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COMMENTARY

Comparing the Roles of Antibodies to Nontyphoidal *Salmonella enterica* in High- and Low-Income Countries and Implications for Vaccine Development

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The article by Trebicka et al. (1) in the current issue of *Clinical and Vaccine Immunology*, on antibodies to *Salmonella* among adults and children in the United States, is paradoxically important for our understanding of immunity to nontyphoidal *Salmonella* (NTS) globally and the development of a much-needed vaccine for Africa. In recent years, there has been a growing awareness of the major public health problem attributable to NTS infections in sub-Saharan Africa (2–5). Unlike the self-limiting gastroenteritis commonly seen in high-income countries (6), the presentation in Africa is often with life-threatening invasive NTS (iNTS) disease (2, 3, 5). This usually manifests as bacteremia, where fever may be the only symptom, but also as meningitis (7).

Incidence levels of iNTS are around 500 cases/100,000 people/year among African children under 2 years (8, 9), with case fatality rates of 20 to 25% (2). Diagnosing iNTS without blood culture facilities is particularly difficult, and there is an increasing frequency of antibiotic resistance, with no vaccine available for use in humans (3). An effective vaccine could have an enormous beneficial impact on health care in the continent. This would make a strong positive contribution to achieving the Millennium Development Goals, particularly goal 4, the reduction of child mortality (10). As with young children, HIV-infected individuals of all ages are highly susceptible to iNTS disease (11, 12), and there are well-recognized clinical associations with malaria (13), anemia (14), and malnutrition (15).

The high prevalence of iNTS disease in Africa and its relative rarity in high-income countries may relate to the specific microbiological features of the circulating strains and the transmission of the bacteria in Africa (3). Recently, NTS isolates in Africa have been shown to be genetically different from those present elsewhere. *Salmonella enterica* serovar Typhimurium, the most common serovar responsible for iNTS disease in Africa, with a distinct multilocus sequence type, ST313, has been implicated in the appearance of epidemic iNTS disease (4, 16). This pathovar is rarely found outside Africa and has genomic features in common with *S. enterica* serovar Typhi, most notably the presence of high levels of genome degradation (16). However, relatively little is known about its phenotypic features that are associated with invasive disease. Transmission of iNTS in Africa also appears to be different from the food-borne or animal-related transmission commonly associated with *Salmonella* infections in high-income countries. There is evidence for human-to-human spread as the main form of transmission in Africa (17, 18). This may be facilitated by the lower levels of sanitation and the lack of availability of clean water in much of the continent.

Apart from the distinct bacterial genotype associated with iNTS, differences in immunological status are likely to have an impact on the occurrence of iNTS disease (3). This is not least because early childhood can represent an immunologically naive state and the clinical associations with iNTS disease in Africa (HIV, malnutrition, malaria, and anemia) can all have an impact on immunity. A proper understanding of immunity to NTS is required for the development of a vaccine against iNTS disease for Africa. Hence, studies of immunity to NTS are important and should be conducted in high-income countries as well as low-income countries. Mechanistic immunological research into *Salmonella* infections in high-income countries has tended to focus on disease in mice, resulting in an unusual paradigm in which the more-recent studies on immunity to iNTS in humans have been conducted in low-income countries (19, 20). The current study by Trebicka et al. represents a welcome step toward redressing this imbalance and attaining a more holistic overview of immunity to NTS infections at a global level.

It is key for us to acquire a fuller understanding of the mechanisms of protective immunity and to identify the relevant target antigens for developing such immunity (21). Cell-mediated immunity has long been viewed as essential for protection against this facultative intracellular pathogen (22). While cell-mediated immunity is important for clearing intracellular disease, it is ineffective at preventing fatal bacteremia. In contrast, bacteremia can be countered by antibody acting both directly through complement-mediated killing (19) and indirectly through opsonic mechanisms and blood cell phagocytes (20). There is strong epidemiological evidence from Africa for the protective effect of antibody, with markedly reduced numbers of cases of iNTS disease being associated with placentally transferred IgG and the acquisition of antibody to NTS with age (19).

Relatively little work on the key targets of protective, acquired immunity to iNTS in Africa has been published. Investigation into the underlying mechanisms responsible for the link between HIV infection and fatal iNTS disease in African adults found that sera from some HIV-infected individuals were unable to kill *S.* Typhimurium *in vitro* (23). That study went on to show that the lack of

Published ahead of print 31 July 2013

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doi:10.1128/CVI.00465-13

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.

killing was associated with the presence of high levels of antibodies targeting the O antigen of *S.* Typhimurium LPS. When purified, such antibodies blocked *in vitro* complement-mediated killing of *Salmonella* by antibodies from healthy individuals.

Interestingly, Trebicka and colleagues have shown bactericidal activity against *S.* Typhimurium in deidentified sera from healthy adults and children (6 months to 5 years of age) ($n = 49$) attending clinics in Boston, as has been found in Africans (19, 23). Comparison with the results of work from Malawi has been facilitated by the use of similar methodologies in the different studies. The main obvious difference between these studies was the use of a common laboratory strain of *S.* Typhimurium (SL1344; ST19, a genotype common worldwide) by Trebicka et al. and the use of the invasive *S.* Typhimurium ST313 isolate, D23580, by the African studies (16, 19, 20, 23). As shown for African children (19), the need for both antibody and complement for cell-free bactericidal activity was confirmed. Although all sera from children in the Boston study were able to kill SL1344, the level of killing was significantly lower than that effected by sera from adults, presumably due to lower levels of antibodies to *S.* Typhimurium.

Trebicka et al. detected IgM and IgG antibodies against *S.* Typhimurium LPS in their sera and speculated that the bactericidal antibodies are specific for LPS. Removal of bactericidal activity after preabsorption with LPS from *S.* Typhimurium, but not LPS from *E. coli*, supports this concept. It suggests that the specificity of these bactericidal antibodies is for the O antigen of LPS, since this is the most variable moiety of LPS among different species and serovars of Gram-negative bacteria. The findings may appear contradictory to those from studies in Malawi that focused on HIV-infected adults (23). However, these HIV-infected individuals had marked immune dysregulation. Many of them had CD4 counts less than 200 cells/μl, and none was on antiretroviral therapy. All had hypergammaglobulinemia. Lack of bactericidal activity and inhibition of the bactericidal activity of control serum was observed in a subset of HIV-infected sera containing the highest concentrations of total and anti-LPS IgG antibodies. The inhibitory effect could be recapitulated using affinity-purified anti-LPS IgG from either HIV-infected or non-HIV-infected bactericidal sera provided they were concentrated to the same high levels present in the HIV-infected inhibitory sera (23).

Further work on the African sera has demonstrated that at concentrations found in non-HIV-infected sera and most HIV-infected sera, these anti-LPS antibodies are bactericidal (24). In addition, absorption studies similar to the ones conducted by Trebicka et al. show that bactericidal activity can be curtailed in the African sera by removal of anti-LPS antibodies (25). Trebicka et al. speculated that the contrast between bactericidal and inhibitory anti-LPS antibodies from Africa and the United States might result from exposure to the ST313 pathovar of *S.* Typhimurium.

Against this, a recent study into the immunogenicity of the *S.* Typhimurium ST313 D23580 isolate demonstrates that bactericidal antibodies are induced in mice immunized with this strain (26) and that glycoconjugates consisting of D23580 O antigen linked to cross-reacting material 197 (CRM_{197}) induce bactericidal antibodies against D23580 (27). Passive transfer to naive mice of immune sera from mice immunized with an *S. enterica* serovar Enteritidis O-antigen flagellin glycoconjugate vaccine has been shown to be protective against an invasive *S.* Enteritidis isolate from Mali, West Africa (28). Furthermore, mice immunized with a live attenuated vaccine strain derived from another African ST313 isolate also developed bactericidal antibodies against strains of this genotype (29).

Overall, the results of this mechanistic study by Trebicka et al., conducted in a high-income country, have remarkable concordance with those of African studies. Although, at very high concentrations, anti-*Salmonella* LPS IgG antibodies can exert an inhibitory effect on *in vitro* complement-mediated killing of *S.* Typhimurium (23), at most concentrations they are bactericidal (24). Perhaps the most surprising finding of the Boston study is the almost-universal presence of anti-*Salmonella* LPS antibodies in a collection of sera from healthy children and adults from that city. The authors speculate two possible reasons for this: first, the development of cross-reactive antibodies against *S.* Typhimurium LPS from exposure to environmental LPSs from other organisms, and second, the occurrence of subclinical infections with *S.* Typhimurium leading to the development of these antibodies. Both explanations are plausible. While the former will require more than exposure to *E. coli* LPS, the latter is not as strange as it may appear. Asymptomatic infections with *Salmonella*, when looked for, are more common than expected (30). Moreover, when HIV/AIDS first emerged in high-income countries, severe disease with NTS was a common presentation (31, 32), suggesting that exposure to these bacteria is more widespread than appreciated.

One of the 49 sera examined in the Boston study was unable to kill *S.* Typhimurium and was able to inhibit the killing of these bacteria by control sera. The likely mechanism appears to be different from that in HIV-infected African adults. Using absorption studies, the authors were able to implicate anti-*Salmonella* LPS IgM, rather than IgG. Surprisingly, this was associated with a decreased level of complement deposition, rather than the high levels of complement deposition seen in the African studies. IgM is normally a potent activator of complement on *Salmonella* (33), and the authors speculate that structural idiosyncrasies of these particular IgM molecules might interfere with complement binding. It will be interesting to see whether such a mechanism can be demonstrated in a future study. The observation suggests that there are different mechanisms by which antibody-mediated killing of *Salmonella* can be blocked.

In conclusion, the findings of the Boston study, together with ongoing emerging work from Africa, indicate an important role for anti-LPS antibodies for complement-mediated killing of *Salmonella* and that the induction of such antibodies may be an effective vaccine strategy. The bactericidal and protective efficacies of antibodies to *Salmonella* outer membrane proteins (23, 34, 35) and flagellin (36) have also been described. The presence of such protein antigens in a vaccine may be advantageous, since they have the added potential benefit of being able to activate *Salmonella*-specific $CD4^+$ T helper cells. Together, these results suggest that glycoconjugate vaccines in which *Salmonella* LPS O antigen is coupled to a *Salmonella*-specific or other protein can elicit protective antibodies and would be effective in reducing iNTS disease in Africa.

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