune hemolytic anemia and renal insufficiency. It occurs most frequently in young children after a prodromal period of bloody diarrhea. The principal cause of HUS is infection with Shiga toxin-producing *Escherichia coli* (particularly serotype O157:H7). The clinical features of HUS have been well described, but its pathophysiology is only partly elucidated and definitive treatment is not available. Approximately 73,000 cases of infection with O157:H7 are thought to occur each year in the United States. About 10 to 15 percent of infected children are at risk of developing HUS.

It is thought that the Shiga toxins (Verotoxins) produced by *E. coli* O157:H7 gain access to the circulation, injure renal glomerular endothelial cells during the early stages of *E. coli* O157:H7 infection, and initiate a pathophysiological cascade that leads to HUS. Studies in animal models have suggested that parenterally administered monoclonal antibodies (MAbs) with the capacity to neutralize the toxins produced by *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC) strains are protective against systemic toxin-mediated disease. The development of humanized mouse monoclonal antibodies reactive with Shiga toxins (Stx) types 1 and 2 provides the potential for their use as therapeutic and/or prophylactic modalities in human illness. NIAID has collaborated in an effort to develop these MAbs as clinically useful products. The MAbs are now at the stage where the IND process could be initiated.

In order to get input about how the use of these MAbs and other similar products could affect the development of HUS, NIAID convened an Expert Panel. The panel was asked to address four questions as the foundation for the discussions. The responses to each of the questions, recommendations and conclusions are summarized below.

Question 1: ***Given what we know about the pathogenic mechanisms of infection with Shiga toxin-producing E. coli (STEC) and the subsequent development of HUS in some children, what is the likelihood that treatment of STEC-infected children with toxin-specific monoclonal (MAbs) could***

potentiate the development and/or severity of watery or bloody diarrhea or HUS?

Although there are a number of hypotheses about the pathophysiological basis of HUS, current understanding of the basis of disease is limited. Studies in humans are difficult and human biopsy material is available principally as retrospective patient specimens. Most nephrologists do not see HUS patients early enough in the course of their illness to enable study of early stage disease. Measurement of circulating factors and/or immune cells can provide some information, but the potential number of measurable factors and alterations in their levels makes such studies complex.

The average seropositivity (by IgG antibody) in an urban population ranging in age from 0-60 years was found to be about 45% to Stx2 and about 12% to Stx1, suggesting that exposure to STEC is more common than initially assumed. The fact that the most susceptible populations for HUS are the very young and the very old suggests that lack of immunity or waning immunity is a susceptibility factor and that natural exposure provides protection. Antibodies that block toxins have been successfully used in a number of human diseases. Data from animal model studies indicate that MAbs to Stx may be useful as therapeutic and prophylactic agents to STEC-associated disease.

From an overall immunopathologic perspective, there is no evidence (with the exception of one observation from the 1980s) that there are autoantibodies in HUS as occur in post-infectious types of pathology such as rheumatic fever. There is no evidence for HUS being an immune complex or serum sickness-like illness. There is limited, at best, evidence for complement-mediated injury to the host as the basis of HUS. However, some data are emerging regarding the coagulation system in HUS. First, the coagulation abnormalities in HUS differ from those observed in classic disseminated intravascular coagulation (DIC), because the concentration of fibrinogen is normal or elevated, and the prothrombin time and partial-thromboplastin time are normal or only slightly prolonged. However, the concentration of circulating prothrombin fragment 1+2 (the peptide that is cleaved from prothrombin when thrombin is generated) increases. Elevated plasminogen-activator inhibitor type 1 (PAI-1) activity and increased tissue plasminogen activator (t-PA) antigen and D-dimer concentrations in plasma further characterize the coagulopathy of STEC-induced HUS. These abnormalities precede the development of renal injury in infected children.

Among the hypotheses for HUS pathogenesis are those that propose an association with complement activation. There was considerable discussion about the possibilities and mechanisms whereby activation of the complement pathways might affect outcomes in patients infected with STECs and about whether treatment with MAbs to Stx might alter disease outcome. It was suggested that the diarrheal stage and HUS be considered separately in terms of

pathology and effects of the MAb treatment. Complement activation in the course of HUS should be considered separately from complement activation due to treatment. It was noted that there are microbial products that can activate complement in the absence of antibody. Whether this pertains to the pathophysiology of STEC-associated illness has yet to be shown. Measurement of complement levels alone is an indirect, and likely insufficient, approach to this issue.

The pathway of toxin trafficking in STEC infection and HUS remains unclear. Studies have shown that the STEC toxins are associated with white blood cells in the circulation; free toxin in the circulation has not been detected. Free toxin can be found in fecal filtrates. The toxins are found for a number of days suggesting that there might be an interval in which MAb can be given, before eukaryotic cell injury occurs. While it is not known whether the toxins can adhere to endothelial cell surfaces, and in that way lead to complement activation and initiation of procoagulant reactions, very little toxin has been demonstrated on the (renal) vessel endothelium or other glomerular tissue, suggesting that large amounts of Stx-antibody complexes might not be formed. Also, toxins are found associated with neutrophils in the blood of healthy family members of HUS cases. However, studies have shown that toxin present on granulocytes is cytotoxic for human glomerular endothelial cells.

Recent studies have suggested that prior to the overt development of HUS many of the pathophysiologic derangements observed during HUS are already manifest in laboratory abnormalities in many patients, including generation of thrombin, inhibition of fibrinolysis and degradation of von Willebrand factor multimers.

In vitro studies indicate that the Shiga toxins can damage cells. Transfer of toxin from neutrophils to glomerular endothelial cells is seen in *In vitro* studies. Anti-Stx antibodies could block this transfer. However, it is difficult to extrapolate these findings to in vivo clinical effects. It is not clear which are the target tissues in the kidney, and how best to minimize Stx-mediated injury to the host. Fc receptors on cells should also be considered for their potential role in immunopathology, especially if an Stx molecule is bound by a monoclonal antibody. However, it should be noted that immune complex deposition in the kidney is not generally observed during STEC-induced HUS.

There was discussion of the use of MAb as intervention modalities in model systems and in other diseases. MAbs have been successfully employed to treat various diseases. However, a theoretical possibility exists that MAb treatment might worsen the disease. It is also possible to modify the MAbs and their properties so as to make them more effective. It was also noted that there are many systems in which MAb don't activate complement. Each system of MAb and its antigen(s) has its own properties and effects.

There have been few (and no controlled) studies of treatment of HUS patients with intravenous pooled gamma globulin. In this very small number of patients, no additional pathology appeared to be caused by this treatment nor was benefit established. In this context, it was also noted that circulating immunoglobulin could suppress unwanted complement effects.

There are various animal models of HUS, all of which differ from human disease. Based on the studies to date of the MAbs in mouse models, protective effects have been demonstrated without indication of potentiation of disease pathology. In the mouse model, in which renal damage is principally tubular and not glomerular, antibody can prevent kidney damage and death even when given 48-72 hours post-infection. Even those animals that fail to survive seem to have less pathological effects than animals not receiving antibody. The mouse model would be amenable to additional studies of tubular effects.

Ferrets were discussed as a possible animal model that might allow for screening for deleterious effects of the MAb. The model relies on oral/enteral administration of STEC to induce disease. The ferrets develop glomerular lesions and thromocytopenia somewhat reminiscent of human HUS. The drawbacks of this model are that because only 20-25% of infected animals develop glomerular lesions, a large number of animals would need to be studied in order to have meaningful results. Additionally, multiple blood draws are necessary, which is technically difficult because of the ferret's small size. Studies with the Stx MAbs have not yet been done in this model. Unpublished observations suggest that more tissue damage can be detected in ferrets when electron microscopy rather than light microscopy is used for assessment. It is not known if ferrets develop coagulopathies and specific immunological reagents for the coagulation system of ferrets are not available, further complicating experiments in this model.

The use of the baboon model was discussed briefly. The unnatural (intravenous) route of toxin delivery in this model makes extrapolations to human HUS and the relevance of effects of MAb in this system uncertain. There is also a rabbit model in which Stx2c-producing strains cause a severe hemorrhagic colitis, but no nephropathy. Thus, no known animal model parallels human HUS after STEC infection.

The experience with the formalin-inactivated respiratory syncytial virus (RSV) vaccine, in which disease was potentiated in vaccinated children, forms the background of concern for intervention in childhood diseases and the basis for trying to predict the potential for deleterious treatment effects. It was pointed out that the product under discussion is a MAb and not a vaccine, and that MAb against RSV are used prophylactically in children without ill effect.

Although studies in the animal models could serve to demonstrate a lack of deleterious effects, the ultimate test of the MAbs would be their use in children.

Question 2: ***What are considered the most important characteristics of a MAb for treatment of STEC-infected children for the purpose of preventing HUS?***

The STEC MAbs are being formulated using methodologies similar to that of MAb products currently on the market. It was suggested that formulation of the product be similar to that of the RSV monoclonal antibody for premature infants. Formulation should recognize that in sick children treatment would be started when the patients have low protein levels. The proposed dosage should take into account the presence of vascular leakage in these children, and the resulting volume of distribution of circulating IgG.

The MAbs to Stx1 and Stx2 would be available in separate vials. It is anticipated that they will be given together with more Stx2 than Stx1 (about 3:1) in order to allow for neutralization of the variants of Stx2. (Coverage of Stx1, Stx2, Stx2c, and Stx2d activatable appeared to be the most clinically relevant.)

The proposed Phase I protocol will include measurements of human anti-chimeric antibody (HACA). With MAb products currently on the market, anti-idiotypic antibodies have not been a problem. Only one or two injections with MAbs are anticipated. The MAb are specific and do not seem to react with other bacterial antigens or with human tissues in the testing performed to date (not discussed at this meeting). The use of both Stx1 and Stx2 MAbs is appropriate given that both toxins can cause severe disease. It was also noted that early in the twentieth century, large amounts of horse anti-diphtheria antibodies had been given to many children without uniformly adverse effects, although there was some occurrence of serum sickness.

The discussion of usage of antibody fragments, rather than whole antibody, concluded that the whole antibody would pose fewer problems in terms of site and rate of clearance. (Fab complexes are more likely to be cleared more rapidly than whole antibody and cleared by the kidney rather than by the liver; however, the site of clearance may not be relevant.)

Question 3: ***What is the definition of the target population, i.e., child presenting with diarrhea caused by STEC?***

There are two populations at high-risk for HUS, young children and the elderly. Data from population-based studies measuring IgG to Stx1 and Stx2 in urban and rural areas show a consistent pattern of lower antibody levels in children and in the elderly, which correlates with higher risk of HUS in these populations. Several overlapping databases on HUS were presented including subject identification in emergency rooms, by stool microbiology, from contacts of those with diarrhea, and from outbreak situations. Most cases of HUS are sporadic rather than outbreak associated. The proportions of cases of diarrhea due to STECs and of these, those who developed HUS, are modest and this will impact

on the design of efficacy studies because of the need to study a very large number of cases prospectively and who will need to be identified early in illness to receive treatment. Given the disease rates, it is difficult to ascertain if there have been trends or changes in incidence. The number of clinical laboratories that test for O157:H7 is only about 60 percent. (There are recent decreases in Campylobacter and Salmonella incidence and so it is possible that O157:H7 could be diminishing, but the current year data are needed to assess this.) In the Pacific Northwest/Seattle area, the number of severe outcomes such as seizures, strokes and fatalities has diminished since 1993. This may be a consequence of the appreciation by clinicians in the Pacific Northwest/Seattle area of the clinical significance of bloody diarrhea and the rapid administration of intravenous fluids to patients presenting with such symptoms.

There was substantial discussion about defining populations appropriate for use of the chimeric MAb. It was noted that it is difficult to define the point for optimal benefit, as intervention in the sickest children could be at a point where the MAb might be less effective or there might be a greater (theoretical) risk of potentiating illness. It appeared that children less than 10 years of age, with bloody diarrhea, brought to the hospital/ER, and having clinical or bacteriological evidence of an *E. coli* O157:H7 infection, could hypothetically benefit from MAb as a therapeutic, as up to 20 percent of these children go on to develop HUS. However, if bloody diarrhea in an Emergency Department setting is the criterion, a much lower percent of subjects will be at risk of developing HUS (probably under 5%), because of inclusion of patients with diarrhea due to pathogens with no or minimal risk of causing HUS.

An appropriate immunoprophylactic usage of the MAbs could be in an institutional outbreak (e.g., day care, nursing home) or when a public-health agency identified point-source exposure (e.g., food-borne outbreak). In this latter situation, the young and elderly, rather than all exposed persons, would be the target groups.

Reliable rapid diagnostic tools for STEC are not currently available, but are being developed. The exact age for inclusion criteria for children might be adjusted depending upon local epidemiological information. For outbreak situations, "ready-to-go" protocols would need to be available for prophylactic use of the MAb during an ongoing epidemiological investigation. In view of the kinetics of outbreak detection, most cases that will subsequently develop HUS, or become symptomatic, are already having symptoms by the time the "at risk" group comes to light. While more people are infected during an outbreak, they often have milder disease than would be seen in a hospital/ER situation evaluating sporadic illness. It should be emphasized that therapies applied to subjects in an outbreak might not be generalizable to all strains of *E. coli* O157:H7.

If toxin is playing a major role in the pathology of HUS and continuing to be present in the gut, on neutrophils, and in the circulation, the administration of the

MAb could ameliorate the disease process if binding to the MAb sequesters toxin away from target sites. On the other hand, there was some concern that without a better understanding of the pathophysiology of disease, perhaps the opposite could occur and disease potentiation would result.

Randomized and blinded studies would be needed to demonstrate therapeutic or prophylactic benefits, but the design of the trial and study protocol were not part of the charge to the expert panel and as such were not discussed in great detail.

Question 4: ***Do the existing preclinical data justify moving the MAb therapy forward into clinical studies in a) Phase I - healthy adults, and b) Phase II - sick children? Please evaluate from the standpoint of both safety and efficacy.***

The proposed Phase I toxicity studies of the chimeric monoclonal antibodies in healthy adults were briefly described to the panel. They appear to be appropriate for consideration for implementation. The current study design of intravenous administration was selected based on the preferred route for therapeutic administration. If the MAbs are eventually used as prophylactic agents, future evaluation of intramuscular administration could be studied as data for that indication are developed. Studies of complement fixation in healthy individuals would be difficult as no antigen (Stx) will be present in probands of the Phase I studies and any changes in complement levels would be quite low as the MAb would be in a "sea of complement." However, there are two circumstances in which one might consider such studies. If the antibody were altered during manufacture or if it encountered an unexpected antigen to which it reacted, measurable changes might occur. Thus, if the MAb disappears from the circulation very rapidly, one might consider doing complement studies, e.g., C4 levels.

Given the current lack of understanding of the pathophysiology of HUS, it may be more appropriate to ascertain the pharmacokinetics of the MAb in healthy children and in sick children without HUS. At this point in time, MAb is not appropriate for compassionate therapeutic use.

The risk for complications in prophylactic studies in children exposed to STEC infections is probably much less than in studies in sick children. However, a substantially larger cohort will be needed to achieve comparable statistical power. Obtaining truly informed consent for studies in sick children will be very difficult and will complicate the logistics of the clinical studies.

General Discussion:

It would be important to understand the normal immune response to the Stx toxins, in terms of immunoglobulin classes and IgG subtypes, and the steps involved between the introduction of toxins into the body and the induction of the

coagulopathies and other pathological effects. This might provide insight about the potential for immunopotentiation by monoclonal antibodies or reassurance that this was not likely.

While the historical observation of disease potentiation with formalinized RSV vaccine provides pause, there is some current experience with clinical use of MAb that suggests that there could be merit to this approach. A monoclonal RSV antibody is currently being used as a prophylactic modality for high-risk children. Although the RSV monoclonal had not been effective when used as a therapeutic modality, it is effective in preventing RSV disease. Monoclonal antibodies to Staphylococcus are currently in Phase I trials in adults and neonates. In addition, polyclonal antibodies to toxin-mediated diseases have a long history of successful medical use.

The long-term consequences of HUS are not known. There is some suggestion that there could be increased risk of kidney problems and hypertension. However, given the interval between HUS and the later development of clinical problems, plausibly related to HUS which may also be affected by other variables (such as smoking, and over-weight) that could impact on pathological consequences; this would be very difficult to study and verify.

It was also noted that STEC have been discussed as potential bioterrorism agents and the monoclonal antibodies, although not initially conceived for this purpose, could have relevance in the biodefense context.

Conclusions:

There is clearly a need to develop therapeutic, and preferably prophylactic, modalities for HUS. Phase I pharmacokinetic studies in adults with intravenous administration of escalating doses of anti-Stx MAb are appropriate at this time with the assumption that the long-term goal is to proceed with studies in at-risk populations. Before proceeding with studies into other phases and other groups, it is important to have more data on the pathophysiology of HUS to determine its underlying basis, and thus be able to better assess the risk for MAb to diminish, rather than enhance disease. Additional *In vitro* and animal model studies may provide more insights into pathogenesis and provide safety assurance for further studies in humans. Characterization of the normal immune response to toxin in naturally occurring infections in both asymptomatic and symptomatic humans could provide important insights into the potential for MAb to parallel such protective antibody responses rather than potentiate pathological responses. While animal studies can provide additional information, as in other diseases, only studies in humans can allow for the determination of the efficacy of the MAbs in treating and/or preventing HUS.

EXPERT PANEL MEMBERS
Prevention of Hemolytic Uremic Syndrome (HUS) Caused by Infection with

Shiga Toxin-Producing Escherichia coli (STEC) with Monoclonal Antibody Therapy
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Invited Expert	Area of Expertise
David Acheson, M.D. Associate Professor University of Maryland School of Medicine Department of Epidemiology and Preventive Medicine	Research in enteric diseases
Martin M. Bitzan, M.D. Department of Pediatrics Pediatric Nephrology Wake Forest University Baptist Medical Center	Pediatric nephrologist Infectious diseases Physician Scientist Endothelial/ renal tubule cell injury and HUS
Michael Frank, M.D. Professor, and Chair Department of Pediatrics Children's Health Center Durham, NC	Immunology Expertise Complement
Patricia Griffin, M.D. MS A38 Foodborne Diseases Epidemiology Section DBMD, NCID, CDC	Epidemiologist
Mohamed Karmali, M.D. Director General Laboratory for Foodborne Zoonoses Health Canada	Medical microbiology Infectious diseases
Alison D. O'Brien, Ph.D. Chair, Department of Microbiology and Immunology Uniformed Services University of the Health Sciences	Microbiology Developed Shiga toxin MAb Animal models of HUS
Marguerite Neill, M.D. Brown University School of Medicine Memorial Hospital, Division of Infectious	Infectious Diseases Public Health Laboratory research

Disease	Epidemiology Clinical Trials
Phillip I. Tarr, M.D. Children's Hospital and Regional Medical Center Department of Pediatrics Seattle, WA	Pediatric Gastroenterologist Clinical and population-based research on diarrheagenic *E. coli*
Mark Taylor, M.D.* Department of Nephrology Birmingham Children's Hospital Kadywood Middleway United Kingdom	Nephrologist Renal immunobiology Rat model Cytokine responses
N.C.A.J. van de Kar, M.D. Dept. Pediatric Nephrology University Hospital Nijmegen The Netherlands	Pediatric Nephrologist
* Dr. Taylor was unable to attend, but provided written comments to the questions distributed to the panel members; his comments were included in the panel discussions.	